FEB - FRESENIUS ENVIRONMENTAL BULLETIN
Founded jointly by F. Korte and F. Coulston
Production by PSP - Vimy Str. 1e, 85354 Freising, Germany in
cooperation with PRT-Parlar Research & Technology
Vimy Str 1e, 85354 Freising

Printed in Germany-ISSN 1018-4619

Fresenius Environmental Bulletin is abstracted/indexed in:
Biology & Environmental Sciences, BIOSIS, CAB International, Cambridge Scientific abstracts, Chemical Abstracts, Current Awareness, Current Contents/Agriculture, CSA Civil Engineering Abstracts, CSA Mechanical & Transportation Engineering, IBIDS database, Information Ventures, NISC, Research Alert, Science Citation Index (SCI), Scisearch, Selected Water Resources Abstracts

CONTENTS

ORIGINAL PAPERS

© by PSP Volume 27 – No. 12B/2018 pages 9433-9440 Fresenius Environmental Bulletin

A STUDY ON AQUATIC HYPHOMYCETES FROM BURSA – ULUDAG MOUNTAIN, KIRAZLI PLATEAU

C Cem Ergul[1,*], Sahsine Muratoglu[1], Hasan Akgul[2], Demet Yilmazkaya[1]

[1]University of Uludag, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey
[2]University of Akdeniz, Faculty of Arts and Sciences, Department of Biology, Antalya, Turkey

ABSTRACT

This study was carried out in Uludağ National Park situated at approximately 1400 m asl on a plateau within the boundaries of Turkey's fifth national park. The Kirazlı Plateau has a small drinking water reservoir with favorable conditions for freshwater mitosporic fungi (Ingoldian fungi, or aquatic hyphomycetes). The purpose of this study was to define the distribution and identities of fungi from the reservoir dam and spores trapped in foam from immediately adjacent areas. It presents the distribution and identification of 5 species, *Alatospora acuminata* Ingold 1942, *Dendrospora erecta* Ingold 1942, *Fontanospora eccentrica* (R.H. Petersen) Dyko, *Tricladium angulatum* Ingold 1942, *Tricladium splendens* Ingold 1942. *Dendrospora erecta, Fontanospora eccentrica* and *Tricladium splendens* are new records for Turkey.

KEYWORDS:
Freshwater fungi, Ingoldian fungi, Marmara region, Turkey, Uludağ National Park

INTRODUCTION

Climate change is negatively affecting many living organisms. The Intergovernmental Panel on Climate Change (IPCC) has presented evidence that in response to current climate change, many species in terrestial, marine and freshwater ecosystems has continue to change their geographical and seasonal distributions as a result of migration and evolving relationships between species. The unavoidable global climate change has affected aquatic ecosystems and may expose the inability of vulnerable species to adapt to the new environmental conditions and eventually result in their extinction [1].

The aquatic habitat provides a suitable environment for many micro- and macroorganisms and may serve as biological reservoirs. In particular, lotic and lentic biotopes serve as important habitats for such fungal groups as the Chytridiomycota, Hyphochytriomycota, Oomycota, Ascomycota and aquatic hyphomycetes (mitosporic fungi). The aquatic hyphomycetes are a dominant mycoflora on submerged decaying allochthonous plant debris lotic and to a lesser extent in lentic systems [2]. Bärlocher [3] stated that aquatic hyphomycetes are believed to be sensitive to pollution and are usually associated with clean and well aerated freshwaters. They convert some of the organic compounds into fungal biomass [4]. Especially in lotic habitats, aquatic hyphomycetes are important in the degradation and recycling of leaf litter. This degradation process results in the loss of litter mass in freshwater ecosystems [5, 6]. They have the ability to degrade the leaf matrix by the activities of cellulose and pectinase (extracellular enzymes), which have different pH optima [3, 7, 8]. Hence, aquatic hyphomycetes comprise a major link in the stream food web. Freshwater ascomycetes which are often endophytes and saprobes on dead plant materials, algae and aquatic macrophytes, can be found in lotic and lenthic ecological habitats [9]. In addition, these fungi colonize submerged leaves of riparian vegetation and also actively function in the energy turnover and trophic dynamics of aquatic ecosystems.

Kirk et al. [10] reported that around the world, hyphomycetes group organisms had 9000 species in 1800 genera. Over 600 species of freshwater fungi and fungal-like organisms have been discovered, and of this the temperate regions tend to have a higher number of species as a contrast to the tropical region [11]. These consist of 300 ascomycetes, 300 mitosporic fungi and a quantity of chytrids and oomycetes [12]. A sexual stage has been recognized in roughly 10% of the 300 species of aquatic hyphomycetes that have been reported [3, 13]. They are a phylogenetically heterogeneous, ecologically defined group and depend entirely or partly on freshwater for their life cycle [14]. Their mycelia usually grow on decomposed leaves whilst their conidia are released from mycelia in the leaves and collect in foam [15]. Lignicolous freshwater fungi include freshwater ascomycetes [16] and anamorphic hyphomycetes and coelomycetes [17]. In aquatic hyphomycetes, the production and dispersal of conidia occur under water. These fungi also develop on submerged wood and twigs, but they have not been observed on coniferous wood [18]. Anamorphic freshwater fungi were first observed by Ingold [19] as a unique group of freshwater fungi that normally exist on partially decomposed

and immersed leaves of angiosperms. They have therefore been referred to as Ingoldian fungi in his honor. Predominately aquatic, Ingoldian hyphomycetes are collected in small to medium-sized, relatively clean, well-ventilated rivers and streams passing through forest or wooded areas with an excessive amount of foam in a conidium-rich environment. High biomass and species diversity of Ingoldian fungi mostly exist on deteriorating woods and leaves in a well - aerated streams [20]. Ingoldian hyphomycetes have adapted to small and fast-flowing river waters in a number of ways, including the conidial form, mucilage release, and appressorium formation. Extracellular enzyme production varies in the presence of rapid colonization and sporulation. In addition, a temperature close to 0 □C is another important factor that affects sporulation and development [21]. They are infrequently observed on leaves of *Betula* and *Fagus*. Moreover, this fungal group was rarely or never observed on *Ulmus* and *Corylus*. In contrast to *Betula, Fagus* and *Quercus*, which have slowly decaying, leathery leaves, the leaves of *Alnus* species undergo a much faster degradation [20]. Fungal species composition varies with latitude [18, 22].

The naturally occurring foams produced by water turbulence normally trap the conidia released underwater. Tetra-radiate conidia are the type most commonly observed in many aquatic hyphomycetes, in which four long and straight branches emerge from a single point [18]. Under turbulent settings the branches of conidia may be very useful in attachment of spores to the substratum or intertwine the spore inside organic debris, which eventually turn out to be a substratum [22]. Tetra-radiate spores usually have three points of contact with the surfaces, for instance leaves, whilst sigmoid spores have two [23]. In addition, as noted by Webster, the sedimentation rates of tetra-radiate conidia are not consistently lower than those of more conventionally shaped conidia [24]. Ingold [19] stated that the dominance of certain spore shapes indicates convergent evolution. A lot of species are distinctively adapted to dispersal in running water. The topic did not receive much experimental attention, but it has been documented in many countries worldwide [11].

Turkey is located at the intersection of three phytogeographical regions (Mediterranean, Euro-Siberian, and Irano-Turanian) and, thus, has different regional proportions and rich diversity of species [25, 26]. Turkey has limited aquatic biodiversity areas. The study area in the Marmara region is at the southern part of the Marmara Sea and a Mediterranean transition climate is dominant in the region. The aquatic fungal biota of the Marmara region and of Turkey is still unexplored. There have been only two studies on the aquatic hyphomycetes in Turkey [27, 28]. Both were conducted using decaying leaves and foam recovered in the eastern region of Anatolia in the Aras River and its tributaries. Species obtained from these studies include *Alatospora acuminata* Ingold 1942, *Anguillospora longissima* (de Wild.) Ingold 1942, *Articulospora inflata* Ingold 1944, *Articulospora proliferata* A. Roldan & W.J.J. van der Merwe 1990, *Clavariopsis aquatica* de Wild. 1895, *Clavatospora longibrachiata* (Ingold) Sv. Nilsson ex Marvanova & Sv. Nilsson 1971, *Flagellospora curvula* Ingold 1942, *Heliscella stellata* (Ingold & Cox) Marvanova 1980, *Heliscus lugdunensis* Sacc. & Therry 1880, *Lemonniera aquatica* de Wild. 1894, *Lemonniera centrosphaera* Marvanova 1968, *Tetracladium furcatum* Descals 1983, *Tetracladium marchalianum* de Wild. 1893, *Tricladium angulatum* Ingold 1942, *Tricladium curvisporum* Descals 1983, *Tricladium giganteum* S.H. Iqbal 1971, *Tricladium gracile* Ingold 1944, and *Triscelophorus monosporus* Ingold 1943.

MATERIALS AND METHODS

The study was carried in a small (average depth approximately 4 m) stream leading into a dam that is fed from the upper zone of Uludağ Mountain on the Kirazlı Plateau (40º07′00″ N, 29º05′55″ E, elev. ca. 1570 m), by a well-aerated stream running through forest areas. The Kirazlı water reservoir is located on the Kirazlı Plateau with its unique biotic and climatic conditions to which it is connected by a rich forest cover. Materials were obtained between April 2009 and July 2010 study period at difference intervals. Due to the harsh winter conditions no material could be obtained in February and March. To the extent possible, decaying angiosperm leaves and detritus material were collected from the dam lake. In particular, *Populus tremula* and *Fagus orientalis* leaves obtained from the dominant riparian vegetation were gathered from the stockpile leaf packs. A great quantity of samples was collected from the bottom layer of the dam, and a quantity of water samples was collected from foam bubbles that formed on the dam overflow. The material was collected and evaluated by partly modified routine laboratory methods [18, 20, 22, 29].

During the study, a total of 97 samples were collected and incubated in the laboratory. A small amount of water was added to the collected samples, which were placed in sterile plastic sealed containers. These containers were then transported to the laboratory and washed in tap water. 30 mL of distilled water was added to 10 cm diameter glass Petri dishes and incubated at approximately 10 □C to induce sporulation. After 24 hours, 5 to 10 cc of methylene blue solution was added to improve visibility on the Petri dishes. Careful microscopic observations were focused on the veins and petioles of the older leaves gathered from the surface layer

and bottom of the reservoir. The samples were examined under a light microscope for the development and emergence of conidiophores at regular intervals over a 15-day observation period. In addition, conidia of aquatic hyphomycetes were collected for isolation from foam formed on the dam overflow. For this purpose, samples were taken from the parts of the dam that produced high amounts of foam due to elevated flow rates. Immediately after sampling, the fixing solution FA was added to the jar to inhibit spore germination. Furthermore, fresh foam samples were transported to the laboratory on the day of the field study and transferred to centrifuge tubes. Microscopic examination for fungal spores was subsequently performed after adding 1-2 drops of methylene blue solution into the mixture. After incubation, for approximately 10 minutes, the pipetted samples were transferred onto a microscope slide in a drop of water. Then, the covered slides were examined under a light microscope. Photographs were made of the foam and decaying leaves of the samples with an Olympus BX 51 microscope. Taxonomic characterizations of the samples were based on microscopic observations of the specimens obtained from semipermanent slides. Taxonomic identifications of the samples were made based on keys by Petersen [30], Nilsson [20], Crane [29], Ingold [18], Subramanian [31] and Gulis et al. [32]. Hence, some of the general characters and related to the microscopic observations and geographic

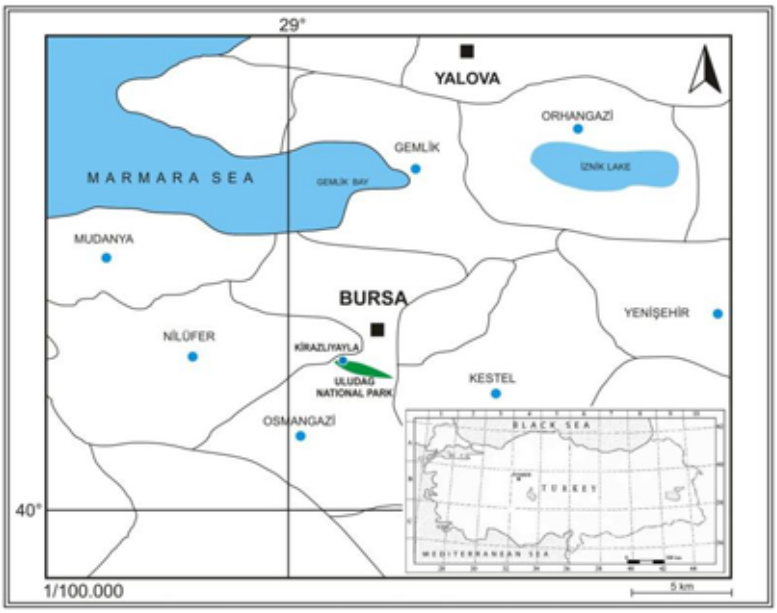


FIGURE 1
The study area on a map of Turkey with a grid system

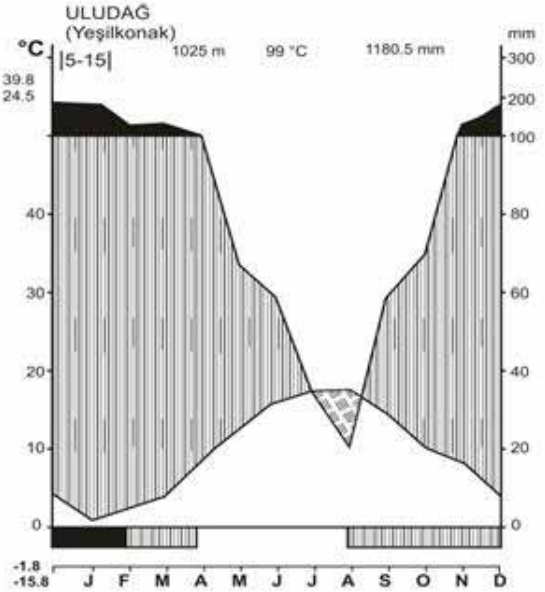


FIGURE 2
Climate diagramme for Uludağ-Yeşilkonak

distributions are derived from authorized publications, with our evaluations based on the lists of species in several studies [18, 20, 32, 33]. A map of the study area (Fig. 1), the seasonal distribution and occurrence of fungal taxa (Table 1) and a climate diagram are shown (Fig. 2) and also microscopical images are presented (Figs. 3–7). New records of taxa for Turkey are indicated by an asterisk.

RESULTS

Uludağ and the Kirazlı Plateau area are in the vicinity of Uludağ National Park in Turkey. The site of the dam of Kirazlı is approximately 1400 m asl. The primary objective of this research was to obtain qualitative data about the distributions and identities of aquatic fungi from Uludağ Mountain, which is under the influence of the Mediterranean climate of the South Marmara region of Turkey. Five species, belonging to four genera, of freshwater hyphomycetes were isolated from the dam, especially in the upper zone, between April 2009 and July 2010. These taxa are *Alatospora acuminata* Ingold, *Dendrospora erecta* Ingold, *Fontanospora eccentrica* (R.H. Petersen) Dyko, *Tricladium angulatum* Ingold, and *Tricladium splendens* Ingold. *Dendrospora erecta, Fontanospora eccentrica* and *Tricladium splendens* are new records for Turkey.

List of species. *Alatospora acuminata* Ingold 1942. This was isolated from foam samples. Most records have been from temperate regions and many of the records are from the UK [18]. This is one of the most common species in Sweden and also appears particularly abundant and dominant in the foam samples. This species is often prevalent in late autumn, winter and early spring in lotic waters and spores are also observed in some mountain rivers in Sweden [20]. The morphological appearance formed from two side arms can be easily identified major axis and a small tetra-radiate phialocondial structure [18]. Phialides develop singly at the tips of short which has two divergent lateral arms arise and extend simultaneously [33]. Fig. 3

FIGURE 3
***Alatospora acuminata* Ingold**

***Dendrospora erecta* Ingold 1943.** This was isolated from leaf samples. This taxon is reported from Europe, Asia and North America [20]. The conidia are easily defined by a straight main axis and a large thalloconidial structure consisting of one or more level side arms. It exhibits a basal branch near the base of the arms [18]. Fig. 4

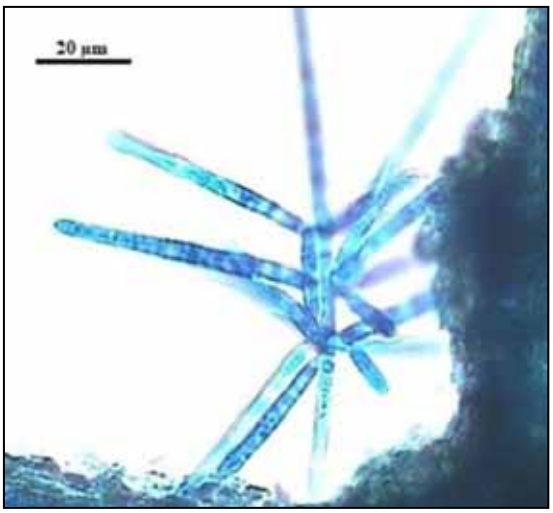


FIGURE 4
***Dendrospora erecta* Ingold**

***Fontanospora eccentrica* (R.H. Petersen) Dyko, Trans. Br. mycol. Soc. 70(3): 412 (1978).** It was isolated from the foam sample. Taxon have been reported from USA, Canada, Ireland and Central European from rivers and lakes in especially from submerged decaying leaves [30, 34]. Conidia with geniculate or curved axis and two branches attached near its middle. Branches subopposite, not aqual in length, axis subconstricted at a septum between branch insertions. Axis typically over 90 mikrometers long, elements cylindrical. Spore production begins with septum formation 20-30 micrometers from the tip of the aleuriospore [30, 32]. Fig. 5

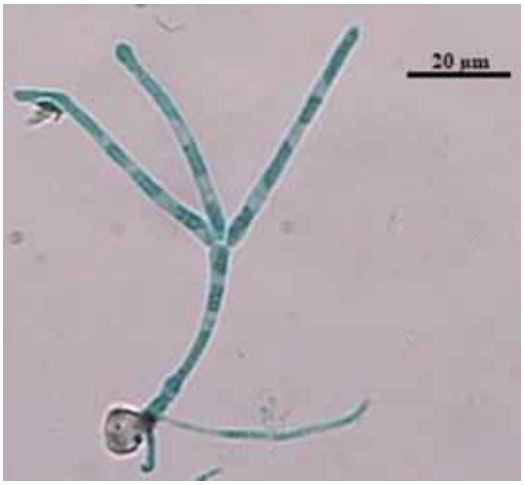


FIGURE 5
***Fontanospora eccentrica* (R.H. Petersen) Dyko**

***Tricladium angulatum* Ingold 1942.** Sample material was isolated from the leaves of undetermined species. Although it was rarely found in the foam specimens, it is a very common and easily identifiable species. It is recorded from Europe, Africa, North America. The main axis of the terminal thalloconidium has a sharp slope at the site of the side arms [18, 20]. Fig. 6

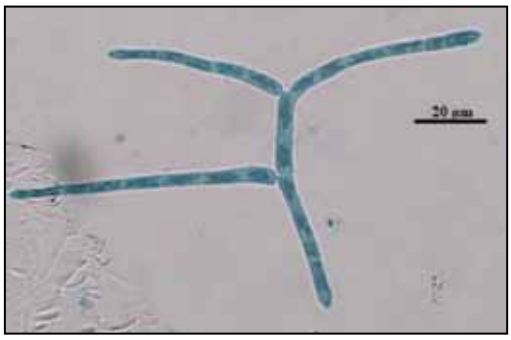


FIGURE 6
***Tricladium angulatum* Ingold**

***Tricladium splendens* Ingold 1942.** The specimen was isolated from the foam sample. This taxon is easily recognized from the leaves or in foam specimens and also shows a very wide distribution in Sweden and the UK. It is probably common in the entire Northern Hemisphere and is also distributed in Africa and Asia. The conidia have a slightly curved terminal thalloconidium branching at different levels in one or more of the long main axes [18, 20]. Fig. 7

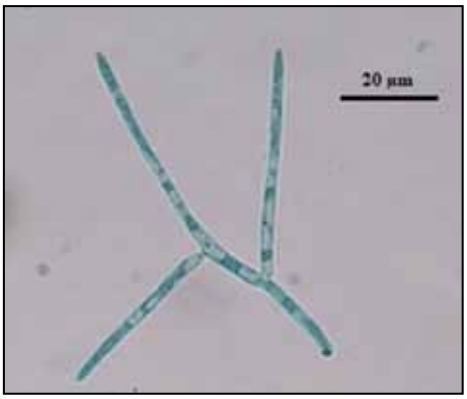


FIGURE 7
***Tricladium splendens* Ingold**

DISCUSSION

Ingold [18] stated that the aquatic hyphomycetes are especially abundant in the end of third and fourth months of the year, when lower water temperatures (less than 20 ºC) provide optimum growth compared to terrestrial species. By the end of the winter season during April and May, significant increases in the number of species and sporulations occur in streams affected by snow melt. In most temperate streams, leaves appear within a few weeks in the fall, disappear within a few months due to drift and invertebrate feeding. They also occur in discrete units, which generally necessitates a reproductive effort by a fungus every time it colonizes a new leaf [35].

This study was carried out in a water reservoir located on a high plateau in the Uludağ National Park, an area with an intense Mediterranean climate, with a short dry period in summer and rainfall in spring and autumn (Fig. 2). In the context of these data and ombrothermic diagrames, the climate of Uludağ Mountain is described as a subsector of rainy Mediterranean bioclimatic stratum [25, 26]. As shown in Table 1, no isolation took place during the winter months (February and March) due to prevailing harsh conditions in the study area. In April of 2009 and 2010 we were able to record the most species and identified 4 different taxa. No sporulation was observed in any samples during June and July. During the summer the amount and condition of water in the dam region was substandard, due to the onset of a drought period. Decreased precipitation, increased temperature, excessive evaporation resulted in a lower water level, which provided unfavorable conditions in these study periods for the development of aquatic fungi. We recorded only two taxa from August to December. In September we recovered one species, two species in October and one species in November. Thus, seasons that allow fungal conidia transported from the upper zone of melting snow and rain water can also carry over to the dam area with a high amount of substrate material. Despite this, in the summer and autumn seasons, local climate conditions negatively impacted the supply of water to the reservoir due to extremely low water flow (Table 1).

In the study area, rainfall and temperature influenced the amount of plant material entering the streams from deciduous trees in the early spring and the autumn. This and similar circumstances that occur in the region, climatic conditions can cause a fungal sporulation increase. In their study, Iqbal & Webster [36] observed high leaf fall in early winter containing fast-flowing rivers. Hence, Descals [37] stated that autumn in cold and temperate climates tends to be the highly productive collection season in streams flowing through deciduous woods even though minor peaks may occur at other times. Our findings are partly compatible with these statements concerning species recoveries from the same periods (Table 1). Descals and Moralejo [38] are also stated that the small number of species in temporary streams is associated with the seasonality of the water flow. As stated by Khan [2], aquatic fungi exhibit a remarkable seasonality; to explain this, a couple of models have been put forward, which tend to establish a strong correlation between their seasonal occurrence and the temperature and pH of the aquatic systems.

TABLE 1
Occurence of conidia in during the study periods *

Species	Year/ Month	1	2	3	4	5	6	7	8	9	10	11	12
Fontanospora	**2009**	-	-	-	-	-	-	-	-	+	+	-	-
eccentrica	**2010**	+	-	-	+	+	-	-	•	•	•	•	•
Tricladium	**2009**	•	•	•	+	+	-	-	+	-	-	+	+
spendens	**2010**	-	-	-	-	-	-	-	•	•	•	•	•
Tricladium	**2009**	•	•	•	-	-	-	-	+	-	+	-	+
angulatum	**2010**	-	-	-	+	-	-	-	•	•	•	•	•
Alatospora	**2009**	•	•	•	+	-	-	-	-	-	-	-	-
acuminata	**2010**	-	-	-	-	-	-	-	•	•	•	•	•
Dendrospora	**2009**	•	•	•	+	-	-	-	-	-	-	-	-
erecta	**2010**	-	-	-	-	-	-	-	•	•	•	•	•

* (+) observed, (-) not observed, (•) untreated period

On the other hand, the regional conditions were adversely affected in other water quality parameters, e.g., salinity, turbidity, and BOD. Hence, this condition was limiting for the proliferation of aquatic hyphomycetes in this extreme period. Without doubt, the occurrence in this season of adverse macroclimatic conditions makes the continued existence of all living organisms in the aquatic environment difficult.

We have identified five species, *Alatospora acuminata*, *Dendrospora erecta*, *Fontanospora eccentrica*, *Tricladium angulatum*, *Tricladium splendens*. *Dendrospora erecta, Fontanospora eccentrica* and *Tricladium splendens* are new records for Turkey, which was a lot less than expected for this region. Potential reasons include the harsh regional climate, but also the periodic cleaning of this reservoir before the autumn rains that occur in the region. All these activities caused the loss of organic substrates that are essential for the development of aquatic hyphomycetes. Hence, the accumulation of decaying debris and suspended material remain at very low levels in subsequent seasons. This also limited the seasonal recovery of conidia from the sample materials for laboratory studies. These interventions interrupted the degradation processes and disrupted nutrient cycling and undoubtedly limited the occurrence of species.

CONCLUSION

Most freshwater habitats are vulnerable to disturbance. Any disruption inside the drainage basin will have an effect on the in-stream communities through wash-off or run-off processes, and due to the downhill flow of water. Thus, any alteration in the headstream areas will eventually affect the downstream reaches [11]. As the freshwater habitats are shrinking due to the activities of human and climate change, it is very important to understand the geographical distribution patterns of freshwater ascomycetes. Worldwide increases in temperature believed due to the greenhouse effect, acidification and exposure of aquatic ecosystems to anthropogenic pollutants are important factors influencing the distribution and composition of species in the aquatic environment, in the short term, it is important to determine which taxa of aquatic organisms exist in the world. Dubey et al. [34] reported that pH alone cannot be a major factor accounting for low species numbers, for appreciable differences also existed for most other chemical characteristics such as high sulfate and/or aluminum concentrations. In order to maintain the ecological processes in aquatic ecosystems, it very important to preserve the biological qualities. Given these considerations, it is crucial to understand and preserve our areas of aquatic diversity.

All of the identified species were specifically recorded from the region for the first time and three of them are also new records for Turkey. The presence of a limited number of species will help us to at least have an understanding of some aspects of the biodiversity of aquatic environments in Turkey, they will also increase our understanding of the world's known biodiversity in the aquatic environment.

ACKNOWLEDGEMENTS

This study is based on the MSc thesis entitled 'Taxonomic Researches on The Aquatic Fungi of Kirazlıyayla Uludağ - Bursa' in Graduate School of Natural and Applied Sciences, Uludağ University. We thank Prof. Dr. Felix Bärlocher, (Mount Allison University, New Brunswick, Canada) and Prof. Dr. Sridhar Kandikere (Mangalore University, Mangalagangotri - Karnataka State, India) for their invaluable suggestions to our manuscript. We would like to acknowledge financial support through a grant from Uludağ University BİLİMAR, project number UAP(F)-2010/29. Editing was carried out by AJA.

This study was presented partly as a poster at the symposium "II. National Days of Mycology, 9-11 September 2015, in İstanbul-Turkey".

REFERENCES

[1] Anonymous (2014) Climate Change IPCC 2014: Mitigation of Climate Change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

[2] Khan, M.A. (1987) Interspecies interactions in aquatic hyphomycetes. Botanical Magazine. 100, 295–303.

[3] Bärlocher, F.T. (1992) The ecology of aquatic hyphomycetes. Springer-Verlag, Berlin.

[4] Gulis, V. and Suberkropp, K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology. 48, 123–134.

[5] Hieber, M. and Gessner, M.O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology. 83, 1026–1038.

[6] Tsui, C.K.M. and Hyde, K.D. (2004) Biodiversity of fungi on submerged wood in a stream and its estuary in the Tai Ho Bay, Hong Kong. Fungal Diversity. 15, 205–220.

[7] Suberkropp, K. and Klug, M.J. (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology. 57, 720–727.

[8] Chamier, A.C. and Dixon, P.A. (1982) Pectinases in leaf degradation by aquatic hyphomycetes: the enzymes and leaf maceration. Microbiology. 128, 2469–2483.

[9] Shearer, C.A. and Raja, H.A. (2010) Freshwater Ascomycetes Database. http://fungi.life.illinois.edu/about/intro.

[10] Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. (2008) Dictionary of the fungi. 10th edn. CABI Publishers, Wallingford, UK.

[11] Michelle, K.M.W., Goh, T.K., Hodgkiss, I.J., Hyde, K.D., Ranghoo, V.M., Tsui, C.K.M., Ho, W.H., Wong, W.S.W., Yuen, T.K. (1998) Role of fungi in freshwater ecosystems. Biodiversity and Conservation. 7(9), 1187–1206.

[12] Goh, T.K. and Hyde, K.D. (1996) Biodiversity of freshwater fungi. Journal of Industrial Microbiology and Biotechnology. 17, 328–345.

[13] Sivichai, S. and Jones, E.B.G. (2003) Teleomorphic-anamorphic connections of freshwater fungi. In: Tsui, C.K.M., Hyde, K.D. (eds.) Freshwater Mycology. Fungal Diversity Press, Hong Kong, 259–272.

[14] Thomas, K. (1996) Australian freshwater fungi. In: Grgurinovic, C.A. (ed.) Introductory, volume to the fungi (part II). Fungi of Australia, vol IB. Australian Biological Resources Study, Canberra, Australia, 1–37.

[15] Huang, Z., Jones, E.B.G., Zhou, D., Bahkali, A.H., Hyde, K.D. (2011) Checklist of freshwater fungi in Thailand. Cryptogamie Mycologie. 32(2), 199–217.

[16] Cai, L., Zhang, K.Q., Hyde, K.D. (2003) Freshwater Ascomycetes. Fungal Diversity Research Series. 10, 275–324.

[17] Goh, T.K. and Tsui, C.K.M. (2003) Key to common dematiaceous hyphomycetes from freshwater. In: Tsui, C.K.M., Hyde, K.D. (eds.) Freshwater mycology. Fungal Diversity Press, Hong Kong, 325–343.

[18] Ingold, C.T. (1975) An illustrated guide to aquatic and water-borne hyphomycetes (Fungi Imperfecti), with notes on their biology. Freshwater Biological Association Scientific Publications. vol. 30, Cumbria, England.

[19] Ingold, C.T. (1942) Aquatic hyphomycetes of decaying alder leaves. Transactions of the British Mycological Society. 25, 339–417.

[20] Nilsson, S. (1964) Freshwater hyphomycetes: Taxonomy, morphology, and ecology. Symbolae Botanicae Upsalienses. 18(2), 1–130.

[21] Tsui, C.K.M., Hyde, K.D. (2003) Freshwater mycology. Fungal Biodiversity Press, Hong Kong.

[22] Webster, J. and Descals, E. (1981) Morphology, distribution and ecology of conidial fungi in freshwater habitats. In: Cole, G.T., Kendrick, B. (eds.) the biology of conidial fungi. vol. 1, Academic Press, New York, 295–355.

[23] Webster, J. (1987) Convergent evolution and the functional significance of spore shape in aquatic and semi-aquatic fungi. In: Rayner, A.D.M., Brasier, C.M., Moore, D. (eds.) Evolutionary biology of the fungi. Cambridge University Press, Cambridge, 191–201.

[24] Webster, J. (1959) Experiments with spores of aquatic hyphomycetes, I Sedimentation, and impaction on smooth surfaces. Annals of Botany. 23, 595–61.

[25] Atalay, İ. (2002) Ecoregions of Turkey. Ministry of Forestry Publication, 163, Meta Publisher, İzmir, Turkey.

[26] Atalay, İ. (2008) Ecosystem ecology and geography. vol I., Ministry of Environment and Forestry Publications, Meta Basımevi, İzmir, Turkey.

[27] Yeşilyurt, S. and Hasenekoğlu, İ. (2004) A study on aquatic hyphomycetes flora of decaying leaves in Aras River within Erzurum Province. Trakya University Journal of Natural Sciences. 5(1), 19–29.

[28] Yeşilyurt S. and Hasenekoğlu, İ. (2004) A study on aquatic hyphomycetes of foams of Aras River within Erzurum. Trakya University Journal of Natural Sciences. 5(1), 29–33.

[29] Crane, J.L. (1968) Freshwater hyphomycetes of the Northern Appalachian Highland including New England, and three coastal Plain States. American Journal of Botany. 55(8), 996–1002.
[30] Petersen, R.H. (1962) Aquatic hyphomycetes from North America. I. Aleuriosporae (Part I) and key to the genera. Mycologia. 54(2), 117–151.
[31] Subramanian, C.V. (1983) Hyphomycetes: Taxonomy and biology. Academic Press, New York.
[32] Gulis, V., Marvanova, L., Descals, E. (2005) An Illustrated key to the common temperate species of aquatic hyphomycetes. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (eds) Methods to study litter decomposition, a practical guide. Springer, Netherlands, 153–167.
[33] Webster, J. and Weber, R. (2007) Introduction to fungi. Cambridge University Press, Cambridge.
[34] Dubey, T., Stephenson, S.L., Edwards, P.J. (1994) Effect of pH on the distribution and occurrence of aquatic fungi in six west Virginia Mountain Streams. Journal of Environmental Quality. 23, 1271–1279.
[35] Sudheep, N.M. and Sridhar, K.R. (2011) Diversity of lignicolous and Ingoldian fungi on woody litter from the River Kali (Western Ghats, India). Mycology. 2(2), 98–108.
[36] Iqbal, S.H. and Webster, J. (1977) Aquatic hyphomycetes spore of some Dartmoor Streams. Transactions of the British Mycological Society. 71, 231–237.
[37] Descals, E. (1997) Ingoldian Fungi: some field and laboratory technique. Bolletí de la Societat d'Història Natural de les Balears. 40, 169–221.
[38] Descals, E. and Moralejo, E. (2001) Water and asexual reproduction in the Ingoldian fungi. Botanica Complutensis. 25, 13–71.

Received: **18.08.2017**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

C Cem Ergul
University of Uludağ,
Faculty of Arts and Sciences,
Department of Biology,
Bursa – Turkey

e-mail: ergulc@uludag.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9441-9446 Fresenius Environmental Bulletin

POTASSIUM AND CALCIUM CONTENT IN POTATO TUBERS DEPENDING ON WAYS OF APPLICATION OF THE UGMAX PREPARATION

Alicja Baranowska[1,*], Krystyna Zarzecka[2], Iwona Mystkowska[1], Marek Gugala[2]

[1]Institute of Agriculture, Pope John II State School of Higher Education in Biala Podlaska, Poland
[2]Department of Agrotechnology, University of Natural Sciences and Humanities in Siedlce, Poland

ABSTRACT

In recent years, dynamic changes occur in agriculture, and the idea of protection of the natural environment and the consumer becomes more and more popular. Care for the natural environment and quality of the harvested crops is reflected in introduction of various kinds of microbiological preparations to agricultural practice, which affect physical and chemical properties of soil and qualitative characteristics of plants. The purpose of the experiment was to identify the effect of using the UGmax microbiological preparation (Soil Fertilizer) on the content of potassium and calcium in edible potato tubers. The experiment was established according to randomized split-block method, in three replications. The examined factors included: 1st factor: edible potato cultivars (Satina and Tajfun), 2nd factor: doses and dates of application of the UGmax Soil Fertilizer.

As a result of the conducted study, it was determined that potato tubers contain more potassium and calcium after application of the UGmax soil preparation as compared to tubers harvested from the control object, where UGmax was not used, but these differences were not confirmed statistically. It was proven that weather conditions and cultivars affect accumulation of potassium and calcium in dry matter of potato tubers. It was demonstrated that the experiment factors had a significant impact on the uptake of the examined macroelements with the yield of potato tubers.

KEYWORDS:
Potato, potassium, calcium, microbiological preparation

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the main cultivated plants, decisive in feeding the world population [1, 2]. It is cultivated in over 125 countries and consumed every day by more than a billion people [3].

The nutritional value of ware potato tubers is determined by the content and quality of chemical substances, such as: starch, complete protein, sugars, vitamins, phenol compounds, dietary fiber, organic acids, lipids, and minerals [4].

Minerals constitute approximately 1-1.2 % of fresh mass of potato tubers. One of the most important elements is potassium, occurring in its ionic form K^+. Consumption of 300g of potatoes covers approximately 48.6 % of the daily demand for this element [5].

In the human body, potassium is the main cation of the intracellular fluid, and it is present in digestive juices and bones. It is responsible for maintenance of the water-electrolyte balance. This element plays a key role in the functioning of the heart, the nervous system and muscles [6].

Potassium also plays an important role in many biochemical and physiological processes taking place in plants. Among others, it plays a major role in osmotic processes, activation of plant enzymes, transport of ions and organic compounds. Therefore, it determines the quality and quantity of harvested crops [7, 8].

Calcium Ca^{2+} is also an important element present in potato tubers. This macroelement fulfills an important framegenic role, is a component of cellular walls and body fluids, and is also the activator of many enzymes and takes part in the process of blood clotting. This element determines correct growth and development of the body [9]. In the body of an adult person, 99 % of calcium can be found in the skeletal system [10].

Few ambiguous results of empirical research on the effect of microbiological preparations on the content of macroelements in ware potato tubers induce to conduct further research. The experiment assumed that the UGmax microbiological preparation (Soil Fertilizer) will favorably affect the content of the concerned macroelements in potato tubers. The purpose of the experiment was to identify the effect of using the UGmax preparation on the content of potassium and calcium in ware potato tubers.

MATERIALS AND METHODS

The research material consisted of edible potato tubers of the Satina and Tajfun cultivars obtained from a three-year field experiment, conducted with the use of the UGmax Soil Fertilizer (microbiological preparation) in the period of 2008-2010 in the Agricultural Experimental Station in the area of central Poland. The experiment was conducted on soil with the granulometric composition of sandy clay, slightly acidic and acidic (4.81-5.91 pH in one n of KCl). The assimilable potassium content in soil ranged from low to high, phosphorus content - from high to very high, and magnesium - from low to average.

The experiment was established according to the randomized split-block method, in three replications. The examined factors included: the cultivar and application of the UGmax Soil Fertilizer. The assessment covered two medium early cultivars of edible potato - Satina and Tajfun, and five ways of application of the UGmax preparation at different doses and application times: (1) control object without UGmax, (2) UGmax applied to soil before planting at a dose of 1.0 $dm^3 \cdot ha^{-1}$, (3) UGmax applied to soil before planting at a dose of 0.5 $dm^3 \cdot ha^{-1}$, when the height of plants is about 10-15cm, and in the flower buds making phase at a dose of 0.25 $dm^3 \cdot ha^{-1}$, (4) UGmax applied before planting at a dose of 1.0 $dm^3 \cdot ha^{-1}$ and when the height of plants is about 10-15cm, and in the flower buds making phase at a dose of 0.5 $dm^3 \cdot ha^{-1}$, (5) UGmax applied to leaves when the height of plants is about 10-15cm and in the flower bud making phase at a dose of 0.5 $dm^3 \cdot ha^{-1}$. The UGmax microbiological preparation is an extract of a special compost containing a vaccine of soil microorganisms. It consists of: yeast, lactic acid bacteria, photosynthetic bacteria, *Azotobacter* spp, *Pseudomonas* spp , *Actinomycetes*, as well as macro- and microelements, such as: potassium (3500 $mg \cdot dm^3$), nitrogen (1200 $mg \cdot dm^3$), sulfur (1000 $mg \cdot dm^3$), phosphorus (500 $mg \cdot dm^3$), sodium (200 $mg \cdot dm^3$), magnesium (100 $mg \cdot dm^3$), zinc (20 $mg \cdot dm^3$), manganese (0.3 $mg \cdot dm^3$) [11, 12].

In autumn, before the experiment was established, manure was applied at a dose of 25 $t \cdot ha^{-1}$, and phosphorus fertilization at 44.0 kg $P \cdot ha^{-1}$ P/ha (superphosphate 46 %) and potassium fertilization at 124.5 kg $K \cdot ha^{-1}$ (potassium salt 60 %) were used. In spring, nitrogen fertilization (ammonium nitrate 34 %) was applied at a dose of 100 kg $N \cdot ha^{-1}$. Potato tubers were planted in the second decade of April.

In order to avoid weed infestation, before the potato plants sprung out, a mixture of herbicides Afalon Dispersive 450 SC (1.0 $dm^3 \cdot ha^{-1}$) and Command 480 SC (0.2 $dm^3 \cdot ha^{-1}$) was applied. During growing season, the plantation was protected against the potato beetle with insecticides Actara 25 WG (80.0 $g \cdot ha^{-1}$) and Apacz 50 WG (40.0 $g \cdot ha^{-1}$) and the potato blight using Dithane 455 SC (2.0 $kg \cdot ha^{-1}$) and Ridomil Gold MZ 68 WG (2.0 $kg \cdot ha^{-1}$). Tubers were harvested in the period of technological maturity, in the first decade of September.

Chemical analyses were performed on dry plant material in three replications. Potassium and calcium content were determined with the use of the Atomic Absorption Spectrometry (ASA) after prior grinding, drying and mineralization of potato tuber samples in a laboratory furnace at the temperature of 450-550°C.

The study results were analyzed statistically using the variance analysis, and the significance of the differences was assessed using Tukey's test, at the significance level of P = 0.05.

Weather conditions during the experiment were diverse (Table 1). In 2008, rainfall was well distributed in particular months of vegetation. Air temperature was close to the temperature in the multiannual period. It was a season stimulating growth and development of potato plants. In 2009, rainfall was spread irregularly, and average air temperature was higher than the multiannual average. 2010 was warmer than the previous seasons, and rainfall was very high (459.7mm) and exceeded the average sum from the multiannual period; it was the most humid season.

TABLE 1
Rainfalls and air temperatures in 2008-2010 vegetation seasons at the Agricultural Experimental Station, Poland

Years	Months						Mean/ Sum IV–IX
	IV	V	VI	VII	VIII	IX	
	Temperature (°C)						
2008	9.1	12.7	17.4	18.4	18.5	12.2	14.7
2009	10.3	12.9	15.7	19.4	17,7	14.6	15.1
2010	8.9	14.0	17.4	21.6	19.8	11.8	15.6
The average over the years 1987-2000	7.8	12.5	17.2	19.2	18.5	13.1	14.7
	Rainfalls (mm)						
2008	28.2	85.6	49.0	69.8	75.4	63.4	371.4
2009	8.1	68.9	145.2	26.4	80.9	24.9	354.4
2010	10.7	93.2	62.6	77.0	106.3	109.9	459.7
The average over the years 1987-2000	38.6	44.1	52.4	49.8	43.0	47.3	275.2

TABLE 2
Content of potassium (K) in dry mass of potato tubers depending on methods of use of UGmax, cultivar and weather conditions in years of study (g·kg $^{-1}$)

Objects	Potato cutivars		Years			Mean
	Satina	Tajfun	2008	2009	2010	
1. Control object	23.82	23.40	24.87	24.47	21.50	23.61
2. UGmax 1.0+0 dm^3·ha^{-1}	24.38	24.51	26.22	25.17	21.95	24.45
3. UGmax 0.5+0.25+0.25 dm^3·ha^{-1}	24.67	24.92	26.70	25.34	22.35	24.80
4. UGmax 1.0+0.25+0.25 dm^3·ha^{-1}	24.90	25.24	27.27	25.45	22.50	25.07
5. UGmax 0+0.5+0.5 dm^3·ha^{-1}	24.22	24.37	25.80	25.09	22.00	24.30
Mean	24.40	24.48	26.16	25.10	22.06	24.44

$LSD_{0.05}$ for: methods of use of UGmax - ns, cultivars - ns, years - ns, interaction between methods of use of UGmax x cultivars x years = ns
ns -not significant at $P_{0.05}$

RESULTS AND DISCUSSION

In the opinion of many authors Emitazi et al. [11]; Rogóż et al. [13]; Trawczyński et Bogdanowicz [14]; Kołodziejczyk [15]; Zarzecka et al. [16], the content of minerals in potato tubers depends on the soil pH, its nutrient content, appropriate humidity, genetic factors, as well as the use of various kinds of microbiological biopreparations (soil fertilizers) improving the properties of soil. These preparations currently generate great interest both among manufacturers themselves, as well as among scientists [17].

Potassium content in dry matter of potato tubers. The authors' research showed that potassium content in dry mass of potato tubers was at the level from 23.40 up to 25.24 g·kg^{-1} and depended on the methods of application of the UGmax preparation, the cultivar and the weather conditions (Table 2).

Potassium content in potato tubers was similar to the values obtained by other authors [18, 19]. As a result of the conducted study, it was determined that the UGmax microbiological preparation caused increase in the content of potassium as compared to tubers harvested from the control object, where UGmax was not used, but these differences were not confirmed statistically (Table 2).

The greatest potassium content was accumulated in tubers harvested from object 4, where the UGmax preparation was applied to soil before planting of tubers in damp soil and twice during the vegetation at the overall dose of 1.5 dm^3·ha^{-1}, and from object 3, where the UGmax Soil Fertilizer was applied on the same dates, but at a smaller dose of 1.0 dm^3·ha^{-1}.

Similar research findings were obtained by Wichrowska et al. [20], who concluded that microbiological preparations intensify the biological activity of soil and contribute to transformation of unavailable forms of nutritional components into forms of available for plants. On the other hand, Trawczyński and Bogdanowicz [11] did not notice any impact of the microbiological preparation on the increase in the potassium content in potato tubers.

The authors' research showed that potassium content depended on cultivar features. Greater content of this component was observed in tubers of the Tajfun cultivar than in the Satina cultivar, but these differences were not confirmed statistically (Table 2). According to Wichrowska et al. [20], White et al. [21], Ekin [22], the content of this element in potato tubers is shaped by the genetic properties of cultivars. On the other hand, Sawicka et al. [23] demonstrated in their studies that the content of minerals in edible potato tubers is not only a cultivar feature, but also depends on environmental factors, one of which is fitoavailability of minerals in the soil.

It was also observed in the experiment that weather conditions in the research period differentiated the potassium content (Table 2). Tubers accumulated the largest quantity of this element in the vegetation period of 2008, when rainfall was well distributed in particular months of vegetation and air temperature was close to the temperature in the multiannual period, which is consistent with the research of Wadas et al. [24]. On the other hand, Kołodziejczyk et Szmigiel [25] demonstrated greater concentration of this element in tubers from cooler and more humid periods.

Calcium content in dry matter of potato tubers. One of the more important macroelements present in potato tubers is calcium. The authors' research showed that calcium content in dry matter of potato tubers was at the level from 0.671 up to 0.764 g·kg^{-1} and depended on the methods of application of the UGmax preparation, the cultivar and the weather conditions (Table 3). The content of this element in potato tubers was similar to the values obtained by Różyło and Pałys [26].

When comparing calcium content in tubers, it was observed that applications of the UGmax preparation substantially contributed to the increase in concentration of this macroelement as compared to

potatoes harvested from the control object, where UGmax was not used. The greatest calcium content was accumulated in tubers harvested from objects 4 and 3 (on average, 0.752 and 0.726 g·kg^{-1}) (Table 3).

Calcium content depended on cultivar features. Greater content of this component was observed in tubers of the Tajfun cultivar than in the Satina cultivar, but these differences were not confirmed statistically (Table 3). The genetic properties of cultivars were indicated by Wichrowska et al. [20]; Miles et Buchman [27] and Zarzecka et Gugała [28].

The authors' research demonstrated significant impact of weather conditions on the calcium content in potato tubers (Table 3). Greater concentration of this element was observed in 2009, which was a warm year with irregularly distributed rainfall, which is consistent with the research of Zarzecka et Gugała [28].

Potassium and calcium uptake with the yield of potato tubers. The uptake of potassium and calcium with the yield of potato tubers was substantially shaped by the methods of application of the UGmax preparation, the cultivar and the humidity and temperature conditions prevailing in the research period (Table 4-5).

Substantially larger uptake of potassium and calcium was recorded on objects 4 and 3, which were sprayed with the UGmax preparation three times, at different doses and application dates. Larger uptake of the examined components was recorded in the case the Tajfun cultivar than in the Satina cultivar. It was proven that weather conditions in the research period had significant impact on the uptake of potassium and calcium. The uptake of the examined macroelements with the yield of potato tubers was the largest in 2008, which was the most beneficial year for the growth and vegetation of potato plants. The study showed the impact of interactions between the application methods of the UGmax preparation and the cultivars, as well as between the application methods of the UGmax preparation and the weather conditions on the uptake of the concerned macroelements with the yield of potato tubers (Table 4-5).

The obtained results of the author's research allow for stating that the performed applications of the UGmax microbiological preparation had a positive influence on the accumulation of potassium and calcium in dry matter of potato tubers, and hence on the qualitative characteristics of the harvested crops.

TABLE 3
Content of calcium (Ca) in dry mass of potato tubers depending on methods of use of UGmax, cultivar and weather conditions in years of study (g·kg^{-1})

Objects	Potato cultivars		Years			Mean
	Satina	Tajfun	2008	2009	2010	
1. Control object	0.671	0.686	0.692	0.714	0.630	0.679
2. UGmax 1.0+0 dm^3·ha^{-1}	0.714	0.720	0.720	0.740	0.692	0.717
3. UGmax 0.5+0.25+0.25 dm^3·ha^{-1}	0.712	0.740	0.739	0.745	0.695	0.726
4. UGmax 1.0+0.25+0.25 dm^3·ha^{-1}	0.739	0.764	0.759	0.780	0.717	0.752
5. UGmax 0+0.5+0.5 dm^3·ha^{-1}	0.710	0.708	0.749	0.730	0.649	0.709
Mean	0.709	0.724	0.732	0.742	0.677	0.717

$LSD_{0.05}$ for: methods of use of UGmax - 0.030, cultivars - ns, interaction between methods of use of UGmax x cultivars = ns; $LSD_{0.05}$ for: methods of use of UGmax - 0.030, years - 0.027, interaction between methods of use UGmax x years = ns.
ns - not significant at $P_{0.05}$

TABLE 4
Uptake of potassium (K) with the yield of potato tubers depending on methods of use of UGmax and weather conditions in years of study (g·kg^{-1})

Objects	Potato cultivars		Years			Mean
	Satina	Tajfun	2008	2009	2010	
1. Control object	130.3	202.9	244.6	141.2	114.0	166.6
2. UGmax 1.0+0 dm^3·ha^{-1}	174.5	265.2	339.9	175.3	144.3	219.8
3. UGmax 0.5+0.25+0.25 dm^3·ha^{-1}	188.0	284.4	341.1	201.3	166.2	236.2
4. UGmax 1.0+0.25+0.25 dm^3·ha^{-1}	212.7	323.3	392.3	234.1	177.6	268.0
5. UGmax 0+0.5+0.5 dm^3·ha^{-1}	150.4	240.1	288.5	162.9	134.3	195.2
Mean	171.2	263.1	321.3	183.0	147.3	217.2

$LSD_{0.05}$ for: methods of use of UGmax - 11.6, cultivars - 6.2, interaction between methods of use of UGmax x cultivars = 11.6; $LSD_{0.05}$ for: methods of use of UGmax - 11.6, years - 9.6, interaction between methods of use UGmax x years = 20.2
ns - not significant at $P_{0.05}$

TABLE 5
Uptake of calcium (Ca) with the yield of potato tubers depending on methods of use of UGmax and weather conditions in years of study (g·kg^{-1})

Objects	Potato cultivars		Years			Mean
	Satina	Tajfun	2008	2009	2010	
1. Control object	3.66	5.90	6.83	4.17	3.35	4.78
2. UGmax 1.0+0 dm^3·ha^{-1}	5.01	7.67	9.33	5.15	4.54	6.34
3. UGmax 0.5+0.25+0.25 dm^3·ha^{-1}	5.36	8.39	9.49	5.94	5.19	6.87
4. UGmax 1.0+0.25+0.25 dm^3·ha^{-1}	6.21	9.66	10.94	7.19	5.67	7.93
5. UGmax 0+0.5+0.5 dm^3·ha^{-1}	4.39	6.97	8.37	4.74	3.94	5.68
Mean	4.92	7.72	8.99	5.44	4.54	6.32

$LSD_{0.05}$ for: methods of use of UGmax - 0.35, cultivars - 0.16, interaction between methods of use of UGmax x , cultivars = 0.35; $LSD_{0.05}$ for: methods of use of UGmax - 0.35, years - 0.26, interaction between methods of use of UGmax x years = 0.62
ns - not significant at $P_{0.05}$

ACKNOWLEDGEMENT

The results of the research carried out under the research theme number 214/04/S were financed from the science grant granted by the Ministry of Science and Higher Education.

REFERENCES

[1] Ezekiel, R., Singh, N., Sharma, S. and Kaur, A. (2013) Beneficial phytochemicals in potato - a review. Food Research International. 50, 487-496.
[2] Hamouz, K., Lachman, J., Hejtmánková, K., Pazderů, K., Čížek, M. and Dvořák, P. (2010) Effect of natural and growing conditions on the content of phenolics in potatoes with different flesh colour. Plant, Soil and Environment. 56, 368-374.
[3] Bishwoyog, B. and Swarnima, K.C. (2016) Effect of potassium on quality and yield of potato tubers - a review. International Journal of Agriculture and Environmental Science. 3, 9-14.
[4] Love, S.L. and Pavek, J.J. (2008) Positioning the potato as primary food source of vitamin C. American Journal of Potato Research. 85, 350-359.
[5] Wichrowska, D., Wojdyła, T. and Rogozińska, I. (2009) Concentrations of some macroelements in potato tubers stored at 4°C and 8°C. Journal of Elementology. 14 (2), 373-382. (in Polish).
[6] Macdonald, J.E. and Struthers, A.D. (2004) What is the optimal serum potassium level in cardiovascular patients. Journal of the American College of Cardiology. 43, 155-161.
[7] Thomas, T.C. and Thomas, A.C. (2009) Vital role of potassium in the osmotic mechanism of stomata aperture modulation and its link with potassium deficiency. Plant Signal Behaviour. 4(3), 240-243.
[8] Patil, R.B. (2011) Role of potassium humate on growth and yield of soybean and black gram. International Journal of Pharma and Bio sciences. 2(1), 242-246.
[9] White, P.J. (2001) The pathways of calcium movement to the xylem. Journal of Experimental Botany. 52, 891-899.
[10] Friedrich, M., Jankowiak, D., Ożgo, M., Skrzypczak, W.F. and Stepanowska, K. (2002) Minerals in human and animal nutrition. Wyd. Akademii Rolniczej, Szczecin. (In Polish).
[11] Trawczyński, C. and Bogdanowicz, P. (2007) The use of Soil Fertilizer with respect to ecological potato cultivation. Journal of Research and Applications in Agricultural Engineering. 52(4), 94-97 (in. Polish).
[12] Kołodziejczyk, M. (2014) Effect of nitrogen fertilization and microbial preparations on potato yielding. Plant, Soil and Environment. 60, 379-386.
[13] Emitazi, G., Nader, A. and Etemadifar, Z. (2004) Effect of nitrogen fixing bacteria on growth of potato tubers. Advance Journal of Food Science and Technology. 26(2), 56-58.
[14] Rogóż, A. and Trąbczyńska, K. (2009) Micronutrients content in soils and selected root crops. Part I. Copper and zinc content. Part II. Manganese and iron content. Zeszyty Problemowe Postępów Nauk Rolniczych. 541(2), 353-373. (in Polish).
[15] Kołodziejczyk, M. (2016) Effect of nitrogen fertilisation and microbial preparations on quality and storage loses in edible potato. Acta Agrophysica. 23(1), 67-78. (in Polish).
[16] Zarzecka, K., Gugała, M., Baranowska, A., Dołęga, H. and Sikorska, A. (2016) Concentrations of copper, zinc and manganese in potato tubers under the influence of herbicides. Jurnal of Elementology. 21(1), 259-267. (in Polish).
[17] Sangakkara, U.R., Wijesinghe, D.B. and Attana Yake, K.B. (2011) Soil quality Phaseolus bean yields as affected by organic matter and EM in degraded tropical soil. In: 16th IFOAM Organic World Congress. Italy, Modena. Organic Crop Protection 1. 102-105.

[18] Wierzbicka, A. and Trawczyński C. (2011) Effect of irrigation and soil's microorganisms on the macro and micronutrient contents in organic potato tubers. Fragmenta Agronomica. 28(4), 139-148. (in Polish).
[19] Šreck, P., Hejcman, M. and Kunzová, E. (2012) Effect of long-term cattle slurry and mineral N, P and K application on concentrations of N, P, K, Ca, Mg, As, Cd, Cr, Cu, Mn, Ni Pb and Zn in peeled potato tubers and peels. Plant, Soil Environmental. 58(4), 167-173.
[20] Wichrowska, D., Wszelaczyńska, E. and Poberężny, J. (2015) Effect of nutrient supply from different sources on some quality parameters of potato tubers. Journal of Elementology. 20(1), 217-230. (in Polish).
[21] White, P.J., Bradshaw, J.E., Finaly, M., Dale, B., Ramsay, G., Hammond, J.P. and Broadley, M.R. (2009) Relationships between yield and mineral concentrations in potato tubers. Horticultural Science. 44(1), 6-11.
[22] Ekin, Z. (2011) Some analytical quality characteristics for evaluating the utilization and consumption of potato (Solanum tuberosum L.) tubers. African Journal of Biotechnology. 10(32), 6001-6010.
[23] Sawicka, B., Hulali Noaema, A., Seead Hameed, T. and Skiba D. (2016) Genotype and environmental variability of chemical elements in potato tubers. Acta Scientarum Polonorum, Agricultura. 15(3), 79-91. (in Polish).
[24] Wadas, W., Jabłońska-Ceglarek, R., Kosterna, E. and Łęczycka, T. (2012) Potassium content in young potato tubers depending on the method of cultivation. Roczniki Akademii Rolniczej w Poznaniu. CCCLXXXIII, Ogrodnictwo. 41, 643-647. (in Polish).
[25] Kołodziejczyk, M. and Szmigiel, A. (2005) Macroelements content in edible potato tubers depending on soil formation, cultivar and fertilization. Fragmenta Agronomica. 1(85), 436-445. (in Polish).
[26] Różyło, K. and Pałys, E. (2006) The impact of fertilization and soil conditions on the chemical composition of potato tubers and their health condition. Zeszyty Problemowe Postępów Nauk Rolniczych. 511, 279-286. (in Polish).
[27] Miles, G.P. and Buchman, J.L. (2009) Impact of zebra chip disease on the mineral content of potatotubers. American Journal of Potato Research. 86, 481-489.
[28] Zarzecka, K. and Gugała, M. (2010) Content and uptake of phosphorus and calcium with the yield of potato tubers depending on cultivation operations. Journal of Elementology. 5(2), 385-392. (in Polish).

Received: **18.8.2017**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Alicja Baranowska
Institute of Agriculture,
Pope John II State School of Higher Education in Biala Podlaska,
Sidorska 95/98, 21–500 Biała Podlaska – Poland

e-mail: alabar@tlen.pl

© by PSP Volume 27 – No. 12B/2018 pages 9447-9452 Fresenius Environmental Bulletin

A GREENHOUSE CONSTRUCTION WITH FIBER-REINFORCED PLASTIC CHORDS AND TRIANGULAR PYRAMID MODELS

Cevdet Saglam[1,*], Mustafa Guzel[2], Necati Cetin[1]

[1]Erciyes University, Faculty of Agriculture, Department of Biosystems Engineering, 38039, Kayseri, Turkey
[2]Gaziosmanpasa University, Faculty of Agriculture, Department of Biosystems Engineering, 60160, Tokat, Turkey

ABSTRACT

This study concerns a prefabricated plastic structure column that can be used as a greenhouse. The plastic construction, having a frame assembly, is a structure in which a series of a Triangular Pyramid Model (TPM) is arranged between four continuous parallel Fiber-Reinforced Plastic (FRP) chords. One of the parallel chords is connected to a TPM by passing through one of its vertex nodes. Thus, each TPM is a connection member between four distinct parallel chords.

The main object of this study is to provide a simple, prefabricated, portable a greenhouse structure with plastic column, one which employs a series of model units of novel design that permits even an inexperienced person to the quickly and easily assemble a building. This new greenhouse structure can be displayed in attractive and functional properties that will work by providing excellent opportunities for the quality, health and safety of constructions. As a result, the system was resistant to two years of severe wind speeds and forces. At the end of the third year, wind speeds reaching 77 km/h has not cause any damage to the model, even though the greenhouse cover material was damaged.

KEYWORDS:
Buildings, construction design, greenhouse, greenhouse structure, triangular pyramid model

INTRODUCTION

Innovative new structural approaches have recently widespread in agricultural activities to provide ecological sustainability. In this sense, new materials and systems have been developed based on physical behaviors of natural materials encountered in nature. Besides durability, esthetics and lightness, antimicrobial, fireproof and environmental friendliness should be taken into consideration while selecting construction materials. Such materials should not pose any chemical and biological threats on plant cover, surrounding environment and local ecology.

To prevent natural resources, some essential factors such as; waste minimization, efficient use of water, recycling of building materials and proper material selection should be considered [1]. Many advances in greenhouse materials and designs have occurred in the last decade. Traditionally, greenhouses were constructed by using galvanized iron pipes and all had a similar structure. Today, however, a great variety exists, and the materials chosen depend on production goals and financial constraints. Materials for greenhouse structures can be classified as wood, metal, plastic or, concrete. Structural members, used for the greenhouse "skeleton", must be strong enough to prevent structural failures during adverse weather conditions but be kept to a minimum size and number to reduce the amount of shading and to provide for maximum light transmission [2]. Another crucial issue is the changes in the greenhouse environmental factors which may have significant effects on growth, development and productivity of crops [3]. Water droplets effect total internal reflection of incident light and consequently reduce the light transmission in greenhouses [4]. Low light transmission, for instance, reduces photosynthetic rates of plants in greenhouse and it may lead to a proportional loss in productivity of crops [3]. Similarly, in a greenhouse ecosystem, the short-wave radiation plays an important role in the overall energy balance of the greenhouse construction (structural parts and cover) [5]. It is understood that greenhouse transmission and daily temperature or daily solar radiation values will be practical to determine ET_0 in low cost plastic greenhouses in Mediterranean climate zones [6].

Greenhouses are glass or plastic-covered structures that allow farmers to grow vegetables and fruits year-round through automatic or manual climate control systems [7]. Prefabricated structures are known in the art and generally are formed of solid panels which are joined in many different ways. Some provide solid panels with adjacent interlocking edges, while others permit prefabrication by utilizing a multitude of unusually shaped members and beams. They very often require special tools for assembling as well as drilling holes for fastening [8]. Kong et al. [9] stated that the

determination of the dynamic characteristic of the double-layer triangle pyramid reticulated dome structure was very important for the basis of structural dynamic analysis. This study concerns a prefabricated plastic structure that can be used as a greenhouse. This invention relates generally to prefabricated structures and more particularly relates to a universal beam construction to prefabricate panels for assembling a structure.

MATERIALS AND METHODS

Steel is expensive, heavy and inflexible; it also damages the glazing during heat waves. Wood is heavy, expensive, and vulnerable to termites and rots quickly. A column made by Fiber-Reinforced Plastic (FRP) chord and Triangular Pyramid Model (TPM) is readily available, also has very high durability and withstand ultraviolet (UV) rays or high temperatures. The bars are wrapped with a clear helical glass fiber chord; surface coated with resin, and rolled in sand to provide enhanced bond properties [10]. Depending on the specific purpose, the material for the greenhouse is generally chosen because of its strength, flexibility, ease of alignment, cost and availability. The ridges of the TPM make spacing of fasteners easy and help prevent incorrect alignment of the panels, because the ridges normally run longitudinally on columns consist of the FRP chords. Regarding the various end subassemblies, all of them are made from fiber-reinforced plastic material that is used on columns. One of the parallel chords is connected to a TPM by passing through one of its vertex nodes. Thus, each TPM model is a connection member between four distinct parallel chords. A structural skeleton used as column made up of a plurality of pairs of TPM units. The structural skeleton is prefabricated, portable, and in which TPM can be used as series. A space frame or truss can be assembled from a structural skeleton by the addition of model units in the four parallel bars. In such systems, plastic chords of trusses advantageously are lighter forms than that of steel and create compression elements by filling the forms and create.

The materials used to cover greenhouse structures must allow for maximum light transmission. They can be rigid or flexible, double-walled or single walled, smooth or corrugated. Most "glazing" materials made today incorporate compounds that inhibit rapid degradation by UV radiation. However, all glazing material will age and they are therefore rated by the number of years they will maintain a certain level of light transmission capability. The glazing material of polycarbonate is lightweight, easy to work with and is resistant to high impacts and fire. Typical light transmission (PAR; Photosynthetically active radiation) is 79 % for double wall and 87 % for single wall. The estimated lifetime is now over 10 years or more, depending on the type. Corrugated polycarbonate sheet, technical specifications and glass fiber rod technical specifications given in Table 1, Table 2 and Table 3 respectively.

TABLE 1
Corrugated polycarbonate sheet

Specifications	Unit	Value	Test Method
Temperature limits (min.-max.)	ºC	-40ºC - +120ºC	-
Linear heat expansion coefficient	mm/(m/ºC)	0.065	-
Specific weight	gr/cm^3	1.2	ISO 1183
Incombustibility class	-	B1 (max. 105ºC)	DIN 4102
Resistance to flood	-	Unbreakable	STL 98101
Vicat softening point	ºC	150	ISO 306
Water absorption (average)	%	25	ASTM-D570

TABLE 2
Technical specifications

Specifications	Unit	Value				Tolerance	Test Method
Number of walls	-	2				-	-
Width	mm	2100				±10	-
Length	m	6 - 14				±0.03	-
Thickness	mm	4	6	8	10	±0.4	-
Weight	gr/m^2	830	1300	1500	1700	±5%	-
Light permeability (transparent)	-	80	80	78	78	-	ARMDM CT 17/TS
K value	W/mK	3.9	3.6	3.4	3.2	-	-
The force at break	N/mm^2	2300	2300	2300	2300	-	ISO-R 527
Cold bending radius	mm	1000	1500	2000	2500	-	-

TABLE 3
Glass fiber rod technical specifications

Color	Diameter	Length	Density	Flash Point
Orange	12 mm	6 m	2.1 gr/m^3	180°C
Tensile Strength	**Modules of Elasticity**	**Distance to copper**	**Shelf Life**	
>1600 MPa	>53000 MPa	3%	Unlimited	

The greenhouse construction process has three phases: 1. preparing the ground, 2. setting up the framework, and 3. installing the glazing. The greenhouses can be readily constructed in two eight-hour days by two adults who have little or no formal education. Each greenhouse embodiment is designed to be field assembled from several sub-assemblies which may be mass produced in a factory. The light weight prefabricated sub-assemblies of the greenhouse are easily assembled. The techniques of assembly are simple, and a relatively inexperienced person may quickly perform the final assembly of the arched roof greenhouse. The free-standing style is often a Quonset (Gothic-shaped Structures), which will accommodate many growing situations but presents height restrictions near the side walls. This type of greenhouse is constructed by using trusses of TPM model and FRP chords for permanent framed structure. Both TPM model and FRP chords are made by fiber-reinforced plastic material.

"Effect of wind speed on the structure" can be estimated by using the following equations [11].

$$w = c_p \times q \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$q = \frac{v^2}{1600} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where;
q: Surface wind pressure or suction (kN/m^2)
v: Wind speed
w: Equivalent static pressure or suction force
c_p: Coefficients dependent on; the position of the building surface; the windward side (pressure: 0.8 kN/m^2); the leeward side (suction: 0.4 kN/m^2); in the direction of the wind and in the angles that make the angle α and in the winds hit (pressure or suction: 1.2 Sinα - 0.4 kN/m^2)

Maximum wind speeds for the years 2013 to 2016 in Kayseri region were obtained from meteorological reports [12].

RESULTS AND DISCUSSIONS

The main objective of this study is to provide a simple, prefabricated, portable greenhouse structure with plastic column, one which employs a series of TPM model units of novel design which is shown in Figure 1 that permits even an inexperienced person to quickly and easily assemble a building.

FIGURE 1
A perspective view of a TPM model

The prefabricated and portable greenhouse structure is made of plastic column which is shown in Figure 2. A space frame or truss can be assembled from a structural column by the addition of model units in the four parallel bars.

FIGURE 2
A structural column by the addition of TPM models in the four parallel bars

FIGURE 3
Plastic-covered, quonset-style greenhouse

Double poly carbonate is used as the covering material of greenhouse. The greenhouse cover is attached to the framed structure with the help of screws. Quonset-style greenhouse which is shown in Figure 3 offers growers and farmers a more cost-effective structure.

The width, length and height of the green-house are 9 ft, 15 ft and 9 ft respectively. It has two doors of dimension 6 ft×3 ft on the either sides. In a double poly carbonate Quonset structure, a plastic column frame forms a large half circle or dome (Figure 3).

The forces due to the wind speeds acting on the structure are given graphically in Figure 4-6. As seen in Tables 4 and 5, the first two years wind speeds and the total forces applied to CTP structure due to wind speeds showed a normal distribution. However, in the third year the wind speed reached 67.1 km/h in March and 77 km/h in December. Depending on the wind speeds, the total forces applied to the CTP structure is 86.9 kN/m^2 and 114.4 kN/m^2 in March and December respectively.

TABLE 4
Max Wind Speed (km/h)

Years	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014	32.8	47.3	56.7	45.00	37.6	34.3	35.0	26.5	31.6	33.2	32.7	48.0
2015	49.5	47.5	58.0	43.75	38.8	33.0	35.0	25.0	32.0	33.7	33.0	56.0
2016	52.0	49.8	67.1	55.0	43.1	35.0	32.3	27.5	33.9	38.7	52.5	77.0

TABLE 5
Total forces applied to CTP structure due to wind speeds (kN/m^2)

Years	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014	20.8	43.2	62.0	39.1	27.3	22.7	23.6	13.6	19.3	21.3	20.6	44.5
2015	47.3	43.5	64.9	36.9	29.1	21.0	23.6	12.1	19.8	21.9	21.0	60.5
2016	52.2	47.8	86.9	58.4	35.9	23.6	20.1	14.6	22.1	28.9	53.2	114.4

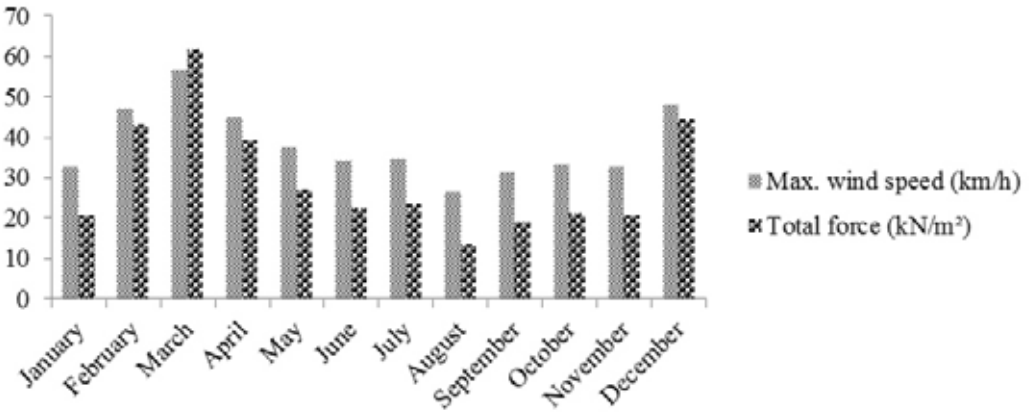


FIGURE 4
Total force due to wind speed in 2014

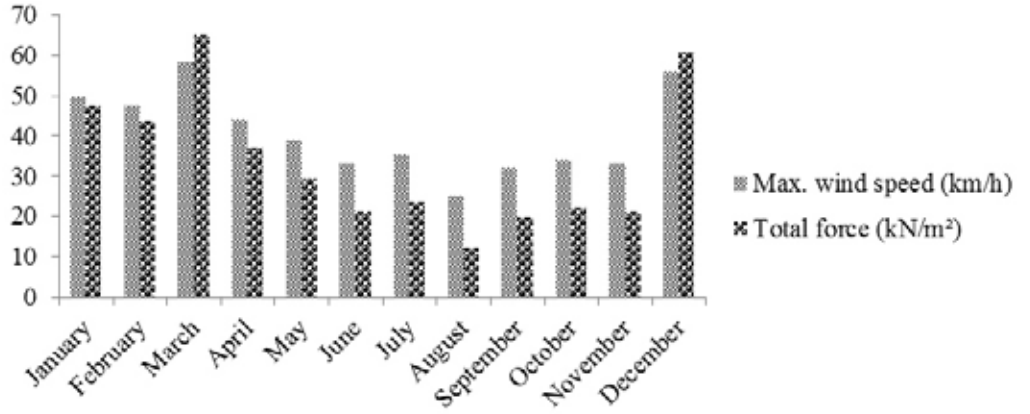


FIGURE 5
Total force due to wind speed in 2015

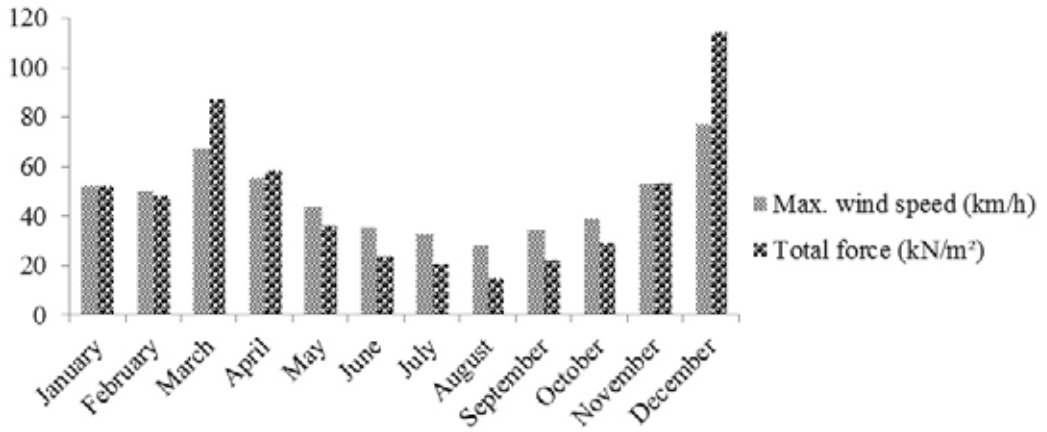


FIGURE 6
Total force due to wind speed in 2016

Due to the severe winds in the region during the third year of the study, as seen in Figure-3 December-2016, the greenhouse cover material was damaged. It can be stronger with a cover material which thicker and more durable than the already used one. In blustering wind over the last year of work only the sera cover material was damaged. There was no damage to the model that made up the greenhouse structure. This problem may be eliminated by using thicker cover material.

CONCLUSIONS

Our technology team continues to streamline the design for easier assembly and is researching low-cost substitutes for greenhouse-grade plastic. The simplicity of the TPM connector coupled with the durable yet flexible FRP chords has kept the greenhouses structurally sound. Through behaving of the coupling elements of TPM by supporting each other due to their geometric structure, the applied force is distributed throughout three dimensions and on all system and increases the resistance force. Structure column has a big force more than other systems to make stand the force applied from one coordinate. TPM model is designed for joist, column and belt in where resistance is basis and main. To bear more force with less material is the claim of this invention. The geometric structure of TPM developed by inspiring from the diamond chemical structure is an important position for both the architecture and the light and powerful structure. According to the claim of using of structure column, its complementary accessories may be changed. This new greenhouse structure can be displayed in attractive and functional properties that will work by providing excellent opportunities for the quality, health and safety of constructions.

ACKNOWLEDGEMENTS

The Authors are grateful to Scientific Research Department of Erciyes University-Turkey for their financial support (Project no: FBA-2013-4531). The authors are also grateful to Assoc. Prof. Dr. Zeki Gökalp (a Certified English Translator and an expert in Biosystems Engineering) for his critical reading and through syntactic corrections of the manuscript.

REFERENCES

[1] Manoliadis, O.G. and Pantouvakis, J.P. (2002) Sustainable Construction In Greece: Harmonization and Implementation. Fresen. Environ. Bull. 11, 763-768.
[2] Hickman, G.W. (2010) Tropical Greenhouses: Design, Construction and Supply Lists (metric units) (also called: polyhouses, hightunnels)- 3 designs. Cuesta Roble Consulting, University of California, USA, 1-2.
[3] Cemek, B. and Demir, Y. (2005) Testing of the condensation characteristics and light transmissions of different plastic film covering materials. Polymer testing. 24(3), 284-289.
[4] Geoola, F. and Peiper, U.M. (1994) Outdoor Testing of The Condensation Characteristics of Plastic Film Covering Materials Using a Model Greenhouse. Journal of Agricultural Engineering Research. 57(3), 167-172.
[5] Papadakis, G., Briassoulis, D., Mugnozza, G. S., Vox, G., Feuilloley, P. and Stoffers, J.A. (2000) Radiometric and Thermal Properties of, and Testing Methods for, Greenhouse Covering Materials. Journal of Agricultural Engineering Research. 77(1), 7-38.

[6] Fernández, M.D., Bonachela, S., Orgaz, F., Thompson, R., López, J.C., Granados, M.R. and Fereres, E. (2010) Measurement and estimation of plastic greenhouse reference evapotranspiration in a Mediterranean climate. Irrigation Science. 28(6), 497-509.
[7] Heidenreich, C., Pritts, M., Kelly, M.J and Demchak, K. (2009) High Tunnel Raspberries and Blackberries. Department of Horticulture Publication. 47, 1-51.
[8] Technical Notes 17A. Reinforced Brick Masonry - Materials and Construction, Technical Notes on Brick Construction 17A, Reston, VA, August. 1997, 1-10.
[9] Kong, B., Qiao, K. and Yuan, J. (2014) The Investigation of Long-span Double-layer Reticulated Dome Greenhouse's Dynamic Characteristics. International Conference on Mechanics and Materials Engineering (ICMME). 338-343.
[10] ACI Committee 440 (1996) State-of-the-Art Report on Fiber-Reinforced Plastic Reinforcement for Concrete Structures. Detroit: ACI, 1-68. https://www.academia.edu/11904101/440R-96_State-of-the-Art_Report_on_Fiber_Reinforced_Plastic_FRP_Reinforcement_for_Concrete_Structures (04.01.2017).
[11] Topçu, A. (2016) Reinforced Concrete Lecture Notes II. Eskisehir Osmangazi University. http://mmf2.ogu.edu.tr/atopcu/index_dosyalar/Betonarme2.htm (04.01.2017).
[12] Anonymous (2017) Maximum wind speeds for the years 2013 to 2016 in Kayseri region https://www.meteoblue.com/tr/hava/tahmin/modelclimate/kayseri_t%C3%BCrkiye_308464 (05.01.2017).

Received: **21.09.2017**
Accepted: **21.11.2018**

CORRESPONDING AUTHOR

Cevdet Saglam
Department of Biosystems Engineering,
Faculty of Agriculture,
Erciyes University,
38039, Kayseri – Turkey

e-mail: cevdetsaglam@erciyes.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9453-9462 Fresenius Environmental Bulletin

DATABASE DRIVEN WEB-BASED MULTI-SPAN GREENHOUSE PLANNING AND CONSTRUCTION APPLICATION

Sevket Yilmaz[1], Kenan Buyuktas[2,*], Ahmet Tezcan[2], Ismail Sari[3]

[1]Pamukkale University, Technology Faculty, Department of Mechatronics Engineering, Denizli, Turkey
[2]Akdeniz University, Agricultural Faculty, Department of Farm Structures and Irrigation, Antalya, Turkey
[3]Pamukkale University, Denizli Technical Sciences and Vocational School, Department of Computer Technology, Denizli, Turkey

ABSTRACT

In this study, it was aimed to develop a web-based application to calculate dimensions and determine the cost of the multi-span greenhouses having different roof types are naturally ventilated by entering some size in computer. This application was aimed to determine the cost of the multi-span greenhouses with base area 480-11500 m^2 depending on the selected values. With this study, different options will be chosen regarding the greenhouse dimensions such as 4-5 m side wall height, 8 and 9.60 m in width, between 2 and 20 block-size for 30-60 m length values. For this purpose, the prepared web-based application is easy to operate, user-friendly, interactive interface with and it is also completing an application process step by step according to user preferences. The application was prepared as a HTML, PHP, PDO, jQuery and Bootstrap-based and in the calculation user-induced errors were reduced to minimum for all options using the form elements. At the end of this study, manufacturers will be able to calculate cost of greenhouse type which is chosen by entering the appropriate data which chosen by themselves and they will be able to see the plan, section and view of greenhouse chosen according to the product grown in.

KEYWORDS:
Cost, designing, multi-span greenhouse, roof-type, web-based application, data base.

INTRODUCTION

The greenhouse structures used in agriculture can be analyzed in three groups such as low plastic tunnels, high plastic tunnels and greenhouses. Low plastic tunnels can be defined as simple greenhouse production areas covered with plastic material of 60 to 200 cm in width, 30 to 200 cm in height and 20 to 50 m in length with half circle skeleton. High plastic tunnels are structures between greenhouses and low tunnels, 300 to 400 cm in width, 150 to 200 cm in height and 50 to 60 m in length with half-circle skeleton. Greenhouses are non-climate dependent structures that provide more favorable conditions for cultivated plants and provide a higher income [1], [2].

When the farmers who want to build a greenhouse want to bid from greenhouse companies, creation of bid can be long time by the company. Because it is a long and troublesome job to calculate the cost of greenhouse. Manufacturers who want to build a greenhouse do not have information about the costs of the greenhouses they will be built. However, they can take advantages and decide criteria such as greenhouse type, dimensions etc. in advance if they know cost of greenhouse that will be built. With this study, greenhouse companies will able to prepare bid much faster and easier and they will able to deliver to the manufacturers who want it. In addition, a manufacturer who want to build greenhouse will able to determine cost of greenhouse easily in a few step by defining some basic data belong to greenhouse [3], [4].

In this study, it was aimed to create a web-based greenhouse cost calculator for the commercial greenhouse manufacturers. Additionally, with this application, the costs of greenhouses with different characteristics can be calculated by defining simple greenhouse properties such as roof type, greenhouse length, width, height, number of blocks.

MATERIALS AND METHODS

Web technologies used to development of this database driven web based application are HTML, PHP [5], PDO [6], jQuery [7] and Bootstrap [8]. HTML used to create static web content and forms. Using PHP form data processed and managed database operations. PDO extension defines an interface for accessing databases in PHP. jQuery used to interaction of user responses on forms. Bootstrap used to design user-friendly interface.

A Web application consists of a set of static HTML pages displayed to the user and (possibly) of server side programs, which perform some computation, finally resulting in the production of dynam-

ic pages transmitted to the browser for display. Moreover, HTML pages may embed client procedures which are either executed during page loading or in response to graphical interface events.

A Web application is a special case of client-server system, in which the Web server plays the role of the server, the Web browser plays the role of the client, and a fixed communication protocol (the HTTP protocol) is established between the client and the server.

Static Web sites consist only of a set of fixed Web pages written in HTML and stored in the file system. They are transmitted to the Web browser upon request. Dynamic Web applications include also a set of server programs, which *build* (part of) the HTML code to be displayed by the browser.

Several server side languages are available for the construction of a Web application (e.g., PHP, Java, Perl, VBscript, etc.). The same is true for the client side code (e.g., Java, JavaScript, etc.). In this paper, we will consider PHP for the server and JavaScript for the client. Such a choice is by no means restrictive, since these languages offer all the basic features available in the others [9].

User interface in the application uses form controls to interact with user. Filled form fields sent to PHP interpreter to calculations. PHP interpreter at the server executes script that evaluates passing parameters from form. Script file calculates and manage database operations. The main components of the system are shown in FIGURE 1.

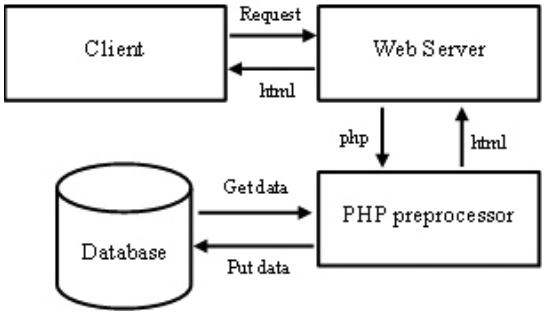


FIGURE 1
Basic structure of PHP-based server system

PHP (recursive acronym for *PHP: Hypertext Preprocessor*) is a widely-used open source general-purpose scripting language that is especially suited for web development and can be embedded into HTML [10].

Since PHP has the feature that its script contexts can be embedded in the HTML language. PHP is an open source language for server side scripting. It is widely used and contains a rich function library code, which makes it ideal for web applications. It is also object oriented so the developed scripts take advantage of object oriented design.

jQuery is a fast, small, and feature-rich JavaScript library. It makes things like HTML document traversal and manipulation, event handling, animation, and Ajax much simpler with an easy-to-use API that works across a multitude of browsers. With a combination of versatility and extensibility, jQuery has changed the way that millions of people write JavaScript [11].

jQuery takes a lot of common tasks that require many lines of JavaScript code to accomplish, and wraps them into methods that you can call with a single line of code. jQuery also simplifies a lot of the complicated things from JavaScript, like AJAX calls and DOM manipulation.

The jQuery library contains the following features:

- HTML/DOM manipulation
- CSS manipulation
- HTML event methods
- Effects and animations
- AJAX
- Utilities

There are lots of other JavaScript frameworks out there, but jQuery seems to be the most popular, and also the most extendable [12].

Bootstrap is a free front-end framework for faster and easier web development, includes HTML and CSS based design templates for typography, forms, buttons, tables, navigation, modals, image carousels and many other, as well as optional JavaScript plugins also gives the ability to easily create responsive designs [13].

Besides properties belong to four different greenhouses chosen as material were given in Figure 2.

RESULT AND DISCUSSION

In the index page shown in Figure 2, there is a general information about the application and default values used in design are given as tables. It is entered to the application with the link of "Project Pages" at the bottom of the pages (Figure 2).

When the "Project Pages" link is clicked, a login form is entered in which users can enter their user name and password for registered users. The "Sign-Up Here" link is used by non-registered users to register (Figure 3).

On the Sign Up page (Figure 4) users can register with the user name, e-mail and password information. The user's name and e-mail are checked to see if it has been used before during registration and users are warned if the same username and e-mail has been registered in the previous recordings. Form based SQL injection is prevented by cleaning harmful codes during processing of form data.

FIGURE 2
Properties belong to greenhouses chosen as material

FIGURE 3
Sign in Form

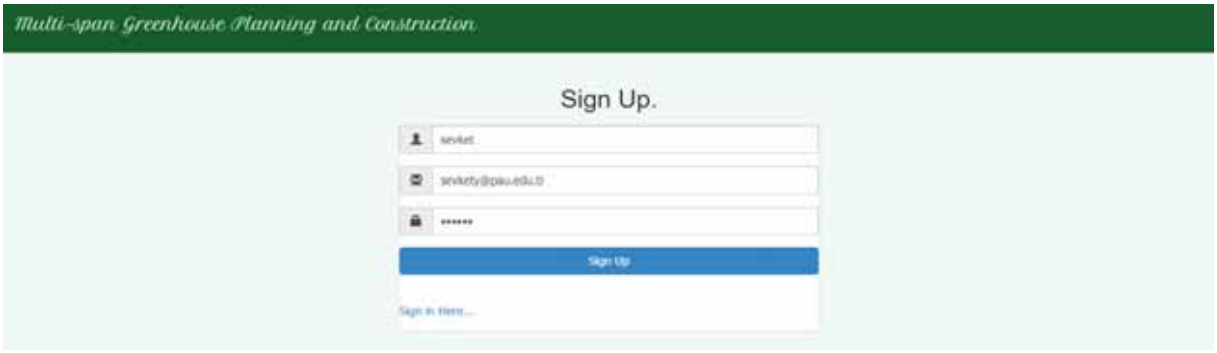


FIGURE 4
Sign Up Form

After the filled the form in Figure 5 and registration is completed, a message is created shown that registration is successful and a link is created that directed to the home page. It is gone to home page shown in Figure 3 by clicking the link of "log-in" in Figure 5 or "Sing in" at the bottom of the pages.

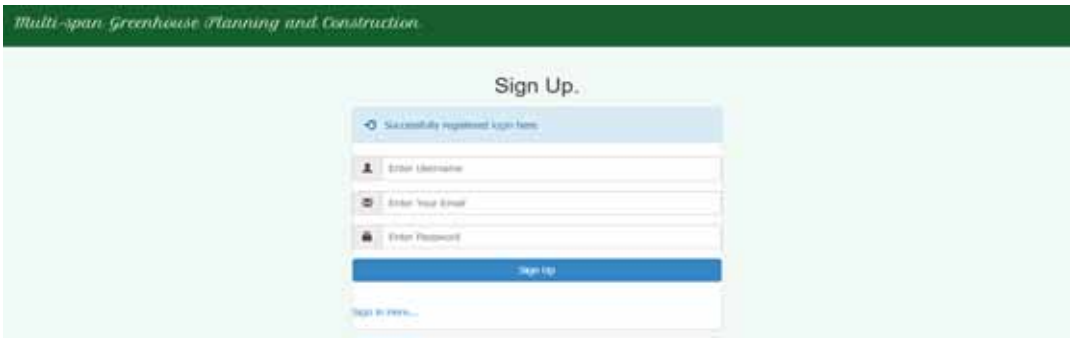


FIGURE 5
Screen where registration is completed

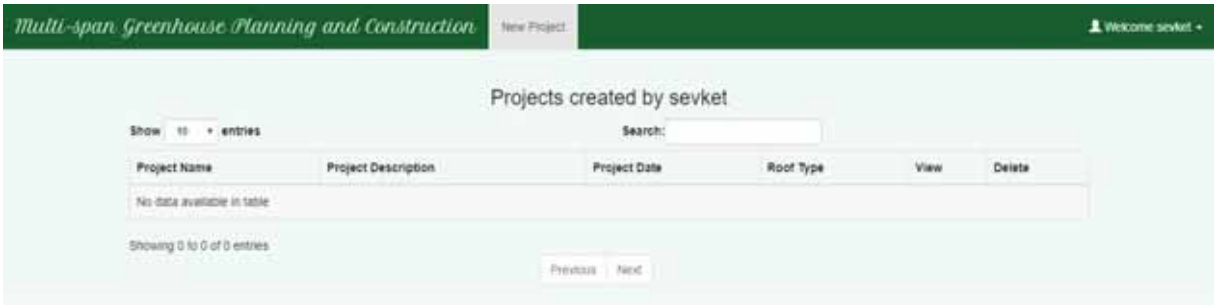


FIGURE 6
Project management page

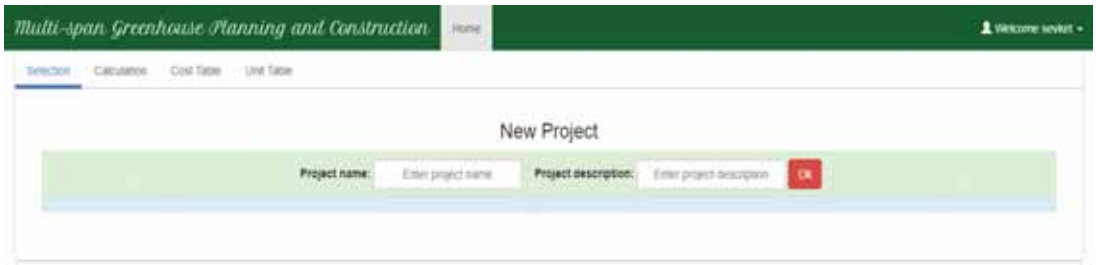


FIGURE 7
New Project page

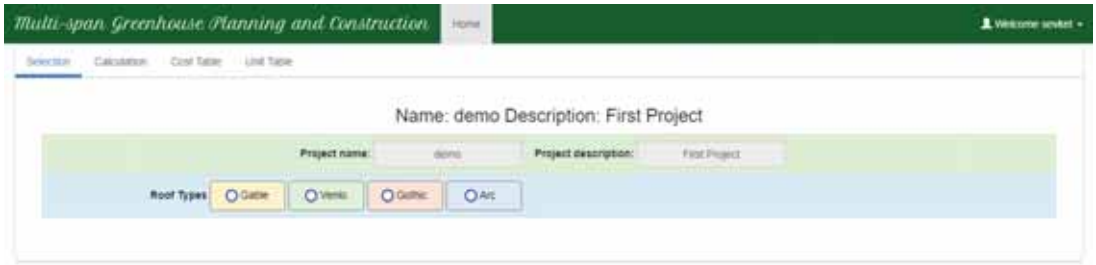


FIGURE 8
Roof type selection

Users reach to the project management page shown in Figure 6 when the users are entered with the registered user information. Projects created by the user in this page are listed as tables with the titles “Project Description”, “Project Date” and “Roof Type”. The listed projects are displayed with the "View" icon and deleted with the "Delete" icon. Here, the table is empty because the user has not created a project. It has to click “New Project” link to create project.

Screen in Figure 7 appears when the "New Project" link is clicked. The first step of creation a project is to give a name and description to the project. It is not passed to the next step before these fields are not filled in. After these fields are filled in and click "OK" button it can be passed to the next step. “Project name” and “Project description” entered are saved in the database and these information is listed in the project management pages.

After project name and description is entered,

roof-type selection is activated. One of the four roof types such as gable, venlo, gothic and arc desired is chosen by clicking radio box button (Figure 8).

After the roof type is selected, sliders are activated where the he greenhouse width and block number can be changed (Figure 9). The user can choose the desired width 5 m intervals between 30-60 m from here. The number of blocks can choose from 2 to 20 blocks. Calculated greenhouse area depending on the selections are displayed in "Area" field with the real time.

Drawings belong to the front and side views of the selected roof type are shown. These drawings change according to number of block selected. In this stage, project is saved by clicking to "Save" button activated after the roof type selection. Before saving, calculation results can be shown by clicking "Calculation" tab where the calculations belonging to the selections are listed, cost results can be shown by clicking "Cost Table" tab or unit price can be shown and update if necessary with the "Unit Table" tab (Figure 9).

Greenhouse drawings which are changing depending on block number selection are shown in Figure 10, 11, 12, 13 for the 3, 4, 5, 6 blocks, respectively. Same drawings are used for the 6 blocks and more.

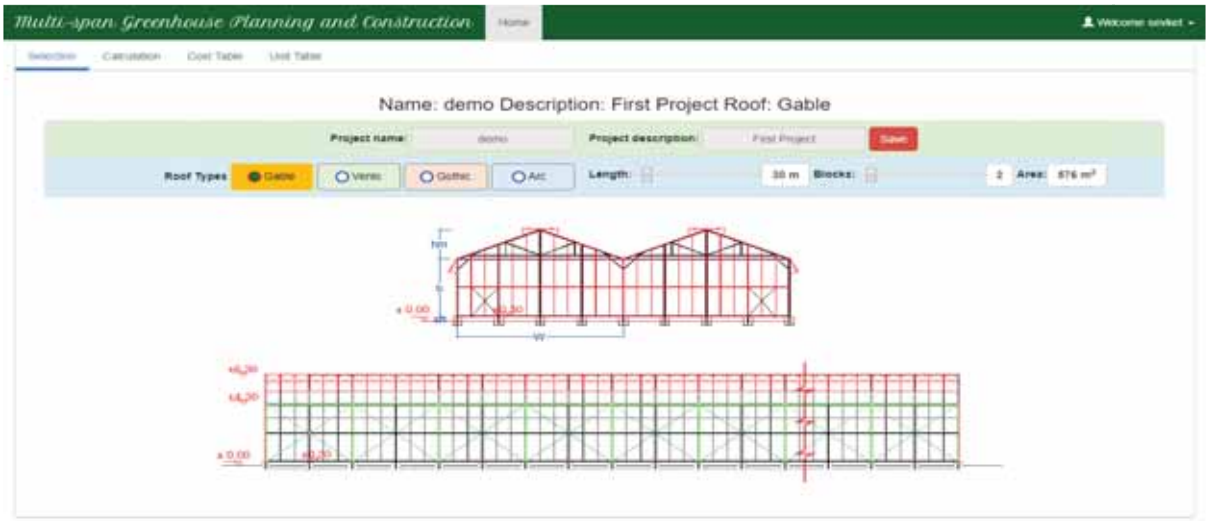


FIGURE 9
Screen where the roof type is selected.

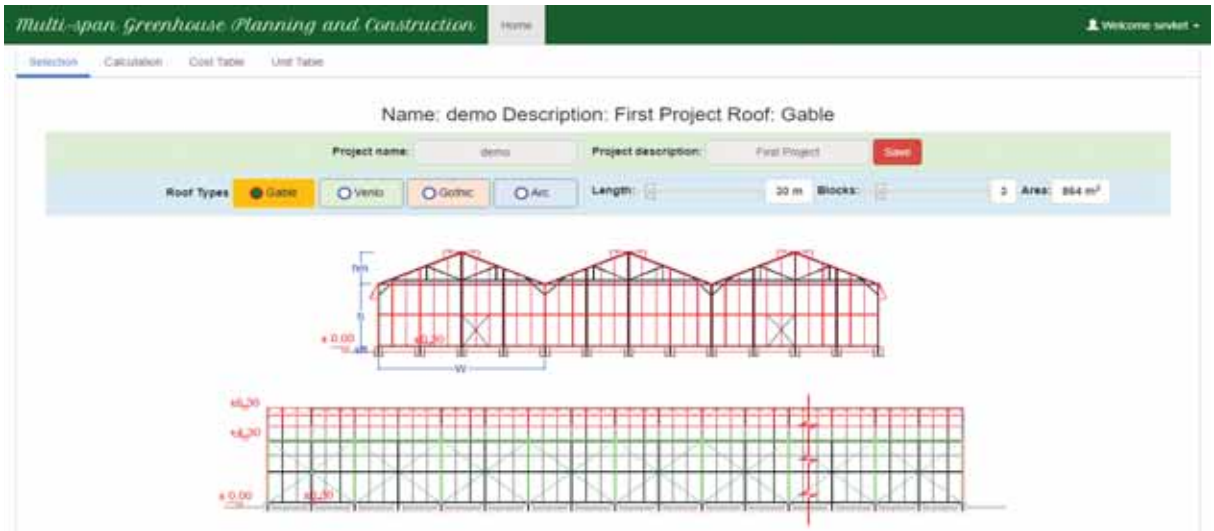


FIGURE 10
3 blocks greenhouse drawings

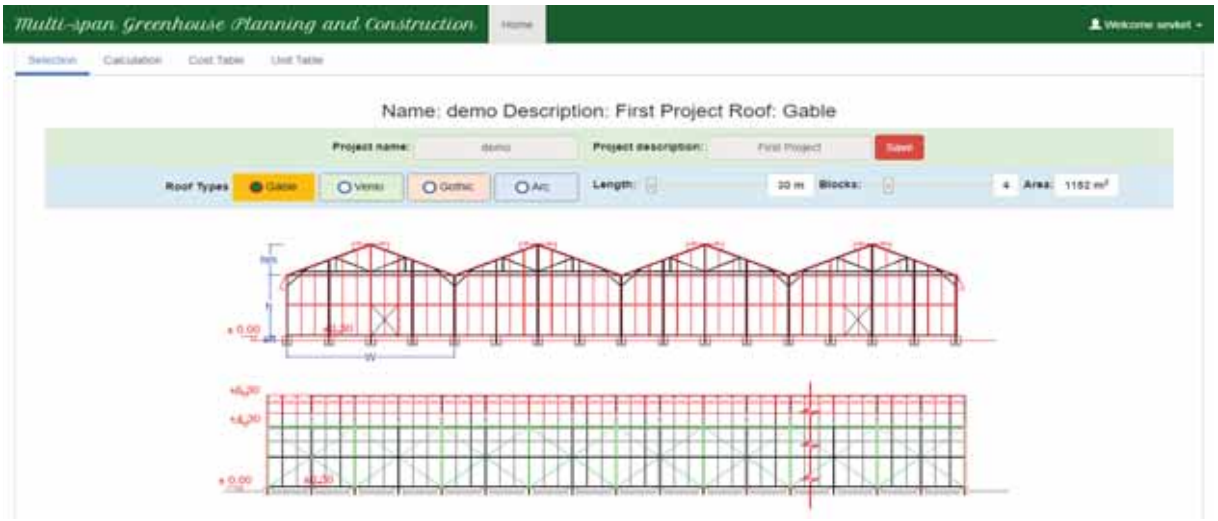


FIGURE 11
4 blocks greenhouse drawings

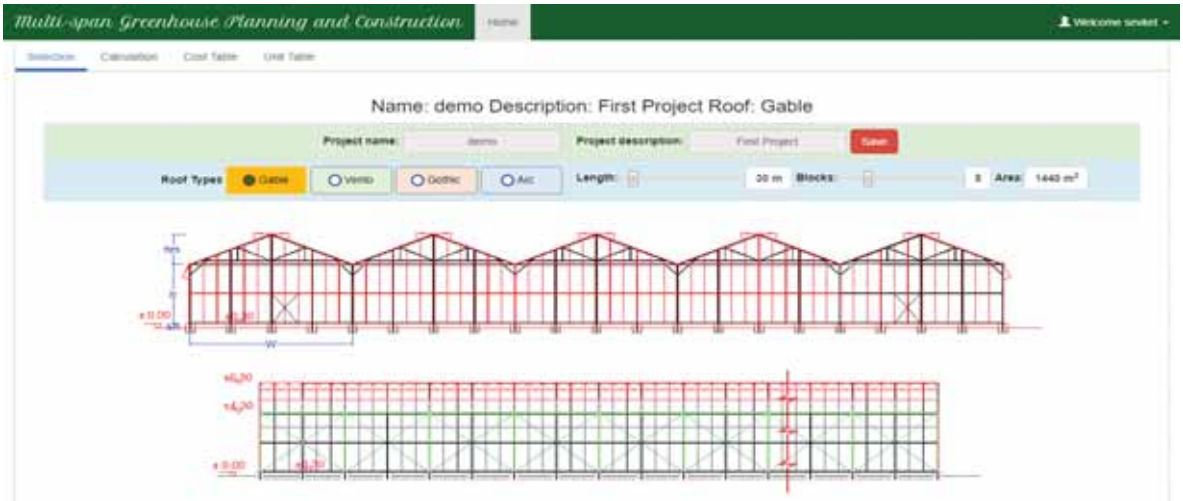


FIGURE 12
5 blocks greenhouse drawings

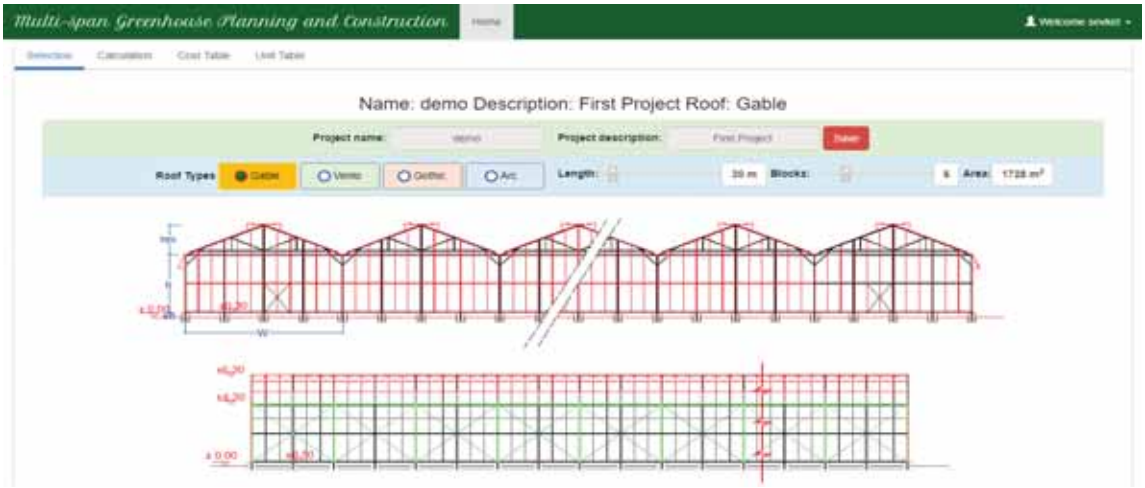


FIGURE 13
6 blocks greenhouse drawings

Calculations which is done according to selections located in “Calculation” tab were given in Figure 14 as table. Detailed information about the profiles to be used in the design can be obtained from the "Details" link on the side.

FIGURE 14
Calculation tab

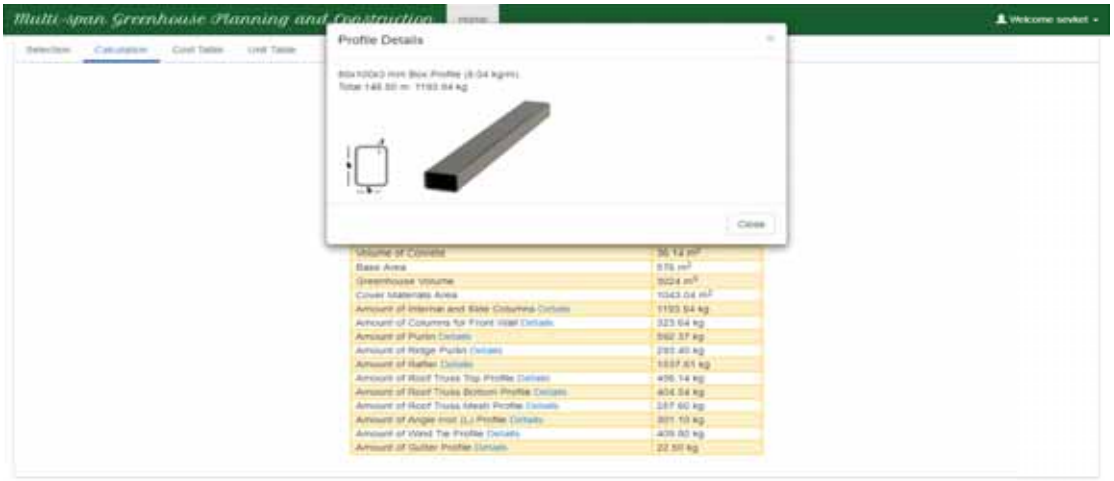


FIGURE 15
Details screen

FIGURE 16
Cost table screen.

For instance, users can see profile type, unit weight, total weight and how many meter should be used from the information screen for the "Internal and Side Columns" (Figure 15).

Volume of Excavation, Volume of Concrete, Amount of Mold, Cover Materials, Irrigation Sys-

tem, quantities, unit prices and their sum belong to required profiles are shown in the table in the "Cost Table" tab. Total cost of greenhouse and unit cost per m^2 are shown at the bottom of the table. Unit prices are selected from the dropdown box. Calculations are performed according to last entered unit prices by default. If desired, recalculations can be made by selecting previous unit prices. Unit prices can be selected as Turkish Lira (₺), Euro (€) or U.S. Dollar ($) (Figure 16).

FIGURE 17
Unit table screen.

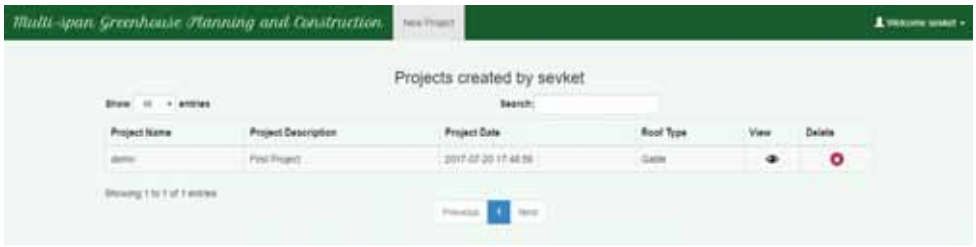


FIGURE 18
Project management page

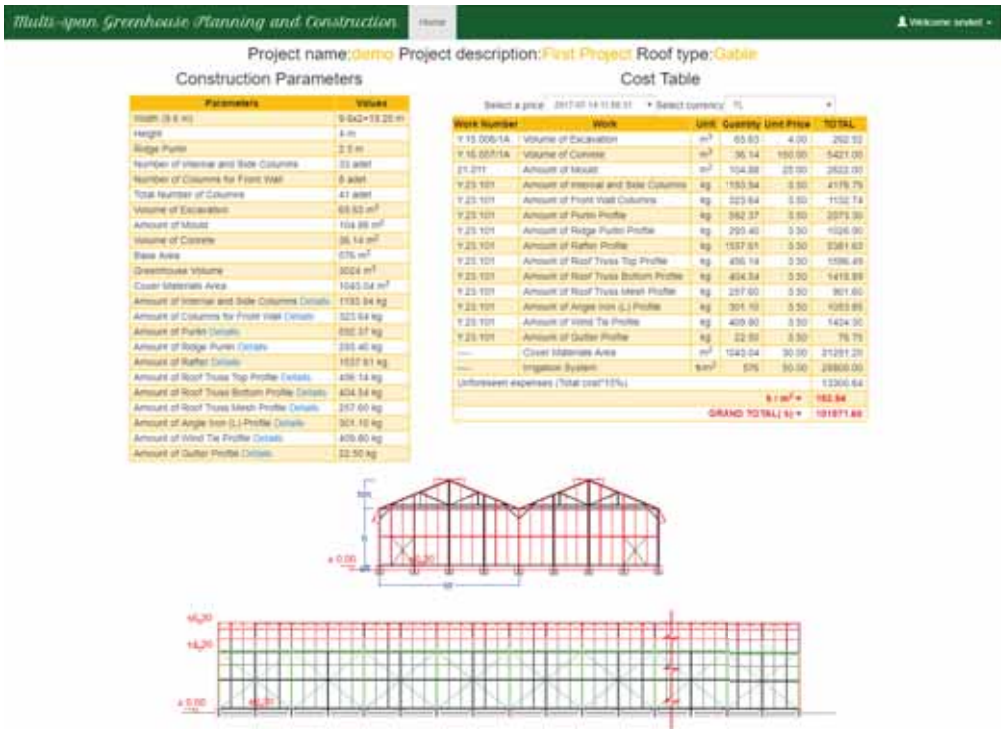


FIGURE 19
View screen

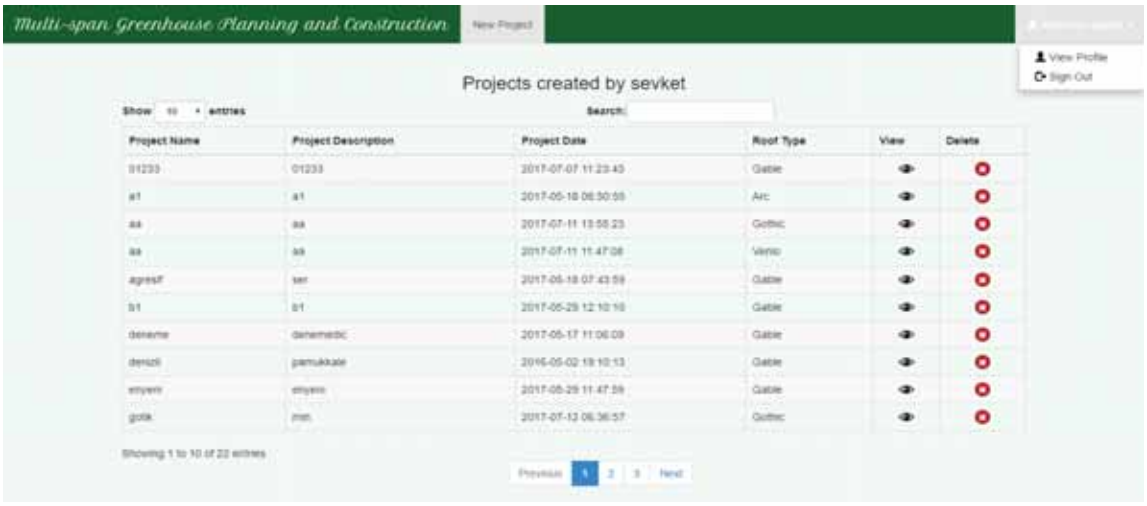


FIGURE 20
Sign out tab

Unit prices of the all components required for the project cost calculation is located at the "Unit Table". Unit prices are updated when requested and saved in the database with the "Save" button. The foreign exchange provisions of unit prices are taken instantaneously from the data of the Central Bank and changed. The unit price table includes unit prices of the profiles used in the all greenhouses (Figure 17).

It is returned to the project management page when the project is saved by clicking the "Save" button at the "Selection" tab. As shown in Figure 18, project name, description, date and roof-type were shown in the table and "View" and "Delete" buttons are activated. Project details are shown as a single page by clicking to the "View" icon and delete the project with the "Delete" icon.

All data belong to project design at the page opened when the view icon is clicked are shown in Figure 19. In this screen, the details of the profiles can be seen by clicking on the related links and results can be calculated instantaneously by changing the currency.

After the project is completed, the "Sign Out" option in the upper left menu is used to exit the project page and return to the main page (Figure 20).

Buyuktas et al., (2011) [14] aimed to determine the cost of singular greenhouses in computer with a software having ground area of 100 to 750 m^2 by entering greenhouse size. They reported that their study would last long and respond to the demands of the greenhouse designers for years. They also specified that it was so practical and advantegous that it could be used both in designing and in education in the fields of civil engineering and agriculture. Guzman et al., (2004) [15] developed a web-based system able of remotely controlling greenhouse climatic conditions and irrigation using different hardware and software platforms. They noted that this web-based system could be used by the student and greenhouse manufacturers to design a greenhouse climate. Büyüktaş et al., 2013) [16] carried out a study to determine the cost of multi-span greenhouses with a ground area of 400 m^2 - 5760 m^2 through computer data entry. They allowed the program to calculate the greenhouse cost according to some defined greenhouse characteristics. They also reported that the manufacturers would be able to select a greenhouse type and its cost according to the product they wanted to grow by entering data they have selected and would be able to see the plan, section and view of the criteria entered in a particular greenhouse. Buyuktas et al., (2010) [17] aimed to calculate the dimensions of necessary measures and to determine the cost of a greenhouse structure, where vegetables are grown, by the computer based on some information provided by the user. They noted that the software developed was easy to use, user friendly and had an interactive interface; and it continues the process step by step according to the user preferences. In practice, all of the options would be used in the calculation to minimize user errors, as selected from the prepared radio box or combo box. Our results were in line with the results of these studies.

CONCLUSION

Manufacturers who want to install a greenhouse can easily calculate the installation cost in a few steps for vegetables or fruit greenhouse with gable, venlo, gothic and arc roofed by using the web-based application. Manufacturers or companies will be able to complete very long bidding process much more quickly using the application with user-friendly interface. In addition, manufacturers will be able to compare the accuracy of the bid they receive from companies with this application.

User registration, user login, project management and new project creation have been successfully accomplished in this web-based database driven application. With this application, the most economical and efficient greenhouse design can be done with program codes by choosing the minimum parameters of the user in project design.

Supervisor operations such as user registration confirmation, user deletion, project limitation, etc. do not add to web-site because application is not considered to be a commercial product at this stage. Hovewer, mobile version of this application can be prepared in the next step.

ACKNOWLEDGEMENTS

The authors would like to thank to Administration Units of Akdeniz University and Pamukkale University for their support.

REFERENCES

[1] TSE. (1996) Greenhouse-Terms and Definitions. Turkish Standards Institute. ICS 65.040.30.I, Ankara (in Turkish).
[2] Ertekin, N. (1991) Low Plastic Tunnel. Greenhouse Resource Institute, Farm Chapter 2, İzmir (in Turkish).
[3] Baytorun, N. (2016) Grenhouses. Greenhouse Types, Equipments and Climates. Nobel Publishing. 417p.
[4] Büyüktaş, K., Atılgan, A. and Tezcan, A. (2016) Agricultural Production Structures. Textbook No:101. Suleyman Demirel University printing office. Isparta/ Turkey. 253p.
[5] PHP. (2017) Retrieved 07 20, 2017, from PHP: http://php.net/
[6] PDO. (2017) Retrieved 07 20, 2017, from PHP Data Objects: http://php.net/manual/en/ book.pdo.php
[7] jQuery. (2017) Retrieved 07 20, 2017, from jQuery: https://jquery.com/
[8] Bootstrap. (2017) Retrieved 07 20, 2017, from Bootstrap: http://getbootstrap.com/
[9] Tonella, P. and Ricca, F. (2002) Construction of the System Dependence Graph for Web Application Slicing. Proceedings of the Second IEEE International Workshop on Source Code Analysis and Manipulation (SCAM'02).
[10] Anonymous. (2017a) What is PHP? Retrieved 07 20, 2017, from PHP: http://php.net/manual/en/ intro-whatis.php
[11] Anonymous. (2017b) jQuery. Retrieved 07 28, 2017, from jQuery: https://jquery.com/
[12] Anonymous. (2017c) jQuery Introduction. Retrieved 07 20, 2017, from w3schools: https://www.w3schools.com/jquery/jquery_intro.asp
[13] Anonymous. (2017d) Bootstrap Get Started. Retrieved 07 20, 2017, from w3schools: https://www.w3schools.com/bootstrap/bootstrap_get_started.asp
[14] Buyuktas, K., Yılmaz, S. and Ertekin, C. (2011) The Comparation of Costs by Compuuture Aided and Interactive Greenhouse Design Application. African Journal of Agricultural Research. 6(14), 3355-3362.
[15] Guzman, J.L., Berenguel, M., Rodriguez, F. and Dormido, S. (2005) Web-Based Remote Control Laboratory Using a Greenhouse Scale Model. Computer Applications in Engineering Education. 13(2), 111-124.
[16] Büyüktaş, K., Yilmaz, S. and Sari, I. (2013) The Comparation of Costs by Compuuture Aided and Interactive Greenhouse Design Application. African Journal of Agricultural Research. 6(14), 3355-3362.
[17] Buyuktas, K., Yılmaz, S. and Ertekin, C. (2010) Computer aided and interactive greenhouse design application. Journal of Food, Agriculture and Environment. 8(3), 340-347.

Received: **18.10.2017**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

Kenan Buyuktas
Akdeniz University,
Agricultural Faculty,
Department of Farm Structures and Irrigation,
Antalya – Turkey

e-mail: kbuyuktas@akdeniz.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9463-9472 Fresenius Environmental Bulletin

A STUDY ON PLANTATION OF TRABZON-SURMENE KUTLULAR SOLID WASTE LANDFILL SITE

Emine Tarakci-Eren, Tugba Duzenli, Duygu Akyol*

Department of Landscape Architecture, Faculty of Forestry, Karadeniz Technical University, 61080 Trabzon, Turkey

ABSTRACT

The present study was conducted to determine the observations and recommendations on planting and landscape restoration and preservation of the nature in Sürmene Kutlular Solid Waste Landfill, where solid wastes and garbage that originated in Trabzon and Rize provinces were dumped for several years. Plant selection in such landfills is similar to the selection of suitable material in landscape design in various areas. Environmental factors that occur in these areas such as toxic gases, wastewater, etc. could directly affect plant growth. Such problems that might be encountered during planting could be prevented by selecting suitable plant species for the site. Furthermore, selection of indigenous species would reestablish broken ecological connections. Planting in landfills are predominantly designed based on ecological and material opportunities. Thus, the selection of woody and herbaceous species that are hardy and problem free, and could resist the hardships related to such fields, should be the priority in plant selection.

KEYWORDS:
Trabzon, Sürmene, Kutlular, Solid waste landfill, Landscape rehabilitation, Planting.

INTRODUCTION

Today, rapid population growth and urbanization led to the problem of waste, in other words, garbage management requirements in urban areas. Based on technological and industrial developments and changes in the materials used, the emergence of waste has differentiated over time [1]. The per capita waste in Turkish provinces was recorded as follows: Aydın 1.60 kg, Aksaray 1.59 kg, Bingöl 1.49 kg, Sivas 1.4 kg, Antalya 1.36 kg, Bolu and Isparta 1.2 kg, Manisa 1.16 kg, Bursa 1.02 kg, Konya 1.01 kg, Adana 1 kg, Samsun 0.94 kg, Mersin 0.84 kg, Erzincan 0.76 kg, Mus 0.64 kg, Trabzon 0.5 kg, Sirnak 0.43 kg and Mardin 0.4 kg [2]. The mean per capita waste in Turkey is 0.6 kg [3].

The term waste refers to all types of used substances that are no longer desirable, and cause harm to the environment. On the other hand, solid waste refers to solid matter and treatment sludge that needs to be disposed by the producer and should be removed regularly in order to protect the society and especially the environment [4, 5].

Classification of Solid Waste. There are several different resources in the literature on the classification of solid waste, but the most commonly referred is the categorization by Baran (1995) [6] based on the quality of solid waste. He classified solid waste as follows:

- Medical and chemical waste including toxic substances and products
- Domestic waste (garbage) [4,7].

Turkish Environmental Problems Foundation (TCSV) classified solid waste as follows: domestic, commercial, construction, agricultural, and hospital waste [8].

Solid Waste Landfills and Classification. Solid waste landfills are sites where most unwanted or unused waste is stored. Until the mid-20th century, waste was dumped in sites with an adequate natural topography in an uncontrolled and open manner. Waste incineration was often observed to reduce the volume of landfills. Topographically low streams, fields with slopes, floodplains and unused mines, sand and gravel quarries were the most common landfill sites. Garbage disposal practices in open areas began to be regulated more carefully during recent years. It is possible to classify the solid waste landfills that are used to eliminate the solid waste problem created by human activities as irregular landfills and regular landfills [9].

Wild Landfills. Wild landfill is a method used in undeveloped or developing countries where solid wastes are removed from the human environment and randomly discharged to open land without any provisions (Figure 1). This method leads to serious problems such as the formation of dust clouds due to wind effect in landfills, air pollution due to the natural gas formation, the environmental and visual pollution due to the solid waste spreading over a wide area, and infectious diseases caused by the animals living and feeding on waste. The landfill at Moloz locality in our region that was closed in 2006 was a wild landfill site [10]. This area was rehabilitated by the Municipality of Trabzon in

2006 with the sponsorship of European Union grant project. This area was converted into a park.

Sanitary Landfills. Solid waste that is needed to be disposed by the manufacturer and removed regularly to protect the environment and solid waste categorized as treatment sludge should be collected systematically based on its physical, chemical and biological effects on the environment and stored accordingly. The aim of sanitary landfills is the treatment of the waste with physical, mechanical, chemical and biological processes, and removal of the solid waste that are not economically viable or created by the above-mentioned processes and is a threat to human health, harmful for other living beings and disrupt environmental aesthetics and damage the environment [12]. Sanitary landfills that are built in accordance with the technical standards such as adequate site selection and environmental protection measures are the most effective way of waste disposal [10]. Trabzon Sürmene Çamburnu Kutlular Solid Waste Landfill in our region is a sanitary landfill site.

Based on Regulation for Solid Waste Control,

- Solid waste landfills should be at least 1 km away from the nearest settlement and at least 3 km away from an airport.
- It should not be constructed in designated preservation areas where drinking, tap and irrigation water are procured or surface water, which could be used for above-mentioned purposes, is present.

FIGURE 1
Waste landfills in coastal areas [11]

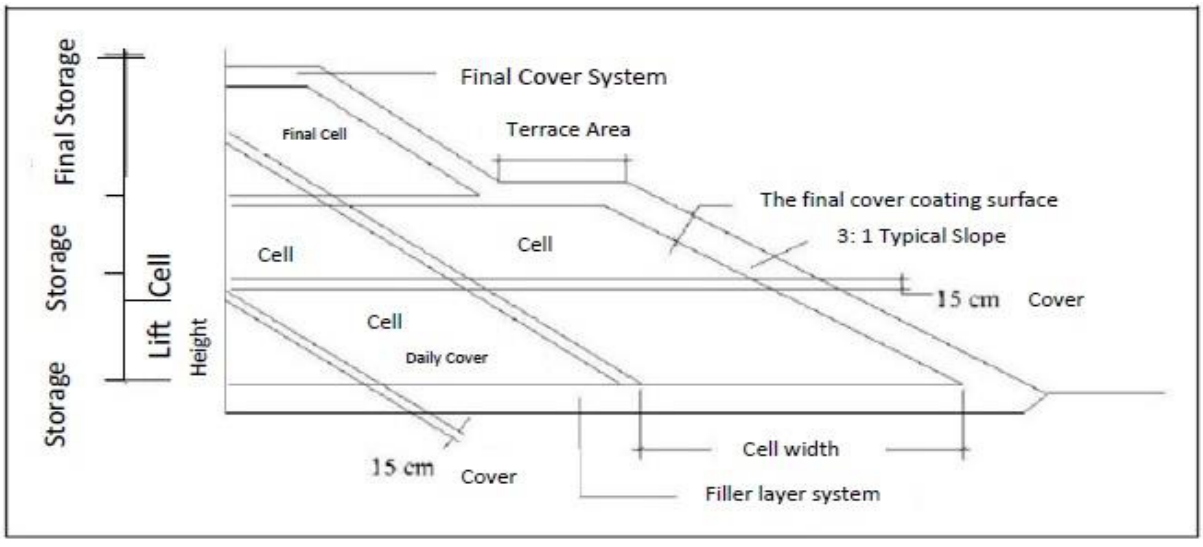


FIGURE 2
A cross section of a typical sanitary solid waste landfill

- Beyond a level of stability that will ensure the continuity of the landfill operation and to ensure that groundwater is not contaminated, the most important feature in the ground is minimum water permeability for landfill sites. Areas with minimum water permeability should be preferred.
- Areas that could be improved by landfills and landscaping such as areas destroyed by previous industrial activities, excavated lands or that do not serve any purpose are suitable for solid waste landfills(Figure 2).
- Landfills should not be built on fault lines in earthquake prone regions.
- Landfills should not be built in areas with a high risk of flood, avalanches, landslides and erosion.
- Access to landfills from the garbage collection areas should be easy. In addition, transport distance is a major factor in site selection. If the transportation distance is short and other conditions are met, this is desirable condition to reduce the transportation costs.
- Based on urban planning principles, solid waste landfills should not be constructed in the direction of predominant winds.
- The landfill capacity should be at least 500,000 m3 in areas with a population greater than 100,000, and it should be sufficient to hold 10 years of waste in settlements with a population of less than 100,000.
- It should be ensured that the landfill is not opened for settlement at least 40 years after the closure of the operations and location of the facility should be detailed in the zoning plan.
- Following the selection, 1/1000 map of the land should be prepared and an EIA report should be developed.
- Plans and projects that should be designed for the landfill are as follows:
- How the land base would be prepared, how the drainage, leak-proof strata and gas control structures would be constructed should be demonstrated.
- The landfill operations, transportation methods, soil deposits should be specified.
- How to abandon the full landfill area and how to green these areas should be mentioned in these projects.
- Projects for infrastructure and service buildings should be provided.

Effects of Solid Waste on Individuals and the Environment. Garbage that is not deposited under adequate conditions or disposed randomly into a field creates a suitable breeding environment for organisms that cause diseases. It is known that hundreds of infectious diseases are transmitted to humans through garbage and solid waste. Thus, landfills are the largest medium of reproduction and propagation that threaten our health.

Solid waste landfill soil is composed of cells that contain a wide variety of materials with different physical, chemical and biological properties when compared to the normal soil conditions. Their growth potential that cause environmental problems such as uncontrolled gas production, irregular ground motion, water pollution and leachate should not be ignored.

The formation of leachate in landfills is a complex process. Solid waste stored on the land is transformed by chemical and biochemical mechanisms. Groups with organic origins, such as food, garden and animal waste, are used by microorganisms and broken via aerobic and anaerobic methods. In the event that excessive amounts of water enter the solid waste piles above a certain water retention capacity, the landfill could not retain the excess water and discharge it. This excess water, which is called leachate, carries various pollutants and degradation products to the surface or groundwater sources as it passes through the garbage. Rainfall water, runoff water or underground water come into contact with incompletely stabilized garbage, resulting in environmental degradation by transporting disintegrating products outside of the garbage storage cells [13]. This is one of the most important problems that threaten public health. In general, the problem of leachate in storage areas in arid regions with low precipitation does not reach significant dimensions. However, in regions with an annual rainfall of more than 400mm, the problem of leachate could lead to dangerous consequences [14, 8]. According to the estimations by TÜİK, the annual precipitation in Trabzon was between 1000-1200mm in 2015. In other words, if the leachate is not controlled, it could cause great hazards [3].

Problem Area and the Aim of the Study. In principle, all the technical problems that could occur in sanitary landfills have been accounted for. But to summarize, the common problems in landfills are as follows:

- Uncontrollable gas production,
- Irregular ground motion (erosion),
- Leachate.

These problems will be addressed and recommendations will be provided with respect to technical and landscape restoration in Kutlular sanitary landfill (Figure 3). In fact, these problems were technically resolved when the field was active. After the closure of the site, the proposed restoration (planting) will completely prevent adverse effects on individuals and the environment. Furthermore, integration of the structure with the environment will be ensured.

The main objective of landscape restoration that would be conducted after the closure of solid waste landfill is to render the landscape elements or parts that would affect the environment negatively harmless and take necessary measures to establish

various rational human activities in the surrounding area. In the plantation studies that would be conducted after the closure of the area, ecologically and aesthetically diverse environmental features should be restored, and in particular the ruptured ecological ties with the environment and ecological sustainability should be reestablished [15]. However, restoration with plants, that is, restoration with live materials, have some disadvantages compared to restoration with inanimate materials. These disadvantages are,

- The conditions for plant growth must be available, or these conditions should be created.
- Planting could only be conducted at certain times of the year.

Effective restoration could be achieved only after a long period of time after the completion of the work [16, 17, 18].

When such sites are within the borders of the urban center, it is very useful for urban areas and urbanites to open these areas for new uses. Despite the increase in the amount of green space per capita in Turkish cities and in Trabzon especially during the recent years (8.5 m2), it is still not at the desired level (10 m2) [20, 21]. If the study area was an urban area, it could be suggested that this area should be utilized as an urban green area. This restoration could be conducted with combined restoration with both living and non-living materials. However, since the study area is a rural area, ecological continuity with the surrounding environment should be maintained rather than a conversion to different uses after closure. Because, in planning or design of rural areas, natural environments that meet the needs of local people, suitable landforms that are compatible with the climate conditions and solutions with an emphasis on cultural values are planned or designed [22]. Thus, the primary goal in this field of study is to make this area compatible with its immediate surroundings. This is only possible through selection of indigenous plants, recreation of the local character and reestablishment of ecological ties with the proposed landscape restoration. However, the negative aspects of the landscape should be taken into consideration in plant selection. As mentioned earlier, when living materials are used in restoration, the presence of proper conditions is significant. Therefore, in addition to indigenous species, suitable species for the restoration of the particular area will be recommended.

Trabzon Sürmene Çamburnu Kutlular Sanitary Solid Waste Landfill will be planted and restored as a natural environment. However, the use of scientific approaches in the restoration of this area, for example in the selection of the plant material, rapid growth of plants, reduction of environmental effects and creating visual effects, should be taken into consideration. In particular, the pH of the soil in question and its permeability are the first limiting factors in the determination of the plant species.

FIGURE 3
Wild landfills before Kutlular sanitary landfill was built in Trabzon [19]

RESULTS AND DISCUSSIONS

Study Area. The study area was the Sanitary Solid Waste Landfill Site in Trabzon Province, Sürmene District Çamburnu Locality (Figure 4). Trabzon has a surface area of 4685 km^2 between 40^o-33^o and 41^o-07^o north latitudes and39^o-07^o and 40^o-30^o east longitudes in the Eastern Black Sea Region. Gümüşhane province is in the south, Giresun province is in the west, Rize province is in the east, and Black Sea is in the north. The population is 214,949. Mountains, hills and highlands are usually found in the higher altitudes. In Trabzon, which has a very rich vegetation, there are 2500 plant species which are rare in Turkey and 440 of these species are indigenous. Trabzon has a humid climate, where the humidity is could reach 99%. The average annual precipitation was 1000-1200 mm in 2015 [3]. The precipitation rate is higher the inner regions. The least rainfall is observed in July and August and the most snow falls in February.

The study field was used as a copper mine site by copper mining enterprises before it became a sanitary solid waste landfill. Later, after the process started in 1995 under the leadership of the Ministry of Environment, it was considered adequate to use this area as a sanitary solid waste landfill after 2006 [23].

After the mine was removed, the landscape needed to be restored, however the area was abandoned and had become a marsh (Figure 5). As a result, the surface waters flowing through the field and into Gökçesu stream polluted the stream due to the high copper content [19].

The construction stages of the study area and the elements (Vehicle Maintenance, Administrative Building, Scale, Tire Washing, Sterilization of Medical Wastes, Nitrification Denitrification, Balancing Pool, Nano Filtration, Ultra Filtration, Methane Gas Power Generation Facility) included in the field after the construction are presented in Figure 6. The study area is surrounded by forests.

FIGURE 4
Study field

FIGURE 5
Picture of the Kutlular Sanitary Solid Waste Landfill when it was used as a copper mine field [18]

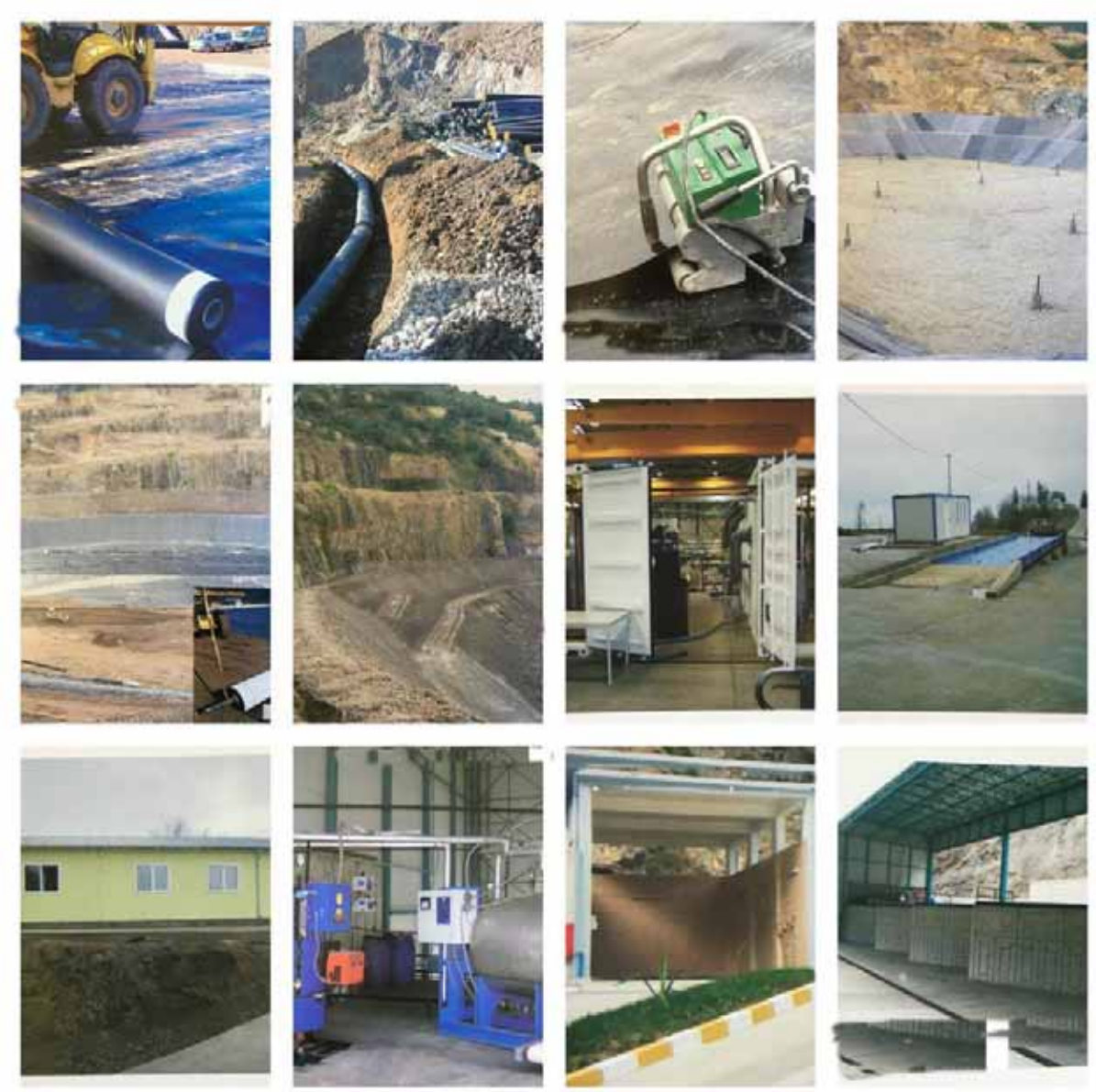


FIGURE 6
Construction stages of the sanitary solid waste landfill site and the units in the site [18]

FIGURE 7
The present state of Kutlular Sanitary Landfill Site

FIGURE 8
Kutlular Sanitary Landfill Site after landscape restoration

Findings on the Identified Problems About the Study Field and Recommended Plant Restoration Measures. Findings and Recommendations on Methane Gas Removal in The Landfill Site. Technical findings on methane production and removal in sanitary landfill site were as follows:

There are 2 gas engines and generator sets, with a capacity of 1,415 MW each in the power generation plant built on the landfill site. As of November 2016, the total installed capacity was 2,8 MW / hour, and the capacity of the plant could be increased by adding an engine based on the amount of gas generated in the site. Furthermore, in case of maintenance or failure of the gas engines, or production of over-capacity gas, the input gas is removed with a burning flare (Figure 7). Thus, even when the plant does not work, it contributes to the emission reduction and eliminates the methane gas without harming the environment and human health and attempts to prevent the formation of a smell.

The botanical measures that could be taken for methane production and disposal even before the closure of the site are as follows:

Fragrant plants could be used to prevent malodor generated in the site. Woody plant species *Laurus nobilis, Tilia platyphyllos, Rosmarinus officinalis, Philadelphus coronarius, Camallia sinensis* and herbaceous species *Allyssum sp., Rosa sp., Salvia sp., Narcissus sp., Lonicera sp*., *Thymus sp*. could be used on the northwestern direction of the study site to improve the impact of their fragrance. Thus, the bad odor will be avoided both by technical means and landscape restoration.

Findings and Recommendations on Irregular Ground Movements. The study area has a northeasterly exposure. The altitude is 450 m. The study site surface area is 12000 m^2. In Trabzon province, there is a frequent risk of erosion. Thus, adequate plant species should be selected in order to prevent surface movements in the study area.

Factors that cause irregular ground movements are water abrasion and transportation (leakage, or rain water), wind erosion, and landslides. In short, the active factors that lead to irregular ground movements are 'water and wind'. In addition to the wind and water, biological factors (collapse, deterioration, decomposition, etc.) can cause irregular ground movements in the study area. As a result of regular measurements in surveys conducted in the study area, it was determined that northwesterly winds are dominant in the area. The study area descends from the south to the north. The garbage is disposed towards the south with a cellular pattern. Thus, the dominant winds do not cause surface movements in the study area. Due to the susceptibility of the soil to wind erosion, wind curtains could be formed with woody evergreens and deciduous species on the northwest boundaries of the study area. This problem could be significantly reduced by plantation on the slopes of the field that are open to the winds. After the landfill was closed, the final ground cover was 1 m deep. This would make it impossible to use woody species initially. Therefore, it is necessary to use indigenous herbaceous species that are resistant to the local climate properties.

Thus, the following herbaceous species could be used: *Sambucus sp., Ranunculus spp., Malva parviflora, woody watery mildew spp., Medicago sativa, Urtica dioica, Mentha sp., Pteridophyta sp., Hedera helix, Xanthium strumarium, Conium maculatum, Pulmonaria officinalis, Taraxacum officinale, Melissa officinalis, Lamium purpureum, Rhododendron ponticum, Corylus avellana, Alnus glutinosa, Ficus carica, Fagus orientalis, Picea orientalis, Pinus sylvestris, Camellia sinensis, Vaccinium sp.* As the area would be rehabilitated using

indigenous species, ecological networks could be reestablished and accelerated in the environment.

Findings and Recommendations on Leachate Management. Technical findings on leachate management in the sanitary landfill site are as follows:

Certain projects were developed in Kutlular Sanitary Landfill Facility to treat the leachate during the construction phase based on site characteristics and meteorological conditions. In order to ensure drainage of waste leachate during the construction of the site, perforated drainage pipes were installed at the bottom of the landfill after it was covered with a leak-proof impermeable layer. These drainage pipes were laid out in the form of tree branches so that they could intake the leachate formed in all parts of the field. Constructed waste leachate drainage pipes were covered with 30 cm thick gravel (Figure 7). Thus, the pipes were protected against the garbage piles, and most importantly, the proper drainage was ensured. Leachate drained from the field via perforated pipes under the ground flows into the pump pit using the gravity. From the pump pit, the leachate is then transferred to the balancing pool, where the treatment is initiated. All parts of the leachate management system are regularly maintained at frequent intervals and kept operational. The pipes are checked regularly and frequently for sand and sludge accumulation, clogging or other obstacles. Factors that could affect the performance of the leachate management system adversely are reviewed and all parts of the leachate management system are checked regularly and frequently for possible damage and required repairs are performed. The leachate management system is based on the principle that maintenance is more efficient than fixing the problems.

Landfill leachate is treated with a biological process and then transferred to the treatment plant for the physical process. UF and NF filters are used for the physical treatment, and when the treated leachate meets the standards of the Water Pollution Control Regulation, it is discharged to the receiving body. Based on the current values, COD pollution of about 20000 mg / l in leachate is lowered to 1400 mg / lt with 90% efficiency in the UF system effluent and to 200 mg / lt in the NF system effluent. The treated leachate is discharged to Gökçesu stream, which is at a distance of 10 m to the treatment plant on the northeast. The measured values in the leachate at the treatment plant are analyzed by special laboratories accredited by the ministry. Furthermore, analysis of waste leachate is conducted routinely in Provincial Environment and Urban Planning Directorate laboratory with ready-to-use kits.

Leachate management system is under considerable control with technical studies conducted in the field. However, after the site is closed, the proposed planting would ensure the continuous control of the leachate. As mentioned earlier, leachate control is more problematic in areas with high rainfall when compared to low-rainfall areas. Because, rainfall, surface waters or underground water interact with garbage, leading to environmental pollution by transportation of the garbage out of the storage cells. This impends public health and the environment. Thus, in the context of the conducted landscape restoration study, the water holding capacity of the soil and access water in the soil were examined by Thorn Thwaite method based on the annual precipitation in Trabzon province and suitable plant species were selected to prevent leachate problem in the site.

Accordingly, water status graph was developed for Trabzon province by superposing evapotranspiration, which is the total water loss in the soil due to the plant intake and evaporation, and the precipitation amount. In Trabzon, rains start to increase after August, and highest precipitation is observed in October. Water is stored in the soil between August and January. In April, the precipitation decreases and the consumption of the water in the reservoir starts. In July, the soil water is consumed and water shortage begins. Thus, irrigation could be commenced by July. However, the species that could be used for the improvement of the areas with damp and wet soil in the presence of leachate problem are *Picea orientalis., Taxodium distichum, Thuja occidentalis, Platycladus ortelis, Thujopsis dolabrata, Alnus glutinosa, Crataegus oxycantha, Eucalyptus sp., Fraxinus sp., Kerria japonica, Lliquidambar orientalis, Liquidambar straciflua, Quercus sp., and Sorbus aucuparia.*

In addition to the recommended species, the species that are generally tolerant in sanitary landfills are *Acacia latifolia, Acer rubra, Arbutus unedo, Eucalyptus lehmannii, Fraxinus pennsylvannii, Ginkgo biloba, Gledtsia triacanthos, Grevillea robusta, Liquidambar styraciflua, Melaleuca quinquenervia, Myoporum laetum, Myrica pensylvanica, Nyssa sylvatica, Picea abiestir.* The species that are sensitive to landfills are *Abies concolor, Acer saccharum, Caria ovata, Cornus Florida, Malus, Picea pungens, Pinus resinosa, Populus nigra italica, Prunus, Pseudotsuga menziesii, Quercus velutine, Salix sp, Sorbus aucuparia* .

DISCUSSION AND RESULTS

Waste is a natural and inevitable consequence of life. Today waste is considered as one of the reasons of environmental problems and this fact is mostly related to the failure of societies to manage waste. The development of solid waste landfill techniques and recycling the resources should provide economic, environmental and energy-related benefits for future forms of landfill reuse.

Garbage landfills lead to negative psychological consequences in public health [24, 25, 26]. People who live near these areas are diagnosed with unsubstantiated health symptoms by the specialists due to the presence of nearby landfill sites. Thus, plantation of these areas, colored with flowering plants, and hence, minimization of the visual pollution would have a positive effect on public health, contributing to the elimination of psychological pressure on individuals and their health concerns [27]. There are no public settlements near Kutlular sanitary landfill site. The Black Sea region geography primarily includes detached buildings surrounded by forests. It is therefore important that it would demonstrate harmony with the surrounding forests in ecological and aesthetic terms.

As a result of the observations and examinations conducted at Trabzon Sürmene Çamburnu Kutlular Sanitary Landfill, it was determined that planting should be conducted to solve the environmental problems that were caused by the mine or after its closure and restoration of the site. The observations conducted in the area demonstrated that it was important to plan the planting in the present site based on economic and ecologic resources and reduction of environmental pollution and restoration of the ecological ties with the surrounding forests. Because, it is not possible for Kutlular site, which has been used for several years as a landfill site for Trabzon and Rize urban settlements, to repair itself if the solid waste deposits were left untouched after closure. Since the soil content would change, regeneration of the vegetation would be difficult in the area. If no measures would be taken and restoration would not be conducted, groundwater pollution, as well as image pollution and unwanted odors would increase. Thus, it is important to plant woody and herbaceous material, which could be grown under harsh conditions, at Kutlular solid waste landfill site.

Limited number of studies were conducted in Turkey on restoring the old landfills to the nature. No studies were conducted on or suggested landscape restoration in the sanitary landfills in Trabzon province. The present study is significant in this regard. In the present study, plant species were suggested for the restoration of the site. However, planting in the area in blocks and monitoring the development of the recommended species, and determination of the species that could facilitate or slow down the restoration process require future studies on the topic.

REFERENCES

[1] Sadowsky, M.J. (1999) Phytoremediation: Past Promises and Future Practises. Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. In: Bell, C.R., Brylinsky M., Johnson-Green, P. (ed.) Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

[2] URL-1 Turkey's Statistical Agency, Environmental Statictics (TUIK). (2016) https://biruni.tuik.gov.tr/medas/?kn=119&locale=tr.

[3] TÜİK. (2006) Turkey Newsletter, Municipal Solid Waste Statistics 2006. T. C. Prime Minister of Turkey Statistical Institute. http://www.tuik. gov.tr/.

[4] Başkaya, F.A., Yıldızcı, A.C. (2011) Evaluation of Solid Waste Areas of Istanbul City in terms of Landscape Planning. ITU Journal Series A: Architecture, Planning, Design. 10(1), 116-124.

[5] Gökçe, G., Aydemir, K.P., Hasanoğlu, P. (2015) Breeding and Plantation of Solid Waste Landfills and Wild Storage Areas. Düzce Üniversitesi Bilim ve Teknoloji Dergisi. 3, 258-271.

[6] Baran, S. (1995) Some Main Considerations in the Selection and Construction of Solid Waste (Garbage) Storage Locations, Issue 46. May, Ankara

[7] Şahin, C., Serin. N. (2008) Researches on the Plantation of Old Landfill Storage Area in Isparta City. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi. 1, 121-133.

[8] TÇSV (Turkey Foundation for Environmental Problems) (1991), Turkey's Environmental Problems'91,Ankara: TÇSV Publications.

[9] Palabıyık, H. (2001) Urban Solid Waste Management in Municipalities: The Case of Izmir Metropolitan Municipality. Ph.D. Thesis published. Dokuz Eylül University, Institute of Social Sciences, İzmir.

[10] Yılmaz, A., Bozkurt, Y. (2010) Urban Solid Waste Management Practices and Kütahya Solid Waste Association of Turkey (KÜKAB) Example. Süleyman Demirel Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi. 15(1), 11-28.

[11] Yılmaz, C. (2007) Effects of the Black Sea Coast Road on Coastal Cities. Retrieved from http://tucaum.ankara.edu.tr/wp-content/uploads/sites/280/2015/08/semp5_15.

[12] Uluatam, S., Semra, M., Yener, Ö.and Wasti,Y. (2008) A Study on Regular Solid Waste Storage and Regeneration of Old Areas. Design Construction. 276p.

[13] Jim, C.Y. (2001) Ecological and Landscape Rehabilitation of a Quarry Site in Hong Kong. Restoration Ecology. 9(1), 85–94.

[14] Nagendran, R., Selvam, A. Kurian, J., Chiemchaisri, C. (2006) Phytoremediation and Rehabilitation of Municipal Solid Waste Landfills and Dumpsites: A brief review. Waste Management. 26(12), 1357-136.
[15] Lovell, S.T., Douglas, M.J. (2009) Designing Landscapes for Performance Based on Emerging Principles in Landscape Ecology Sarah. Ecology and Society. 14(1), 44.
[16] EPA (Environmental Protection Agency) (2000) Introduction to Phytoremediatio., EPA/600/r-99/107, Cincinati, Ohio, U.S.A, 72, http://www.clu-in.org.
[17] Henry, J.R. (2000) An Overview of The Phytoremediation of Lead and Mercury. U.S. EPA, Office of Solid Waste and Emergency Response Technology Innovation Office. Report May-Aug., 51.
[18] Sutherson, S.S. (ed.) (1999) Phytoremediation: Remediation Engineering: Design Concepts. CRC Press LLC. Boca Raton.
[19] URL-3Kutlular Regular Storage Area, (2016) http://www.trabrikab.org.tr/S.aspx?id=11.
[20] World Health Organization. (2016) Urban Green spaces and health. Copenhagen, Denmark.
[21] URL-2 Green area per person in Trabzon. (2016) http://www.samsunhaber.tc/trabzon/trabzonda-kisi-basi-dusen-yesil-alan-859-metrekare-h135226.html,
[22] Eminağaoğlu, Z., Çevik, S. (2006) Nature Rural Settlements. Kafkas Üniversitesi Artvin Orman Fakültesi Dergisi. 7(1), 28-40.
[23] Trabzon Province 2015 Environmental Situation Report. http://webdosya.csb.gov.tr/db/ced/editordosya/Trabzon2015_.pdf.
[24] Dunne, M.P., Burnet, P., Lawton, J. (1990) The Health Effects of Chemical Waste in an Urban Community. The Med. Jour of Australia. 152(4), 592-597.
[25] Lipscomb, J.A., Goldman, L.R., Satin, K.P., Smith, DF., Vance, W.A., Neutra, R.R. (1991) A Follow-up Study of the Community near the McColl Waste Disposal Site. Environ. Health pers. 94, 15-24.
[26] Baker, D.D., Greenland, S., Mendelein, J., Harmon, P. (1994) A Health Study of Two Communities near the Stringfellow Waste Disposal Site. Arch. of Environmental Health. 43(5), 325-334.
[27] Erdoğan, R., Uzun, G. (2007) An Example for Herbal Breeding of Solid Waste Storage Areas: Adana Sofulu Waste Storage Area. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi. 20(1), 71-82.

Received: **30.10.2017**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

Duygu Akyol
Department of Landscape Architecture,
Faculty of Forestry,
Karadeniz Technical University,
61080 Trabzon – Turkey

e-mail: duyguakyol@ktu.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9473-9480 Fresenius Environmental Bulletin

DESIGNING NATURE FRIENDLY SCHOOL GARDENS: IMPLEMENTING VISIONS OF STUDENTS

Makbulenur Bekar, Nilgun Guneroglu*

Department of Landscape Architecture Karadeniz Technical University, Trabzon, Turkey

ABSTRACT

Education is one of the most important factors for a child to become an individual. School and school gardens are indispensable elements of child education. But in our country school gardens are not as important as school buildings. However, school gardens are an important part of children's physical and mental development. The information that children gain by playing and living in school gardens is as important as the information they earn through the education they receive from the schools. Lack of institutional management, inadequate design of green areas and unplanned settlements lead to construction of gardens with solid textures in school campuses. However, children should be offered the opportunity to be involved with nature. They should be given a chance to live by learning and feeling the nature.

In this study, it is aimed to determine the items and qualities that should be in school gardens from the children's point of view. The study was conducted with a group of 100 students consisting of second, third and fourth grade students in a randomly selected primary school. The methodology is based on semi-structured interviews and drawing exercises with students. The aim was to catch the imagination of the students which are direct users of school gardens on daily basis. As a result, it is determined that children prefer more green space in their schools' gardens and imagine interior spaces with items such as water, sand and soil. Suggestions have been made on how school gardens should be in line with pupils' views.

KEYWORDS:
Elementary school gardens, Nature, Child, Design

INTRODUCTION

Today, the vast majority of the world's population lives in cities due to technological developments and industrialization. The number of children in this dense urban population is too high to neglect. Nevertheless, children are ignored in urban planning studies and the necessary spaces for children are not designed [1, 2]. Scientific research emphasized that children's positive or negative behavior are directly related to the environment they live in [3]. It should not be forgotten that individuals who do not spend their childhood in spaces that affect their physical and social development positively, cannot become healthy adults. In other words, children need spaces and activities where they can develop their social, physical, emotional and mental skills to be a positive individual in the future [4]. Children's playgrounds and school gardens are the most important of these spaces and the most important activity is to play.

Playing games, the most important event in childhood, is a universal concept. The game playing is an activity that conveys excitement and happiness and allows children to have fun and learn. Play activity contributes to the development of children's physical, mental and intelligence in all cultures [5, 6]. Nature is the prominent teacher in child development and contributes to children's learning by experience [7]. It was emphasized in the previous studies that children who spend time in nature get less ill and develop more physical abilities [8, 9].

However, in today's cities, losses in green areas and increasing daily construction activities result in the destruction of natural playgrounds for children [10, 11]. Children are trapped in malls, and homes, while they could learn and develop within the nature. They go to their school which is at walking distance using school shuttles due to heavy vehicle traffic [12].

School and school gardens are the best place for playing games in the contemporary times. Children spend a large part of their daily lives at school. The school gardens, where most of the breaks are spent, are especially important for children. Previous studies have indicated that school gardens could be used for different purposes other than those known by all. These studies also emphasized that school gardens enhance students' sense of responsibility, understanding nature, and improve their sense of being in the nature [13, 14]. Malone and Tranter [15] suggested that school gardens should enable activities that allow children to move freely and experience unconstructed experiences. They argued that these activities would be effective in the development of children's cognitive and social intelligence. Thus, school gardens should not be regarded as ineffective areas where children spend

their free time in the recess, but should be part of the education, and the focus should be on the regulation of school gardens as much as other structural elements in the school [16]. However, it seems like the school gardens are often composed of paved surfaces and the possibilities for green spaces are limited. These gardens do not provide opportunities for children to relax and experience the nature [9, 17]. However, school gardens should offer children several possibilities as spaces where they can learn the nature by experience and recognition by touching. Several studies were conducted on the design of nature friendly school gardens.

The Learning Through Landscapes institution has used natural facilities to educate and teach the children (Figure 1). It aims to explore children's abilities with educational features of the nature. The institution utilized school gardens that it supported with natural resources for these implementations [18, 19, 20].

The Nursery Fields Forever project, created by a group of Italian and Dutch architects, is an example where children are trained through direct observation of farm work and contact with animals [21], (Figure 2).

Similar initiatives were realized with the programs titled Learning Grounds in Canada and Skolans Uterum in Sweden [12]. In the nature-based schools of Canada Grandview Elementary School (Figure 3), Windsor School, Monica's Catholic School and Dartmouth High School, butterfly gardens, hobby gardens and a variety of other activities are utilized directly with the facilities that the nature provides [22, 23, 24].

In Turkey, the compulsory elementary education includes the children in the 6-13 age group and the education commences at the end of September of the year that the child is five years old and ends at the end of the school year when the child is 13 years old. Thus, the school and its garden, the child's first socialization institution, plays a significant role in the development of the child's mental-physical intelligence. However, it is also true that the schools and school gardens that are necessary for mental-physical intelligence development of children are not designed with nature-based planning approaches in Turkey. School gardens in Turkey reflect several missing natural landscape elements. Moreover, these gardens do not fulfil the recreational demands of children adequately [25].

FIGURE 1
School activities implemented with Learning Through Landscapes

FIGURE 2
Children working at the garden in Nursery Fields Forever Project

FIGURE 3
Some activities in Grandview Elementary School

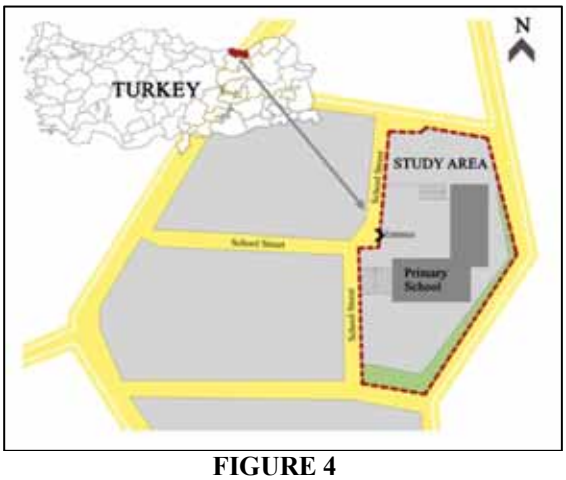


FIGURE 4
Study area plan

The effects of natural elements such as plants, water and soil on children's learning skills should not be overlooked. Especially plants in school gardens affect children's creativity and provide play facilities. Studies demonstrated that children in schools with natural landscape facilities are more creative and their learning skills are more active [26].

Thus, in the present study, the primary school children were asked to draw a picture of a nature-friendly school garden that they imagine. The differences between the current state of their current school garden and the nature-friendly school garden they imagined were identified. It was aimed to determine the desires of the children on the type of the school and school garden they would like to attend and thus, to contribute to the future school garden designs.

MATERIALS AND METHODS

The study was conducted in an elementary school located in Ortahisar district of Trabzon province (Figure 4). The school, which has an area of 6030 m², includes a main building and an auxiliary building (Figure 5). The main school building has 3 floors, 12 classrooms, 3 administrative offices, a 6-cabin bathroom, and the auxiliary building has 5 floors. It includes 21 classrooms, 1 laboratory, a dining hall and a 6-cabin bathroom on each floor. It is an elementary school with a student population of 800-850 in the 1st, 2nd, 3rd and 4th grades.

The school garden is divided into three different gardens due to its topography. Stairs were used as transition elements between the elevation differences. Activities such as ceremonies, demonstrations and playing tag are conducted in the school garden, which is paved with asphalt material. It is not used as a car park besides the vehicles that cater the school which is totally dedicated to the student use. The study group included 100 students randomly selected from the 2nd, 3rd and 4th grades in the 2016-2017 academic year. Of 100 students, 34 were 7-8 years old students, 34 were 8-9 years old students and 32 were 9-10 years old students, 52 were males and 48 were females.

A three-step method was used in the study. In the first stage, the landscaping requirements for an elementary school garden and the studies on nature friendly school gardens were analyzed. Other stages included interviews which were the data collection methods. A personal-semi-structured interview and drawing work were conducted with the children in the selected primary school (Figure 6). In semi-structured interviews usually open-ended questions are asked. Thus, the interviewer has the opportunity to ask questions and redirect the same when the answers are not satisfactory. As a result, the observer could explain the topic better for the subjects and obtain clear responses [27].

At the interview stage, the children were asked about the landscape elements, activities and qualities they desired in their nature-friendly school garden, and thus, the landscape elements, activities and qualities that primary school gardens should possess based on the views of the children were determined. Furthermore, children were also asked to draw the dream-friendly school garden they imagine at this stage.

FIGURE 5
Study area: front and back gardens

FIGURE 6
Students drawing the imaginary nature-friendly school garden

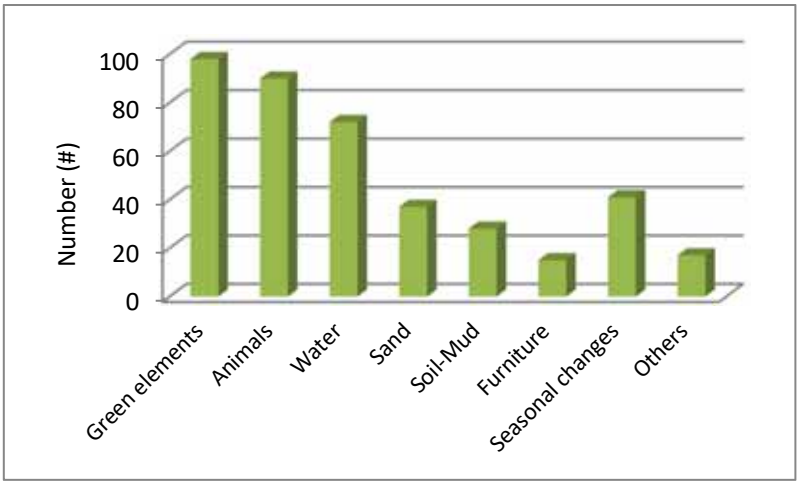


FIGURE 7
Landscape elements and their preference rates determined in the interviews

As a result of the drawings and the interviews, the desired landscaping items are obtained. By comparing the data obtained with semi-structured interviews and drawings, basic landscape items were identified. The identified items were also supported by other studies on the topic, and the research questions were answered.

RESULTS

With the semi-structured interview, students were asked how they imagined the school garden should be. At the end of the study, all the answers given by the students were classified by associating these responses with the landscaping items and these are presented in Figure 7. Eight active landscape elements (green elements, animals, water, sand, soil-mud, furniture, seasonal changes and others), and preference rates of these elements were determined.

The 98% of the answers obtained in the interviews included green elements. These elements included trees and especially fruit trees. Also colorful flowers, vines and green hills were also preferred. Fragrant plants and large grasslands were also among the elements that students wanted to see in a school garden. 90% of the results included the element of animals. The children especially mentioned the activities of playing with animals and feeding them. Among the animals, it was determined that butterflies and birds were the most preferred. 72% preferred the water element. Water elements include ornamental pools, water slides, fountains, puddles, lakes, water parks. 37% preferred the element of sand. Building sand castles and opening pits in the sand were the most requested activities. Furthermore, 28% preferred soil-sludge element and the activity of playing with mud was preferred the most. 41% preferred the seasonal changes. Under this topic, answers that reflect longing for the nature such as sliding on the snow instead of holing up in buildings when it snows, playing with snowballs, touching fallen leaves, dripping in the rain, touching the wet soil were obtained. Furthermore, running under the sun and in the areas where the sun is reflected were also identified as other notable information obtained. It was determined that hammocks, wooden swings, tents, etc. were among the least desirable items with a rate of 15%. In addition, activities such as climbing, wall painting, flying kites, sliding, swinging were identified under the title others with a ratio of 17%.

In addition to the results obtained in the interviews, children's drawings demonstrated that they desired lines drawn on the grounds for games, natural green hills, running water, bridges over the running water, tree climbing, fruit gathering, caverns

for hiding, planting saplings, animal shelters, log houses, feeding aquatic animals and an area where they could see the rainbow (Figure 8).

As a result of interviews and children's drawings, it was determined that the school garden that children desired was an inartificial garden. Thus, the school garden was redesigned as a result of the conducted literature review and the review of existing school gardens where natural elements are used (Figure 9). This predominantly natural design constructed spaces that utilize ecological design approaches to provide the curiosity of the children and allow them to learn by experimentation, rather than guiding children to play certain games. Green areas are the most frequently used element in a natural school garden. Thus, the amount of green spaces in the study area was increased and associated with other areas. In the first section of the school garden, green hills, fruit gardens, butterfly gardens, fragrance and color gardens, shelters for animals such as rabbits, squirrels, ducks, labyrinths, sand ponds, still-running water ponds and sitting elements with canopies were considered. The second section where the school entrance is located is the most suitable area for ceremonial and celebratory events. Various spaces were constructed in section 3 based on the slope of the land in this section. Especially the hills, caves and climbing tracks are the most effective spaces to be built in this area. Furthermore, this area, which is the sunniest spot in the garden, was considered for large lawn areas designed for activities such as picnicking, kite flying and outdoor courses. Also, designed for use in cold weather was a winter garden and sitting elements with canopies. Also, a space where birdhouses could be built and fish could be fed, and includes mud pools, dry pools and other spaces for games drawn on the floor was designed.

DISCUSSION AND CONCLUSION

Primary school gardens in Turkey are used as spaces where children spend their free time when not in the classroom. Paved surfaces are usually asphalt or concrete, and these spaces are passive areas, which cannot contribute to the development of the body and mind of the children. These school gardens, which do not include natural areas and elements, cause children to be insensitive to the environment. However, school gardens should be at least as important as the school buildings. School gardens should offer thought-provoking, natural experience opportunities and should not be used only for ceremonies and playing. Children attend the school for 261 days every year and spend an average of 1827 hours at school. This extended period of time, which is quite long to be underestimated, supports the fact that school gardens must be changed in the positive direction.

In the study, it was determined that the school garden that the children imagined was very different from the school garden they experience. The predominance of the desire for green spaces reflects children's longing for nature. These findings were consistent with the argument by Özdemir and Yilmaz [25] that there should be less paved surfaces in school gardens. Thus, the green elements

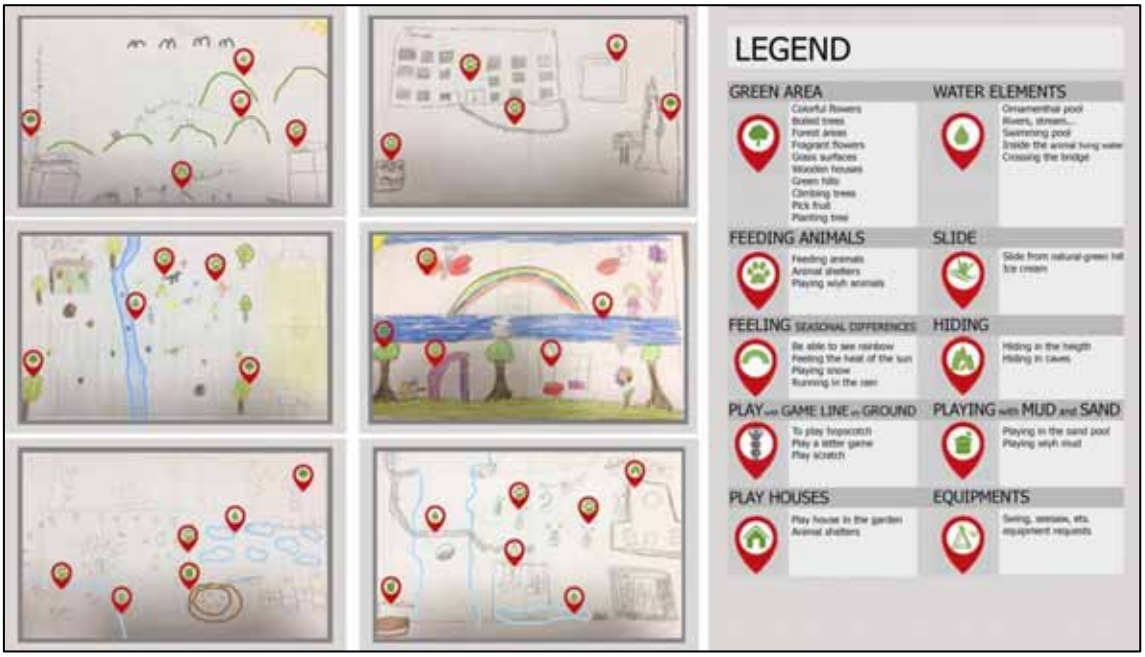


FIGURE 8
Certain activities determined by children's drawings

FIGURE 9
The designed school garden

such as trees, shrubs, groundcover plants and grass should be predominant in school gardens. Such plant elements should be selected based on the functional features such as shading, noise and wind control as well as the aesthetic features that are of interest for children, as Pamay [28] stated. Features such as color, texture, form and size of the plants should be taught to children with games. Thus, children will be able to perceive the morphological features of plants such as flower-foliage colors, flowering-leafing periods and whether they are evergreen or not, and the appearances of seasonal differences in nature. It should not be forgotten that every plant species cannot be used in school gardens; prickly species, those with allergenic pollens, toxic fruits and leaves should not be used. In addition to the educational features of the plants, their properties as attractive and intriguing playgrounds should not be forgotten [29]. Thus, the amount of plant elements in school gardens should be increased. The responses obtained such as the desire to play with animals and feeding them are supported by the findings of a study conducted by Titman [30], who stated that presence of animals at school would provide opportunities for children and teach them several things. It is obvious that children are interested in all living organisms such as plants, because children love to explore and learn new things. It is

important not to forget the effects of events such as a caterpillar turning into a butterfly, the industrious ants, and how the bees create honey on the future of children. It could be quite surprising and remarkable for children that butterflies prefer scented and colorful plants. Thus, butterfly gardens exist in many projects in Europe. The butterfly garden, also practiced in Dartmouth High School, is one of the projects that recognized the interests of the children [24]. Water is one of the other important items desired. Water games improve motor skills by helping children to relax. It was observed that children are not distracted while playing with water. It should not be forgotten that water positively affects the environment, not only in school gardens, but everywhere it is used [5]. Children who play with water will be able to learn by experiencing various rules of physics such as buoyancy of water, its solid-liquid-gas forms, its property to take the shape of the container it was placed in [7]. Sand, soil and mud were among the other preferred elements. Items that could be shaped like sand and soil encourage children to produce. Children can produce their own toys by constructing the objects they imagine with elements such as sand and mud. These elements develop manipulative skills as well as the coordination of hand and mind. Seasonal differences also played an active role in the obtained results. This finding was different from the landscaping activities found in other studies in the literature. The fact that most of today's children spend their time in structural elements revealed that they dream of spaces where they could experience natural phenomena. The results of the present study demonstrated that children long for many activities such as getting wet in the rain, feeling the sunshine and touching the soil. Especially the desire to be under the sun and in sunny areas was determined as the most mentioned seasonal element. This result could be related to the negative effects of the schools on the children, which are established in random places in neighborhoods in Turkey, without considering their geographical position and exposure. It is inevitable for children to want to see and touch the natural transformations that the seasons offer. Also, children's preferences for green hills and caves to hide are an indication of their desire to use natural topography. The statement by Fjørtoft [31] that the natural topography of the rocks and hills are dynamic playgrounds for children supports this preference. As a result, these responses demonstrated that children desired more intriguing and surprising, and curious elements [15, 25, 30]. The fact that the furniture was desired the least could be explained by the fact that children do not prefer the spaces created with artificial elements, but the areas where surprises await them, as Titman [30] argued. Furthermore, as Kaplan [32] notes, unlike artificial elements, natural elements have the potential to respond to children's desires and educate them. It should not be forgotten that nature is the main resource for several activities.

In addition, security in natural school spaces, where children will have a lot of mobility, is one of the most important concerns. It should not be forgotten that 70% of child injuries occur due to falls [7]. Thus, children should be observed by teachers during the time they spend in nature-friendly school gardens. It should also be noted that children playing in the garden may dirty the school and the classroom environment. Based on this fact, cleaning staff and related costs should be taken into consideration. It should be kept in mid that all these factors would result in extra expenditures for the school administration. Since the schools could hardly find adequate funding for several needs, the environmentally friendly school garden is a service that requires financial support.

As a result, the loss of natural areas as a result of urbanization affects the children as well as all living beings. School gardens, the most important playgrounds for the children where they spend most of their time, are also becoming more and more artificial. Thus, it is necessary to implement the procedures utilized in Europe and planning similar to that of several organizations in our country as well. Our school gardens should be rehabilitated with directive design decisions, and implementation should be conducted with planning proposals using nature friendly approaches for new schools. In future studies, design criteria that specify the qualities of a nature-friendly school garden must first be established. The criteria should be defined by experts such as teachers, psychologists and sociologists, child development specialists and landscape architects. The application of these criteria must be ensured by laws and legislation. Furthermore, the necessary funds should be allocated and support should be provided to implement and sustain natural gardens at schools. The opportunities to learn and play within the nature in environmentally friendly school gardens they create in their imagination should be provided for Children, who are our future, as a community health policy.

REFERENCES

[1] Birol, G. (2009) Where is the child friendly city? Megaron Balıkesir, Journal of the Chamber of Architects Balıkesir Branch. 10-13.

[2] Tandoğan, O. (2014) More livable urban space for children: practices around the world. Megaron. 9(1), 19-33.

[3] Barker, R.G. (1968) Ecological Psychology: Concepts and Methods for Studying the Environment of Human Behavior. Stanford University Press. 242p.

[4] Karakaya, B., Kiper, T. (2013) According to landscape design principles determination of current situations of orchards of some elementary school in Edirne city center. Journal of Tekirdag Agricultural Faculty. 10(1), 59-71.
[5] Ünal, M. (2009) The place and importance of playgrounds in child development. Inonu University Journal of The Faculty of Education. 10(2), 95-109.
[6] Şişman, E.E., Özyavuz, M. (2010) Distribution and Use of Proficiency of Playgrounds: A Case Study Tekirdağ. Journal of Tekirdag Agricultural Faculty. 7(1), 13-22.
[7] Turgut, H., Yılmaz, S. (2010) Building ecology-based playgrounds for kids. 3rd National Black Forestry Congress. 4, 1618-1630.
[8] Lindholm, G. (1995) Schoolyards: the significance of place properties to outdoor activities in schools. Environment and Behavior. 23(3), 259-293.
[9] Özdemir, A., Yılmaz, O. (2008) Assessment of outdoor school environments and physical activity in Ankara's primary schools. Journal of Environmental Psychology. 28, 287-300.
[10] Talay, İ., Aslan, F., Belkayalı, N. (2010) Nature friendly and child participation based outdoor design approaches in preschool institutions: a project proposal. Kastamonu Education Journal. 18(1), 317-322.
[11] Alat, Z., Akgümüş, Ö., Cavalı, D. (2012) Teacher's opinion and applications on outdoor activities in pre-school education. Mersin University Journal of the Faculty of Education. 8(3), 47-62.
[12] Rivkin, M. (1997) The schoolyard habitat movement: what it is and why children need it. Early Childhood Education Journal. 25(1), 61-66.
[13] Bowker, R., Tearle, P. (2007) Gardening as a learning environment: A study of children's perceptions and understanding of school gardens as part of an international project. Learning Environ Res. 10, 83-100.
[14] Karatekin, K., Çetinkaya, G., Evaluation of school gardens in terms of environmental education (Manisa province sample). The Journal of International Social Research. 6(27), 307-315.
[15] Malone, K., Tranter, P. (2003) Schoolgrounds as sites for learning: making the most of environmental opportunities. Environmental Education Research. 9(4), 283–303.
[16] Özgüç Erdönmez, İ.M. (2007) Landscape designing norms for elementary school gardens. Journal of the Faculty of Forestry Istanbul University. 57(1), 107-122.
[17] Özdemir, A. (2011) Change of a school garden: Bartın Akpınar elementary school landscape project. Inonu University Journal of Art and Design. 1(3), 267-276.
[18] URL-1, http://www.ltl.org.uk/resources/index.php.
[19] URL-2, http://www.ltl.org.uk/spaces/PlayTreesInSchool.php.
[20] URL-3, https://thepsychologist.bps.org.uk/volume-28/september-2015/big-picture-learning-through-landscapes.
[21] URL-4, http://www.archdaily.com/781867/nursery-fields-forever-reconnects-early-childhood-education-with-nature.
[22] URL-5, https://www.evergreen.ca/our-impact/children/greening-school-grounds/.
[23] URL-6, http://www.grandviewelementary.org/album/40025.jpg.
[24] URL-7, https://natureedmonton.files.wordpress.com/2015/09/heather-kerr.jpg.
[25] Özdemir, A., Yılmaz, O. (2009) Assessment of primary school gardens for physical activity. Milli Eğitim. 181, 121-130.
[26] URL-8, http://www.fao.org/docrep/013/i1689e/i1689e00.pdf. Available at: 30.01.2013.
[27] Çepni, S. (2007) Introduction to Research and Project Studies. Trabzon Celepler Press. 365p.
[28] Pamay, B. (1979) Park garden and landscape architecture. Istanbul University Faculty of Forestry Publication. 157-158.
[29] Acar, H. (2003) A Study on the determining the plant preferences of users in children playgrounds: an example of Trabzon city. M.Sc. Thesis. Karadeniz Technical University. Trabzon, Turkey.
[30] Titman, W. (1992) Play, Playtime and Playgrounds: Key issues for Teachers, Supervisors and Governors of Primary Schools. Cambridge: Learning through Landscapes/World Wide Fund for Nature, UK.
[31] Fjørtoft, I. (2001) The natural environment as a playground for children: the impact of outdoor play activities in pre-primary school children. Early Childhood Education Journal. 29(2), 111-117.
[32] Kaplan, R. (1973) Some psychological benefits of gardening. Environment and Behavior. 5(2), 145.

Received: **03.11.2017**
Accepted: **13.11.2018**

CORRESPONDING AUTHOR

Nilgun Guneroglu
Department of Landscape Architecture
Karadeniz Technical University,
61080, Trabzon – Turkey

e-mail: nayhan@ktu.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9481-9486 Fresenius Environmental Bulletin

IN VITRO ASSESSMENT OF DROUGHT TOLERANCE RESPONSES IN STRAWBERRY

Nafiye Adak[1,*], Ilhami Tozlu[1], Ayse Gul Nasircilar[2], Kamile Ulukapi[3]

[1]Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya, Turkey
[2]Akdeniz University, Faculty of Education, Department of Mathematic and Science Education, Antalya, Turkey
[3]Akdeniz University, Vocational School of Technical Sciences, Antalya, Turkey

ABSTRACT

Global warming, intensifying problem in Mediterranean climate, has affect on yield and quality of crops. Strawberry is a sensitive crop to abiotic stresses (drought, salinity etc.) and this sensitivity is further increases in greenhouse production conditions. Hence, determinations to tolerance level to abiotic stress conditions of cultivars and genotypes are important for their cultivation and breeding. This tolerance level can be determined through morphological, physiological and biochemical analysis. In this study, tolerance levels of Osmanli and Festival strawberry cultivars have been determined through biochemical analysis (SOD- superoxide dismutase, CAT-catalase, MDA- malondialdehyde) of callus tissues and physiological analysis (callus formation ratio, callus fresh and dry weight, callus dry mass ratio) under *in vitro* drought conditions (0, 3, 6, 9 and 12% PEG). There was a linear relation between PEG concentration and oxidative enzyme activities (SOD and CAT) and lipid peroxidation (MDA). Moreover, there was no callus development at high PEG conditions (9-12% PEG 6000). The highest SOD, CAT and MDA values were obtained from Osmanli cultivar. In addition, the highest callus formation rate and fresh weight values were determined to Festival strawberry cultivar. Callus formation rates, callus fresh and dry weight decreased when PEG 6000 concentrations were increased on tried strawberry cultivars.

KEYWORDS:
Fragaria x ananassa, in vitro, callus, SOD, CAT, MDA, callus dry weight.

INTRODUCTION

Strawberry has the advantage of being grown in a vast range of ecological conditions because of a wide range of species and cultivar richness. However, the tolerance of strawberries to abiotic stress factors considerably vary among cultivars. Indeed, Johnson [1] reported that there were significant differences among cultivars in tolerance to high temperatures, and, especially, the Camarosa cultivar in greenhouse cultivation hindered flowering at high temperatures above 30°C, increased vegetative growth, and considerably decreased the fruit size. Ledesma and Sugiyama [2] stated that the Nyoho strawberry cultivar was more resistant to stress of high temperature than the Toyonoka cultivar, and Klamkowski and Treder [3] reported that, in strawberries grown in greenhouse under drought stress conditions, the efficiency of water use increased, photosynthesis and transpiration rate decreased, and leaf area and yield decreased by 30 – 35% as compared to control.

The *in vitro* and *in vivo* techniques can be used in researches on resistance to abiotic stress factors, and particularly, the *in vitro* callus culture technique is fast, easy and an effective method for stress parameters such as salinity and drought [4]. Large molecular weighted polyethylene glycol (PEG) used *in vitro* studies for this purpose increases the solution viscosity, reduces the amount of oxygen in the environment of plant roots and provides osmotic water stress [5]. In addition, Hassanein [6] stated that Mannitol and PEG were the osmotic agents promoting the drought at *in vitro* conditions.

Drought stress directly or indirectly affects several metabolic activities in plants, and triggers numerous physiological, biochemical and molecular responses in plant structure [7]. The antioxidant enzyme activities are initiated under stress conditions, which were resulted in the synthesis of free radicals and the destruction of cell membranes by hazardous chemicals [8, 9, 10,11]. Hence, in many studies carried out on vegetables, the stress – resistant cultivars and genotypes are identified to contain more activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) enzymes during the stress period than susceptible ones [4]. In addition, the amount of malondialdehyde (MDA), a chemical substance indicating lipid degradations in cell membrane, increases under stress conditions [11]. Increase in callus weight and color change in callus cultures are also at remarkable levels under stress conditions. In the studies carrying out the resistance of cherry rootstocks at *in vitro* conditions, 1%, 2% and 4% of PEG 8000 concentrations were used and the MDA, SOD, CAT, POX, APX and GR enzyme activities were

determined to rise in parallel with increasing PEG concentrations [12]. Yong et al. [13] reported that after the short term low temperature stress applications (0, 12, 24, 48 and 72 hours at 0 ºC) in strawberries, the activities of antioxidant enzymes such as the SOD, CAT, POD and APX along with the MDA and the content of hydrogen peroxide (H_2O_2) increased as compared to the control plants.

Sensitivity of plants to drought parameters depends on stress duration, plant species, and plant developmental stages [14]. In strawberries consisting of a vast gene pool, identification of tolerance level of cultivars to drought stress is among the important topics for both breeding studies and cultivation purposes. Hence, it is important to use of the tolerant cultivars to water stress both particularly soilless cultivation and in breeding studies. Therefore, the aim of this study was to determine the mechanism of drought stress among tolerant and less tolerant strawberry cultivars, examining the physiological and biochemical characteristics of the calluses at *in vitro* conditions inducing drought stress.

MATERIALS AND METHODS

Material. This research was carried out in the tissue culture laboratories of a private research institution (2K VEG Inc.) between 2013 and 2015 years. In the research, two strawberry cultivars, which were identified in previous studies as drought tolerant (Festival) and less tolerant (Osmanli), were used.

Method. In the experiment, stolon tips collected in June were used, surface sterilized materials were isolated so as to contain 1–2 leaf primordia under a binocular microscope, and they were transferred to Murashige and Skoog medium [15]. (30 g/l sucrose, 2.4 g/l Gelrite and 4.43 g/l MS). After meristems were cultured in the MS medium containing 1.5 mg/l TDZ+1 mg/l IAA for one month, they were transferred into MS medium containing 1.5 mg/l TDZ+1 mg /l IAA + 0.1 mg/l GA_3 in order to stimulate the shoot and leaf explants [16]. Later on, the explants grown in this medium were transferred into callus development medium (4 mg/l ά-naphthalene acetic acid-NAA) containing different concentrations of PEG 6000 (0, 3, 6, 9, 12 %). Then, the explants were subjected to a subculture in the same medium in order to achieve callus development [17]. Preliminary studies have revealed that the use of 4 mg/l NAA is sufficient for callus development in strawberries (Table 1). During all experimental stages, cultures were maintained in a growth chamber with controlled temperature (25°C±1°C), photoperiod (16-8 h) and light (3000 $\mu mol \cdot s^{-1} \cdot m^{-2}$).

TABLE 1
The experimental calendar used under *in vitro* conditions

Time	Treatments
15.06.2014	Surface sterilization of the starting materials. Isolation of the meristems and transfer into MS medium containing 1.5 mg/l TDZ + 1 mg/l IAA
15.07.2014	Multiplication and sub – culturing (1.5 mg/l TDZ+1 mg/l IAA+0.1 mg/l GA_3)
15.08.2014	Culturing of the explants at different PEG concentrations containing 4 mg/l NAA.
15.09.2014	Callus development at 4 mg/l NAA+ PEG+MS environment
15.10.2014	Biochemical analyses on calluses

The lipid peroxidation (MDA) and enzyme activity (SOD, CAT) values on calluses were determined in the study. At the end of callus development period, the amount of malondialdehyde (MDA), a product of lipid peroxidation that can be entitled as cell membrane damage, was measured as described by Madhave and Sresty [18]; and the other biochemical analyses given below were performed based on the following literature; the antioxidant enzyme activities and the enzyme extractions, Hossain et al. [19]; the SOD enzyme activity, Tang et al., [20]; the CAT enzyme activity, Levya et al. [21], and the MDA analysis, Lutts et al. [22], respectively. Also, callus formation rate, callus fresh and dry weight, callus dry mass rate were recorded at end of the *in vitro* stages.

The study was planned on two factors, two strawberry cultivars (Festival and Osmanli) and five different PEG 6000 concentrations (0, 3, 6, 9, 12 %), and the experimental design was set based on the randomized split plots in factorial design with three replication, each containing six explants.

RESULTS AND DISCUSSION

SOD, CAT, MDA. The effects of different PEG 6000 concentrations on the SOD, CAT antioxidant enzyme activities and the MDA contents were given in Table 2. As seen in Table 2, the SOD and CAT enzyme activities increased depending on the elevation of the PEG concentrations. In experiment, while the highest SOD activity value was determined in 6 % PEG 6000 concentration (90.17 U mg^{-1}), the lowest in control treatment (29.96 U mg^{-1}). Similarity, the highest CAT activity value was founded in 6 % PEG 6000 concentration (119.49 U mg^{-1}) (Table 2). However callus can not survive in 9% and 12% PEG 6000 concentration. Consequently, the increased *in vitro* stress conditions triggered enzyme activities. Differences among the cultivars in terms of enzyme activities were also identified, and the enzyme activities in the Osmanli strawberry cultivar were determined higher values than the Festival cultivar (Table 2). Regarding to interactions, the highest enzyme activities were founded in

6% PEG concentration for the Osmanli strawberry cultivar (Table 3).

The effect of PEG concentrations on the callus MDA contents was found statistically significant in the experiment (Table 2). As a matter of fact, the MDA contents increased depending on the elevation of the PEG concentration. In addition, difference between the cultivars was also observed, and the MDA contents in the Osmanli cultivar were determined higher than the Festival cultivar (Table 2). The 'PEG concentrations x cultivar' interactions were also at a significant level, and as the highest MDA contents were identified in the Osmanli cultivar with high concentration PEG applications. Therefore, both the antioxidant enzyme activities and the malondialdehyde contents were further stimulated in Osmanli cultivar than Festival cultivar (Table 3).

TABLE 2
Effects of different PEG concentrations and strawberry cultivars on the antioxidative enzyme (SOD and CAT) activities and the malondialdehyde (MDA) content

Factors	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	MDA (µmol g^{-1})
PEG Conc. (%)			
0	29.96 c	33.04 c	0.04 c
3	54.39 b	71.71 b	0.07 b
6	90.17 a	119.49 a	0.09 a
9	-	-	-
12	-	-	-
$LSD_{\%5\ (PEG)}$	**5.121**	**5.798**	**0.0102**
Cultivars			
Festival	52.95 b	70.01 b	0.053 b
Osmanli	63.40 a	79.49 a	0.081 a
$LSD_{\%5\ (cultivar)}$	**4.181**	**4.734**	**0.0083**

*PEG 6000 Concentrations

TABLE 3
Effects of different PEG concentrations on the antioxidative enzyme (SOD and CAT) activities and the malondialdehyde (MDA) content of the Osmanli and Festival cultivars

Cultivars	PEG Conc. (%)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	MDA (µmol g^{-1})
	0	29.90 e	31.78 e	0.026 d
	3	44.81 d	63.93 d	0.056 c
Festival	6	84.15 b	114.32 b	0.076 b
	9	-	-	-
	12	-	-	-
	0	30.03 e	34.30 e	0.053 c
	3	63.97 c	79.49 c	0.085 b
Osmanli	6	96.19 a	124.67 a	0.106 a
	9	-	-	-
	12	-	-	-
$LSD_{\%5(PEGx\ Cultivar)}$		**7.242**	**8.200**	**0.0144**

In our study, the malondialdehyde (MDA) content, a by – product of lipid damage, and SOD and CAT enzyme activities in calluses were significantly affected by the PEG concentrations. The SOD and CAT activities increased in higher PEG concentrations, and this increment was particularly observed in the Osmanli cultivar (Table 2 and Table 3). In addition, the MDA contents also showed an increase in stress conditions as well as the enzyme activities. This situation, as the enzyme activities, was yet striking in the Osmanli cultivar.

Our findings were in agreement with those of Wang [23], Yasar et al. [24], Yong et al. [13] and Erturk et al. [25]. Indeed, Erturk et al. [25] reported in a study carried out with cherry rootstocks grown *in vitro* saline environment that the antioxidant enzyme activities such as the MDA, CAT, SOD, GR, and the POX increased depending on elevation of the NaCl concentrations. Moreover, Uzal [26] reported that the MDA content in strawberries grown in saline environment increased; however, that increase was slowed down by the jasmonic acid application. To the knowledge, there is no study reporting the malondialdehyde, antioxidant enzyme activities on stressed strawberry cultivars.

TABLE 4
Effects of different PEG concentrations and strawberry cultivars on the callus formation rate, callus fresh weight, callus dry weight and callus dry mass ratio.

Factors	Callus formation (%)	Callus fresh weight (g)	Callus dry weight (g)	Callus dry mass (%)
PEG* Conc. (%)				
0	92.65 (4.53** a)	4.05 a	2.83 a	71.14 (4.26**)
3	67.46 (4.16 a)	2.93 b	2.01 b	68.65 (4.21)
6	23.61 (3.02 b)	2.25 b	1.25 c	59.02 (4.04)
9	-	-	-	-
12	-	-	-	-
$LSD_{\%5\ (PEG)}$	**0.450**	**0.983**	**0.706**	**NS**
Cultivars				
Festival	41.30 (4.09 a)	3.51 a	2.24	62.90 (4.12)
Osmanli	32.19 (3.72 b)	2.64 b	1.82	69.63 (4.22)
$LSD_{\%5\ (cultivar)}$	**0.367**	**0.802**	**NS**	**NS**

*PEG 6000 Concentrations
** Logarithmic transformation values.

Callus formation rate, Callus fresh weight, Callus dry weight, Callus dry mass rate. The effects of different PEG 6000 concentrations on the callus formation rate, fresh, dry weight and dry mass rate values were given in Table 4. As can be seen in Table 4, the highest callus formation rate, fresh and dry weight values were determined control treatment. As a matter of fact that callus formation rates, callus fresh and dry weight decreased while PEG 6000 concentrations were increased on tried strawberry cultivars. In case of callus dry mass ratio, there were no statistical differences among the treatments. Between the cultivars, highest callus formation rate (41.30 %) and fresh weight values (3.51 g) were founded in Festival (Table 4).

TABLE 5
Effects of different PEG concentrations on the callus formation rate, callus fresh weight, callus dry weight and callus dry mass ratio in Osmanli and Festival cultivars.

Cultivars	*PEG Conc. (%)	Callus formation (%)	Callus fresh weight (g)	Callus dry weight (g)	Callus dry mass (%)
	0	95.00 (4.55)**	4.83	3.17	65.20 (4.17**)
	3	80.48 (4.38)	3.43	2.40	69.18 (4.21)
Festival	6	31.02 (3.33)	2.27	1.17	54.35 (3.98)
	9	-	-	-	-
	12	-	-	-	-
	0	90.30 (4.49)	3.27	2.50	77.08 (4.34)
	3	54.44 (3.93)	2.43	1.63	68.12 (4.22)
Osmanli	6	16.20 (2.71)	2.23	1.33	63.70 (4.10)
	9	-	-	-	-
	12	-	-	-	-
$LSD_{\%5(PEGxCultivar)}$		**NS**	**NS**	**NS**	**NS**

*PEG 6000 Concentrations
** Logarithmic transformation values.

In this study, 'PEG concentration x Cultivar' interactions were examined and the datas obtained were shown in Table 5. Statistical analysis showed that there were not significant differences in terms of 'PEG concentration x Cultivar' interactions. Nevertheless, callus formation rate, fresh, dry weight and dry mass rate values increased depending on the elevated PEG concentrations in tried two strawberry cultivars. These results were similar to the values reported by other authors [12, 27]. As stated Sivritepe et al. [12], found the dry mass ratio increased significantly by increasing PEG concentration from 0% to 4% in cherry. Celik and Atak [27] reported shoot and root length, fresh weight decreases, but MDA increases by increasing NaCl concentration under *in vitro* nutrient solution.

CONCLUSION

Strawberry is a sensitive species to abiotic stress factors, and its tolerance level show differences depending on cultivars, growing conditions and stress parameters. The level of tolerance of cultivars and genotypes to biotic and abiotic stress factors can be determined morphologically, physiologically and biochemically at *in vivo* and *in vitro* conditions. In this study, determination of the tolerance level of cultivars through *in vitro* drought technique in a short time and effectiveness of methods in strawberries were presented. Furthermore, as a result of this study, identification of the antioxidative enzyme activities and physiological parameters in calluses obtained from *in vitro* conditions were also advised as effective selection parameter.

ABBREVIATIONS

APX: ascorbate peroxidase*; CAT:* catalase; *GR:* glutathione reductase*; PEG:* polyethylene glycol*; POX:* peroxidase*; SOD:* superoxide dismutase; *MDA:* malondialdehyde.

ACKNOWLEDGEMENTS

This research was partly supported by The Scientific Research Projects Coordination Unit of Akdeniz University, Antalya-Turkey. Project Number: 2014.01.0104.005

Partly of this study was presented in the congress entitled 'The International Conference on Agriculture, Forest, Food Sciences and Technologies (ICAFOF) on 15 – 17 May 2017 and published as an Abstract.

REFERENCES

[1] Johnson, G. (2011) High temperatures can affect strawberry yields. Available: http://agdev.anr.udel.edu/weeklycropupdate/?tag=abiotic-stress&paged=2 [22.09.2013].
[2] Ledesma, N. and Sugiyama, N. (2005) Pollen quality and performance in strawberry plants exposed to high-temperatures stress. J. Amer Soc Hort Sci. 130(3), 341-347.
[3] Klamkowski, K. and Treder, W. (2008) Response to drought stress of three strawberry cultivars grown under greenhouse conditions. J Fruit Ornam Plant Res. 16, 179-188.
[4] Kuşvuran, Ş., Uslu Kıran, S. and Ellialtıoğlu, Ş. (2011) Bitkilerde kuraklığa toleransın belirlenmesinde kullanılabilecek tarama yöntemleri. II. Ulusal Toprak ve Su Kaynakları Kongresi, 22-25 Kasım 2011, Ankara, Bildiriler Kitabı. 2, 1113-1121. [In Turkish].
[5] Shavrukoz, Y., Genc, Y. and Hayes, J. (2013) The use of hydroponic in abiotic stress tolerance research. 39-66. Available: http://www.intechopen.com/books/hydroponics-a-standard-methodology-for-plant-biological-researches/the-use-of-hydroponics-in-abiotic-stress-tolerance-research 2013 [22.09.2013].

[6] Hassanein, A.M.A. (2010) Establishment of efficiency *in vitro* method for drought tolerance evaluation in pelargonium. Journal of Horticultural Science and Ornamental Plants. 2(1), 8-15.
[7] Kalefetoglu, T. and Ekmekci, E. (2005) The effects of drought on plants and tolerance mechanisms. G.Ü. Fen Bilimleri Dergisi. 18(4), 723-740, 2005 [In Turkish with English abstract].
[8] Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. 7, 405-410.
[9] Miller, G., Suzuki, N., Ciftci-Yilmaz, S. and Mittler, R. (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 33, 453-467.
[10] Doniak, M., Kazmierczak, A., Byczkowska, A. and Glinska, S. (2017) Reactive oxygen species and sugars may be the messengers in kinetin-induced death of field bean root cortex cells. Biologia Plantarum. 61(1), 178-186.
[11] Kaur, G. and Asthir, B. (2017) Molecular responses to drought stress in plants. Biologia Plantarum. 61(2), 201–209.
[12] Sivritepe, N., Ertürk, U., Yerlikaya, C., Turkan, I., Bor, M. and Özdemir, F. (2008) Response of the cherry rootstock to water stress induced *in vitro.* Biologia Plantarum. 52(3), 573-576.
[13] Yong, Z., Hao-Ru, T. and Ya, L. (2008) Variation in antioxidant enzyme activities of two strawberry cultivars with short-term low temperatures stress. World Journal of Agricultural Sciences. 4(4), 458-462.
[14] Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M. and Feller, U. (2009) Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. – Acta Physiol Plant. 31, 1129-1138.
[15] Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.
[16] Adak, N., Kaynak, L., Pekmezci, M. and Gubbuk, H. (2009) The effect of various hormone types on *in-vitro* propagation of strawberry. Acta Hortic. 829, 305-308.
[17] Gerdakaneh, M., Mozafari, A.A., Khaligni, A. and Siosehmardah, A. (2009) The effects of carbonhydrate source and concentration on somatic embryogenesis of strawberry. American-Eurasian J Agric and Environment Sci. 6(1), 76-80.
[18] Madhave, R.K.V. and Sresty, T.V.S. (2000) Antioxidative parameters in the seedlings of pigeonpea (Cajanus Cajan L.Millspaugh) in response to Zn and Ni stress. Plant Sci. 157,113-128.
[19] Hossain, M.A., Hasanuzzaman, M. and Fujita, M. (2010) Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiology and Molecular Biology of Plants. 16, 259-272.
[20] Tang, S.Y., Whiteman, M., Peng, Z.F., Jenner, A., Yong, E.L. and Halliwell, B. (2004) Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. Free Radical Biology and Medicine. 36, 1575-1587.
[21] Leyva, R., Sánchez-Rodríguez, E., Ríos, J.J., Rubio-Wilhelmi, M.M., Romero, L., Ruiz, J.M. and Blasco, B. (2004) Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. Plant Science. 181, 195-202.
[22] Lutts, S., Kinet, J.M and Bouharmont J. (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann Bot. 78, 389-398.
[23] Wang, S.Y. (1999) Methyl jasmonate reduces water stress in strawberry. Journal of Plant Growth Regulation. 18(3), 127-134.
[24] Yasar, F., Ellialtioglu, S., and Kusvuran, S. (2006) Ion and lipid peroxide content in sensitive and tolerant eggplant callus cultured under salt stress. Europ J Hort Sci. 71(4), 169-172.
[25] Erturk, U., Sivritepe, N., Yerlikaya, C., Bor, M., Ozdemir, F. and Turkan, I. (2007) Responses of the cherry rootstock to salinity *in vitro*. Biologia Plantarum. 51(3), 597-600.
[26] Uzal, Ö. (2009) Tuz stresi altında yetiştirilen bazı çilek çeşitlerinde jasmonik asitin bitki gelişimi ve antioksidant enzim aktiviteleri üzerine etkisi. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı Yüksek Lisans Tezi. 97p. (MSc Thesis) [In Turkish with English abstract].
[27] Celik, O., Atak, C. (2012) The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turk J Biol. 36, 339-356.

Received: **10.11.2017**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Nafiye Adak
Akdeniz University,
Faculty of Agriculture,
Department of Horticulture,
Antalya – Turkey

e-mail: nafiyeadak@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9487-9490 Fresenius Environmental Bulletin

IN VITRO EFFECTS OF OLEUROPEIN ON MELONOMA CELLS

Vesile Duzguner*

Health Sciences School, Ardahan University, 75000 Ardahan, Turkey

ABSTRACT

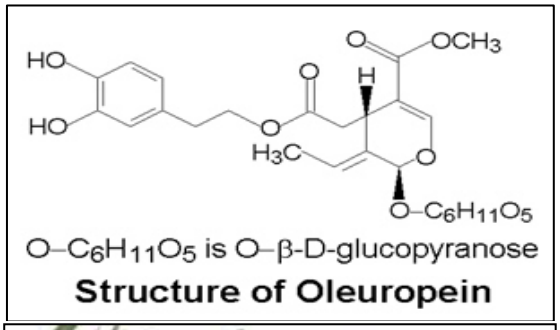
Oxidative stress is considered to be involved in the pathophysiology of all cancers. Melanoma is the main cause of death in patients with skin cancer. It is caused by neural crest-derived melanocytes -pigmented cells normally presented normally in the epidermis and, dermis. Oleuropein is a heterosidic ester of elenolic acid and hydroxytyrosol and possesses beneficial effects on human health. The aim of this study was to determine *in vitro* effects of oleuropein on melanoma cells. Viability of the **c**ells was quantified by MTT assay in a time and dose response manner. Melanoma cells were treated with 100 µmol for three hours. Glutathione (GSH), Total oxidant capacity and total antioxidant capacity (TOC, TAC) and nitric oxide levels were identified using specific colorimetric methods. Oleuropein treatment decreased cell viability in melanoma cell line in a dose-dependent manner. Oleuropein inhibits the activation of nitric oxide and prevented decreased levels of GSH. Also, total oxidant capacity decreased significantly after treatment. Oleuropein decreased cell viability and triggered antioxidant system positively. These findings suggested that oleuropein has potent anticancer and antioxidant properties on melanoma cells. However, further *in vivo* studies are required to determine the exact potential of this agent.

KEYWORDS:
Oleuropein, Melanoma, Oxidative stress

INTRODUCTION

Olive oil is high-value edible oil which is considered important for its flavour and its preventive effects against important diseases such as coronary heart diseases, neurological disorders and some types of cancer [1]. This beneficial food's compounds are attracted attention with nutraceutical uses and significant properties [2].

Oleuropein is a phenolic secoiridoid glycoside and one of major phenolic compound of Olea europae. It has significant antioxidant, anti-tumoural, anti-inflammatory, antidiabetic, hypolipidaemic, anti-angiogenic, antiatherosclerotic and platelet anti-aggregate activities when compared with other oil compounds. [3, 4, 5, 6, 7].



PICTURE 1
Structure of Oleuropein and the Image of Olive Plant

Oxidative stress is the main part of the pathogenesis and complications of many diseases including cancer [8]. Particular attention has been focused on the therapeutic activities of oleuropein and its constituents about its antioxidant activity. Oleuropein act as an antioxidant such as free radical scavenging, radical chain breaking. The catecholic structure it has is able to eradicate peroxyl radicals and break oxidative chain reactions [9]. This study is carried out aim of this study was to determine *in vitro* effects of oleuropein on melanoma cells.

MATERIALS AND METHODS

Human melanoma cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penincillin and 100 µg/ml streptomycin, at 37°C with 5% CO_2. Briefly, cells were plated in 24-well plates (0.4×10^5cells) and pre-treated with 100 µM oleuropein for 3h. After the incubation, the supernatant was replaced by fresh medium.

Cell survival was quantified by colorimetric MTT assay [10]. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Fluka, USA) measures the mitochondrial activity of viable cells by quantifying the conversion of the tetrazolium salt to its formazan product. Microglia (2×10^6/2 ml culture medium/well) were placed in a 12- well flat-bottom plate in triplicate and cultured at various times containing the indicated doses of agent. Following culture at 37 °C, 1 ml/well of MTT (5 mg/ml) was added to the wells, followed by incubation for an additional 2 h for each experimental time interval. The viable cells produced a dark blue formazan product, whereas no such staining was formed in the dead cells. The resulting formazan product was solubilized in 1 ml/well of acidic isopropanol, and absorbance was read at 570 nm with ELISA reader (µQuant-USA). Cell viability was calculated by normalization of optical densities (OD) to the negative control.

GSH levels were determined according to reaction with 5,5-dithiobis-2-nitrobenzoic acid resulting in the formation of a product with has a maximal absorbance at 410 nm. The results were expressed as mmol/L [11].

Nitric oxide concentration in plasma and tissue samples was analysed indirectly by measuring the nitrite levels based on Griess reaction.

Total antioxidant level of the sample was calculated according to ABTS (dark blue coloured radical) reducing capacity of antioxidants at 660 nm. Results were given as Trolox equivalent (mmol/lt) which is a vitamin E analog. Additionally, oxidants in the sample oxidize ferrous-ion chelator complex to ferric ion. Briefly, total oxidant level was measured by colorimetric methods according to absorbance change of formed coloured complex at 530 nm. Results were given as H_2O_2 equivalent (mmol/lt).

Statistical analysis. The one-way analysis of variance (ANOVA) and post hoc Duncan tests were performed on the data to examine the differences among groups using the SPSS statistical software package. The results are presented as average ±SE. A value of $p<0.05$ was considered significant.

RESULTS

We first evaluated the effects of increasing concentrations (10%, 50% and 100%) of oleuropein for 1h and 3 hours on skin cancer cells proliferation using MTT assay. We observed that treatment for 3 h and 100% concentration with oleuropein reduced cell viability in melanoma cells in a time and dose dependent manner (Fig. 1). The NO oxide levels approximately 9.40 % decreased in oleuropein treated samples (Table 1)

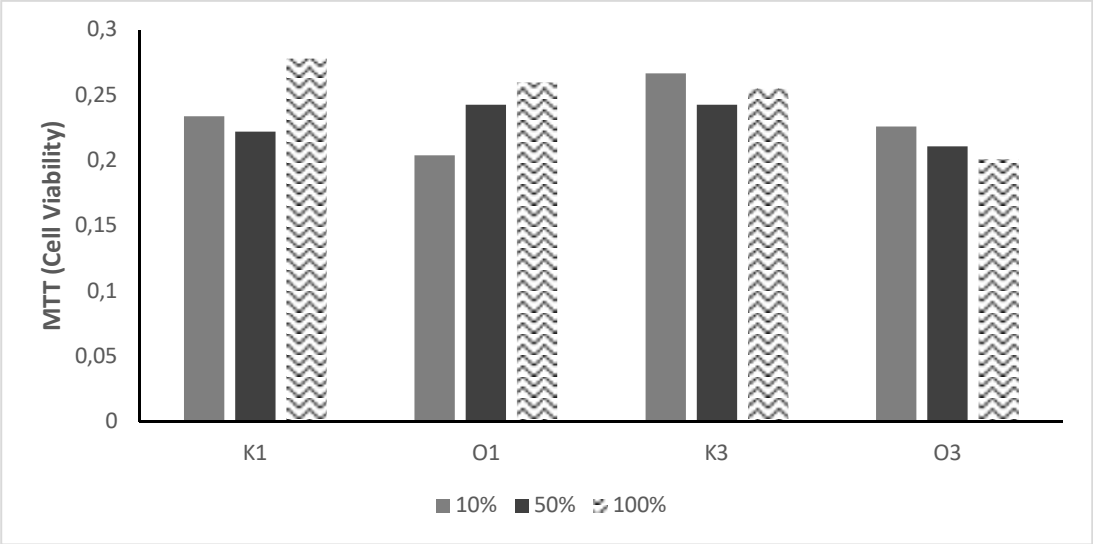


FIGURE 1
Time and Dose Response of Cell Viability Test (MTT)

TABLE 1
The Effect of Oleuropein on The Levels Oxidant and Antioxidant Parameters

	Control	Oleuropein (100 µM)
Nitric oxide (µmol/L)	197.4 ± 1.47	168.84±0.12
Glutathione (mmol/L)	8.56 ± 0.02	22.85 ± 0.02
Total Antioxidant Status (mmol/L)	1.258 ± 0.0004	1.715 ± 0.0008
Total Oxidant Status (mmol/L)	7.430 ± 0.0007	6.900 ± 0.0005

GSH levels increased 3 times after 100µl oleuropein administration for 3 hours (Table 1). Total oxidant capacity was found very high in melanoma cells but treatment of oleuropein was fixed the decreased levels of TOC as shown in Table 1. In addition, oleuropein administration enhanced total antioxidant capacity as well (Table 1).

DISCUSSION

Oleuropein, the main compound of a phenolic content of olive leaves and drupes, exhibited antiproliferative effects against some in vitro cancer cell lines [12, 13, 14] like the cell line which is used for recent study. The antioxidant effects of oleuropein may reduce cancer risk. Oxidation of major macromolecules (DNA, proteins, lipids) have been shown to trigger to cancer. It's clearly become evident that antioxidants reduce the risk of mutagenesis and carcinogenesis [15]. In vitro and in vivo studies have shown that, among the phenolic component of olive oil, oleuropein exhibits significant antioxidant action [16, 17].

It is well known that the antioxidant effect of oleuropein and its constituents depends on its catecholic effects [18]. This catecholic property of oleuropein may earn free radical scavenging and metal-chelating activities which are responsible for the ability of protect membranes from lipid oxidation. Also, Kimura and Sumiyoshi [19] showed that the therapeutic effects of the oleuropein on UVB-induced skin cancer may be due to inhibition of the gene expression of proteolytic enzymes and VEGF. Bulotta et al. [20] reported that oleuropein may lead to cell apoptosis and modulate pro- and anti-oncogenic signalling pathways. Investigations about the structure activity relationships with scavenging activity of oleuropein in different experimental oxidative models like total antioxidant capacity are reported that potential antioxidant activity of oleuropein may due to the presence of hydroxyl groups which are prevent oxidation. In addition, Quiñones et al. [21] established that oleuropein has anti-inflammatory action that may be associated particularly to its antioxidant potential. The study about oral administration in mice reported that oleuropein regressed tumours in 9-12 days [22]. Also, it is well known that oleuropein modulate several oncogenic signalling by regulating pathways HER2, interfering with MAPK pathway, modulation of apoptosis and PI3K/AKT as well as by preventing ROS production [23].

As we found in recent study, the study with Mongolian gerbils with cerebral ischemia revealed that oleuropein was decreased levels of nitric oxide and lipid peroxidation and increased SOD activity [24].

Reduced glutathione (GSH) plays an important role in metabolic processes, transport and cellular protection in nearly all cells of the body by way of its thiol groups. So, the low levels of GSH fixed by oleuropein treatment. Al-Alzawie and Alhamdani, [25] demonstrated that oleuropein treatment showed significant increase in glutathione levels in diabetic rabbits. Also, another study about spinal cord injury reported that also reported increased glutathione levels after oleuropein administration [26].

In conclusion, this study demonstrated the beneficial effect of using oleuropein as an effective antioxidant agent in preventing oxidative stress and free radicals as well as in enhancing antioxidant system.

REFERENCES

[1] Hassen, I., Casabianca, H., Hosni, K. (2015) Biological activities of the natural antioxidant oleuropein: Exceeding the expectation-A mini-review. Journal of Functional of Foods. 18, 926-940.

[2] Zbakh, H., El Abbassi, A. (2012) Potential use of olive mill wastewater in the preparation of functional beverages: A review. Journal of Functional Foods. 4, 53-65.

[3] Fuentes, E., Palomo, I. (2014) Antiplatelet effects of natural bioactive compounds by multiple targets: Food and drug interactions. Journal of Functional Foods. 6, 73-81.

[4] Nakbi, A., Tayeb, W., Dabbou, S., Chargui, I., Issaoui, M., Zakhama, A., Miled, A., Hammami, M. (2012) Hypolipidimic and antioxidant activities of virgin olive oil and its fractions in 2,4-diclorophenoxyacetic acid-treated rats. Nutrition. 28, 81-91.

[5] Fortes, E., García-Vilas, J.A., Quesada, A.R., Medina, M.A. (2012) Evaluation of the anti-angiogenic potential of hydroxytyrosol and tyrosol, two bio-active phenolic compounds of extra virgin olive oil, in endothelial cell culture. Food Chemistry. 134, 134-140.

[6] Fernández-Arroyo, S., Gómez-Martínez, A., Rocamora-Reverti, L., Quirantes-Piné, R., Segura-Carretero, A., Fernández-Guttiérez, A., Ferragut, J.A. (2012) Application of nanoLC-ESI-TOF-MS for the metabolomic analysis of phenolic compounds from extra-virgin olive oil in treated colon-cancer cells. Journal of Pharmaceutical and Biomedical Analysis. 63, 128-134.

[7] Scognamiglio, M., D'Abrsosca, B., Pacifico, S., Fiumano, V., De Luca, P.F., Monaco, P., Fiorentino, A. (2012) Polyphenol characterization and antioxidant evaluation of *Olea europaea* varieties cultivated in Cilento National Park (Italy). Food Research International. 46, 294-303.

[8] Duzguner, V., Erdogan, S. (2012) Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver & central nervous system of rats. Pesticide Biochemistry and Physiology. 104, 58-64.
[9] Czerwińska, M., Kiss, A.K., Naruszewicz, M. (2012) A comparison of antioxidant activities of oleuropein and its dialdehydic derivative from olive oil, oleacin. Food Chemistry. 131, 940-947.
[10] Hansen, M.B., Nielsen, S.E., Berg, K. (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. Journal of Immunology Methods. 119-203.
[11] Sedlak, J., Lindsay, R.H. (1968) Estimation of Total, Protein-Bound, and Nonprotein Sulfhydryl Groups in Tissue with Ellman's Reagent. Anal Biochem. 25, 192.
[12] Goulas, V., Exarchou, V., Troganis, A. N., Psomiadou, E., Fotsis, T., Briasoulis, E., Gerothanassis, I.P. (2009) Phytochemicals in olive-leaf extracts and their antiproliferative activity against cancer and endothelial cells. Molecular Nutrition and Food Research. 53, 600-608.
[13] Han, J., Talorete, T. P., Yamada, P., Isoda, H. (2009) Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. Cytotechnology. 59, 45-53.
[14] Sirianni, R., Chimento, A., De Luca, A., Casaburi, I., Rizza, P., Onofrio, A., Lacopetta, D., Puoci, F., Ando, S., Maggiolini, M., Pezzi, V. (2010) Oleuropein and hydroxytyrosol inhibit MCF-7 breast cancer cell proliferation interfering with ERK1/2 activation. Molecular Nutrition and Food Research. 54(6), 833-840.
[15] Colomer, R., Menéndez, J.A. (2006) Mediterranean diet, olive oil and cancer. Clinical and Translational Oncology. 8(1), 15-21.
[16] El, S.N., Karakaya, S. (2009) Olive tree (Olea europaea) leaves: Potential beneficial effects on human health. Nutrition Reviews. 67(11), 632-638.
[17] Poudyal, H., Campbell, F., Brown, L. (2010) Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate, high fat-fed rats. The Journal of Nutrition. 140(5), 946-953.
[18] Visioli, F., Poli, A., Galli, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews. 22, 65-75.
[19] Kimura, Y., Sumiyoshi, M. (2009) Olive leaf extract and its main component oleuropein prevent chronic ultraviolet B radiation-induced skin damage and carcinogenesis in hairless mice. Journal of Nutrition. 139, 2079-2086.
[20] Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Pujia, A., Russo, D. (2014) Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: Focus on protection against cardiovascular and metabolic diseases. Journal of Translational Medicine. 12, 219.
[21] Quiñones, M., Miguel, M., Aleixandre, A., (2013) Beneficial effects of polyphenols on cardiovascular disease. Pharmacological Research. 68, 125-131.
[22] Bulotta, S., Corradino, R., Celano, M., Maiuolo, J., D'Agostino, M., Oliverio, M., Procopio, A., Filetti, S., Russo, D. (2013) Antioxidant and antigrowth action of peracetylated oleuropein in thyroid cancer cells. J. Mol. Endocrinol. 51, 181-189.
[23] Farooqi, A.A., Fayyaz, S., Silva, A.S., Sureda, A., Nabavi, S.F., Mocan, A., Nabavi, S.M., Bishayee, A. (2017) Oleuropein and Cancer Chemoprevention: The Link is Hot. Molecules. 22, 705-718.
[24] Dakanski, D., Selaković, V., Piperski, V., Radulović, Ž., Korenić, A., Radenović, L., (2011) Protective effect of olive leaf extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology. 18, 1137-1143.
[25] Al-Azzawie, H.F., Alhamdani, S. (2006) Hypoglycaemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sciences. 78, 1371-1378.
[26] Khalatbary, A.R., Ahmadvand, H. (2012) Neuroprotective effect of oleuropein following spinal cord injury in rats. Neurological Research. 34, 44-51.

Received: **11.11.2017**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Vesile Duzguner
Health Sciences School,
Ardahan University,
75000 Ardahan – Turkey

e-mail: vesileduzguner@ardahan.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9491-9499 Fresenius Environmental Bulletin

EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL AND ANTIMUTAGENIC PROPERTIES IN *EREMURUS SPECTABILIS* BIEB. GROWN IN DIFFERENT ECOLOGICAL REGIONS

Zehra Tugba Murathan[1], Mehmet Arslan[2], Nurcan Erbil[2,*]

[1]Ardahan University, Engineering Faculty, Food Engineering Department, 75000, Ardahan, Turkey
[2]Ardahan University, Health Sciences College, Department of Nursing, 75000, Ardahan, Turkey

ABSTRACT

The leaves of *Eremurus spectabilis* are consumed as a vegetable in some regions of Turkey. The current study investigated the total phenolic content (TPC), pigment content, antimicrobial, antioxidant, and antimutagenic activities in aerial parts of *E. spectabilis* Bieb. The samples were collected from Bingöl, Kahramanmaras (the cities of Turkey) and Nakhcevan. The results showed that TPC of methanolic extracts of *E. spectabilis* ranged between 60.3 and 115.8 mg gallic acid equivalents (GAE)/100g of dry weight (DW). The results of antioxidant activity correlated with TPC, both of which were determined to be highest in the sample collected from Bingöl. The sample collected from Kahramanmaras recorded the lowest values for 2,2-diphenyl-1-picrylhydrazyl (DPPH) (17.21%), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (42.81%), and Ferric Reducing Antioxidant Power (FRAP) (263.1 mmol Fe(II)/g DW). It was observed that the pigment content was too high in samples collected from Nakhcevan as compared with others. The strongest antibacterial activity was detected in the sample obtained from Nakhcevan against *Enterobacter aerogenes* with an inhibition zone of 14.970 mm. Additionally, antifungal activity was recorded only against *Saccharomyces cerevisiae*. Four different concentrations (10, 20, 40, and 80 µL/plate) of *E. spectabilis* extracts were used to analyze the antimutagenic activity. The tests were designed in *Salmonella typhimurium* TA 98 and TA 100 strains. We found that all doses of Bingöl extracts and the highest dose (80 µL/plate) of Kahramanmaras extracts exhibited antimutagenic activity in *Salmonella typhimurium* TA 98. However, only 40 µL/plate dose of Nakhcevan extracts was found to be statistically significant in *Salmonella typhimurium* TA 100 strain. Therefore, the present research suggested that the consumption of *E. spectabilis* plant might prove to be beneficial for human health.

KEYWORDS:
Antimicrobial, Antimutagenic, Antioxidant, *Eremurus spectabilis* Bieb., Phenolic

INTRODUCTION

Factors such as environmental pollution, ionizing and ultraviolet (UV) radiations, and smoking, cause oxidative stress [1]. Free radicals and reactive metabolites are produced during stress. Many diseases, especially cancer, is strongly related to oxidative stress. In addition, a positive relationship exists between the frequency of chronic diseases and the oxidative stress [2]. Antioxidants are produced as a part of the defense mechanism of higher organisms and prevent oxidative damage related to diseases [3]. Therefore, antioxidants have an important role in the protection of human health [4].

It is known that bioactive compounds produced by plants possess antioxidant activity. Factors such as genetic, environmental, maturity time, postharvest time, storage time, etc., influence the amount of bioactive compounds produced in plants [5]. Recently, many studies have been conducted to determine the antioxidant capacity of plants [6, 7, 8]. Phenolics and flavonoids in plants are the major bioactive compounds, which are a significant source of antioxidants [9]. It is think that phenolic acids are useful to prevent from various diseases such as Alzheimer's disease [10, 11]. Some phenolics also posses antimicrobial and antimutagenic potentials [12, 13]. Recently, an indiscriminate use of antibiotics has given rise to problems such as antibiotic resistance at a global level.

Genus *Eremurus,* which is a member of the family Liliaceae, includes nearly 50 species [14]. The leaves of this plant are used as a vegetable, and roots are used as a source of glue [15]. Two species of this genus, namely *E. spectabilis,* and *E. cappadocicus* are widely present in Turkey. *E. spectabilis* is one of the most important species in this genus, and it is popularly called Çiriş or Gulik in Turkey [16]. It has been used in the treatment of rheumatism, gastrointestinal diseases, eye pain, fungal infections,

eczema, hemorrhoids, diabetes, skin inflammatory disorders, and as antidysuria and antihypertensive [17, 18, 19, 20]. In earlier studies, it was determined that *E. spectabilis* contains polysaccharides, flavonoids, vitamin K, Vitamin D, potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and copper (Cu) [19, 21, 22]. It has been reported that methanolic extracts of roots of *E. spectabilis* blocked the growth of several Gram-positive and Gram-negative bacteria [23].

Various studies on chemical content, antioxidant and antimicrobial activities of leaf and root extracts of *E. spectabilis* have been performed [19, 21, 22, 23]. However, samples used in these studies were collected from different regions. Moreover, the antimutagenic activity of the *E. spectabilis* has not yet been reported. The present study aimed determining the total phenolic content, pigment content, antioxidant, antimicrobial, and antimutagenic properties in aerial parts of *E. spectabilis* collected from Bingöl, Kahramanmaras (the cities of Turkey) and Nakhcevan.

MATERIALS AND METHODS

Plant Material. The aerial parts of *E. spectabilis* were collected from Bingöl (1170 m), Kahramanmaras (1200 m), and Nakhcevan (875 m) (Figure 1), which are taxonomically identified according to Davis (1965–1985) [24]. A portion of aerial parts was dried for the analysis of antioxidant, antimicrobial, and antimutagenic activity and the rest was frozen at –80ºC for analysis of pigment content.

Extraction. Dried aerial parts were ground with an electrical blender. The powdered aerial parts (30 g) were placed in Soxhlet apparatus, and extraction was performed in 300 mL of methanol (polarity index: 5.1) for 6 h. The solution was filtered and concentrated at 40°C by vacuum (SCILOGEX RE100-Pro, USA). Extracts were frozen (–18°C) until used for determining the total phenolic content (TPC), antioxidant, antimicrobial, and antimutagenic activities.

Total Phenolic Content (TPC). TPC of aerial parts of *E. spectabilis* was determined using Folin–Ciocalteu procedure described by Spanos and Wrolstad [25] with slight modifications. The diluted extract (0.4 mL) and 2 mL of Folin–Ciocalteu's reagent (10%) were combined. After an incubation of 2–3 min, 1.6 mL sodium carbonate (Na_2CO_3) (7.5%) was added, following which the mixture was left for 1 h in the dark. The absorbance was measured by UV-Vis spectrophotometer (UNICO S1205, USA) at 765 nm. The results are expressed as milligrams of gallic acid equivalents (GAE) per 100g of dry weight (DW).

Antioxidant Activity. The antioxidant activity of aerial parts of *E. spectabilis* was determined according to 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and Ferric Reducing Antioxidant Power (FRAP) methods.

DPPH Method. The diluted extract (50µL) was mixed with DPPH solution (950 µL, 0.1 N). The mixture was placed in a shaker at room temperature in the dark for 30 min. The sample was then measured at 515 nm by UV-Vis spectrophotometer. The inhibition of the DPPH radical by the sample was calculated using the formula: (Absorbance control-absorbance sample/Absorbance control)×100 [26].

FIGURE 1
Geografical location of the research area
1: Kahramanmaraş/Turkey, 2: Bingöl/Turkey, 3: Nakhcevan (www.google.com.tr/maps)

ABTS Method. This assay was carried out as described earlier [27]. ABTS solution and 2.45 mM potassium persulfate solution were stirred (1:1 v/v). The mixture was left for 12–16 h at room temperature in the dark. After an absorbance value of 0.70 at 734 nm was reached, the mixture was diluted with methanol. The diluted extract (0.15 mL) was then mixed with 2.85 mL of diluted ABTS solution followed by incubation for 2 h at room temperature in the dark. Absorbance was measured at 734 nm by spectrophotometer. Percentage of ABTS was calculated using the formula: (Absorbance control – absorbance sample/Absorbance control)×100.

FRAP Method. FRAP assay was performed according to the procedure described by Benzie and Strain [28] with slight modifications. To prepare the FRAP reagent, 25 mL of 300 mM sodium acetate buffer (pH 3.6), 2.5 mL of 2,4,6-tripyridil-s-triazin(TPTZ), 10 mM of 40 mM HCl, and 2.5 mL of iron(III) chloride hexahydrate ($FeCl_3 6H_2O$) (20 mM) were mixed. The initial absorbance value of 900 µL of reagent was measured at 593 nm. The diluted extract (20 µL) and 2.98 mL of FRAP reagent were mixed followed by incubation for 10 min at room temperature. Absorbance was measured at 593 nm using spectrophotometer. The ferric ion reducing ability of aerial parts of *E. spectabilis* was determined using the calibration curve and reported as mmol of $FeSO_4$ equivalents per gram of sample.

Pigment Analysis. The extraction of pigments from the aerial parts of *E. spectabilis* was carried out according to the method described by De-Kok and Graham [29]. A sample of 1 g was homogenized (Wise Tis HG–15A, Germany) in 50 mL of acetone for 5 min followed by shaking for 30 min. It was refrigerated at 4ºC for 2 h. The sample was then filtered, and 1/5 volume of distilled water was added to it. The sample was shaken for 15 min and centrifuged at 3000 rpm for 10 min. Absorbance was measured at 470, 645, and 662 nm. Chlorophyll a (Cha), Chlorophyll b (Chb), Total chlorophyll and Total Carotenoid (Cx) contents were calculated using standard equations.

Antimicrobial Activity. The antimicrobial activity of methanolic extracts of aerial parts of *E. spectabilis* was determined by the agar well diffusion method. Eight strains of bacteria (*Pseudomonas aeroginosa* ATCC 9027, *Bacillus subtilis, Enterobacter aerogenes, Bacillus licheniformis, Klebsiella pneumoniae, Staphylococcus aureus* ATCC 6538, *Bacillus megaterium* DSM 32, and *Escherichia coli*) and three strains of yeast (*Yarrowia lipolytica, Candida albicans,* and *Saccharomyces cerevisiae*) were used to evaluate antimicrobial activity of *E. spectabilis*. Agar wells of 11 mm diameter were prepared with a sterilized cork borer to which 150 µL of extract was added. A microbial suspension (1%) of each strain having 10^6–10^7 colony forming units (CFU/mL) was added to 15 mL of sterile media (For bacteria, Muller-Hintone Agar and for yeast, Sabouraud 2% Glucose Agar were used.) [30, 31]. Erythromycin was used as a positive control and inhibition zones were measured using a digital ruler.

Antimutagenicity Test. In this study, *Salmonella typhimurium* TA 98 and TA 100 strains were used to examine the antimutagenic activity of aerial parts of *E. spectabilis* according to Ames test with slight modifications. *S. typhimurium* TA 98 and TA 100 strains were routinely checked to confirm the genetic properties according to Maron and Ames [32].

The Ames test was used to assay the antimutagenic activity of aerial parts of *E. spectabilis* [32]. Four different concentrations of methanolic extracts (10, 20, 40, and 80 µL/plate) were used. Antimutagenic experiments were performed in the absence of S9 mix on *Salmonella typhimurium* TA 98 and TA 100 strains. 4-Nitro-o-phenylenediamine (4-NPD) and sodium azide (SA) were used as positive controls for TA 98 (10µg/plate) and TA 100 strains (100 µg/plate) respectively. After plating the bacterial culture (100 µL/plate), leaf extract and the mutagen (4-NPD for TA 98 and SA for TA 100) were added to 2 mL of top agar. After gently mixing each plate, minimal glucose agar (MGA) was poured onto each plate. The plates were incubated at 37ºC for 48–72 h.

Data Analyses. In this study, all experiments were performed in triplicates. Results are represented as mean ± standard deviation. Statistical analysis was performed using SPSS (version 16) software. One-way analysis of variance (ANOVA) and significant differences between the groups were detected by multiple comparison procedures according to Duncan [33]. The results were considered statistically significant at $p < 0.05$. The antimutagenic data were tested for normal distribution (Shapiro–Wilkmethod) and analyzed with ANOVA and Dunnett's test.

RESULTS AND DISCUSSION

Total Phenolic Content and Antioxidant Activity. Phenolic compounds produced by plants have many biological effects such as antimicrobial, anti-inflammatory, antioxidant, and anti-tumor [34, 35]. Total phenolic content and antioxidant activity of methanolic extracts of aerial parts of *E. spectabilis* obtained from three different regions (Bingöl, Kahramanmaras, and Nakhcevan) are shown in Table 1. Statistically significant differences among samples were observed. According to the data obtained, total phenolic content of *E. spectabilis* extracts was between 60.3 and 115.8 mg GAE/100g DW. TPC was found to be highest in the sample

from Bingöl, whereas the lowest TPC was recorded for the sample obtained from Kahramanmaras.

It is important to choose a suitable method for the detection of antioxidant activity of plants. The DPPH, FRAP, and ABTS methods are simple and easy for the assessment of antioxidant activity [36]. The DPPH method is based on the ability of antioxidants to interact with DPPH free radicals. The FRAP method depends on the reduction of ferric to ferrous ions in a complex catalyzed by antioxidants [37]. The ABTS method is based on the inhibition of production of ABTS radical cation and does not involve a substrate [38]. In the present study, the reactivity of methanolic extracts of *E. spectabilis* was analyzed with DPPH, ABTS, and FRAP assays. The antioxidant activity in the DPPH, ABTS, and FRAP assays correlated with TPC (Table 1). The sample from Bingöl had the highest antioxidant activity according to all methods, while it was observed that sample from Kahramanmaras recorded the lowest DPPH (17.21%), ABTS (42.81%), and FRAP (263.1 mmolFe (II)/g DW) values ($p < 0.05$). Several studies similar to ours have been conducted by other researchers. Tosun et al. [19] reported that DPPH and TPC values in *E. spectabilis* were 53.43–86.66 µg/mL and 178–259 mg/100g, respectively. Another study [20] observed the total phenolic content to be 44.93–49.84% in *E. spectabilis*. According to Bircan and Kirbag [22], percentage inhibition of DPPH radical was between 5 and 60.57% at *E. spectabilis* plant extracts. It is thought that the results obtained from different studies are caused by different planting locations and different climatic conditions.

Chlorophyll a, Chlorophyll b, Carotenoid, and Total Chlorophyll Content. Chlorophyll regulates many physiological functions in plants. The changes in the levels of chlorophyll in plants are an important indicator of stress, environmental quality, and nutritional status [39, 40, 41]. Many abiotic factors affect pigment contents of plants [42]. The values of different pigments (Chlorophyll a (Cha), chlorophyll b (Chb), total chlorophyll (TC) and carotenoid (Cx)) in aerial partsof *E. spectabilis*are listed in Table 2. Statistically significant differences in these parameters were observed among aerial parts obtained from different regions ($p < 0.05$). Cha was higher than Chb in all the samples. We found that the content of all pigments was too high in the samples obtained from Nakhcevan as compared with others. The Cha content was between 1.51 and 14.85 µg/g, and Chb content was between 0.46–7.40 µg/g. The lowest values of Cha, Chb, Cx, and TC contents were recorded in the sample collected from Kahramanmaras. The pigment content of *E. spectabilis* has not yet been identified before. The content of Cha and Chb in the some medicinal herbs are given below. For example, a study [43] reported that *Mentha piperita*, *Melissa officinalis*, *Ginkgo biloba*, *Camellia sinensis*, and *Salvia officinalis* have 774.1, 492.9, 334.9, 147.5, 194.8, and 213.2 mg/100g Cha content, and 356.8, 239.6, 149.5, 68.5, 54.4, and 102.1 mg/100g Chb content, respectively. According to the results of Loranty et al. [44], Cha was detected in *Equisetum arvense* (9.7 mg/g of dry tea), *Melissa officinalis* (15.7 mg/g of dry tea), *Viola tricolor* (38.6 mg/g of dry tea), and *Urtica dioica* (58.7 mg/g of dry tea). The Chb content varied from 2.3 mg/g to 75.2 mg/g of dry tea. This may be due to a variation between species, use of different methods, differences in environmental conditions, locality, and altitude.

Antimicrobial Activity. The agar well diffusion method was used to identify the antimicrobial activities of methanolic extracts of aerial parts of *E. spectabilis* (Table 3). The standard antibiotic (erythromycin) was used as a positive control (Table 3). In the present study, it was observed that methanolic extracts exhibited antibacterial activity against different test microorganisms at different rates. The strongest antibacterial activity was observed in the sample obtained from Nakhcevan against *Enterobacter aerogenes* with an inhibition zone of 14.970 mm. However, the extracts exhibited antifungal activity only against *Saccharomyces cerevisiae*. No growth inhibition zone was observed for the other two yeast strains. The lowest activity was recorded in the sample collected from Nakhcevan against *Bacillus licheniformis* with an inhibition zone of 12.270 mm.

TABLE 1
Total phenolic contents and antioxidant activities of *E. spectabilis* areal parts

Samples	Total phenolic content (mgGAE/100g DW)	DPPH (%)	ABTS (%)	FRAP (mmol Fe II/g DW)
Bingöl/Turkey	115.8±10.2^{a}	73.38±5.30^{a}	48.33±2.01^{a}	297.1±12.1^{a}
Kahramanmaraş /Turkey	60.3±6.9^{c}	17.21±9.8^{c}	42.81±0.71^{c}	263.1±19.9^{c}
Nakhcevan	90.1±4.4^{b}	39.46±5.19^{b}	46.26±1.70^{b}	291.3±9.2^{b}

All values are presented as means ± SD (n = 3). Different letters (a-c) within the columns indicate statistically significant differences by Duncan's multiple range test at $p<0.05$.

TABLE 2
Chlorophyll a, chlorophyll b and carotenoid contents of *E. spectabilis* aereal parts

Samples	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Total Chlorophyll (µg/g)	Carotenoid (µg/g)
Bingöl/Turkey	3.38±0.14[b]	1.45±0.31[b]	4.83±0.26[b]	1.39±0.10[b]
Kahramanmaraş/Turkey	1.51±0.14[c]	0.46±0.19[c]	1.97±0.17[c]	0.52±0.04[c]
Nakhcevan	14.85±0.98[a]	7.40±1.20[a]	22.25±1.09[a]	5.6±0.89[a]

All values are presented as means ± SD (n = 3). Different letters (a-c) within the columns indicate statistically significant differences by Duncan's multiple range test at $p<0.05$.

TABLE 3
Antimicrobial activities of *E. spectabilis* areal parts and standard antibiotic (mm)

	Bingöl/ Turkey	Kahramanmaraş/ Turkey	Nakhcevan	Erythromycin
Pseudomonas aeroginosa ATCC 9027	13.300±0.030	14.026±0.494	13.280±0.455	26.00±0.577
Bacillus subtilis	13.846±0.451	13.280±0.649	13.793±0.396	19.33±0.333
Enterobacter aerogenes	14.663±0.055	13.913±0.288	14.970±0.665	25.33±0.333
Bacillus licheniformis	-	-	12.270±0.334	30.00±0.0
Klebsiella pneumoniae	14.753±0.513	13.036±0.430	13.300±0.635	21.33±1.856
Staphylococcus aureus ATCC 6538	13.716±0.060	13.023±0.124	13.070±0.136	23.33±0.333
Bacillus megaterium DSM 32	12.966±0.401	12.970±0.276	14.196±0.728	25.67±0.333
Escherichia coli	14.326±0.255	12.903±0.152	14.276±0.319	23.67±0.333
Yarrovia lipolytica	-	-	-	-
Candida albicans	-	-	-	-
Saccharomyces cerevisiae	12.620±0.050	12.635±0.175	12.830±0.320	-

All values are presented as means ± SD (n = 3).

TABLE 4
Antimutagenicity of *E. spectabilis* areal parts in *Salmonella typhimurium* TA 98 strain

***E. spectabilis* extracts**	**Concentration (µL/plate)**	**Revertant colonies Mean±Sd****
Bingöl/Turkey	Control	13.00±1.00
	Positive control(4-NPD)[+]	684±159
	Methanol	15.67±1.86
	10 µL/plate	318.0±89.8 *
	20 µL/ plate	339.3±27.4 *
	40 µL/ plate	316.3±15.6 *
	80 µL/ plate	255.7±15.2 *
Kahramanmaraş/ Turkey	Control	13.00±1.00
	Positive control(4-NPD)	684±159
	Methanol	15.67±1.86
	10 µL/plate	398±108
	20 µL/ plate	324.7±38.6
	40 µL/ plate	326.7±44.5
	80 µL/ plate	200.0±43.5 *
Nakhcevan	Control	13.00±1.00
	Positive control(4-NPD)	684±159
	Methanol	15.67±1.86
	10 µL/plate	751.0±93.7
	20 µL/ plate	331.3±61.9
	40 µL/ plate	445±128
	80 µL/ plate	306.0±23.2

[+]4-NPD: 4-nitro-o-phenylenediamine; **Sd: Standard deviation, *Significant difference between positive control, P≤0.05

TABLE 5
Antimutagenicity of *E. spectabilis* areal parts in *Salmonella typhimurium* TA 100 strain

E. spectabilis extracts	Concentration (µL/plate)	Revertant colonies Mean±Sd**
Bingöl/Turkey	Control	370.0±47.6
	Positive control (SA)+	5843±440
	Methanol	204.7±68.7
	10 µL/plate	4534±634
	20 µL/ plate	3919±1100
	40 µL/ plate	3564±570
	80 µL/ plate	5721±887
Kahramanmaraş/ Turkey	Control	370.0±47.6
	Positive control (SA)	5843±440
	Methanol	204.7±68.7
	10 µL/plate	4419±1207
	20 µL/ plate	3345±480
	40 µL/ plate	7531±1216
	80 µL/ plate	3009±256
Nakhcevan	Control	370.0±47.6
	Positive control (SA)	5843±440
	Methanol	204.7±68.7
	10 µL/plate	5897±431
	20 µL/ plate	4432±683
	40 µL/ plate	2823±253 *
	80 µL/ plate	3513±1047

+SA: Sodium azide, **Sd: Standard deviation, *Significant difference between positive control, P≤0.05

To the best of our knowledge, only a few studies have been published on the antimicrobial activity of *E. spectabilis*. However, our samples were collected from different regions of Turkey. Kanaani and Sani [23] did not observed any growth inhibition by the agar diffusion method in the methanolic root extracts of *E. spectabilis*. Instead, they observed an antibacterial activity by microdilution broth assay method against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella enterica,* and *Escherichia coli*. According to Taskin et al. [45], chloroform and aqueous extracts of *E. spectabilis* exhibited an antifungal activity against *Candida albicans*. In addition, ethyl acetate and aqueous extracts displayed antibacterial effects on *Klebsiella pneumoniae* and *Staphylococcus aureus*. Bircan and Kirbag in 2015 [22] reported that the extracts of *E. spectabilis* led to a zone of inhibition of 12 mm for *Staphylococcus aureus,* 14 mm for *Escherichia coli,* 9 mm for *Candida albicans,* and 8 mm for *Epidermophyton* spp. Some differences can be observed between different studis. This may be due to reasons such as the different extraction method, the different solvent using the extraction, the different bacterial strain used in study, the different geographical region of plant or the different amount of the used material.

Antimutagenic Activity. In the present study, we determined the antimutagenic activity of aerial parts of *E. spectabilis*. Four different concentrations (10, 20, 40, and 80 µL/plate) of *E. spectabilis* extracts were used based on the results of preliminary experiments. The tests were performed in *Salmonella typhimurium* TA 98 and TA 100 strains. We found that all doses of Bingöl extracts and the highest dose (80 µL/plate) of Kahramanmaras extracts exhibited the antimutagenic activity in *Salmonella typhimurium* TA 98 strain. None of the concentrations of Nakhcevan extracts displayed antimutagenic effect against TA 98 strain (Table 4).

Only 40 µL/plate dose of Nakhcevan extract was found to be statistically significant form *Salmonella typhimurium* TA 100 strain. All other doses did not exhibit antimutagenic effect against TA 100 strain (Table 5). As far as we know, the antimutagenic activity of *E. spectabilis* has not yet been reported. Ipek et al. [46] observed that the oil of *Origanum onites* is antimutagenic against *Salmonella typhimurium* TA 98 and TA 100 strains. In another study, antimutagenic activity of *Melaleuca alternifolia*, *Lavandula angustifolia*, *Spinacia oleracea* L., *Lepidium sativum* L., and *Sisymbrium officinale* Scop. was determined in *Salmonella typhimurium* TA 98 and TA 100 [47, 48, 49].

CONCLUSION

The present study concluded that the extracts of aerial parts of *E. spectabilis* have a high total phenolic content. It is known that the phenolic compounds have antioxidant effects. Additionally, we determined *E. spectabilis* extracts to have a significant antioxidant and free radical scavenging ability using three methods (DPPH, ABTS, and FRAP).

Therefore, the consumption of this plant may protect the body against oxidative damage. Furthermore, chlorophyll is a good source of antioxidants and aerial parts of *E. spectabilis*, especially the sample from Nakhcevan had high pigment (Cha, Chb, Cx, and TC) content. The results of this study revealed that the extracts of aerial parts of *E. spectabilis* possess antimicrobial and antimutagenic properties. Therefore, the consumption of *E. spectabilis* plant may prove to be beneficial for human health.

REFERENCES

[1] Dey, T.B., Chakraborty, S., Jain, K.K., Sharma, A., Kuhad, R.C. (2016) Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review. Trends in Food Science and Technology. 53, 60-74.

[2] Sosa, V., Moline, T., Somoza, R., Paciucci, R., Kondoh, H., LLeonart, M.E. (2013) Oxidative stress and cancer: An overview. Ageing Research Reviews. 12(1), 376-390.

[3] Adebayo, E.A., Oloke, J.K., Ayandele, A.A., Adegunlola, C.O. (2012) Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonariuse* LAU 09 (JF736658). Journal of Microbiology and Biotechnology Research. 2, 366-374.

[4] Lambert, J.D., Yang, C.S. (2003) Mechanisms of cancer prevention by tea constituents. Journal of Nutrition. 133, 3262-3267.

[5] Olsson, M.E., Ekvall, J., Gustavsson, K., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B., Nyman, M.G.L. (2004) Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria* × *ananassa*): Effects of cultivar, ripening, and storage. Journal of Agriculture and Food Chemistry. 52, 2490-2498.

[6] Bajalan, I., Mohammadi, M., Alaei, M., Pirbalouti, A.G. (2016) Total phenolic and flavonoid contents and antioxidant activity of extracts from different populations of lavandin. Industrial Crops and Products. 87, 255-260.

[7] Jin, L., Li, X.B., Tian, D.Q., Fang, X.P., Yu, Y.M., Zhu, H.Q., Ge, Y.Y., Ma, G.Y., Wang, W.Y,, Xiao, W.F., Li, M., (2016) Antioxidant properties and color parameters of herbal teas in China. Industrial Crops and Product. 87, 198-209.

[8] Lima, A., Bento, A., Baraldi, I., Malheiro, R., (2016) Selection of grapevine leaf varieties for culinary process based on phytochemical composition and antioxidant properties. Food Chemistry. 212, 291-295.

[9] Zhang, L., Tu, Z., Yuan, T., Wang, H., Xie, X., Fu, Z. (2016) Antioxidants and -glucosidase inhibitors from Ipomoea batatas leaves identified by bioassay-guided approach and structure-activity relationships. Food Chemistry. 208, 61-67.

[10] Akinmoladun, A.C., Obuotor, E.M., Farombi, E.O. (2010) Evaluation of antioxidant and free radical scavenging capacities of some nigerian indigenous medicinal plants. Journal of Medicinal Food. 13, 441-451.

[11] Elekofehinti, O.O., Kade, I.J. (2012) Aqueous extract of *Solanum anguivi* Lam. fruits (African egg plant) inhibit Fe2þ and SNP induced lipid peroxidation in Rat's brain e in vitro. Der Pharmacia Lettre. 4, 1352-1359.

[12] Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews. 56, 317-333.

[13] Daglia, M. (2012) Polyphenols as antimicrobial agents. Current Opinion in Biotechnology. 23, 174-181.

[14] Li, C., Shi, J.G., Zhang, Y.P., Zhang, C.Z., (2000) Constituents of *Eremuruschinensis*. Journal of Natural Product. 63, 653-656.

[15] Kamenetsky, K., Rabinowitch, E., (1999) Flowering response of Eremurus to post-harvest temperature. Scientia Horticulturae. 79, 75-86.

[16] Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present). 2nd ed. Nobel Medicine Publication, Istanbul, 118-119.

[17] Altundag, E., Ozturk, M. (2011) Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Procedia Social and Behavioral Sciences. 19, 756–777.

[18] Ozturk, F., Olcucu, C. (2011) Ethnobotanical features of some plants in the district of Semdinli (Hakkari–Turkey). International Journal of Academic Research. 3, 120-125.

[19] Tosun, M., Ercisli, S., Ozer, H., Turan, M., Polat, T., Ozturk, E., Padem, H., Kılıçgün, H. (2012) Chemical composition and antioxidant activity of foxtail lily (*Eremurus spectabilis*). Acta Scientiarum Polonorum Hortorum Cultus. 11, 145-153.

[20] Gaggeri, R., Rossi, D., Mahmood, K., Gozzini, D., Mannucci, B., Corana, F., Daglia, M., Avanzini, A., Mantelli, M., Martino, E., Collina, S. (2015) Towards elucidating *Eremurus* root remedy: Chemicalprofiling and preliminary biological investigations of *Eremurus persicus* and *Eremurus spectabilis* rootethanolic extracts. Journal of Medicinal Plants Research. 8, 1038-1048.

[21] Hakemi, V.M., Asgarpanah, J., Hedayati, M., Shirali, J., Bagheri-Bejestani, F. (2011) Antibacterial and cytotoxic activity of *Eremurus persicus* (Jaub and Spach) Boiss. African Journal of Microbiology Research. 5, 23-49.

[22] Bircan, B., Kirbag, S. (2015) Determination of antioxidant and antimicrobial properties of *Eremurusspectabilis* Bieb. Artvin Coruh University Journal of Forestry Faculty. 16, 176-186.
[23] Kanaani, S., Sani, A.M. (2015) Chemical Composition of Essential Oils and in vitro Antibacterial Activity of Methanolic Extract of Sonchus arvensis and Eremurus spectabilis against Foodborne Pathogenic Bacteria. Journal of Essential Oil Bearing Plants. 18, 1093-1099.
[24] Davis, P.H. (ed.) (1965-1985) Flora of Turkey and the East Aegean Islands. vol.1-9, Edinburgh University Press, Edinburgh, UK.
[25] Spanos, G.A., Wrolstad, R.E. (1992) Phenolic of apple, pear and white grape juices and their changes with processing and storage. Journal of Agriculture and Food Chemistry. 40, 1478-1487.
[26] Bakhshi, D., Arakawa, O. (2006) Effects of UV-B irradiation on phenolic compound accumulation and antioxidant activity in 'Jonathan' apple influenced by bagging, temperature and maturation. Journal of Food Agriculture and Environment. 4, 75-79.
[27] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 26, 1231-1237.
[28] Benzie, I.F.F., Strain, J.J. (1996) The ferric reducing Ability of plasma (FRAB) as a measure of "Antioxidant power": The FRAP assay. Analytical Biochemistry. 239, 70-76.
[29] De-Kok, L., Graham, M. (1980) Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of Arabidopsis thaliana during dark induced and natural senesence. Plant Physiology and Biochemistry. 27, 133-142.
[30] Collins, C.M., Lyne, P.M., Grange, J.M. (1989) Antimicrobial sensitivity and assay tests. Collins and Lyne's Microbiological Methods. Butterworths, London, UK.
[31] Bradshaw, L.J. (1992) Laboratory Microbiology. 4th Ed., Saunders College Publishing, New York, USA.
[32] Maron, D.M., Ames, B.N. (1983) Revised methods fort he *Salmonella* mutagenicity test. Mutation Research. 113, 173-215.
[33] Duncan, D.B. (1955) Multiple range and multiple F Tests. Biometrics. 11, 1-14.
[34] Prior, R.L. (2003) Fruits and vegetables in the prevention of cellular oxidative damage. The American Journal of Clinical Nutrition. 78, 570-578.
[35] Bataglion, G.A., Da Silva, F.M.A., Eberlin, M.N., Koolen, H.H.F. (2014) Simultaneous quantification of phenolic compounds in buriti fruit (*Mauritia flexuosa* L.f.) by ultra-performance liquid chromatography coupled to tandem mass spectrometry. Food Research International. 66, 396-400.
[36] Karadag, A., Ozcelik, B., Saner, S. (2009) Review of methods to determine antioxidant capacities. Food Analytical Methods. 2, 41-60.
[37] Parejo, L., Codina, C., Petrakis, C., Kefalas, P. (2000) Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH center dot (2,2-diphenyl-1- picrylhydrazyl) free radical assay. Journal of Pharmacological and Toxicological Methods. 44, 507-512.
[38] Mbaebie, B.O., Edeoga, H.O., Ajelayan, A.J. (2012) Phytochemical analysis and antioxidant activities of aqueous bark extract of Schoba lahtolic JACQ. Asian Pacific Journal of Tropical Biomedicine. 2, 118-124.
[39] Filella, I., Serrano, L., Serra, J., Penuelas, J. (1995) Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. Crop Science. 35, 1400-1405.
[40] Carter, A.G., Spiering, B.A. (2002) Optical properties of intact leaves for estimating chlorophyll concentration. Journal of Environmental Quality. 31, 1424-1432.
[41] Wu, C., Niu, Z., Tang, Q., Huang, W. (2008) Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. Agricultural and Forest Meteorology. 48, 1230-1241.
[42] Wang, S., Li, H., Lin, C. (2013) Physiological, biochemical and growth responses of Italian ryegrass to butachlor exposure. Pesticide Biochemistry and Physiology. 106, 21-27.
[43] Zilic, S., Jankovic, M., Basic, Z., Vancetovic, J., Maksimovic, V. (2016) Antioxidant activity, phenolic profile, chlorophyll and mineral matter content of corn silk (*Zea mays* L): Comparison with medicinal herbs. Journal of Cereal Science. 69, 363-370.
[44] Loranty, A., Rembialkowska, E., Rosa, E.A.S., Bennett, R.N. (2010) Identification, quantification and avaliability of carotenoids and chlorophylls in fruit, herb and medicinal teas. Journal of Food Composition and Analysis. 23, 432-441.
[45] Taskin, T., Bitis, L., Birteksöz-Tan, A.S. (2012) Antioxidant and antimicrobial activities of different extracts from *Eremurus spectabilis* leaves. Spatula. 2, 213-217.

[46] Ipek, E., Zeytinoglu, H., Okay, S., Tuylu, B., Kurkcuoglu, M., Can, B.K.H. (2005) Genotoxicity and antigenotoxicity of *Origanum* oil and carvacrole valuated by Ames Salmonella/microsomal test. Food Chemistry. 93, 551-556.
[47] Kalaycıoğlu, A., Oner, C. (1997) Bazı Bitki Ekstraktlarının Antimutajenik Etkilerinin Amest-Salmonella Test Sistemiyle Arastırılması. Turkish Journal of Botany. 18, 117-122.
[48] Evandri, M.G., Battinelli, L., Daniele, C., Mastrangelo, S., Bolle, P., Mazzanti, G. (2005) The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. Food and Chemical Toxicolology. 43, 1381-1387.
[49] Sotto, A.D., Vitalone, A., Nicoletti, M., Piccin, A., Mazzanti, G. (2010) Pharmacological and phytochemical study on a *Sisymbrium officinale* Scop. Extract. Journal of Ethnopharmacology. 127, 731-736.

Received: **11.11.2017**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Nurcan Erbil
Ardahan University,
Health Sciences School,
Department of Nursing,
75000, Ardahan – Turkey

e-mail: nurcanerbil@ardahan.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9500-9508 Fresenius Environmental Bulletin

COMPARING SEVEN DIFFERENT REFERENCE EVAPOTRANSPIRATION EQUATIONS WITH PENMAN-MONTEITH FOR ANTALYA, TURKEY

Cihan Karaca[1], Dursun Buyuktas[1,*], Ruhi Bastug[1], Koksal Aydinsakir[2], Kenan Buyuktas[1]

[1]Department of Agricultural Structures and Irrigation, Faculty of Agriculture, Akdeniz University, Antalya, Turkey
[2]Bati Akdeniz Agricultural Research Institute, Antalya, Turkey

ABSTRACT

Evapotranspiration (ET) is one of the most difficult processes in nature. The most accurate and reliable method to determine ET is lysimeter, which is difficult, expensive and time-consuming. On the other hand, ET can be estimated using reference crop evapotranspiration (ET_o) and crop coefficient (k_c). easily Many ET_o equations using meteorological data have been developed. The most widely accepted equation for ET_o is FAO-56 Penman-Monteith (FAO-56 PM) equation. However, FAO-56 PM equation needs numerous meteorological data and is hard to measure for farmers. For this reason, equations which need less meteorological data have been gaining importance. Characteristic Mediterranean climate prevails in coastal areas of Antalya, Turkey. In the present study, long term data (1985 to 2015) obtained from meteorological stations was used to compute ET_o in Antalya. Seven different ET_o equations (Penman, FAO 24 Penman, Hargreaves, Turc, Priestley-Taylor, FAO 24 Pan, Christiansen Pan) were compared with FAO-56 PM. It was shown that the Turc equation gives results close to FAO-56 PM equation.

KEYWORDS:
ET_o, Penman-Monteith, Humid conditions, Turkey, Turc

INTRODUCTION

Determining the amount of evapotranspiration for optimal irrigation programming is important because of declining water resources. But determining of ET is one of the most difficult processes in nature. The most accurate and reliable method to determine ET is lysimeter, which is difficult, expensive and time-consuming. On the other hand, ET can be estimated with meteorological data and crop coefficient easily. ET quantification frequently must be preceded by the determination of reference evapotranspiration (ET_o) [1, 2].

Although FAO-56 PM is considered as a standard and most accurate model under various climatic conditions to estimate ET_o [3, 4, 5, 6, 7, 8, 9, 10] many models have been developed in accordance with various climate and geographical conditions. The major limitation to FAO-56 PM model is that it requires many meteorological inputs, thereby limiting its utility in data-sparse areas. It is expensive to equip meteorology stations to measure these data essentially in developing countries. Therefore, it is recommended to apply simpler models because they need parameters that are readily available from station observing meteorological data [11]. For this reason, many researchers had investigated the performance of ET_o models that need less climate parameters.

Penman [12], FAO-24 Penman [13], Hargreaves [14], Turc [15], Priestley-Taylor [16], FAO-24 Pan [13], Christiansen Pan [17, 18] models require less climate parameters than the FAO-56 PM [4] model. However, the accuracy of these models relative to the FAO-56 PM varies with climate, region and altitude.

Djaman et al. [10] evaluated sixteen reference evapotranspiration methods under Sahelian conditions in the Senegal River Valley. They revealed that the Hargreaves equations systematically overestimated ET_o with the highest percentage error of estimate (PE) while Turc equations systematically underestimated ET_o. Sheikh and Mohammadi [19] tested different estimation methods of ET_o in semi-arid regions. Their results showed that the Hargreaves-Samani equation gave the smallest difference compared to the FAO-56 PM.

Tabari et al. [2] compared 31 ET_o models against FAO-56 PM under humid conditions. The results indicated that all of the pan evaporation-based methods had a tendency to underestimate FAO-56 PM ET_o. In contrast with the pan evaporation-based, the temperature-based and radiation-based equations overestimated against FAO-56 PM.

According to Bogawski and Bednorz [20], the best estimations were obtained using the radiation-based models, followed by pan coefficient-based and temperature-based models at the border between two humid climates in Poland. In addition, the authors indicated that the pan evaporation methods were not applicable in Poland and that the Class A Pan was not routinely used for ET_o calculation. Also, the Hargreaves methods produced the least accurate

monthly ET_o estimates.

Fernandes et al. [21] compared six different equations against FAO-56 PM. The results showed that the Hargreaves, Priestley-Taylor and Makkink methods presented the highest agreements with respect to the FAO-56 PM equation. These methods achieved better performances due to the approach of improving the representation of the effects of the radiative process, since they use solar radiation. Xystrakis and Matzarakis [8] evaluated thirteen different ET_o equations and compared with FAO-56 PM in order to determine the equation that gives closest results to FAO-56 PM in Mediterranean region. The radiation-based equations generally performed better than those that included only temperature-related input variables.

Several researchers [6, 11, 22, 23, 24, 25, 26] indicated that the Turc model performs well in humid climate. Similarly, Tukimat et al. [26] found that radiation based methods (Turc, Priestley-Taylor) gave better performance compared to temperature-based methods (Hargreaves) in estimation of ET_o in the humid area. They also said that Turc method needs less number of parameters to estimate ET_o compared to Priestley-Taylor method and therefore, much easier to use.

Tabari [11] evaluated four models (Makkink, Turc, Priestley–Taylor and Hargreaves) commonly used to estimate monthly ET_o values for different climates and altitude. The author concluded that Hargreaves and Turc model presented the best estimates for warm humid climate. Lu et al. [25] reported that the radiation based equations (Priestley-Taylor and Turc) showed best performance for the southeast side of the United States. Trajkovic and Kolakovic [6] tested five commonly used models to estimate ET_o under humid condition which were compared to corresponding values estimated using the standardized FAO-56 PM equation. They indicated that the Turc equation was the most suitable model for estimating ET_o at humid locations. Fontenot [24] evaluated seven commonly used ET_o equations (FAO-24 Radiation, Blaney-Criddle, Hargreaves and Samani, Priestley-Taylor, Makkink and Turc) against ET_o values computed by the FAO-56 PM model in humid area. The results of this study indicated that the Turc model was the most accurate model in estimating daily and monthly ET_o.

The aim of this study was to assess the performance of seven ET_o equations models against FAO-56 PM model which was accepted as a standard method. By using FAO-56 PM model as reference for these models, multiple regression equations were developed in Antalya conditions.

MATERIALS AND METHODS

Study Area and Climate Dataset. In the present study, long term data (1985 to 2015) obtained from meteorological stations was used to compute ET_o in Antalya (Fig 1). All climatic data were obtained from the Turkish State Meteorological Service.

Climatic data used in ET_o calculation was collected daily during the period between January of 1985 and December of 2015. The long term average data used is given in Table 1.

Antalya is under the influence of the Mediterranean climate with hot summers and rainy winters. Since this site lies within the coastal plane, the solar radiation values are very high in summer and rather low in the winter periods. The solar radiation is under the influence of humidity due to excessive evaporation in this region.

FIGURE 1
Location of Antalya on map of Turkey.

TABLE 1
Monthly means of the main climatic variables at Antalya station from 1985 to 2015.

Station no: 17302		Latitude: 36.8851				Longitude: 30.6828							Altitude: 47.0
Month		1	2	3	4	5	6	7	8	9	10	11	12
T_{max}	(°C)	15.06	15.55	18.06	21.24	26.15	31.63	34.52	34.46	31.26	27.19	21.02	16.35
T_{min}	(°C)	5.54	5.86	7.70	10.86	15.24	19.83	23.05	22.92	19.28	15.47	10.22	7.23
RH	(%)	63.35	61.05	64.60	68.00	66.10	58.70	58.22	60.64	60.27	60.55	63.32	66.39
u_2	(m s^{-1})	2.35	2.59	2.32	2.01	1.76	2.01	1.85	1.76	1.90	1.85	1.97	2.13
Rs	(MJ $m^{-2}day^{-1}$)	8.71	12.05	16.25	19.76	23.16	25.95	25.25	22.78	19.49	14.23	9.70	7.38
N	(hours)	5.56	6.11	7.02	8.00	9.87	11.78	11.91	11.25	9.67	8.02	6.41	5.09
E_{pan}	(mm)	1.64	1.97	2.60	3.14	4.22	6.03	6.45	5.92	4.77	3.24	1.95	1.40

T_{max} is maximum air temperature; T_{min} is minimum air temperature; RH is relative humidity; u_2 is wind speed at 2 meter; R_s is solar radiation; n is sunshine hours and E_{pan} is pan evaporation.

Reference Evapotranspiration Estimation Methods. FAO-56 Penman-Monteith. The FAO Penman-Monteith [4] method for calculating ET_o is given in Eq. 1:

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T_{mean}+273} u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34\, u_2)} \quad (1)$$

where ET_o is the reference crop evapotranspiration (mm day^{-1}); R_n is the net radiation (MJ m^{-2} day^{-1}); G is the soil heat flux (MJ m^{-2} day^{-1}); γ is the psychometric constant (kPa $^oC^{-1}$); e_s is the saturation vapor pressure (kPa); e_a is the actual vapor pressure (kPa); and Δ is the slope of the saturation vapor pressure temperature curve (kPa $^oC^{-1}$); T_{mean} is the average daily air temperature (^oC) and u_2 is the mean daily wind speed at 2 m (m s^{-1}).

Combination-Based ET_o equations. Penman 1963. Penman's formula [12] combined the energy balance with the mass transfer method and derived an equation to compute the evaporation from an open water surface based on standard climatic records of sunshine, temperature, humidity and wind speed [27].

The Penman equation (Eq. 2) is expressed as:

$$ET_o = \left[\frac{\Delta}{\Delta+\gamma} R_n + \frac{\gamma}{\Delta+\gamma} E_a\right] 0.8 \quad (2)$$

where ET_o is reference evapotranspiration (mm day^{-1}); Δ is the slope of saturation vapour pressure temperature curve (kPa $^oC^{-1}$); γ is the psychometric constant (kPa $^oC^{-1}$); R_n is the net radiation (mm day^{-1}); E_a is aerodynamic term (mm day^{-1}). The aerodynamic term is given in equation (Eq. 3):

$$E_a = 0.35(1 + 0.22u_2)(e_s - e_a)7.4 \quad (3)$$

where u_2 is wind speed measured at 2 m (m s^{-1}); e_s is the saturation vapor pressure (kPa); e_a is the actual vapor pressure (kPa).

FAO 24 Penman. The FAO-24 Penman equation [13] is expressed as Eq. 4:

$$ET_o = \left[0.408\frac{\Delta}{\Delta+\gamma}(R_n - G) + 2.7\frac{\gamma}{\Delta+\gamma}(1 + 0.864u_2)(e_s - e_a)\right] \quad (4)$$

Where ET_o is reference evapotranspiration (mm day^{-1}); R_n is the net radiation (MJ m^{-2} day^{-1}); G is the soil heat flux (MJ m^{-2} day^{-1}); γ is the psychometric constant (kPa $^oC^{-1}$); e_s is the saturation vapor pressure (kPa); e_a is the actual vapor pressure (kPa); and Δ is the slope of the saturation vapor pressure temperature curve (kPa $^oC^{-1}$); and u_2 is the mean daily wind speed at 2 m (m s^{-1}).

Temperature-based ET_o equations. Hargreaves. The Hargreaves model is not truly temperature-based method because it contains a radiation term. Since measurement is not needed for extraterrestrial radiation, this method may be classified as a temperature-based method [28]. The Hargreaves model [14] is expressed as Eq. 5.

$$ET_o = 0.0023(T_{mean} + 17.8)(T_{max} - T_{min})^{0.5} R_a \quad (5)$$

where ET_o is reference evapotranspiration (mm day^{-1}); T_{mean} the daily mean air temperature (^oC); T_{max} the daily maximum air temperature (^oC); T_{min} the daily minimum air temperature (^oC) and R_a is the extraterrestrial radiation (MJ m^{-2} day^{-1}).

Radiation-Based ET_o Equations. Turc. The Turc model [15] developed in the Netherlands is given below by Eq. 6-7.

For RH ≥ 50%
$$ET_o = 0.013\frac{T_{mean}}{T_{mean}+15}\frac{23.88xR_s + 50}{\lambda} \quad (6)$$

For RH < 50%
$$ET_o = \left(1 + \frac{50 - RH}{70}\right) 0.013\frac{T_{mean}}{T_{mean}+15}\frac{23.88xR_s + 50}{\lambda} \quad (7)$$

where ET_o is reference crop evapotranspiration (mm day^{-1}); T_{mean} is the mean monthly air temperature (^oC), R_s is solar radiation (MJ m^{-2} day^{-1}), and λ is the latent heat of vaporization (MJ kg^{-1}). If the mean daily relative humidity (RH mean) was greater than or equal to 50%, then equation 6 is used. If the mean daily relative humidity was less than 50%, then equation 7 is used.

Priestley-Taylor. The Priestley-Taylor equation [16] which is a simplification of the Penman equation, is expressed as Eq. 8;

$$ET_o = 1.26\frac{\Delta(R_n - G)}{\Delta + \gamma} \quad (8)$$

where ET_o is the reference crop evapotranspiration (mm day^{-1}); R_n is the net radiation (MJ m^{-2} day^{-1}); G is the soil heat flux (MJ m^{-2} day^{-1}); γ is the psychometric constant (kPa $^oC^{-1}$) and Δ is the slope of the saturation vapor pressure temperature curve (kPa $^oC^{-1}$).

Pan Evaporation-Based Estimation. For the most of countries, Class 'A' pan is used at monitoring stations to measure evaporation from free surface water.

FAO 24 Pan. E_{pan} can be used to estimate ET_o when the k_{pan} coefficient is applied as expressed by Eq. 9.

$$ET_o = k_{pan} x E_{pan} \quad (9)$$

k_{pan} values were determined from the FAO-24 table prepared by Doorenbos and Pruitt [13].

Christiansen Pan. The following equation (Eq. 10) was developed by Christiansen and Hargreaves [18] to estimate ET_o using Class 'A' pan and various climate parameters.

$$ET_o = 0.755 E_{pan} C_{T2} C_{w2} C_{H2} C_{S2} \quad (10)$$

where E_{pan} is class A pan evaporation (cm d^{-1}), C_{T2}, C_{W2}, C_{H2} and C_{S2} are estimated by following set of equations:

$$C_{T2} = 0.862 + 0.719\left(\frac{T_{mean}}{20}\right) - 0.041\left(\frac{T_{mean}}{20}\right)^2 \quad (11)$$

$$C_{W2} = 1.189 - 0.240\left(\frac{u}{6.7}\right) + 0.051\left(\frac{u}{6.7}\right)^2 \quad (12)$$

$$C_{H2} = 0.499 + 0.620\left(\frac{RH_{mean}}{60}\right) - 0.119\left(\frac{RH_{mean}}{60}\right)^2 \quad (13)$$

$$C_{S2} = 0.904 - 0.0080\left(\frac{\frac{n}{N}}{0.80}\right) + 0.088\left(\frac{\frac{n}{N}}{0.80}\right)^2 \quad (14)$$

where T_{mean} is the mean air temperature (°C); u is wind speed (km/hr), RH_{mean} is the mean relative humidity (%,) n is the actual sunshine hours (h) and N is maximum possible sunshine hours.

Statistical Analysis. The FAO-56 PM model was assumed as the equation giving the most accurate ET_o value. Other seven methods were compared and evaluated to determine the ET_o equation that provides the best fit with the FAO-56 PM model on daily time step. For this purpose, some performance criteria were used including coefficient of determination (R^2), root mean square error (RMSE), relative error (RE), mean bias error (MBE), The Willmott index [29] of agreement. These criteria are defined as in Eq. 15, 16, 17, 18 and 19.

$$R^2 = \frac{\left[\sum_{i=1}^{n}(X_i - \overline{X})(Y_i - \overline{Y})\right]^2}{\sum_{i=1}^{n}(X_i - \overline{X})^2 \sum_{i=1}^{n}(Y_i - \overline{Y})^2} \quad (15)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(X_i - Y_i)^2}{n}} \quad (16)$$

$$RE = \frac{RMSE}{\overline{Y}_i} \quad (17)$$

$$MBE = \frac{\sum_{i=1}^{n}(X_i - Y_i)}{n} \quad (18)$$

$$d = 1 - \frac{\sum_{i=1}^{n}(X_i - Y_i)^2}{\sum_{i=n}^{n}\left((X_i - \overline{Y}_i) + (Y_i - \overline{Y}_i)\right)^2} \quad (19)$$

Where n is number of observations, X_i is estimated ET_o by other equations, Y_i is estimated ET_o by FAO-56 PM and $\overline{Y}_i$ is mean value of ET_o by FAO-56 PM. The most accurate result is obtained when RMSE, RE and MBE were equal to 0 and d and R^2 equal 1.

RESULTS AND DISCUSSION

Seven methods were compared and evaluated against the reference evapotranspiration data calculated using the FAO-56 PM model. These results are given in table 2.

Combination-Based ET_o equations. The results of the statistical analysis of the combination-based methods versus the FAO-56 PM model are given in Table 2. According to the MBE values and figure 2-3, all of the combination-based methods overestimated in comparison with FAO-56 PM model. Although the combination methods had high R^2 values, these methods gave the worst results in all other statistical cases.

Similarly, some researchers [30, 31] reported that Penman's method estimated the ET_o values higher in humid region. However, it had been determined that ET_o value is less predicted by Penman method in arid regions. According to Lopez-Urrea et al. [1] and Jensen et al. [3] the Penman method underestimated the lysimetric measurement in arid region. Also, they declared that FAO-24 Penman estimated higher ET_o value.

Temperature-based ET_o equations. According to Table 2 and Figure 4, our study showed that Hargreaves method did not give good result (R^2= 0.758, RMSE= 0.811mm day^{-1}, RE= 0.247 and d= 0.931).

The result obtained by the Hargreaves method is similar to previous studies of different researchers [6, 21, 30, 31, 32, 33]. As a result of many studies Hargreaves equation is not recommended for humid areas because Hargreaves equation was developed in the dry climate region of California [11, 25, 33, 34]. According to Temesgen et al. [35] higher wind speed

TABLE 2
Statistical performance of the seven different ET_o models versus the FAO-56 PM model for estimating daily ET_o during study period (1985-2015)

	R^2	RMSE (mm day^{-1})	RE	MBE (mm day^{-1})	d
Penman	0,997	0,689	0,210	-0,647	0,959
FAO-24 Penman	0,995	1,212	0,370	-1,167	0,889
Hargreaves	0,758	0,811	0,247	-0,131	0,931
Turc	0,973	0,267	0,081	-0,059	0,993
Priestley-Taylor	0,965	0,494	0,151	0,165	0,979
FAO-24 Pan	0,945	0,486	0,148	0,319	0,974
Christiansen Pan	0,931	0,674	0,206	0,535	0,952

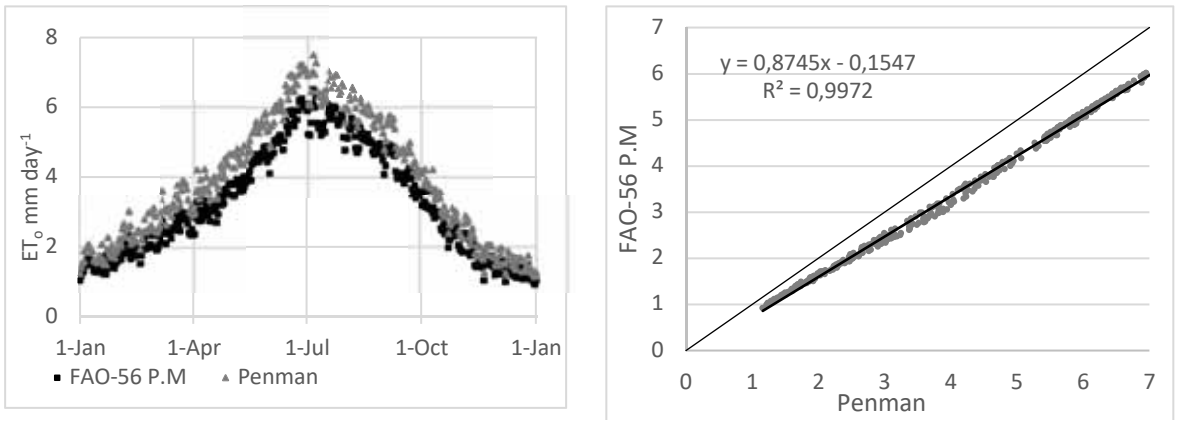


FIGURE 2
Daily ET_o comparison between the Penman and the FAO-56 PM equation

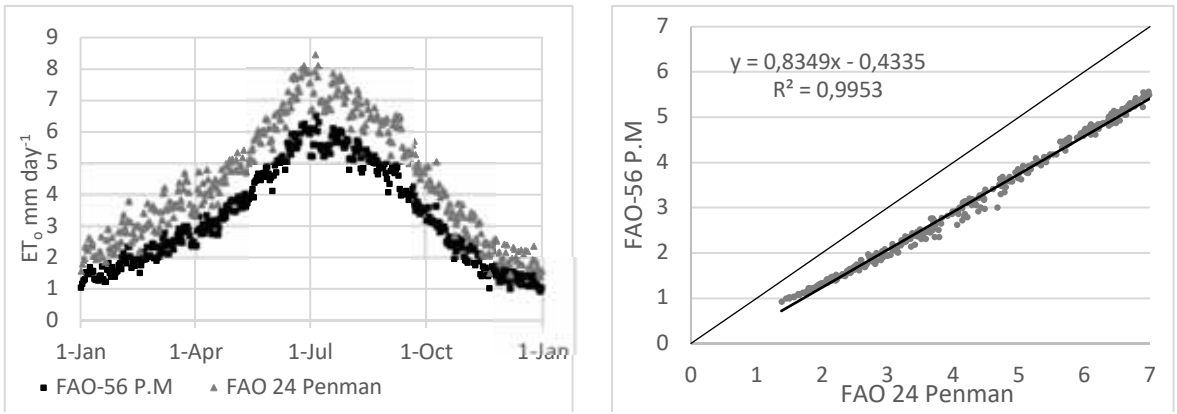


FIGURE 3
Daily ET_o comparison between the FAO-24 Penman and the FAO-56 PM equation

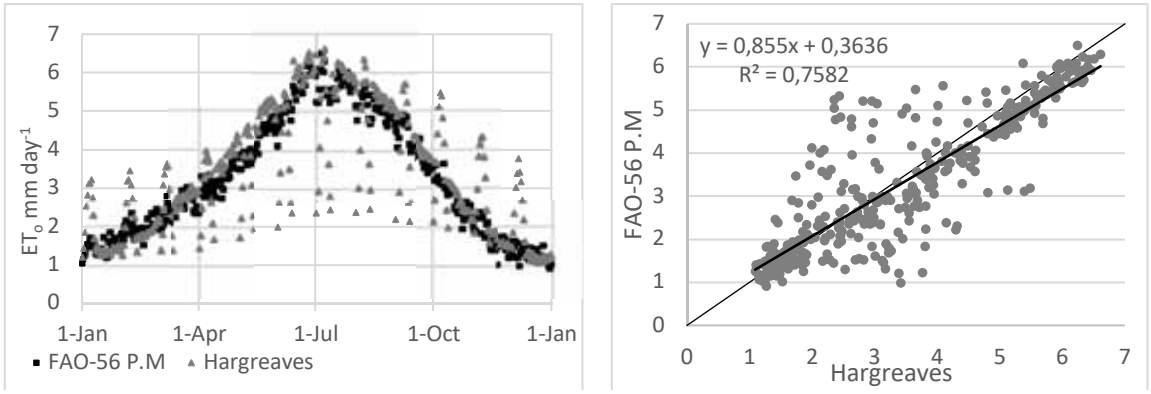


FIGURE 4
Daily ET_o comparison between the Hargreaves and the FAO-56 PM equation

combined with lower humidity resulted in lower values of Hargreaves ET_o compared to FAO-56 PM ET_o. Also, lower wind speed combined with higher humidity resulted in higher values of Hargreaves ET_o compared to FAO-56 PM ET_o. This is probably due to the lack of explicit wind speed and humidity terms in the Hargreaves et al. [14] equation.

Radiation-Based ET_o Equations. According to the results obtained, radiation-based ET models made the closest estimates to FAO-56 PM (Figure 5-6) for our region. In particular, the Turc model (R^2=0.973, RMSE=0.267, RE=0.081, RE=-0.059, d=0.993) achieved the best result compared to all other methods (Table 2). Also Turc method needs less number of parameters to estimate ET compared to Priestley-Taylor method and therefore, much easier to use [26].

Trajkovic and Kolakovic [6] indicated that the Turc equation is most suitable for estimating reference evapotranspiration at humid locations when weather data are insufficient to apply the FAO-56 PM equation. Similarly, Lu et al. [25] stated that radiation based methods that were developed for warm, humid climate conditions (Priestley-Taylor and Turc methods) perform well for the southeastern United States. Kashyap and Panda [30] and Tukimat et al. [26] also declared that the Turc model gave the closest results to the FAO-56 PM model.

Similar to Trajkovic and Kolakovic [6] Priestley-Taylor method gave reasonable results in our study (R^2= 0.965, RMSE= 0.494, RE=0.151, MBE=0.165, d=0.979). Fernandes et al. [21] achieved the good performances by Priestley-Taylor method with high coefficients of determination (over 0.9) and indices of agreement close to 1.

Pan Evaporation-Based Estimation. The results showed that Pan Evaporation-Based models underestimated in comparison with FAO-56 PM (Table 2 and Figure 7-8). In this study, the FAO-24 model gave more significant results than the Christiansen model.

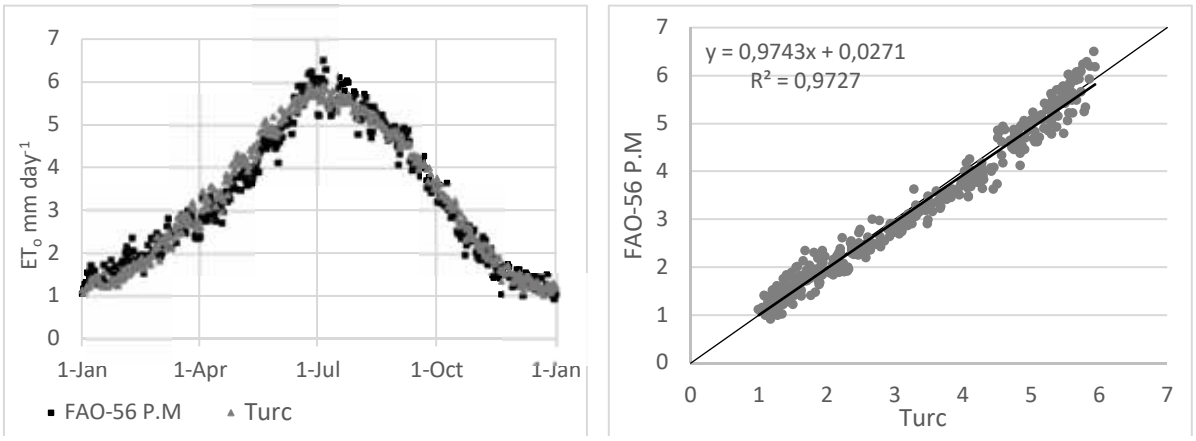


FIGURE 5
Daily ET_o comparison between the Turc and the FAO-56 PM equation

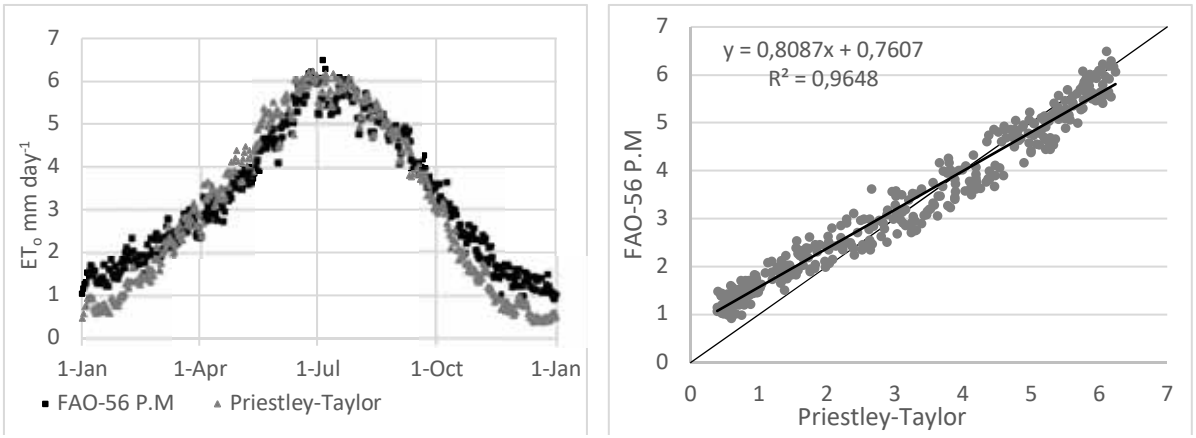


FIGURE 6
Daily ET_o comparison between the Priestley-Taylor and the FAO-56 PM equation

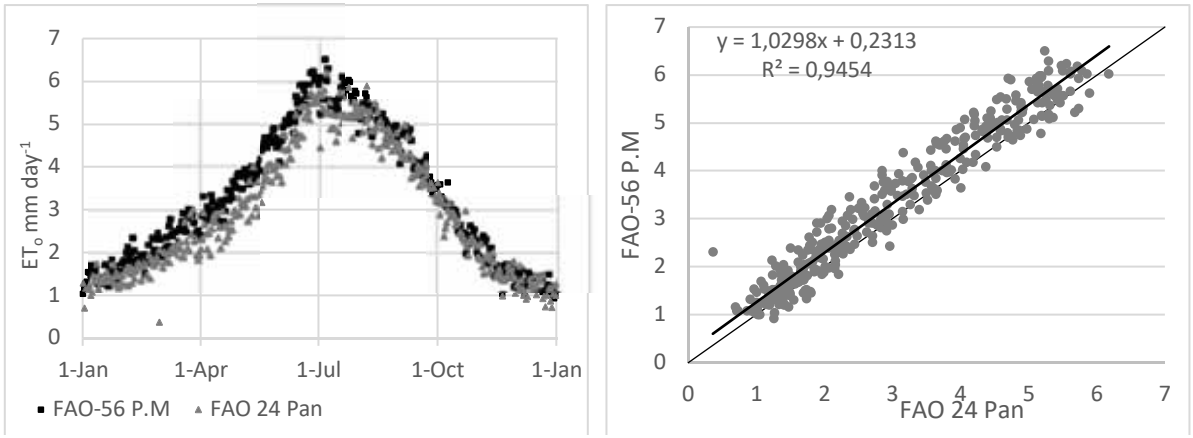


FIGURE 7
Daily ET_o comparison between the FAO-24 Pan and the FAO-56 PM equation

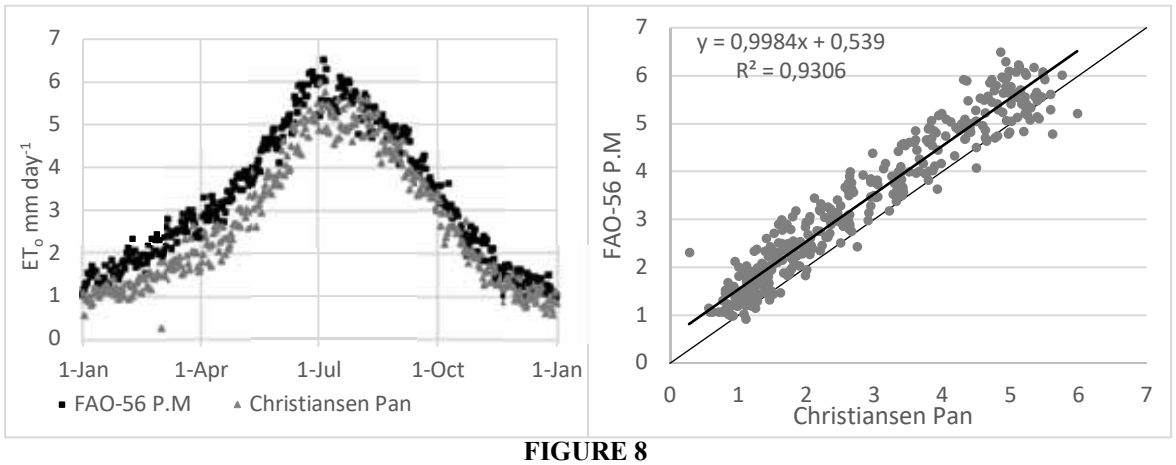


FIGURE 8
Daily ET_o comparison between the Christiansen Pan and the FAO-56 PM equation

Figure 7 and Table 2 compares the daily ET_o values computed by FAO-24 Pan model and the obtained values by FAO-56 PM. As shown, the FAO-24 Pan model presented the good results at our region. But Christiansen Pan model did not present good results in comparison with other models.

According to Irmak and Haman [36] Christiansen model significantly and consistently underestimated E_{pan} throughout the year. The results obtained from this study are similar to previous studies in this regard.

CONCLUSIONS

Although the FAO-56 PM model is considered the standard model, it requires a large number of climatic parameters. For this reason, seven simple commonly used ET_o equations evaluated against FAO-56 PM as the standard equation of estimating reference evapotranspiration.

FAO-24 Penman model gave the worst result in comparison with other models. Because of high evaporative demand periods Hargreaves model did not give good results. Similar to previous studies, it is not recommended to use the Hargreaves equation for this region. The FAO-24 Pan model gave better results comparison with Christiansen model when the Pan Evaporation-Based models were examined.

In this study, the best results were obtained with radiation-based equations. Turc model was the best estimating ET_o model. Also Priestley-Taylor model gave good result. Because it needs less number of parameters to estimate ET_o and much easier to use it is recommended to use Turc model against Priestley-Taylor.

REFERENCES

[1] Lopez-Urrea, R., Martin de Santa Olalla, F., Fabeiro, C. and Moratalla, A. (2006) Testing Evapotranspiration Equations Using Lysimeter Observations in a Semiarid Climate. Agricultural Water Management. 85(1-2), 15-26.

[2] Tabari, H., Grismer, M.E. and Trajkovic, S. (2013) Comparative Analysis of 31 Reference Evapotranspiration Methods under Humid Conditions. Irrig. Sci. 31(2), 107-117.
[3] Jensen, M.E., Burman, R.D. and Allen, R.G. (1990) Evapotranspiration and Irrigation Water Requirements. ASCE Manual and Reports on Engineering Practice No. 70. ASCE, New York, 1-360
[4] Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. (1998) Crop Evapotranspiration Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization, Rome, 1-300.
[5] Irmak, S., Allen, R.G. and Whitty, E.B. (2003.) Daily Grass and Alfalfa-Reference Evapotranspiration Estimates and Alfalfa to Grass Evapotranspiration Ratios in Florida. Journal of Irrigation and Drainage Engineering. 129(5), 360-370.
[6] Trajkovic, S. and Kolakovic, S. (2009) Evaluation of Reference Evapotranspiration Equations under Humid Conditions. Water Resources Management. 23(14), 3057-3067.
[7] Martinez, C.J. and Thepadia, M. (2010) Estimating Reference Evapotranspiration with Minimum Data in Florida. Journal of Irrigation and Drainage Engineering. 136(7), 494-501.
[8] Xystrakis, F. and Matzarakis, A. (2011) Evaluation of 13 Empirical Reference Potential Evapotranspiration Equations on the Island of Crete in Southern Greece. J. Irrig. Drain. Eng. ASCE. 137(4), 211-222.
[9] Azhar, A.H. and Perera, B.J.C. (2011) Evaluation of Reference Evapotranspiration Estimation Methods under Southeast Australian Conditions Journal of Irrigation and Drainage Engineering. 137(5), 268-279.
[10] Djaman, K., Balde, A.B., Sow, A., Muller, B., Irmak, S., N'Diaye, M.K., Manneh, B. and Saito, K. (2015) Evaluation of Sixteen Reference Evapotranspiration Methods under Sahelian Conditions in the Senegal River Valley. Journal of Hydrology. 3, 139-159.
[11] Tabari, H. (2010) Evaluation of Reference Crop Evapotranspiration Equations in Various Climates. Water Resources Management. 24(10), 2311-2337.
[12] Penman, H.L. (1963) Vegetation and Hydrology. Soil Science. 96(5), 1-357.
[13] Doorenboos, J., Pruitt, W.O. (1977) Guidelines for Predicting Crop Water Requirements, Irrigation and Drainage Paper 24. L. Water Dev. Div., FAO Rome. 24, 1-144.
[14] Hargreaves, G.H. and Samani, Z.A. (1985) Reference Crop Evapotranspiration from Temperature. Applied Engineering in Agriculture. 1(2), 96-99.
[15] Turc, L. (1961) Estimation of Irrigation Water Requirements, Potential Evapotranspiration: A Simple Climatic Formula Evolved up to Date. Ann. Agronom. 12, 13-14.
[16] Priestley, C.H.B. and Taylor, R.J. (1972) On the Assessment of Surface Heat Flux and Evaporation Using Large Scale Parameters. Monthly Weather Review. 100(2), 81-92.
[17] Christiansen, J.E. (1968) Pan Evaporation and Evapotranspiration from Climatic Data. Journal of Irrigation and Drainage Engineering, ASCE. 94(2), 243-265.
[18] Christiansen, J.E. and Hargreaves, G.H. (1969) Irrigation Requirements from Evaporation. Transaction of the International Commission on Irrigation and Drainage. 23(3), 569-596.
[19] Sheikh V. and Mohammadi M. (2013) Evaluation of Reference Evapotranspiration Equations in Semi-Arid Regions of Northeast of Iran. International Journal of Agriculture and Crop Sciences. 5(5), 450-456.
[20] Bogawski, P. and Bednorz, E. (2014) Comparison and Validation of Selected Evapotranspiration Models for Conditions in Poland (Central Europe). Water Resources Management. 28(14), 5021-5038.
[21] Fernandes, L.C., Paiva, C.M. and Filho, O.C.R. (2012) Evaluation of Six Empirical Evapotranspiration Equations-Case Study: Campos dos Goytacazes/RJ. Revista Brasileira de Meteorologia. 27(3), 272-280.
[22] Amatya, D.M, Skaggs, R.W. and Gregory, J.D. (1995) Comparison of Methods for Estimating REF-ET. Journal of Irrigation and Drainage Engineering. 121(6), 427-435.
[23] George, B.A., Reddy, B.R.S., Raghuwanshi, N.S. and Wallender, W.W. (2002) Decision Support System for Estimating Reference Evapotranspiration. Journal of Irrigation and Drainage Engineering. 128(1), 1-10.
[24] Fontenot, R.L. (2004) An Evaluation of Reference Evapotranspiration Models in Louisiana. Master Thesis. Louisiana State University and A&M College, Louisiana, 1-83.
[25] Lu, J.B., Sun, G., McNulty, S.G. and Amatya, D.M. (2005) A Comparison of Six Potential Evapotranspiration Methods for Regional Use in the Southeastern United States. American Water Resources Association. 41(3), 621-633.
[26] Tukimat, N.N.A., Harun, S. and Shahid, S. (2012) Comparison of Different Methods in Estimating Potential Evapotranspiration at Muda Irrigation Scheme of Malaysia. Journal of Agriculture and Rural Development in the Tropics and Subtropics. 113(1), 77-85.
[27] Şen, Z. (2014) Practical and Applied Hydrogeology. Elsevier, New York, 1-406.
[28] Abtew, W. and Melesse, A. (2013) Evaporation and Evapotranspiration. Measurements and Estimations. Springer. 1-206.

[29] Willmott, C.J. (1982) Some Comments on the Evaluation of Model Performance. Bull. Am. Meteorol. Soc. 63, 1309-1313.
[30] Kashyap, P.S. and Panda R.K. (2001) Evaluation of Evapotranspiration Estimation Methods and Development of Crop-Coefficients for Potato Crop in a Sub-Humid Region. Agricultural Water Management. 50(1), 9-25.
[31] Aydinşakir, K., Baştuğ, R. and Büyüktaş, D. (2003) Antalya Yöresinde Çim Kıyas Bitki Su Tüketimini Veren Bazı Ampirik Eşitliklerin Tarla Ve Lizimetre Koşullarında Kalibrasyonu. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi. 16(1), 107-119.
[32] Emekli, Y. and Baştuğ, R. (2007) Antalya'da Tarla Koşullarında Bermuda Çiminin Su Tüketimi ve Bazı Kıyas Bitki Su Tüketimi Eşitliklerinin Geçerliliğinin Belirlenmesi. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi. 20(1), 45-57.
[33] Lopez-Moreno, J.I., Hess, T.M. and White, S.M. (2009) Estimation of Reference Evapotranspiration in a Mountainous Mediterranean Site Using the Penman Monteith Equation with Limited Meteorological Data. Pirineos. 164, 7-31.
[34] Martinez-Cob A. and Tejero-Juste, M. (2004) A Wind-Based Qualitative Calibration of the Hargreaves ET_o Estimation Equation in Semiarid Regions, Agricultural Water Management. 64(3), 251-264.
[35] Temesgen, B., Eching, S., Davidoff, B. and Frame, K. (2005) Comparison of Some Reference Evapotranspiration Equations for California. Journal of Irrigation and Drainage Engineering, ASCE. 131(1), 73-84.
[36] Irmak, S. and Haman, D.Z. (2003) Evaluation of Five Methods for Estimating Class A Pan Evaporation in a Humid Climate. HortTechnology. 13(39), 500-508.

Received: **11.11.2017**
Accepted: **22.08.2018**

CORRESPONDING AUTHOR

Dursun Buyuktas
Department of Agricultural Structures and Irrigation,
Faculty of Agriculture,
Akdeniz University,
Antalya – Turkey

e-mail: dbuyuktas@akdeniz.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9509-9519 Fresenius Environmental Bulletin

TEMPORAL AND SPATIAL VARIABILITY OF HYDRAULIC CONDUCTIVITY OF A STREAMBED IN A TYPICAL CONTINENTAL RIVER, CHINA

Guangpeng Zhang[1,2], Hailiang Xu[1], Hongbo Ling[1,*], Xiaoya Deng[3]

[1]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences (CAS), Urumqi 830011, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]State Key Laboratory of Simulation and Regulation of Water Cycle in River Basin, Institute of Water Resources and Hydropower Research, Engineering and Technology Research Center for Water Resources and Hydro-ecology of the Ministry of Water Resources, Beijing, 100038, China

ABSTRACT

The hydraulic conductivity (*K*) of streambed sediment is a basic parameter in studying the exchange of water between surface sources and ground sources, the transport of solutes, etc. Inadequate understanding of *K* upper stream of the Tarim River makes it difficult to allocate water optimally. Particle sizes of streambed sediment upstream and midstream of the Tarim River were analysed and, coupled with data on water temperature and run-off, the spatial and temporal variation in *K* was studied. The results of the study were as follows. (1) In 132 sediment samples, the particle size was less than 5 mm; the sediment was predominantly sandy; and the Sauerbrei's formula proved to be the most reliable empirical formula. (2) At any given water temperature, *K* increased along the course of the river at locations upstream whereas *K* at midstream locations showed no obvious regularity. Changes in *K* with depth showed no consistent pattern. (3) At 20 °C, the mean value of *K* across all the locations was 44.29 m/d, that in the upper reaches was 64.53 m/d, and that in the middle reaches was 37.82 m/d; the difference was largely due to the differences in the process of sediment deposition.

KEYWORDS:
Streambed sediment, hydraulic conductivity, variability, particle-size analysis, Tarim River

INTRODUCTION

The hydraulic conductivity (*K*) of streambed sediment influences the movement of water in that porous medium, a crucial parameter affecting many hydrogeological processes such as the exchange of water between surface sources and ground sources and the transport of solutes [1-4]. In an arid inland river basin, groundwater plays a vital role in maintaining the stability of oases and of agricultural production [5, 6]. In arid areas, by definition, precipitation is scanty and evaporation is high, and leakage from river run-off becomes the main source of the recharge of groundwater [7-9]. Hydraulic conductivity is the basis of research on the exchange of water between surface sources and ground sources and the research on it is extremely important in arid inland river basins.

In recent years, many scholars have used different methods to study the hydraulic conductivity of streambeds and obtained significantly different results [10-15], although it is generally accepted that the hydraulic conductivity of streambed sediment shows a clear temporal and spatial variation [10, 13, 14]. The most common methods of the determination of hydraulic conductivity can be divided into two main types: in situ measurement including pumping method [16, 17], the slug and bail test [10, 12] and in situ standpipe [11, 18], and laboratory determination including grain-size analysis and permeameter test [19-21]. Compared to other methods, grain-size analysis is not restricted by field conditions, and the determination process is relatively concise. Based on the classification of the soil sediments by size, the hydraulic conductivity can be determined more reliably by using appropriate calculations, and research on related aspects shows that temporal variation in hydraulic conductivity can be determined more accurately by particle size than by any other method [19].

The Tarim River flows through an arid region in north-western China. The area, characterized by a dry climate, scanty rainfall, and water supply through glacial melt, is a typical arid area. Since the 1950s, the increase in population has resulted in a rapid increase in the area under cultivation, and the expansion of water resources increased and use of water have adversely affected the run-off in the lower reaches. The result was a series of serious environmental problems such as the drying up of rivers and lakes, the degradation of the green cover, and desertification [22]. In order to restore the ecological destruction and to balance the supply of water with

the demand for it in the Tarim River basin, Chinese government has invested 10.7 billion yuan since 2001 in the Ecological Water Conveyance Project. The project transferred 350 million m^3 of water from the Kongque River to downstream locations of the Tarim River and restored the flow of water into the Taitema Lake. The ecology of areas downstream of the Tarim River has improved significantly [23, 24]. Over the years, scholars have studied hydraulic conductivity, water exchange, etc. downstream of the Tarim River, and much has been achieved including important insights into scientific and rational conveyance of water and measures to optimize the effects of such conveyance [24, 25]. However, hydrological processes upstream and midstream of the Tarim River have not received as much attention; in particular, the hydraulic conductivity has been largely ignored. Such lack of related research has made it difficult to manage the water resources equitably. Allocation of water resources appears to be particularly imbalanced in the dry season. For instance, in 2008, because run-off decreased in the area of origin and water resources were not allocated equitably in upstream and midstream stretches of the Tarim River, no water could flow into the downstream stretches. This break in the flow affected ecological restoration downstream. In order to ensure that water conveyance is sustainable and the ecological damage downstream is restored, the present paper selected some stretches upstream and midstream of the Tarim River as the study area, determined the hydraulic conductivity of the streambed sediment by analysing the particle size of the sediment, and examined the temporal and spatial variability of the hydraulic conductivity. The study can provide a more scientific basis for further research on hydrological processes in the upstream and midstream stretches of the Tarim River.

STUDY AREA

The Tarim River basin (34.20°–43.39° N, 71.39°–93.45° E) lies in south Xinjiang, China, and abuts the Takliamkan desert, which is surrounded by nine great rivers, namely Aksu, Yarkand, Kashgar, Hotan, Keriya, Weigan–Kuqa, Dina, Kaidu–Kongque, and Cheerchen rivers (Fig.1). The long-term average annual run-off is 39.8 billion m^3, and total water resources amount to 42.9 billion m^3. The total area of the basin is 1.02 million km^2 and covers five prefectures, 42 counties, and 55 regiments of the Construction and Production Corps of Xinjiang, with a population of 4.81 million.

The Tarim River is the longest inland river in China, with a total length of 1321 km [23, 25]. The upper reach (447 km) extends from Alar to Yingbazha and the middle reaches (398 km) from Yingbazha to Qiala (Fig.1). Over this area, the mean annual precipitation is only 20 mm whereas the mean annual evaporation is 2500–3000 mm, because the area, which forms the hinterland of Eurasia, is far away from the sea. The climate is typically continental arid desert. The Tarim river, a dissipative type of continental river, produces no run-off of its own but relies mainly on its tributaries. Since the 19th century, the Kashgar, Weigan, Keriya-Kuqa, Dina, Kaidu–Kongque, and Cheerchen rivers have been cut off from the Tarim River because of climate change and human activities. Only the Aksu, Hotan, and Yarkand rivers remain the tributaries of the Tarim River, contributing 73.2%, 23.2%, and 3.6% of its total resources, respectively, as monitored at the Alar section. The natural vegetation along the Tarim River is a desert riparian forest, consisting mainly of *Populus euphratica* and *Tamarix* trees and of *Phragmites australis*, *Alhagi sparsifolia*, and *Karelinia caspica* as the main herbs.

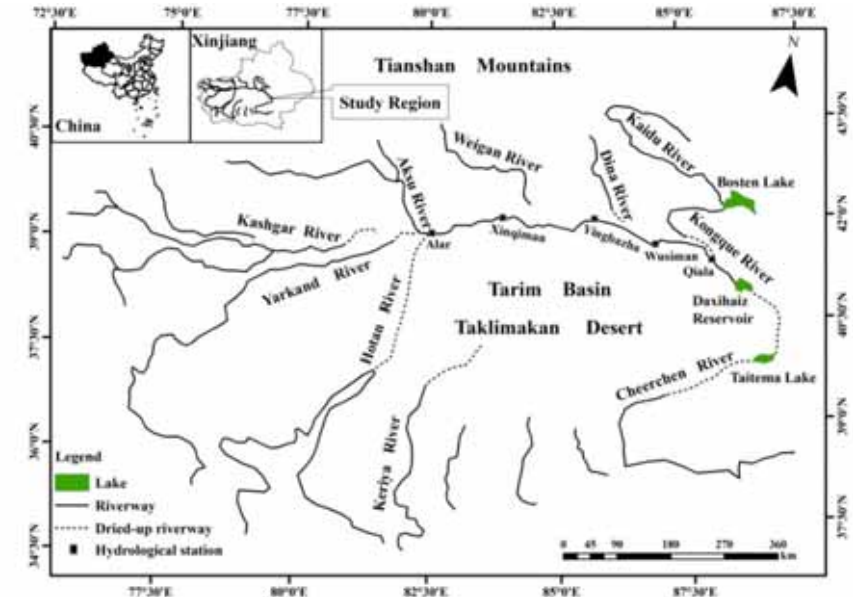


FIGURE 1
The study area in the Tarim River Basin

METHODS

Data. The present paper draws on the data from the analysis of 33 sampling sites (8 upstream and 25 midstream; 132 soil samples). The water temperature is the monthly average temperature from the hydrological station at Xinqiman for the upstream locations and that at Wusiman for the midstream locations from 1957 to 2016, provided by the Xinjiang Tarim River Basin Management Bureau. Run-off was measured by the hydrological stations in Alar and Yingbazha covering the same period.

Sediment sampling and particle-size analysis. The sampling work was in May 2015 when was dry season. It's difficult to enter the river channel of upstream because of thick vegetation, so only 8 sampling sites were set. In the midstream, the width of vegetation is narrow, and the vehicle can travel along the river embankment in parallel. Accordingly, sampling was relatively simple so that 25 sampling sites were set. The sampling sites are uniformly distributed along the river with the information of vehicle odometer and GPS. Song et al. [11] studied hydraulic conductivity at depths of 50–70 cm and 90–100 cm from the streambed surface along the Elkhorn River, Nebraska, USA. However, to study the effect of depth more carefully, we measured the hydraulic conductivity at four depths, namely 30 cm, 60 cm, 100 cm, and 150 cm, using a soil auger (catalogue no. 04.02.SC, Eijkelkamp, Giesbeek, The Netherlands) to collect the sample. Each sample weighed about 50 g so that enough quantity was available for the follow-up experiment.

Impurities, salts, and organic matter were removed from the samples [26] and a dispersant, namely Sodium Hexametaphosphate ($(NaPO_3)_6$), was added to the processed samples. The mixtures were cleaned for 10 minutes using an ultrasonic cleaner (SB-4200DTDS, Shanghai Jingxin, Shanghai, China). Particle sizes were analysed using a particle-size analyser (Mastersizer 2000, Malvern Instruments, Malvern, UK).

Classification of sediment. The standards for soil classification differ with the subject discipline and region; the classification used in the present study was the one based on DL/T5355-2006 [27] and consisted of determining the content of each grade (gravel, sand, silt, etc.) of particles in each sample, determining the size gradation characteristics of each sample, and classifying the samples with reference to the standard (Table 1).

Determination of *K* from particle-size analysis. Vukovic and Soro [19] summarized the following general formula for calculating *K*:

$$K = (g/v) \cdot C \cdot \varphi(n) \cdot d_e^2 \quad (1)$$

where *K* is the hydraulic conductivity; *g* is the acceleration due to gravity (9.81 m/s^2 in the present study); *v* is the water viscosity coefficient (m^2/s); *C* is a dimensionless coefficient; *n* is the porosity, $\varphi(n)$ is the dimensionless porosity (*n*) function; and d_e is the effective particle diameter at a cumulative weight percentage *e* of the smaller particle size (Table 2).

The value of the water viscosity coefficient depends on the water temperature (*t*, unit: °C), which is calculated by the following equation [28]:

$$v = \frac{0.01775}{1 + 0.0337t + 0.000221t^2} \quad (2)$$

TABLE 1
Soil classification standard based on DL/T5355-2006

		Content of coarse grain group (0.075mm＜*d*≤60mm)	Content of fine grain group (0.075mm≤*d*)	Name of soil type
Coarse-grained soil	＞50%	Gravel (The content of gravel (2mm＜*d*≤60mm) is greater than the content of sand (0.075mm＜*d*≤2mm))	≤5%	
			＞5%，≤15%	gravel containing fine-grained (GF)
			Clay(*d*≤0.005mm) ＞5%，＜50%	Clayey gravel (GC)
			Silt (0.005mm＜*d*≤0.075mm) ＞5%，＜50%	Silty gravel (GM)
		Sand (The content of sand (0.075mm＜*d*≤2mm) is greater than the content of gravel (2mm＜d≤60mm))	≤5%	
			＞5%，≤15%	Sand containing fine-grained (SF)
			Clay (d≤0.005mm) ＞5%，＜50%	Clayey sand (SC)
			Silt (0.005mm＜d≤0.075mm) ＞5%，＜50%	Silty sand (SM)
Fine-grained soil			≥50%	

TABLE 2
Formula selections of coefficients C and $\varphi(n)$ in the process of calculating hydraulic conductivity

Method	Function of porosity ($\varphi(n)$)	Effective grain diameter (d_e)	Value of coefficient (C)	Domain of applicability
Hazen	1+10(n-0.26)	d_5	6×10^{-4}	0.1mm< d_e <3mm, η<5
Slichter	$n^{3.278}$	d_{10}	6.1×10^{-3}	0.01mm< d_e <5mm
Terzaghi	$\left(\frac{n-0.13}{\sqrt[3]{1-n}}\right)^2$	d_{10}	$6\times10^{-4}\log(500/\eta)$	Large-grain sands
Beyer	1	d_{10}	3.75×10^{-3}	0.06mm< d_e <0.6mm; 1<η<20
Sauerbrei	$n^3/(1-n^2)$	d_{17}	6×10^{-4}	Sand and sandy clay, d_e <0.5mm
Kozeny	$n^3/(1-n^2)$	d_{10}	8.3×10^{-4}	Large-grain sands
USBR	1	d_{20}	$4.8\times10^{-4}\, d_{20}^{0.3}$	Medium-grain sands, η<5

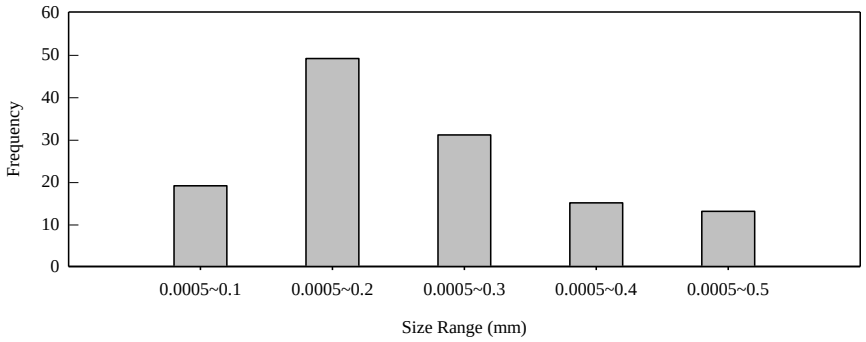


FIGURE 2
Distribution of samples' size range

The dimensionless coefficient and the dimensionless porosity function should be determined by the soil texture (Table 1). The formula for calculating the porosity is as follows:

$$n = 0.255 \cdot (1 + 0.83^{\eta}) \quad (3)$$

$$\eta = d_{60} / d_{10} \quad (4)$$

where, η is the coefficient of particle uniformity, d_{60} and d_{10} are the effective particle diameters at 60% and 10% of the cumulative weight of the smaller particles.

RESULTS

Size range. The maximum particle size of the 132 sediment samples was less than 5 mm (Fig. 2); in 49 samples (approximately 37% of the samples), it ranged from 0.0005 mm to 0.2 mm and in 13 (10%), from 0.0005 mm to 0.5 mm. The samples thus consisted mainly of smaller particles fairly uniform in size.

Characteristic particle size. The characteristic particle size reflects the structure of the sediment and is also the basis for classifying the sediments and calculating hydraulic conductivity. In the present study, the following sizes were analysed: effective size (d_{10}), median size (d_{50}), constrained grain size (d_{60}), and d_{17}.The changes in the mean particle size of the samples from each site along the river were complex (Fig. 3 and Table 3): the size increased along the course of the river in its upper reaches but showed no regular pattern of change in the middle reaches. Statistical results of 132 samples show that the mean of characteristic particle size of upstream and midstream is approximately equal. The size range showed clear differences. As the cumulative volume increased, so did the standard deviation, which shows a greater dispersion.

Type of sediments. Based on the soil classification standard (Table 1), the contents of gravel, sand, clay, and silt in each sample were analysed. Gravel was entirely missing from all the samples, and fewer than 15% of the samples showed the presence of fine particles: 73 samples showed clayey sand, 47 showed silty sand, and 12 showed fine particles.

Spatial and temporal variation in streambed hydraulic conductivity. The particles of streambed sediments were smaller than 0.5 mm and the soil was predominantly sandy. Therefore, the coefficients C and φ(n) of Sauerbrei were selected for calculating the streambed hydraulic conductivity. Further, the monthly K of each sample site as it varied with the water temperature was also calculated.

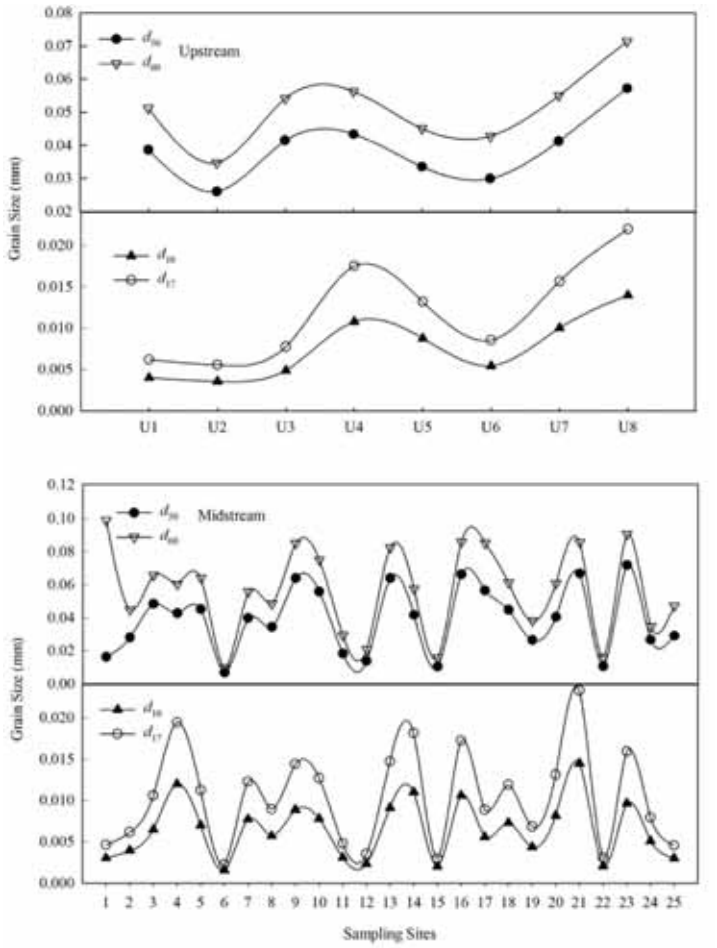


FIGURE 3
Variation curves of characteristic diameter value along the river

TABLE 3
Statistical characteristics values of sediment's particle grain size (Unit: mm)

		Mean	Minimum	Maximum	Range	Standard deviation (σ)
d_{10}	Upstream	0.007454	0.001489	0.024070	0.022581	0.000038
	Midstream	0.006346	0.001442	0.036243	0.034801	0.000036
	All	0.006681	0.001442	0.036243	0.034801	0.000037
d_{17}	Upstream	0.011672	0.002135	0.039112	0.036977	0.000093
	Midstream	0.010159	0.002042	0.060899	0.058857	0.000101
	All	0.010628	0.002042	0.060899	0.058857	0.000099
d_{50}	Upstream	0.037738	0.007172	0.080105	0.072933	0.000555
	Midstream	0.037948	0.005843	0.117693	0.111850	0.000854
	All	0.038185	0.005843	0.117693	0.111850	0.000773
d_{60}	Upstream	0.049756	0.010510	0.100340	0.089830	0.000783
	Midstream	0.055864	0.008116	0.328098	0.319982	0.002024
	All	0.054717	0.008116	0.328098	0.319982	0.001708

Temporal variation in hydraulic conductivity. The conductivity of every layer of the sample varied even within a given year (Fig. 4). The mean of hydraulic conductivity at different depths of each sampling site is used to analysis the temporal variation in hydraulic conductivity. The changes in *K* and water temperature during a year were parallel and unimodal. The water temperature peaked in July, and so did *K*; the water temperature was the lowest in January, and so was *K*, although it varied across the sampling sites at any given time. At site U4, *K* was 175.8 m/d in July, which was the maximum value upstream; in January, the value was 90.6 m/d. At U1, *K* was 6.2 m/d in July; in January it was only 3.2 m/d—the lowest value upstream. At M4, *K* was relatively high among all the midstream sites: 225.8 m/d in July and 116.4 m/d in January. On the other hand, at M6 *K* was 1.2 m/d in July and only 0.64 m/d in January—the lowest value midstream.

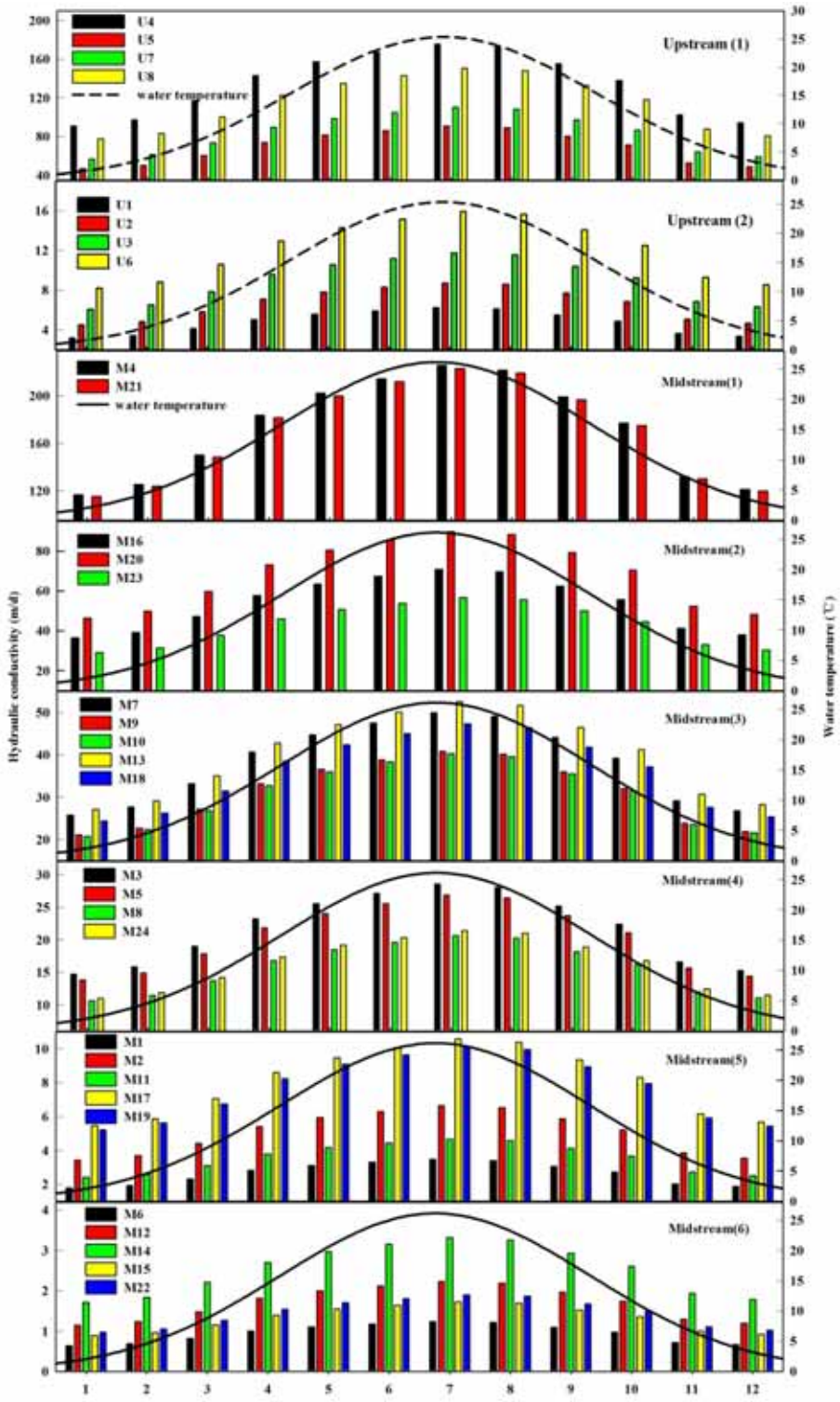


FIGURE 4
The changes of K of all sampling sites and water temperature during the year

Variation in hydraulic conductivity with depth. Water temperature influences *K* greatly (Fig. 5). For analysing the variation in *K* with depth, *K* at 20 ℃ is used as a standard. Upstream, the *K* of each layer did not differ much across some sites: U1 (σ = 2.75), U2 (σ =13.32), U3 (σ = 11.96), and U6 (σ = 10.84) but differed substantially at some other sites, with the standard deviation (σ) more than 90. In particular, *K* at U4 (σ = 249.54) at the depth of 100 cm was up to 526.7 m/d; at 30 cm, it was only 2.8 m/d. Midstream, the *K* of each layer was different: M6 (σ = 0.11), M12 (σ = 0.56), M15 (σ = 0.27), and M22 (σ = 0.78). The differences with depth were most significant at M4 (σ = 239.74): up to 469.3 m/d at the

depth of 30 cm but only 1.0 m/d at 100 cm. We observed the mean *K* at 30 cm and 60 cm to be the *K* at shallower depths and that at 100 cm and 150 cm to be the *K* at greater depths. At upstream sites U1, U2, U5, U7, and U8, the values of *K* at shallower depths were higher than those at greater depths. The same pattern was seen at midstream sites M2, M3, M4, M6, M8, M10, M12, M18, M19, and M23. However, at the remaining sites, K at shallower depths was less than that at the greater depths. Between the upstream sites and the midstream sites, changes in *K* with depth showed no consistent pattern.

Variation in conductivity along the river. The Tarim River is a seasonal river: from May to October, the run-off is more. During this period, the water temperature is more than 15 °C. It is important to study *K* during this period, and the mean values of *K* at 30 cm, 60 cm, 100 cm, and 150 cm at water temperatures of 15 °C, 20 °C, and 25 °C were used in analysing the variation in *K* along the river (Fig. 6). At any given water temperature, *K* upstream increased along the course of the river, being the minimum at U1 and the maximum at U4; the corresponding values were as follows: at U1, K was 5.0

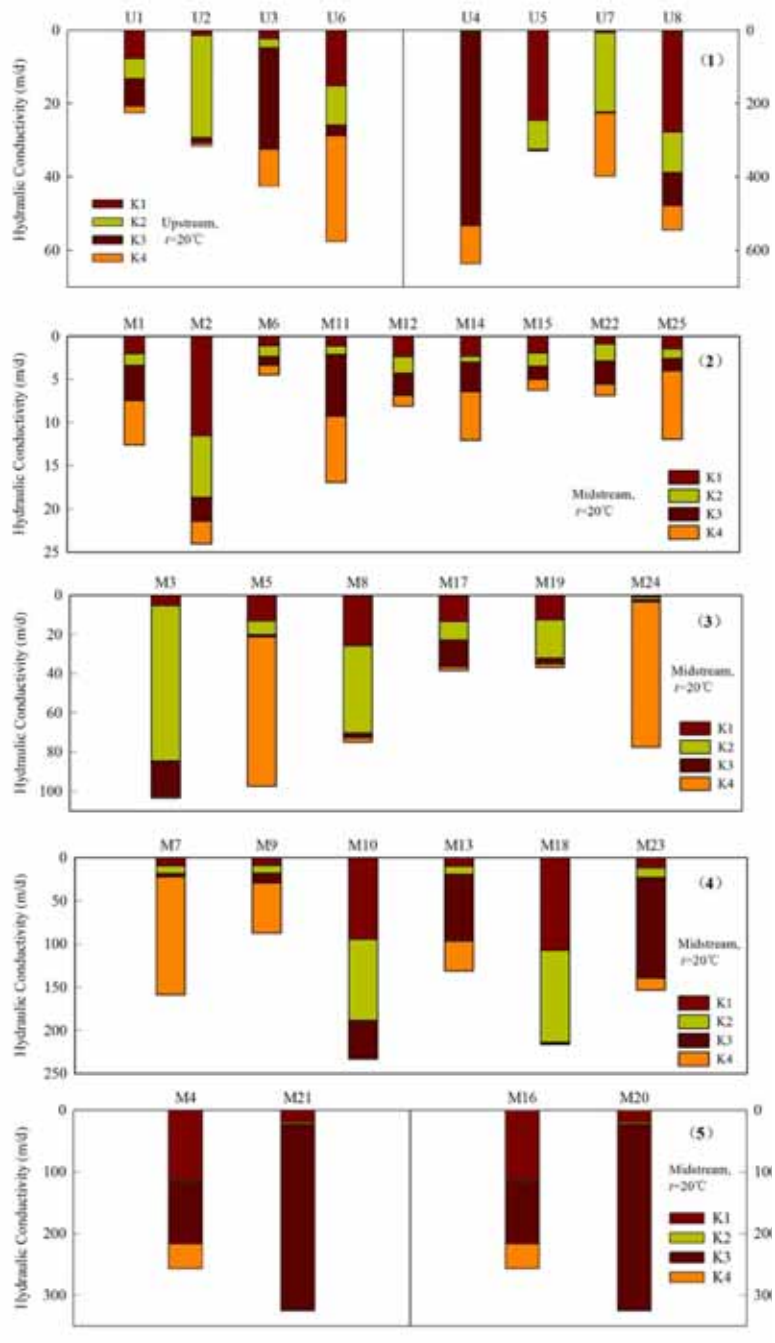


FIGURE 5
Variation of *K* of each sample site with depth, at 20°C

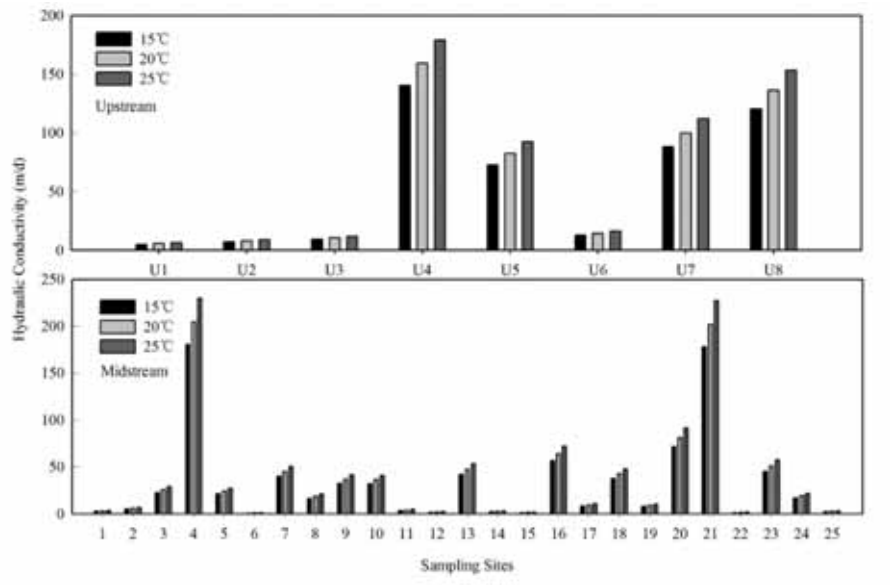


FIGURE 6
Variation of *K* along the river

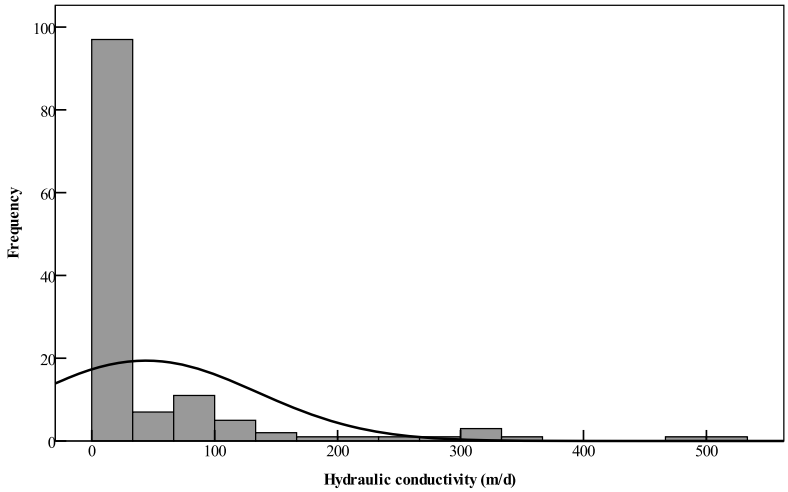


FIGURE 7
Histogram of *K* and logarithmic *K* of 132 samples from all sites, at 20 ℃

TABLE 4
Statistical characteristics of the *K* of 132 samples at 20 ℃, (Unit: m/d)

	Means	Range	Standard deviation（σ）
Upstream	64.53	0.74~525.99	114.17
Midstream	37.82	0.67~468.67	80.78
All	44.29	0.67~526.06	90.27

m/d at 15 °C, 5.6 m/d at 20 °C, and 6.3 m/d at 25 °C; at U4, it was 140.5 m/d at 15 °C, 159.2 m/d at 20 °C, and 178.9 m/d at 25 °C. Midstream, the changes in *K* followed a more complex pattern along the river: maximum at M4, minimum at M21, but showing neither consistent increase nor consistent decrease at intermediate locations. The high and low values were as follows: 180.4 m/d at 15 °C, 204.5 m/d at 20 °C, and 229.8 m/d at 25 °C (M4) and 1.0 m/d at 15 °C, 1.1 m/d at 20 °C, and 1.2 m/d at 25 °C (M21).

DISCUSSION

As can be seen from the above analysis, at any given water temperature, changes in *K* showed no obvious and clear-cut pattern, irrespective of the depth and the location (whether closer or father along the river). When the value of *K* in all the 132 samples at 20 °C was analysed statistically (Table 4, Fig. 7), it became apparent that *K* upstream was greater than that in the middle reaches. Although the

range of *K* showed no obvious difference, whether upstream or midstream, its dispersion upstream was greater than that midstream. The mean of all the 132 samples was 44.29 m/d at 20 °C and that of most samples fell within 0–100 m/d (Fig. 7). In particular, the number of samples with *K* from 0 m/d to 33.33 m/d was as high as 97 (74.5% of all samples).

The complexity of the spatial variation of *K* was related to the complexity of the particle size distribution of the streambed sediment. Upstream, the variation in particle size along the course of the river was parallel with the variation in *K*. Midstream, the complex pattern of changes in the permeability coefficient along the course of the river was due to the disorderly distribution of the particle size. The process of deposition of the streambed sediment is influenced by the run-off velocity, the sediment-carrying capacity of the run-off, the geotechnical characteristics of the river and of the run-off flow, etc [29]. In the Tarim River, the inflow upstream is heavier than that midstream [30], the run-off velocity is higher, and the channel is straighter [29-32]: therefore, the run-off carries larger quantities of sediment. The main sediment deposition area is upstream of the Tarim River [30]. The process of sediment deposition is fairly simple. Reaching the midstream, as the strength of the current begins to decrease, the run-off also decreases and so does its sediment-carrying capacity. The quantity of sediment carried by the run-off therefore falls off and the process of sediment deposition becomes slower. The course of the river midstream is not straight, punctuated by many levees. The many tributaries also influence run-off, and the process of sediment deposition becomes more complex [30].

Hydraulic conductivity is influenced by water temperature, which is higher in July, August, and September, and therefore *K* is also higher. Meanwhile, run-off in the three months is most abundant upstream and midstream (Fig. 8). Because of abundant water and greater hydraulic conductivity, water leakage increases significantly during this period. The leakage from run-off is the main source of groundwater in the arid area and is important for the stability and regeneration of the desert riparian forest. Studying the recharge of groundwater from run-off under different water conditions can provide useful insights for ensuring optimal allocation of water resources of the Tarim River. The study of temporal and spatial variability of the hydraulic conductivity of the streambed sediment makes the study of the process and the results of such recharge of groundwater by run-off more reliable.

CONCLUSIONS

The present study analysed the characteristics of particle size of the sediment in the Tarim River. Based on that analysis and on the temporal and spatial variability of the hydraulic conductivity of the streambed sediment, the following main conclusions are drawn.

(1) The maximum particle size of streambed sediment in the upper reaches of the Tarim River is less than 0.5 mm. The soils are predominantly sandy. The Sauerbrei's formula proved to be the most reliable empirical formula for calculating the conductivity.

(2) As water temperature increases, so does the hydraulic conductivity in the upper reaches of the Tarim River. During the year, the hydraulic conductivity shows a clear single peak, reaching its maximum value in July; the lowest value is reached in January. At the time, the conductivity varied greatly across the sampling sites.

(3) At any given water temperature, *K* upstream increased along the course of the river, whereas the middle reaches of the river showed no obvious regular pattern. Both upstream and midstream, the patterns of variability of *K* with depth were complex and displayed no regularity.

(4) At 20 °C, the mean value of *K* of all the samples was 44.29 m/d. The mean value upstream was 64.53 m/d and that midstream was 37.82 m/d. The discrete degree of *K* was significant, the standard deviation (σ) of which was 90.27 m/d. The values of *K* in the upper middle reaches of the river were due mainly to the differences in the process of deposition of the sediment.

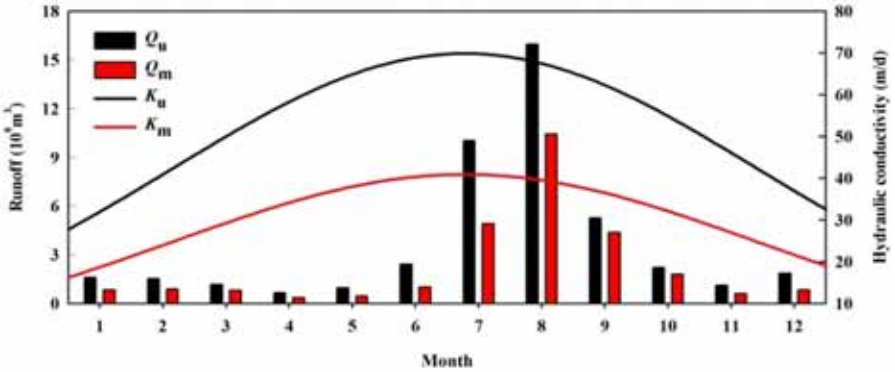


FIGURE 8
Annual variation of runoff and *K* of streambed sediment

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (41471099, 41561103 and 41761014), State Key Laboratory Project of Desert and Youth Innovation Promotion Association Project (CAS) (2014389).

REFERENCES

[1] Jury, W.A. (1982) Simulation of solute transport using a transfer function model. Water Resour. Res. 18(2), 363–368.

[2] Xie, X.L., Feng, H., Dong, X., and Wu, Z.B. (2010) Hydrodynamic aspects of particle clogging in the simulated vertical flow constructed wetland using river sands as substrate. Fresen. Environ. Bull. 19, 2567-2575.

[3] Chen, X.H., Burbach, M. and Cheng, C. (2008) Electrical and hydraulic vertical variability in channel sediments and its effects on streamflow depletion due to groundwater extraction. J. Hydrol. 352, 250–266.

[4] Feng, G.X., Zhang, Z.Y., Kong, L.L. and Bakhtawar, W. (2017) Modeling and parameters sensitivity analysis of nitrogen loss through runoff from upland in the Yangtze River downstream. Fresen. Environ. Bull. 26, 2025-2032.

[5] Sibanda, T., Nonner, J.C. and Uhlenbrook, S. (2009) Comparison of groundwater recharge estimation methods for the semi-arid Nyamandhlovu area, Zimbabwe. Hydrogeol. J. 17, 1427-1441.

[6] Huo, Z.L., Feng, S.Y., Kang, S.Z., Mao, X.M. and Wang, F.X. (2011) Numerically modelling groundwater in an arid area with ANN-generated dynamic boundary conditions. Hydrol. Process. 25, 705–713.

[7] Walter, M.T., Kim, J.S., Steenhuis, T.S., Parlange, J.Y., Heilig, A., Braddock, R.D., Selker, J.S. and Boll, J. (2000) Funneled flow mechanisms in a sloping layered soil: Laboratory investigation. Water Resour. Res. 36, 841-849.

[8] Hogan, J.F., Phillips, F.M., and Scanlon, B.R. (2004) Evaluation of Methods of Estimating Recharge in Semiarid and Arid Regions in the Southwestern US. Groundwater Recharge in a Desert Environment: The Southwestern United States. American Geophysical Union. 235-354.

[9] Yao, Y., Zheng, C., Liu, J., Cao, G.L., Xiao, H.L., Li, H.T., and Li, W.P. (2015) Conceptual and numerical models for groundwater flow in an arid inland river basin. Hydrol. Process. 29, 1480–1492.

[10] Springer, A.E., Petroutson, W.D. and Semmens, B.A. (1999) Spatial and Temporal Variability of Hydraulic Conductivity in Active Reattachment Bars of the Colorado River, Grand Canyon. Groundwater. 37, 338–344.

[11] Song, J.X., Chen, X.H., Cheng, C., Wang, D.M., Lackeya, S. and Xu, Z.X. (2009) Feasibility of grain-size analysis methods for determination of vertical hydraulic conductivity of streambeds. J. Hydrol. 375, 428–437.

[12] Landon, M.K., Rus, D.L. and Harvey, F.E. (2001) Comparison of Instream Methods for Measuring Hydraulic Conductivity in Sandy Streambeds. Groundwater. 39, 870–885.

[13] Hatch, C.E., Fisher, A.T., Ruehl, C.R. and Stemler, G. (2010) Spatial and temporal variations in streambed hydraulic conductivity quantified with time-series thermal methods. J. Hydrol. 389, 276–288.

[14] Genereux, D.P., Leahy, S., Mitasova, H., Kennedy, C.D. and Corbett, D.R. (2008) Spatial and temporal variability of streambed hydraulic conductivity in West Bear Creek, North Carolina, USA. J. Hydrol. 358, 332–353.

[15] Chen, X.H. (2007) Streambed hydraulic conductivity for rivers in south-central Nebraska. Journal of the American Water Resour. Res. 40, 561–573.

[16] Hvorslev, M.J. (1951) Time lag and soil permeability in ground-water observations. U.S. Army Corps of Engineers Waterway Experimental Station Bulletin. 36, 1-50.

[17] Kennedy, C.D., Genereux, D.P., Mitasova, H., Corbett, D.R., and Leahy, S. (2008) Effect of sampling density and design on estimation of streambed attributes. J. Hydrol. 355, 164–180.

[18] Chen, X.H. (2000) Measurement of streambed hydraulic conductivity and its anisotropy. Environ. Geol. 39, 1317-1324.

[19] Vukovic, M. and Soro, A. (1992) Determination of hydraulic conductivity of porous media from grain-size composition. Water Resources Publications, Littleton, Colorado.

[20] Alayamani, M.S., and Sen, Z. (1993) Determination of Hydraulic Conductivity from Complete Grain-Size Distribution Curves. Groundwater. 31, 551–555.

[21] Kelly, S.E. and Murdoch, L. (2003) Measuring the Hydraulic Conductivity of Shallow Submerged Sediments. Groundwater. 41, 431–439.

[22] Chen, Y.N., Li, W.H., Xu, C.C., Ye, Z.X. and Chen, Y.P. (2015) Desert riparian vegetation and groundwater in the lower reaches of the Tarim River basin. Environ. Earth Sci. 73, 547-558.

[23] Xu, H., Ye, M., and Li, J. (2008) The water transfer effects on agricultural development in the lower Tarim River, Xinjiang of China. Agric. Water Manage. 95(1), 59-68.

[24] Hao, X.M., Li, W.H., Huang, X., Zhu, C.G. and Ma, J.X. (2010) Assessment of the groundwater threshold of desert riparian forest vegetation along the middle and lower reaches of the Tarim River, China. Hydrol. Process. 24, 178–186.

[25] Chen, Y.N., Takeuchi, K., Xu, C.C., Chen, Y.P., and Xu, Z.X. (2006) Regional climate change and its effects on river runoff in the Tarim Basin, China. Hydrol. Process. 20, 2207–2216.
[26] Okatan, A., Aydin, M., Usta, A., and Yilmaz, M. (2010) Effects of land use type on hydro-physical properties of soils in the Torul Dam Basin-Gumushane, Turkey. Fresen. Environ. Bull. 19, 3230-3241.
[27] DL/T5355-2006. (2007) Code for Soil Tests for Hydropower and Water Conservancy Engineering. China Electric Power Press, Beijing, 4-9.
[28] Huang, R.Q. (2007) Hydraulics. Southwest Jiaotong University Press. Chengdu, 5-6.
[29] Qian, N. and Wan, Z.H. (1983) Sediment transport mechanics. Science Press. Beijing, 57-68.
[30] Wang, J. Gong, W.H., Shen, Y.P., Gao, Q.Z., and Wang, S.D. (2009) Effects of Riverbed Deposition and Water Consumption on Ecological Environment in Middle and Lower Sections of Upper Reaches of Mainstream of Tarim River. Journal of Glaciology and Geocryology. 31, 1086-1093. (In Chinese).
[31] Wang, Y.G., Hu, C.H., Zhou, W.H. and Zhu, B.S. (2003) Evolution characteristics of Tarim River. Journal of Hydraulic Engineering. 34, 27-33. (In Chinese).
[32] Hu, C.H. and Wang, Y.G. (2006) Study on comprehensive regulation measures of the Main Stem Tarim River—Fluvial Processes. Journal of Sediment Research. 4, 30-38. (In Chinese).

Received: **14.11.2017**
Accepted: **04.11.2018**

CORRESPONDING AUTHOR

Hongbo Ling
Xinjiang Institute of Ecology and Geography,
Chinese Academy of Sciences
Urumqi, 830011 – China

e-mail: linghongbo0622@163.com

© by PSP Volume 27 – No. 12B/2018 pages 9520-9528 Fresenius Environmental Bulletin

HEAVY METALS IN THE RHIZOSPHERE OF ABSINTHIUM (*ARTEMISIA ABSINTHIUM* L.) IN CONDITIONS OF TECHNOGENESIS

Elena N Polivkina[1], Elena N Artamonova[1], Zhanar S Kassymova[2], Elena P Evlampiyeva[1], Kulbanu K Kabdulkarimova[2], Meruyert Kaygusuz[3,*], Nuriya M Omarova[4]

[1]Department of Ecology and Environment, Faculty of Engineering and Technology, Shakarim State University of Semey, 071412 Semey, Kazakhstan
[2]Chemistry Department, Faculty of Engineering and Technology, Shakarim State University of Semey, 071412 Semey, Kazakhstan
[3]Denizli Vocational School of Technical Sciences, Pamukkale University, 20013 Denizli, Turkey
[4]Chemistry Department, Faculty of Natural Sciences, Eurasian National University named after L.N. Gumilyov, Astana, Kazakhstan

ABSTRACT

The soil covering the zone of the industrial complex of Ust-Kamenogorsk city in Kazakhstan is characterized by very high concentrations of copper and zinc of technogenic origin. Research on the distribution of mobile forms of copper and zinc in the rhizosphere of plants under such conditions is of great importance for understanding the mechanisms of plant defense reactions. Maximum levels of total content and concentrations of the mobile forms of heavy metals (HM) in the rhizosphere of absinthium (*Artemisia absinthium* L.) were investigated in the area of the Ulba Metallurgical Plant and Lead-Zinc Plant. Accumulation of exchange and acid-soluble forms of zinc and copper was not statistically significant in the rhizosphere of absinthium (*A. absinthium*) The strength of the correlations between the content of heavy metals in the plant biomass and the concentration of mobile compounds depended on the nature and form of occurrence of HM, and the type and extent of soil contamination.

KEYWORDS:
Heavy metals, *Artemisia absinthium*, rhizosphere, root, soil pH

INTRODUCTION

The modern environmental situation is characterized by a rapid growth of heavy metal (HM) concentration in the environment, especially in the soil. The interrelation in the soil-plant system relative to invading HM in the plant body is a complex and controversial problem. Higher plants are able through various morphological and physiological properties to adapt to the increased content of heavy metals in the soil. In the course of evolution, the roots of plants have had repeated contact with an excess of particular elements in the soil, and they have developed protection mechanisms to ensure the preservation of a constant internal environment. The rhizosphere, the soil adjacent to the root, can be thought of as an external safety mechanism that controls the absorption of HM by the plant. Detoxification of HM ions occurs when they are bound to organic products secreted by the roots or by rhizosphere microflora, resulting in less toxic complexes, or in compounds which are not easily accessible.

Plants accomplish a huge transformative function in the environment, since they change the forms of elements. The soil of the rhizosphere is a unique eco niche and a zone of active interaction between the soil, microorganisms and roots, and performs the key role in this process [1-7]. The study of hidden mechanisms of this interaction, especially in conditions of technogenic pollution of soil by HM, is important, since an essential characteristic in predicting the accumulation of heavy metals in plants is the direction of the transformation of elements and compounds entering the soil and the change in the degree of their accessibility to the root system [8].

Also, there are numerous studies regarding the use of plants as bioindicators in environmental investigations due to their ability to accumulate chemical substances [9]. Because of this characteristic, researchers have identified them as a group potentially useful for bioremediation and biomonitoring [10].

The purpose of this research was to explore the total content and concentration of mobile forms of Cu, Zn and pH in the rhizosphere of absinthium (*Artemisia absinthium* L.) in the technogenesis conditions of the example of Ust-Kamenogorsk city. The objectives of the study were: 1. to determine the total content and concentration of mobile forms of HM, and the pH in the rhizosphere of absinthium (*A. absinthium*), in conditions of technogenic pollution in the area of Ust-Kamenogorsk city; 2. to determine the effects of the rhizosphere on HM invasion in plants.

TABLE 1
Total content of copper and zinc in the rhizosphere of *A. absinthium* in Ust-Kamenogorsk city and its environments (mg/kg)

Exploration area	TMS	RS	RSS
Copper			
Titanium-magnesium Plant	266.7±0.03 4.0	266.9±0.06 3.5	267.0±0.05 2.8
Cement Plant	458.1±0.03 2,4	458.0±0.04 2.4	458.4±0.03 1.9
Ulba Metallurgical Plant	489.2±0.03 2.2	490.5±0.02 3.1	490.5±0.03 4.2
Lead-zinc Plant	379.4±0.03 2.8	379.6±0.03 2.1	379.9±0.04 2.5
Irtysh polymetallic Plant	385.9±0.03 2.8	386.3±0.02 2.3	386.4±0.03 2.1
Ablaketka	180.8±0.03 6.4	181.2±0.03 5,2	181.9±0.03 5,0
Processing Plant	280.2±0.05 3.7	280.6±0.04 5.2	181.9±0.03 5.0
Zinc			
Titanium-magnesium Plant	488.0±0.05 2.3	489.2±0.06 1.4	488.0±0.05 1.7
Cement Plant	2671.6±0.06 1.1	2671.8±0.05 1.3	2671.4±0.04 1.5
Ulba Metallurgical Plant	3342.0±0.04 1.2	3342.2±0.03 1.0	3342.3±0.06 1.2
Lead-zinc Plant	3821.4±0.05 1.2	3822.0±0.03 1.2	3821.9±0.05 1.5
Irtysh polymetallic Plant	1983.5±0.03 1.3	1982.5±0.04 1.5	1982.1±0.05 1.5
Ablaketka	420.6±0.04 2.4	421.0±0.05 1.1	420.9±0.05 1.2
Processing Plant	2180.8±0.06 1.2	2181.5±0.07 1.0	2181.2±0.05 1.0

Note: The numerator is the arithmetic average and its error; the denominator is the coefficient of variation (%).

MATERIALS AND METHODS

The study of the total content and composition of the mobile forms of Cu and Zn and pH in the rhizosphere of absinthium (*A. absinthium*) was carried out in the territory of Ust-Kamenogorsk city in conditions of real technogenic pollution. Soil and plant samples were taken at a distance of 1 km from the Titanium-Magnesium Plant, Lead-Zinc Plant, Cement Plant, Ulba Metallurgical Plant, Belo-usovskoe Mining and Processing Mill and Ust-Kamenogorsk Hydroelectric Power Station (Ablaketka).

The prevailing wind direction was taken into account in sampling in the city area. The control was samples selected within 60 km of the city limits in the direction opposite to the direction of prevailing winds. The objects of the study were soil samples from two areas of the rhizosphere of plants: the rhizosphere (RS) and root surface soil (RSS), and samples taken from the total mass of soil (TMS).

In the selection of soil samples, the root system of the plants was dug out of the soil monolithically, and carefully freed from the surrounding soil so that a thin soil layer remained on the roots (1-3 mm). Then, the roots were separated from the shoot and dried. After drying, the soil layer (RS) was gently shaken off, and soil particles adhering to the root surface (RSS) were removed with a brush. TMS free of plants was selected for comparative analysis on the same site [11-13]. Measuring the pH of aqueous suspensions of the soil samples was performed using a pH meter-ion meter (Ecotest-120, Russia). Determination of soil physico-chemical properties was carried out in accordance with conventional methods [14]. For analysis on the total content of Cu and Zn, chemical decomposition of the soil was performed. Mobile forms of the metals were extracted using the most common and conventional extractants, dissolved in double distilled water: exchanged ammonium-acetate buffer at pH 4.8 and acid-soluble 1N HCl. The concentration of metals in the extracts was determined by the photo colorimetric dithizone method [15]. Determination of the concentrations of the metals in the plant organs was determined by using an atomic absorption spectrometer (Quantum AAS - 2A). Statistical analysis of experimental data was conducted according to standard formulas and indicators [16-18] using the software package Excel. Environmental assessment of the results was realized with the use of basic ecological and biogeochemical parameters (bioavailability factor - BF, accumulation factor - AF) [19-21]. The accumulation factor was calculated as the ratio of the content of the heavy metals in the organs of the plants (mg/kg dry basis) to the content of the mobile forms of the heavy metals in the soil (mg/kg). The bioavailability factor

was calculated as the ratio of the concentrations of mobile forms of HM compounds to the total content of the heavy metals in the soil (mg/kg) [21].

RESULTS AND DISCUSSION

Ust-Kamenogorsk city is the regional center of East Kazakhstan region and its surrounding neighborhood is saturated with large, environmentally dangerous industrial facilities of non-ferrous and ferrous metallurgy, and nuclear and thermal power systems. It was for this reason that research was carried out on the total content and composition of mobile forms of Cu and Zn compounds in the rhizosphere of absinthium (*A. absinthium*) in the area of Ust-Kamenogorsk city and environs. The territory of Ust-Kamenogorsk city and its environs are represented by common loamy black humus earth. The common features of this group of soils are the high capacity of the soil profile and the humus horizon, loamy composition, significant humus content, and neutral or mildly alkaline reaction in the upper horizon and alkaline in the lower horizons. Physical-chemical characteristics of this type of soil are: humus 3 to 6%, cation exchange capacity 15-22 mg/100 g soil, pH (water) from 6.8 to 8.1. Black soils, specific to the area of Ust-Kamenogorsk city and its surroundings have a very high sorption capacity of the soil absorbing complex and are very resistant to contamination by HM [22-23]. According to the results of the study, the total content of Cu and Zn was almost the same in the areas of the rhizosphere as in the total mass of soil, and it varied within the following limits (mg/kg): copper 180-490 (average 335); zinc 420-3821 (2129.7) (Table 1).

As can be seen from Table 1, the total metal content in the rhizosphere of absinthium (*A. absinthium*) and in the common soil is highest in the area of the Ulba Metallurgical Plant, and lowest in the area of Ablaketka. From the standpoint of environmental assessment, the total content of metals in the investigated soil fractions of the rhizosphere and the total mass of soil exceeded the regional Clarke copper by 5.7 times on average, and zinc by 17 times. It was found that the excess of maximum allowable concentration (MAC) [24] of the total content of copper and zinc in the rhizosphere of absinthium (*A. absinthium*) and in the total mass of the soil was 1.3-3.7 times and 1.9-15.2 times respectively in all research areas.

TABLE 2
The concentration of the acid-soluble form of copper and zinc in the rhizosphere of absinthium (*A. absinthium*) of Ust-Kamenogorsk city and the surrounding neighborhoods (mg/kg)

Exploration area	TMS	RS	RSS
Copper			
Titanium-Magnesium Plant	11.5±0.10(4.3) 3.4	12.8±0.08(4.8) 3.8	12.9±0.09(4.8) 4.2
Cement Plant	15.5±0.07(3.4) 4.2	15.4±0.04(3.4) 4.4	15.5±0.06(3.4) 4.0
Ulba Metallurgical Plant	14.7±0.04(3.0) 5.2	15.1±0.05(3.1) 4.4	15.4±0.05(3.1) 4.0
Lead-Zinc Plant	14.4±0.06(3.8) 3.6	14.0±0.05(3.8) 4.2	14.5±0.06(3.8) 4.6
Irtysh Polymetallic Plant	14.5±0.05(3.8) 3.5	14.8±0.09(3.9) 3.5	14.6±0.03(3.8) 3.0
Ablaketka	9.8±0.07(5.4) 5.1	10.5±0.06(5.8) 4.4	10.6±0.04(5.8) 5.2
Processing Plant	13.7±0.06(4.9) 6.2	14.0±0.04(5.0) 5.2	14.5±0.06(5.2) 5.1
Zinc			
Titanium-Magnesium Plant	36.1±0.04(7.4) 8.2	38.9±0.06(8.0) 9.0	39.2±0.05(8.0) 8.5
Cement Plant	610.7±0.07(22.9) 10.2	637.2±0.04(23.8) 12.5	638.0±0.06(23.9) 10.2
Ulba Metallurgical Plant	821.9±0.06(24.6) 9,7	846.9±0.05(25.3) 10.8	847.0±0.08(25.3) 11.5
Lead-Zinc Plant	921.5±0.07(24.1) 12.5	930.8±0.09(24.3) 10.7	930.6±0.05(24.3) 9.7
Irtysh Polymetallic Plant	361.7±0.06(18.2) 7.7	366.7±0.04(18.5) 10.2	366.8±0.04(18.5) 9.7
Ablaketka	68.3±0.07(16.2) 10.2	76.4±0.08(18.1) 10.2	77.0±0,05(18.3) 9.5
Processing Plant	439.1±0.07(20.1) 15.2	454.0±0.06(20.8) 15.0	454.8±0.05(20.8) 14.5

Note: The numerator is the arithmetic average and its error, with the percentage of the total content in brackets; the denominator is the coefficient of variation (%).

TABLE 3
The concentration of exchangeable forms of copper and zinc in the rhizosphere of absinthium (*A. absinthium*) of Ust-Kamenogorsk city and the surrounding neighborhoods (mg/kg)

Study area	TMS	RS	RSS
Copper			
Titanium-Magnesium Plant	1.6±0.04(0.6) 2.4	1.8±0.06(0.7) 3.7	2.1±0.04(0.8) 2.6
Cement Plant	5.5±0.05(1.2) 1.7	5.7±0.06(1.2) 2.2	6.2±0.07(1.4) 1.5
Ulba Metallurgical Plant	7.0±0.03(1.8) 9.6	7.1±0.06(1.9) 10.5	7.0±0.04(1.8) 8.5
Lead-Zinc Plant	6.9±0.04(1,8) 5.5	7.0±0.04(1.8) 5.0	7.2±0.06(1.8) 6.2
Irtysh Polymetallic Plant	5.7±0.07(1.5) 10.,2	4.8±0.04(1.2) 12.4	5.0±0.05(1.3) 14.,2
Ablaketka	1.8±0.04(1.0) 16.7	2.4±0.05(1.3) 15.6	2.6±0.04(1.4) 15.6
Processing Plant	6.9±0.04(2.5) 5.9	7.4±0.05(2.6) 4.2	7.7±0.07(2.7) 5.7
Zinc			
Titanium-magnesium Plant	23.3±0.04(4.8) 5.6	34.3±0.06(7.0) 7.5	35.0±0.06(7.2) 7.0
Cement Plant	353.8±0.03(13.2) 10.7	362.5±0.04(13.6) 11.1	363.0±0.06(13.6) 12.2
Ulba Metallurgical Plant	659.3±0.04(19.7) 8.7	667.4±0.06(20.0) 10.7	667.7±0.05(20.0) 10.8
Lead-Zinc Plant	344.4±0.05(9.0) 4.7	348.7±0.06(9.1) 5.1	348.8±0.04(9.1) 8.1
Irtysh Polymetallic Plant	126.5±0.09(6.4) 17.8	142.0±0.04(7.2) 14.4	142.8±0.08(7.2) 15.4
Ablaketka	28.5±0.04(6.8) 7.7	36.0±0.05(8.6) 8.4	36.5±0.04(8.7) 10.1
Processing Plant	96.0±0.06(4.4) 10.7	119.0±0.05(5.4) 14.6	119.7±0.03(5.8) 12.6

The content of mobile forms of metals in the rhizosphere of absinthium and in the total mass of the soil varied widely. According to the data obtained, the concentrations of the acid forms of the metals were (mg/kg): Cu, 10.6-15.5 (average 13.8) in the soil from the root surface, 10.5-15.1 (13.8) in the rhizosphere and 9.8-15.5 (13.4) in the total mass of the soil; and Zn, 39.2-930.0 (479) in the soil from the root surface, 38.9-930.0 (478.7) in the rhizosphere and 36.1-921.0 (465.6) in the total mass of the soil (Table 2).

The proportion of acid forms of HM compounds from the total content in the soil fractions investigated was (%): 3-5.8 for copper (average 4.2), and 7.4-25 (19.8) for zinc. The range coefficient of variation of the acid form of HM was (%) 3.4-6 for copper, and 8-15 for zinc. From an environmental point of view the concentration of the acid form of copper in the rhizosphere and in the total mass of soil in all areas of research did not exceed its maximum allowable concentration (MAC) and was lower by 5-10 times. The content of the similar form of zinc exceeded MAC in all soil fractions by 1.3-15.5 times, except for the area of the Titanium-Magnesium Plant.

In general, the maximum concentration of acid-soluble forms of metals was observed in the rhizosphere and in the total mass of the soil near the Ulba Metallurgical Plant and the Lead-Zinc Plant. Analysis of the results showed an accurate ($P_{0.1-0.5}$) increase by 1-1.1 times in the concentration of the acid form of zinc in the rhizosphere of absinthium relative to the total mass of the soil in all study areas, whereas no significant accumulation in the rhizosphere was established for the similar form of copper.

Research on the concentration of the exchangeable form of metals revealed the following limits of variation (mg/kg): Cu, 2.6-7.7 (average 5.4) in soil from the root surface, 1.8-7.4 (5.1) in rhizosphere, and 1.6-7.0 (5,1) in the total mass of the soil; Zn, 35-667 (244.8) in soil from the root surface, 34-667 (244.2) in the rhizosphere, and 23.3-659 (233) in the total mass of soil (Table 3).

The ratio of the exchangeable form of HM to the total content in the rhizosphere and in the total mass of the soil was (%) 0.6-2.7 for copper, and 4.8-20 for zinc. The variation coefficient of the exchangeable form in the investigated soil fractions varied within the following limits (%): 1.5-16 for copper, and 4.7-15 for zinc. The maximum content of the exchange form of heavy metals in the rhizosphere of absinthium and in the total mass of the soil was noted close to Ulba Metallurgical Plant and the Lead-Zinc Plant, and the minimum was in the area of the Titanium-Magnesium Plant and Ablaketka. Comparison of the concentrations of the exchangeable form of metals in the rhizosphere and in the total

mass of soil with MAC revealed an excess, by 1.8-2.3 times for copper, except for the area of the Titanium-Magnesium Plant and Ablaketka; and by 1.5-29 times for zinc in all areas of the research.

According to our research, only the content of the exchangeable form of zinc in the rhizosphere of absinthium was significantly increased by 1-1.5 times ($P_{0.1}$) compared to the total mass of the soil. The concentration of the soluble form of the metals, according to the data obtained, constituted (mg/kg) for copper, 3.5-4.4 (average 4.0) in soil from the surface of the root, 3.5-4.2 (3,8) in the rhizosphere, and 3.4-4.0 (3.7) in the total mass of the soil; for zinc, 9.8-12.7 (10.5) in the soil from the surface of the root 9.5-11.8 (10.2) in the rhizosphere, and 8.6-12.9 (9.7) in the total soil mass (Table 4).

The proportion of water-soluble compounds in the total amount varied within the following limits (%): 1.3-2.2 for copper, and 0.3-4.7 for zinc. The range of variation of coefficients of the water-soluble forms of HM (%) was 7.8-16 for copper, and 6.4-17 for zinc. Analysis of the study results did not reveal a statistically significant accumulation of water-soluble metal compounds in the rhizosphere of absinthium (*A. absinthium*) relative to the total weight of the soil on the territory of all the studied areas.

There was no statistically significant difference in the results of the studies between the areas of the rhizosphere in the total content and concentration of mobile forms of metals, or between the total content of heavy metals in the rhizosphere and in the total mass of the soil. The levels of bioavailability factor of HM in the rhizosphere were 1-1.5 times higher than in the total mass of the soil, or were equal. The exchangeable form of zinc was characterized by maximum availability in the rhizosphere and in soil total mass; the exchangeable form of copper was characterized by minimum availability in the rhizosphere and in the total soil. The levels of bioavailability factor of the exchangeable form of HM in the rhizosphere and in the total mass of the soil in the areas of maximum technogenic contamination were higher than with a water soluble form: for copper by 1.5-1.8 times and for zinc by 3.5-50 times. The soils of the study area are exposed to powerful anthropogenic pollution (especially HM), so that the plants are experiencing strong chemical stress. In times of chemical stress, as is known, the barrier functions of the root system are broken, and there is an active absorption of pollutants, including mobile compounds of HM. Increasing the concentration of mobile forms of zinc in the rhizosphere can be explained in this situation by the plants being able to eject an excess of zinc from their organism through the roots [25, 26].

TABLE 4
Concentration of the water soluble forms of copper and zinc in the rhizosphere of absinthium (*A. absinthium*) in Ust-Kamenogorsk city and the surrounding neighborhoods (mg/kg)

Exploration area	TMS	RS	RSS
Copper			
Titanium-Magnesium Plant	3.4±0.06(1.3) 10.2	3.5±0.05(1.3) 10.2	3.5±0.05(1.3) 10.2
Cement Plant	3.5±0.05(0.8) 11.1	3.7±0.04(0.8) 12.7	3.9±0.04(0.9) 14.2
Ulba Metallurgical Plant	3.9±0.06(0.8) 10.5	4.2±0.04(0.9) 10.5	4.4±0.05(0.9) 11.2
Lead-Zinc Plant	3.7±0.05(1.0) 12.7	4.1±0.07(1.1) 15.7	4.4±0.06(1.2) 16.4
Irtysh Polymetallic Plant	4.0±0.04(1.0) 7.8	3.7±0.06(1.0) 8.1	4.0±0.04(1.0) 9.1
Ablaketka	3.6±0.03(2.0) 10.2	4.0±0.03(2.2) 12.6	4.2±0.05(2.2) 14.2
Processing Plant	3.7±0.05(1.3) 8.6	3.8±0.04(1.3) 9.1	4.0±0.05(1.3) 8.7
Zinc			
Titanium-Magnesium Plant	8.6±0.04(1.8) 11.2	9.8±0.03(2.0) 15.7	10.0±0.03(2.0) 14.6
Cement Plant	9.5±0.05(0.4) 8.4	10.0±0.04(0.4) 6.4	10.4±0.07(0.4) 7.2
Ulba Metallurgical Plant	12.9±0.05(0.4) 10.2	11.8±0.06(0.4) 8.6	12.7±0.07(0.7) 7.1
Lead-Zinc Plant	9.4±0.04(0.2) 14.4	10.1±0.06(0.3) 17.5	10.5±0.04(0.3) 16.7
Irtysh Polymetallic Plant	9.7±0.08(0.5) 11.2	10.1±0.07(0.5) 14.4	10.1±0.05(0.5) 15.7
Ablaketka	9.2±0.04(2.2) 7.2	9.8±0.06(2.3) 9.7	10.0±0.06(2.3) 10.2
Processing Plant	8.9±0.04(4.4) 10.7	9.5±0.04(4.7) 11.2	9.8±0.05(4.7) 12.7

TABLE 5
The pH levels in the rhizosphere of absinthium (*A. absinthium*)

Study area	TMS	RS	RSS
Titanium-Magnesium Plant	7.86±0.07(1.2)	7.75±0.04(2.2)	7.72±0.06(1.3)
Cement Plant	7.70±0.07(0.9)	7.60±0.05(1.0)	7.60±0.07(0.9)
Ulba Metallurgical Plant	7.90±0.04(1.4)	7.70±0.06(1.7)	7.68±0.05(1.2)
Lead-Zinc Plant	7.80±0.05(2.0)	7.65±0.05(1.3)	7.60±0.06(1.7)
Irtysh Polymetallic Plant	7.71±0.07(1.3)	7.60±0.05(1.2)	7.61±0.07(1.5)
Ablaketka	7.70±0.04(1.1)	7.60±0.07(1.4)	7.60±0.05(1.7)
Processing Plant	7.84±0.06(2.1)	7.74±0.07(2.1)	7.73±0.06(1.9)

Note: The arithmetic mean and its error, with the coefficient of variation in brackets.

TABLE 6
Content of copper in the organs of absinthium (mg/kg dry basis)

Study area	Leaves	Stems	Roots
Titanium-Magnesium Plant	14	6.1	12
Cement Plant	35.5	7.4	22
Ulba Metallurgical Plant	72	13	58
Lead-Zinc Plant	51	19	43.5
Irtysh Polymetallic Plant	52	15	48
Ablaketka	11	5.3	14
Processing Plant	55	20	48
MAC – 15[17]			

TABLE 7
Content of zinc in absinthium organs (mg/kg on dry basis)

Study area	Leaves	Stems	Roots
Titanium-Magnesium Plant	79	36	36
Cement Plant	418	71	194
Ulba Metallurgical Plant	1326	291	663
Lead-Zinc Plant	434	112	214
Irtysh Polymetallic Plant	260	87	239
Ablaketka	30	18	35
Ore Mining and Processing Mill	2454.5	80	227
MAC – 50[17]			

The results indicate that the rhizosphere soil does not have a constant behavior [27]. In addition, the mobilization of heavy metals in the rhizosphere involved microflora metabolites, but such a high level of soil contamination by HM leads to a drastic reduction in the quantitative and qualitative composition of the rhizoidmicrobial community. The soil cover in the area of influence of the industrial complex of Ust-Kamenogorsk city and the surrounding neighborhoods is characterized by a neutral or weakly alkaline reaction of the area of 7.7-7.9. The limits of fluctuations of pH in the rhizosphere of absinthium (*A. absinthium*) were 7.57-7.72 in the soil from the surface of the root, and 7.60-7.75 in the rhizosphere itself (Table 5).

As can be seen from Table 5, pH decreased in the zones of the rhizosphere of absinthium (*A. absinthium*), i.e. the solution acidified, compared to the total mass of the soil. Thus, the pH value in the rhizosphere of absinthium (*A. absinthium*) in the neighborhood of the Titanium-Magnesium Plant was 1.8% lower than in the total mass of the soil; in the territory of the Cement Plant it was 1.3% lower; near the Ulba Metallurgical Plant it was 2.3% lower; near the Lead-Zinc Plant it was 2.6% lower; near the Irtysh Polymetallic Plant it was 1.3% lower; near Ablaketka it was 1.3% lower, and near the Ore Mining and Processing Mill it was 1.4% lower. Thus, the maximum reduction of pH was found in the rhizosphere of absinthium (*A. absinthium*) in the area of the Lead-Zinc Plant and Ulba Metallurgical Plant.

There was no significant correlated dependence of the concentration of mobile forms of metals and pH in the soil fractions studied. The content of heavy metals in the biomass of absinthium, according to the data obtained, is characterized by wide fluctuations in range. Thus, the concentration of copper in the organs of absinthium was (mg/kg dry basis): 11-72 (average 41.5) in the leaves, 5.3-19 (12.3) in the stems, and 12-58 (35.1) in the roots (Table 6).

Table 6 shows that the maximum content of copper in plants was observed in the area of the Ulba Metallurgical Plant, the Lead-Zinc Plant and the Irtysh Polymetallic Plant, and that the minimum was

in the area of the Titanium-Magnesium Plant and Ablaketka. The distribution of copper in absinthium bodies in all areas of study except Ablaketka has the following form: leaves > roots > stems. From the ecological point, copper content exceeded MAC by 3-5 times in the leaves of absinthium and by 1.5-3 times in the roots in the area of the Cement Plant and Ulba Metallurgical Plant, the Lead-Zinc Plant and Irtysh Polymetallic Plant, as well as near the Ore Mining and Processing Mill. The zinc concentration in the absinthium organs was (mg/kg dry basis): 30-1326 (714.5) in the leaves, 18-291 (99.3) in the stems, and 35-663 (229.7) in the roots (Table 7).

The maximum zinc content in the bodies of absinthium was noted near the Ulba Metallurgical Plant, and the minimum in the area of Ablaketka. The distribution of zinc in absinthium organs was similar to that of copper, and had basically the following form: leaves > roots > stems. Comparing the zinc content in the bodies of the test plants with the MAC, it was established to be in excess by 1.6-26.5 times in the leaves and by 3.9-13.3 times in the roots in all areas except Ablaketka. Differences between the contents of the elements analyzed in the absinthium body were smaller in the leaves and roots than in the stems. The maximum values of the metals analyzed were higher for leaves and roots in almost all areas with the exception of Albaketka than for stems.

Quantitative assessment of HM transition from soil to plants using the accumulation factor (AF) revealed the following. The maxima of the accumulation factors of copper and zinc were by leaves, and the minima by stems. Revealed regularity, as well as the basipetal nature of the distribution of the metals, was apparently caused by significant environmental contamination of the plants [28, 29].

Regarding the mobile form of HM compounds, the accumulation factors were of the following decreasing series: AF accumulation factor (water soluble form) > AF accumulation factor (exchange form) > AF accumulation factor (water soluble form). In most cases, the accumulation factors calculated with respect to the rhizosphere and the total mass of soil were virtually identical. Correlations were established between the content of heavy metals in absinthium organs and the concentration of mobile forms of heavy metals in the rhizosphere.

The content of HM in the leaves of absinthium is closely correlated with the concentration of acid-soluble and exchangeable forms of metals in the rhizosphere, and there was a very strong positive correlation in the total mass of the soil (r = 0.7-0.99). There was a very close correlation between the concentrations of copper and zinc in the leaves of absinthium and the concentrations of water-soluble compounds in the rhizosphere and in the total mass of the soil (r = 0.57-0.97). The correlation between the content of copper in the roots and the concentration of the acid form in the rhizosphere was very close (r = 0.8), but in the total mass of the soil it was practically absent (r = 0.1). The correlation between the copper content in the roots and the concentration of its exchangeable form in all soil fractions had an average strength (r = 0.5). Cu and Zn content in the roots of absinthium was closely correlated with the concentration of the water-soluble compounds of these metals in the rhizosphere and in the total mass of the soil (r = 0.82-0.94).

CONCLUSIONS

On the territory of Ust-Kamenogorsk city, the maximum of total content and concentrations of mobile forms of HM in the rhizosphere of absinthium were found in the area of the Ulba Metallurgical Plant and the Lead-Zinc Plant. The total content of copper and zinc was nearly identical in areas in the rhizosphere and in the total mass of the soil. It was found that the accumulation of exchange and acid-soluble forms of zinc and the accumulation of similar copper forms in the rhizosphere of absinthium were not statistically significant. The increase in the concentration of mobile zinc in the rhizosphere can be explained in this situation by the fact that plants are able to remove the excess of zinc from the body through the roots. On the territory of all the areas studied, no statistically significant accumulation of soluble metal compounds was established in the rhizosphere of absinthium relative to the total mass of the soil. A drop in pH was observed in the rhizosphere of absinthium compared to the total mass of the soil in the study area in a range of 1.3-2.6%. In most cases, the accumulation coefficients calculated with respect to the rhizosphere and total soil were virtually identical. The bioavailability factor of heavy metals in the rhizosphere of the studied plant varied depending on the nature and form of HM occurrence and the level and nature of soil contamination. There were positive correlations between the concentration of heavy metals in absinthium organs and the content of mobile forms of these metals in the rhizosphere and in the total mass of the soil. The strength of the correlations between the content of heavy metals in the plant biomass and the concentration of mobile connections depended on the nature and mode of occurrence of HM, and the nature and extent of soil contamination.

REFERENCES

[1] Nye, P.H. (1981) Changes of pH across the rhizosphere induced by roots. Plant and Soil. 61(1-2), 7-26.

[2] Raven, J.A., Edwards, D. (2001) Roots: evolutionary origin and biogeochemical significance. Journal of Experimental Botany. 52, 381-408.

[3] Chaignon, V., Bedin, F., Hinsinger, P. (2002) Copper bioavailability and rhizosphere pH changes as affected by nitrogen supply for tomato and oilseed rape cropped on an acidic and a calcareous soil. Plant and Soil. 243(2), 219-228.
[4] Read, D.B., Bengough, A.G., Gregory, P.J., Crawford, J.W., Robinson, D., Scrimgeour, C.M., Young, I.M., Zhang, K., Zhang, X. (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytologist. 157(2), 315-326.
[5] Read, D.J., Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in ecosystems a journey towards relevance. New Phytologist. 157, 475-492.
[6] Raynaud, X., Jaillard, B., Leadley, P.W. (2008) Plants may alter competition by modifying nutrient bioavailability in rhizosphere: a modeling approach. The American naturalist. 171(1), 44-58.
[7] Philippot, L., Hallin, S, Börjesson, G., Baggs, E.M. (2009) Biogeochemical cycling in the rhizosphere having an impact on global change. Plant and Soil. 321, 61-81.
[8] McGrath, S.P., Shen, Z.G., Zhao, F.J. (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. Plant and Soil. 188(1), 153-159.
[9] Papa, S., Bartoli, G., Alvarez-Romero, M., Barbato, G., Vitale, A., Ferrante, C., Fioretto, A. (2017) Trace metals accumulation and their translocation in *Phragmites australis* (Cav.) collected along the Sarno river. Fresen. Environ. Bull. 26, 467-474.
[10] Salt, D.E., Smith, R.D., Raskin, I. (1998) Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology. 49, 643-668.
[11] Gobran, G.R., Glegg, S. (1996) A conceptual model for nutrient availability in the soil-root system. Canadian Journal Soil Science. 76(2), 125-131.
[12] Gobran, G.R., Glegg, S., Courchesne, F. (1999) The rhizosphere and trace element acquisition in soils. In: Iskandar, I.K., Magdi Selim, H. (eds.) Fate and Transport of Heavy metals in the Vadose Zone. 1st Ed. 226-249.
[13] Gobran, G.R., Wenzel, W.W., Lombi, E. (2000) Trace elements in the rhizosphere. CRC Press, 344p.
[14] Arinushkina, E.V. (1970) Guidance on chemical analysis of soil. MSU, Moscow, 488p. (in Russian).
[15] Rinkis, G.Ya., Ramane, H.K., Kunitskaya, T.A. (1987) Methods of analysis of soils and plants. Zinatnee, Riga, 174p. (in Russian).
[16] Lakin, G.F. (1980) Biometrics. Moscow, Higher School, 293p. (in Russian).
[17] Dospekhov, B.A. (1985) Methods of field experience (with the fundamentals of statistical processing of the results of research). Moscow, Agropromizdat, 351p. (in Russian).
[18] Dmitriyev E.A. (1995) Mathematical Statistics in Soil science. MSU, Moscow, 320p. (in Russian).
[19] Ilyin, V.B. (1991) Heavy metals in soil-plant system. Science, Novosibirsk, 151p. (in Russian).
[20] Kirpichnikov, N.A., Chernych, N.A., Chernychand, I.N., Tsyganok, S.I. (1991) Controlling the flow of elements in plants. Chemization in agriculture. 10, 45-47. (in Russian).
[21] Knox, A.S., Seaman, J., Adriano, D.S., Pierzynski, G. (2000) Chemo-phytostabilization of metals in contaminated soils. In: Wise, D.L., Trantolo, D.J., Cichon, E.J., Inyang, H.I., Stottmeister, U. (eds.) Bioremediation of contaminated soils. Marcel Dekker Inc., New York, 811-836.
[22] Obukhov, A.I., Yefremova, L.L. (1988) Protection and remediation of soil contaminated with heavy metals. Heavy metals in the environment and nature protection. Science, Moscow, 1-21. (in Russian).
[23] Obukhov, A.I. (1989) Stability of black soil to heavy metal contamination. Problems of protection, management and remediation of black soil. Science, Moscow, 33p. (in Russian).
[24] Anonymous (1987) Guidelines for assessing the danger of soil contamination by chemicals. Higher School, Moscow, 24p. (in Russian).
[25] Stefansky, K.S. (1984) Phytotoxicity of soil and plants in the expansion of the oxide of heavy metal oxides. Agricultural biology. 8, 105-110. (in Russian).
[26] Teng, Y., Yang, J., Song, L, Wang, J. (2013) Fractionation and bioavailability of vanadium in alfalfa rhizosphere and bulk soils by greenhouse experiment. Fresen. Environ. Bull. 22, 1837-1844.
[27] Alidoust, D., Suzuki, S., Matsumura, S., Yoshida, M. (2010) Distribution and chemical forms of cadmium in the fractionated rhizosphere soils of *Helianthus annus* during citric acid-assisted phytoextraction. Fresen. Environ. Bull. 19, 1585-1593.
[28] Korzeniowska, J., Stanislawska-Glubiak, E. (2013) A comparison of the suitability of several methods to estimate the bioavailability of elements in soils to plants. Fresen. Environ. Bull. 22, 943-963.
[29] Mazur, Z., Radziemska, M., Maczuga, O., Makuch, A. (2013) Heavy metal concentrations in soil and moss (*Pleurozium schreberi*) near railroad lines in Olsztyn (Poland). Fresen. Environ. Bull. 22, 957-963.

Received: **21.12.2017**
Accepted: **03.11.2018**

CORRESPONDING AUTHOR

Meruyert Kaygusuz
Denizli Vocational School of Technical Sciences,
Pamukkale University,
20013 Denizli – Turkey

e-mail: meruyert_k@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9529-9534 Fresenius Environmental Bulletin

EFFECTS OF FOUR PESTICIDES ON BROILERS GLUTATHIONE-S-TRANSFERASE ACTIVITY

Mikail Arslan[1], Tugba Tak[2], Adem Ergun[2], Nahit Gencer[2,*], Oktay Arslan[2]

[1]Susurluk Technical Vocational School of Higher Education, Balikesir University, 10100 Balikesir, Turkey
[2] Faculty of Science and Letters, Department of Chemistry, Balikesir University, 10100 Balikesir, Turkey

ABSTRACT

GST is the most important detoxifying enzyme of environmental pollutants, such as carcinogenic matters, heavy metals, some of drugs and pesticides. In this study, hydrophobic gel having a chemical structure of Sepharose 4B-L-tyrosine naphthylamine (STN), for the first time, was used for the purification of Broiler chicken glutathione S-transferase (bcGST). The effects of pesticides namely, Delmetrin®, Mamba Turbo®, Alpgor® and Petra® on purified bcGST activity were determined. Among the pesticides tested, Delmetrin® and Petra® had very strong inhibitory effects on bcGST with IC_{50} values of 28 μM, 45 μM, respectively, but the activity of enzyme was poorly inhibited by Mamba Turbo®. On other hand, Alpgor® with dimethoate had no inhibitory effect on bcGST at the working concentration (20-60 μM).

KEYWORDS:
Glutathione S-transferase, Pesticides, Hydrophobic Interaction, Broilers.

INTRODUCTION

Living organisms are continuously exposed to foreign chemicals compounds in the environment [1]. These chemicals, also known as xenobiotics, can interact with biomolecules, causing serious damage. Concerns about the ecological effects of pesticides which are the most important xenobiotics are increasing almost everywhere in the world. Among many purposes, pesticides are especially used to increase crop yield in agriculture industry [2-4]. Many of the pesticides have very stable chemical structures. For this reason, they have a potential for bioaccumulation with long half-lives in the environment. It has been noted that the bioaccumulation of pesticides in the food chain can lead to significant side effects in all non-target organisms, including the human [5]. It is well known that pesticides and other pollutants induce the intracellular production of ROS as well as altering the activity of many enzymes [6-8].

Almost all living organisms have highly sophisticated detoxification systems against toxic xenobiotics [9, 10]. Detoxification of xenobiotics occurs in two phases, phase I and phase II. There are two different enzyme systems for detoxification of xenobiotics. With these systems, toxic chemicals are transformed into more hydrophilic and more easily excreted compounds. Glutathione S-transferase (GST) is a phase II enzyme that plays critical role in the metabolism of xenobiotics [11-14]. GST catalyzes the conjugation reaction of electrophilic xenobiotics with reduced glutathione. Conjugated compounds are more soluble forms that are easily excreted in the urine. It is estimated that GST is found in almost all living organisms. Numerous GST isoenzymes have been determined in animal tissues, including chicken liver [15-20].

There has been a serious concern for years about bioaccumulation of pesticides in the feed of animals that are the main protein source of humans. It is known that animal feeds consisting of numerous additives contain pesticide residues [21]. Chickens, also known as broilers, are raised for meat in large quantities throughout the world. Broiler chicken is most preferred as a protein source with more protein and less fat [22]. The chicken proteins are of high quality because they contain high levels of essential amino acids. In addition, fat in chickens has a high degree of unsaturated fatty acid residues, which protects against diseases. Chicken meat is relatively low in cost compared to other meats. For these reasons, chicken meat is coming more popular in order to provide the protein needs of the growing world population [23].

Reduced activity of the GST enzyme in chicken liver is an important risk for its xenobiotic metabolism. As a consequence, chicken can accumulate xenobiotics in various tissues and constitutes a major danger for human health. Numerous chemicals have been reported in the literature as GST inhibitors. Some of these compounds are glutathione analogues, phenolic, α-chloroacetamide derivatives, cibacron blue, bromosulfophthalein, compounds containing benzothiazole groups, flavonoids, chloroacetamide derivatives, ethacrynic acid and pesticides [24-29]. Commercial formulations with lambda cyhalothrin, glyphosate dimethylamine salt, dimethoate and deltamethrin are among the most commonly used pesticides worldwide and pose an important danger to non-target organisms. Effects of these compounds have been studied on

GSTs from different sources such as carp (*Cyprinus carpio* L.) [30], fungus *Beauveria bassiana* [31], mice [32], earthworm *Eisenia andrei* [33], midge *Chironomus riparius* [34], *Daphnia magna* [35], different earthworm species [36].

However, no information has been found in literature about the effects of these pesticides on chicken liver glutathione S-transferase which is an important enzymatic detoxification. It is clear that the determination of the effects of these compounds on bcGST is important, as can be understood from the statements discussed above. In this study, the bcGST enzyme was purified from the chicken liver by hydrophobic interaction chromatography method. The main purpose of the study is to determine the effect of several pesticides on purified bcGST enzyme activity.

MATERIALS AND METHODS

Chemicals. Commercial preparations of the pesticides used in our research were obtained from the market. These preparations were Delmetrin 25 EC® (25 g Deltamethrin /L), Mamba Turbo® (608 g Glyphosate dimethylamine salt /L), Alpgor 40 EC® (400 g Dimethoate /L) and Petra 5 EC® (50 g Lambda- cyhalothrin /L). All other reagents used in the study were purchased from Sigma-Aldrich.

Protein Determination. Protein quantities were achieved spectrophotometrically at 595 nm according to Bradford's method. In this procedure serum albumin was used as standard [37].

Enzyme assays. bcGST activity was assayed spectrophotometrically at 340 nm by the procedure of Habig and Jakoby. For this purpose, the reaction medium contained 100 mM phosphate buffer (pH 6.5), 20 mM GSH and 25 mM 1-Chloro-2,4-dinitrobenzene (CDNB), ethanol (final concentration %3) and enzyme solution. Under assay conditions, activity measurements without enzyme were used as controls. All measurements were repeated at least three times [38].

Synthesis of Hydrophobic Gel. The hydrophobic gel used for bcGST purification was synthesized in three steps. First, the hydroxyl groups on Sepharose 4B are activated by cyanogen bromide. In the next step, Sepharose-4B-L-tyrosine gel was obtained by the reaction of L-tyrosine with the CNBr activated Sepharose 4B. Finally, the hydrophobic matrix was synthesized by coupling of diazotized 1-naphthylamine to the Sepharose 4B-L-tyrosine [39].

Purification of bcGST. Chicken livers were immersed in liquid nitrogen and powdered with a blender. The powdered sample was suspended in 50 mM Tris buffer (pH 7.5) containing 1 mM EDTA and than centrifuged for 15 min (13,000×*g*). The hydrophobic column, namely Sepharose 4B-Ltyrosine-1-naphthylamine, was equilibrated with the solution 25 mM Tris/HCl (pH 8.0)/10 mM $CaCl_2$/3 M NaCl and than, the sample containing the bcGST enzyme was applied to this hydrophobic column. bcGST enzyme was eluted with the solution of 25 mM Tris/HCl (pH 8.0). 1.5 mL fractions were collected.

In vitro Effects of Pesticides. The inhibitory effects of pesticides on bcGST enzyme activity were determined at five different concentrations of these pesticides by using GST and CDNB as substrates. IC_{50} values (the inhibitor concentration, which reduces enzyme activity by 50%) for this pesticide compounds were calculated from percent activity versus pesticide concentration graphs. In the absence of pesticide percent activity was accepted as 100.

RESULTS

GSTs were purified from many different sources by affinity chromatography having a chemical structural of GS-Sepharose. Some of these sources are turkey [15], cattle [17], human [18], mouse [19], rat [20], chicken [29], midge [34], rabbit [40] and fish [41].

In this study, hydrophobic gel having a chemical structure of Sepharose 4B-L-tyrosine-naphthylamine (STN), for the first time, was used for the purification of bcGST. According to structural analyzes on GSTs, they have two different domain. Domain I, designated GSH binding site, is located in the N-terminal region of the enzyme. Domain II is composed of a helix structure and nonspecifically interacts with hydrophobic xenobiotics. This region is also called H-region [42]. Considering the H-region of GST, we preferred the hydrophobic interaction chromatography technique for the purification of this enzyme. Purification of bcGST was accomplished by liver homogenate 7 fold and a recovery of about 7.2%. In previous studies, the purchased GS-Sepharose affinity column was used for GST purification from different sources [15, 17, 18 and 19]. The STN gel used in this study was previously synthesized in our laboratory for the purification of PON enzyme [37]. We have considered as an advantage of using the same gel mentioned above to purify the bcGST enzyme without buying another new gel.

This research was carried out to determine the effect of pesticides namely, Delmetrin ®, Mamba Turbo®, Alpgor® and Petra® on bcGST activity which is one of the detoxification enzymes. Pesticides with Deltamethrin, Dimethoate and Lambda-cyhalothrin are widely used in agriculture because

of efficiency against a wide spectrum of insect pests [31, 33, 34, 43 and 44]. They were previously thought to be less toxic to non-targets. However, there are many studies showing that it is extremely toxic to many mammals, including human [30].

As shown in Figure 1, Alpgor® with dimethoate had no inhibitory effect on bcGST at the working concentration (20-60 µM). Van Preat et al. studied the effect of dimethoate on midge *Chironomus riparius* GST enzyme [34]. They reported that pesticide did show inhibition effect on enzyme activity. On the other hand, it was found that pesticide with same compound activated the *Eisenia andrei* GST [36]. It was determined that purified cGST was inhibited by Delmetrin® and Petra® at different levels (Figure 1). We found the IC_{50} values for Delmetrin® and Petra® as 28 µM, 45 µM, respectively. According to results, Delmetrin® was the most potent inhibitor of the tested compounds. This pesticide with Deltamethrin is a widely used compound because it has broad spectrum and is very effective against insects. Several pesticides containing deltamethrin have been studied for their effects on the GST enzymes [30]. Our result was similar to that reported by Rehman et al. [32] who found a significant decrease in the activity GST in the liver of deltamethrin-treated mice. In addition, Yamamoto et al. noted that GSTs from different sources was inhibited by fenitrothion, permethrin, and deltamethrin [45]. But in another study it was found that liver GST activity was increased in fish exposed to high pesticide concentration [30]. On the other hand, Itabajara et al. reported the inhibition effects of some pesticides including deltamethrin on the *Boophilus microplus* GST [45]. In addition, it was found that fenitrothion and lambda-cyhalothrin mixture inhibited rat kidney GST [44].

DISCUSSION

bcGST is a multifunctional enzyme with an important role in xenobiotic metabolism, as described in the introduction section. It is known that GST is the most important detoxifying enzyme of environmental pollutants, such as carcinogenic matters, heavy metals, some of drugs and pesticides [25-28]. It is evident that the reduction of liver GST activity will result in the accumulation of these compounds in living organism. This may be not very important for the health of the broilers because their lives are only a few weeks. However, chicken meat with xenobiotic remnants is an important threat to human health [23]. It was reported that methyl parathion and copper oxychloride lead to some toxic effects on the GST enzyme activity. It was significantly affected by these pesticide applications on earthworms [47]. Benradia et al. [48] reported that the activity of GST was measured in Palaemon adspersus. The activity of the biomarkers was determined during the summer and autumn seasons during two consecutive years 2013 and 2014.

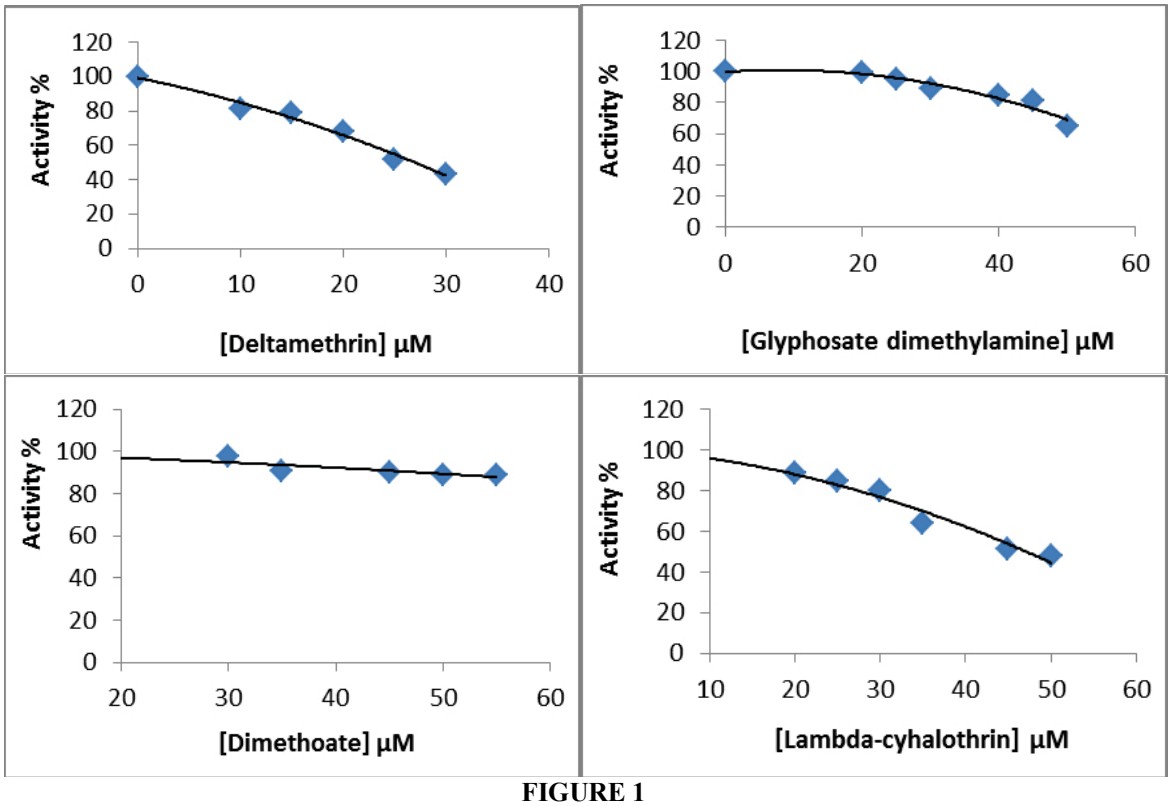


FIGURE 1
Graphs of percentage inhibition of bcGST enzyme activity some pesticide concentrations

Chicken meat is one of the most popular and most common protein sources in the world because of the taste, price, and easy to cook. Consumption of chicken meat has been increased dramatically in recent years in Turkey. Concerns about the potential that exists in adulterating poultry products with pesticide residues have been increase in recent years. This problem has international implications because chicken feeds and chicken products are among the most important sectors that are traded between different countries in the world [22].

Unlike other pesticides used in our research, the glyphosate dimethylamine is a glyphosate-based herbicide that is used intensively in agriculture struggle [49]. We found the glyphosate dimethylamine at the concentration of 50 µM showed approximately an inhibition effect of 20% on GST activities. Glyphosate dimethylamine is one of the most popular compounds among these herbicides as an active ingredient. This compound acts specifically in plants by inhibiting the enzyme, which plays an important role in the synthesis of aromatic amino acids in plants [50]. Many pesticides, at relatively low dosages affect the metabolism of biota by altering normal enzyme activity. For instance, we previously reported the inhibitory effects of some pesticides incorporating lead on the activity of paraoxonase [51] and carbonic anhydrase [52].

REFERENCES

[1] Ezeji, E.U., Ernest, A.A., Ezejiofor, T.N., Udensi, J.U. (2012) Determination of reduced glutathione and glutathione S-transferase of poultry birds exposed to permethrin insecticide. American Journal of Biochemistry. 2(3), 21-24.

[2] Song, Y., Chen, M., Zhou, J. (2017) Effects of three pesticides on superoxide dismutase and glutathione-S-transferase activities and reproduction of *Daphnia magna.* Archives of Environmental Protection. 43(1), 80–86.

[3] Wiszniowski, J., Terhalle, A., Richard, C., Bonnemoy, F., Bohatier, J. (2011) Toxicity of sulcotrione photoproducts mixture towards Vibrio fi sheri in the environment. Archives of Environmental Protection. 37, 15–22.

[4] Hayasaka, D., Korenaga, T., Suzuki, K., Saito, F., Sánchez-Bayo, F., Goka, K. (2012) Cumulative ecological impacts of two successive annual treatments of imidacloprid and fi pronil on aquatic communities of paddy mesocosms. Ecotoxicology and Environmental Safety. 80, 355–362.

[5] Yang, D., Qi, S., Zhang, J., Wue, C. (2013) X. Organochlorine pesticides in soil, water and sediment along the Jinjiang River mainstream to Quanzhou Bay, southeast China. Ecotoxicology and Environmental Safety. 89, 59-65.

[6] Uçkun, A.A. (2017) Ecotoxicological Evaluation of Pesticide Pollution in Ataturk Dam Lake (Euphrates River), Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 17(2), 313-321.

[7] Bukalasa, J.S., Brunekreef, B., Brouwer, M., Vermeulen, R., Jongste, J.C., Rossem, L.M., Vonk, J., Wijga, A., Huss, A. (2017) Proximity to agricultural fields as proxy for environmental exposure to pesticides among children: The PIAMA birth cohort. Science of The Total Environment. 595, 515–520.

[8] Jiang, P., Wang, J., Sheng, N., Wei, D., Dai, J. (2017) Effects of pentachlorophenol on the quail (Coturnix japonica) liver detoxification pathway. Chemosphere. 177, 44-50.

[9] Antos, P.A., Blachuta, M., Hrabia, A., Grzegorzewska, A.K., Sechman, A. (2015) Expression of aryl hydrocarbon receptor 1 (AHR1), AHR1 nuclear translocator 1 (ARNT1) and CYP1 family monooxygenase mRNAs and their activity in chicken ovarian follicles following in vitro exposure to 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD). Toxicology Letter. 237, 100-111.

[10] Mortensen, A.S., Braathen, M., Sandvik, M., Arukwe, A. (2007) Effects of hydroxy-polychlorinated biphenyl (OH-PCB) congeners on the xenobiotic biotransformation gene expression patterns in primary culture of Atlantic salmon (Salmo salar) hepatocytes. Ecotoxicology and Environmental Safety. 68, 351–360.

[11] Atkinson, H.J. and Babbitt, P.C. (2009) Glutathione transferases are structural and functional outliers in the thioredoxin fold. Biochemistry. 48, 11108–11116.

[12] Ketterer, B. (2001) A bird's eye view of the glutathione transferase field. Chemico Biological Interactions. 138, 27–42.

[13] Dalmizrak, O., Kulaksiz E.G., Ozer, N. (2012) Possible prenatal impact of sertraline on human placental glutathione S-transferase-p. Human Experimental Toxicology. 31, 457–464.

[14] Rodeiro, I., Donato, M.T., Lahoz, A., Garrido, G., Delgado, R., Gomez- Lechon, M.J. (2008) Interactions of polyphenols with the P450 system: possible implications on human therapeutics. Mini Rev Med Chem. 8, 97–106.

[15] Akkemik, E., Taser, P., Bayindir, A., Budak, H., Ciftci, M. (2012) Purification and characterization of glutathione S-transferase from turkey liver and inhibition effects of some metal ions on enzyme activity. Environmental Toxicology and Pharmacology. 34(3), 888-894.

[16] Aydemir, T., Kavrayan, D. (2009) Purification and characterization of glutathione-S-transferase from chicken erythrocyte. Artificial Cells, Blood Substitutes, and Biotechnology. 37(2), 92-100.

[17] Guvercin, S., Erat, M., Sakiroglu, H. (2008) Determination of some kinetic and characteristic properties of glutathione S transferase from bovine erythrocytes. Protein Peptide Letter. 15, 6–12.
[18] Kamisaka, K., Habig, W.H., Ketley, J.N., Arias, I.M., Jakoby, W.B., (1975) Multiple forms of human glutathione S-transferase and their affinity for bilirubin. European Journal Biochemistry. 60, 153–161.
[19] Lee, C.Y., Johnson, L., Cox, R.H., McKinney, J.D., Lee, S.M. (1981) Mouse-liver glutathione S-transferases – biochemical and immunological characterization. Journal of Biological Chemistry. 256, 8110–8116.
[20] Friedberg, T., Milbert, U., Bentley, P., Guenther, T.M., Oesch, F. (1983) Purification and characterization of a new cytosolic glutathione S-transferase (glutathione-S-transferase-X) from rat-liver. Biochemical Journal. 215, 617–625.
[21] Jayaraman, K., Munira, H., Dababrata, C., Iranmanesh, M. (2013) The preference and consumption of chicken lovers with race as a moderator-An empirical study in Malaysia. International Food Research Journal. 20(1), 165-174.
[22] Nestle, M. (1999) Meat or wheat for the next millennium? Proceedings of the Nutrition Society. 58, 211-18.
[23] Jia, B., Yoon, S. C., Zhuang, H., Wang, W., Li, C. (2017) Prediction of pH of fresh chicken breast fillets by VNIR hyperspectral imaging. Journal of Food Engineering. 208, 57-65.
[24] Sau, A., Pellizzari, T.F., Valentino, F., Federici, G., Caccuri, A.M. (2010) Glutathione transferases and development of new principles to overcome drug resistance. Archives of Biochemistry and Biophysics. 500, 116-122.
[25] Ramkumar, K., Samanta, S., Kyani, A., Yang, S., Tamura, S., Ziemke, E., Debnath, B. (2016) Mechanistic evaluation and transcriptional signature of a glutathione S-transferase omega 1 inhibitor. Nature Communications. 7, 13084-13097.
[26] Jiang, P., Wang, J., Sheng, N., Wei, D., Dai, J. (2017) Effects of pentachlorophenol on the quail (Coturnix japonica) liver detoxification pathway. Chemosphere. 177, 44-50.
[27] Hayeshi, R., Chinyanga, F., Chengedza, S., Mukanganyama, S. (2006) Inhibition of human glutathione transferases by multidrug resistance chemomodulators in vitro. J Enzyme Inhibition Med Chem. 21, 581-587.
[28] Musdal, Y., Bolelli, T.E., Bolelli, K., Yılmaz, S., Ceyhan, D., Hegazy, U., Aksoy, Y. (2012) Inhibition of human glutathione transferase P1-1 by novel benzazole derivatives. Turkish Journal of Biochemistry/Turk Biyokimya Dergisi. 37(4), 431-436.
[29] Tian, X., Song, E., Pi, R., Zhu, X., Liu, L., Ma, X., Song, Y. (2012) Polychlorinated biphenyls and their different level metabolites as inhibitors of glutathione S-transferase isoenzymes. Chemico-biological interactions. 198(1), 1-8.
[30] Ensibi, C., Perez-Lopez, M., Rodríguez, F.S., Miguez-Santiyan, M.P., Yahya, M.D., Hernández-Moreno, D. (2013) Effects of deltamethrin on biometric parameters and liver biomarkers in common carp (Cyprinus carpio L.). Environmental Toxicology and Pharmacology. 36(2), 384-391.
[31] Forlani, L., Juárez, M.P., Lavarías, S., Pedrini, N. (2014) Toxicological and biochemical response of the entomopathogenic fungus Beauveria bassiana after exposure to deltamethrin. Pest Management Science. 70(5), 751-756.
[32] Rehman, H., Ali, M., Atif, F., Kaur, M., Bhatia, K., Raisuddin, S. (2006) The modulatory effect of deltamethrin on antioxidants in mice. Clinica Chimica Acta. 369(1), 61-65.
[33] Velki, M., Hackenberger, B.K. (2013) Inhibition and recovery of molecular biomarkers of earthworm Eisenia andrei after exposure to organophosphate dimethoate. Soil Biology and Biochemistry. 57, 100-108.
[34] Van Praet, N., De Jonge, M., Stoks, R., Bervoets, L. (2014) Additive effects of predator cues and dimethoate on different levels of biological organisation in the non-biting midge Chironomus riparius. Aquatic toxicology. 155, 236-243.
[35] Song, Y., Chen, M., Zhou, J. (2017) Effects of three pesticides on superoxide dismutase and glutathione-S-transferase activities and reproduction of Daphnia magna. Archives of Environmental Protection. 43(1), 80-86.
[36] Velki, M., Hackenberger, B.K., Lončarić, Ž., Hackenberger, D.K. (2014) Application of microcosmic system for assessment of insecticide effects on biomarker responses in ecologically different earthworm species. Ecotoxicology and Environmental Safety. 104, 110-119.
[37] Sinan, S., Kockar, F., Arslan, O. (2006) Novel purification strategy for human PON1 and inhibition of the activity by cephalosporin and aminoglikozide derived antibiotics Biochimie. 88(5), 565-574.
[38] Pabst, M.J., Habig, W.H., Jakoby, W.B. (1974) Glutathione S-transferase A: a novel kinetic mechanism in which the major reaction pathway depends on substrate concentration. Journal of biological chemistry. 249, 7140–7148.
[39] Bradford, M.M. (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Analytical Biochemistry. 72, 248–254.

[40] Miyaura, S., Isono, H. (1986) Purification and characterization of multiple forms of rabbit hepatic glutathione S-transferase. Chemical and Pharmaceutical Bulletin. 34(1), 194-205.
[41] Aksoy, M., Ozaslan, M.S., Kufrevioglu, O.I. (2016) Purification of glutathione S-transferase from Van Lake fish (Chalcalburnus tarichii Pallas) muscle and investigation of some metal ions effect on enzyme activity. Journal of Enzyme Inhibition and Medicinal Chemistry. 31(4), 546-550.
[42] Sheehan, D., Meade, G., Foley, V.M. (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochemical Journal. 360(1), 1-16.
[43] Fetoui, H., Makni, M., Garoui, E.M., Zeghal, N. (2010) Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: involvement of oxidative stress and protective role of ascorbic acid. Experimental and Toxicologic Pathology. 62(6), 593-599.
[44] El-Demerdash, F.M. (2012) Cytotoxic effect of fenitrothion and lambda-cyhalothrin mixture on lipid peroxidation and antioxidant defense system in rat kidney. Journal of Environmental Science and Health, Part B. 47(4), 262-268.
[45] Yamamoto, K., Teshiba, S., Aso, Y. (2008) Characterization of glutathione S-transferase of the rice leaffolder moth, Cnaphalocrocis medinalis (Lepidoptera: Pyralidae): Comparison of its properties of glutathione S-transferases from other lepidopteran insects. Pesticide Biochemistry and Physiology. 92, 125–128.
[46] da Silva Vaz, I., Lermen, T.T., Michelon, A., Ferreira, C.A.S., de Freitas, D.R.J., Termignoni, C., Masuda, A. (2004) Effect of acaricides on the activity of a Boophilus microplus glutathione S-transferase. Veterinary Parasitology. 119(2), 237-245.
[47] Yologlu, E. and Ozmen, M. (2013) Effects of Methyl Parathion And Copper Oxychloride Application On Earthworms (Lumbricus Terrestris) In An Apricot Orchard. Fresen. Environ. Bull. 22, 3442-3447.
[48] Benradia, H., Berghiche, H., and Soltani, Noureddine. (2016) Measure of Environmental Stress Biomarkers In The Shrimp Palaemon Adspersus From The Mellah Lagoon (Algeria): Spatial and Temporal Variations. Fresen. Environ. Bull. 25, 2563-2566.
[49] Rissoli, R.Z., Abdalla, F.C., Costa, M.J., Rantin, F.T., McKenzie, D.J., Kalinin, A.L. (2016) Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles. Chemosphere. 156, 37-44.
[50] Kamijo, Y., Takai, M., Sakamoto, T. (2016) A multicenter retrospective survey of poisoning after ingestion of herbicides containing glyphosate potassium salt or other glyphosate salts in Japan. Clinical toxicology. 54(2), 147-151.
[51] Gencer, N. and Arslan O. (2011) In vitro effects of some pesticides on PON1Q192 and PON1R192 isoenzymes from human serum. Fresen. Environ. Bull. 20, 590-596.
[52] Gencer, N., Ergun, A., Demir, D. (2012) In Vitro Effects of Some Herbicides And Fungicides on Human Erythrocyte Carbonic Anhydrase Activity. Fresen. Environ. Bull. 21, 549-552.

Received: **02.01.2018**
Accepted: **16.10.2018**

CORRESPONDING AUTHOR

Nahit Gencer
Faculty of Science and Letters,
Department of Chemistry,
Balikesir University,
10100 Balikesir, Turkey

e-mail: ngencer@balikesir.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9535-9541 Fresenius Environmental Bulletin

APPLICATION OF NEURAL NETWORKS IN WASTEWATERS MANAGEMENT IN A COASTAL KARST AREA

Milena Tadic*, Stanka Filipovic

University of Montenegro, Faculty of Metallurgy and Technology, George Washington bb, Podgorica, Montenegro

ABSTRACT

A complex research of coastal karst basin waters is the topic of this research. The aim of the research is to develop a model for the wastewaters management of the basin's area in order to protect waters in the karst underground.

As the research spot a particular karst basin is chosen. Municipial wastewaters are discharged into the basin without any purification and, after holding up in the underground, they appear at the spring of the river which supplies water of the biggest lake on the Balkans.

Results determined by the simulation of neural network by inserting qualitative indexes of wastewaters and spring waters, indicate a satisfactory compatibility with measurements.

Scientific importance of the shown research is a contribution to development of the karst waters usage and protection methods through an application of the developed model. This model has been proven as a reliable control/management element in the process of defining and prompt implementation of the necessary protective measures, particularly in accidental water pollutions.

KEYWORDS:
Wastewaters, basin, coastal karst, neural network.

INTRODUCTION

Karst systems require specific investigation methods that capture their duality due to their particular dynamics, i.e., both slow/diffuse and fast/concentrated flow and storage dynamics [1].

Knowledge of the behaviour of waters in karst areas, in both quantitative and qualitative terms, is one of the issues of great importance. This is due to their specific behaviour, as well as to the difficulty in making a sufficiently reliable and at the same time easy to use model of control of discharge of pollutants in karst terrains [2]. Hydrologic models require an adequate representation of karst specific processes, like the strong subsurface hydraulic heterogeneity of karstified rocks [3].

Contamination control of karst systems as natural phenomena becomes one of the most important aspects of water wealth protection, because it can be easily contaminated but hardly "cleaned" in a relatively short period of time. The quality of karst water has important differences compared to the water quality from other hydrogeological environments, not only because of its natural state, but also because of the contamination [4, 5].

The protection from the sources of contamination, especially from industrial and communal wastewaters from settlements in karst terrains, is a very complex approach to problem solving. In this regard, water management at the basin level using information technology is a challenge, especially regarding karst hydrological systems [6].

The objective of the Water Framework Directive is to remedy the damage done so far and to achieve good chemical and ecological status of waters [7]. This is difficult to achieve without the complex monitoring of the quality of all water body types in the basin, within the existing technological and socio-economic development. In addition, the application of artificial intelligence, such as models and expert systems, also has a great role and importance when it is based on a reliable database, such as data on the processes in the area of the affected basin, the boundary flow rate of inlet wastewater load, its effect on quality of the recipient and others.

The subject of this paper is the testing of the waters of the Crnojevica River Basin, oriented towards the development of a model of wastewaters management in a coastal karst area. Given the specificity of the coastal karst, where the quantity of inlet and outlet water is not completely known, which is the case with the selected basin, a better knowledge of the reactions of the basin in question from the aspect of contamination will be obtained by properly combining the data on the variable water quality and quantity at the inlet and outlet using neural networks for identification. The results of the research will enable the proposal of appropriate measures for the protection of the waters of the karst system, an invaluable water wealth, which is also of practical importance for the already established strategy of sustainable development of Montenegro.

MATERIALS AND METHODS

The basin of the Crnojevica River taken as an example of coastal karst, belongs to a highly holokarstic area with typical surface and underground karstic forms. These terrains have high differences in height and the cotes range from 1600 to 20 m a.s.l. The geological material of the basin is dominated by limestone, dolomit limestones and dolomites form from the upper trias and lower Jurassic ages. Intense karstification has destroyed the surface river net and moved it underground [8].

In the identification of karst systems and estimation of parameters, the mathematical - statistical approach input – response – output is of particular importance. This implies the determination of the character of the system's response, using the calculated data on variables at the inlet and outlet of karst formations (channel system, aquifer, complex basins, etc.). However, due to the insurmountable difficulties in understanding and describing the karst aquifers and the underground channel systems as a whole, especially due to the inaccessibility of these regions, the modeling of karst systems has shown relatively modest results. This is because the inlet is insufficiently defined, in contrast to the outlet, since it is mainly focused on concentrated outflows that are easily accessible for measurement and observation.

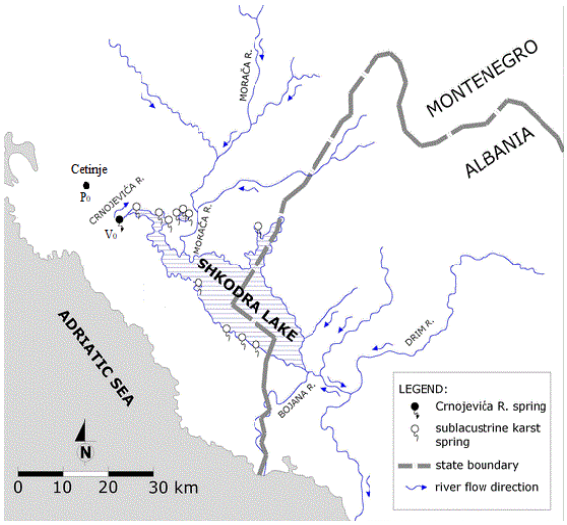


FIGURE 1
Location map of measuring point karst swallet P_0 i karst spring V_0 [9]

First, the sources of contamination were identified and through the series of measurements the quantitative and qualitative characteristics of wastewater were determined. The wastewaters from settlement Cetinje mostly flow into the karst swallet - the inlet (measuring point – P_0), and after a relatively short holding up time of 55-78 hours spent in the karst underground [8], depending on the hydrological conditions, they reach the spring of the watercourse - the outlet (measuring point – V_0), whose recipient is Shkodra Lake (Fig.1) [9].

The dynamics and range of the investigation were appropriate for the investigation of karst hydrological systems [10], while physical, chemical and bacterial examinations were performed using standard methods [11].

The instantaneous samples were taken at measuring points P_0 and V_0 in five series. The series of investigations at these points have been performed every two hours in 7 days time. Sampling at point V_0 was in each series moved in time for the predicted period of retention time of wastewaters in karst underground [8].

The terrain measurements include water flow (Q), temperature (t), electrolytic conductivity (EC) and dissolved oxygen (DO), while other parameters: pH, BOD_5, Na^+, K^+, Ca^{2+}, NH_3, NO_3^-, NO_2^-, Cl^-, SO_4^{2-}, PO_4^{3-}, total coliform bacteria and fecal coliform bacteria were determined in laboratory.

Neural Network Toolboxes module, an integral part of the MATLAB software package, was used to design and simulate neural networks. Immediately prior to importing data into the Grafical User Interface (GUI), intended for work with neural networks, the data must be normalized with no boundaries for the inlet values, while the outlet values must be in the range of -1 and 1.

Neural networks are trained with the results obtained for the following parameters: temperature, electrolytic conductivity, pH, BOD_5, total N (TN) and total P (TP). As emphasized, there are no defined boundaries for the inlet values, thus the obtained numerical values were used for training and simulation of the network. In contrast, the outlet values are defined by a certain interval, so the values obtained by measurements at the spring i.e. outlet for the corresponding parameter, were adjusted to the required interval. Namely, the obtained outlet values for the selected parameters, used for training of the neural networks, are divided with the maximum measured value for the given parameter and thus the outlet data were obtained in the interval from 0 to 1, which is in accordance with the requested interval.

After the data normalization was completed, the training of the neural network was initiated individually for each parameter obtained during four series and the results were verified on the basis of data obtained from the fifth series of testing.

Based on the analysis of types of neural networks, the choice was reduced to the Feed-forward Backpropagation Neural Network, which will be specially trained, with different performances, on data for the specified parameters.

The results are shown in Tables 1 and 2 and in Fig. 2-8.

RESULTS AND DISCUSSION

The data obtained by field and laboratory testing of wastewater at the measuring point P_0 and waters at the spring of the Crnojevica River watercourse - exit V_0 are presented in Table 1, 2. Due to their high amount, only the ranges of the obtained data are presented.

Measuring the flow and sampling every two hours enables very good monitoring of the changes in the wastewater flow and the concentration of foreign components contained therein. The data obtained by this method basically include all oscillations in the contamination load so that their summing up can provide a realistic data on the average amount of contamination during the day.

TABLE 1
Range of values of the quality indicator for wastewaters at the entrance to the karst swallet (measuring point P_0)

Statistics		Q, l/s	t, °C	pH	EC, µS/cm	DO, mg/l	BOD_5 mg/l	Na^+, mg/l	K^+, mg/l	Cl^-, mg/l	SO_4^{2-} mg/l	PO_4^{3-} mg/l	TP, mg/l	TN, mg/l
I SERIES	min	5.5	9.3	6.46	130	10	0	3.70	1.7	6.60	5.2	4.3	2.8	0.5
	max	90	20	8.75	1110	350	8.9	194	38.9	40.40	499.4	680	442	14.3
	stdev	12.81	1.8	0.39	180.54	71.9	2.1	42.57	4.7	7.73	81.95	131.6	85.6	3.6
	median	23.80	17	7.54	438	65	4.8	29.80	7.1	22.35	38.1	23.9	15.6	1.3
II SERIES	min	6.10	4	6.46	90	32.6	1	6	2	8.2	7.1	1	0.65	0.1
	max	177	13	8.26	540	317	9.8	123	8.4	110.2	133.1	1753	1139.4	1.4
	stdev	33.66	1.9	0.40	82.10	50.4	2.5	15.78	1.4	16.46	19.31	252.6	85.6	0.2
	median	26	8	7.43	310	90.1	6.2	20	5.3	24.85	25.3	7.6	15.6	0.15
III SERIES	min	3	9	6.92	160	26	0	6.2	1.2	2	13	1.1	0.7	0.8
	max	220	15	7.90	430	210	9.5	48	55.6	54.6	73.6	31.8	20.7	18.1
	stdev	36.89	1.7	0.21	62.56	37.6	1.9	9.04	5.8	7.94	11.47	4.3	2.8	4.8
	median	43	11	7.37	347.5	103.5	6.2	24.5	4.6	19	26.95	4.7	3.1	5.4
IV SERIES	min	3	10	6.95	260	15	0.2	13.5	4.5	10	12.8	0.8	0.5	2.1
	max	63	20	8.85	840	315	7.9	310	15	95	75	51.8	33.6	30.3
	stdev	13.77	2.3	0.37	102.12	62.3	2.1	57.62	2.1	16.93	12.51	13.9	9.1	7.1
	median	30	14	7.35	466.50	112.5	4.1	42.5	9	25.25	33.3	6.4	4.2	12.1
V SERIES	min	4	8	7	20	15	0	4.5	2.5	6	5.2	0.3	0.2	0
	max	170	15	8.40	1440	360	9.7	266	26	640	85.8	28.6	18.6	1.5
	stdev	39.68	1.9	0.27	228.98	66.5	3.1	39.02	5.2	76.21	17.09	5.7	3.7	0.3
	median	52	10	7.65	355	96	1.8	27	8	18.35	20.5	7.4	4.8	0.11

TABLE 2
Range of values of the quality indicator for waters at the exit from the karst underground (measuring point V_0)

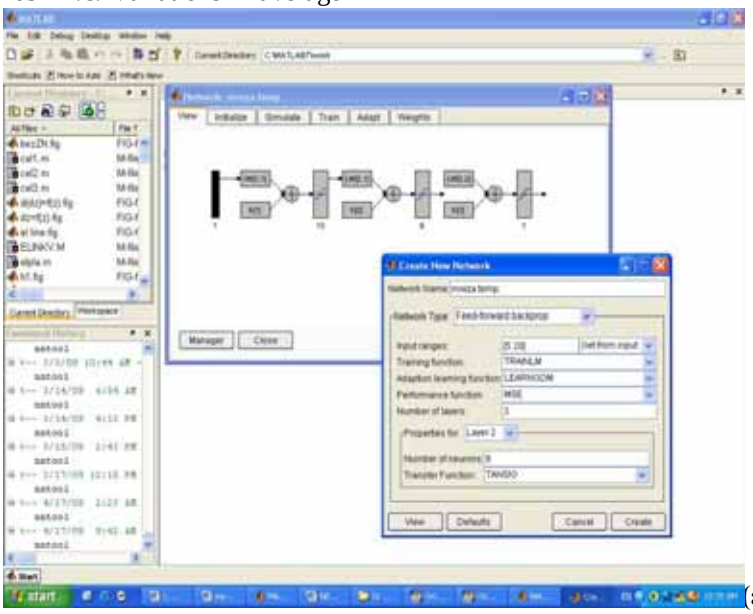
Statistics		Q, l/s	t,°C	pH	EC, µS/cm	DO, mg/l	BOD_5 mg/l	Na^+ mg/l	K^+, mg/l	Ca/Mg, mol ratio	Cl^-, mg/l	SO_4^{2-} mg/l	PO_4^{3-}, mg/l	TP mg/l	TN, mg/l
I SERIES	min	505	10	7.70	220	0	2.9	0.8	4	0.54	7.7	2.6	4.3	0.65	0.1
	max	505	17	8.53	285	8.1	6.2	1.7	12	14.28	65.3	14.4	680	13.8	0.7
	stdev	0	1.4	0.89	13.90	1.9	0.65	0.2	1.35	1.75	10.15	2	131.6	2.1	0.2
	median	505	12	8.05	270	1.6	5.2	1.3	9.3	1.57	14.1	10.2	23.9	1.7	0.4
II SERIES	min	4 680	10	7.25	120	0.3	2	0.2	3	0.47	3.9	7.2	1	0.03	0.05
	max	$11 \cdot 10^3$	13	8.17	210	13.5	6.4	1.7	11	6.31	39.7	13.9	1753	1.3	0.4
	stdev	$24 \cdot 10^3$	0.8	0.13	22.66	1.8	0.52	0.3	1.42	1.07	7.73	1.3	252.6	0.2	0.1
	median	7 690	11	7.84	180	3.7	2.85	0.6	5.6	1.21	16	11.1	7.6	0.1	0.05
SE-III SERIES	min	1 200	11	7.45	200	0.8	1.7	0.8	4	0.54	5.1	7.5	1.1	0.03	0.05
	max	$11 \cdot 10^3$	13.4	8.3	260	6	6.2	4	8.9	27.89	46.7	14.1	31.8	5.2	0.3
	stdev	2 375.9	0.6	0.15	15.99	0.9	0.7	0.6	0.85	3.37	6.57	0.9	4.3	0.6	0.1
	median	2 570	11.2	7.84	230	1.9	2.2	1	5.95	1.91	14.1	10.8	4.7	0.1	0.1
IV	min	460	10	7.8	245	0.6	2	1	7	1.47	8.98	3.1	0.8	0.05	0.02
	max	460	14	8.6	300	5.7	9.6	3.9	9.1	12.27	26.9	13.3	51.8	0.9	0.3
	stdev	0	0.8	0.18	14.43	1.1	1.49	0.4	0.53	1.70	4.79	1.9	13.9	0.2	0.05
	median	460	10	8.15	279	2.1	3.7	1.5	8	1.99	14.1	10.2	6.4	0.2	0.1
V SERIES	min	0.32	10	7.8	140	1	3.5	0.3	5	1.11	4.5	8	0.3	0	0.02
	max	24.55	12	8.4	285	6	10.1	5.9	32	3.74	38.4	12.5	28.6	14.3	0.4
	stdev	5.51	0.6	0.14	30.52	1.1	1.43	1.1	6.01	0.62	4.62	0.9	5.7	1.6	0.1
	median	3.86	10	8.15	200	2.1	5.6	1.9	7.25	2.09	10.9	10.8	7.4	0.1	0.1

Regardless of the annual period and very poor system for drainage of municipal wastewaters and atmospheric precipitations into the swallet P_0, the data obtained during the research period (Table 1) indicate relatively uniform quantities of water released into the swallet. High concentrations of contaminants are best reflected in the range of values of water temperature, pH, electrolytic conductivity, sodium, chloride, phosphate, etc.

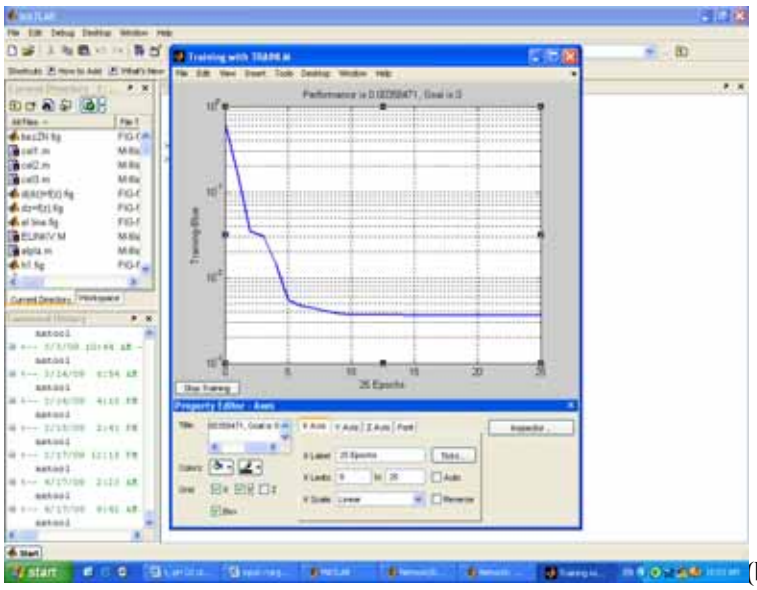
It can be noticed that the maximum values of most components, i.e. their contents are more evident during the morning working hours, while after midnight, when daily technical and technological processes are completed, significantly lower values of these components are recorded.

As a consequence of different hydrological periods of the year, the flow of water at the outlet (Table 2) from the karst underground was in the range of 0.32 l/s to 109 m^3/s. Variations in average values of temperature during the research period did not exceed 2°C, which is at the same time a characteristic of strong and permanent karst springs [12]. Based on the range of values for total hardness, the waters of the Crnojevica River spring are classified into soft to moderate hard waters.

The increased content of nitrogen compounds and organic matter in the period of high waters is a characteristic of karst springs [13]. The situation is different at the Crnojevica River spring. The slightly increased content of nitrogen compounds and organic matter was recorded in the period of low waters. Ammonia and nitrites - nitrogen assimilators - are formed by degradation of nitrogenous organic substances using enzymes of ammonification bacteria in certain ecological conditions [14]. The waters of this spring were constantly loaded with organic substances, as indicated by BOD_5.



(a)



(b)

FIGURE 2
Performance and convergence diagram of the neural network for temperature

The microbiological investigations showed that the spring waters were loaded with bacteria (24000 bacteria in 100 ml of water).

Testing neural networks using water quality indicators. The data normalized according to the previously defined method were incorporated into the neural network and the network performance was selected to make the output results for the selected parameters as close as possible to the actual ones. For each parameter a certain network performance was obtained and afterwards the simulation of such trained network was performed on the fifth series of data.

The Fig. 2 shows characteristics the of the network that has given the best results for temperature, for example. The selected neural network consists of three layers, of which the first one has 13, the other 9, and the last output layer according to rule 1 is a neuron (Fig. 2a). Although the set number of epochs (iteration) for this network was 100, it achieved this goal in 25 epochs with an accuracy of 10^{-3} (Fig. 2b).

A comparison of the results obtained by measurements and neural network for the selected parameters is shown in the Figure 3-8.

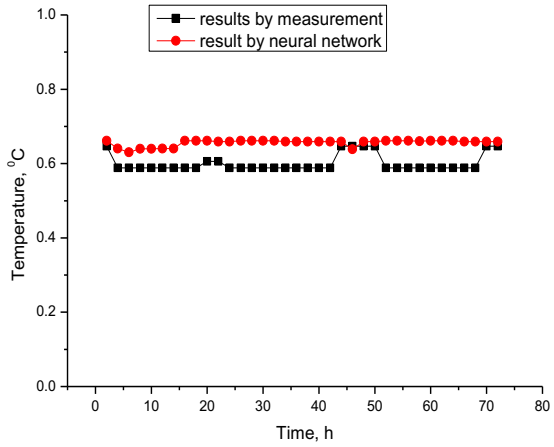


FIGURE 3
Comparison of the results obtained by measurements and neural network for the temperature

Temperature. The results obtained by simulation correspond with the results obtained by measurements, as shown in Fig. 3. Since water temperature is a crucial factor affecting many natural processes that occur in water, such trained neural network that yields satisfactory results can indicate industrial emissions, cooling water intake or other inputs that are the result of accidents.

Electrolytic conductivity. By simulation of neural network the obtained results (Fig. 4) follow the dynamics of the change in electrolytic conductivity values obtained by measurements. Electrolytic conductivity is an important summary parameter for dissolved, dissociated substances and, therefore, it is suitable for tests that determine changes in ion concentrations over time, as indicated by the neural network trained in this manner.

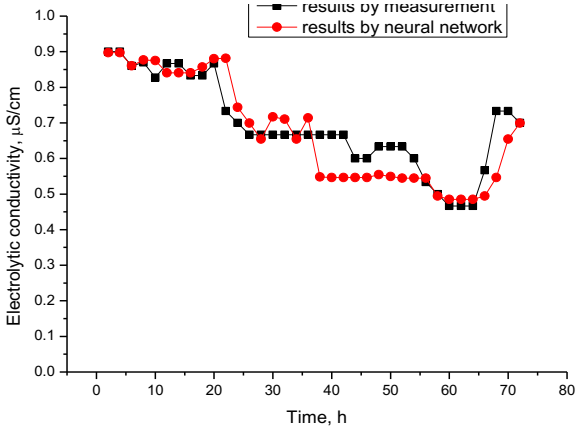


FIGURE 4
Comparison of the results obtained by measurements and neural network for the electrolytic conductivity

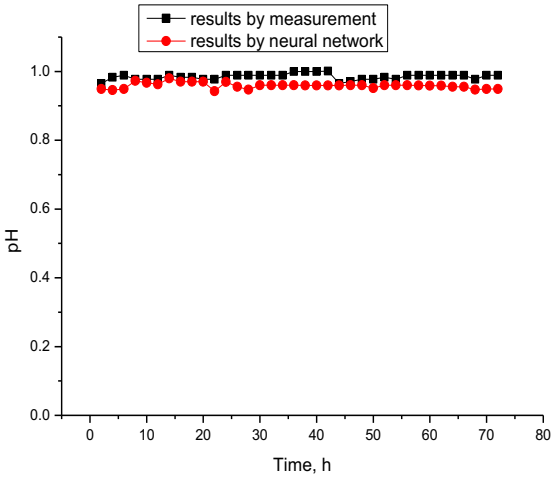


FIGURE 5
Comparison of the results obtained by measurements and neural network for the pH

pH. The trained neural network gave satisfactory output data for the pH value (Fig. 5). This gives us the possibility of timely action in case of accidents caused by the absorption of acid and alkaline wastewater, since the water is retained for a certain period of time in the karst underground. With an increase in the pH value, the NH_4^+/NH_3 balance is displaced in favour of ammonia. If the pH value exceeds 9, then ammonium ion (NH_4^+), which is almost non-toxic, is mostly converted into ammonia (NH_3), a strong cell toxin. The pH value is influenced by numerous exogenous factors (e.g. alkaline solutions, limewater, washing) and endogenous factors derived from metabolic activity of plants (especially macrophytes and phytoplankton), and the buffer capacity of the water body is naturally defined by the chemical activity of its basin.

BOD$_5$. The results in a certain degree deviate from the results obtained by measurements (Fig. 6). However, there is a relatively uniform trend in increase and decrease in the concentration of BOD_5 in both cases, which is somewhat satisfactory, since such trained neural network can indicate the influence of the contaminants at the inlet which influence the change of natural BOD_5 value in the water of the Crnojevica River spring. Naturally, the obtained results also point to the fact that the network cannot register the effects of some other contaminants that do not reach the major swallet, but are washed from the basin into the spring through groundwater.

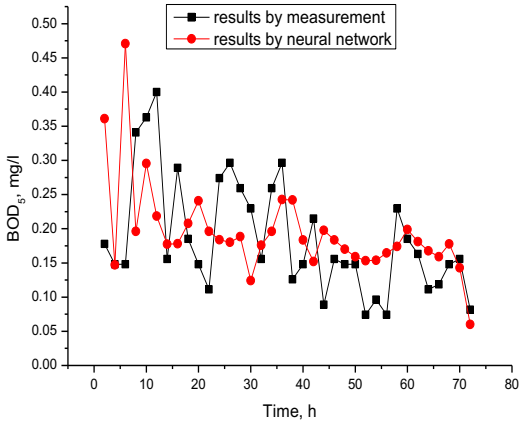


FIGURE 6
Comparison of the results obtained by measurements and neural network for the BOD_5

Total N. The results obtained by simulation of trained neural network in the case of total nitrogen show maximum compatibility with the measurement results (Fig. 7) compared to other included indicators of the quality of wastewater and spring water. This is of great importance in case of accidents, because we could take timely measures to protect the waters of the Crnojevica River spring, since water in the underground is retained for at least 55 hours.

Total P. The simulation results for total P show deviations from the measurement results (Fig. 8), indicating very complex processes of transferring phosphorus into karst underground waters, as opposed to other pollutants that are washed from the basin into the spring waters. However, the change trend of total P concentration obtained by simulation follows to a certain extent the values obtained by measurements, and such trained neural network will indicate the value expected at the outlet, on the basis of the inlet total P value in wastewater.

In order for neural networks to achieve even better results for all included quality indicators of wastewater and spring water, it is necessary to increase the number of training samples, i.e. much more training data are needed to make the output data as close as possible to the actual ones.

The automatic measuring stations for continuous measurement of water quantity and quality parameters can provide enough data to make the use of neural networks as efficient as possible.

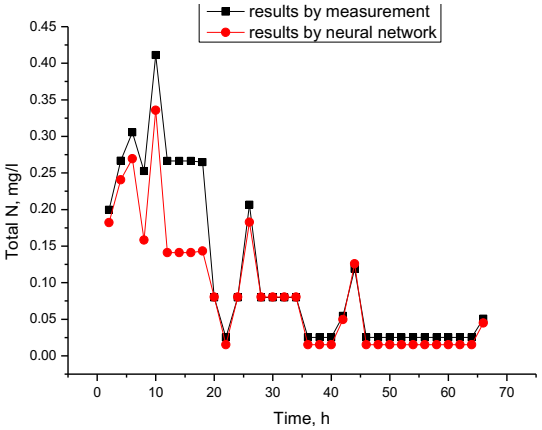


FIGURE 7
Comparison of the results obtained by measurements and neural network for the total N

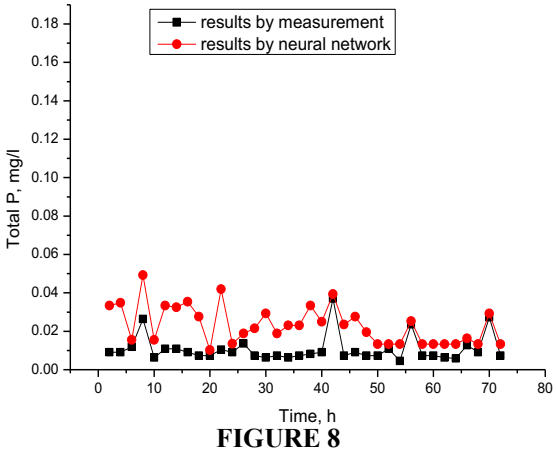


FIGURE 8
Comparison of the results obtained by measurements and neural network for the total P

CONCLUSIONS

Waters, especially in the Mediterranean karst area, are usually the most important natural resource that influences economic and general development of the area. The problems of using and protection of water resources are extremely important in these conditions.

The method applied in this paper enabled a realistic view of the relation between contaminants and recipient by studying the transfer of contaminants in karst underground waters, as a precondition of the created wastewater management model for settlements using neural networks.

The analysis of the obtained results at the swallet and spring and their statistical analysis enabled the training of neural networks. The results obtained by simulation of neural networks, on the basis of the included indicators of wastewater and spring water quality, indicate maximum compatibility with the measurements results in the case of total nitrogen, while to a lesser extent in the case of all other tested indicators.

The research presented in this paper is a contribution to the development of methods for the use and protection of karst waters through the development of the applied model. This model proved to be a reliable control element in defining and timely undertaking the necessary protection measures, especially in case of accidental contamination of the Mediterranean karst waters.

REFERENCES

[1] Goldscheider, N., Drew, D. (2007) Combined use of methods. In: Book Methods in Karst Hydrogeology. 1st ed. Taylor and Francis Group, Leiden, Netherlands. 266p.

[2] Hartmann, A., Goldscheider, N., Wagener, T., Lange, J., Weiler, M. (2014) Karst water resources in a changing world: Review of hydrological modeling approaches. AGU Publications, Reviews of Geophysics. 218-242.

[3] Bakalowicz, M. (2005) Karst groundwater: A challenge for new resources. Hydrogeology Journal. 13(1), 148–160.

[4] Vesper, J.D., Loop M.C., White B.W. (2003) Contaminant transport in karst aquifers. Speleogenesis and Evolution of Karst Aquifers. 1(2), 1-11.

[5] Kacaroglu, F. (1999) Review of groundwater pollution and protection in karst areas. Water, Air and Soil Pollution. 113, 337-356.

[6] Ford, D., Williams, P. (2007) Karst Water Resources Management. In: book Karst Hydrogeology and Geomorphology. John Wiley and Sons, England, 441-469.

[7] European Council (2000) Water Framework Directive 2000/60/EC.

[8] Tadić, M., Filipović, S. (2011) Basin Water Management in a Coastal Karst Area. Polish J. of Environ. Stud. 20(2), 461-467.

[9] Bonacci, O. (2015) Karst hydrogeology/hydrology of dinaric chain and isles, Environmental Earth Sciences. 74 (1), 37-55.

[10] Milanović, S. (2007) Hydrogeological characteristics of some deep siphonal springs in Serbia and Montenegro karst. Environmental Geology. 51(5), 755.

[11] APHA-AWWA-WEF (2005) Standard methods for the examination of water and wastewater. 21st ed.; Andrew D. Eaton, Lenore S. Clesceri, Eugene W. Rice, Arnold E. Greenberg, American Public Health Association: 800 I Street, NW Washington, Volume 1.

[12] Luhmann, A.J., Covington M.D., Peters A.J., Alexander S.C., Anger C.T., Green J.A., Runkel A.C., Alexander E.C. (2011) Classification of thermal patterns at karst springs and cave streams. Ground Water. 49(3), 324-335.

[13] Preka, N., Preka-Lipold, N., Avdagić, I. (1985) The selfpurification Capability of Karst Underground Watercourses With Respect to the pollution of the Mediterranean Sea, Pollution of the Mediterranean sea, Yugoslav Society for water pollution control. Split, Yugoslavia. 267-277.

[14] Herbert, R.A. (1999) Nitrogen cycling in coastal marine ecosystems. FEMS Microbiology Reviews. 23, 563-590.

Received: **11.10.2018**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Milena Tadic
University of Montenegro,
Faculty of Metallurgy and Technology
George Washington bb,
81000 Podgorica – Montenegro

e-mail: milenak@ac.me

© by PSP Volume 27 – No. 12B/2018 pages 9542-9548 Fresenius Environmental Bulletin

IMPORTANT PARAMETERS IN MECHANICAL MANAGEMENT OF CAROB MOTH [*APOMYELOIS* (=*ECTOMYELOIS*) *CERATONIAE* ZELLER (LEP.: PYRALIDAE)] IN POMEGRANATE ORCHARDS: DETERMINATION OF OVERWINTERING POPULATION DENSITY AND INFESTATION RATE

Mehmet Mamay*

Harran University, Agricultural Faculty, Department of Plant Protection, Şanlıurfa, Turkey

ABSTRACT

Carob Moth [*Apomyelois (=Ectomyelois) ceratoniae* Zeller (Lepidoptera: Pyralidae)] is a key pest in pomegranate orchards, *Punica granatum* L. (Myrtales: Punicaceae) in the Southeastern of Turkey. The pest causes significant damage and reduces marketability of fruits and is difficult to control using insecticides. The pest generally overwinters at different larval stages in infested pomegranate fruits. Therefore, infested fruits hanging on the trees or fallen on the ground are important for the Carob Moth's summer population in pomegranate orchards. This study was aimed to determine the infestation rate of *A. ceratoniae* and overwintering larvae population density of the pest on pomegranate fruits remaining on or under the trees. This study was carried out in two pomegranate orchards located in Şanlıurfa during 2015-2016 and 2016-2017 winter periods. In the study, 20 hanging fruits and 20 fallen fruits (200 fruits per orchard per year) were collected in each row. The infested and non-infested fruits registered individually. The infested rate with Carob Moth was then calculated. Also, overwintering larvae numbers were determined from collected infested pomegranate fruits. As a result of the study, the maximum infestation rate of fallen fruits was determined as 52% while infested hanging fruits were determined as 25% in Central County in 2016-2017 winter. The minimum infestation rate of fallen and hanging fruits rate were determined as 26% and 12% in Suruç County in 2015-2016 and 2016-2017 winter seasons respectively. The maximum overwintering larvae density of the pest was determined from fallen fruits as 120 larvae/100 infested fruits in Suruç in 2016-2017 winter. Conversely, the minimum overwintering larvae density of the pest was determined from hanging fruits in Suruç pomegranate orchard in the first working year with 40 larvae/100 infested fruits. Removal of those infested pomegranate fruits from the orchards in terms of mechanical management of *A. ceratoniae* in winter is very important to reduce the population density of the pest in spring and summer period in pomegranate orchards.

KEYWORDS:
Ectomyelois ceratoniae, pomegranate, overwintering, population density, environmentally friendly pest management

INTRODUCTION

Turkey is one of the leading pomegranate growing countries. The crop popularity is growing and production areas have expanded in Turkey. Pomegranate is widely grown in Aegean, Mediterranean and Southeast Anatolia regions of Turkey. Turkey owns 16.000.000 pomegranate trees and pomegranate production was 383.000 tons in 2013. Şanlıurfa province had 1.325.000 pomegranate trees and the annual pomegranate production was 6.400 tons [1].

There are many pests that impede pomegranate production and reduce yield and quality. The Carob moth is one of the major pests that can cause significant damage on pomegranate fruits. Carob moth is a key pest in pomegranate orchards in Southeastern Anatolia Region of Turkey and this pest causes up to 80% of damage [2].

Carob Moth larvae first feed on petals and then bore into fruits. Larval feeding causes brown lesions on pomegranate fruit skin near petals. Advanced lesions cause hollow, cracked and rotten fruits that are not marketable. Inside heavily damaged pomegranate fruits are black in color and can be completely covered in mold [3, 4, 5, 6].

The pest causes significant damage and reduces marketability of fruits, and is difficult to control using insecticides. Despite 4-5 conventional pesticides available for application, the pest remains a significant problem in pomegranate production in Turkey. Therefore, the use of insecticides is not alone appropriate because the larvae are protected from insecticides inside the pomegranate fruit [7].

TABLE 1
Description of pomegranate orchards and their locations where included in the study

District	Orchard Location	Coordinates	Cultivar	Tree age (Year)	Area(m^2)	Altitude (m)
Şanlıurfa-Central	Bakımlı	N37°10′26,00″ E39°02′07,50″	Local variety	50-60	20.000	482
Şanlıura-Suruç	Aligör	N37°01′13,90″ E38°26′18,70″	Local variety	20-30	10.000	511

The pest overwinters at different larval stages in infested pomegranate fruits. Therefore, infested fruits hanging on the trees or fallen on the ground are important for the Carob Moth's summer population in pomegranate orchards. In addition, removing infested fruits from orchards through mechanical management is vital in order to control the pest. This study aimed to determine the infestation rate of *A. ceratoniae* and overwintering larvae population density of the pest on pomegranate fruits remaining on or under the trees in pomegranate orchards in Southeastern Anatolia.

MATERIAL AND METHODS

Material. The main analyzed material of the study was fallen and hanging pomegranate fruits in winter season. This study was carried out during 2015-2016 and 2016-2017 winter periods in two pomegranate orchards established 5x5 m within and between rows with local cultivars and located in Şanlıurfa Central and Suruç County. Description of the pomegranate orchards included the study are given in Table 1. Sampling and counting procedures were carried out in December and January of corresponding years.

Weather data were acquired from the Şanlıurfa Regional Directorate of Meteorology Station in order to compare both years' ecological conditions.

Method. The trial was designed in a randomized complete block design with five replications. For this purpose, 20 hanging fruits on trees and 20 fallen fruits on the ground were collected in each pomegranate row. Since some trees had no pomegranate fruits, rows are accepted as replication instead of trees. In this way, 100 hanging and 100 fallen pomegranate fruits were collected and controlled in total per orchard and per year.

Fruits were brought to the laboratory for evaluation of the percentage of the infested fruits and larvae numbers. The infested and non-infested fruits registered individually and the infested rate with Carob Moth is calculated for each orchard in terms of fallen and hanging fruits individually using the following equation:

$$\textit{Infestation rate}\ (\%) = \frac{\textit{Infested fruit number}}{\textit{Examined total fruit number}} \times 100 \quad (1)$$

Also overwintering larvae numbers were determined from collected infested pomegranate fruits in both locations and corresponding years. Calculation was done only in terms of infested fruits. Therefore, in order to objective comparison, it was converted to an average for 100 infested fruits (Equation 2).

$$\textit{Larva number} = \frac{\textit{Total larva number}}{\textit{Total infested fruit number}} \times 100 \quad (2)$$

Statistical Analysis. Collected data of infestation rates and larva population density were tested in terms of normality and due to normality of data no transformation was needed. Infestation rates and overwintering larva population density of fruits fallen on the ground and hanging on the trees were tested by Independent T-Test in corresponding locations and years. Results are presented herein as means per treatment (SEM). All data were compared by locations, years, criterion and methods using the statistical computer packet program JMP 8 (SAS Institute Inc.).

RESULTS AND DISCUSSION

The mean infestation rate of fruits by *A. ceratoniae* according to locations in 2015-2016 and 2016-2017 winter seasons are shown in Fig. 1.

As a result of the study, the wintertime infestation rate of fallen fruits in Şanlıurfa-Central County and Suruç County were determined as 50% and 52% in 2015-2016 and 2016-2017 respectively while hanging fruits were found to be infested at a rate of 17% and 25% respectively. In the same way, infestation rate of fallen fruits in Şanlıurfa-Suruç County were determined as 31% and 26% in 2015-2016 and 2016-2017 wintertime while hanging fruits were 12% and 15% according to the corresponding years (Fig. 1). It has been reported that this pest causes up to 80% of damage in pomegranate orchards in the summer season [2]. Similarly, some researchers stated that Carob Moth damage rate can be even higher (between 20-80%) in uncared for pomegranate orchards [8].

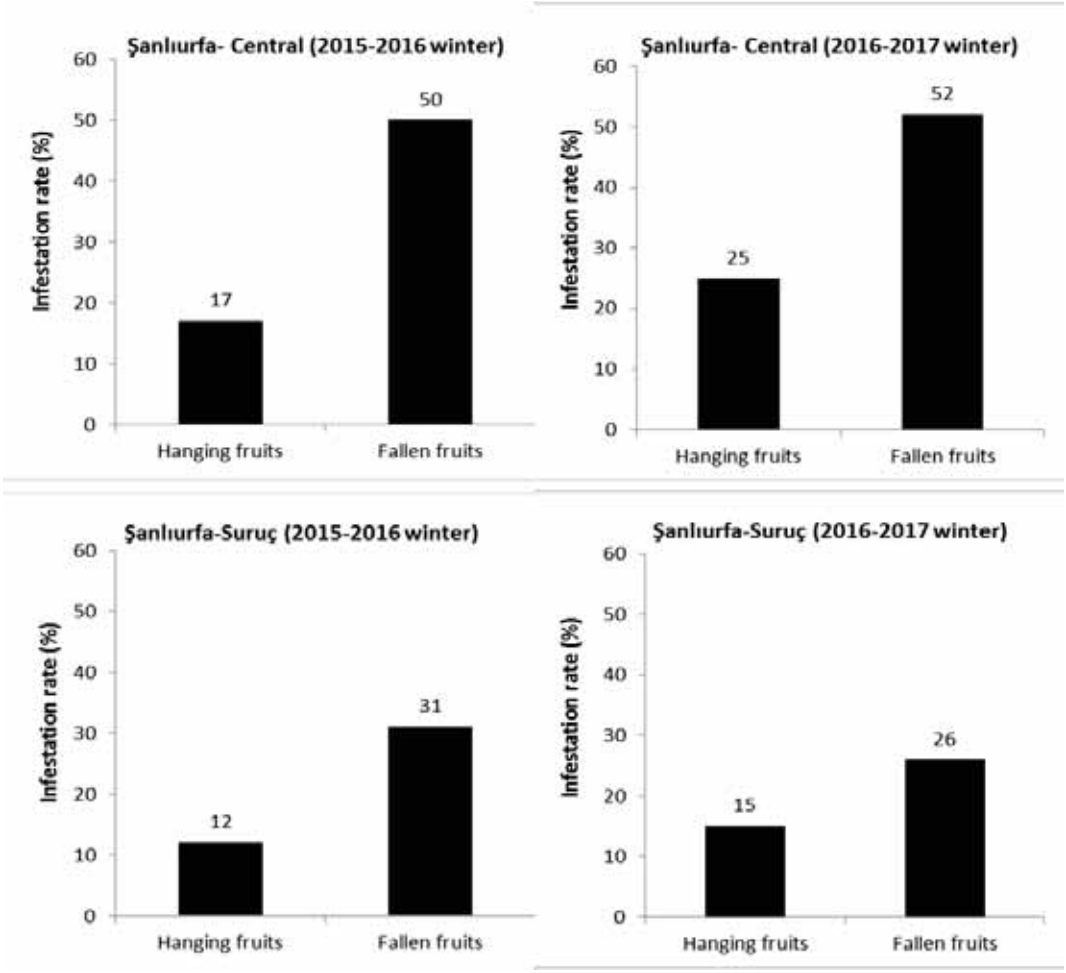


FIGURE 1
The infestation rate of pomegranate fruits by Carob moth in 2015-2016 and 2016-2017 winter season in Şanlıurfa province.

TABLE 2
Carob moth infestation rate of fallen and hanging fruits by years and locations according to Independent T-Test

METHOD	YEARS	N	$\bar{X}$	SD	SEM	df	t	p
Infestation rate of Fallen Fruits	2015-2016	10	40.50	15.3569	4.8563	18	-0.21669	0.8309 (p>0.05)
	2016-2017	10	39.00	15.5991	4.9329			
	LOCATIONS							
	Central	10	51.00	11.2546	3.5590	18	-5.4167	0.0001 (p<0.05)
	Suruç	10	28.50	8.5147	2.6926			
METHOD	YEARS	N	$\bar{X}$	SD	SEM	df	t	p
Infestation rate of Hanging Fruits	2015-2016	10	14.50	4.97214	1.5723	18	2.087939	0.0525 (p>0.05)
	2016-2017	10	20.00	6.68331	2.1134			
	LOCATIONS							
	Central	10	21.00	5.79272	1.8318	18	-3.19963	0.0052 (p<0.05)
	Suruç	10	13.50	4.62481	1.4625			

Unfortunately, there is no study conducted on overwintering infestation rate and larva density in pomegranate orchards in literature except [9]. Therefore we couldn't discuss the study with literatures sufficiently. This study aims to contribute to this field. According to the data above, the maximum infestation rate was determined from fallen fruits rather than those of hanging fruits not only in both locations but also at both years. In parallel with our study, [9] reported that the remaining fruits underneath of the trees were more infested by *A. ceratoniae* than the fruits on the trees in two consecutive years in Iran significantly. This means that fallen fruits dropped because of Carob Moth infestation. The hanging fruits generally are the fruits from the last flowers and are small to collect during harvest. Also, they cannot ripen because of insufficient time from last flowers to harvest.

According to Shapiro Wilk Normality Test, infestation rate of fallen and hanging fruits were of a normal distribution in both years and also both locations (Shapiro Wilk: W>%90; p>0.05). A significant difference was determined between Şanlıurfa Central and Suruç locations on the basis of Carob Moth infestation rate of fallen fruits ($t_{(18)}$= -5.4167; p=0.0001<0,05), while there was no significant difference between years on the basis of fallen fruits infestation ($t_{(18)}$= -0.21669; p=0.8309>0.05) (Figure 1; Table 2). In the same way, there is no difference between years ($t_{(18)}$= 2.087939; p=0.0525>0.05) on the basis of the infestation of hanging fruits and there is significant difference between Central and Suruç Counties ($t_{(18)}$= -3.19963; p= 0.0052<0.05).

In a study conducted in the same locations, it was determined that pomegranate fruits were infested by Carob Moth 48% and %28.5 in summer in Şanlıurfa Central and Suruç Counties respectively [2]. Another study, conducted in different three regions of Iran, showed that infestation rate of Carob Moth on pomegranate fruit was significantly lower in Saveh than that in Varamin and Qom but there was no significant difference between Qom and Varamin [9]. In another study, carried out in 9 pomegranate orchards in Hatay Province of Turkey in 2008-2009 summer season in order to determine the infestation rate of the Carob Moth, it was determined that the pomegranate fruit infestation rate with *A. ceratoniae* was varied from 13% to 40% [10].

The overwintering larval population density of *A. ceratoniae* according to the fallen and hanging infested fruits, in terms of locations in 2015-2016 and 2016-2017 winter seasons are shown in Figure 2.

According to the data of the study, both maximum and minimum overwintering larva density were determined in Suruç County. The maximum overwintering larvae density of the pest was determined from fallen fruits as 120 larva/100 infested fruits in Suruç in 2016-2017 wintertime. Surprisingly, the minimum overwintering larvae density of the pest was determined from hanging fruits in the first working year with 40 larvae/100 infested fruits in the same orchard (Fig. 2). Moreover, the larva number we determined to be higher in terms of infested hanging fruits in the second working year in contrast to the infested fallen fruits in both locations.

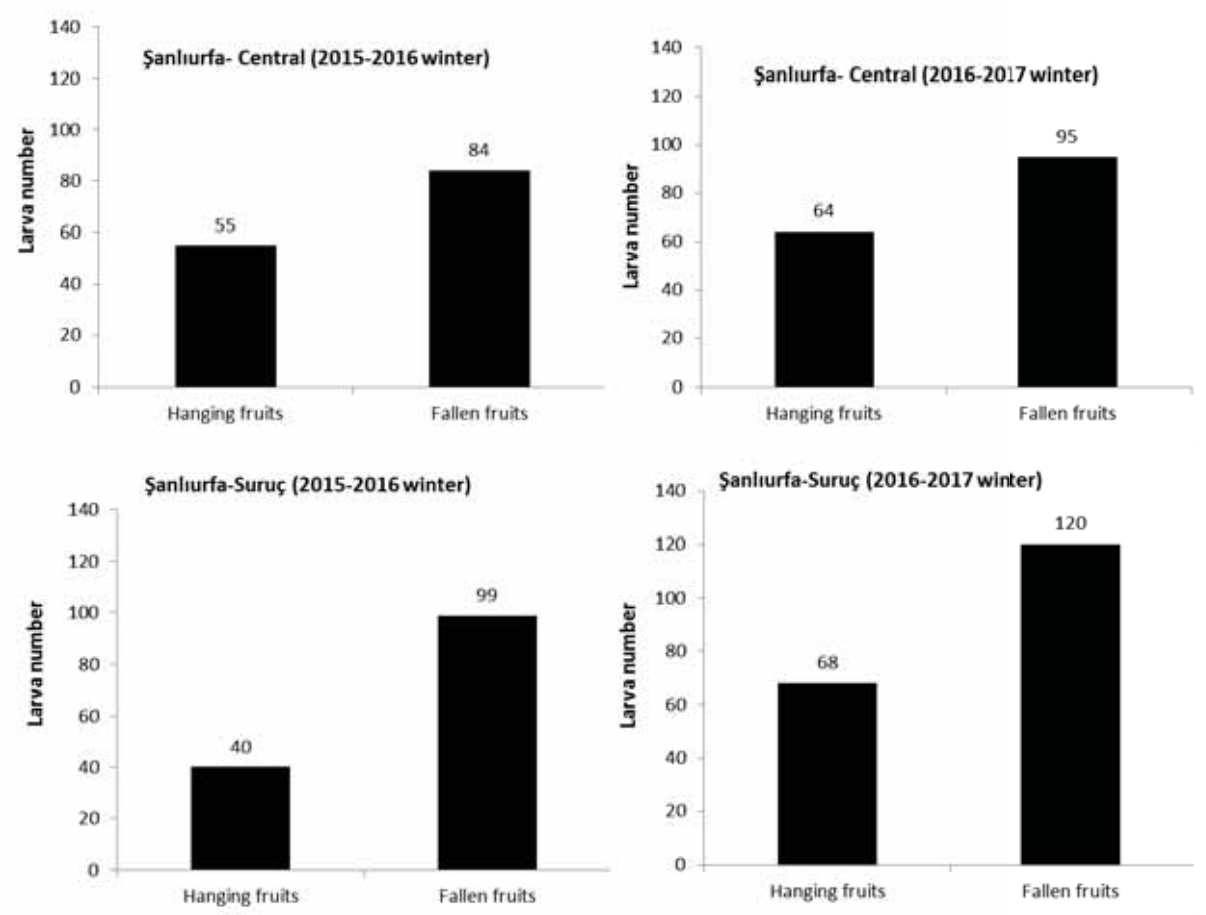


FIGURE 2
Carob moth overwintering larva density in pomegranate fruits in Şanlıurfa province in 2015-2016 and 2016-2017 winter seasons.

The collected data of overwintering larva density were tested in terms of normality by Shapiro Wilk Normality Test. The data were normal distribution both years and locations (Shapiro Wilk: W>%90; p>0.05). And due to normality of data no transformation was needed. Independent T-Test Analysis indicated that the overwintering larva density was no different in terms of years ($t_{(18)}$= -0.61967; p=0.5440>0.05) and locations ($t_{(18)}$= -0.34837; p=0.7318>0.05) on the basis of fallen fruits (Table 3). However, it was determined that the overwintering larva density was difference significantly in terms of years ($t_{(18)}$= 2.433661; p=0.0261<0.05) on the basis of hanging fruits, on the contrary there is no difference by locations ($t_{(18)}$= 0.99909; p=0.3327>0.05) (Table 3).

Table 4 can be concluded statistically in terms of sample method as fallen and hanging fruits according to infestation rate and population density ignoring years and locations.

As a result of the study, Independent T-Test analysis indicated that the infestation rate of Carob Moth is different between fallen and hanging fruits significantly ($t_{(38)}$= -6.14201; p<0.05). Conversely, there is no significant difference between fallen and hanging fruits in terms of overwintering larva density ($t_{(38)}$= -1.28947; p>0.05) (Table 4).

Average weekly temperature in Şanlıurfa for 2015-2016 and 2016-2017 winter seasons are shown in Figure 3.

TABLE 3
Carob moth overwintering larva density in infested fallen and hanging fruits by years and locations according to Independent T-Test

METHOD	YEARS	N	$\bar{X}$	SD	SEM	df	t	p
Overwintering Larva Density in Infested Fallen Fruits	2015-2016	10	91.69	42.0955	13.312	18	-0.61967	0.5440 (p>0.05)
	2016-2017	10	81.50	30.5305	9.655			
	LOCATIONS							
	Central	10	89.48	32.4920	10.275	18	-0.34837	0.7318 (p>0.05)
	Suruç	10	83.71	41.0804	12.991			
METHOD	YEARS	N	$\bar{X}$	SD	SEM	df	t	p
Overwintering Larva Density in Infested Hanging Fruits	2015-2016	10	47.50	45.1175	14.267	18	2.433661	0.0261 (p<0.05)
	2016-2017	10	92.00	36.1649	11.436			
	LOCATIONS							
	Central	10	59.50	36.6517	11.590	18	0.99909	0.3327 (p>0.05)
	Suruç	10	80.00	53.5426	16.932			

TABLE 4
Carob moth infestation rate and overwintering larva density in infested fallen and hanging fruits according to Independent T-Test

CRITERION	METHODS	N	$\bar{X}$	SD	SEM	df	t	p
Infestation Rate	Fallen fruits	20	39.7500	15.0853	3.3732	38	-6.14201	0.0001 (p<0.05)
	Hanging fruits	20	17.2500	6.3898	1.4288			
Population Density	Fallen fruits	20	86.5950	36.1695	8.088	38	-1.28947	0.2055 (p>0.05)
	Hanging fruits	20	69.7500	45.8789	10.259			

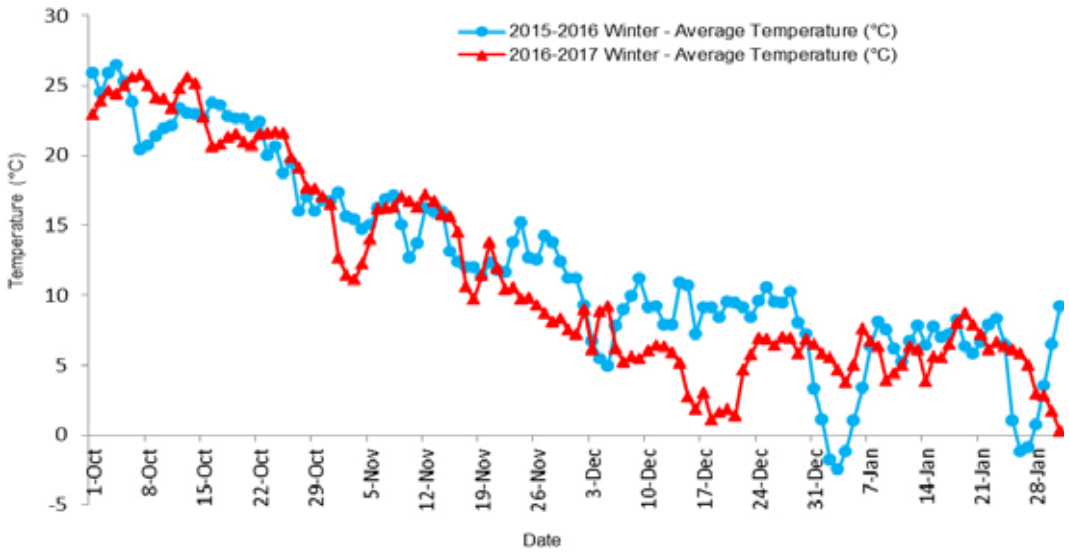


FIGURE 3
Average weekly temperature in Şanlıurfa for 2015-2016 and 2016-2017 winter seasons.

As shown in Fig. 3, winter average weekly temperature pattern wasn't so different between the two years. Nevertheless, the temperature in December was higher in 2015 than in 2016 year. Also, the minimum temperature was measured in January in 2016 as 2.5 degrees below zero while it was never measured below zero during the second year of the study. This means the greatest temperature fluctuation occurred in the 2015-2016 winter season.

Several researchers conducted studies to control the *A. ceratoniae* damage in pomegranate orchards such as chemical management [11, 12, 7]; biotechnological management including mating disruption and mass trapping [13, 14, 7, 15]; biological control [16, 17] and mechanical management [16, 9].

Unfortunately, from these management methods, there isn't any method which can be recommended alone. Therefore [7] suggested that integration of mating disruption technique with a chemical or biological pesticide application, weekly destruction of infested fruits and prevention fruit cracks are all important for success of integrated pest management. Many studies confirm these thoughts such as [9] and [2]. For instance, [9] reported that the most recommended procedure to control this pest is collecting and destroying infested fruits in the orchards at the end of cropping season to eliminate overwintering sites.

CONCLUSION

In contrast to the above authors, some researchers expressed their concerns about collecting and destroying infected fruits in the orchards at the end of cropping season due to negative effects on naturel enemies such as parasitoids and a decrease in biodiversity. This idea has been shared by us to some extent since larval parasitoids of Carob Moths in overwintering seasons might pressure the population of the pest at the beginning of the following cropping season. However, this can only be expected in pomegranate orchards in Southeastern Anatolia.

Infested fruits hanging on the trees or fallen on the floor are important in the management of Carob moth. Since the pest overwinters at different larval stages in infested pomegranate fruits, removal of those infested pomegranate fruits from the orchards during the winter season through mechanical management is very important to reduce the Carob Moth population density in the spring and summer periods.

ACKNOWLEDGEMENT

We are grateful to Bjorn Betzler (am.bekaa@drclebanon.dk, native speaker in English, USA,) from Danish Refugee Council for critically, grammatically and linguistically reading this manuscript.

The first year data of this study was presented as poster and published abstract in the Turkey 6th Plant Protection Congress with International Participation in Konya, Turkey at 5-8 May 2016 while the second year data was presented as oral presentation and published in abstract book in the Symposium on EuroAsian Biodiversity in Minsk, Belarus at 5-8 July 2017.

REFERENCES

[1] Turkstat, (2015) Turkish Statistical Institute. The Turkey Statistical Institute data. (https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul). (Access date: November 2017).

[2] Mamay, M., Ünlü, L. Yanık, E. and İkinci, A. (2014a) Infestation and prevalence situation of Carob moth *Apomyelois ceratoniae* Zell. (Lepidoptera: Pyralidae) in pomegranate orchards in Şanlıurfa. Turkish Bulletin of Entomology. 4(1), 47-54.

[3] Mamay, M., Ikinci, A., Ünlü, L. and Doğan, E. (2014b) Infestation rate and host preference of carob moth [*Apomyelois* (=*Ectomyelois*) *ceratoniae* Zeller (Lepidoptera: Pyralidae)] on different pomegranate cultivars. Turkish Journal of Entomology. 38(1), 101-110.

[4] Mamay, M. and Ünlü, L. (2013) Determination of adult population development and harmful rate of carob moth, *Apomyelois ceratoniae* Zell. (Lepidoptera: Pyralidae) in pomegranate orchards in Sanlıurfa province. Turkish Bulletin of Entomology. 3,121-131.

[5] Mozaffarian, F., Sarafrazi, A. and Ganbalani, G.N. (2007) Host plant-associated population variation in the carob moth *Ectomyelois ceratoniae* in Iran: A geometric morphometric analysis suggests a nutritional basis. Journal Insect Sciense. 7 (2), 11.

[6] Norouzi, A., Talebi, A.A. and Fathipour, Y. (2008) Development and demographic parameters of the Carob moth *Ectomyelois ceratoniae* on four diet regimes. Bulletin of Insectology. 61 (2), 291-297.

[7] Mamay, M., Ünlü, L., Yanık, E., Doğramacı, M. and İkinci, A. (2016) Efficacy of mating disruption technique against carob moth, *Apomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) in pomegranate orchards in Southeast Turkey (Şanlıurfa). International Journal of Pest Management. 62(4), 295-299.

[8] Al-Izzi, M.A. Al-Maliky, J.S., Younis, K.M. A. and Jabbo, N.F. (1985) Bionomics of *Ectomyelois ceratoniae* (Zell.) (Lep.: Pyralidae) on pomegranate in Iraq. Environmental Entomology. 14, 149–153.

[9] Kishani-Farahani, H., Goldansaz S.H. and Sabahi, Q. (2012) A survey on the overwintering larval parasitoids of *Ectomyelois ceratoniae* in three regions in Iran. Crop Protection. 36, 52-57.
[10] Uluç, F.T. and Demirel, N. (2011) "Detecting and monitoring the Carob Moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) by using sex pheromone traps to determine its distribution, population density and damage level in pomegranate orchards in Hatay province, 202". Türkiye IV. Bitki Koruma Kongresi (28-30 Haziran 2011, Kahraman-maraş) Bildirileri. 496 s.
[11] Warner, R.L., Barnes, M.M., and Laird, E.F., (1990) Chemical control of a Carob Moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyrali-dae) and various Nitidulid Beetles (Coleoptera) on 'Deglet Noor' dates in California. Journal of Economic Entomology, 83 (6), 2357-2361.
[12] Shakeri, M., (2004) A review on investigations on pomegranate neck worm in Iran. In: A Proceeding on Evaluation of Finding and Current Problems Associated with Spectrobates ceratoniae Management in Pomegranate. Ministry of Jihad-e-Agriculture, Organization of Research and Education.
[13] Kehat, M., Blumberg, D., Dunkelblum E. and Anshelevich, L. (1995) Experiments with synthetic sexpheromones for the control of the raisin moth, and for monitoring the carob moth in date plantations. Alon Hanotea. 49, 284e290.
[14] Vetter, R.S., Millar, J.G., Vickers, N.J. and Baker, T.C. (2006) Mating Disruption of Carob Moth, *Ectomyelois ceratoniae,* with a sex pheromone analog. Southwestern Entomologist, 31 (1), 33-47.
[15] Mamay, M. and Dağ, E. (2016) "Efficacy of Mass Trapping Technique against Carob moth [*Apomyelois* (=*Ectomyelois*) *ceratoniae* Zell. (Lepidoptera: Pyralidae)] in pomegranate orchards, 2:36-42".II.International Multidisciplinary Congress Of Eurasia (11-13 July 2016, Odessa-Ukrayna) proceedings. 773 pp.
[16] Tokmakoğlu, C., Soylu O.Z. and Devecioğlu, H., (1967) Myelois ceratoniae Zeller'in biyolojisi ve mücadele metotları üzerinde araştırmalar. Plant Protection Bulletin. 7 (3), 91-106.
[17] Nasrollahi, A.A., Shojai, M. and Ziaii, M., (1998) "Large scale production and application of Trichogramma wasps for biological control of pomegranate moth Ectomyelois ceratoniae in Yazd province, 167". 13th Iranian Plant Protection Congress, Proceeding of Junior College of Agriculture, Karaj, p. 167.

Received: **18.01.2018**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

Mehmet Mamay
Harran University, Agricultural Faculty,
Department of Plant Protection
Osmanbey Campus, 63100, Şanlıurfa, Turkey

e-mail: mehmetmamay@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9549-9556 Fresenius Environmental Bulletin

STUDIES ON THEORETICAL CALCULATIONS OF CORROSION INHIBITION BEHAVIOR OF PYRIDAZINE AND PYRAZOLE DERIVATIVES

Erdem Ergan[1,*], Esvet Akbas[2]

[1]Department of Property Protection and Security, Van Vocational School of Security, University of Van Yuzuncu Yil, 65080 Van, Turkey
[2]Department of Chemistry, Faculty of Science, University of Van Yuzuncu Yil, 65080 Van, Turkey

ABSTRACT

The corrosion inhibition behaviors were investigated earlier synthesized pyridazine and pyrazole derivatives. The quantum chemical parameters of compounds calculated using density functional theory (DFT) at B3LYP/6-31G (d, p) level. Theoretical calculations showed that the compound BMPPCN could be used as a corrosion inhibitor.

KEYWORDS:
Pyrazole, Pyridazine, Corrosion Inhibition, Molecular Modeling, DFT.

INTRODUCTION

The deterioration of materials due to corrosion causes economic loss. A wide variety of research is conducted to prevent this harmful process. One of the excellent methods to protect materials against corrosion is use of heterocyclic compounds containing π-electrons [1]. The physicochemical parameters of the molecules are important because they determine adsorption on the metal surface. Most effective inhibitor molecules behave as both electron donor and electron acceptor [2]. When using heterocyclic compounds for corrosion inhibition are sharing the lone pairs on hetero atoms or π electrons to over metal surface and the molecule is adsorbing on the metal surface [3].

The quantum chemical calculations have been widely used to the reactivity of organic compounds for corrosion inhibition [4]. The inhibitor activities are involved molecular geometry and orbitals of the organic compounds and correlated with frontier orbital energy. The highest occupied molecular orbital energy (E_{HOMO}) is associated with the electron donating ability of the molecule. There is a good correlation between the speed of corrosion and E_{HOMO}. The adsorption of the molecule on the metal surface can occur on the basis of donor–acceptor interactions between the lone pairs on hetero atoms or π electrons [5]. The high E_{HOMO} value has a molecule tendency to give electrons, while a low E_{LUMO} value indicates the ability of the molecule to accept electrons. The difference between E_{LUMO} and E_{HOMO} energies is called energy gap. Larger values of the energy gap will provide low reactivity to a chemical interaction and inhibition efficiency. But the lower values of the ΔE will render good reactivity to a chemical interaction and inhibition efficiency [6].

The ionization potential (I) and electron affinity (A) of the inhibitor molecule and E_{HOMO} and E_{LUMO} are related to each other. The absolute electronegativity (χ) and the global hardness (η) depending on the ionization potential and electron affinity of the inhibitor molecules, the following can be given (1) [7].

$$\chi = -\mu = -\left(\frac{\partial E}{\partial N}\right)_{v(\vec{r})}$$

$$\eta = \left(\frac{\partial^2 E}{\partial N^2}\right)_{v(\vec{r})} = \left(\frac{\partial \mu}{\partial N}\right)_{v(\vec{r})} \quad (1)$$

The chemical softness (S) is a chemical descriptor measuring the molecular stability and reactivity. The relation between chemical softness (S) and chemical hardness can be given as follows (2) [7].

$$S = \frac{1}{\eta} \quad (2)$$

The global electrophilicity index (ω) is a measure of energy lowering due to maximal electron flow between donor and acceptor. It can be given as a function of the dipole moment and the chemical hardness as follows (3) [7].

$$\omega = \frac{\mu^2}{2\eta} \quad (3)$$

The global electrophilicity index measures the tendency of molecules to accept electrons. A molecule with a high ω value has an electrophile and with a low ω value has a nucleophilic character.

The transferred electrons fraction index (ΔN) measures the stabilization in energy when the sys-

tem acquires an additional electronic charge from the environment. Thus the fraction of electrons transferred from the inhibitor to metallic surface (4) [7].

$$\Delta N = \frac{\chi_{Fe} - \chi_{inh}}{2(\eta_{Fe} + \eta_{inh})} \quad (4)$$

According to the simple charge-transfer model there are occurring governing the interaction between the inhibitor molecule and the metal surface (5) [8].

$$\Delta E_{back\ donation} = -\frac{\eta}{4} \quad (5)$$

The $\Delta E_{\text{back donation}}$ implies that when $\eta > 0$ and $\Delta E_{\text{back donation}} < 0$ the charge-transfer to a molecule, followed by a back donation from the molecule, is energetically favored.

METHOD OF CALCULATION

The quantum chemical parameters of the earlier synthesized 2*H*-pyridazin-3-one, 1*H*-pyrazole-3-carboxylic acid and derivatives [9] were calculated using DFT based on Beck's three parameter exchange functional and Lee–Yang–Parr nonlocal correlation functional (B3LYP) and the 6-31G (d, p) orbital basis sets in Gaussian09 program (Table 1)[10-12].

RESULTS AND DISCUSSION

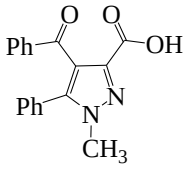
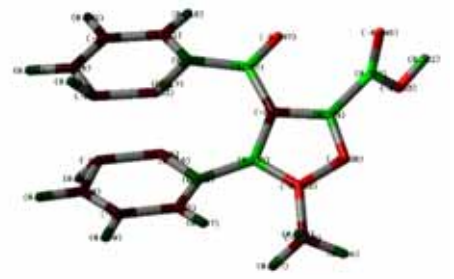
The quantum chemical calculations of the all molecules have been performed using DFT based on Beck's three parameter exchange functional and Lee–Yang–Parr nonlocal correlation functional (B3LYP) and the 6-31G (d, p) orbital basis sets in Gaussian09 program (Fig.1). This method has been widely implemented to study the relationship between corrosion inhibition efficiency of the molecules and their electronic properties [13].

The quantum chemical parameters of all compounds such as the energies of highest occupied molecular orbital (E_{HOMO}) and the lowest unoccupied molecular orbital (E_{LUMO}), the energy gap (ΔE), dipole moment (μ), ionization potential (I), electron affinity (A), absolute electronegativity (χ), chemical hardness (η), global electrophilicity index (w), chemical softness (S), fraction of electrons transferred (ΔN) and back donation energy ($\Delta E_{\text{back donation}}$) were calculated (Table 1).

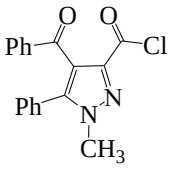
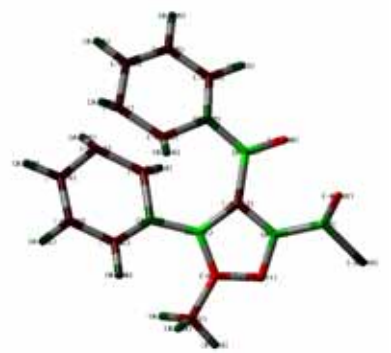
According to the frontier molecular orbital theory of the chemical reactivity is depends interaction between highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). The energy of the highest occupied molecular orbital (E_{HOMO}) measures the tendency towards the donation of electron by a molecule. Therefore, higher values of E_{HOMO} indicate better tendency towards the donation of electron, enhancing the adsorption of the inhibitor on metal and therefore better inhibition efficiency. E_{LUMO} indicates the ability of the molecule to accept electrons. The binding ability of the inhibitor to the metal surface increases with increasing of the HOMO and decreasing of the LUMO energy values [14]. Frontier molecular orbital diagrams of BMPPC, BMPPCC, BMPPCA, BBMPPC, NBMPPC, BMPPCN and BHMPP is represented in Fig. 2.

TABLE 1
Calculated quantum chemical parameters of the studied molecules

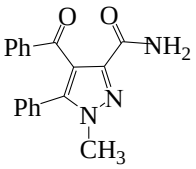
	BMPPC	BMPPCC	BMPPCA	BBMPPC	NBMPPC	BMPPCN	BHMPP
E_{HOMO} (eV)	-5.7499	-5.9159	-5.7499	-5.7145	-5.7499	-5.4097	-6.1227
E_{LUMO} (eV)	-2.0716	-2.2760	-2.0708	-2.0327	-2.0736	-2.4464	-1.8449
Ionization potential: I (eV)	5.7499	5.9159	5.7499	5.7145	5.7499	5.4097	6.1227
Electron affinity: A (eV)	2.0716	2.2760	2.0708	2.0327	2.0736	2.4464	1.8449
Electronegativity: χ(eV)	3.9106	4.0959	3.9104	3.8736	6.7867	3.9281	3.9838
Chemical hardness: η(eV)	3.6783	3.6399	3.6791	3.6818	3.6763	2.9633	4.2778
Chemical softness: S	0.2719	0.2747	0.2718	0.2716	0.2720	0.3374	0.2338
Electrophilicity index: (ω)	6.0433	12.2303	6.0419	5.4430	10.7047	17.1377	1.7775
Dipole moment: μ(debye)	6.6677	9.4358	6.6677	6.3309	8.8717	10.0781	3.8997
Transferred electrons fraction: (ΔN)	0.4199	0.3989	0.4199	0.4246	0.0290	0.5183	0.3525
Energy gap: $\Delta E = E_{LUMO} - E_{HOMO}$ (eV)	3.6783	3.6399	3.6791	3.6818	3.6763	2.9633	4.2778
$\Delta E_{\text{back donation}}$	-0.9196	-0.9099	-0.9198	-0.1705	-0.9191	-0.7408	-1.0695

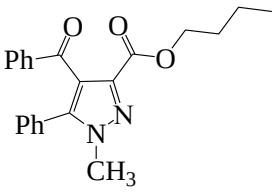
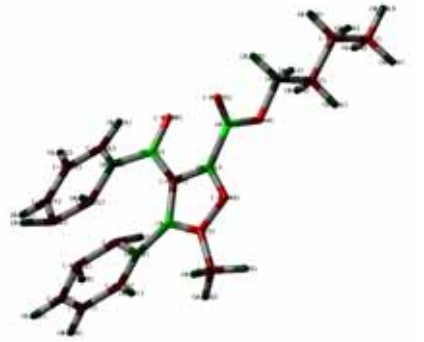
4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid (BMPPC) [9]

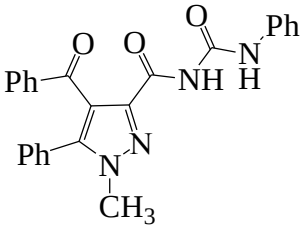
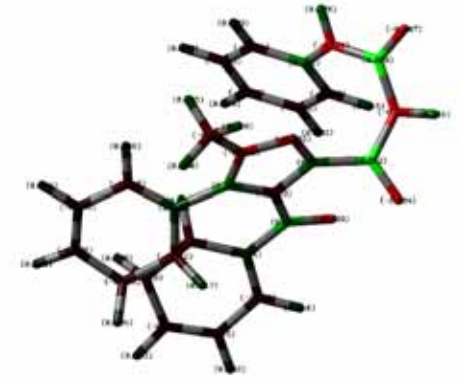
4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carbonyl chloride (BMPPCC) [9]



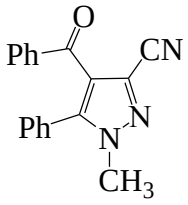

4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide (BMPPCA) [9]

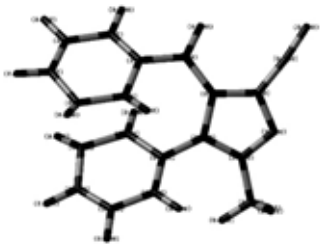



butyl-4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (BBMPPC) [9]

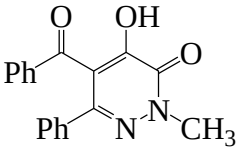



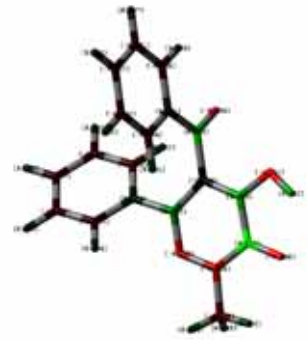
N-[4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carbonyl]-N′-phenyl urea (NBMPPC) [9]





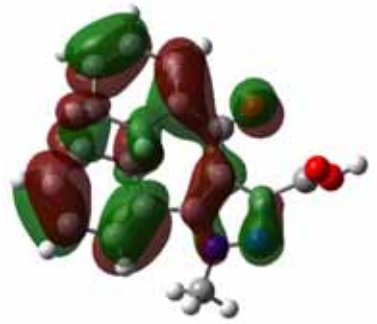
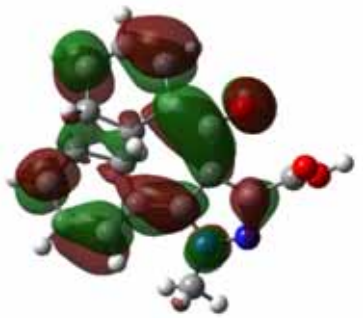
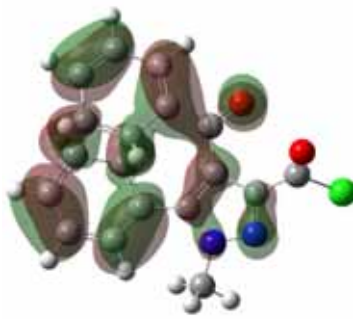
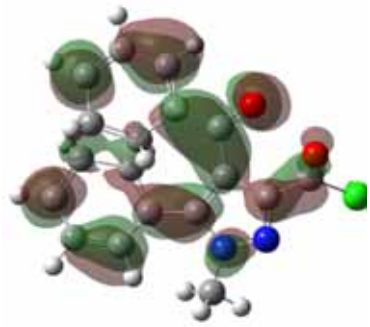
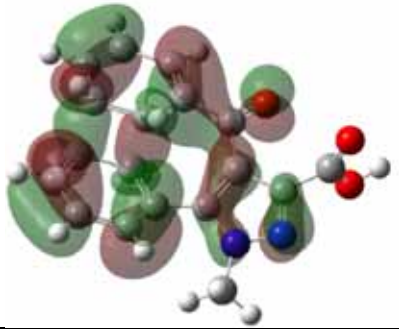
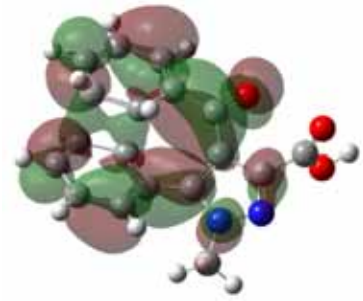
4-benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carbonitrile (BMPPCN) [9]





5-benzoyl-4-hydroxsy-2-methyl-6-phenyl-2Hpyridazin-3-one (BHMPP) [9]

FIGURE 1
Names, molecular and optimized structures of the compounds

	HOMO	LUMO
BMPPC		
BMPPCC		
BMPPCA		

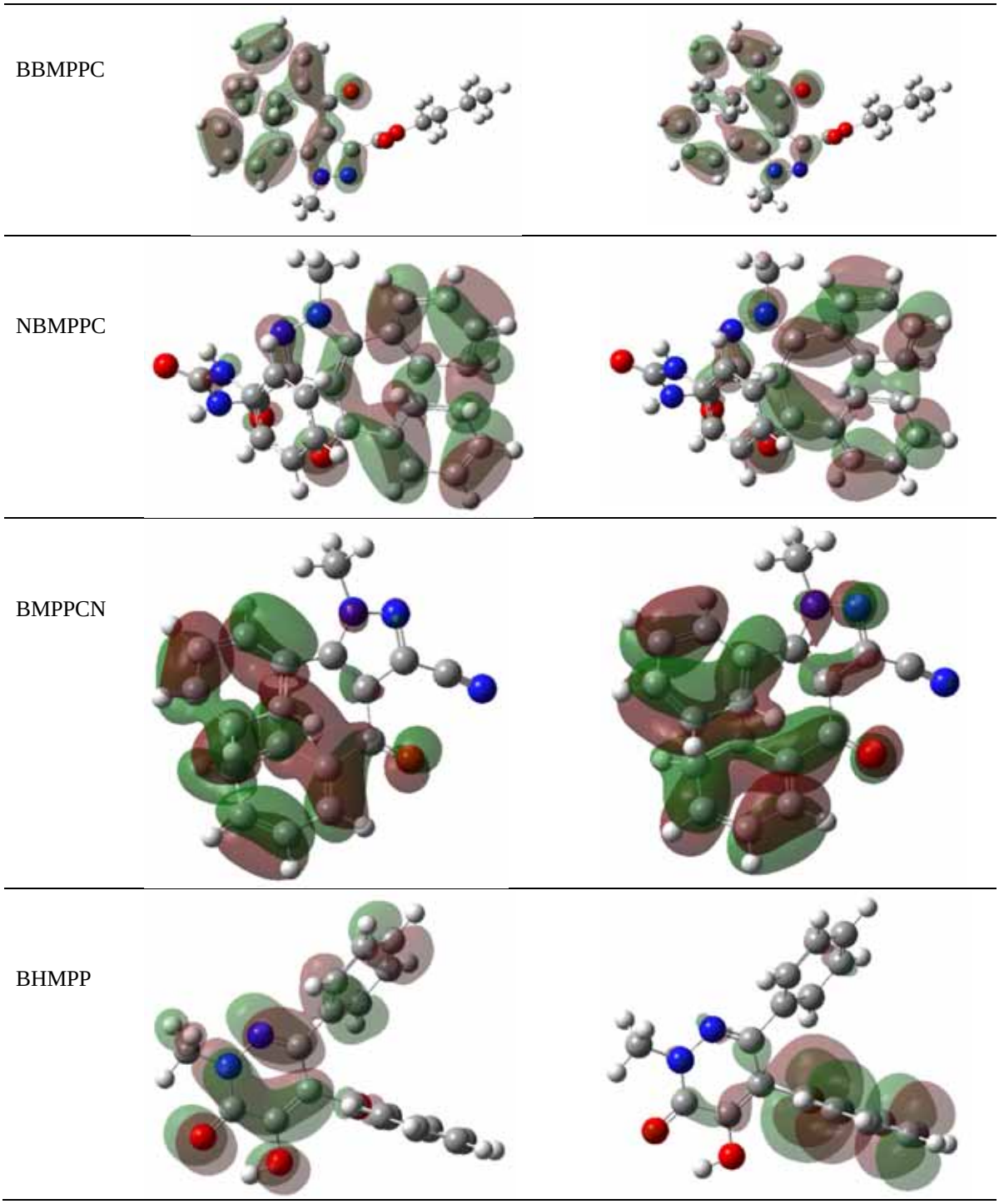


FIGURE 2
Frontier molecular orbital diagrams of BMPPC, BMPPCC, BMPPCA, BBMPPC, NBMPPC, BMPPCN and BHMPP

E_{HOMO} for the seven compounds follows the order; BMPPCN> BBMPPC >BMPPC= NBMPPC=BMPPCA>BMPPCC>BHMPP which implies that BMPPCN has the highest tendency to donate electrons (Table 1). High value of E_{HOMO} is likely to a tendency of the molecule to donate electrons to appropriate acceptor molecule of low empty molecular orbital energy.

The inhibitor does not only donate electron to the metal ion but can also accept electron from the metal leading to the formation of a feedback bond. In this case the E_{LUMO} value of the molecule is also important for interaction. E_{LUMO} for the all molecules are; BHMPP > BBMPPC > BMPPCA> BMPPC > NBMPPC > BMPPCC > BMPPCN which implies that BMPPCN has the highest tendency to acceptor electrons (Table 1).

The energy gap (ΔE) between the E_{HOMO} and E_{LUMO} energy levels of the molecules is an important parameter as a function of reactivity of the

inhibitor molecule. When ΔE decreases, the reactivity of the molecule increases. There is a relationship between chemical hardness and chemical softness with energy gap. The soft molecules have low energy gap and thus these inhibitors are the most effective for metals [15]. In this study, the results indicated that the compound BMPPCN has the lowest energy gap (table 1). This means that the compound BMPPCN could have better performance as corrosion inhibitor.

The ionization energy is one of a fundamental descriptor the chemical reactivity of molecules. High ionization energy indicates high stability and chemical inertness and small ionization energy indicates high reactivity of the molecules [16]. The compound BMPPCN has the lowest ionization energy when compared with other compounds. This indicates that BMPPCN has a high inhibitory effect.

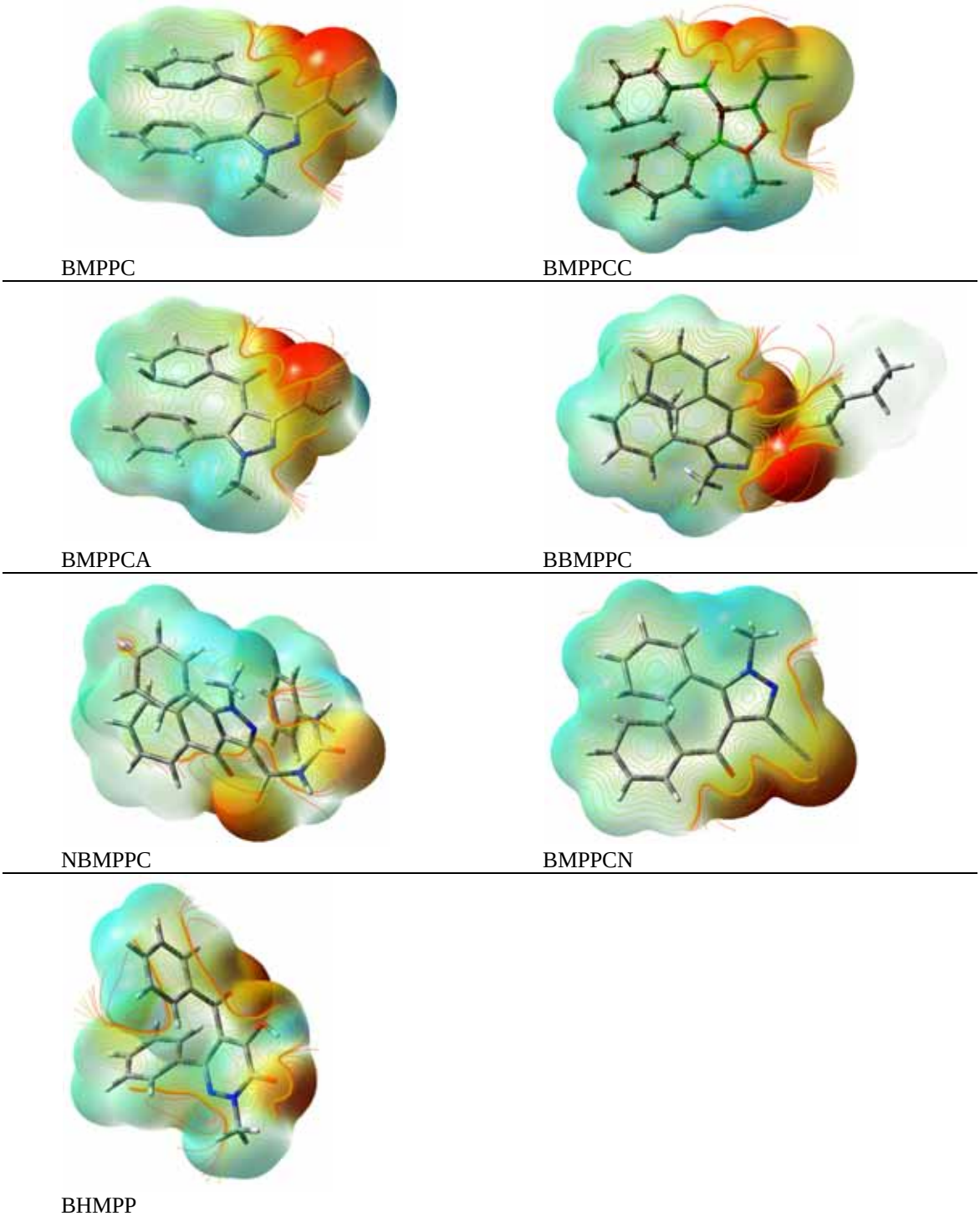


FIGURE 3
The molecular electrostatic potentials (MEPs).

The global electrophilicity index (ω) is the measure of the electrophilic tendency of a molecule. In our work, the compound BMPPCN with high electrophilicity index value than the other compounds has the highest inhibition efficiency.

The electron transferred (Δ*N*) and back-donation *($\Delta E_{back\text{-}donation}$)* were also calculated (table 1). If $\Delta N < 3.6$, the inhibition efficiency increases by increasing electron-donating ability of these inhibitors to donate electrons to the metal surface [17]. The highest fraction of electrons transferred is associated with the best inhibitor.

The calculated $\Delta E_{back\ donation}$ values for the inhibitors reveal that back donation is favored for the molecule BMPPCN which is the best inhibitor (table 1).

The molecular electrostatic potential (MEP) provides information about reactive sites for electrophilic and nucleophilic attack in order to predict reactive sites for electrophilic and nucleophilic attack in all molecules, the MEP maps were also fixed in figure 3. The electrostatic potential on the surfaces have been represented by colors. The blue regions of MEP maps shows electrophilic, while the red areas show nucleophilic reactivity.

CONCLUSION

The 2*H*-pyridazin-3-one, 1*H*-pyrazole-3-carboxylic acid and derivatives [9] were investigated as corrosion inhibitors using density functional theory (DFT) at B3LYP/6-31G(d,p) level. From the results, it can be concluded that the compound BMPPCN is good inhibitors for the corrosion.

ACKNOWLEDGEMENTS

This work was financially supported by Van Yuzuncu Yil University Scientific Research Projects Coordination Department (No. FBA-2018-7182).

REFERENCES

[1] Elmors, M.A., Hassanein, A.M. (1999) Corrosion inhibition of copper by heterocyclic compounds. Corrosion Science. 41(12), 2337.

[2] Ikpi, M.E., Okonkwo, B.O. (2017) Electrochemical investigation on the corrosion of API 5L X52 carbon steel in simulated soil solutions. Journal of Materials and Environmental Sciences. 8(11), 3809.

[3] Lagrenée, M., Mernari, B., Bouanis, M., Traisnel, M., Bentiss, F. (2002) Study of the mechanism and inhibiting efficiency of 3,5-bis(4-methylthiophenyl)-4H-1,2,4-triazole on mild steel corrosion in acidic media. Corrosion Science. 44(3), 573.

[4] Khalil, N., (2003) Quantum chemical approach of corrosion inhibition. Electrochimica Acta. 48(18), 2635.

[5] Caliskan, N., Akbas, E. (2012) Corrosion inhibition of austenitic stainless steel by some pyrimidine compounds in hydrochloric acid. Materials and Corrosion. 63(3), 231.

[6] Karzazi, Y., Belghiti, M.E.A., Dafali, A., Hammouti, B. (2014) A theoretical investigation on the corrosion inhibition of mild steel by piperidine derivatives in hydrochloric acid solution. Journal of Chemical and Pharmaceutical Research. 6(4), 689.

[7] Chattaraj, P.K., Sarkar, U., Roy, D.R. (2006) Electrophilicity Index. Chemical Reviews. 106, 2065.

[8] Ramírez-Ramírez, J.Z., Rubicelia Vargas, R., Garza, J., Gázquez, J.L. (2010) Simple Charge-Transfer Model for Metallic Complexes. The Journal of Physical Chemistry A. 114(30), 7945.

[9] Akbas, E., Berber, I., Sener, A., Hasanov, B. (2005) Synthesis and antibacterial activity of 4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid and derivatives. IL Farmaco, 60, 23.

[10] Becke, A.D. (1993) A new mixing of Hartree–Fock and local density-functional theories. The Journal of Chemical Physics. 98, 1372.

[11] Lee, C., Yang, W., Parr, R.G. (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Physical Review B. 37(2), 785.

[12] Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A., Jr., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, N.J., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A. J., Cammi, R., Pomelli, C., Ochterski, J. W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Ö., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J. (2009) Gaussian 09, Revision A.02. Gaussian Inc., Wallingford, CT.

[13] Young, D.C. (2001) A practical guide for applying techniques to real world problems in Computational Chemistry. New York: John Wiley and Sons Inc. 630p.
[14] Zarrouk, A., Hammouti, B., Dafali, A., Bouachrine, M., Zarrok, H., Boukhris, S., Al-Deyab, S.S. (2014) (2011) A theoretical study on the inhibition efficiencies of some quinoxalines as corrosion inhibitors of copper in nitric acid. Journal of Saudi Chemical Society. 18, 450.
[15] Kadhim, A., Al-Okbi, A.K., Jamil, D.M., Qussay, A., Al-Amiery, A.A., Gaaz, T.S., Kadhum, A.A.H., Mohamad, A.B., Nassir, M.H., (2017) Experimental and theoretical studies of benzoxazines corrosion inhibitors. Results in Physics. 7, 4013.
[16] Su, N.Q., Zhang, I.Y., Wu, J., Xu, X. (2011) Calculations of ionization energies and electron affinities for atoms and molecules: A comparative study with different methods. Frontiers of Chemistry in China. 6(4), 269.
[17] Lukovits, I., Kalman, E., Zucchi, F. (2001). Corrosion Inhibitors-Correlation between Electronic Structure and Efficiency. Corrosion. 57, 3-8

Received: **25.01.2018**
Accepted: **19.11.2018**

CORRESPONDING AUTHOR

Erdem Ergan
Department of Property Protection and Security,
Van Vocational School of Security,
University of Van Yuzuncu Yil,
65080 Van – Turkey

e-mail: erdemergan@yyu.edu.tr
erdem_ergan@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9557-9563 Fresenius Environmental Bulletin

GC-MS ANALYSIS OF VOLATILE COMPONENTS OF SAFRANBOLU AND KIRIKHAN SAFFRON (*CROCUS SATIVUS* L.) PREPARED BY ULTRASONIC EXTRACTION

Hasan Asil*

Medicinal and Aromatic Plants, Altinozu Vocational School of Agricultural Sciences, Mustafa Kemal University, 31001, Hatay, Turkey

ABSTRACT

Extraction of the saffron (Crocus sativus L.) stigma grown in Kırıkhan-Hatay and Karabük-Safranbolu (which is the most common saffron farming area in Turkey) with methanol / ethyl acetate solvent mixture was carried out and volatile components of the saffron were detected by GC-MS (gas chromatography-mass spectroscopy) analysis. According to the obtained results, fifteen compounds were identified in Safranbolu Saffron stigma and fourteen components were detected for Kırıkhan Saffron stigma. Some of these compounds including β-Isophorone, α-Isophorone, 4-Ketoisophorone, Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one, 2,4-bis(1,1-dimethylethyl)phenol, Nonadecane and tricosane have also been obtained in previous studies according to the literature. Some of compounds in Kirikhan Saffron stigmas including; 3-chlorophenylhydrazine, 2,2-dimethyl-3-(2-methyl-1propenyl)cyclopropanecarboxylic acid, 9,12-octadecadienoic acid, Bis-2-ethylhexyladipate, heneicosane, 1-eicosanol, heptacosane and hexacosane have been detected for the first time in a Saffron stigma according to the literature.

KEYWORDS:
Crocus sativus L, GC-MS, ultrasonic extraction, saffron, stigma

INTRODUCTION

Crocus Sativus L. is a member of iridaceae family. C. Sativus L. is cultivated plant with an onion flower blooming in the autumn. It is height around 20-30 cm long [1, 2]. Acreages of Saffron (*Crocus sativus* L.) in Turkey greatly reduced and dropped to 20-30 per hectare due to production difficulties. In the near future, the production of this plant seems to be abandoned in Turkey. Saffron is an important plant for food, paint, cosmetic and pharmaceutical applications. It is one of the most expensive spices in the world due to using it in the treatment of cancer [3-6]. Saffron has been cultivated for about 4000 years and is used as a spice due to its fragrances, colors and healing properties [4, 7, 8].

It is important that the cultivation of this plant is to be sustainable because application area is more and important. There is an important problem in the growth of Crocus Sativus L. Saffron can not be grown from the seed because it is an infertile plant. Growth of this plant occurs vegetatively with bulbs. However, reproduction with bulbs takes a quite long time (at least needed to have two-three years). Since it requires a lot of work to growth this plant, it is not seemed to be economically satisfactory [9-11].

Saffron has been known in Anatolia soils since Hittites and exported to abroad in Ottoman period. Today, very little space is being produced and efforts are being made to develop it for production in different regions [5,6]. Saffron agriculture is spreading in tropical and subtropical climate regions in the northern hemisphere. Nowadays, saffron is grown in countries such as Iran, Spain, India, Greece, Azerbaijan and Italy. Iran is the most important producer country in the world and has a production of 190 tons per year. Iran is also the leading exporter country in world trade [12]. Although one of the origin countries of saffron is Turkey, there is a very limited research on saffron. Therefore, to increase the number of research on saffron in Turkey is of great importance [13].

Safranal ($C_{10}H_{14}O$) is the principle essential oil responsible for the aroma of saffron and it designates the quality of saffron. Safranal had various pharmacological impacts in prevention of stomach ulcers and it is also used as anti-nociceptive, anticonvulsant, antidepressant, antitussive, antimicrobial and antitumor. Crocin is an unstable molecule responsible for the color of saffron and it has some medicinal impacts as antitumor, antidepressant, hypotensive, hippocampus and etc. [14-16].

The amounts of crocin and picrocrosin in Saffron stigma may decrease during the gathering, drying out and storage processes. The volatile components, spice flavor and chemical components of saffron must be identified by using Gas Chromatography (GC) and Gas Chromatography-Mass Spec-

trometry (GC-MS). Ultrasonic solvent extraction (USE) method using organic solvents is used for the isolation of volatile, bioactive and flavor compounds of plants at room temperature. Ultrasonic solvent extraction (USE) method provides highly efficient contact between the sample matrix and solvent. Acoustic cavitations, mechanical and thermal functions have a direct effect on the efficiency of ultrasonic extraction [17-23].

In this study, an USE extraction method for determining separations and detections of volatile components of saffron grown in Kırıkhan-Hatay and Karabük-Safranbolu (which is the most common saffron farming area in Turkey) was developed. This study applies extraction of the volatile components of the saffron and then uses GC-MS technique to determine volatile components of this plant.

EXPERIMENTAL

Saffron sample and chemicals. Saffron stigmas used in the study were obtained from saffron cultivated area of Kırıkhan/Hatay and Karabük-Safranbolu in the provinces of Turkey in 2017. Stigmas were dried at room temperature, and stored in the absence of light. Methanol (Isolab, catalog # 947.043.2500) and ethyl acetate (Tekkim, catalog # TK.050140.02500) were purchased and used as received.

Extraction procedure. The extraction of Saffron stigmas was accomplished by ultrasonic-assisted solvent extraction method in an ultrasound-cleaning bath (Bandelin, Germany) as reported in the literature, with input power 180 W, by the mode of indirect sonication at the frequency of 35 kHz at 25 ◦C. Each batch of saffron sample was extracted as further described.

1 g of Saffron stigmas was first powdered to make a uniform blend. Then, the powdered stigmas were put into a flask. The flask was charged with a mixture of (18–42 mL) methanol: ethylacetate (70:30) as the extraction solvent. Sonication was performed for 15 min. After sonication, organic extract colored as orange was put into a centrifuge tube and centrifuged in 5000 rpm for 3 min. 10 mL of the solvent mixture was then added to the residual of extract, and sonicated for 10 more min. New formed organic extract was centrifuged for 3 min at 5000 rpm. After decantation, the organic extracts were combined together and concentrated up to 10 mL. Obtained organic extract was stored at 4 oC in the fridge and the absence of light before GC–MS analysis. 1 µL was used for GC–MS analysis [23].

Gas chromatography-mass spectrometry (GC–MS) analysis. GC–MS analyses were performed using a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 60 m 0.25 mm i.d., film thickness 0.25 µm), interfaced with a Hewlett-Packard mass selective detector 6890.

GC-MS analyses were performed according to the procedure reported before. The oven was first heated at 60 oC and held for 1 min at that temperature; the temperature was then increased at 5 oC/min to 200 oC and held for 1 min. Finally, the temperature was raised at 20 oC/min to 280 oC with final hold for 21 min. Helium as a carrier gas (99.999%) was used with a flow rate of 1 mL/min. Injector temperature was at 200 oC.

RESULTS AND DISCUSSION

Characterization of the volatile components of Kırıkhan and Safranbolu saffron. The chemical composition of the volatile components of Kırıkhan and Safranbolu saffron was obtained by USE method with GC-MS. The total ion chromatogram (TIC) obtained by USE-GC-MS method of Kırıkhan saffron is shown in Fig. 1 and the results are also summarized in Table 1. The total ion chromatogram (TIC) of Safranbolu saffron is shown in Figure 2 and the results for that are also summarized in Table 2. GC-MS analysis results of the volatile components of saffrons were compared with the standard mass spectra found in NIST library.

According to the results obtained from Kırıkhan and Safranbolu saffron in table 1 and 2; the name of the chemical components and their formula, MW- molecular weight, tR retention time, % Relative area percent (peak area relative to the total peak area) and MF Match Factor in library are shown in Table 1 and 2.

The ratio of the peak areas of extracted compounds to the total peak areas is given as percentage in Table 1 and 2. Fourteen compounds are defined for Kırıkhan saffron and their total peak areas are shown in Table 1. Fifteen components have been identified for Safranbolu saffron and their total peak areas are given in Table 2. Similarity relations of the compounds found in the GS-MS library are also given in Table 1 and 2.

Kırıkhan saffron contains fourteen compounds and the highest peak areas of the compounds were found in the order of Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), 2-(1,1-dimethylethyl) phenol, **α**-Isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) and 1-eicosanol (Table 1).

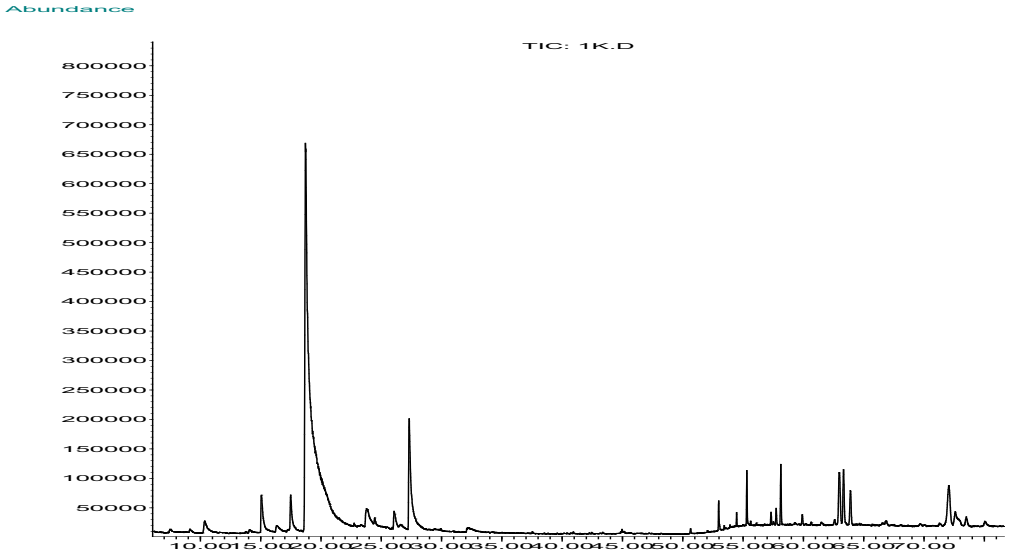


FIGURE 1
Total ion chromatogram (TIC) of Kırıkhan saffron obtained by USE–GC–MS in optimized conditions. Numbers are related to the components in Table 1.

TABLE 1
Chemical components of Kırıkhan saffron

No.	Chemical name	Formula	MWg/ mol	*t*R	%	MF
1	*β*-Isophorone	$C_9H_{14}O$	138.21	10.36	0.31	70
2	α-Isophorone (3,5,5-trimetyl-2-cyclohexen-1-one)	$C_9H_{14}O$	138.21	15.07	0.82	91
3	2,2,6-Trimethyl-1,4-cyclohexanedione	$C_9H_{12}O_2$	154.21	17.49	0.60	86
4	Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde)	$C_{10}H_{14}O$	150.221	18.72	16.20	98
5	3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexane-2-dione	$C_9H_{14}O_2$	154.2063	23.78	0.47	50
6	2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadiene-1-one	$C_{10}H_{12}O_3$	180.2005	26.07	0.37	64
7	2-(1,1-dimethylethyl)Phenol	$C_{14}H_{22}O$	206.329	27.32	2.69	72
8	Bis(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	370.57	52.99	0.22	83
9	Heneicosane	$C_{21}H_{44}$	296.583	54.46	0.08	87
10	Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390.56	55.32	0.35	90
11	Nonadecane	$C_{19}H_{38}$	266.513	57.32	0.47	90
12	1-Eicosanol	$C_{20}H_{42}O$	298.555	57.75	0.93	81
13	Heptacosane	$C_{27}H_{56}$	380.745	58.13	0.48	98
14	Hexacosane	$C_{26}H_{54}$	366.718	59.92	0.11	50

MW- molecular weight, tR retention time. % Relative area percent (peak area relative to the total peak.

A total of fifteen components have been detected for Safranbolu saffron and similarity relations of components compared to that of found in GC-MS library and their total peak areas are given in Table 2. The highest peak areas of the compounds were found in the order of Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), 2-(1,1-dimethylethyl) phenol, 2,4,4-carboxaldehyde-5-hydroxy-2,5-cyclohexadiene-1-one and 1-eicosanol (Table 2).

There are four compounds ((3-chlorophenyl) hydrazine, 2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylic acid, 9,12-octadecadienoic acid and tricosane) in Safranbolu saffron which are not found in Kırıkhan saffron. There are three compounds (β-Isophorone, Heneicosane and Nonadecane) in Kırıkhan saffron which are not found in Safranbolu saffron. Despite the same extraction method, the same extraction solvent and the same amounts of saffrons were used, different volatile components and different amounts of the components were detected for Safranbolu and Kırıkhan saffrons. Safranbolu saffron has 70.3% of safranal compound in 30.4 total peak area, whereas safranal in Kırıkhan saffron is obtained in 67.2% of 24.10 total peak area. Safranbolu saffron has more

amount of safranal than that of Kırıkhan saffron. Kırıkhan saffron has more amount of 2,4-bis (1,1-dimethylethyl)phenol compound compared to that of Safranbolu saffron. When we look at the percentages of the components, both saffron samples have different amounts for each compounds. This can be a reason of the effects of geographical and climate differences.

According to the literature; *β*-Isophorone, **α**-Isophorone, 4-Ketoisophorone, Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), 3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexane-2-dione, 2,4-bis(1,1 dimethylethyl) phenol, 2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadiene-1-one, Nonadacene and Tricosane. Similar studies have also been accomplished by several research groups such as [23-28]. According to the literature, some compounds found in the samples have been detected in saffron for the first time ((3-chlorophenyl) hydrazine, 2,2-Dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylic acid, 9,12-octadecadienoic acid, Bis-(2-ethylhexyl) adipate, heneicosane, Bis-(2-ethylhexyl)phthalate, 1-Eicosanol, Heptacosane ve hexacosane) in Table 3.

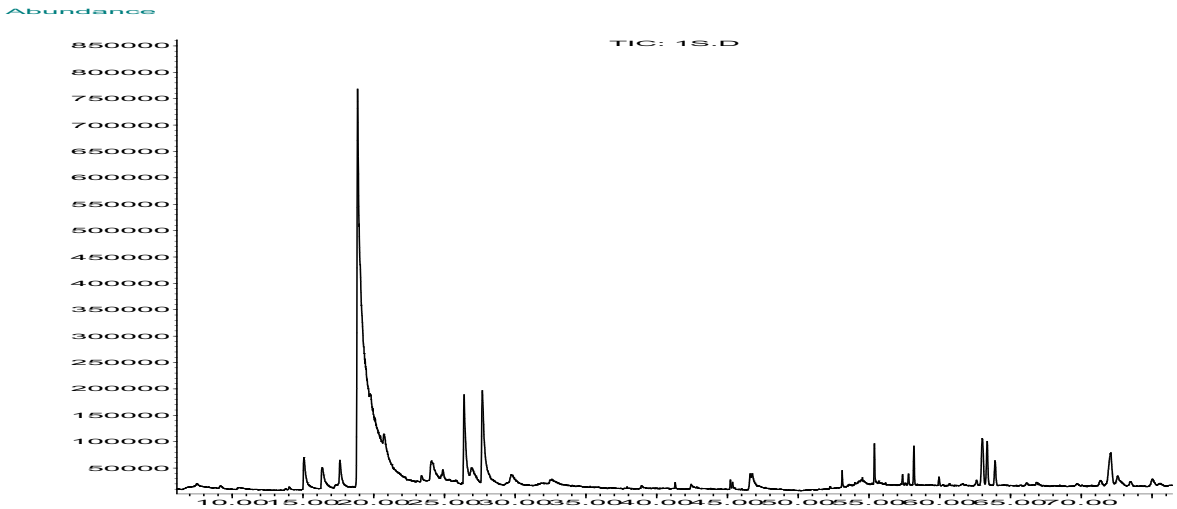


FIGURE 2
Total ion chromatogram (TIC) of Safranbolu saffron obtained by USE–GC–MS in optimized conditions.
Numbers are related to the components in Table 2.

TABLE 2
Chemical components of Safranbolu saffron

No.	Chemical name	Formula	MW g/mol	*t*R	%	MF
1	**α**-Isophorone (3,5,5-trimetyl-2-cyclohexen-1-one)	$C_9H_{12}O$	138.21	15.07	0.68	91
2	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	152.19	16.36	0.56	91
3	Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde)	$C_9H_{14}O_2$	154.20	18.87	26.72	98
4	3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexanone-2-ene	$C_9H_{14}O_2$	154.20	24.10	0.75	64
5	2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadiene-1-one	$C_{10}H_{12}O_3$	180.20	26.40	1.80	1.80
6	(3-Chlorophenyl) hydrazine	$C_6H_7ClN_2$	142.58	26.91	0.44	22
7	2-(1,1-dimethylethyl)phenol	$C_4H_6O_2$	86.09	27.68	2.42	55
8	2,2-Dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylic acid	$C_{10}H_{16}O_2$	168.23	29.74	0.11	47
9	9,12-Octadecadienoic acid	$C_{19}H_{34}O_2$	294.47	45.21	0.57	98
10	Bis(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	370.57	53.10	0.12	80
11	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	55.38	0.29	86
12	Tricosane	$C_{23}H_{48}$	324.63	57.38	0.09	57
13	1-Eicosanol	$C_{20}H_{42}O$	298.55	57.79	0.73	76
14	Heptacosane	$C_{27}H_{56}$	380.74	58.18	0.36	98
15	Hexacosane	$C_{26}H_{54}$	366.72	59.95	0.10	50

*MW- molecular weight, t*R retention time. % Relative area percent (peak area relative to the total peak area), MF match factor in library.

TABLE 3
Comparison of percentage ratios of total chemical contents of the main compounds of Kırıkhan saffron with Safranbolu saffron.

No.	Chemical name	Kırıkhan	Safranbolu
		% (24.1)*	% (30.4)**
1	*β*-Isophorone	1.3	Nf
2	**α**-Isophorone	3.4	2.2
3	4-Ketoisophorone	2.5	1.8
4	Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde)	67.2	70.4
5	3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexane-2-dione	2.0	2.5
6	2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadiene-1-one	1.5	5.9
7	(3-Chlorophenyl) hydrazine	nf	1.4
8	2,4-bis(1,1-dimethylethyl)phenol	11.2	8.0
9	2,2-Dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylic acid	nf	0.4
10	9,12-Octadecadienoic acid	nf	1.8
11	Bis-(2-ethylhexyl) adipate	0.9	0.4
12	Heneicosane	0.3	Nf
13	Bis-(2-ethylhexyl)phthalate	1.5	0.9
14	Nonadecane	2.0	Nf
15	Tricosane	nf	0.3
16	1-Eicosanol	3.9	2.4
17	Heptacosane	2.0	1.2
18	Hexacosane	0.5	0.3

* GC-MS analysis of Kırıkhan saffron (24.1 peak area of total 26.8 peak area (89.8% of which is shown in the table))
** GC-MS analysis of Safranbolu saffron (30.4 peak area of total 33.23 peak area (93.8% of which is shown on the table))

CONCLUSION

The aim of this study is to explore the volatile components of saffron samples grown in two different regions (Kirikhan/Hatay and Safranbolu/Karabuk) of Turkey using ultrasonic solvent extraction method with the GC-MS analysis and to identify differences between them. After characterization of volatile compounds of these saffrons, chemical compounds and their components were found to be different for each regions. There is still a need of research to discover components of saffons in different ecological conditions in Turkey. As a result of this research, differences between Safranbolu saffron and Kırıkhan saffron in terms of chemical components and components ratios have been shown. While Safranbolu saffron has more amount of safranal compound compared to that of Kırıkhan saffron, Kırıkhan saffron has more 2,4-bis(1,1-dimethylethyl)phenol compound compared to that of Safranbolu saffron. When we look at the percentages of the components, both saffron samples have some differences between each other. The reason of these differences might be the effect of geographical and climate differences. New compounds in the components of saffron samples were also detected compared to the previous reports. According to the literature, compounds found in the samples have been detected in saffron for the first time ((3-chlorophenyl) hydrazine, 2,2-Dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid, 9,12-octadecadienoic acid, Bis-(2-ethylhexyl) adipate, heneicosane, Bis-(2-ethylhexyl)phthalate, 1-Eicosanol, Heptacosane ve hexacosane).

REFERENCES

[1] Arslan, N., Gürbüz, B., Ipek, A., Özcan, S., Sarihan, E., Daeshian, A.M. Moghadassi, M.S. (2007) The effect of corm size and different harvesting times on Saffron (Crocus sativus l.) regeneration. Acta Horticulturae. 739, 113-117.

[2] Bakhtavari, A.S. (2010) Effect of different saffron (crocus sativus L.) bulb (corm) sizes and plant density on the yield and factors affecting yield. Ph.D. Thesis. Ankara University, Graduate School of Natural and Applied Sciences Department of Field Crops. Ankara, 79p.

[3] Gümüşoğlu, İ. (2002) Plant of Safranbolu (Crocus sativus L.). Safranbolu Service Union Cultural Publications No: 10 First Edition

[4] Çavuşoğlu, A., Erkel, İ.A. (2009) Saffron (Crocus sativus L.) Growing without Removing of Mother Corms under Greenhouse Condition. Turkish J. of Field Crops. 14(2), 170-180.

[5] Ünaldı, Ü.E. (2007) A Plant Exposed to Danger: Saffron (Crocus Sativus). Fırat University Journal of Social Science. 17(2), 53-67.

[6] Rezaeieh, K.A.P., Vaziri, P. (2012) Enhanced Plantlet Induction from Cultured Segments of Saffron (Crocus sativus L.) through In vitro Micropropagation: Background and Prospects. Türk Bilimsel Derlemeler Dergisi. 5(2), 29-31.
[7] Arslan, N., Sarıhan, E.O., İpek, A. (2008) Effects of Different Bulb Cutting Methods on Some Characteristics of Fritillaria persica L. Ankara University Faculty of Agriculture Journal of Agricultural Sciences (JAS). 14(3), 246-250.
[8] Aytekin, A., Açıkgöz, A.O. (2008) Hormone and Microorganism Treatments in the Cultivation of Saffron (Crocus sativus L.) Plants. Molecules. 13(5), 1135-1147.
[9] İpek, A., Arslan, N., Sarıhan, E.O. (2009) Effects of Different Planting Depth and Bulb Sizes on Yield and Yield Components of Saffron (Crocus sativus L.). Ankara University Faculty of Agriculture Journal of Agricultural Sciences (JAS). 15(1) 38-46.
[10] Karaoğlu, C., Cocu, S., İpek, A., Parmaksız, I., Sarıhan, E., Uranbey, S., Arslan, N., Kaya, M.D., Sancak, C., Özcan, S., Gürbüz, B., Mirici, S., Er, C., Khawar, K.M. (2007) In Vitro Micropropagation of Saffron. ActaHorticulturae. 739, 223-228.
[11] Yıldırım, M.U., Hajyzadeh, M., Küçük, G., Sarıhan, E.O. (2017) Effects of Different Manures on Growth of Saffron Plant (Crocus sativus L.) and Some Characteristics. KSU J. Nat. Sci. 20(Special Issue), 327-331.
[12] Valizadeh, N., Arslan, N. (2014) Saffron Agriculture and Industry in Iran, II. Medical and Aromatic Plants Symposium, 23-25 September 2014, Poster 63, Yalova.
[13] Taghiloofar, A.H., Bayram, E. (2014) Izmir, Turkey and Iran saffron Origin Populations Conditions on Agro-Morphological Studies II. Medical and Aromatic Plants Symposium, 23-25 September 2014, Poster 8, Yalova.
[14] D'Archivio, A.A., Maggi, M.A. (2017) Geographical identification of saffron (Crocus sativus L.) by linear discriminant analysis applied to the UV-visible spectra of aqueous extracts. Food Chemistry. 219, 408-413.
[15] Siracusa, L., Gresta, F., Avola, G., Albertini, E., Raggi, L., Marconi, G., Ruberto, G. (2013) Agronomic, chemical and genetic variability of saffron (Crocus sativus L.) of different origin by LC-UV-vis-DAD and AFLP analyses. Genetic Resources and Crop Evolution. 60(2), 711-721.
[16] Rubert, J., Lacina, O., Zachariasova, M., Hajslova, J. (2016) Saffron authentication based on liquid chromatography high resolution tandem mass spectrometry and multivariate data analysis. Food Chemistry. 204, 201-209.
[17] Lozano, P., Delgado, D., Gomez, D., Rubio, M., Iborra J.L. (2000) A non-destructive method to determine the safranal content of saffron (Crocus sativus L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. J. Biochem. Biophys. Methods. 43, 367-378.
[18] Alonso, G.L., Varon, R., Gomez, R., Navaro, F., Salinas, M.R. (1990) Auto-oxidation in Saffron at 40°C and 75% Relative Humidity. J. Food sci. 55, 595.
[19] Pan, J., Xia, X.X., Liang, J. (2008) Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry. Ultrasonics Sonochemistry. 15, 25-32.
[20] Jerkovic, I., Mastelic, J., Marijianovic, Z., Klein, Z., Jelic, M. (2007) Comparison of hydrodistillation and ultrasonic solvent extraction for the isolation of volatile compounds from two unifloral honeys of Robinia pseudoacacia L. and Castanea sativa L. Ultrasonics Sonochemistry. 14, 750-756.
[21] Alissandrakis, E., Daferera, D., Tarantilis, P.A., Polissiou, M., Harizanis, P.C. (2003) Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. Food Chem. 82, 575.
[22] Kanakis, C.D., Daferera, D.J., Tarantilis, P.A., Polissiou, M.G. (2004) Qualitative determination of volatile compounds and quantitative evaluation of safranal and 4-hydroxy-2,6,6-trimethyl-cyclohexene-1-carboxaldehyde (HTCC) in Greek saffron. Journal of Agricultural and Food Chemistry. 52(14), 4515-4521.
[23] Heravi, M.J., Parastar, H., Najafabadi, H.E. (2009) Characterization of volatile components of Iranian saffron using factorial-based response surface modeling of ultrasonic extraction combined with gas chromatography-mass spectrometry analysis. Journal of Chromatography A. 1216, 6088-6097.
[24] Amanpour, A., Sönmezdağ, A.S., Kelebek, H., Selli, S. (2015) GC-MS-olfactometric characterization of the most aroma-active components in a representative aromatic extract from Iranian saffron (Crocus sativus L.). Food Chemistry. 182, 251-256.
[25] Culleré, L., San-Juan, F., Cacho, J. (2011) Characterisation of aroma active compounds of Spanish saffron by gas chromatography–olfactometry: Quantitative evaluation of the most relevant aromatic compounds. Food Chemistry. 127, 1866-1871.
[26] Masi, E., Taiti, C., Heimler, D., Vignolini, P., Romani, A., Mancuso, S. (2016) PTR-TOF-MS and HPLC analysis in the characterization of saffron (Crocus sativus L.) from Italy and Iran. Food Chemistry. 192, 75-81.

[27] D'Archivio, A.A., Giannito, A., Maggi, M.A., Ruggieri, F. (2016) Geographical classification of Italian saffron (Crocus sativus L.) based on chemical constituents determined by high-performance liquid-chromatography and by using linear discriminant analysis. Food Chemistry. 212, 110-116.

[28] Melnyk, P.J., Wang, S., Marcone, M.F. (2010) Chemical and biological properties of the world's most expensive spice: Saffron. Food Research International. 43, 1981-1989.

Received: **19.06.2018**
Accepted: **19.11.2018**

CORRESPONDING AUTHOR

Hasan Asil
Mustafa Kemal University,
Altınözü Vocational School of
Agricultural Sciences
Medicinal and Aromatic Plants,
31001, Hatay – Turkey

e-mail: hasanasil@mku.edu.tr
hasan.asil@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9564-9572 Fresenius Environmental Bulletin

DETERMINATION OF SOME QUALITY PROPERTIES OF WHEAT GENOTYPES SUITABLE FOR YUFKA FLOUR PRODUCTION IN IZMIR

Nihan Bas-Zeybekoglu[1,*], Kemal Kemahlioglu[2], Gulden Ova[3]

[1]Aegean Agricultural Research Institute, Menemen, Izmir
[2]Ege University, Vocational College, Bornova, Izmir
[3]Ege University, Faculty of Engineering, Department of Food Engineering, Bornova, Izmir

ABSTRACT

In this study, physicochemical, chemical and rheological properties of 13 genotypes were determined in the İzmir province to develop a yufka flour producing wheat genotype. In this context, "genotype x year interactions" of some characteristics of wheat, wheat flour, and dough from the year 2015-2016 and correlations of these analyses were examined. The analyses that are regarded as indicative for the yufka quality criteria are wet gluten, modified zeleny sedimentation, alveoconsistograph W (40), extensograph 45′ energy value and Rmax**.** The prominent genotypes are EBVD1-16-E, EBVD 1-8-E, Efe-E, EBVD 2-48-E, EKM 129-Ü, Kaşifbey-E, and Saggitario-Ü.

KEYWORDS:
Yufka, quality, wheat.

INTRODUCTION

Wheat is the primary source of food for approximately 35% of the world population. The total grain production in the world was 2048 million tons in 2015. However, the wheat production is 730 million tons and trade is 153 million tons [1].

Numerous studies have been carried out to determine the qualities of bread and durum wheat. Most of these studies were carried to investigate the effects of genotypes on different quality parameters while some others were carried on to determine the effects of cultivation conditions, as plant nutrition studies, on these parameters [2], [3], [4], [5], [6] and [7].

The development and production of fortified, special-purpose flours and flours for specific products and the development of target-oriented varieties from the field stage will gain further importance in the future.

Currently, the flat breads from the bread in ancient times are still produced (chapatti, yufka, oatcake,etc.) Yufka is registered in UNESCO's "Representative List of Intangible Cultural Heritage of Humanity" in 2016 and it represents the culture of Turkey [8]. The total yufka production in Turkey is 1 million 200 thousand tons, the export is 400 thousand tons and the yufka export corresponds to 600 million TL. [9]. Although numerous studies have been carried out to determine the qualities of bread wheat flour, studies on the determination of the qualities of yufka flour and special-purpose flours, which are the group in which yufka flour is found, are limited. However, for yufka flour, usually crops cultivated at low temperature ecologies are preferred for their quality criteria [10]. One of our aim is to investigate the performance of spring wheat genotypes in Izmir, which has respectively a high temperature ecology.

This study aimed to determine the chemical, physicochemical, and rheological properties of the Aegean coastal region wheat genotypes and determine yufka wheat genotypes suitable for improvement.

MATERIALS AND METHODS

In this study, 13 genotypes were selected from the Aegean Agricultural Research Institute and Ege University Faculty of Agriculture Field Crops Department. Wheat samples were stored in glass jars at -20 °C until the time of analysis, avoiding exposure to moisture. After the samples were annealed, they were milled in CD1 mill and rested for 3 weeks until the analyses were performed.

Thirteen genotypes used in the present study are listed below (Table 1). From these wheat genotypes, the physicochemical, chemical and rheological characteristics of 2015 and 2016 harvests were analyzed and compared.

Method. The characteristics of the 13 wheat genotypes (heading days, plant height, yield, and hectoliter weight) were determined by the units from which the samples were obtained.

TABLE 1
Genotypes used in the study

EBVD1-16-E*	EKM 129-U*
EBVD 2-37-E	EKM 340-U
EVD 2.1.2013-E	EKM 342-U
EBVD 1-8-E	GOLİA-U
EBVD 2-48-E	SAGGİTARİO-U
KAŞİFBEY-E	
KAYRA-E	
EFE-E	

*E: Genotypes taken from Aegean Agricultural Research Institute, U: Genotypes taken from Ege University Faculty of Agriculture Field Crops Department

Analyses conducted on wheat flour:

- **Humidity** ICC 110/1 [11]; **Protein** ICC 167 [12]**; Ash** ICC 104/1 [13]; **Starch Damage** AACC Method 76.33.01 [14]; **Wet gluten, dry gluten, gluten index** ICC 155 [15]; **Zeleny Sedimentation** ICC 116/1 [16]; **Modified Zeleny Sedimentation** [17]; **SDS (sodium dodecyl sulfate) sedimentation** ICC 151 [18]; **Falling Number** ICC 107/1 [19]. Among these analyses, wet gluten and modified zeleny sedimentation analyses, which determine protein quantity and protein quality, were taken into consideration.

- **Analyses conducted on the dough:**

- **Farinograph** 115/1 [20]; **Extensograph** ICC 114/1 [21]; **Glutograph** [22]; **Mixograph** AACC 54-40.02 [23]; **Alveoconsistograph** ICC 121 [24]. Although large amounts of data were obtained according to the metadata method in this analysis, only the data that were important in terms of yufka characteristics were taken into consideration.

Three replications for each sample were taken in two years. During these two years, the analysis results of 13 genotypes were evaluated by the JMP7 statistical program, based on the genotype * year interaction.

RESULTS AND DISCUSSION

Table 2 shows the field data of 13 genotypes, Table 3 shows the physicochemical, chemical and rheological properties, and Table 4 shows the correlation between wheat flour and dough properties.

Examining "genotype x year" interaction data values in Table 2, the earliest genotypes in terms of heading days were Ebvd 1-16-E, Kayra-E, and Evd 2.1.2013-E, while the genotypes with high yield values were Efe-E, Kayra-E, Ebvd 2-48-E, Kaşifbey-E, Ebvd 2-37-E, Ebvd 1-16-E, Ebvd1-1-8-E and Evd 2.1.2013-E. The genotypes with high hectoliters were Kayra-E, Ebvd 2-37-E, Efe-E, Ekm129-U, Evd 2.1.2013-E, Ebvd1-8-E and Ebvd 1-16-E. The flour yields of genotypes with high hectoliters are expected to be high.

TABLE 2
Field data's of genotypes

	Heading days			Plant Height (cm)			Yield (kg/da)			Hl (kg/Hl)		
Genotype	2015	2016	Average	2015	2016	Average	2015	2016	Average	2015	2016	Average
EBVD 1-16-E	94.33^{j}	89.00^{m}	91.66^{i}	90.33^{l}	100.33^{jk}	95.33^{de}	697.33^{g}	835.00^{d}	766.16^{b}	77.66^{k}	84.33^{c}	80.95^{e}
EBVD 2-37-E	104.33^{e}	94.33^{j}	99.33^{d}	109.66^{b-e}	100.33^{jk}	105.00^{b}	827.00^{d}	890.00^{bc}	858.50^{a}	78.90^{h}	86.33^{a}	82.62^{a}
EVD 2.1.2013-E	94.66^{ij}	92.00^{kl}	93.33^{h}	105.00^{g-i}	105.33^{f-h}	105.16^{b}	700.33^{g}	756.00^{ef}	728.16^{c}	78.03^{j}	84.90^{b}	81.47^{c}
EBVD 1-8-E	93.33^{jk}	96.00^{hi}	94.66^{g}	89.66^{l}	105.33^{f-h}	97.50^{d}	780.00^{e}	732.00^{fg}	756.00^{bc}	78.16^{j}	84.23^{c}	81.20^{d}
EBVD 2-48-E	104.66^{de}	91.33^{l}	98.00^{ef}	110.00^{a-d}	100.33^{jk}	105.16^{b}	760.33^{ef}	1001.00^{a}	880.66^{a}	77.53^{k}	83.90^{d}	80.72^{f}
KAŞİF-BEY-E	99.66^{g}	97.00^{h}	98.33^{de}	110.00^{a-d}	100.00^{jk}	105.00^{b}	824.66^{d}	894.00^{bc}	859.33^{a}	77.13^{l}	83.83^{d}	80.48^{g}
KAYRA-E	91.66^{l}	91.33^{l}	91.50^{i}	103.00^{g-j}	100.33^{jk}	101.66^{c}	853.66^{cd}	919.00^{b}	886.33^{a}	78.93^{h}	86.23^{a}	82.58^{a}
EFE-E	96.66^{h}	97.33^{h}	97.00^{f}	110.00 $^{a-d}$	110.33^{a-c}	110.16^{a}	859.66^{cd}	914.00^{b}	886.83^{a}	78.66^{i}	84.86^{b}	81.77^{b}
EKM 129-U	114.66^{b}	99.66^{g}	107.16^{c}	108.66^{c-f}	102.33^{h-k}	105.50^{b}	447.66^{h}	391.33^{i}	419.50^{d}	78.03^{j}	85.00^{b}	81.52^{c}
EKM 340-U	120.33^{a}	101.33^{f}	110.83^{b}	113.00^{ab}	101.33^{i-k}	107.16^{b}	329.33^{jk}	364.33^{ij}	346.83^{ef}	77.00^{l}	82.00^{e}	79.50^{h}
EKM 342-U	121.00^{a}	101.33^{f}	111.16^{b}	113.33^{a}	106.00^{e-g}	109.66^{a}	246.33^{m}	322.33^{kl}	284.33^{g}	75.20^{m}	79.03^{h}	77.12^{j}
GOLİA-U	121.00^{a}	106.33^{c}	113.66^{a}	83.33^{m}	106.33^{d-g}	94.83^{e}	356.00^{ijk}	395.33^{i}	375.66^{e}	73.96^{n}	80.00^{g}	76.98^{k}
SAGGİTARİO-U	121.00^{a}	106.00^{cd}	113.50^{a}	98.66^{k}	106.00^{efg}	102.33^{c}	288.33^{l}	388.66^{i}	338.50^{f}	74.03^{n}	81.33^{f}	77.68^{i}
Average	105.95^{a} ±0.19	97.15^{b} ±0.19		103.43 ^{a} ±0.62	103.41^{a} ±0.62		613.13^{b} ±6.95	677.15^{a} ±6.95		77.16^{b} ±0.02	83.54^{a} ±0.02	
	CV 0.9 Genotype *LSD 1.06 Year *LSD 0.52 Genotype*Year *LSD 1.53			CV 1.96 Genotype *LSD 2.35 Year LSD : N.S. Genotype*Year *LSD 3.66			CV 3.86 Genotype *LSD 28.88 Year*LSD 19.76 Genotype*Year *LSD 43.94			CV 0.11 Genotype*LSD0.1 Year *LSD 0.04 Genotype*Year *LSD 0.14		

N.S.: Not Significant *: Significant at p<0.05

The study of the physicochemical, chemical and rheological properties (Table 3) in terms of "genotype x year" interaction showed that the protein values were 10.18-12.58 d.m%, the wet gluten was 22.82-32.42 (14% m.b), modified zeleny sedimentation was 16.12-47.07 ml, MTAL 369.77-435.05 Tq%*min, water absorption was 54.05-62.85%, dough development time was 1.40-13.90 min, 45′energy was 40.75-99.25 cm^2, 45′ Rmax was 144.00-477.25 B.U. Moreover, the 45′ extensibility was 153.50-195.50 mm, Alveograph P was 46.75-97.25 mm, Ie was 35.20-52.37%, G was 16.47-27.50 mm, W was 135.50-272.00 $*10^{-4}$ J, W(40) was 70.75-157.00$*10^{-4}$ J, glutograph relaxation was 108.42-256.92 B.U. The wet gluten, sedimentation and dry gluten values showing the protein quality of genotypes with high protein content were also found to be high, similar to the results of Erekul and Köhn [25] and Barutcular et al. [26]. No similar studies on the mixograph have been found. The farinograph water absorption values determined in our study are in agreement with the studies on yufka and flat breads [1], [27], [28], [29], [30], [31] and [32]. However, Kundu [33], Shalini and Laxmi [34], Rao et al., [35] reported higher water absorption values in their study on chapatti. Yeyinli [29] conducted interviews with flour factories, and concluded that the wheat flour must have a water absorption rate of over 60%. The duration of the dough development is consistent with the study on yufka and flat breads [1], [27], [28], [29], [30], [31], [32], [33], [34] and [35]. In our study, extensograph 45′ values, 45′ energy 57.7-166.7 cm^2, 45′ Rmax 285-700 B.U. and 45′ extensibility 110-233 mm values were found between the values found between those reported by Yeyinli [29] and Olçay [32]. Alveograme values were found to be compatible with Acar [1] and Olçay [32], but higher than Gül et al. [36], Li et al. [37], Agyare et al. [38] and Indrani et al. [39]. Acar [1], in their study, have reported that flour samples for baklava production obtained from pure wheat varieties and samples of commercial baklava flour showed the glutograph stretching times of 6 - 125 s and relaxation values of 245-650 B.U. These values were corroborated in our study (Table 3).

TABLE 3
Genotype*year interactions of some chemical, physicochemical and rheological characteristics of wheat flour and dough

	Protein (%d.m)**			WG- Wet Gluten (%14 m.b)**			MZS- Modified zeleny sedimentation (ml)		
Genotype	2015	2016	Average	2015	2016	Average	2015	2016	Average
EBVD 1-16-E	13.68^{ab}	11.49^{d-f}	12.58^{a}	32.17^{c-e}	30.68^{d-g}	31.43^{ab}	44.33^{d}	64.33^{b}	54.33^{a}
EBVD 2-37-E	12.98^{bc}	9.39^{i-k}	11.19^{b}	31.6^{c-f}	25.62^{h-j}	28.61^{c}	24.33^{jk}	19.33^{m}	21.83^{j}
EVD 2.1.2013-E	10.66^{f-h}	11.97^{c-e}	11.31^{b}	21.88^{kl}	36.36^{a}	29.12^{bc}	30.00^{h}	30.33^{h}	30.17^{g}
EBVD 1-8-E	12.53^{cd}	12.11^{c-e}	12.32^{a}	29.72^{d-g}	32.66^{b-d}	31.19^{ab}	40.00^{f}	42.33^{e}	41.16^{d}
EBVD 2-48-E	10.05^{h-j}	10.63^{f-h}	10.34^{c}	19.61^{l}	26.01^{h-j}	22.82^{e}	25.66^{ij}	67.66^{a}	46.67^{b}
KAŞİFBEY-E	10.71^{f-h}	11.15^{e-g}	10.93^{bc}	25.55^{h-j}	29.99^{d-g}	27.77^{c}	36.00^{g}	39.67^{f}	37.83^{e}
KAYRA-E	13.71^{ab}	8.81^{k}	11.26^{b}	34.44^{a-c}	22.67^{j-l}	28.56^{c}	60.67^{c}	27.00^{i}	43.83^{c}
EFE-E	14.69^{a}	9.67^{h-k}	12.18^{a}	36.07^{ab}	28.77^{e-h}	32.42^{a}	61.67^{c}	29.33^{h}	45.50^{b}
EKM 129-U	12.91^{bc}	9.09^{jk}	11.00^{bc}	32.14^{c-e}	25.88^{h-j}	29.01^{bc}	35.00^{g}	24.66^{j}	29.83^{g}
EKM 340-U	12.12^{c-e}	10.24^{g-i}	11.18^{b}	30.43^{d-g}	25.87^{h-j}	28.15^{c}	34.33^{g}	21.33^{l}	27.83^{h}
EKM 342-U	12.34^{cd}	8.98^{jk}	10.66^{bc}	27.37^{g-i}	21.79^{kl}	24.58^{de}	30.33^{h}	35.00^{g}	32.67^{f}
GOLİA-U	11.17^{e-g}	10.34^{g-i}	10.76^{bc}	25.05^{i-k}	28.69^{f-h}	26.87^{cd}	22.67^{kl}	29.33^{h}	26.00^{i}
SAGGİTARİO-U	11.53^{d-f}	9.14^{jk}	10.33^{c}	30.44^{d-g}	26.07^{h-j}	28.26^{c}	35.33^{g}	40.33^{f}	37.83^{e}
Average	12.24^{a} ±0.15	10.23^{b} ±0.15		28.96^{a} ±0.31	27.77^{a} ±0.31		36.95^{a} ±0.23	36.21^{a} ±0.23	
	CV 5.56			CV 7.40			CV 2.87		
	Genotype *LSD 0.76			Genotype *LSD 2.43			Genotype *LSD 1.23		
	Year*LSD 0.46			Year N.S.			Year N.S.		
	Genotype*Year *LSD1.14			Genotype*Year *LSD 3.41			Genotype*Year *LSD 1.79		

N.S.: Not Significant *: Significant at p<0.05 **%14 m.b.: %14 moisture basis, %d.m: % dry matter basis.

Continues:

	Extensograme 45′ Energy (cm^2)			Extensograme 45′ Maximum Resistance-Rmax (BU)			Extensograme 45′ Extensibility-Ext. (mm)		
Genotype	2015	2016	Average	2015	2016	Average	2015	2016	Average
EBVD 1-16-E	78.50^{g-i}	120.00^{b}	99.25^{b}	281.00^{g-i}	486.00^{b}	383.50^{b}	207.00^{abc}	184.00^{d-f}	195.50^{a}
EBVD 2-37-E	49.00^{n}	32.50^{pq}	40.75^{i}	160.50^{kl}	127.50^{l}	144.00^{i}	208.00^{ab}	162.50^{g-k}	182.25^{ab}
EVD 2.1.2013-E	22.50^{r}	44.00^{no}	33.25^{j}	87.00^{m}	183.50^{jk}	135.25^{i}	170.00^{f-j}	154.00^{i-l}	162.00^{de}
EBVD 1-8-E	70.50^{ij}	83.00^{fg}	76.75^{d}	268.00^{h-i}	307.50^{efg}	287.75^{de}	192.50^{b-e}	190.00^{c-e}	191.25^{ab}
EBVD 2-48-E	25.50^{qr}	111.00^{c}	68.25^{ef}	90.50^{m}	398.00^{cd}	244.25^{g}	175.00^{e-h}	201.00^{a-d}	188.00^{ab}
KAŞİFBEY-E	69.50^{ijk}	98.50^{de}	84.00^{c}	281.00^{g-i}	395.00^{cd}	338.00^{c}	178.00^{e-g}	182.00^{ef}	180.00^{bc}
KAYRA-E	99.00^{d}	45.00^{no}	72.00^{de}	370.00^{d}	180.50^{k}	275.25^{ef}	206.50^{a-c}	171.50^{f-i}	189.00^{ab}
EFE-E	156.50^{a}	61.00^{kl}	108.75^{a}	661.00^{a}	293.50^{f-h}	477.25^{a}	190.50^{b-e}	146.00^{kl}	168.25^{cd}
EKM 129-U	90.00^{ef}	58.00^{lm}	74.00^{de}	324.00^{ef}	250.50^{i}	287.25^{de}	210.50^{a}	159.00^{h-l}	184.75^{ab}
EKM 340-U	51.50^{mn}	70.00^{ij}	60.75^{gh}	171.50^{k}	292.50^{f-h}	232.00^{g}	211.50^{a}	168.00^{f-j}	189.75^{ab}
EKM 342-U	21.00^{r}	60.50^{l}	40.75^{i}	85.00^{m}	273.50^{g-i}	179.25^{h}	160.50^{g-k}	153.50^{j-l}	157.00^{de}
GOLİA-U	38.50^{op}	73.00^{h-j}	55.75^{h}	173.50^{k}	331.50^{e}	252.50^{fg}	150.50^{kl}	156.50^{i-l}	153.50^{e}
SAGGİTARİO-U	51.50^{mn}	79.50^{gh}	65.50^{fg}	215.50^{j}	405.00^{c}	310.25^{d}	169.00^{f-j}	143.00^{l}	156.00^{de}
Average	63.34^{b}±0.65	72.00^{a}±0.65		243.73^{b}±0.73	301.88^{a}±0.73		186.88^{a}±1.13	167.00^{b}±1.13	
	CV 6.42			CV 6.28			CV 4.90		
	Genotype *LSD 6.17			Genotype *LSD 24.36			Genotype*LSD 3.22		
	Year*LSD 1.83			Year *LSD 2.07			Year*LSD 12.32		
	Genotype*Year*LSD 8.60			Genotype*Year *LSD 33.17			Genotype*Year *LSD 17.04		

N.S.: Not Significant *: Significant at p<0.05

Continues:

	Alveograme P-tenacity (mm)			Alveograme Ie-elasticity (%)			Alveograme G-index of swelling (mm)		
Genotype	2015	2016	Average	2015	2016	Average	2015	2016	Average
EBVD 1-16-E	59.00^{g-i}	90.50^{b}	74.75^{c}	50.65^{b-f}	54.10^{a-e}	52.37^{a}	27.35^{ab}	24.60^{bc}	25.97^{ab}
EBVD 2-37-E	47.50^{jk}	46.00^{kl}	46.75^{h}	47.20^{d-h}	36.25^{i-l}	41.72^{cd}	26.95^{ab}	22.75^{c-g}	24.85^{bc}
EVD 2.1.2013-E	39.00^{m}	79.50^{cd}	59.25^{e}	29.65^{l}	40.75^{h-k}	35.20^{e}	21.15^{e-j}	20.15^{g-l}	20.65^{e}
EBVD 1-8-E	39.50^{lm}	60.50^{gh}	50.00^{gh}	57.45^{ab}	43.85^{f-i}	50.65^{ab}	28.15^{a}	26.85^{ab}	27.50^{a}
EBVD 2-48-E	59.00^{g-i}	77.00^{c-e}	68.00^{d}	44.90^{e-h}	59.65^{a}	52.27^{a}	19.55^{h-m}	20.55^{f-k}	20.05^{e}
KAŞİFBEY-E	69.50^{f}	81.50^{c}	75.50^{c}	54.75^{a-d}	48.40^{c-g}	51.57^{ab}	19.75^{h-m}	23.05^{c-f}	21.40^{e}
KAYRA-E	74.00^{d-f}	76.00^{c-f}	75.00^{c}	52.05^{b-f}	41.60^{g-k}	46.82^{bc}	24.50^{b-d}	17.00^{m}	20.75^{e}
EFE-E	103.50^{a}	91.00^{b}	97.25^{a}	60.45^{a}	36.60^{i-l}	48.55^{ab}	23.40^{c-e}	18.25^{k-m}	20.82^{e}
EKM 129-U	80.50^{cd}	74.50^{c-f}	77.50^{c}	55.70^{a-c}	40.25^{h-k}	47.97^{ab}	22.70^{c-g}	18.85^{j-m}	20.77^{e}
EKM 340-U	61.00^{g}	59.00^{gh}	60.00^{e}	40.15^{h-k}	40.10^{h-k}	40.12^{de}	21.75^{d-i}	21.60^{e-j}	21.67^{de}
EKM 342-U	52.00^{i-k}	54.00^{h-j}	53.00^{fg}	34.40^{kl}	42.10^{g-j}	38.25^{de}	17.70^{lm}	22.05^{c-h}	19.87^{e}
GOLİA-U	79.50^{cd}	89.50^{b}	84.50^{b}	36.10^{j-l}	45.65^{e-h}	40.87^{d}	13.50^{n}	19.45^{h-m}	16.47^{f}
SAGGİTARİO-U	39.50^{lm}	71.00^{ef}	55.25^{ef}	54.95^{a-c}	45.15^{e-h}	50.05^{ab}	28.15^{a}	18.95^{ijklm}	23.55^{cd}
Average	61.80^{b}±0.36	73.07^{a}±0.36		47.57^{a}±0.55	44.19^{b}±0.55		22.66^{a}±0.21	21.08^{b}±0.21	
	CV 5.04			CV 8.06			CV 6.35		
	Genotype *LSD 4.82			Genotype*LSD 5.25			Genotype *LSD 1.97		
	Year *LSD 1			Year* LSD 1.55			Year* LSD 0.58		
	Genotype*Year *LSD 6.63			Genotype*Year *LSD 7.3			Genotype*Year *LSD 2.73		

N.S.: Not Significant *: Significant at p<0.05

Continues:

	Alveograme W-baking strength (10^{-4}J)			AlveogrameW(40) – baking strength at 40. second (10^{-4} J)			Glutograme Relaxation (BU)		
Genotype	2015	2016	Average	2015	2016	Average	2015	2016	Aver-age
EBVD 1-16-E	222.50^{d-f}	327.00^{b}	274.75^{a}	96.50^{f}	151.50^{b}	124.00^{b}	205.50^{ef}	225.00^{de}	215.25^{c}
EBVD 2-37-E	162.00^{i-k}	111.00^{l-n}	136.50^{fg}	74.00^{ij}	67.50^{j}	70.75^{g}	166.00^{g-i}	260.00^{a-c}	213.00^{c}
EVD 2.1.2013-E	94.00^{n}	177.00^{g-j}	135.50^{fg}	54.50^{k}	119.00^{e}	86.75^{ef}	225.50^{de}	249.33^{b-d}	237.42^{ab}
EBVD 1-8-E	177.50^{g-j}	197.50^{f-h}	187.50^{cd}	69.00^{j}	93.50^{fg}	81.25^{f}	153.50^{g-j}	271.67^{ab}	212.58^{c}
EBVD 2-48-E	133.50^{k-m}	243.50^{cd}	188.50^{cd}	93.00^{fg}	136.00^{cd}	114.50^{cd}	145.50^{h-j}	71.33^{k}	108.42^{g}
KAŞİFBEY-E	190.50^{f-i}	235.50^{c-e}	213.00^{b}	118.50^{e}	127.00^{de}	122.75^{bc}	129.00^{j}	246.00^{b-d}	187.50^{de}
KAYRA-E	249.50^{cd}	153.50^{jk}	201.50^{bc}	120.50^{e}	119.00^{e}	119.75^{b-d}	175.50^{f-h}	227.00^{de}	201.25^{cd}
EFE-E	368.50^{a}	175.50^{g-j}	272.00^{a}	179.50^{a}	134.50^{cd}	157.00^{a}	159.00^{g-j}	278.33^{a}	218.67^{bc}
EKM 129-U	262.00^{c}	155.50^{jk}	208.75^{bc}	137.50^{cd}	115.00^{e}	126.25^{b}	147.00^{h-j}	271.33^{ab}	209.17^{cd}
EKM 340-U	144.50^{j-l}	144.00^{j-l}	144.25^{f}	90.50^{f-h}	90.50^{f-h}	90.50^{e}	240.50^{cd}	273.33^{ab}	256.92^{a}
EKM 342-U	95.50^{n}	136.00^{k-m}	115.75^{g}	75.50^{ij}	82.00^{g-i}	78.75^{fg}	160.50^{g-j}	180.67^{fg}	170.58^{ef}
GOLİA-U	109.50^{mn}	209.00^{e-g}	159.25^{ef}	80.05^{h-j}	143.50^{bc}	111.77^{d}	140.00^{ij}	162.67^{g-i}	151.33^{f}
SAGGİTARİO-U	177.50^{g-j}	164.00^{h-k}	170.75^{de}	69.00^{j}	115.00^{e}	92.00^{e}	172.50^{f-i}	246.67^{b-d}	209.58^{c}
Average	183.61^{a}±2.91	186.84^{a}±2.91		96.77^{b}±0.99	114.92^{a}±0.99		170.76^{b}±3.01	227.94^{a}±2.46	
	CV 8.95			CV 5.85			CV 8.18		
	Genotype *LSD 24.06			Genotype *LSD 8.78			Genotype *LSD 21.79		
	Year : N.S.			Year * 2.81			Year *LSD 7.82		
	Genotype*Year *LSD 33.71			Genotype*Year *LSD 12.28			Genotype*Year *LSD 33.75		

N.S.: Not Significant *: Significant at p<0.05

Table 4 shows the correlations of some chemical, physicochemical and rheological properties of wheat genotypes and doughs. In our study, significant positive correlations were determined between protein content x water absorption, protein x dough development time, Protein x Alveogram G, Protein x Alveogram W, Protein x Alveogram Ie (r=+0.8181, +0.7544, +0.5707, +0.5165, +0.4881, p<0.05). Yeyinli [29] found positive correlations between protein x water absorption and protein x dough development time (r = + 0.51, +0.45, p <0.0001) in their study on correlations of some properties of yufka dough flour.

Extensograph is a quality analysis that shows the producibility of yufka dough in the pastry. While 135' values were generally used in studies on flat breads, in our study, 45′ values were preferred since yufka is not a leavened dough [32], [39] and [40]. Correlations were determined between 45′ energy x wet gluten, 45′ energy x modified zeleny sedimentation (r=+0.4943, +0.8421; p<0.05), 45′ extensibility x wet gluten, 45′ extensibility x relaxation, 45′ extensibility x modified zeleny sedimentation,, 45′ extensibility x 45′ energy protein (r=+0.4614, -0.3134, +0.4670, +0.4116, p<0.05), 45′ Rmax x wet gluten, 45′ Rmax x modified zeleny sedimentation, and 45′ Rmax x 45′ energy (r=+0.4271, +0.7787, +0.9713; p<0.05).

Extensograph 45′ values corroborated those reported by Yeyinli [29] and Olçay [32]. Yeyinli [29], in their study on the relation of extensograph and chemical-physicochemical properties of flour samples sold for special purposes in the market, found r= +0.72, +0.60, +0.66, +0.51, +0.59, +0.59 between 45′ energy& modified zeleny sedimentation, 45′ energy x total gluten, 45′ Rmax x modified zeleny sedimentation, 45′ Rmax x total gluten, 45′ extensibility x modified zeleny sedimentation, 45′ extensibility x total gluten, respectively (p<0.0001). The correlations were positive and close to our values. Olçay [32], in their study, found correlation between 45′ energy values and wet gluten, zeleny sedimentation and alveograme W (r=0.58, 0.49, 0.55; p≤0.01), 45′ extensibiliy x wet gluten, 45′ extensibiliy x zeleny sedimentation, 45′ extensibility x alv W (r=0.65, 0.52, 0.39, ; p≤0.01), 45′ Rmax x zeleny sedimentation, 45′ Rmax x alv P, 45′ Rmax x alv W (r=0.19, 0.24, 0.46; p≤0.01), correlations determined in the flour samples obtained from pure wheat genotypes were reported as 45′ extensibility x wet gluten (r=0.82; p≤0.05), 45′ extensibility x alveograme P (r=-0.68; p≤0.05); 45′ energy x wet gluten, 45′ energy x zeleny sedimentation, 45′ energy x dough development time, 45′ energy x alveograme W (r=0.61, 0.61, 0.57, 0.64; p≤0.05), and Rmax x dough development time (r= 0.75; p≤0.05). In our study, extensograph and wet gluten analyses demonstrated positive correlations with each other but were slightly lower than in Olçay's [32] study.

In our study, positive correlation was determined between water absorption and wet gluten (r=+0.6917, p<0.05), water absorption and 45′ extensibility (r=+0.3842, p<0.05); dough development time and wet gluten, dough development time and modified zeleny sedimentation, dough development time and 45′ energy, dough development time and 45′ extensibility, dough development time and 45′ Rmax, dough development time and water absorption (r= +0.6727, +0.6087, +0.5436, +0.5403,

TABLE 4
Correlations of some chemical, physicochemical and rheological characteristics of wheat flour and dough

	Wet gluten	Protein %	MZS	Relaxation (BU)	45′ Energy	45′ Ext.	45′ Rmax	WA	DDT	Alv P	Alv G	Alv W	Ie	W(40)
Wet gluten	1													
% protein	0.7999	1		-0.2733	0.3850	0.6850		0.8181	0.7544		0.5707	0.5165	0.4881	
MZS	0.2923		1	-0.3314										
Relaxation (BU)				1										
45'energy	0.4943		0.8421		1									
45'Ext.	0.4614		0.4670	-0.3134	0.4116	1								
45 ' Rmax	0.4271		0.7787		0.9713		1							
WA	0.6917					0.3842		1						
DDT	0.6727		0.6087		0.5436	0.5403	0.4637	0.6163	1					
Alv P	0.3095		0.4194		0.6168		0.6681			1				
Alv G	0.5511		0.3890		0.3442	0.6207			0.4979	-0.3408	1			
Alv W	0.6165		0.8088		0.9296	0.4833	0.8792	0.3567	0.6519	0.6563	0.4083	1		
Alv Ie	0.4566		0.7151	-0.5264	0.7370	0.5887	0.6509		0.4309	0.2788	0.5581	0.7935	1	
Alv W(40)	0.3785		0.5758		0.7679		0.7907		0.3593	0.9303		0.8192	0.5126	1
MTAL	0.3773		0.2797		0.3383		0.3207			0.5822		0.3748		0.5318

WA:Water Absorbtion, DDT: Dough Development Time, MTAL: Mixograme Total Area

+0.4637, +0.6163, p<0.05). Olçay [32] found that the values of water absorption x zeleny sedimentation, water absorption x wet gluten, water absorption x alveogram P values for commercial yufka flour samples were ((r= 0.44, 0.40, 0.50; p≤0.01) positive correlations. For pure wheat genotypes, Olçay [32] reported correlations similar to those found in our study as water absorption x zeleny sedimentation, dough development time x zeleny sedimentation, dough development time x 45′ energy (r=0.54, 0.55, 0.57; p≤0.05).

Alveogram values (Table 3) were similar to those reported by Acar [1] and Olçay [32]. In our study, correlations Alveogram P and various factors including wet gluten, modified zeleny sedimentation, 45′ Energy, 45′ Rmax (r=+0.3095, +0.4194, +0.6168, +0.6681, p<0.05). The correlation of Alveogram G with wet gluten, modified zeleny sedimentation, 45′ Energy, 45′ Extensibility, dough development time and alveogram P was also determined (r=+0.5511, +0.3890, +0.3442, +0.6207, +0.4979 -0.3408, p<0.05). Alveogram W and various factors such as Alveogram W x wet gluten, Alveogram W x modified zeleny sedimentation, Alveogram W x 45′ Energy, Alveogram W x 45′ Extensibility, Alveogram W x 45′ Rmax, Alveogram W x water absorption, Alveogram W x Dough development time, Alveogram W x Alveogram P, Alveogram W x Alveogram G (r= +0.6165, +0.8088, +0.9296, + 0.4833, +0.8792, +0.3567, +0.6519, +0.6563, +0.4083, p<0.05); The values further determined were: Alveogram Ie x wet gluten, Alveogram Ie x relaxation (BU), Alveogram Ie x modified zeleny sedimentation, Alveogram Ie x 45′ Energy, Alveogram Ie x 45′ Extensibility, Alveogram Ie x 45′ Rmax, Alveogram Ie x Dough development time, Alveogram Ie x Alveogram P, Alveogram Ie x Alveogram G, Alveogram Ie x Alveogram W (+0.4566, -0.5264, +0.7151, +0.7370, +0.5887, +0.6509, +0.4309, +0.2788, +0.5581, +0.7935, p<0.05); Alveogram W(40) x wet gluten, Alveogram W(40) x modified zeleny sedimentation, Alveogram W(40) x 45′ Energy, Alveogram W(40) x 45′ Rmax, Alveogram W(40) x Dough Development Time, Alveogram W(40) x Alveogram P, Alveogram W(40) x Alveogram W, Alveogram W(40) x Alveogram Ie (r= +0.3785, +0.5758, +0.7679, +0.7907, +0.3593, +0.9303, +0.8192, +0.5126, p<0.05). Acar [1] determined that alveograph P and Ie values indicate wheat flour quality for commercial flours used for baklava production. In the same study, it was revealed that the quality of yufka flour can be estimated by using alveograph Ie and gluten index values. In the Olçay [32] study, there was as relation between 45′energy value x Alveogram W (r = 0.64, p≤0.05). Olçay [32] also reported correlations between alveograph P value and farinogram water absorption (r=0.50, p≤0.01) and alveogram W x wet gluten (r=0.54; p≤0.01) for yufka flour samples, and correlations between Alveogram P x wet gluten (r=-0.50, p≤0.05), alveogram W x zeleny sedimentation (r=0.60, p≤0.05) for pure wheat genotypes. These results were similar to those obtained in our study.

In our study, correlations were found between Mixogram MTAL x wet gluten, MTAL x modified zeleny sedimentation, MTAL x 45′ Energy, MTAL x 45′ Rmax, MTAL x Alveogram P, MTAL x Alveogram W, and MTAL x Alveogram W(40) (r=+0.3773, +0.2797, +0.3383, +0.3207, +0.5822, +0.3748, +0.5318; p<0.05)

CONCLUSION

When the correlations obtained in our study were examined, alveoconsistographe was found to be a guiding device in the selection of yufka wheat varieties. Particularly, alveoconsistographe exhibited high correlations with modified zeleny sedimentation and 45′ extensogrametric data.

As a consequence of the study, wet gluten modified zeleny sedimentation and alveoconsistogrametric W (40), Ie, 45′ extensogrametric energy and Rmax values were determined to be significant in terms of yufka quality. As a result of genotype-year interactions evaluation for two years in Table 3, Ebvd 1-16-E, Ebvd1-8-E, Efe-E, Ebvd 2-48-E are the prominent genotypes due to their protein content and high quality. Likewise, it is seen that the genotypes of Kaşifbey-E, Saggitario-U and Ekm-129-U are suitable for mixogrametric MTAL, 45 'extensogrametric, alveogrametric values. As a result of these evaluations, it can be seen that genotypes Ebvd 1-16-E, Ebvd 1-8-E, Efe-E, Ebvd 2-48-E and Ekm 129 -U, Kaşifbey-E, Saggitario-U can be suggested as yufka wheat varieties. It is also thought that Ebvd 1-16-E, Ebvd 1-8-E, Efe-E, and Ebvd 2-48-E genotypes are also good in terms of quality and yield values, and the use of these varieties in Aegean and Mediterranean coast, where has similar ecologic conditions with İzmir, can be suggested for widespread usage, by the farmers. In the subsequent studies, yufka will be produced using these genotypes, yufka quality analyses will be conducted, and the genotypes that are most suitable for yufka production will be determined.

ACKNOWLEDGEMENTS

This project was supported by the Republic of Turkey Food, Agriculture and Livestock Ministry General Directorate of Agricultural Research and Policy (TAGEM). For their technical support in our study, we thank Aegean Agricultural Research Institute Field Crops Department Wheat Branch, Ege University Agricultural Faculty Field Crops Department, Yüksel Tezcan Food Co. and Stern Ingredients Turkey.

REFERENCES

[1] Acar, O. (2012) An investigation on baklava quality characteristics and suitability of flours obtained from various wheat cultivars for baklava production. Hacettepe University Graduate School of Natural and Applied Science, Ankara.

[2] Erdem, H., Torun, M.B. (2017) Assesment of sulphur efficiency of durum and bread wheat genotypes. Fresen. Environ. Bull. 26, 5891-5899.

[3] Galantini, J.A., Landriscini, M.R., Iglesias, J.O., Miglierina, A.M., Rosell, R.A. (2000) The effects of crop rotation and fertilization on wheat productivity in the Pampean semiarid region of Argentina 2. Nutrient balance, yield and grain quality. Soil and Tillage Research. 53, 137-144.

[4] Pislea, D., Boldea, M., Sala, F. (2013) Assesing the gluten content in wheat as an expression of the nutrition deficit, through beta distribution. AIP Conference Proceedings. AIP Publishing, Sydney, Australia, 1558-1571.

[5] Cho S-W., Kang C-S., Kang T-G., Cho, K-M., Park, C.S. (2018) Influence of different nitrogen application on flour properties, gluten properties by HPLC and end-use quality of Korean wheat. Journal of Integrative Agriculture. 17(5), 982-993.

[6] Klikocka, H., Cybulska, M., Barczak, B., Narolski, B., Szostak, B., Kobiałka, A., Nowak, A., Wójcik, E. (2016) The effect of sulphur and nitrogen fertilization on grain yield and technological quality of spring wheat. Plant Soil Environ. 62(5), 230-236.

[7] Liu, H.E., Wang, Q.Y., Rengel, Z., Zhao P. (2015) Zinc fertilization alters flour protein composition of winter wheat genotypes varying in gluten content. Plant Soil Environ. 61(5), 195-200.

[8] www.aregem.kulturturizm.gov.tr, accessed on December 8th,2017

[9] Çelebi, E., (2011) The machine took the place of the oklavan, 4.2 billion TL yufka has reached 6 months shelf- life. www.hurriyet.com.tr, accessed on September 15th, 2013

[10] Yüksel Tezcan Food Co., personal communication in 2013.

[11] Anonymous, (1982) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 110/1.

[12] Anonymous, (2000) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 167.

[13] Anonymous, (1990) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 104/1

[14] Anonymous, (2006) AACC International Method 76– 33.01. Damaged starch - amperometric method by SDmatic, Approved Methods of Analysis, 11th ed. St. Paul: AACC International. ISBN: 978-1-891127-68-2.

[15] Anonymous, (1994) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 155.

[16] Anonymous, (1994) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 116/1.
[17] Pinckney, A.J., Greenaway, W.T, Lawrence, Z. (1957) Further developments in the sedimentation test for wheat quality. Cereal Chem. 34:16 – 26 (From, Yeyinli, 2011).
[18] Anonymous, (1990) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 151.
[19] Anonymous, (1995) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 107/1.
[20] Anonymous, (1992) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 115/1.
[21] Anonymous, (1992) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 114/1.
[22] Glutograph- E Manual, (2007) Brabender Measurement and Control Systems.
[23] Anonymous, (1999) AACC International Method 54-40.02. Mixograph method, approved methods of analysis, 11th ed. St. Paul: AACC International. ISBN:978-1-891127-68-2.
[24] Anonymous, (1992) Standard Methods of the International Association for Cereal Chemistry (ICC), Verlag Moritz Scläfer Detmold. ICC No: 121.
[25] Erekul, O and Köhn, W. (2006) Effect of weather and soil conditions on yield components and bread-making quality of winter wheat (Triticum aestivum L.) and winter triticale (Triticosecale Wittm.) varieties in North-East Germany. J. Agronomy and Crop Science. 192, 452-464.
[26] Barutcular, C., Yildirim, M., Koc, M., Dizlek, H., Akinci, C., El Sabagh, A., Saneoka, H., Ueda, A., Islam, M. S., Toptas, I., Albayrak, O., Tanrikulu, A., (2016) Quality traits performance of bread wheat genotypes under drought and heat stress conditions. Fresen. Environ. Bull. 25, 6159-6165.
[27] Kaur, A., Singh, N., Ahlawat, A.K., Kaur, S., Singh, A.M., Chauhan, H., Singh, G.P., (2013) Diversity in grain, flour, dough and gluten properties among Indian wheat cultivars varying in high molecular weight subunits (HMW-GS). Food Research International. 53, 63-72.
[28] Guo, G., Jackson, D.S., Graybosch, R.A., Parkhurst, A.M., (2013) Wheat tortilla quality: impact of amylose content adjustment using waxy wheat flour. Cereal Chem. 80(4), 427-436.
[29] Yeyinli, N. (2011) Determination of some physical, chemical and technological properties of some special purpose wheat flours. Celal Bayar University Graduate School of Natural and Applied Science, Manisa.
[30] Barros, F., Alviola, J.N., Rooney, L.W. (2010) Comparison of quality of refined and whole wheat tortillas. J. Cereal Sci. 51, 50-56.
[31] Mosharraf, L., Kadivar, M., Shahedi, M. (2009) Effect of hydrothermaled bran on physicochemical, rheological, and microstructural characteristics of sangak bread. J.Cereal Sci. 49, 389-404.
[32] Olçay, M. (2000) The impact of variety and grinding technology on the quality of yufka. Ege University, Graduate School of Natural and Applied Science, İzmir.
[33] Kundu, M., Khatkar, B.S., Gulia, N., (2017) Assesment of chapatti quality of wheat varieties based on physicochemical, rheological and sensort traits. Food Chem. 226, 95-101.
[34] Shalini, K.G., Laxmi, A., (2007) Influence of additives on rheological characteristics of whole wheat dough and quality of chapatti (Indian unleavened flat bread) Part 1-hydrocolloids. Food Hydrocolloids. 21, 110-117.
[35] Rao, P.H., Leelavathi, K., Shurpalekar, S.R. (1989) Effect of damaged starch on the chapatti-making quality of whole wheat flour. Cereal Chem. 66(4), 329-333
[36] Gul, H., Hayit, F., Bicakci, S., Acun, S. (2018) Effect of heat stabilized wheat germ on some properties of cookies. Fresen. Environ. Bull. 27, 1145-1151.
[37] Li, J., Hou, G.G., Chen, Z., Chung, A-L., Gehring, K. (2014) Studying the effects of whole-wheat flour on the rheological properties and the quality attributes of whole-wheat saltine cracker using SRC, alveograph, rheometer, and NMR technique. LWT-Food Sci. and Tech. 55, 43-50.
[38] Agyarea, K.K., Addo, K., Xiong, Y.L., Akoh, C.C. (2005) Effect of structured lipid on alveograph characteristics, baking and textural qualities of soft wheat flour. J. Cereal Sci. 42, 309-316.
[39] Indrani, D., Manohar, R.S., Rajiv, J., Rao, G.V. (2007) Alveograph as a tool to assess the quality characteristics of wheat flour for parotta making. J. Food Eng. 78, 1202-1206.
[40] Taşdemir, B. (2005) Determination of suitability of different passages to some traditional bread varieties. Ankara University Graduate School of Natural and Applied Science, Ankara.

Received: **08.02.2018**
Accepted: **25.07.2018**

CORRESPONDING AUTHOR

Nihan Bas-Zeybekoglu
Aegean Agricultural Research Institute
Izmir – Turkey

e-mail: nihanbas@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9573-9580 Fresenius Environmental Bulletin

RISK ASSESSMENT AND CONCENTRATIONS OF HEAVY METALS IN MARKET-PURCHASED VEGETABLES IN HANGZHOU, CHINA

Xiaoqin Zhang[1], Xiachen Lv[1], Yunxia Fang[1], Hua Jiang[2], Dawei Xue[1,*]

[1]College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China
[2]Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Science, Hangzhou 310021, China

ABSTRACT

To investigate the extent of heavy metal contamination in market-purchased vegetables in Hangzhou, China, and assess the health risk of eating these vegetables, 19 vegetable samples were purchased at three major wholesale vegetable markets in Xiaoshan, Gouzhuang, and Xiasha. The concentrations of Cd, Pb, Cr, Cu, and Zn were measured, and the extent of heavy metal contamination in the vegetables was assessed according to related Chinese standards. The health risk of heavy metals in market-purchased vegetables was also assessed using the exposure assessment equation from the MMSOILS model of the United States Environmental Protection Agency. Results indicated that Pb and Zn, with standard-exceeding rates of 94.7% and 100%, respectively, are the main metal contaminants in the vegetables sold in Hangzhou. All vegetables sold in Hangzhou in the summer of 2016 were seriously contaminated. According to our analysis using the exposure assessment equation, only the HQ_{veg} of Cu was greater than 1 among the five metals studied. The results of the present study indicated that consuming vegetables sold in vegetable markets in Xiaoshan, Gouzhuang, and Xiasha may have adverse effects on human health because of their excessive Cu content. Based on the exposure assessment equation, the pollutant, Cu is considered hazardous to human health. The situation requires more attention not only from the consumers but also from the administrators.

KEYWORDS:
Vegetables, heavy metals, pollution, health risk assessment

INTRODUCTION

Vegetables, as a major food group in human diet, provide the human body with abundant vitamins, mineral salts, and cellulose. The quality of vegetables has a direct effect on human health [1, 2]. However, with the rapid development of industry and agriculture, vegetables are often contaminated by various heavy metals because of the unreasonable application of agricultural chemicals and fertilizers containing heavy metals.

Characterized by non-reversibility and incomplete decomposition, heavy metals are easily accumulated in the roots, stems, leaves, and seeds of plants [3]. Then, they are absorbed into the human body through the food chain and threaten human health [4]. Vegetables from various regions have been found to be contaminated by different quantities of heavy metals, particularly Pb, Cd, Cr, Cu, and Zn [1, 2, 5-8]. Once the concentrations of these five heavy metals reach a certain level in the human body, serious illnesses may develop. For example, lead is difficult to eliminate from the human body and can directly damage brain cells [9]. This metal also has carcinogenic and mutagenic effects.

Assessments on heavy metal contaminations in vegetables sold in Hangzhou are lacking [10]. To provide references to monitor the heavy metal contamination in vegetables in Hangzhou, aid government decision-making, and provide consumers with a safe eating guide, the heavy metal concentrations in vegetable samples obtained from wholesale markets in Hangzhou were measured. The pollution status of these vegetables was also assessed according to food hygiene standards.

MATERIALS AND METHODS

Sample collection. In the summer of 2016, 19 types of vegetables were obtained from the main vegetable wholesale markets in Hangzhou and divided into nine major categories (Table 1).

To ensure that the sampling sites were equally distributed, the vegetables samples were acquired from three major vegetable markets, namely, Hangzhou Xiaoshan agricultural products wholesale market, Hangzhou Gouzhuang vegetable wholesale market, and Xiasha agricultural byproduct market (Fig. 1). Sampling was performed over two time periods; one in July 2016, during which three collections were performed, and the other in August 2016, during which another three collections were performed. During each sampling, 1 kg of each vegetable type in each market was collected.

TABLE 1
Vegetables used in the present research

Major categories	Name			
Leafy vegetable	Lettuce	Spinach		
Brassica sp.	Cauliflower			
Tuber vegetable	Carrot			
Allium sp.	Garlic			
Stem vegetable	Celery	*Zizania latifolia*	Lotus root	
Melons and solanum	Cucumber	Tomato	Green pepper	Eggplant
Beans	Asparagus bean	Green bean	Kidney bean	
Edible mushrooms	Enoki mushroom	*Pleurotus geesteranus*		
Tomato, potato and cassava	Taro	Potato		

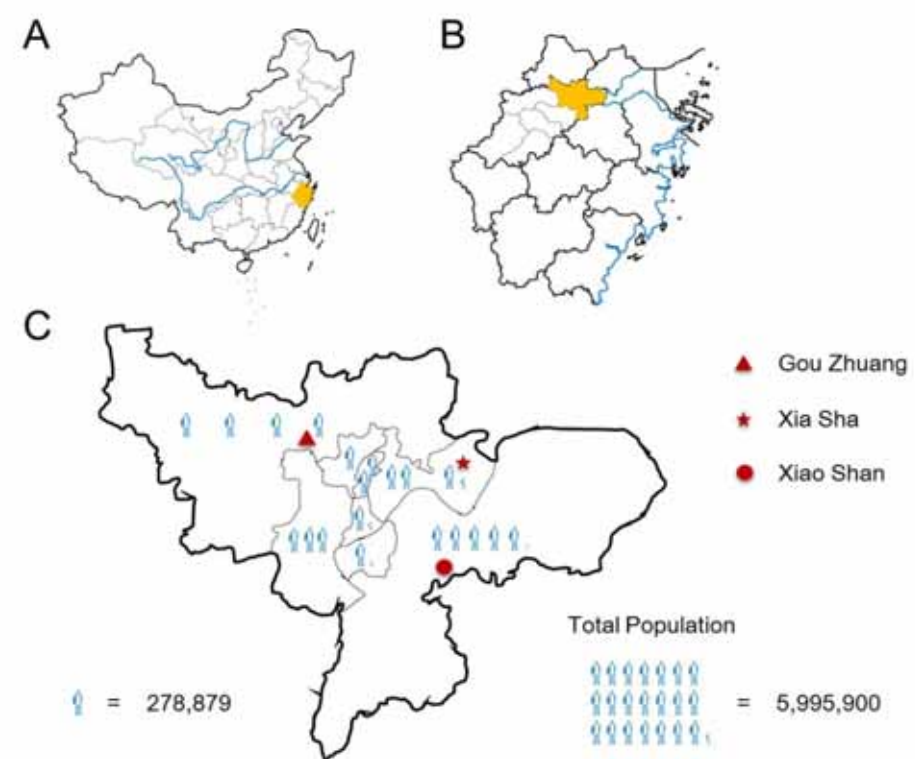


FIGURE 1
Sampling sites in Hangzhou

A) Map of China; the yellow region indicates Zhejiang Province; B) Map of Zhejiang province; the yellow region indicates Hangzhou City; C) Map of Hangzhou; represents the population and ▲, ★ and ● represents the sampling sites

Sample handling and analysis: The edible parts of the vegetables were obtained after repeated washing using deionized water. After the surface water had been removed using filter paper, the samples were placed in a drying oven at 105 °C for 30 min and then heated to a constant weight at 70 °C. The dry samples were grounded using a micro pulverizer, passed through a nylon sieve of 100 mesh, and stored in plastic bags. The samples were measured as follows: The processed sample (0.5 g) was put into a digestion tube and then mixed with 0.5 mL of HF, 1 mL of H_2O_2, and 5 mL of HNO_3. The solution was digested using a microwave digester (CEM, MARS) [11]. The concentrations of Cd, Pb, Cr, Cu, and Zn in the digestion solution were measured by graphite furnace atomic absorption spectrophotometry (PE Company, A800). Each group included 39 samples and one blank control.

Assessment method and standards for heavy metal determination in vegetables: Heavy metals in vegetables may be represented by the standard-exceeding rate. The standard-exceeding rate is expressed as

$$C_i\ (\%) = (n_i / Ni) \times 100, \quad (1)$$

where C_i is the standard-exceeding rate of a certain pollutant in a certain vegetable type, n_i is the number of the samples containing a certain pollutant for a certain type of vegetable, and N_i is the total number of samples collected for a certain type of vegetable [12].

TABLE 2
Standards of maximum concentration limits for heavy metal pollution in the present research

Heavy metal	Vegetables	Concentration limit (mg/kg)	Criterion
Pb	Fresh vegetables (except *Brassica* sp., leafy vegetables, beans and potatoes)	0.1	GB 2762-2012
	Brassica sp. and leafy vegetables	0.3	
	Beans and potatoes	0.2	
Cd	Fresh vegetables (except foliage vegetables, beans, root and tuber vegetables, and stem vegetables)	0.05	
	Leafy vegetables	0.2	
	Beans, root and tuber vegetables, stem vegetables (except celery)	0.1	
	Celery	0.2	
Cr	All vegetables	0.5	
Cu	All vegetables	≤10 mg/kg	GB 13199-1991
Zn	All vegetables	≤20 mg/kg	GB 13106-1991

TABLE 3
Quality classification standards of vegetables

Classification	1	2	3	4	5
Integrated pollution index	P≤0.7	0.7<P≤1.0	1.0<P≤2.0	2.0<P≤3.0	P>3.0
Pollution degrees	Safe	Vigilance	Mild pollution	Moderate pollution	Serious pollution
Pollution levels	Clean	Moderately clean	Start to be polluted	At moderate level of pollution	At serious level of pollution

TABLE 4
Rf_D values recommended by the WHO

Heavy metal	Cr	Pb	Cd	Cu	Zn
Rf_D (reference dose) [μg/(kg·d)	15	3.5	1	500	300

The extent of heavy metal contamination in vegetables was assessed according to national food safety standards GB 2762-2012 and GB13106-1991 (Table 2).

Assessment of heavy metal contamination in market-purchased vegetables in Hangzhou: The heavy metal contamination in vegetables was assessed using a single-factor pollution index and an integrated pollution index according to the average concentrations of heavy metals [13].

(1) Single-factor pollution index method:

$$P_i = C_i / S_i \quad (2)$$

where P_i is the single-factor pollution index of a certain heavy metal, C_i is the measured concentration of certain heavy metal, and S_i is the standard concentration of each assessment.

(2) The degree of contamination in the vegetable samples was assessed according to the integrated pollution index and the quality classification standard of vegetables (Table 3).

The integrated pollution index is calculated as follows:

$$P_z = [(P^2_{avg} + P^2_{max}) / 2]^{1/2}, \quad (3)$$

where P_z is the integrated pollution index, P_{avg} is the average of each single-factor pollution index (P_i), and P_{max} is the maximum of P_i.

Health risk assessment of heavy metals in the vegetables in Hangzhou: The average daily intake of heavy metals from the edible vegetables was calculated using the simplified exposure assessment equation (generally applied in determining exposure risks from water, food, and air) from the MMSOILS model of the United States Environmental Protection Agency (USEPA) [14].

$$CDI_{veg} = (C \bullet I \bullet 10^3) / BW \quad (4)$$

$$C \cdot I = \sum (c_i \cdot D_i \cdot F_d) \quad (5)$$

where CDI_{veg} is the average daily intake of heavy metal contaminants from vegetables [μg/(kg•d)], C is the concentration of heavy metals in the vegetables (mg/kg), I is the contact rate, BW is the body weight (kg), considered as 60 kg, c_i is the average concentration of heavy metals in a certain type of vegetable (mg/kg), D_i is the daily consumption of a certain type of vegetable (kg), and F_d is the proportion of dry weight calculated from the fresh weight of vegetable, considered as 0.1 [15]. According to research on the diet, nutrition, and health situations of Chinese residents, the consumption of vegetables per capita is 311.63g•d^{-1} [16].

In this research, the exposure risk index of heavy metals from edible vegetables is expressed as HQ_{veg} (hazard quotient), and the maximum permitted uptake from this pathway is $0.5R_fD$ [17]. The

average daily uptake of vegetables (CDIveg) was compared against 50% of the reference dose of exposure to toxic pollutants (R_fD, Table 4).

If HQ_{veg} is greater than 1, the pollutant is considered hazardous to human health. The value of HQ_{veg} is proportional to the hazard brought about by the pollutant [4]. HQ_{veg} was calculated using the following formula:

$$HQ_{veg} = CDI_{veg} / 0.5\ R_fD \quad (6)$$

RESULTS

Analysis of heavy metal concentrations in vegetables: Of the 19 types of vegetables tested, leafy vegetables generally had the highest concentrations of heavy metals (except Cu), whereas beans had the lowest concentrations (Fig. 2). This finding concurred with a previous report [18]; that heavy metal concentrations and the integrated pollution index in foliage vegetables were higher than that in other types of vegetables, such as beans, roots and tubers, melons, and solanum, in representative regions of Guangdong Province. Leafy vegetables have strong accumulative ability. Heavy metal elements in smoke and dust may be absorbed by leaves and accumulate in vegetables [19].

By comparing the heavy metal concentrations in vegetables collected over different months, the concentrations of five metals across the months were not found significant difference (data not shown). Several differences in the heavy metal concentrations of vegetables from different sampling sites were observed, but these differences were generally small (data not shown).

Analysis of heavy metal excess in vegetables. To understand the extent of heavy metal pollution in different types of vegetables, the amounts of heavy metals in vegetables sold in Hangzhou were investigated and assessed according to Chinese limitation standards. Pb and Zn, which showed standard-exceeding rates of 94.7% and 100%, respectively, were the heavy metal pollutants with the highest concentrations in the vegetables (Table 5). The standard-exceeding rates of Pb in all types of vegetables except garlic were close to 100%; Pb in garlic did not exceed the specified limits. The concentrations of Cu in the vegetable samples fell below the maximum permitted concentrations according to Chinese national standards. The general standard-exceeding rates of Cr and Cd (36.8% and 63.2%, respectively) were relatively high, but their concentrations varied among the different vegetables. For example, the standard-exceeding rates of Cr and Cd in foliage vegetables reached 100%. The concentrations of Cr in stem vegetables, melons, solanum, and edible mushrooms were much higher than the specified limits. By contrast, Cd concentrations in all vegetables except *Brassica* species were very high. The standard-exceeding rates of heavy metals in vegetables from different sampling sites slightly differed, but these differences were not significant (Table 5).

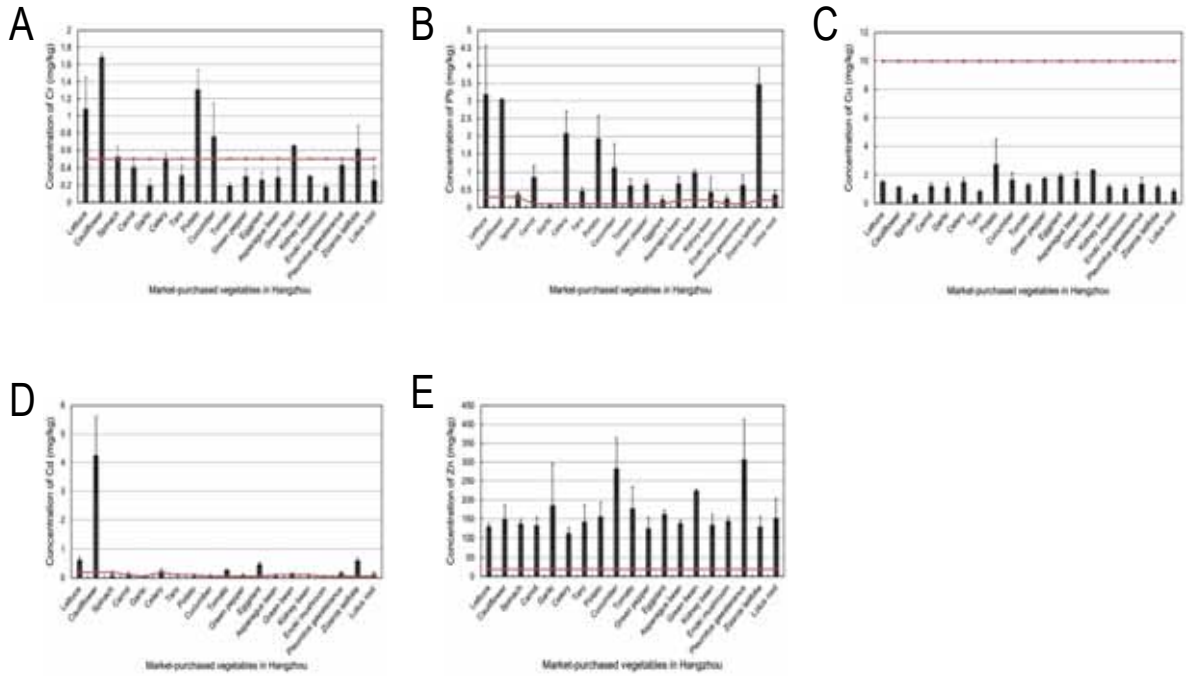


FIGURE 2
Heavy metal concentrations in vegetables and their distribution characteristics
A) Cd; B) Pb; C) Cr; D) Cu; E) Zn.
The red lines indicate maximum concentration limits according to Chinese national standards in vegetables.

TABLE 5
Standard-exceeding rates of various vegetables in the present research

Major categories *	Cr			Pb			Cu			Cd			Zn		
	Gouzhuang	Xiaoshan	Xiasha	Gouzhuang	Xiaoshan	Xiasha	Gouzhuang	Xiaoshan	Xiasha	Gouzhuang	Xiaoshan	Xiasha	Gouzhuang	Xiaoshan	Xiasha
Leafy vegetables (2)	100	100	100	100	100	100	0	0	0	100	100	100	100	100	100
Brassica sp. (1)	100	100	0	100	100	100	0	0	0	0	0	0	100	100	100
Tuber vegetable (1)	0	0	0	100	100	100	0	0	0	100	0	0	100	100	100
Allium sp. (1)	0	0	-	0	0	-	0	0	0	0	100	-	100	100	100
Stem vegetables (3)	33.3	33.3	0	100	100	100	0	0	0	0	0	33.3	100	100	100
Melons and solanum (4)	25	25	0	100	100	100	0	0	0	100	75	75	100	100	100
Beans (3)	0	0	33.3	100	100	66.7	0	0	0	0	0	33.3	100	100	100
Edible mushrooms (2)	0	100	0	100	100	100	0	0	0	0	100	50	100	100	100
Potatoes (2)	0	0	0	100	100	100	0	0	0	100	100	100	100	100	100

Note: *Figures in the brackets represent the number of species included in each category; '-' indicates that the vegetable is not available in the specified location.

TABLE 6
Single-factor and integrated pollution indices of various vegetables

Categories	Vegetables	Single-factor pollution index					Integrated pollution index	Pollution degrees
		Cr	Pb	Cu	Cd	Zn		
Leafy vegetable	Lettuce	2.1700	10.6117	0.1500	3.1192	6.4700	8.1515	Serious
	Spinach	3.3780	10.1667	0.1174	21.2825	7.5200	16.2030	Serious
Brassica sp.	Cauliflower	1.0513	1.2600	0.0594	0.3367	6.8700	5.0431	Serious
Tuber vegetable	Carrot	0.8223	8.6083	0.1232	1.2567	6.6558	6.5691	Serious
Allium sp.	Garlic	0.4010	0.4875	0.1148	0.7250	9.2963	6.7558	Serious
Stem vegetable	Celery	0.9780	20.8067	0.1494	1.1308	5.5975	15.2607	Serious
	Zizania latifolia	0.6297	4.6633	0.0852	0.0867	7.1133	5.3352	Serious
	Lotus root	2.6197	19.3183	0.2750	0.2783	7.7892	14.3156	Serious
Melons and solanum	Cucumber	1.5210	11.3800	0.1656	1.2400	14.1733	10.8011	Serious
	Tomato	0.3903	6.2517	0.1281	5.4000	8.8842	6.9520	Serious
	Green pepper	0.6123	6.6850	0.1753	1.8867	6.2192	5.2152	Serious
	Eggplant	0.5300	2.2150	0.1924	9.4000	8.1408	7.2503	Serious
Beans	Asparagus bean	0.5870	3.4117	0.1709	0.3217	6.8975	5.1363	Serious
	Green bean	1.3100	4.9450	0.2319	1.6100	11.2350	8.4016	Serious
	Soybean	0.6130	2.2038	0.1176	0.1500	6.7075	4.9409	Serious
Edible mushrooms	Enoki mushroom	0.3605	2.6075	0.1052	0.2900	7.2263	5.3247	Serious
	Pleurotus geesteranus	0.8580	6.4000	0.1370	3.3550	15.3663	11.4761	Serious
Tomato, potato and cassava	Taro	1.2343	17.3358	0.1155	11.9700	6.4517	13.3343	Serious
	Potato	0.5217	1.8558	0.0854	2.5867	7.5908	5.6574	Serious

Pollution indices of heavy metals in vegetables. To determine the extent of heavy metal contamination in vegetables sold in Hangzhou, the single-factor and integrated pollution indices were applied (Table 6). The single-factor pollution indices of foliage vegetables were generally higher than the standard-exceeding rates of heavy metals, and the Pb pollution index of each type of vegetables was also higher because of serious Pb excess. Differences in the pollution indices of various vegetables, which may be related to the habitats of the vegetables and pesticide usage, were also observed. The integrated pollution indices of all vegetables were greater than 3, implying that these vegetables were seriously contaminated. The highest integrated pollution indices were observed in spinach, celery, and lotus root.

TABLE 7
Heavy metal concentrations in edible vegetables and associated health risks

Assessment index	Cr	Pb	Cu	Cd	Zn
CI	0.0169	0.0353	0.0443	0.0121	5.1240
CDI	0.2814	0.5878	0.7378	0.2019	85.4001
HQ of vegetables	0.0375	0.3359	1.4756	0.0008	0.5693

Health risk assessment of heavy metals in vegetables in Hangzhou. The health risk associated with heavy metal pollution in vegetables in Hangzhou was assessed according to the simplified assessment equation of exposure (risk from water, food, and air) from the MMSOILS model of USEPA (Table 7).

The health risk indices of the five heavy metals (Table 7) showed that all the HQ_{veg} values of Cr, Pb, Cd, and Zn were smaller than 1, whereas the HQ_{veg} value of Cu was greater than 1. These results indicate that market-purchased vegetables in Hangzhou may promote health risks among local residents because of excessive Cu content.

DISCUSSION

Heavy metal concentrations in 19 types of vegetables (nine major categories) purchased from the main vegetable wholesale markets in Hangzhou in the summer of 2016 were analyzed and assessed in this study. The present work is the first systematic investigation on heavy metal concentrations in vegetables in Hangzhou. The Xiaoshan agricultural products and Gouzhuang vegetable wholesale markets are the major wholesale markets in Hangzhou that provide the majority of the vegetable supply in the region, and have important roles to ensure the vegetable supply of Hangzhou City. Census data reveal that the population of the urban area of Hangzhou is 5.9959 million (Fig 1). If the average weight of vegetables consumed daily by each person is 0.3 kg, then 1798 tons of vegetables are consumed every day in Hangzhou. Therefore, to ensure that vegetables are safe to consume is a matter of great concern [20].

Our study indicated that vegetables sold in Hangzhou are seriously contaminated by Pb and Zn, with standard-exceeding rates close to 100%. Among the five heavy metals determined, only Cu was found at concentrations lower than the upper limit of national food safety standards. Small differences in heavy metal concentrations across sampling times and sites were further observed. Serious heavy metal excesses were detected in all samples [21].

Large differences in heavy metal concentrations were found among the sample vegetables. The heavy metal concentrations in beans, for example, were relatively low, whereas that in leafy vegetables were relatively high [22]. Differences in the concentration of the same heavy metal in various vegetables, as well as in the concentration of different heavy metals in the same vegetable were further observed. These findings indicated that absorption of heavy metal elements from the soil by the vegetables is influenced by multiple factors [14]. Heavy metal concentrations in vegetables are related not only to the degree of soil contamination, but also to the absorption ability of the vegetables. Therefore, differences in heavy metal concentrations in vegetables are the result of synergistic effects of multiple factors [17].

The single-factor index reflected the degree of pollution of each pollutant, but it failed to comprehensively reflect the extent of contamination in vegetables. The integrated pollution index highlighted the significance of pollutants with high concentrations because its calculation involved the average and maximum of the single-factor pollution index [5]. The integrated pollution indices of all samples were greater than 3, and the integrated indices of some samples were greater than 10. These findings indicated that all samples have serious levels of contamination. Health risk assessment using the USEPA model showed that the Cu concentrations in all vegetable samples were not above the national standards. However, safety risks from Cu pollution were relatively high because of the low dose exposure to the metal [23].

The concentration of heavy metals accumulated in vegetables is closely related to the heavy metal concentration in the habitat. The heavy metal concentration in soil solutions around plant roots is generally an important factor that influenced the metal absorption of vegetables. When the concentrations of heavy metals in the soil solutions increase, the amount of heavy metals absorbed by vegetables also increased [24]. The vegetable samples in the present research were seriously contaminated by heavy metals, which may be related to the fact that the soils of vegetable-producing areas are also polluted. Major factors that influence the increase in heavy metal accumulation in soil include sewage irrigation, sludge and city garbage for agricultural usage, application of fertilizers and pesticides, and atmospheric sedimentation [25]. Heavy metal contamination in vegetables is related to the extent of pollution in the soil, water, and air in a given location [26, 27]. As a representative aquatic vegetable, lotus root showed excessive heavy metal concentrations because of water pollution in its habitat. The sewage yield every year in China is estimated to reach tens of billions, but only 24% of the industrial sewage and 4% of the domestic sew-

age are processed. Therefore, 80% of the water sources in China are polluted. Industrial and domestic waste and pesticide usage further increase the heavy metal concentrations in water and lead to contamination of aquatic vegetables [28]. As such, ensuring vegetable quality and safety by improving the detection and supervision of heavy metal contamination in vegetable-producing areas is of utmost importance.

Unreasonable use of fertilizers is an important factor that increases heavy metal concentrations in vegetables. Excessive use of nitrogen fertilizers is a common practice because farmers desire high yields and early maturation of vegetables [15]. When the pH of soil declines, its H^+ increases and the exchange capacity between H^+ and heavy metal cations adsorbed on the surface of colloids and clay mineral particles increases. These phenomena induce desorption of heavy metal cations into the soil solution. Declines in pH value also disrupt the precipitation-dissolution equilibrium of heavy metal cations, which enhances the activity of some heavy metals in soil and promotes their release [18]. Consequently, the heavy metal absorption of plants increases. As such, reasonable fertilization is an important measure to prevent heavy metal pollution in vegetables.

The purchase channel of wholesale vegetable markets in Hangzhou is not confined to Zhejiang Province [12]. Vegetables from other provinces, such as Anhui, Shandong, and Fujian, occupy a considerable proportion of imported vegetables. Aside from local vegetables in Hangzhou, vegetables from other provinces must also be strictly inspected [19]. Logistics and storage management practices must be strengthened to ensure the safety of vegetables.

CONCLUSION

Vegetables are important sources of nutrition for people, especially trace elements. However, the heavy metal contamination has increased the risk of vegetable consumption. We collected different vegetable samples from different wholesale markets, and the concentrations of Cd, Pb, Cr, Cu, and Zn were measured. All vegetables were seriously contaminated in Hangzhou. According to our results, the standard-exceeding rates of Pb and Zn were 94.7% and 100%, respectively, which are the main metal contaminants in the tested vegetables. Based on the exposure assessment equation, the pollutant, Cu is considered hazardous to human health. The situation requires more attention not only from the consumers but also from the administrators.

ACKNOWLEDGEMENTS

This work was financially supported by Undergraduate Innovation Project of HZNU (CX2017105), and partly supported by National Key Technology Research and Development Program (2015BAD01B02).

REFERENCES

[1] Krivokapic, S. and Petrovic, D. (2018) Trace metals in vegetable plants (*Allium cepa* L., *Capsicum annum* L., *Lycopersicon esculentum* MILL. and *Beta vulgaris* L.) from the Zeta Valley of Montenegro. Fresen. Environ. Bull. 27, 2690-2696.

[2] Avci, H. (2013) Heavy metals in vegetables irrigated with wastewaters in Gaziantep, Turkey: a review of causes and potential for human health risks. Fresen. Environ. Bull. 22, 146-151.

[3] Wu, J., Meng, X. and Li, K. (2005) Phytoremediation of soils contaminated by lead. Soils. 37, 258-264.

[4] Wang, B., Liu, X. and Feng, C. (2011) Concentration and health risk of heavy metals in vegetables from the markets of Wuhu City. Chinese Agr. Sci. Bull. 27, 143-146.

[5] Chen, Y., Huang, S., Liu, S., Wang, G., Ding, F., Shao, Z. and Shen, Z. (2006) Study of the heavy metal contamination in soils and vegetables in Nanjing area. Resour. Environ. Yangtze Basin. 15, 356-360.

[6] Leblebici, Z., Aksoy, A. and Akgul, G. (2017) Accumulation and effects of heavy metals on potatoes (*solanum tuberosum* l.) in the Nevsehir, Turkey. Fresen. Environ. Bull. 26, 7083-7090.

[7] Ahmad, M., Khan, Z., Ahmad, K., Batool, A., Hamid, S., Sher, M., Noorka, I., Hayat, Z., Bashir, H., Sohail, M., Ejaz, A., Sheikh, Z., Muneeb, A., Mehmood, N., Muqaddas, H., Zubair, F., Amjad, N., Taj, F., Iqbal, S. and Batool, F. (2017) Accretion and dispersion of heavy metals in a vegetable grown in contaminated soil with sewage water: a matter of great concern and community health consequences. Fresen. Environ. Bull. 26, 4345-4349.

[8] Sun, C., Xiang, S., Zhang, Q., Xiao, W. and Xu, P. (2017) Heavy metal content in broccoli (*Brassica oleracea* L. var. italic plenck) and potential health risk in Zhejiang Province, China. Fresen. Environ. Bull. 26, 2340-2347.

[9] Sharma, R., Agrawal, M. and Marshall, F. (2008) Heavy metal (Cu. Zn. Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. Environ. Pollut. 154, 254-263.

[10] Wang, Y., Zhu, W., Jin, J., Guo, D. and Yuan, Y. (2010) Researches on pollution evaluation and enrichment characteristics of Cu, Zn and Pb in farmland vegetable in Hangzhou. J. Hangzhou Norm. Univ. (Nat. Sci. Edit.). 9, 65-70.
[11] Mou, R., Chen, M., Ying, X., Shi, Y. and Zhu, Z. (2003) Determination of trace arsenic in rice by microwave digestion-HG-AFS. Chinese J. Spectrosc. Lab. 20, 852-855.
[12] Zhang, L. and Liu, L. (2006) Analysis and evaluation on heavy metal contents of commercial vegetables in Guiyang. Guizhou Agr. Sci. 34, 59-60.
[13] Fang, Y., Sun, X., Yang, W., Ma, N., Xin, Z., Fu, J., Liu, X., Liu, M., Mariga, A., Zhu, X. and Hu, Q. (2014) Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. Food chem. 147, 147-151.
[14] Chen, H., Liu, Z. and Li, G. (2006) A study of the establishment and evaluation method of the soil risk guideline of contaminated sites. Hydroge. Eng. Geol. 33, 84-88.
[15] Xie, H., Liao, X., Chen, T. and Lin, J. (2005) Arsenic in plants of farmland and its healthy risk: a case study in an As-contaminated site in Dengjiatang. Chenzhou City, Hunan province. Geogr. Res. 24, 151-159.
[16] Huang, L., Zhang, R., Gu, F., Zhang, H., He, Q., Meng, J. and Huang, E. (2011) Survey on dietary nutrition and health status among residents in two areas of Zhejiang province. Zhejiang J. Preven. Med. 23, 1-4.
[17] Liu, F., Wu, J., Gao, H., Zhao, Y. and Tang, Y. (2007) A survey of cadmium exposure in vegetables and soils and the potential risks to Human Health. J. Agro-Env. Sci. 26, 1860-1864.
[18] Yang, G., Luo, W., Gao, J., Zhang, T., Wan, H. and Gao Y. (2008) Heavy metal contents and pollution evaluation in vegetables in Guangdong Province. Chinese J. Soil Sci. 39, 133-136.
[19] Yang, H. and Zhang, B. (2007) Major problems and countermeasures of pollution in vegetable production in China. J. Anhui Agr. Sci. 35, 1431-1433.
[20] Lang, I., Scarlett, A., Guralnik, J., Depledge, M., Melzer, D. and Galloway, T. (2009) Age-related impairments of mobility associated with cobalt and other heavy metals: data from NHANES 1999–2004. J.Toxicol. Env. Heal. A. 72, 402-409.
[21] Lee, J., Wu, S., Lu, L., Yang, Y. and Jeng, S. (2009) Cadmium concentration and metallothionein expression in prostate cancer and benign prostatic hyperplasia of humans. J. Formos. Med. Assoc. 108. 554-559.
[22] Li, M., Tang, H., Tang, R. and Zhang, Y. (2005) The investigation and evaluation of heavy metal state form soil and vegetable in the suburb of Changsha region. Hunan Agr. Sci. 3, 34-36.
[23] Cui, Y., Zhu, Y., Zhai, R., Huang, Y., Qiu, Y. and Liang, J. (2005) Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. Environ. Int. 31, 784-790.
[24] Zhou, J. and Chen, TB. (2002) Situation and prospect of research on heavy metal pollution in vegetables and soils for vegetable cultivation in urban areas of China. J. Hubei Agr. Coll. 22, 476-480.
[25] Zhao, X., Yang, X., Chen, G., Li, H. and Wang, C. (2010) Assessment of heavy metal enrichment in vegetables of Chengdu Plain, Southwest China. J. Agr. Sci. 23, 1142-1146.
[26] Teng, L. (2007) Health risk assessment of Cd on drinking waters in Ningbo. Guangdong Trace Elem. Sci. 14, 44-46.
[27] Duian, L. (2017) Comparative assessment of heavy metals in agricultural soils under different land uses: a case study from China. Fresenius Environ. Bull. 26, 5323-5330.
[28] Wang, X., Sato, T., Xing, B. and Tao, S. (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci. Total Environ. 350, 28-37.

Received: **18.02.2018**
Accepted: **19.11.2018**

CORRESPONDING AUTHOR

Dawei Xue
College of Life and Environmental Sciences,
Hangzhou Normal University,
No. 16 Xuelin Street,
Hangzhou 310036, Zhejiang – China

e-mail: dwxue@hznu.edu.cn

© by PSP Volume 27 – No. 12B/2018 pages 9581-9589 Fresenius Environmental Bulletin

TAXONOMIC IMPLICATION OF TRICHOMES ON SILICULES IN *ALYSSUM L.* (BRASSICACEAE) SPECIES IN TURKEY

Ali Savas Bulbul[1,*], Kader Varlik[1], Metin Armagan[2]

[1]Bartin Faculty of Science, Department of Molecular Biology and Genetics, Bartin, Turkey
[2]Adnan Menderes University Buharkent Vocational School Medical and Aromatic Plants Programme, Aydin, Turkey

ABSTRACT

This study includes detailed trichomes morphological analysis of *Alyssum* which are distributed in Turkey. Trichome shapes were investigated fruit surface of 16 species of *Alyssum* L., 4 of which are endemic to Turkey, by scanning electron microscopy (SEM). The shapes of trichomes vary from stellate to lepidote, tuberculate and bifurcate. The stellate is the most common type. Some of species have two or three kinds of trichomes on their silicules. The trichome types recognized in this study provided useful taxonomic evidence that can be used to identify the different taxa of *Alyssum.*

KEYWORDS:
Trichome, Silicule, *Alyssum*, Brassicaceae, Micromorphology, SEM.

INTRODUCTION

The Brassicaceae (Cruciferae) or mustard family comprising approximately 338 genera with 3709 species distributed worldwide [1]. The Brassicaceae are cosmopolitan but especially temperate areas, with the highest diversity in the western North America Irano-Turanian region and Mediterranean area [2]. The genus *Alyssum* L. comprises about species in the 113 and 56 of these are endemic to Turkey [3]. The Brassicaceae family has characteristics siliques, floral and fruit morphology by the cruciform corolla and tetradynamous stamens, that are readily distinguished from other flowering plant families [4].

The genus *Alyssum L.* contain annual and perennial herbaceous plants and (rarely) small shrubs, with oblong-oval leaves and yellow or white flowers (pink and purple in a few species), stellate, stalked or sessile trichomes, and dehiscent silicle fruit [5].

The taxonomic significance of trichomes has been used by many authors as a character in the classification and identification of the Brassicaceae. Trichome morphology, first emphasized by Dennert [6] then trichome variation has been used to a classification of Brassicaceae by Prantl [7]. The taxonomic significance of trichomes has been emphasized by many authors [8, 9, 10, 11, 12, 13]. More recently, Oran [14] studied the taxonomic significance of trichomes types of 12 species of the genus *Alyssum* in Jordan by light and scanning electron microscope (SEM) and 16 types of trichomes were recognized. Khalik [15] noted that, trichomes morphology, structure, and taxonomic significance of 82 species belonging to tribes of Brassicaceae from Egypt were investigated aid of light microscopy (LM) and scanning electron microscopy (SEM). İn this study three species which are also present in Iran including *Alyssum desertorum* Stapf, *A. homalocarpum* (Fisch. & C.A.Mey.) Boiss. and *A. marginatum* Steud. ex Boiss. were identified as stellate trichomes. Ancev and Goranova [16] studied the taxonomic significance of silicule trichomes of 8 species of tribe Alysseae in Brassicaceae. The trichomes of silicules and pedicels in 37 *Alyssum* species of Iran were studied by endemihefi and Assadi [17] to identify trichome types. In this research shapes of trichomes vary from star-shape to simple and dendroid. The star-shape is the most common type.

The aim of this research is to study the variation of silicule trichomes of *Alyssum* species for expanding the knowledge of the trichome diversity in Brassicaceae and possible use of it in the taxonomy of the genus.

MATERIALS AND METHODS

Trichome morphology of 16 species of *Alyssum* was studied using scanning electron microscopy of material from field collections made by the third author and from herbarium specimens. A list of taxa with full voucher data is provided in (Table 1). The basic terminology used in trichome classification and description used here follows Payne [18] but simple self-explanatory terms are included to identify each specific type of trichome.

For scanning electron microscopy, dried specimens were used. Samples were mounted using double adhesive tape on aluminum stubs, sputter-coated with gold and examined with a Jeol Tescan scanning electron microscope at the Bartın University Central Research Laboratory.

TABLE 1
List of investigated taxa and localities

Collector Number	Taxon	Collection Date	Habitat	Phyto geographic Region	Localities
Armağan 4807	*Alyssum murale* Waldst. & Kit. subsp. murale var. alpinum Boiss. ex Nyár.	18 06 2014	Step Road side	Irano-Turanian Endemic	Tunceli: Pülümür, 2 km east of village Kırklar (by road)
Armağan 6577	*Alyssum bulbotrichum* Hausskn. & Bornm.	13 06 2015	Step	Endemic	Van: Gürpınar, east of the village of Doluçıkın, Kepir mountain
Armağan 4381	*Alyssum condensatum* Boiss. & Hausskn. subsp. flexibile (Nyar) T.R.Dudley	5 06 2014	Step	- - -	Tunceli: Pülümür, Çakırkaya village
Armağan 4182	*Alyssum filiforme* Nyár.	3 06 2014	Step	Irano-Turanian Endemic	Tunceli: Nazımiye, Nazımiye - Tunceli road, 3rd km
Armağan 7794	*Alyssum lepidotum* Boiss.	27 07 2017	Between calcareous rocks	Endemic	Muğla: Kavaklıdere, Menteşe village, 3.5 km south of Gökçukur highland
Armağan 7977	*Alyssum masmenaeum* Boiss.	30 07 2017	Step	Endemic	Mugla: Köyceğiz, north mountain of Sandras (Çiçek Baba) mountain
Armağan 7961	*Alyssum masmenaeum* Boiss.	30 07 2017	Step	Endemic	Muğla: Köyceğiz, 4 km north of village Sazak
Armağan 4123	*Alyssum* callichroum Boiss. & Balansa	2 06 2014	Step	- - -	Tunceli: Mazgirt, Akpazar, Yenice south of village Yenice north of hamlet)
Armağan 7443	*Alyssum discolor* T.R.Dudley & Hub.-Mor.	15 05 2017	Step	Eastern mediterranean endemic	Muğla: Marmaris, Beldibi neighborhood, Muğla highway exit
Armağan 4274	*Alyssum ochroleucum* Boiss. & A.Huet	4 06 2014	Step	Irano-Turanian Endemic	Tunceli: Ovacık, 3 km north of Isikvuran village Munzur Mountains
Armağan 7353	*Alyssum sibiricum* Willd.	12 05 2017	Rocky area	- - -	Muğla: Center, Karşıyakaneighborhood
Armağan 4412	*Alyssum strictum* Willd.	5 06 2014	Step	Irano-Turanian	Tunceli: Pülümür, between Dereboyu and Saglamtas village
Armağan 6803	*Alyssum strigosum* Banks & Sol.	15 04 2017	Step Hillsides	- - -	Siirt: Kurtalan, 500 m south-west of village Yeşilkonak
Armağan 5043	*Alyssum pateri* Nyár. subsp. prostratum (Nyár.) T.R.Dudley	20 06 2014	Step	Irano-Turanian Endemic	Tunceli: Between Ovacık, Yazıören - Gözeler villages
Armağan 7673	*Alyssum baumgartnerianum* Bornm. ex Baumg.	18 07 2017	Rocky area	- - -	Muğla: Köyceğiz, Sandras western side(Çiçek Baba) mountain
Armağan 4477	*Alyssum simplex* Rudolph	6 06 2014	Step	- - -	Tunceli: Hozat, 2 km northeast of village Akpınar
Armağan 5907	*Alyssum murale* Waldst. & Kit. subsp. murale var. murale	8 08 2014	Step	Eastern mediterranean	Tunceli: Ovacık, Tunceli - 2.5 km from the village of Yakatarla on the road to Ovacık

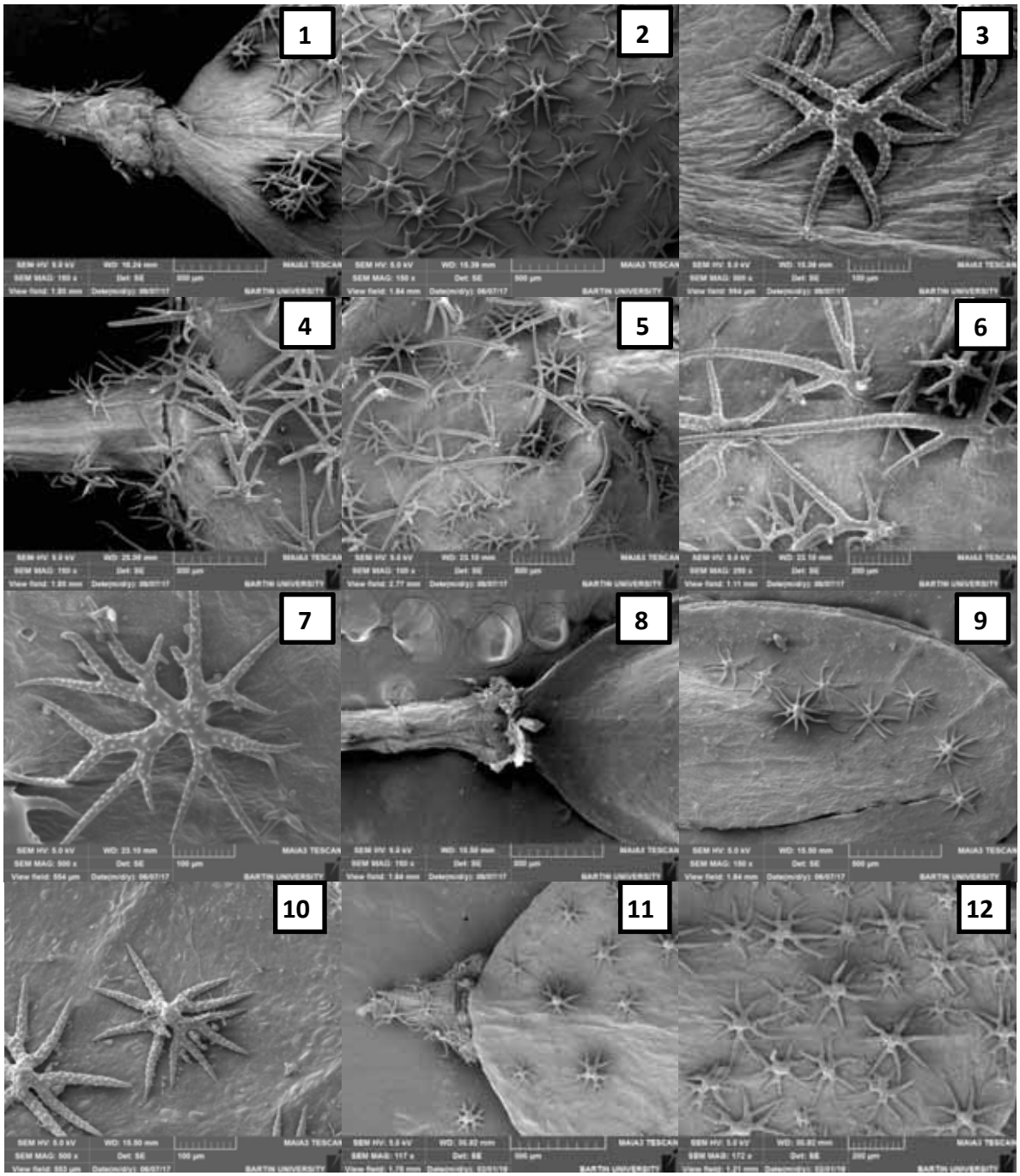


FIGURE 1
Scanning electron micrographs of the fruit trichomes. *Alyssum murale* subsp. *murale* var. *alpinum* (1-3); *Alyssum bulbotrichum* (4-7); *Alyssum condensatum* subsp. *flexibile* (8-10); *Alyssum murale* subsp. *murale* var. *murale* (11-13).

RESULT

The main types of the trichomes and their density among the *Alyssum* species studied are summarized in (Table 2). Selected SEM micrographs of trichome types are presented in Figure 1-5. Fruit trichomes of 16 *Alyssum* species can be separated into four types; stellate, lepidote, tuberculate and bifurcate. In most species, only stellate trichome is observed. Only two species are found to have two or three trichome types *A. bulbotrichum* has stellate and tuberculate trichomes, *A. strigosum* has stellate, bifurcate and tuberculate trichomes. The trichome types in *A. lepidotum* and *A. baumgartnerianum* were lepidote but in *A. lepidotum* had widely webbed trichomes whilst *A. baumgartnerianum* had webbed trichomes at the base.

Trichomes density is different in the species studied, but the most cramped trichomes are found *A. ochroleucum* and *A. simplex Rudolph.* Trichomes

on the indumentum of the *A. condensatum* subsp. *flexibile*, *A. filiforme* ve *A. pateri. subsp. prostratum* are relatively more sparse *A. masmenaeum* and *A. discolor* have only trichomes on pedicel.

End of branches of trichomes was similar in different varieties. The number of rays was 2-22 in studied *Alyssum* taxa. *A. ochroleucum* has 18-22 branches while *A. strigosum* has only 2-6 branches on trichomes. Rays length different in all species.

Trichome surface ornamentation was similar in the species studied. *A. callichroum* has more prominent tuberculate ornamentation while *A.discolor* has obscure tuberculate on trichomes. Some of these stellate hairs are characterised by the presence of primary and secondary branches in the stellate hairs and others by multibranches.

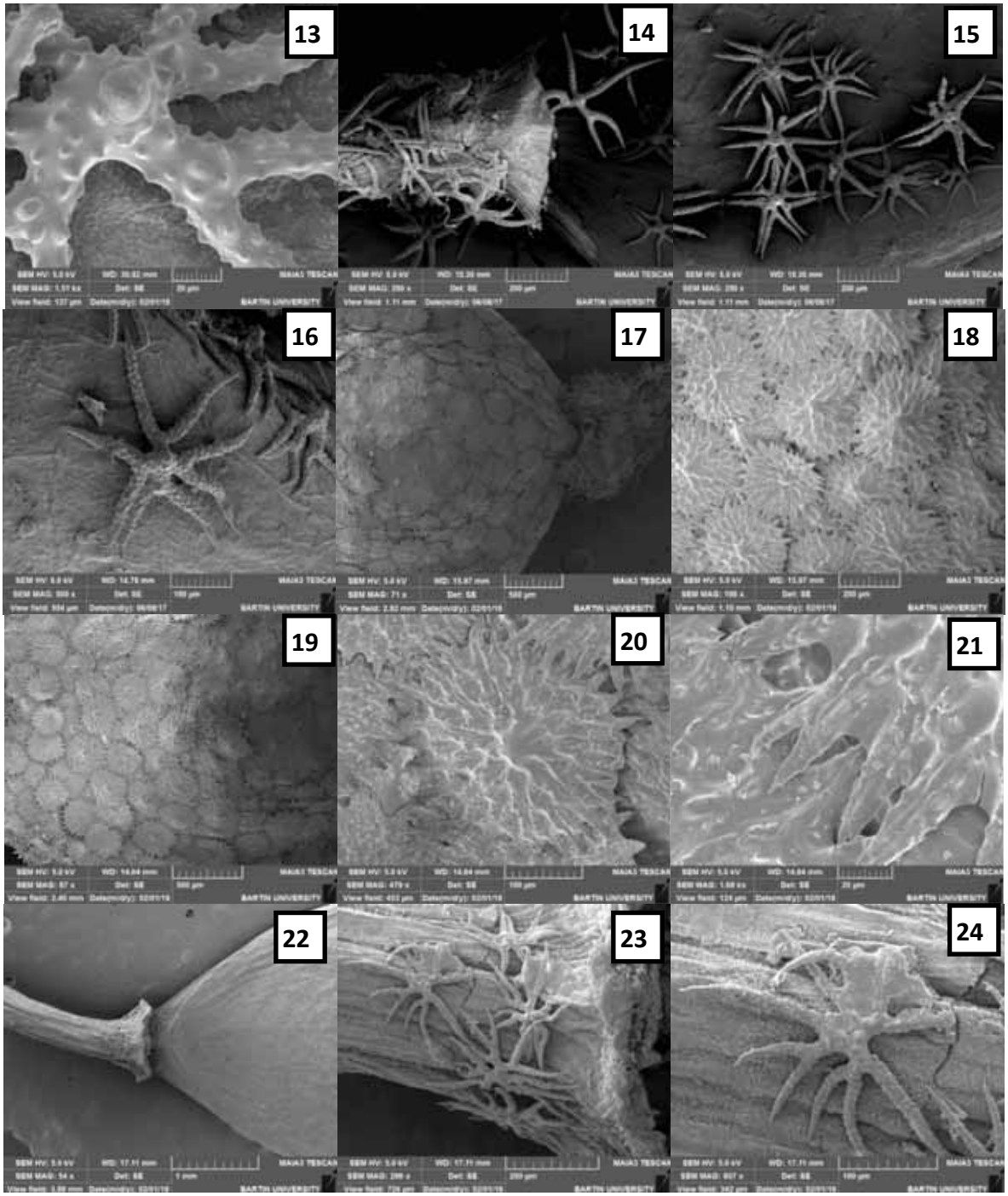


FIGURE 2
Scanning electron micrographs of the fruit trichomes. *Alyssum murale* (11-13); *Alyssum* filiforme (14-16); *Alyssum lepidotum* (17-21); *Alyssum masmenaeum* (22-24).

The most common type which has been observed is the sessile trichomes but stalked type only have been observed in *A. bulbotrichum* and *A. strigosum.* Some of the stellate trichomes have inflation at the centers, especially in *A. callichroum* and *A. murale* subsp. *murale* var. *murale.*

Alyssum strigosum has asymmetric rays on pedicel that make it different from the others.

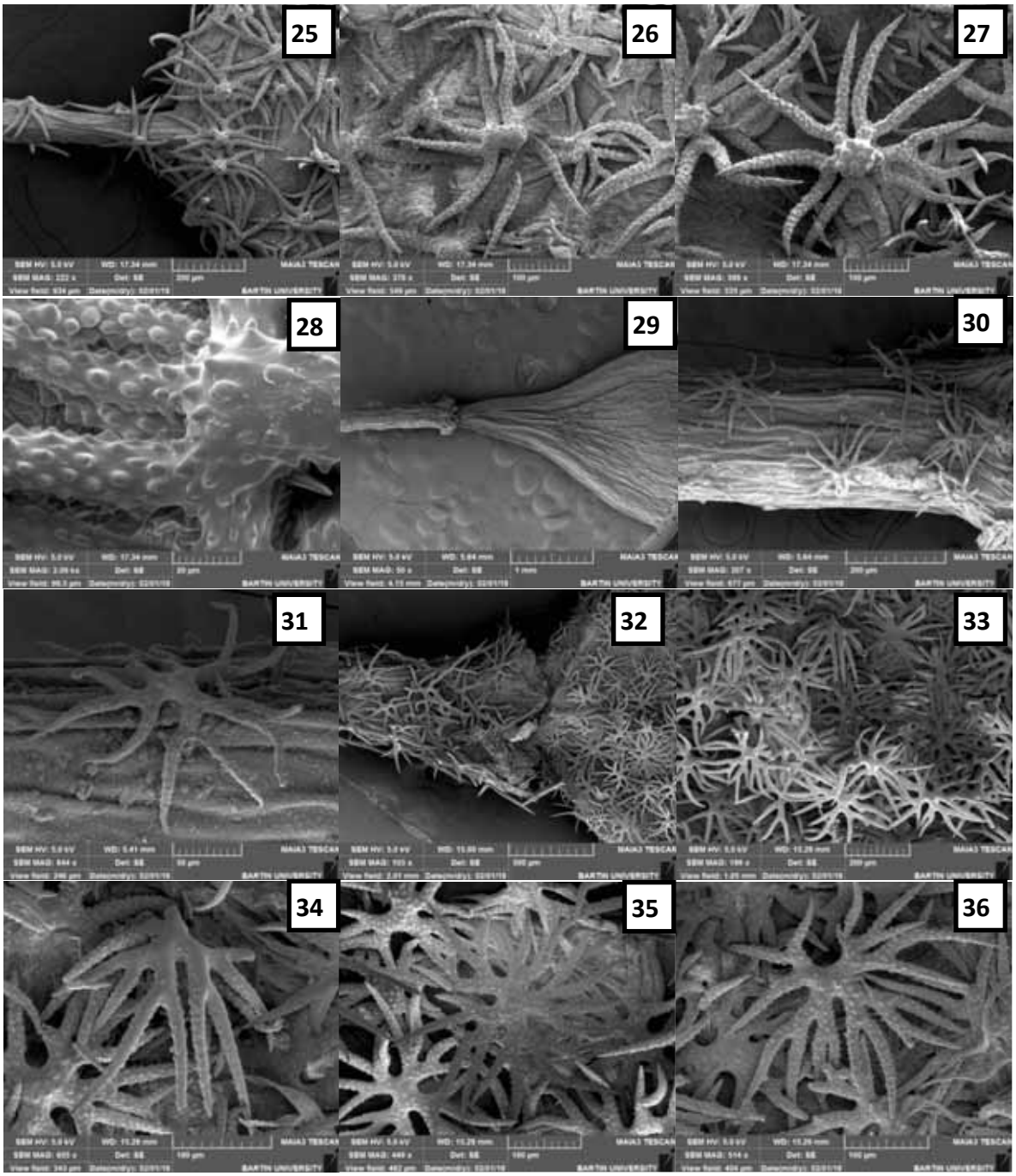


FIGURE 3
Scanning electron micrographs of the fruit trichomes. *Alyssum callichroum* (25-28); *Alyssum discolor* (29-31); *Alyssum ochroleucum* (32-36).

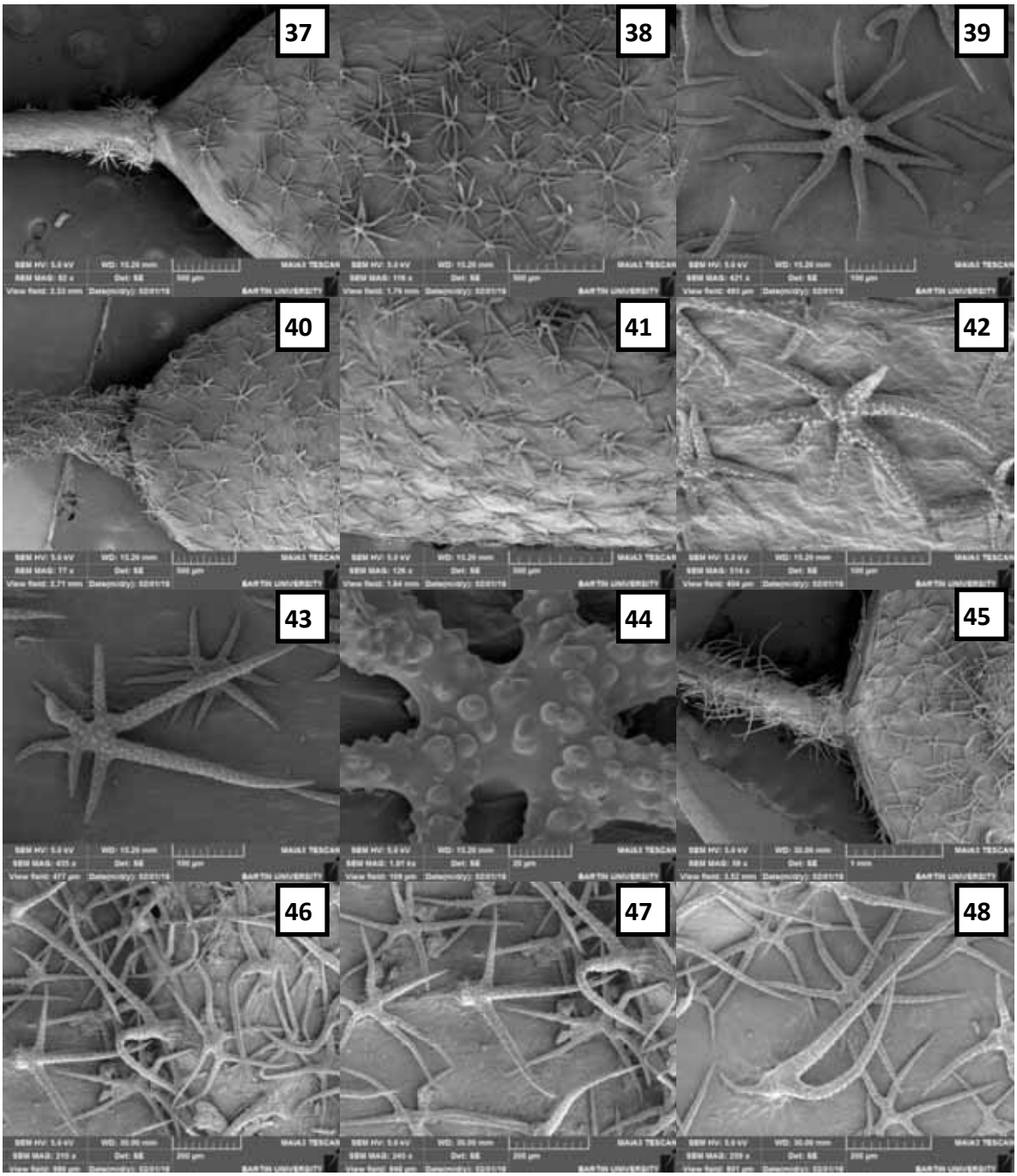


FIGURE 4
Scanning electron micrographs of the fruit trichomes. *Alyssum sibiricum* (37-39); *Alyssum strictum* (40-44); *Alyssum strigosum* (45-48).

DISCUSSION

Alyssum trichome morphology has been generally used as a diagnostic character. Trichome branching has a complex pattern of evolution in the Brassicaceae [19]. Among the 16 species we observed, 5 species were also observed earlier by Vaghefi et al., [17]. Compared to their works, trichomes of *Alyssum strigosum, Alyssum baumgartnerianum, Alyssum murale, Alyssum sibiricum* and *Alyssum strictum* are characterized as similar to their observation. But we observed flat center on the stellate trichomes of *Alyssum strictum* and we find that dentate, stellate and bifurcate trichome on the indumentum of *Alyssum strigosum.*

A.lepidotum and *A. baumgartnerianum* are distinguished by their characteristic indumentum consisting of lepidote hairs. Furthermore, *A.lepidotum* is distinct from *A.baumgartnerianum* by its larger webbed parts. Lepidote trichomes differs from the stellate type by the fusion.

Among the 16 *Alyssum* species studied, 14 have sessile trichomes and 2 species has stalked trichomes.

In *Alyssum*, surface of fruit covered with tuberculate protruding and branches are tapering at tip. The shape of these protrudings and trichomes sculpturing vary among studied populations. It is more prominent in some populations, while it is more obscure in others.

Studied *Alyssum* species can be easily distinguished from each other by trichome type. In conclusion, the present study supports the use of fruit trichomes morphological characters as a parameter for species identification.

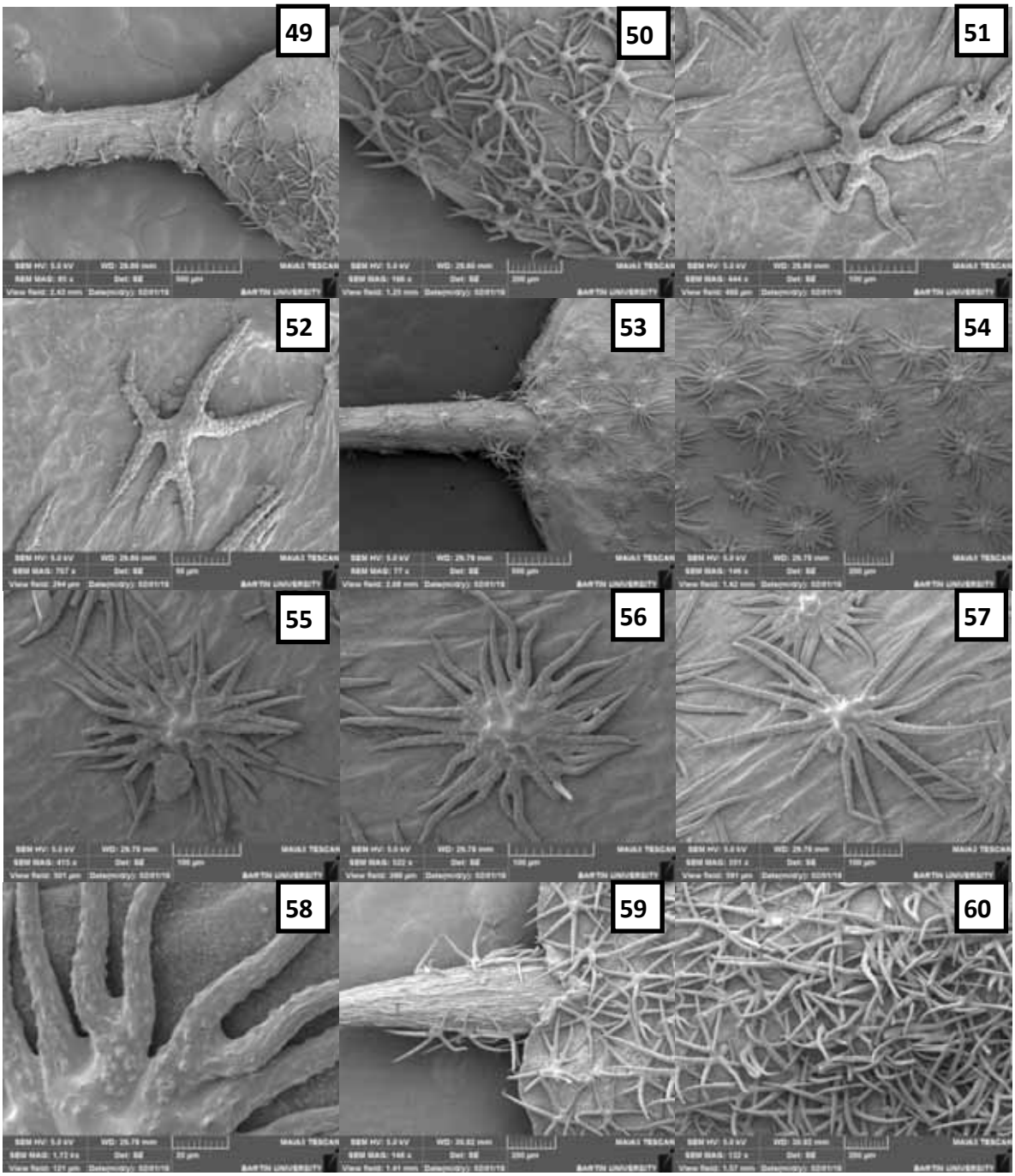


FIGURE 4-CONTINUED

Scanning electron micrographs of the fruit trichomes. *Alyssum pateri.* subsp. *prostratum* (49-52); *Alyssum baumgartnerianum* (53-58); *Alyssum simplex* (59-62).

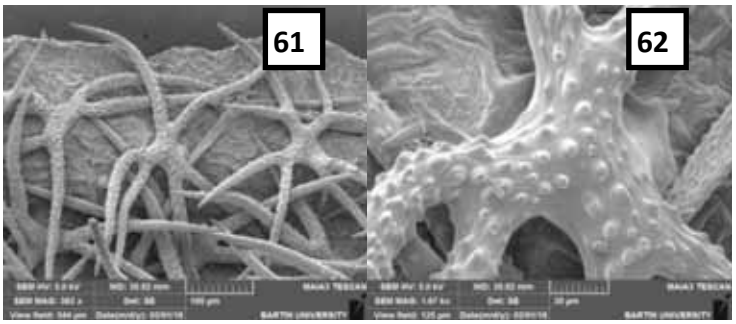


FIGURE 5
Scanning electron micrographs of the fruit trichomes. *Alyssum simplex* (59-62).

TABLE 2
Morphological data of silicule in *Alyssum* varieti

Taxon	Trichome types	Tri-chome distri-bution	Number of rays	Trichome surface	Branching	Centre	Sessile / stalked	Web-bing
Alyssum murale subsp. *murale* var. *alpinum*	Stellate	Dense	10-16 equal rays	Tuberculate	Multibranches	Inflated	Sessile	Absent
Alyssum bulb-otrichum (Endemic)	Stellate Tuberculate	Dense	3-6 une-qual rays	Tuberculate	Multibranches	Flat	Stalked	Absent
Alyssum condensa-tum subsp. *flexibile*	Stellate	Rare	9-13 Slightly equal rays	Tuberculate	Multibranches	Inflated	Sessile	Absent
Alyssum murale subsp. murale var. murale	Stellate	Dense	6-10 equal rays	Tuberculate	Primary and Sec-ondary branches	Prominent Inflated	Sessile	Absent
Alyssum filiforme	Stellate	Rare	10-14 equal rays	Tuberculate	Primary and Sec-ondary branches	Inflated	Sessile	Absent
Alyssum lepidotum (Endemic)	Lepidote	Dense	-	Tuberculate	-	Flat	Sessile	Widely Webbe d
Alyssum masme-naeum. (Endemic)	Stellate	Rare on pedi-cel	8-12 Slightly equal rays	Tuberculate	Multibranches	Flat	Sessile	Absent
Alyssum callichroum	Stellate	Dense	10-14 equal rays	Prominent Tu-berculate	Primary and Sec-ondary branches	Prominent Inflated	Sessile	Absent
Alyssum discolor (Endemic)	Stellate	Rare on pedicel	12-16 equal rays	Obscure Tuberculate	Multibranches	Flat	Sessile	Absent
Alyssum ochroleucum	Stellate	Very Dense	18-22 equal rays	Tuberculate	Multibranches	Flat	Sessile	Absent
Alyssum sibiricum	Stellate	Dense	7-12 equal rays	Tuberculate	Mostly Primary and Secondary branches	Inflated	Sessile	Absent
Alyssum strictum	Stellate	Dense	6-9 un equal rays	Tuberculate	Primary and Sec-ondary branches	Flat	Sessile	Absent
Alyssum strigosum	Stellate Bifurcate Tuberculate	Dense	2-6 unequal rays	Tuberculate	Multibranches	Flat	Stalked	Absent
Alyssum pateri. subsp. *prostratum* (Endemic)	Stellate	Rare	6-10 equal rays	Tuberculate	Mostly Primary and Secondary branches	Inflated	Sessile	Absent
Alyssum baumgart-nerianum	Lepidote	Dense	12-22 equal rays	Tuberculate	Mostly Second-ary branches	Flat	Sessile	Webbe d only at base
Alyssum simplex	Stellate	Very Dense	6-10un equal rays	Tuberculate	Primary and Sec-ondary branches	Flat	Sessile	Absent

REFERENCES

[1] Warwick, S.I., Francis, A. and Al-Shehbaz, I.A. (2006) Brassicaceae: species checklist and database on CD-Rom. Plant Systematics and Evolution. 259(2-4), 249-258.

[2] Schweingruber, F.H., Börner, A. and Schulze, E.D. (2011) Atlas of stem anatomy in herbs, shrubs and trees. Vol. 1. Springer Science and Business Media.

[3] Yılmaz, Ö. (2012) Alyssum kaynakiae sp. nov. (sect. Gamosepalum, Brassicaceae) from southwest Anatolia, Turkey. Nordic journal of botany. 30(2), 190-194.

[4] Warwick, S.I., Sauder, C.A. and Al-Shehbaz, I.A. (2008) Phylogenetic relationships in the tribe Alysseae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. Botany. 86(4), 315-336.

[5] Ball, P.W. and Dudley, T.R. (1964) Alyssum L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A. (eds.) Flora of Europeae. Vol. 1. Lycopodiaceae to Platanaceae. Cambridge Univ. Press, 359–369.

[6] Dennert, E. (1884) Beiträge zur vergleichenden Anatomie des Laubstengels der Cruciferen. Universität Marburg.

[7] Prantl, K. (1891) Cruciferae. In: Engler, A., Prantl, K. (ed.) Die Natürlichen Pflanzenfamilien. vol. 3. Wilhelm Engelmann, Leipzig. 145–206.

[8] Janchen, E. (1942) Das system der Cruciferen. Österreichische Botanische Zeitschrift. 91(1), 1-28.

[9] Nyárády, E.I. (1955) Alyssum. Flora republicii populare Romîne. 3, 318-355.

[10] Dudley, T.R. (1964) Synopsis of the genus Alyssum. Journal of the Arnold Arboretum. 45(3), 358-373.

[11] Rollins, R.C. and Banerjee, U.C. (1976) Trichomes in studies of the Cruciferae. In: Vaughan, J.G., Macleod, A.J., Jones, B.M.G. (eds.) The Biology and Chemistry of the Cruciferae. Academic Press.

[12] Inamdar, J.A., Rao, N.V. (1983) Light and scanning electron microscopic studies on trichomes of some Brassicaceae. Feddes Rep. 94, 183–190.

[13] Al-Shehbaz, I.A. (1987) The genera of Alysseae (Cruciferae; Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum. 68(2), 185-240.

[14] Oran, S. (1996) Trichomes of the genus Alyssum L. (Cruciferae) in Jordan. Webbia. 50 (2), 237-245.

[15] Abdel, K.K. (2005) Morphological studies on trichomes of Brassicaceae in Egypt and taxonomic significance. Acta Botanica Croatica. 64(1), 57-73.

[16] Ančev, M. and Goranova, V. (2006) Trichome morphology of eleven genera of the tribe Alysseae (Brassicaceae) occurring in Bulgaria. Willdenowia. 36(1), 193-204.

[17] Vaghefi, S.S.M, Assadi, M. and Sheidai, M. (2015) Taxonomic implication of trichomes on pedicels and silicules in Alyssum L. (Brassicaceae) species in Iran. Iran. J. Bot. 21(2), 133-141.

[18] Payne, W.W. (1978) A glossary of plant hair terminology. Brittonia. 30(2), 239-255.

[19] Beilstein, M.A., Al-Shehbaz, I.A. and Kellogg, E.A. (2006) Brassicaceae phylogeny and trichome evolution. American journal of botany. 93(4), 607-619.

Received: **23.02.2018**
Accepted: **03.09.2018**

CORRESPONDING AUTHOR

Ali Savas Bulbul
Bartın Faculty of Science,
Department of Molecular Biology and Genetics,
Bartın – Turkey

e-mail: asavasbulbul@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9590-9594 Fresenius Environmental Bulletin

PREPARATION OF POTASSIUM PERMANGANATE-MODIFIED SHRIMP SHELL WASTE AND ITS APPLICATION IN CYANOBACTERIAL BLOOM REMOVAL

Hongqiang Wang[1], Qingliang Wang[1], Eming Hu[1], Lieyu Zhang[2,*]

[1]School of Resource & Environment and Safety Engineering, University of South China, Hengyang 421001, China
[2]State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

ABSTRACT

Shrimp shell waste is a good source of chitin, which represents 17.8% of the dry weight of shrimp offal. Therefore, the use of a prepared shrimp shell adsorbent has good application prospects. This work aimed to study the preparation process and potential use of a potassium permanganate-modified shrimp shell adsorbent for *Microcystis aeruginosa* cell removal. In addition, the effects of the potassium permanganate concentration, modification reaction time and amount of added modified shrimp shell adsorbent on the cell removal efficiency were also evaluated. Under the optimal modification conditions, the potassium permanganate-modified shrimp shell adsorbent concentration was 1.0 g/L, and it achieved a 76.32% removal efficiency. The present study provides a promising technique for the removal of toxic cyanobacterial blooms.

KEYWORDS:
Microcystis aeruginosa, microcystin, sodium hydroxide, modified shrimp shell, adsorbent

INTRODUCTION

The eutrophication of lakes or reservoirs can lead to cyanobacterial blooms; when a cyanobacteria bloom breaks out, cyanobacteria will reproduce at a rapid rate and multiply quickly in a short time, turning the lake or reservoir green locally or even globally [1]. Cyanobacteria may produce and release toxic compounds, resulting in disastrous effects on the aquatic organisms, wild life, domesticated animals and humans that drink or come into contact with water containing the toxic compounds [2]. Therefore, the control and removal of harmful cyanobacterial blooms are urgent issues worldwide [3].

To date, many measures have been proposed to remove cyanobacterial blooms in aquatic ecosystems. These measures can be categorized into three groups. The first group is biological treatments, which include the use of algae-lysing bacteria, allelopathic compounds, and zooplankton, silver carp and bighead carp that graze on the algae [4-10]. The second group consists of physical treatments, such as shading and the mechanical salvaging method [11]. The third group is chemical treatments, which are the most widely used algae removal techniques around the world; these treatments include flocculation, adsorption, and algicide [12-14]. Adsorption is presently considered one of the best curative methods. Hence, it is necessary to look for a new adsorbent that is effective, inexpensive and eco-safe.

Shrimp shell waste, a kind of high-quantity food waste, is an inexpensive and reliable biomass source that can be used without increasing the competition for food [15]. Shrimp shells have been used as a source of extracted chitin and chitosan [16-17]. In addition, non-treated shrimp shells have been used to remove textile dyes from wastewater through adsorption [18]. The current work describes the preparation process and influence factors of potassium permanganate-modified shrimp shell waste, as well as its removal characteristics on *Microcystis aeruginosa* cells.

MATERIALS AND METHODS

Algal culture. An axenic unicellular *M. aeruginosa* culture was obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were cultured in sterilized BG11 medium (pH 7.4) at 25 ℃ with a light intensity of 2500 lux and a 12:12 h light: dark cycle. The algae were cultured for 4 days, until reaching the exponential phase at a density of 10^6 cells/mL, and they were then used to assay the adsorptive properties of a potassium permanganate-modified shrimp shell adsorbent. The growth medium for all cultures was BG11 [19].

Preparation of modified shrimp shell. Shrimp shells were obtained from the supermarket of Pingdingshan in Henan Province, China. The

shells were first washed free of debris with tap water and then washed by deionized water before they were oven-dried on trays at 105°C for 2 h. After drying, the shells were smashed and sieved using an 80-mesh screen.

The specific preparation process of the potassium permanganate-modified shrimp shells is as follows. First, 0.5-15 g/L of a potassium permanganate solution was prepared. Next, 4 g of the pretreated shrimp shells was slowly added to 400 mL of the potassium permanganate solution, and the mixed suspension was slowly stirred with a magnetic stirrer. Finally, the suspension was filtered with qualitative filter paper (10-15 μm), and the modified shrimp shells were washed four to five times with distilled water and then oven-dried to a constant weight at 60℃. The dried, modified shrimp shells were the final adsorbent.

Removal of *M. aeruginosa*. The ability of the potassium permanganate-modified shrimp shell absorbent to remove harmful algae blooms was tested using *M. aeruginosa*. The potassium permanganate-modified shrimp shell absorbent was added to 50 mL of the algal culture in a 100-mL beaker and allowed to stand for 24 h. In the control groups, the potassium permanganate-modified shrimp shell absorbent was not added. At the end of the settling period, a sample was collected from each beaker 2 cm below the surface for analysis.

Analysis methods for determining the concentration of *chlorophyll-a*. The concentration of chlorophyll-*a* was measured as an indicator of the change in the concentration of *M. aeruginosa* cells during the flocculation experiment. The chlorophyll-*a* concentration was determined using standard methods [20].

The clearance of algae (r, %) in every sample, based on the chlorophyll-*a* concentration, was determined after the 24 h exposure period by the following formula:

$$r = \frac{T_2 - T_1}{T_2} \times 100\% \quad (1)$$

where T_1 and T_2 are the chlorophyll-*a* concentrations for the culture after the adsorption treatment and the control, respectively.

Data analysis. Differences between the groups were evaluated using one-way analysis of variance with the removal efficiency of algae as a factor. A probability level of 0.05 was used to establish significance ($P< 0.05$). All statistical analyses were performed using SPSS 19.0 and Origin 8.0 software.

RESULTS AND DISCUSSION

Effect of the potassium permanganate concentration. In preliminary experiments, different concentrations of potassium permanganate were used as modifiers to investigate the removal efficiency of the modified adsorbents on *M. aeruginosa* cells (Fig. 1). The removal efficiency increased with an increase in the potassium permanganate concentration in the range of 0.5-6.0 g/L. After the potassium permanganate concentration exceeded 6.0 g/L, the removal efficiency decreased. A concentration of 6.0 g/L was found to be an optimum level for the removal of *M. aeruginosa* cells.

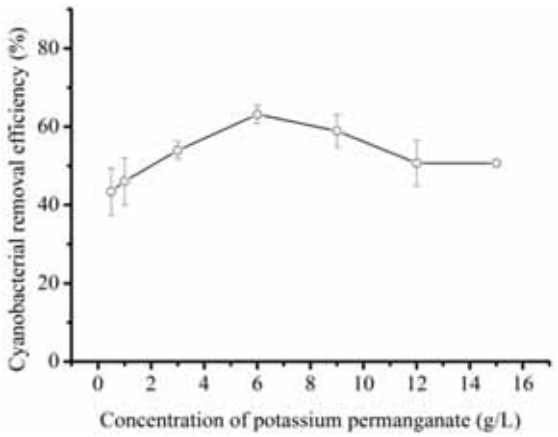


FIGURE 1
Effect of the potassium permanganate concentration on the removal of *M. aeruginosa* cells

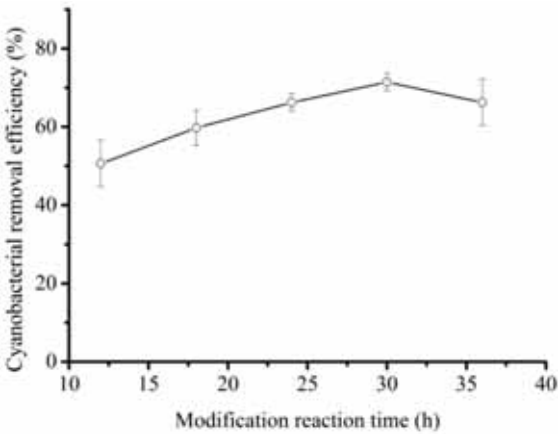


FIGURE 2
Effect of modification reaction time on *M. aeruginosa* cell removal

Effect of modification reaction times. The effect of modification reaction times on the removal of *M. aeruginosa* cells was tested at five different times: 12, 18, 24, 30 and 36h. The results shown in Fig. 2 indicate that the *M. aeruginosa* cell removal efficiency first increased with the increasing modification reaction time, reached a peak, and then dropped with further increases. The highest removal

efficiency, 71.43%, occurred at 30 h. Therefore, we can conclude that 30 h is the optimal time for the experimental study on *M. aeruginosa* cell removal.

Effect of the amount of modified shrimp shell adsorbent. To understand the effect of the amount of modified shrimp shell adsorbent on *M. aeruginosa* cell removal, experiments were conducted with five different amounts of added potassium permanganate-modified shrimp shell adsorbent. The samples were analyzed after each experiment to estimate the effect of the modified adsorbent concentration on the cyanobacteria removal efficiency. Fig. 3 shows that the efficiency of cyanobacteria removal increased steadily with increases in the modified adsorbent concentration, reached a peak, and then tended to decrease with further increases in the adsorbent concentration. Therefore, the most effective modified adsorbent concentration was found to be 1.0 g/L, at which 76.32% removal efficiency was achieved.

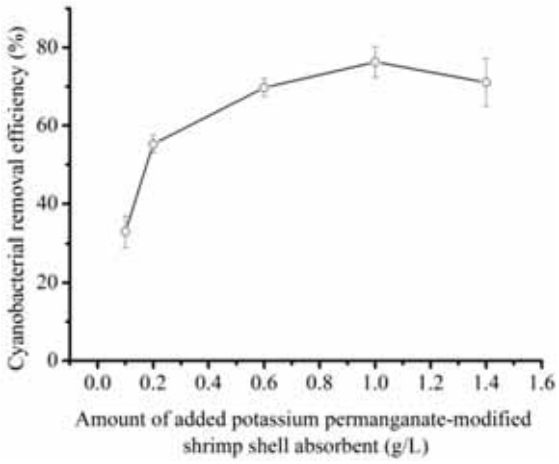


FIGURE 3
Effect of the amount of potassium permanganate-modified shrimp shell on *M. aeruginosa* cell removal

In our previous work, potassium permanganate was used as a pre-oxidant to enhance the removal efficiency, and the results indicated that it could improve algae coagulation removal by 9.44% when *M. aeruginosa* was pre-oxidized by potassium permanganate at a concentration of 0.9 mg/L [21]. Ou et al. [22] also reported that an appropriate dose of $KMnO_4$ can reduce the photosynthetic capacity of *M. aeruginosa* cells and degrade their extracellular MC-LR, resulting in low cell activity and intact cells in suspension. In addition, some studies on the use of potassium permanganate as a modifier report that when potassium permanganate-modified activated carbon was used to adsorb Cu and Sb (III) effectively improved the adsorption efficiency of the activated carbon [23-24]. In the current work, potassium permanganate was employed to modify shrimp shells, and the algae removal efficiency of the modified shrimp shells was lower than those of chemical sorbents. The reason for this result may be that the hydrated MnO_2 was washed. Some researchers have shown that the hydrated MnO_2 from potassium permanganate acts synergistically with coagulants to improve the algae removal efficiency [22]. Therefore, the effect of hydrated MnO_2 on the modification reaction will be investigated in further research.

CONCLUSIONS

According to the above mentioned results and discussion, we can recommend the following procedure: 1) 6.0 g/L of potassium permanganate solution is prepared, and then,4 g of the pretreated shrimp shells is slowly added to 400 mL of the potassium permanganate solution; 2) the mixed suspension is slowly stirred with a magnetic stirrer for 30 h; 3) the suspension is filtered with qualitative filter paper (10-15 μm), and then, the modified shrimp shells were washed four to five times with distilled water and oven-dried to a constant weight at 60℃. The dried, modified shrimp shells were the final adsorbent. Under the optimal modification conditions, the removal efficiency of the potassium permanganate-modified shrimp shell adsorbent on the *M. aeruginosa* cells was 76.32% when the added concentration of the modified adsorbent was 1.0 g/L.

ACKNOWLEDGEMENTS

This work was supported by the National Science-technology Support Plan Projects of China (2015BAL04B01), the National Natural Science Foundation of China (51108447; 51208485; 51678549), the China Postdoctoral Science Foundation Project (20100471208), the China Postdoctoral Science Special Foundation (201104499), the Key Science and Technology Project of Henan Province of China (172102310720), the Key Scientific Research Foundation of the Higher Education Institutions of Henan Province, China (16A610006) and the Double First Class Construct Program of USC (2017SYL05).

REFERENCES

[1] Sheng, H., Liu, H., Wang, C.Y., Guo, H.C., Liu, Y., Yang, Y.H. (2012) Analysis of cyanobacteria bloom in the Waihai part of Dianchi Lake, China. Ecological Informatics. 10, 37-48.

[2] Liu, Y.M., Chen, W., Li, D.H., Huang, Z.B., Shen, Y.W., Liu, Y.D. (2011) Cyanobacteria-/cyanotoxin-contaminations and eutrophication status before Wuxi Drinking Water Crisis in Lake Taihu, China. Journal of Environmental Sciences. 23(4), 575-581.

[3] Xiong, Y., Wang, H.Q., Zhang, L.Y., Wang, L., Che, W. (2017) Preparation of sodium alginate/ grapheneoxide /gallic acid complex gel beads and their algae removal performance. Fresen. Environ. Bull. 26, 2926-2930.

[4] Lee, S., Kato, J., Takiguchi, N., Kuroda, A., Ikeda, T., Mitsutani, A., Ohtake, H. (2000) Involvement of an extracellular protease in Algicidal activity of the marine bacterium *Pseudoalteromonas sp*. strain A28. Applied and Environmental Microbiology. 66(1), 4334-4339.

[5] Wang, S.X., Shen, Y.C., Qu, Y.F., Zhu, Y.P. (2010) Distribution and Control of Phytoplankton in Chenhang Reservoir in Shanghai. China Water and Wastewater. 26(12), 8-11.

[6] Ma, J.M., Jin, P., Wang, C.L., Jin, T.X., Yang, C., Xv, T., Qin, X.M. (2013) Study on the interactions among Chlorella vulgaris, Daphnia magna and *Ceratophyllum demersum* in simulated natural water. Acta Scientiae Circumstantiae. 33(2), 528-534.

[7] Gao, Y.N., Ge, F.J., Zhang, L.P., He, Y., Lu, Z.Y., Zhang, Y.Y., Liu, B.Y., Zhou, Q.H., Wu, Z.B. (2017a) Enhanced toxicity to the cyanobacterium *Microcystis aeruginosa* by low-dosage repeated exposure to the allelochemical N-phenyl-1-naphthylamine. Chemosphere. 174, 732-738.

[8] Gao, Y.N., Dong, J., Fu, Q.Q., Wang Y.P., Chen, C., Li, J.H., Li, R., Zhou, C. J. (2017b) Allelopathic effects of submerged macrophytes on phytoplankton. Allelopathy Journal. 40(1), 1-22.

[9] Wang, H.Q., Zhang, L.Y. (2017b) Allelopathic Activity of ethyl acetate extracts from typical emergent plants against *Microcystis aeruginosa* Kütz. Bangladesh Journal of Botany. 46(3), 1025-1029.

[10] Liang, F., Wang, H.Q. (2017) Composition of the ethyl acetate extracts from *Portulaca oleracea* and determination of their antialgal activity. Fresen. Environ. Bull. 26, 7101-7104.

[11] Wu, C., Chang, X.X., Dong, H.J., Li, D.F., Liu, J.Y. (2008) Allelopathic inhibitory effect of *Myriophyllum aquaticum* (Vel.l) Verdc. On Microcystis aeruginosa and its physiological mechanism. Acta Ecologica Sinica. 28(6), 2595-2603.

[12] Zhou, S.Q., Shao, Y.S., Gao, N.Y., Deng, Y., Qiao, J.L., Ou H., Deng, J. (2013) Effects of different algaecides on the photosynthetic capacity, cell integrity and microcystin-LR release of Microcystis aeruginosa. Science of the Total Environment. 463-464, 111-119.

[13] Ma, C.X., Hu, W.R., Pei, H.Y., Xu, H.Z., Pei, R.T. (2016) Enhancing integrated removal of *Microcystis aeruginosa* and adsorption of microcystins using chitosan-aluminum chloride combined coagulants: Effect of chemical dosing orders and coagulation mechanisms. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 490, 258-267.

[14] Li, L., Pan, G. (2013) A universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand. Environmental Science and Technology. 47(9), 4555–4562.

[15] Qu, J.Y., Geng, C., Lv, S.Y., Shao, G.H., Ma, S.Y., Wu, M.B. (2015) Nitrogen, oxygen and phosphorus decorated porous carbons derived from shrimp shells for supercapacitors. Electrochimica Acta. 176, 982-988.

[16] Kumari, S.A., Sri Hari Kumar, A.S.H., Sahoo, A., Rath, P. (2017) Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. International Journal of Biological Macromolecules. 104, 1697-1705.

[17] Seghir, B.B., Benhamza, M.H. (2017) Preparation, optimization and characterization of chitosan polymer from shrimp shells. Journal of Food Measurement and Characterization. 11(3), 1137-1147.

[18] Fabbricino, M., Pontoni, L. (2016) Use of non-treated shrimp-shells for textile dye removal from wastewater. Journal of Environmental Chemical Engineering. 4(4), 4100-4106.

[19] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology. 111(1), 1-61.

[20] Chinese, E.P.A. (2002) The determination of aquatic biological communities. In: Methods for water and wastewater analysis. Fourth ed. Environmental Science Publishing House of China, Beijing, 1-784.

[21] Wang, H.Q., Mao, T.G., Xi, B.D., Zhang, L.Y., Zhou, Q.H. (2015) $KMnO_4$ pre-oxidation for *Microcystis aeruginosa* removal by a low dosage of flocculant. Ecological Engineering. 81, 298-300.

[22] Ou, H., Gao, N. Y., Wei, C. H., Deng, Y., Qiao, J.L. (2012) Immediate and long-term impacts of potassium permanganate on photosynthetic activity, survival and microcystin-LR release risk of *Microcystis aeruginosa*. Journal of Hazardous Materials. 219-220, 267-275.

[23] Ding, C.S., Zou, B.W., Miao, J., Zhu, Q.F. (2012) Characterization and Cu^{2+}adsorption capability of activated carbon modified by $KMnO_4$. Journal of Central South University (Science and Technology). 43(5), 2016-2022.

[24] Wang, X.H., Yu, T.C., Li, C., Ye, M.M. (2012) The adsorption of Sb (III) in aqueous by $KMnO_4$-modified activated carbon. Journal of Zhejiang University (Engineering Science). 46(11), 2028-2034.

Received: **13.03.2018**
Accepted: **21.08.2018**

CORRESPONDING AUTHOR

Lieyu Zhang
State Key Laboratory of Environmental Criteria and Risk Assessment,
Chinese Research Academy of Environmental Sciences,
Beijing 100012 – China

e-mail: zhanglieyu@163.com

© by PSP Volume 27 – No. 12B/2018 pages 9595-9601 Fresenius Environmental Bulleti

FUNCTIONALIZATION OF GRAPHENE OXIDE AND ITS EXTRAORDINARY ADSORPTION PROPERTY TOWARD LEAD ION

XiuYu Liu[1], Hui Huang[1], Ying Zhu[1], RanRan Shan[1,*], Houyi Ma[2]

[1]Qilu University of Technology (Shandong Academy of Sciences), Advanced Materials Institute, Shandong Academy of Sciences, Jinan, 250014, China
[2]Department of Chemistry, Shandong University, Jinan 250100, China

ABSTRACT

Well defined graphene oxide (GO) nanosheets were synthesized via a facile synthetic method and further functionalized by silanization treatment. Adsorption property of GO toward heavy metal ions was evaluated by using Pb(II) ions as model ions. The results showed that modified GO exhibit high adsorption ability for Pb(II) ion in aqueous solution and the removal efficiency is nearly 99.5%, which indicating modified GO-based carbon nanostructures provide a promising class of adsorbents for heavy metal removal.

KEYWORDS:
Graphene oxide, Silanization, Lead, Adsorption

INTRODUCTION

Heavy metals are widespread in the environment; especially the lead pollution is becoming more and more serious due to the increased demand for industrial use of lead. The form of lead in water is mainly Pb^{2+}, which can affect blood, neural system, urinary system and immune system in human body, as well as carcinogenicity.

There are many ways to adsorb lead ions (Pb^{2+}) from aqueous solution. For high levels of Pb^{2+} in wastewater, chemistry precipitation (hydroxide, sulphide) is an economic method [1-2]. For low levels of Pb^{2+} in wastewater, ion exchange process, reverse osmosis and adsorption method could be used [3-5]. However, the maintenance cost of exchange process and reverse osmosis is higher, and the recent researches indicated that adsorption strategy has been widely applied [6]. A lot of materials such as activated carbon, zeolites, dust, metal oxides, chitosan and agricultural byproducts have been developed as adsorbents to remove Pb^{2+} [7, 8].

Graphene-based materials, one kind of high efficiency adsorbents, have been manufactured come from graphite oxide (GO) in ton quantities at low cost [9]. This means that graphene has moved from the laboratory research to the marketplace and sold by the ton. The theoretical specific surface area (SSA) of graphene is 2630 m^2/g, which is much higher than single-walled CNTs (SWNTs), an ideal limit SSA is 1300 m^2/g [10-12].

It has been demonstrated that the adsorption of heavy metals onto carbonaceous materials primarily takes place at acidic functional groups as shown in Fig. 1 [13-15]. The hydrophily of GO derive from oxygen functional groups (carboxyl, hydroxyl and epoxy groups in Fig. 2) is helpful to adsorb heavy metal ions. GO also possesses novel physical properties, such as high thermal and superior chemical stability, which enable it to act as an ideal material for adsorption of lead ion [16-18].

In this paper, we used GO as adsorbent to remove Pb^{2+} in water. Simultaneously, we developed a modified GO taking advantage of a kind of silicohydride compound. This simple and economic approach holds the potential to solve the problem of lead pollution in water.

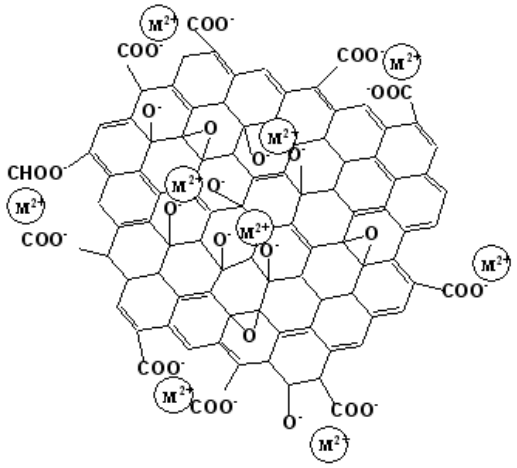


FIGURE 1
Schematic diagram of the adsorption mechanism of heavy metal ions at acidic functional groups of GO

EXPERIMENTAL SECTION

Materials Synthesis of GO. Graphite oxide is produced by the oxidative treatment of graphite, and the commonly used method is Hummers method [19]. Firstly, 3.0 g graphite and 1.5 g $NaNO_3$ were mixed together in a flask, then 100 ml H_2SO_4 (98%) was added to the mixture, which was kept in an ice bath. While maintaining agitation, 300 g $KMnO_4$ was added to the suspension little by little to avoid overheating. After that, the ice bath was removed and the suspension was stirred at room temperature for 2 h. The color of the suspension would become bright brown. Then, 90 ml of distilled water was added slowly, causing an increase in temperature of the suspension to about 90 ℃. The dilute suspension would change to yellow in color. The suspension was stirred at 98 ℃ for 12 h and treated with 30 ml 30% H_2O_2 to reduce the $KMnO_4$ and MnO_2. For purification, the mixture was washed with 5% HCl and then distilled water for several times. After that the suspension was centrifuged at 3000 r.p.m for 5 min, followed by drying in vacuum. As-synthesized graphite oxide is black powders and when suspended in water it gives a brown dispersion. After the above process, there were still some residual salts and acids, which was ought to dialysis in elaborate experiments. Ultrapure Milli-Q water was used in all experiments.

Synthesis of modified GO. Under N_2 flow, 20mL of MeOH was added slowly to a mixture of APMS (3-aminopropyltrimethoxysilane, 2 g, 11 mmol) and methyl acrylate (17.2 g, 200 mmol) at ambient temperature and stirred for 24 h-36 h. Then the solution was heated at 40-60 °C for 30 min (Scheme 1a). Then, the product was heated at 100-150 °C adding DMF under nitrogen flow (Scheme 1b). As-synthesized product was hydrolyzed in water (Scheme 1c). MeOH and methyl acrylate were evaporated at ambient temperature. Then we obtained a colorless product in 97% yield [20, 21].

Adsorption of Pb^{2+}. Different concentrations of lead ions were used for each of four synthetic wastewaters investigated. After each experiment, the composite materials were separated from the solution by centrifugation and then prepared for further analysis. The supernatant was analyzed by flame atomic absorption spectrophotometer (AAS） using a Perkin-Elmer 2380 atomic absorption spectrophotometer. The sediment was vacuum drying and then analyzed by scanning electron microscopy (SEM) and automatic scanning type of X-ray fluorescence spectrometer (ZSX Primus II, The Japanese Company).

RESULTS AND DISCUSSION

Characterization of GO. Graphite oxide is produced by the commonly used Hummers method by exfoliation treatment of graphite, which results in two dimensional GO with an excellent source of oxygen functional groups including carboxyl, hydroxyl and epoxy groups [22]. It has been demonstrated that the adsorption of heavy metals onto carbonaceous materials primarily takes place at acidic functional groups [23]. The oxygen functional groups can interact with Pb^{2+} by electrostatic forces.

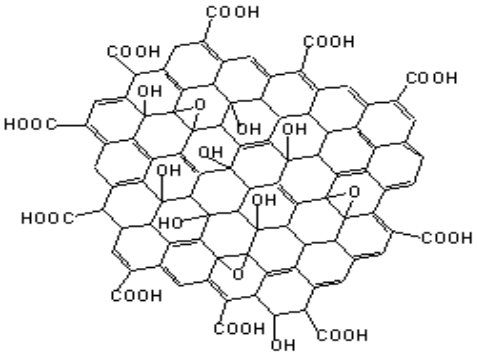


FIGURE 2
The structure of GO

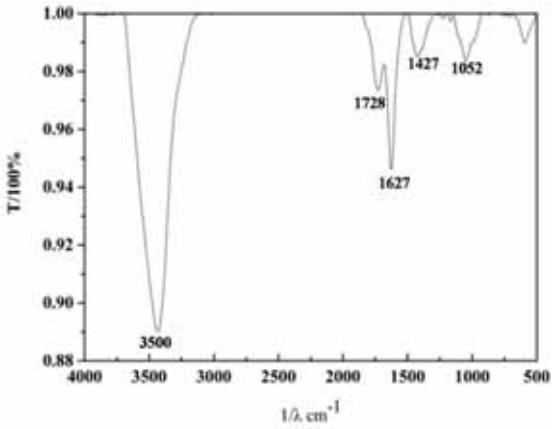
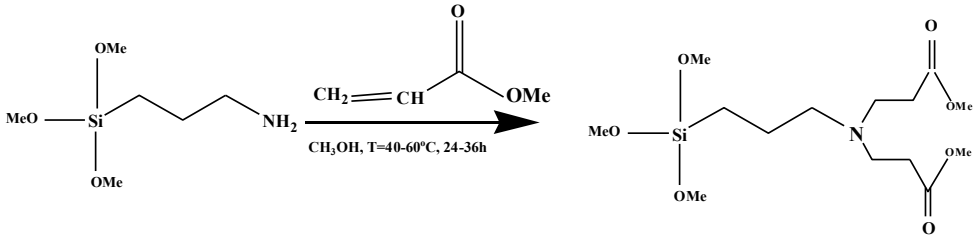
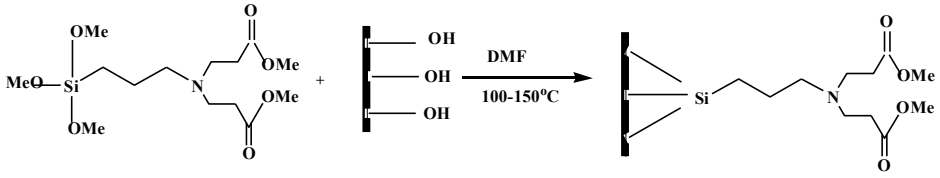


FIGURE 3
The FTIR of GO with acidic functional groups

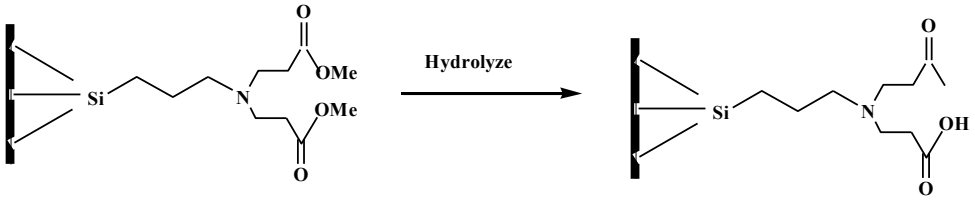
Characterization of the product by Fourier transform infrared spectroscopy (FTIR) in Fig.3 demonstrates that we have successfully prepared GO. The absorption peaks at 3500 cm^{-1} implied the formation of hydroxyl (O-H). The absorption peaks at 1728 cm^{-1} and 1627 cm^{-1} assigned to the C=O stretching and C=C stretching peak. At the same time, the absorption peak at 1052 cm^{-1} indicated the introduction of epoxy groups(C-O-C). The absorption peak at 1427 cm^{-1} showed that the hydrogen (O-H) shock on carboxyl group.



SCHEME 1a
A schematic diagram of reaction equation using APMS and methyl acrylate



SCHEME 1b
A schematic diagram of reaction equation using APMS and methyl acrylate



SCHEME 1c
A schematic diagram of the hydrolyzed process of as-synthesized product in water.

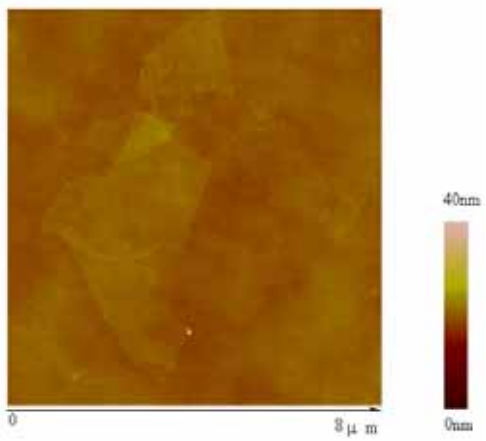


FIGURE 4
The AFM of GO with acidic functional groups

Tapping mode Atomic force microscopy (AFM) (AutoProbe CP/MT Scanning Probe Microscope (MultiTask), Veeco Instruments) image of GO sheets exfoliated by the ultrasonic treatment at concentrations of 0.1 mg/mL in water with a height profile taken along the red line. The sample was prepared by drop-casting a dilute GO dispersion onto a silicon wafer, with a mean thickness of 1 nm in Fig. 4.

Silylanization of graphene oxide (GO). APMS (3-aminopropyltrimethoxysilane) is a common silane coupling agent, which can bridge the inorganic and organic materials--two very different materials. The essence of the reaction is that APMS has the chemical groups which can react with inorganic materials and organic materials. When as-synthesized MGO was dissolved in water, the ester groups were hydrolyzed and the acidic functional groups were formed. At the same time, it is commonly known that the nitrogen atoms can offer lone pair electrons, which could attract lead ion effectively; accordingly, nitrogen-containing groups have been widely used for complexing lead ions. So, the modified GO (MGO) with APMS is expected to increase the adsorption efficiency for Pb^{2+} [24, 25].

The adsorption characteristics of Pb^{2+}. All of the supernatants were analyzed by flame atomic absorption spectrophotometry (AAS) and the data were reported in Table 1. The removal efficiency (η) and the amount of Pb^{2+} adsorbed q (mg/mg) was given according to the formula:

$$\eta = \frac{C_0 - C_1}{C_0} \times 100\% \quad (1)$$

$$q = \frac{C_0 - C_1}{C} \quad (2)$$

Where C_0 (mg/mL) is the initial concentration of Pb^{2+}, C_1(mg/mL) is the concentration of Pb^{2+} after adsorption, C (mg/mL) is the concentration of GO.

Table 1 shows the relationship between adsorption efficiency of lead ion and concentrations of lead ion, MGO and GO. With the increase of Pb^{2+} concentration, the adsorption efficiency decreased, but the adsorption capacity increased. And the adsorption efficiencies of MGO were all higher than that of GO for the same concentration of Pb^{2+}. For instance, at a concentration of 100 mg/mL of Pb^{2+}, the adsorption efficiency was 55.6% of GO, but when we used modified GO (other conditions remain unchanged), the adsorption efficiency was 92.2% in table 1, almost doubled. Otherwise, the surplus Pb^{2+} in lead solution achieves the requirement of drinking water after the adsorption of MGO when the initial concentration of Pb^{2+} is 1mg/mL.

The U.S. Food and Drug Administration sets an action level of 500 ppb (500μg/L) for lead in products intended for use by infants and children. The U.S. Environmental Protection Agency's (EPA) action levels for total lead in soils range from 100 to 400 ppm (0.5 to 1.5 mg/L) [26].

SEM images (Fig. 5) shows that GO crimps with Pb^{2+}. Surface morphologies of metallic architectures were observed by field emission scanning electron microscope (FESEM, Zeiss EVO-MA10), EDS analyses (Fig.6) were recorded with an INCAX-sight energy dispersive X-ray spectrometer equipped on the FESEM. The SEM and EDS show the relationship between adsorption efficiency to lead ion and concentrations of lead ion and MGO. The results of SEM showed that when reacting with Pb^{2+}, the crimps emerged on the surface of GO, which indicated that the Pb^{2+} was adsorbed on the surface of GO [27]. From SEM and EDS measurements, it is clear that there were a weight ratio of 25.7% of Pb^{2+} adsorbed on MGO surface as shown in table 2. Moreover, these data reveal information about the removal Pb((II) mechanism. Hence we conclude that this simple and economic approach holds the potential to solve the problem of lead pollution in water.

TABLE 1
The data of adsorption of lead ion onto GO and MGO

adsorbent	C (mg/mL)	C_0(mg/mL)	C_1(mg/mL)	η(%)	q(mg/mg)
MGO	0.5	1000	370	63.0	1260.0
MGO	0.5	100	7.8	92.2	184.4
MGO	0.5	10	0.21	97.9	19.58
MGO	0.5	1	0.005	99.5	1.99
GO	0.5	1	0.19	81.1	1.62
GO	0.5	10	2.5	75.0	15.0
GO	0.5	100	44.4	55.6	111.2
GO	0.5	1000	780	22.0	440.0

TABLE 2
The EDS data of the GO adsorption of Pb

Element	C K	O K	Si K	S K	Pb K	totals
Weight%	40.05	32.42	0.52	1.31	25.70	100%
Atomic%	60.15	36.55	0.33	0.74	2.24	100%

TABLE 3
the data of scanning X-ray fluorescence (XRF) analyses.

Elements	Mg	Al	Si	P	S	Cl	K	Ca	Fe	Pb
wt%	0.082	1.812	8.489	0.895	16.466	6.847	0.466	0.908	1.534	62.496

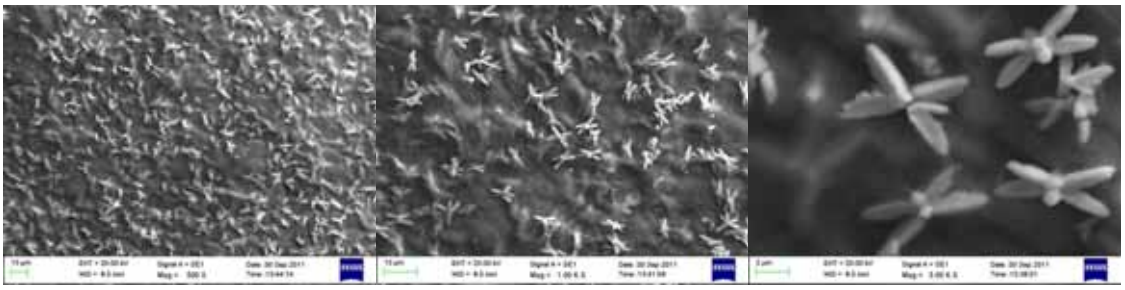


FIGURE 5
SEM of the GO after adsorption of Pb(II)

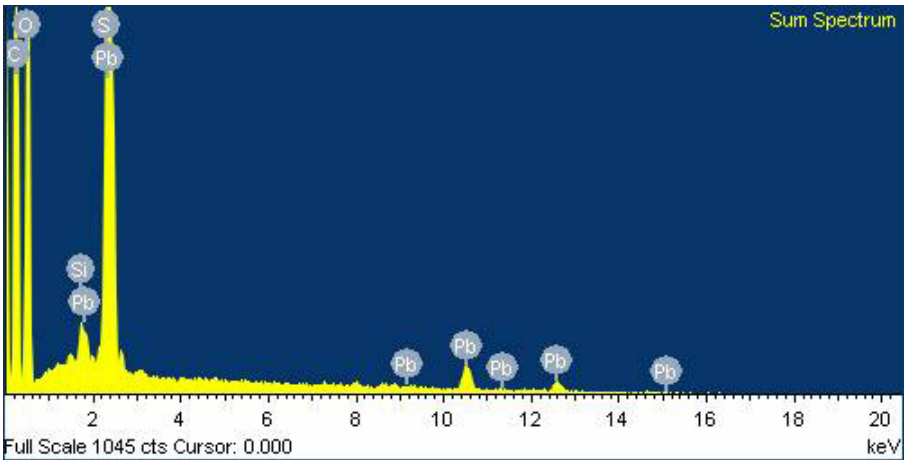


FIGURE 6
EDS analysis of the GO with Pb

X-ray fluorescence (XRF) core scanning is increasingly accepted as an effective method of obtaining high-resolution elemental records because of its non-destructive and nearly continuous measurements.

In this paper we use automatic scanning type of X-ray fluorescence spectrometer (XRF，ZSX Primus II, The Japanese Company) to analyze the surface of GO after the adsorption of Pb^{2+}. Although the results in table 3 suggest that nine elements (Mg, Al, Si, P, S, Cl, K, Ca, Fe and Pb) can be detected using scanning XRF method, only five elements (C, O, Si, S and Pb) can be employed by EDS. These two elements (Pb, C) cannot be sufficiently detected by the XRF core scanner. So the results of XRF data are found to be in conformance with the results of EDS of the weight of the content Pb and C elements.

CONCLUSIONS

Graphite oxide, the high efficiency adsorbent, has been reported that it can be used to remove heavy metals from aqueous solution efficiently. In this work, the GO and MGO were synthesized and used to remove Pb^{2+} from water. A kind of facile method of preparation of MGO was developed. In addition, it was found that MGO showed high adsorption ability in Pb^{2+}, even more than 99% adsorption efficiency in our investigation. The results suggest a potential application of this simple and economic approach to solve the problem of lead pollution in water.

ACKNOWLEDGEMENTS

This work is supported by the Natural Science Foundation of Shandong Province (ZR2015YL004, ZR2017MEE064, ZR2017PEE012) and the National Natural Science Foundation of China (21603125).

REFERENCES

[1] Li, J., Guo, S.J, Zhai, Y.M, Wang, E.K. (2009) High-sensitivity determination of lead and cadmium based on the Nafion-graphene composite film. Analytica Chimica Acta. 649, 196-201.

[2] Bridgewater, B.M., Parkin, G. (2000) Lead poisoning and inactivation of 5-aminolevulinate dehydratase as modeled by the tris (2-mercapto-1-phenylimidazolyl)hydro- borato lead complex, {[Tm^{Ph}]Pb}[ClO_4]. Journal of the American Chemical Society. 122, 7140-7141.

[3] Lewis, J.A., Cohen, S.M. (2004) Addressing lead toxicity: complexation of lead(II) with thiopyrone and hydroxypyridinethione O, S mixed chelators. Inorganic Chemistry. 43, 6534-6536.

[4] Wei, Y., Yang, R. (2011) High adsorptive AlOOH(boehmite)@SiO_2/Fe_3O_4 porous magnetic microspheres for detection of toxic metal ions in drinking water. Chemical Communications. 47,11062-11064.

[5] Zhang, Y.X., Yu, X.Y, Jin, Z., Jia, Y., Xu, W.H, Luo, T., Zhu, B.J, Liu, J.H., Huang, X.J. (2011) Ultra high adsorption capacity of fried egg jelly fish-like-AlOOH(Boehmite)@SiO_2/Fe_3O_4 porous magnetic microspheres for aqueous Pb(II) removal. Journal of Materials Chemistry. 21, 16550-16557.

[6] Hu, X.J., Liu, Y.G., Zeng, G.M., You, S.H., Wang, H., Hu, X., Guo, Y.M., Tan, X.F., Guo, F.Y. (2014) Effects of background electrolytes and ionic strength on enrichment of Cd(II) ions with magnetic graphene oxide–supported sulfanilic acid. Journal of Colloid and Interface Science. 435, 138-144

[7] Stoller, M.D., Park, S., Zhu, Y., An, J., Ruoff, R.S. (2008) Graphene-based ultracapacitors, Nano Letters. 8, 3498-3502.

[8] Samarghandi, M.R., Poormohammadi, A., Nazemi, F. (2015) Removal of Acid orange 7 from Aqueous solution using activated carbon and grapheme as adsorbents. Fresen. Environmen. Bull. 24, 1841-1851.

[9] Wang, B., Chang, Y.H., Zhi, L.J. (2011) High yield production of graphene and its improved property in detecting heavy metal ions. New Carbon Materials. 26, 31-35.

[10] Zhu, Y.W., Murali, S., Stoller, M.D., Ganesh, K.P., Cai, W.W., Ferreira, P.J., Pirkle, A., Wallace, R.M., Cychosz, K.A., Thommes, M., Su, D., Stach, E.A., Ruoff, R.S. (2011) Carbon-Based Supercapcitor produced by activation of graphene. Science. 332, 1537-1541.

[11] Peng, W.J., Li, H.G., Liu, Y.Y., Song, S.X., (2016) Comparison of Pb(II) adsorption onto graphene oxide prepared from natural graphites: Diagramming the Pb(II) adsorption sites. Applied Surface Science. 364, 620-627.

[12] Shao, Y.Y., Wang, J., Engelhard, M., Wang, C.M., Lin, Y.H. (2010) Facile and controllable electrochemical reduction of graphene oxide and its applications. Journal of Materials Chemistry. 20, 743-748.

[13] Madadrang, C.J., Kim, H.Y., Gao, G.H., Wang, N., Zhu, J., Feng, H., Gorring, M., Kasner, M.L., Hou, S.F. (2012) Adsorption Behavior of EDTA-Graphene Oxide for Pb (II) Removal, Appllied Material Interfaces. 4, 1186-1193.

[14] Qi, P.F., Vermesh, O., Grecu, M.H., Javey, A., Wang, Q., Dai, H.J., Peng, S., Cho, K.J. (2003) Toward Large Arrays of Multiplex Functionalized Carbon Nanotube Sensors for Highly Sensitive and Selective Molecular Detection. Nano Letters. 3, 347-351.

[15] Kikuchi, Y., Qian, Q., Machida, M., Tatsumoto, H. (2006) Effect of ZnO loading to activated carbon on Pb(II) adsorption from aqueous solution. Carbon. 44, 195-202.

[16] Shim, J.W., Park, S.J., Ryu, S.K. (2001) Effect of modification with HNO3 and NaOH on metal adsorption by pitch-based activated carbon fibers. Carbon. 39,1635-1642.

[17] Machida, M., Mochimaru, T., Tatsumoto, H. (2006) Lead(II) adsorption onto the graphene layer of carbonaceous materials in aqueous solution. Carbon. 44,2681-2688.

[18] Madadrang, C.J., Kim, H.Y., Gao, G.H., Wang, N., Zhu, J., Feng, H., Gorring, M., Hou, S.F. (2012) Adsorption Behavior of EDTA- Graphene Oxide for Pb (II) Removal. ACS Applied Material Interfaces. 4, 1186-1193.

[19] Hummers, W.S., Offeman, R.E. (1958) Preparation of Graphitic Oxide. Journal of the American Chemical Society. 80,1339.

[20] Moussodia, R.O., Balan, L., Merlin, C., Mustin, C., Schneider, R. (2010) Biocompatible and stable ZnO quantum dots generated by functionalization with siloxane-core PAMAM dendrons. Journal of Materials Chemistry. 20, 1147-1155.

[21] Wang, B., Luo, B., Liang, M.H., Wang, A., Wang, J., Fang, Y., Chang, Y.H., Zhi, L.J. (2011) Chemical amination of graphene oxides and their extraordinary properties in the detection of lead ions. Nanoscale. 3, 5059-5066.

[22] Zhao, Z.Q., Chen, X., Yang, Q., Liu, J.H., Huang, X.J. (2012) Selective adsorption toward toxic metal ions results in selective response: electrochemical studies on a polypyrrole/reduced graphene oxide nanocomposite. Chemistry Communications. 48, 2180-2182.

[23] Guinea, E. , Centellas, F., Brillas, E., Canizares, P., Saez, C., Rodrigo, M.A. (2009) Electrocatalytic properties of diamond in the oxidation of a persistant pollutant. Applied Catalysis B: Environmental. 89, 645-650.

[24] Szpyrkowicz, L., Ricci, F. (2005) Characterization of the catalytic films formed on stainless steel anodes employed for the electrochemical treatment of cuprocyanide wastewaters. Journal of Hazardous Materials. 119,145-152.

[25] Huang, H., Chen, T., Liu, X.Y., Ma, H.Y. (2014) Ultrasensitive and simultaneous detection of heavy metal ions based on three-dimensional graphene-carbon nanotubes hybrid electrode materials. Analytica Chimica Acta. 852, 45-54.

[26] Huang, H., Zhu, W., Gao, X., Liu, X.Y., Ma, H.Y. (2016) Synthesis of a novel electrode material containing phytic acid-polyaniline nanofibers for simultaneous determination of cadmium and lead ions. Analytica Chimica Acta. 947, 32-41.

[27] Hao, L.Y, Song, H.J., Zhang, L.C., Wan, X.Y., Tang, Y.R., Lv, Y. (2012) SiO_2 /graphene composite for highly selective adsorption of Pb(II) ion. Journal of Colloid and Interface Science. 369, 381-387

Received: **15.03.2018**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

RanRan Shan
Qilu University of Technology
(Shandong Academy of Sciences),
Advanced Materials Institute,
Shandong Academy of Sciences,
Jinan, 250014 – China

e-mail: s_claire@163.com

© by PSP Volume 27 – No. 12B/2018 pages 9602-9610 Fresenius Environmental Bulletin

THE COMPARATIVE TOXICITY OF NANO AND MICRO BORON PARTICLES TO FRESHWATER MICROALGA (*DESMODESMUS MULTIVARIABILIS*): THE IMPORTANCE OF PARTICLES SIZE

Yesim Daglioglu*

Ordu University Department of Molecular Biology and Genetics, Ordu, Turkey

ABSTRACT

Nanoparticles gain new characteristics, which are related to their nano-scale structure. Thanks to these features, they have found wide applications in the industry and as well as in the daily life. However, with the increase in the release of nanoparticles into the environment, the world of science has attracted attention. In this study, an effect on photosynthetic pigment content and oxidative stress of boron particles on *Desmodesmus multivariabilis* was investigated. Acute exposure tests were conducted in the BG-11 medium for 24, 48, and 72 hours on algal cells with the 0.1, 0.01 and 0.001 mg/L concentrations. Results revealed that oxidative stress and photosynthetic pigments content in the algal cells changed with time-varying exposure. Nano and micro boron particles were roughly indicated the same toxic effects on the pigments. Reactive oxygen species activity exhibited nonlinear changes between their treatment groups in the both boron particles. Consequently, after time-varying exposure of B particles at various concentrations, *Desmodesmus multivariabilis* displayed different toxicity.

KEYWORDS:
Boron, *Desmodesmus multivariabilis*, nanoparticles, oxidative stress, nanotoxicology

INTRODUCTION

Though nanoparticles (NPs) have many beneficial applications, the release of the NPs in the environment may cause them to affect the health of the ecosystem. Many experimental studies to date have proved that metal NPs containing silver, zinc, copper, gold and boron have anti-microbial and anti-bacterial properties and are therefore used in various medical applications and consumer products. Nano boron is produced industrially, especially because it is used in numerous applications, including potential energy source, medical, military and civilian [1-3]. However, the inclusion of these beneficial NPs in the natural environment may pose a risk to soil and water environments [4]. NPs have many sources which are generally categorized as natural and anthropogenic sources. Natural sources of NPs; dust storms, forest fires, volcanoes, ocean, water evaporation and organisms (body parts such as skin and hair). Anthropogenic sources; diesel and engine exhaust NPs, indoor pollution, cigarette smoke, building demolition, cosmetics and other consumer products, engineered nanomaterials [5]. These NPs may find their way into aquatic environments. NPs entering the aquatic environment may become components of previously known natural colloids. Colloids are complex mixtures in water containing natural organic matter (humic acids, protein, polysaccharide exudates), inorganic matter (iron, manganese, aluminum), viruses and bacteria [6-7]. Fate, behavior and carrying of NPs after incorporation with these colloids will depend on both the physicochemical properties of the aqueous environment and their interaction with other colloidal ingredients [8]. These are the effects the behavior of NPs in aquatic environments, relation to sediment and colloidal particles, connecting with lipophilic organic and metal contaminants, transition pathways to biota, a contribution of surface properties and particle size to toxicity and organism health and ecosystem integrity [9]. NPs have a wide range of physicochemical characteristics and this influence their toxic degree of NPs. These physicochemical characteristics are chemical composition, density, shape, crystal structure, chemical reactivity, surface area, surface energy solubility, dielectric constants, surface roughness, conductivity, melting and boiling points, hardness, and optical properties. Among these properties are the small dimensions of the NPs, which are particularly noteworthy [10]. Due to the nanoscale nature of the NPs, their extraordinary properties can interfere with living organisms and cause effects within the environment. These effects can be overdone or unforeseen when compared to more bulky equivalents [8]. Numerous studies have shown that nano-size significantly changes biological effects of NPs [11]. NPs can interact easily with biological systems via internalization by cells. Therefore, NPs may cause harmful biological effects on living organisms [12]. As was stated by Borm et al., [13] although there

are several advantages of the nanotechnology, ultrafine particles may pose a risk to living organisms. One of those risks is oxidative stress which is the major factor of the toxicity induced by NPs. Oxidative stress which the production of ROS are the states of oxidizing (redox) imbalance that reduces the antioxidant defense capacity of the cell, which may result in negative biological effects in living organisms [14]. However, the effects of boron NPs on the environment are still entirely unknown. In addition, the behavior of boron NPs in aquatic environments varies depending on many factors such as aggregation characteristics and natural organic matter relationships [15]. Although the boron element is useful in trace amounts for plants, it has been reported in previous studies that it is toxic and even fatal in large quantities [16]. Therefore, removal of toxic metals from water is an important area of research. For this purpose, the data of toxicity tests made with different organisms (bacteria, seaweed, and sea chestnut) from aquatic environments compare different environments. [17-18]. For example, in a few studies; *Artemia salina* exposed to micro boron and nano boron particles. Accordingly, the mortality rate increased for both particles when the exposure time longer at all concentrations. However, this increase is much higher for micro boron particles [19]. Dağlıoğlu et al. Assessed the toxicity of nano and micro boron NPs to *Apis mellifera* and reported that the toxic effects of the particles increased during the long-term exposure period [20]. Strigul et al. *Daphnia magna* reported that the boron NPs was toxic [21]. In another study, they reported that nano and micro boron particles in *Desmodesmus multivariabilis* exhibited different amounts of accumulation [22]. In this study, algae were used as indicator organism. Because, in an aquatic environment, green algae constitute the main source of biomass production required for animals at high ecological trophic levels. Algae are used as bioindicator species to monitor the health of aquatic ecosystems due to the accumulation of metallic pollutants in their biomass [23]. Also, responses of algae species to different toxic chemicals are different [24]. In this study, single-celled green algae called *Desmodesmus multivariabilis* was used as a model organism to determine the comparative toxicity and characterization of nano boron (NB) and micro boron (MB) particles. In order to evaluate photosynthetic pigment and oxidative stress levels, the algae cells were left for a 72 hours exposure to NB and MB particles. Moreover, by minimizing the boron particle down to a nano size, a comparison as to the variations in the response of the algae cells to toxicity and oxidative stress could be made.

MATERIALS AND METHODS

Algae culture and cultivation conditions. The test organism *Desmodesmus multivariabilis* was collected from freshwater (from Eber Lake in Afyon, Turkey). Isolated and pure culture was grown according to the procedure given by Rippka, on BG-11 medium commonly used for growing blue-green algae in flasks. This medium contains only trace amounts of metal ions and allows rich growth [25]. The cells were grown in sterile shake flasks containing 100 mL of BG-11. The algal cultures were grown under the cool white fluorescent light intensity of 3000 lux light intensity at 25±1 °C, in 12 h–12 h light–dark cycle and were incubated for 15–20 days in an incubator which is suitable for photosynthesis (Figure 1A). The obtained algal cells were later done species identification using morphological and molecular methods. The DNA of our research material was insulated with the help of the Qiagen marked DNA isolation Kit.

Exposure of algae to boron particles and determination of pigment contents. The exposure test was conducted following the "Alga, Growth Inhibition Test" Guideline OECD No. 201 [26]. The boron particles suspensions were prepared in 200 mg/L stock solution with BG-11 medium previous to the experiments. Inoculums were grown in flask containing 100 ml of BG-11 medium in the same conditions on a shaking at 100 rpm. Three concentrations, 0.1, 0.01, 0.001 mg/L (nominal concentration, (0.01, 0.001 and 0.0001 mg/L) of B particles were added to flasks from the stock solution of particles suspensions, aliquots of 90 mL of the with an initial cell density (2.1×10^5 cells mL) of algal culture in the exponential growth phase. Chlorophyll a (C_a), Chlorophyll b (C_b), and carotenoids (C_c) were extracted with 80% (v/v) acetone and determined according to the method of Lichtenthaler and Wellburn [27]. Light absorbance of the supernatant liquid was measured with a UV-1200 spectrophotometer (Hange- Lange brand DR 2800) at 663 nm (A663), 646 nm (A646), and 470 nm (A470), respectively [28]. C_a, C_b and C_c were calculated using the following equations [29].

$$C_a = 12.21A663 - 2.81A646$$
$$C_b = 20.13A646 - 5.03A663$$
$$C_c = (1000A470 - 3.27C_a - 104C_b)/229$$

TEM and SEM analysis of the boron particles and the algae. Nano boron (NB) and micro boron (MB) powders were purchased from Pavezyum Chemicals Industry (Istanbul, Turkey). According to the manufacturer, the diameter particles of NB particles were <100 nm, purity was 98.5% and MB particles were <1 μm, purity was 95-97 % (Figure 2). SEM images of the cells exposed to B particles after ethanol pretreatment is in

Figure 2. For this, *Desmodesmus multivariabilis* cells (about $5x10^7$ cell/ml) exposed to 72 hours 0.1, 0.01 and 0.001 mg/L concentrations were collected into falcon tube containing Phosphate Buffer Sodium (PBS) by centrifugation. Then these collected cells, after washed twice, were centrifuged at 1000 g for 10 minutes. And then, cells prepared in 0.1 M PBS was taken up in 2.5% glutaraldehyde. After 1 overnight (4 °C) cells were detected washing with buffer and taken up in 1% osmium tetroxide was subjected to secondary fixation. Then, the samples were dehydrated in a graded ethanol series (50%, 70%, 90%, and 100%) with 10 min intervals. Images were taken up after sample coating with gold (Figure 1B). The surface of *Desmodesmus multivariabilis* was observed with SEM (JEOL JSM5600).

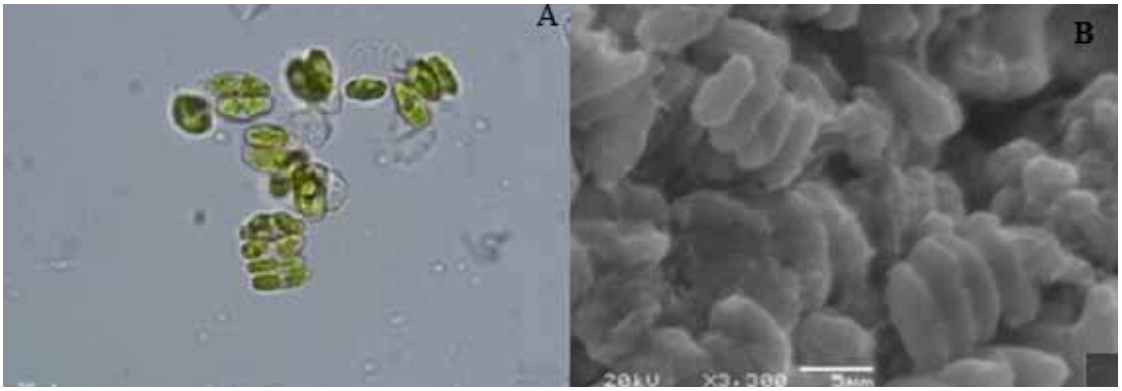


FIGURE 1

A) Light micrographs of the young and mature vegetative algal cells B) SEM images of algal cells after exposure to particles for 72 h

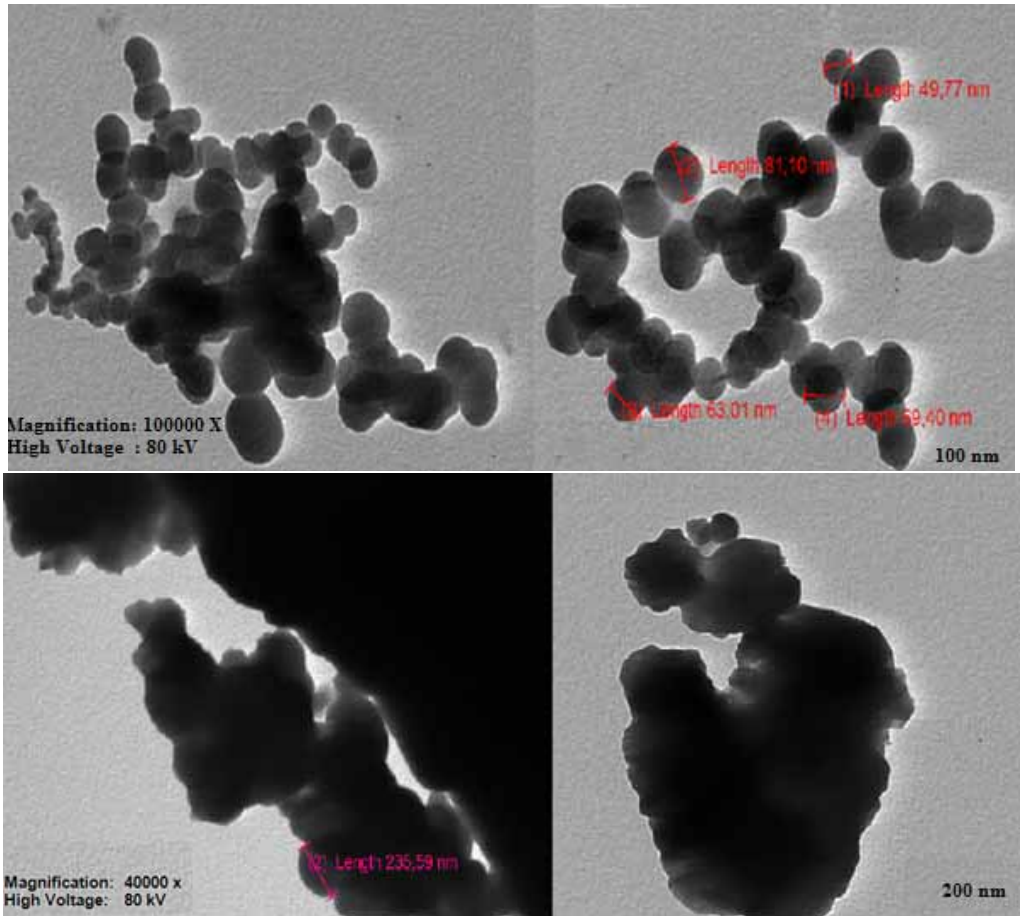


FIGURE 2

TEM image of nano and micro boron particles (10 mg/L) in pure water

Oxidative stress evaluation. Cellular oxidative stress reactive oxygen species (ROS) formation was measured by using the cell permeable indicator 20,70-dichlor-odihydro fluorescein diacetate (H_2DCFDA) [30]. Cellular esterases hydrolyze the probe to the non-fluorescent 20,70-dichlorodihydrofluorescein (H_2DCF), which is better protected in the cells. In the entity of ROS and cellular peroxidases, H_2DCF is converted to the extremely fluorescent 20,70-dichlorofluorescein (DCF). The treatment groups and the control group were related with 5 mM of H_2DCFDA in 1 ml of solution. The DCF fluorescence was measured by using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. All the fluorescence data were collected using a fluorescence platereader. Samples were diluted nine times with potassium phosphate buffer (pH 7.8). Afterwards, homogenization and centrifugation were performed (10.000 rpm, 20 min) and the supernatant was removed.

Statistical Analyses. All experiments were independently repeated three times and the data were recorded as the mean value with standard deviation. For acute toxicity and ROS tests, a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons was used to detect significant differences between the control and treated groups.

RESULTS

Characterisation of boron particles. TEM images confirm that NB and MB particles have a spherical shape. Analysis by TEM showed NB and MB particles suspensions in pure water media to have an average diameter of particle size distribution of 10-80 nm and 250-300 nm, respectively (Figure 2).

Photosynthetic pigment content. *Desmodesmus multivariabilis* was exposed to BG-11 medium containing NB and MB particles at various concentrations for 24, 48, and 72 hours. The photosynthetic pigment levels were measured after this time-varying exposure. In this study, *Desmodesmus multivariabilis* was exposed with BG-11 medium containing boron particles in the range of 0.1 mg/L-0.001 mg/L and the medium without boron particles (untreated control group).

As shown in Figue 3, compared to control groups, NB and MB particles were demonstrated very different reactions depending on the time-varying exposure and various concentrations, and these reactions are parallel to each other. For example, the values of C_a have decreased rather a great deal at all treatment groups in the 48 h. 0.1 mg/L, 0.01 mg/L and 0.001 mg/L for these values have decreased by 80%, 94% and 99% for NB

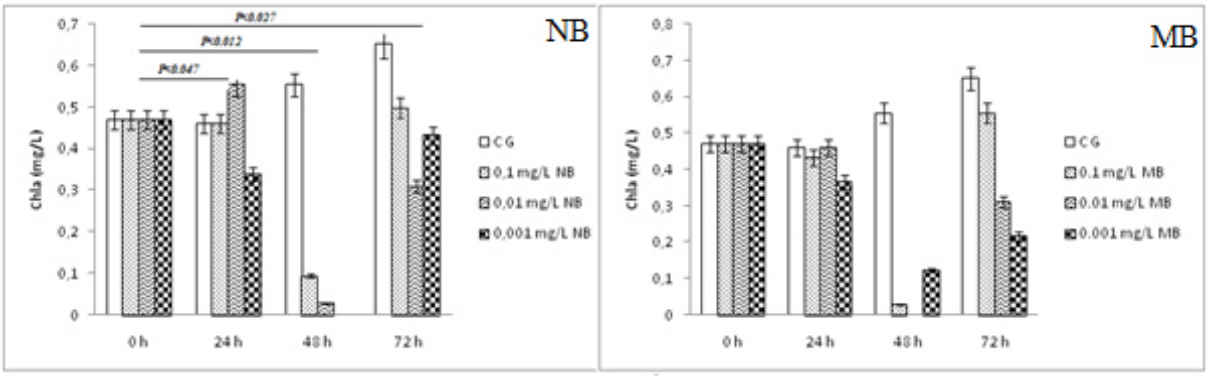


FIGURE 3
Chlorophyll a content according to time-varying exposure

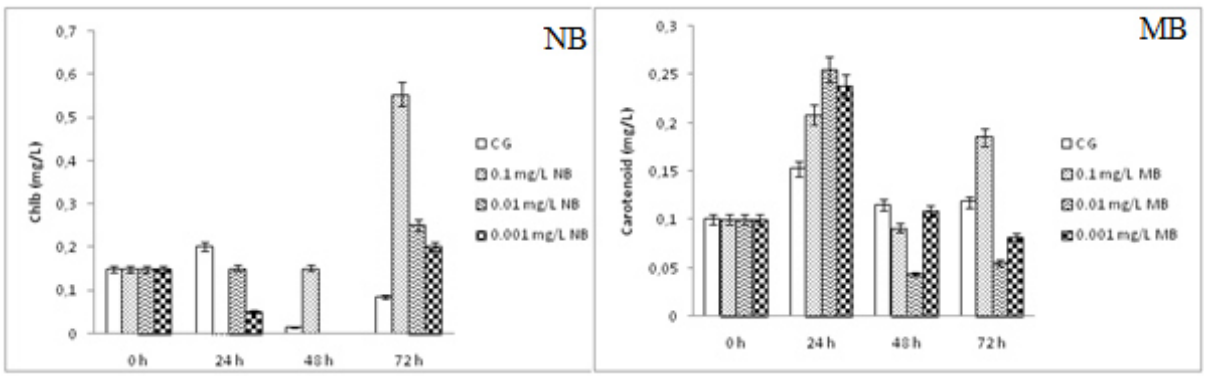


FIGURE 4
Chlorophyll b content according to time-varying exposure

particles, 81%, 99%, and 75% for MB particles respectively. When the exposure time was extended to 72 hours, the C_a values have increased again in the both particle groups. Generally, both nano and MB particles have a negative effect on the amount of C_a compared to the control groups, and a significant have decreased occurred. In the statistical study, there was no significant difference in the C_a amount of the MB in terms of exposure time and concentration ($P>0.05$). Only, the C_a amount of the NB is important depending on exposure time ($P<0.05$).

NB particles have shown a toxic effect on the C_b pigment for 24 and 48 hours and reduced the amount of C_b. When the exposure time was extended to 72 hours, the amount of C_b increased again. This increase is considerably higher than the initial amount of C_b. At concentrations of 0.1, 0.01 and 0.001 mg/L these values are 266%, 66% and 13 % for NB and 5%, 66% and 33% for MB, respectively. Accordingly, the amount of C_b in NB is higher than that of MB (Figure 4). It was found that B particles did not exhibit any significant difference on C_b depending on the exposure time and concentration ($P>0.05$).

The amount of C_c for nano and MB particles increased in algae at 24 hours exposure time compared to the control groups. At 48 hours of exposure time, the amount of C_c decreased in all treatment groups, including the control groups, compared to the 24 hours exposure period (Figure 5). Generally, at 72 hours exposure time, the amount of C_c decreased very seriously in treatment groups (except 0.1 mg/L) for NB and MB particles (30% for 0.01mg/L of MB and 57 % for 0.001 mg/L of NB). While there was a significant difference in C_c value in terms of time-varying exposures of boron particles ($P<0.05$), there was no significant difference in terms of exposure concentration ($P>0.05$).

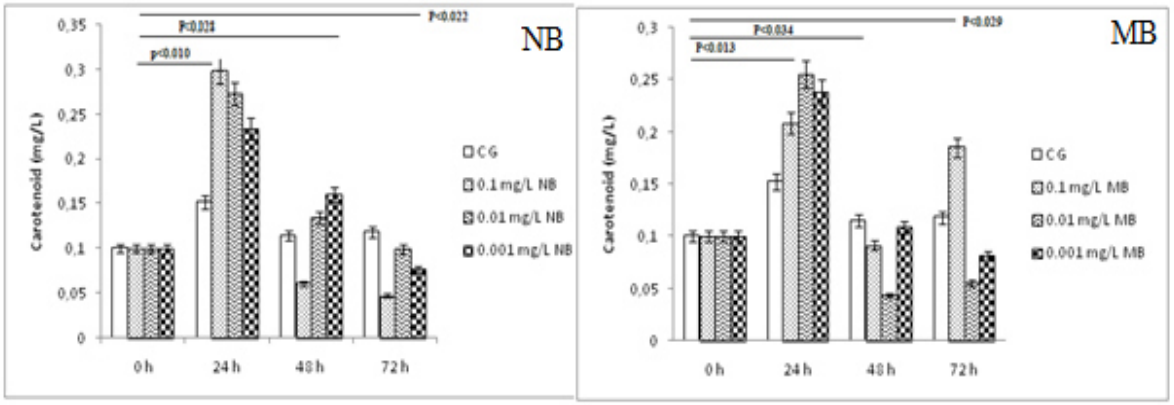


FIGURE 5
Carotenoid content according to time-varying exposure

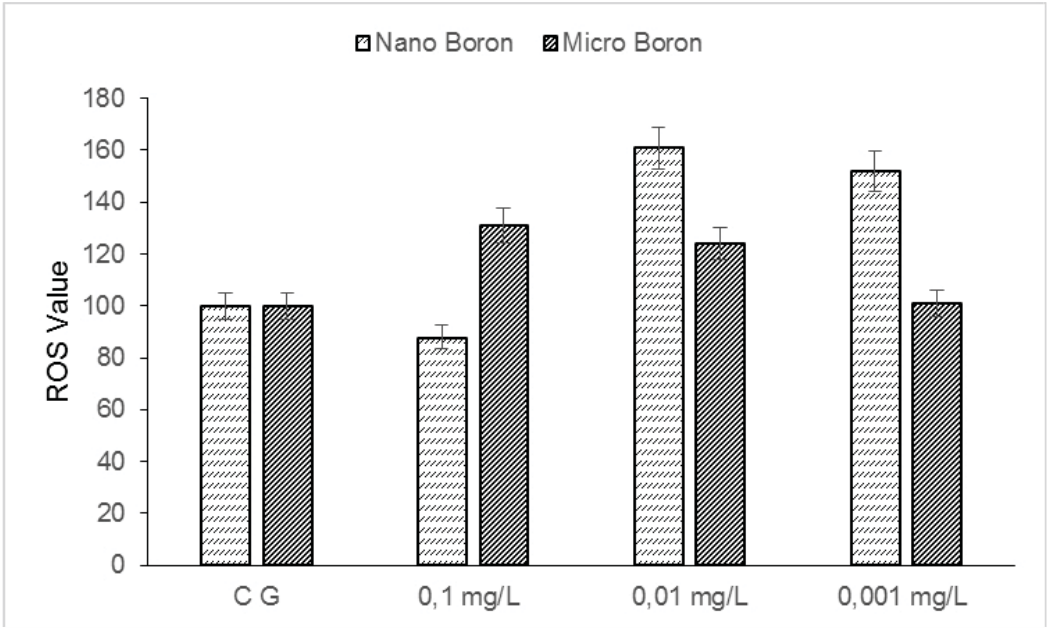


FIGURE 6
The ROS values of the nano nd micro boron within the algae cells at 72 h

Reactive oxygen species production after exposure to boron particles in algae. At the 72 hours exposure to all the treatment groups (0.1, 0.01 and 0.001 mg/L) of the NB and MB particles in algal cells, there has a significant difference at $P<0.01$ level in terms of ROS level in a general means, which was, at most, seen in the 0.001 mg/L of the MB. When all the groups exposed to B particles were compared with the control groups the ROS level proved to be high in all the algae cell suspensions (excluding 0.1 mg/L) (Figure 6). When we compared the groups exposed to NB and MB particles among themselves, the ROS level was higher in MB than in NB ($P<0.05$). Contrary to expectations, the MB was observed to have the highest level of ROS at the lowest concentration (0.001 mg/L). Furthermore, there was no definite relationship between the treatment groups and ROS levels in algae exposed to boron particles.

DISCUSSION

In the usage of NPs, the environmental release is increasingly important. Because, they have rapidly increasing applications in a wide range of fields such as economics, textile, electronics, pharmacy, cosmetics and environmental improvement [31]. Therefore, risk assessment of NPs should be performed. The assessment of the environmental risks of NPs is made by comparing the environmental concentrations of NPs that are toxic to organisms. In aquatic ecosystems, it can affect the productivity of algae either because of direct toxicity or of indirect toxic effect due to physical constraints. As a direct effect, NPs internalized by algae may cause it to shade or their increase of cellular weight eventually sinks [31]. As an indirect effect, the aqueous suspensions of the NPs are opaque and thus can inhibit algal productivity by absorbing light from the algae [32]. Our aim was to determine the toxic effect of nano and MB particles on *Desmodesmus multivariabilis* which is the indicator organisms of the aquatic ecosystem. In this study, the quantities of photosynthetic pigments (C_a, C_b and C_c) were comparatively evaluated after exposure to nano and MB particles, according to their exposure concentrations (0.1, 0.01 and 0.001 mg/L) and varying-time (24, 48, 72 and 96 h). One of the methods used to investigate the effects of stress factors on algae is to determine photosynthetic pigment contents. In our study, NPs have adversely affected the photosynthesis mechanisms of algae. C_a and C_b are separate by molecular solubility and absorption towards the light. Therefore, despite the decreasing amount of C_a the amount of C_b increases; this increase is caused by the induction of the conversion of C_a to C_b. When we compare NB and MB in terms of C_a pigment, there is no différence in either of them as seen in Figure 3. The amount of C_a decreased according to control groups when the exposure time was prolonged. However, at 72 hours the amount of C_a has increased again compared to 24 and 48 hours. In C_b, on the other hand, the MB particles were seen to be more toxic than the NB at 24 h. That is recorded to exhibit toxic effects on *Desmodesmus multivariabilis* at 48 hours of exposure to NB and MB particles. Within this exposure period, the particles most effectively interacted with the algae and showed the highest toxic effect, which is why the development of the defense mechanisms of algal cells can be interpreted as the reduction of particulate uptake by the agglomeration of particles over time. In C_a, C_b and C_c pigments, the most toxic exposure time for NB and MB particles is 48 hours. The reason for this can be interpreted as the fact that the algal cells had interacted with these particles in the most active way. At the same time, considering C_a/C_b ratio, there was an increase in the algae exposed to NB particle at all the concentrations in 24 h when compared to the control groups, whereas a gradual decrease was observed at the 48th and 72nd h. Considering the MB particle, on the other hand, there was a similar situation at the 48th and 72nd h, however, this rate only increased at 0.01 mg/L at the 24th h while diminishing in treatment groups. As stated by Fang et al., [33], as the first step for degradation, C_b transforms into C_a. While C_a exists only at the reaction center of photosystem, C_b exists both in the reaction centers and in the light-capturing complexes. A change in the C_a/C_b rate stands for the adaptation mechanism to keep the amount of light held by a leaf in equilibrium as well as the use of it for a photochemical way [34]. It was reported that the algal cells were affected by these particles and that the chlorophyll content of the cells had changed. Compared to the control groups, the C_c value of the B particles reached their maximum values at 24 h and their minimum at 72 h of exposure. At 24 hours exposure, the C_c increase rates were 0, 0.1, 0.01 and 0.001 mg/L, respectively; 190%, 170% and 130 for NB particles, 100%, 155% and 130% for MB particles. In this study, all photosynthetic pigments of *Desmodesmus multivariabilis* were shown the most toxic effect at 48 hours. This is the indication that B particles are most effective with algae and that exposure time is 48 hours. Previous studies, as in our study, have reported an increase in C_c pigment levels in the presence of stress. Chromium stress, aquatic plant species such as *Spirodela polyrrhiza* and *Vallisneria spiralis*, and *Brassica juncea* caused an increase in the content of carotenoids [35].

The presence of oxygen in cells produces continuous oxidative stress for cells and mechanisms [36]. Although oxygen itself is a completely innocent molecule, it is partially degradable and can form harmful reactive oxygen species [37]. In plants, ROS is generally formed by the infiltration

of molecular oxygen from the electron transporting activities of chloroplasts, mitochondria and plasma membranes, and by a variety of environmental stresses (high light, temperature and salt concentration, drought, heavy metal and NPs. The resulting ROS species may react with certain biomolecules to alter or inactivate biochemical activities, resulting in toxicity. Plant cells have developed various enzymatic and non-enzymatic antioxidant mechanisms to struggle the stress created by ROS. Carotenoids are in non-enzymatic antioxidant mechanisms [37]. In this study, the level of carotenoid pigment increased in *Desmodesmus multivariabilis* as a result of exposure to B particles. Likewise, the increase in ROS values parallel to C_c pigment is evidence of the defense mechanism of algal cells against NB and MB particle toxicity. For example, the highest ROS value for NB and MB was found at 0.01 mg / L and the highest carotenoid levels were observed at 24 hours. However, the NB has a 6% higher level of carotenoid than the MB. Furthermore, NB was below the control group of ROS level at 0.1 mg/L (highest treatment group). At ROS analysis, when the control groups are compared with those under exposure, it is seen that the NB and MB particles cause an oxidative stress on the algae. ROS level was formed on a different level for each concentration in NB and MB particles. In general, the MB particles have posed more ROS in all the concentrations. So far, some studies have been carried out to evaluate the toxicity of NPs on algae. For example, Navarro et al. [38] evaluated the short-time toxic effect of silver NPs and ionic silver on *Chlamydomonas reinhardtii*. $AgNO_3$ toxicity was reported to be 18 times higher than AgNP according to total Ag concentration. However, when the function of Ag^+ concentration is compared, the toxicity of AgNP is much higher than the toxicity of $AgNO_3$.

CONCLUSIONS

I) When traditional materials are reduced to nano size, their physicochemical properties change. In this study, it has been observed that the size of the boron has different toxicity due to the different physicochemical properties of micro and nano size.

Also, when we look at the ROS level, we can conclude that the nano bo-run is more toxic.

II) In alg cells, various stress factors lead to the production of reactive oxygen species. In this study, different sized boron particles, which are stress factors, produced oxidative stress in algal cells. Depending on the size (nano and nano B), these effects are quite different. The resulting ROS activated the defense mechanisms of the algae. The non-enzymatic antioxidant defense mechanisms have increased with increasing levels of ROS in the carotenoids.

ACKNOWLEDGEMENTS

A special thanks to my father Murat Özkan for his exertion. May his soul rest in peace.

REFERENCES

[1] Risha, G.A., Evans, B.J., Boyer, E., Wehrman, R.B., Kuo, K.K. (2003) Nano-sized aluminum and boron-based solid-fuel characterization. 39th AIAA/ASME/SAE/ASEE joint propulsion conference and exhibit. Huntsville, AL.

[2] Kuo, K.K., Risha, G.A., Evans, B.J., Boyer, E. (2003) Potential usage of energetic nano-sized powders for combustion and rocket propulsion. MRS Online Proceedings Library Archive, 800.

[3] Kaste, P.J., Rice, B.M. (2004) Novel energetic materials for the future force: the army pursues the next generation of propellant and explosives. AMPTIAC Q, 8, 84-90.

[4] Simonin, M., Richaume A. (2015) Impact of engineered nanoparticles on the activity, abundance, and diversity of soil microbial communities: a review. Environ. Sci. Pollut. Res. 1–14.

[5] Buzea, C., Pacheco, I.I., Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases. 2(4), MR17-MR71.

[6] Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R. (2008) Nanomaterials in the environment: Behavior, fate, bioavailability and effects Environ Toxicol Chem. 27, 1825–1851.

[7] Lead, J.R., Wilkinson, K.J. (2006) Aquatic colloids and nanoparticles: current knowledge and future trends. Environmental Chemistry. 3(3), 159-171.

[8] Scown, T.M., Van Aerle, R., Tyler, C.R. (2010) Review: Do engineered nanoparticles pose a significant threat to the aquatic environment? Critical Reviews in Toxicology. 40(7), 653-670.

[9] Moore, M.N. (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment international. 32(8), 967-976.

[10] Gatto, N.M., Henderson, V.W., Hodis, H.N., John, J.A.S., Lurmann, F., Chen, J.C., Mack, W.J. (2014) Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. Neurotoxicology. 40, 1-7.

[11] Kononenko, V., Narat, M., Drobne, D. (2015) Nanoparticle interaction with the immune system. Arhivza higijenu rada i toksikologiju. 66(2), 97-108.

[12] Chen, L., Zhou, L., Liu, Y., Deng, Wu, H., Wang, G. (2012) Toxicological effects of nanometer titanium dioxide (nano-TiO_2) on *Chlamydomonas reinhardtii*. Ecotocixology and Environmental Safety. 84, 155-162.
[13] Borm, P.J., Schins, R.P., Albrecht, C. (2004) Inhaled particles and lung cancer, part B: paradigms and risk assessment. International Journal of Cancer. 110(1), 3-14.
[14] Halliwell, B., Gutteridge, J.M.C. (1999) Free radicals in biology and medicine. Oxford University Press. Inc, New York.
[15] Liu, X., Wazne, M., Han, Y., Christodoulatos, C., Jasinkiewicz, K.L. (2010) Effects of natural organic matter on aggregation kinetics of boron nanoparticles in monovalent and divalent electrolytes. Journal of Colloid and Interface Science. 348(1), 101-107.
[16] Huertas, E., Herzberg, M., Oron, G., Elimelech, M. (2008) Influence of biofouling on boron removal by nanofiltration and reverse osmosis membranes. Journal of Membrane Science. 318(1-2), 264-270.
[17] Manfra, L., Onorati, F., AndreaTornambé, C.M., Nonnis, O., Gabellini, M. (2013) Worst case method, qualitative assessment and quantitative assessment to interpret toxicity data. Fresen. Environ. Bull. 22, 895-903.
[18] Jian-Gang, H., Di-Wu, F., Xiu-Zhi, L., Yong-li, Z., Sajjad, A. (2016) Mechanism of Cr^{6+}, Mn^{2+}, Cu^{2+} and Cd^{2+} adsorption by a low-cost rice husk-derived biochar in aqueous solutions. Fresen. Environ. Bull. 25, 2736.
[19] Dağlioğlu, Y. (2016) Acute toxicity of nano and micro boron particles in *Artemia salina*. 1st International Mediterranean Science and Engineering Congress (IMSEC) October 26-28, 2016 Adana, Turkey.
[20] Dağlioğlu, Y., Kabakçi, D., Akdeniz, G. (2015) Toxicity of nano and non-nano boron particles on *Apis mellifera* (honey bee). Research Journal of Chemical and Environmental Sciences. 3(3), 06-13.
[21] Strigul, N., Vaccari, L., Galdun, C., Wazne, M., Liu, X., Christodoulatos, C., Jasinkiewicz, K. (2009) Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to *Daphnia magna* and *Vibrio fischeri*. Desalination. 248(1-3), 771-782.
[22] Dağlioğlu, Y., Öztürk, B.Y. (2008) The assessment of biological accumulation on exposure in boron particles of Desmodesmus multivariabilis. Biological Diversity and Conservation. 9(3), 204-209.
[23] Barhoumi, L., Dewez, D. (2013) Toxicity of superparamagnetic iron oxide nanoparticles on green alga *Chlorella vulgaris*. BioMed research international. 2013, 11p.
[24] Boyle, T.P. (1984) Effect of environmental contaminants on aquatic algae. Algae as Ecological Indicators. Academic Press, New York 1984. 237-256.
[25] Rippka, R. (1988) Isolation and purification of cyanobacteria. Methods in enzymology. 167, 3-27.
[26] Organisation for Economic Cooperation and Development. (1984) Organisation for Economic Cooperation and Development Algal growth inhibition test OECD guidelines for testing of chemicals. 201.
[27] Lichtenthaler, H.K., Wellburn, A.R. (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions. 11(5), 591-592.
[28] Vajpayee, P., Rai, U.N., Ali, M.B., Tripathi, R.D., Yadav, V., Sinha, S., Singh, S.N. (2001) Chromium-induced physiologic changes in *Vallisneria spiralis* L. and its role in phyto remediation of tannery effluent. Bulletin of Environmental Contamination and toxicology. 67(2), 246-256.
[29] Shi, D., Zheng, X., Li, L., Lin, W., Xie, W., Yang, J., Chen, S., Jin, W. (2013) Chlorophyll deficiency in th maize longated mesocotyl mutant is caused by a defective heme oxygennase and delaying grana stacking. PloS one. 8(11), e80107.
[30] Gerber, I.B., Dubery, I.A (2004) Fluorescence microplate assay for the detection of oxidative burst products in tobacco cell suspensions using 2′, 7′-dichlorofluorescein. Methods in Cell Science. 25(3-4), 115-122.
[31] Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Filser, J., Miao, A. J., Quigg, A., Santschi, P. H., Sigg, L. (2008) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 17(5), 372-386.
[32] Aruoja, V., Dubourguier, H.C., Kasemets, K., Kahru, A. (2009) Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Science of the Total Environment. 407(4), 1461–1468.
[33] Fang, Z., Bouwkamp, J.C., Solomos, T. (1998) Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. Journal of Experimental Botany. 49(320), 503-510.
[34] Lin, Y.M., Zou, X.H., Liu, J.B., Guo, Z.J., Lin, P., Sonali, S. (2005) Nutrient, chlorophyll and caloric dynamics of *Phyllostachys pubescens* leaves in Yong chun County, Fujian, China. Journal of Bamboo and Rattan. 4(4), 369-385.

[35] Vajpayee, P., Rai, U.N., Ali, M.B., Tripathi, R.D., Yadav, V., Sinha, S., Singh, S.N. (2001) Chromium-induced physiologic changes in *Vallisneria spiralis* L. and its role in phyto remediation of tannery effluent. Bulletin of Environmental Contamination and toxicology. 67(2), 246-256.
[36] Alscher, R.G, Donahue, J.L., Cramer, C.L. (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant. 100, 224-233.
[37] Mallick, N., Mohn, F.H. (2000) Reactive oxygen species: response of algal cells. Journal of Plant Physiology. 157(2), 183–193.
[38] Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L. (2008) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 17(5), 372-386.

Received: **15.03.2018**
Accepted: **31.10.2018**

CORRESPONDING AUTHOR

Yesim Daglıoglu
Ordu University
Faculty of Science & Literature
Department of Molecular Biology and Genetics
Ordu 52200 - Turkey

e-mail: yozkan52@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9611-9616 Fresenius Environmental Bulletin

THE IMPACTS OF DIFFERENT AUXINS ON PHENOLIC CONTENTS AND THE TOTAL ANTIOXIDANT CAPACITY OF *HYPERICUM RETUSUM* AUCHER

Sureyya Namli[1], Yesim Kara[2,*], Cigdem Isikalan[1], Filiz Akbas[3], Havser Ertem Vaizogullar[2]

[1]Department of Biology, Faculty of Science, The University of Dicle, Diyarbakir, Turkey
[2]Department of Biology, Faculty of Science and Art, University of Pamukkale, Denizli, Turkey
[3]Department of Biology, Faculty of Science and Art, The University of Batman, Batman, Turkey

ABSTRACT

The study was aimed to evaluate the effects of different auxins on phenolic contents and antioxidant potential of methanolic extract of *Hypericum retusum* Aucher (Clusiaceae*)* plantlets grown under *in vitro* conditions. After seed sterilisation and germination, shoot proliferations were performed. In this study, shoots were separately cultured in the medium containing BAP (0.5 mgl^{-1}) combined with three different auxins (0.25 mgl^{-1} IAA, NAA, IBA) and the highest number of shoots was obtained on medium supplemented with 0.5 mgl^{-1} BAP. The highest total antioxidant capacity and phenolic contents were observed auxins. The methanol extracts of plantlets grown *in vitro* conditions showed the strongest free radical scavenging capacities at concentrations of 100 and 150 µg/ml. It has been found that auxins and *in vitro* growth conditions have a significant effect on the total antioxidant capacity and phenolic contents.

KEYWORDS:
Hypericum retusum Aucher, auxins, antioxidant, phenolic.

INTRODUCTION

The species Hypericum is the genus Hypericaceae, usually found in a lower family in Clusiaceae, and covers more than 450 species separated into 36 sections. In recent years, the use of Hypericum species has increased due to the antiviral and antidepressant activities found in the extracts of these plants [1]. Hypericum is known to contain a number of chemical compounds according to previous drugs. These compounds are defined as flavonoids, tannins, phenolics, hyperizine, essential oils, and pseudohyphe- sine. Antisepsis is reported to have multifunctional effects including antimicrobial, anti-inflammatory, antitumor and antioxidant capacities [2-5]. In both in vivo and *in vitro* systems, auxins have been found to exhibit a strong biological activity at very low concentrations and are essential for maintaining of physiological processes in plants [6]. Recent studies have focused on the health functions of phenolics, including anthocyanins and flavonoids [7-9[. Some researchers found that wheat Pseudomonas sp. Ile has been reported to be inoculated. It stimulates plant growth through reduction of toxic ion uptake, increases in oxine content, and the formation of strase specific proteins in plants under stress caused by toxic ions [10]. Several studies have been published about the antioxidant properties of MEL (Melatonin) and of other plant indoles, such as indole-3-acetic acid (IAA), indole-3-butyric acid, indole-3-methanol, indole-3-propionic acid and tryptophan, and found that they all showed good antioxidant properties [11]. In the present paper, we study the effects of different auxins on the total antioxidant capacity and phenolic contents of *Hypericum retusum* Aucher raised under *in vitro* conditions.

MATERIALS AND METHODS

Chemicals. DPPH (1,1-diphenyl-2-picryl-hydrazyl), BHT (butylated hydroxytoluene), BHA (butylated hydro-xyanisole), ascorbic acid and all of the chemicals used in the MS medium were purchased from Sigma (Sigma, Aldrich). Potassium hydroxide (KOH), methanol and the other reagents and chemicals were purchased from Sigma (Steinheim, Germany**).**

Collection of Plant Material. The seeds of *H. retusum* (Clusiaceae) were collected from South of East of Turkey, in Diyarbakır. Voucher specimen have been deposited at the Herbarium of the Department of Biology, Faculty of Science (DUF-2513-c, voucher no). The taxonomic identification of plant material was confirmed by Prof. Dr. A. Selçuk Ertekin.

Preparation of Plant Material. The seed germination and sterilization procedures were carried out as described in our previous study[12]. Following germination, micro-shoots (0.5–1.0 cm length) were separately transferred to Murashige &

Skoog medium supplemented with 0.5 mgl^{-1} N-6-benzylaminopurine (BAP) [13]. Based on the results of our previous experiments, media containing BAP (0.5 mg l-1) were separately supplemented with various auxins (0.25 mgl^{-1} IAA, IBA, NAA) for shoot proliferation [14]. All media were supplemented with 30 gl^{-1} sucrose and solidified with agar (5.464 gl^{-1}, Agar-Agar (Sigma). The pH was set to 5.8 before autoclaving (120ºC for 20 min). The *in vitro* cultures were maintained at 25 ± 2ºC for a 16 h photoperiod (40 µmol m-2 s-1) provided by mercury fluorescent lamps. All experiments were repeated two times

Preparation of extracts. Plantlets were removed from gels by washing them with distilled water. Plant materials were dried at room temperature for 10 days (air dry weight); then, they were powdered and extracted with 1:10 (w/v) methanol for 48 hours on a 125 rpm rotary shaker. The crude extracts were obtained by filtering and a rotary evaporator (BUCHI R3) was used to remove the methanol. All tests were performed in triplicate.

Determination of Total Antioxidant Capacity. The free radical scavenging effects of the metanol extracts were estimated according to the method of Blois (1958) with minor modifications [15]. The DPPH solution was placed in a freshly prepared, aluminum foil-coated bottle each day and stored at + 4 ° C in the dark. 1 ml of each sample prepared at different concentrations (5, 10, 25, 50, 100 and 150 µg/ml), were added to 4 ml of a 100 µM DPPH radical solution. The mixture was agitated and allowed to stand in the dark for 30 minutes at room temperature and then the absorbance was measured at 517 nm with a spectrophotometer. The same procedure was repeated with Ascorbic acid, BHA and BHT as a positive control. All experiments were performed three times. The percentage inhibition activity was calculated by the following equation:

Scavenging effect (%) =[(A_{517} of control − A_{517} of sample/A_{517} of control)] × 100.

where A_{517} *of control* and A_{517} *of sample* are the absorbance values of the control sample and the test sample, at particular times, respectively.

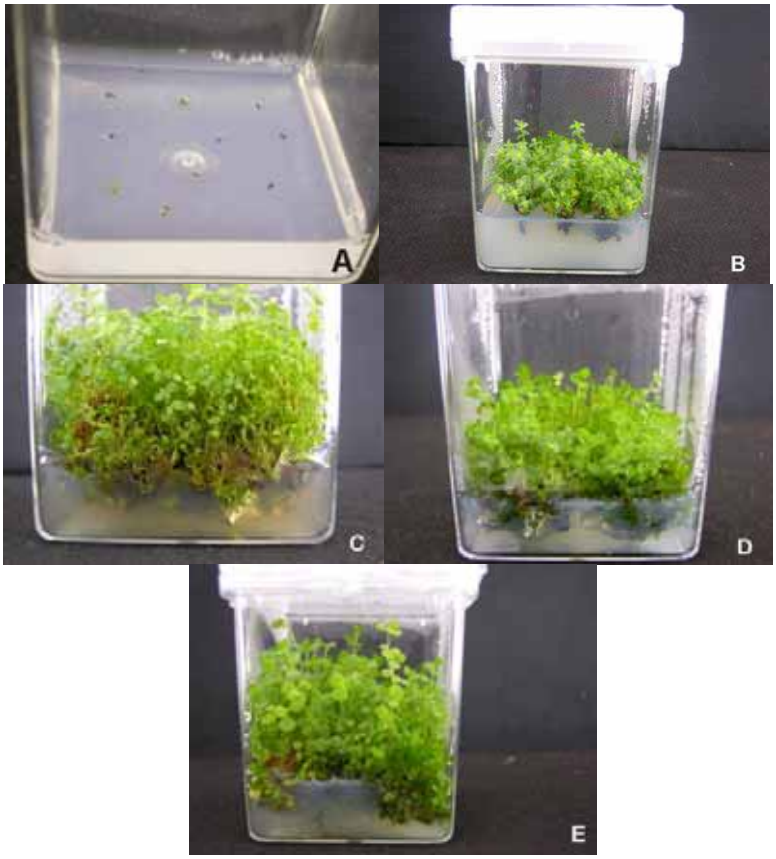


FIGURE 1
Morphological aspects of the effects of different auxins on *Hypericum retusum* Aucher raised under in vitro conditions.
224 x 420 mm (96 x 96 DPI), 1A) Aspect of shoots were germinated from mature seeds of *H. retusum* Aucher, 1B) Development of shoots on MS medium containing 0.5 mgl^{-1} BAP, 1C) Development of shoots on MS medium containing 0.5 mgl^{-1} BAP+0.25 mgl^{-1} NAA, 1D) Development of shoots on MS medium containing 0.5 mgl^{-1} BAP+0.25 mgl^{-1} IAA, 1E) Aspect of shoots on MS medium containing 0.5 mgl-1 BAP+0.25 mgl-1 IBA.

Determination of Total Phenolic Contents. The concentration of total phenolics of methanol extracts were determined by using Folin-Ciocalteu reagent and external calibration with gallic acid [16]. Gallic acid was obtained from Sigma (Steinheim, Germany). About 0.1 ml of extract solution, 4.5 ml of distilled water and 0.1 ml of Folin-Ciocalteu reagent were added and the contents mixed vigorously. After shaking 3 min, 0.3 ml of 2% Na_2CO_3 was added, and finally the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm using UV-VIS spectrophotometer. The concentration of the total phenolics was estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The quantification of phenolic compounds in all the fractions were carried out in triplicate and the results were averaged.

Absorbance= 0.010 gallic acid (µg) + 0.041 (R^2 = 0.991).

RESULTS AND DISCUSSION

In vitro germination of the *H.retusum* seeds were standardized in Murashige and Skoog (MS) hormone-free medium [17]. Cultures were initiated from shoots inoculated onto MS medium supplemented with 0.5 mg l^{-1} BAP (Fig 1A, 1B).

Shoots were cultured on MS medium supplemented with cytokinin and combination of different auxins (0.5 mg l^{-1} BAP + 0.25 mgl^{-1} IAA, IBA, NAA) (Fig 1C,1D,1E). In our previous study, the best results in view of number and length of shoots obtained on MS medium supplemented with 0.25 mgl^{-1} IBA (54.12 shoot/explant, 3.36 length of shoot)+ 0.5 mgl^{-1} BAP [15]. The ascorbic acid (AA) had higher capacity than BHT (butylated hydroxytoluene) and butylated hydroxyanisole (BHA) at all concentrations (5, 10, 25, 50, 100 and 150 µg/ml) of the DPPH solution. Ascorbic acid the highest had compared to BHT and BHA at all concentrations (5, 10, 25, 50, 100 and 150 µg/ml) of the DPPH solution (Fig. 2, 3).

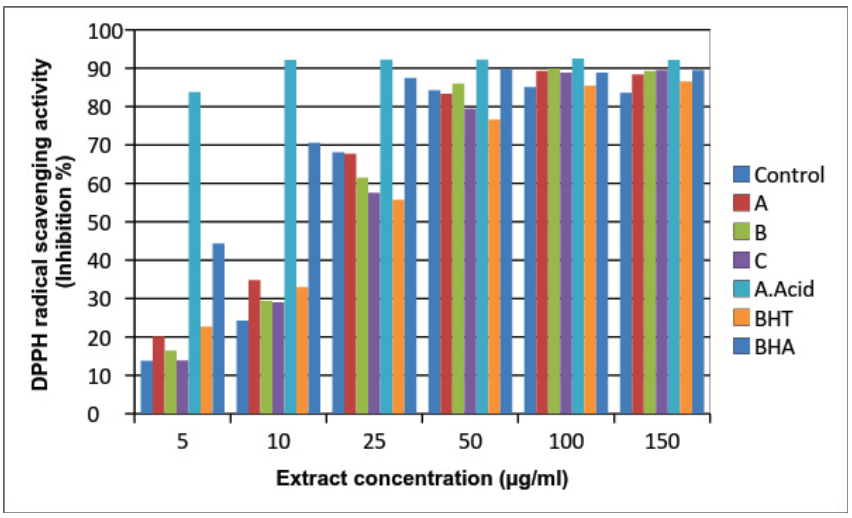


FIGURE 2
The free radical scavenging effects of the methanolic extracts of *H. retusum* standard, AA, BHT, BHA.

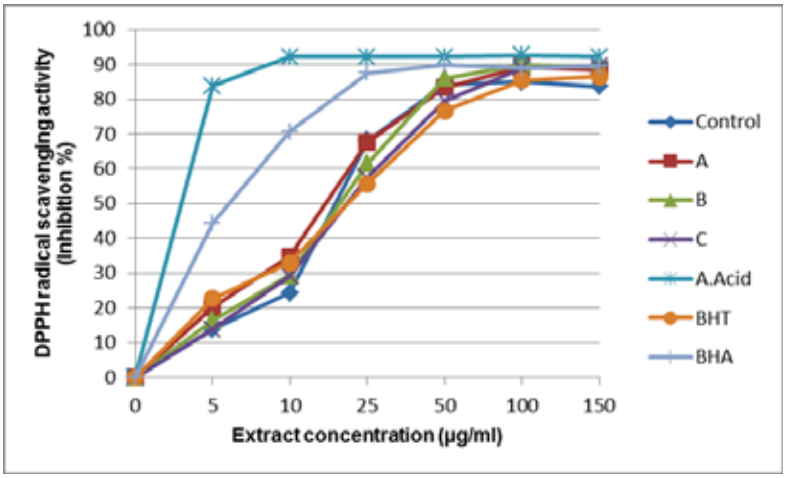


FIGURE 3
Calibration curve of free radical scavenging effects of methanolic extracts of *H. retusum* standard, AA, BHT, BHA.

TABLE 1
The total antioxidant capacity of *Hypericum retusum* Aucher raised under the effects of different auxins in vitro conditions and A.Acid, BHT, BHA

Concentrations	Control	A(NAA)	B(IAA)	C(IBA)	A.Acid	BHT	BHA
5µg/ml	13,81±0,57	20,15±0,07	16,45±1,26	13,85±1,28	83,84±1,69	22,7±1,15	44,39±1,61
10µg/ml	24,25±0,86	34,84±1,69	29,36±1,15	29,05±0,81	92,18±0,07	32,93±0,13	70,63±0,61
25 µg/ml	68,08±1,39	67,73±1,88	61,54±2,87	57,56±2,02	92,23±0,07	55,77±1,47	87,51±0,20
50 µg/ml	84,32±1,75	83,35±0,39	86,01±0,70	79,51±0,70	92,23±0,07	76,62±2,58	89,90±0,65
100 µg/ml	85,08±0,27	89,34±0,61	90,01±0,06	88,85±0,13	92,58±0,65	85,43±1,27	88,90±0,92
150 µg/ml	83,62±0,07	88,41±0,13	89,28±0,24	89,54±0,06	92,18±0,15	86,58±0,92	89,55±0,53

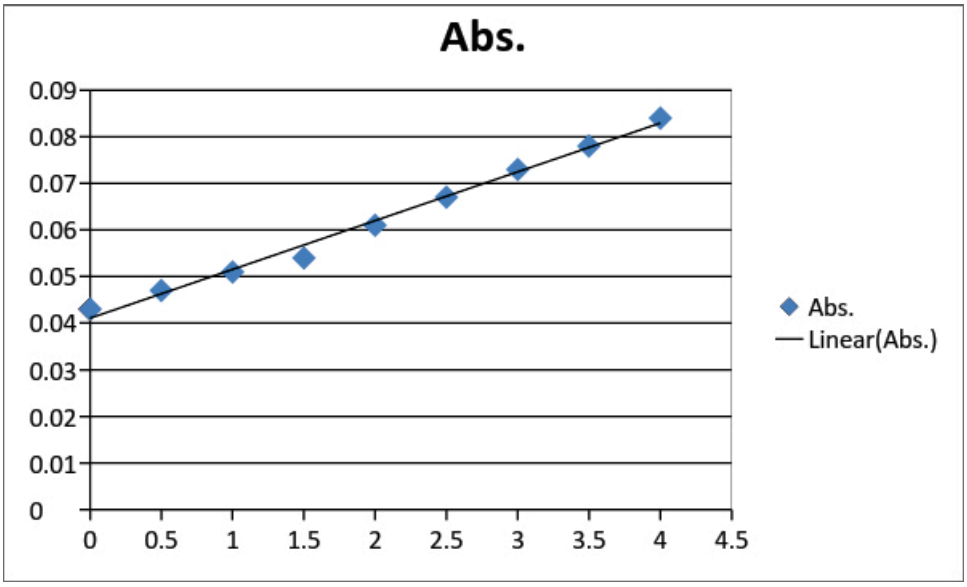


FIGURE 4
Calibration curve of the concentration of total phenolics of methanolic extracts of *H. Retusum*

However, the free radical scavenging ability and phenolic contents of *H. retusum* extract was increased after auxin treatment. The results of the present investigation showed that the free radical scavenging ability and phenolic contents changed, depending on different auxins and concentrations of the DPPH solution. The lowest value of control group and IBA had the antioxidant capacity of at 5 µg/ml of the DPPH solution (13,81±0,57 and 13,85±1,28) respectively. IAA (90,01±0,06) the highest antioxidant capacity had at 100 µg/ml of the DPPH solution (Table 1).

The lowest of phenolic contents was observed in the control group (481,66±0,28 µgGAE/mg ekstre). The best results in view of phenolic contents obtained on MS medium supplemented with 0.5 mgl^{-1} BAP + 0.25 mgl^{-1} IAA (576,66±0,97 µg/mg) as shown in Table 2.

TABLE 2
The total phenolic contents of Hypericum retusum Aucher raised under the effects of different auxins in vitro conditions

Numunes	Phenolic contents (µg GAE/mg ekstre)
Control group	481,66±0,28
A (NAA)	560±1,08
B (IAA)	576,66±0,97
C (IBA)	541,66±0,15

The total amount of phenolic compounds of plant extracts was determined as the microgram of gallic acid equivalent by using an equation obtained from the standard gallic acid graph (Fig 4).

Concentrations of secondary metabolits of the plants may vary substantially with auxins. The highest on the total antioxidant capacity and phenolic contents were obtained on medium containing BAP+IAA when compared with the contents of control plants. The researchers found that both exogenous IBA and NAA stimulated ascorbate peroxidase activity in tomato, but no significant difference was found in plants treated with IAA [16]. Furthermore, there was no significant difference when compared to control in catalase activity of all bioregulator treated plants. Our results were obtained from Tyburski et al. The presence of exogenous auxin in culture suggests an increase in ascorbate levels at the roots of the tomato seedlings [17]. Our results demonstrate the accuracy of the data obtained in experiments on *Caulerpa prolifera* when IAA affects optimal growth stimulation [18]. Our findings correspond to other studies in which IAA significantly reduces *Chlorella pyrenoidosa* cell division at high concentration [19]. The effects of auxin group of hormones on the total antioxidant capacity and phenolic contents have been discussed in several studies. The auxin group of phytohormones have an important effect in different pro-

cesses, such as leaf expansion, cell division, differentiation and elongation [20-22]. Auxin was showed to stimulate antioxidant enzyme activities; It has been shown that the activity of peroxidase is increased by auxine in the distal and proximal parts of the organ, where catalase activity is induced in the root distal region [23]. The aim of this study was to find out the effect of plant bioregulators on the total antioxidant capacity and phenolic contents. IBA and IAA are two naturally founding auxins found in plants. NAA is a synthetic auxin analog. Three compounds; IBA, NAA, and IAA were used in our study to explore the auxin effects on the total antioxidant capacity and phenolic contents of *H.retusum.* The previous studies and based on our results, we propose that auxin may possess an efficient function on the total antioxidant capacity and phenolic contents of *H.retusum.* In many studies, it has been shown that auxins play an important role in plant growth and development [22, 24].

CONCLUSIONS

In this study, predictive models for the content of pharmaceutically important secondary metabolites, namely, antioxidant, phenolic, were developed for *H.retusum* Aucher. The identification of chemical content in phytochemical studies on Hypericum species is known to be an important issue. The results of our study are important for providing information about pharmacological activities associated with *Hypericum retusum*. The results provide useful information on pharmacological activities associated with free radicals of these traditional folk remedies. Further studies are underway to identify in vitro antioxidant capacity and to identify active compounds found in this plant.

ACKNOWLEDGEMENTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Robson, N.K.B. (2001) Studies in the genus Hypericum L. (Guttiferae). 4(1). Sections 7. Roscyna to 9. Hypericum sensu lato (part 1). Bulletin of The Natural History Museum, London (Botany) 31, 37–88.

[2] Stolz, E.D., Viana, A.F., Hasse, D.R., Von Poser, G.L., Do Rego, J.C., Rates, S.M. (2012) Uliginosin B presents antinociceptive effect mediated by dopaminergic and opioid systems in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 39(1), 80-87.

[3] Meinke, M.C., Schanzer, S., Haag, S.F., Casetti, F., Müller, M.L., Wölfle, U., Kleemann, A., Lademann, J., Schempp, C.M. (2012) In vivo photoprotective and anti-inflammatory effect of hyperforin is associated with high antioxidant activity in vitro and ex vivo. European Journal of pharmaceutics and Biopharmaceutics. 81(2), 346-350.

[4] Mortensen, T., Shen, S., Shen, F., Walsh, M.K., Sims, R.C., Miller, C.D. (2012) Investigating the effectiveness of St John's wort herb as an antimicrobial agent against mycobacteria. Phytotherapy Research. 26(9), 1327-1333.

[5] Barış, D., Kızıl, M., Aytekin, Ç., Kızıl, G., Yavuz, M., Çeken, B., Ertekin, A.S. (2011) In vitro antimicrobial and antioxidant activity of ethanol extract of three hypericum and three achillea species from Turkey. International Journal of Food Properties. 14, 339–355.

[6] Radwanski, E.R., Last, R.L. (1995) Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. Plant Cell. 7, 921–934.

[7] Karaogul, E., Kirecci, E., Alma, M.H. (2016) Determination of phenolic compounds from Turkish Kermes Oak (Quercus coccifera L.) roots by high performance liquid chromatography: Its antimicrobial activities. Fresen. Environ. Bull. 25, 2356-2363.

[8] Bayan, Y., Genç, N., Kusek, M., Gul, F., Imeciki Z. (2017) Determination of chemical compositions, antifungal, antibacterial and antioxidant activity of Thymbra spicata L. from Turkey. Fresen. Environ. Bull. 26, 7595-7599.

[9] Emsen, B., Kocabaş, A., Kaya, A., Cinar, S., Aasim, M., Sadi, G. (2017) In vitro cytotoxicity, antibacterial and antioxidant pro-perties of various extracts from Schizophyllum commune. Fresen. Environ. Bull. 26, 1144-1143.

[10] Hasnain, S., Sabri, A.N. (1996) Growth stimulation of Triticum aestvum seedlings under Cr-stress bynonrhizospheric Pseudomonas strains. In: Abstract Book of 7th Int. Symp. On Nitrogen Fixation with Non- legumes. Faisalabad, Pakistan, 36p.

[11] Cano, A., Alcaraz, O., Arnao, M.B. (2003) Free radical-scavenging activity of indolic compounds in aqueous and ethanolic media. Anal Bioanal Chem. 376, 33–37.

[12] Namlı, S., Akbaş, F., Işıkalan, Ç., Ayaz Tilkat, E., Başaran, D. (2010) The effect of different plant hormones (PGRs) on multiple shoots of Hypericum retusum Aucher. Plant omics journal. 3(1), 12- 17.

[13] Murashige, T., Skoog, F.A. (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15, 473–479.

[14] Blois, M.S. (1958) Antioxidant determination by the use of stable free radicals. Nature. 181, 1199-2000.

[15] Slinkard, K., Singleton, V.L. (1977) Total phenol analyses: Automation and comparison with manual methods, Am. J. Enol. Viticult. 28, 49-55.
[16] El-Gaied, L.F., El-Heba, A.A., El-Sherif, A.N. (2013) Effect of growth hormones on some antioxidant parameters and gene expression in tomato. GM Crops and Food, 4(1), 67-73.
[17] Tyburski, J., Krzemin´ski Ł., Tretyn, A. (2008) Exogenous auxin affects ascorbate metabolism in roots of tomato seedlings. Plant Growth Regul. 54, 203–215.
[18] Jacobs, W.P. (1993) A search for some angiosperm hormones and their metabolites in Caulerpa paspaloides (Chlorophyta). Journal of Phycology. 29, 595–600.
[19] Vance, B.D. (1987) Phytohormone effects on cell division in Chlorella pyrenoidosa Chick (TX-7-11-05) (Chlorellaceae). J Plant Growth Regul. 5, 169–173.
[20] Estelle, M. (1992) The plant hormone auxin: insight in sight. Bioessays. 14, 439-444.
[21] Aydın, A.A., Zerbes, V., Parlar, H., Letzel T. (2013) The medical plant butterbur (Petaistes): Analytical and physiological review. Journal of Pharmaceutical and Biomedical Analysis. 75, 220-229.
[22] Goldsmith, M.H.M. (1993) Cellular signaling: new insights into the action of the plant growth hormone auxin. Proc Natl Acad Sci U.S.A. 90, 11442-5.
[23] Tyburski, J., Dunajska, K., Mazurek, P., Piotrowska, B., Tretyn, A. (2009) Exogenous auxin regulates H_2O_2 metabolism in roots of tomato (Lycopersicon esculentum Mill.) seedlings affecting the expression and activity of CuZn-superoxide dismutase, catalase, and peroxidase. Acta Physiol Plant. 31, 249-60.
[24] Cooke, T.J., Poli, D.B., Sztein, A.E., Cohen, J.D. (2002) Evolutionary patterns in auxin action. Plant Mol Biol. 49, 319–338.

Received: **20.03.2018**
Accepted: **14.09.2018**

CORRESPONDING AUTHOR

Yesim Kara
Department of Biology,
Faculty of Science and Art,
University of Pamukkale,
Denizli – Turkey

e-mail: eylul@pau.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9617-9625 Fresenius Environmental Bulletin

VISUAL LANDSCAPE EVALUATION OF KASTAMONU CLOCK TOWER ENVIRONMENT AS A HISTORICAL URBAN AREA

Sevgi Ozturk, Elif Ayan, Oznur Isinkaralar*

Kastamonu University, Faculty of Engineering and Architecture, Department of Landscape Architecture

ABSTRACT

Historic environments remain in rapid and irregular urbanization of in today's cities and cannot adapt to urbanization process. Historical fabrics are damaged in time and they may even become neglected areas. Planning and design are attempted by taking into account the protection and utilization balance in these areas that are important for the image and cultural heritage of the city., Visual quality, which historical sites offer to the urban and urban dwellers has started to be prioritized when looking at the increasingly modernizing city. In this context, it is aimed to evaluate the visual quality in the historical area. The study is carried out around the Clock Tower, which is situated on the slope of the Sarayüstü hill at the center of the city of Kastamonu and inholds important landmark and nodal points of the city.

The study area is examined by dividing into 3 zones depending on the usage of the area and the buildings in its borders. The visual quality evaluation in the study was done using photo questionnaire method based on the Legibility, Consistency, Complexity, Temporality/Continuity, Definability, Ownership/Belonging, Historicity, Naturality, Visual Scale and Sense of Space criteria by experts and users with a total of 100 points. As a result of expert and user group e valuation, it is found that the zone with housing areas has lower visual quality value, whereas the visual quality value in the zones with historical buildings were located is found higher.

KEYWORDS:
Visual Landscape Quality, Historical Environment, Clock Tower, Kastamonu-Turkey

INTRODUCTION

Historical areas that remain under the pressure of dense housing today are considered as an important part of the urban image. However, spatial perception of historical urban areas gradually deteriorates and their visual quality is affected adversely as a result of unplanned housing. Evaluation of visual quality in historical areas is to define whether a scenery is aesthetically appropriate, and identify certain factors and physical landscape components. In various studies, visual quality is defined as the product of certain (visible) properties of landscape that interacts with relevant psychological (perceptional, cognitive and emotional) processes of human observer [1] and it is mentioned that visual elements do not only reflect aesthetical values but also mutual relations among cultural, economic and biological values have a balance [2,3]. In the evaluation of visual quality, there are two main approaches used, namely the objectivity based on a physical paradigm and subjectivity based on a psychological paradigm [4,1,5]. The objective approach considers aesthetic quality as a feature inherent to a scenery. On the other hand, subjective approach assumes that aesthetic quality is a subjective value derived from the eyes of viewer [4,6]. In aesthetical context, the environment is generally evaluated considering the criteria of form, proportion, rhythm, scale, complexity, color, light, shading, order, hierarchy, spatial relationships, conflict, ambiguity, confusion and innovation [7,8,9,10]. Visual quality of an environment is a perceptual and objective concept that involves environmental/ecological, socio-cultural and psychological factors. What matters in the concept of visual quality is visual criteria (form, line, color, vitality, harmony, unity etc.) and organization, positioning, ratios and especially physical structures and associations of these visual criteria are the main elements that constitute visual quality [11,12]. When studying the concept of visual quality in historical areas, the concept of preservation should be prioritized and criteria should be established and scaled based on characteristics of the space and evaluations should be made separately [13].

This study aims to present visual landscape quality of Clock Tower and its surroundings involving historically rich civil architectural examples, covering popular spots of visitors but exposed to dense housing in recent times in Kastamonu, and to develop recommendations on visual quality with respect to decisions to be taken about the space.

STUDY AREA

The study area is the Clock Tower and its periphery located in the east of Karaçomak Stream in the urban area of Kastamonu in the Western Black Sea Region of Turkey (Figure 1). Kastamonu has limited settlement areas due to its physical structure. The first settlements positioned in line with the topography in the east and west of Karaçomak Stream that passes through the center of city.

It was found that the Clock Tower and its surroundings have different functional uses (Figure 2). For this reason, the study was conducted in terms of historical layers and urban functions, and visual quality evaluation was performed in three different zones, namely landmark (Zone1), residential district (Zone2) and square (Zone3) (Figure 3).

Historical ground of the area selected as sample in the study dates back to the Principality of Jandar. Architectural settlement in the area can be examined in three layers, especially in the scale of monumental structures. Zone1 is within the borders of historic urban area and has two registered buildings. The Clock Tower located in Zone1 is in the second architectural layer of the area as it was built in 1885 [14,15]. The landscaping was carried out in 2000 (Figure 4). The Sarayüstü hill situated in the silhouette of city just behind the area is one of the important natural areas of the city. This natural hill provides a clearer view of the Clock Tower, which is an important landmark of the city.

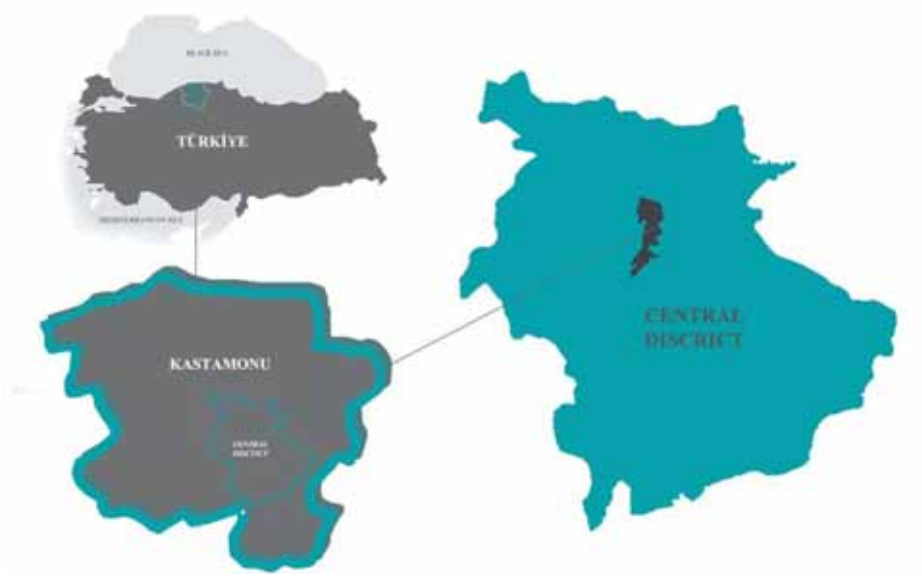


FIGURE 1
Location of the study site

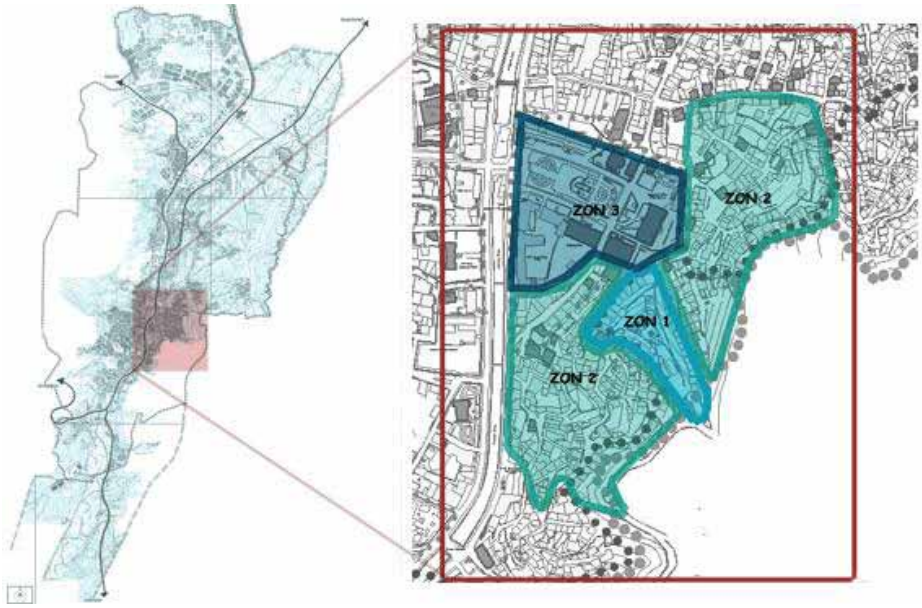


FIGURE 2
Zones of the study site

FIGURE 3A
Views from the study site (Zone 1)

FIGURE 3B
Views from the study site (Zone 2)

FIGURE 3C
Views from the study site (Zone 3)

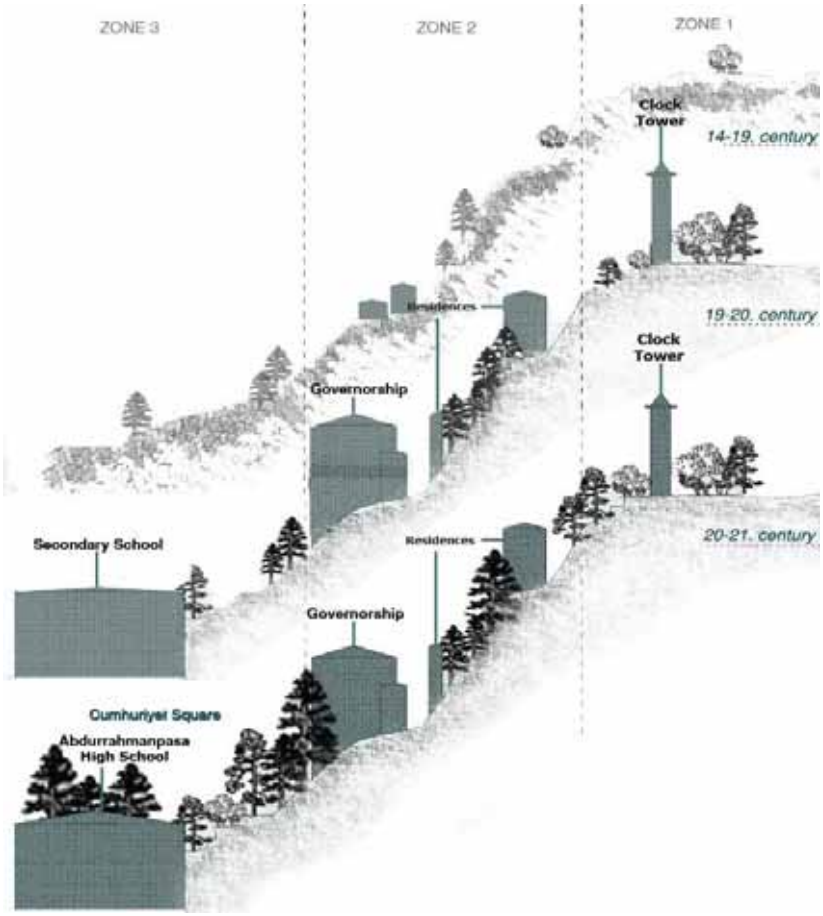


FIGURE 4
Study Area Historical Layers

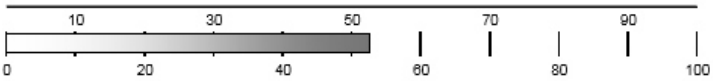


FIGURE 5
Visual Landscape Quality Score Scale

METHOD

In the study, necessary literature review was made and data related to the area were collected in line with the purpose and scope of study. Based on these data, the indicators used to evaluate visual quality of the study area were established. When defining these indicators, especially the studies [6,17,18,19,20] were used and 10 criteria, namely Legibility, Consistency, Complexity, Temporality/Continuity, Definability, Ownership/Belonging, Historicity, Naturality, Visual Scale and Sense of Space, and their 24 sub-criteria were defined. In order to reveal the degree of importance of these criteria in terms of visual quality, an expert group consisting of a landscape architect, an urban designer, a city and region planner, a photographer, a sociologist and a forest engineer was asked to grade them from 1 to 5 by using the multi-criteria evaluation method. Arithmetic mean of these scores was taken and indicator multipliers of the criteria were defined. An evaluation table with a total of 100 points was prepared on the basis of the indicator multiplier created and the number of indicators in each concept. Then, the photo-questionnaire method [21] was used by selecting 9 photographs out of 320 that were taken in the semi-sunny times of area during the spring. The questionnaire was applied to a total of 275 people, which consisted of 250 users and 25 experts. Expert and user group completed the evaluation in the survey by making positive (+) and negative (-) markings based on each parameter for 3 different zones. Calculations at the end of the evaluation were multiplied by the weight score to find the value of that criterion if the value marked by user and expert groups was (+). Arithmetic mean of the sum of criterion value marked by participant groups for each criterion was taken and visual landscape quality score was obtained accordingly (Figure 5).

Problems and deficiencies in the whole area and specific to the zones were identified based on all data obtained as a result of the questionnaire. In this context, urban design recommendations were made with a holistic approach within the theory of environmental and urban aesthetics with respect to visual quality of this space that is situated at an important strategic point of the city.

RESULTS

In the findings of Zone1, Zone2 and Zone3; Zone1 got 61 points from the expert group and 58 points from the user group. According to the score scale given in Figure 3, the area was evaluated as high visual quality by the expert group and moderate visual quality by the user group. In terms of total scores, Zone1, which includes the Republic Square and its vicinity, had a score in visual quality lower than Zone3 but higher than Zone2.

Visual quality of Zone2, which got the lowest points from both user and expert groups in the evaluation, was evaluated as moderate with 51 points from the expert group and low level with 46 points from the user group according to the Figure 3. In the study area, Zone2 has a low visual quality. Zone3 received 81 points out of 100 in the evaluation of both user and expert groups and was found to have very high visual quality. In all zones, both groups seem to support each other in terms of their responses to the criteria.

When the criteria were evaluated by the expert group it was found that for Zone1, 15 sub-criteria received positive opinions, the main criteria of consistency, temporality/continuity, ownership/ belonging and historicity were positive with all sub-criteria, but the main criteria of legibility, naturality and sense of space were negative with all sub-criteria.

For Zone2, 12 out of 24 sub-criteria received positive responses. From among these, all sub-criteria in the main criteria of temporality/continuity and historicity were positive, while all sub-criteria of legibility, definability, naturality and sense of space received negative responses.

It was found that for Zone3, 20 sub-criteria received positive opinions; main criteria of legibility, temporality/continuity, ownership/belonging and historicity, visual sale and sense of space were positive with all sub-criteria, and only the naturality criterion received negative response.

There are no significant differences between responses given by those who live and know the area and those involved in relevant professional groups. In general, it was found for Zone3 that there is no harmony between the spaces, diversity of inanimate materials is insufficient, watching points at the Clock Tower are not clearly perceived, and presence of natural flora is not preserved enough. On the other hand, it is believed that existing flora shows seasonal diversity depending on climatic change, and presence of historical housing fabrics, historical elements and layers continue to exist in the area.

TABLE 1
The results of visual landscape criteria and sub criteria regarding the groups of users and experts

Concept	Indicators	Users' Opinion						Experts' Opinion					
		Value			Score			Value			Score		
		Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3
Consistency Factor: 3	Harmony	-	-	-	6	6	6	+	-	-	9	3	6
	Balance (balance on scale, dimensional balance)	+	+	+				+	+	+			
	Color (harmony of colors, repetition of colors and harmony of shades)	+	+	+				+	-	+			
Readability Factor: 5	Perceptibility of location	-	-	+	0	0	15	-	-	+	0	0	15
	Clear directions	-	-	+				-	-	+			
	Road hierarchy	-	-	+				-	-	+			
Complexity Factor: 5	Presence of different objects	+	-	+	10	5	10	+	+	+	10	10	10
	Variety of inanimate materials	-	-	-				-	-	-			
	Variety of animate materials	+	+	+				+	+	+			
Temporariness/Permanence Factor: 4	Use of vegetation that allows transition through seasonal changes	+	+	+	4	4	4	+	+	+	4	4	4
	Climatic change	+	+	+				+	+	+			
Describability Factor: 3	Presence of vantage points	-	-	-	3	0	3	-	-	-	3	0	3
	Landmarks	+	-	+				+	-	+			
Sense of Protection/Belonging Factor: 4	Sense of Care (Absence of desolate areas)	+	+	+	12	8	12	+	+	+	12	8	12
	Well maintained buildings and linear objects (walls-roads-fences)	+	-	+				+	-	+			
	Absence of infrastructural issues	+	+	+				+	+	+			
Historicity Factor: 5	Sense of historical buildings	+	+	+	15	15	15	+	+	+	15	15	15
	Presence and variety of linear historical elements (fountains, roads, monuments)	+	+	+				+	+	+			
	Visibility of historical timelines	+	+	+				+	+	+			
Naturalness Factor: 5	Presence and use of natural vegetation as a landscape element	-	-	-	0	0	0	-	-	-	0	0	0
Visual Scale Factor: 4	Sufficiency of openness and enclosure within the location	+	+	+	8	8	12	+	+	+	8	8	12
	Unobstructed view	+	+	+				+	+	+			
	Sense of depth and spaciousness	-	-	+				-	-	+			
Sense of Location Factor: 4	Perception of location's identity	-	-	+	0	0	4	-	-	+	0	0	4
TOTAL					58	46	81				61	51	81

DISCUSSION

The study attempted to find answers on how to design an environment or space and which design criteria should be used when designing. To that end, a visual quality evaluation was made and it was found that Zone3 has a visually good quality with a rate of 81%, while Zone2 has the lowest visual quality. Since historical structures reflect traditional architecture in visual quality and constitute important criteria for urban identity, they receive high points both from experts and users. In the study in Kemaliye district [3], urban sceneries with traditional architecture received high visual quality score. It was observed that visual quality decreases in areas with high human intervention [22], but areas with historical structures that have been preserved until today are an important element in terms of visual quality. In a visual quality study conducted on coastline of Trabzon, [23] found that presence of historical structures has an important value in visual quality of the area and the area shows a consistency. Also, in the study conducted by [24], historical fabric received more positive results in terms of visual quality in the evaluation of gardens in different areas of use in Bartın based on new and old fabric. In our study, it was found that since there are historical structures around the Clock Tower and Republic Square, the historicity value received positive responses from both user and expert groups.

One of the important findings of the study is that although the area is situated on a natural hill and has presence of vegetation, naturality indicator of the entire area received zero point from user and expert groups. Unplanned housing and insufficiency of green areas caused this indicator of area to get zero point. In the improvements and preservation efforts, decisions should be taken with the awareness that naturality is an important indicator of visual quality [25,26,27,28]. In the study [29], found that areas that have been subject to human intervention have very low visual quality value and presence of vegetation has a positive impact on quality [30,31,32]. In the study conducted by Cengiz et al. [33] on rural road routes, they found that preferences and liking of people differ by closure and openness of vegetation. In naturality perception of the area, intensity of vegetation is an important criterion. Unplanned housing, lack of green areas, neglect and lack of fittings especially in Zone 2 cause the area to have a low visual quality. Differences in texture, color and height in the housing decrease the visual quality of area. Legibility is examined in various studies as it creates clarity and balance in a space and embodies certain landmarks [34,35]. From this perspective, improvement efforts that have been performed at the Republic Square are clearly perceived by participants due to location of area and various landmarks it embodies, while legibility of other zones is low.

In terms of total points received by different zones in the area, Zone3 stands out as an area with "very high visual landscape quality" with 81 points. In the study conducted by Öztürk et al. [36] with a similar method, Taşköprü district, which is known with its historical nature, got 75 points, falling behind the Republic Square despite the fact that it is an area defined as "with high landscape quality". Zon1 was found as an area with high quality by the expert group with 61 points and moderate quality by the user group with 58 points. Zon2 was rated as having a moderate level of landscape quality with 46 points from users and 48 points from experts. At this point, the results of expert and user opinions are close to each other.

CONCLUSION

Land prices are very expensive because of rapid urbanization and crowded population. There are lots of historic garden and green areas which are under the pressure of urbanization. These historic gardens and open spaces are very sensitive to use so that the need of the protection is a vital key point in the urban planning decisions [37]. Accordingly, it is very important to evaluate visual quality of today's cities, which are increasingly growing vertically and horizontally.

In city center of Kastamonu, there are many architectural examples that still exist today, especially the structures that were built for commercial and residential purposes since the Principality of Jandar. The Clock Tower and its vicinity, which constitute the subject of this study, has many historical structures and hosts today's civil architectural examples as well. Different floor heights and parceling forms in the area cause diversity. On the other hand, the fact that the city is in a process of fast housing and transformation made this study essential to preserve historical environments and study their visual perception.

When an environment or space is evaluated in a better, more beautiful, more aesthetic and holistic way, it is required to identify insufficient aspects in order to make sure that the visual quality, which is the common point, is higher. In the study, the data were obtained in relation to the design principles that can be used in restoration and rehabilitation activities at historical urban spaces in Kastamonu.

In this context, although the Clock Tower is a space with a historical value and is preferred by tourists in Zone1, it lacks direction signs for vehicle and pedestrian access. This area lacks in terms of presence of vegetation and falls short in terms of perceptibility. In landscaping activities, use of vegetation and fittings that reflect characteristics of the city should be preferred. For example, paving stone used in the pavement does not reflect the

traditional material of city. The children's playground is both inadequate and disharmonious with the area in terms of color and material details. Plastic materials are used in the seats of tea gardens in the area. The vegetation choice and similar fittings have negative impact on harmony of spaces and naturality criteria of the space. On the other hand, preferring plant species that reflect historical spaces of the city, such as *Tilia sp., Salix sp., Acer sp., Platanus orientallis, Buxus sempervirens*, will make spatial perception of the Clock Tower and its vicinity clearer. Also, use of granite and wood in pavements and fittings will be more suitable for Kastamonu city, which has a forested area of 80%.

The fact that Zone2 is located right next to the city's focal point, the Republic Square, and its landmark, the Clock Tower, and residential areas with high land prices but low building quality and transformation into desolated areas caused to have the lowest visual landscape quality value. There is not road hierarchy with open direction in the streets where organic transport links exist and there is no sense of building and parceling relations. Components of buildings such as external balconies, windows and doors have surveying differences and there are non-restored low quality registered buildings. The street is not perceived in a holistic way in terms of pavements, walls, signs and other fixtures and all street components are non-maintained. The area which is situated on a very rugged terrain lacks the sense of depth and comfort. Historical structures are mainly wooden structures built on stone foundations and there are today's reinforced concrete structures as well. Different housing typologies, building heights and deteriorations in historical street details as well as lack of maintenance result in poor visual quality.

Street improvement works should be carried out in these residential areas, which are located an access point to a touristic watching point. A restoration activity should be carried out to restore former identity of Kuleli Street, where non-Muslims (Armenians) lived once and which provides connection to the Clock Tower and was made of uniquely harmonious colors. The architectural design on second and third floors of building is quite convenient for using ceiling beams. Ruined houses and idle parcels should be restored for re-use and external parts of structures can be used as design components to improve the visual quality. In street design, children's playgrounds, sitting/recreation areas should be planned spatially in order to revive the street culture which is the basis of traditional social relations. That plan that is developed to make these spaces more perceivable and legible should be supported with a vegetation design. As a result, it will serve as a buffer between crowded housing structures, clean the air and act as shadow elements between the spaces. When selecting the materials of urban fixtures (garbage cans, sitting benches, signs, walls, fences, etc.), characteristics of the city should be taken into consideration. Materials (granite, cobblestone, wood, etc.) that are more compatible with the Republic Square located right in the west should be preferred. Existing registered buildings in the area should be restored, and existing commercial areas and religious and social facilities should be maintained.

The Zone 3, which includes architectural structures from the Republican Period, the Recent Era and the Seljukians, received negative responses in 4 sub-criteria. The area, which is completely surrounded by historical buildings, is considered inadequate in terms of natural vegetation elements. Landscape elements specially designed for historical spaces (living and non-living) are even more important in this zone. Bus stops that are located right next to the zone cause a complexity in the area and have adverse impact on its visual quality.

In conclusion historical spaces are important in urban planning considering that urban heritage is a historical layer of values created by successive and living cultures and the accumulation of socially accepted traditions and experiences. However, such rapid and often uncontrolled housing can cause the disintegration and destruction of urban heritage. In this context, it is necessary for the study area, which hosted many civilizations, to be evaluated in a way to reflect the identity of city with sustainable planning approaches. On the other hand, there is an ongoing work for preparation of urban archaeological databases and it is very important to evaluate and protect the spaces with historical fabric until such databases are prepared.

The study, which was conducted to evaluate visual landscape quality, is expected to contribute greatly to better designing of Zone1, Zone2 and Zone3. Also planning and future researches where people's satisfaction and quality of life is considered at a high level and to reveal the impact of visual landscape quality on the value of space as well as the identity of city, as it presents the evaluation process which is the preliminary stage of design.

REFERENCES

[1] Daniel, T.C. (2001). Whither scenic beauty? Visual landscape quality assessment in the 21st century. Landscape and urban planning. 54(1-4), 267-281.

[2] Angileri, V., & Toccolini, A. (1993). The assessment of visual quality as a tool for the conservation of rural landscape diversity. Landscape and Urban Planning. 24(1-4), 105-112.

[3] Bulut, Z., & Yilmaz, H. (2008). Determination of landscape beauties through visual quality assessment method: a case study for Kemaliye (Erzincan/Turkey). Environmental Monitoring and Assessment. 141(1-3), 121-129.
[4] Lothian, A. (1999). Landscape and the philosophy of aesthetics: is landscape quality inherent in the landscape or in the eye of the beholder?. Landscape and urban planning. 44(4), 177-198.
[5] Fuante de Val, G.D.L.F., Atauri, J.A., & de Lucio, J.V. (2006). Relationship between landscape visual attributes and spatial pattern indices: a test study in Mediterranean-climate landscapes. Landscape and urban planning. 77(4), 393-407.
[6] Tveit, M.S. (2009). Indicators of visual scale as predictors of landscape preference; a comparison between groups. Journal of Environmental Management. 90(9), 2882-2888.
[7] Wohlwill, J.F. (1976). Environmental aesthetics: The environment as a source of affect. In Human behavior and environment Springer, Boston, MA. 37-86.
[8] Lang, J. (1988). Symbolic aesthetics in architecture: toward a research agenda. Environmental aesthetics: Theory, research, and application. 45-55.
[9] Groat, L.N., & Després, C. (1991). The significance of architectural theory for environmental design research. In Advances in environment, behavior, and design Springer, Boston. MA. 3-52.
[10] Swanwick, C. (2002). Landscape Character Assessment: Guidance for England and Scotland: Prepared for the Countryside Agency and Scottish Natural Heritage by Carys Swanwick. Countryside Agency.
[11] Litton, R.B. (1968). Forest landscape description and inventories-a basis for land planning and design. Res. Paper PSW-RP-049. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture. 88-49.
[12] Daniel, T.C., & Vining, J. (1983). Methodological issues in the assessment of landscape quality. In Behavior and the natural environment. Springer, Boston, MA., 39-84.
[13] Cohen, N. (2001). Urban planning, conservation, and preservation (Vol. 1). McGraw Hill Professional.
[14] Eyüpgiller, K. (1999). A City History: Kastamonu, Eren Publishing. (in Turkish)
[15] Karasalihoğlu. M. (2005). Innovation In The Architecture Of The City Of Kastamonu At The Beginning Of The 19th Century And The Beginning Of The 20th Century, Social History (140), 76-81. (in Turkish)
[16] Tveit, M., Ode, Å., & Fry, G. (2006). Key concepts in a framework for analysing visual landscape character. Landscape research, 31(3), 229-255.
[17] Ode, Å., Tveit, M.S., & Fry, G. (2008). Capturing landscape visual character using indicators: touching base with landscape aesthetic theory. Landscape research. 33(1), 89-117.
[18] Tüfekçioğlu K.H. (2008). Historical Landscape Quality Assessment In Historical Environment Istanbul Yedikule Example. Istanbul Technical University, Institute Of Science And Technology, Master Thesis, İstanbul, 136 s. (in Turkish).
[19] Fry, G., Tveit, M.S., Ode, Å., & Velarde, M.D. (2009). The ecology of visual landscapes: Exploring the conceptual common ground of visual and ecological landscape indicators. Ecological indicators. 9(5), 933-947.
[20] Öztürk, S. and Ayan E. (2016). The Determination of Visual Quality Assessment In Landscape In Urban Environment: "A Case Study of Kastamonu Castle Environment". International Forestry Symposium.
[21] Düzgüneş, E., & Demirel, Ö. (2015). Evaluation of rural areas in terms of landscape quality: Salacik Village (Trabzon/Turkey) example. Environmental monitoring and assessment. 187(6), 310.
[22] Alpak, E.M., Özkan, D.G., Mumcu, S., & Özbilen, A. (2016). Complexity, Historicity and Coherence: Preference and Quality of the Changes in the Urban Scene. The Anthropologist. 24(3), 762-777.
[23] Cengiz, C., Bekci, B., & Cengİz, B. (2014). A comparative study of public green spaces in the changing urban texture in terms of preferences for ornamental plants and visual quality: the case of Bartin (Turkey). Fresen. Environ. Bull. 23, 2326-2342.
[24] Kaplan, S., Kaplan, R., & Wendt, J.S. (1972). Rated preference and complexity for natural and urban visual material. Perception & Psychophysics. 12(4), 354-356.
[25] Van den Berg, A.E., Vlek, C.A., & Coeterier, J.F. (1998). Group differences in the aesthetic evaluation of nature development plans: a multilevel approach. Journal of environmental psychology. 18(2), 141-157.
[26] Simonič, T. (2006). Urban landscape as a restorative environment: preferences and design considerations. Acta Agricolturae Slovenica. 87, 325-332.
[27] Bernasconi, C., Strager, M.P., Maskey, V., & Hasenmyer, M. (2009). Assessing public preferences for design and environmental attributes of an urban automated transportation system. Landscape and Urban Planning. 90(3-4), 155-167.

[28] Polat, A.T., Güngör, S., & Adıyaman, S. (2012). The Relationship Between Visual Quality of Urban Recreation Areas and Demographic Characteristics of Users in the Surrounding Area of Konya City. KSÜ Journal of Natural Sciences Special Issue. 70-79.
[29] Arriaza, M., Cañas-Ortega, J.F., Cañas-Madueño, J.A., & Ruiz-Aviles, P. (2004). Assessing the visual quality of rural landscapes. Landscape and urban planning. 69(1), 115-125.
[30] Ulrich R.S. (1986). Human responses to vegetation and landscapes Landscape and Urban Planning. 13, 29-44.
[31] Tzolova, G.V. (1995). An experiment in greenway analysis and assessment: the Danube River. Landscape and Urban Planning. 33(1-3), 283-294.
[32] Misgav, A. (2000). Visual preference of the public for vegetation groups in Israel. Landscape and Urban Planning. 48(3-4), 143-159.
[33] Cengiz, B., Cengiz, C., & Bekci, B. (2016). Assessıng The Vısual Qualıty Of Rural Hıghway Landscape Reclamatıon In The Western Black Sea Regıon Of Turkey. Fresen. Environ. Bull. 25, 933-952.
[34] Kaplan, S. (1987). Aesthetics, affect, and cognition: Environmental preference from an evolutionary perspective. Environment and behavior. 19(1), 3-32.
[35] Brown, T. (1994). Conceptualizing smoothness and density as landscape elements in visual resource management.
[36] Öztürk S., Bulan Ö., & Kravkaz Kuşçu İ.S. (2017). Visual Landscape Quality Evaluation in Urban Environment: "Taşköprü Case", Taşköprü Symposium.
[37] Altıncekıc, H.S.C., Erdogan, R., Dırık, H., & Oktay, E. (2016). Sustainable Management and Protection of Historical Landscape: The Case of Bakırköy Mental and Psychiatric Diseases Hospital Garden, Istanbul-Turkey. Environmental Sustainability and Landscape Management. 81.

Received: **21.03.2018**
Accepted: **21.10.2018**

CORRESPONDING AUTHOR

Sevgi Ozturk
Kastamonu University,
Engineering and Architecture Faculty,
Kastamonu/ Türkiye,

e-mail: sevgiozturk37@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9626-9633 Fresenius Environmental Bulletin

BIOLOGICAL CONTROL OF *RHIZOCTONIA* ROOT ROT OF WHEAT WITH BINUCLEATE *RHIZOCTONIA* FUNGI

Filiz Unal[1,*], F Sara Dolar[2]

[1]Central Plant Protection Research Institute, Ankara, Turkey
[2]Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, Turkey

ABSTRACT

The current study was conducted to identify the effect of six non-pathogen binucleate *Rhizoctonia* (BNR) anastomosis groups (AG A, AG C, AG E, AG G, AG H and AG I) as a biological control agent against *Rhizoctonia* spp. causing root rot and stunting in Central Anatolian wheat fields in Turkey. The pathogen isolates represented anastomosis groups *R. solani* AG 4 HG II, AG 5, AG 8, *R. cerealis* AG D I, *Waitea circinata* var. *circinata, Waitea circinata* var. *zeae.* All six BNR isolates, when combined with AG 4, AG 8, *R. cerealis* AG D, *W. cir*. var. *circinata* and *W. cir.var zeae,* saw a greater reduction in disease severity when compared to the effect of the isolates alone. It was also detected that three of the six BNR isolates (AG H, AG E and AG C) significantly suppressed disease on wheat caused by *R. solani* AG 5 in greenhouse conditions and *Rhizoctonia* AG H provided a high degree of protection against all pathogen groups. These BNR isolates may have potential use in the management of the pathogen *Rhizoctonia* anastomosis groups on wheat, but will require rigorous testing under field conditions.

KEYWORDS:
Biological control, binucleate, *Rhizoctonia* anastomosis groups, wheat

INTRODUCTION

Rhizoctonia is an agriculturally important fungal genus which contains diverse species that are found wordwide. It is a species complex composed of several pathogenic and non-pathogenic anastomosis groups (AGs). To date, 14 AGs of *Rhizoctonia solani* (AGs 1-13 and BI) have been identified on the basis of hyphal anastomosis interactions, cultural and morphological characters, host range, pathogenicity, biochemical reactions and molecular studying in *R. solani. R. zeae* and *R. oryzae* were assigned to *Waitea* anastomosis groups WAG-Z and WAG-O, respectively [1, 2]. Up until now, Binucleate *Rhizoctonia* (BNR) (teleomorph: *Ceratobasidium* sp.) isolates were grouped into 18 AGs (AG A-W) [3, 4, 5].

Soilborne fungi of the genus *Rhizoctonia* are known to be important pathogens in numerous field crops causing damping off, foliar blight, and root and stem rot. In Turkey, wheat is the largest cereal crop, and *Rhizoctonia* is one of the main causal agents of wheat root rot. Wheat is susceptible to infection by several anastomosis groups (AGs) and subgroups of *Rhizoctonia. R. solani* AG 1 IB, 2-1, 2-2, 3, 4, 5, 6, 8, 11, *Waitea circinata* var *circinata, Waitea circinata* var *zeae, Waitea circinata* var *oryzae, Rhizoctonia cerealis* AG D were determined as pathogen on wheat [1, 6, 7, 8]. *Rhizoctonia solani* AG 2-1, 3, 4, 5, 8, AG 11 and *Waitea circinata* var *circinata* and *W. c.* var *zeae* have been reported previously to be wheat pathogens in Turkey [9, 10, 11].

Currently, *Rhizoctonia* disease is managed by cultural practices, such as crop rotation and methods that minimize prolonged contact of the plant with the pathogen, and the use of chemicals. However, current cultural and chemical controls are not completely effective, and *Rhizoctonia* disease remains a persistent problem. Control of these pathogens is rather difficult due to their ecological behavior, extremely broad host range and high survival rate of resistant forms such as sclerotia under different environmental conditions [12]. The use of chemical plant protection results in the contamination of soil and increases pathogen resistance to commonly-used fungicides. These chemical effects are beneficial to microorganisms as well, and destroy the biological balance in soil. It has become necessary to develop alternative management methods-especially against soil pathogens-since excessive fungicide applications damage the ecosystem over time. Biological control is an alternative and environmentally-safe method of plant protection. Several genera of fungi, including binucleate *Rhizoctonia* [13,14], *Trichoderma* [15,16], *Gliocladium* [16] and *Cladorrhinum* [17] can control the development of pathogen isolates of *R.solani*. Binucleate *Rhizoctonia* spp. controlled *Rhizoctonia* diseases on creeping bentgrass [18], bean [13], potato [19], bedding plants [20], sugarbeet [21], cotton [22], cabbage [23], cucumber [24]. Also, BNR has been effective in controlling *Pythium* and *Alternaria* diseases [22, 25].

TABLE 1
Anastomosis groups, geographic origins, sources of isolation and accesion numbers of pathogen *Rhizoctonia* isolates used in this study

Isolate Number	Origin (Province)	Isolation Source	AG/Subgroup	Accesion Number
6684	Yozgat	Plant	*R. solani* AG 4 HG II	KC590602
0672	Ankara	Soil	*R. solani* AG 5	KC590597
0610	Ankara	Plant	*R. solani* AG 8	KC590583
6629	Yozgat	Soil	*Waitea circinata* var. *circinata*	KC590532
4226	Konya	Plant	*Waitea circinata* var. *zeae*	KC590515
0653	Ankara	Plant	*R. cerealis* AG D I	KC590591

BNR AG A, C, E, H, I, G, K, Bb anastomosis groups isolated from wheat [11, 26, 27]. AG I and AG K were reported on wheat in Turkey by Demirci [9]. None of them are pathogenic on wheat [28]. Although there are few studies reporting that binucleate *Rhizoctonia* spp. shows pathogenity in some other hosts, these species generally show saprophytic character in the soil. Among them, low-virulent or non-virulent species are exhibiting hypo-virulent properties [1,29]. Regarding the genetic and physiological factors involved in hypovirulence in the different groups of *Rhizoctonia*, the possession of enzymes and the ability to synthesize melanin have been revealed as important. Melanin has been found to be essential in pathogenicity processes in several fungi. Several authors have pointed out that hyphae from hypovirulent isolates from the genus *Ceratobasidium* are usually hyaline, while hyphae from pathogenic isolates of *R. solani* are usually pigmented with brown or grey tones, due to the accumulation of melanin in their cell walls [30, 31]. The effect mechanism of binucleate *Rhizoctonia* AGs and hypovirulent *Rhizoctonia* AGs used in the control of virulent *Rhizoctonia* species is in the form of the systemic promotion of host resistance and food or habitat competition [1,18]. However, few reports have been published that document the ability of non-pathogen *Rhizoctonia* species to control diseases caused by pathogen *Rhizoctonia* species on wheat in the world [6].

The objectives of present work are to evaluate the effect of non-pathogen binucleate *Rhizoctonia* anastomosis groups potential use for biological control against pathogen *Rhizoctonia* anastomosis groups isolated from wheat fields in greenhouse conditions.

MATERIALS AND METHODS

Fungal Materials and Isolation. Binucleate *Rhizoctonia* species were isolated from 1256 wheat rhizosphere soil samples collected from Konya, Ankara, Yozgat, Eskişehir, Kırıkkale, Kayseri, Kırşehir, Nevşehir and Aksaray provinces in the Central Anatolian Region of Turkey. *R. solani* AG 4 HG II (isolate num.: 6684), AG 5 (0672), AG 8 (0610), *R. cerealis* AG D I (0653), *Waitea circinata* var. *circinata* (6629), *Waitea circinata* var. *zeae* (4226) isolates, were used as pathogens throughout this study (Table 1). These isolates belong to six anastomosis groups which were previously isolated and identified from wheat root by Ünal [10, 11, 32].

Sterile wheat straws were used for binucleate *Rhizoctonia* spp. isolation from soil samples. Soil samples from the respective fields were transferred to pots on a greenhouse bench (20 ± 2^0C). Pots were then watered to field capacity. Four internodal segments of mature, dried wheat straws, approximately 4 cm long, were inserted vertically into each pot, and left for 3 to 4 days. After that, the straws were removed, washed, blotted and placed on acidified water agar (WA). Isolates of *Rhizoctonia* were transferred to potato dextrose agar (PDA, Merck, Germany) [33].

Identification of *Rhizoctonia* spp. Anastomosis grouping. In order to determine hyphal diameter and the number of nuclei per cell of the BNR isolates, they were maintained on PDA at 25ºC in the dark. Developing mycelia of the isolates were stained with safranin O (Sigma, USA) and 3% KOH [34] and observed at x400 using phase contrast microscopy. Hyphal diameter was determined by measuring 10 cells. Nuclei were counted in 15 cells.

Anastomosis was tested by pairing isolates with representative testers. *Rhizoctonia* isolates were activated on PDA at 25^0C in the dark. Coverslips, which were sterilized by dipping in 95% ethyl alcohol and flaming, were coated with a thin layer of 0.5% PDA and placed on water agar plates. Agar plugs with mycelia of *Rhizoctonia* isolates and the tester isolates were cut at the margin of a growing colony and transferred to water agar plates on the opposite sides of the coverslip. After incubation at 25^0C in the dark for 24-48 hours, when overlapping mycelia of the two isolates were observed, the coverslip was removed from the plate and placed on the mixture of one drop of safranin O and one drop of 3% KOH on a slide. Stained hyphae were then examined microscopically (x 400) and anastomosing hyphae were traced back to their source in order to confirm the anastomosis between our isolates and the tester isolates [35]. For the anastomosis testing, all the pairs were examined twice.

DNA extraction. Approximately 300 mg mycelium were harvested and ground with liquid nitrogen in a sterile mortar for DNA extraction from culture medium. Genomic DNA was extracted using a Qiagen DNeasy ® Plant Mini Kit, as specified by the manufacturer, and stored at -20^{0}C prior to use [32].

DNA sequencing and data analysis. PCR reaction mixtures and condition were modified from previous studies [36, 37]. The reaction mixtures of PCR, a final volume of 50 µl, contained 5 µl of 10X buffer [75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM $(NH4)_2SO4$], 2 µl of 5 µM each primer, 5 µl of 1.5mM $MgCl_2$, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs), 1 U Taq polymerase (Fermentas), 5 µl of DNA template for each reaction and 5 µl of bovine serum albumin (BSA: 10 mg/ml). DNA amplifications were carried out in a Techne TC-5000 thermal cycler by the following program: 94 C for 2 min, followed by 34 cycles of (1) denaturation (94ºC for 30 s), (2) annealing (60ºC for 30 s) and (3) extention (72ºC for 30 s), and a final extension step 10 min at 72ºC.

The ITS region of the isolate was amplified using the universal primers ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3' [38]. The PCR products were separated in 1.5% agarose gels stained with ethidium bromide, and visualized under UV light. They were sequenced by REFGEN (Gene Research and Biotechnology Company, Ankara, Turkey).

Pathogenicity Tests. Pathogenicity tests were performed with agar plate assay with six binucleate isolates belonging to AG A, C, E, G, H, I anastomosis groups (Table 2). Isolates were incubated on PDA at 25^{0}C for 2 days, mycelial discs (4 mm) from an actively growing edge of the fungal culture were transferred to 2% WA and incubated in the same conditions for 2 days. Seeds of the susceptible wheat cultivar (cv. Kate A-1) were disinfected by dipping in 1% NaOCl for 5 mins, rinsed with sterile distilled water and aseptically blotted, then six seeds were placed adjacent to the growing edge of the isolates in each petri plate. Five plates were used for each isolate. Untreated plates were used as a control. After incubation for 7–8 days at 26±1^{0}C under a photoperiod of 12 hours, disease assessment was rated on a scale of 0-5, based on the relative size of a necrotic area on the hypocotyl as follows : 0= no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80% and 5= the entire hypocotyl was infected [39]. These scale values were converted to disease severity values using the following formula [40].

Disease Severity= [∑ (no. of plant in category x category value)] x 100 / Total no. of plants x max. category value).

Isolates that produced a disease severity between 0-10% were considered essentially non pathogen.

Effectiveness of BNR in the Control of Pathogen *Rhizoctonia* Anastomosis Groups. BNR isolates used in biocontrol studies were obtained by randomly selecting from among the isolates belonging to each different BNR anastomosis group isolated in this study.

Cultures of pathogen and non pathogen *Rhizoctonia* isolates were grown on PDA at 25^{0}C in the dark for 9 days. The mycelial mats were rinsed with sterile distilled water, blotted dry, and weighed. Then 4 g dried fungus cultures were blended in 200 ml sterile water. The suspension of non pathogen fungus culture was thoroughly mixed with 4 kg of sterile soil mix (soil, sand, farm manure and bran at 2:1:1:1/2 v/v) and placed in plastic pots (10 cm x 8,5 cm) [39]. In addition, four seeds infested with each non pathogen BNR isolates (Table 2) were put in each pot. Infested seed inoculums were produced in 1-liter flasks filled with 250 ml of wheat grain soaked in water and autoclaved twice. The grain was inoculated with 10 discs of 1-cm^2 pieces from nine-day-old potato dextrose agar (PDA) plate culture of each isolate. The flasks were shaken once each week, and the inoculum was harvested after 4 weeks [41].

Plastic pots were incubated in the greenhouse at 26±2^{0}C with a 12 hr photoperiod at 6000 lux. After 5 days of incubation, eight wheat seeds per pot were sown. Three days after the seeds were sown in soil infested with the non pathogen isolates, a mycelial suspension of each virulent isolate was spread on the soil (1g mycelium per 1kg of soil) and mixed in soil. In addition, eight seeds infested with each pathogen isolate mycelium were put in each pot. Twenty-two days after sowing, roots and hypocotyles of seedlings were examined and disease assessment was rated according to a modified 0-5 scale 0= no disease, 1= 1-10%, 2= 11-30%, 3=31-50%, 4= 51-80%, 5= the entire hypocotyl/root is infected and/or stunting. These scale values were converted to disease severity values [40]. In addition, percent protection values of BNR isolates were calculated using the following formula at the end of the study [39].

Percent protection=[(C-B)/(A-B)]X100)] in which A: percentage of healthy plants in the non-infested control, B: percentage of symptomless plants in soil infested with the virulent strain, and C: percentage of symptomless plants in soil infested with the avirulent strain and then challenged with the virulent strain.

Non-infested soil mix, infested soil with only non-pathogen isolate and infested soil with only pathogen isolate were used as a control. Experiments were conducted with five replications (one pot per replication) and repeated twice.

TABLE 2
Anastomosis groups, geographic origins, sources of isolation and accesion numbers of Binucleate *Rhizoctonia* isolates used in this study

Isolate Number	Origin (Province)	Isolation Source	AG/Subgroup	Accesion Number
0680	Ankara	Soil	AG A	KC590599
26114	Eskişehir	Soil	AG C	KC590578
06100	Ankara	Soil	AG E	KC590572
0615	Ankara	Soil	AG G	KC590522
0660	Ankara	Soil	AG H	KC590560
0616	Ankara	Soil	AG I	KC590588

TABLE 3
Non-pathogen + pathogen *Rhizoctonia* isolates application with the results observed in disease severity values

	Disease Severity (%)					
	R.solani AG 4	*R.solani* AG 5	*R.solani* AG 8	*R.cerealis* AG D	*Waitea cir.* var. *circinata*	*Waitea cir.* var. *zeae*
AG A	8,5	24,0	1,5	8,5	7,5	17,5
AG C	2,5	14,0	7,5	17,0	2,0	15,0
AG E	4,0	4,5	14,3	0,0	32,5	13,0
AG G	0,0	14,5	9,0	13,5	26,5	30,0
AG H	2,0	1,5	0,0	9,0	8,0	13,0
AG I	6,5	24,0	31,0	22,0	20,0	21,5
No BNR [a]	86,0	76,0	73,5	78,0	82,0	73,0
Uninfested Control [b]	0,0	0,0	0,0	0,0	0,0	0,0

[a]No BNR=soil mix infested with pathogen, not infested with BNR
[b] Uninfested Control=soil mix not infested with pathogen and BNR

Data analysis. Statistical analyses were performed with Minitab and MSTAT statistical software. The data were evaluated by analysis of variance using Fisher's least significant difference test ($P < 0.05$).

RESULTS AND DISCUSSION

A total of 112 BNR isolates were obtained from 1256 soil samples collected from 9 provinces in Central Anatolia. According to the cellular nucleus number, width of the main runner hyphae, colony morphology and the anastomosis test, the isolates were identified as binucleate *Rhizoctonia*. As a result of the anastomosis test, these isolates anastomosed with high fusion frequency (C 3 and C2 reactions) with tester isolates. These isolates characterized into 6 anastomosis groups (*Rhizoctonia* AG I, A, C, E, G and H) with the help of their vegetative hyphae, nuclear condition and hyphal anastomosis reaction with known tester isolates and rDNA-ITS sequence analysis. All isolates produced ≈650 bp amplicons during amplification with ITS1 and ITS4 primers. Sequences were submitted to GenBank at NCBI and their accession numbers were obtained (Table 2). The isolates' resulting sequences used in this study were compared to other sequences and were 90-100% identical to other *Rhizoctonia* sequences in GenBank.

In pathogenicity studies, isolates with pathogenicity values 0-10% were evaluated as non-pathogenic. All of the 112 BNR isolates were detected as non-pathogenic. Generally, the values of disease severity in these groups changed between 0,0% and 9,5%. All six BNR isolates, when combined with AG 4 HG II, AG 8, *R. cerealis* AG D I, *W. cir.* var. *circinata* and *W. cir.*var *zeae*, saw a greater reduction in disease severity when compared to the effect of the isolates alone. It was also detected that non-pathogen AG H group decreased the values of disease severity in all pathogen AG groups significantly; however, in combinations of AG I group with other pathogen groups except for *R. solani* AG 4 HGII, the values of disease severity varied between 20% and 31% (Table 3).

It was also detected that according to the protection values (%) calculated on the basis of the number of symptomless seedlings, *Rhizoctonia* AG H provided a high degree of protection (between 75% to 100%) against all pathogen groups. Protection values of AG C, AG E, AG G against all pathogen groups ranged from between 60% and 95%, 50% and 100%, and 57% and 100%, respectively. Although AG I significantly prevented infection of *R. solani* AG 4 HG II, it prevented the infections caused by AG 5 and AG 8 only at the rates of 44% and 35%. It was also determined that AG A provided protection against five pathogen groups except for *R. solani* AG 5 at the rates varying between 80% and 96% (Table 4). All of the non-pathogen isolates provided a high rate of protection against *R. solani* AG 4 HGII. The most successful results against *R. solani* AG 5 were obtained by AG H and AG E. AG I prevented the infection caused by *R. solani* AG 8 at the rate of

35%, and therefore, it was considered the most ineffective group against this pathogen. All non-pathogen groups provided an effective protection against *R. cerealis* AG D I and *Waitea cir.* var. *zeae*. The most effective group against these pathogens was AG E. The groups with *Rhizoctonia* AG C, AG A and AG H saw the highest degree of protection (85%, 80% and 80%, respectively) against *Waitea cir*. var. *circinata* (Table 4).

In this study, the effect of 6 non-pathogen BNR anastomosis groups (AG A, C, E, G, H and I) against 5 pathogen *Rhizoctonia* anastomosis groups on wheat was investigated and hopeful results were obtained in greenhouse conditions. In the present study, all BNR isolates induced no disease symptoms on wheat and were evaluated as non-pathogens. Aside from some exceptions, BNR isolates used for biocontrol of *R. solani* and some other pathogens are also pathogen some other hosts. In this study BNR AG A was found to be non-pathogenic on wheat, but pathogenic to some other plants. Ogoshi [33] stated that AG A is pathogenic on strawberry, sugar beet, peanut and potato. It was also reported on bean and soybean in Turkey as non-pathogenic [42, 43, 44, 45]. In this study, AG A provided strong protection against five pathogen groups except for *R. solani* AG 5. Similarly, Bell [46] reported that heat treated soil artificially infested with *R. solani* AG 4 plus AG A and planted with snap bean had a significantly higher percentage of normal bean plants 2-5 weeks later than did soil infested only with AG 4. Also, AG A has been reported to be effective biocontrol agents against *Fusarium* wilt of spinach and tomato. The isolate significantly reduced disease severity [47, 48]. In the present study, AG C induced no disease symptoms and the isolates controlled only AG 4, *W.c*. var. *circinata* and *W.c*. var. *zeae.* It is known that AG C is symbiotic with terrestrial orchids and no important pathogens have been reported about it [28, 49]. AG E have previously been recognized on beans in Turkey [43, 44, 50] and some the other plants including wheat around the world [28]. AG E reduced disease severity on wheat caused by AG 4 HG II, AG 5, AG 8, AG D and *W.c*. var *zeae*. AG G group provided protection 75%, 90% and 100% against AG D I, AG 8 and AG 4 HG II, respectively in this study. Similarly, non-pathogenic AG G provide effective protection to young bean seedlings against root rot caused by *R. solani* AG 4 [51,52]. Escande and Echandi [19] also found effective AG G in control of *Rhizoctonia* canker of potato. BNR AG H provided a high degree of protection (between 75% and 100%) against all pathogen groups in this study. They are symbiotic on orchids. AG H was also reported on wheat by Ogoshi [26]. AG I controlled only AG 4 HG II group in our study. Previously, it was detected on wheat by Demirci [9] in Turkey and in the other countries [27,53, 54]. Its hosts are strawberry, sugar beet, wheat, apple, orchids, and fragaria x ananassa [28] and it is pathogenic on strawberry [52].

There is a limited number of studies on the control of non-pathogen *Rhizoctonia* species with pathogen *Rhizoctonia* species on wheat. Roberts and Sivasithamparam [6] reported that in wheat fields with soil infested with *R. solani*, the center of disease patches was dominated by this fungus, while the populations of binucleate *Rhizoctonia* were distributed along the margins of these patches, suggesting that these isolates could be involved in controlling the spread of these disease patches by means of antagonistic phenomena against pathogenic *R. solani* strains. Similarly, Ichielevich-Auster et al. [39] indicated a hypovirulent *R. solani* AG-4 isolate as an effective biocontrol agent for suppression of damping-off in cotton, radish and wheat seedlings caused by virulent isolates of *R. soluni* AG-4 and *R. zeae.* There are many studies reporting that BNR groups, with the exception the BNR isolated in this study, also control many important diseases. Cardoso and Echandi [55], determined that BNR AG R isolates significantly reduce the severity of the root rot disease in bean seedlings caused by *R. solani* under greenhouse conditions. Pascual et al. [56] indicated that the hypovirulent, binucleate *Rhizoctonia* Rhv7 strain effectively controlled *R. solani* AG1-IA isolate in corn. González et al. [57] reported a new binucleate *Rhizoctonia* species *Ceratobasidium albasitensis* capable of protecting several plant species against *R. solani* and other pathogen fungal species such as *Fusarium solani, Alternaria alternata, Penicillium digitatum* and *P. expansum*. It known that BNR isolates prevented the damping off caused by *Rhizoctonia solani* AG-4 and AG 8 in cucumber [20] and also root and crown rot caused by AG 2-2 isolates of *R. solani* in sugar beet [29].

TABLE 4
Protection values of non-pathogen *Rhizoctonia* isolates (%)

	Protection (%)					
	R.solani AG 4	*R.solani* AG5	*R.solani* AG8	*R.cerealis* AG D	*Waitea cir.* var. *circinata*	*Waitea cir.* var. *zeae*
AG A	90,0	40,0	96,0	90,0	90,0	80,0
AG C	94,0	60,0	72,0	66,6	95,0	80,0
AG E	92,5	82,0	70,0	100,0	57,0	85,0
AG G	100,0	50,0	90,0	75,0	65,0	67,5
AG H	97,5	95,0	100,0	90,0	90,0	75,0
AG I	90,0	44,0	35,0	60,0	60,0	65,0
LSD (p=0,05)	0,53	4,87	5,71	3,12	1,93	1,28

The defense reactions in plants treated with biocontrol agents might be the same as the ones that occurred in resistant cultivars against pathogen attack. Nutrient competition and/or host-induced resistance may play an important role in the mechanism of protected effect of BNR against virulent *Rhizoctonia* species [18, 55].

The study conducted by Honeycutt and Benson [14] investigated the effect of two binucleate *Rhizoctonia* spp. in Pesta and rice flour formulations and were evaluated for control of preemergence damping-off of impatiens caused by *R. solani*. In the study, effective results were obtained about control of disease, but the conclusion was reached that improved shelf life of BNR isolates is needed before formulated products can be developed for biocontrol of preemergence damping-off.

Results of our study show that BNR isolates suppressed disease on wheat caused by *R. solani* AG 4 HG II, 5, 8, *R. cerealis* AG D I, *Waitea circinata* var. *circinata* and *Waitea circinata* var. *zeae* in greenhouse conditions. AG H among binucleate *Rhizoctonia* groups have shown strong protection against all pathogen groups in this study. These results suggest its greater potential use in the management of the pathogen *Rhizoctonia* anastomosis groups on wheat, but will require rigorous testing under field conditions and formulations.

ACKNOWLEDGEMENTS

We are grateful to Dr. A. Ogoshi (Hokkaido University, Japan), Dr. M. Mazzola (Tree Fruit Research Laboratory, Western Ave., USA), Dr. Erkol Demirci (Karadeniz Tecnical University, Turkey), Dr. Takeshi Toda (Akita Prefectural University, Japan), Dr. Grzegorz Lemańczyk (University of Technology and Life Sciences, Poland) and Dr. Patricia Okubara (Washington State University, USA), who provided us binucleate tester isolates, and the Scientific and Technological Research Council of Turkey (TUBITAK) for the grant (Project No:TOVAG-110O622).

REFERENCES

[1] Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (1996) *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and diseases control. Kluwer Academic Publishers. Netherlands, Dordrecht. 1-578.

[2] Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S. and Sneh, B. (2008) Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysissupports the genetic basis of the classical anastomosis grouping. Mycoscience. 49(2), 93–114.

[3] Yang, Y.G., Zhao, C., Guo, Z.J. and Wu, X.H. (2015) Characterization of a new anastomosis group (AG-W) of binucleate *Rhizoctonia*, causal agent for potato stem canker. Plant Diseases. 99(12), 1757-1763.

[4] Dong, W., Li, Y., Duan, C., Li, X., Naito, S., Conner, R.L., Yang, G. and Li, C. (2017) Identification of AG-V, a new anastomosis group of binucleate *Rhizoctonia* spp. from taro and ginger in Yunnan province. European Journal of Plant Pathology. 148(4), 839-851.

[5] Ogoshi, A., Oniki, M., Araki, T. and Ui, T. (1983) Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. J. Fac. Agr. Hokkaido Univ. 61(2), 244-260.

[6] Roberts, F.A. and Sivasithamparam, K. (1986) Identity and pathogenicity of *Rhizoctonia* spp. associated with bare patch diseases of cereals at field site in Western Australia. Europan Journal of Plant Pathology. 92(5), 185-195.

[7] Meyer, L., Nel, L.H. and Carling, D.E. (1997) Anastomosis grouping and molecular genetic typing of *Rhizoctonia solani* causing crater disease of wheat in South Africa. Diagnosis and identification of plant pathogens. In: Proceedings 4th International Symposium of the European Foundation for Plant Pathology, 9-12 September 1996, 53-57, Bonn, Germany.

[8] Tomaso-Peterson, M. and Trevathan, L.E. (2007) Characterization of *Rhizoctonia*-like fungi isolated from agronomic crops and turfgrasses in Mississippi. Plant Disease. 91(3), 260-265.

[9] Demirci, E. (1998) *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology. 47(1), 10-15.

[10] Ünal, F. and Dolar, F.S. (2012) First report of *Rhizoctonia solani* AG 8 in Turkey. Journal of Phytopathology. 160(1), 52–54.

[11] Ünal, F. (2013) Determination of *Rhizoctonia* species, anastomosis groups and the reactions of cultuvars on wheat production areas of Central Anatolia Region. Ankara University Graduate School of Natural and Applied, Ankara, Turkey. PhD Thesis.

[12] Yangui, T., Rhouma A., Triki M.A., Gargouri K., Bouzid J. (2008) Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Protection. 27(2), 189-197.

[13] Cardoso, J.E. and Echandi, E. (1987a) Biological control of *Rhizoctonia* root rot of snap bean with binucleate *Rhizoctonia*-like fungi. Plant Disease. 71, 167-170.

[14] Honeycutt, E.W. and Benson, D.M. (2001) Formulation of binucleate *Rhizoctonia* spp. and biocontrol of *Rhizoctonia solani* on impatiens. Plant Disease. 85(12), 1241-1248.

[15] Elad, Y., Hadar, Y., Hadar, E. and Chet, I. (1981) Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation. Plant Disease. 65, 675-677.
[16] Lewis, J.A. and Papavizas, G.C. (1985) Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. Phytopathology. 75, 812-817.
[17] Lewis, J.A., Fravel, D.R. and Papvizas, G.C. (1995) *Cladorrhinum foecundissimum*: a potential biological control agent for the reduction of *Rhizoctonia solani*. Soil Biology and Biochemistry. 27(7), 863-869.
[18] Burpee, L.L. and Goulty, L.G. (1984) Suppression of brown patch disease of creeping bentgrass by isolates of nonpathogenic *Rhizoctonia* spp. Phytopathology. 74, 692-694.
[19] Escande, A.R. and Echandi, E. (1991) Protection of potato from *Rhizoctonia* canker with binucleate *Rhizoctonia* fungi. Plant Pathology. 40(2), 197-202.
[20] Harris, A.R., Schisler, D.A., Neate, S.M. and Ryder, M.H. (1994) Suppression of damping-off caused by *Rhizoctonia solani*, and growth promotion, in bedding plants by binucleate *Rhizoctonia* spp. Soil Biology and Biochemistry. 26(2), 263-268.
[21] Herr, L.J. (1988) Biocontrol of *Rhizoctonia* crown and root rot of sugar beet by binucleate *Rhizoctonia* spp. and *Laetisaria arvalis*, Annuals of Applied Biology. 113, 107-118.
[22] Jabaji-Hare, S. and Neate, S.M. (2005) Nonpathogenic binucleate *Rhizoctonia* spp. and benzothiadiazole protect cotton seedlings against *Rhizoctonia* damping-off and *Alternaria* leaf spot in cotton. Phytopathology. 95, 1030-1036.
[23] Ross, R.E., Keinath, A.P. and Cubeta, M.A. (1998) Biological control of wirestem on cabbage using binucleate *Rhizoctonia* spp. Crop Protection. 17(2), 99-104.
[24] Villajuan-Abgona, R., Katsumo, N., Kageyama, K. and Hyakumachi, M. (1993) Isolation of hypovirulent strains of *Rhizoctonia* from soil and their cross protection effect. Annals of the Phytopathological Society of Japan. 59, 61.
[25] Burns, J.R. and Benson, D.M. (2000) Biocontrol of damping-off of *Catharantus roseus* caused by *Pythium ultimum* with *Trichoderma virens* and binucleate *Rhizoctonia* fungi. Plant Disease. 84, 644.648.
[26] Ogoshi, A., Cook, R.J. and Bassett, E.N. (1990) *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology. 80(9), 785-788.
[27] Tewoldemethin, Y., Lamprecht, S.C., Mclead, A. and Mazzola, M. (2006) Characterization of *Rhizoctonia* spp. recovered from crop plants used in rotational cropping systems in the Western Cape Province of South Africa. Plant Disease. 90(11), 1399-1406.
[28] Sneh, B., Burpee, L. and Ogoshi, A. (1998) Identification of *Rhizoctonia* species. The APS, St. Paul, Minesota. 377p.
[29] Herr, L.J. (1995) Biological control of *Rhizoctonia solani* by binucleate *Rhizoctonia* spp. and hypovirulent *R. solani* agents. Crop Protection. 14(3), 179-186.
[30] Sneh, B., Ichielevich-Auster, M. and Shomer, I. (1985) Comparative anatomy of colonization of cotton hypocotyls and roots by virulent and hypovirulent isolates of *Rhizoctonia solani*. Canadian Journal of Botany. 67(7), 2142-2149.
[31] Villajuan-Abgona, R., Kageyama, K. and Hyakumachi, M. (1996) Biological control of *Rhizoctonia* damping-off of cucumber by non-pathogenic binucleate *Rhizoctonia*. European Journal of Plant Pathology. 102(3), 227-235.
[32] Ünal, F., Dolar, F.S. and Özben, S. (2013) First report of *Rhizoctonia zeae* causing stunting and root rot of wheat in Turkey. IV International Symposium "Agrosym 2013" Jahorina, 3-6 October 2013, Bosnia and Herzegovina.
[33] Ogoshi, A. (1985) Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia.* Fitopatologia Brasi-leira. 10, 371- 390.
[34] Bandoni, R.J. (1979) Safranin O as a rapid stain for fungi. Mycologia. 71, 873–874.
[35] Kronland, W.C. and Stanghellini, M.E. (1988) Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathology. 78, 820–822.
[36] Aroca, A. and Raposo, R. **(**2007) PCR-based stragety to detect and identify species of *Phaeoacremonium* causing grapevine diseases. Applied and Environmental Microbiology. 73, 2911-2918.
[37] Cobos, R. and Martin, M.T. (2008) Molecular characterisation of *Phaeomoniella chlamydospora* isolated from grapevines in Castilla y León (Spain). Phytopathologia Mediterranea. 47, 20–27.
[38] White, T.J., Bruns, T., Lee, S. and Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds.) PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York. 315-322.
[39] Ichielevich-Auster, M., Sneh, B., Koltin, Y. and Barash, I. (1985) Suppression of Damping-Off Caused by *Rhizoctonia* Species by a nonpathogenic isolate of *R. solani*. Phyto-pathology. 75, 1080-1084.

[40] Karman, M. (1971) Bitki Koruma Araştırmalarında Genel Bilgiler. Denemelerin Kuruluşu ve Değerlendirme Esasları, Bornova-İzmir, 279p.
[41] Paulitz, T.C., Smith, J.D. and Kidwell, K.K. (2003) Virulence of *Rhizoctonia oryzae* on wheat and barley cultuvars from the Pacific Northwest. Plant Disease. 87(1), 51-55.
[42] Tuncer, G. and Erdiller, G. (1990) The identification of *Rhizoctonia solani* Kühn anastomosis groups isolated from potato and some other crops in Central Anatolia. Journal of Turkish Phytopathology. 19(2), 89–93.
[43] Demirci, E. and Döken, M.T. (1995) Anastomosis groups of *Rhizoctonia solani* Kühn and binucleate *Rhizoctonia* isolates from various crops in Turkiye. Journal of Turkish Phytopathology. 24(2), 57–62.
[44] Eken, C. and Demirci, E. (2004) Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from bean in Erzurum, Turkey. Journal of Plant Pathology. 86(1), 49–52.
[45] Erper, İ., Özkoç and İ., Karaca, G.H. (2011) Identification and pathogenicity of *Rhizoctonia* species isolated from bean and soybean plants in Samsun, Turkey. Archives of Phytopathology and Plant Protection. 44(1), 78-84.
[46] Bell, D.K., Sumner, D.R. and Mullinix, B.G. Jr. (1984) Antagonism of *Laetisaria arvalis* and binucleate *Rhizoctonia-like* CAG-2 and CAG-4 to *Rhizoctonia solani* AG-4 and AG-2 type 2 on snapbean and corn. Phytopathology. 74, 625.
[47] Muslim, A., Horinouchi, H. and Hyakumachi, M. (2003a) Biological control of *Fusarium* wilt of tomato with hypovirulent binucleate *Rhizoctonia* in greenhouse conditions. Mycoscience. 44, 77-84.
[48] Muslim, A., Horinouchi, H.and Hyakumachi, M. (2003b) Suppression of *Fusarium* wilt of spinach with hypovirulent binucleate *Rhizoctonia.* Journal of General Plant Pathology. 69(2), 143–150.
[49] Di Pardo, A.F., Chiocchio, V.M., Barrera, V., Colombol, R.P., Martinez, A.E., Gasoni, L. and Godeas, A.M. (2015) Mycorrhizal fungi isolated from native terrestrial orchids of pristine regions in Córdoba (Argentina). Revista de Biologia Tropical Journal. 63(1), 275-283.
[50] Karaca, G.H., Özkoç, İ. and Erper, İ. (2002) Determination of the anastomosis grouping and virulence of *Rhizoctonia solani* Kühn isolates associated with bean plants grown in Samsun/Turkey. Pakistan J Biol Sci. 5(4), 434–437.
[51] Leclerc-Potwin, C., Balmas, V., Charest, P.M. and Jabaji, H.S. (1999) Development of reliable molecular markers to detect non-pathogenic binucleate *Rhizoctonia* isolates (AG-G) using PCR. Mycological Resarch. 103 (9), 1165-1172.
[52] Martin, S.B. (1988) Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology. 78, 379–384.
[53] Ravanlou, A. and Banihashemi, Z. (2002) Isolation of some anastomosis groups of *Rhizoctonia* associated with wheat root and crown in Fars Province. Iranian Journal of Plant Pathology. 38 (3/4), 151-157.
[54] Schroeder, K.L., Shetty, K.K. and Paulitz, T.C. (2011) Survey of *Rhizoctonia* spp. from wheat soils in the U.S. and determination of pathogenicity on wheat and barley. Phytopathology. 101, S161.
[55] Cardoso, J.E. and Echandi, E. (1987b) Nature of protection of bean seedlings from *Rhizoctonia* root rot by a binucleate *Rhizoctonia*-like fungus. Phytopathology. 77, 1548-1551.
[56] Pascual, C.B., Raymundo, A.D. and Hayakumachi, M. (2000) Efficacy of hypovirulent binucleate *Rhizoctonia* sp. to control banded leaf and sheath blight in corn. Journal of General Plant Pathology. 66, 95-102.
[57] González, V., Salazar, O., Julıán, M.C., Acero, J., Portal, M.A., Muñoz, R., López-Córcoles, H., Gómez-Acebo, E., López-Fuster, P. and Rubio, V. (2002) *Ceratobasidium albasitensis*, a new *Rhizoctonia*-like fungus isolated in Spain. Persoonia. 17, 601-614.

Received: **29.03.2018**
Accepted: **03.11.2018**

CORRESPONDING AUTHOR

Filiz Unal
Directorate of Plant Protection Central Research Institute,
06172, Ankara – Turkey

e-mail: fucar06@yahoo.com

© by PSP Volume 27 – No. 12B/2018 pages 9634-9641 Fresenius Environmental Bulletin

DIVERSITY OF SOIL MICROARTHROPODS IN HABITATS CONTAINING DIFFERENT TREE SPECIES IN THE SPRING SEASON

Ahmet Duyar*

Department of Forest Engineering, Faculty of Forestry, Karabuk University, Karabuk, Turkey

ABSTRACT

This study aims to determine the diversity and community structure of microarthropods in habitats dominated by different tree species. For this purpose, the microarthropod contents of the soil and litter samples collected in May 2017 from habitats dominated by the Arnold/Black pine (*Pinus nigra* (Arnold)), Oriental beech (*Fagus orientalis* (Lipsky.)) and Uludag fir (*Abies nordmanniana* (Stev.) subsp. *bornmulleriana* (Mattf.)) tree species were investigated. Soil and litter samples were collected from Arac, Karabuk and Safranbolu forests for Black Pine, Oriental Beech and Fir, respectively. Litter samples used in the extraction of microarthropods were collected using a cylinder of 5 cm diameter and were as thick as the litter cover, while 5 cm thick soil samples were collected from the upper mineral soil to a depth of 5 cm. Moreover, extra samples were collected from the same locations to determine certain physical properties of the litter and soil in those locations. Microarthropods were extracted from the litter and soil samples using the Berlese funnel method and were counted and classified using a microscope. According to the analyses, there were 74 different taxa throughout the three different ecosystems, and there was no statistically significant difference in terms of biological diversity. Based on tree species, the Fir forest had the highest average taxonomic richness (26 taxa), while the Pine forest had the lowest tree species-based taxonomic richness (19 taxa). Similarly, the Shannon's diversity index (H′) was the highest in the Fir forest (2.65), while it was the lowest in the Pine forest (2.34). In addition, the litter layer (H′ = 2.64) had a significantly higher biological diversity than that in the soil (H′ = 2.39). The study is still in progress and any seasonal changes in the relationships will also be revealed with new samples collected during other seasons.

KEYWORDS:
Soil, litter, Berlese funnel, ecosystem, biological diversity

INTRODUCTION

Some features such as geographic location caused by the physical structure of the World, distances to the seas, landscape forms, aspects and parent rock are the principle factors having certain impacts on the distribution of terrestrial plant species and the formation of ecosystems. Various forest sites created by those factors generate forest habitat dominated by the appropriate tree species that specifically grow on those conditions [1, 2]. Moreover, forests may be the habitats dominated by different tree species and the natural gen resources of various biodiversity characteristics [3, 4].

The most promising organisms indicating the diversity/richness of other species in forest ecosystems and the status of forest biodiversity are the invertebrate soil fauna living in the soil [5]. The soil invertebrates include taxa varying from protozoa (5 -30 μm) to the giant Australia worms, few meters in sizes with different body sizes [6, 7]. The population size and the breeding potentials are quite high. These small sized organisms provide the effective monitoring of the details of important changes affecting the quality of the sites. It is possible to determine the population sizes with small samples using the proportionate and statistical methods. It is also ideal to detect changes over time, as the generation time is quite short. Therefore, they constitute the widest diversity of micro habitat and their niches in ecosystems. The soil invertebrates provide better ecological role than other vertebrates in ecosystems as they contribute to accelerate the decomposition of surface litter, the nutrient network, soil structure and other activities in ecosystems [8 - 10].

When the use of the indicator species is evaluated, the status of a whole forest ecosystem cannot be represented enough with a few indicator species due to the limitation of the extent of geographic area of single indicator species. Thus, the biodiversity indicators of richness or abundance of species are quite effective to confirm the relationships of other easily measured parameters (stand age, amount of dead wood, rate of exotic or introduced species and the forested area) [5].

Data are generated with the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests)

[11]. The ICP Forest Programme, having been implemented in European countries since 1985, initiated in Turkey in 2006. General Directorate of Forestry (GDF) is responsible to manage the programme under the "Forest Ecosystems Monitoring Programme" [12]. 52 level 2 permanent sample plots or observation points were established to represent the whole country and observations and measurements started afterwards [13]. Determination and monitoring of the diversity of soil fauna in those areas have the potential to contribute to the forest ecosystems monitoring programme in Turkey.

This primary aim of this study is to determine the diversity and community structure of microarthropods in forest sites dominated by different forest trees. For this purpose, the microarthropod content of soil and litter samples collected in May 2017 (during spring) from habitats dominated by Black pine (*Pinus nigra* (Arnold.)), Oriental beech (*Fagus orientalis* (Lipsky.)) and Uludag fir (*Abies nordmanniana* (Stev.) subsp*. bornmulleriana* (Mattf.)) tree species were investigated.

MATERIALS AND METHODS

This study was conducted in Oriental beech (*Fagus orientalis*), Uludag fir (*Abies nordmanniana* subsp. *bornmulleriana*) and Black pine (*Pinus nigra*) dominated pure stands. Samples from both soils and surface litter were taken to determine the microarthropods diversity of soils under those forest habitats. Furthermore, additional investigations were carried to determine some features of soils and surface litter within the habitats. Further details are provided in the following sections.

The study area. The case study area holds some permanent observation plots of ICP Forests Level-2 located in Karabuk (Beech), Safranbolu (Fir) and Arac (Pine) (Figure 1). The plots were selected from the forests rising between 900 and 1400 meters in elevation across the western Black Sea region of Turkey (Table 1). All three sample plots are located in the region beyond the Black Sea dominated by the semiterrestrial climate conditions. The general characteristics of climate in the study area resemble to the typical terrestrial climate. The area experiences a dryer climate with hot (22.1 °C) and dry summers, and cold (2.2 °C) and snowy winters. The annual total precipitation is mostly in winter and spring periods. Mean precipitations are 603 mm for Karabuk, 597 mm for Safranbolu and 570 mm for Arac [14].

The Level 2 plots of ICP Forests established in 2007 - 2013 within the study area were selected carefully to represent the habitats of the species in question. A square area with 100 m by 100 m sizes was marked in the selected forest landscape and a level 2 permanent plot was established [13]. The sample points were taken from each outside corner of the corresponding permanent observation plots. Thus, 12 samples were taken from each sample types (3 plots × four corners).

In this study, the surface litter and soil layers (0 - 5 cm upper soil) in the sample points were assumed to be different habitats (the living spaces) and the habitat of each tree species was evaluated differently. The samplings and the corresponding evaluations were conducted accordingly.

FIGURE 1
Map of study sites and location of plots

TABLE 1
Characteristics of Level 2 sample plots of ICP Forests

Plot number of ICP Forest Level-2	5	49	50
Tree species	Beech	Fir	Pine
Stand type	($Kncd_3$) Fully covered mature-overmature pure Beech stands	(GA) Large sized pure Fir stands under uneven-aged management	(Ckc_3) Fully covered mature pure Pine stands
Altitude	1368	1386	933
Coordinates of Plots	41°02'15.6"N 32°43'54.3"E	41°22'15.7"N 32°41'07.9"E	41°16'20.8"N 33°20'01.7"E
Regional Directorate of Forestry	Zonguldak	Zonguldak	Kastamonu
Forest District Directorate	Karabuk	Safranbolu	Arac
Forest Range and Compartment	Karatepe - 124	Safranbolu - 2	Arac - 122

Characteristics of surface litter and soils. The litter samples taken from 25 cm × 25 cm sized plots were labelled and put into the polyethylene pockets for analysis. The amount ($g \cdot m^{-2}$) and the moisture contents (%) of litter samples were determined both in fresh and dry (24 hours 65 °C) in the laboratory. The soil samples were taken from the 0 - 5 cm thickness of mineral soils in the middle of litter plots using volume cylinders with 5 cm in dimeter and 5 cm in height, only from. The bulk density and soil moisture were determined using the fresh and dry (24 hours 105 °C) mass of the soil samples. The samples were pounded down to a powder and the gravels/stones and roots were eliminated using a 2 mm sized sieve. Then, the mass of fine soils and the amount of soil skeleton were determined.

Sampling microarthropods. Steel cylinders with 5 cm in dimeter were used to take the fresh litter and soils samples during the sampling microarthropods in the forests [15]. The litter samples were taken with the steel cylinder pressed up to the level of mineral soil and perpendicularly located on the surface litter. Similarly, the soil samples were taken with 5 × 5 cm cylinder from the 0 - 5 cm depth of mineral soil where the litter samples were identified [16]. The sample cylinder was pressed gently by hand and a hammer was not used. One litter sample and soil sample were taken from each sampling point. The cylinders sampled with microarthropods were carefully wrapped with polyethylene folio in order to protect soil moisture, natural porosity of soils and the gallery of soil microorganisms. The samples were labelled, put into the cases and transported to the labs. Without any delay, the extraction process was initiated immediately after the samples reached to the labs.

Extracting microarthropods from litter and soil samples. The modified Tullgren-Berlese method, performing under the principles that the soil microorganisms escape from the light and temperature into the depth of soils, was used in this study [16]. The litter and soil samples taken from the ground were placed into the Tullgren-Berlese funnel upside-down with the surface layer at the bottom and left over 6 days under the 25 watts lamb providing light and temperature [17]. Therefore, the microarthropods using the galleries in soils were dumped into the collecting cup placed underneath and filled with the liquid consisting of 70% ethyl alcohol and + 2% glycerin [18].

Identifying and classifying microarthropods. The identification, classification and counting the microarthropods samples gathered from both the soil and litter samples were conducted in the laboratory. All samples were identified, classified and counted using 7x - 180x magnified and stereo zoom microscope, as the microarthropods were so small that could not be identified with bare eyes [19]. The taxonomical classification was performed at the hierarchical level of class, order and family as functional groups [15, 18 - 22]. Rare species or taxa are identified and evaluated at only class level. The identification key in various literature such as the Soil Biology Guide by [23], invertebrates of the HJ Andrews Experimental Forest by [24], Kwik-Key to Soil-Dwelling Invertebrates of [25] and the Keys to Insects of the European USSR by [26] was used.

Statistical analysis. The abundance of microarthropods identified from the samples were evaluated as individuals per·m² [27]. The statistical analysis was performed with the SPSS statistical software. The correlation among the parameters was statistically analyzed and the relationships were identified at ($P < 0.05$) level of confidence. The differences within the data showing the changes with respect to the independent variables of habitat and the living spaces were analyzed with ANOVA and any significant differences ($P < 0.05$) were further analyzed

with Duncan test. Furthermore, the Shannon-Wiener diversity index (H′) and Taxonomic Richness (S′) index showing the number of taxa were used to determine the diversity of microarthropods.

RESULTS

The soil and litter properties of tree habitats. The variations of habitat characteristics of soil and litter were detected depending on the tree species habitats. The soil moisture is quite similar in both Beech and Fir habitats (around 47%). However, soil moisture in Pine habitat is 25%, and less than other habitats. Conversely, litter mass, soil bulk density and amount of soil skeleton were all found to be the highest in Pine habitat compared to the other habitats. Soil pH (6.05) was weakly acidic than litter pH (5.64). However, litter moistures with a mean of 119%, were found to be similar in all habitats (Table 2).

Abundance of microarthropods. Within the scope of this study, 12 soil and litter samples were taken from the field to determine the diversity of microarthropods over the spring seasons. Based on the extracted 24 samples, 3088 individuals including 74 taxa were identified and classified.

When the microarthropods soil fauna in all habitats were evaluated together, the average number of individuals per square meter is found to be about 142,173 (Table 3).

TABLE 2
The characteristics of living spaces with respect to tree species

Characteristics of Living Spaces	Tree Species					
	Pine	Beech	Fir	Mean	St. Dev.	P
Soil Moisture (%)	25% a	47% b	46% b	39%	16%	0.000
Soil Bulk Density (g·l□¹)	1120 b	903 a	830 a	951	227	0.003
Soil Skeleton (g·l□¹)	312 b	148 ab	63 a	174	230	0.022
Soil pH	6.77 b	5.63 a	5.76 a	6.05	0.7	0.047
Soil EC (µmhos)	280 b	151 a	386 b	273	122	0.006
Sand (%)	31 a	47 c	37 b	38	7	0.040
Silt (%)	56 b	40 a	38 a	45	9	0.038
Clay (%)	13 a	13 a	25 b	17	7	0.046
Litter Moisture (%)	115%	117%	124%	119%	28%	0.706
Litter Mass (g·m□²)	5596 b	2353 a	3035 a	3661	2206	0.000
Litter pH	5.18 a	5.86 b	5.89 b	5.64	0.4	0.002

TABLE 3
The distribution of abundance of soil microarthropods over tree species habitats and living spaces (individuals per m²).

Taxa	Tree Species						Grand Totals						
	Pine Forest		Beech Forest		Fir Forest		Tree Species				Living Spaces		
	Soil	Litter	Soil	Litter	Soil	Litter	Pine	Beech	Fir	P	Soil	Litter	P
Neanurinae	0	0	0	0	0	138	0	0	138	0.385	0	46	0.328
Onychiuridae	3866	0	6352	3452	9667	3314	3866	9804	12981	0.220	6628	2255	0.039
Hypogastruridae	276	828	828	2485	2209	5524	1104	3313	7733	0.058	1104	2946	0.121
Entomobryidae	414	4004	414	3728	1795	5386	4418	4142	7181	0.467	874	4373	0.000
Isotomidae	690	10633	5385	8009	6628	24858	11323	13394	31486	0.134	4234	14500	0.019
Tomoceridae	0	138	0	276	0	552	138	276	552	0.720	0	322	0.117
Neelidae	138	2486	552	0	0	138	2624	552	138	0.074	230	875	0.189
Sminthuridae	0	690	276	276	138	1104	690	552	1242	0.629	138	690	0.063
Oribatida	8284	48054	12564	19604	47226	34519	56338	32168	81745	0.099	22691	34059	0.240
Mesostigmata	2623	4694	2761	3037	6627	12565	7317 a	5798 a	19192 b	0.002	4004	6765	0.138
Prostigmata	16294	23888	4004	13117	18503	6489	40182	17121	24992	0.110	12934	14498	0.741
Astigmata	0	0	138	414	0	552	0	552	552	0.432	46	322	0.163
Araneae	0	138	0	0	0	0	138	0	0	0.385	0	46	0.328
Pseudoscorpiones	0	0	0	276	138	276	0	276	414	0.450	46	184	0.308
Chilopoda	138	0	138	138	276	0	138	276	276	0.800	184	46	0.143
Diplopoda	0	0	0	552	414	0	0	552	414	0.258	138	184	0.752
Symphyla	276	0	690	138	4833	138	276	828	4971	0.083	1933	92	0.051
Pauropoda	0	0	0	0	552	0	0	0	552	0.385	184	0	0.328
Protura	414	276	276	414	3176	828	690 a	690 a	4004 b	0.032	1289	506	0.211
Diptera	138	0	0	0	138	276	138	0	414	0.296	92	92	1.000
Insect Larvae	1795	1657	1519	276	828	690	3452	1795	1518	0.302	1381	874	0.362
Coleoptera	0	0	0	276	0	276	0	276	276	0.614	0	184	0.152
Hymenoptera	0	0	0	138	0	138	0	138	138	0.614	0	92	0.152
Isopoda	0	0	0	138	0	138	0	138	138	0.614	0	92	0.152
Total	**35346**	**97486**	**35897**	**56744**	**103148**	**97899**	**132832**	**92641**	**201047**	**0.272**	**58130**	**84043**	**0.380**

While some taxa such as Neanurinae and Pauropoda exit only in a specific habitat, most of the other taxa such as Hypogastruridae, Entomobryidae, Isotomidae, Oribatida, Mesostigmata, Prostigmata, Chilopoda, Symphyla, Protura, Diptera and insect larvae belonging to Collembolans, Acarina, Miriapoda and Insecta groups do not seem to select a specific habitat and they occur commonly in all other habitats (Table 3). When the distribution of microarthropods over the tree species habitat was evaluated, the average number of individuals per square meter is found to be 201,047 in Fir forests, 132,832 in Black pine forests and 92,641 in Beech forests (Table 3).

The site or habitat of each tree species was also evaluated separately. On an average, 84,043 individuals per·m^{-2} were found in the surface litter habitat. Specifically, the average number of microarthropods is 97,899 per·m^{-2} within the litter habitat of Fir forests, 97,486 per·m^{-2} within the litter habitat of Pine forests and 56,744 per·m^{-2} within the litter habitat of Beech forests. While the average number of microarthropods is 103,838 per·m^{-2} within the soil habitat, it is about 35,500 per·m^{-2} in two other habitats. Although the distribution of microarthropods over the tree species habitats or sites varies in diversity, only the diversity of the Mesostigmata and Diptera taxa was found to be statistically significant. These two taxa were also found to be higher in Fir habitats than Beech and Pine habitats (Table 3).

Diversity of microarthropods. The changes of tree species habitats did not cause any significant differences on the diversity of soil microarthropods according to Shannon's Diversity and Taxa Richness indexes. The average Shannon's Diversity Index was calculated to be H′ = 2.52 and Taxa Richness to be S′ = 22. Table 4 indicates that both Shannon's Diversity index and Taxa Richness index gradually increase with respect to Pine, Beech and Fir habitats.

TABLE 4
Diversity of soil microarthropods within the tree species habitats.

	Habitats of Tree Species				
	Pine	Beech	Fir	Mean ± SD	P
Shannon's Diversity (H′)	2.34	2.57	2.65	2.5 ± 0.3	0.113
Taxa Richness (S′)	19	21	26	22 ± 7	0.113

Both Shannon's Diversity index and Taxa Richness index indicate significant differences when the distributions of microarthropods to their habitats are investigated carefully. The surface litter has more diversity (H′ = 2.64 and S′ = 26) whereas the soil has less diversity (H′ = 2.34 and S′ = 18) within the associated habitats (Table 5).

TABLE 5
Diversity of soil microarthropods within the living spaces

	Living Spaces			
	Soil	Litter	Mean ± SD	P
Shannon's Diversity (H′)	2.39	2.64	2.5 ± 0.3	0.039
Taxa Richness (S′)	18	26	22 ± 7	0.005

DISCUSSION

The properties of soil and litter except litter moisture change according to the tree species habitats. Although the amount of soil skeleton and soil bulk density do not vary based on seasonal characteristics, both the soil and litter moisture do change according to the precipitation and the seasonal characteristics [28, 29]. Furthermore, the increase in soil skeleton has negative effects on the soil moisture [30].

Almost all of 74 taxa identified during this study were also detected more or less in all three sites. These taxa can survive under the ecosystems of dominated by different tree species. This indicates that the identified soil fauna has a widespread ecology. We found that microarthropods abundance in surface litter habitat are quite higher than that in soil habitat. Cakir and Makineci [31] reported that the abundance of microarthropods in Pine stands is 100,966 per·m^{-2} and 46,473 per·m^{-2} in Oak stands, based on a research conducted in spring season in Belgrad forests, Turkey. As in our results, their results seem to be quite higher in coniferous stands and lower in deciduous stands. Furthermore, similar to our results, [32] presented a similar other research, conducted in spring season, indicating that the abundance of microarthropods is about 167,895 per·m^{-2} in the litter habitat under Fir forests and 83,038 per·m^{-2} in the soil. In the same line of studies, [28] showed that there were 36,992 microarthropods per·m^{-2} in Beech stands and 53,278 individuals per·m^{-2} in surface litter.

Our results indicated that the abundance of microarthropods in surface litter habitat are quite similar to each other in Fir and Pine stands/habitats (about 97,500 individuals per·m^{-2}). However, the abundance of microarthropods in Beech stands is almost half the size in those two habitats. This could be attributed to the fact that the coniferous litter is accumulated and vertically layered with more porosity structure while the surface litter in Beech stands is layered vertically to hinder/decrease the capacity of nesting and movements of microarthropods. Moreover, while the litter habitats in both Pine stands and Fir stands have similar number of microarthropods, the soils in Pine stands accommodate only one third of living organisms compared to the soils in Fir stands. The principle reason may come from the fact that the soil moisture in Pine stands is

half the amount of soil moistures of both of other stands and the rate of soil skeleton is far above in Pine stands than that in other two coniferous stands. As known, the appropriate rate of soil moisture is quite important to feed and shelter the soil microarthropods [33, 34]. Moreover, the increase of soil skeleton causes to lower the amount of porosity and gaps in soil bulk density per unit, limiting the movement, shelter and feeding areas of microarthropods. Besides, being decreased the soil moisture in bulk density is thought to cause limitation in the sheltering capacity of soil microarthropods.

The measurements of biodiversity of soil fauna may be used as indicators in evaluating the natural status of forest habitats [35]. Our study results indicated that the diversity of soil microarthropods in each of the three tree species habitats is quite similar in each habitat (average $S' = 22$; $H' = 2.52$). The Collembolans extracted from litter and upper soil in Fir forests at 1400-1600 m elevation in France were identified at species level to investigate the effects of anthropogenic disturbances of habitat on the biodiversity. Menta et al. [35] showed that the number of taxa was 24 and the Shannon-Wiener index was 2.51 in managed Fir forests. In another study conducted by [36] in Italy, Shannon-Wiener index was found to be a bit lower ($H' = 1.2$) in 3 - 10 years fire affected Maritime pine forests. The results have clearly indicated that anthropogenic disturbances in natural environments have negative effects on biodiversity. The forests under investigation are naturally managed forests. The biodiversity values indirectly measured by two important fragmentation or biodiversity indicators reasonably coincide with those found by [35] in managed Fir forests. As such, it would obviously be stated that any disturbances to the natural forests whether managed or not should carefully be planned for management in order to maintain the biological diversity of forest ecosystems.

CONCLUSION

This research presented the preliminary results gathered during the spring season of an ongoing project designed to be conducted over a vegetation period. Significant differences were found between the soil and litter properties of tree species habitats. The soil moisture in Pine forests was lower than that in the other two tree species habitats. However, the associated soil bulk density, soil skeleton and the surface litter mass were found to be quite higher. The abundance of microarthropods differed among the habitats investigated in this study. Specifically, the number of individuals per square meter was about 201,047 in Fir forests, 132,832 in Pine forest and 92,641 in Beech forests. While the differences in microarthropods abundance exist, there were no significant differences in biodiversity values among the habitats. It is expected that the future results from the samples to be taken in both summer and fall seasons during the progress of the project would shed some lights on the understanding of microarthropods biodiversity within various habitats. Furthermore, any prospective studies to be conducted in various other geographic locations of the same tree species would undoubtedly enlighten the specific structure and biodiversity of habitats of the tree species and thus would be expected to contribute to the science in general.

ACKNOWLEDGEMENTS

This work was conducted with a support from the research project (Grant No: KBUBAP-17-KP-244) by the Coordinator of Scientific Research Projects in Karabuk University.

REFERENCES

[1] Sevik, H., Topacoglu, O., Umur, R. and Ciftcioglu, S. (2013) Genetic Variation Between Populations of Abies nordmanniana subsp. bornmulleriana Matff According to Morphological Features of 2+1 Years Seedling. The Black Sea Journal of Sciences. 3(9), 91-102.

[2] Iverson, L.R. and McKenzie, D. (2013) Tree-species range shifts in a changing climate: detecting, modelling, assisting. Landscape Ecology. 28(5), 879-889.

[3] Muscolo, A., Bagnato, S., Sidari, M. and Mercurio, R. (2014) A review of the roles of forest canopy gaps. Journal of Forestry Research. 25(4), 725-736.

[4] Kaynas, B.Y., Dogukan, D., Akcan, A., Ozkan, R. and Yucel, T. (2016) Evaluation of Different Habitats with regard to Arthropod Communities: Vegetations of Black Pine (Pinus nigra Arnold) and Almond (Prunus dulcis Mill.). The Journal of Graduate School of Natural and Applied Sciences of Mehmet Akif Ersoy University. 7(1), 38-43.

[5] Weidema, B.P. (2007) Framework for and review of biodiversity indicators for forest management in the context of product life cycle assessment. Draft report prepared for TetraPak. Hørsholm: 2.-0 LCA consultants.

[6] Jones, C.G., Lawton, J.H. and Shachak, M. (1994) Organisms as ecosystem engineers. Oikos. 373-386.

[7] Matz, C. and Kjelleberg, S. (2005) Off the hook-how bacteria survive protozoan grazing. Trends in Microbiology. 13(7), 302-307.

[8] Nakamura, A., Proctor, H. and Catterall, C.P. (2003) Using soil and litter arthropods to assess the state of rainforest restoration. Ecological Management and Restoration. 4(s1), 20-28.

[9] Fu-sheng, C., De-hui, Z., Xiao-fei, H., Guang-sheng, C. and Zhan-yuan, Y. (2007) Soil animals and nitrogen mineralization under sand-fixation plantations in Zhanggutai region, China. Journal of Forestry Research. 18(1), 73-77.

[10] Sridhar, K.R., Kadamannaya, B.S. and Karamchand, K.S. (2013) Nutrient composition of pill millipede manure of the Western Ghats, India. Journal of forestry research. 24(3), 539-545.

[11] ICP Forests, (2010) Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. UNECE, ICP Forests, Hamburg. www. icp-forests. org/Manual.htm (Access: 1 June 2017).

[12] Tasdemir, C., Aytar, F. and Ozturk, S. (2014) Implementation and Evaluation in Turkey of Crown Condition and Damage Agents within the Scope of International Co-Operative Programme (ICP Forests) on Assessment and Monitoring of Air Pollution Effects on Forests. II. National Mediterranean Forest and Environment Symposium: Future of Mediterranean forests: Sustainable society and environment. 22-24 October 2014 – Isparta.

[13] General Directorate of Forestry (GDF) (2013) The Healthy Situation of Turkey's Forests (2008 – 2012), Technical Report of ICP Forests.

[14] Anonymous. (2018) https://en.climate-data.org (Accessed on 8 March 2018)

[15] Meehan, T.D., Drumm, P.K., Schottland, F.R., Oral, K., Lanier, K.E., Pennington, E.A., Pennington, L.A., Stafurik, I.T., Valore, D.V. and Wylie, A.D. (2006) Energetic equivalence in a soil arthropod community from an aspen-conifer forest. Pedobiologia. 50, 307-312.

[16] Coleman, D.C., Crossley, D.A. and Hendrix, P.F. (2004) Fundamentals of Soil Ecology. Academic press, USA.

[17] Dombos, M. (2002) A Tullgren–type extractor for sampling springtails populations from small volume soil cores in high sample size. Tiscia. 33, 3-7.

[18] Joo, S.J., Yim, M.H. and Nakane, K. (2006) Contribution of microarthropods to the decomposition of needle litter in a Japanese cedar (Cryptomeria japonica D. Don) plantation, Forest Ecology and Management. 234, 192-198.

[19] Salmon, S., Mantel, J., Frizzera, L. and Zanella, A. (2006) Changes in humus forms and soil animal communities in two developmental phases of Norway spruce on an acidic substrate. Forest Ecology and Management. 237, 47-56.

[20] Bird, S.B., Coulson, R.N. and Fisher, R.F. (2004) Changes in soil and litter arthropod abundance following tree harvesting and site preparation in a loblolly pine (Pinus taeda L.) plantation. Forest Ecology and Management. 202, 195-208.

[21] Bird, S., Coulson, R.N. and Crossley Jr., D.A. (2000) Impacts of silvicultural practices on soil and litter arthropod diversity in a Texas pine plantation. Forest Ecology and Management. 131(1-3), 65-80.

[22] Santos, S.A.P., Cabanas, J.E. and Pereira, J.A. (2007) Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. European Journal of Soil Biology. 43, 77-83.

[23] Dindal, D.L. (1990) Soil Biology Guide. Wiley, New York.

[24] Moldenke, A.R., Fichter, B.L. and Forest, H.A.E. (1988) Invertebrates of the HJ Andrews Experimental Forest, western Cascade Mountains, Oregon. IV, the oribatid mites (Acari: Cryptostigmata), Portland, Or.: US Dept. of Agriculture, Forest Service, Pacific Northwest Research Station.

[25] Meyer, J.R. (1994) Kwik-key to soil-dwelling invertebrates. Raleigh N.C., Vision Press.

[26] Bei-Bienko, G.Y., Blagoveshchenskii, D.I., Chernova, O.A., Datsig, E.M., Emel'yanov, A.F., Kerzhner, I.M., Loginova, M.M. and Martynova, E.F. (1967) Keys to Insects of the European USSR. Akademiya Nauk, USSR.

[27] Wiwatwitaya, D. and Takeda, H. (2005) Seasonal changes in soil arthropod abundance in the dry evergreen forest of north-east Thailand, with special reference to collembolan communities. Ecological Research. 20, 59-70.

[28] Cakır, M. (2013) Seasonal Changes of Soil Arthropods in Beech and Oak Ecosystems and Their Effects on Litter Decomposition. Istanbul University, Science Institute. Istanbul (In Turkish with English Summary).

[29] Duyar, A. (2014) Seasonal variatıon of soil arthropods (Arthropoda) in fir (Abies bornmulleriana Mattf.) ecosystems in Bolu-Aladag. Istanbul University, Science Institute, Istanbul (In Turkish with English Summary).

[30] Lazorík, M. and Kula, E. (2015) Impact of weather and habitat on the occurrence of centipedes, millipedes and terrestrial isopods in mountain spruce forests. Folia Oecologica. 42(2), 103-112.

[31] Cakır, M. and Makineci, E. (2013) Humus characteristics and seasonal changes of soil arthropod communities in a natural sessile oak (Quercus petraea L.) stand and adjacent Austrian pine (Pinus nigra Arnold) plantation. Environmental Monitoring and Assessment. 185, 8943-8955.

[32] Duyar, A. and Makineci, E. (2016) Seasonal and altitudinal variations of soil arthropods in Abies nordmanniana subsp. bornmulleriana forests. Bosque. 37(2), 335-345.

[33] Vannier, G. (1970) Response of micro-arthropods to variations of soil-moisture conditions. Techniques for extracting arthropods from soil. Revue d'Ecologie et de Biologie du Sol. 7, 289-309.
[34] Shakir, M.M. and Ahmed, S. (2015) Seasonal abundance of soil arthropods in relation to meteorological and edaphic factors in the agroecosystems of Faisalabad, Punjab, Pakistan. International journal of biometeorology. 59(5), 605-616.
[35] Cassagne, N., Gauquelin, T., Bal-Serin, M.C. and Gers, C. (2006) Endemic Collembola, privileged bioindicators of forest management. Pedobiologia. 50(2), 127-134.
[36] Menta, C., Conti, F.D. and Pinto, S. (2018) Microarthropods biodiversity in natural, seminatural and cultivated soils-QBS-ar approach. Applied Soil Ecology. 123, 74-743.
[37] Lisa, C., Paffetti, D., Nocentini, S., Marchi, E., Bottalico, F., Fiorentini, S. and Travaglini, D. (2015) Impact of wildfire on the edaphic microarthropod community in a Pinus pinaster forest in central Italy. iForest-Biogeosciences and Forestry. 8(6), 874.

Received: **30.03.2018**
Accepted: **04.11.2018**

CORRESPONDING AUTHOR

Ahmet Duyar
Department of Forest Engineering,
Faculty of Forestry,
Karabuk University,
Karabuk – Turkey

e-mail: ahmetduyar@karabuk.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9642-9649 Fresenius Environmental Bulletin

THE EFFECTS OF FOREST CANOPY COVER AND ALTITUDE ON SNOW ACCUMULATION AND MELTING IN THE UPPER WATERSHEDS

Ahmet Duyar*

Karabuk University, Faculty of Forestry, Department of Forestry Engineering, Karabuk, Turkey

ABSTRACT

A significant amount of water falls down on the ground with snowfall. Therefore, the snow cover accumulated in the upper watersheds can reservoir very large amounts of water. Snow masses may melt at different speeds depending on climate conditions. These reservoirs can regularly feed water resources in slow melting conditions or cause floods with sudden melts. Knowing some of the effective environmental characteristics on the accumulation of snow masses can render possible the estimated amount of water reserves and the extent of flood risks. For this purpose, the snow accumulation and the amount of snow water equivalent at the open area and under the different forest canopies were investigated in the upper watersheds. The selected study area is located in southeast of Bolu province (northwestern Turkey) and consists sampling points on route of Kartalkaya Ski Center (2000 m a.s.l.). In total 11 permanent sample areas were determined between 936 m and 1930 m (a.s.l.) altitudes. Three sampling points were selected as the open area, the semi closed and the fully closed forests at each sample area. The samples were collected three times in a week from December to April. The snow depths (SD) were measured at all sampling points. Snow densities and snow water equivalents (SWE) were determined in the laboratory by using snow core samples taken from the sites. In the evaluation of the samples, Correlation, ANOVA and Duncan analysis were applied to the data, such as independent variables of time, forest cover, altitude and aspect affecting the snow accumulation and dependent variables of SD, SWE and snow density. When the data obtained is evaluated, SD and SWE negatively correlated with forest closures, and positively correlated with altitudes. SD (762 mm) and SWE (170 mm) reached maximum values at open areas in February. Forest canopies significantly reduced the snow accumulations compared to the open areas. The average snow depth was observed in different values within open areas (371 mm), semi closed forests (229 mm) and fully closed forests (165 mm). Similarly, the water equivalent of snow was observed as 86 mm in open areas, 53 mm in semi closed forest, and 38 mm in fully closed forest. Although the snow density did not show a significant difference depending on the forest canopies, it increased based on altitudes and dates (221 g/l in December and 368 g/l in April). The amounts of SD and SWE in open areas were formulated according to altitudes by using the findings. In this way, it is considered that snow accumulation, water reserve and possible water regimes in the upper watersheds can be estimated depending on the snowfall information in a region.

KEYWORDS:
Bolu, altitude, snow density, snow depth, snowfall, snow-water equivalent

INTRODUCTION

The water is the most important condition for the maintenance of land ecosystems. The water which is mixed with the atmosphere through evaporation from the earth returns to the earth in various forms of precipitation [1]. Snow is one of the important precipitation forms. From the beginning of the winter months, the snow falling at varying amounts and intervals covers the earth. Even if snow cover melts and disappears in lower altitudes in a short time, it accumulates and deepens due to altitude increase. Snowfall and accumulation in the upper watersheds are important in terms of water balance of the forests, drinking and tap water resources [2, 3] and natural disasters such as floods and avalanche [4, 5]. The snow masses that accumulate in the upstream of watersheds and are able to store water in very high quantities both can feed water resources regularly under slow melting conditions and they are important factors that can cause to floods [6] and erosion [7] due to sudden melting. Throughout the season, although the snowfall and melting continue constantly, melting is accelerated by heating the air in the spring. However, snow mass accumulation and melting rate can also vary according to the aspect and topography [8].

The amount of snowfall is increasing in direct proportion to the altitude [3, 9]. The forest canopy cover is effective on the amount of snow that accumulates on the ground by holding some of the falling snow [10, 11]. These studies have been

made about the water balance of the basin, melting and sublimation in snow cover and its influence on the stand structure and emphasized that the snow cover depth, the duration of snowy days and snow-water equivalent (SWE) amounts have some of the uncertainties.

For the estimation of water efficiency of the basins, various models including the amount of precipitation, topography, vegetation and climate characteristics are used [12, 13]. The snow depth can be estimated by a mathematical model which uses topographical factors such as altitude, slope and aspect [8]. By using the mathematical models to be formed for snow accumulation, the probable snow depth and SWE values can be estimated. In this view, basin's flow forecasts can be accomplished.

The aim of this study was to determine the effect of altitude, aspect and forest cover on snow characteristics in the upper watersheds. Another aim was to compare the snow water equivalent (SWE) for under canopy areas compared with open areas. In this study, it was made use of a road route of Kartalkaya ski resort which provides working and sampling opportunities since the road is always open during the winter season. Here, the effects of the duration of snow, altitude, slope, aspect and the forest canopy cover on the depth and density of snow cover and SWE snow-water equivalent have been investigated.

MATERIALS AND METHODS

Study area. This study was conducted in a mixed forest 38 kilometers southeast of Bolu, Turkey in the Kartalkaya ski center (40°35'00"-40°45'00" N, 31°43'00"-31°50'00" E); (Figure 1). Forests are dominated by larch from 900 to 1300 m, at upper altitudes and fir on north aspects, and Scotch pine on south aspects. Sample sites ranged in altitude from 936-1930 m a.s.l. and are annually snow covered from December 2007 to April 2008. This study area was chosen due to easy road access throughout the winter.

Data collection method. The sample areas at which the snow measurements are to be made are shown in Figure 1. In total, 11 sample areas were selected between 936 m and 1930 m (a.s.l.) where 3 of them with south aspect and remains with north aspect. Three sampling points are specified at each sample area. Sampling points were selected from forests that are semi closed (SC) at canopy cover of 40-70% and fully closed (FC) forests at canopy cover of 71-100% and non-forested open area (OA). The permanently snow poles scaled in the length of 1.75 m were set up on these specified sampling points [14].

Both depth and density samplings were repeated three times a week during December to April. The depth of the snow layer was measured at all points [14]. Bulk density snow samples taken from the undisturbed snow cover with a cylinder tube in 70 mm diameter were examined in the laboratory and snow densities and snow water equivalents were determined [15].

Data analysis. In the evaluation of the samples, the correlation of the independent variables of date, forest cover, altitude and aspect affecting the snow accumulation and dependent variables of snow depth, snow density and SWE were examined. Moreover, the change of measured values according to independent variables have been revealed by one-way variance analysis. The differences between means were separated by multiple range test of Duncan.

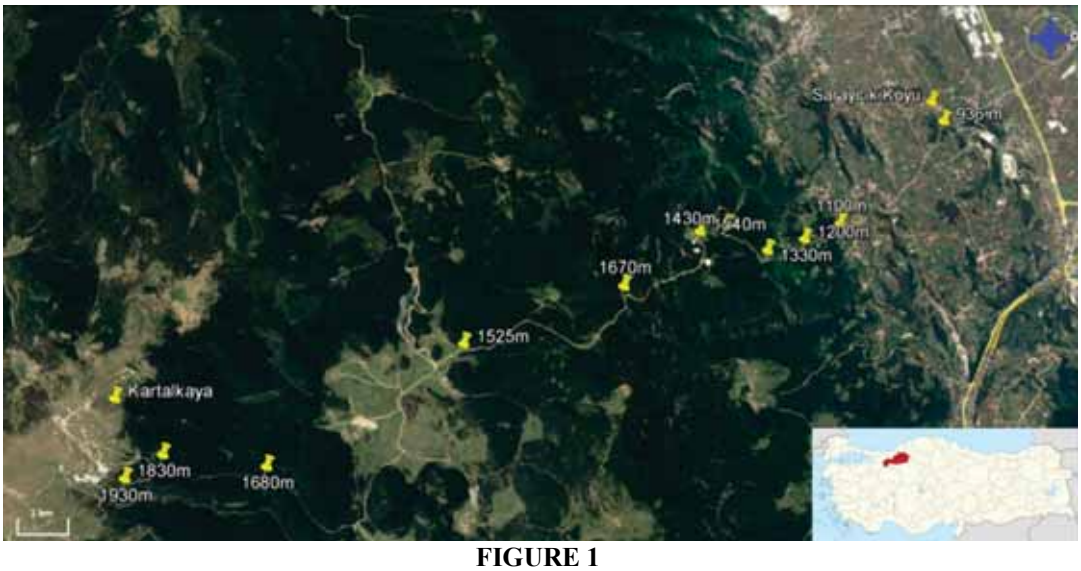


FIGURE 1
Study area and sampling sites near Bolu, Turkey (Google Earth) since each sampling site is marked with a pin in the image.

RESULTS

Relation of snow parameters and site conditions. Snow density is positively associated with date, altitude and north. Snow depth and SWE were found to be positively correlated with elevation and north and negatively correlated with forest canopy cover (Table 1). These relationships are discussed in detail below.

Temporal variations of Snow Measurement Parameters. First snow accumulation in the field of study started in the first week of December and disappeared from the second week of April (Figure 2). While snow depth is increasing over time with new snowfalls throughout winter season, it tends to decrease with ongoing melting and compression processes on the other hand. Within these processes, the average snow depth gradually increased from December to the highest amount (762 mm) in February. From the beginning of March, it has decreased to an average depth of 150 mm with a rapid melting and compacting first. Snow cover which continued its presence despite decreasing until the end of March with the effect of snowfall declined rapidly in April. However, at high altitudes in the north, snow blocks in fragments were seen even in June.

The average density of the snow layer has generally decreased in fresh snowfall periods, as can be seen in the graph in Figure 2. This case resulted from the effect of new snow cover having lower density that accumulates on is due to the effect on the average density of the new snow layer of lower density that accumulates on the relatively denser old snow layer. The SWE of the snow cover (the amount of water stored) increased from the beginning of the snowy season and reached to the highest value (170 mm) at the end of February. The snow cover in the field of study started decreasing from March and it disappeared completely in the middle of April. Due to snow density that is lower in the first months of the winter, although SWE of thicker snow layers are lower, the snow masses in the same depth compacted more in date towards the end of the season have more snow water equivalent (Figure 2).

TABLE 1
Correlation analysis of measured snow parameters and site conditions

	Snow Density	Snow Depth	Snow-Water Equivalent
Date	0.397**	0.077**	0.153**
Altitude	0.210**	0.558**	0.565**
Aspect (Northness)	0.154**	0.209**	0.231**
Canopy cover	-0.040	-0.307**	-0.295**
Snow Density	1	0.094**	0.365**
Snow Depth		1	0.945**

* Correlation is significant at the 0.05 level and ** correlation is significant at the 0.01 level (2-tailed).

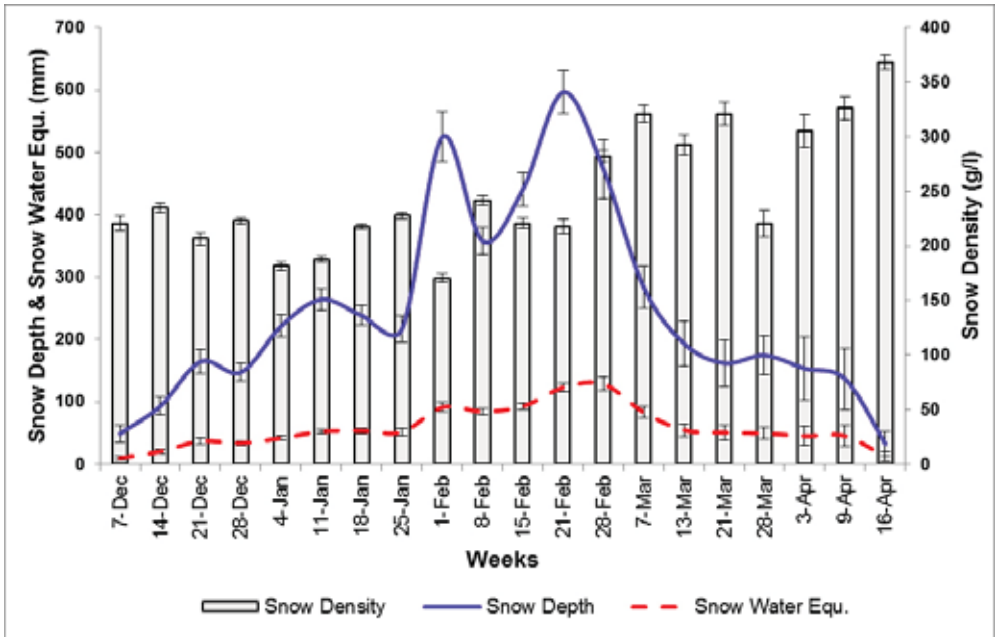


FIGURE 2
Temporal variations of mean values of snow depth, snow-water equivalent and snow density for all sampling points.

Variation of Measured Snow Parameters Due to Altitude and Aspect. As can be seen in Figure 3, snow depth with increases altitude. Northern aspects tended to collect more snow than southern aspects at similar altitudes. SWE, as a derivative of depth and density followed the same trend. Northern aspects tended to collect more snow than southern aspects at similar altitudes. SWE, as a derivative of depth and density followed the same trend. Northern aspects tended to collect more snow than southern aspects at similar altitudes. SWE, as a derivative of depth and density followed the same trend.

Variation of Measured Snow Parameters Depending on Canopy Cover. For each altitude, the snowfall characteristics measured at OA are considered normal of that altitude. In the other SC and FC, measures related to snow parameters were evaluated by comparing with OA.

It has been determined that the forest canopy cover has a significant effect on snow depth accumulated on the ground. 63% of snow cover in OA was observed in SC forest and 43% of it in FC forest. The aspect also had an impact on snow accumulation with canopy cover. When evaluated one by one according to the aspect, snow depth occurred to be 68% in SC south aspect and 60% in north aspect; while 43% in FC south aspect and 41% in north aspect (Table 2).

Based on the open areas in north aspect, the equation that expressing change of snow depth depending on the altitude was found as follows (R^2 = 0.83);

$$SD = 0.0009 \times A^2 - 1.10199 \times A + 587.93 \quad (1)$$

Here; SD displays open area snow depth (mm), A displays altitudes (m). The other snow depths accumulated under forest can be calculated by considering and proportioning canopy cover level.

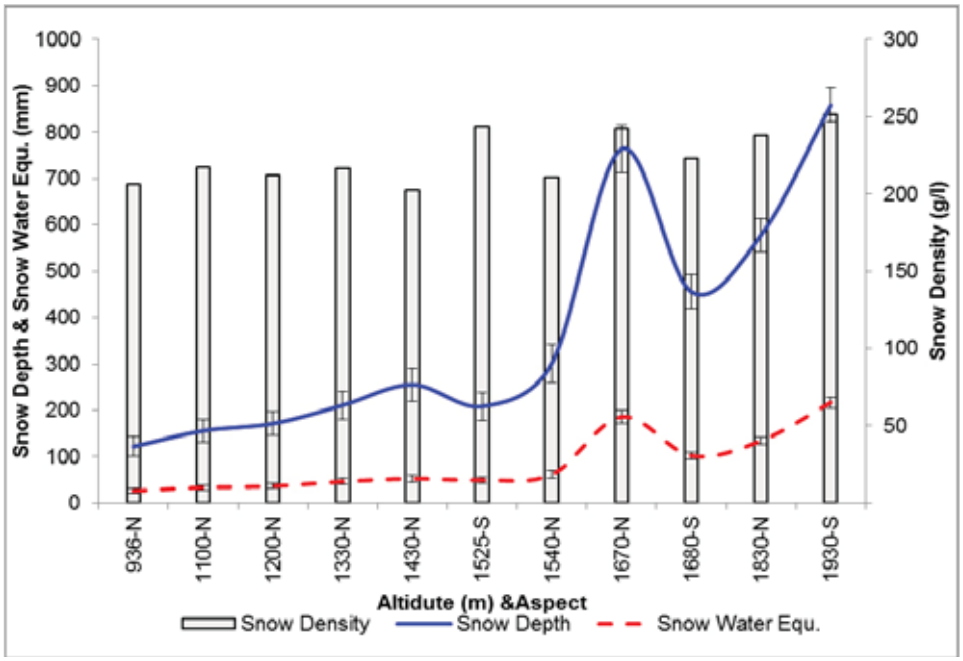


FIGURE 3
Effect of altitude and aspect on snow depth, snow-water equivalent and snow density.

TABLE 2
Snow depth variations vs altitude and canopy cover closure

		Snow Depth (mm)				
Altitudes	**Aspect**	**Open Area**	**Semi Closed**	**Fully Closed**	**Mean**	**P**
936	N	122 b	53 a	37 a	71±103	0.001
1100	N	156 b	64 a	49 a	90±118	0.001
1200	N	172 b	140 b	72 a	128±147	0.004
1330	N	211 b	163 b	60 a	145±166	0.001
1430	N	256 b	122 a	85 a	154±183	0.001
1525	S	208 b	202 b	108 a	173±174	0.009
1540	N	302 b	169 a	218 ab	230±223	0.017
1670	N	764 c	360 b	212 a	445±351	0.001
1680	S	456 b	207 a	170 a	278±227	0.001
1830	N	578 b	526 b	394 a	499±252	0.002
1930	S	858 c	517 b	410 a	595±295	0.001
Mean		**371±334**	**229±238**	**165±189**	**255**	
P		0.001	0.001	0.001		

TABLE 3
Variations of SWE with altitude and aspect

		Snow Water Equivalent (mm)				
Altitudes	**Aspect**	**Open Area**	**Semi Closed**	**Fully Closed**	**Mean**	**P**
936	N	26 b	11 a	8 a	15±20	0.001
1100	N	34 b	14 a	10 a	19±21	0.001
1200	N	36 b	30 b	16 a	27±31	0.009
1330	N	46 b	36 b	14 a	32±33	0.001
1430	N	52 b	25 a	17 a	31±34	0.001
1525	S	49 b	51 b	27 a	42±42	0.015
1540	N	62 b	34 a	46 ab	47±43	0.015
1670	N	186 c	86 b	50 a	107±64	0.001
1680	S	102 b	43 a	35 a	60±38	0.001
1830	N	134 b	122 b	91 a	116±54	0.001
1930	S	217 c	130 b	102 a	150±62	0.001
Mean		**86±83**	**53±57**	**38±44**	**59**	
P		0.001	0.001	0.001		

TABLE 4
Variations mean snow depth vs months and canopy closure

	Snow Depth (mm)				
Months	**Open Area**	**Semi Closed**	**Fully Closed**	**Mean**	**P**
December	190 c	112 b	63 a	122±128	0.001
January	390 c	237 b	160 a	262±176	0.001
February	621 c	422 b	328 a	457±250	0.001
March	316 b	175 a	135 a	209±296	0.001
April	191 b	85 ab	47 a	108±224	0.046
Mean	**342±294**	**206±200**	**147±151**	**232±215**	
P	0.001	0.001	0.001		

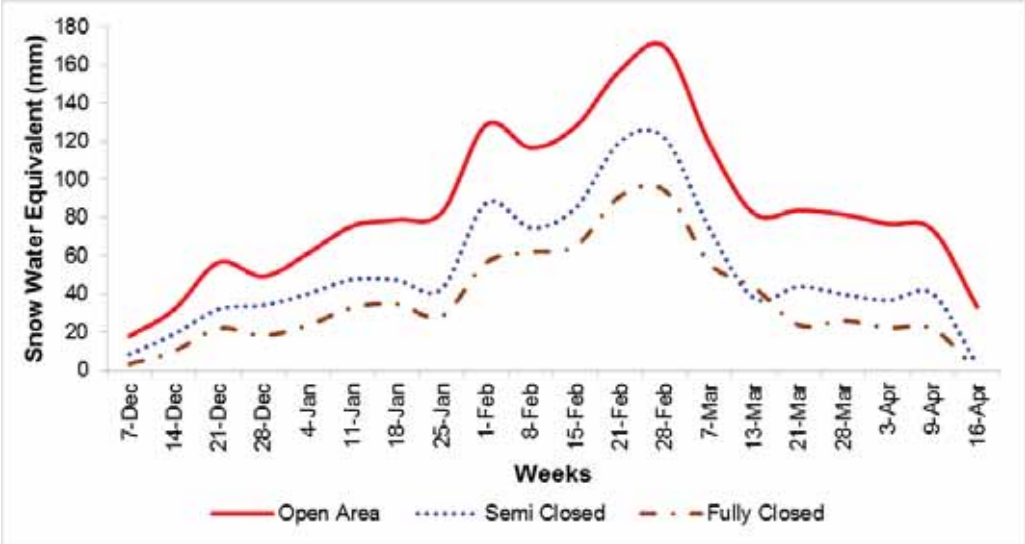


FIGURE 4
SWE variations vs date and canopy closure

SWE accumulated on the basis of forest changes depending on canopy closure. Inversely proportionally to the increase in canopy closure density, SWE amount decreases (Table 3).

In order to express the altitude-dependent change of snow-water equivalent, this equation (R^2 = 0.82) has been formed by considering the sampling points in the open areas.

$SWE = 0.0003 \times A^2 - 0.5419 \times A + 319.32$ (2)

Here; SWE displays Snow Water Equivalent (mm) and A displays altitudes (m). The other snow depths accumulated under forest can be calculated by considering canopy cover level (Table 3).

The change of snow cover depth depending on the months and canopy cover level is significantly different (Table 4). Compared to monthly average snow depth of open areas, SC forests accumulated 58% less average monthly snow and only 39% accumulated in FC forests. As it is detected in the monthly change, the highest average snow depth was detected to be highest (457 mm) in February and the lowest in April (108 mm). Besides, the snow depth ratio under forest was found to be highest in February compared to the open area and it

was observed to be 68% under SC and 53% under FC. The lowest ratio appeared in April. The lowest snow depth was detected to be 45% under SC and 25% under FC compared to OA (Table 4).

SWE stored on the ground displayed significant differences depending on the canopy closure level. Compared to the open area, water-snow equivalent stored under forest is lower at the ratio of 40% under SC and 60% under FC (Figure 4).

SWE peaked up to 170 mm at open area in February. In respect of water efficiency of the basins the water stock in the open area is about 1700 m^3 per hectare in February.

DISCUSSIONS

Snow accumulation started in December on Bolu Mountains melts up to the middle of April and disappears. Early period snow was less dense and become denser over the last period melt season holding a greater quantity of water with less depth. During this period, snow cover depth peaked within February. This value was found to be 762 mm in the OA, 574 mm in SC forest and 454 mm in FC forest. Similar results were found in a study conducted in the Spanish Pyrenees. The snowfall started in December and peaked up to (about 900 mm) at open area in February. Besides, the open area accumulated much more snow (average 42%) than occurred under the canopy. The snow cover was completely melted and disappeared in May [16]. Kremsa et al. [7] stated that the time of snow cover in Finland lasted for 6-8 months and the highest average snow depths is 1150 mm under forest in March- April and 935 mm in OA. Similarly, in a study carried out in Colorado (U.S.A.), Broxton et al. [17], it is stated that the snowfall period is from November to April. Annual cumulative snowfall reaches up to 537 mm in the open area.

Although less snow accumulates under forest on Bolu Mountains depending on the level of canopy cover closures compared to the open area in the same altitude, thicker snow cover accumulates under forest in semi-boreal forests in Finland [7]. Besides, when compared in terms of weekly SWE, SWE was found to be 134 mm in OA in the forests of Bolu in February, 93 mm in SC forest and 71 mm in FC forest while it is found to be 215 mm in the OA in Finland, 286 mm under forest [7]. The reason of this is considered that the forests of Bolu (Turkey) that is located in the mild temperature zone, reach to the temperature level enough for the melting and sublimation of the snow clanged to the tree crowns even in winter, in Finland which is located in boreal climate zone, the snow accumulated on tree branches could not find the opportunity to melt and accumulates by falling on the forest ground.

In the observations made during the measurements, it can be seen that old snow cover compacted by transforming into ice layers and ice crystals. Therefore, in March and April, when the new snowfall was less, and melting was more, the snow intensity reached to the highest values. Snow density tends to increase with the ongoing time of snowy season and the depth of snow (Figure 1). Sexstone and Fassnacht [18] state that the snow density tends to increase step by step with the crystallization, subsiding and compacting of snow during snowy season. At the same time, altitude is also effective on snow density [19]. Since topography and forest cover affect insolation also affect snow density. Local differences in land conditions and canopy cover closure can change snowpack [18].

Forest canopy cover closure played an important role in the distribution of snow amount (Table 2 and 3). Sexstone and Fassnacht [18] list precipitation amount, sun radiation, wind, topography and vegetation cover as main factors that are effective on the distribution of snow and the feature of snowpack. The sublimation of snow clinging to forest canopy cover by inception is one of the main components within total water balance [20]. Melting and sublimation ratio of the snow is directly correlative with heat and relative air humidity [12].

SWE, density, depth, northness and altitude was found to be positively correlated with each other. However, canopy cover was found to be negatively correlated with SWE and snow depth (Table 1). Similar to the findings, Sexstone and Fassnacht [18] canopy cover and SWE are expressed as negatively correlated. In mountainous lands, being able to be completely expressed of the spatial distribution of SWE has a critical importance in the evaluation of snow as a water source [21-23]. Although high flow rates are observed in the rivers fed by high basins in associated with spring and autumn [6], flow rates of precipitation remain lower in winter months in proportion to the falling precipitation because of snowpack. The distribution of snow in the basin and inception, melting and sublimation ratios and rates show differences under the effect of local factors [24]. Depending on these facts, the depth of the snow, the duration of remaining on the ground and SWE amount include some uncertainties [25, 26].

In order to estimate the water efficiency of the basins, various models have been used including precipitation amount, topography, vegetation and climate characteristics [12, 13, 26-29]. In this study, regression equation for each was formed to express snow depth and SWE depending on the altitude. The equations are appropriate for explaining the data belonging to the open areas in the north aspect. The effect of canopy cover closure can be estimated proportionally over these values [30-32]. But they stated that it is possible to make more effective evaluations by including topographic factors such as aspect and slope in the equation formed by [6].

CONCLUSION

On the route of Bolu – Kartalkaya route, between the altitudes of 936 – 1930 m, snowy season continues from the beginning of December to the middle of April. The period when snow depth is the highest is the last week of February. In this period, it reaches to the highest value in SWE. Forest canopy cover closure decreases snow accumulation ratio. Snow depth and SWE increases depending on the altitude. This increase is expressed by a regression equation and this makes possible to calculate the water amount contained by it. So, it is possible to determine basin water efficiency and possible flood possibilities.

In the next studies, it is required to determine compound effects of radiation on regional basis, climatic data such as wind rate, relative air humidity etc. various topographic factors on snow accumulation. Besides, interception amounts of basic forest species must be revealed. This will allow making more precise estimations for broader areas.

REFERENCES

[1] Kocak, K. (2011) Short Story of a Water Drop. Bilim ve Aklın Aydınlığında Eğitim. 132, 10-20. [In Turkish].

[2] Marks, D., Kimball, J., Tingey, D. and Link, T. (1998) The sensitivity of snow melt processes to climate condition sand forest cover during rain on snow: a case study of the 1996, Pacific Northwestflood. Hydrological Processes. 12, 1569-1587.

[3] Sugiura, K., Kadota T., Zhang, Y., Ishikawa M., Iijima Y., Yamazaki T., Purevdagva K., Davaa G. and Ohata, T. (2006) Altitudinal Distribution of Snow Water Equivalent in the Tuul River Basin, Mongolia, 2002-2006.

[4] Kose, N., Aydin, A., Akkemik, U., Yurtseven, H. and Guner, T. (2010) Using tree-ring signals and numerical model to identify the snow avalanche tracks in Kastamonu, Turkey. Natural Hazards. 54(2), 435-449.

[5] Volk, G., Aydın, A. and Eker, R. (2015) Avalanche Control with Mitigation Measures: A Case Study from Karaçam-Trabzon (Turkey). Eur J Forest Eng. 1(2), 61-68.

[6] Zheng, Z., Kirchner, P.B., and Bales, R.C. (2016) Topographic and vegetation effects on snow accumulation in the southern Sierra Nevada: a statistical summary from lidar-data. The Cryosphere. 10(1), 257-269.

[7] Kremsa, J., Křeček, J. and Kubin, E. (2015) Comparing the impacts of mature spruce forests and grasslands on snow melt, water resource recharge, and run-off in the northern boreal environment. International Soil and Water Conservation Research. 3(1), 50-56.

[8] Buldur, A. and D. Sari, S. (2012) Çarşamba River's Overflow on the date of 15 December 2010 and It's Effect in Bozkır (Konya). Marmara Coğrafya Dergisi. 25, 81-107. [In Turkish].

[9] Putkonen, J.K. (2004) Continuous snow and rain data at 500 to 4400 m altitude near Annapurna, Nepal, 1999–2001. Arctic, Antarctic, and Alpine Research. 36(2), 244-248.

[10] Suzuki, K., Kodama, Y., Yamazaki, T., Kosugi, K. and Nakai, Y. (2008) Snow accumulation on evergreen needle-leaved and deciduous broad-leaved trees. Boreal Environment Research. 13, 403-416.

[11] Storck, P. and Lettenmaier, D.P. (2002) Measurement of snow interception and canopy effects on snow accumulation and melt in a mountainous maritime climate, Oregon, United States. Water Resources Research. 38(11), 1223.

[12] Feiccabrino, J., Graff, W., Lundberg, A., Sandström, N. and Gustafsson, D. (2015) Meteorological Knowledge Useful for the Improvement of Snow Rain Separation in Surface Based Models, Hydrology. 2(4), 266-288.

[13] Troin, M., Arsenault, R. and Brissette, F. (2015) Performance and Uncertainty Evaluation of Snow Models on Snowmelt Flow Simulations over a Nordic Catchment (Mistassibi, Canada). Hydrology. 2(4), 289-317.

[14] Anonymous (2005) Avalanche Observations Handbook, Ministry of Public Works and Settlement, General Directorate of Disaster Affairs, Ankara. [In Turkish].

[15] Ozkaya, M., Kaya, Y., Goveli, M. and Urgenç, B. (2007) Snow Observations [1966 – 2005], General Directorate of Electrical Power Resources Survey and Development Administration, Ankara, ISBN 978/975/8964/68/0. [In Turkish].

[16] Revuelto, J., López-Moreno, J.I., Azorin- Molina, C. and Vicente-Serrano, S.M. (2015) Canopy influence on snow depth distribution in a pine stand determined from terrestrial laser data. Water Resources Research. 51(5), 3476-3489.

[17] Broxton, P.D., Harpold, A.A., Biederman, J.A., Troch, P.A., Molotch, N.P. and Brooks, P.D. (2015) Quantifying the effects of vegetation structure on snow accumulation and ablation in mixed-conifer forests. Ecohydrology. 8(6), 1073-1094.

[18] Sexstone, G.A. and Fassnacht, S.R. (2014) What drives basin scale spatial variability of snow pack properties in northern Colorado? The Cryosphere. 8(2), 329-344.

[19] Singh, K.K., Negi, H.S., Kumar, A., Kulkarni, A.V., Dewali, S.K., Datt, P. and Kumar, S. (2017) Estimation of snow accumulation on Samudra Tapu glacier, Western Himalaya using airborne ground penetrating radar. Current Science. 112(6), 1208-1218.
[20] Roth, T.R. and Nolin, A.W. (2017) Forest impacts on snow accumulation and ablation across an elevation gradient in a temperate montane environment. Hydrology and Earth System Sciences. 21(11), 5427-5442.
[21] Wetlaufer, K., Hendrikx, J. and Marshall, L. (2016) Spatial Heterogeneity of Snow Density and Its Influence on Snow Water Equivalence Estimates in a Large Mountainous Basin. Hydrology. 3(1), 3.
[22] Rittger, K., Bair, E.H., Kahl, A. and Dozier, J. (2016) Spatial estimates of snow water equivalent from reconstruction. Advances in water resources. 94, 345-363.
[23] Schneider, D. and Molotch, N.P. (2016) Real-time estimation of snow water equivalent in the Upper Colorado River Basin using MODIS-based SWE Reconstructions and SNOTEL data, Water Resources Research. 52(10), 7892-7910.
[24] Devia, G.K., Ganasri, B.P. and Dwarakish, G.S. (2015) A review on hydrological models. Aquatic Procedia. 4, 1001-1007.
[25] Tayefeh Neskili, N., Zahraie, B. and Saghafian, B. (2017) Coupling snow accumulation and melt rate modules of monthly water balance models with the Jazim monthly water balance model. Hydrological Sciences Journal. 62(14), 2348-2368.
[26] Dickerson-Lange, S.E., Gersonde, R.F., Hubbart, J.A., Link, T.E., Nolin, A.W., Perry, G.H. and Lundquist, J.D. (2017) Snow disappearance timing is dominated by forest effects on snow accumulation in warm winter climates of the Pacific Northwest, United States. Hydrological processes. 31(10), 1846-1862.
[27] Middelkoop, H., Daamen, K., Gellens, D., Grabs, W., Kwadijk, J.C., Lang, H. and Wilke, K. (2001) Impact of climate change on hydrological regimes and water resources management in the Rhine basin. Climatic change. 49(1-2), 105-128.
[28] Leavesley, G.H. (1994) Modeling the effects of climate change on water resources - a review, In: Assessing the Impacts of Climate Change on Natural Resource Systems. Springer, Dordrecht. 159-177.
[29] Morán-Tejeda, E., Zabalza, J., Rahman, K., Gago-Silva, A., López-Moreno, J.I., Vicente-Serrano, S. and Beniston, M. (2015) Hydrological impacts of climate and land-use changes in a mountain watershed: uncertainty estimation based on model comparison. Ecohydrology. 8(8), 1396-1416.
[30] Kirchhoff, M.D. and Schoen, J.W. (1987) Forest cover and snow: implications for deer habitat in southeast Alaska. The Journal of wildlife management. 51(1), 28-33.
[31] Stevens, J.T. (2017) Scale-dependent effects of post-fire canopy cover on snowpack depth in montane coniferous forests. Ecological Applications. 27(6), 1888-1900.
[32] Hubbart, J.A., Link, T.E. and Gravelle, J.A. (2015) Forest canopy reduction and snowpack dynamics in a northern Idaho watershed of the continental-maritime region, United States. Forest Science. 61(5), 882-894.

Received: **31.03.2018**
Accepted: **04.11.2018**

CORRESPONDING AUTHOR

Ahmet Duyar
Karabük University,
Faculty of Forestry,
Department of Forestry Engineering
Karabük – Turkey

e-mail: ahmetduyar@karabuk.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9650-9661 Fresenius Environmental Bulletin

ENVIRONMENTAL DEGRADATION ANALYSIS USING GIS IN TEKIRDAG PROVINCE, TURKEY

Emre Ozsahin*, Ilker Eroglu

Department of Geography, Faculty of Art and Science, Namik Kemal University, Tekirdag, Turkey

ABSTRACT

In this study, we analyzed environmental degradation using Geographic Information Systems (GIS) techniques. Within the scope of this study, the Environmentally Sensitive Areas (ESA) index method was employed to determine the degrees of influence and the scoring of environmental classifications. These classifications are based on three different critical environmental factors, specifically Soil Quality Index (SQI), Climatic Quality Index (CQI), and Vegetation Quality Index (VQI). Thematic maps, soil analyses, and meteorological records were used as secondary data. The degradation of the study area was explored and mapped based the analysis of the factors influencing environmental sensitivity. The results indicated that in the Tekirdag province that has no classes of low and medium sensitivity areas, the largest area (62%) is subject to environmental degradation.

KEYWORDS:
Environmentally Sensitive Areas (ESA) Index, Soil Quality Index (SQI), Climatic Quality Index (CQI), Vegetation Quality Index (VQI), Tekirdag.

INTRODUCTION

Increased human settlement and population demands have led to increased pressure on landscape and natural resources [1]. Environmental equilibrium has become unbalanced due to inappropriate or excessive human activities, which have increased in recent decades [2]. This process, which has persisted since ancient times, had been underestimated, and the severity of the problems had been understood only after they started to threaten world ecosystems [3].

Environmental degradation refers to the deterioration of ecosystems, which is an important environmental problem that has emerged as a result of negative human influence [4]. It is limited not only to developing countries or arid areas, but also to other countries worldwide. Environmental degradation influences one-third of agricultural areas around the world at either moderate or high levels [5] and directly affects the lives of more than 1.5 billion people [6].

The Mediterranean basin has been described as a "love story" between humans and nature [7]. The Mediterranean is very suitable for habitation; hence, the region became exposed to many anthropogenic influences throughout history [8]. This anthropogenic pressure has led to significant environmental degradation [9, 10].

Various projects, such as DeMon [11], Mediterranean Desertification, and Land Use [12], DISMED [13], DESERTLINKS [14], and LADAMER [15], have sought to detect and understand this sensitive regional situation [16]. One such project, Mediterranean Desertification and Land Use (MEDALUS), conducted with the help of the European Environment Agency (EEA), covers the five ecosystems covered in the European Topic Centre on Terrestrial Environment (ETC-TE), making it possible to detect and map areas sensitive to degradation in Mediterranean countries. The Environmental Sensitivity Areas (ESA) index method was developed within the scope of this project. This method has been applied in various areas [17, 18, 19, 20], with Mediterranean Europe being the first region to have yielded highly efficient results [21, 22].

The ESA index is often implemented along with Geographic Information Systems (GIS) [23, 24], which allows for efficient data management, spatial analysis, and dynamic monitoring [25]. The analysis of the relationship between these results and environmental factors (topographic, climatic, edaphic, and biotic factors) provides evidence regarding the scope of environmental deterioration, its causes, and its generalized inflections [26].

Turkey is highly sensitive to environmental degradation due to its geographical location, natural conditions, and human settlement patterns [27]. In this study, we attempted to conduct an environmental degradation analysis using GIS techniques and the ESA index method. We hope that decision makers can use the findings of this study as a guide to environmental remediation policy implementation.

This study revealed the interaction between humans in the area and the natural environment based on the principle of spatial distribution of data. Therefore, this is an important study, as it shows

the extent of anthropogenic influence on the natural environment. Hence, considering the fact that environmental problems encountered in various areas across the world may create a domino effect and influence the entire world, the need for a better analysis of the interaction and relationship between human activities and natural phenomena becomes ever more obvious. In addition, this study is important because it provides results that are consistent with those reported in other studies focusing on Turkey's conditions.

DESCRIPTION OF THE STUDY AREA

The study area is the Tekirdag province located on the Thrace Peninsula in northwest Turkey (Figure 1), southeast of the Strandja Massif and Thrace Basin. The substratum is from the Precambrian period. The land shows undulatory plateau relief with average altitude of 152 m. The climate is dry with low humidity, the coastal area is semi-humid while the mountains are humid (Köppen-Geiger climate classification). The most important fluvial feature of Tekirdag province is the Ergene River. Six types of soils are evident in the Tekirdag province: Entisols, Alfisols, Inceptisol, Mollisol, Vertisol, and Andisol. The vegetation includes dry forest in the interior, semi-humid forest in the coastal areas, and humid forests in the mountains. With a population of 906,732, the Tekirdag province is considered a metropolis. The largest land use is agriculture (58.84%). Furthermore, a large part of the remaining space of the province consists of non-agricultural fields that are used as residential areas (19.49%) [28].

MATERIALS AND METHODS

The primary materials used in this study were topography maps with a scale of 1:100.000 prepared by the Turkish General Command of Mapping (2015) and data on the soil, climate, and vegetation in the study area. In addition, thematic maps prepared by various organizations/institutions were used (Table 1).

The ESA index was employed in this study together with the Soil Quality Index (SQI), Climate Quality Index (CQI), and Vegetation Quality Index (VQI).

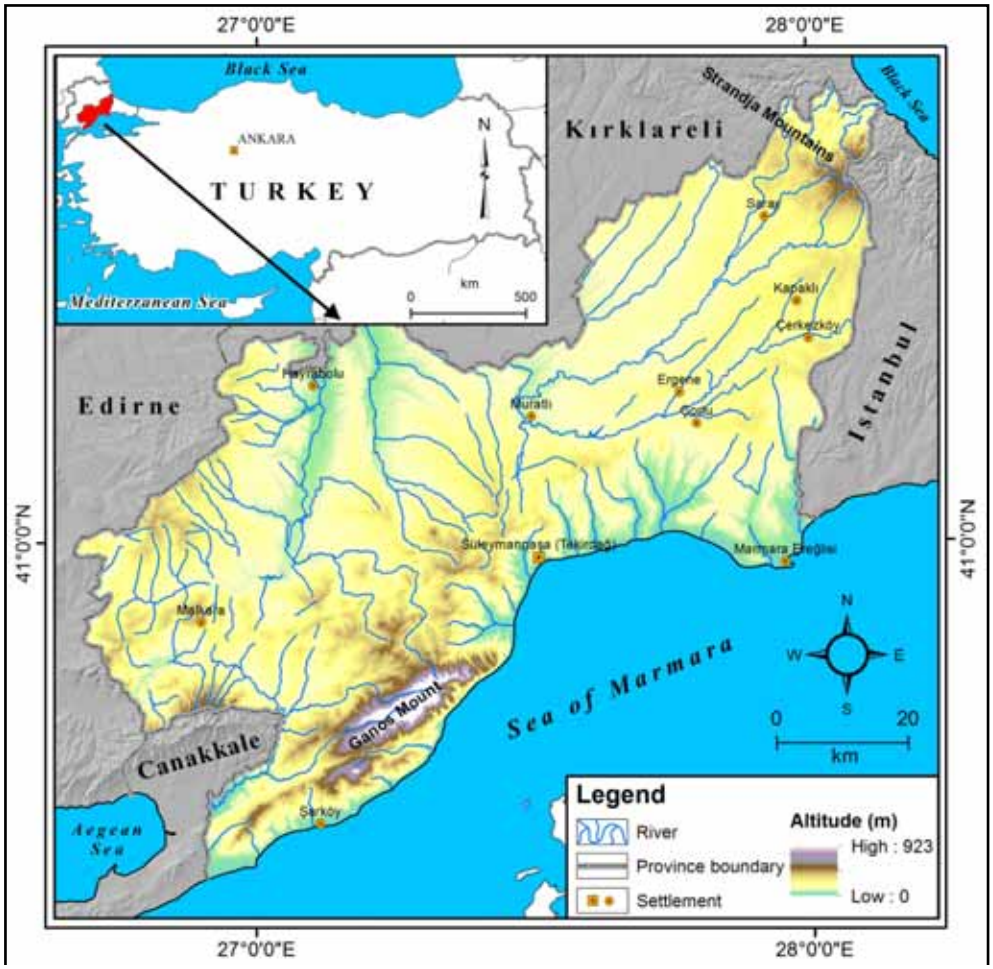


FIGURE 1
The location map of the study area

TABLE 1
Characteristics of the data used

Data types	Data source
Topography map (Scale: 1/100.000)	[47]
Geological map (Scale: 1/100.000)	[48]
The meteorological observation data	[30]
Soil map (Scale: 1/100.000)	[35]
Land Use-Land Cover (LU/LC) map (Scale: 1/100.000)	[28]

TABLE 2
Parameters and scores used to determine the SQI

Class of parent material (Ip)		Description	Score
Coherent parental material: limestone, dolomite, non-friable sandstone, hard limestone layer		Good	1.0
Parental material moderately coherent: Marno-limestone, friable sandstone		Moderate	1.5
Parental material soft to friable: Calcareous clay, clay, sandy formation, alluvium and colluviums		Poor	2.0
Class of soil depth (Id)	**Description**		**Score**
Very deep	Soil thickness is more than 1 meter		1.0
Moderately deep	Soil thickness ranges from <1m to 0.5 m		1.33
Not deep	Soil thickness ranges from <0.5m to 0.25 m		1.66
Very thin	Soil thickness 0.15 m		2.0
Texture Classes (It)	**Description**		**Score**
Not very light to average	L, SCL, SL, LS, CL		1.0
Fine to average	SC, SiL, SiCL		1.33
Fine	Si, C, SiC		1.66
Coarse	S		2.0
Slope (%) Classes (Is)	**Description**		**Score**
< - 6	Gentle		1.0
6 – 18	Not very gentle		1.33
19 – 35	Abrupt		1.66
35 - >	Very abrupt		2.0

TABLE 3
Classes, description and scores of SQI

Class	Description	Score
1	High quality	< - 1.13
2	Moderate quality	1.13 – 1.45
3	Low quality	1.46 - >

TABLE 4
Coordinates, altitudes and years of observation of meteorology stations

No	Meteorology stations	Coordinates	Altitude (m)	Years of observation
1	Babaeski	41°N – 27°E	50	1969-1983
2	Banarlı	41°N – 27°E	150	1985-1990
3	Camlıca	40°N – 26°E	65	1988-2003
4	Cerkezkoy	41°N – 28°E	160	1984-1997
5	Corlu	41°N – 27°E	183	1950-2014
6	Copkoy	41°N – 26°E	75	1987-1996
7	Dambaslar	41°N – 27°E	50	1987-2000
8	Hayrabolu	41°N – 27°E	40	1965-1989
9	İbriktepe	41°N – 26°E	150	1987-1998
10	İnecik	40°N – 27°E	200	1984-1990
11	Kesan	40°N – 26°E	185	1965-1976
12	Kıyıkoy- Midye	41°N – 28°E	20	1985-1997
13	Luleburgaz	41°N – 27°E	46	1950-2014
14	Malkara	40°N – 26°E	283	1980-2014
15	Marmara Ereglisi	40°N – 27°E	5	1987-1997
16	Muratlı	41°N – 27°E	80	1965-1991
17	Murefte	40°N – 27°E	15	1972-1993
18	Saray	41°N – 27°E	140	1977-1982
19	Sarkoy	40°N – 27°E	10	1965-1992
20	Tekirdag	40°N – 27°E	4	1950-2014
21	Uzunkopru	41°N – 26°E	52	1962-2014
22	Vize	41°N – 27°E	210	1985-1992

TABLE 5
Classes, description and scores of CQI

Class	Description	CQI	Score
1	Hyper-Arid	< - 0.05	2.0
2	Arid	0.05 – 0.20	1.75
3	Semi-Arid	0.20 – 0.50	1.50
4	Dry Sub-Humid	0.50 – 0.65	1.25
5	Humid	0.65 - >	1.0

The index results were distributed using the inverse distance weighted (IDW) technique, a common geostatistical method. The raster data were fixed at the common data scale (100x100 pixels) of the input factors and were combined with the GIS techniques within the framework of the grid-based analysis methodology.

SQI was determined based on the composite material, thickness, texture, and slope characteristics of 174 samples taken randomly from the large soil groups in the study area in equal amounts (26 pcs). Texture was identified using the Bouyoucos hydrometer method [29]. The results were graded according to the ranges reported by Kosmas et al. [12], based on which we were able to derive the indices (Table 2).

SQI was derived using the following formula:

SQI = (Ip * It * Id * Is) ¼ (Eq. 1)

Here, "SQI" refers to Soil Quality Index, "Ip" refers to the main material index, "It" refers to the soil texture index, "Id" refers to the soil depth index, and "Is" refers to the slope index. The class and score of each parameter differ from one another. The values generally range from 1 to 2. The SQI ranges from 1 (high quality) to 3 (low quality) (Table 3).

In this study, CQI was calculated from the long-term records of meteorology stations affiliated with the Turkish State Meteorological Service [30]. The calculations were made according to the following formula:

CQI = (P / PET) (Eq. 2)

Here (Eq. 2), "CQI" refers to Climate Quality Index, "P" refers to mean annual precipitation, and "PET" refers to mean annual potential evapotranspiration. The results were grouped according to defined classes (Table 5), and they were distributed spatially.

The relevant parameters were evaluated based on land use-land cover (LU/LC) classes determined by the CORINE system, which has been widely used in previous studies with similar content [17, 22].

VQI was evaluated within the parameters of the LU/LC classes [17, 22]. To this end, we used data from the LU/LC map collected by Sarı and Ozsahin [28]. The VQI value was calculated based on the following formula;

$VQI = (IEp * IDr * IVc)^{1/3}$ (Eq. 3)

Here (Eq. 3), "VQI" refers to Vegetation Quality Index, "IEp" refers to the index of erosion protection, "IDr" refers to the index of drought and resistance, and "IVc" refers to the vegetation index. According to the results, VQI ranged from good (< - 1.2) to very poor (1.6 - >) (Table 6).

The raster data obtained after the calculation of the indices were combined according to the formula below:

$ESA = (SQI * CQI * VQI)^{1/3}$ (Eq. 4)

Here (Eq. 4), "ESA" refers to Environmental Sensitivity Areas, "SQI" refers to Soil Quality Index, "CQI" refers to Climate Quality Index, and "VQI" refers to the Vegetation Quality Index. The resultant map was sorted and classified according to the =ESA classification into non-affected or very low sensitive areas (< - 1.2), low sensitive areas ($1.2 \leq ESA < 1.3$), medium sensitive areas ($1.3 \leq ESA < 1.4$), sensitive areas ($1.4 \leq ESA < 1.6$), and very sensitive areas ($ESA \geq 1.6$) [22].

TABLE 6
Classes, description and scores of VQI

Class	Description	Score
1	Good	1.0 - 1.2
2	Average	1.2 – 1.4
3	Weak	1.4 – 1.6
4	Very weak	1.6 – 2.0

In the final phase, zonal statistics were collected to detect the factors influencing spatial difference. In this regard, the ESA index for Environmental degradation characterizes the relationship between environmental sensitivity levels and interactions with biotic (LU/LC) factors [31].

The imaging and analysis phases of the study were performed using ArcGIS 10.5 software.

RESULTS AND DISCUSSION

Basic phenomena, defined as geosystems, were traditionally divided into certain globes, which constitute the parts of a whole [32]. The ESA index method, which examines the identification, diagnosis, and treatment processes of environmentally sensitive areas, revealed interactions among soil, climate, and vegetation [33, 34].

SQI (Soil Quality Index). The SQI in the study area was examined for the qualities of thickness, texture, and slope. In the study area, descriptions of the classes of parent material are generally poor and moderate (Table 2, Figure 2). Good description of class of parent material is in urban areas close to the Strandja Mountains. These areas comprise generally massive rocks. The soil is mostly very deep in the study area (Table 2, Figure 2). The relationship between slope and soil thickness is inverse in that as slope increases, soil thickness decreases (Table 2, Figure 2).

As a reflection of pedologic evolution, clay-structure and heavy soil are more common. These texture types, found in areas where vertisol ordo is more common, is classified as "good" in the SQI parameter classification. The average slope is 9.31% in the study area. According to the SQI slope classification, this slope would be categorized as "not very gentle" (Table 2, Figure 2). Although the site nearer Ganos Mountain and the mountainous

area leading to the Black Sea has higher slope values, thus it should be classified as "not very gentle," it is categorized as "gentle" thanks to decreasing slope values in the western part of the area (Table 2, Figure 2).

The average SQI value for the Tekirdag province was found to be 1.32 (Moderate quality). The SQI value increases near Ganos Mountain and the Hayrabolu and Muratlı settlements while it decreases around the Sarkoy and Corlu settlements (Table 2, Figure 2). The value increases depending on the intensity of the anthropogenic activities, such as the unbalanced use of fertilizer and chemicals, overirrigation, inadequate drainage conditions, use of heavy machines and equipment, and overgrazing in a directly proportional way [35].

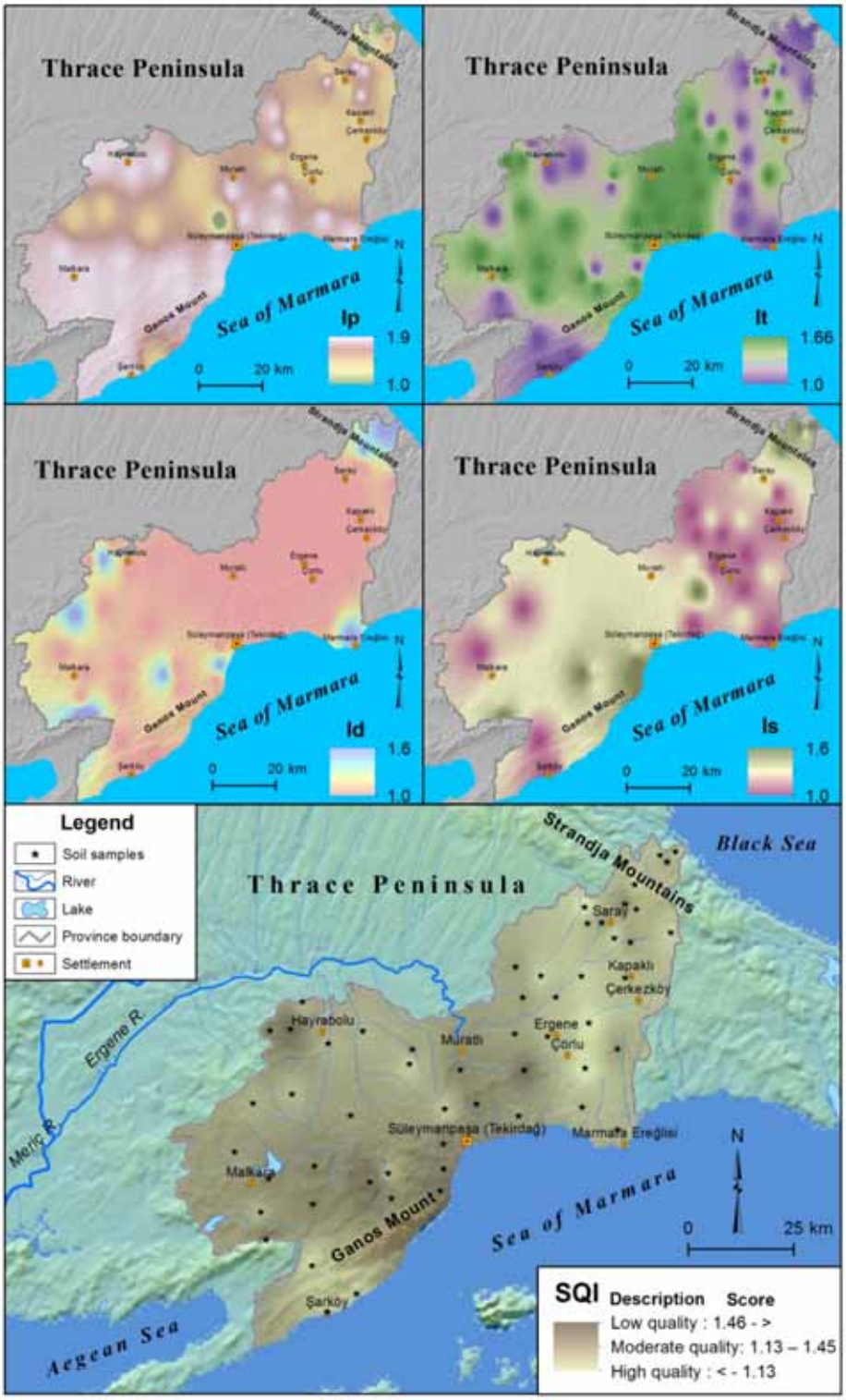


FIGURE 2
Distribution map of SQI area

CQI (Climate Quality Index). Climate conditions also influence environmental degradation processes and provide insights into the process of degradation in the Mediterranean [36]. In this sense, mean annual precipitation (P) and mean annual potential evapotranspiration (PET) are the main indicators used in the CQI.

In the study area, the mean annual precipitation is 531.2 mm. The highest precipitation value was recorded at the Saray station (866.6 mm) while the lowest value was recorded at the Marmara Ereglisi station (371.4 mm) (Table 7).

As a reflection of pedologic evolution, clay-structure and heavy soil are more common. This texture type, found in areas where vertisol ordo is more common, is classified as "good" in the SQI parameter classification. The average slope is 9.31% in the study area. According to the SQI slope classification, this falls under the "not very gentle" category (Table 2, Figure 2). Although the site nearer Ganos Mountain and the mountainous area leading to the Black Sea has higher slope values, which correspond to "not very gentle" classification, it is categorized as "gentle" thanks to decreasing slope values in the western part of the area (Table 2, Figure 2).

The average SQI value for the Tekirdag province was found to be 1.32 (Moderate quality). The SQI value increases near Ganos Mountain and the Hayrabolu and Muratlı settlements while it decreases around the Sarkoy and Corlu settlements (Table 2, Figure 2). The value increases depending on the intensity of the anthropogenic activities, such as the unbalanced use of fertilizer and chemicals, overirrigation, inadequate drainage conditions, use of heavy machines and equipment, and overgrazing in a directly proportional way [35].

CQI (Climate Quality Index). Climate conditions are another influencing factor in environmental degradation processes. They are considered as key data points in revealing the process of degradation in the Mediterranean [36]. In this sense, mean annual precipitation (P) and mean annual potential evapotranspiration (PET) are the main indicators used in the CQI.

In the study area, the mean annual precipitation is 531.2 mm, and the highest precipitation value was recorded at the Saray station (866.6 mm) while the lowest was recorded at the Marmara Ereglisi station (371.4 mm) (Table 7).

The mean potential evapotranspiration value was found to be 725.1 mm. After proportioning the mean annual precipitation and potential evapotranspiration, the CQI value was approximately 0.70 (Table 7). Accordingly, in the study area where different climates can be observed, the mean climate quality value was 0.73 (Table 7). Hence, this area belongs to the humid category overall. Although the CQI value increases near Marmara Ereglisi, it decreases in the northern and western parts of the province (Figure 3).

VQI (Vegetation Quality Index). Vegetation plays an important role in decreasing degradation [36]. The relationship between vegetation, erosion, and drought are among the most important factors in evaluating environmental sensitivity [19]. VQI was determined in association with erosion protection, drought resistance, and vegetation indices (Table 8).

TABLE 7
P, PET and P/PET values of meteorology stations

Meteorology stations	P	PET	P/PET
Babaeski	576.4	721.1	0.8
Banarlı	393.7	709.4	0.6
Camlıca	531.1	752.3	0.7
Cerkezkoy	517.5	671.3	0.8
Corlu	540.3	716.3	0.8
Copkoy	463.5	719.8	0.6
Dambaslar	480.0	662.2	0.7
Hayrabolu	490.7	736.7	0.7
İbriktepe	488.7	731.9	0.7
İnecik	510.4	724.9	0.7
Kesan	576.2	762.7	0.8
Kıyıkoy- Midye	626.4	702.5	0.9
Luleburgaz	565.4	741.6	0.8
Malkara	620.6	725.9	0.9
Marmara Ereglisi	371.4	743.0	0.5
Muratlı	518.4	718.3	0.7
Murefte	442.4	761.9	0.6
Saray	866.6	693.8	1.2
Sarkoy	471.9	782.3	0.6
Tekirdag	590.5	737.4	0.8
Uzunkopru	618.6	745.2	0.8
Vize	426.8	692.5	0.6

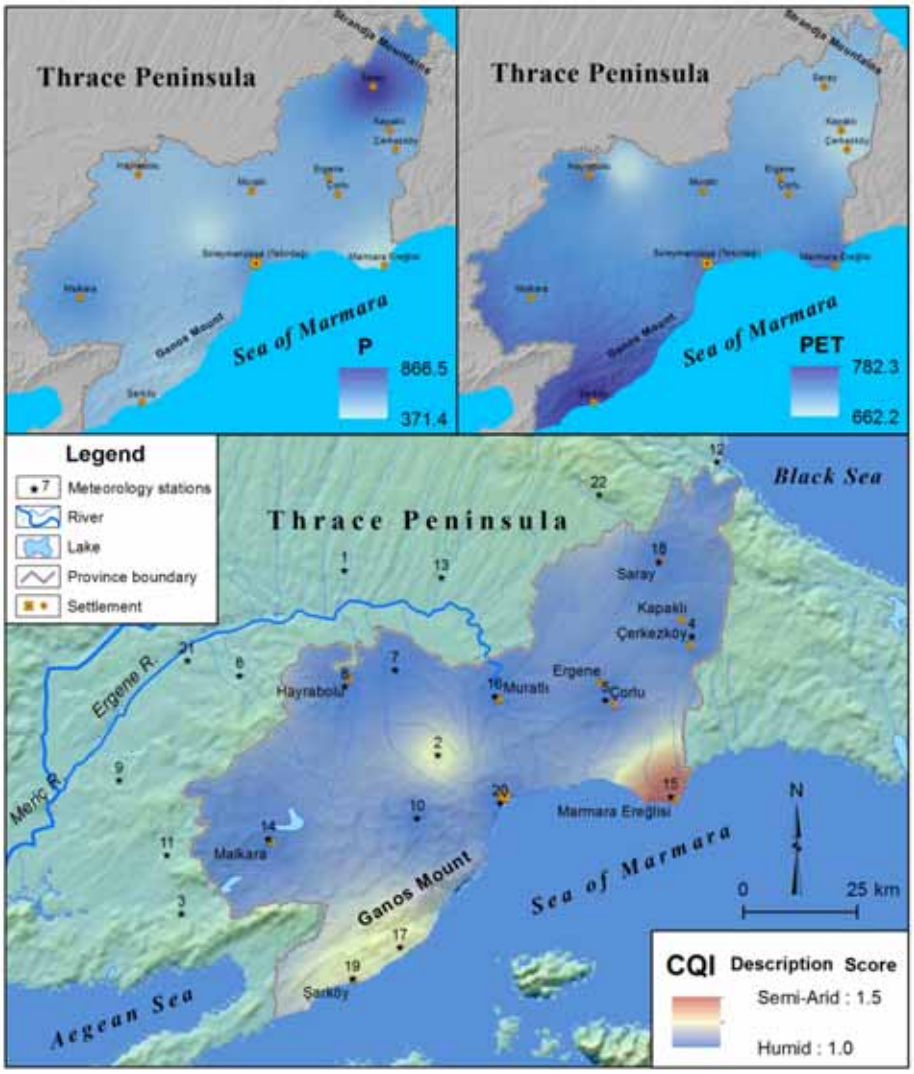


FIGURE 3
Distribution maps of CQI

TABLE 8
Classes, description and scores of VQI based on LU/LC classification

Class	Description	IEp	IDr	IVc	VQI
1	Artificial surfaces (settlements, etc.)	2.0	2.0	2.0	2.0
2	Barren land (areas of little or no vegetation areas, water bodies etc.)	2.0	2.0	2.0	2.0
3	Vineyard and Gardens	1.8	1.4	1.8	1.66
4	Non-irrigated arable land	2.0	2.0	2.0	2.0
5	Permanently irrigated land	2.0	2.0	2.0	2.0
6	Pastures	1.3	1.7	1.8	1.58
7	Natural grasslands	1.3	1.7	1.33	1.43
8	Bushes	1.3	1.0	1.33	1.20
9	Mixed forests	1.0	1.2	1.0	1.06
10	Broad-leaved forests	1.0	1.0	1.0	1.0
11	Coniferous forests	1.3	1.2	2.0	1.46

Soil loss in the Tekirdag province, where a mild risk of erosion is widely observed, was calculated as 5.26 t ha^{-1} y^{-1} [37]. The province is covered mostly by dry farming areas. The mean erosion protection index was found to be 1.4. The drought resistance index is characterized by vegetation types. The mean VQI value in the study area was found to be 1.40 (average). Similarly, the areas classified as "very weak" are generally in artificial surfaces (settlements, etc.), barren land (areas of little or no vegetation areas, water bodies etc.), non-irrigated arable land, and permanently irrigated land whereas the areas classified as "good" are generally broad-leaved and mixed forests (Table 8). The highest VQI was recorded in broad-leaved forests (Figure 4).

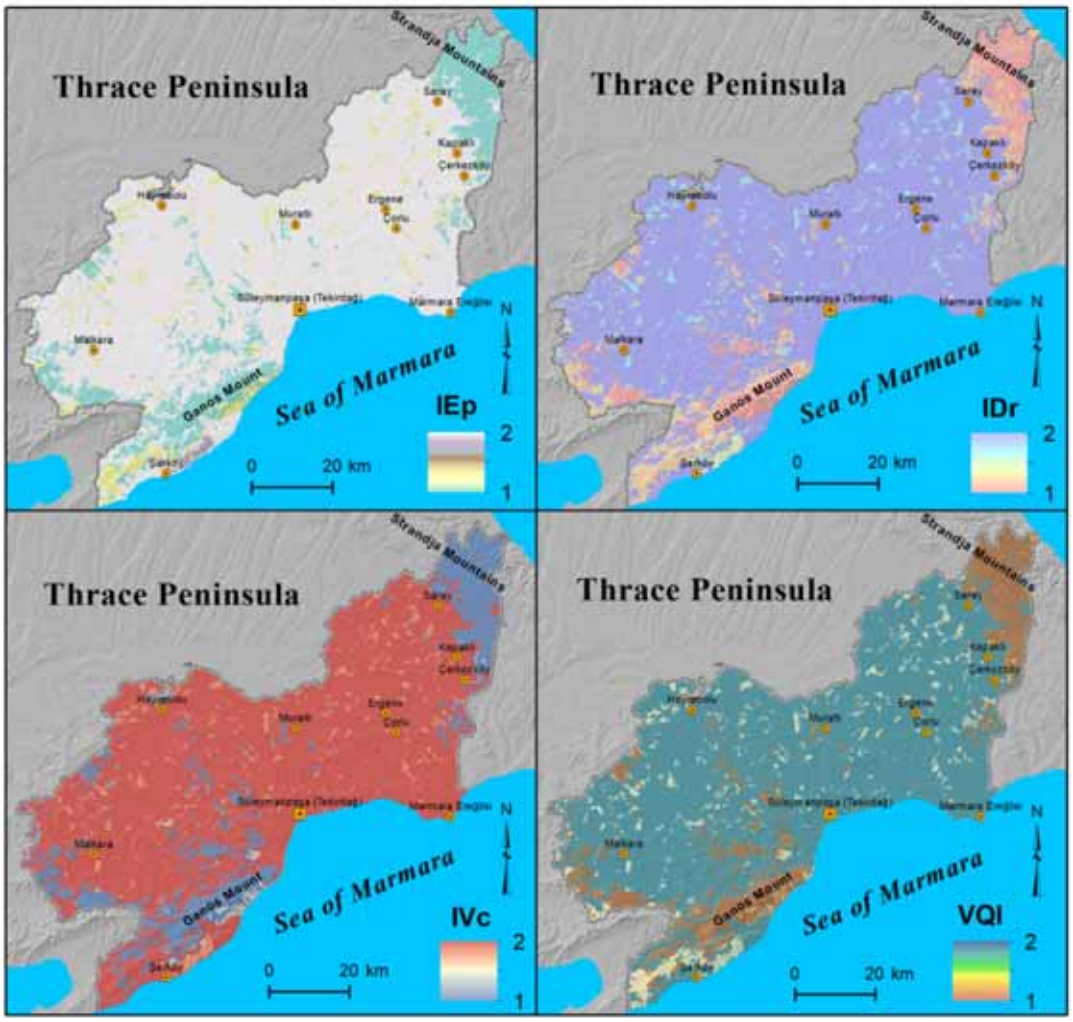


FIGURE 4
Distribution maps of VQI

CONCLUSIONS

Environmental degradation is one of the most important global problems caused by the complex relationship between humans and the natural environment [3]. This phenomenon divided into two categories including anthropogenic and natural has taken on a negative character over time due to changes in both biophysical natural ecosystem and socio-economic conditions [34; 38]. It has also grown in importance, especially as a result of the recent increase in various causal factors, such as climate changes, land use/land cover (LU/LC) changes, and human-dominated land management [39, 40, 41, 42].

Environmental degradation is a more serious problem especially in arid and semi-arid areas due to the sensitivity of these ecosystems [43]. Thus, it is also commonly observed in the Mediterranean basin characterized by a semi-arid climate. Environmental degradation is clearly visible in Turkey that has witnessed intense anthropogenic activities from ancient times to the present day, especially in the study area.

According to ESA index analyses, the mean value of the study area (1.36) represents "medium sensitive areas" ($1.3 \leq ESA < 1.4$) (Table 9). ESA values for low and medium sensitive areas were not found in urban areas. The largest area (52.43%) covers regions with sensitive ESA values (Figure 5; Table 9). These spread through nearly the entire artificial surfaces and barren land. The second highest rate (27.84%) represents medium sensitive areas, which are observed around west of Hayrabolu settlement and the southern part of the Strandja Mountains (Figure 5; Table 9). The third highest rate (15.89%) represents non-affected areas or very low sensitive areas. These places are found near the Malkara settlement and Ganos Mountain as well as their immediate surroundings. The smallest ESA class (3.85%) represents low sensitive areas covering forested areas near the mountainous regions of the study area (Figure 5; Table 9).

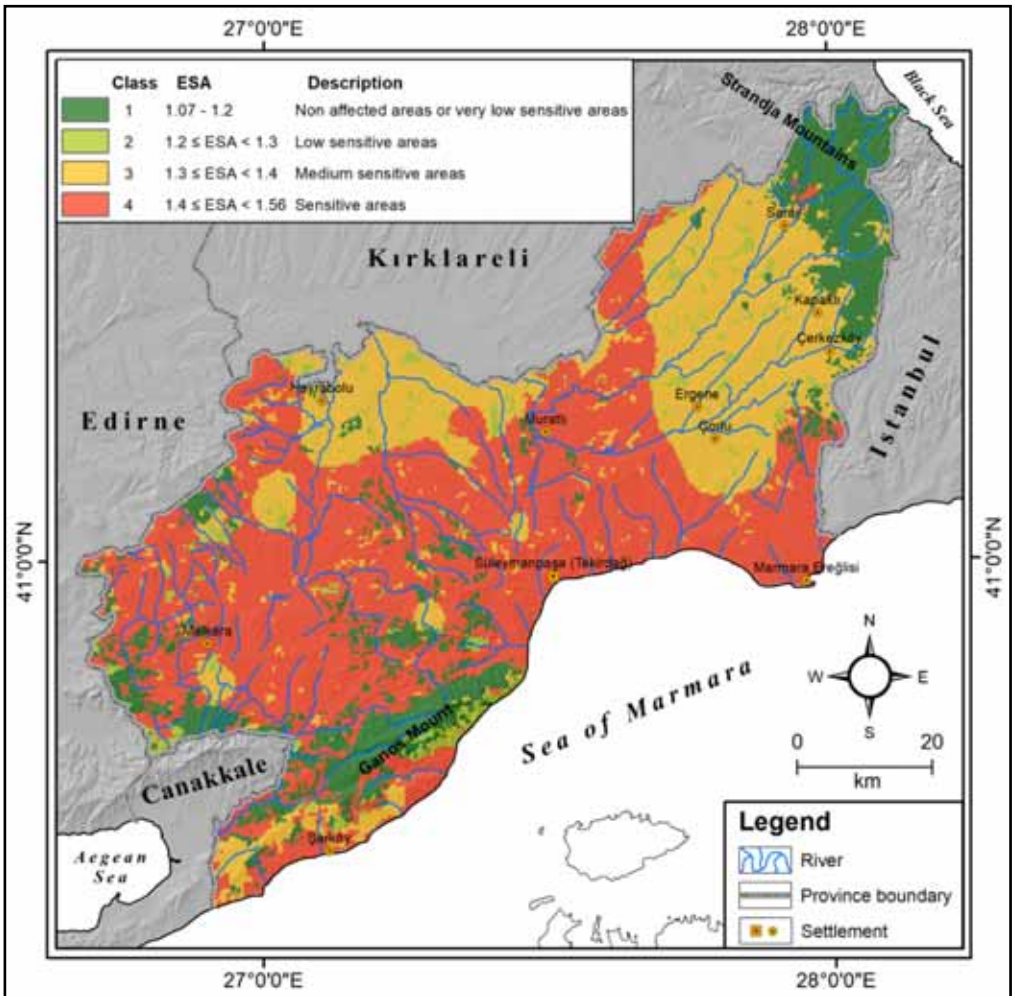


FIGURE 5
Distribution maps of the ESA index

TABLE 9
Areal distribution of the ESA index and rate (%)

Class	ESA	Description	Area (km²)	Rate (%)
1	1.07 - 1.2	Non-affected areas or very low sensitive areas	982.58	15.89
2	1.2 ≤ ESA < 1.3	Low sensitive areas	237.70	3.85
3	1.3 ≤ ESA < 1.4	Medium sensitive areas	1720.76	27.84
4	1.4 ≤ ESA < 1.56	Sensitive areas	3240.95	52.43
TOTAL			**6182**	**100**

Environmental degradation is a problem that occurs along with some ecological processes, particularly LU/LC changes [43, 44, 45]. Thus, in order to understand the environmental degradation in the study area, it is important to analyze the relationship between LU/LC and the ESA index. Among LU/LC classes identified in the study area, the highest ESA impact value (63.78%) was calculated in agricultural areas (Table 10).

According to the ESA classes, these agricultural areas comprise the greatest percentage of areas classified as sensitive e (90.18%) (Table 10). This indicates that agricultural areas are more sensitive to environmental degradation compared to other LU/LC classes. Frattaruolo et al. [46] showed that high levels of degradation in agricultural lands developed due to human agricultural mismanagement and abuse of water resources. Forests and semi-natural areas belong to another LU/LC class with high ESA impact values (31.86%) (Table 10). According to the ESA classes, these areas comprise the largest percentage (85.07%) of areas categorized as non-affected areas or very low sensitive (Table 10). This finding suggests that forests and semi-natural areas are less sensitive to environmental degradation compared to other LU/LC classes. Gad and Lotfy [17] stated that environmental sensitivity to degradation has less influence in forested areas. For the other LU/LC classes, the ESA impact values have a proportional distribution across artificial surfaces (3.87%), water bodies (0.49%), and wetlands (0.01%) respectively (Table 10).

TABLE 10
Zonal statistical values of effective factors based on ESA index (%)

Class of effective factors	Zonal statistical Class of ESA index			Class of effective factors	Zonal statistical Class of ESA index		
Slope (%)	**1**	**4**	**5**	**Soil**	**1**	**4**	**5**
< - 6	23.5	39.3	40.8	Alfisol	34.6	15.3	23.7
6 – 18	50.4	48.6	54.5	Andisol	0.5	0.1	0.9
19 – 35	20.1	9.6	4.5	Entisol	26.3	32.9	18.1
35 - >	5.9	2.5	0.2	İnceptisol	22.2	17.5	18.9
Standard deviation: 20.7; Mean: 25.0				Mollisol	10.7	17.6	11.1
Aspect				Vertisol	5.6	16.6	27.1
North	38.6	31.0	31.8	**Standard deviation: 16.7; Mean: 10.4**			
South	37.4	43.9	41.3	**LULC**			
East	10.6	14.6	9.5	Insensible areas (settlements, water bodies etc.)	25.7	0.1	0.1
West	13.2	10.2	17.2	Open spaces (Open spaces with little or no vegetation)	0.02	0.2	0.01
Flat	0.2	0.3	0.2	Vineyard and Gardens	0.02	3.7	0.01
Standard deviation: 20.0; Mean: 15.8				Permanently irrigated land	0.1	80.5	0.1
Temperature (°C)				Non-irrigated arable land	0.6	0.4	92.9
<-12	6.5	1.3	2.6	Pastures	0.1	0.1	6.7
12--13	43.5	29.4	48.4	Natural grasslands	0.03	2.7	0.01
13--14	38.5	54.0	46.1	Bushes	0.03	3.1	0.01
14->	11.5	15.3	2.9	Mixed forests	34.7	0.1	0.1
Standard deviation: 25.0; Mean: 20.3				Broad-leaved forests	38.7	0.04	0.1
Precipitation (mm)				Coniferous forests	0.03	9.1	0.01
<-500	26.7	40.8	28.4	**Standard deviation: 7.7; Mean: 20.6**			
500-600	45.1	55.3	62.0				
600-700	21.0	1.3	6.2	**TOTAL**			
700->	7.1	2.5	3.4	**Standard deviation: 19.9; Mean: 18.2**			
Standard deviation: 25.0; Mean: 21.7							

Thus, according to the ESA assessment, the agricultural regions in the study area have a very high environmental sensitivity in terms of environmental degradation while this sensitivity decreases in the forests. The ESA values in the study area show a distribution according to the sensitivity of the LU/LC classes to environmental degradation. Thus, the most important indicator or the key parameter of environmental degradation in this area is LU/LC rather than other factors, such as soil and climate.

This study used quantitative data based on the principle of spatial distribution to examine areas of environmental sensitivity and degradation. Hence, this study is important in that it shows the existing conditions of the natural environment, and it can be used as a guide to encourage or restrict human action. It further indicates the need to further explore the interaction and relationship between human activities and natural phenomena. The findings of this study, based on accepted scientific methodologies, provide insight into one Turkish region, suggesting that although human influence in this region is quite extreme, it could be mitigated with concrete public policies.

REFERENCES

[1] Ellis, E.C. (2015) Ecology in an anthropogenic biosphere. Ecological Monographs. 85(3), 287-331.

[2] Williams, J.R. (1991) Search for sustainability: agriculture and its place in the natural ecosystem. Agricultural Science. 4, 32-39.

[3] Riebsame, W.E., Meyer, W.B. and Turner, B.L. (1994) Modeling land-use and cover as part of global environmental change. Climate Change. 28, 45-64.

[4] UNCCD (The United Nations Convention to Combat Desertification) (2015) Climate change and land degradation: Bridging knowledge and stakeholders. The UNCCD 3rd Scientific Conference Scientific Advisory Committee & The UNCCD Science-Policy Interface, 9-12 March 2015. Cancun, Mexico: United Nations Convention to Combat Desertification.

[5] UNCCD (The United Nations Convention to Combat Desertification). (2014a) Desertification The Invisible Frontline. Switchboard: UNCCD publications.

[6] UNCCD (The United Nations Convention to Combat Desertification). (2014b) Land Degradation Neutrality: Resilience at Local, National and Regional Levels. Switchboard: UNCCD publications.

[7] Blondel, J. and Aronson, J. (2004) Biology and Wildlife of the Mediterranean Region. New York, USA: Oxford University Press.
[8] Tzatzanis, M., Wrbka, T. and Sauberer, N. (2003) Landspace and vegetation responses to human impact in sandy coasts of Western Crete, Greece. J Nature Conservation. 11, 187-195.
[9] Thornes, J. (1996) Desertification in the Mediterranean, In: Brandt, J. and Thornes, J. (Eds.) Mediterranean Desertification and Land Use. Chichester: Wiley, 1-12.
[10] Irshad, M., Inoue, M., Faridullah, M.A., Delower, H.K.M. and Tsunekawa, A. (2007) Land desertification-an emerging threat to Environment and Food Security of Pakistan. J Applied Science. 7, 1199-1205.
[11] Lacaze, B., Caselles, V., Coll, C., Hill, J., Hoff, C., De Jong, S., Mehl, W., Negendank, J. F.W., Riezebos, H., Rubio, E., Sommer, S., Teixeira Filho, J. and Valor, E. (1996) Integrated Approaches to Desertification Mapping and Monitoring in the Mediterranean Basin. In: Hill, J. (editors), Final Report of the DeMon-1 Project, Luxembourg: Office for Official Publications of the European Communities.
[12] Kosmas, C., Kirkby, M. and Geeson, N. (1999) The Medalus project Mediterranean desertification and land use, Manual on key indicators of desertification and mapping environmentally sensitive areas to desertification. http://www.medalus.demon.co.uk/. (Accessed February 29, 2015).
[13] Enne, G. and Zucca, C. (2000) Desertification indicators for the European Mediterranean region. ANPA, Rome: State of the art and possible methodological approaches.
[14] Imeson, A.C. (2004) Desertlinks-Combating desertification in Mediterranean Europe linking science with stakeholders: Functional indicators for monitoring Desertification. http:// www .kcl. ac.uk/projects/ desertlinks/ (Accessed February 29, 2015).
[15] Hill, J. (2005) LADAMER: Land and Soil Degradation Assessments in Mediterranean Europe. http://www.ladamer.org/ladamer/ index.php?category=home, (Accessed February 29, 2015).
[16] Silleos, N., Cherif, I., Kolejka, J., Karydas, C., Katsogiannos, F. and Silleos, G. (2008) Desertification assessment in the Lesvos Island in Greece and methodology for mitigation action planning. International Advanced Workshop on Information and Communication Technologies for Sustainable Agri-production and Environment, 22-23 August 2008. Alexandroupolis: Proceedings, 241-254.
[17] Gad, A. and Lotfy, I. (2006) Use of Remote Sensing and GIS in Mapping the Environmental Sensitivity Areas for Desertification of Egyptian territory. The 2nd International Conf. on Water Resources and Arid Environment, 1-16.
[18] Gad, A. and Lotfy, I. (2008) Use of remote sensing and GIS in mapping the environmental sensitivity areas for desertification of Egyptian territory. Earth Discuss. 3, 41-85.
[19] Abdel Kawy, W.A. and Belal. A.Z. (2011) GIS to Assess the Environmental Sensitivity for Desertification in Soil Adjacent to El-Manzala Lake, East of Nile Delta, Egypt. American-Eurasian J Agric and Environ Sci. 10(5), 844-856.
[20] Khebour Allouche, F. and Gad, A. (2014) A Comparison of Methods for Mapping the Environmental Sensitivity Areas for Desertification of a Mediterranean Landscape Using Remote Sensing and GIS Applications. European Academic Research I. (8), 10233-10253.
[21] Basso, F., Bove, E., Dumontet, S., Ferrara, A., Pisante, M., Quaranta, G. and Taberner, M. (2000) Evaluating environmental sensitivity at the basin scale through the use of geographic information systems and remotely sensed data: an example covering the Agri basin (Southern Italy). Catena. 40, 19-35.
[22] Domingues, F. and Fons-Esteve. J. (2008) Mapping sensitivity to desertification (DISMED). Final report Version 2. Gentile AR, project manager. European Topic Centre Land Use and Spatial Information.
[23] Anderson, J.R., Hardy, E.E., Roach, J.T. and Witmer, R.E. (1976) A Land Use and Land Cover Classification System for Use with Remote Sensor Data. USA: U.S. Geological Survey Professional Paper 964.
[24] Hinton, J.C. (1996) GIS and remote sensing integration for environmental applications. International Journal of Geographical Information Systems. 10, 877-890.
[25] Lai, J., Luo, J. and Zhang, M. (2012) Design and realization of the intangible cultural heritage information management system based on web map service. Advances in Information Technology and Industry Applications. 136, 605-612.
[26] Duro, A., Piccione, V., Ragusa, M.A. and Veneziano, V. (2014) New environmentally sensitive patch index-ESPI-for MEDALUS protocol. In: Sivasundaram, S. (Ed.) 10th International Conference on Mathematical Problems in Engineering, 15-18 July 2014, Narvik, Norway: Aerospace and Sciences: ICNPAA 2014, AIP Conf. Proc. 1637, 305-318.

[27] Kutiel, H. and Turkes, M. (2017) Spatial and temporal variability of dryness characteristics in Turkey. International Journal of Climatology. 37, 818-828.
[28] Sarı, H. and Ozsahin, E. (2016) Spatiotemporal change in the LULC (Landuse/ Landcover) characteristics of Tekirdag Province based on the CORINE (Thrace, Turkey). Fresen. Environ. Bull. 25, 4694-4707.
[29] Bouyoucos, G.J. (1953) An improved type of soil hydrometer. Soil Science. 76(5), 377-378.
[30] Turkish State Meteorological Service. (2015) Long-term observation records of meteorology stations in Tekirdag Province. Ankara: Turkish State Meteorological Service.
[31] Bai, Z.G., Van Lynden, G.W.J., Sahin, S., Cotteleer, G., Van Ierland, E. and Bindraban, P.S. (2011) Mapping land degradation and risk in Turkey. Wageningen, Netherlands: World Soil Information, ISRIC Report 2011/05.
[32] Christopherson, R.W. and Birkeland, G.E. (2017) Geosystems: An Introduction to Physical Geography (10th Edition). USA: Pearson Publishing.
[33] Nkonya, E., Gerber, N., Von Braun, J. and De Pinto, A. (2011) Economics of land degradation. IFPRI Issue Brief 68, USA: International Food Policy Research Institute.
[34] Li, Z., Deng, X., Yin, F. and Yang, C. (2015) Analysis of Climate and Land Use Changes Impacts on Land Degradation in the North China Plain. Hindawi Publishing Corporation Advances in Meteorology. 1-11.
[35] Ozsahin, E., Eroglu, I. and Pektezel, H. (2017) Soil Quality Index (SQI) Analysis of Tekirdag Province Using GIS (Thrace, Turkey). Fresen. Environ. Bull. 26, 3005-3014.
[36] Motroni, A., Canu, S., Blanco, G. and Loj, G. (2006) Monitoring Sensitive Areas to Desertification in Sardinia: The Contribute of the Regional Agrometeorological Service. Desertification and Risk Analysis Using High and Medium Resolution Satellite Data (Tozeur, Tunisia), In: Marini, A. and Talbi, M. (Eds.) Training Workshop on Mapping Desertification. NATO Science for Peace and Security Series-C: Environmental Security. The Netherlands: Springer.
[37] Ozsahin, E. (2014) Using GIS Based RUSLE Model in Erosion Risk Assessment in Tekirdag Province. Journal of Tekirdag Agricultural Faculty. 11(3), 45-56.
[38] Millennium Ecosystem Assessment. (2005) Ecosystems and Human Well-being: Desertification Synthesis. USA: World Resources Institute.
[39] Sivakumar, M.V. and Ndiang'ui, N. (Eds.) (2007) Climate and Land Degradation. Berlin: Springer.
[40] Symeonakis, E., Calvo-Cases, A. and Arnau-Rosalen, E. (2007) Land use change and land degradation in southeastern Mediterranean Spain. Environmental Management. 40, 80-94.
[41] Pienaru, A., Iancu, P. and Căzănescu, S. (2009) Desertification and its effects on environment and agricultural production in Romania. Annals. Food Science and Technology. 10(2), 624-629.
[42] Bajocco, S., De Angelis, A., Perini, L., Ferrara, A. and Salvati, L. (2012) The impact of land use/land cover changes on land degradation dynamics: a Mediterranean case study. Environmental Management. 49(5), 980-989.
[43] Hadeel, A.S., Jabbar, M.T. and Chen, X. (2011) Remote Sensing and GIS Application in the Detection of Environmental Degradation Indicators. Geo-spatial Information Science. 14(1), 39-47.
[44] Thomas, D.S.G. and Middleton, N.J. (1993) Salinization: new perspectives on a major desertification issue. Journal of Arid Environments. 24, 95-105.
[45] Warren, A. (2002) Land degradation is contextual. Land Degradation and Development. 13, 449-459.
[46] Frattaruolo, F., Pennetta, L. and Piccarreta, M. (2009) Desertification Vulnerability Map of Tavoliere, Apulia (Southern Italy). Journal of Maps. 5(1), 117-125.
[47] Turkish General Command of Mapping. (2015) Topography map (Scale: 1/100.000) in Tekirdag Province. Ankara: GCM Publications.
[48] Turkish General Directorate of Mineral Research and Exploration. (2015) Geological map (Scale: 1/100.000) in Tekirdag Province. Ankara: MTA Publications.

Received: **02.04.2018**
Accepted: **03.11.2018**

CORRESPONDING AUTHOR

Emre Ozsahin
Department of Geography,
Faculty of Art and Science,
Namık Kemal University,
Tekirdag – Turkey

e-mail: eozsahin@nku.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9662-9673 Fresenius Environmental Bulletin

USE OF BIOMONITOR EUROPEAN ASH (*FRAXINUS EXCELSIOR* L.) TREE FOR MONITORING TRAFFIC RELATED HEAVY METAL POLLUTION IN BISHKEK/KYRGYZSTAN: IMPACTS ON PLANT NUTRITION

Zeki Severoglu*

Marmara University, Faculty of Sciences and Arts, Department of Biology, 34722, Goztepe, Istanbul, Turkey

ABSTRACT

Biomonitor organisms can be used to determine pollution levels and their regional distributions. In this sense, this study investigated traffic-related heavy metals pollution and its impact on plant nutrition by using biomonitor plant European ash (*Fraxinus excelsior* L.) tree. For this aim, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb and Zn levels were analyzed by using ICP-OES in plant and soil samples taken from eight different locations in Bishkek/Kyrgyzstan. Ca and Mg levels were highest at Shabdan Baatry Street (St2) while other remaining elements were highest at Chuy Street (St4). Chuy Street has been an international transit road with heavy traffic, thus increased levels of most elements were reasonable. Heavy metals Cd and Pb in soil, and Cd, Cr and Pb in plants were above the acceptable ranges but not toxic levels in plant samples. Removal rates of Cd, Cu, Mn and Pb between washed and unwashed leaves were higher than other elements at all stations. Except Ca and Na, all test metals demonstrated strong positive correlations. In light of these findings, the biomonitor plant *F. excelsior* could effectively reflect the pollution levels in Bishkek/Kyrgyzstan.

KEYWORDS:
Heavy metal, mineral nutrition, pollution, anthropogenic activity, toxicity

INTRODUCTION

Pollution has been very serious problem at ever-increasing rates all over the world. Especially in big cities, various types of pollutants contaminated air, residential and agricultural areas [1]. Pollutants were primarily introduced into ecosystems as results of anthropogenic activities [2]. Pesticides, herbicides, fertilizers, heavy metals, oils, mining residues, acid rains, ash, road debris, and nuclear, industrial and electronic wastes were among major pollutants [3, 4]. From those, heavy metals were regarded as most deleterious elemental contaminants due to their toxic and ecotoxic impacts on environment and living organisms. So, many study disciplines *e.g.* ecology, toxicology canalized their search interests on those toxic heavy metals [5]. Some heavy metals such as Cu, Fe and Zn are accepted as essential for plant growth and development, some are non-essential but beneficial like Ni whereas As, Cd, Hg and Pb are regarded as toxic [6, 7]. So, excess levels of essential and beneficial elements and less concentration of toxic heavy metals could cause serious metabolic problems in living organisms, even leading to death [8, 9].

Heavy metal emission could occur either by naturally (geochemical) or results of anthropogenic impacts mainly related to unplanned urbanization and industrialization, population growth and extravagant consumption [10-12]. Especially in major cities, heavy metal pollution has reached to such alarming levels [13]. So, monitoring of heavy metal levels has been an inevitable subject for authorities; measurement and risk assessments must be urgently done and necessary legislative regulations be implemented [14]. There have been a number of analytical techniques that can be used in monitoring pollution levels, from which one included the use of biomonitor organisms as pollution indicator. For example, plants can be used as biomonitors to figure out the presence of heavy metals and their concentrations in an environment [3, 10, 15]. However, an organism being used as biomonitor must have following characteristics: (i) being present all over the test area, (ii) having wide geographical distribution, (iii) being able to discriminate airborne heavy metals from soil born and (iv) having easy sampling and identification features [16, 17]. The powerful side of using organisms in pollution monitoring was that biomonitor organism has been already part of that ecosystem/s. In this regard, employing plants in biomonitoring activities could provide further advantages like they were relatively cheap, easily available and gave quantitative results [3].

FIGURE 1
Map of the studied area in Bishkek.
Numbers and yellow lines refer to stations which are 1. Akhunbaev Street, 2. Shabdan Baatyr Street, 3. Jibek Jolu Street, 4. Chuy Street, 5. Moskovskaya Street, 6. Bokonbaeva Street, 7. Gorky Street and 8. Yusup Abdrahmanov & Baitik Baatyr Streets.

Fraxinus excelsior, European ash or common ash- belongs to Oleaceae family. It is mainly native to Europe and distributed from Spain to Russia and to Caucasia, and from southwestern Asia to Turkey [18]. This tree species could grow on various soil types and had a lifespan up to 300 years and was drought tolerant but sensitive to cold [19]. With these features, ash tree was planted for forestry and landscape purposes, used in animal feeding, buildings, shipbuilding and musical instruments, and used as firewood, ornamental purposes, shelter and timber. Besides, plant leaves and barks have been used as traditional medicine in treatment of various diseases since Hippocrates [18]. In addition, biomonitoring features of *F. excelsior* tree for indication of certain heavy metals have been clearly established so far [20].

In this regard, this study aimed to determine heavy metal pollution levels and to evaluate the impact of heavy metals on mineral nutrition of biomonitor plant *F. excelsior*.

MATERIALS AND METHODS

Study area. The study area was located at Bishkek, Kyrgyzstan's capital city. Bishkek has been economic and cultural center of the country with its about 1 million population [21]. It has a continental Mediterranean climate with average 440 mm precipitation per year and lush vegetation and mostly tree-lined streets [22]. Plant and soil samplings were done from eight main streets (showed in Figure 1) in Bishkek; Akhunbaev street as Station 1 (hereafter St1), Shabdan Baatyr street as Station 2 (St2), Jibek Jolu street as Station 3 (St3), Chuy street as Station 4 (St4), Moskovskaya street as Station 5 (St5), Bokonbaeva street as Station 6 (St6), Gorky street as Station Yusup Abdrahmanov 7 (St7) and & Baitik Baatyr street as Station 8 (St8).

Sample collection, preparation and analysis. Samplings were done with extreme caution to avoid contamination and mistake in allocation and labeling. Eight plants and eight soil samples were collected, taking one sample from each location with three repetitions. Soil samples (approximately 500 g) were taken from about 10 cm depth using a stainless

shovel. Similar leaf and bark samples were taken to ensure standardization. Leaf samples were divided into two groups: one group was washed with distilled water to remove airborne elements while other group was kept unwashed. Then, all plant samples were oven-dried at 80°C for 48 h and dried samples were grinded using mortar and pestle. 0.2-0.25 g plant sample was transferred into Teflon vessels and added with 8 ml 65% HNO_3 (Merck). Soil samples were passed through 2 mm sieve and weighted 0.2 g sample was transferred into Teflon vessels and added with 9 ml 65% HNO_3, 3 ml 37% HCl and 2 ml 48% HF (Merck). Mineralization was done by using microwave digestion system (Berghof Speedwave MWS-2) as 5 min at 145°C, 5 min at 165°C and 20 min at 175°C. Then, samples were filtered by using Whatman filters (Macherey-Nagel), added up to 50 ml volume with ultrapure water and stored in 50 ml falcon tubes until measurement. Standard solutions were prepared by using multi-element stock solutions 1000 mg/l (Merck). Element measurements Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb and Zn were done by using ICP-OES (PerkinElmer-Optima 7000 DV).

Statistical analysis and calculations. All analysis and calculations are based on dry weight (dw) basis. Multivariate analysis of variance (MANOVA) with Tukey's post-hoc HSD and Pearson correlation was performed by using IBM SPSS Statistics v20. Paired-Samples *t*-test was used to compare means of heavy metals in unwashed and washed leaf samples. Removal ratio and airborne element levels were calculated using element concentrations in unwashed and washed leaves.

RESULTS AND DISCUSSION

To reveal traffic related heavy metal pollution levels by using biomonitor plant *F. excelsior* and its impacts on plant nutrition, concentrations of some heavy metals and mineral nutrients such Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb and Zn in *F. excelsior* plant parts (washed leaf, unwashed leaf and bark) and their co-located soil samples collected from eight different stations (streets) in Bishkek/Kyrgyzstan were analyzed. The measured mean concentrations of tested mineral elements and heavy metals in soil samples (Table 1) and plant parts (Table 2) were given. In addition, expressions "washed leaf", "unwashed leaf" and "bark" are hereafter abbreviated as WL, UwL and B, respectively because of their convenience in use.

TABLE 1
The mean concentrations of mineral elements and heavy metals (mg kg^{-1}, dw) in soil samples

Element	Station	Soil	Element	Station	Soil	Element	Station	Soil
Ca	1	13743.89±137.581	Cd	1	4.992±0.053**	Cr	1	28.662±0.642^{**e}
	2	14944.961±193.192		2	4.694±0.054**		2	26.951±0.69^{**e}
	3	12361.068±148.261		3	5.307±0.066**		3	30.721±0.633^{**d}
	4	8650.093±93.215		4	7.054±0.076**		4	40.172±0.433^{**a}
	5	10604.926±126.999		5	5.962±0.067**		5	34.233±0.513^{**c}
	6	11674.658±128.76		6	5.564±0.059**		6	32.201±0.582^{**d}
	7	9292.296±82.377		7	6.515±0.076**		7	37.081±0.441^{**b}
	8	9859.639±108.6		8	6.185±0.08**		8	35.244±0.431^{**c}
Cu	1	39.117±0.745^{**e}	Fe	1	4497.821±60.334^{**ef}	K	1	2456.841±47.716^{**f}
	2	36.773±0.714^{**f}		2	4221.711±58.337^{**f}		2	2310.353±46.19^{**f}
	3	41.928±0.703^{**d}		3	4786.23±66.343^{**de}		3	2633.423±52.593^{**e}
	4	55.264±0.596^{**a}		4	6359.542±68.531^{**a}		4	3471.645±37.394^{**a}
	5	47.083±0.701^{**c}		5	5377.133±76.488^{**c}		5	2934.823±41.537^{**c}
	6	43.942±0.654^{**d}		6	5017.044±74.811^{**d}		6	2739.112±48.566^{**d}
	7	51.416±0.703^{**b}		7	5870.329±83.087^{**b}		7	3230.254±35.869^{**b}
	8	48.867±0.656^{**c}		8	5577.936±91.816^{**c}		8	3046.413±44.596^{**c}
Mg	1	3626.306±54.195**	Mn	1	185.115±2.916^{**e}	Na	1	98.432±0.876**
	2	3914.906±54.784**		2	173.792±2.736^{**f}		2	91.811±0.928**
	3	3261.661±49.487**		3	196.764±3.077^{**d}		3	104.689±1.044**
	4	2267.602±24.421**		4	259.41±2.796^{**a}		4	136.899±1.475**
	5	2778.544±35.109**		5	221.037±3.193^{**c}		5	116.661±0.929**
	6	3058.867±43.232**		6	206.272±3.016^{**d}		6	108.863±1.072**
	7	2435.289±21.376**		7	239.496±2.09^{**b}		7	127.398±1.003**
	8	2565.166±30.76**		8	227.62±2.574^{**c}		8	120.142±1.275**
Pb	1	71.479±0.861**	Zn	1	186.474±2.31^{**f}			
	2	67.134±0.857**		2	172.874±2.863^{**f}			
	3	75.98±0.66**		3	201.638±2.446^{**e}			
	4	99.73±1.075**		4	268.004±2.888^{**a}			
	5	84.266±0.701**		5	224.457±2.678^{**c}			
	6	79.658±0.72**		6	211.116±2.741^{**d}			
	7	92.698±0.796**		7	247.061±2.505^{**b}			
	8	87.455±0.558**		8	233.141±1.763^{**c}			

Note: Statistical analyses such as multivariate analysis of variance (MANOVA) with Tukey's post hoc HSD were performed. The mean differences is significant at $P < 0.01$ (**) level. a, b, c, d, e, f: different letters indicate different averages within same column, which are significant in terms of averages ($P < 0.01$).

TABLE 2
The mean concentrations of mineral elements and heavy metals (mg kg^{-1}, dw) in plant parts (washed leaf, unwashed leaf and bark) and removal rates

Elements	Stations	Bark (B)	Unwashed leaf (UwL)	Washed leaf (WL)	Removal (%)
Ca	1	15586.756±131.124	14942.684±300.875	14561.957±198.973	2.55
	2	16828.364±147.106	16137.549±345.817	15789.064±126.073	2.16
	3	14120.866±139.342	13530.738±238.336	13459.174±195.329	0.53
	4	9956.991±100.586	9535.839±108.963	9291.708±95.159	2.56
	5	12204.961±186.386	11686.007±160.321	11466.658±136.013	1.88
	6	13341.899±166.131	12778.315±193.756	12622.582±169.348	1.22
	7	10697.239±137.221	10315.114±121.919	10051.487±110.526	2.56
	8	11348.261±174.601	10865.253±156.639	10585.458±140.866	2.58
Cd	1	1.340±0.023**	1.346±0.023**	1.155±0.027**	14.19
	2	1.251±0.022**	1.256±0.023**	1.083±0.026**	13.77
	3	1.436±0.024**	1.442±0.021**	1.229±0.028**	14.77
	4	1.899±0.019**	1.888±0.022**	1.621±0.017**	14.14
	5	1.603±0.020**	1.611±0.029**	1.369±0.023**	15.02
	6	1.496±0.023**	1.503±0.023**	1.288±0.026**	14.30
	7	1.753±0.024**	1.757±0.024**	1.496±0.019**	14.85
	8	1.654±0.023**	1.658±0.026**	1.421±0.020**	14.29
Cr	1	1.512±0.025^{**e}	3.733±0.042^{**e}	3.408±0.045^{**e}	8.71
	2	1.411±0.027^{**e}	3.509±0.039^{**e}	3.179±0.053^{**e}	9.40
	3	1.608±0.022^{**d}	3.971±0.049^{**d}	3.65±0.039^{**d}	8.08
	4	2.119±0.021^{**a}	5.237±0.06^{**a}	4.85±0.05^{**a}	7.39
	5	1.807±0.022^{**c}	4.428±0.061^{**c}	4.069±0.042^{**c}	8.11
	6	1.686±0.026^{**d}	4.131±0.059^{**d}	3.827±0.033^{**d}	7.36
	7	1.973±0.024^{**b}	4.835±0.067^{**b}	4.476±0.049^{**b}	7.43
	8	1.875±0.023^{**c}	4.561±0.072^{**c}	4.223±0.053^{**c}	7.41
Cu	1	8.161±0.13^{**e}	8.588±0.09^{**e}	7.08±0.085^{**e}	17.56
	2	7.611±0.129^{**f}	8.01±0.111^{**f}	6.605±0.09^{**f}	17.54
	3	8.676±0.104^{**d}	9.133±0.125^{**d}	7.59±0.1^{**d}	16.89
	4	11.349±0.115^{**a}	12.241±0.14^{**a}	10.01±0.103^{**a}	18.23
	5	9.673±0.13^{**c}	10.259±0.107^{**c}	8.399±0.114^{**c}	18.13
	6	9.027±0.123^{**d}	9.574±0.136^{**d}	7.899±0.116^{**d}	17.50
	7	10.561±0.142^{**b}	11.297±0.162^{**b}	9.242±0.086^{**b}	18.19
	8	9.963±0.142^{**c}	10.65±0.13^{**c}	8.714±0.101^{**c}	18.18
Fe	1	130.348±1.817^{**ef}	145.496±2.572^{**ef}	141.251±2.185^{**ef}	2.92
	2	121.595±1.602^{**f}	136.607±2.237^{**f}	132.748±2.042^{**f}	2.82
	3	139.648±1.804^{**de}	155.974±3.033^{**de}	150.281±2.447^{**de}	3.65
	4	184.278±1.862^{**a}	205.572±2.349^{**a}	196.841±2.016^{**a}	4.25
	5	155.697±2.279^{**c}	172.643±2.69^{**c}	167.762±2.574^{**c}	2.83
	6	146.408±1.961^{**d}	162.275±2.901^{**d}	156.579±2.77^{**d}	3.51
	7	171.255±1.79^{**b}	189.866±2.547^{**b}	183.181±2.307^{**b}	3.52
	8	161.503±1.989^{**c}	179.115±2.471^{**c}	174.068±2.522^{**c}	2.82
K	1	301.446±3.389^{**f}	1877.756±44.279^{**f}	1811.625±25.045^{**f}	3.52
	2	283.523±4.219^{**f}	1752.01±44.752^{**f}	1689.931±21.087^{**f}	3.54
	3	320.437±2.679^{**e}	1996.193±44.469^{**e}	1942.281±18.866^{**e}	2.70
	4	415.700±4.199^{**a}	2631.271±30.067^{**a}	2560.869±26.227^{**a}	2.68
	5	357.247±4.327^{**c}	2224.233±42.263^{**c}	2146.924±22.756^{**c}	3.48
	6	333.325±3.783^{**d}	2092.726±40.870^{**d}	2019.75±19.882^{**d}	3.49
	7	386.888±3.818^{**b}	2428.165±33.993^{**b}	2363.218±19.634^{**b}	2.67
	8	367.833±4.142^{**c}	2289.697±37.038^{**c}	2228.231±24.117^{**c}	2.68
Mg	1	787.579±12.344**	2254.776±25.960**	2207.471±35.896**	2.10
	2	856.426±14.234**	2451.640±29.240**	2382.789±35.844**	2.81
	3	713.573±12.114**	2028.758±24.468**	1999.104±27.875**	1.46
	4	506.359±5.115**	1419.800±16.222**	1370.743±14.040**	3.46
	5	616.309±9.601**	1740.405±23.971**	1703.413±25.065**	2.13
	6	673.860±9.204**	1915.477±22.054**	1875.018±23.996**	2.11
	7	543.926±7.054**	1525.457±20.877**	1482.569±17.921**	2.81
	8	572.941±7.178**	1617.992±18.319**	1572.785±23.037**	2.79
Mn	1	8.786±0.122^{**e}	10.44±0.046^{**e}	7.899±0.099^{**e}	24.34
	2	8.202±0.123^{**f}	9.803±0.031^{**f}	7.372±0.102^{**f}	24.80
	3	9.414±0.157^{**d}	11.102±0.057^{**d}	8.399±0.075^{**d}	24.35
	4	12.214±0.123^{**a}	14.532±0.166^{**a}	11.088±0.114^{**a}	23.70
	5	10.414±0.119^{**c}	12.279±0.139^{**c}	9.377±0.1^{**c}	23.63
	6	9.794±0.136^{**d}	11.548±0.092^{**d}	8.748±0.082^{**d}	24.25
	7	11.372±0.113^{**b}	13.512±0.138^{**b}	10.235±0.111^{**b}	24.25
	8	10.808±0.12^{**c}	12.745±0.169^{**c}	9.656±0.12^{**c}	24.24

Elements	Stations	Bark (B)	Unwashed leaf (UwL)	Washed leaf (WL)	Removal (%)
Na	1	95.93±1.476**	193.686±2.652**	187.474±2.088**	3.21
	2	89.464±1.183**	180.615±2.427**	176.27±2.295**	2.41
	3	102.81±1.328**	205.976±2.673**	200.991±2.312**	2.42
	4	135.629±1.37**	271.681±3.104**	260.689±2.67**	4.05
	5	114.595±1.154**	227.736±2.962**	224.05±3.346**	1.62
	6	106.92±1.239**	214.245±2.65**	210.756±2.795**	1.63
	7	125.143±1.246**	250.766±3.473**	242.605±3.211**	3.25
	8	118.949±0.944**	236.405±3.014**	230.663±3.423**	2.43
Pb	1	8.563±0.088**	12.729±0.186**	10.087±0.13**	20.76
	2	7.988±0.092**	11.871±0.179**	9.483±0.122**	20.12
	3	9.177±0.096**	13.643±0.174**	10.808±0.103**	20.78
	4	12.122±0.122**	18.016±0.206**	14.26±0.146**	20.85
	5	10.238±0.101**	15.206±0.158**	12.059±0.112**	20.70
	6	9.555±0.097**	14.304±0.188**	11.333±0.105**	20.77
	7	11.188±0.111**	16.648±0.223**	13.169±0.162**	20.90
	8	10.621±0.089**	15.701±0.177**	12.513±0.137**	20.30
Zn	1	37.146±0.465**f	42.943±0.412**f	36.696±3.517**f	14.55
	2	34.859±0.584**f	40.145±0.372**f	34.583±3.283**f	13.85
	3	39.997±0.356**e	45.567±0.441**e	43.229±0.233**e	5.13
	4	52.033±0.526**a	60.41±0.69**a	56.183±0.575**a	7.00
	5	44.669±0.284**c	50.886±0.441**c	48.213±0.277**c	5.25
	6	41.697±0.32**d	47.458±0.493**d	45.324±0.174**d	4.50
	7	48.421±0.363**b	55.744±0.503**b	52.275±0.475**b	6.22
	8	46.021±0.301**c	52.843±0.486**c	49.697±0.293**c	5.95

Note: Statistical analyses such as multivariate analysis of variance (MANOVA) with Tukey's post hoc HSD were performed. The mean differences is significant at $P < 0.01$ (**) level. a, b, c, d, e, f: different letters indicate different averages within same column, which are significant in terms of averages ($P < 0.01$).

Calcium (Ca) is an essential macroelement for plants. It involves in intracellular signaling, symbiotic and immune responses [23], maintains cell integrity, membrane stability, osmoregulation, cation/anion balance and plant growth [24]. It is also a structural component for cell wall and stimulates production of cell wall precursors [25]. Its natural concentrations in plants were reported between 1000 and 50000 mg kg^{-1} [24]. In this study, lowest-to-highest Ca levels (mg kg^{-1}) in soil were found as 8650.093±93.215 (St4)-14944.961±193.192 (St2) while in plant parts it ranged as 9291.708±95.159 (St4/WL)-16828.364±147.106 (St2/B). Removal rates were calculated between lowest 0.53% at St3 and highest 2.58% at St8. In addition, St3 (0.53%) and St6 (1.22%) were also noted with relatively lower removal rates compared to other stations. In a similar study, *F. excelsior* plants under different nutrient and water statuses from forest sites of Bavaria/Germany were investigated and Ca contents in leaves were found 7000-31100 mg kg^{-1} [26]. This was higher than findings of this study and also suggested that variations could be caused by different soil properties at study sites. In another work from an abandoned coal mine site at Indiana/USA, Ca levels in *F. pennsylvanica* leaves were 10300 mg kg^{-1} at control site and 12000 mg kg^{-1} at mine site [27]. However in this study, Ca content was slightly higher than Jensen et al. [27] reports. The physiological variations between *F. excelsior* and *F. pennsylvanica* plants, and negative impacts of mine site were thereby considered to have some impacts. In other similar study conducted by Severoglu et al. [21], Ca levels in *Juniperus virginiana* from Bishkek/Kyrgyzstan were reported as 9296.613-16283.677 mg kg^{-1} in soil, 1657.342-2841.166 mg kg^{-1} in bark, 2461.592-4161.037 mg kg^{-1} in unwashed leaves and 2369.536-4092.177 mg kg^{-1} in washed leaves. In the same study, removal ratio of Ca was calculated as 1.61-3.74%. Leaf Cd levels reported by Severoglu et al. [21] were higher than findings of this study while in soil it was slightly higher. Overall, Ca levels were implicated to show variations depending on soil type, plant parts and plant species.

Cadmium (Cd) is a toxic heavy metal and its presence and accumulation are regarded as serious threat for ecosystem/s [28]. Its emission is mainly caused by anthropogenic activities like industrial and agricultural applications, mining, waste combustion etc. [29]. The expected natural Cd range in soil and plant tissues are reported as 0.06-1.1 mg kg^{-1} and 0.05-0.5 mg kg^{-1} respectively [30]. In this study, lowest-to-highest Cd contents (mg kg^{-1}) in soil samples were detected as 4.694±0.054 (St2)-7.054±0.076 (St4) whereas in plant parts were ranged as 1.083±0.026 (St2/WL)-1.899±0.019 (St4/B). The removal ratio of Cd was lowest 13.77% at St2 and highest 15.02% at St5. Figuring out that Cd levels in plant and soil samples at all stations were higher than the normal limits. In addition, a negative correlation was also present between Ca and Cd levels and this may be arising from a competition between them. Recently, Huang et al. [29] stated that due to chemical similarity between those elements, Ca could mediate Cd-triggered metabolic

alterations in plants. Colak et al., [61] reported that the nearly 40% of Cd comes from industrial facilities and dust sourced from traffic. Increase in population of Bishkek city have affected the pollution negatively. For example increase in vehicles and traffic jams caused emission of more amount of pollutants in to ecosystem [28].

Relevant studies reported that Cd content in *F. excelsior* leaves and dredged brackish sediment were 0.3±0.3 and 5.7±0.8 mg kg^{-1} respectively [31]. Cd contents from different sites in Kayseri/Turkey were 2.02-3.43 mg kg^{-1} in soil and 0.428-0.243 mg kg^{-1} in leaves, and removal ratio of Cd was 18.52-41.35% [20]. Cd content in leaves of *Fraxinus* spp. in Xinxiang/China were 0.090±0.001 mg kg^{-1} in clean area and 0.113±0.003 mg kg^{-1} in polluted area [32]. Cd content in *F. manshurica* leaves from road sides was 0.053 mg kg^{-1} [33]. Cd content in *F. rotundifolia* soil and leaves from roads with heavy traffics in Karaj/Iran was 3.7±0.3 mg kg^{-1} and 2.4±0.2 mg kg^{-1} respectively [34]. Cd content in soil and leaf samples of *F. sogdiana* from mining-influenced sites in Jinchang/China was 9.2 mg kg^{-1} and 2.08 mg kg^{-1} respectively [35].

Chromium (Cr) is a phytotoxic heavy metal and its toxicity gives damage to DNA and cell membranes, decreases activity of enzymes related to nitrogen and starch metabolism, causes structural alterations and reductions in biomass and growth, and interferes with respiration, photosynthesis, water and minerals uptake [36 - 38]. In literature, soil Cr contents showed great variability, nevertheless it is also stated as average 54 mg kg^{-1} [30]. In addition, maximum allowable limit for protection of human health and environment is reported as 64 mg kg^{-1} [39, 40]. In present work, lowest-to-highest soil Cr contents (mg kg^{-1}) were 26.951±0.690 (St2)-40.172±0.433 (St4). Thus, soil Cr levels at stations were below the world average and maximum allowable limits. The average plant Cr content was stated 0.1-0.5 mg kg^{-1} and 5-30 mg kg^{-1} of Cr were defined as toxic [41]. In this study, lowest-to-highest Cr levels (mg kg^{-1}) in plant parts were 1.411±0.027 (St2/B)-5.237±0.060 (St4/UwL) and removal rate of air-borne Cr was 7.36% (St6)-9.40% (St2). It appeared that soil Cr level was lower than the average but in plants it was higher than normal limits without demonstrating any toxicity symptoms. Li-qiang et al. [33] reported Cr content in leaves of *F. manshurica* as 6.04 mg kg^{-1} [33]. Cr contents in *F. excelsior* soil and leaves were demonstrated as 124.42 mg kg^{-1} and 1.7-1.1 mg kg^{-1} respectively and Cr removal ratio was 18.8-43.55% [20]. Cr content in *F. pennsylvanica* barks ranged as 1.7-5.4 mg kg^{-1} [42]. Bing et al. [32] demonstrated Cr content in leaves of *Fraxinus* plants from roadsides as 1.138±0.049 mg kg^{-1} in clean areas and 1.787±0.017 mg kg^{-1} in polluted areas.

Copper (Cu) is an essential micronutrient for plants and it involves in redox reactions, cellular respiration, photosynthesis and protection mechanisms related to oxidative damage [43, 44]. Natural Cu content in soil is reported as 25-75 mg kg^{-1} [30]. In the present work, lowest-to-highest Cu contents (mg kg^{-1}) in soil were found as 36.773±0.714 (St2)-55.264±0.596 (St4) and this was within the normal range. However, natural Cu levels in plants are reported as 5-30 mg kg^{-1} and many plants show toxicity symptoms above 30 mg kg^{-1} [43]. In this study, lowest-to-highest Cu content (mg kg^{-1}) in plant parts were noted as 6.605±0.09 (St2/WL)-12.241±0.14 (St4/UwL) and removal rates of Cu were 16.89% (St3)-18.23% (St4). In a similar study, Mertens et al. [31] reported Cu content in *F. excelsior* soil and leaves as 54.2±6.3 mg kg^{-1} and 12.4±1.8 mg kg^{-1} respectively. Aksoy and Demirezen [20] reported Cu levels as 21.06 mg kg^{-1} in soil and 16.21-6.62 mg kg^{-1} in *F. excelsior* leaves. In the same study, Cu removal ratio was calculated as 10.27-32.69% [20]. Faggi et al. [42] showed Cu content in *F. pennsylvanica* barks as 36.9-94.4 mg kg^{-1}.

Iron (Fe) is an essential plant micronutrient. It participates in various metabolic processes including photosynthesis, respiration, DNA replication, chlorophyll and hormone synthesis and nitrogen fixation [45 - 47]. Fe content is reported to range 5000-50,000 mg kg^{-1} in soil [41] and 50-250 mg kg^{-1} in plants and above 500 mg kg^{-1} concentrations plants mainly show toxicity symptoms [43, 50]. In this study, lowest-to-highest soil Fe levels (mg kg^{-1}) were measured as 4221.711±58.337 (St2)-6359.542±68.531 (St4) and these values were close/under the sublimit whereas in plant parts they were 121.595±1.602 (St2/B)-205.572±2.349 (St4/UwL). So, Fe levels in *F. excelsior* plants were within the normal range. The removal rates of Fe were estimated lowest 2.82% at St2 and 8, and highest 4.25% at St4. In earlier studies, Fe content in *F. excelsior* leaves was stated as 51-140 mg kg^{-1} [26]. Fe levels in *F. pennsylvanica* leaves was reported as 82.8-75.1 mg kg^{-1} [27]. Faggi et al. [42] showed Fe content in ash tree barks as 111.3-1105.1 mg kg^{-1}. Gałuszka et al. [48] demonstrated Fe contents as 11300-23700 mg kg^{-1} in soil, 100-200 mg kg^{-1} in leaves and 300-320 mg kg^{-1} in bark.

Potassium (K) is an essential, highly mobile and most abundant cation in plant cytosol. It has crucial roles in soil-water-plant interactions and it regulates stomata guard cell activity and involves in osmotic and membrane potential mechanisms. It also participates in photosynthesis, enzyme activation, protein synthesis, photonastic and seismonastic movements, phloem transport, carbohydrate production and translocation, and fruit development and growth [24, 49]. The natural K content in soil is reported to be about 12000 mg kg^{-1} [50] and in plants it ranges as 20000-50000 mg kg^{-1} in vegetative parts [24]. In present work, lowest-to-highest K content (mg kg^{-1}) in soil was determined as 2310.353±46.190 (St2)-3471.645±37.394 (St4) and in plant parts it ranged as 283.523±4.219 (St2/B)-

2631.271±30.067 (St4/UwL). So, both in soil and plant parts potassium levels were quite below the expected range. Besides, removal rates of K were estimated lowest 2.68% at St4 and 8, and highest 3.54% at St2. In relevant works, K content in *F. excelsior* leaves was reported in ranges 6000-27900 mg kg^{-1} [26]. Jensen et al. [27] showed K content in *F. pennsylvanica* leaves as 18200-25600 mg kg^{-1}. K contents were estimated 1100-2200 mg kg^{-1} in soil, 8300-14400 mg kg^{-1} in leaves and 1500-6700 mg kg^{-1} in bark [48]. Reduced K levels could be the results of slightly higher accumulation of toxic metals [51].

Magnesium (Mg) is an essential macronutrient and serves as an important divalent cation. It is required by various enzymes as cofactor and it is also a component of chlorophyll molecules, and involves in DNA replication and protein synthase mechanisms [52]. The range of Mg levels is reported as 300-8000 mg kg^{-1} in soil [53] and 15000-35000 mg kg^{-1} in plants [24]. Present work revealed that lowest-to-highest Mg content (mg kg^{-1}) in soil was 2267.602±24.421 (St4)-3914.906±54.784 (St2) and in plant parts it was 506.359±5.115 (St4/B)-2451.640±29.240 (St2/UwL). Based on above-literature, soil Mg levels were within normal range whereas they were below the normal limits in plant parts. In addition, removal rates of Mg were estimated lowest 1.46% at St3 and highest 3.46% at St4. Similar studies reported that Mg content in *F. excelsior* leaves was 2200-6900 mg kg^{-1} [26]. Mg content in *F. pennsylvanica* leaves ranged as 3820-3050 mg kg^{-1} [27]. Gałuszka et al. [48] reported Mg contents as 1300-4900 mg kg^{-1} in soil, 1500-3500 mg kg^{-1} in leaves and 1100-2100 mg kg^{-1} in bark.

Manganese (Mn) is an essential plant micronutrient and it involves in various metabolic processes such as photosynthesis, various enzymatic reactions, protein, lignin and carbohydrate synthesis, and cell division and extension [43, 54]. The range of Mn contents in soil is reported to be as 10-9000 mg kg^{-1} with average 437 mg kg^{-1} [30] whereas in plant parts, it is demonstrated as 30-300 mg kg^{-1} [41]. In this study, lowest-to-highest detected Mn content (mg kg^{-1}) in soil was 173.792±2.736 (St2)-259.41±2.796 (St4) and in plant parts it ranged 7.372±0.102 (St2/WL)-14.532±0.166 (St4/UwL). Inferring to above-literature, Mn in plant parts was indicated below the normal range. In addition, Mn removal rates were estimated lowest 23.63% at St5 and highest 24.80% at St2. This also revealed Mn has the highest proportion of air-emission. Studies also demonstrated that Mn content in *F. pennsylvanica* leaves was 32.9-48.7 mg kg^{-1} [27]. Mn level in *F. pennsylvanica* bark was 19.2-26.5 mg kg^{-1} [42]. Gałuszka et al. [48] reported Mn contents as 342-1570 mg kg^{-1} in soil, 9-36 mg kg^{-1} in leaves and 16-75 mg kg^{-1} in bark.

Sodium (Na) is classified as a beneficial element due to its essentiality for some plants and to some extent it can take over K functions in case of K-deficiency [55, 56]. Soil and plant Na concentrations are reported to show great variability, ranging as 1000-10000 mg kg^{-1} in soil and 100-100000 mg kg^{-1} in plants [50]. This study showed that lowest-to-highest Na content (mg kg^{-1}) in soil ranged as 91.811±0.928 (St2)-136.899±1.475 (St4) and in plant parts it was 89.464±1.183 (St2/B)-271.681±3.104 (St4/UwL). In addition, Na removal rates were noted lowest 1.62% at St5 and highest 4.05% at St4. In earlier studies, Na content in *F. pennsylvanica* leaves was reported as 23.1-23.2 mg kg^{-1} [27]. Gałuszka et al. [48] reported Na contents as 100-800 mg kg^{-1} in soil, 50-370 mg kg^{-1} in leaves and 140-1240 mg kg^{-1} in bark.

Lead (Pb) is considered as second toxic substance after arsenic according to Agency for Toxic Substances and Disease Registry report [59, 60]. It adversely affects plants from biochemical, structural, metabolic, morphological and physiological aspects. Pb levels in soil are reported to range 10-40 mg kg^{-1} with a grand average 25 mg kg^{-1} whereas in plants 5-10 mg kg^{-1} is accepted as normal but above 30 mg kg^{-1} is considered toxic [41]. In this study determined that lowest-to-highest Pb content (mg kg^{-1}) in soil was 67.134±0.857 (St2)-99.73±1.075 (St4) and in plant parts it was 7.988±0.092 (St2/B)-18.016±0.206 (St4/UwL). Referencing to Kabata-Pendias and Pendias [41], Pb levels in soil and plants were above the normal range but it was not at toxic level for plants. In addition, Pb removal rates were measured lowest 20.12% at St2 and highest 20.90% at St7. Similar studies also reported that Pb contents in soil and *F. excelsior* leaves were 75.2 mg kg^{-1} and 5.0 mg kg^{-1} respectively [31]. Aksoy and Demirezen [20] reported Pb content as 9.12-57.62 mg kg^{-1} in soil and 8.02-18.42 mg kg^{-1} in leaves and removal ratio of Pb was 9.57-46.11% [20]. Pb content in *F. pennsylvanica* bark was 0.7-43.0 mg kg^{-1} [42]. Pb content in *F. pennsylvanica* leaves was 0.129-0.130 mg kg^{-1} [27]. Pb contents in soil and *F. rotundifolia* leaves were as 7.1±1.4 mg kg^{-1} and 9±1.4 mg kg^{-1} respectively [34].

Zinc (Zn) is an essential plant micronutrient. It has crucial role in enzyme structure and function. It also involves in tryptophan and indole acetic acid (IAA) synthesis, membrane integrity and lipid peroxidation, carbohydrate metabolism and protein synthesis [43, 57]. The natural Zn contents in soil is reported as 3-770 mg kg^{-1} with a grand average of 65 mg kg^{-1} [10, 43, 53, 58] whereas in plants it is ranged as 27-150 mg kg^{-1} [41]. Present study demonstrated that lowest-to-highest Zn content (mg kg^{-1}) in soil was 172.874±2.863 (St2)-268.004±2.888 (St4) and in plant parts it was 34.583±3.283 (St2/WL)-60.410±0.690 (St4/UwL). Inferring to above-reference, soil Zn levels were noted above average while in plant parts levels were within normal ranges. In addition, Zn removal rates were calculated lowest 4.50% at St6 and highest 14.55% at St1. Studies showed that Zn contents in soil and *F. excelsior*

leaves were 358 mg kg^{-1} and 26 mg kg^{-1} respectively [31]. Aksoy and Demirezen [20] showed Zn levels as 92.64 mg kg^{-1} in soil and 13.22-29.41 mg kg^{-1} in leaves, and Zn removal ratio was reported as 9.92-38.16% [20]. Zn content in *F. pennsylvanica* bark was demonstrated as 2.7-189.1 mg kg^{-1} [42]. Zn level in *F. pennsylvanica* leaves was determined as 23.9-41.7 mg kg^{-1} [27].

Overall estimations revealed that Cd, Cr, Cu, Fe, K, Mn, Na, Pb and Zn elements demonstrated their highest concentrations at St4 (Chuy Street) and their lowest values were at St2 (Shabdan Baatry Street). In contrary, Ca and Mg levels were highest at St2 (Shabdan Baatry Street) and lowest at St4 (Chuy Street). Low element levels at St2 (Shabdan Baatry Street) were understandable because this street has been well exposed to breezes from mountain site and it has also low traffic density. However, St4 (Chuy Street) has been the busiest road, as it is an international transit road passing through the city. The city's largest automotive industrial zone was also located on this road. Besides, air flow was low at this site because street was away from mountain and closed to the mountain breezes.

Moreover, in this study work also analyzed the correlations between soil and washed leaf samples (Table 3). Results clearly demonstrated that there has been a definite positive (>0.86, >0.99) or negative (->0.86, ->0.96) correlation between the quantities of soil and plant elements. This significant correlation once more showed the suitability of using *F. excelsior* plants as biomonitor organism and also further corroborated the similar reports in this regard.

In addition, self-correlations of all soil and plant elements showed that all test metals except Ca and Na demonstrated strong positive correlations (>0.61, >0.99) with each other (Table 4).

Furthermore, paired-sample *t*-test results for unwashed and washed leaf samples demonstrated that atmospheric particles deposited on leaves could increase a significant amount of elemental concentrations in unwashed leaves (Table 5).

TABLE 3
Correlation matrix (*R*) data between quantities of soil and washed leaf elements. Matrix data are obtained by Pearson correlation method.

Correlation matrix (*R*)												
Pearson correlation		Soil										
		Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Zn
Washed Leaf (WL)	Ca	.986**	-.955**	-.939**	-.948**	-.942**	-.934**	.988**	-.943**	-.960**	-.952**	-.957**
	Cd	-.916**	.975**	.955**	.965**	.972**	.966**	-.911**	.988**	.971**	.973**	.966**
	Cr	-.939**	.992**	.986**	.991**	.989**	.980**	-.942**	.985**	.997**	.987**	.988**
	Cu	-.939**	.989**	.972**	.979**	.987**	.985**	-.930**	.993**	.987**	.980**	.986**
	Fe	-.923**	.993**	.987**	.993**	.993**	.985**	-.922**	.991**	.991**	.975**	.986**
	K	-.946**	.983**	.969**	.979**	.984**	.978**	-.945**	.985**	.988**	.993**	.984**
	Mg	.995**	-.942**	-.914**	-.926**	-.926**	-.918**	.991**	-.930**	-.947**	-.948**	-.943**
	Mn	-.941**	.997**	.979**	.986**	.988**	.977**	-.938**	.994**	.993**	.983**	.986**
	Na	-.938**	.986**	.978**	.983**	.992**	.990**	-.929**	.987**	.987**	.978**	.988**
	Pb	-.948**	.982**	.968**	.977**	.981**	.976**	-.947**	.984**	.987**	.992**	.981**
	Zn	-.857**	.889**	.896**	.896**	.873**	.856**	-.886**	.876**	.901**	.886**	.885**

**Correlation is significant at 0.01 levels (2-tailed)

TABLE 4
Self-correlation matrix (*R*) data of soil and plant elements. Matrix data are obtained by Pearson correlation method.

Correlation matrix (*R*)										
Pearson correlation	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Zn
Ca	-.371**	-.322**	-.351**	-.297**	-.443**	.122	-.300**	-.299**	-.336**	-.367**
Cd		.992**	.999**	.994**	.614**	.630**	.995**	-.472**	.996**	.997**
Cr			.995**	.997**	.675**	.712**	.996**	-.428**	.998**	.997**
Cu				.997**	.623**	.650**	.997**	-.472**	.997**	.998**
Fe					.618**	.684**	1.000**	-.493**	.997**	.996**
K						.736**	.618**	.359**	.656**	.648**
Mg							.683**	-.104	.688**	.659**
Mn								-.492**	.998**	.996**
Na									-.444**	-.444**
Pb										.998**

**Correlation is significant at 0.01 levels (2-tailed)

TABLE 5
Paired-sample *t*-test results for unwashed and washed leaf samples

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	**Ca UwL - Ca WL**	245.426725	366.954833	58.020653	128.068876	362.784574	4.230	39	.000
Pair 2	**Cd UwL - Cd WL**	.225175	.035620	.005632	.213783	.236567	39.981	39	.000
Pair 3	**Cr UwL - Cr WL**	.340400	.060668	.009592	.320997	.359803	35.486	39	.000
Pair 4	**Cu UwL - Cu WL**	1.776475	.311402	.049237	1.676884	1.876066	36.080	39	.000
Pair 5	**Fe UwL – Fe WL**	5.604650	2.495342	.394548	4.806601	6.402699	14.205	39	.000
Pair 6	**K UwL – K WL**	66.152800	52.940551	8.370636	49.221590	83.084010	7.903	39	.000
Pair 7	**Mg UwL - Mg WL**	45.051700	21.709391	3.432556	38.108700	51.994700	13.125	39	.000
Pair 8	**Mn UwL - Mn WL**	2.898225	.366023	.057873	2.781165	3.015285	50.079	39	.000
Pair 9	**Na UwL - Na WL**	5.951525	5.489910	.868031	4.195767	7.707283	6.856	39	.000
Pair 10	**Pb UwL - Pb WL**	3.050725	.497776	.078705	2.891529	3.209921	38.761	39	.000
Pair 11	**Zn UwL - Zn WL**	3.724725	3.880366	.613540	2.483724	4.965726	6.071	39	.000

Unwashed Leaf (UwL); Washed leaf (WL).

CONCLUSION

Findings showed that mineral element contents in *F. excelsior* plants are affected by heavy metal pollution. From stations, Chuy (St4) and Shabdan Baatry (St2) Streets were noted with highest and lowest pollution rates, respectively. Cd, Cr, Cu, Fe, K, Mg, Mn, Pb and Zn demonstrated strong positive correlations with each other. Heavy metals Cd and Pb in soil, and Cd, Cr and Pb in plants were above the acceptable limits. Removal rates of Cd, Cu, Mn and Pb were higher than other elements and demonstrated similarities between all stations. Overall, *F. excelsior* plants compensate the characteristics that must be found in biomonitor organisms and they reflects the pollution levels at the test stations, appropriately.

ACKNOWLEDGEMENTS

This investigation was funded by Kyrgyz-Turkish Manas University, Commission of Scientific Research Project under grant KTMU-BAP 2013.FEB.03.

REFERENCES

[1] Xu, X., Zhao, Y., Zhao, X., Wang, Y., Deng, W. (2014) Sources of heavy metal pollution in agricultural soils of a rapidly industrializing area in the Yangtze Delta of China. Ecotoxicology and Environmental Safety. 108, 161-167.

[2] Osma, E., Ozyigit, I.I., Yasar U., Demir G. (2014) Assessment of some heavy metals in wild type and cultivated purslane (*Portulaca oleracea* L.) and soils in Istanbul, Turkey. Fresen. Environ. Bull. 23, 2181-2189.

[3] Akguc, N., Ozyigit, I.I. and C. Yarci, (2008) *Pyracantha coccinea* Roem. (Rosaceae) as a biomonitor for Cd, Pb and Zn in Mugla Province (Turkey). Pakistan Journal of Botany. 40(3), 1767-1776.

[4] Ozyigit, I. ., Dogan, I., Igdelioglu, S., Filiz, E., Karadeniz, S., Uzunova, Z. (2016) Screening of damage induced by lead (Pb) in rye (*Secale cereale* L.)–a genetic and physiological approach. Biotechnology and Biotechnological Equipment. 30(3), 489-496,

[5] Armah, F.A., Quansah, R., Luginaah, I. (2014) A systematic review of heavy metals of anthropogenic origin in environmental media and biota in the context of gold mining in Ghana. International Scholarly Research Notices. 2014, Article ID 252148, 37p.

[6] Osma, E., Ozyigit, I.I., Leblebici, Z., Demir, G., Serin, M. (2012) Determination of heavy metal concentrations in tomato (*Lycopersicon esculentum* Miller) grown in different station types. Romanian Biotechnological Letters. 17(1), 6962-6974.

[7] Ozyigit, I.I., Yalcin, B., Turan, S., Saracoglu, I. A., Karadeniz, S., Yalcin, I. E., Demir, G. (2018) Heavy metal and mineral nutrient status of widely used medicinal plants' leaves in Turkey and their health implications. Biological Trace Element Research. 182, 387-406.

[8] Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B., Mishra, S. (2010) Effect of toxic metals on human health. The Open Nutraceuticals Journal. 3(1), 94-99.

[9] Ebah, E., Tersagh, I., Okpokwasili, G.C. (2016) Studies on Seasonal Variation and Effect of Heavy Metal Pollution on Microbial Load of Marine Sediment. American Journal of Marine Science. 4(1), 4-10.

[10] Sahin, I., Akcicek, E., Guner, O., Dogan, Y., and Ugulu, I. (2016) An investigation on determining heavy metal accumulation in plants growing at Kumalar Mountain in Turkey. Eur-Asian Journal of BioSciences, 10, 22-29.

[11] Alloway, B.J. (2013) Sources of heavy metals and metalloids in soils. In: Heavy metals in soils. Springer, Dordrecht. 11-50.

[12] Ozturk, A., Yarci, C., Ozyigit, I.I. (2017) Assessment of heavy metal pollution in Istanbul using plant (*Celtis australis* L.) and soil assays. Biotechnology and Biotechnological Equipment. 31(5), 948-954.

[13] Chen, T.B., Zheng, Y.M., Lei, M., Huang, Z.C., Wu, H.T., Chen, H., Fan, K.K., Wu, X., Tian, Q.Z. (2005) Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. Chemosphere. 60(4), 542-551.

[14] Davidson, C.M. (2013) Methods for the determination of heavy metals and metalloids in soils. In: Alloway, B.J. (Ed.) Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioavailability. 3rd edition. Springer Netherlands, 97-140.

[15] Martin, M.H. (2012) Biological monitoring of heavy metal pollution: land and air. Springer Science and Business Media.

[16] Wittig, R. (1993) General aspects of biomonitoring heavy metals by plants. In: Markert, B. (ed.) Plants as Biomonitors: indicators for heavy metals in the terrestrial environment. Weinheim VCH Publisher, 3-28.

[17] Yilmaz, R., Sakcali, S., Yarci, C., Aksoy, A., Ozturk, M. (2006) Use of *Aesculus hippocastanum* L. as a biomonitor of heavy metal pollution. Pakistan Journal of Botany. 38(5), 1519-1527.

[18] Thomas, P.A. (2016) Biological Flora of the British Isles: *Fraxinus excelsior*. Journal of Ecology. 104(4), 1158-1209.

[19] Pautasso, M., Aas, G., Queloz, V., Holdenrieder, O. (2013) European ash (*Fraxinus excelsior*) dieback-a conservation biology challenge. Biological Conservation. 158, 37-49.

[20] Aksoy, A., Demirezen, D. (2006) *Fraxinus excelsior* as a Biomonitor of Heavy Metal Pollution. Polish Journal of Environmental Studies. 15(1), 27-33.

[21] Severoglu, Z., Ozyigit, I.I., Dogan, I., Kurmanbekova, G., Demir, G., Yalcin, I.E., Kari, G.K. (2015) The usability of *Juniperus virginiana* L. as a biomonitor of heavy metal pollution in Bishkek City, Kyrgyzstan. Biotechnology and Biotechnological Equipment. 29(6), 1104-1112.

[22] Hofmann, P., Strobl, J., Nazarkulova, A. (2011) Mapping green spaces in Bishkek-how reliable can spatial analysis be? Remote Sensing. 3(6), 1088-1103.

[23] Yuan, P., Jauregui, E., Du, L., Tanaka, K., Poovaiah, B.W. (2017) Calcium signatures and signaling events orchestrate plant-microbe interactions. Current Opinion in Plant Biology. 38, 173-183.

[24] Hawkesford M., Horst W., Kichey T., Lambers H., Schjoerring J., Møller I.S. and White P. (2012) Functions of Macronutrients. In: Marschner, P. (ed.) Marschner's Mineral Nutrition of Higher Plants. Third Edition. Academic Press, San Diego. ISBN 9780123849052, 135-189.

[25] Hepler, P.K., Winship, L.J. (2010) Calcium at the Cell Wall-Cytoplast Interface. Journal of integrative plant biology. 52(2), 147-160.

[26] Weber-Blaschke, G., Heitz, R., Blaschke, M., Ammer, C. (2008) Growth and nutrition of young European ash (*Fraxinus excelsior* L.) and sycamore maple (*Acer pseudoplatanus* L.) on sites with different nutrient and water statuses. European journal of forest research. 127(6), 465.

[27] Jensen, R.R., Brake, S.S., Wolf, S.F., Bekker, M.F., Hardin, P.J., Jackson, M.W. (2010) Chemical element concentrations in black locust (*Robinia pseudoacacia* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) leaves at the reclaimed Green Valley coal Mine, Indiana, USA. Environmental Earth Sciences. 60(7), 1391-1405.

[28] Dogan I., Ozyigit I.I., Tombuloglu G., Sakcali M.S. Tombuloglu H. (2016) Assessment of Cd-induced genotoxic damage in *Urtica pilulifera* L. using RAPD-PCR analysis. Biotechnology and Biotechnological Equipment. 30(2), 284-291.

[29] Huang, D., Gong, X., Liu, Y., Zeng, G., Lai, C., Bashir, H., Zhou, L., Wang, D., Xu, P., Cheng, M., Wan, J. (2017) Effects of calcium at toxic concentrations of cadmium in plants. Planta. 245, 863–873.

[30] Kabata-Pendias, A., Mukherjee, A.B. (2007) Trace elements from soil to human. New York: Springer-Verlag.

[31] Mertens, J., Vervaeke, P., De Schrijver, A., Luyssaert, S. (2004) Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. Science of the total Environment. 326(1-3), 209-215.

[32] Bing, L., Zhou, Y., Li, Y., Al Azzam, K.M., Aboul-Enein, H.Y. (2015) A Comparative Study of the Contents of Cadmium and Chromium in Leaves of Seventeen Kinds of Road Greening Trees. World. 3(1A), 1-5.

[33] Li-qiang, M., Hai-yan, S., Ning, Z. (2004) Absorption capacity of major urban afforestation species in northeastern China to heavy metal pollutants in the atmosphere. Journal of Forestry Research. 15(1), 73-76.

[34] Monfared, S.H., Matinizadeh, M., Shirvany, A., Amiri, G.Z., Fard, R.M., Rostami, F. (2013) Accumulation of heavy metal in *Platanus orientalis, Robinia pseudoacacia* and *Fraxinus rotundifolia*. Journal of Forestry Research. 24(2), 391-395.

[35] Lu, Y., Li, X., He, M., Zeng, F., Li, X. (2017) Accumulation of heavy metals in native plants growing on mining-influenced sites in Jinchang: a typical industrial city (China). Environmental Earth Sciences. 76(13), 446.

[36] da Conceicao Gomes, M.A., Hauser-Davis, R.A., Suzuki, M.S., and Vitoria, A.P. (2017) Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations. Ecotoxicology and environmental safety, 140, 55-64.

[37] Singh, H.P., Mahajan, P., Kaur, S., Batish, D.R., Kohli, R.K. (2013) Chromium toxicity and tolerance in plants. Environmental chemistry letters. 11(3), 229-254.

[38] Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P., Yang, S., Ali, S., Zhou, W. (2015) Chromium-induced physio-chemical and ultra-structural changes in four cultivars of Brassica napus L. Chemosphere. 120, 154-164.

[39] CCME (2015) Canadian soil Quality Guidelines for the Protection of Environmental and Human Health. Canada Council of Ministers of the Environment, Winnipeg.

[40] Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N.K., Dumat, C., Rashid, M.I. (2017) Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. Chemosphere. 178, 513-533.

[41] Kabata-Pendias, A., Pendias, H. (2001) Trace Elements in Soils and Plants. Third Edition. CRC Press, Boca Raton, USA.

[42] Faggi, A.M., Fujiwara, F., Anido, C., Perelman, P.E. (2011) Use of tree bark for comparing environmental pollution in different sites from Buenos Aires and Montevideo. Environmental monitoring and assessment. 178(1-4), 237-245.

[43] Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F. (2012a) Function of nutrients: micronutrients. In: Marschner's Mineral Nutrition of Higher Plants. Third Edition. Academic Press, San Diego, 191-248.

[44] Vatansever, R., Ozyigit, I.I., Filiz, E. (2017) Genome-wide identification and comparative analysis of copper transporter genes in plants. Interdisciplinary Sciences: Computational Life Sciences. 9(2), 278-291.

[45] Kobayashi, T. and Nishizawa, N.K. (2012) Iron uptake, translocation, and regulation in higher plants. Annual Review of Plant Biology. 63, 131-152.

[46] Briat, J.F., Dubos, C., Gaymard, F. (2015) Iron nutrition, biomass production, and plant product quality. Trends in Plant Science. 20(1), 33-40.

[47] Vatansever, R., Filiz, E., Ozyigit, I. I. (2015) Genome-wide analysis of iron-regulated transporter 1 (IRT1) genes in plants. Horticulture, Environment, and Biotechnology. 56(4), 516-523.

[48] Gałuszka, A., Migaszewski, Z. M., Podlaski, R., Dołęgowska, S., Michalik, A. (2011) The influence of chloride deicers on mineral nutrition and the health status of roadside trees in the city of Kielce, Poland. Environmental Monitoring and Assessment. 176(1-4), 451-464.

[49] Meena, V.S., Bahadur, I., Maurya, B.R., Kumar, A., Meena, R.K., Meena, S.K., Verma, J.P. (2016) Potassium-solubilizing microorganism in evergreen agriculture: an overview. In: Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, 1-20.

[50] Kacar, B., Katkat, A.V. (2007) Plant nutrition. Nobel publication. Istanbul.

[51] Dogan, I., Ozyigit, I.I., Demir, G. (2014) Influence of aluminum on mineral nutrient uptake and accumulation in *Urtica pilulifera* L. J Plant Nutr. 37(3), 469-481.

[52] Chen, Z.C., Peng, W.T., Li, J., Liao, H. (2017) Functional dissection and transport mechanism of magnesium in plants. In: Seminars in cell and developmental biology. Academic Press.

[53] Barker, A.V., Pilbeam, D.J. (Eds.) (2007) Handbook of plant nutrition. Vol. 117. CRC press.

[54] Vatansever, R., Filiz, E., Ozyigit, I.I. (2016a) In silico analysis of Mn transporters (NRAMP1) in various plant species. Molecular biology reports. 43(3), 151-163.

[55] Broadley, M., Brown, P., Cakmak, I., Ma, J. F., Rengel, Z., Zhao, F. (2012b) Beneficial elements. In: Marschner's Mineral Nutrition of Higher Plants. Third Edition. Academic Press, San Diego, 249-269.

[56] Maathuis, F.J. (2013) Sodium in plants: perception, signalling, and regulation of sodium fluxes. Journal of Experimental Botany. 65(3), 849-858.

[57] Vatansever, R., Ozyigit, I.I., Filiz, E. (2016b) Comparative and phylogenetic analysis of zinc transporter genes/proteins in plants. Turkish Journal of Biology. 40, 600-611.

[58] Alloway, B.J. (Ed.) (2013) Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioavailability. 3rd edition. Springer, Amsterdam, The Netherlands.
[59] ATSDR (2003) Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/
[60] Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E. (2011) Lead uptake, toxicity, and detoxification in plants. Reviews of Environmental Contamination and Toxicology. 213, 113-136.
[61] Colak, M., Gumrukcuoglu, M., Boysan, F., Baysal, E. (2016) Determination and mapping of cadmium accumulation in plant leaves on the highway roadside, Turkey. Archives of Environmental Protection. 42(3), 11-16.

Received: **03.04.2018**
Accepted: **07.11.2018**

CORRESPONDING AUTHOR

Zeki Severoglu
Marmara University,
Faculty of Sciences & Arts,
Department of Biology, 34722,
Goztepe, Istanbul – Turkey

e-mail: zseveroglu@marmara.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9674-9685 Fresenius Environmental Bulletin

STUDY ON CHARACTERISTICS OF SIMULATED DYE WASTEWATER TREATMENT USING COUPLED BIO-ELECTROCHEMICAL AF-BAF

Luting Pan, Xinjue Xie, Ajun Wan*, Yue Han, Guijiao Lin, Yixuan Liu

Institute of Modern Agricultural Science and Engineering, Tongji University, Shanghai 200092, China

ABSTRACT

A pilot scale experiment was conducted to analyze the characteristics and factors of contaminant removal in the coupled bio-electrochemical AF-BAF process treating dyeing wastewater. Results showed that the optimum conditions of coupled system were: HRT=7h, pH =6, and DO=4mg·L^{-1}. And the influent COD and dye concentration controlled at 300 mg·$L^{-1}$and 100 mg·L^{-1} respectively were beneficial for pollutants removal. After treatment under the optimum conditions, removal efficiencies of COD, dye and chroma reached 92.2%、97.7% and 99.0% respectively. The system can remove highly concentrated azo dyes efficiently with low COD concentration through multiple mechanisms (e.g. biochemical and internal electrolysis reactions), and simultaneously produce less intermediate products (aromatic amine). The conclusions can provide reference for practical engineering and theoretical research.

KEYWORDS:
AF, azo dye, BAF, coupling system of electrochemistry and biochemistry, textile wastewater

INTRODUCTION

Dyeing wastewater is one of the most refractory industrial sewage because it has a complex composition, contains many refractory substances and produces carcinogenic intermediate products (aniline) during the treatment process. According to the "Textile Dyeing and Finishing Industry Water Pollutant Discharge Standard" (GB4287-2012) implemented in 2015, the effluent COD (chemical oxygen demand) concentration must be less than 50mg·L^{-1}, chroma less than 50 times, and aniline must not be detected, which proposes a higher treatment requirement.

Currently, approaches for treating dyeing wastewater are mainly divided into two types. The first type is physicochemical method, e.g. adsorption, membrane separation, chemical precipitation, chemical oxidation & reduction and electrochemical technology [1, 2]. Adsorption process has difficulty regenerating adsorption materials and is easy to cause secondary pollution [3]. Membrane separation technology requires high operation and maintenance costs, and may cause membrane pollution. Chemical oxidation-reduction process has disadvantage of selective oxidation and incomplete processing [4]. For electrochemical oxidation method, the consumption of a huge amount of energy and electrode materials produces large quantities of sewage. The second type is biological method, and it is of high economic feasibility and widely used. The most common one utilized for the actual treatment is anaerobic-aerobic combination process; however, this process also has some shortcomings, such as the unstable removal effect of aniline compounds and poor impact resistance.

Aiming at these problems, an anaerobic filter (AF) – biological aerated filter (BAF) coupling process filled with internal electrolytic packings was introduced. The principle of catalytic internal electrolysis method is: wastewater serves as electrolyte solution, where numerous iron-carbon primary cell reactions [5] occur after adding inert substances (e.g. activated carbon and graphite) and high-potential metals to cast iron scraps. The underlying mechanisms can be summarized as:

(1) Electro chemistry. The basic electrode reaction is:

anode (Fe):$Fe \rightarrow Fe^{2+}+2e$ $E_0(Fe^{2+}/Fe)$＝-0.44V (1)

cathode (C):$2H^{+}+2e \rightarrow H_2$ E_0 (H^{+}/H_2) =0V (2)

When O_2 exists, the cathode reaction is:

$O_2+4H^{+}+4e \rightarrow 2H_2O$ (acidic solution) $E_0(O_2)$=1.23V (3)

$O_2+2H_2O+4e \rightarrow 4OH^{-}$(neutral or alkaline solution) $E_0(O_2/OH^{-})$ =0.40 (4)

During electrode reaction, unsaturated chromophores of dye molecules acquire electrons easily, thereby changing the structure of chromophore and reducing chroma in wastewater[6]. Moreover, low oxidation-reduction potential gained by electrochemical reaction develops an excellent reductive environment, helping to reduce azo dye molecules to hydrogenated azo or aromatic amine compounds.

(2) Oxidation and reduction of hydrogen. Under acidic condition, nascent hydrogen [H] the

electrode produces can destroy chromophore of dye molecules and strengthen decolorization and decomposition performance. Moreover, H_2 produced on filler surface can be used as electron donors for microbial degradation, energy sources for microbial growth, and reduction equivalents for dye degradation [7].

(3) Reduction of iron. Iron, with strong electronegativity ($E_0(Fe^{2+}/Fe) = -0.44V$), can degrade pollutants under certain conditions.

(4) Adsorption and flocculation of iron ions. Fe^{2+} and Fe^{3+} are produced during electrochemical reaction. Their hydrates have strong adsorption and flocculation activity which can make fine dispersed particles and flocculent colloidal organic compounds precipitate. Moreover, iron elements contribute to microbial growth and enzyme activity enhancement [8-10], thereby strengthening biodegradation and improving shock resistance capacity [11].

Compared with ordinary biological or physicochemical methods, this coupling process has great ability to strengthen biodegradability, maintain a stable acid-base environment and accelerate dye degradation [12]. It can remove pollutants at a low organic concentration in a short time and reduce microbial dependence on symbiotic metabolism, with less intermediates (aromatic amines) produced. In this paper, we analyzed the factors of this coupling process and determined its optimum operating parameters. Also, comparative experiments were conducted to study its unique advantages, and investigate the element migration in both the effluent and biofilm to discuss the pollutant removal characteristics and underlying reaction mechanisms. The conclusions can provide reference for practical engineering and theoretical research, and they have both significant economic benefits and ecological efficiency toward dyeing industry.

MATERIALS AND METHODS

Experimental Water. In this experiment, reactive red X-3B (99% purity) azo dye wastewater was taken as the research object, which was obtained from the Shanghai Jiaying Chemical Co. Ltd. $C_6H_{12}O_6$, NH_4Cl and KH_2PO_4 were added in proportion (concentration ratio of C:N:P=200:5:1) as the carbon, nitrogen and phosphorus source of the influent respectively. And $NaHCO_3$ and H_2SO_4 were chosen as the acidity regulator to adjust the influent pH value. Moreover, 0.3ml nutrient solution was added to each liter of stimulated wastewater influent [13].

Apparatus and Experimental Procedures. The experimental apparatus used in this experiment, a bench scale system consisted of an AF and a BAF, is shown in Figure 1. Considering factors such as hydraulic detention time (HRT) and height of filter layer, bioreactor parameters were designed: external diameter of 108mm, wall thickness 4mm and height 1600mm with working volume of 2.4 L for AF, and external diameter of 80mm, wall thickness 4mm and height 1000mm with working volume of 1.2L for BAF. Both biofilters were made of plexiglass, filled with internal electrolysis composite fillers which were prepared based on our preliminary research results [14, 15] and engineering experiences. This fillers have spherical shape with diameter of 10 to 20mm, consisting of iron powder (mass ratio 85~90%), activated carbon powder (mass ratio 10~15%) and a small amount of binder and catalyst (rare earth metal powder). Before being used, fillers were activated by diluted (5%) hydrochloride acid for 1 h firstly, and then soaked in diluted (5%) sodium hydroxide for 1 h.

Characterization and analysis methods. The analytical methods used are shown in Table 1.

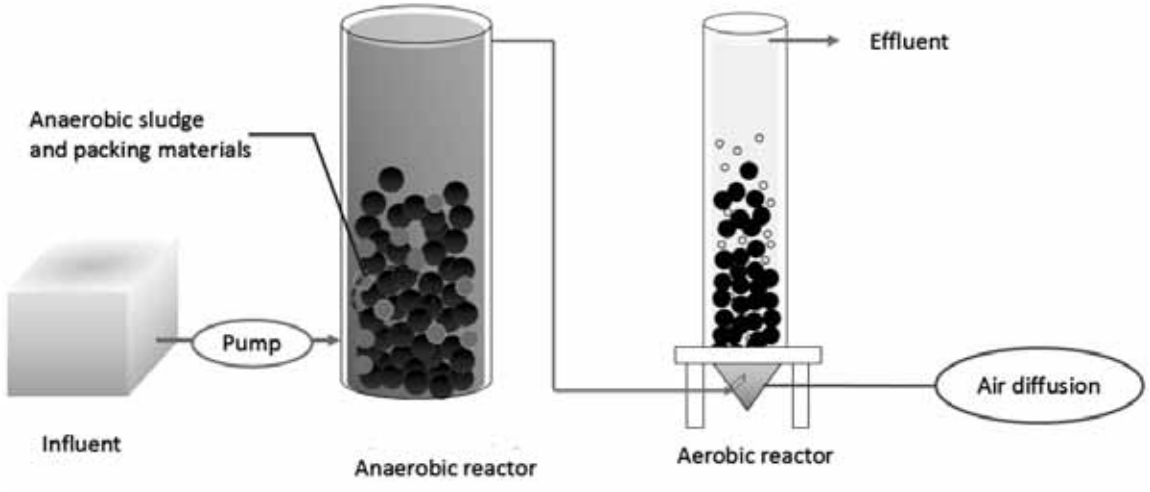


FIGURE 1
Flow chart of the test system

TABLE 1
Analytical methods

Parameters	Analytical methods
COD	Potassium chromate method
NH^{4+}-N	Nessler 's reagent spectrophotometric method
dye concentration and removal rate	UV - Vis spectrophotometric method
chroma	Dilution multiple method
aniline compounds	N- (1- naphthyl) ethylenediamine azo spectrophotometric method
pH	Glass - electrodes method
DO	Portable dissolved oxygen meter

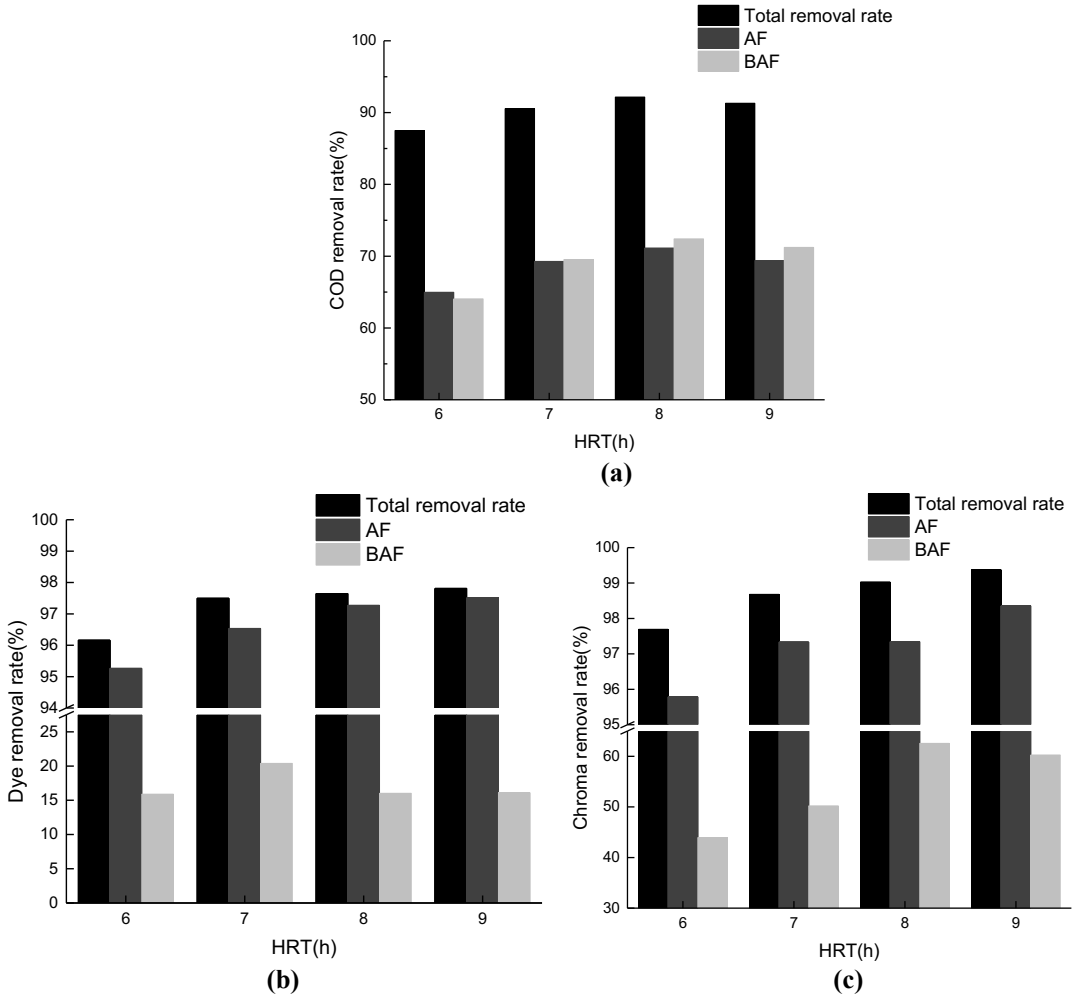


FIGURE 2
Effect of HRT on pollutant removal rate:(a)COD (b)dye (c)chroma

RESULTS AND DISCUSSION

Effect of HRT. To what degree the chemical reaction proceeds, whether complete or not, it is subject to length of HRT. In the experiment, effects of HRT on pollutants removal was studied when HRT was 6h, 7h, 8h and 9h respectively while the initial COD 275~300mg·L^{-1}, initial pH 6.5~7.5 and DO 4.0±0.3 mg·L^{-1} all the time.

Effect of HRT on COD removal. Results in Figure 2(a) indicated that proper prolongation of HRT is beneficial for organics degradation. Because the highly concentrated non-biodegradable dyes exist in the influent, proper reduction of hydraulic load can create excellent growing conditions for microorganisms. Also, it can ensure sufficient contact between wastewater and biofilm through reducing the shearing force of influent. However, excessive HRT can be harmful to microorganisms too. Because toxic intermediates (aromatic amines) into which dyes are usually degraded in AF would remain in system for a long time. Moreover, results also demonstrated that COD removal mainly occurred in AF, which helped to stabilize the system operation, reduce the impact of hydraulic load fluctuation and relieve load pressure of the subsequent BAF process.

Effect of HRT on chroma removal. From Figure 2(b), it is obvious that AF played a dominant role in dye removal. When HRT was 9h, 8h, 7h and 6h, the corresponding dye removal rate in AF were 97%, 96.9%, 96.2% and 95.1% respectively. While dye removal rate in BAF did not change regularly with HRT, but hit the top when HRT was 7h. This was due to the promotion of driving force for dye degradation in BAF resulting from the rise of influent dye concentration when HRT was decreasing. Also, decreasing HRT brought about enhanced water shearing force and accelerated aging biofilm renewal, which is favorable for corrosion and redox action of iron on filler surface. However, dye removal capacity in BAF decreased when HRT was 6h, since wastewater and microorganisms were not able to contact fully and adequately.

From Figure 2(c), it demonstrated that the average chroma removal efficiency in AF decreased with HRT decreasing, while that in BAF fluctuated between 45% and 63%. However, as for the whole system, there was no distinct difference in chroma removal when HRT varied.

In summary, when HRT was decreasing, even though the contaminant (COD, dye and chroma) removal declined variably, the overall system still achieved a comparatively outstanding degradation effect. It is because the coupling effect of microorganism and internal electrolysis reaction offer the system, especially AF, a strong resistance to HRT variation and hydraulic load fluctuation. Thus, the optimum HRT is 7h through synthetical consideration.

Effect of initial pH value. During treatment, pH can not only affect the performance of internal electrolysis fillers and corrosion rate of iron, but also influence the growth and activity of microorganisms. The internal electrolysis reaction served a double purpose of degrading pollutants and keeping acid-base balance by producing OH^- or consuming extra H^+ under different pH condition, which was beneficial for microorganism growth. Meanwhile, in neutral and alkalescent environment, Fe^{2+} and Fe^{3+} of strong flocculating activity derived from internal electrolysis reaction contributed to pollutant removal. It is also worth noticing that the loss of fillers was dramatically slow and almost negligible, because the internal electrolysis reaction, as an auxiliary coupling reaction strengthening biological metabolism, was relatively faint and weak.

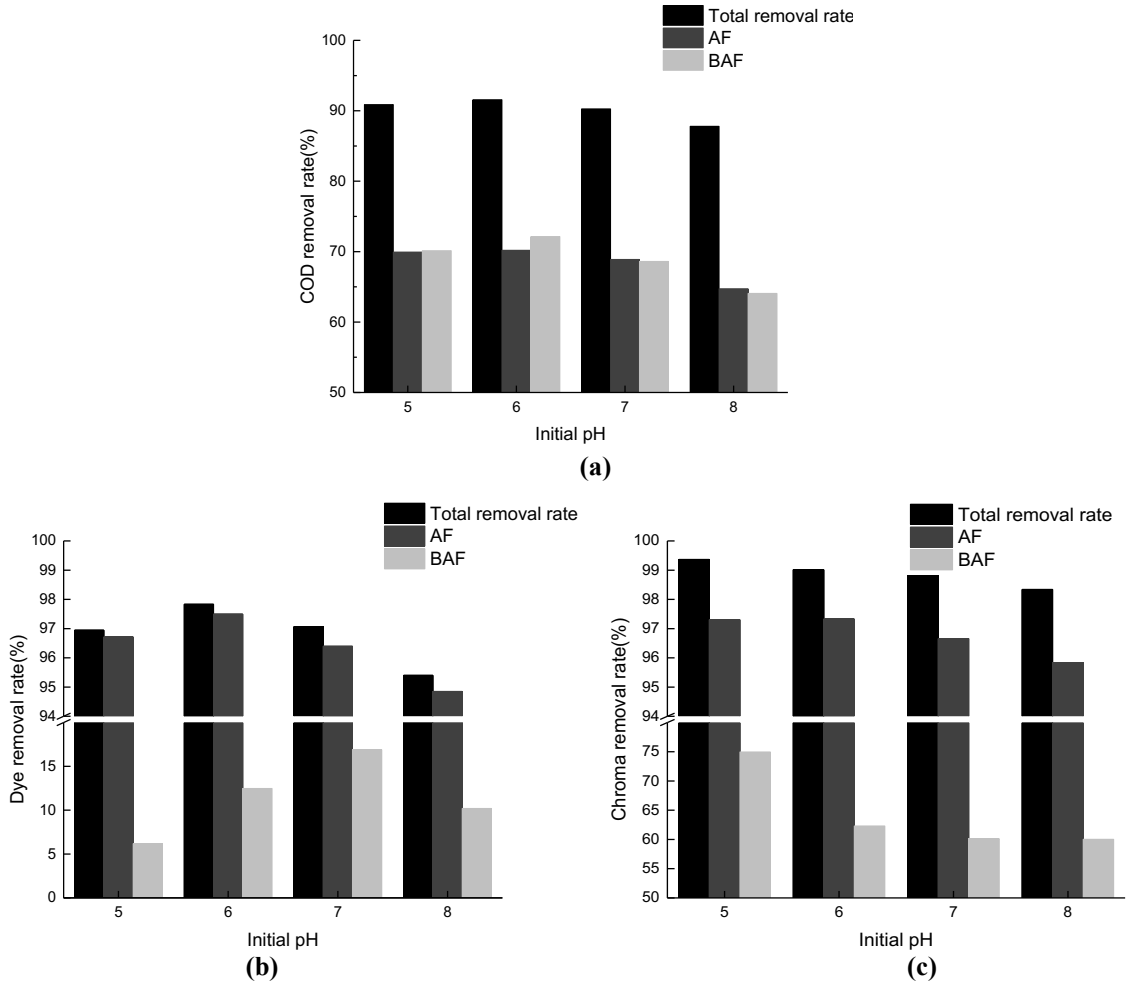


FIGURE 3
Effect of initial pH value on pollutant removal rate:(a) COD (b) dye (c) chroma

In the experiment, the effects of initial influent pH on pollutants removal as well as the effluent pH value after each processing stage were studied. The initial influent pH was set at 5, 6, 7 and 8 respectively while the initial COD 275~300mg·L^{-1}, HRT 7h and DO 4.0±0.3 mg·L^{-1} all the time.

Effect of initial pH value on COD removal Figure 3(a) showed that the total COD removal efficiency firstly increased and then decreased as pH decreasing, and reached a high level in slightly acid condition, because the corrosion on filler surface and organics degradation through electrochemical action were strengthened. Meanwhile, exsolved Fe^{2+} from filler surface, as an essential mineral element for microorganisms, favored the improvement of microbial enzymes activity [8] and ability of microorganism to resist pH fluctuation.

Effect of initial pH value on chroma removal. As shown in Figure 3(b), the total chroma removal efficiency increased firstly and decreased after as pH decreased, and it peaked at 97.8% when pH was 6.

When pH value was 6, a better organics degradation in AF was achieved due to the improved internal electrolysis action, which resulted from accelerated corrosion on filler surface. Moreover, two substances released during process contributed to dye degradation, i.e. electron donor H_2 which is essential for microbial metabolism, and Fe^{2+} which promotes microbial metabolic enzyme activity. With pH decreasing, biofilm attached to filler surface suffered visible abscission, and part of the adsorption and degradation effect produced by microorganisms got inhibited. Nevertheless, dye degradation still remained at a high level due to the stable acid-base environment owing to internal electrolysis action.

As shown in Figure 3(c), the total chroma removal rate declined notably with initial pH value rising. It demonstrated that acid condition helps increase the internal electrolysis activity and thus enhances the chroma degradation [16].

In summary, 6 is considered as the optimum initial pH value.

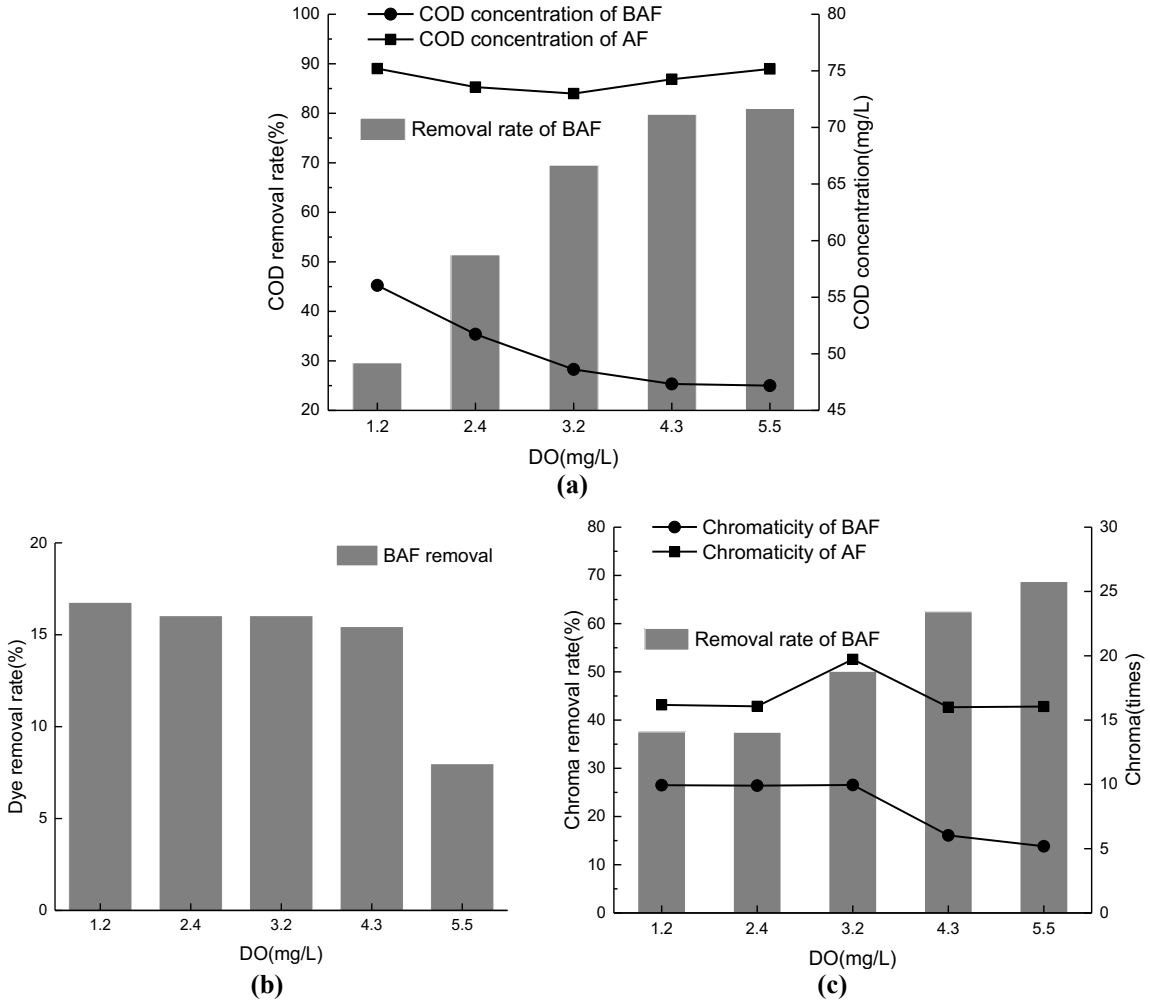


FIGURE 4
Effect of dissolved oxygen concentration on pollutant removal rate:(a) COD (b) dye (c) chroma

Effect of DO on COD removal. Most recalcitrant organics were reduced into intermediate products in AF, and oxygen was essential for complete oxidation decomposition in the following BAF. From Figure 4(a), the COD removal rate in BAF increased gradually as DO increased, and almost unchanged when DO rose from 4.3 mg·L^{-1} to 5.5 mg·L^{-1}. And high COD removal rate was achieved when DO was around 4 mg·L^{-1}. Beyond a certain range, however, decreasing DO had little effect on organics removal.

Effect of DO on chroma removal. Figure 4(b) showed that the highest average dye removal rate in BAF was only 16.7%, suggesting that dye removal mainly occurred in AF and stabilized when DO was 1.2~4.3mg·L^{-1}. DO has little effect on dye removal, because dye degradation relies heavily on reduction in AF. While DO in the subsequent BAF mainly helps to oxidize the intermediate metabolites produced in AF.

Under proper DO concentration, intermediate products generated in AF can be decomposed in BAF, thus achieving a deeper overall removal of chroma. From Figure 4(c), the chroma removal efficiency increased with DO increase within lower DO concentration range, under which condition microorganisms were stimulated to fully break down intermediate products. Besides, higher removal efficiency in BAF was also due to the physical adsorption and interception action of fillers, as well as the flocculation action of biofilm and bioiron on filler surface [18].

In summary, 4mg·L^{-1} is considered as the optimum DO concentration.

Effect of initial substrate concentration. The anaerobic degradation of azo dyes belongs to cometabolism process, suggesting that organics are essential as the primary electronic donors in dye degradation process. In this study, internal electrolysis fillers were applied into bioreactors, and it was speculated that the electrochemical effect in coupled system could keep the removal system more stable through reducing the dependence of biochemical degradation of dye on organics.

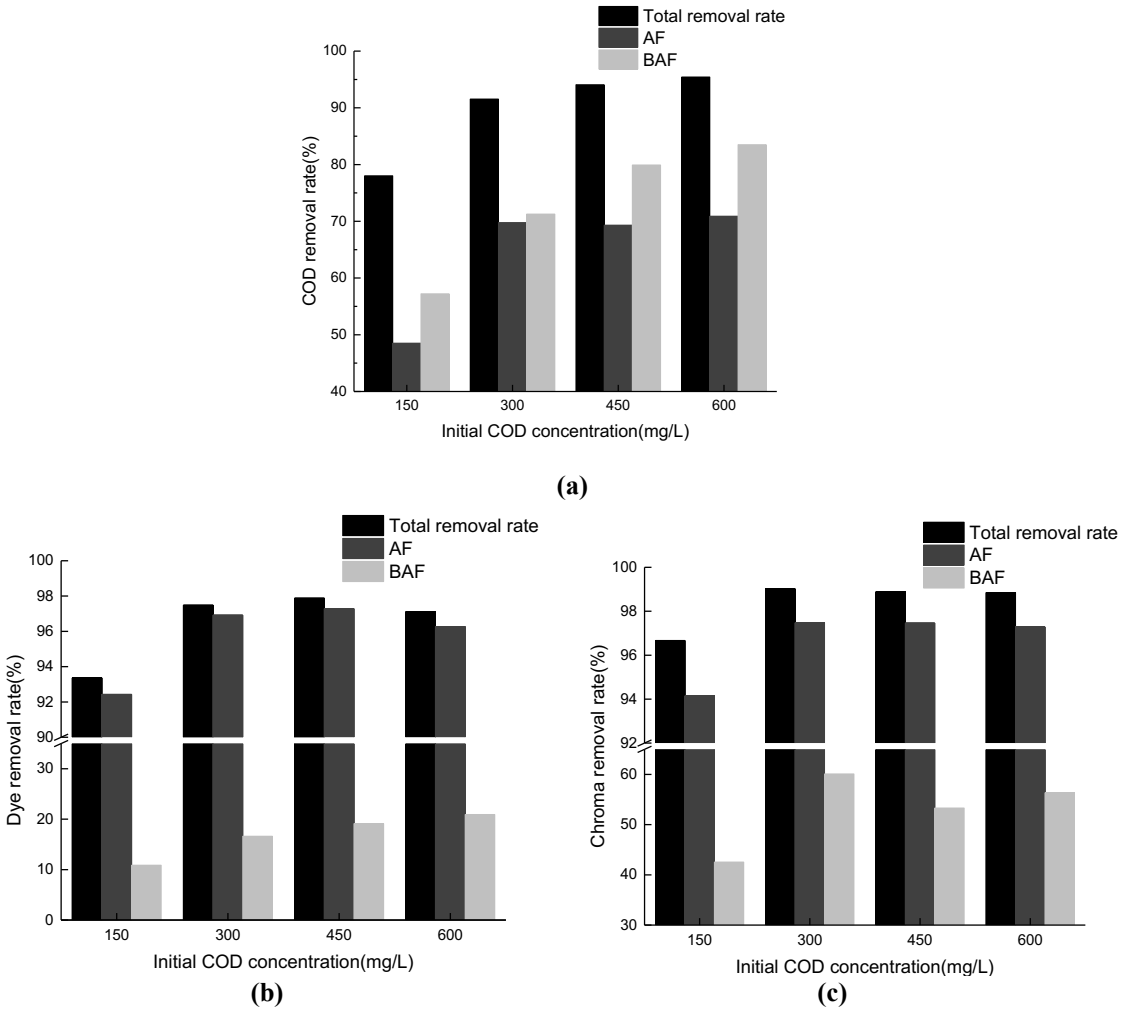


FIGURE 5
Effect of initial substrate concentration on pollutant removal rate:(a) COD (b) dye (c) chroma

The effects of different initial substrate concentration on pollutants removal were investigated in the experiments, carried out at four initial COD concentrations: 150 mg·L^{-1}、300 mg·L^{-1}、450 mg·L^{-1}、600 mg·L^{-1}. And the influent dye concentration was set at 100 mg·L^{-1}, HRT 7h, initial pH 6±0.3 and DO 4±0.3mg·L^{-1}.

Effect of initial substrate concentration on COD removal. Figure 5(a) suggested that the overall COD removal efficiency increased as initial substrate concentration increased. It is because with influent COD concentration increasing, the effluent COD concentration in AF increased accordingly, which greatly improved the microbial enzyme activity and increased COD removal rate in BAF. However, when COD concentration exceeded 300mg. L^{-1}, increasing influent substrate no longer influenced COD removal greatly. It suggested that this coulped dye wastewater treatment had less dependence on organics due to its electrochemical action, compared to those traditional single biochemical approaches.

Effect of initial substrate concentration on chroma removal. The effect of microorganisms degrading azo dyes can be strengthened by raising organic substrate concentration appropriately [19]. As shown in Figure 5(b), dye removal rate in BAF increased as influent organic substrate(glucose) concentration increased, owing to the cometabolism action produced by dye, glucose and anaerobic microorganisms. However, dye removal rate presented a slight decline when organic substrate concentration increased to 600mg·L^{-1}. That is because microorganisms utilize degradable substrates (glucose) preferentially rather than reactive red X-3B for growth energy when both substrates coexist [20].

From Figure 5(c), the change in chroma removal is similar to that of dye, which revealed an increasing and subsequent slightly decreasing tendency with the growth of organic substrate concentration.

In summary, efficient removal capacity of refractory azo dyes in coupling system was achieved under lower organic substrate concentration, and the activity of microbial decolorization enzymes could be improved within a certain glucose concentration range. It concluded that the optimum initial

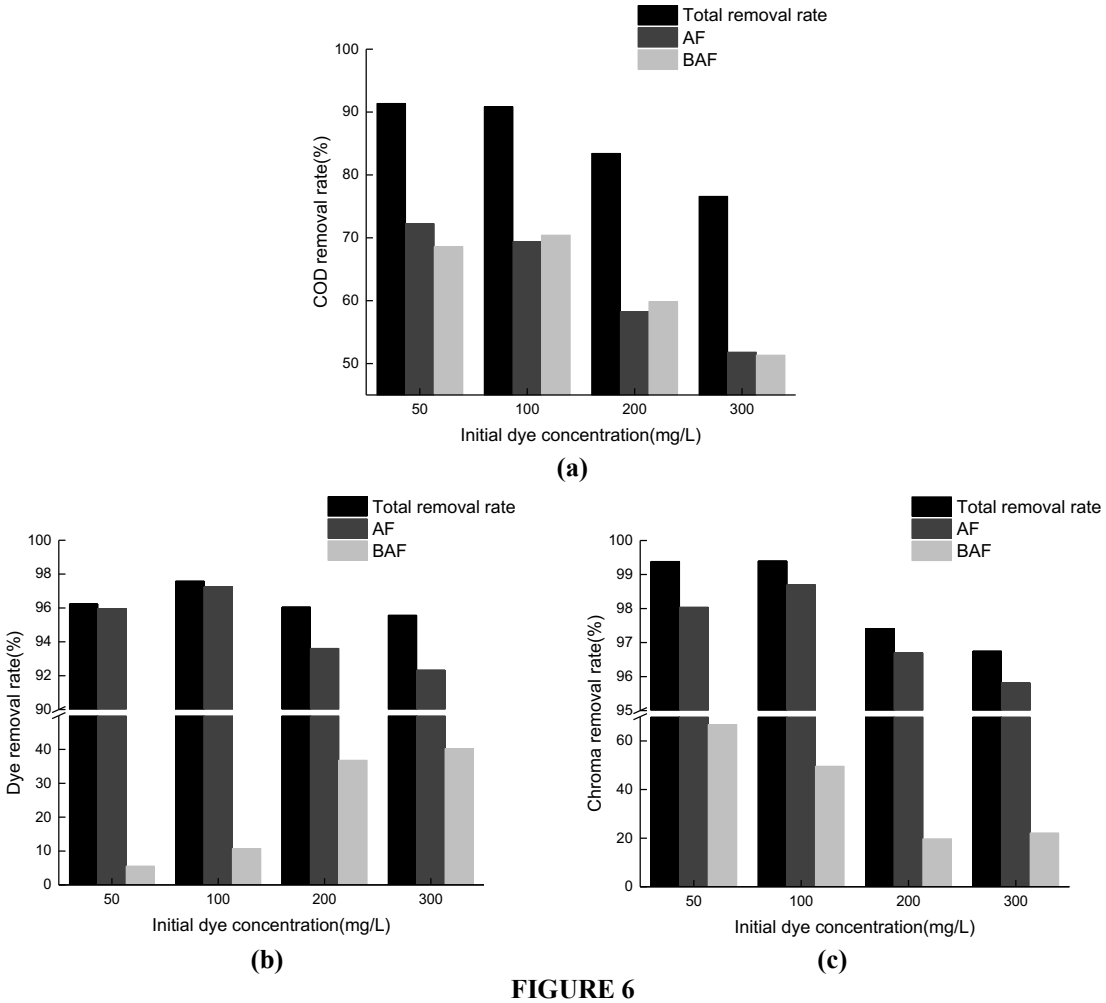


FIGURE 6
Effect of initial dye concentration on pollutant removal rate: (a)COD (b) dye (c) chroma

COD concentration is 300mg·L^{-1}, under which condition the decolorization efficiency of the system has already reached a high and stable level.

Effect of initial dye concentration. There usually appears a great fluctuation of dye loss in the actual dyeing process. In this paper, the effect of initial dye concentration on treatment performance was investigated.

The experiments were conducted at four initial dye concentrations: 50mg·L^{-1}, 100mg·L^{-1} 200mg·L^{-1}, 300mg·L^{-1}, while the influent COD was kept at 270~300 mg·L^{-1}, HRT 7h, initial pH 6±0.3 and DO 4±0.3mg·L^{-1}.

Effect of initial dye concentration on COD removal. Figure 6(a) showed that the average final effluent COD concentration varied from 25mg·L^{-1} to 68mg·L^{-1}. COD removal rate decreased when initial dye concentration increased from 100 mg·L^{-1} to 200mg·L^{-1}, because microbial activity was inhibited by toxic intermediates produced in dye degradation process [21].

Effect of initial dye concentration on chroma removal. As shown in Figure 6(b), within initial dye concentration range of 50mg·L^{-1} to 300mg·L^{-1}, the overall removal efficiency experienced an upward then downward trend, which is mainly caused by the changes occurred in AF. Firstly, the dye removal rate in AF increased slightly when initial dye concentration rose from 50mg·L^{-1} to 100mg·L^{-1}, owing to the adaptation to influent dye through preceding experiment processes. Afterwards, the continuously increase of initial dye beyond a certain range had a negative impact on dye removal, because the activity of decolorizing anaerobic bacteria decomposing dye groups was inhibited. By contrast, dye removal in BAF was improved as initial dye concentration grew. That is because, on the one hand, when influent concentration of dye was low, the effluent dye concentration of BAF remained almost unchanged after dye being removed thoroughly in AF. On the other hand, biofilms on filler surface developed adsorption and degradation ability after being adapted to the environment. Moreover, $Fe(OH)_3$ with high flocculation activity produced on filler surface under aerobic alkaline conditions is conductive to dye removal.

From Figure 6(c), weakened chroma removal performance was found as initial dye concentration increased from 50mg·L^{-1} to 300mg·L^{-1}. Changes in chroma removal in either AF or the whole system followed a similar pattern. In BAF, chroma removal rate maintained approximately 55% and dropped to around 20% subsequently when initial dye concentration rose from 50mg·L^{-1} to 200mg·L^{-1}. This revealed that excess dye had a negative effect on chroma removal in BAF.

In summary, strong resistance to dye concentration fluctuation was shown in AF. When dye concentration varied from 50mg·L^{-1} to 300mg·L^{-1}, an excellent COD and chroma removal performance was achieved despite a modest decline resulting from the inhibited microorganism activity caused by increasing dye and toxic intermediates [22]. Overall, the optimum influent dye concentration is 100mg·L^{-1}.

Comparative experiments of steady operation. Based on the results above, the optimum processing condition is: HRT=7h, initial pH=6, DO=4mg·L^{-1}, influent COD=300 mg·L^{-1} and influent dye concentration=100 mg·L^{-1}. In order to further discuss the pollutant removal characteristics, a comparative experiment was conducted in the identical process using normal ceramsite as packing material instead. Both the experimental group (using coupling system) and contrast group (using normal ceramsite) were operated stably for 15 days under the optimum conditions, during which time no packing replacement was necessarily required. If in long-term daily operation, however, fillers should be renewed and sludge discharge and backwashing cycle be set according to the actual situations. In the comparative experiments, changes of pollutant removal, pH, ORP and accumulation and degradation of aniline were studied.

Comparison and analysis of operation results. The COD, dye and chroma removal of both experimental group (Group-e) and contrast group (Group-c) were tested, and results were displayed in Table 2.

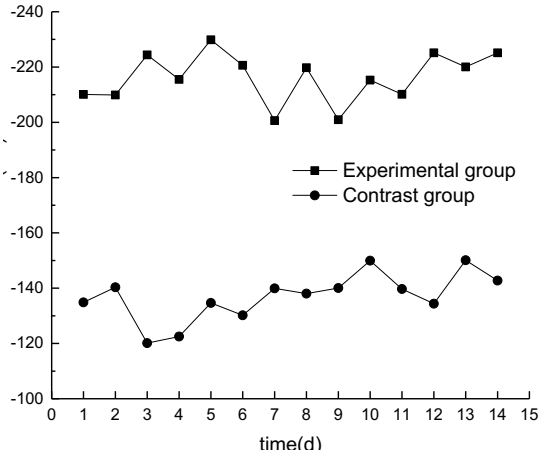
TABLE 2
Stable operation effect

Processing Unit		COD				dye				chroma			
		C-in (mg·L^{-1})	C-e (mg·L^{-1})	RR (%)	TRR (%)	C-in (mg·L^{-1})	C-e (mg·L^{-1})	RR (%)	TRR (%)	Inflow (times)	Effluent (times)	RR (%)	TRR (%)
Group-e	AF	290.2	88.5	69.5	92.2	101.5	2.7	97.3	97.7	600	12	98	99
	BAF	88.5	22.6	74.5		2.7	2.3	14.8		12	6	52.6	
Group-c	AF	290.2	165.1	43.1	87.3	101.5	7.1	93	95.6	600	28	95.3	97
	BAF	165.1	37.0	77.6		7.1	4.5	36.6		28	18	35.7	

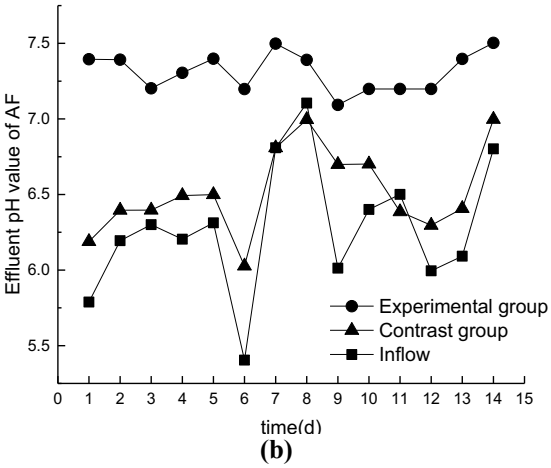
Note: C-in: inflow concentration. C-e: effluent concentration. RR: removal rate. TRR: total removal rate

Table 2 illustrated that under the same conditions, both COD and chroma removal rate were higher in experimental group than that in contrast group.

In terms of COD removal, coupling system has a superior removal capacity. When treating refractory toxic wastewater under low COD concentration, organics removal mainly occurs in BAF, while AF mostly play the role of degrading macromolecular refractory substances through hydrolysis and acidification simply, and that can be also proven in experimental results of contrast group. Nevertheless, in coupling system, the degradation ability in either BAF or AF is quite similarly excellent. Massive refractory substances and organics can be degraded and removed within a short HRT, about 4h, in AF. It can be revealed that the coupling system contributes to shortening HRT, reducing power consumption and lightening organic load of the subsequent aerobic treatment.



(a)



(b)

FIGURE 7
Changes of ORP and effluent pH in AF: (a) ORP (b) pH

In terms of chroma removal, experimental results showed that the effluent dye concentration in AF of contrast group was 2.6 times that in experimental group, and the final effluent concentration of the former was 2 times as much as that of the latter. It is obvious that the coupling system had a superior dye removal capacity which mainly occurred in AF. Moreover, the chroma removal performance in coupling system was better too.

Changes of pH value and ORP value in the system. Dye is degraded by accepting electrons and subsequently breaking azo bonds down mainly in AF. Therefore, it is of great significance to maintain low redox potential for anaerobic degradation of dye. Under the same conditions, the changes of ORP (oxidation and reduction potential) and effluent pH of AF over operation time were tested. Results were shown in Figure 7 (a), (b).

Figure 7 showed that AF in coupled system had a lower ORP and higher pH value, which were -218mv and 7.5 respectively. It suggested that AF filled with electrolytic packings was more favorable for electrons transmission during dye degradation and thus led to acceleration of reduction reaction. From Figure 7(b), pH value of AF in coupling system was seldom affected by the influent pH, and the effluent always remained neutral and alkaline. This is because that iron corrosion on internal electrolysis filler surface produced OH^- and thus maintained pH stability of the system.

Accumulation and degradation of aniline. During dyeing wastewater treatment process, attention should not only be paid to contaminant removal, but also carcinogenic aromatic amines compounds generated in AF. Therefore, in the comparative experiments, the optimum HRT range for dye removal in AF was selected to study the aniline accumulation in AF and aniline removal in the subsequent BAF. Results were shown in Figure 8.

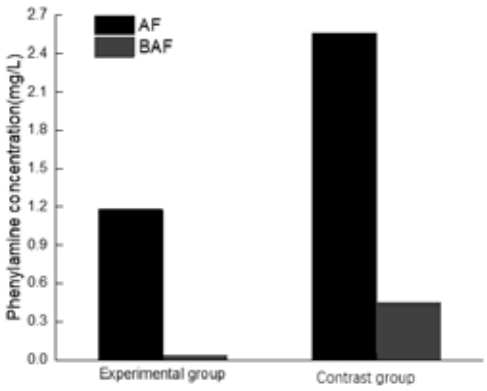


FIGURE 8
Effluent aniline concentration of each group

From Figure 8, the average aniline concentration in final effluent of experimental group was within the detection limit, less than 0.05mg/L, while that of contrast group was about 0.3mg/L, which failed to meet discharge standards.

TABLE 3
XRF analysis results (excluding C, O elements)

		Fe	S	Si	Ca
Coupled system	AF	70.8%	15.7%	5.2%	2.3%
	BAF	89.8%	0.6%	3.2%	2.8%
Ordinary filter	AF	10.8%	1.9%	24.9%	33.3%
	BAF	10.2%	1.2%	23.6%	41%

TABLE 4
XRF analysis results

		Fe	C	O	S
Coupled system	AF	30.2%	32.0%	22.8%	8.2%
	BAF	42.3%	21.6%	30.5%	0.3%
Ordinary filter	AF	2.9%	24.0%	35.1%	0.7%
	BAF	2.5%	29.0%	33.6%	0.5%

As for AF, firstly, sulfate reduction action and possible denitrification were produced by sulfonic acid groups and amino groups of reactive Brilliant Red X-3B Dye during dye reduction, both of which contributed to aniline degradation [23]. Secondly, a part of dyes were removed through flocculation and adsorption of Fe^{3+} and Fe^{2+} [24] or adsorption of internal electrolytic fillers surface [25], thus controlling aniline compounds from the source.

As for BAF, aniline removal rate of experimental group (96.4%) was far higher than that of contrast group (82.1%) because of glucose effects, that is, aniline compounds were preferentially degraded when organics concentration was lower. Moreover, after thorough aniline degradation in AF of experimental group, the inhibition action against microorganisms caused by aniline in the subsequent BAF was alleviated, resulting in a better aniline removal.

Migration and transformation of important elements. Biofilm in coupled system was more compact with better sedimentation property compared with that in contrast group, because substantial Fe^{3+}, Fe^{2+} or $Fe(OH)_x$ existing in coupled system stimulated microorganism growth. The biofilm element composition was analyzed by X-ray fluorescence spectrometry (XRF), and results were displayed in Table 3 and Table 4.

The adsorption of dyes or their metabolites in biofilm was evaluated by sulfur content, because sulfur existed exclusively in dye molecules in the influent. From Table 3, 1.9% of sulfur was observed in biofilm in ordinary AF, while that in coupled AF was 15.7%. Moreover, Fe of extremely high concentration was discovered in biofilm in coupled system. The newly dissolved Fe^{2+} or Fe^{3+} has strong flocculation activity that could remove dye directly. Also, FeS and Fe(OH)x, formed by reaction between Fe^{2+} or Fe^{3+} and S^{2-} produced during dye reduction, could remove metabolites through flocculation.

From Table 4, massive Fe and O elements with a few S elements were observed in aerobic sludge, suggesting that a large number of iron oxide compounds were produced, and also proving that dye removal occurred mainly in AF.

Through the analysis of element composition and effect comparison of pollutant removal, it concluded that strong pollutant removal mainly occurred in the internal electrolysis filler biofilm. Its underlying mechanisms included not only microbial redox, but also adsorption, coprecipitation and net capture of iron flocs in biofilm. In AF, more and more Fe^{3+} and Fe^{2+} were dissolved out through microorganism metabolism and biological corrosion on filler surface because of its high reducing capacity gained by internal electrolysis. In conclusion, removal efficiency of organic compounds and dyes in AF of coupling system has obvious advantages over that of ordinary ceramsite filter system.

CONCLUSION

In this study, internal electrolysis fillers were applied innovatively to electrochemical and biological coupled AF-BAF process to treat simulated dyeing wastewater. The optimum operation parameters were determined through processing effect study under different working conditions, and technology advantages were investigated through comparative experiments and dynamic analysis. Overall, conclusions are drawn as follows:

1) When treating simulated dyeing wastewater by coupled process, the optimum processing parameters were: HRT=7h, initial pH =6, and DO=4mg·L^{-1}. Under the optimum conditions, removal efficiencies of COD, dye and chroma were as high as 92.2%、97.7% and 99.0% respectively. Influent COD and dye concentration of 300 mg·$L^{-1}$and 100 mg·L^{-1} respectively was beneficial to the treatment. Moreover, a strong ability to resist the influence of dye concentration fluctuation was

observed in AF.

2) The comparative experiments suggest: a superior effect of COD removal and decoloration were obtained by the coupled process compared with traditional ceramsite methods; through multiple enhanced biological actions, the high contaminant removal efficiency in a very short time and less aromatic intermediate products can be achieved simultaneously.

3) The biofilm element composition analysis suggests: enhanced pollutant removal effect of the coupled process mainly occurred in the internal electrolysis filler biofilm; its underlying removal mechanisms includes not only microbial redox, but also adsorption, coprecipitation and net capture of iron flocs.

REFERENCES

[1] Wang, H., Zheng, X., Su, J., Tian, Y., Xiong, X., Zheng, T. (2009) Biological decolorization of the reactive dyes Reactive Black 5 by a novel isolated bacterial strain Enterobacter sp EC3. Journal of Hazardous Materials. 171(1-3), 654-659

[2] Chou, W.L, Wang, C.T., Chang, C.P., Chung, M.H., Kuo, Y.M. (2011) Removal of color and COD from dyeing waste water by paired electrochemical oxidation Fresen. Environ. Bull. 20, 78-85.

[3] Vimonses, V., Lei, S., Jin, B., Chow, C.W., Saint, C. (2009) Kinetic study and equilibrium isotherm analysis of Congo Red adsorption by clay materials. Chemical Engineering Journal. 148(2-3), 354-364

[4] Ren, N., Zhou, X., Guo, W., Yangshanshan, S. (2013) A review on treatment methods of dye wastewater. CIESC Journal. 01, 84-94.

[5] Lv, J., Cui, Y. (2015) Treatment of reactive red X-3B dye wastewater with Cu-Fe inner electrolysis method. China Dyeing and Finishing. 07,17-20

[6] Mao, X., Tian, F., Gan, F., Zhang, X., Peng, T. (2006) A study of electrochemical degradation of azo-dye AO(7) on Na_2SO_4 medium. Fresen. Environ. Bull.15, 1307-1315

[7] Guo, Y., Xiao, L., Deng, Z., Tang, W. (2010) Laboratory study on the decolorization of reactive brilliant blue X-BR treated by an integrated Fe~0 anaerobic biological system. China Environmental Science. 04, 510-515.

[8] Vardanyan, Z., Trchounian, A. (2012) Fe(III) and Fe(II) ions different effects on Enterococcus hirae cell growth and membrane-associated ATPase activity. Biochemical and Biophysical Research Communications. 417(1), 541-545

[9] Zhang, Z., Wang, Y., Leslie, G.L., David Waite, T. (2015) Effect of ferric and ferrous iron addition on phosphorus removal and fouling in submerged membrane bioreactors. Water Research. 69, 210-222

[10] Ozkan, O., Uyanik, I. (2018) Removal of COD from medical waste sterilization plant wastewater by electrocoagulation. Fresen. Environ. Bull. 27, 204-209.

[11] Qin, L., Zhang, G., Meng, Q., Xu, L., Bosheng, L.V. (2012) Enhanced MBR by internal microelectrolysis for degradation of anthraquinone dye wastewater. Chemical Engineering Journal. 210, 575-584

[12] Li, Q., Wang, Y. (2017) Dyes wastewater treatment via electrochemical oxidation. Fresen. Environ. Bull. 26, 141-144

[13] Tsuneda, S., Ohno, T., Soejima, K., Hirata, A. (2006) Simultaneous nitrogen and phosphorus removal using denitrifying phosphate-accumulating organisms in a sequencing batch reactor. Biochemical Engineering Journal. 3(27), 191-196

[14] Pan, L.T., Han, Y. (2016) A novel anoxic-aerobic biofilter process using new composite packing material for the treatment of rural domestic wastewater. Water Science and Technology. 73(10), 2486-2492.

[15] Tongji University (2015) Method for preparing filler material of biofilter for removing nitrogen and phosphorus; China, 201410048193.8[P]. 2015-08-12.

[16] Ozyonar, F., Muratcobanoglu, H., Gokkus, O. (2017) Taguchi approach for color removel using electrocoagulation with different electrode connection types. Fresen. Environ. Bull. 26, 7600-7607.

[17] Dong, X., Wang, Y., Zhang, P., Tan, Z., Jiao, Z., Jin, Q. (2016) Degradation of isatin and anthranilic acid by achromobacter xylosoxidans N4 isolated from denim-manufacturing mill sludge. Biochemical and Biophysical Research Communications. 25(8), 2784-2794

[18] Zhang, S. (2006) Study on organic wastewater deep treatment using biological aerated filter (BAF). Donghua University, Shanghai.

[19] Murali, V., Ong, S.A, Ho, L.N, Wong, Y.S (2013) Evaluation of integrated anaerobic–aerobic biofilm reactor for degradation of azo dye methyl orange. Bioresource Technology. 143, 104-111

[20] Qiu, L. (2010) Study on biodegradability and decolorization of reactive black KN-B by anaerobic granular sludge. Donghua University, Shanghai.

[21] Liu, Y., Zhang, Y., Quan, X., Zhang, J., Zhao, H., Chen, S. (2011) Effects of an electric field and zero valent iron on anaerobic treatment of azo dye wastewater and microbial community structures. Bioresource Technology. 102(3), 2578-2584.
[22] Yemashova, N., Kalyuzhnyi, S. (2006) Microbial conversion of selected azo dyes and their breakdown products. Water Science and Technology. 53(11),163-171.
[23] Pereira, R., Pereira, L., Van der Zee, F.P., Madalena Alves, M. (2011) Fate of aniline and sulfanilic acid in UASB bioreactors under denitrifying conditions. Water Research. 45(1), 191-200.
[24] Venkatesh, S., Quaff, A.R., Pandey, N.D., Venkatesh, K. (2016) Decolorization and mineralization of CI direct red 28 azo dye by ozonation. Desalination and Water Treatment. 57(9), 4135-4145.
[25] Mantha, R., Taylor, K.E., Biswas, N., Bewtra, J.K. (2001) A continuous system for Fe(0) reduction of nitrobenzene in synthetic wastewater. Environmental Science and Technology. 35(15), 3231-3236.

Received: **08.04.2018**
Accepted: **26.08.2018**

CORRESPONDING AUTHOR

Ajun Wan
Institute of Modern Agricultural Science and Engineering,
Tongji University,
Shanghai 200092 – China

e-mail: wanajun@tongji.edu.cn

© by PSP Volume 27 – No. 12B/2018 pages 9686-9695 Fresenius Environmental Bulletin

IN VITRO ASSESSMENT OF ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY, AND IDENTIFICATION OF PHENOLIC PROFILE OF *GYPSOPHILA LEPIDIOIDES* BOISS. EXTRACTS FROM TURKEY

Ahmet Altay*

Erzincan University, Faculty of Arts and Science, Department of Chemistry, Erzincan, Turkey

ABSTRACT

The aim of this study was to evaluate the antioxidant and anti-proliferative activities of *Gypsophila lepidiodies* Boiss. together with investigation of phenolic content. Antioxidant activity of water and methanol extracts were evaluated by four different methods namely, DPPH and ABTS radical scavenging activity, cupric ion reducing antioxidant capacity (CUPRAC), and Fe^{2+} chelating assay. Antiproliferative activities of the extracts were assessed against various human cancer cell lines namely, breast adenocarcinoma (MCF-7), colorectal adenocarcinoma (HT-29) and hepatocellular carcinoma (HepG2) cells. HPLC analysis indicated the presence of eight phenolic compounds in eighteen phenolic standards scanned. Antioxidant activity results showed the superiority of methanol extract to water extract according to DPPH, CUPRAC and Fe^{2+} chelating tests, while the water extract displayed more antioxidant activity by ABTS assay. Antiproliferative activity results demostrated that methanol extract displayed about 2.5-fold, 3.3-fold and 6-fold more activity against MCF-7 cells, HepG2 cells and HT-29 cells, respectively. However, both water and methanol extracts were observed to display moderate biological activity compared to positive controls. The obtained data suggest that *Gypsophila lepidioides* may be evaluated as a promising source for food and nutraceutical industries due to its good antioxidant and moderate antiproliferative potentials together with its rich phytochemical profile.

KEYWORDS:
Gypsophila lepidiodies, RP-HPLC, antioxidant activity, anti-proliferative activity, cell culture

INTRODUCTION

Medicinal plants, including biologically active phytochemicals have been commonly used for many years in a diverse array of purposes such as medicine, nutrition, flavorings, beverages and cosmetics [1]. The action modes of the phytochemicals in the plants and/or plant-derived products might be attributed to their antioxidant behaviors since they can stop or prevent the oxidation of many significant macromolecules in the cell [2, 3]. In addition, phenolic compounds behaving as antioxidants have commonly been used in food and pharmaceutical industries for contribution to protection against oxidative degradation of foods [4]. Besides, phenolic compounds from plants possess important biological activities such as anti-inflammatory, antibacterial, anti-proliferative, anti-mutagenic, anticarcinogenic activities, [5]. However, many plants, which are used as food or traditional medicine may become mutagenic, cytotoxic or genotoxic for healthy cells, resulting from the long-term usage [6]. Therefore, cytotoxicity tests are very useful to determine the concentration range to be used, and also these tests may contribute to more detailed studies to provide meaningful information on parameters such as cytotoxicity, genotoxicity, induction of mutations or programmed cell death [7]. Many studies have been carried out on natural sources to unravel the phenolic components having high antioxidant potential and strong antiproliferative activity against cancerous cells [6]. Because of diverse biological roles, the identification and quantification of phytochemicals in different plant species are very essential [8].

The genus Gypsophila L. having 126 species worldwide is mainly distributed in the Irano-Turanian and Mediterranean regions, and it is the third biggest genus of Caryophyllaceae family in Turkey, possessing 55 species, represented by 58 taxa, 33 of which are endemic [9]. *Gypsophila* species have been widely used in folk medicine throughout the world due to its diverse medicinal purposes such as spermicidal, hypocholesterolaemic, anti-inflammatory and antiviral activities [10], antioxidant activity [11], cytotoxic activity [12]. They are also used to treat fever, consumptive disease, and infantile malnutrition syndrome [13]. Additionally, some *Gypsophila* species are used in food industry to produce soaproot, ice-cream, liquor, herby cheese [14], and also some species are added to halva in order to give crispness, [9]. Moreover; because of their good sparkling proper-

ties and high saponin contents they are used in various industrial applications such as soap, detergent and expectorant production [9]. Furthermore, the saponin sources of some Gypsophila species are used in gastronomy in Arabic countries [15].

Gypsophila lepidioides is a local endemic plant to Erzincan, Turkey, and the living area of the plant continues along the gypsy soils between Kemah and Ilic (Erzincan) [16]. It has a very nice appearance with the congested white flowers. The closest relative of the plant is eriocalyx and shows spread in the same area. However, seasonal differences are observed between the two species due to earlier flowering [17]. In the present study we aimed to investigate the *in vitro* antioxidant and anti-proliferative activity potentials of methanol and water extracts of endemic plant *Gypsophila lepidioides* as well as its phenolic content.

MATERIALS AND METHODS

Plant material and chemicals. The endemic plant material *G. lepidioides* was collected from gypsy slopes of Kuruçay-İliç, Erzincan, Turkey, on 25 June 2017, at an altitude of 990 m. The samples were taxonomically identified by Dr. Mustafa Korkmaz. The voucher specimens were prepared according to the herbarium techniques, enumerated (4355) and preserved at the Laboratory of Plant Systematic, Department of Biological Sciences, Erzincan University, Erzincan, Turkey.

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Calbiochem Co. (San Diego, CA). Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulfate, foline-ciocalteu phenol reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, ethanol, and methanol were purchased from Sigma-Aldrich. All phenolic standard compounds and solvents being HPLC grade were also obtained from both Sigma-Aldrich and *Merck* (Darmstadt, Germany).

Sample preparation. *Gypsophila lepidioides* was extracted and analyzed shortly after the collection step. Briefly, the aerial parts of *Gypsophila lepidioides* protected from direct sun light were dried at room temperature. Briefly, the aerial parts the samples were mixed with 250 mL of extraction solvents (methanol and water) in a ration of 1:5 (w:v), and then left to shaken over night. This process was applied three times. In order to get ultra-dry powders, the extracts were filtered through Whatman No. 4 filter paper, and then concentrated under vacuum with rotary evaporator (Heidolph, Germany) at 40 ˚C, and then lyophilised using a Scanvac Cool Safe™ freeze-dryer (CoolSafe 55, Lynge, Denmark). The extracted powder was weighed and stored at -20°C in a brown bottle until use.

Determination of total phenolic and flavonoid contents. Total phenolic content of the extracts were determined according to the Folin-Ciocalteu method [18] with some modifications [19]. Briefly, 20 µL of the extract was mixed with 1N, 100 µL of 20 % Folin-Ciocalteu's phenol reagent and 80 µL of 10 % sodium carbonate solution in the 96 well plate and shaken vigorously. After 30 minutes of incubation at room temperature, absorbance values were recorded at 750 nm against blank that contain 20 µL ethanol without any sample using Elisa reader. Results were evaluated using gallic acid standart curve and recorded as milligrams of total phenolics (TP) per gram of extract, as the gallic acid equivalents (GAE). Analyses were run in three replicates and the results were expressed as mean ± standard deviation (SD).

Total flavonoid content of the extracts were determined by Aluminium chloride colorimetric method [20], which some modifications [19]. Briefly, 20 µL of extract or standart solution of catechin was added into 96 well plate containing 80 µL of distilled water. 6 µL of 5% sodium nitrite was added to the wells. After 5 minutes later, 6 µL of 10 % aluminium chloride solution was added. At 6th minute, 40 µL of 1 M sodium hydroxide was added and the total volume was completed up to 200 µL with distilled water. The solution was mixed well and the absorbance was measured against prepared blank at 510 nm using Elisa reader. Results were calculated by using the quercetin standart curve and recorded as milligrams of total flavonoids in gram of extract, as the quercetin equivalents (QE). Analyses were run in three replicates and the results were expressed as mean ± standard deviation (SD).

RP-HPLC analysis. The HPLC analysis was performed on a Dionex UltiMate 3000 HPLC system equipped with UltiMate 3000 Pump, UltiMate 3000 Autosampler Column Compartment, UltiMate 3000 Photodiode Array Detector and Chromeleon software. Separation was carried out using an Agilent Zorbax SB-C18 (250mm x 4,6mm x 5µm) with a guard column packed with the same material. The column was maintained at 30 °C throughout the analysis, and 280 nm was selected as the wavelength for UV detection.

Gradient elution was carried out at a flow rate of 1.0 mL/min at 30°C. The mobile phase consisted of (A) methanol:water [50% (v/v)] and (B) water:acetic acid [98:2 by volume]) using a gradient elution as follows: 0–3 min, 0% B; next 3-5 min, 8% B; next 5-57 min, linear change from A-B (92:8) to A-B (28:72), then back to 100% A at 57-60 min. All samples were filtered through a 0.45µm membrane filter (Millipore, Milford, MA). An aliquot of 20 µL of the filtrate was injected into HPLC

for analysis. Three HPLC replicate injections were performed for each standard phenolics and plant extracts.

DPPH radical scavenging activity. DPPH radical scavenging activity was determined according to DPPH assay [21] with some modifications [19]. Briefly, 140 µL of 0.05 mg/mL DPPH solution in ethanol and 10 µL of extract were mixed in 96 well plate and reaction mixture was shaken vigorously. Decrease of absorbance in the mixture was determined after 20 minute at 517 nm due to depletion of DPPH radical by using Elisa reader. α- tocopherol, trolox, BHA and BHT were employed as reference control. According to the results, RSA% vs final concentrations of the extracts (mg/mL) were plotted and IC_{50} (50% effective concentration) values were calculated. All measurements were performed three times.

ABTS radical scavenging activity. ABTS radical scavenging activities of the extracts were performed according to ABTS cation radical assay [22] with some modifications [19]. Briefly, 2.5 µL of extract or standart solution was added to 96 well plate including 250 µL of ABTS radical solution. After that, the absorbance of reaction mixture was monitored at 734 nm using Elisa plate reader. After the initial mixing of the reactants, time was recorded every minute from 1st to 6th. The results were expressed as IC_{50} value. All measurements were performed three times.

CUPRAC assay. Cupric ions' (Cu^{2+}) reducing capacities of the extracts was performed according to the CUPRAC method of [23]. Briefly, different sample extract concentrations (10–30 µg/mL) were added to a premixed reaction mixture containing 0.25 mL of CH_3COONH_4 buffer solution (1.0 M), 0.25 mL of ethanolic neocuproine solution (7.5 × 10−3 M), 0.25 mL of $CuCl_2$ solution (0.01 M). After adjusting the final volumes to 2 mL with distilled water, absorbances were measured at 450 nm after 30 min incubation in room temperature. Increased absorbance was considered as increased reducing capacity.

Determination of metal chelating activity. Ferrious ions (Fe^{+2}) chelating activity of the extracts was monitored according to Dinis method [24] with some modifications. Different concentrations of the investigated extracts (0.5 to 4 mg/mL) and EDTA (0,093 to 3.72 mg/mL) were prepared, respectively. 50 µL of the working solutions of the extracts/ EDTA were placed to 96 well plate containing 185 µL of dH_2O, and 5 µL of 2 mM $FeCl_2$ solution was added. After 5 min, the reaction was initiated by adding 10 µL of 5 mM ferrozine solution. Absorbance at 562 nm was recorded after 10 min of incubation at room temperature. A reaction mixture containing methanol (50 µL) instead of substance solution was used as a control. EDTA was used as the chelating standard.

Antiproliferative activity assay. The cell lines; HT-29, HepG2, and MCF-7 were obtained from the ATCC (American Type Culture Collection, LGC Promochem, UK). MCF-7 and HepG2 cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 1% L- Glutamine, 10% fetal bovine serum, 1% penicillin-streptomycin (Pen Strep) solution and 1% Na-pyruvate. HT-29 cells were grown in a medium McCoys's 5A containing 10% fetal bovine serum, 1% L- Glutamine and 1% penicillin-streptomycin solution. Cultures were incubated at 37°C with 5% carbon dioxide (CO2) and 95% humidity in incubator (NUVE, Turkey). Studies were performed in Metisafe Class II Safety Cabinet.

The cytotoxic potentials of the extracts on the cancer and healthy cells were evaluated by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide (XTT) assay, which is based on the extracellular reduction of tetrazolium salt XTT by NADH produced in the mitochondria *via* trans-plasma membrane electron transport and an electron mediator [25]. The cells were seeded at a concentration of $1x10^5$ cells/mL into 96-well culture plates and allowed to adhere overnight at 37 °C. After 24 h, cells were treated with the test compounds at different concentrations, in triplicate, or with a solvent control (0.5 % DMSO) in complete medium. After 48 h incubation, the plates were gently shaken for 1 min and absorbance was measured at 415 nm by Epoch Microplate Reader (BioTek, USA). 5-Fluorouracil (5-FU) was used as a standard reference drug.

Statistical analysis. Statistical analyses were performed for evaluation of antioxidant and cytotoxic activity results by unpaired Student's t-test by using statistical program of GraphPad Prism 6 (GraphPad, La Jolla, CA) Software 7.0). All results were expressed as means with their standard deviation (SD). $p<0.05$ was taken as the minimum level of significance.

RESULTS AND DISCUSSION

Phytochemical composition. Many herbs and formulations are still used today for their therapeutic effects. Their diverse biological actions might be attributed to their rich phytochemical contents [26]. Therefore, it is very important to establish a correlation between the biological activity potentials and phytochemical composition of the samples. In the present study, we tried to clarify the phytochemical composition of *G. lepidioides* extracts. The amounts of total phenolics and flavonoids in meth-

anol extract were determined as 116.35 mg GAE/g extract and 39.75 mg QE/ g extract, respectively, while those of water extract were 90.86 mg GAE/g extract and 46.02 mg QE/ g extract, respectively. These results show that total phenolic content of methanol extract was higher than that of water extract while the flavonoid level was lower than water extract. Also, the results indicated that flavonoids constitute about half of the total phenolic content in both extracts. In the literature, it was reported that the total phenolic content of *G. pilulifera* as 6.5 µg GAE/mg extract [27]. In another study, total phenolic contents of *Gypsophila arrostii, G.pilulifera and G.simonii* from Turkey were reported as 0.26, 0.54 and 15.15 µg GAE/mg extract, respectively [28]. Compared to the literature, the superiority of *G. lepidioides* in terms of phenolic content comes to the forefront.

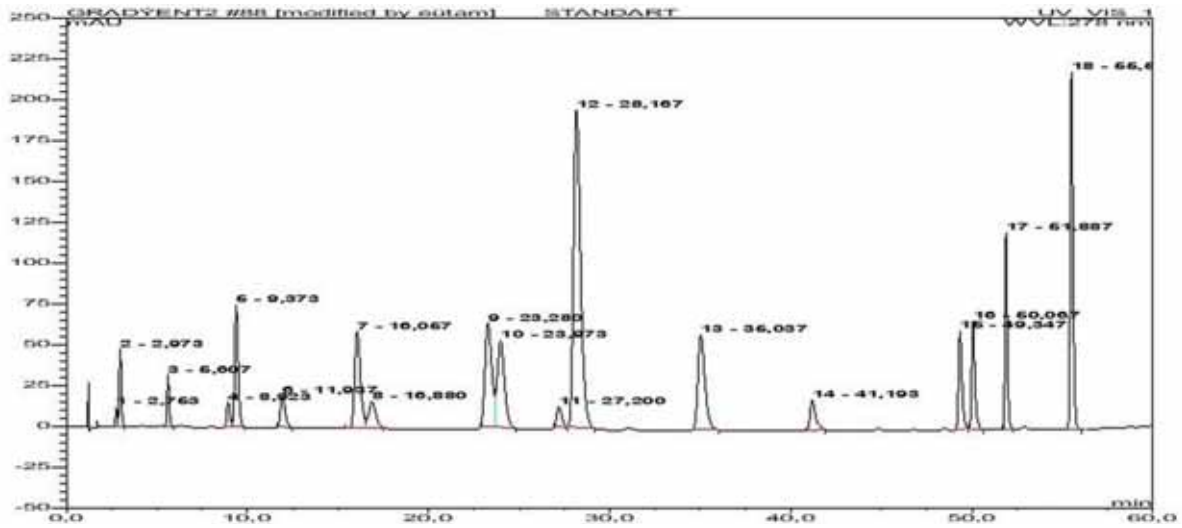


FIGURE 1

HPLC chromatogram of phenolic standards at 280 nm with their retention times (min.)

1- Pyrogallol, 2- gallic acid, 3-3,4-Dihydroxybenzoic acid, 4-2,3-Dihydroxybenzoic acid, 5- p-Hydroxybenzoic acid, 6-(+)-Catechin, 7- Caffeic acid, 8- Chlorogenic acid, 9- Vanillin, 10- Syringic acid, 11- (-)-Epicatechin, 12- p-Coumaric acid, 13-Taxifolin, 14- Sinapic acid, 15- Resveratrol, 16- Rutin, 17- Rosmarinic acid, 18- Naringenin.

TABLE 1

Phenolic composition of *G. lepidioides* extracts (mean ± SD)[x]

No.	Retention time (min.)	Phenolics and flavonoids	Concentration (µg/g dry plant)		Analytical characteristics		
			Methanol	Water	R^2	LOD (mg per L)[y]	LOQ (mg per L)[z]
1	2.75	Pyrogallol	0.72±0.018	0.16±0.04	0.998	0.003	0.01
2	2.97	Gallic acid	0.42±0.009	nd	0.999	0.002	0.007
3	5.60	3,4-Dihydroxybenzoic acid	0.17±0.03	0.13±0.02	0.999	0.0003	0.001
4	8.92	2,3-Dihydroxybenzoic acid	nd	nd	0.997	0.0003	0.001
5	9.37	p-Hydroxybenzoic acid	0.026±0.008	0.009±0.002	0.999	0.003	0.001
6	11.94	(+)-Catechin	nd	nd	0.997	0.003	0.009
7	16.06	Caffeic acid	nd	nd	0.996	0.002	0.008
8	16.88	Chlorogenic acid	nd	nd	0.998	0.001	0.004
9	23.28	Vanillin	0.016±0.002 [a]	0.05±0.04 [b]	0.995	0.002	0.008
10	23.97	Syringic acid	nd	1.45±0.05	0.996	0.002	0.009
11	27.20	(-)-Epicatechin	nd	nd	0.999	0.0001	0.0005
12	28.17	p-Coumaric acid	nd	2.05±0.07	0.998	0.004	0.001
13	35.04	Taxifolin	nd	nd	0.997	0.002	0.009
14	41.19	Sinapic acid	nd	nd	0.998	0.002	0.009
15	49.35	Resveratrol	nd	nd	0.996	0.003	0.01
16	50.07	Rutin	0.36±0.04 [a]	0.42±0.03 [b]	0.997	0.001	0.005
17	51.89	Rosmarinic acid	nd	0.96±0.07	0.998	0.001	0.005
18	55.50	Naringenin	nd	nd	0.996	0.001	0.005

[x]Data marked with different superscripts (a and b) within the same row indicate significant difference statistically ($p < 0.05$).
[y]LOD, limit of detection.
[z]LOQ, limit of quantification.
[w]nd, not detected

In parallel to the total phenolic and flavonoid contents, eighteen individual phenolic compounds were also screened by HPLC analysis (Figure 1), and the results of the analytical parameters were tabulated in Table 1. As seen from the Table 1, The most abundant compound in the methanol extract of *G. lepidioides* was pyrogallol which is followed by gallic acid, rutin, 3,4-dihydroxybenzoic acid, p-hydroxybenzoic acid and vanillin, respectively. On the other hand, water extract of *G. lepidioides* was found to contain syringic acid at the highest level, which is followed by rosmarinic acid, pyrogallol, 3,4-dihydroxybenzoic acid and p-hydroxybenzoic acid, respectively. Upon comparing both extracts considering Table 1, gallic acid, vanillin and rutin were found only in methanol extract, while syringic acid and rosmarinic acid were detected only in water extract. This is not unexpected phenomenon since the polarity of the extraction solution affects the phenolic content of the plant extracts [29–31].

In the literature, there are many studies reporting that saponarin (apigenin-6- C-glucosyl-7-O-glucoside) is the main phenolic compound found in *Gypsophil*a species [11, 27, 28, 32]. Apart from the reports mentioned above, no further study is available in the literature regarding their individual phytochemical composition to compare that of *G. lepidioides*. By this study, we reported the first detailed phenolic content of *G. lepidioides.*

Antioxidant activity. There are many ways for evaluating the antioxidant potential of a source to have complete understanding of the mechanism of action. In the present study, the antioxidant potential of the methanol and water extracts of *G. lepidioides* were evaluated by DPPH, ABTS, reducing CUPRAC and metal chelating assays. The results of radical scavenging and metal chelating assays were tabulated in Table 2, while the CUPRAC assay results were shown in Figure 2.

DPPH and ABTS free radicals have been widely used to evaluate the radical scavenging ability of antioxidants [33]. Therefore, radical scavenging power potential of the extracts was examined by using two different test systems, namely DPPH and ABTS. As can be seen from the results presented in Table 2, while methanol extract of *G. lepidioides* showed lower DPPH radical scavenging activity than water extract, it displayed higher ABTS radical scavenging activity with an IC_{50} value than water extract. On the other hand, both water and methanol extracts displayed moderate radical scavenging activity compared to all of the standard antioxidants tested (Table 2). The results obtained show us that the phytochemicals, scavenging DPPH and ABTS radicals are distributed differently in water and methanol extracts.

The findings are closely consistent with the literature. Serteser et al. (2009) reported a study, where five *Gypsophila* species were tested for their DPPH radical scavenging activities, showing the IC_{50} values of the species change between 3.1 mg/mL to 3.6 mg/mL [34]. In another study, IC_{50} values of different *Gypsophila pilulifera* extracts for DPPH radical scavenging activity were reported as 446 µg/mL and 4.56 mg/mL, respectively [27, 32]. Different antioxidant activities from different species can be expected due to differences in methodology and experimental conditions used in the different studies [35]. Moreover, the presence of potential antioxidant compounds, such as vitamins, flavonoids, phenolic acids and sulphur compounds present in plants, of cource, influence the antioxidant activity of the extract [36, 37].

TABLE 2
Radical scavenging activity, reducing power and metal chelating potentials of *G. lepidioides* extracts (mean ± SD) [x]

Test samples	Assays		
	Radical Scavenging		Metal Chelating
	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	Fe^{2+} chelating IC_{50} (µg/mL)
G. lepidioides methanol ext.	802.6± 3.55[a]	118.2±2.35[a]	149.2±3.48 [a]
G. lepidioides water ext.	696.4±0.042[b]	249.3±2.95 [b]	166.5±2.52 [b]
[q]BHA	8.2±0.52 [c]	15.6±0.85 [c]	nt
[y]BHT	21.6±1.02 [d]	7.2±0.56 [d]	nt
Trolox	18.8±0.75 [d]	12.4±0.56 [c]	nt
α-Tocopherol	28.4±0.45 [e]	18.7±0.78 [c]	nt
[z]EDTA	nt	nt	3.47±0.27

[x]Data marked with different superscripts (a, b, c, d and e) within the same column indicate significant difference statistically ($p < 0.05$).
[q]BHA, Butylated Hydroxyanisole.
[y]BHT, Butylated hydroxytoluene.
[z]EDTA, Ethylenediaminetetraacetic acid.
nt, not tested.

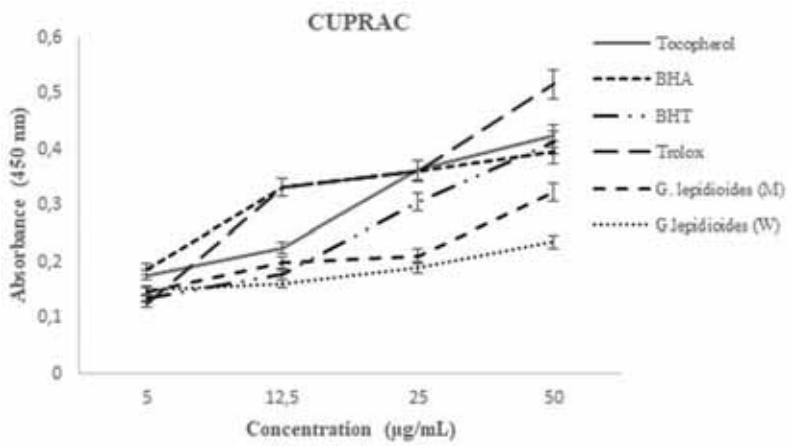


FIGURE 2

Cu^{2+} reducing power of *G. lepidioides* water (W) and methanol (M) extracts and standard antioxidants. BHA; Butylated hydroxyanisole, BHT; Butylated hydroxytoluene, trolox and α-tocopherol. Each point represents the average of three independent measurements, each done in triplicate, with the standard deviation of the mean.

TABLE 3
Antiproliferative activities of *G. lepidioides* extracts against human-derived tumor cell lines

Tested samples	IC_{50} (mg/mL)[x]		
	HepG2 [y]	HT-29 [y]	MCF-7 [y]
water extract	3.19±0,032[a]	3.85±0.47 [a]	2.66±0.55 [a]
methanol extract	1.02±0,015[b]	0.64±0.25 [b]	1.11±0.32 [b]
[z]5-FU	0.047±0.002[c]	0.023±0.006 [c]	0.018±0.004 [c]

[a]Human tumor cell lines: HepG2, hepatocellular carcinoma; HT-29, colorectal adenocarcinoma; MCF7, breast carcinoma.
[x]IC_{50} (mg/mL): Data marked with different superscripts (a, b and c) within the same column indicate significant difference statistically ($p < 0.05$).
[y]5-FU: 5-Fluorouracil (positive control).

Transition metals can stimulate lipid peroxidation by generating hydroxyl radicals via Fenton reaction and also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, and thus leading to persistence of the chain reaction of lipid peroxidation. Therefore, the evaluation of antioxidant activities of the plant species on metal chelating capacities is indispensable. Antioxidant activity of the extracts, in this line, was screened by Fe^{2+} chelating assay. Metal chelating activity results showed the superiority of the methanol extract to the water extract extract. However, the metal chelating capacities of both extracts were below that of EDTA (Table 2). In the literature, Fe^{2+} chelating capacities of five gypsophila species was reported to vary between 17.2% and 24.3% [34]. However, in this study, *G. lepidioides* methanol and water extracts could be able to exhibit high chelating activity up to 51 %and 63%, respectively.

The CUPRAC assay is commonly used to screen the antioxidant capacity of plant extracts, because of the requirements of a small number of equipment, as well as fast and reproducible results [38]. In order to examine the reducing power of the extracts, the Cu^{2+} to Cu^{+} reduction in the presence of the extracts was investigated. As shown in Figure 2, both extract of *G. lepidioides* displayed a good cupric ions (Cu^{2+}) reducing capacity. Even, both extract of *G. lepidioides* showed higher reducing avtivity than standard antioxidants BHT and trolox at 5 µg/mL concentration. As the concentrations increased, the methanol extract showed higher activity than the water extract. However, both extracts exhibited moderate reducing activity, compared to all standard antioxidants at these high concentrations. Regarding reducing power of *G. lepidioides*, there are limited number of studies in the literature. One of them was *Gypsophila bitlisensis*, possessing moderate reducing power compered to the standard antioxidants trolox and α-tocopherol [39].

All the antioxidant activity assays performed in this study indicate that a plant extract, exhibiting low antioxidant activity by a method could not be labelled as a poor source of antioxidant, because an extract is composed of chemicals with different functional groups and polarities and may behave differently depending on the reaction mixture [38]. Detected phenolic compounds in the plant extracts, of course, are not responsible alone for the antioxidant activity. Contribution of other phytochemicals and their synergetic effects should also be taken into consideration.

Antiproliferative activity. To evaluate the antiproliferative activities of the *G. lepidioides* water and methanol extracts against human-derived can-

cer cell lines HT-29 (colorectal adenocarcinoma cell line), HepG2 (hepatocellular carcinoma cell line) and MCF-7 (breast adenocarcinoma cell line), cytotoxicity XTT assay was carried out. Growth inhibition percent was calculated by comparing to a negative control growth after 48h incubation time and IC_{50} values of the extracts and 5-Flourouracil (5-FU) used as a positive control were tabulated in Table 3.

The experimental results showed that the proliferation of three tested cell lines were significantly inhibited by *G. lepidioides* extracts in a dose-dependent manner depending on the cell types. The results also indicated the superiority of cytotoxic activity of methanol extract to water extract against all the tested cancer cell lines with its lower IC_{50} values. The water extract displayed the highest anti-proliferative activity against MCF-7 cells with its lower IC_{50} value. Statistically, there were no difference between the activities of the water extract against HepG2 cells and HT-29 cells. On the other hand, the most susceptible cell line to methanol extract of *G. lepidioides* was HT-29 cells with its lower IC_{50} value. The cytotoxic activity of the methanolic extract against HepG2 cells and MCF-7 cells was similar. As shown in Table 3, both methanol and water extracts showed moderate cytotoxic activity against all tested cell lines compared to positive control 5-Fluorouracil.

These *in vitro* results are in accordance with the literature. For instance, the cytotoxic activities of methanolic extracts of three Gypsophila species against HepG2, HT-29, MCF-7 as well as A549 *(*lung cancer cell line) and MDBK (median- darby bovine kidney) were reported, and none of these extracts showed cytotoxic activity on the tested cell lines up to concentration of 0.1 mg/mL, while only *G. bicolor* had cytotoxicity on MDKB cells [40]. In another study, methanol extract of *G. capillaris* was reported to inhibit the cell proliferations of MCF-7, HCT-116, HepG2 and A-549 cells by 32.5%, 10.3%, 24% and 10.8%, respectively at the concentration of 0.1 mg/mL [41]. Furthermore, methanolic extract of *G. trichotoma* was reported to induce the inhibition of BV-173 leukemia cell proliferation by 2.2% at the concentration of 0.1 mg/mL [42]. Against the moderate cytotoxicity of the family Caryophyllaceae extracts, the saponins they possess was reported to enhance the cytotoxic potentials of the extracts [43–45]. For instance, studies on saponins of Gypsophila species have illustrated their anti-carcinogenic properties, including cytotoxicity, immune-modulating effects and normalization of carcinogen induced cell proliferation [46]. The saponins in the extracts exploit their cytotoxic effects through either apoptosis inducement or non-apoptotic cell death stimulation. In fact, there are some well-known process leading to cell death, but having different mechanisms of action such as stimulation of autophagic cell death, decrease in nitric oxide (NO) production and disassembly of cytoskeleton integrity [47].

CONCLUSION

The purpose of this study was to explore the phenolic content and biological activity potential of *G. lepidioides* for functional food and medicinal uses. The obtained results showed that methanol extract of *G. lepidioides* had higher antioxidant and anti-proliferative activity than the water extract because of its higher percentage of biologically active compounds determined by HPLC analysis. Accordingly, methanol extract of *G. lepidioides* may have a potential for possible applications in food and pharmaceutical industries. Hovewer, further studies should be performed for the discovery of new bioactive metabolites via biological activity guided chromatographic techniques.

ACKNOWLEDGEMENTS

This work was financially supported by grants from Erzincan University Scientific Research Projects Coordination Commission (EU-BAP) (Project number: FBA-2017-470).

REFERENCES

[1] Kontogianni, V.G., Tomic, G., Nikolic, I. Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I.P., Tzakos, A.G. (2013) Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. Food Chem. 136, 120–129.

[2] Farzaneh, V., Carvalho, I.S. (2015) A review of the health benefit potentials of herbal plant infusions and their mechanism of actions. Ind. Crops Prod. 65, 247–258.

[3] Köksal, E., Tohma, H., Kılıç, Ö., Alan, Y., Aras, A., Gülçin, I., Bursal, E. (2017) Assessment of antimicrobial and antioxidant activities of nepeta trachonitica: Analysis of its phenolic compounds using HPLC-MS/MS. Sci. Pharm. 85, 1–14.

[4] Ngo, D.H., Wijesekara, I., Vo, T.S., Van Ta, Q., Kim, S.K. (2011) Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. Food Res. Int. 44, 523–529.

[5] dos Santos, H.F., Campos, J.F., dos Santos, C.M., Balestieri, J.B.P., Silva, D.B., Carollo, C.A., Souza, K. de P., Estevinho, L.M., dos Santos, E.L. (2017) Chemical profile and antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities of geopropolis from the stingless bee Melipona orbignyi. Int. J. Mol. Sci. 18.

[6] Poyraz, İ.E., Çiftçi, G.A., Öztürk, N. (2017) Phenolic Contents, in vitro antioxidant and cytotoxicity activities of salvia aethiopis l. and s. ceratophylla l. (lamiaceae). Rec. Nat. Prod. 11, 345–355.

[7] Guérard, M., Baum, M., Bitsch, A., Eisenbrand, G., Elhajouji, A., Epe, B., Habermeyer, M., Kaina, B., Martus, H.J., Pfuhler, S., Schmitz, C., Sutter, A., Thomas, A.D., Ziemann, C., Froetschl, R. (2015) Assessment of mechanisms driving non-linear dose-response relationships in genotoxicity testing, Mutat. Res. - Rev. Mutat. Res. 763, 181–201.

[8] Gülçin, İ. (2011) Antioxidant Activity of Eugenol: A Structure–Activity Relationship Study. J. Med. Food. 14, 975–985.

[9] Korkmaz, M., Özçelik, H. (2011) Economic importance of Gypsophila L., Ankyropetalum Fenzl and Saponaria L. (Caryophyllaceae) taxa of Turkey. African J. Biotechnol. 10, 9533–9541.

[10] Primorac, M., Sekulovic, D., Antonic, S. (1985) In vitro determination of the spermicidal activity of plant saponins. Pharmazie. 40, 585.

[11] Vitcheva, V., Simeonova, R., Krasteva, I., Yotova, M., Nikolov, S., Mitcheva, M. (2011) Hepatoprotective effects of saponarin, isolated from *Gypsophila trichotoma* Wend. on cocaine-induced oxidative stress in rats. Redox Rep. 16, 56–61.

[12] Bai, H., Zhong, Y., Xie, Y.Y., Wang, Y.S., Liu, L., Zhou, L., Wang, J., Mu, Y.L., Zuo, C.X. (2007) A major triterpenoid saponin from Gypsophila oldhamiana. Chem. Biodivers. 4, 955–960.

[13] Yao, S., Ma, L., Luo Jian, G., Wang Jim, S., Kong Ling, Y. (2010) Triterpenoid saponins from the roots of Gypsophila paniculata. Chin. J. Nat. Med. 8, 28–33.

[14] Koyuncu, M., Kiliç, C.S., Güvenç, A. (2008) Doğu Anadolu'da Çöven Elde Edilen Bitkiler ve Bunlarin Doğadaki Potansiyeli, Turk. J. Botany. 32, 489–494.

[15] Yücekutlu, A.N., Bildacı, I. (2008) Determination of Plant Saponins and Some of Gypsophila Species : A review of the literature. Hacettepe J. Biol. Chem. 36, 129–135.

[16] Korkmaz, M., Özçelik, H. (2013) Soil-plant relations in the annual Gypsophila (Caryopyhllaceae) taxa of Turkey. Turk. J. Botany. 37, 85–98.

[17] Özdemir, C., Özkan, M., Kandemir, A. (2010) The morphological and anatomical properties of gypsophila lepidioides boiss (caryophyllaceae) endemic to Turkey. Int. Res. J. Plant Sci. 1, 69–74.

[18] Singleton, V.L., Rossi, J.A. (1965) Colorunetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

[19] Altay, A., Celep, G.S., Yaprak, A.E., Baskose, I., Bozoglu, F. (2017) Glassworts as Possible Anticancer Agents Against Human Colorectal Adenocarcinoma Cells with Their Nutritive, Antioxidant and Phytochemical Profiles. Chem. Biodivers. 14, 1-10.

[20] Zhishen, J., Mengcheng, T., Jianming, W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.

[21] Blois, M.S. (1958) Antioxidant determinations by the use of a stable free radical. Nature. 181, 1199–1200.

[22] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.

[23] Apak, R., Güçlü, K., Özyürek, M., Çelik, S.E. (2008) Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. Microchim. Acta. 160, 413–419.

[24] Dinis, T.P.C., Madeira, V.M.C., Almeida, L.M. (1994) Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers. Arch. Biochem. Biophys. 315, 161–169.

[25] Trendowski, M., Yu, G., Wong, V. Acquafondata, C., Christen, T., Fondy, T.P. (2014) The real deal: Using cytochalasin B in sonodynamic therapy to preferentially damage leukemia cells, Anticancer Res. 34, 2195–2202.

[26] Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., Armand, R. (2015) The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. Int. J. Clin. Med. 6, 635–642.

[27] Yazici, S.Ö., Özmen, İ. (2018) Effect of the Crude Saponin Extract from Gypsophila pilulifera Boiss. & Heldr. on Protease from Bacillus subtilis ATCC 6633 and Antioxidant Properties of the Extract. Iran. J. Sci. Technol. Trans. A Sci. 42, 1707-1713.

[28] Arslan, I., Çelik, A. (2013) Saponin Rich Fractions (SRPs) from Soapwort Show Antioxidant and Hemolytic Activity. APCBEE Procedia. 7, 103–108.

[29] Fu, Z.F., Tu, Z.C., Zhang, L., Wang, H., Wen, Q.H., Huang, T. (2016) Antioxidant activities and polyphenols of sweet potato (Ipomoea batatas L.) leaves extracted with solvents of various polarities. Food Biosci. 15, 11–18.

[30] Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., Ju, Y.H. (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J. Food Drug Anal. 22, 296–302.

[31] Ammar, I., Ennouri, M., Attia, H. (2015) Phenolic content and antioxidant activity of cactus (Opuntia ficus-indica L.) flowers are modified according to the extraction method. Ind. Crops Prod. 64, 97–104.

[32] Chima, N.K., Nahar, L., Majinda, R.R.T., Celik, S., Sarker, S.D. (2014) Assessment of free-radical scavenging activity of Gypsophila pilulifera: Assay-guided isolation of verba-scoside as the main active component. Brazilian J. Pharmacogn. 24, 38–43.

[33] Zhao, G.R., Xiang, Z.J., Ye, T.X., Yuan, Y.J., Guo, Z.X. (2006) Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. Food Chem. 99, 767–774.

[34] Serteser, A., Kargıoğlu, M., Gök, V., Bağci, Y., Özcan, M.M., Arslan, D. (2009) Antioxidant properties of some plants growing wild in Turkey. Grasas Aceites. 60, 147–154.

[35] Tohidi, B., Rahimmalek, M., Arzani, A. (2017) Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem. 220, 153–161.

[36] Embuscado, M.E. (2015) Spices and herbs: Natural sources of antioxidants - A mini review. J. Funct. Foods. 18, 811–819.

[37] Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., Sochor, J. (2015) Bioactive compounds and antioxidant activity in different types of berries. Int. J. Mol. Sci. 16, 24673–24706.

[38] Tusevski, O., Kostovska, A., Iloska, A., Trajkovska, L., Simic, S.G. (2014) Phenolic production and antioxidant properties of some Macedonian medicinal plants. Cent. Eur. J. Biol. 9, 888–900.

[39] Işık, M., Korkmaz, M., Bursal, E., Gülçin, İ., Köksal, E., Tohma, H. (2015) Determination of Antioxidant Properties of Gypsophila bitlisensis Bark. Int. J. Pharmacol. 11, 366–371.

[40] Naghibi, F., Irani, M., Hassanpour, A., Pirani, A., Hamzeloo-Moghadam, M. (2014) Cytotoxic effects of selective species of Caryophyllaceae in Iran. Res. J. Pharmacogn. 1, 29–32.

[41] Moustafa, S.M., Menshawi, B.M., Wassel, G.M., Mahmoud, K., Mounier, M. (2014) Screening of some Plants in Egypt for their Cytotoxicity against four Human Cancer cell lines. Int. J. PharmTech Res. 6, 1074–1084.

[42] Voutquenne-Nazabadioko, L., Gevrenova, R., Borie, N., Harakat, D., Sayagh, C., Weng, A., Thakur, M., Zaharieva, M., Henry, M. (2013) Triterpenoid saponins from the roots of Gypsophila trichotoma Wender. Phytochemistry. 90, 114–127.

[43] Holmes, S.E., Bachran, C., Fuchs, H., Weng, A., Melzig, M.F., Flavell, S.U., Flavell, D.J. (2015) Triterpenoid saponin augmention of saporin-based immunotoxin cytotoxicity for human leukaemia and lymphoma cells is partially immunospecific and target molecule dependent. Immunopharmacol. Immunotoxicol. 37, 42–55.

[44] Kamal, Z., Ullah, F., Ayaz, M., Sadiq, A., Ahmad, A. Zeb, A. Hussain, M., Imran, M. (2015) Anticholinesterase and antioxidant investigations of crude extracts, subsequent fractions, saponins and flavonoids of atriplex laciniata L.: potential effectiveness in Alzheimer's and other neurological disorders. Biol. Res. 48.

[45] Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Subhan, F., Khan, M.A., Ahmad, W., Ali, G., Imran, M., Ahmad, S. (2016) Molecularly characterized solvent extracts and saponins from Polygonum hydropiper L. show high anti-angiogenic, anti-tumor, brine shrimp, and fibroblast NIH/3T3 cell line cytotoxicity. Front. Pharmacol. 7, 1–13.

[46] Navarro del Hierro, J., Herrera, T., Fornari, T., Reglero, G., Martin, D. (2018) The gastrointestinal behavior of saponins and its significance for their bioavailability and bioactivities. J. Funct. Foods. 40, 484–497.

[47] Podolak, I., Galanty, A., Sobolewska, D. (2010) Saponins as cytotoxic agents: A review, Phytochem. Rev. 9, 425–474.

Received: **16.04.2018**
Accepted: **12.10.2018**

CORRESPONDING AUTHOR

Ahmet Altay
Department of Chemistry,
Faculty of Arts and Science,
Erzincan University,
24100, Erzincan – Turkey

e-mail: aaltay2013@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9696-9705 Fresenius Environmental Bulletin

PRE AND POST-HARVEST CALCIUM CHLORIDE TREATMENTS MAINTAIN THE OVERALL QUALITY OF SWEET CHERRIES

Muhammad Mazahir, Yasser Durrani, Ihsan Mabood Qazi, Majid S Hashmi*, Ali Muhammad

Department of Food Science and Technology, The University of Agriculture Peshawar, Pakistan

ABSTRACT

Cherry fruits are abundantly grown in Gilgit Baltistan province of Pakistan. To investigate the impact of Calcium chloride ($CaCl_2$) on cherries, two experiments were carried out, in first experiment 0.5 and 1% $CaCl_2$ solutions were sprayed on sweet cherry fruits as pre-harvest foliar within 10, 20 and 30 days of intervals. Fruit were then harvested after optimum maturity and were analyzed for cracking index. Sound fruits were packed in foam trays and stored at room temperature. In second experiment fresh cherry fruits were harvested at commercial maturity, graded and dipped in 0.5 and 1% $CaCl_2$ solution for 5-10 minutes. The treated fruit were packed in foam trays and stored at room temperature for 20 days. Both pre and post-harvest treated fruit samples were studied for selected physico-chemical and sensory attributes within 4 days of interval. Pre-harvest application of $CaCl_2$ had significantly reduced the cracking index and moisture loss, subsequently reducing weight loss and decay index of the fruit. Moreover, these treatments maintained Total Soluble Solids (TSS) and Vitamin-C content. Data regarding sensory characteristics revealed that $CaCl_2$ had significantly retained the color, flavor, texture and overall acceptability of the fruits. However, expectedly texture of the fruit was more positively influenced with $CaCl_2$ application than rest of the sensory attributes. Hence, concluded that application of 1 % $CaCl_2$ solution as pre and post-harvest treatment had overcome the issues related to cherry fruits especially cracking index and is recommended to the cherry growers for application. Further research is needed to explore the effect of $CaCl_2$ treatment on the physiology of cherry fruit.

KEYWORDS:
Sweet cherry fruit, Calcium chloride, Pre-harvest, Post-harvest.

INTRODUCTION

Sweet cherry (*Prunus avium* L.) belongs to the genus *Prunus* is one of the most famous fleshy, stone, pulpy and temperate fruit. Cherry fruit is normally eaten in fresh and dried form but also processed into jam, marmalade and pickle etc. There are several species of cherry grown in the world such as sweet cherry (*Prunus avium*), citrus, pie or tart cherries (*Prunus cerasus*), black cherry (*Prunus serotina*), West Indian cherry *Prunus myrtifolia* [1]. Sweet cherry is of considerable importance for maintenance of healthy life because it prevents certain diseases [2]. The health benefits are associated with strong antioxidant activities responsible for weight loss, neuro protective effects; prevent arthritis pain and inflammation [3,4]. Gilgit Baltistan (GB) is located in extreme north of Pakistan where it borders the Xinjang province of China in north, Chitral in west and Kaghan valley in south. It lies in temperate regions and provides favorable atmospheric conditions for the growth of and various high quality fruits including cherry, apricot, and mulberry. In GB cherries cultivated in an area of 1,302 hectares with total production of 2,387 tones [5].

Calcium (Ca) plays very important role in the fruits regarding the cell wall structure, because of its ability to strengthen plasma membrane, structure rigidity and improve cellular signaling responses [6, 7]. Pre-harvest $CaCl_2$ treatments retard the aging, softening and hence delay senescence in majority of thin skin fruits [8, 9] and reduce disintegration and disorders such as bitter pit [10, 11]. The Ca treatment before picking of fruits is the safest and most effective method to improve the quality and extend the shelf life of fresh fruit. For cherries Ca treatments were used many years ago to reduce cracking of the fruit [12]. Earlier the post-harvest application of $CaCl_2$ delayed the senescence in fruit with no detrimental effect on consumer acceptance and it also stabilized fruit cell wall from degradation. Furthermore, post-harvest $CaCl_2$ treatments minimized fruit softening and enhanced the shelf life stability [13]. Post-harvest calcium dips efficiently increase the Ca content in pericarp as well as in mesocarrp of the fruit and stabilize the membrane by making cross linking which strengthens the cell wall [14]. The Ca

application improve fruit superiority by delaying the fruits ripening, slow down the respiration and enhance the fruits shelf life and marketability [15].

In GB heavy losses of cherry fruits may occur due to lack of knowledge about fresh fruits handling and preservation, improper pre-harvest farming practices, post-harvest processing, storage conditions, transportation facilities and financial support. For improving the quality of fresh fruit, application of inexpensive pre and post-harvest $CaCl_2$ treatments might be helpful in reducing cherry fruit losses further it will improve the financial condition of cherry growers and will also save time to transport the produce to market. Keeping in view the above various aspects, this research was designed to study the influence of both pre and post-harvest application of $CaCl_2$ on cracking issues subsequently storage stability and overall quality of cherry fruit grown in GB province of Pakistan.

MATERIALS AND METHODS

Sweet cherries (*Prunus avium* L.) were divided into pre and post-harvest lots in the orchard. This research work was carried out during (2016-17) in the analytical laboratory of PCSIR (Pakistan Council of Scientific and Industrial Research Center) in Skardu (GB).

Calcium Chloride Treatment. Two experiments were conducted in which, cherry fruit were treated before and after harvesting with different solutions of $CaCl_2$. In first experiment, 0.5 and 1% $CaCl_2$ solutions were sprayed as pre-harvest foliar at an interval of 10, 20 and 30 days. Cherries were harvested after obtaining optimum maturity and cracking index was measured then the fruits were packed in foam trays and stored at room temperature. In second experiment fresh sweet cherry fruits were harvested at commercial maturity from the local orchard and transferred to the PCSIR laboratories complex (GB). The fruit were graded and dipped in 0.5 and 1% $CaCl_2$ solution for 5-10 minutes in three replications each. The treated fruit were packed in foam trays and stored at room temperature. Fruit samples of both the experiment were studied for selected physico-chemical and sensory characteristics.

Chemical Analysis. Moisture content, ascorbic acid, total soluble solids and titratable acidity of the cherry fruit samples were determined by the approved standard method of AOAC [16] .

Cracking Index. Cracking indexes of cherries were calculated as per the recommended method of Bilgener et al. (1999) [17]. Cracking indexes were calculated according to the following formula.

$$Cracking\ index\ (\%) = (5a + 3b + c) \times 100/200$$

Decay index (%). The decay index of cherry fruit was determined by using the recommended method of Wang et al. 2005 [18]. The percent decay index was calculated by using the following formula:

$$Decay\ index\ (\%) = [(1 \times N1 + 2 \times N2 + 3 \times N3) \times 100/(3 \times N)]$$

Where N is the total number of fruits and N_1, N_2 and N_3 is the number of decay fruits.

Weight Loss (%). Weight loss of the cherry fruit was determined by using the calibrated digital weight balance according to the standard method [18] (Wang et al., 2005). Fruits were weighed after 4 days interval and percent weight loss calculated by using the given formula:

$$Weight\ loss\ (\%) = \frac{(W1 - W2)}{W1} \times 100$$

Sensory Analysis. Both pre and post treated fruits were subjected to sensory evaluation by panel of judges according to Larmond (1997) [19]. The judges were asked to rank the fruit samples from 1(dislike extremely) to 9 (like extremely) as per hedonic scale. Fruits were examined for color, flavor, texture, taste and overall acceptability.

Statistical Analysis. Data regarding cracking index of the pre-harvest $CaCl_2$ treated cherry fruits was analyzed statistically by using one way ANOVA and the rest of the data of both pre and post-harvest treated fruits were analyzed by using Complete Randomized Design with two factors ($CaCl_2$ treatment and storage interval) and the means were compared by LSD test [20].

RESULTS AND DISCUSSION

The main purpose of this research was to study the effects of pre and post-harvest $CaCl_2$ treatments on the shelf life of sweet cherries grown in Gilgit Baltistan - Pakistan. The fresh fruit contain moisture content (81%), pH (4.41), TSS (13.91°Brix), non-reducing sugar (1.54 %), reducing sugar (9.86%), acidity (1.54%) and ascorbic acid (9.51 mg/ 100g). The fruit were also studied for various selected physico-chemical characteristics including, weight loss, decay index and sensory characteristics.

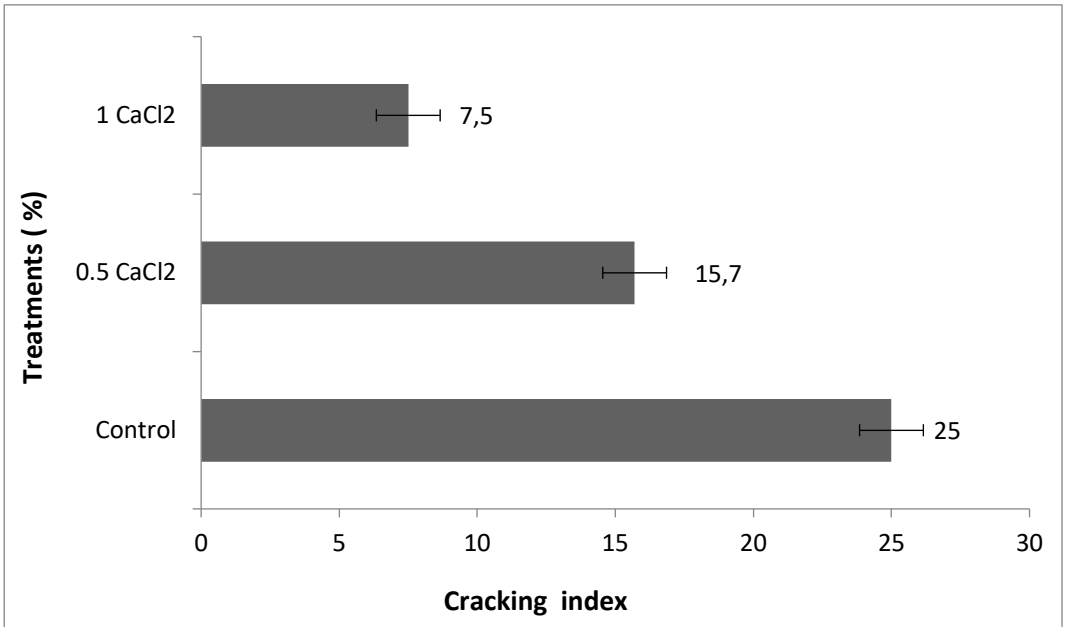


FIGURE 1
Cracking index of pre-harvest 0.5 and 1 % calcium chloride treated sweet cherries.
The horizontal line shows standard error bar of the mean value.

Cracking Index of Pre-harvest treated fruits. Fruit cracking is the major problem that limits the successful production of cherries in most area of the GB attributed to extreme environmental factors. Cherry fruit were late sprayed three times with 0.5 and 1 % calcium chloride solution before harvesting within 10 days of intervals. Previously it was noted that early $CaCl_2$ pre-harvest spray was less effective in increasing calcium content of apple fruit than late spray [21]. This study showed that cracking index declined from 25 % (control) to 7.5 % (1 % $CaCl_2$) (Fig 1). Similarly, it was demonstrated that pre-harvest application of $CaCl_2$ decreased 11 to 33% cracking index in sweet cherry fruit [22]. The pre-harvest application of $CaCl_2$ can also improve the post-harvest shelf life of fruits because Ca delays moisture loss, respiration process and control weight loss during post-harvest storage. It was noticed that pre-harvest application of $CaCl_2$ significantly reduced cracking in cherries which lead to control fungal growth. In some cracked fruits, a visible green mould colonization mainly of *Aspergillus flavus* was observed and similarly, cracked cherries are prone to different storage diseases and have shorter shelf-life [23]. Pre-harvest $CaCl_2$ spray significantly reduced rain-induced cracking in sweet cherries [24]. $CaCl_2$ has the ability to retard the softening of sweet cherries and control cracking index, it may be due to fact that Ca stabilized the membrane, increase integrity and maintain cell wall structure by interacting with pectic acid in cell wall to make calcium pectate [12]. Hence, concluded that the pre-harvest application of $CaCl_2$ can strengthen the peel which subsequently improves the stability of the cherry fruit against the harsh environmental condition before picking. However, it is expected that $CaCl_2$ application would significantly improve the shelf life of the cherry fruits.

Post-harvest quality of treated fruit. As it is evident that pre-harvest treatment with calcium chloride minimized the indices of cracking in cherry fruit, therefore in second experiment fruit were dipped in $CaCl_2$ solution because previous studies showed that post-harvest calcium treatment also extended the shelf life of fresh fruits by maintaining fruits cell turgor, tissues firmness and delayed its membrane lipid catabolism [25, 9]. Therefore, the comparative effect of both pre and post-harvest treatments on the physico-chemical, physiological and sensory aspects of cherry fruits during certain time period of storage was studies.

Physicochemical Analysis. Moisture content. The moisture content of sweet cherry samples was analyzed at every 4 days interval during storage. Initially moisture content of all cherry fruit samples was in the range of 80.10 – 81.75 %, in control and C_{2pre}, respectively, which reduced to 51.08 % (control) to 65.05 % (C_{4post}) (Table 1). Study showed that $CaCl_2$ had significantly ($P \leq 0.05$) reduced moisture loss of cherry fruits as compared to control samples while non-significant difference was observed among treated samples. This is also clear form mean values that cherry fruits with 1% $CaCl_2$ (post treated) retained higher moisture (72.88%). Moisture loss in control samples might be due to its poor skin resistance to water vapor movement, air current, warm temperature, low relative humidity and temperature gradient between the air and fruits [26] (Chen and

Hong, 1992). Conversion of pectin to calcium pectate in pre and post- harvest cherry fruits might have resulted in minimum moisture loss, weight loss, respiration rate and fruits decay [27]. Earlier, it was observed that moisture content might be affected due to the permeability of the fruit skin, which make it vulnerable to quick water loss during storage [28].

Weight loss (%). As it is evident from the data of moisture content that wilting of fruits due to removal of 5-10% moisture content increases weight loss with the passage of time. The statistical data clarified that calcium chloride treatments and storage had significantly ($P \leq 0.05$) influenced the weight loss of sweet cherry fruits in comparison with untreated sample, while both treatments had non-significant effect on fruits in comparison with each other during storage at room temperature (Table 2). Weight loss in cherry fruit noticed on 4th day of storage in all the treatments which is finally increased to 8.21% C_0 and 1.43% in C_{4post}. Previously it was reported that calcium treated samples had lower mean weight losses (2.63 %) as compared to untreated apricot (3.42%) during 70 days of storage [29]. Similarly, fruit treated with 6% $CaCl_2$ comparatively showed lowest weight loss [30]. Calcium salts delay the natural physiological process such as respiration, moisture loss and ripening of the cherry fruits so, calcium treated samples retained higher fruits weight as compared to untreated cherries. Previously minimum weight loss was noted in fruit samples treated with $CaCl_2$ which might have retained tissue rigidity and fruit firmness by lowering the activity of enzymes responsible for breakdown of cellular structure [31, 32]. Further $CaCl_2$ treatment may also contribute in fruits cell functions including cell growth and its division which eventually retain moisture content and minimize weight loss in fruits [33].

Decay index (%). Sweet cherry samples were analysed at every 4 days of intervals and according to expectations untreated cherry fruits significantly ($P \leq 0.05$) decayed up to (19.02%) in comparison with pre (8.65%) and post-harvest (5.10%) $CaCl_2$ treated cherries (Table 3). Earlier calcium treated apple fruit showed had lower (1.42%) decay-index in comparison with untreated apple (9.80%) during storage [34]. Similarly, apple and potato dipped in $CaCl_2$ solution showed negligible post-harvest decay [35]. The results are also in line with the studies carried out on strawberries dipped in $CaCl_2$ solution and stored at 18◦C [23]. Post-harvest

TABLE 1
Effect of pre and post-harvest Calcium chloride treatments and storage period on Moisture content (%) of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	80.10±1.16	74.18±0.71	68.66±0.64	63.34±1.78	57.01±0.98	51.08±1.17	65.62b
$C_{1\ pre}$	81.41±0.80	77.23±1.24	73.03±2.30	68.79±1.15	64.50±1.74	59.42±0.62	70.73a
$C_{2\ pre}$	81.75±1.17	77.76±1.73	73.75±0.57	69.78±1.73	65.79±1.15	61.80±1.74	71.77a
$C_{3\ post}$	80.34±1.03	76.93±1.46	73.52±0.58	70.11±1.32	66.70±1.15	63.29±2.31	71.81a
$C_{4\ post}$	80.69±0.62	77.57±1.76	74.45±1.15	71.33±1.78	68.21±1.02	65.05±1.18	72.88a
Means	80.85a	76.73b	72.68c	68.67d	64.44e	60.12f	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment), values were expressed as ± standard deviation. Means with different letters are significantly different at $P \leq 0.05$.

TABLE 2
Effect of pre and post-harvest Calcium chloride treatments and storage period on Weight loss of sweet cherry fruit

Treatments	Storage Interval (4 days)						Means
	Initial	4	8	12	16	20	
C_0	0±0.00	0.61±0.05	1.43±0.28	2.98±0.58	3.43±0.61	8.21±0.58	2.77a
$C_{1\ pre}$	0±0.00	0.37±0.02	0.55±0.04	0.73±0.05	1.19±0.06	2.41±0.11	0.87b
$C_{2\ pre}$	0±0.00	0.31±0.03	0.49±0.04	0.58±0.04	0.83±0.02	1.89±0.10	0.68b
$C_{3\ post}$	0±0.00	0.27±0.04	0.35±0.03	0.43±0.05	0.71±0.04	1.60±0.11	0.56b
$C_{4\ post}$	0±0.00	0.21±0.04	0.31±0.02	0.38±0.03	0.65±0.03	1.43±0.11	0.49b
Means	0b	0.35b	0.62b	1.02b	1.36b	3.10a	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 3
Effect of pre and post-harvest Calcium chloride treatments and storage period on Decay index of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	0±0.00	0.41±0.11	3.51±0.60	6.31±1.17	11.57±1.15	19.02±0.88	6.80a
$C_{1\ pre}$	0±0.00	0±0.00	1.29±0.59	3.83±0.62	8.76±0.57	11.09±0.58	4.16b
$C_{2\ pre}$	0±0.00	0±0.00	1.25±0.58	3.12±0.57	6.45±1.15	8.65±1.15	3.24c
$C_{3\ post}$	0±0.00	0±0.00	1.20±0.23	2.85±0.58	4.53±0.60	6.87±1.20	2.57d
$C_{4\ post}$	0±0.00	0±0.00	1.17±0.24	2.09±0.57	3.81±1.10	5.10±1.19	2.02d
Means	0d	0.08d	1.68cd	3.64c	7.02b	10.14a	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 4
Effect of pre and post-harvest Calcium chloride and storage period on TSS content (ºBrix) of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	13.90±0.60	14.81±0.58	15.72±1.15	16.63±1.18	17.54±0.57	18.45±1.15	16.17a
$C_{1\ pre}$	13.91±0.60	14.62±1.15	15.32±1.15	16.02±0.58	16.72±1.15	17.42±0.57	15.66b
$C_{2\ pre}$	13.92±1.15	14.60±0.58	15.29±0.62	15.97±1.15	16.65±1.16	17.33±0.58	15.62b
$C_{3\ post}$	13.94±0.57	14.51±1.22	15.08±0.57	15.65±0.57	16.22±0.61	16.79±1.15	15.36bc
$C_{4\ post}$	13.95±0.58	14.42±0.63	14.87±1.15	15.33±0.58	15.70±0.62	16.25±0.60	15.08c
Means	13.92f	14.59e	15.25d	15.92c	16.56b	17.24a	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

treatment of fruits with calcium salts may slow down their respiration rate, maintains fruits tissues stability, lowers the decay rate and microbial growth [36]. Mean values revealed that pre and post-harvest $CaCl_2$ treatments are statistically different from each other. However, $CaCl_2$ levels have significant effect when applied before harvesting although in post-harvest application the $CaCl_2$ levels showed insignificant effect. Regardless the pre and post-harvest treatments $CaCl_2$ prominently reduced the cherry fruits decay. The greater efficacy of pre and post-harvest application of $CaCl_2$ observed in this research work might have directly destroyed the pathogen, and also inhibited the polygalacturonase enzyme activity [37, 38], and indirectly risen the calcium tissue concentrations, which in response stimulated endogenous resistance mechanisms, through the synthesis of phenolic compounds and phytoalexins [39]. Previous studies further, verified that calcium has ability to inhibit fungal growth and infections in fruits by reducing pectinolytic activity of *P. expansum* and *B. cinerea* while calcium application on apple fruit can stop the polygalacturonase activity caused by Glomerella cingulate which eventually reduces the apple decay [38, 40]. In another study it was concluded that calcium-dependent enzymes were directly activated by the influx of Ca_2+ occurring concomitantly with leakage of cell constituents [39].

Total soluble solids (ºBrix). This experiment shows influence of calcium chloride pre and post-harvest treatments on TSS of cherry fruits. Statistical analysis indicated that $CaCl_2$ treatment had significantly ($P \leq 0.05$) affected the TSS of the fruits during storage. It is observed that untreated cherry fruits showed higher increase in TSS 13.90 to 18.54 ºBrix, while minimum increase was recorded in C_{4post} from 13.95 to 16.25 ºBrix during storage of 20 days. Data regarding levels of $CaCl_2$ applied before and after harvesting gave similar results (Table 4). Similarly minimum decrease was noted in TSS of berries dipped in 1 and 2% calcium chloride solution which might be due to the delay in polysaccharide degradation by the formation of $CaCl_2$ protective thin layer on the outer surface of the fruit [41]. Previously it was explained that the enzymatic conversion of polysaccharides such as pectin and starch into simple sugars are responsible for increase in TSS content of fruits but calcium slow down the hydrolysis of polysaccharide into simple sugar so it may be the reason of lower increment in TSS of calcium treated cherries during storage [42].

Percent Acidity. The result showed that the percent acidity of all the samples decreased significantly ($P \leq 0.05$) during storage (Table 5). According to the data initially percent acidity ranged from 1.12 % (control) to 1.19 % (C_{4post}). During 20 days of storage percent acidity of untreated and treated cherry fruits decreased to 0.51 % and 0.89 % (C_{4post}). Similarly decreasing trend in acidity was observed in control and Ca treated strawberries during storage at

18ºC [23] Garcia et al., (1996). Furthermore, mean values showed that untreated samples had higher loss (0.83 %) and C_{4post} showed minimum loss (1.04 %) respectively. Here the data clarifies the role of $CaCl_2$ which might have reduced the utilization of frutaric acids by restricting the respiration and ripening process of cherries [43]. Calcium has the ability to strengthen the fruit skin by forming calcium pectate which might have reduced oxidation and metabolic activities responsible for depletion of organic acids [44, 45, 46]. While $CaCl_2$ treated apricot fruits also retained significantly higher acidity up to 0.74% in comparison with untreated sample 0.65% during ten days of storage [47].

Ascorbic Acid Content (mg/100g). The application of calcium chloride retained the ascorbic acid content of sweet cherries during entire storage period. The mean values showed that ascorbic acid significantly ($P \leq 0.05$) declined from (9.82) to (3mg/100g) in C_0, (5.32 mg/100gm) C_{3pre} and (5.77mg/100gm) C_{4post} during 20 days of storage at room temperature in all the samples (Table 6). Ascorbic acid is highly unstable during oxidation process and it is considered very important nutrient among other fruits quality parameters during storage [48]. However, comparatively pre and post-harvest treatment significantly retained the ascorbic acid content in sweet cherries. While post-harvest treatments insignificantly affected the ascorbic acid content during storage. The higher retention of ascorbic acid in cherry fruits might be due to the application of calcium chloride which reduced internal break down of tissues, minimized the intra and extracellular processes responsible for retaining total acid content and fruit quality [49, 10]. Similarly, higher amount of ascorbic acid in fruits dipped in 9% $CaCl_2$ solution was observed in earlier studies [33], while some other studies also confirmed the existence of vitamin C in calcium treated fruit samples during storage [50, 51].

Sensory Evaluation. Colour. Color is the main attribute of the fruits that attract the consumer and overall acceptability during storage. Sweet cherry fruit samples were observed for color at 4 days interval during storage at room temperature as shown in Table 7. Initially color scores were noticed in range of 8.0 (C_{4post}) to 8.40 (C_{2pre}). The panelists observed degradation in colour of the stored samples in range of 3.70 (C_0) to 7.40 (C_{4post}). It has been observed that untreated cherries suffered from greater moisture loss during storage which in response lessens the bright red color of cherries; while later on darkening occurred may be due to oxidative browning reactions [52]. Statistical analysis showed that treated samples got significantly ($P \leq 0.05$) higher color score than C_0 (6.0). The control of moisture loss due to pre and post-harvest $CaCl_2$ treatment should have minimized the changes in cherry fruit skin color in fully ripe cherries while higher and lower color scores allotted to untreated and treated samples might have been accentuated by the storage temperature. However, this is also indicated from the means values that levels of $CaCl_2$ and their application stage had non-significantly influenced the red color of the sweet cherries. Previously it was reported that the application of 2% calcium chloride retained maximum scores (1.61 to 3.42) as compared to untreated peach fruits samples [53]. These results are also related to a study which showed that calcium treatments increase integrity of cell wall during pectic polymers cross linking in fruits that is why it imparts the color of fruits rather than untreated fruit [13].

TABLE 5
Effect of pre and post-harvest Calcium chloride treatments and storage period on Titratable acidity (%) of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	1.12±0.05	1.08±0.04	0.88±0.02	0.75±0.04	0.64±0.05	0.51±0.03	0.83c
$C_{1\ pre}$	1.14±0.03	1.04±0.02	0.94±0.04	0.85±0.02	0.76±0.04	0.66±0.02	0.89b
$C_{2\ pre}$	1.15±0.03	1.06±0.01	0.97±0.03	0.88±0.04	0.79±0.02	0.70±0.04	0.92b
$C_{3\ post}$	1.17±0.04	1.10±0.03	1.03±0.02	0.96±0.01	0.89±0.05	0.82±0.03	0.99a
$C_{4\ post}$	1.19±0.02	1.13±0.04	1.07±0.03	1.01±0.02	0.95±0.03	0.89±0.05	1.04a
Means	1.15a	1.08b	0.97c	0.89d	0.80e	0.71f	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 6
Effect of pre and post-harvest Calcium chloride treatments and storage period on Ascorbic Acid content (mg/100g) of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	9.81±0.60	8.41±0.61	6.98±0.58	5.34±0.59	4.29±0.58	3.00±0.57	6.30c
$C_{1\ pre}$	9.80±0.58	8.81±0.60	7.82±0.36	6.84±0.58	5.83±0.70	4.85±0.62	7.32b
$C_{2\ pre}$	9.81±0.57	8.91±0.60	8.02±0.58	7.11±0.56	6.21±0.81	5.32±0.57	7.56ab
C_{3post}	9.82±0.61	8.95±0.58	8.08±0.59	7.21±0.57	6.34±0.60	5.47±0.62	7.64a
C_{4post}	9.81±0.57	9.01±0.61	8.20±0.58	7.39±0.60	6.58±0.57	5.77±0.61	7.79a
Means	9.81a	8.81b	7.82c	6.77d	5.85e	4.88f	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 7
Effect of pre and post-harvest Calcium chloride treatments and storage period on Color of sweet cherry fruit

Treatments	Storage Interval (4 days)						Means
	Initial	4	8	12	16	20	
C_0	8.20±0.34	7.50±0.57	6.80±0.58	5.30±1.10	4.60±0.58	3.70±0.60	6.01b
$C_{1\ pre}$	8.30±0.28	8.10±0.51	7.70±0.57	7.40±0.62	7.10±0.58	6.80±0.58	7.55a
$C_{2\ pre}$	8.40±0.34	8.20±0.51	7.80±0.62	7.50±0.62	7.30±0.62	7.00±0.57	7.71a
$C_{3\ post}$	8.20±0.46	8.00±0.54	7.80±0.61	7.60±1.15	7.40±0.60	7.30±0.61	7.72a
$C_{4\ post}$	8.00±0.40	8.10±0.52	7.90±0.58	7.70±0.59	7.60±0.61	7.40±0.57	7.78a
Means	8.22a	7.98ab	7.62abc	7.10bcd	6.80cd	6.42d	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 8
Effect of pre and post-harvest Calcium chloride treatments and storage period on Flavor of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	8.40±0.32	7.50±0.61	6.30±0.57	5.40±0.57	5.00±0.58	3.60±0.61	6.05c
$C_{1\ pre}$	8.30±0.34	7.70±0.57	7.40±0.58	6.60±1.15	6.20±1.15	5.70±0.57	6.98b
$C_{2\ pre}$	8.30±0.17	7.90±0.58	7.60±0.60	7.30±0.58	6.90±0.57	6.60±0.58	7.43ab
$C_{3\ post}$	8.10±0.40	8.00±0.43	7.80±0.58	7.60±0.62	7.40±0.60	7.20±0.13	7.68a
$C_{4\ post}$	8.20±0.28	8.10±0.42	7.90±0.59	7.70±0.57	7.60±0.37	7.50±0.62	7.83a
Means	8.26a	7.84ab	7.40bc	6.92cd	6.64d	6.12d	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

TABLE 9
Effect of pre and post-harvest Calcium chloride treatments and storage period on Texture of sweet cherry fruit

Treatments	Initial	4	8	12	16	20	Means
C_0	8.20±0.28	7.30±0.61	6.40±0.58	5.50±0.58	4.60±0.58	3.70±0.60	5.95c
$C_{1\ pre}$	8.30±0.23	7.70±0.58	7.10±0.60	6.50±0.57	5.90±1.15	5.30±0.59	6.80b
$C_{2\ pre}$	8.40±0.28	8.10±0.57	7.50±0.57	6.90±0.61	6.60±0.58	6.20±0.58	7.28ab
$C_{3\ post}$	8.10±0.24	7.80±0.62	7.50±0.61	7.10±0.57	6.80±1.15	6.40±0.60	7.29ab
$C_{4\ post}$	8.20±0.31	8.00±0.57	7.80±0.58	7.60±0.58	7.40±0.57	7.20±0.61	7.70a
Means	8.24a	7.78ab	7.26bc	6.72cd	6.26de	5.76e	

C_0 (untreated cherry fruit), C_{1pre} (Cherry fruit 0.5% $CaCl_2$ treatment), C_{2pre} (Cherry fruit 1% $CaCl_2$ treatment), C_{3post} (Cherry fruit 0.5% $CaCl_2$ treatment) and C_{4post} (Cherry fruit 1% $CaCl_2$ treatment)

Flavor. Flavor is one of the most important sensory attributes which is responsible for the characteristic aroma and taste of the fruits. Flavor of the sweet cherry samples significantly reduced from 8.40 to 3.60 during 20 days of storage (Table 8). The highest mean value (7.83) was noted in C_{4post} while lowest (6.05) recorded in untreated cherry sample. Mean-while it has been observed that $CaCl_2$ treated samples have slight salty lip feel and bitter taste might be due to residual chlorine remaining on the surface of cherries during 20 days of storage. This hypothesis is confirmed from earlier studies that development of bitterness and salts flavors in food system is associated with the usage of $CaCl_2$ [54, 55].

Our results are in conformity with earlier research findings which clarifies that $CaCl_2$ treatment retained the natural flavor of apple, plum and cherries, may be by providing firmness to the outer skin of fruits which in response slow down the respiration rate and oxidation reaction finally responsible for browning of color and production of off- flavors [49, 56, 57].

Texture. Sound texture of fruits influence the consumer acceptability even the fruit is eye appealing due to its colour. This study showed significant effect of $CaCl_2$ on cherry fruit from 8.40 to (6.20) pre and (7.20) post-harvest treated samples in comparison with untreated cherries (3.70) during 20 days of storage at room temperature. The mean values showed that both treatments impact are statistically in close comity with each other (Table 9). Higher sensory mean scores 6.32 and 3.42 for texture of calcium treated and untreated apricot fruit samples was observed during 10 days of storage [46]. Fruits texture can be retained with calcium treatment because calcium salts maintain the cell membrane integrity and firmness or longer period of time [58]. The higher sensory scores obtained by $CaCl_2$ treated samples clarifies that calcium application retained both external (texture) and internal (flavor) contents, dependent on soluble solids, titratable acidity and their ratio [59, 60, 61].

CONCLUSION

This research was conducted to study the effect of calcium chloride treatments before and after harvesting on sweet cherry fruits. In first experiment fruits were treated before harvesting with $CaCl_2$ solution and studied for cracking index. Results showed that calcium salt application significantly ($P \leq 0.05$) reduced the cracking index by improving the peel strength and integrity. In second experiment both pre and post-harvest $CaCl_2$ treated fruits were examined for selected physico-chemical and sensory characteristics and comparatively post-harvest treated cherry fruits with 1 % $CaCl_2$ showed superior quality.

ACKNOWLEDGEMENTS

The authors are thankful for the support of Director General PCSIR lab Skardu, The Director Agriculture Department of Skardu Gilgit Baltistan and The University of Agriculture Peshawar. All the authors have equal contribution for planning of research work, arrangement of lab facilities, statistical analysis and support to finalize the research goals.

The authors have no conflict of interest.

REFERENCES

[1] Looney, N.E., Webster, A.D., Kupperman, E.M. (1996) Harvest and handling sweet cherries for the fresh market, cherries: Crop physiology, production and uses. CAB Inter. Cambridge, UK, 45, 109-117.

[2] Yilmaz, K.U., Ercisli, S., Zengin, Y., Sengul, M., Kafkas, E.Y. (2009) Preliminary characterization of Cornelian cherry (*Cornus mas* L.) genotypes for their physicochemical properties. Food Chem. 114, 408-412.

[3] Jakcobek, L., Seruga. M., Kosanovic. M., Novak, I. (2007) Anthocyanin content and antioxidant activity of various red fruit juices. Deutsche Lebensmittel Rundschau. 103, 59-64.

[4] Mamani-Matsuda, M., Kauss, T., Al-Kharrat, A., Rambert, J., Fawaz, F., Thiolat, D., Moynet, D., Coves, S., Mossalay, M.D. (2006) Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators. Biochem Pharma. 72(10), 1304-1310.

[5] Agriculture, Statistics of Pakistan. (2015) Department of Agriculture, Gilgit Baltistan, Pakistan.

[6] Poovaiah, B.W., Reddy, A.S.N. (1993) Calcium and signal transduction in plants. Critical Rev in Plant Sci. 12, 185-211.

[7] Tsantili, E., Rouskas, D., Christopoulos, M.V., Stanidis, V., Akrivos, J., Papanikolaou, D. (2007) Effects of two pre-harvest calcium treatments on physiological and quality parameters in 'Vogue' cherries during storage. J. Hort. Sci. Biotech. 82, 657-66.

[8] Poovaiah, B.W., Glenn, G.M., Reddy, A.S.N. (1998) Calcium and fruit softening: Physiology and biochemistry. Hort. Rev. 10, 107-152.

[9] Picchionii, G.A., Watada, A.E., Conway, W.S., Whitaker, B.D., Sams, C.E. (1998) Postharvest calcium infiltration delays membrane lipid catabolism in apple fruit. J. Agri. and Food Chem. 46, 2452-2457.

[10] Conway, W.S., Sams, C.E., Kelman, A. (1989) Enhancing the natural resistance of plant tissues to post-harvest diseases through calcium applications. Hort. Sci. 29, 751-754.

[11] Fallahi, E., Conway, W., Hicky, S.K.D., Sams, C.E. (1997) The role of calcium and nitrogen in postharvest quality and disease resistance of apples. Hort Sci. 32, 831-835.

[12] Meheriuk, M., Neilsen, G.H., Mckenzie, D.L. (1991) Incidence of rain splitting in sweet cherries treated with calcium or coating materials. Canadian J. Plant Sci. 71, 231-234.

[13] White, P.J., Broadley, M.R. (2003) Calcium in plants. J. Annals of Bot. 92, 487-511.

[14] Ippolito, A., Schana, L., Pentimone, I., Nigro, F. (2005) Control of post-harvest applications of sweet cherries by pre and postharvest applications of *Aureobasidiumpullulansn* in combination with calcium chloride or sodium bicarbonate. Postharvest Biol. Tech. 36, 245-252.
[15] Ishaq, S., Ahmed, R., Masud, T., Ali, S. (2009) Influence of post-harvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot (*Prunusarmeniaca* L.) fruit during storage. Pak. J. Nutr. 8, 861-865.
[16] AOAC (2012) Association of Official and Analytical Chemists. Official methods of analysis. Washington D.C.
[17] Bilgener, L., Demirsoy, H. (1999) The effect of vapor gard, GA_3 and calcium hydroxide applications on fruit cracking in sweet cherry. National Horticultural Congress. Turkey, 828-884.
[18] Wang, Y., Tian, S.P., Xu, Y. (2005) Effect of high oxygen concentration on antioxidant enzymes in peach fruits during post-harvest periods. J. Food Chem. 91, 99-104.
[19] Larmond, E. (1977) Laboratory methods of survey evaluation of food publication in Canada. Dept. of Agri. Ottawa, 30, 78-88.
[20] Steel, R.G.D., Torrie, J.H. (1980) Principles and procedures of statistics. Mc. Graw Hill Pub. Co. Inc. New York.
[21] Stahly, E.A. (1986) Time of application of calcium sprays to increase fruit calcium and reduce fruit pitting of apples sprayed with TIBA. Hort Sci. 21, 95-96.
[22] Fernandez, R.T., Flore, J.A. (1998) Intermittent application of calcium chloride to control rain cracking of sweet cherry. Acta Hort. 468, 683-689.
[23] Ono, T., Oyazizu, W., Suzuki, K. (1954) Studies on the reduction of cracking in sweet cherries (1). J. Hortic. Japan. 22, 239-243 (In Japanese).
[24] Long, K.S., Jiwan, S., James, S.B., Pilar, M., (2013) Handbook of Fruits and Fruit Process. Published John Wiley and Sons, Inc. 2, 435-446.
[25] Garcia, J.M., Herrera, S., Morilla, A. (1996) Effects of post-harvest dips in calcium chloride on strawberry. J. Agric and Food Chem. 44, 30-33.
[26] Chen, W.J., Hong, Q.Z. (1992) Studies on senescence and browning of Litchi fruit pericarp during storage. Acta Hortic. Sic. 19(3), 227-232.
[27] Hussain, P.R., Meena, R.S., Dar, M.A., Wani, A.M. (2012) Effect of post-harvest calcium chloride dip treatment and gama irradiation on storage quality and shelf life extension of red delicious apple. J. Food Sci. and Tech. 49, 415-426.
[28] Gonzalo, M., Berna, A., Gonzalez, R., Mulet, A. (2014) The storage of dried apricots: The effect of packaging and temperature on the changes of texture and moisture. J. Food Process Preser. 38, 565-573.
[29] Akhtar, A., Abbasi, N.A., Hussain, A. (2010) Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. Pak. J. Botany. 42(2), 181-186.
[30] Gupta, N., Jawandha, S.K., Gill, P.S. (2011) Effect of calcium on cold storage and post-harvest storage quality of peach. J. Food. Sci. 48, 225-229.
[31] Wani, A.M., Hussain, P.R., Meena, R.S., Dar, M.A. (2008) Effect of gamma irradiation and refrigerated storage on the improvement of quality and shelf life extension of pear fruits. Rad Phy. Chem. 77, 983-989.
[32] Levy, D., Poovaiah, B.W. (1979) Effect of calcium infiltration of senescence of apples. Hortic. Sci. 14, 466.
[33] Johnson, R.S. (2008) Nutrient and water requirements of peach trees. In: Lyne, D.R. and Bassi, D. (eds.) The Peach Botany, Production and Uses. CAB International, 310-311.
[34] Jan, I., Rab, A., Sajid, M. (2013) Effect of calcium chloride on physical characteristics and soft rot incidence on fruit of apple cultivars. The J. Animal and Plant Sci. 23(5), 1354-1359.
[35] Conway, W.S., Sams, C.E. (1983) Calcium infiltration of golden delicious apples and its effect on decay. Phytopath. 73, 1068-1071.
[36] Lara, I., Garcia, P., Venderll, M. (2004) Modifications in cell wall composition after cold storage of calcium treated strawberry fruit. J. Postharvest Biol and Tech. 34, 331-339.
[37] Conway, W.S., Abbott, J.A., Bruton, B.D. (1991) Postharvest calcium treatment of apple fruit to provide broad-spectrum protection against postharvest pathogens. Plant Disease. 75, 620-622.
[38] Droby, S., Wisniewski, M.E., Cohen, L., Weiss, B., Touitou, D., Eilam, Y., Chalutz, E. (1997) Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue, and bio control activity of *Pichia guilliermondii*. Phytopath. 87, 310-315.
[39] Kohle, H., Jblick, W., Poten, F., Blaschek, W., Kauss, H. (1985) Chitosan-elicited cellulose synthesis in soy bean cells as a Ca2+ dependent process. Plant Physiology. 77, 544-551.
[40] Conway, W.S., Greene, G.M., Hickey, K.D. (1987) Effects of pre-harvest and post-harvest calcium treatments of peaches on decay caused by *Monilinia fructicola*. Plant Disease. 71, 1084-1086.
[41] Turmanidze, T., Levan, G., Merab, J., Louise, W. (2016) Effect of calcium chloride treatments on quality characteristics of blackberry, raspberry and strawberry fruits after cold storage. Turkish Journal of Agriculture-Food Science and Technology. 4(12), 1127-1133.
[42] Cordenunsi, B.R., Nascimento, J.R.O., Lajolo, F.M. (2003) Physio-chemical changes related to quality of five strawberry fruit cultivars during cool storage. J. Food Chem. 83, 167-173.

[43] Raese, J.T., Drake, S.R. (1993) Effect of preharvest calcium sprays on apple and pear quality. J. Plant Nutr. 16(9), 1807-1918.
[44] Rahmat, K.V.R., Setty, G.R., Murthy, N.V.N., Saroj, S., Nanjundaswamy, A.M. (1979) Effect of ethephone, benomyl, thiobendazoleans wax on color and shelf life of coorg mandarin (Citrus reticulate Blanco). J. Trop. Sci. 21, 265-272.
[45] Daske, S.R., Spayed, S.E. (1983) Influence of calcium treatment on Golden Delicious apple quality. J. Food. Sci. 48(2), 403-405.
[46] Ding, C.K., Chachin, Y., Hamauzu, Y., Imahori, Y. (1998) Influence of storage temperatures on physiology and quality of loquat fruit. Postharvest Bio and Technol. 14(3), 308-316.
[47] Saira, I., Ahmed, H., Masud, T., Ali, S. (2009) Influence of post-harvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot fruit during storage. Pak. J. Nutr. 8(6), 861-865.
[48] Veltman, R.H., Kho, R.M., Van Schaik, A.C.R., Sanders, M.G., Oosterhaven, J. (2000) Ascorbic acid and tissue browning in pears (*Pyrus communis* L. cvs Rocha and Conference) under controlled atmosphere conditions. Postharvest Biology and Technology. 19(2), 129-137.
[49] Bangerth, F., Dilley, D.R., Dewey, D.H. (1972) Effect of postharvest calcium treatment on internal breakdown and respiration of apple fruits. J. Amer. Soc. Hort. Sci. 97, 679-682.
[50] Ruoyi, K., Zhifang, Y., Zhoxin, L.Z. (2005) Effect of coating and Intermittent warming on enzymes, soluble pectin substances and ascorbic acid of Prunuspersica (cv. Zhonghuashoutao) during refrigerated storage. J. Food Res. Int. 38, 331-336.
[51] Davey, M.W., Montagu, M.V., Inze, D., Sanmarita, M., Fletcher, J. (2000) Plants ascorbic acid, chemistry, function, metabolism, bioavailability and effect of processing. J. Sci. Food Agric. 80, 825-860.
[52] Nunes, M.C.N., Brecht, J.F., Morais, A.M.M.B., Sargent, S.A. (2005) Possible influences of water loss and polyphenol oxidase activity on anthocyanin content and discolouration in fresh ripe strawberry (cv. Oso Grande) during storage at 1ºC. J. Food Sci. 70, S79-S84.
[53] Lysiak, G., Florkowski, W.J.S.E., Prussia, S.E., (2008) Post-harvest calcium chloride application and moisture barrier influence on peach fruit quality. J. Hort Tech. 18(1), 100-106.
[54] Bolin, H.R., Huxsoll, C.C. (1989) Storage stability of minimally processed fruit. J. Food Process and Preser. 13, 281-292.
[55] Ohlsson, T. (1994) Minimal processing-preservation methods of the future-an overview. Trends in Food Sci and Tech. 5, 341-344.
[56] Faust, M. (1978) Evolution of fruit nutrition during the 20th century. J. Horti. Sci. 14, 321-324.
[57] Soliva-Fortuny, R.C., Martin-Belloso, O., (2003) New advances in extending the shelf-life of fresh-cut fruits: a review. Trends in Food Sci. and Tech. 14, 341-353.
[58] Prakash, A., Inthajak, P., Huibregst, H., Caporaso, F., Foley, D.M., (2000) Effect of low dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Chem. 32(2), 314-321.
[59] Shewfelt, R.L. (2009) Measuring quality and maturity. In: Florkowski, W.J., Shewfelt, R.L., Brueckner, B., Prussia, S.E., (eds.) Postharvest Handling: A System Approach. Second ed. Academic Press, Elsevier, Amsterdam, Netherlands. 461-481.
[60] Chen, L., Opara, U.L. (2013a) Texture measurement approaches in fresh and processed foods - a review. Food Res. Int. 51, 823-835.
[61] Chen, L., Opara, U.L., (2013b) Approaches to analysis and modelling texture in fresh and processed foods - a review. J. Food Eng. 119, 497-507.

Received: **17.04.2018**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

Majid S Hashmi
Department of Food Science and Technology,
The University of Agriculture Peshawar,
Pakistan

e-mail: majidsuhail@aup.edu.pk

© by PSP Volume 27 – No. 12B/2018 pages 9706-9716 Fresenius Environmental Bulletin

DEBRIS FLOW SUSCEPTIBILITY MAPPING USING AN IMPROVED INFORMATION VALUE MODEL BASED ON A COMBINED WEIGHTING METHOD FOR JILIN PROVINCE, CHINA

Junjun Chen[1], Chen Cao[1], Shengwu Qin[1,*], Shuaiying Peng[1], Qiang Ma[2], Xu Liu[1], Jianjian Zhai[1]

[1]College of Construction Engineering, Jilin University, Changchun 130026, China
[2]Jilin Province Department of Land & Resources, Changchun, 130000, China

ABSTRACT

The main purpose of this study is to generate a debris flow susceptibility zoning map for Jilin Province, which is in northeast China. An improved information value model (IVM) based on a combined weighting method (IVM-CWM) was proposed as the main susceptibility evaluation method. To begin, a debris flow inventory map of Jilin Province was prepared using a total of 1407 investigated debris flow events that occurred before 2012. The debris flow events were randomly divided into two groups: 70% for model training and 30% for verifying. Ten debris flow influencing factors were initially prepared, and the relativity between these factors and the occurrence of debris flow hazards was analysed by calculating the information value index of each layer using the 70% training data. The weight of each influencing factor was calculated based on a linear combined weighting method (CWM), which itself was based on an analytic hierarchy process (AHP) and entropy weighting method (EWM). Four debris flow susceptibility maps of Jilin Province were generated based on the IVM, IVM based on AHP (IVM-AHP), IVM based on EWM (IVM-EWM), and IVM-CWM. According to the receiver operating characteristic (ROC) curve, the new proposed IVM-CWM was determined to be ideal for this study. The percentage area of very low, low, moderate, high and very high in the optimal susceptibility map are 23.75%, 28.39%, 21.16%, 17.38%, and 8.32%, respectively.

KEYWORDS:
Debris flow, susceptibility mapping, information value model, combined weighting method, Jilin Province

INTRODUCTION

Debris flow is one of the most frequent geological hazards in Jilin Province, which is an agricultural province in Northeast China. With the development of local agriculture and urbanization, the demand for land has increased. Many woodlands have been developed into cultivated land, which has resulted in an increase in loose deposits on the surface. Every year, during the rainy season, large quantities of loose deposits are eroded under the action of rainwater, with some erosion ultimately generating debris flows. This means that the entire area is under a high susceptibility of debris flow hazards. Based on the geological hazards' investigation data supported by the Department of Land & Resources of Jilin Province, there were 1407 debris flow events that have been investigated throughout the entire province, which account for 35.94% of the total geological hazard events in the area. In addition, debris flow hazards in this province caused 48 deaths, destroyed 24968 houses, and led to direct economic losses of approximately 137.6 million dollars. Therefore, a predictive study for debris flow hazards is critical for engineering construction and disaster prevention and reduction.

Geological hazard susceptibility mapping is an efficient and feasible method for predictive disaster studies, which is also suited for debris flow prediction. The occurrence of geological hazards are the outcomes of various internal and external factors. Based on previous studies, the internal factors mainly include the engineering geological condition, hydrogeological condition, geologic structure, vegetation coverage, land-use types and geomorphic types. The external factors mainly include precipitation, earthquake events and human activities [1-4]. All the above factors have different interactions with the occurrence of geological hazards in various regions. The relationships and correlations between influencing factors and geological hazards are quite complicated. Geological hazard susceptibility expresses the likelihood of a geological hazards' occurrence in an area based on the regional geological environment. The generation of susceptibility maps based on field investigations and regional geological data has become a popular approach for urbanization planning.

Currently, geological hazard susceptibility assessments have been widely and quantitatively studied due to the rapid development of mathematical theories and geographic information systems

(GIS) [5]. These evaluation models mainly include logistic regression models [6,7], decision tree algorithms [8, 9], artificial neural network (ANN) models [10-12], the deterministic coefficients model [13, 14], a support vector machine (SVM) model [15-17], cellular automata methods [18-20], and the certainty factor (CF) method [21, 22]. All of the above models performed well in susceptibility modelling studies. However, different methods give different accuracy results for different conditions [23]. For example, the prediction accuracy of ANN is more reliable when the training data is sufficiently large. Additionally, it is difficult to obtain the necessary geological environmental data for those mathematical analysis models. Finally, the accuracy of the methods was also influenced by the scale of a study area [4]. The information value model (IVM), as a statistical data-driven method, is widely applied and highly recommended by researchers for large scale geological hazard susceptibility zonation due to its easy operation [24]. Xu [4] studied the susceptibility of debris flow hazards in Sichuan Province, China, based on the information value model. Che [25] assessed landslide susceptibility in Limbe, SW Cameroon, based on a field calibrated seed cell and information value method. Chen [26] compared the information value model with a logistic regression model for landslide susceptibility mapping. The accuracy validation results showed that the information value model is the more effective method in this area. Zhu [27] made a comparison between the information value model and the weights-of-evidence method for landslide susceptibility mapping. The results demonstrate that the information value model had a higher prediction accuracy. However, the common information value model did not consider factor weights and regards all factors as contributing equally to the development of geological hazards. Hence, the geological hazard susceptibility maps generated based on the common information value model cannot reflect the differences between each influencing factor. To ensure the results are more reliable, some improved information value models based on weighting methods have been proposed. Jiang [28] assessed landslide susceptibility in a 10-degree slope region of the Wenchuan earthquake and used an improved information value model based on analytic hierarchy (AHP). Sharma [29] combined the information value model with an entropy weighting method to assess the susceptibility and zonation in the Sikkim Himalayas in India. However, each of these single weighting analysis methods has its drawbacks. For example, the results calculated using the AHP method were varied among studies. In addition, the entropy weighting method (EWM) is not applicable to studies with a small sample size.

As Jilin Province is a large area, the information value model (IVM) is applicable for its debris flow susceptibility mapping. Additionally, a combined weighting method (CWM) based on AHP and EWM was proposed to improve the IVM. The IVM, IVM based on AHP (IVM-AHP), IVM based on EWM (IVM-EWM) and IVM based on CWM (IVM-CWM) were all adopted for Jilin Province debris flow susceptibility mapping. The accuracy of the above four models were validated according to the receiver operating characteristic (ROC) curves.

STUDY AREA

The study area is in the north-eastern part of China and is roughly bounded by longitudes of 121°35′E and 131°25′E and latitudes of 40°51′N and 46°23′N. This area covers approximately 187,400 km^2.

The overall topography of the study area declined from southeast to northeast, with an altitude of 5.0 m~2691.0 m. The study area is bounded by the Huifahe-Gudonghe deep fault and spans two first-order geological structural units, namely, the Sino-Korean quasi-platform area and the Tianshan-Xingan geosyncline area. The exposed strata are all from the Archaeozoic to Cenozoic Erathem. The lithologies mainly consist of granite, basalt, pyroclastic rocks, slate, phyllite, schist, various kinds of loose deposits, etc.

There are thousands of rivers distributed in Jilin Province, with a river net density of 0.19 km/km^2. All of these rivers belong to five basins, including the Second Songhua River Basin, the Liao River Basin, the Yalu River Basin, the Tumen River Basin, and the Suifenhe River Basin.

The study area has a temperate continental climate: it is hot and rainy in the summer and cold and snowy in winter. The annual mean precipitation is approximately 500-600 mm. More than 60% of the annual precipitation is concentrated in the summer because of monsoons. The precipitation shows a decreasing trend from southeast to northwest, and the area of the most abundant precipitation is in the southeast mountainous area.

Due to its unique geological environment and climatic characteristics, Jilin Province is highly susceptible to geological hazards, and debris flows are one of the most extensive and serious geological hazards. Every year, during the rainy season, which occurs between June and August, hundreds of debris flows occur and threaten roads, farmlands, houses, lives, etc.

DATA COLLECTION

Debris flow Inventory Data. In this study, a database with 1407 total debris flow locations occurring prior to 2012 in Jilin Province was provided by the Department of Land and Resources of Jilin Province. All debris flow locations were represent

ed by point features with geographic coordinates (Figure 1). As shown in Figure 1, the debris flows were mostly distributed in the southeast mountainous areas and some areas in the northwest.

Influencing Factors of Debris Flows. The occurrence of a debris flow hazard requires specific terrain conditions, material conditions and rainfall conditions [1-4]. According to this principle, ten influencing factors were chosen as debris flow susceptibility evaluation indexes, including slope gradient, slope aspect, topographic relief, annual precipitation, vegetation coverage, population density, land types, lithology group, distance from faults and distance from the hydrographic net. The original data used for this study is shown in Table 1.

The slope gradient has a direct effect on debris flow formation. According to previous studies, debris flows have different susceptibilities on different gradient slopes [30-32]. The ability of the slope to accumulate solid, loose mass and initiate a debris flows varies based on gradient. The slope gradient in this study was divided into 5 classes, including 0°-7°, 7°-14°, 14°-21°, 21°-28°, and > 28° (Figure 2).

Slope aspect is another important influencing factor for debris flows [33]. Different slope aspects lead to different water distributions, sunshine, heat, human activities, etc. [34]. In this study, the slope aspect was classified as north (0-22.5° and 337.5°-360°), northeast (22.5°-67.5°), east (67.5°-112.5°), southeast (112.5°-157.5°), south (157.5°-202.5°), southwest (202.5°-247.5°), west (247.5°-292.5°), and northwest (292.5°-337.5°) (Figure 2).

Topographic relief reflects the micro-geomorphology characteristics of the study area. It determines whether the loose deposits can migrate smoothly and form destructive disasters [35,36]. The value of the topography in the study area varies from 0-206 m per 30 m×30 m area. In addition, topography was divided into five classes, including 0-10 m, 10-26 m, 26-44 m, 44-68 m and >68 m (Figure 2).

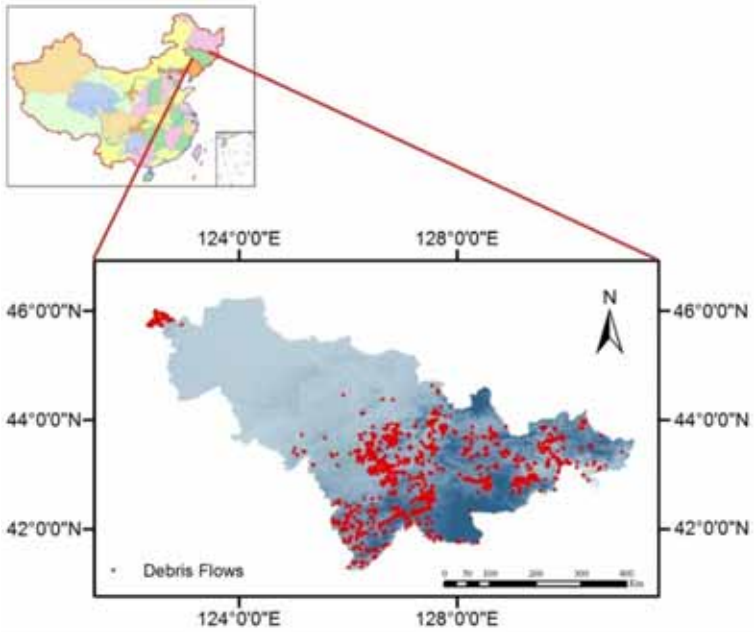


FIGURE 1
Topographic map of Jilin showing the location of research area. Points indicate debris flow locations before 2010.

TABLE 1
A list of data sources of each influencing factor

Influence factor	Data sources
slope gradient	Generated from Digital Elevation Model (30 m×30 m) based on GIS platform
slope aspect	
topographic relief	
annual precipitation	Annual precipitation distribution map in a scale of 1:500,000
vegetation coverage	Raster data (50 m×50 m) of China Vegetation Coverage
population density	Raster data (50 m×50 m) of China Population Density Distribution
land types	GlobeLand 30-2010
lithology group	Extract from the Geological map of Jilin province on a scale of 1:500,000.
distance from faults	
distance from hydrographic net	Raster data (50 m×50 m) of hydrographic net in Jilin Province

Rainfall is one of the major initiating factors of debris flows [37, 38]. Most of the debris flow hazards in the study area occurred during the rainy seasons. Therefore, rain is of great significance for debris flow susceptibility analysis. The historical debris flow hazards in this study resulted from multiple occurrences of rainfall, so it is better to use the annual precipitation as the rainfall factor. The annual precipitation was classified into six classes: 0-400 mm, 400-500 mm, 500-600 mm, 600-700 mm, 700-800 mm, and >800 mm (Figure 2).

Vegetation coverage plays an important role in controlling the development and occurrence of debris flows [39]. Plant roots can consolidate soil and rocks, which increases the external forces for the initiation of debris flows. The vegetation coverage was classified into four classes: very high, high, moderate and low (Figure 2).

Population density reflects the intensity of human activities, including plant destruction, mine exploitation, and unreasonable stacking of waste stones and soil. These activities make an area more prone debris flow hazard, either directly or indirectly [40, 41]. The population density was grouped into five classes in this study: very high, high, moderate, low, and very low (Figure 2).

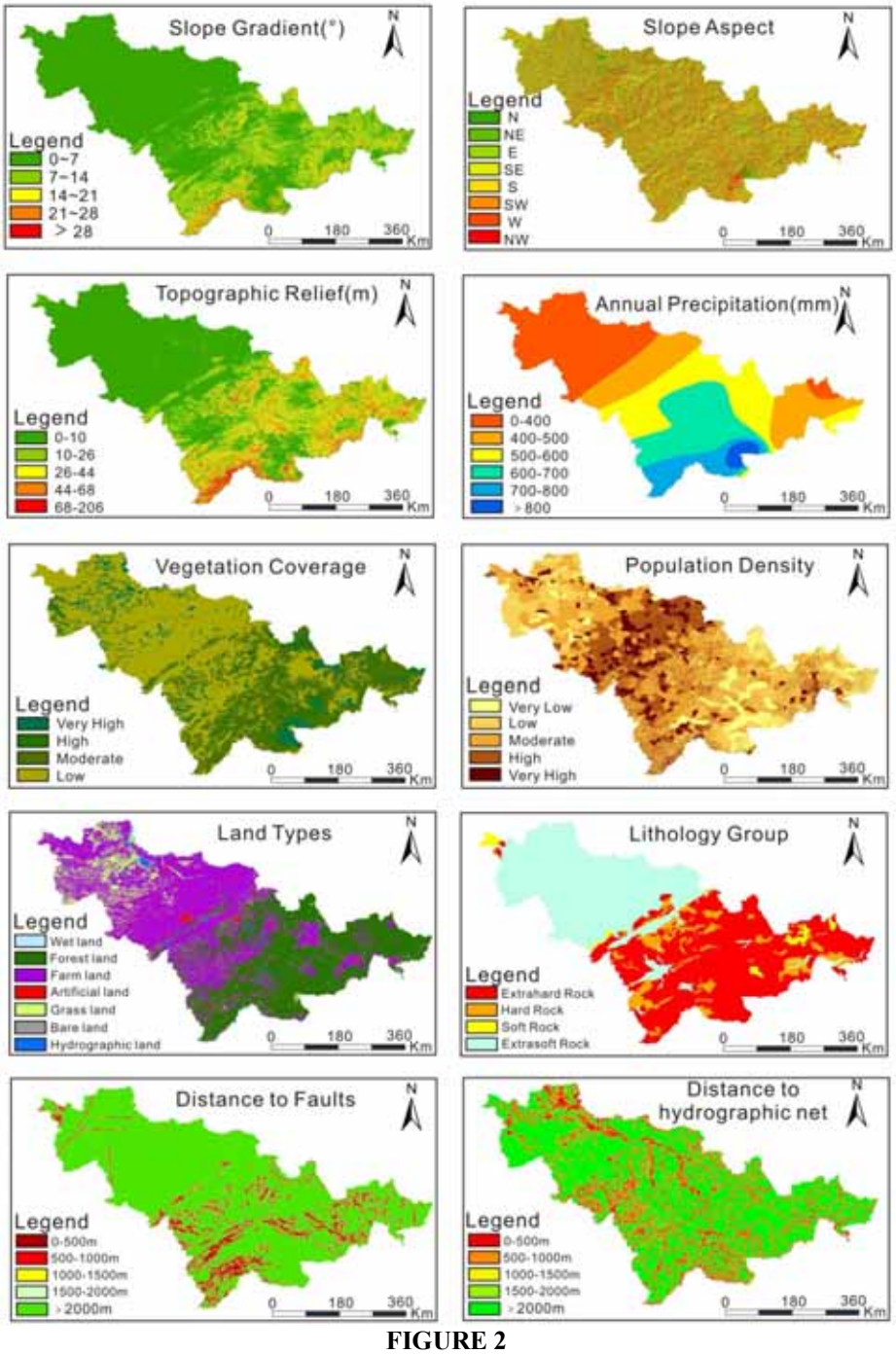


FIGURE 2
Debris flow influencing factors of the study area

The susceptibility of an area to experience a debris flow is also based on land types [42]. Different types of land provide different types and volumes of loose deposits, and these areas can vary dramatically in their initial conditions. There are seven types of land in Jilin Province, including hydrographic land, forest land, wet land, artificial land, grass land, bare land and farmland (Figure 2).

The lithology determines the main material sources for debris flows. Debris flow hazards are more likely to occur where the lithology is easily weathered, which is because the weathering crust on the rock surface is easily eroded under the action of external forces. This leads to an increased presence of loose deposits [43]. The lithology in this study was divided into four groups according to the engineering geology dividing principle: extra-hard rock, hard rock, soft rock and extra-soft rock (Figure 2).

The geological structure is an important factor controlling the regional stability. In the fault developed areas, the rocks were always broken, and the bottom of the valley had accumulated massive amounts of loose deposits. Therefore, it is necessary to consider faults as an influencing factor in debris flow susceptibility [39,44]. This influencing factor was divided into five classes according to the distance from faults: 0-500 m, 500-1000 m, 1000-1500 m, 1500-2000 m, and >2000 m (Figure 2).

Distance to the hydrographic net is also important for the development of debris flows [45]. Erosion occurs due to water fluctuations, and loose deposits closer to the river or lake have lower consolidation degrees. In this paper, the distance to the hydrographic net was classified as follows: 0-500 m, 500-1000 m, 1000-1500 m, 1500-2000 m, and >2000 m (Figure 2).

METHODS

In this study, a grid cell of 30 m×30 m was set as the study unit. Approximately 70% of the total debris flow points were randomly selected for model training. The remaining 30% were used for model validation. The four models, i.e., the IVM, IVM-AHP, IVM-EWM, and IVM-CWM were used to assess the susceptibility of debris flows in Jilin Province. Finally, the calculated results were divided into five classes, very low, low, moderate, high, and very high, based on the principle of Jenks natural breaks optimization.

Information Value Model (IVM). The information value model is a widely used assessment method based on the theory of statistics [4]. In this model, the information value for each influencing factor was calculated based on historical debris flow hazards. Finally, the possibility of a debris flow hazard occurrence can be obtained from the total information value. The information value $I(x_i, H)$ for each influencing factor x_i (i=1,2,…,n) can be calculated by the following formula:

$$I\left(x_i, H\right) = \ln \frac{N_i / N}{S_i / S} \quad (1)$$

where S is the total amount of pixels in for the study area, N is the total number of debris flows used for model training, S_i is the pixels' amount of influence on factor x_i in the study area, and N_i is the number of debris flows under the condition of factor x_i.

The total information value of the study area can be obtained by a weighted sum of each influencing factor.

$$I_w = \sum_{i=1}^{n} w_i I_i = \sum_{i=1}^{n} w_i \ln \frac{N_i / N}{S_i / S} \quad (2)$$

where w_i (i=1,2,…,n) is the weight of each influencing factor. In the common information value model, $w_1=w_2=\ldots w_n$.

Weight analysis method. To find the reliable weight values (w_i) in Equation (2), a combined weighting analysis method based on an analytic hierarchy process (AHP) and entropy weighting method (EWM) was proposed. The AHP is a multiple criteria decision-making method based on expert experience [46], while the EWM is based on objective reality [47]. A combined weight method can balance expert experience and objective laws.

In this paper, the combined weights were determined by the linear combination rule, which can be expressed as follows:

$$W^C=\alpha W^A+\beta W^E \quad (3)$$

where the weight distribution coefficients α and β are satisfied by $\alpha+\beta=1$.

To ensure that the differences between W^A and W^E are consistent with the difference between α and β, the exact values of α and β can be obtained by constructing the distance function equation:

$$d\left(w_i^A, w_i^E\right) = \left[\frac{1}{2}\sum_{i=1}^{n}\left(w_i^A - w_i^E\right)^2\right]^{\frac{1}{2}} \quad (4)$$

Differences between the distribution coefficients can be expressed as follows:

$$D=|\alpha-\beta| \quad (5)$$

Hence, the value of α and β can obtained by solving the following equations:

$$\begin{cases} d\left(w_i^A, w_i^E\right) = \left(\alpha-\beta\right)^2 \\ \alpha+\beta = 1 \end{cases} \quad (6)$$

For the methodology of AHP and EWM, refer to reference [28, 29].

RESULTS

Based on the evaluation index of the debris flow disaster in Jilin Province and the methodology proposed in this paper, this study mainly includes the following four steps:

(1) Calculation of the information value for each influencing factor layer;

(2) Weight analysis for each debris flow influencing factor;

(3) Debris flow susceptibility mapping;

(4) Results validation.

Calculation of Information Value. Using the randomly selected 70% debris flow points, the information value of each class of debris flow influencing factors were calculated based on the IVM theory introduced in the METHODOLOGY chapter, as well as the spatial analysis function of ArcGIS. The results are shown in Table 2.

TABLE 2
Information values of debris flow influencing factors

Factors	Classed	Si/S (%)	Ni/N (%)	Information Value
Slope Gradient	0-7°	70.87%	70.25%	-0.008786
	7°-14°	17.48%	21.02%	0.184282
	14°-21°	8.65%	7.01%	-0.210667
	21°-28°	2.41%	0.91%	-0.971636
	>28°	0.59%	0.81%	0.326804
Slope Aspect	N	19.38%	16.14%	-0.182588
	NE	11.66%	13.71%	0.162058
	E	9.98%	11.07%	0.103282
	SE	11.82%	15.03%	0.239939
	S	13.87%	17.97%	0.258787
	SW	10.79%	9.14%	-0.165823
	W	9.83%	8.12%	-0.190875
	NW	12.68%	8.83%	-0.361724
Topographic Relief	0-10 m	57.63%	26.96%	-0.759891
	10 m-26 m	17.72%	26.32%	0.395387
	26 m-44 m	14.41%	11.45%	-0.229870
	44 m-68 m	8.16%	4.20%	-0.664886
	>68 m	2.08%	1.14%	-0.601461
Annual Precipitation	0-400 mm	29.35%	6.40%	-1.522764
	400 mm-500 mm	20.53%	9.67%	-0.752701
	500 mm-600 mm	22.25%	12.30%	-0.592464
	600 mm-700 mm	18.75%	32.43%	0.547952
	700 mm-800 mm	7.35%	9.17%	0.221501
	>800 mm	1.76%	0.07%	-3.211337
Vegetation Coverage	Very High	8.62%	0.92%	-2.232760
	High	4.00%	0.43%	-2.238074
	Moderate	31.09%	22.19%	-0.337152
	Low	56.29%	46.51%	-0.190720
Population Density	Very High	10.39%	1.00%	-2.344840
	High	24.73%	14.44%	-0.538261
	Moderate	32.98%	33.07%	0.002822
	Low	22.48%	15.15%	-0.394853
	Very Low	9.42%	6.40%	-0.385988
Land Types	Hydrographic land	1.20%	0.57%	-0.743263
	Forest Land	38.54%	10.24%	-1.325193
	Wet Land	1.00%	1.00%	-0.009029
	Artificial Land	3.47%	5.41%	0.442048
	Grass Land	8.85%	2.99%	-1.086241
	Bare Land	4.70%	3.77%	-0.220912
	Farmland	42.23%	46.09%	0.087362
Lithology Group	Extra-hard Rock	45.97%	43.95%	0.317698
	Hard Rock	9.09%	14.51%	0.856514
	Soft Rock	1.86%	8.75%	1.857930
	Extra-Soft Rock	43.08%	2.84%	-2.514515
Distance to Faults	0-500 m	5.64%	10.31%	0.604218
	500 m-1000 m	5.10%	7.61%	0.400324
	1000 m-1500 m	4.59%	6.83%	0.397386
	1500 m-2000 m	4.21%	5.26%	0.224325
	>2000 m	80.47%	40.04%	-0.697938
Distance to Hydrographic Net	0-500 m	13.56%	18.71%	0.321338
	500 m-1000 m	10.00%	12.45%	0.218981
	1000 m-1500 m	9.23%	8.04%	-0.138580
	1500 m-2000 m	8.34%	7.04%	-0.169703
	>2000 m	58.86%	53.77%	-0.090473

Weight Analysis Results. The weights of each influencing factor are calculated based on AHP and EWM, shown in Table 5. Therefore, the coefficients α and β were calculated according to Equation (6) whose results are α=0.402 and β=0.598. Therefore, the combined weights can be obtained according to Equation (3); the results are shown in Table 3.

Debris Flow Susceptibility Mapping. According to the result of IV and the weights analysed above, four debris flow susceptibility maps were generated based on IVM, IVM-AHP, IVM-EWM and IVM-CWM. Each was divided into five classes, very low, low, moderate, high, and very high, based on the principle of Jenks natural breaks optimization (Figure 3).

Discussion and Results Validation. According to IV shown in Table 4, the following combination of debris flow influencing factors had the largest total IV and contributed the most to the occurrence of debris flows: the south slope aspect, >28° slope gradient, 10-26 m topographic relief, 600-700 mm mean annual precipitation, low vegetation coverage, moderate population density, artificial land, soft rock group, <500 m distance from the faults, and <500 m distance from the hydrographic net. The general distributions of susceptibility values were similar, which is shown in the four resulting maps. In addition, the statistical results of the areas of each class in the four maps are demonstrated in Figure 4. As shown in Figure 4, the low-class areas account for the largest percentage in all four maps, which is followed by the moderate classes, except for the map generated by IVM, in which the high areas are slightly larger than the moderate areas. The areas of very high class were almost the smallest, except for IVM, which was slightly larger than the very low areas. The percentage of high-class areas for IVM's was 24.95%, which was significantly different from the other methods' results. The percentage of very low class in each of the debris flow susceptibility maps were quite different. Most areas in Jilin Province were in classes of low-moderate debris flow susceptibility.

TABLE 3
Weight analysis results based on AHP, EWM, and CWM

Factors	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
AHP	0.020	0.056	0.073	0.168	0.226	0.030	0.073	0.097	0.229	0.029
EWM	0.172	0.119	0.128	0.071	0.139	0.047	0.017	0.067	0.108	0.113
CWM	0.111	0.094	0.106	0.110	0.174	0.040	0.039	0.079	0.156	0.091

Notes: X_1: slope aspect; X_2: slope gradient; X_3: topographic relief; X_4: annual precipitation; X_5: lithology group; X6: population density; X_7: vegetation coverage; X_8: land types; X_9: distance from faults; X_{10}: distance from hydrographic net.

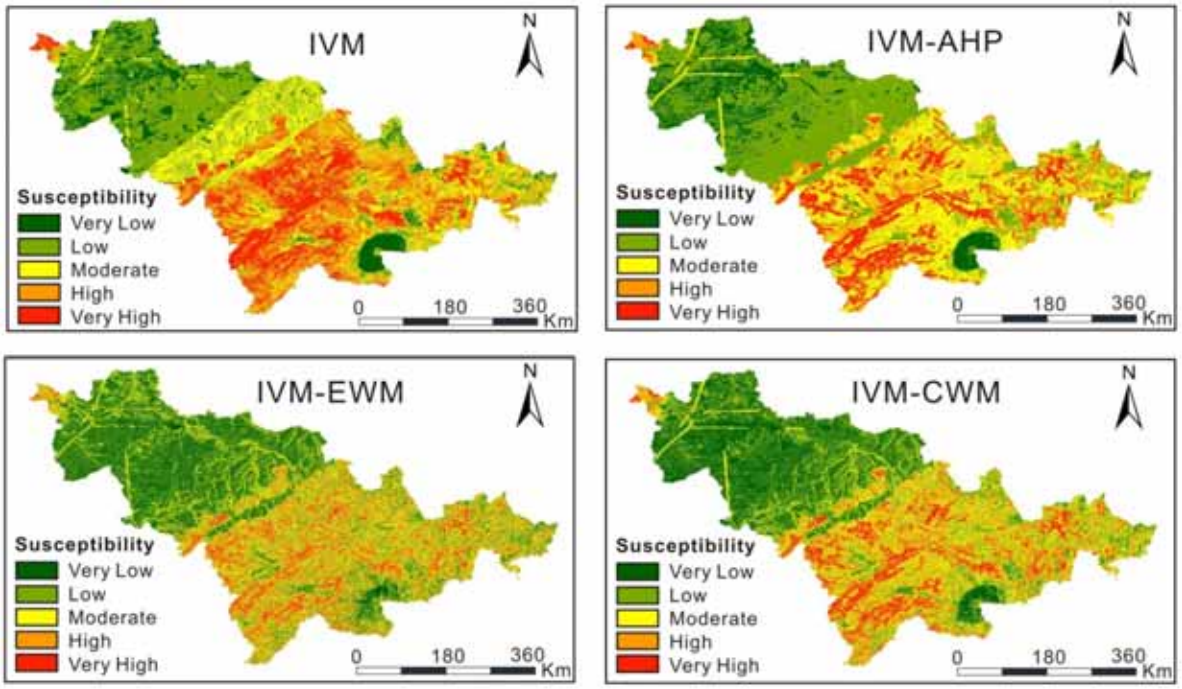


FIGURE 3
The debris flows susceptibility maps based on IVM, IVM-AHP, IVM-EWM and IVM-CWM

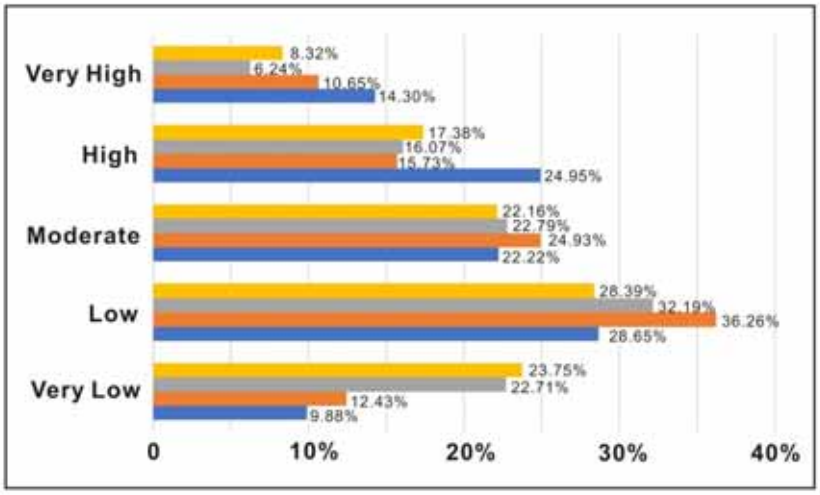


FIGURE 4
Columnar statistical graph for the percentage of each classes' areas in the four susceptibility maps

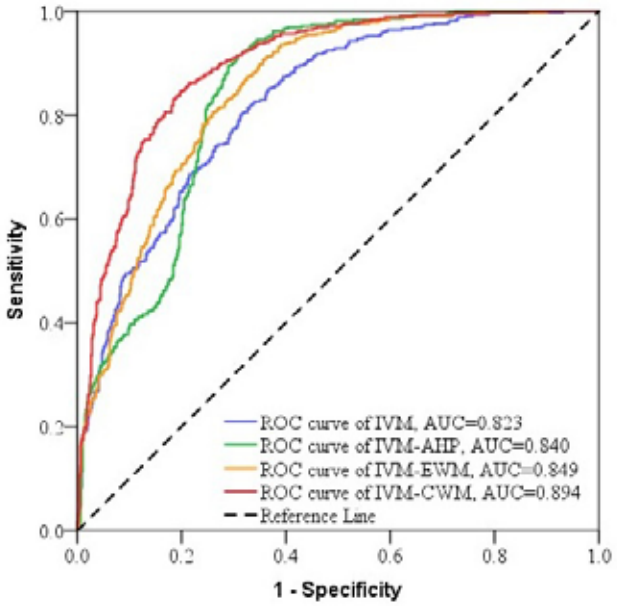


FIGURE 5
The ROC curve of the four models: (Generated using the 30% debris flow validation group)

To validate the accuracy of the four above-mentioned models and to determine the most reliable debris flow susceptibility map of Jilin Province, the receiver operating characteristic (ROC) curve was selected, which was widely used for assessing model validation. The accuracy of above maps and prediction ability of the four models could be assessed by comparing their prediction rates [25].

In this study, the remaining 30% (422) of the total debris flow hazards points were used for generating the ROC curves (Figure 5). The prediction rate value was obtained by calculating the area under the ROC curve (AUC). As shown in Figure 5, the prediction rates of IVM, IVM-AHP, IVM-EWM and IVM-CWM were 0.823, 0.840, 0.849 and 0.894, respectively. Therefore, the IVM improved by the CWM (IVM-CWM) has the optimal effect for Jilin Province debris flow susceptibility map among these methods. Its corresponding map is the most reliable for debris flow hazard prevention and mitigation of Jilin Province.

CONCLUSIONS

In this paper, an improved information value model for Jilin Province was proposed, which is based on the AHP and entropy combined weighting method for debris flow susceptibility analysis. Taking the slope aspect, slope gradient, topographic relief, mean annual precipitation, vegetation coverage, population density, land types, lithology group, distance from faults and distance from the hydrographic net as debris flow influencing factors, the

debris flow susceptibility maps of the study area were generated based on four models, i.e., IVM, IVM-AHP, IVM-EM, and IVM-CWM.

The four resulting susceptibility maps illustrated that the high susceptibility areas were mainly distributed in the southeast mountainous area of Jilin Province, whereas the low susceptibility areas were concentrated in the north-western plain areas. According to the prediction rate obtained from the AUC, all four models had high accuracy in the susceptibility analysis of debris flows in Jilin Province. In addition, the proposed IVM-CWM had the best effect for debris flow susceptibility mapping in this area. The percentage area of very low, low, moderate, high and very high in the optimal susceptibility map were 23.75%, 28.39%, 21.16%, 17.38%, and 8.32%, respectively. IVM-CWM obtained the weights of each of the influencing factors through a combination of AHP and entropy weighting methods. This method well-balanced the advantages and disadvantages of the subjective and objective factors.

However, in the newly improved IVM-CWM, the classification of each influencing factor was based on the principle of equal division, and the experience of researchers may not be the most reasonable. Therefore, an objective and reasonable factor classification method should be studied in the future. The seismic belt should be considered an influencing factor if there are sufficient relative data. The debris flow susceptibility based on the basin unit in this case is worth studying in the future. Furthermore, the safety area should be taken into consideration for model training to ensure the results are more reliable.

ACKNOWLEDGEMENTS

This study was supported by the Jilin Provincial Science and Technology Department (Grants 20170101001JC); the National Natural Science Foundation of China (Grants 41202197); Key Projects of the National Natural Science Foundation of China (Grants 41330636); China Postdoctoral Science Foundation Funded Project (Project No.2017M621212); and China Geological Survey: Comprehensive Geological Survey of Lanzhou-Xining Economic Zone (Grant No.DD20160262). Thanks to the anonymous reviewers for their valuable feedback on the manuscript.

REFERENCES

[1] Wilford, D.J., Sakals, M.E., Innes, J.L., Sidle, R.C. and Bergerud, W.A. (2004) Recognition of debris flow, debris flood and flood hazard through watershed morphometrics. Landslides. 1(1), 61-66.

[2] Cui, P., Zou, Q., Xiang, L.Z. and Zeng, C. (2013) Risk assessment of simultaneous debris flows in mountain townships. Progress in Physical Geography. 37(4), 516-542.

[3] Kazakova, E., Lobkina, V., Gensiorovskiy, Y. and Zhiruev, S. (2017) Large-scale assessment of avalanche and debris flow hazards in the Sakhalin region, Russian Federation. Natural Hazards. 88(S1), 237-251.

[4] Xu, W., Yu, W., Jing, S., Zhang, G. and Huang, J. (2013) Debris flow susceptibility assessment by GIS and information value model in a large-scale region, Sichuan Province (China). Natural Hazards. 65(3), 1379-1392.

[5] Chen, X., Chen, H., You, Y., Chen, X.and Liu, J. (2016) Weights-of-evidence method based on GIS for assessing susceptibility to debris flows in Kangding County, Sichuan Province, China. Environmental Earth Sciences. 75(1), 70.

[6] Lee, S. (2005) Application of logistic regression model and its validation for landslide susceptibility mapping using GIS and remote sensing data. International Journal of Remote Sensing. 26(7), 1477-1491.

[7] Hadji, R., Rais, K., Gadri, L., Chouabi, A.and Hamed, Y. (2017) Slope Failure Characteristics and Slope Movement Susceptibility Assessment Using GIS in a Medium Scale: A Case Study from Ouled Driss and Machroha Municipalities, Northeast Algeria. Arabian Journal for Science and Engineering. 42(1), 281-300.

[8] Yeon, Y.K., Han, J.G., Ryu and K.H. (2010) Landslide susceptibility mapping in Injae, Korea, using a decision tree. Engineering Geology. 116(3–4), 274-283.

[9] Park, I. and Lee, S. (2014) Spatial prediction of landslide susceptibility using a decision tree approach: a case study of the Pyeongchang area, Korea. International Journal of Remote Sensing. 35(16), 6089-6112.

[10] Lee, S., Ryu, J.H., Lee, M.J. and Won, J.S. (2003) Use of an artificial neural network for analysis of the susceptibility to landslides at Boun, Korea. Environmental Geology. 44(7), 820-833.

[11] Lee, S., Ryu, J.H., Min, K. and Won, J.S. (2003) Landslide susceptibility analysis using GIS and artificial neural network. Earth Surface Processes and Landforms. 28(12), 1361-1376.

[12] Lee, S. and Evangelista, D.G. (2006) Earthquake-induced landslide-susceptibility mapping using an artificial neural network. Natural Hazards and Earth System Sciences. 6(5), 687-695.

[13] Jia, N., Mitani, Y., Xie, M. and Djamaluddin, I. (2012) Shallow landslide hazard assessment using a three-dimensional deterministic model in a mountainous area. Computers and Geotechnics. 45(3), 1-10.
[14] Liu, B., Yin, K. (2012) GIS-based Landslide Susceptibility Mapping Using Infinite Slope Model for the New Site of Badong County, China. Disaster Advances. 5(4), 1552-1557.
[15] Pourghasemi, H.R., Jirandeh, A.G., Pradhan, B., Chong, X.U. and Gokceoglu, C. (2013) Landslide susceptibility mapping using support vector machine and GIS at the Golestan Province, Iran. Journal of Earth System Science. 122(2), 349-369.
[16] Feizizadeh, B., Roodposhti, M.S., Blaschke, T. and Aryal, J. (2017) Comparing GIS-based support vector machine kernel functions for landslide susceptibility mapping. Arabian Journal of Geosciences. 10(5), 122.
[17] Lee, S., Hong, S.M. and Jung, H.S. (2017) A Support Vector Machine for Landslide Susceptibility Mapping in Gangwon Province, Korea. Sustainability. 9(1), 48.
[18] Iovine, G., Gregorio, S.D. and Lupiano, V. (2003) Debris-flow susceptibility assessment through cellular automata modeling: an example from 15–16 December 1999 disaster at Cervinara and San Martino Valle Caudina (Campania, southern Italy). Natural Hazards and Earth System Sciences. 3(5), 457-468.
[19] D'Ambrosio, D., Gregorio, S.D. and Iovine, G. (2013) Simulating debris flows through a hexagonal cellular automata model: SCIDDICA S3–hex. Natural Hazards and Earth System Sciences. 3(6), 545-559.
[20] Sakieh, Y., Salmanmahiny, A. and Mirkarimi, S.H. (2017) Tailoring a non-path-dependent model for environmental risk management and polycentric urban land-use planning. Environmental Monitoring and Assessment. 189(2), 91.
[21] Devkota, K.C., Regmi, A.D., Pourghasemi, H.R., Yoshida, K., Pradhan, B., Ryu, I.C., Dhital, M.R. and Althuwaynee, O.F. (2013) Landslide susceptibility mapping using certainty factor, index of entropy and logistic regression models in GIS and their comparison at Mugling–Narayanghat road section in Nepal Himalaya. Natural Hazards. 65(1), 135-165.
[22] Wang, Q., Wang, Y., Niu, R. and Peng, L. (2017) Integration of Information Theory, K-Means Cluster Analysis and the Logistic Regression Model for Landslide Susceptibility Mapping in the Three Gorges Area, China. Remote Sensing. 9(9), 938.
[23] Pourghasemi, H.R. and Rahmati, O. (2018) Prediction of the landslide susceptibility: Which algorithm, which precision? Catena. 162, 177-192.
[24] Ba, Q., Chen, Y., Deng, S., Wu, Q., Yang, J., Zhang, J. (2017) An Improved Information Value Model Based on Gray Clustering for Landslide Susceptibility Mapping. ISPRS International Journal of Geo-Information. 6(1), 18.
[25] Che, V.B., Kervyn, M., Suh, C.E., Fontijn, K., Ernst, G.G.J., Marmol, M.A.D., Trefois, P. and Jacobs, P. (2012) Landslide susceptibility assessment in Limbe (SW Cameroon): A field calibrated seed cell and information value method. Catena. 92(1), 83-98.
[26] Chen, T., Niu, R. and Jia, X. (2016) A comparison of information value and logistic regression models in landslide susceptibility mapping by using GIS. Environmental Earth Sciences. 75(10), 1-16.
[27] Zhu, C. and Wang, X. (2009) Landslide susceptibility mapping: A comparison of information and weights-of-evidence methods in Three Gorges Area. In: International Conference on Environmental Science and Information Application Technology. 2009, 342-346.
[28] Jiang, L., Liu, D., Jiang, Y. and Fang, H. (2014) Landside susceptibility assessment based on weighted information value model: A case study of Wenchuan earthquake 10 degree region. In: International Conference on Agro-Geoinformatics. 2014, 1-4.
[29] Sharma, L.P., Patel, N., Ghose, M.K. and Debnath, P. (2015) Development and application of Shannon's entropy integrated information value model for landslide susceptibility assessment and zonation in Sikkim Himalayas in India. Natural Hazards. 75(2), 1555-1576.
[30] Blaisstevens, A. and Behnia, P. (2016) Debris flow susceptibility mapping using a qualitative heuristic method and Flow-R along the Yukon Alaska Highway Corridor, Canada. Natural Hazards and Earth System Sciences. 3(5), 3509-3541.
[31] Horton, P., Jaboyedoff, M., Rudaz, B. and Zimmermann, M. (2013) Flow-R, a model for susceptibility mapping of debris flows and other gravitational hazards at a regional scale. Natural Hazards and Earth System Sciences. 13(4), 869-885.
[32] Rogelis, M.C. and Werner, M. (2014) Regional debris flow susceptibility analysis in mountainous peri-urban areas through morphometric and land cover indicators. Natural Hazards and Earth System Sciences. 14(11), 3043-3064.
[33] Wang, J., Yan, Y.U., Yang, S., Gui-Hong, L.U. and Guo-Qiang, O.U. (2014) A Modified Certainty Coefficient Method(M-CF) for Debris Flow Susceptibility Assessment: A Case Study for the Wenchuan Earthquake Meizoseismal Areas. Journal of Mountain Science. 11(5), 1286-1297.

[34] Cao, C., Wang, Q., Chen, J., Ruan, Y., Zheng, L., Song, S. and Niu, C. (2016) Landslide Susceptibility Mapping in Vertical Distribution Law of Precipitation Area: Case of the Xulong Hydropower Station Reservoir, Southwestern China. Water. 8(7), 270.
[35] Dibiase, R.A. and Whipple, K.X. (2011) The influence of erosion thresholds and runoff variability on the relationships among topography, climate, and erosion rate. Journal of Geophysical Research Earth Surface. 116, F04036-F04052.
[36] Stock, J. and Dietrich, W.E. (2003) Valley incision by debris flows: Evidence of a topographic signature. Water Resources Research. 39(4), ESG 25.
[37] Berenguer, M., Semperetorres, D. and Hürlimann, M. (2015) Debris-flow hazard assessment at regional scale by combining susceptibility mapping and radar rainfall. Natural Hazards and Earth System Sciences. 15(3), 587-602.
[38] Stancanelli, L.M., Peres, D.J., Cancelliere, A. and Foti, E. (2017) A combined triggering-propagation modeling approach for the assessment of rainfall induced debris flow susceptibility. Journal of Hydrology. 550, 130-143.
[39] Cama, M., Lombardo, L., Conoscenti, C. and Rotigliano, E. (2017) Improving transferability strategies for debris flow susceptibility assessment: Application to the Saponara and Itala catchments (Messina, Italy). Geomorphology. 288, 52-65.
[40] Hereford, R., Thompson, K.S., Burke, K.J. and Fairley, H.C. (1996) Tributary debris fans and the late Holocene alluvial chronology of the Colorado River, eastern Grand Canyon, Arizona. Geological Society of America Bulletin. 108(1), 3-19.
[41] Ni, H.Y. (2015) Experimental study on initiation of gully-type debris flow based on artificial rainfall and channel runoff. Environmental Earth Sciences. 73(10), 6213-6227.
[42] Cheng, J.D., Huang, Y.C., Wu, H.L., Yeh, J.L. and Chang, C.H. (2005) Hydrometeorological and landuse attributes of debris flows and debris floods during typhoon Toraji, July 29–30, 2001 in central Taiwan. Journal of Hydrology. 306(1–4), 161-173.
[43] Carrara, A., Crosta, G. and Frattini, P. (2008) Comparing models of debris-flow susceptibility in the alpine environment. Geomorphology. 94(3–4), 353-378 (2008).
[44] Li, Y., Wang, H., Chen, J. and Shang, Y. (2017) Debris Flow Susceptibility Assessment in the Wudongde Dam Area, China Based on Rock Engineering System and Fuzzy C-Means Algorithm. Water. 9(9), 669.
[45] Wang, Q., Kong, Y., Zhang, W., Chen, J., Xu, P., Li, H., Xue, Y., Yuan, X., Zhan, J. and Zhu, Y. (2016) Regional debris flow susceptibility analysis based on principal component analysis and self-organizing map: a case study in Southwest China. Arabian Journal of Geosciences. 9(18), 718.
[46] Saaty, T.L. (1978) Modeling unstructured decision problems — the theory of analytical hierarchies. Mathematics and Computers in Simulation. 20(3), 147-158.
[47] Cano, A., Jurado, F. and Sánchez, H. (2014) Optimal sizing of stand-alone hybrid systems based on PV/WT/FC by using several methodologies. Journal of the Energy Institute. 87(4), 330-340.

Received: **23.04.2018**
Accepted: **25.10.2018**

CORRESPONDING AUTHOR

Shengwu Qin
College of Construction Engineering
Jilin University,
Changchun 130026 – China

e-mail: qinsw@jlu.edu.cn

© by PSP Volume 27 – No. 12B/2018 pages 9717-9722 Fresenius Environmental Bulletin

FACTORS INFLUENCING THE HEALTH OF EMPLOYEES IN THE FURNITURE MARKETS

Junliang Tao[1,2], Yongyi Bi[1], Yonghong Man[1,2], Dongxian Zhang[3,*]

[1]School of Public Health, Wuhan University, Wuhan, P.R. China
[2]Nanyang Medical College, Nanyang, P.R. China
[3]School of Management, Hainan Medical University, Hainan, P.R. China

ABSTRACT

This study was to determine indoor air pollution and to assess the health impact of factors on workers in the furniture markets. Formaldehyde is the major indoor air pollutants and the concentration of formaldehyde was significantly higher in summer than it in winter. The exceeding ratios were 76.19% and 92.47%. In the furniture market, the mean values of employees' awareness of occupational health, attitude towards occupational health and occupational health behavior were 44.9 ± SD 12.87, 69.5 ± SD 12.24 and 34.1 ± SD 11.15 respectively. There were significant differences in age, education, residential location, economic condition and working years. Our results showed factors influencing on occupational health in the furniture markets through using a KAP survey, which revealed the importance of creating awareness about the health of employees in the furniture markets and taking effective measures to protect those employees against indoor air pollution.

KEYWORDS:
Occupational health, furniture market, indoor air pollution

INTRODUCTION

According to statistics, nearly half of the world's population suffers from indoor pollution, which has become one of risk factors endangering public health. Most recently, with the improvement of people's living standard, novel and fashion room furnishing has being expected, resulting in the change of furniture. In addition, numerous artificially synthesized materials have been widely used for furniture. Therefore, furniture pollution, construction pollution and indoor air after decoration are the most common sources of indoor pollution.

Indoor air pollution caused by furniture has been attached great importance [1-3], but its influence on employees in furniture market has not been received enough attention [4]. Nevertheless, diverse furniture is stored in the furniture markets leading to poor air quality, few researches on air pollution air pollution have been conducted in furniture market. The existing articles have substantiated that the concentration of formaldehyde in the furniture market exceed the standard. Especially, its concentration is much higher in summer than in winter [5-7].

In order to determine the air quality and health status of employees in the furniture market, we carried out an investigation on indoor air pollution and health status of staff in the furniture markets in the summer and the winter. We also made field test for harmful substances in indoor air, which is referential for ameliorating air pollution in the furniture market, preventing the occupational diseases, strengthening the professionally occupational health education, and establishing effective measures [8, 9].

METHODS

Test of Indoor Air Quality. Several aspects of indoor air were tested including temperature, humidity, wind speed, and concentration of the most important substances (formaldehyde, benzene series, carbon monoxide (CO), and carbon dioxide (CO_2)). Hygienic Standard for Shopping Centre and Book Store (GB9670-1996) was used to assess microclimate, CO, CO_2 and formaldehyde. Indoor Air Quality Standard (GB/T 18883-2002) was used to evaluate benzene, toluene, and 2, 2'-p-phenylenebis (5-phenyloxazol) (POPOP).

Sampling. This cross-sectional and oblique-sectional study was conducted every week. Sampling method is suitable for measuring the microclimate and air quality in the chosen furniture market. In accordance with the standard of Health Monitoring Technique Specifications in Public Places, sampling was carried out between the time of 09:00-11:00 a.m. and 02:00-04:00 p.m.

Population Survey. A total of 196 employees who worked in eight large-scale furniture markets were selected as respondents, using a clustered random-sampling procedure and the on-site investigation. They were completed questionnaires consisting of the basic information (name, gender, age,

educational degree, economic condition, working years, and working hours), 7 questions about knowledge of indoor air pollution, 8 questions about attitudes of indoor air pollution in furniture market, 8 questions about the occupational health behaviors, and 12 questions about the personal health status. Questionnaires referred to the domestic and international references correlated with this research, as well as the advices of experts. In order to make comprehensive estimate for respondents, we designed different scoring methods. All the data were recorded and transferred to centesimal system. The actual data were used to assess employees' knowledge, attitude, behavior and health status.

Statistical analysis. Data were expressed as mean ± SD. All the data were analyzed using SPSS 13.0 statistical analysis software package. Chi-square (χ^2) tests were used to identify differences in categorical data. A p-value of < 0.05 was considered significant. The multiple linear stepwise regression was applied to analyze the influential factors of the health status. Polychotomous variables in the independent variables were analyzed by dummy variables.

RESULTS

Indoor Air Condition in the Furniture Market. On average, the temperature, humidity, and wind speed in furniture market in summer were 26.8 °C, 53.6 % and 0.26 m/s respectively, which were higher than those in winters. In winter, they were 16.7 °C, 31.9% and 0.07m/s respectively. All the data were consistent with the national ambient air quality standards.

During heating period, the exceeding ratios of formaldehyde, benzene, toluene, and POPOP were 77.8%, 36.72%, 6.47% and 5.00% respectively. During non-heating period, the exceeding ratios of formaldehyde and benzene were 80.82% and 24.22% respectively. The concentrations of toluene and POPOP were not over the standard of indoor air quality. The concentration of formaldehyde was not only higher in winter than it in summer (χ^2 = 13.99, p = 0.00018), but also it exceeded this standard. The concentration of benzene in summer almost kept the same with that in winter (χ^2 = 2.314, p = 0.128), yet the mean value was higher than it prescribed by the national ambient air quality standards. The concentrations of toluene and POPOP were below the standard of indoor air quality. There were no statistically significant differences between groups.

Basic information of employees. While a total of 196 individuals responded to the survey, the questionnaires of 185 employees which constituted 94.4% of all the respondents were collected for analysis. Among participants, 43 (21.9%) were male, and 142 (78.1%) were female; 97 (52.4%) were migrant workers. A large majority of participants had low levels of education. Of these, 77.7% graduated from junior high and senior high school; 76.4% had worked in the same occupation over 2 years.

TABLE 1
Employees' states of knowledge about occupational health in the furniture markets

			Educational degree & awareness rate						Working years & awareness rate					
	Number of the aware	Awareness rate (%)	Primary school and below	Awareness rate (%)	Junior high	Awareness rate (%)	Senior high and above	Awareness rate (%)	<2 years	Awareness rate (%)	2 | 5 years	Awareness rate (%)	>5 years	Awareness rate (%)
Existing poisonous and hazardous materials	126	68.1	20	47.6	58	69.9	48	80.0	22	47.8	48	75.0	56	74.7
Self -protection	99	53.5	14	33.3	44	53.0	41	68.3	15	32.6	36	56.3	48	64.0
Major indoor pollutants														
formaldehyde	88	47.6	10	23.8	35	42.2	43	71.7	18	39.1	31	48.4	39	52.0
benzene series	86	46.5	13	31.0	37	44.6	36	60.0	16	34.8	29	45.3	41	54.7
Ammonia, CO_2, CO	34	18.4	6	14.3	14	16.7	14	23.3	7	15.2	12	18.8	15	20.0
Cognition on health hazard														
Irritating to eyes	148	80.0	32	76.2	67	80.7	49	81.7	30	65.2	54	81.3	62	82.7
Irritating to respiratory system	162	87.6	36	85.7	74	89.2	52	86.7	36	78.3	62	96.9	64	85.3
Dizziness, headache, chest distress	135	73.0	25	59.5	59	71.1	51	85.0	27	58.7	50	78.1	58	77.3
Skin allergy	83	44.9	18	42.9	37	44.6	28	46.7	14	30.4	31	48.4	38	50.7
Lower immunity	59	31.9	12	28.6	23	27.7	24	40.0	13	28.3	20	31.3	26	34.7
carcinogenicity	32	17.3	8	19.0	23	27.7	21	35.0	10	21.7	17	26.6	25	33.3

Employees' states of knowledge about occupational health. The study showed that 68.1% of participants expressed awareness of poisonous and hazardous materials existing in the furniture market (Table 1). The awareness rate was related to educational degree and working years ($\chi^2_{\text{educational degree}}$ = 8.25, p<0.05; $\chi^2_{\text{working years}}$ = 8.13, p < 0.05). 53.5% of participants had the consciousness of self-protection, which was also associated with education and working years ($\chi^2_{\text{educational degree}}$ = 7.62, p< 0.05; $\chi^2_{\text{working years}}$ = 6.81, p<0.05). The mean value of employees' awareness of occupational health was 44.9 ± SD 12.87.

Employees' attitude towards occupational health. Table 2 revealed that the majority of employees dealt with occupational health in a positive way. Over 60% of participants approved the individual viewpoint about occupational health. Especially, above 90% accepted the items, including having the better working environment (96.2%) and receiving periodic health examination (94.1%). However, 26% of the employees hold the negative attitudes. The mean value of employees' attitude towards occupational health was 69.5 ± SD 12.24.

Employees' occupational health behaviors. As seen in Table 3, participants had no good behaviors to ensure occupational health and safety in the furniture markets. In other words, few participants took effective measures to keep healthy and safety. 55.1% of participants ventilated the rooms, which was the most common measure. Besides, 67.6% of participants did not take self-protection; 64.3% of participants never initiatively learned the occupational health; 60% of participants never learned occupational health and reported pollution. The mean value of occupational health behavior of employees in the furniture markets was 34.1 ± SD 11.15.

Health status of Employees. Table 4 represented that the morbidity of employees who worked in the furniture markets was higher than that in the shopping centers. There were statistically significant differences in the occurrence rates of several symptoms between these two groups (p < 0.01), including suffocation, dizziness, coldness, respiratory tract irritation, and skin diseases. The significant differences between the groups were also found in the occurrence rates of irregular menstruation, epistaxis, bleeding gums, and getting the same diseases with colleagues (p < 0.05). For employees who worked in the furniture markets, the occurrence rate of neurological problems (suffocation and dizziness) was the highest among all the symptoms. It accounted for 44.3%, which was higher than that of the upper respiratory tract (38.4%). By contrast, the occurrence rates of reduction in blood cells and frequent hemorrhage were lowest, accounting for 9.2%.

Factors Influencing Employees' Health. In this study, Gender, age, marital status, educational degree, residential location, economic condition, working years, and working hours per day were used as independent variable. The score of health knowledge, health attitude, and health behavior are used as dependent variable. By analysis of multiple linear stepwise regression, the results presented in Table 5 showed that factors influenced employees' health including age, educational degree, residential location, economic condition, working years, health knowledge, health attitude, and health behavior (b' was 0.169, 0.144, 0.067, 0.097, 0.068, 0.185, 0.171, 0.291, and 0.494 respectively; the mean value of p < 0.05).

TABLE 2
Employees' attitude towards occupational health in the furniture markets

Items	Number of approvers	Approval ratio (%)
It's important to know occupation health knowledge	125	67.6
Taking protective measures	116	62.7
Having the better working environment	178	96.2
Receiving occupational health education	123	66.5
Receiving periodic health examination	174	94.1
Selling environmentally-friendly furniture	112	60.5
Strengthen supervision and inspection	129	69.7

TABLE 3
Employees' occupational health behaviors in the furniture markets

Items	Often		Sometimes		None	
	Number	Rate (%)	Number	Rate (%)	Number	Rate (%)
Utility of protective equipment	21	11.4	39	21.1	125	67.6
Learning occupational health knowledge	24	13.0	42	22.7	119	64.3
Keeping ventilated	102	55.1	46	24.9	37	20.0
Interruption of work once having any signs of illness	52	28.1	63	34.1	70	37.9
Timely inspection and treatment	54	29.2	74	40.0	57	30.8
Requiring the better working environment	35	18.9	62	33.5	88	47.6
Reporting indoor pollution to authorities	21	11.4	43	23.2	121	65.4

TABLE 4
Major symptoms of employees in the furniture markets

Symptoms or feeling	Employees in furniture market			Employees in shopping centers			χ^2	P
	No. of cases	frequency	Occurrence rate (%)	No. of cases	frequency	Occurrence rate (%)		
Suffocation, sickness when working, even dizziness, symptoms are alleviated after work	185	82	44.3	106	25	23.6	12.47	0.0004
Easy to get cold	185	67	36.2	106	18	17.0	12.06	0.0005
Do not smoke, seldom contact smoking environment, but often have a sore throat, foreign body sensation, disturbance in respiration	185	76	41.1	106	23	21.7	11.28	0.0008
Often cough, sneeze, low immunity, easy to get sick	185	71	38.4	106	15	14.2	18.99	0.0001
Frequent skin itch, skin allergy	185	53	28.6	106	11	10.4	13.11	0.0003
Get same disease with colleague, symptoms are alleviated after leaving the environment	185	28	15.1	106	6	6.7	5.86	0.0155
Long time infertility after marriage; can not find the reason	79	8	10.1	38	3	7.9	0.0024	0.9608
Fetal malformation or easy to miscarry	62	7	11.3	27	2	7.4	0.31	0.8602
irregular menstruation	142	41	28.9	91	13	14.3	6.63	0.0100
Frequent epistaxis, bleeding gums after taking up the occupation	185	17	9.2	106	2	1.9	4.75	0.0293
Contact trachitis, bronchitis or pulmonary disease after taking up the occupation	185	19	10.3	106	8	7.5	0.59	0.4410
Blood cells reduces after taking up the occupation	65	6	9.2	36	2	5.6	1.31	0.2517

TABLE 5
Linear Stepwise Regression Analysis of Factors Influencing Employees' Health

Basic variables	No.	*b*	S_b	*b'*	t statistic	*p* value
Gender	X_1	2.858	1.867	0.047	1.531	0.216
Age	X_2	4.394	0.692	0.169	6.353	0.0001
Marital status	X_3	0.699	0.987	0.027	0.708	0.479
Educational degree	X_4	4.196	0.841	0.144	4.986	0.0001
Residential location	X_5	1.779	0.761	0.067	2.336	0.020
Economic condition	X_6	7.539	3.115	0.068	2.420	0.016
Working years	X_7	5.520	0.820	0.185	6.735	0.0001
Working hours per day	X_8	1.429	0.837	0.046	1.707	0.088
Health knowledge	X_9	8.462	1.496	0.171	5.655	0.0001
Health attitude	X_{10}	0.388	0.134	0.291	2.903	0.004
Health behavior	X_{11}	0.456	0.092	0.494	4.943	0.0001

DISCUSSION

In the furniture production, chemical materials have been widely used contributing to indoor air pollution [10]. Among these, formaldehyde and benzene, as the major pollutants, are hazardous substances from the application of board tackifier, paints and adhesives, which are extensively used in the latest furniture. Indeed, present investigations have reported a large amount of adhesives are applied in extrusion forming of wooden boards [11-13]. The adhesives are formed by polymerization from formaldehyde and urea, especially in winter, which are easy to release gaseous formaldehyde. Therefore, the pollution of formaldehyde and benzene is correlated with the quality of wooden, proportions of boards, storage time after board production, and storage environment [14].

Due to fund shortage, the actual number of samples was only for 4% of the total workers who employed in the furniture markets of Nanyang. Nonetheless, the submitted quantity was adequate for the present study. In this study of workers employed in the furniture markets, the symptoms about nerve system, the upper respiratory tract and skin were hypothesized to be associated with the high concentration of formaldehyde. Moreover, the high incidences of irregular menstruation and abnormal pregnancy can be related with benzene. There was no significant difference in the reduction

of blood cells among employees engaged in the furniture markets compared to those who worked in the shopping center, which was probably caused by changing the concentration of benzene at the same time [15]. The cause of changes on blood cells was not known for certain, whereas the problem could be solved satisfactorily by testing the individual and increasing the sampling amount. Furthermore, there are other personal factors influencing the results, such as aperiodic health examination, unawareness of health and self-protection, and the lack of health management from industry.

A Knowledge, Attitude and Practices (KAP) survey, as a quantitative method, was applied to determine factors influencing on the occupation health in the furniture markets. This study pinpointed that most employees lacked the awareness of occupation health. 31.9% of employees were unaware that they were being exposed to poisonous and hazardous gases at work, and 46.5% of employees lacked a consciousness of self-protection. The awareness rate was significantly connected to educational degree and working years ($\chi^2_{\text{educational degree}} = 8.25$, $p < 0.05$; $\chi^2_{\text{working years}} = 8.13$, $p < 0.05$). Employees with high educational background not only easily obtain knowledge of occupational health from many sources, but also have strong abilities to fully understand new information. Additionally, due to the long working years, senior workers paid much more attention to occupation health by learning from experience compared to newcomers. Because occupational health was poorly publicized, only 30.2% of employees obtained it from the related health and safety departments, which emphasized the importance of providing occupational health education in furniture enterprises [16].

Despite the fact that employees with low-level cognition still hold positive attitude toward occupational health, the approval rate of taking protective measures were low (62.7%). It reveals that safety and health education should be popularized in workplace, which is the crucial for employees to have a strong demand for occupational health.

It has been proven that health behavior of employees depends largely on their health knowledge and positive attitude. On the other hand, culture, custom, public opinion, morality, law and regulations could both have an impact on behavior. Therefore, it is hard to define exactly the change of occupational behavior. The present study showed that few employees keep on using the facilities for self-protection. 64.3% of employees hardly learned the knowledge of occupational health, which was relevant to low educational background, causing difficulty in reading and lack of time. Thereby, the health and safety departments should adopt common and lively methods to make occupational health accessible. 30.8% of employees kept on working in spite of illness, which was associated with misconception and low income. 65.4% of employees never reported indoor pollution to authorities [17]. These results were found to be related to lack of legal education and social security. In order to figure out these problems, the departments for the administration of industry are supposed to attach importance to vulnerable groups, strengthening management, and safeguarding employees' legitimate rights.

In our study, occupational health of employees was significantly influenced by age, educational degree, residential location, economic condition, working years, health knowledge, attitude, and behavior. Older employees had more experience and knowledge of occupational health than did young employees. Likewise, employees with long working years did good in maintaining safety and health in workplace. Similar results were found in the study preformed by Jinling Liu et al [1]. Employees with higher educational degree have strong abilities in their acquirement of new knowledge and greatly concerned about occupational health. Residential location provides a circumstance of general education for people, leading to fostering correct views on life and values [18], which may indirectly affect occupational health.

Establishing the rules and regulations could be helpful to supervise indoor air quality of furniture market and to provide effective measures to reduce indoor air pollution from hazardous materials, such as limiting the application amount of formaldehyde and ammonia in adhesive, keeping ventilated, and utilizing biological technology. Our data emphasized the need to develop health education programs that enhance occupational health knowledge among employees in the furniture markets. For instance, furniture enterprise should enforce occupational health education and check employees' health behavior in workplace by examination. Besides, employees who work in the furniture markets should receive a regular health checkup every year.

CONCLUSION

We investigated indoor air condition in the furniture markets and the health status of employees. campus. The high risks of formaldehyde and benzene were demonstrated to be a concern to the employees. Besides, occupational health of employees was significantly influenced by age, educational degree, residential location, economic condition, working years, health knowledge, attitude, and behavior, which emphasized occupational health in the furniture markets and contributed to take effective measures to protect employees against indoor air pollution.

ACKNOWLEDGEMENTS

We have list all third-party financial support for the work in the submitted manuscript. The author declare there have no any conflict of interest

REFERENCES

[1] Liu, J.L. and Zhang, H. (2008) Method to reduce pollution of in-door furniture. Furniture and Interior Design. 3, 108-109.
[2] Yu, H.F., Li, X.Y. and Lu, J. (2000) The survey of air quality in furniture market. Environment and Health. 17(4), 224-227.
[3] Chu, J.H., Yin, X.J., Xiao, Z.X.M. and Jing, S.A. (1996) Formaldehyde pollution in furniture market affect health. Management and technology of environment evaluation. 8(1), 18-19.
[4] Wang, X.Y., Pan, H. and Xiong, W. (2002) Pollution control of civil engineering construction. Beijing: China Planning Press.
[5] Xu, L. and Zhao, Y.L. (2005) Prevention and controlling countermeasure of in-door air pollution. Technology of Pollution control. 22(2), 150.
[6] Yi, Z.G. and Gong, Y. (2007) Prevention and controlling countermeasure of in-door Environment pollution. Industrial safety, environmental protection. 33(4), 37-38.
[7] Mage, D.T. and Gammage, R.B. (1985) Evaluation of changes in indoor air quality occurring over the past several decades. In: Gammage R.B., Kaye, S.V. (eds.) Indoor Air and human health. Michigan: Lewis Publisher, 5-7.
[8] Nandate, K., Ishidao, T., Hori, H., Morimoto, Y. and Ogata, M. (2003) The effect of formaldehyde exposure on cytokine production in murine alveolar macrophages. JUOEH. 25(2), 197-205.
[9] Guo, X.N., Yu, F.S. and Ding, H.Y. (2003) Prevention and controlling countermeasure of formaldehyde pollution after in-door interior decoration. Journal of Preventive Medicine Information. 25(6), 415-416.
[10] Zhan, P. and Wang, J. (2003) Formaldehyde pollution after interior decoration. Journal of Preventive Medicine Information. 19(4), 296-298.
[11] Han, L.F., Li, Q. and Zhao, J.Q. (1996) Research on effects of in-door formaldehyde on health. Chinese Journal of Public Health. 12(4), 151-152.
[12] Zhang, Y.F., Liu, B. and Chao, H.M. (1999) Research on effects of in-door are formaldehyde on health. Heilongjiang medicine science. 22(4), 20.
[13] Zhao, S. (2008) research on formaldehyde pollution of furniture market in Hengshui city. Journal of environment and health. 5(25), 449.
[14] Wang, G.P., Zhou, Z.P. and Fu, L.X. (2003) Research on feature of in-door formaldehyde and Benzene. Shanghai environment science. 22(12), 977-978.
[15] Giese, M., Bauer-Doranth, U., Langebartels, C. and Sandermann, H. Jr. (1994) Detoxification of Formaldehyde by the Spider Plant (Chlorophytum comosum L.) and by Soybean (Glycine max L.). Cell-Suspension Cultures Plant Physiol. 104(4), 1301-1305.
[16] Mills, A. and Le Hunte, S. (1997) An over view of semiconductor photocatalysis. Journal of Photochemistry and Photobiology: A Chemistry. 108, 1-35.
[17] Aguado, S., Polo, A.C., Bernal, M.P., Coronas, J. and Santamaría, J. (2004) Removal of pollutants from indoor air using zeolite membranes. Journal of Membrane Science. 240, 159-166.
[18] Jiao, Z., Luo, P.C., Wu, Y.T., Ding, S. and Zhang, Z. (2006) Absorption of lean formaldehyde form air with Na_2SO_3 solution. Journal of Hazardous Materials B. 134, 176-182.

Received: **25.04.2018**
Accepted: **26.10.2018**

CORRESPONDING AUTHOR

Dongxian Zhang
School of Management,
Hainan Medical University,
NO.3 Xueyuan Road, Haikou City – China

e-mail: yongyibi2015@sina.com

© by PSP Volume 27 – No. 12B/2018 pages 9723-9738 Fresenius Environmental Bulletin

EVALUATION OF WASTEWATER QUALITY AT THE INLET-OUTLET OF THE MOST MODERN WASTEWATER TREATMENT PLANT IN BULGARIA

Gergana Kostadinova[1], Diyana Dermendzhieva[1], Georgi Petkov[1,*], Georgi Beev[2], Koycho Koev[3]

[1]Trakia University, Faculty of Agriculture, Department of Applied Ecology and Animal Hygiene, 6000 Stara Zagora, Bulgaria
[2]Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria
[3]Trakia University, Faculty of Veterinary Medicine, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, 6000 Stara Zagora, Bulgaria

ABSTRACT

The study was carried out during the period 2015-2016 based on 24 physicochemical and 5 microbiological wastewater (WW) parameters. WW samples were collected twice per month from both monitoring points (MPs) of the Municipal Wastewater Treatment Plant (MWWTP) - MP-1 (inlet) and MP-2 (outlet) and screened parameters were analyzed according to Bulgarian standards complied with ISO standards. For the estimation of total and specific microbial load, selective chromogenic culture medium sheets were used. Multivariate statistical technique was applied to analyze the data for different parameters. It was found the ranges of variability and trends of inlet-outlet WW values changes. The MWWTP demonstrated different removal efficiency (8.31-97.8%) referring to different WW parameters. 127 strong positive and negative correlations exist between controlled WW parameters. The parameters involved in the most numerous statistically significant correlations were T°C and Cl. EC at inlet/outlet affected at a great extent Factor 1 of Rotated factor loading matrix. The factor analysis determined MP as a factor influencing the largest number of parameters (14), followed by factors Month (7) and Year (2). PCA revealed different WW parameters at inlet-outlet that were affected by F1 and F2. The treated WW did not meet the requirements for discharge in the receiving water body (with respect to the total P content) and for irrigation (as fats content and the number of total Coliforms, *E. coli*, *Enterobacteriaceae* and *Salmonella* spp. was concerned).

KEYWORDS:
Wastewater Treatment Plant, wastewater, physicochemical and microbiological parameters, heavy metals, multivariate analysis

INTRODUCTION

Urbanization, progressive population growth, industrial and agricultural development are global processes related to constant increase in the generated wastewater amount. Wastewater from settlements, both within a country and between the different countries in the world are characterized with extremely diverse physicochemical composition, microorganisms' content and invasive forms of parasites [1-3]. The level of wastewater purification determines whether they will pose an ecological risk to the receiving water bodies where they discharged [4-6] or to the soil [7-10] and the crops grown when used for irrigation (11-16].

Wastewater discharges can deteriorate the physicochemical and biological properties of water of the receiving water bodies when discharge quality does not comply with the norms [17-19]. Achieving the required quality of treated wastewater depends to a large extent on the purification methods and equipment used in the MWWTPs [20-26]. In order to solve the wastewater problems, Bulgaria strictly follows the EU Directives concerning urban wastewater treatment - for the construction of WWTPs in all settlements with a population equivalent of more than 10 000 [27] and emission-based regulation for effluent quality in terms of COD, BOD, total N and total P [28]. Currently, 174 MWWTPs (7 with primary, 97 with secondary and 109 with tertiary treatment, respectively) are operating in the country. They purify about 75% of the generated wastewater, which in the period 2010-2017 consisted of 786.6-811.2 mill.m^3/y [29]. The most modern MWWTP in Bulgaria was put into operation in April 2011. The final treated effluent is discharged into Bedechka River, left tributary of Maritsa River (main transborder source of irrigation in the Trakia valley [19]) that flows into the Aegean Sea. Therefore, the WWTPs effluent quality is of great ecological (protection of the water purity of the receiving water bodies) and agronomic (when used for irrigation) importance. Based on the above mentioned, the purpose of this study was to make inlet-outlet wastewater quality characterization and ecological

assessment from the most modern Municipal Wastewater Treatment Plant in Bulgaria, using key physicochemical and microbiological parameters.

MATERIALS AND METHODS

Study Area. The study was conducted from January 2015 to December 2016 into the most modern MWWTP in Bulgaria (capacity 3 174 m^3 wastewater per hour), town of Stara Zagora (136000 inhabitants), situated in Central-South part of the country (N42.425777°; E25.634484°). It treats urban wastewater (WW), including domestic (about 85%) and industrial (about 15%) wastewater (from households, brewery, vegetable oil refinery, industrial enterprises, other human activities) and surface rainfall water.

Study Design. MWWTP includes three stages of wastewater treatment: I^{st} stage - mechanical treatment (gratings and filters); II^{nd} stage - biological treatment (aeration tanks, secondary settling tanks and methane tank); III^{rd} stage - additional treatment to limit the amounts of phosphorus and nitrogen by physico-chemical and biological methods. Treated WW and dehydrated sludge are obtained at the outlet of the plant station. Treated WW are discharged into Bedechka River (water receiver), which is defined as semi-mountainous type of water body - type R5 [30]. Its location is in a sensitive area with high anthropogenic impact, according to Order of Minister of Environment and Water [31].

Monitoring Points. For the purpose of the study, two monitoring points (MPs) of MWWTP were identified:

- Inlet (MP-1) - raw municipal wastewater (RMW);
- Outlet (MP-2) - treated municipal wastewater (TMW).

Sampling and Sample Preparation. During the experimental period (January 2015 - December 2016), wastewater samples were collected every two months from both MPs (a total of 24 samples). For wastewater sampling and sample preparation for analyses, international references (ISO 5667-1; ISO 5667-3; ISO 5667-10) were used. The samples for physicochemical analysis were collected in dark (to eliminate photo-oxidation processes) chemically clean glass containers (3L) and for the microbiological analysis - in sterile glass containers (0.25L). The collected wastewater samples were transported to the chemical and microbiological laboratories at Faculty of Agriculture, Trakia University in a cool bag (at 4-6°C), and processed for analysis up to 2 h after the collection.

Parameters and Methods for Analysis. The following 24 physicochemical and 5 microbiological parameters charactaraizing wastewater quality were determined.

Physicochemical parameters. All analyses were performed in triplicate, as followed: temperature (T °C) and pH – ISO 10523, by pH-meter Lab 850 with built-in temperature sensor; electrical conductivity (EC) – EN 27 888, by Multi 340i/SET; total hardness (TH) – Bulgarian State Standard (BSS) – 3775, titrimetric method with complexon III; suspended solids (SS) – BSS 17.1.4.04, by determining the mass of SS in a certain volume of wastewater captured on a filter paper, after its drying at 105°C; COD – ISO 6060; BOD_5 – EN 1899-1, 2 by BOD-System OxiDirect; total nitrogen (TN), ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $N_{org.}$) – EN 25663, by the method of Kjeldahl and using a steam distiller UDK 146; total phosphorus (TP) – EN 6878-1 and sulfate (SO_4^{2-}) – BSS 3588 by UV-VIS Spectrophotometer JENWAY 6705; chlorides (Cl^-) – ISO 9297; fats – EPA Method 1664; iron (Fe), manganese (Mn), chromium (Cr), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb) and nickel (Ni) by ISO 15586, respectively; potassium (K) and sodium (Na) by ISO 9964; calcium (Ca) and magnesium (Mg) by ISO 7980 with an AAS (AAnalyst 800 Perkin-Elmer), with graphite furnace HGA or on flame.

Microbiological parameters. Number of cultivable microorganisms in wastewater samples was enumerated by performing serial dilutions (from 1:10 to 1:1000000) in phosphate-buffered saline which was vortexed for 1 min prior to plating. For the quantitative determination of the aerobic mesophilic microorganisms (AMO), sanitary-indicator microorganisms (coliforms, *E. coli, Enterobacteriaceae*) and pathogens (*Salmonella* spp.) in the wastewater, medium sheets (Rida®Count Total; Rida®Count *E. coli/Coliform*; Rida®Count *Salmonella/Enterobacteriaceae*, R-Biopharm AG, Germany) coated with selective, chromogenic culture medium were used. Transparent cover film was opened and 1 mL of the sample solutions with appropriate dilutions was applied onto the nonwoven fabric of the medium sheet with a pipette. The sheets were inoculated in duplicate, incubated at 35°C for 24-48 h and the colonies were counted. Specific microorganisms formed colonies with different colors on the specific test cards. The results are expressed in colony forming units (CFU/mL).

Removal Efficiency (RE) Assessment of MWWTP. It was determined on the basis of differences between respective inlet and outlet values of the investigated WW parameters expressed as mg/L for physicochemical parameters, CFU/ml for microbial indices and in percentage (%) for both groups of parameters.

Treated Municipal Wastewater Quality Status Assessment. It was made by the method of comparative analysis in which the results obtained for different parameters were compared with the respective stipulated norms in two aspects as followed:

- TMW quality status assessment from an environmental point of view: It was carried out by 5 parameters (COD, BOD, SS, total N and total P) according to emission norms for permissible levels of harmful and hazardous substances in the wastewater discharged into the receiving water bodies [32].
- TMW quality status assessment as a source for irrigation in agriculture: It was made on the basis of 16 physicochemical parameters, 8 trace elements and 5 microbiological parameters according to requirements of the irrigation water quality [33].

Statistical Analysis. All data ware analyzed by Statistical software and data analysis tool XLSTAT, Version 2016.02, Addinsoft.

RESULTS AND DISCUSSION

Physicochemical Parameters. Temperature. WW temperature corresponded the temperature changes during the different seasons and varied from 10.3 to 23.0°C in 2015 and from 10.2 to 26.6°C in 2016 (Table 1). WW average annual temperatures at both MPs changed differently during the survey period - in 2015 the inlet WW temperature was higher as compared to that of outlet WW (on the average by 0.5°C), and in 2016 the opposite tendency was observed - higher temperature at outlet than at inlet (by 0.6°C on the average). These differences were not statistically significant and showed that plant treatment processes did not affect WW temperature. Comparable temperatures to the measured maximum were reported by Zema et al. [34] for TMW from San Lorenzo WWTP, Calabria, Italy – 23.0±4.32°C for the period April-September, a fact that confirms the seasonal influence on the WW temperature.

TABLE 1
Physicochemical parameters of treated wastewater (n=6)

Parameters	Unit	MP*	2015			2016			SL [32]	SL [33]
			C_x±SD	C_{min}	C_{max}	C_x±SD	C_{min}	C_{max}		
T	°C	Inlet	16.6±4.62	10.3	22.9	16.1±6.03	10.2	25.3	-	-
		Outlet	16.1±4.81	9.50	23.0	16.7±5.94	11.4	26.5	-	28
pH	Units	Inlet	7.68±0.39	7.30	8.41	7.57±0.13	7.35	7.75	-	-
		Outlet	7.28±0.36	7.06	8.00	7.49±0.15	7.29	7.72	-	6-9
EC	µS/cm	Inlet	734.0±73.3	649.0	851.0	697.0±52.8	630.0	759.0	-	-
		Outlet	727.0±178.7	601.0	1081.0	638.0±51.4	569.0	699.0	-	2000
TH	mgeqv/L	Inlet	4.22±0.35	3.44	5.73	4.47±0.41	3.87	4.88	-	-
		Outlet	4.08±0.33	2.92	5.55	4.86±0.29	4.44	5.30	-	14
Ca	mg/L	Inlet	70.4±6.32	64.8	77.8	67.4±5.28	59.1	73.7	-	-
		Outlet	66.4±4.35	60.2	72.3	68.3±4.20	61.6	74.1	-	400
Mg	mg/L	Inlet	20.8±2.04	18.2	21.8	17.8±1.97	15.0	20.4	-	-
		Outlet	19.3±3.45	15.8	20.2	17.7±3.45	13.7	22.3	-	300
SS	mg/L	Inlet	60.6±19.5^a	29.8	84.8	59.9±20.0^a	36.0	81.6	-	-
		Outlet	6.31±4.88^a	3.46	16.1	5.62±4.12^a	3.15	15.3	35	50
COD	mg/L	Inlet	200.7±41.4^b	157.2	280.1	196.1±56.0^b	145.0	279.2	-	-
		Outlet	20.7±7.66^b	13.2	32.4	20.2±9.15^b	9.00	34.3	125	100
BOD_5	mg/L	Inlet	88.8±33.8^b	33.6	130.2	79.5±26.2^a	52.2	121.0	-	-
		Outlet	5.17±2.64^b	2.03	8.01	3.33±1.21^a	2.00	5.04	25	25
Total N	mg/L	Inlet	24.6±7.56^a	15.5	35.0	65.7±46.7^a	28.6	153.0	-	-
		Outlet	5.56±2.18^a	2.54	8.50	5.84±1.58^a	4.29	8.58	10	5**/20***
Total P	mg/L	Inlet	49.7±20.6^a	27.8	80.3	38.6±11.0^b	26.4	58.0	-	-
		Outlet	0.98±0.10^a	0.83	1.09	1.08±0.24^b	0.65	1.29	1	3
Total K	mg/L	Inlet	7.03±3.02	4.29	11.6	7.59±3.20	4.79	13.6	-	-
		Outlet	7.12±2.41	4.86	10.1	7.42±2.39	5.07	11.8	-	350
Total Na	mg/L	Inlet	43.1±8.58	29.5	46.1	39.4±8.32	25.8	49.3	-	-
		Outlet	36.3±8.24	27.3	42.4	34.9±7.99	25.1	46.8	-	300
Cl^-	mg/L	Inlet	-	-	-	36.3±5.25	29.3	49.3	-	-
		Outlet	-	-	-	34.1±3.79	29.0	39.9	-	300
SO_4^{2-}	mg/L	Inlet	3.88±0.96	2.66	5.38	3.36±0.71	2.38	4.47	-	-
		Outlet	2.92±0.74	2.20	3.55	2.79±0.62	2.01	3.39	-	300
Fats	mg/L	Inlet	14.8±3.23^b	10.4	22.1	16.1±3.08^b	11.3	19.1	-	-
		Outlet	3.43±1.08^b	2.06	5.43	3.93±1.14^b	2.24	5.21	-	5.0

*MP - Monitoring point; **Standard Limit for $N\text{-}NH_4$;
***Standard Limit for $N\text{-}NO_3$; ****Differences between values by columns are significant at P<0.05 – aa, P < 0.01 – bb.

pH. Inlet/outlet pH values varied within a relatively narrow range, 7.06-8.41 in 2015 and 7.29-7.75 in 2016 (Table 1). A similar trend of change was observed over the monitored period – pH outlet values were averagely 1.05 and 1.01 times lower than those at inlet, in 2015 and 2016, respectively. These small differences in pH values between the two MPs give grounds to conclude that WW treatment processes had almost no influence on this indicator. A contrary trend is reported by Kushwah et al. [2] for Badwai sewage treatment plant (Bhopal, India), where influent pH values were 1.12 to 1.23 times lower than enfluent pH values during different seasons.

It is noteworthy that the data from other investigations all over the world are very diverse. Some of them [9, 26, 35-39] are in the range of the measured pH values (7.3-8.0). Others authors [16, 40-44] reported slightly higher values (8.1-8.4) or slightly lower ones (5.53-7.28) [5, 45, 46]. Data analysis showed that untreated and treated WW had a neutral to slightly alkaline reaction regardless of the country and the plant treatment technology used. The only exceptions of that rule are the treated WW from some cities in Bangladesh, which are slightly acidic [45]. Probably, in those cases there are some specific conditions (WW composition, WW treatment, etc.) affecting WW pH.

EC. Values obtained varied from 601.0 to 1081.0 µS/cm in 2015 and from 569.0 to 759.0 µS/cm in 2016 (Table 1). In both MPs EC was slightly higher in 2015 than in 2016, averagely 1.05 times at inlet and 1.13 times at outlet, showing that the factors influencing WW ions concentrations can vary from year to year. Regardless of the year of measurement, EC was slightly lower in outlet compared to inlet, 1.01 times (2015) and 1.09 times (2016) on the average. A similar trend is found out by Kushwah et al. [2] for Badwai sewage treatment plant, Bhopal, India.

The small and inconsistent differences between the input and output EC values do not imply any influence of plant treatment processes on this parameter. Data similar to our results (955.0-1063 µS/cm) were reported by Zavadil [47] and Pereira et al. [43]. Other authors established higher (1200-2300 µS/cm) [16, 26, 41, 46] or lower (450-630 µS/cm) [38, 42] values than ours. Obviously, WW EC in different countries demonstrates a wide range of fluctuation, probably due to the different conditions for WW salt concentration formation and the methods of treatment.

TH, Ca and Mg. These parameters are interrelated and thus analyzed together. In both MPs, TH varied between 2.92 and 5.73 mgeqv/L, values characterizing WW hardness as medium (8-12°dH) to moderate (12-18°dH) (Table 1). In the scientific literature, more attention is devoted to Ca and Mg content than to TH. Ca and Mg concentrations fluctuated at different levels, higher for Ca 59.1-77.8 mg/L and lower for Mg 13.7-22.3 mg/L (Ca content was 3.38 to 3.85 times higher compared to Mg content). Inlet/outlet Ca and Mg concentrations were very close, which gave reason to assume that plant treatment processes did not affect these minerals. Similar Ca and Mg concentrations were reported by: for Ca (51.4-64.0 mg/L) - Ahmed and Al-Hajri [41], Pereira et al. [43] and Heidarpour et al. [48]; for Mg (15 mg/L) - Zavadil [47]. Lower values for Mg (10.3-11.1 mg/L) were found by Gatta et al. [39] and Pereira et al. [43]. Significantly higher concentrations for both elements (Ca: 78-168 mg/L; Mg: 41.2-94 mg/L) were established by Shakir et al. [16], Zema et al. [34], Bedbabis et al. [38] and Petousi et al. [44]. Obviously, the calcium and magnesium content in urban wastewater from different parts of the world is determined by specific and diverse factors.

SS. The established concentrations fluctuated within a relatively wide range - between 29.8 and 84.8 mg/L in untreated and between 3.15 and 16.1 mg/L in treated WW (Table 1). Outlet SS content was drastically reduced compared to inlet ($P<0.05$), 9.60 times in 2015 and 10.7 times in 2016 on the average. Comparable results for TMW (referring to maximum values 16.2-17.5 mg/L) were obtained by Gatta et al. [39] and Pereira et al. [43]; much lower (<1 mg/L) – by Zavadil [47] and much higher (22.0-56.4 mg/L) – by Shakir et al. [16], Zema et al. [34], Panoras et al. [49], Petousi et al. [44] and Heidarpour et al. [48]. These data characterized SS as a very changeable inlet/outlet WW parameter.

COD. The parameter values varied from 145.0 to 280.1 mg/L at inlet and from 9.00 to 34.3 mg/L at outlet (Table 1). The average COD concentration in the treated WW was 9.7 times lower than in untreated WW for both monitored years and the difference was statistically significant ($P<0.01$). Similar COD values for treated WW (7.6-26.5 mg/L) were established by Rojas-Valencia et al. [12], Shakir et al. [16], Alikhasi et al. [37] and Aiello et al. [40], while the majority of other authors [34, 38, 39, 43, 44, 46-49] reported higher COD levels (39.7-438 mg/L). Apparently, with respect to COD, MWWTPs in many countries cannot provide effective WW treatment.

BOD_5. The fluctuations observed in BOD_5 were similar to those observed for COD and varied between 33.6 and 130.2 mg/L at MP-1 and between 2.00 and 8.01 mg/L at MP-2 (Table 1). BOD_5 values were drastically lower in outlet compared to inlet WW, 17.2 times in 2015 and with 23.9 times in 2016 ($P<0.01$). Data reported by Rojas-Valencia et al. [12], by Alikhasi et al. [37], by Aiello et al. [40] and by Petousi et al. [44] (4.0-7.8 mg/L) were comparable to obtained TMW results. Other authors [16, 38, 39, 43, 46, 48, 49] found much higher levels (13.9-

128 mg/L). Therefore, data about this key parameter of WW quality are quite heterogeneous and contradictory. This requires taking account of specific conditions on a case-by-case basis.

Total Nitrogen (TN). The values of the indicator varied significantly both by study year and by MPs. Average TN inlet content in 2016 was 2.7 times higher than in 2015. Probably the reason was the larger amount of nitrogen substances occurring in WW in 2016 compared to 2015. Nevertheless, it is noteworthy that nitrogen content in the treated WW was almost the same in both monitored years. The TN values differences between inlet and outlet were statistically significant ($P<0.05$). The TN results obtained for TMW are the same as those reported by Aiello et al. [40], by Pereira et al. [43] and by Zavadil [47] - 6.3-8.6 mg/L; higher than the data of Gatta et al. [39] - 1.66 mg/L, and lower than the concentrations found out by Rojas-Valencia et al. [12], by Zema et al. [34], by Bedbabis et al. [38] and by Petousi et al. [44] - 23.6-575.0 mg/L.

Total Phosphorus (TP). The TP quantity was higher in untreated WW in 2015 compared to 2016 (1.29 times on the average), but at the same time its concentration was almost equal in treated WW for both years of study (Table 1). The differences of average TP values between the two MPs for both years were statistically significant ($P<0.05$). A number of other authors [12, 16, 34, 37, 38, 40, 42, 44, 47, 48] reported higher TP levels in TMW (3.47-13.5 mg/L) than ours. Lower than our values are published by Gatta et al. [39] - 0.29 mg/L, while Mojid et al. [45] established a very wide range of parameter fluctuation in TMW from 10 cities in Bangladesh (0.2-17.9 mg/L). This heterogeneous picture is a result of the specific conditions under which each of the above mentioned studies were conducted, the most important of which are the generated WW composition, the adopted technology of their treatment, climatic and other factors.

Total Potassium (TK). TK content, in contrast to that of TN and TP, did not change between inlet and outlet, and varied between 4.29 and 13.6 mg/L and between 4.86 and 11.8 mg/L, respectively (Table 1). The results show that the WW treatment processes had no impact on this indicator. At this stage, we have no acceptable explanation for this fact. Future research would help to clarify this phenomenon. Our TK ranges agreed with those reported by Pereira et al. [43], by Mojid et al. [45] and by Heidarpour et al. [48] - 3.85-7.71 mg/L, and partly with the results published by Shakir et al. [16] - 12.1-19.4 mg/L. Much higher values (17.1-47.6 mg/L) than our data were established by Zema et al. [34], Alikhasi et al. [37], Bedbabis et al. [38], Gatta et al. [39], Galavi et al. [42], Pereira et al. [43], Petousi et al. [44] and Zavadil [47], while much lower – by Singh et al. [9] - 0.31±0.07 mg/L.

Total Na (TNa). The measured concentrations were close by years of measurement for both MPs – inlet 25.8-49.3 mg/L and outlet 25.1-46.8 mg/L (Table 1). Slightly lower TNa content was observed in TMW in comparison to RMW, 1.10 times in 2015 and 1.13 times in 2016 on the average. These results partly match the data reported by Mojid et al. [45] – 44-60 mg/L; they are much higher than the results published by Singh et al. [9] – 1.42±0.2 mg/L and significantly lower than maximum values (90.0-470.0 mg/L) established by many other authors [16, 34, 38, 39, 42, 43, 47, 48]. Obviously, WW TNa concentrations from urban territories all over the world depend on specific conditions, which are of different type and degree of influence.

Cl. RMW and TMW chlorides content was relatively low (29.0-49.3 mg/L) with small differences between inlet-outlet. The values of the parameter were slightly higher at MP-1 compared to MP-2, by 1.06 times on the average. Higher concentrations (61.0-1999.0 mg/L) than ours are presented by Shakir et al. [16], Bedbabis et al. [38], Ahmed and Al-Hajri [41], Galavi et al. [42] and Pereira et al. [43], and lower - by Singh et al. [9] - 5.10±2.0 mg/L.

SO_4. The established concentrations were negligibly low (2.01-5.38 mg/L). Notwithstanding this, the sulfate content was lower in treated than in untreated WW, 1.33 times in 2015 and 1.20 times in 2016 on the average. This indicates that plant treatment processes affect the amount of sulfate, as they reduce it. The reviewed literature sources [38, 39, 41-43, 46] show significantly higher sulfate levels in urban wastewater (24.5-915.4 mg/L) than those in our results.

Fats. Fat content varied within different ranges in RMW and in TMW: 10.4-22.1 mg/L and 2.06-5.43, respectively (Table 1). Fat quantity decreased significantly (>4.0 times) in outlet compared to inlet, a fact that shows the high effectiveness of plant treatment processes. A comparative assessment of the obtained results with data from other authors was not made due to a lack of information in the reviewed literature sources. In our opinion, this parameter deserves attention, as fats are a typical organic pollutant of urban wastewater.

Heavy Metals (Fe, Mn, Cr, Cu, Zn, Cd, Pb, Ni). Inlet/outlet heavy metals concentrations of the investigated elements were negligibly low and varied between <0.01 mg/L for Pb and 0.1 mg/L for Ni (Table 2). In contrast with Cr, Cu, Cd, Pb and Ni levels, which are without fluctuations in both MPs, a significant difference between the maximum and the minimum values is observed at Fe (3.7-5.0 times),

Zn (2.75-4.0 times) and Mn (1.33-1.50 times). The established heavy metal concentrations are: similar to these reported by Panoras et al. [49] for Fe, Cu, Pb, Mn, Ni and Zn content (from <0.1 mg/L for Fe to <0.05 mg/L for Mn), by Surdyk et al. [50] for Fe, Cu, Cd and Pb (0.015-0.02 mg/L), by Petousi et al. [44] for Cu and Ni (not detected), and for Zn – 0.0072 mg/L; higher than the data published by Mojid et al. [45] for Fe (0.12-0.98 mg/L) and for Mn (0.3-1.1 mg/L), and lower than the results found by Zavadil [47] - from 0.009 mg/L for Cr to 0.0026 mg/L for Ni and by Pereira et al. [43] - for Cd/Cr (not detected) to 0.02 mg/L for Ni.

The heavy metal concentrations ranking in both MPs was as followed: inlet - Ni>Zn>Fe>Cr/Cu> Mn>Cd>Pb; outlet - Ni>Cr/Cu>Zn>Fe/Mn>Cd>Pb. The inlet-outlet concentrations of three of the elements (Ni, Cd and Pb) remained equal. The outlet heavy metal ranking partly coincided with the ranking of TMW heavy metal content in the study of Pereira et al. [43] - Ni>Pb>Zn>Fe/Cu>Mn>Cd/Cr. Another order was reported by Zavadil [47] – Fe>Mn>Cr>Ni>Cd>Cu>Pb/Zn. The wide variety of heavy metals content in urban WW, as well as their ranking in descending order by elements, suggests that many different factors have an influence on their level. Further research is warranted to clarify all these aspects.

Microbiological Parameters. All monitored parameter values were time-dependent and varied within a very wide range between both MPs (P>0.05) (Table 3). The microbial load reducing property of the treatment plant has been clearly demonstrated by the fact that at the outlet, the average concentration of all studied microbial groups had significantly decreased compared to inlet values in the following order: AMO – 69.7 times; Coliforms – 41.1 times; *Escherichia coli* –75.4 times; *Enterobacteriaceae* – 61.5 times and *Salmonella* spp. – 48.3 times.

TABLE 2
Heavy metals content of treated wastewater, 2016 (n=4)

Parameters	MP*	C_x±SD mg/L	C_{min} mg/L	C_{max} mg/L	Standard Limit [33]
Fe /total/	Inlet	0.06±0.04	0.03	0.11	-
	Outlet	0.03±0.02	0.01	0.05	5.0
Mn	Inlet	0.03±0.01	0.03	0.04	-
	Outlet	0.023±0.005	0.02	0.03	0.2
Cr /total/	Inlet	<0.05	-	-	-
	Outlet	<0.05	-	-	0.05 (Cr^{6+})/0.5 (Cr^{3+})
Cu	Inlet	<0.05	-	-	-
	Outlet	<0.05	-	-	0.2
Zn	Inlet	0.07±0.04	0.04	0.11	-
	Outlet	0.04±0.03	0.02	0.08	2.0
Cd	Inlet	<0.02			-
	Outlet	<0.02	-	-	0.01
Pb	Inlet	<0.01			-
	Outlet	<0.01	-	-	0.05
Ni	Inlet	<0.1			-
	Outlet	<0.1	-	-	0.2

*MP – Monitoring points - inlet (1) and outlet (2) of MWWTP

TABLE 3
Content of microorganisms in treated wastewater, 2016 (n=6)

Parameters	Unit	MP*	C_x±SD	C_{min}	C_{max}	Standard Limit [33]
Aerobic mesophilic microorganisms	CFU/mL ($x10^3$)	Inlet	308.7±172.2	124.0	600.0	-
		Outlet	4.43±3.13	0.98	9.40	-
Total Coliforms	CFU/mL ($x10^2$)	Inlet	174.5±187.3	60.0	540.0	-
		Outlet	4.25±3.02	0.11	7.60	1–10 CFU/mL
Escherichia coli	CFU/mL ($x10^2$)	Inlet	118.3±82.3	20.0	240.0	-
		Outlet	1.57±1.10	0.10	3.00	Up to 1 CFU/mL
Enterobacteriaceae	CFU/mL ($x10^2$)	Inlet	460.0±312.7	150.0	1000.0	-
		Outlet	7.48±2.7	3.00	10.0	Not allowed
Salmonella spp.	CFU/mL ($x10^2$)	Inlet	100.0±68.7	40.0	210.0	-
		Outlet	2.07±1.08	1.00	4.00	Not allowed

*MP – Monitoring points - inlet (1) and outlet (2) of MWWTP

The ranking of microbiological parameters by absolute count of microorganisms was similar for both MPs: *Enterobacteriaceae*>AMO>Coliforms> *E. coli*>*Salmonella* spp. The positions of the last two groups at MP-2 (outlet) were interchanged.

The RMW values for *E. coli* found in the present study were lower than the *E. coli* values (91–140.10^3 MPN/mL) and higher than the *Enterobacteriaceae* values (190–340.10^2 MPN/mL), established by Lukas et al. [3] in two MWWTPs, located along the Seine River in the Parisian area, France. In this connection, Manania et al. [51] argues that the quantity of enterobacteria (CFU/100 mL) is significantly higher in WWTPs receiving industrial influents as compared to WWTPs receiving only domestic influents. Considering that the study station receives mixed wastewater (about 85% domestic and 15% industrial), perhaps this is one of the reasons for the greater number of enterobacteria in RMW of our plant than in French WWTPs.

The TMW results obtained for total coliforms were comparable to those reported by Alikhasi et al. [37] - 81.2 CFU/ml, Heidarpour et al. [48] - 577.5±17.5 CFU/mL, Petousi et al. [44] – 40.9±13.8 MPN/mL and Panoras et al. [49] – 1.5-40 CFU/mL; much lower than the data published by Rojas-Valencia et al. [12] – 1200 CFU/mL, Al-Jaboobi et al. [46] – 160 000-310 000 CFU/mL and Zavadil [47] – 32 694 CFU/mL, and higher than the results found by Aiello et al. [40] – 1.32 CFU/mL and Galavi et al. [42] – 0.85 CFU/mL. Similar data for *E. coli* were published by Aiello et al. [40] – 1.32 CFU/mL, while lower values – by Pereira et al. [43] and Petousi et al. [44] - 0.23±0.18 MPN/mL. With regard to *Salmonella* spp. the established values coincided with those of Al-Jaboobi et al. [46] – 210 CFU/mL, while other authors (Aiello ey al. [40]) have not detected *Salmonella* in treated urban wastewater (cited authors' values were recalculated in CFU/mL).

The above-mentioned data from our and other studies revealed a large quantitative variation in the monitored microbiological parameters. This is logical because the factors affecting WW microorganisms species diversity and their counts are different by type and by degree of impact.

Removal Efficiency of MWWTP. Treatment plant removal efficiency was calculated on the basis of the difference between respective inlet and outlet concentrations of the monitored WW parameters (Table 4). Parameters whose concentrations did not change or changed insignificantly in both MPs (T °C, pH, EC, Ca, Mg, Cr, Cu, Cd, Pb and Ni) are not presented in the table. MWWTP demonstrated different removal efficiency with respect to different monitored parameters. According to the level of concentrations reduction, they can be divided in four groups: Ist group – with very high removal efficiency (94.1-97.8%): BOD_5, TP and all microbiological parameters; IInd group – with high removal efficiency (74.9-89.8%): SS, COD, total N and fats; IIIrd group - with moderate removal efficiency (40.4-53.9%): Fe/total/ and Zn, and IVth group – with low removal efficiency (8.31-27.9%): total Na, Cl, SO_4 and Mn.

TABLE 4
Removal efficiency of MWWTP

Parameters	2015		2016	
	Reduction		Reduction	
	mg/L	%	mg/L	%
SS	54.3±23.4	85.4±19.4	53.6±17.7	89.8±5.14
COD	180.0±43.9	89.3±4.47	176±51.3	89.6±4.10
BOD_5	83.7±32.5	94.1±2.52	76.2±26.0	95.5±2.46
Total N	19.7±7.85	76.7±8.82	59.9±47.5	87.1±9.36
Total P	48.7±20.5	97.8±0.75	37.5±11.0	97.0±1.00
Total Na	6.55±6.00	15.0±12.7	4.53±4.06	11.2±10.1
Cl^-	-	-	3.08±2.67	8.31±6.63
SO_4^{2-}	0.61±0.76	15.1±13.3	0.57±0.40	16.8±10.2
Fats	11.4±2.81	76.8±4.65	12.1±3.08	74.9±9.08
Fe /total/	-	-	0.03±0.02	53.9±17.1
Zn	-	-	0.02±0.01	40.4±13.5
Mn	-	-	0.01±0.01	27.9±7.10
	CFU/mL	%	CFU/mL	%
AMO*	-	-	304.3 (x10^3)	97.7±2.30
Coliforms	-	-	170.3 (x10^2)	96.3±4.23
Escherichia coli	-	-	116.7 (x10^2)	97.8±1.96
Enterobacteriaceae	-	-	452.5 (x10^2)	97.5±2.25
Salmonella spp.	-	-	97.9 (x10^2)	97.1±2.59

*AMO- Aerobic mesophilic microorganisms

TABLE 5
Pearson's correlation matrix for the physicochemical parameters - Inlet, 2015-2016

	T°C	pH	EC	SS	BOD_5	TN	TP	COD	TH	Ca	Mg	TK	Cl	SO_4	Fats	TNa
T°C	1.000															
pH	-0.509*	1.000														
EC	-0.568*	0.341	1.000													
SS	0.129	-0.251	-0.232	1.000												
BOD_5	-0.250	0.400	0.514*	-0.019	1.000											
TN	0.121	-0.402	-0.057	0.052	0.216	1.000										
TP	0.141	-0.338	0.202	-0.161	0.002	-0.193	1.000									
COD	0.347	-0.136	0.331	0.300	0.522*	0.183	0.306	1.000								
TH	-0.668*	0.595*	0.205	-0.116	0.461*	-0.301	-0.064	-0.104	1.000							
Ca	-0.364	0.457*	0.248	-0.217	0.315	-0.278	-0.031	-0.224	0.305	1.000						
Mg	-0.149	0.264	0.138	-0.044	0.338	-0.263	0.224	0.044	0.313	0.878*	1.000					
TK	-0.461*	-0.160	0.486*	0.036	-0.077	0.349	-0.011	-0.227	-0.308	0.156	-0.055	1.000				
Cl	0.315	-0.430	-0.032	-0.275	-0.456*	0.033	-0.006	-0.121	-0.523*	-0.367	-0.476*	0.182	1.000			
SO_4	-0.741*	0.245	0.739*	-0.274	0.173	-0.001	0.279	-0.177	0.218	0.441*	0.333	0.724*	-0.159	1.000		
Fats	-0.576*	-0.012	0.519*	-0.297	-0.105	0.304	-0.054	-0.194	0.028	-0.314	-0.537*	0.638*	0.319	0.555*	1.000	
TNa	0.357	-0.579*	-0.006	0.178	-0.353	-0.154	0.405	0.235	-0.334	-0.277	-0.005	0.037	0.520*	-0.042	-0.053	1.000

* Marked correlations are significant at p < .05000 N=12 (Casewise deletion of missing data)

Rupali et al. [5] reported similar COD (90.1-93.1%) and BOD_5 (85.6-91.6%) removal efficiency, and a slightly higher one - for SS (93.8-96.4%) for Vithalwadi MWWTP, Pune region, India. The efficiency of carbonaceous (TH) and nitrogenous matters removal in studied MWWTP is confirmed by the TMW COD and total N values, which were less than 100 mgO_2/L and 10 mgN/L, respectively, according to effluent standard. The effectiveness of suspended matter removal was less than 90% (on average 85.4%-89.8% for 2015 and 2016), i.e. below the lower limit value by the standard [52]. BOD_5 removal efficiency reached the planned levels (80-95%) for a WW treatment plant with aerations tanks and biofilters [1]. Removal of all groups of monitored microorganisms was slightly lower than 99%, a level of decontamination that can be achieved at primary and secondary treatment of urban WW [53].

Despite the high treatment plant removal efficiency with regard to microorganisms content, it was not possible to achieve complete decontamination of treated wastewater. About 2.2-3.7% of AMO, total coliforms, *E. coli*, *Enterobacteriaceae* and *Salmonella* spp. remained in the effluents, posing a potential risk for water pollution of Bedechka River (receiver), where TMW are discharged or for soil and cultivated plants if wastewater is used as a source for irrigation.

Plant treatment processes did not affect Cr, Cu, Cd, Pb and Ni concentrations and their levels were identical at the input and output. The most likely cause were the very low concentrations of the elements, below the sensitivity threshold of the plant treatment facilities. At the same time, Fe and Mn contents, which were of the same magnitude as the elements mentioned above, decreased significantly (P>0.05). Total Fe concentration in TMW at the outlet decreased, irrespective of the addition of coagulant ($FeCl_3.6H_2O$) in bio-basins, because in the wastewater treatment processes, most of the Fe, together with the WW phosphorus compounds, pass into the activated sludge. We assume that a similar mechanism was involved in Mn content reduction, albeit to a lesser extent compared to Fe. Future research and the accumulation of more data would contribute to find a convincing explanation of the observed trends.

Statistical Analysis. Correlation Matrix. The correlation matrices revealed 127 strong positive and negative relationships (P<0.05-0.001) between monitored WW parameters on inlet and on outlet of MWWTP as followed:

Inlet. Physicochemical parameters (Table 5): pH – TH and Ca, EC – BOD_5, TK, SO_4 and fats, BOD_5 – COD and TH, Ca – Mg and SO_4, TK – SO_4 and fats, Cl – TNa, SO_4 – fats (r = 0.441 – 0.878), T °C – pH, EC, TH, TK, SO_4 and fats, pH – TNa, Cl – BOD_5, TH and Mg, Mg – fats (r = -0.456 – -0.741); heavy metals (Table 7): Zn – Mn, Fe and Cu, Mn – Fe and Cu, Fe – Cu (r = 0.822 – 0.999); microbial parameters (Table 8): AMO – *Enterobacteriaceae*, *E.coli* – *Salmonella* spp. and Coliforms, *Salmonella* spp. – Coliforms (r = 0.684 – 0.835), AMO – *E.coli* and *Salmonella* spp. (r = -0.665 – -0.715); all parameters: T °C – *E.coli*, *Salmonella* spp. and Coliforms, TH – AMO and Zn, Mg – Zn, *Enterobacteriaceae* – Cl and TNa (r = 0.509 – 0.844), T °C – AMO, pH – *E.coli* and *Salmonella* spp., TH – *E.coli*, *Salmonella* spp. and Coliforms, Ca/Mg – *Enterobacteriaceae* and Coliforms, Cl – Zn, Mn and Fe, *Enterobacteriaceae* – Zn, Mn and Fe (r = -0.523 – -0.986).

Outlet. Physicochemical parameters (Table 6): T °C – TP, COD and Cl, EC – SS, TK and fats, SS – TK and fats, BOD_5 – TH, TN – Cl and TNa, TP – Cl, TH – Ca, Mg and SO_4, Ca – Mg and SO_4, Mg – SO_4 and Cl – TNa (r = 0.479 – 0.877), T °C – pH, EC, SS, SO_4 and fats, pH – COD, TK, Cl and TNa, SO_4 – TN, TP, Cl and TNa, EC – TN and TNa, BOD_5 – TP, Mg – Cl and fats – TNa (r = -0.487 – -0.788); heavy

metals (Table 7): Cu – Zn, Mn and Fe, and Zn – Fe (r = 0.813 – 0.999); microbial parameters (Table 8): AMO – *E.coli* and Coliforms, *E.coli* – *Enterobacteriaceae* and *Salmonella* spp. – Coliforms (r = 0.474 – 0.632); all parameters: T °C – AMO, *Salmonella* spp. – TN, Cl and TNa, Mn – TNa, *E.coli* – TK and Coliforms – Cl (r = 0.584 – 0.944), Coliforms – SO_4, Ca, Mg, Zn and Fe, *E.coli* – Zn and Fe, *Salmonella* spp. – Mn and SO_4, Cl – Zn, Mn and Fe, and TH – Mn (r = -0.576 – -0.854). Unlike our results, Iordache and Dunea [4] reported different and mostly positive correlations between physicochemical parameters (T°C, pH, COD, BOD, nitrites, nitrates, ammonium, phosphates, sulfates, chlorides and detergents) of municipal treated wastewater. Exceptions are the associations T °C – pH, COD and Cl, pH – SO_4, COD – Cl and phosphates – Cl, which coincide with ours.

TABLE 6
Pearson's correlation matrix for the physicochemical parameters - Outlet, 2015-2016

	T°C	pH	EC	SS	BOD_5	TN	TP	COD	TH	Ca	Mg	TK	Cl	SO_4	Fats	TNa
T°C	1.000															
pH	-0.499*	1.000														
EC	-0.563*	-0.027	1.000													
SS	-0.512	-0.263	0.713*	1.000												
BOD_5	-0.157	0.361	-0.185	-0.380	1.000											
TN	0.362	-0.427	-0.649*	-0.298	0.017	1.000										
TP	0.494*	-0.351	-0.211	-0.326	-0.528*	0.303	1.000									
COD	0.479*	-0.557*	0.225	0.047	-0.250	-0.185	0.274	1.000								
TH	-0.335	0.419	-0.074	-0.242	0.464*	-0.263	-0.210	-0.101	1.000							
Ca	-0.342	0.123	0.064	0.127	0.258	-0.201	-0.289	0.036	0.852*	1.000						
Mg	-0.184	0.371	-0.179	-0.243	0.356	-0.216	-0.254	-0.070	0.856*	0.877*	1.000					
TK	-0.280	-0.487*	0.460*	0.800*	-0.215	-0.005	-0.154	0.191	-0.019	0.285	-0.193	1.000				
Cl	0.496*	-0.626*	-0.194	-0.144	-0.109	0.527*	0.486*	0.364	-0.333	-0.402	-0.604*	0.291	1.000			
SO_4	-0.588*	0.408	0.286	0.266	0.291	-0.536*	-0.559*	-0.154	0.718*	0.820*	0.804*	0.114	-0.788*	1.000		
Fats	-0.699*	0.261	0.575*	0.728*	-0.314	-0.370	-0.271	-0.275	-0.284	-0.114	-0.252	0.290	-0.431	0.248	1.000	
TNa	0.425	-0.642*	-0.478*	-0.198	0.009	0.805*	0.243	0.178	-0.080	-0.012	-0.181	0.255	0.764*	-0.487*	-0.562*	1.000

* Marked correlations are significant at p < .05000 N=12 (Casewise deletion of missing data)

TABLE 7
Pearson's correlation matrix for the heavy metal concentrations, 2016

Parameters	Zn	Mn	Fe	Cu
		Inlet		
Zn	1.000			
Mn	0.955*	1.000		
Fe	0.944*	0.999*	1.000	
Cu	0.991*	0.822*	0.963*	1.000
		Outlet		
Zn	1.000			
Mn	0.487	1.000		
Fe	0.999*	0.442	1.000	
Cu	0.933*	0.799	0.853*	1.000

*Marked correlations are significant at p < .05000 N=6 (Casewise deletion of missing data)

TABLE 8
Pearson's correlation matrix for the microbiological parameters, 2016

Parameters	AMO	*E.coli*	*Enterobacteriaceae*	*Salmonella* spp.	Coliforms
			Inlet		
AMO	1.000				
E.coli	-0.665*	1.000			
Enterobacteriaceae	0.684*	-0.110	1.000		
Salmonella spp.	-0.715*	0.835*	0.018	1.000	
Coliforms	-0.377	0.805*	0.199	0.743*	1.000
			Outlet		
AMO	1.000				
E.coli	0.535*	1.000			
Enterobacteriaceae	-0.013	0.632*	1.000		
Salmonella spp.	-0.225	-0.170	-0.200	1.000	
Coliforms	0.474*	0.331	0.208	0.568*	1.000

AMO- Aerobic mesophilic microorganisms
*Marked correlations are significant at p < .05000 N=6 (Casewise deletion of missing data)

Data analysis shows a slightly higher number of correlations at outlet than those at inlet – 61 (32 positive and 29 negative) and 66 (35 positive and 31 negative), respectively. The majority of the established correlations at inlet (21 positive and 21 negative) differed from those at outlet (24 positive and 23 negative). This greater diversity with increase of outlet correlations compared to inlet ones is due to the different character of the change in parameters' values during wastewater treatment. This way, conditions are created for the emergence of new relationships between the WW parameters. Another part of the inlet correlations were retained also at outlet – 11 positive (EC –TK and fats, Ca – Mg and SO_4, Cl – TNa, BOD – TH, Cu – Fe, Zn and Mn, Zn – Fe, *Salmonella* spp. – coliforms) and 8 negative (T^oC – pH, EC and fats, pH – TNa, Cl – Mg, Zn, Mn, Fe). These correlations can be differentiated into three groups. The first group includes associations between parameters that did not change or changed insignificantly their inlet-outlet values (T^oC – pH and EC, pH – TNa, EC –TK, Cl – Mg and TNa, Ca – Mg), which explains their preservation. The second group refers to relationships between pairs of parameters that reduce their values in a similar way between both MPs (Zn – Fe, *Salmonella* spp. – coliforms), so they are also logical and understandable. The third group of correlations addresses parameters that changed significantly their outlet values compared to inlet values (BOD – TH, EC – fats, Cu – Fe, Zn and Mn) – a fact that is difficult to be explained at this stage.

Parameters involved in the most numerous correlations were T^oC and Cl (each of them with 19 correlations, 7/7 positive and 12/12 negative), followed by TH, coliforms and Zn (14, 7/6/7 and 7/8/7, respectively), pH (11, 2 and 9), EC and *Salmonella* spp. (10, 6/6 and 4/4), SO_4, *E.coli*, *Enterobacteriaceae*, Mg, Mn and Fe (9, 5/5/4/4/4/4 and 4/4/5/5/5/5), AMO (8, 5 and 3), Fats and Ca (7, 4/3 and 3/4), BOD_5 (6, 4 and 2), COD (3, 2 and 1), etc. This progression largely reveals the role and importance of the cited parameters for the occurrence of associations between all WW parameters groups - physicochemical, heavy metals, microbiological. The key importance of WW temperature, EC and pH is logical and understandable, as these factors form the substrate environment and thus affect positively or negatively the relationships between the rest of WW parameters. The leading position of TH, coliforms and Zn as well as the comparatively more backward positions of the BOD_5 and COD still remain unexplained.

Factor Analysis (FA). Multivariate analysis reduced the number of factors that explained the variability in the data set of the 21 WW physicochemical and microbiological parameters to 11 at inlet and outlet (Figures 1 and 2). Eight factors (F1-F8) for each of the two MPs met the Kaisser criteration (>1) which accounted for 98.2% (inlet) and 99.2% (outlet) of the data set variability, respectively. According to Nagaraju et al. [54] when factors variance exceeds 70%, they are sufficient to explain the mechanisms of water composition formation. Therefore, it can be argued that the factors with the most significant influence were limited to two at inlet (F1 – eigenvalue 44.770, 62.0% and F2 eigenvalue 10.391, 14.4%, total 76.4%) (Figure 1) and one at outlet (F1 – eigenvalue 85.450, 72.3%) (Figure 2).

WW quality parameters affected differently the specific inlet-outlet factors. Inlet EC was the most significantly loaded on Factor 1 of the Rotated factor loading matrix (Table 9). A group of 12 parameters also influenced Factor 1, but 1.34 to 3.29 times less than EC did (SO_4>TN>TK>TH>Fats>Ca> BOD_5> COD>TP>Mg>Cl>SS). The last 8 parameters (*E. coli*, *Salmonella* spp., coliforms, AMO, T^oC, TNa, pH and *Enterobacteriaceae*) had negligible impact on F1 (<0.976). Factor 2 was the most considerably affected by AMO, *E. coli*, coliforms and *Salmonella* spp.

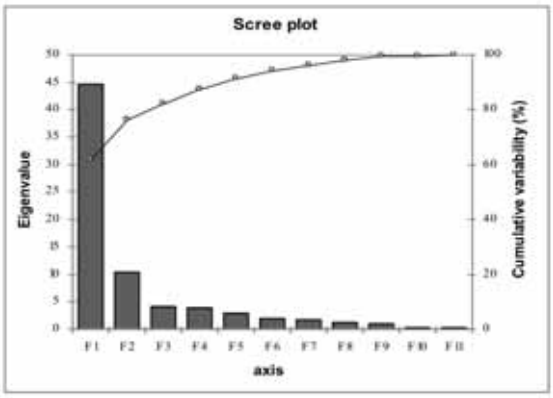


FIGURE 1
Scree plot of the inlet eigenvalues

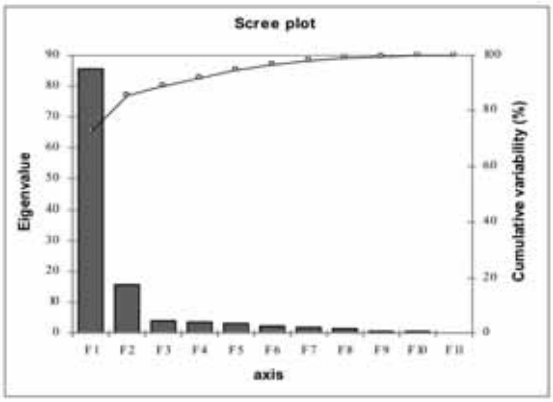


FIGURE 2
Scree plot of the outlet eigenvalues

TABLE 9
Rotated factor loading matrix

Parameters	Inlet		Parameters	Outlet	
	Factor 1	Factor 2		Factor 1	Factor 2
EC	3.341	-0.155	EC	5.726	0.491
SO_4	2.487	-0.635	*Salmonella* spp.	2.797	-0.696
Total N	1.941	-0.359	Total P	2.786	2.241
Total K	1.751	-0.326	*E. coli*	2.763	-0.621
TH	1.673	-0.431	Coloforms	1.989	-0.508
Fats	1.596	-0.106	SO_4	1.849	-0.476
Ca	1.428	-0.044	SS	1.668	1.577
BOD_5	1.410	0.014	Fats	1.598	-0.427
COD	1.409	0.091	Total N	1.562	0.663
Total P	1.372	-0.372	Ca	1.438	-1.764
Mg	1.151	-0.177	Chlorides	1.276	-0.820
Chlorides	1.039	-0.067	Total K	1.254	-0.469
SS	1.015	-0.032	AMO	1.215	-0.548
E. coli	0.976	1.420	TH	1.163	0.071
Salmonella spp.	0.825	1.067	BOD_5	1.155	0.273
Coloforms	0.784	1.385	*Enterobacteriaceae*	1.024	-0.412
AMO*	0.692	1.842	COD	0.979	-0.533
Temperature	0.680	-0.141	Mg	0.979	-0.808
Total Na	0.650	0.231	Temperature	0.964	0.133
pH	0.407	-0.062	Total Na	0.761	-0.662
Enterobacteriaceae	0.364	0.894	pH	0.618	0.095

*AMO- Aerobic mesophilic microorganisms

Outlet Factor 1 as inlet Factor 1 was also the most influenced by EC, even to a greater extent. The other parameters that loaded on F1 (>1.000) were 15 and were arranged as followed: *Salmonella* spp. > TP > *E. coli* > coliforms > SO_4 > SS > Fats > TN >Ca > Cl > TK > AMO > TH > BOD_5 > *Enterobacteriaceae*. Total phosphorus and SS kept their influence and on Factor 2 and were the only parameters with such an impact. Parameters influencing Factor 2 differed also between inlet and outlet, 4 microbiological parameters with loading 1.067-1.842 compared to 2 chemical parameters with loading 1.577-2.241, respectively.

Factorial analysis for the physicochemical parameters based on the factors MP, Month and Year, and for microbiological parameters based on the factors MP and Month showed that the factor MP had the greatest influence on pH, SS, COD, BOD_5, TN, TP, TK, TNa and fats (46.0-89.1% in all variations) ($P<0.001$); a moderate one - on SO_4 (40.6% in all variations) ($P<0.01$); low – on Cl, AMO, *E. coli* and *Salmonell*a spp. (16.7-54.9% in all variations) ($P<0.05$), and insignificant - on T°C, EC, total coliforms and *Enterobacteriaceae* (0.26-36.6% in all variations) ($P>0.05$). The greatest impact of the factor Month was on T°C, EC, TK and TNa (42.0-98.6% in all variations) ($P<0.001$); moderate - on Cl^- (57.8% in all variations) ($P<0.01$); low - on pH and SO_4 (7.82-34.1% in all variations) ($P<0.05$) and insignificant - on SS, COD, BOD_5, TN, TP, fats and all microbial parameters (1.15-34.1% in all variations) ($P>0.05$). Year was a factor significantly influencing EC (12.9% in all variations) ($P<0.01$) and TP (6.15% in all variations) ($P<0.05$), while the influence on the rest of the parameters was not statistically significant.

Data analysis shows that the factor MP had the most statistically significant influence on the studied parameters (14) followed by factor Month (7) and factor Year (2). The strength of the factor MP was determined by the fact that the process of WW treatment from inlet to outlet of the treatment plant significantly reduced the levels of a large part of the controlled parameters. The factor Month influenced mainly T°C and some physicochemical parameters as the WW temperature and salt content, changed during the different months of the year (alternation of working days and weekends, summer and winter holidays, etc.). The factor Year had the least impact on WW parameters, giving reason to believe that the municipal wastewater composition had not changed significantly over the years.

PCA. The contribution of Factors 1 and 2 (factor scores) on WW physicochemical and microbiological variability at inlet-outlet are presented on Figures 3 and 4. Inlet scatter plot (Figure 3) showed that TNa, COD, BOD_5 and all microbiological parameters were positively affected by the two factors (F1 and F2), e.g. these factors had an active role on RMW quality. Temperature, pH, SS, Cl, Ca, Mg, fats, TH, TP, TK, TN, SO_4 and EC were positive by F1, describing 61.04% of variations and negative by

F2. Consequently, F1, which significantly dominated F2 (14.4%), had a positive impact on these parameters. It should be noted that the two factors' strong influence was well emphasized on TNa and *Enterobacteriaceae*. The effect of F1 on pH and T°C was considerable, while at the other parameters the factors effect decreased.

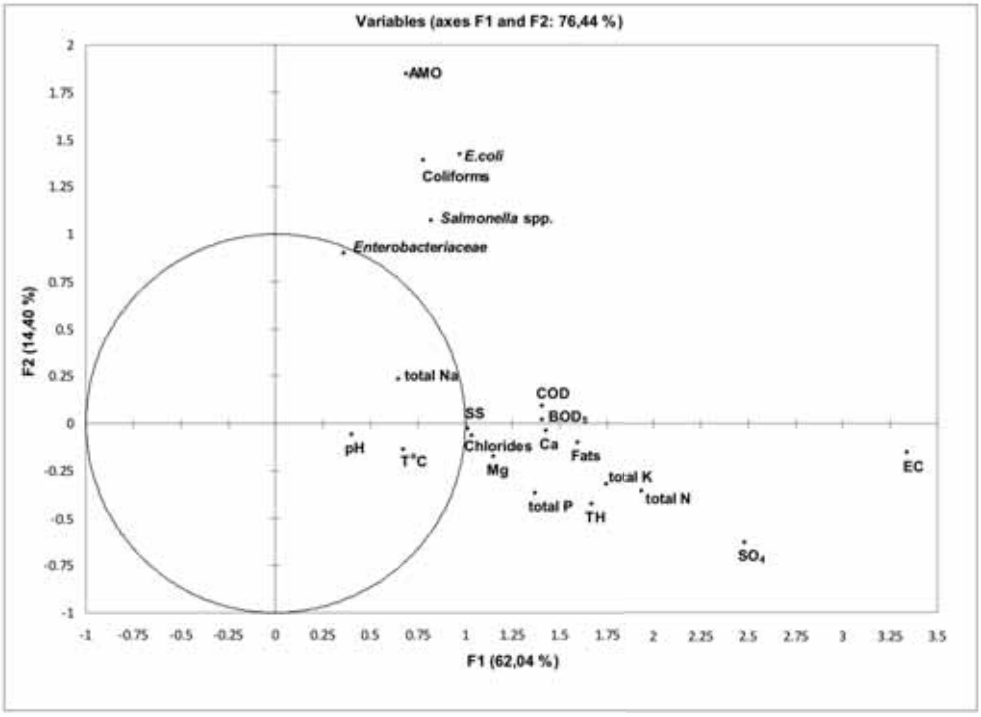


FIGURE 3
Scatter plot of wastewater parameters determined by PCA - inlet

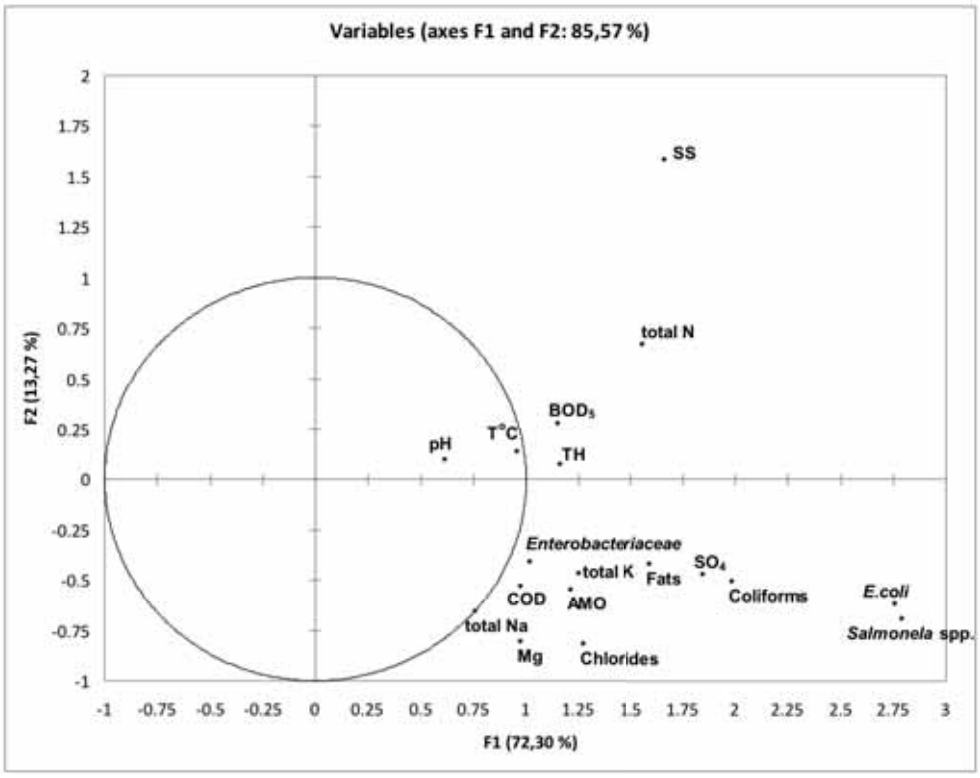


FIGURE 4
Scatter plot of wastewater parameters determined by PCA - outlet

Outlet scatter plot (Figure 4) presents the same picture, but with a different distribution of the TMW parameters. PCA showed that the pH, TºC, TH, BOD_5, TN and SS were the parameters influenced positively by the two factors, while the rest of the parameters were positively affected by F1, describing 72.30% of the variations and negatively by F2 (13.27%). The positive influence of both factors was most pronounced on pH and TºC, and less on TH, BOD_5, TN and SS. The positive influence of F1 was the strongest on TNa and gradually decreases with regard to *Enterobacteriaceae*, COD, Mg, AMO, TK, Cl, fats, SO_4, coliforms, *E. coli*, and *Salmonell*a spp.

TMW Quality Status Assessment According to Requirements for Discharge in the Water Receiver (Bedechka River). This assessment, carried out by 5 chemical parameters (SS, COD, BOD, total N and total P) included in Bulgarian legislation [32] showed that SS, COD, BOD and total N maximum concentrations were 2.17, 3.64, 3.12 and 1.17 times lower, respectively than the relevant standard limit (Table 1). Exceptions were the maximum total phosphorus concentrations, which exceeded the standard limit 1.09-1.29 times during January-July in 2015 and March-December (without May) in 2016. For that reason, the treatment plant can not guarantee the removal of excess phosphate amounts from wastewater for most of the year. Therefore, during the months with total phosphorus levels over the standard limit, treated wastewater did not meet the requirements for discharge in Bedechka River. It is important to note that the treatment plant is designed and constructed to remove excess nitrogen and phosphorus levels in the third stage of wastewater treatment. Obviously, for phosphates, this was not the case. The reasons may be both constructive and technological (inappropriate coagulant or deviations in the mode of its application) flaws. Whatever the reason, the problem needs to find an appropriate solution.

TMW Quality Status Assessment as a Source for Irrigation. Assessment of TMW quality as a source for irrigation was carried out by five groups of parameters [33]: Group A – salinity (EC), Group B - water infiltration rate (total Na, Ca, Mg, total K), Group C - toxicity (Fe, Mn, Cr, Cu, Zn, Cd, Pb, Ni), Group D – sanitary quality indices (total coliforms, *E. coli*, *Enterobacteriaceae* and *Salmonella* spp.), Group E – miscellaneous (TºC, pH, TH, SS, COD, BOD_5, total N, total P, chloride, sulphates, fats). Parameters, which values were lower than the limit values stipulated in the standard were as followed: EC from 1.84 to 3.51 times, total Na from 6.41 to 11.9 times, Ca from 5.40 to 6.64 times, Mg from 13.4 to 21.9 times, total K from 29.7 to 72.0 times, Fe from 100 to 500 times, Mn from 6.67 to 10.0 times, Cu 4.0 times, Zn from 25 to 100 times, Pb 5.0 times, Ni 2.0 times, TºC from 1.05 to 2.94 times, TH from 2.52 to 4.79 times, SS from 3.10 to 15.9 times, COD from 2.91 to 11.1 times, BOD_5 from 3.12 to 12.5 times, total N from 2.33 to 7.87 times, total P from 2.32 to 4.61 times, chloride from 7.51 to 10.3 times and sulphates from 84.5 to 149.2 times. pH values were in the optimum range from 6 to 9 (Tables 1, 2 and 3). In terms of cadmium, it can be assumed that the total Cr content was also below the permissible limit since it did not exceed the Cr^{6+} and Cr^{3+} limit levels specified in the standard.

Above the permissible standard limits were the maximum values for fats (1.04-1.08 times), all values for total coliforms (from 1.1 to 7.6 times), *E. coli* (from 10 to 300 times) as well as for *Enterobacteriaceae* and *Salmonella* spp., which are not allowed in water for irrigation (Tables 2 and 3). Bulgarian legislation [33] allows such waters to be used for irrigation only after decontamination. Given that global drought-related climate change affects also Bulgaria, providing opportunities for using treated wastewater for irrigation will improve soil fertility and protect water quality in the receiving water body and other associated water bodies.

CONCLUSION

Results obtained for inlet-outlet wastewater quality of the most modern MWWTP in Bulgaria showed:

(1) Different ranges of variability and trends of inlet-outlet values changes of the monitored wastewater parameters: TºC, pH, EC, TH, Ca, Mg, SS, COD, BOD_5, total N, total P, total K, total Na, Cl, SO_4, fats, Fe, Mn, Cr, Cu, Zn, Cd, Pb, Ni, aerobic mesophilic microorganisms, total coliforms, *E.coli*, *Enterobacteriaceae* and *Salmonella* spp.;

(2) The treatment plant demonstrated different removal efficiency with respect to different wastewater parameters: very high for BOD_5, total P and all microbiological parameters; high for SS, COD, total N and fats; moderate for Fe/total/ and Zn, and low for total Na, Cl, SO_4 and Mn;

(3) 127 strong positive and negative correlations ($P<0.05$-0.001) between controlled wastewater parameters – 61 correlations (32 positive and 29 negative) at inlet and 66 correlations (35 positive and 31 negative) at outlet;

(4) The parameters involved in the most numerous correlations were TºC and Cl (each of them in 19 correlations), TH, coliforms and Zn (14), pH (11), EC and *Salmonella* spp. (10), SO_4, *E. coli*, *Enterobacteriaceae*, Mg, Mn and Fe (9), AMO (8), fats and Ca (7) and BOD_5 (6);

(5) EC was the most significantly loaded on Factor 1 of Rotated factor loading matrix both at the influents and the effluents, the other parameters influencing Factor 1 (>1.000) were 12 (SO_4, TN, TK, TH, fats, Ca, BOD_5, COD, TP, Mg, Cl, SS) at inlet and 15 (*Salmonella* spp., TP, *E. coli*, coliforms, SO_4,

SS, fats, TN, Ca, Cl, TK, AMO, TH, BOD_5, *Enterobacteriaceae*) at outlet;

(6) Factor analysis determined Monitoring point as a factor with significant influence on pH, SS, COD, BOD_5, TN, TP, TK, TNa, fats SO_4, Cl, AMO, *E. coli* and *Salmonell*a spp.; factor Month - on T°C, EC, TK, TNa, Cl and SO_4, and factor Year - on EC and TP;

(7) PCA at inlet wastewater determined TNa, COD, BOD_5 and all microbiological parameters as positively affected by two factors (F1 and F2), while T°C, pH, SS, Cl, Ca, Mg, fats, TH, TP, TK, TN, SO_4 and EC were positive by F1, and negative by F2; at outlet - pH, T°C, TH, BOD_5, TN and SS were the parameters influenced positively by two factors, while the rest of the parameters were positively affected by F1 and negatively by F2;

(8) Treated wastewater did not meet the requirements for discharge in receiving water body (as total P content was concerned) and for irrigation (with respect to fats content and the number of total coliforms, *E. coli*, *Enterobacteriaceae* and *Salmonella* spp.).

REFERENCES

[1] Taraschenko, S. (2009) Wastewater treatment in Antarctica. (Supervisor N. Gilbert). GCAS, 18-31.

[2] Kushwah, R.K., Bajpaia, A., Malik, S., Singh, A. (2011) Seasonal variation of physicochemical parameters of wastewater from a sewage treatment plant, Bhopal (India). Int. J. Chem. Sci. 9(3), 1545-1552.

[3] Lukas, F.S., Therial, C., Gonçalves, A., Servais, P., Rocher, V., Mouchel, J.-M. (2014) Variation of raw wastewater microbiological quality in dry and wet weather conditions. Environ. Sci. Pollut. Res. 21, 5318-5328.

[4] Iordache, S., Dunea, D. (2013) Multivariate analysis applied to physicochemical parameters of treated wastewater effluents discharged in Teleajen River. Rev. Roum. Chim. 58(7-8), 717-723.

[5] Rupali, A.J., Soumya, B.S., Thanedar, R.P. (2015) Statistical evaluation of wastewater characteristics at the inlet - outlet of an activated sludge process. Int. Journal of Engineering Research and Applications. 5(1), 82-96.

[6] Velichkova, K.N., Sirakov, I.N., Beev, G.G., Denev, S.A., Pavlov, D.H. (2016) Treatment of wastewater originated from aquaculture and biomass production in laboratory algae bioreactor using different carbon sources. Sains Malaysiana. 45(4), 601-608.

[7] Heidarpour, M., Mostafazadeh-Fard, B., Abedi Koupai, J., Malekian R. (2007) The effects of treated wastewater on soil chemical properties using subsurface and surface irrigation methods. Agricultural Water Management. 90, 87-94.

[8] Jalali, M., Merikhpour, H., Kaledhonkar, M.J., Van Der Zee, S.E.A.T.M. (2008) Effects of wastewater irrigation on soil sodicity and nutrient leaching in calcareous soils. Agricultural Water Management. 95, 143-153.

[9] Singh, P.K., Deshbhratar, P.B., Ramteke, D.S. (2012) Effects of sewage wastewater irrigation on soil properties, crop yield and environment. Agricultural Water Management. 103, 100-104.

[10] Najafi, P., Tabatabaei, S.H., Taheri-Sodejani, H. (2015) Long-term effects of different irrigation methods with treated wastewater on soil chemical properties. Journal of Water and Soil. 29(1), 12-22.

[11] Westcot, D.W. (1997) Quality control of wastewater for irrigated crop production. Water reports -10. FAO, Rome, 6-16.

[12] Rojas-Valencia, M.N., Orta de Velásquez, M.T., Franco, V. (2011) Urban agriculture, using sustainable practices that involve the reuse of wastewater and solid waste. Agricultural Water Management. 98, 1388-1394.

[13] Amerasinghe, P., Bhardwaj, R.M., Scott, Ch., Jella, K., Marshall, F. (2013) Urban wastewater and agricultural reuse challenges in India. International Water Management Institute (IWMI) Research Report 147. PO Box 2075, Colombo, Sri Lanka, 3-24.

[14] Salama, Y., Chennaoui, M., Sylla, A., Mountadar, M., Rihani, M., Assobhei, O. (2014) Review of wastewater treatment and reuse in the Morocco: Aspects and perspectives. International Journal of Environment and Pollution Research. 2(1), 9-25.

[15] Myszograj, S., Qteishat, O., Sadecka, Z., Jędrczak, A., Suchowska-Kisielewicz, M. (2014) Possibilities of reuse of treated wastewater for irrigation purposes in the northern Jordan valley. Environment Protection Engineering. 40(2), 17-31.

[16] Shakir, E., Zahraw, Z., Hameed, A., Al-Obaidy, M.J. (2017) Environmental and health risks associated with reuse of wastewater for irrigation. Egyptian Journal of Petroleum. 26(1), 95-102.

[17] Templeton, M., Butler, D. (2011) An Introduction to wastewater treatment. Ventus Publishing ApS. UK, 8-16.

[18] Kostadinova, G., Georgieva, N., Yaneva, Z., Petkov, G., Todorova, M., Miteva, Ch. (2013) Tundzha River water quality as a source for irrigation in agriculture. Bulg. J. of Agr. Sci. 19(4), 635-643.

[19] Kostadinova G., Dermendjieva, D., Beev, G., Petkov, G., Pavlov, D., Valkova, E. (2017) Quality assessment of Maritsa River water as a main source for irrigation in Thracian valley. Fresen. Environ. Bull. 26, 4320-4324.
[20] Sotirakou, E., Kladitis, G., Diamantis, N., Grigoropoulou, H. (1999) Ammonia and phosphorus removal in municipal wastewater treatment plant with extended aeration. Global Nest: The international Journal. 1(1), 47-53.
[21] Oleszkiewicz, J.A., Barnard, J.L. (2006) Nutrient removal technology in North America and the European Union: A Review. Water Qual. Res. J. of Canada. 4, 449-462.
[22] Manav-Demir, N., Demir, S. (2017) An MS Excel tool for wastewater treatment modeling in undergraduate education: Activated sludge model No. 1. Fresen. Environ. Bull. 26, 7008-7017.
[23] Tas, D.S., Tas, O., Tascioglu, S. (2017) Determination of Pb^{2+} ions in wastewater by differential pulse polarography combined with surfactants and membrane filtration process. Fresen. Environ. Bull. 26, 7128-7136.
[24] Chen, P., Zheng, X., Ren, T., Cheng, W., Sun, J. (2017) Vertical flow artificial wetland system of waste bricks removes phosphorus in rural domestic wastewater and phosphorus transport characteristics. Fresen. Environ. Bull. 26, 7411-7418.
[25] Ozturk, E. (2018) Determination of best treatment alternatives for di-(ethylhxexyl) phthalate in drinking water and wastewater employing multiple criteria decision making methods. Fresen. Environ. Bull. 27, 2456-2467.
[26] Wang, H., An, X., Zhang, Z. (2018) Effect of advanced treatment on ammonia nitrogen contained in secondary effluent from wastewater treatment plant. Fresen. Environ. Bull. 26, 2043-2050.
[27] Council Directive 91/271/EEC of 21 May 1991 concerning urban wastewater treatment. Official Journal of the European Communities. 34, 30 May 1991.
[28] Commission Directive 98/15/EC of 27 February 1998 amending Council Directive 91/271/EEC with respect to certain requirements. Official Journal L 067, 07 March 1998.
[29] SYRB (2017) Statistical Yearbook. Republic of Bulgaria, National Statistical Institute. Sofia.
[30] Regulation No. H-4 (2012) On characterization of surface water. State Gazette. No. 22, 14.09.2012.
[31] Order of Minister of Environment and Water. No. RD-970, 28.07.2003.
[32] Regulation No. 6 (2000) On emission limits for the permissible content of harmful and dangerous substances in the wastewater discharged into water bodies. State Gazette. No. 97, 09.11.2000.
[33] Regulation No. 18 (2009) On water quality for irrigation of agricultural crops. State Gazette. No. 43, 09.06.2009.
[34] Zema, D.A., Bombino, G., Andiloro S., Zimbone, S.M. (2012) Irrigation of energy crops with urban wastewater: Effects on biomass yields, soils and heating values. Agricultural Water Management. 115, 55-65.
[35] Pescod, M.B. (1992) Wastewater treatment and use in agriculture-FAO irrigation and drainage. Paper 47, Rome, 18-36.
[36] Zavadil, J. (2009) The effect of municipal wastewater irrigation on the yield and quality of vegetables and crops. Soil and Water Res. 4(3), 91–103.
[37] Alikhasi, M., Kouchakzadeh, M., Baniani, E. (2012) The effect of treated municipal wastewater irrigation in non-agricultural soil on cotton plant. J. Agr. Sci. Tech. 14, 1357-1364.
[38] Bedbabis, S., Trigui, D., Ben Ahmed, Ch., Clodoveo, M.L., Camposeo, S., Vivaldi, G.A., Ben Rouina, B. (2015) Long-terms effects of irrigation with treated municipal wastewater on soil, yield and olive oil quality. Agricultural Water Management. 160, 14-21.
[39] Gatta, G., Libutti, A., Gagliardi, A., Beneduce, L., Brusetti, L., Borruso, L., Disciglio, G., Tarantino, E. (2015) Treated agro-industrial wastewater irrigation of tomato crop: Effectson qualitative/quantitative characteristics of production and microbiological properties of the soil. Agricultural Water Management. 149, 33-43.
[40] Aiello, R., Cirelli, G.L., Consoli, S. (2007) Effects of reclaimed wastewater irrigation on soil and tomato fruits: A case study in Sicily (Italy). Agricultural Water Management. 93, 65-72.
[41] Ahmed, T.A., Al-Hajri, H.H. (2009) Effects of treated municipal wastewater and sea water irrigation on soil and plant characteristics. International Journal of Environmental Research. 3(4), 503-510.
[42] Galavi, M., Jalali, A., Ramroodi, M., Mousavi, S.R., Galavi, H. (2010) Effects of treated municipal wastewater on soil chemical properties and heavy metal uptake by sorghum (*Sorghum Bicolor* L.). J. of Agricultural Science. 2(3), 235-241.
[43] Pereira, B.F.F., He, Z.L., Stoffella P.J., Melfi, A.J. (2011) Reclaimed wastewater: Effects on citrus nutrition. Agricultural Water Management. 98, 1828-1833.
[44] Petousi, I., Fountoulakis, M.S., Saru, M.L., Nikolaidis, N., Fletcher, L., Stentiford, E.I., Manios, T. (2015) Effects of reclaimed wastewater irrigation on olive (*Olea europaea L.* cv. 'Koroneiki') trees. Agricultural Water Management. 160, 33-40.

[45] Mojid, M.A., Wyseure, G.C.L., Biswas, S.K., Hossain, A.B.M.Z. (2010) Hossaina Farmers' perceptions and knowledge in using wastewater for irrigation at twelve peri-urban areas and two sugar mill areas in Bangladesh. Agricultural Water Management. 98, 79-86.
[46] Al-Jaboobi, M., Bouksaim, M., Tijane, M., El-Arigi, S. (2013) Agricultural quality evaluation of wastewater, used in Yemen vegetables production. Middle-East J. of Scientific Research. 16(5), 667-677.
[47] Zavadil, J. (2009) The effect of municipal wastewater irrigation on the yield and quality of vegetables and crops. Soil and Water Res. 4(3), 91-103.
[48] Heidarpour, M., Mostafazadeh-Fard, B., Abedi Koupai, J., Malekian R. (2007) The effects of treated wastewater on soil chemical properties using subsurface and surface irrigation methods. Agricultural Water Management. 90, 87-94.
[49] Panoras, A., Evgenidis, G., Bladenopoulou, S., Melidis, V., Doitsinis A., Samaras, I., Zdragkas A., Matsi, Th. (2003) Corn irrigation with reclaimed municipal wastewater. Global Nest: The Int. J. 5(1), 39-45.
[50] Surdyk N., Cary, L., Blagojevic, S., Jovanovic, Z., Stikic, R., Vucelic-Radovic, B., Zarkovic, B., Sandei, L., Pettenati, M., Kloppmann, W., (2010) Impact of irrigation with treated low quality water on the heavy metal contents of a soil-crop system in Serbia. Agricultural Water Management. 98, 451-457.
[51] Manaia, C.M., Novo, A., Coelho, B., Nunes, O.C. (2010) Ciprofloxacin resistance in domestic wastewater treatment plants. Water Air. Soil. Pollut. 208, 335-343.
[52] Fulazzaky, M. (2009) Carbonaceous, nitrogenous and phosphorus matters removal from domestic wastewater by an activated sludge reactor of nitrification-denitrification type. Journal of Engineering Science and Technology. 1, 69-80.
[53] Servais, P., Garcia-Armisen, T., George, I., Billen, G. (2007) Fecal bacteria in the rivers of the Seine drainage network: source, fate and modeling. Sci. Total. Environ. 375, 52-167.
[54] Nagaraju, A.K., Nagaraju, A., Sunil-Kumar, K., Thejaswi, A., Sharifi, Z. (2014) Statistical analysis of the hydrogeochemical evolution of groundwater in the Rangampeta area, Chittoor District, Andhra Pradesh, South India. American Journal of Water Resources. 3, 63-70.

Received: **26.04.2018**
Accepted: **12.11.2018**

CORRESPONDING AUTHOR

Georgi Petkov
Trakia University,
Faculty of Agriculture,
Department of Applied Ecology and
Animal Hygiene,
6000 Stara Zagora – Bulgaria

e-mail: gpetkov@af.uni-sz.bg

© by PSP Volume 27 – No. 12B/2018 pages 9739-9746 Fresenius Environmental Bulletin

FLUORIDE ADSORPTION ON ARTIFICIAL ZEOLITE IN WATER

Xun Fang, Shibiao Wu*, Yejun Ge, Yaqin Wang, Jie Yang, Xi Li, Haiyan Xu

School of Materials and Chemical Engineering, Anhui Jianzhu University, Hefei, Anhui, 230601, China

ABSTRACT

Artificial zeolite was synthesized by hydrothermal method and activated by soaking in saturated aluminum sulfate aqueous solution. The activated zeolite was characterized by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy(FT-IR); the fluoride adsorption on the zeolite from water was investigated; the effects of pH, temperature on F^- ions adsorption was also discussed. Results showed that the zeolite had excellent fluoride adsorption capacity and rapid regeneration speed; the maximum fluoride adsorption capacity was 65.36mg/g and the time needed for the used adsorbent to be regenerated in saturated aluminum sulfate aqueous solution was only one minute. The adsorption mechanics were ascribed to hydrogen bonds attraction between hydroxyl groups and the fluoride ions in water.

KEYWORDS:
Artificial zeolite, adsorption, fluoride, isotherms, kinetics

INTRODUCTION

Fluoride is one of the essential trace elements of humans and animals. It is the main component of teeth and bones and plays an important role in the formation of bone tissue and enamel [1] .When the human body takes a moderate amount of fluoride, it has a positive effect on preventing cavities, especially during the calcification of the teeth. However, excessive fluoride can damage the normal calcium and phosphorus metabolism of the human body. Absorbing excessive inorganic fluoride for a long time can cause dental fluorosis, periosteum hyperplasia [2]. According to WHO (World Health Organization), the permissible upper limit of F^- ions in drinking water is 1.0 mg/L, and the excessive F^- in the water must be removed [3].

Adsorption is an convenient and energy-saving method which is especially suitable to treat a small amount of drinking water containing low concentration pollutant [4]. In recent years, a variety of adsorbents like activated alumina [5], ion exchange resin [6-8], chitosan beads [9, 10], activated carbon [11], oxides containing rare earth metals [12], hydrotalcite [13-15] etc., have been identified as the promising defluoridating agents. However, there is still a great demand for more simple, low-cost and effective adsorbents for fluoride removal from water [16, 17].

Zeolite is a kind of aluminum silicate mineral with water or alkaline earth metals, and the ion exchange and adsorption characteristics of zeolite are derive from their unique structural characteristics [18, 19]. In this paper, artificial zeolite was synthesized by hydrothermal method and activated by soaking in saturated aluminum sulfate aqueous solution. The fluoride adsorption isotherm, kinetics and regeneration of the zeolite were examined; the effects of pH, temperature on F^- ions adsorption was also discussed. The results showed that the zeolite had excellent fluoride absorption capacity and regeneration performance. The adsorption mechanics were ascribed to hydrogen bonds attraction between hydroxyl groups and the fluoride ions in water.

MATERIALS AND METHODS

Materials. Nano silicon dioxide (SiO_2, 99.5% metals basis, 15 nm, Aladdin), sodium Aluminate ($NaAlO_2$, AR, Macklin), aluminum sulfate octadecahydrate ($Al_2(SO_4)$•18 H_2O, 99.5%, AR), sodium hydroxide (NaOH, AR), nitric acid (HNO_3, 65%, AR) and sodium fluoride (NaF, AR) were purchased from China National Pharmaceutical Group Corporation. Ultra-pure water (18 MΩ•cm) was produced using a PSDK System (ZI-HANSHIJI, Beijing, China).

Preparation of zeolite. Synthesis:1.335g NaOH, 0.3899g $NaAlO_2$,0.8575g SiO_2 and 12.5mL ultra-pure water were mixed and stirred at 800 rpm by a magnetic stirrer for 12h until uniform white colloid was obtained. Then the white colloid was transferred to an autoclave, heated at 105 ℃ or 120℃ for 24h. White precipitate was obtained and washed by deionized water and centrifuged for several times until the pH value was between 7 and 8.

Activation: saturated aluminum sulfate solution, which volume was ten times that of precipitation, was uniformly mixed with the precipitate for

1-2 minutes, and the supernatant was abandoned after centrifugation. Then, the precipitate was washed by deionized water and centrifuged for several times until the pH value was between 6 and 7.

Drying: the clean precipitate was dried in oven at 65 ℃ for 24 hours and the powder of zeolite was obtained.

Characterization. X–ray diffraction was measured on a XRD meter (X'Pert PRO) with a Cu *Kα* source (λ=1.541 Å). The powder morphology was investigated through scanning electron microscopy on a field-emission SEM (Sirion 200, FEI Company, USA) operated at 5 kV voltage value. The molecular structure of the products was detected with Fourier transform infrared (FT-IR) spectra (Nexus-870). A Rex pH meter (PHB-4, Shanghai, China) was used for pH value detection.

Batch adsorption experiments. NaF was dissolved in ultra-pure water to prepare fluoride aqueous solution as simulated water polluted by fluoride. Initial fluoride solutions in batch adsorption were all adjusted to desired pH values by adding HNO_3 or NaOH aqueous solution, and the default initial pH value of the solutions were 6.5 unless otherwise specified.

Adsorption kinetics experiments. 100 mL 3ppm(mg/L) fluoride solution was mixed with an appropriate amount of adsorbent was stirred by an electromagnetic agitator (800 rounds per minute) in a 250 mL sealed plastic conical flask at 298 K for 24h. The ratio of adsorbent mass (m) vs. solution volume (v) was m/v=1 or 2 g/L. The mixture was sampled on schedule and the sample was separated by syringe-driven filter (0.22μm). The concentration of fluoride in the initial solution and filtered clear liquid were determined by a fluorine ion selective electrode.

Adsorption isotherms experiments. A specified volume of fluoride aqueous solution with different fluoride concentration mixed with an appropriate amount of adsorbent was shaken in sealed plastic test tubes at 170 rpm by an oscillator at 298, 313 and 338 K for 24h. The ratio of m/v=1g/L. The initial and final concentrations of fluoride were detected as above. The adsorption percentage and the distribution coefficient (K_d) were calculated from the following equations:

$$Adsorption(\%) = \frac{C_0 - C_e}{C_0} \times 100\% \quad (1)$$

$$Q_e = (C_0 - C_e) \times \frac{V}{m} \quad (2)$$

$$K_d = \frac{C_0 - C_e}{C_e} \times \frac{V}{m} \quad (3)$$

where, C_0 is the initial concentration (mg/L), C_e is the equilibrium concentration (mg/L), m (g) is the mass of the adsorbent, and V (mL) is the volume of the suspension, Q_e (mg/g) is the equilibrium adsorption capacity.

pH influence experiments. The effect of pH on the fluoride adsorption was studied using 14ppm fluoride solution with 1g/L of adsorbent at different pH value, and the experiment temperature was 298K. The equipment of experiment and testing the adsorption capability of the adsorbent was the same as the experiments above.

Adsorbent regeneration experiments. Experimental steps were as followings: 200mg fresh adsorbent and 20ppm fluoride ion solution were mixed in a sealed plastic conical flask in which the ratio m/v=1g/L; the flask was shaken at 170 rpm at 25 ℃ for 12 hours. The sample method and fluoride concentration measurement were same as above. After sampling, remained mixture was centrifugal washed one time by deionized water which volume was 10 times of the precipitation. Then, the precipitation was mixed uniformly with regeneration agent i.e. saturated aluminum sulfate solution which volume was also 10 times of the precipitation for 1 minute and centrifugal washed by deionized water until pH was between 6-7. The drying of regenerated adsorbent was same as the drying process in section 2.2. After drying, next round fluoride adsorption and regeneration repeated until the end of the fifth round.

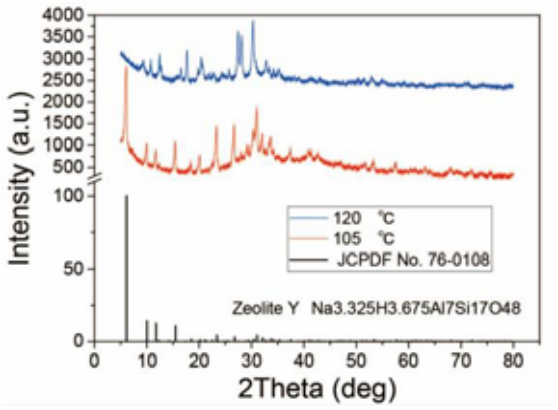


FIGURE 1
XRD pattern of zeolite products and standard PDF

RESULTS AND DISCUSSION

Adsorbent characterization. Figure 1 shows the XRD patterns of the zeolite powder samples prepared at 105 ℃,120 ℃ and standard PDF card

(NO.76-0108, Zeolite Y, $Na_{3.325}H_{3.675}Al_7Si_{17}O_{48}$). According to the figures, the sample prepared at 105 ºC are more accordant with NO.76-0108 standard card and the composition of Si-Al ratio is close to 3. The XRD pattern proved that zeolite was synthesized successfully in this paper. The main diffraction peaks of zeolite prepared at 105 °C are more sharp and stronger than 120 °C, indicating the former product was more well-crystallized than the latter; the temperature of 105 °C was more reasonable process temperature. Therefore, the zeolite characterization samples and adsorbent mentioned in the sections below were all prepared at 105°C.

The morphology of the as-prepared zeolite product was observed by SEM. In Figure 2(A), the zeolite grains showed coil shapes in different sizes. According to Figure 2 (B), the size of zeolite crystal grains was in the micrometer range, about 5 μm in diameter. The adsorbent grain had a rough surface, which was full of nano scale prismatic structure. This surface state was favorable for the fast adsorption of adsorbent on fluorine.

The FT-IR spectra of the sample before and after adsorbing fluoride were showed in Figure 3. The red and black curves show the FT-IR of sample before and after fluoride adsorption, respectively. Comparing to red curve, there is an obvious blue shift of hydroxyl group stretching vibration peak at 3500 cm^{-1} in the black curve, implying inductive effect [20] induced by adsorbed fluoride which combined with hydroxyl groups on the surface of the zeolite by hydrogen bonds.

Adsorption kinetics. The initial fluoride concentration($[F^-]_{initial}$), pH and temperature were 3ppm, 6.5 and 298K, respectively. The ratio of adsorbent mass (m) vs. solution volume (v) was m/v=1 or 2 g/L. The experiment results were showed in Figure 4. Horizontal axis represents the reaction time t (min), and vertical axis represents the F^- adsorption capacities Q (mg/g). According to Figure 4(A), it can be seen that the adsorption capacity measured at m/v=1g/L is better than that m/v = 2g/L.

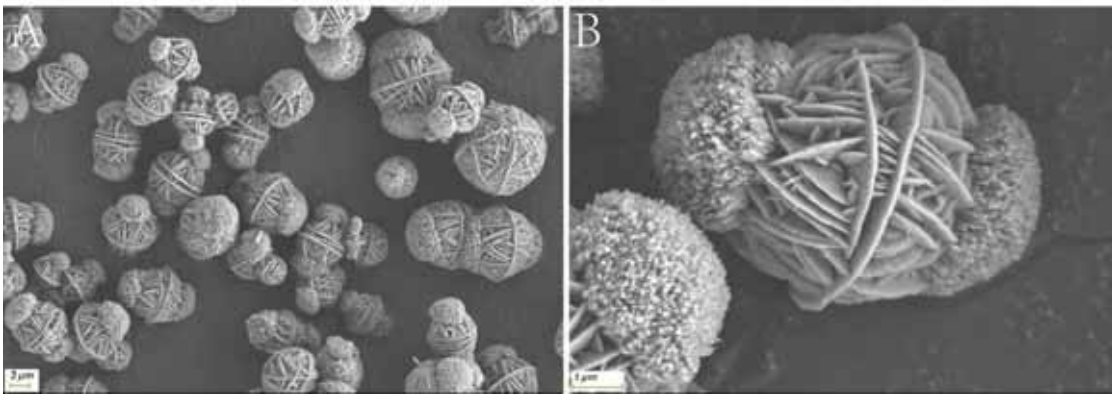


FIGURE 2
SEM image of zeolite. (A) full view, (B) enlarged zeolite grains.

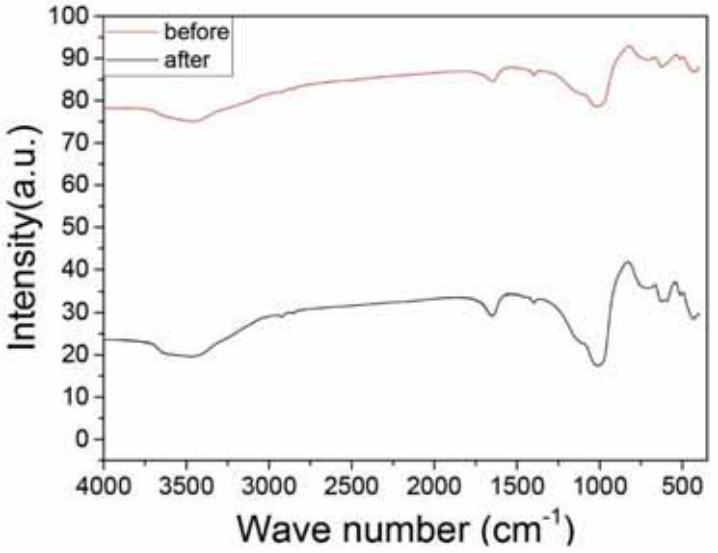


FIGURE 3
FT-IR spectra of the zeolite before and after absorbing fluoride.

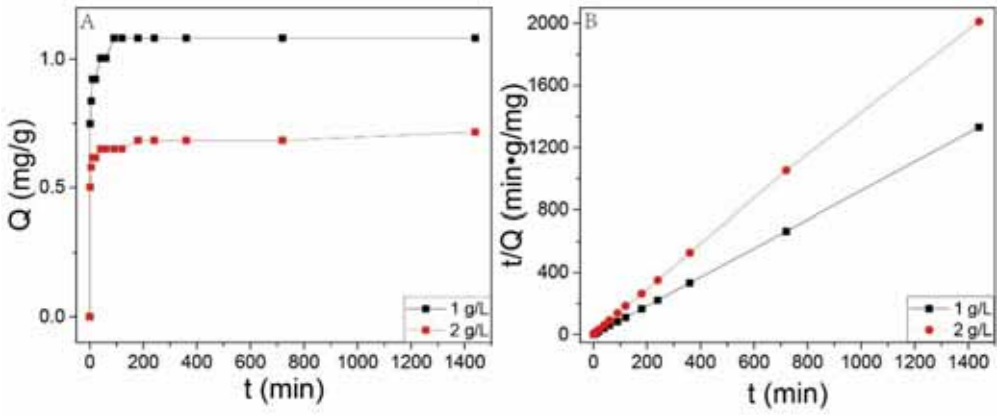


FIGURE 4
Adsorption kinetic curve(A) and quasi-second-order kinetic model(B). $[F^-]_{initial}$=3ppm, Initial pH=6.5, 298K.

The adsorption kinetic data were analyzed using a pseudo-second-order kinetics model which is based on the assumption that chemisorptions are the rate determining step. The pseudo-second-order kinetic model can be described by equation (4) :

$$Q = \frac{k_2 Q_e^2 t}{1 + k_2 Q_e t} \quad (4)$$

Here, k_2 (g mg^{-1} min^{-1}) is the pseudo-second-order rate constant. Q_e and Q are the F^- adsorption capacity at equilibrium and time (t), respectively. A plot of t/Q versus yields the values of Q_e and k_2. The initial adsorption rate r_0 (mg g^{-1} min^{-1}) can be calculated by using equation (5) :

$$r_0 = k_2 Q_e^2 \quad (5)$$

Figure 4(B) shows the relation between t/Q and t. The rate constants and correlation coefficients of the pseudo second-order kinetic models are listed in Table 1. Two coefficients of determination (R^2) are all very close to 1, which implies that F^- captured by the adsorbent follows the pseudo-second order kinetics model very well.

TABLE 1
Rate constants and correlation coefficients of the pseudo second-order kinetic models

m/V(g/L)	Q_e(mg/g)	k_2(g/(mg·min))	r_0 (mg/(g·min))	R^2
1	1.084	0.589	0.692	0.999
2	0.712	0.200	0.102	1.000

Adsorption isotherms. Adsorption isotherms are usually used to determine the capacities of adsorbents. Figure 5 shows the isotherm of F^- adsorption on the adsorbent.

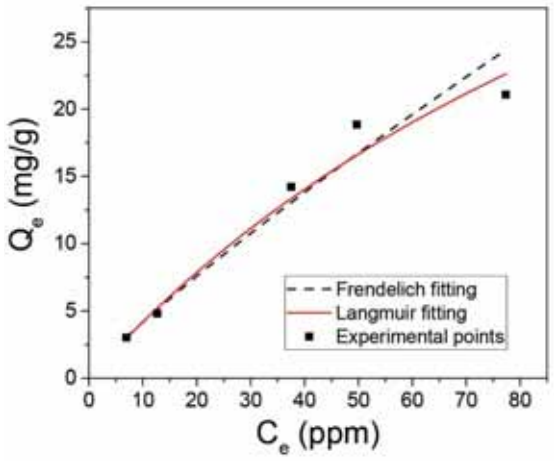


FIGURE 5
Fluoride adsorption isotherms on zeolite. Initial pH=6.5, m/v=1g/L, 298K.

In order to explain the results of the adsorption isotherms experiments, the adsorption isotherms are divided into various types. The common adsorption isotherms models include Langmuir, Freundlich, etc.[21, 22]. The parameters of the models can be obtained by the treatment of adsorption isotherm data.

Langmuir adsorption isotherm. The Langmuir isotherm model is suitable for single layer adsorption onto a surface with a finite number of identical sites and uniform energies of adsorption with no transmigration of adsorbate in the plane of the surface[23]. The Langmuir isotherm model can be described by equation (6):

$$Q_e = \frac{Q_m K_L C_e}{1 + K_L C_e} \quad (6)$$

C_e is the equilibrium concentration of F^- in aqueous solution (mg L^{-1}). Q_e is the amount of F^- adsorbed on adsorbents (mg g^{-1}). Q_m is the maximum amount of F^- adsorbed per unit weight of

adsorbents to form a complete monolayer coverage on the surface. K_L represents the ratio of the rate constants of adsorption and desorption and should vary with temperature. Langmuir model fitting [24] curve was plotted by solid line in Figure 5.

Freundlich adsorption isotherm. The Freundlich type model is used when the adsorption process is assumed to take place on a heterogeneous surface that varies with surface coverage. The Freundlich isotherm model can be described by equation (7):

$$Q_e = K_F C_e^{1/n} \quad (7)$$

K_F and n are the Freundlich constants related to the adsorption capacity and adsorption intensity, respectively. Freundlich model fitting curve was also plotted by dash line in Figure 5.

The parameters of Langmuir and Freundlich models were listed in Table 2.

Two coefficients of determination (R^2) are all very close to 1, which implies that the isotherms were fitted to the Langmuir and Freundlich models, respectively. According to the coefficients of determination (R^2), it is shown that the Langmuir model describes F^- adsorption on the zeolite better than Freundlich model, suggesting that F^- adsorption on the zeolite was monolayer coverage. In Langmuir model, the max value Q_m of fluoride adsorption on the zeolite was 65.36 mg g^{-1}, which was a very good performance.

Adsorption thermodynamics. The effects of temperature on fluoride ion adsorption on the zeolite in water was investigated. The effects of temperature on fluoride ion adsorption are shown in Figure 6. Figure 6(A) shows three adsorption isotherms which experiments were carried out at 293K, 313K and 333K, respectively. The experimental data show that the temperature increase is beneficial to fluoride adsorption on the zeolite in water.

The standard free energy change (ΔG^0) can be calculated according to equation (8).

$$\Delta G^0 = -RT\ln K^0 \quad (8)$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature in Kelvin. K^0 is the adsorption equilibrium constant. $\ln K^0$ can be obtained by plotting $\ln K_d$ (distribution coefficient) versus C_e and extrapolating C_e to zero. Figure 6(B) describes the linear plot of $\ln K_d$ versus C_e for the adsorption of F^- on the zeolite at 293, 313, and 333 K. Figure 6(C) demonstrates the linear plot of $\ln K_o$ versus 1/T. The standard enthalpy change (ΔH^0) and the standard entropy (ΔS^0) are then figure out from the linear plot of $\ln K^0$ versus 1/T in the following relationship equation (9):

$$\ln K^0 = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \quad (9)$$

The obtained thermodynamic parameters of F^- adsorption on the zeolite are listed in Table 3. From Table 3, it can be found that the entropy change ΔS^0=66.60 J $mol^{-1}K^{-1}$, the enthalpy change ΔH^0=4.48kJ mol^{-1}, and the standard free energy change ΔG^0= -15.04kJ mol^{-1} at 293 K, -16.37kJ mol^{-1} at 313 K, and -17.70 kJ mol^{-1} at 333 K, respectively. The positive ΔH^0 value suggests that F^- adsorption on the surface of the zeolite is an endothermic process. Negative ΔG^0 value indicates that F^- adsorption is a spontaneous process.

TABLE 2
Parameters for Langmuir and Freundlich models of F^- adsorption on adsorbent

Adsorbent	Langmuir $Q_e = \frac{Q_m K_L C_e}{1 + K_L C_e}$			Freundlich $Q_e = K_F C_e^{1/n}$		
	Q_m(mg/g)	K_L(L/mg)	R^2	K_F($mg^{1-n}L^n/g$)	n	R^2
Zeolite	65.36	0.006835	0.9936	0.5677	1.156	0.9843

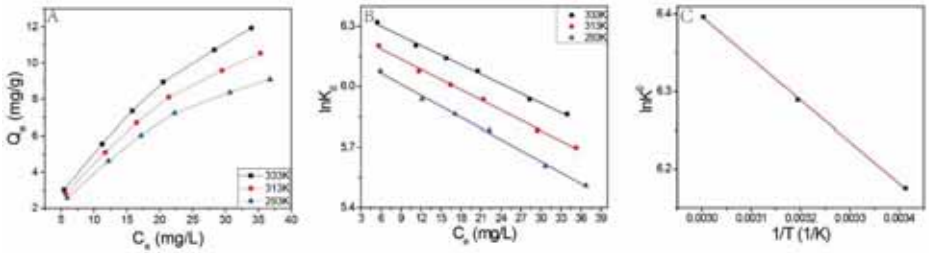


FIGURE 6
The effects of temperature on fluoride ion adsorption. (A) F^- adsorption isotherms on the zeolite at 293K, 313K and 333K; (B) Linear fitting of $\ln K_d$ vs. C_e; (C) Linear plot of $\ln K^o$ vs. 1/T. Initial pH=6.5, m/v=1g/L.

TABLE 3
The obtained thermodynamic parameters of F^- adsorption on the zeolite.

T(K)	ΔH^0 (kJ/mol)	ΔS^0 (J/(mol·K))	ΔG^0 (kJ/mol)
293			-15.0405
313	4.4745	66.60	-16.3726
333			-17.7047

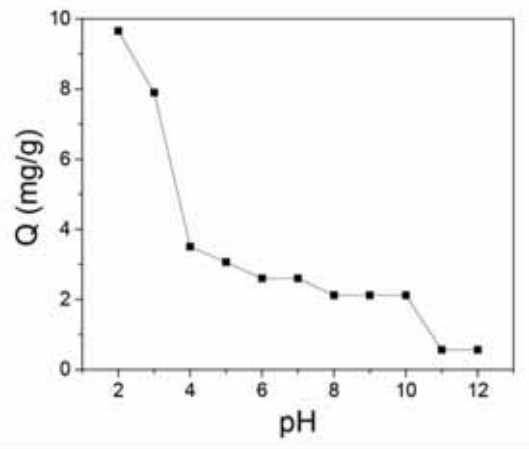


FIGURE 7
The effect of initial pH on the amount of F^- absorbed on zeolite.
$[F^-]_{initial}$=14ppm, m/v=1g/L, 298K.

Influence of pH value on F^- adsorption. Initial solution pH is one of the important parameters to determine the adsorption property of an adsorbent due to its effect not only on surface charge of the adsorbent but also on the degree of ionization and speciation of adsorbate [25]. Figure 7 shows the variation of adsorption capacity of fluoride adsorbed by the zeolite at various initial pH values. The F^- adsorption capacity (Q) shows a decreasing trend when pH rises in Figure 7, which indicates that the acid condition is obvious beneficial to the zeolite defluorination performance. When pH is 2 and 12, the adsorption capacity is the maximum and minimum; the adsorption capacity sharply decreases from pH=2 to 4, slightly drops from4 to 10 and obviously decline from 10 to 12. The decrease of the adsorption capacity in alkaline solution may be attributed to the competition for surface adsorption sites between the negative hydroxyl and F^- anions [26].

Regeneration experiments. 5 rounds regeneration experiments were made. According to the experimental data, the capacity column chart of fluoride ions adsorption on the zeolite is shown in Figure 8.

Regenerative agent was aluminum sulfate, which was neutral and friendlier for water environment than strong basic or acid agents [27, 28]. Initial adsorption capacity was 4.17mg/g; the deterioration of the zeolite adsorbent was slight and the adsorption capacity retained 81% after 5 rounds regeneration which indicated that the adsorbent was reusable. Moreover, in this paper, the regeneration process was very convenient and time-saving, because regeneration time was only one minute in every round.

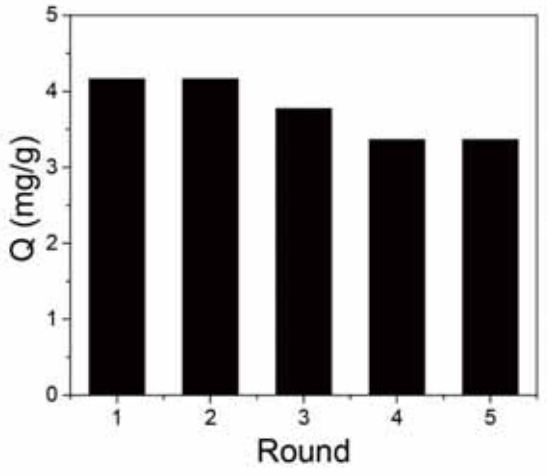


FIGURE 8
Adsorption column chart of regenerative experiments. m/v=1g/L, initial pH=6.5, $[F^-]_{initial}$=20ppm, 298K; regeneration agent was saturated aluminum sulfate solution.

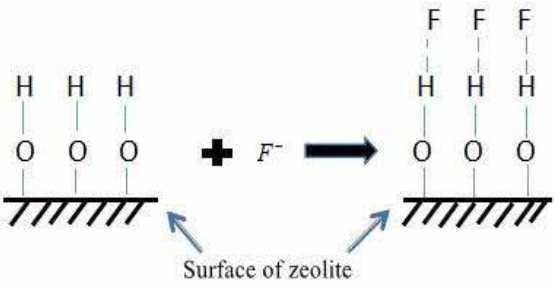


FIGURE 9
Mechanism of fluoride adsorption on the zeolite

Adsorption mechanism. According to the characterization and experimental results, the adsorption mechanism is proposed here. There are abundant surface hydroxyl groups on the surface of the zeolite [29] which provide highly dispersed electroactive sites. The adsorption mechanics is electroactive attraction i.e. hydrogen bonds between hydroxyl groups and the fluoride ions (Figure 9). When the pH value is lower, the more hydroxyl content on the surface of the zeolite, the higher fluoride adsorption capacity achieved (Figure 7). In the FT-IR spectrum (Figure 3), after fluoride adsorption, the sample was blue-shifted at the peak of

3500 cm^{-1}, which proved that the hydroxyl group on the surface of the zeolite combined with fluoride by hydrogen bond during the adsorption process.

CONCLUSIONS

Artificial zeolite was synthesized by hydrothermal process and activated by soaking in saturated aluminum sulfate aqueous solution. The activated zeolite was characterized and investigated its adsorption performance for fluoride from water. The maximum adsorption capability for fluoride approached 65.36mg/g, indicating that the zeolite was one of the very suitable materials in environmental pollution management. The used zeolite could also be regenerated in aluminum sulfate aqueous solution in one minute, and the adsorption capacity deterioration was slight. The adsorption mechanics was hydrogen bond attraction between hydroxyl groups on the surface of zeolite and the fluoride ions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the natural science research project and key project from Education Department of Anhui Province (KJ2017A492, KJ2015JD14) and Natural Science Foundation of Anhui Province (1608085MB37).

REFERENCES

[1] Smith, F.A., Hodge, H.C., Dinman, B.D. (1979) Airborne fluorides and man: Part II, C R C Critical Reviews in Environmental Control. 9, 1-25.

[2] Soni, R., Modi, S. (2013) A Review on Effects of Fluoride on Human Health in Rajasthan. International Journal of Innovative Research and Development. 2(4), 638-646.

[3] Wu, S., Zhang, K., He, J., Cai, X., Chen, K., Li, Y., Sun, B., Kong, L., Liu, J. (2016) High efficient removal of fluoride from aqueous solution by a novel hydroxyl aluminum oxalate adsorbent. J Colloid Interface Sci. 464, 238-245.

[4] Ali, I. (2012) New generation adsorbents for water treatment. Chemical reviews. 112, 5073-5091.

[5] Hao, O.J., Huang, C.P. (1986) Adsorption Characteristics of Fluoride onto Hydrous Alumina, Journal of Environmental Engineering, 112, 1054-1069.

[6] Inoue, K. (2004) The Removal of Fluoride Ion by Using Metal(III)-Loaded Amberlite Resins. Solvent Extraction and Ion Exchange. 22, 305-322.

[7] Chikuma, M., Nishimura M. (1990) Selective sorption of fluoride ions by anion-exchange resin modified with alizarin fluorine blue-praseodymium(III) complex. Reactive Polymers. 13, 131-138.

[8] Zhou, Y., Yu, C., Shan, Y. (2004) Adsorption of fluoride from aqueous solution on La 3+ - impregnated cross-linked gelatin. Separation and Purification Technology. 36, 89-94.

[9] Yu, Q., Deng, S., Yu, G. (2008) Selective removal of perfluorooctane sulfonate from aqueous solution using chitosan-based molecularly imprinted polymer adsorbents. Water Research. 42, 3089-3097.

[10] Chiou, M.S., Li, H.Y. (2003) Adsorption behavior of reactive dye in aqueous solution on chemical cross-linked chitosan beads. Chemosphere. 50, 1095-1105.

[11] Qiang, Y., Zhang, R.Q., Deng, S.B., Huang, J., Gang, Y. (2009) Sorption of perfluorooctane sulfonate and perfluorooctanoate on activated carbons and resin: kinetic and isotherm study. Water Research. 43, 1150-1158.

[12] Wu, X., Zhang, Y., Dou, X., Yang, M. (2007) Fluoride removal performance of a novel Fe-Al-Ce trimetal oxide adsorbent. Chemosphere. 69, 1758-1764.

[13] Mandal, S., Mayadevi, S. (2008) Cellulose supported layered double hydroxides for the adsorption of fluoride from aqueous solution. Chemosphere. 72, 995-998.

[14] Lv, L., He, J., Wei, M., Evans, D.G., Zhou, Z. (2007) Treatment of high fluoride concentration water by MgAl-CO3 layered double hydroxides: kinetic and equilibrium studies. Water Research. 41, 1534-1542.

[15] Lv, L. (2006) Adsorption behaviour of calcined layered double hydroxides towards removal of fluoride from aqueous solution. Journal of Water Supply: Research and Technology - AQUA. 55, 413-418.

[16] Viswanathan, N., Meenakshi, S. (2009) Role of metal ion incorporation in ion exchange resin on the selectivity of fluoride. Journal of Hazardous Materials. 162, 920.

[17] Bhatnagar, A., Kumar, E., Sillanpää, M. (2011) Fluoride removal from water by adsorption—A review. Chemical Engineering Journal. 171 811-840.

[18] Furukawa, H., Ko, N., Yong, B.G., Aratani, N., Sang, B.C., Choi, E., Yazaydin, A.Ö., Snurr, R.Q., O'Keeffe, M., Kim, J. (2010) Ultrahigh Porosity in Metal-Organic Frameworks. Science. 329, 424-428.

[19] Chen, Y., Pan, B., Li, H., Zhang, W., Lv, L., Wu, J. (2010) Selective removal of Cu(II) ions by using cation-exchange resin-supported polyethyleneimine (PEI) nanoclusters. Environmental Science and Technology. 44, 3508-3513.

[20] Lang, J., Mohrbach, J., Dillinger, S., Hewer, J.M., Niedner-Schatteburg, G. (2016) Vibrational blue shift of coordinated N2 in [Fe3O (OAc)6(N2)n](+): "non-classical" dinitrogen complexes. Chemical Communications. 53, 420-423.
[21] Reed, B., Matsumoto, M. (1993) Modeling Cadmium Adsorption by Activated Carbon Using the Langmuir and Freundlich Isotherm Expressions. Separation Science. 28, 2179-2195.
[22] Jossens, L., Prausnitz, J.M., Fritz, W., Schlünder, E.U., Myers, A.L. (1978) Thermodynamics of multi-solute adsorption from dilute aqueous solutions. Chemical Engineering Science. 33, 1097-1106.
[23] Langmuir, I. (1918) The Adsorption of Gases On Plane Surfaces Of Glass, Mica And Platinum. Journal of Chemical Physics. 40, 1361-1403.
[24] Tiwari, J.N., Mahesh, K., Le, N.H., Kemp, K.C., Timilsina, R., Tiwari, R.N., Kim, K.S. (2013) Reduced graphene oxide-based hydrogels for the efficient capture of dye pollutants from aqueous solutions. Carbon. 56, 173-182.
[25] Salifu, A., Petrusevski, B., Ghebremichael, K., Modestus, L., Buamah, R., Aubry, C., Amy, G.L. (2013) Aluminum (hydr)oxide coated pumice for fluoride removal from drinking water: Synthesis, equilibrium, kinetics and mechanism. Chemical Engineering Journal. 228, 63-74.
[26] Li, Y., Zhang, P., Du, Q., Peng, X., Liu, T., Wang, Z., Xia, Y., Zhang, W., Wang, K., Zhu, H., Wu, D. (2011) Adsorption of fluoride from aqueous solution by graphene. Journal of Colloid and Interface Science. 363, 348-354.
[27] Gómez-Hortigüela, L., Pérez-Pariente, J., García, R., Chebude, Y., Díaz, I. (2013) Natural zeolites from Ethiopia for elimination of fluoride from drinking water. Separation and Purification Technology. 120, 224-229.
[28] Sun, Y., Fang, Q., Dong, J., Cheng, X., Xu, J. (2011) Removal of fluoride from drinking water by natural stilbite zeolite modified with Fe(III). Desalination. 277, 121-127.
[29] Samanta, S., Bhunia, K., Pradhan, D., Satpati, B., Srivastava, R. (2018) Ni and Cu ion-exchanged nanostructured mesoporous zeolite: A noble metal free, efficient, and durable electrocatalyst for alkaline methanol oxidation reaction. Materials Today Energy. 845-56.

Received: **04.05.2018**
Accepted: **21.10.2018**

CORRESPONDING AUTHOR

Shibiao Wu
School of Materials and Chemical Engineering,
Anhui Jianzhu University,
Hefei, Anhui, 230601 – China

e-mail: 13956063193@139.com

© by PSP Volume 27 – No. 12B/2018 pages 9747-9754 Fresenius Environmental Bulletin

INTEGRATED FRAMEWORK FOR ASSESSMENT OF BLENDED HIGH-RESOLUTION SATELLITE RAINFALL ESTIMATES OVER COMPLEX ENVIRONMENTAL REGIONS

Muhammad Masood[1], Abdul Sattar Shakir[2], Muhammad Waseem[1,*], Habib-u-Rehman[2], Aftab-H-Azhar[3], Adnan Abbas[4]

[1]Center of Excellence in Water Resources Engineering, University of Engineering and Technology, Lahore 54890, Pakistan
[2]Department of Civil Engineering, University of Engineering & Technology GT-Road, 54890, Lahore, Pakistan
[3]Pakistan Water and Power Development Authority (WAPDA), MDO-WAPDA-Mangla, Pakistan
[4]College of Engineering, China Agricultural University, Beijing 100083, China

ABSTRACT

High-resolution satellite precipitation products are considered as potential sources of precipitation information for studying the hydrologic processes in mountainous areas with sparse gauging networks. Three high-resolution products including Tropical Rainfall Measuring Mission (TRMM), and Integrated Multi-satellite Retrievals for Global precipitation measurement (IMERG) products (i.e. IMERG Late run (hereafter IM-L) and IMERG Final run (hereafter IM-F) are evaluated based on point to pixel analysis using gauged observations of diverse topography of Pakistan. On an average, the IM-F with an average correlation coefficient (CC) of 0.23 dominated, followed by IM-L with an average CC of 0.16 and finally TRMM with an average CC of 0.14. However, inconsistent performances in diverse climatic and topographic regions were observed for all products. Hence, a new framework for the estimation of integrated satellite precipitation estimates (IFP) was introduced, aimed at consistent performance throughout the study area. The IFP was based on the merging algorithm, which includes the satellite product estimates, CC and performance connection weights. The comprehensive analyses showed that IFP provided a consistent, improved efficiency and better agreement with the gauged datasets over the entire study area, as compared to TRMM, IM-L and IM-F.

KEYWORDS:
IMERG, Integrated Framework, Satellite precipitation, TRMM

INTRODUCTION

During last few years, like others countries, Pakistan is also gradually affected by climate change influences in terms of increased temperatures and spatiotemporal variability in precipitation patterns [1, 2], which is providing a base for numbers of emerging challenges and inter-related issues. Reliable information on these notable factors (e.g., precipitation) is vital for accurate assessment and prediction of natural disasters, food security and sustainable agriculture. Typically, a rain gauge or meteorological radar are used to provide reliable precipitation data. However, in developing countries like Pakistan, hydrometric networking is sparse and spatial distribution is inconvenient. Hence, high-resolution satellite precipitation products could be considered as an alternative source for precipitation estimates. Several high-resolution satellite precipitation products have been widely applied and evaluated globally [3-9]. Examples include NOAA's Climate Prediction Center Morphing technique (CMORPH), the Tropical Rainfall Measuring Mission (TRMM), Multi-satellite Precipitation Analysis products (TMPA) and the Integrated Multi-satellite Retrievals for Global precipitation measurement (IMERG).

High-resolution satellite precipitation products have high spatial resolutions of $\leq 0.1^0$ and temporal resolutions of ≤ 30 minutes over nearly quasi-global scales. These satellites have been comprehensively used for numerous hydrological applications, including drought projection, flood forecasting, early warning and hydrological simulation at ungauged basins [10]. High-resolution satellite precipitation products contain uncertainties that ensued from indirect observations measurement inaccuracies, regional and seasonal systematic biases, random miscalculations, retrieval algorithms and sampling processes of bias corrections [11-15]. These errors can proliferate into hydrological prediction [16]. Subsequently, a statistical, quantitative, and hydrological modeling-based evaluation is crucial to substantiate the quality and applicability of the satellite precipitation estimates [17-19]. If proven, the expedient tools would further enhance the retrieval algorithms of a satellite [20] and influence the selection of the hydrological application product [21].

Numerous individual and group-based (i.e., a pilot program launched by the International Precipitation Working Group) evaluations of these products have been conducted at regional and global scales, and at various spatial and temporal resolutions [10]. Based on inclusive evaluations, the preceding studies reported variable accuracy and applicability of these products in different physical, climatic, geographic regions and seasons [22].

Tang et al. [7] and Yuan et al. [23] resulted that though the satellite product may possibly capture the spatiotemporal variability of precipitation, nevertheless, contains substantial errors (e.g., regional biasness) and there is still room for ancillary enhancement of capturing capability in a dry climate and high-altitude regions. According to Maggioni et al. [10], rain detection efficacy of most satellite products showed weak performance in complex terrain and regions with rapid precipitation gradients. This may be due to the poor ability to distinguish between raining and non-raining clouds. Hence, over precipitous regions, further validation research could provide a better understanding of the limitations of using satellite products for flash flood applications. Moreover, topographic diversity, climatological features, and seasonality also play a vital role in the performance of these products, specifically in terms of mean errors and the probability of detection [6]. In addition, the summer (warm) season is associated with the convective structure and the cold season with light precipitation, which could affect the performance of these products [6]. More specifically, the product performance predominantly depends on the variability of climatic and topographic diversity, timescales and precipitation intensities [10, 24]. Irrespective of significant improvements in most satellite precipitation products, a region-specific assessment is of utmost importance before any hydrological simulation, assessment, projection or outlook can be conducted [24].

Therefore, the primary objective of the current study is to introduce a framework for quantification of integrated satellite precipitation estimates based on the relative performances and final estimates of different well-known satellite products (i.e., IMERG Late run (IM-L), IMERG Final run (IM-F) and TRMM). The primary objective was to provide blended regional precipitations estimates - targeting better performance irrespective of climatic and topographic diversity in Pakistan.

MATERIALS AND METHODS

Study Area. Pakistan is a developing country, covering 79.6 million ha, 61°–77° East longitude and 23.5°–37° North latitude, with assorted climatic conditions. Pakistan contains significant landscape diversity, ranging from coastlines along the Arabian Sea in the south, to plains, deserts and plateaus in the center, to the snow-covered mountainous region in the north. The geographical and water resources include the Indus riverine covering a major area of Punjab, Khyber Pakhtunkhwa, and Sindh province, the Himalayan mountainous region (northeast), Northern Highland and drought-prone arid climatic regions (southwest). The majority Pakistan is arid to the semi-arid with comparatively higher rainfalls in the northern regions and lesser in the center and the south. Monsoon and western disturbances are two major sources of rainfall from June to September and in the winter, respectively. Nonetheless, significant seasonal and annual variability in precipitation has been observed, as alternation between drought and flooding.

Weather station network has been established across the country by the Pakistan Metrological Department (PMD). Based on the PMD rainfall and temperature trend database, Pakistan was divided into five different climatic zones (hereafter referred to as Zones 1 through 5). Zone 1 comprises a region featuring cold climate and high mountain range in the north. Zone 2 contains mild cold climate and partially sub-mountainous range, located between 31° N and 34° N. Zone 3 includes cold climate in the winter and hot in summer covering an area between 27° N to 32° N and 64° E to 70° E. Zone 4 is the hottest plain and the driest zone of the country with the highest maximum temperature. Zone 5 is a region covering a coastal area as depicted in Fig. 1. For the current study, 72 stations across all the regions were chosen for evaluation of the satellite precipitation products and development of the proposed framework.

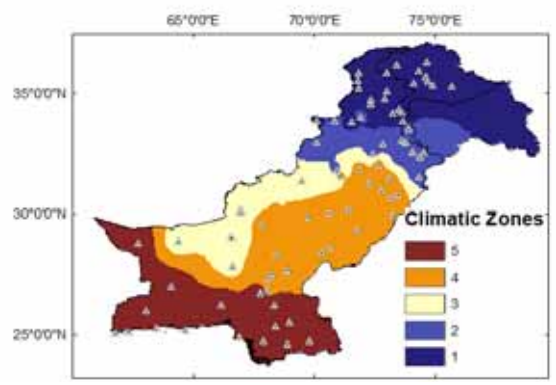


FIGURE 1
Selected gauged stations in the study area

Satellite Products: TRMM-Tropical Rainfall Measuring Mission. The Tropical Rainfall Measuring Mission (TRMM) (https://pmm.nasa.gov/data-access/downloads/trmm) is a combined space mission of Japan's National Space Development Agency and NASA, aimed at monitoring and studying tropical and subtropical precipitation and the associated energy release. The TRMM consists of five different instruments, including CERES-

Cloud and Earth Radiant Energy System, MI-Microwave Imager, PR-Precipitation Radar, VIRS-Visible Infrared Scanner and LSI-Lightning Imaging Sensor. PR and TMI are the key instruments involved in precipitation quantification algorithms. The most commonly used TRMM products include TCI-TRMM Combined Calibration database (TRMM 2B31) and Multi-Satellite Precipitation Analysis (TMPA) products, i.e., 3B43 and 3B42. The 3B43 and 3B42 are generally available as monthly, daily and sub-daily averages at a spatial resolution of 0.25° with coverage at a quasi-global scale (50°N to 50°S). The TRMM-TMPA 3B42 v7 (hereafter referred to as TRMM) was used to evaluate its performance for quantification of regional precipitation estimates in Pakistan.

IMERG- Integrated Multi-Satellite Retrievals for GPM (IMERG). The evolution of the TRMM mission products to the Global Precipitation Measurement (GPM) products (i.e., IMERG) began in mid-March 2014. The IMERG V.03, level 3 is a quasi-global (60° N to 60° S) multi-satellite precipitation product (https://pmm.nasa.gov/GPM), providing different datasets, including IMERG-real-time. This contains the IMERG-Early real-time database (6 h after minimal observation time) for warnings of probable floods or landslides, and the IMERG-Late real-time (IM-L) observation with 18 hours' latency for drought monitoring or agricultural forecasting. The latter is based on the combined use of passive microwave (PMW) sensors, infrared (IR) satellites and ground-based precipitation data. After receiving gauge observation and calibration by the Global Precipitation Climatology Centre (GPCC)'s gauge analysis, the IMERG-Final (IM-F) cycle is run to generate the database three-months later using the observation month. The level 3 products are comprised of a precipitation and snowfall catalog, with a 0.1° × 0.1° spatial resolution and a temporal resolution of 30-minutes [23, 25].

Point-to-Pixel Analysis for Assessment of Satellite Products. The selected satellite products have different spatial resolutions; 0.1° × 0.1° in the case of IMERG and 0.25° × 0.25° in the case of TRMM. To produce consistency, the aggregating technique was used, where areal weights of 0.16, 0.08 and 0.04 were assigned to four IMERG grid cells of 0.1° resolution, laying completely inside a TRMM grid cell, four falling halfway on the cell and nine cells coving one-fourth, respectively [23]. The IMERG and TRMM V7 precipitation observations at the grid cell (box) where at least one weather station falls were compared with the corresponding gauged observations from 7 March 2015 to 31 December 2016. To further ensure the data quality for comparison, the amount of satellite precipitation in the pixel against the exact location of each rain gauge was extracted using the latitude/longitude of the respective rain stations. After preliminary analyses, the accuracy, applicability and performance of the selected satellite products were estimated, based on different statistical indices including: (i) the correlation coefficient,

$$CC = \frac{\sum_{i=1}^{N}\left(P_i - \overline{P}\right)\left(O_i - \overline{O}\right)}{\sqrt{\sum_{i=1}^{N}\left(P_i - \overline{P}\right)^2}\sqrt{\sum_{i=1}^{N}\left(O_i - \overline{O}\right)^2}}, \quad \text{(ii)}$$

Mean Square Error, $E = \left[\frac{\sum_{i=1}^{N}\left(P_i - O_i\right)^2}{N}\right]$, (iii)

the probability of detection, $\mathrm{P} = \frac{\mathrm{H}}{\mathrm{H}+\mathrm{M}}$, (iv) false alarm ratio, $\mathrm{F} = \frac{\mathrm{FA}}{\mathrm{H}+\mathrm{FA}}$ and (v) the critical success index, $\mathrm{C} = \frac{\mathrm{H}}{\mathrm{H}+\mathrm{M}+\mathrm{FA}}$.

where, P_i and O_i represent satellite precipitation product, and gauge-based observation for the *i*th time step. $\overline{P}$ and $\overline{O}$ are average values, *N* indicates the sample size of the selected time series, H is hit, M is miss and FA is a False Alarm.

Projected Conceptual Algorithmic Framework. The projected integrated framework-based precipitation (IFP) estimates were conceptualized to produce blended precipitation estimators using a performance weighting algorithm for the best two estimators in proportion to performance. The performances were evaluated based on the correlation coefficient (CC), the weighting algorithm used for the proportional ratio (φ) and the Langmuir equation as described below.

Let a specific gauge $O_i = \left[O_1, O_2, O_3, \ldots\ldots\ldots\ldots O_N\right]$ be the donor based on an observed time series of size *N*. $P_i^j = \left[P_1, P_2, P_3, \ldots\ldots\ldots\ldots P_N\right]$ is the *j*th (*j* = 1, 2, 3….n)) satellite product (i.e., TRMM, IM-L and IM-F) in the observation time series obtained from the product source. By using O_i and P_i^j time series of a selected gauge, the correlation coefficient for each selected satellite product was estimated.

Based on the CC outcome, the three satellite products were individually ranked, i.e., high (CC_H), medium (CC_M) and low (CC_L). The product with the CC_L value was eliminated by retaining the k (where k=1, 2, 3….m) products (here two out of three) having CC_H and CC_M value for further quantification.

To get achieve consistency in the results, IFP was conceptualized using Eq. (1).

$$IFP = \tau P_i^{CC_H} + (1-\tau) P_i^{CC_M} \quad (1)$$

where, $P_i^{\rho_H}$, $P_i^{\rho_M}$ are the precipitation estimates from the products having CC_H and CC_M, respectively. τ is the connection weight assigned to a respective product based on its performance.

The weights (τ) were assigned to retained *k* products using Eqs. (2) and (3) to favor the more significant product, resulting in a better performance score at an individual gauge station.

$$\varphi = \left(\frac{(CC_M - 1)^2}{(CC_H - 1)^2} \right) \quad (2)$$

$$\tau = \left(\frac{\varphi}{\varphi + 1} \right) \quad (3)$$

Lastly, the developed $IFP_i = [IFP_1, IFP_2, IFP_3, ...IFP_N]$ time series was evaluated by $O_i = [O_1, O_2, O_3, ...O_N]$ of assumed ungauged stations, using Eqs. (1-5) to validate the efficacy and projected improvement compared to selected *j* satellite products.

It is worth mentioning that the proposed regional IFP framework was based on the leave-one-out cross-validation (LOOCV). In LOOCV, the data record of one station (assumed ungauged station) was held out from the calibration database. The weights associated with the performance of the best two products were then estimated using the calibration results of the remaining stations (assumed donors). The LOOCV was repeated for all stations considered in this study.

RESULTS

By using the aforementioned statistical tests, the efficacy of the three selected products (i.e., TRMM, IM-L, IM-F) and the projected regional product (IFP) were evaluated based on individual points/stations and described the zones.

Point-Based Quantitative Assessments. The inter-comparison of selected TRMM, IM-L, and IM-F determined that the IM-F produced a significant correlation between the gauged database and satellite-based estimates. On average, the IM-F (average CC: 0.23; P: 0.46; F: 0.36; C: 0.26 and E: 90.23) product dominated, followed by IM-L (average CC: 0.16; P: 0.40; F: 0.37; C: 0.23 and E: 92.23), and TRMM (average CC: 0.14; P: 0.33; F: 0.40; C: 0.21 and E: 94.23). Based on all statistical assessment measures, TRMM, IM-L, and IM-F dominated in 11, 17 and 46 stations, respectively, out of the selected 74 stations. However, when compared with the proposed IFP (average CC: 0.31; P: 0.60; F: 0.36; C: 0.29 and E: 68.57), better performance over the entire study area was seen for IFP, except for a few stations. The resulting spatial variation of respective statistical measures is depicted in Fig. 2.

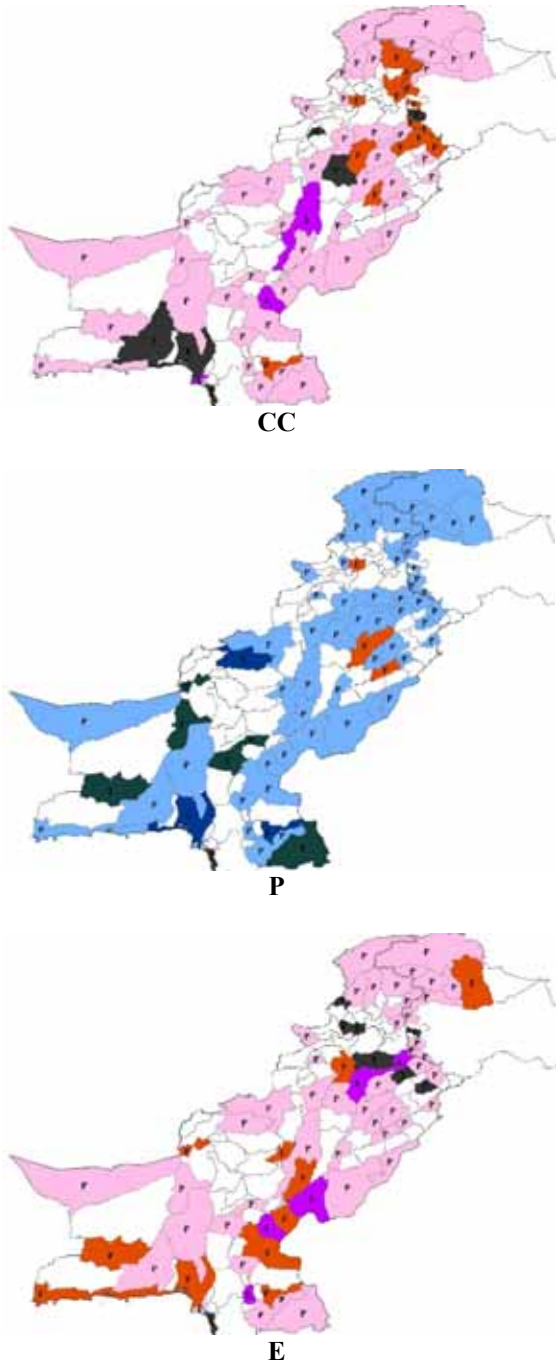


FIGURE 2
The spatial distribution of performance-based domination of respective satellite product
(where P: IFP; F: IM-F; L: IM-L; T: TRMM are represented by rose, gray, blue and red colors respectively) based on measures CC: correlation coefficient; P: Probability of detection; E: Mean square error

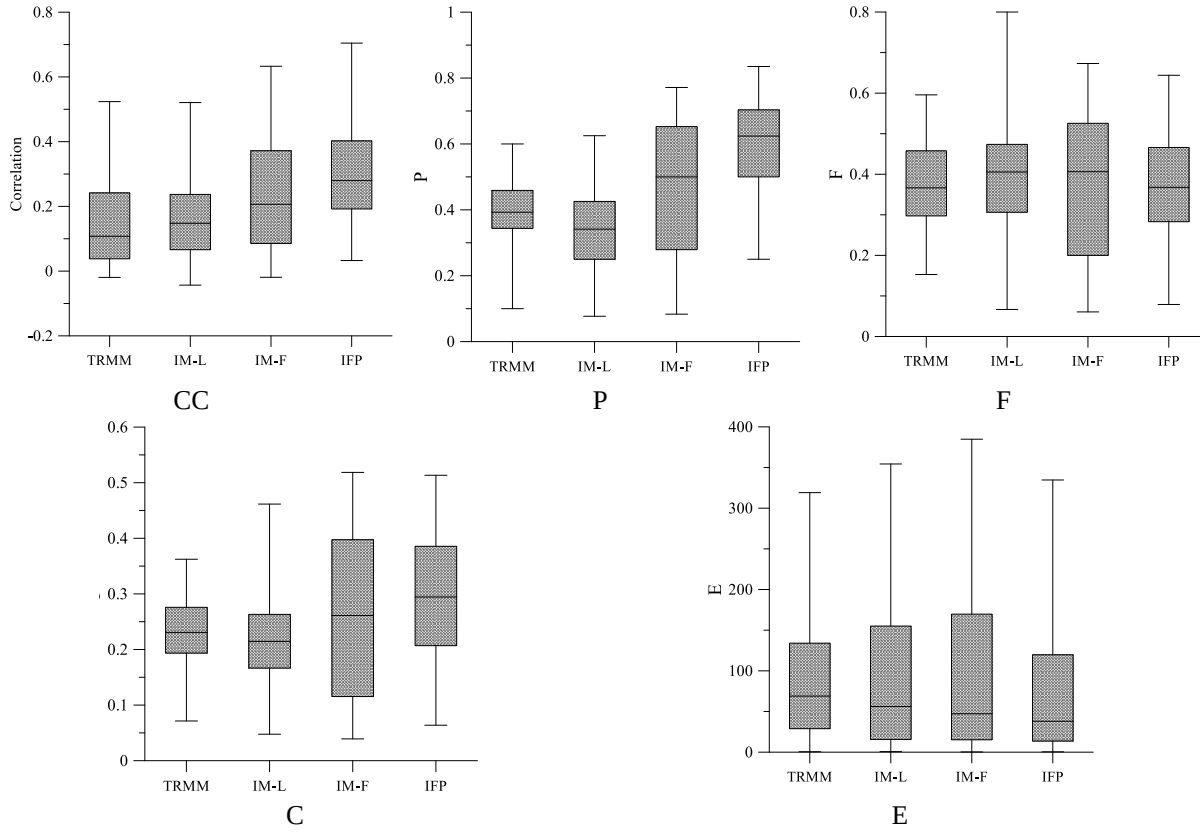


FIGURE 3
Statistical summary of the point-based performance of IFP, IM-F, IM-L and TRMM

Figure 3 describes the overall statistical summary of findings for the evaluation of TRMM, IM-L, IM-F and IFP based on CC, P, F, C, and E. The box-plot in Fig. 3 indicates the minimum (lower line) value, the 1st and 3rd quartile (represented by a central rectangle), the whisker (section in the rectangle) and the maximum (top line) value, by considering 72 stations. The overall summary clearly shows the significant improvement using IFP and the agreement with gauge data compared to the TRMM, IM-L and IM-F.

Zone-Based Quantitative Assessments. In order to probe the aptitude of capturing the precipitation spatial patterns of TRMM, IM-L and IM-F, the assessment was also carried out at a zonal scale. It was noted that the efficacy of these products varied over different regions of Pakistan. It was anticipated, as the nature of the topography would have a significant influence on the quality of satellite-based estimates. Figure 4 indicates that on average, the performance of IM-F and TRMM dominate in zone 1 and zone 2, IM-L and IM-F in zone 3, a mix in zone 4 and TRMM and IM-F in zone 5. However, when IFP was considered, its performance dominated irrespective of the zone, and measurable quality enhancement resulted.

CONCLUSIONS

Precipitation is a most important component of the global hydrological cycle and plays a vital role in the interaction of the atmosphere, hydrosphere and biosphere. Precipitation measurement accuracy is critical in water resource management, drought assessment and projection, flood forecasting and warning systems. However, obtaining an accurate observation is a challenging task. The mainstream methods used are gauge observation, weather radar and satellite-based observation. Recently, satellite products with different temporal and spatial resolution have been released. Among others, the Global Precipitation Measurement (GPM) mission was widely used for precipitation observation. As the successor of TRMM and in the transition era from TRMM to GPM, GPM developed a new calibration algorithm (IMERG) to improve efficacy at a finer scale. However, the inconsistency in performance was observed over varied climatic and topographic regions, timescales and precipitation intensities.

In this study, an integrated framework for quantification of unified satellite precipitation estimates, based on the relative performances and final estimates of well-known satellite products was introduced. It was hypothesized that could maximize the

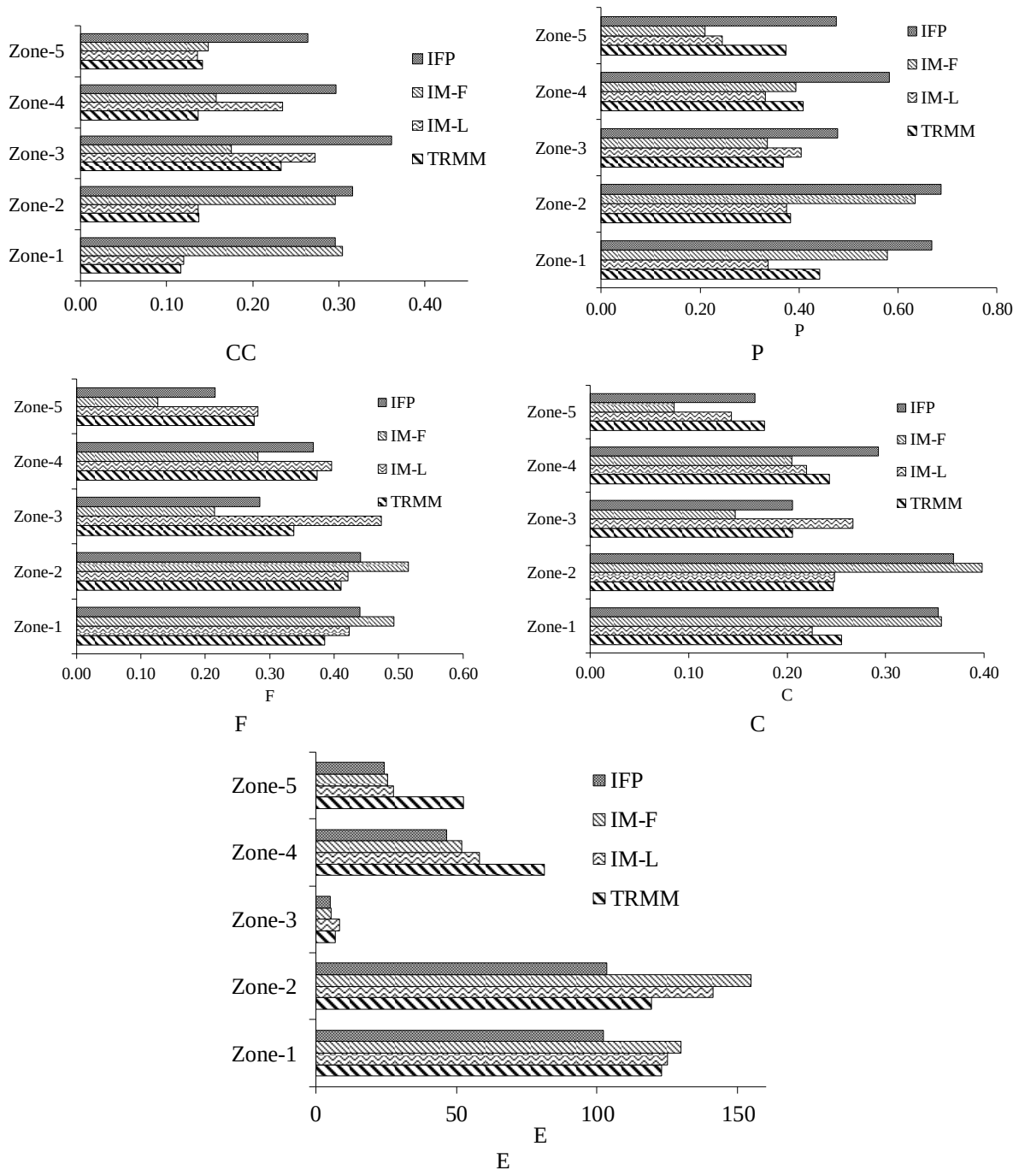


FIGURE 4
Statistical summary of zone-based performance assessment based on the average value

advantages and minimize the disadvantages of considered satellite products. The proposed IFP framework is mainly based on automatic ensemble algorithm to aggregate the product observations, based on the performance (i.e., the value of ρ) of the selected products, connection weights.

The results indicated that the TRMM, IM-L and IM-F have significant capability to capture the gauge's observation, but efficiency was inconsistent in different climatic regions of Pakistan. The TRMM, IM-L and IM-F resulted in significant outcomes in terms of CC, F, P, C, and E - the performance evaluation metrics in low altitude regions. However, in the case of high-altitudes, dry areas and regions with fewer rainfall events, performance decreased. There could be numerous reasons; e.g., uncertainty in verification of results in a developing country like Pakistan (e.g., station density, the impact of wind, random static errors), complex topography and climate, scarce use of the gauge in the production of GPM gauge observation estimation).

In spite of the low performance (average CC: 0.31; P: 0.60; F: 0.36; C: 0.29 and E: 68.57), the projected IFP framework provided comparatively better agreement with the gauged data through the entire study area. This was expected, as the projected framework has the advantage of choosing the set of suitable products based on performance. Hence, IFP is a step forward in the applicability of satellite-based estimates in the data-sparse region like Pakistan.

ACKNOWLEDGEMENTS

The authors would like to thank the Center of Excellence in Water Resource Engineering. The authors are also grateful to the Pakistan Meteorological Department (PMD) for providing the rain gauge data used in the study.

REFERENCES

[1] Moin, E., Shakir, A.S., Zhang, F., Sonia, Z., Nabi, G., Ijaz, A. (2018) Spatiotemporal Variability in Hydro-Meteorological Time Series Data Using Non-Parametric Tests Over Hindu Kush, Himalayan and Karakoram Ranges in Pakistan. Fresen. Environ. Bull. 27, 3666-3677.

[2] Zaman, M., ShouqiYuan, J.L., Usman, M., Ahmad, I., Chandio, F.A., Saifullah, M., Adnan, M. (2017) Quantifying the effect of climate change on precipitation and temperature patterns by using variant of non-parametric techniques. Fresen. Environ. Bull. 26, 7419-7431.

[3] Gao, Y.C., Liu, M.F. (2013) Evaluation of high-resolution satellite precipitation products using rain gauge observations over the Tibetan Plateau. Hydrology and Earth System Science. 17(2), 837-849.

[4] Derin, Y., Yilmaz, K.K. (2014) Evaluation of multiple satellite-based precipitation products over complex topography. Journal of Hydrometeorology. 15, 1498-1516.

[5] Castro, L.M., Miranda, M., Fernández, B. (2015) Evaluation of TRMM multi-satellite precipitation analysis (TMPA) in a mountainous region of the central Andes range with a Mediterranean climate. Hydrology Research. 46(1), 89-105.

[6] Ringard, J., Becke, M., Seyler, F., Linguet, L., (2015) Temporal and Spatial Assessment of Four Satellite Rainfall Estimates over French Guiana and North Brazil. Remote Sensing. 7(12), 16441-16459.

[7] Tang, G., Ma, Y., Long, D., Zhong, L., Hong, Y. (2016) Evaluation of GPM Day-1 IMERG and TMPA Version-7 legacy products over Mainland China at multiple spatiotemporal scales. Journal of Hydrology. 533, 152-167.

[8] Zhang, D., Zhang, Q., Werner, A.D., Gu, R. (2016) Assessment of the reliability of popular satellite products in characterizing the water balance of the Yangtze River Basin China. Hydrology Research. 47(1) 8-23.

[9] Akinsanola, A.A., Ogunjobi, K.O., Ajayi, V.O., Adefisan, E.A., Omotosho, J.A., Sanogo, S. (2017) Comparison of five gridded precipitation products at climatological scales over West Africa. Meteorol Atmos Phys. 129, 669-689

[10] Maggioni, V., Meyers, P.C., Robinson, M.D. (2016) A Review of Merged High-Resolution Satellite Precipitation Product Accuracy during the Tropical Rainfall Measuring Mission (TRMM) Era. Journal of Hydrometeorology. 17(4), 1101-1117.

[11] Ebert, E.E., Janowiak, J.E., Kidd, C. (2007) Comparison of near-real-time precipitation estimates from satellite observations and numerical models. Bulletin of the American Meteorological Society. 88(1), 47-64.

[12] Gebremichael, M., Bitew, M.M., Hirpa, F.A., Tesfay, G.N. (2014) Accuracy of satellite rainfall estimates in the Blue Nile Basin: lowland plain versus Highland Mountain. Water Resources Research. 50(51), 8775-8790.

[13] Falck, A., Maggioni, V., Tomasella, J., Vila, D.A., Diniz, F.L.R. (2015) Propagation of satellite precipitation uncertainties through a distributed hydrologic model: A case study in the Tocantins - Araguaia basin in Brazil. Journal of Hydrology. 527, 943-957.

[14] Mantas, V.M., Liu, Z., Caro, C., Pereira, A.J.S.C. (2015) Validation of TRMM Multi-satellite Precipitation Analysis (TMPA) products in the Peruvian Andes. Atmospheric Research. 163, 132-145.

[15] Sun, R., Yuan, H., Liu, X., Jiang, X. (2016) Evaluation of the latest satellite-gauge precipitation products and their hydrologic applications over the Huaihe River basin. Journal of Hydrology. 536, 302-319.

[16] Yan, D., Liu, S., Qin, T., Weng, B., Li, C., Lu, Y., Liu, J. (2017) Evaluation of TRMM precipitation and its application into distributed hydrological model in Naqu River Basin of the Tibetan Plateau. Hydrology Research. 48(3), 822-839.

[17] Bitew, M.M., Gebremichael, M. (2011) Evaluation of satellite rainfall products through hydrologic simulation in a fully distributed hydrologic model. Water Resources Research. 47(6).

[18] Bitew, M.M., Gebremichael, M., Ghebremichael, L.T., Bayissa, Y.A. (2012) Evaluation of high-resolution satellite rainfall products through streamflow simulation in a hydrological modeling of a small mountainous watershed in Ethiopia. Journal of Hydrometeorology. 13(1), 338-350.

[19] Peng, B., Shi, J., Ni-Meister, W., Zhao, T., Ji, D. (2014) Evaluation of TRMM multi-satellite precipitation analysis (TMPA) products and their potential hydrological application at an arid and semiarid basin in China. IEEE J-STARS. 7(9), 3915-3930.

[20] Yong, B., Liu, D., Gourley, J.J., Tian, Y., Huffman, G.J., Ren, L., Hong, Y. (2015) Global view of real-time TRMM multi-satellite precipitation analysis: implications for its successor global precipitation measurement mission. Bulletin of the American Meteorological Society. 96(2), 283-296.

[21] Thiemig, V., Rojas, R., Zambrano-Bigiarin, M., De-Roo, A. (2013) Hydrological evaluation of satellite-based rainfall estimates over the Volga and Baro-Akobo Basin. Journal of Hydrology. 499, 324-338.

[22] Toté, C., Patricio, D., Boogaard, H., Van-der-Wijngaart, R., Tarnavsky, E., Fun, C. (2015) Evaluation of Satellite Rainfall Estimates for Drought and Flood Monitoring in Mozambique. Remote Sensing. 7(2), 1758-1776.

[23] Yuan, F., Zhang, L., Win, K.W.W., Ren, L., Zhao, C., Zhu, Y., Jiang, S., Liu, Y. (2017) Assessment of GPM and TRMM Multi-Satellite Precipitation Products in Stream Flow Simulations in a Data-Sparse Mountainous Watershed in Myanmar. Remote Sensing. 9(3), 302.

[24] Zambrano-Bigiarini, M., Nauditt, A., Birkel, C., Verbist, K., Ribbe, L. (2016) Temporal and spatial evaluation of satellite-based rainfall estimates across the complex topographical and climatic gradients of Chile. Hydrology and Earth System Science. 21(2), 1295-1320.

[25] Chen, F., Li, X. (2016) Evaluation of IMERG and TRMM 3B43 Monthly Precipitation Products over Mainland China. Remote Sensing. 8(6), 472.

Received: **10.05.2018**
Accepted: **05.11.2018**

CORRESPONDING AUTHOR

Waseem Muhammad
Center of Excellence in
Water Resources Engineering,
University of Engineering and Technology,
Lahore 54890 – Pakistan

e-mail: engr.jatoi@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9755-9761 Fresenius Environmental Bulletin

INVESTIGATION OF THE EFFECTIVENESS OF THE FAN-PAD COOLING SYSTEM AND THE HORIZONTAL TEMPERATURE AND RELATIVE HUMIDITY CHANGES IN THE GREENHOUSE

Sedat Boyaci*

Kirsehir Ahi Evran University, Faculty of Agriculture, Department of Biosystems Engineering, 40200 Kirsehir, Turkey

ABSTRACT

This study was conducted to investigate the effectiveness of the fan-pad cooling system and the temperature and relative humidity changes throughout the horizontal distance in the greenhouse. For this purpose, a total of 6 temperature and relative humidity sensors are installed inside and outside of the greenhouse. In the study, it was determined that the system efficiency was the highest when the external relative humidity is the lowest. When the highest outside temperature measured during the day is considered, the outside temperature at 14:00 was 34.98 °C, while the average internal temperature values in the greenhouse cooled by fan-pad reached to 26.37 °C. The internal temperature in the greenhouse at that hour was found to be 8.61 °C lower than the outside temperature. When the sensor positions are considered, the inside-outside temperature difference measured in front of the pad was 12.27 °C and the cooling effect measured in front of the pad at that hour was 84.60%, while these values in front of the fan were measured as 8.10 °C - 55.85%, respectively. The average temperature increase from the pad to the fan was 5.90 °C and the relative humidity difference was by 16% between the cooling hours of 08:00-19:00. In the statistical analyses performed, it was determined that the temperature-dependent relative humidity difference along the horizontal distance is significantly different from the pad to fan ($p<0.01$).

KEYWORDS:
Greenhouse, fan-pad, temperature, relative humidity

INTRODUCTION

In temperate climate regions, the internal temperature values can exceed the optimum temperature values required for the plant due to the excessive amount of heat energy obtained from solar radiation in the greenhouse environment in open and sunny days. This increase affects the production and marketable fruit quality of the plant [1]. The higher air temperature negatively influence the normal growth and development of crops, including damaging the chloroplast, decreasing photosynthesis rate and closing the leaf stoma. The excessive transpiration cause by high air temperature result in water deficiency [2]. When developing irrigation scheduling for greenhouse crops, environmental factors are important [3]. Moreover, the high temperature increase the consumption of respiration, thus reduce the nutrient uptake of crop plant [2]. In order to reduce the plant stress and provide high quality products for the market, greenhouses need to be cooled. One of the most effective ways of cooling inside a greenhouse is the fan-pad cooling system, which is one of the evaporative cooling methods that allows the sensible heat to turn into a latent heat at the basis of its operation principle. This method was used by different researchers to cool the greenhouses and it was proved that successful results are obtained. In their study that the fan-pad system was used, [4] found that the internal temperature value was 10 °C lower than the outside temperature value and the system efficiency was by 80%. [5] stated that the internal temperature of the greenhouse was cooled to 15 ºC compared to the outdoor temperature using the fan-pad system and that this system has a better cooling efficiency than the other conventional cooling systems by as much as 5 ºC. In their study, in which fans and pads were used, [6] determined a 2 ºC decrease in plant temperature and they accomplished to reduce the temperature inside the greenhouse by 15 ºC compared to the outside temperature. [7] stated that natural ventilation in the greenhouse provides a temperature difference of 2 ºC and that the internal temperature could be lowered to the outside temperature if the shading+ventilation measures were taken together. In addition, they reported that the outside temperature can be reduced to below 8 ºC by conducting evaporative cooling (fan-pad) depending on the external relative humidity and the number of ventilators. The advantage of this method is that the system provides ease of operation and control, and at the same time, it does not lead to the wetting of leaves. On the other hand, the disadvantages of this method are high cost, no uniform cooling in the

greenhouse, and a lower cooling effect compared to the fogging method [8].

This study was carried out to determine the performance of the fan-pad cooling system used to cool the interior of the greenhouse during periods when temperatures rise in the commonly used plastic greenhouses in our country and to determine the temperature and relative humidity changes that occur depending on the distance along the long axis of the greenhouse.

MATERIALS AND METHODS

General characteristics of the studied greenhouse. In the study, 350 µm-thick and 36 months-old UV+IR reinforced PE plastic covering material was used in the greenhouse, in which the floor area is 150 m^2 (7.5 m x 20 m), the undergroove height is 3 m, and the ridge height is 5 m.

A pad material made of cardboard, which is 7.5 m^2 in total (50-m-height and 100x60x10 cm) and dispenser honeycombs ensuring the distribution of the water coming on the pad were placed in the greenhouse floor in the air inlet openings on the short side facing the northern part of the greenhouse. The pads were kept constantly wet by providing water flow at intervals of 10 cm by means of a pipe with 2 mm diameter holes on the dispenser honeycombs. Water was sent to the dampening pads by means of a centrifugal pump that moves from the electric motor to a water tank located outside the greenhouse. A water flow with a flow rate of 10 lt/min per 1 meter pad towards the pads was provided by a water pump [9]. The water that accumulates at the bottom of the pad unit returns to the tank again. A fan at the height of 3 m above the basin was placed opposite to the dampening pads in the greenhouse compartment. The features of the fan used to absorb air through the pad unit were HP: 1.5, KW: 1.1, Volt: 380, HZ:50, and the air velocity in the pads was 1.40 m/s.

Determination of effectiveness of cooling systems. The efficiency of the cooling systems used in the greenhouses is calculated by Equation 1 suggested by [10, 11, 12].

$$CE = \frac{T_{out}-T_{in}}{T_{out}-T_{wb}} \times 100 \qquad [1]$$

Where; CE: Cooling efficiency (%); T_{out}: Outside dry-bulb temperature of entering air to Pad (°C); T_{in}: The dry-bulb temperature of leaving air from Pad (°C); $T_{wb:}$ Outside wet-bulb temperature of entering air to Pad (°C).

The temperature and relative humidity changes along the greenhouse horizontal axis were measured along the horizontal axis between the fan and the pad for 24 hours. When the sensor positions inside and outside the greenhouse are considered, the placement was carried out as follows: the distances of the outer sensor (T_{out}, RH_{out}) and the inner sensors from the pad (T_1, RH_1) in front of the pad, (T_2, RH_2) 2.5 m, (T_3, RH_3) 7.5 m, (T_4, RH_4) 12.5 m, and (T_5, RH_5) 17.5 m (2.5 m away from the fan). The sensor positions in the study are shown in Figure 1.

Instruments and statistical analyzes used in meteorological data measurement. The temperature and relative humidity values in the greenhouse were measured by HOBOs every 30 minutes and the values were recorded. Then these values were transferred to the Microsoft Excel program with BoxCar Pro 4.3 program and graphics were created and the differences between the sensors were analyzed by the SPSS statistical package program, and the differences between the sensors were determined.

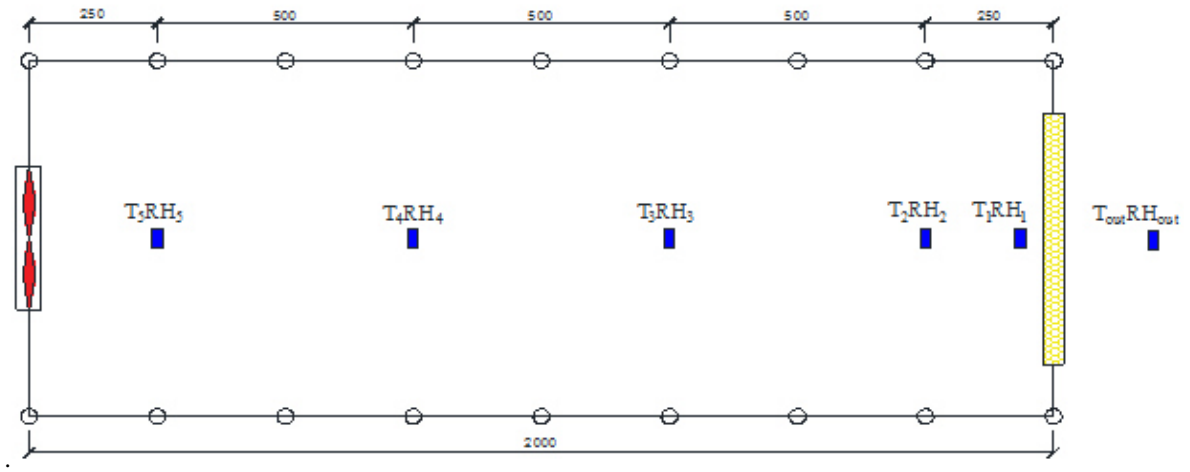


FIGURE 1
Sensor positions inside and outside the greenhouse

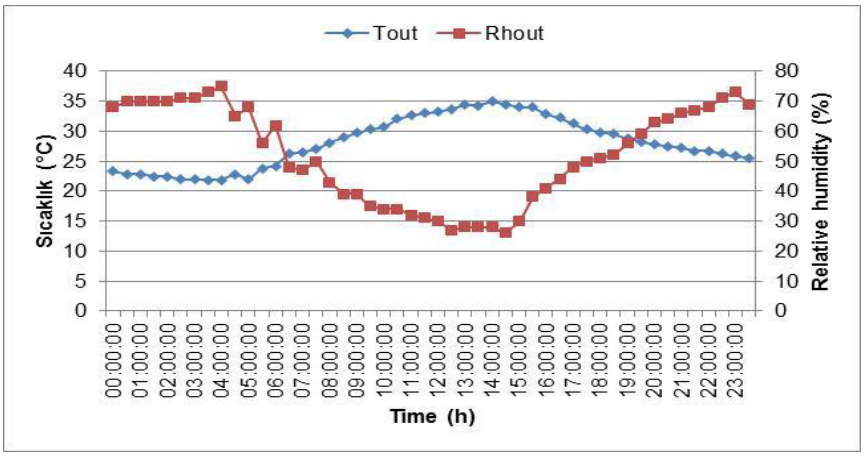


FIGURE 2
The temperature and relative humidity measured in and out of the greenhouse

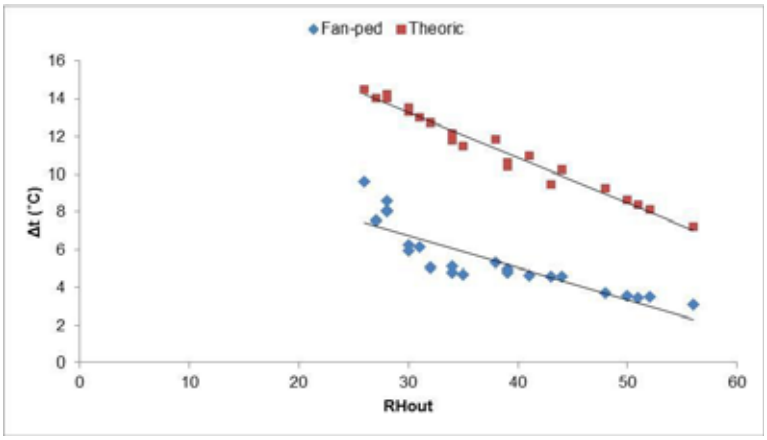


FIGURE 3
The external and internal temperature difference reached due to external relative humidity

RESULTS

In the study, the measured temperature and relative humidity values inside and outside of the greenhouse are given in Figure 2. During the day, the cooling application started to operate between 08:00-19:00. The measured outside temperature values between the hours of cooling varied from 28.02 °C to 34.98 °C and the average temperature value was found to be 31.87 ºC. The internal temperature values were varied between 20.79 °C and 30.47 °C in the FP applied greenhouse and the average temperature values were measured as 26.39 °C. When the external relative humidity values measured during the cooling hours are considered, these values ranged from 26% to 56%. The average external relative humidity value was measured as 38%. The internal relative humidity values ranged from 57% to 76% in the FP. The average relative humidity values were measured as 66%. When the highest outside temperature measured during the day is considered, the outside temperature value at 14:00 was found 34.98 °C, while the mean internal temperature values in the greenhouse in which the FP applied reached to the value of 26.37 °C. At this hour, the internal temperatures in the greenhouse were found to be 8.61 °C lower than the outdoor temperature.

In the study, the values of the inside-outside temperature differences reached in the greenhouses are shown on the graph depending on the external relative humidity, while the theoretical line given shows the external relative humidity-dependent change of the theoretically calculated temperature difference based on the psychrometric diagram (Figure 3). Accordingly, a relationship of R^2 =80 was found between the external relative humidity and inside-outside temperature difference. Accordingly, when the external relative humidity was 26%, the mean internal temperature value was found to be 9.61 °C less than the outside temperature value. With the increase of the external relative humidity, the difference of the inside-outside temperature started to decrease. Therefore, it was seen that the cooling application works more efficiently with the decreasing external relative humidity during the day. Accordingly, the average cooling efficiency during the day was calculated as 47.00%. Increasing the temperature along the horizontal axis from the pad to the fan in the greenhouse caused the

system efficiency to decrease.

The temperature and relative humidity values measured depending on the sensor positions in the greenhouse are given in Figure 4 and Figure 5. With the start of the cooling application between 08:00-19:00, when the temperature inside the greenhouse starts to rise, the FP internal temperature values start to decrease below the outside temperature values at all sensor positions. Accordingly, the measured temperature and relative humidity values in front of the pad in the greenhouse (T_1-RH_1) had the lowest temperature and the highest relative humidity. It was seen that the temperature values increased as the distance from the pad increases, while the relative humidity decreases. The average outside temperature measured at the time of cooling was measured as 31.87 °C and the relative humidity was measured as 38%, while the average temperature and relative humidity from in the front pad to the fan were measured depending on the sensor positions were measured as T_1=23.81˚C, T_2=25.22 ˚C, T_3=26.89 ˚C, T_4=28.14 ˚C, T_5=27.90 ˚C) and RH_1=74%, RH_2=69%, RH_3=66%, RH_4=61%, RH_5=62%, respectively.

Accordingly, the average internal and externainside-outside temperature difference reached was measured depending on the sensor positions as ΔT_1=8.07 ˚C, ΔT_2=6.65 ˚C, ΔT_3=4.98 ˚C, ΔT_4=3.74 ˚C, ΔT_5=3.97 ˚C, respectively. During the day, the measured inside and outside temperature difference reached to the maximum value when the outside relative humidity value was 26%. Accordingly, the inside-outside temperature difference reached was measured depending on the sensor positions as (ΔT_1=12.27 ˚C - ΔT_2=10.79 ˚C - ΔT_3=9.06 ˚C - ΔT_4=7.88 ˚C - ΔT_5=8.10 ˚C), respectively.

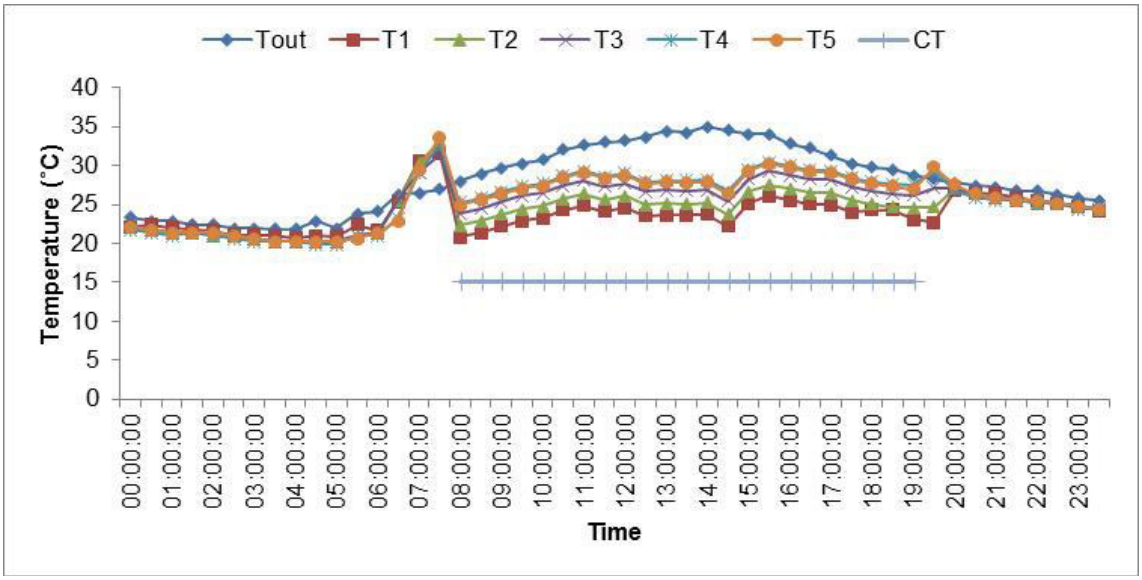


FIGURE 4
The temperature values measured at different distances in the greenhouse

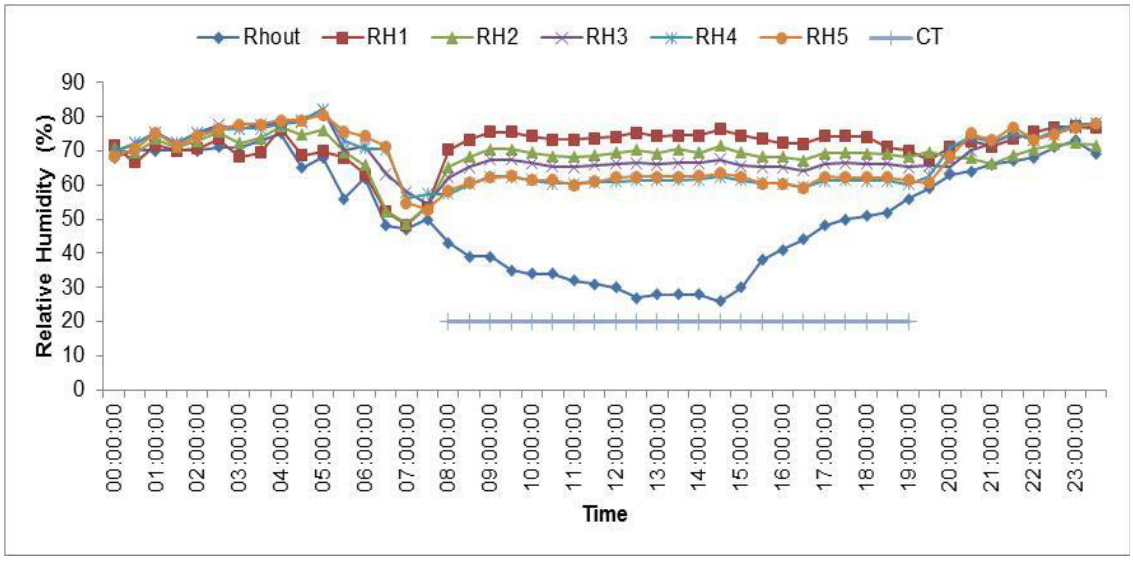


FIGURE 5
The relative humidity values measured at different distances in the greenhouse

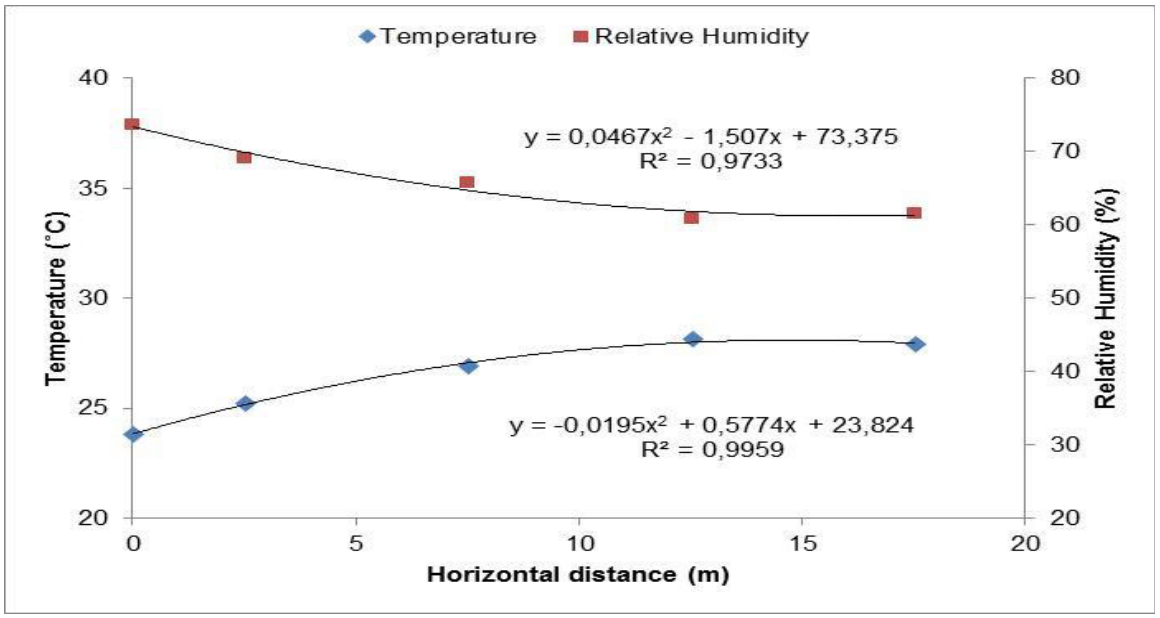


FIGURE 6
Temperature and relative humidity change depending on the horizontal distance

The change in temperature and relative humidity values in the greenhouse, depending on the horizontal distance is given in Figure 6. Accordingly, temperature and relative humidity are found as a second-degree polynomial along the horizontal axis.

DISCUSSION

In the study area having a Mediterranean climate, the internal temperature of the greenhouse started to rise after 8:00 am and it remains above the outdoor temperature until sunset in the evening. Considering that tomato, pepper, cucumber etc. grown in the greenhouses adapted to 17-27 ºC, the average daily temperature in the greenhouse should be 12-22 ºC.

In case that the daily average temperature falls below 12 ºC, the greenhouses should be heated at night, when it rises above 22 ºC, they should be cooled or the greenhouses should be kept empty. The relative humidity values should be between 60-70% [13, 14]. The plant growth continues, and blooming occurs at temperatures above 30 °C however, the pollen germination worsens, the pollen tube does not grow well enough and because there is no fertilization, flowers shed, parthenocarpic small fruit grows and yield decreases [15, 16]. In the study, the measured temperature values outside the greenhouse varied between 28.02 °C and 34.98 °C and the average temperature value was found to be 31.87 ºC. In this case, it is necessary to cool the greenhouse as suggested by the researchers. Otherwise, it does not seem possible to obtain a yield by carrying out cultivation.

In the study conducted by [4], they obtained a decrease of 10 ºC compared to the outdoor temperature in the greenhouse temperature in the fan-pad cooling system and the system efficiency was calculated as about 80%, while [17] calculated the cooling efficiency in his study as 53.3%, between 32.4% and 76.6%. [5] reported that the fan-pad system cooled the internal temperature of the greenhouse by 15 °C compared to the outdoor temperature and that this system has a better cooling efficiency than the other conventional cooling systems by as much as 5 °C. [6] managed to reduce the internal temperature by 15 ºC compared to the outdoor temperature, while [18] reduced the internal temperature by 10-12 ºC. In the study, the maximum inside-outside temperature difference was found 12.27 °C, while the cooling efficiency was found 84.60%. The average inside-outside temperature difference was found 5.48 °C, while the cooling efficiency was found 47.00%.

Although the results obtained show similar characteristics to those of these researchers, the cooling effect in the greenhouse is reduced due to the increase in temperature because of the distance of moist cool air between the pad and the fan in the greenhouse. [8] and [17] stated that one of the negative aspects of the fan-pad system is that there is no uniform cooling in the greenhouse (the amount of humidity decreases in the airflow direction along the building height as the temperature increases) due to the temperature difference between the fan and the pad. [19] stated that there was a difference of 6 ° C between pad and fan, while [20], stated that there was a difference of 7 °C in their study. It was determined that there was a difference of 5.90 °C from the pad to the fan, which is similar to the

study conducted by these researchers.

As a result, it was determined that the effectiveness of the system depends on the external relative humidity value, and the system efficiency is increased when the external relative humidity value is low. In addition, it is very important in terms of system efficiency and a uniform distribution of temperature and relative humidity to use circulation fans inside the greenhouse in order to reduce the increasing temperatures along the path between the pad and the fan along the horizontal axis, to select the fan in the appropriate capacity to provide sufficient air exchange, to place fan and pads on long axis instead of greenhouse short axis and to control unwanted openings in the greenhouse.

REFERENCES

[1] Hanan, J.J., Holley, W.L. and Goldberry, K.L. (1978) Greenhouse management. Advances Series in Agricultural Sciences. Vol. 5. Springer-Verlag, Berlin.

[2] Karaali, R. and Ozturk, I.T. (2016) Thermodynamic optimization of a zero CO_2 emission cogeneration cycle. Fresen. Environ. Bull. 25, 5729-5738.

[3] Fenglin, A., Qiang, S., Shubin, W., Biying, L., Xiangzhu, Z., Ru, X., Iouzen C. and Yizhang, L. (2017) Precision irrigation scheduling for greenhouse tomato based on analysis of environmental characters and the penman-monteith model. Fresen. Environ. Bull. 26, 6938-6944.

[4] Kittas, C., Bartzanas, T. and Jaffrin, A. (2003) Temperature gradients in a partially shaded large greenhouse equipped with evaporative cooling pads. Biosystems Engineering. 85(1), 87-94.

[5] Davies, P.A. (2005) A solar cooling system for greenhouse food production in hot climates. Solar Energy. 79, 661-668.

[6] Fuchs, M., Dayan, E., and Presnov, E. (2006) Evaporative cooling of a ventilated greenhouse rose crop. Agricultural and Forest Meteorology. 138, 203–215.

[7] Baytorun, A.N., Tokgöz, H., Üstün, S. and Akyüz, A. (1994) Air conditioning facilities in greenhouses. 3. Cooling and Air Conditioning Congress. In: Turkey. 1: 303-313 (in Turkish).

[8] Arbel, A., Barak, M. and Shklyar. A. (2003) Combination of forced ventilation and fogging systems for cooling greenhouses. Biosystems Engineering. 84(1), 45-55.

[9] ASAE. (1994) Plants: greenhouses, growth chambers and other facilities. ASAE Fundamentals Handbook (SI).

[10] Bottcher, R.W., Baughman, G.R. and Kesler, D.J. (1989) Evaporative cooling using a pneumatic misting system. Trans. ASAE. 32, 671-676.

[11] Al-Amri, A.M.S. (2000) Comparative use of greenhouse cover materials and their effectiveness in evaporative cooling systems under conditions in eastern province of Saudi Arabia. Agricultural Mechanization in Asia, Africa and Latin America. 31(2), 61-66.

[12] Kittas, C., Katsoulas, N. and Baille, A. (2001) Influence of greenhouse ventilation regime on the microclimate and energy portioning of a rose canopy during summer conditions. Journal of Agricultural Engineering Research. 79(3), 349–360.

[13] Baytorun, N., Abak, K., Üstün, S. and İkiz, Ö. (1996) Greenhouse agriculture potential in GAP areas and comparison with coastal areas. GAP 1. Vegetable Agriculture Symposium. In: Turkey. 1, 367-374. (in Turkish)

[14] Zabeltitz, C.V. (2011) Integrated greenhouse systems for mild climates. ISBN: 978-3-642-14581-0, 29, p, 2011.

[15] Abak, K. and Çürük. S. (1995) Moisture, high temperature adaptation, flower dust vitality and germination capabilities of some tomato genotypes in Çukurova conditions. II. National Horticulture Congress Proceedings. In: Turkey. 2: 177-178. (in Turkish).

[16] Vural, H., Eşiyok, D. and Duman, İ. (2000) Culture plants. Ege University, İzmir, 440p. (in Turkish)

[17] Öztürk, H.H. (2004) The efficiency of fan and pad cooling system, sensible and latent heat transfer in a venlo glasshouse. Journal of Agricultural Sciences. 10(4), 381-388.

[18] Atılgan, A. and Öz, H. (2007) Cooling of greenhouses in cool climate regions by fan-pad system. Derim. 24(1), 11-18. (in Turkish)

[19] Oz, H., Atilgan, A., Buyuktas, K. and Alagoz, T. (2009) The efficiency of fan-pad cooling system in greenhouse and building up of internal greenhouse temperature map. African Journal of Biotechnology. 8(20), 5436-5444.

[20] Dayıoğlu, M.A. and Silleli, H.H. (2015) Performance analysis of a greenhouse fan-pad cooling system: gradients of horizontal temperature and relative humidity. Journal of Agricultural Sciences. 21, 132-143.

Received: **10.05.2018**
Accepted: **10.09.2018**

CORRESPONDING AUTHOR

Sedat Boyaci
Kırşehir Ahi Evran University,
Faculty of Agriculture,
Department of Biosystems Engineering,
40200 Kırşehir – Turkey

e-mail: sedat.boyaci@ahievran.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9762-9767 Fresenius Environmental Bulletin

STUDY ON PERFORMANCE OF WATER TYPE HEAT TRANSFER AND RESISTANCE PROPERTIES OF PLATE HEAT EXCHANGER OF R410A

Qing Luo[1,2,3,4], Jinping Li[1,2,3,*], Yuping Deng[5]

[1]Western China Energy & Environment Research Center, Lanzhou University of Technology, Lanzhou, 730050, China
[2]Key Laboratory of Energy Supply System Drived by Biomass Energy and Solar Energy of Gansu Province, Lanzhou, 730050, China
[3]College of Energy and Power Engineering, Lanzhou University of Technology, Lanzhou, 730050, China
[4]School of Civil Engineering, Sichuan University of Science & Engineering, Zigong, 643000, China
[5]Qingdao Hisense Hitachi Air-conditioning Systems Co., Ltd., Qingdao, 266510, China

ABSTRACT

The plate heat exchanger has become a very important heat exchanger due to its high heat transfer performance and compact structure. In recent years, the research and structural improvement of the plate heat exchanger have made the application range of the plate heat exchanger more and more extensive.

Based on water type heat transfer, this paper studies the performance of water type heat transfer and resistance properties of plate heat exchanger of R410A. The method and model for the simulation calculation of refrigeration system are given.

Based on the experiment, the relationship between the heat transfer area, corrugation depth, the thickness of the plate and the relative error of the heat balance (η) is found through the change of the operating parameters.

KEYWORDS:
R410A, Plate heat exchanger, Heat transfer, Exergetic efficiency

INTRODUCTION

On account of their compactness and high heat transfer coefficient (HTC), plate heat exchanger (PHE) has been increasingly used in various industries in the past decades. There is a large amount of research activities concerning heat transfer of plate heat exchanger in recent years. Among these studies, a few literatures of experimental investigation on condensation heat transfer in PHE can be found. For steam condensation, Wang and Zhao [1] carried out watersteam test of complete and partial condensation at counterflow and at concurrent flow in a PHE, the steam inlet pressure of the PHE was maintained at 0.13 MPa, 0.16 MPa and 0.2 MPa. The experimental result indicated that the main factors affecting the condensation HTC in PHE were total mass velocity, condensation pressure, and Prandtl number of the liquid. Wang et al. [2] set up a test rig for investigation of steam condensation in PHEs, seven different PHEs had been tested. Based on the experimental data, although they did not take any local measurement, they proposed a modified equation to predict the local HTC of steam condensation in PHEs.

Condensation in PHE is a very complicated process, involve various parameters such as quality [3-6], fluid property, mass flux, local flow regimes, and oil effect [7-12]. Furthermore, the attention almost is focused on refrigeration industry [13-15].

Based on the experiment, the relationship between the heat transfer area (A), corrugation depth (B), the thickness of the plate (C) and the relative error of the heat balance (η) is found through the change of the operating parameters, which is an experiment of the circulating cooling water fouling in the R410A plate heat exchanger. Research provides reference.

EXPERIMENTAL

An experimental apparatus was designed incorporating corrugated PHE to probe into the heat transfer phenomenon under different operating conditions. The M3–FG model of corrugated PHE, used in this experiment, was purchased from USA. The operational data were as follows: design pressure was 1 MPa, test pressure and design temperatures were 1.3 MPa and 100°C respectively. The material of plate was SA 240 GR.316 with 0.5 mm thickness. The shape of corrugation in plates was of chevron type. The area of heat exchanger was 1.2 m^2.

The experiment setup mainly consists of two fluid loops. The cold fluid loop and hot fluid loop. The cold fluid loop comprised four components i.e. nanofluid tank (25 liter volume), gear pump, gate valve and Coriollis flow meter. Here, the nanofluid was working as cold fluid/coolant. Similarly, the hot fluid loop incorporated a DM water tank (25 liter volume) with 4 kW heater, a hot fluid pump, a gate valve and a flow meter. Water was the working fluid

in this loop. There was provision for insulation to minimize the heat loss.

In this experimentation, first of all, the nanofluid solution of requisite concentration was prepared and nanofluid tank was filled with it. Afterward hot water was filled in DM water tank. The inlet temperature of nanofluid and hot DM water was set by human machine interface (HMI). The desired inlet temperatures were 20°C and 50°C for nanofluid and hot DM water respectively. The volume flow rates were set by Coriollis flow meter. The experimentation unit was run till the set temperature of DM water and nanofluid was achieved. In this experiment, the four port temperatures for PHE were automatically displayed on the screen of HMI under steady state condition. Pressure between the entrance and exit port of the PHE for hot DM water and nanofluid was recorded manually by Pressure indicators. The volume flow rate of nanofluid varied from 0.5 to 2.0 lpm while the volume flow rate of hot DM water was kept constant at 3 lpm. The experiment setup mainly consisted of two fluid loops. The cold fluid loop and hot fluid loop. The cold fluid loop comprises four components i.e. nanofluid tank (25 liter volume), gear pump, gate valve and coriollis flow meter. Here, the nanofluid was working as cold fluid/coolant. Similarly, the hot fluid loop incorporated the DM water tank (25 liter volume) with 4 kW heater, hot fluid pump, gate valve and flow meter. Water was the working fluid in this loop. There was proper insulation to minimize heat loss.

RESULTS AND DISCUSSION

Relative error of heat balance = 1.7533 +0.1488A +0.0988B +0.1675C -0.2679A*A -0.3329B*B -0.1354C*C +0.0775A*B -0.0950A*C +0.0700B*C

High heat transfer coefficient, small footprint, light weight, low fouling factor, flexible assembly, easy maintenance and other advantages, compared with the conventional shell and tube heat exchanger, under the same flow resistance and pump power consumption, heat transfer coefficient should be about twice higher.

For Fig.1, contour plot of relative error of heat balance vs C, B is present. the relative error of the heat balance (η) is between 1.65-1.80 and its absolute value is < 5%, which means experimental data is reasonable.

For Fig.2, contour plot of relative error of heat balance vs B, A is present. the relative error of the heat balance (η) is between 1.4-1.6 and its absolute value is < 5%, which means experimental data is reasonable.

For Fig.3, contour plot of relative error of heat balance vs C, A is present. the relative error of the heat balance (η) is between 1.6-1.8 and its absolute value is < 5%, which means experimental data is reasonable.

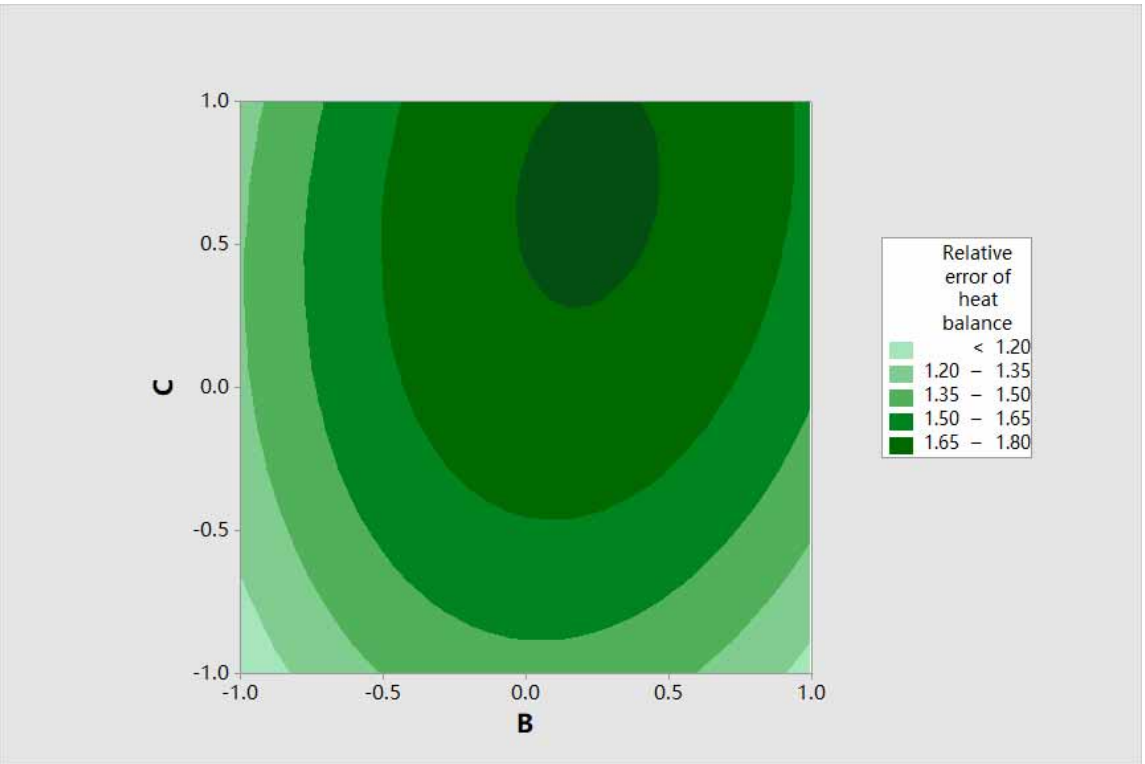


FIGURE 1
Contour Plot of Relative error of heat balance vs C, B

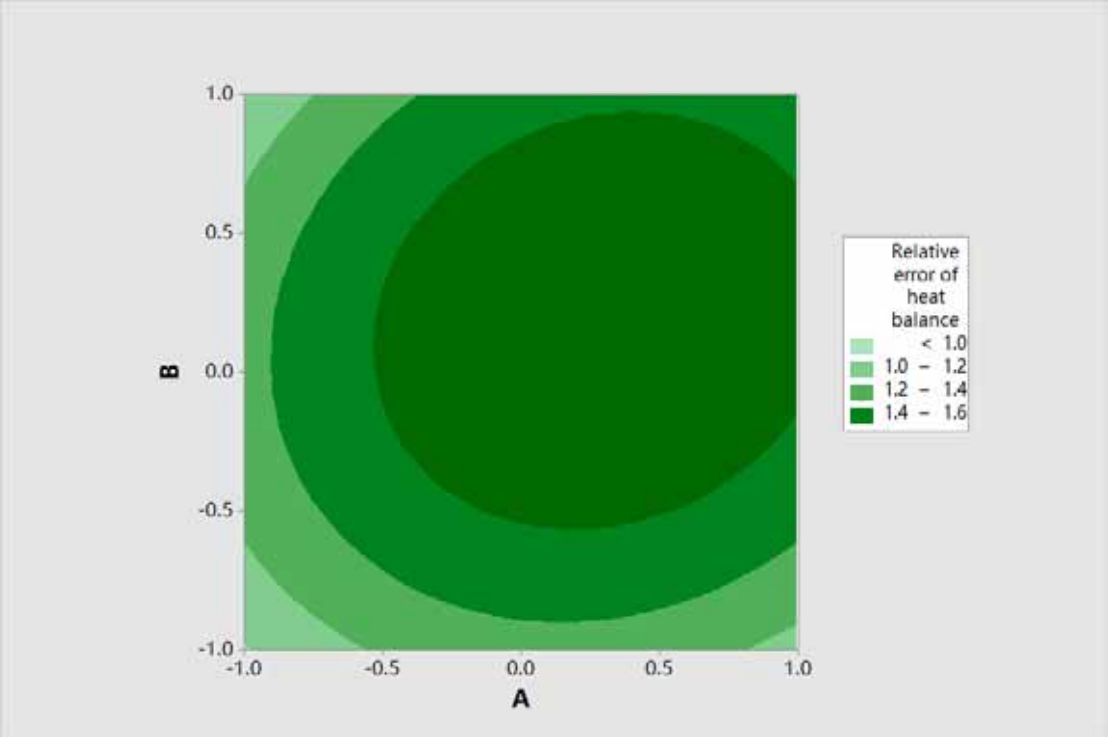


FIGURE 2
Contour Plot of Relative error of heat balance vs B, A

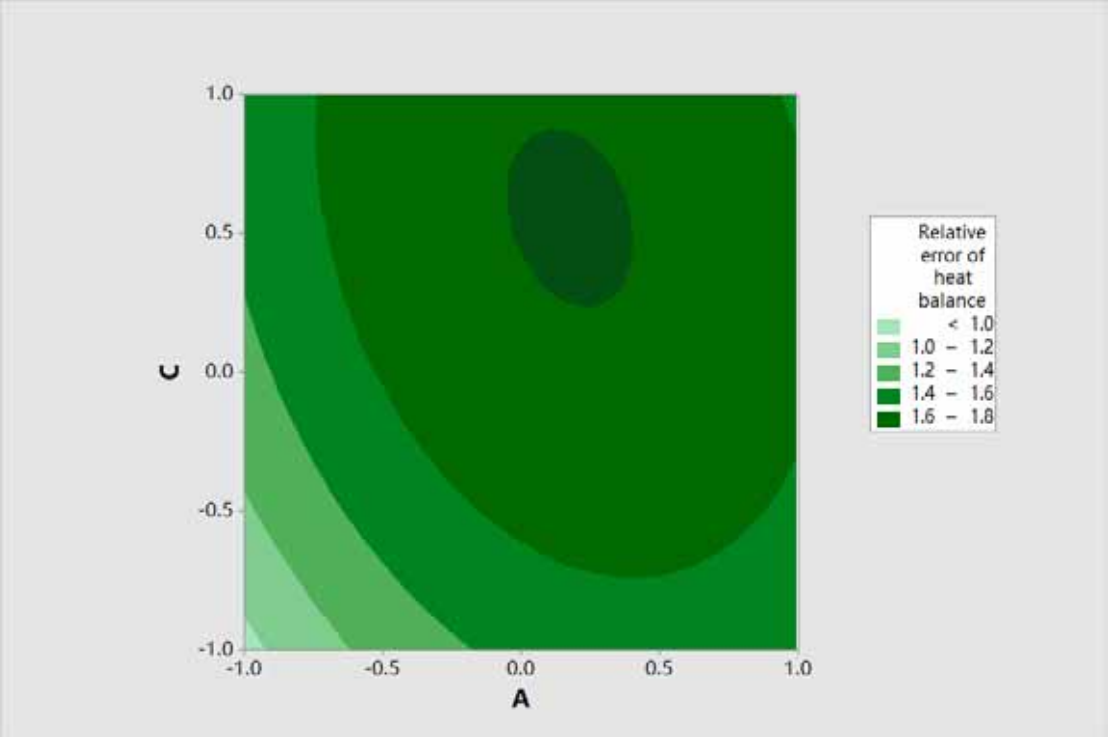


FIGURE 3
Contour Plot of Relative error of heat balance vs C, A

For Fig.4, it is seen that the relative error of heat balance increased when thickness of the plate increased. The relative error of heat balance is maximum when thickness of the plate is 0.6 mm.

Therefore, in the selection of heat exchangers, according to actual needs, comprehensive factors should be integrated to select the appropriate model, so that the fluid reaches the best flow state, and the heat exchanger performance and economy are optimized.

For Fig.5, it is seen that the relative error of heat balance increased when thickness of the plate

increased. The relative error of heat balance is maximum when thickness of the plate is 0.6 mm.

It is seen that the relative error of heat balance increased when heat transfer area increased. The relative error of heat balance is maximum when heat transfer area is 0.3 m^2.

Water type heat transfer has high heat transfer efficiency, using herringbone corrugated sheet in water. The heat exchange coefficient under water exchange can reach 6000W/(m^2K), and it can reach 2000-3000W/(m^2K) under normal circumstances.

It is seen that the relative error of heat balance increased when corrugation depth increased. The relative error of heat balance is maximum when corrugation depth is 2 mm.

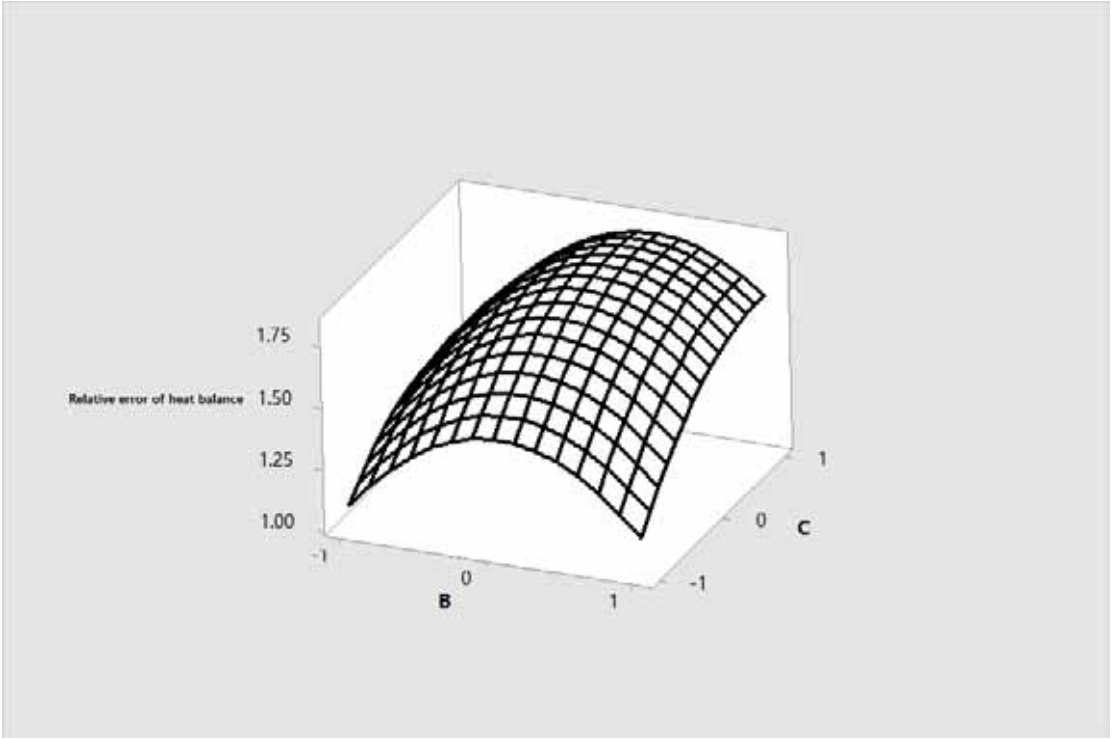


FIGURE 4
Surface Plot of Relative error of heat balance vs C, B

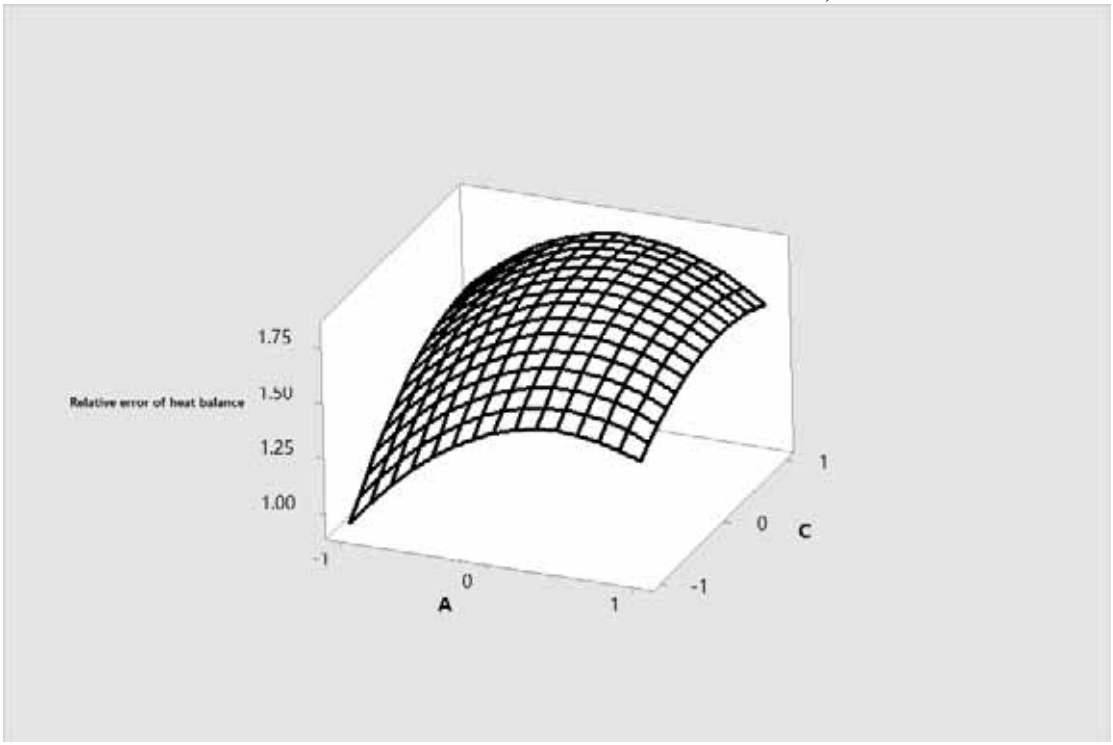


FIGURE 5
Surface Plot of Relative error of heat balance vs C, A

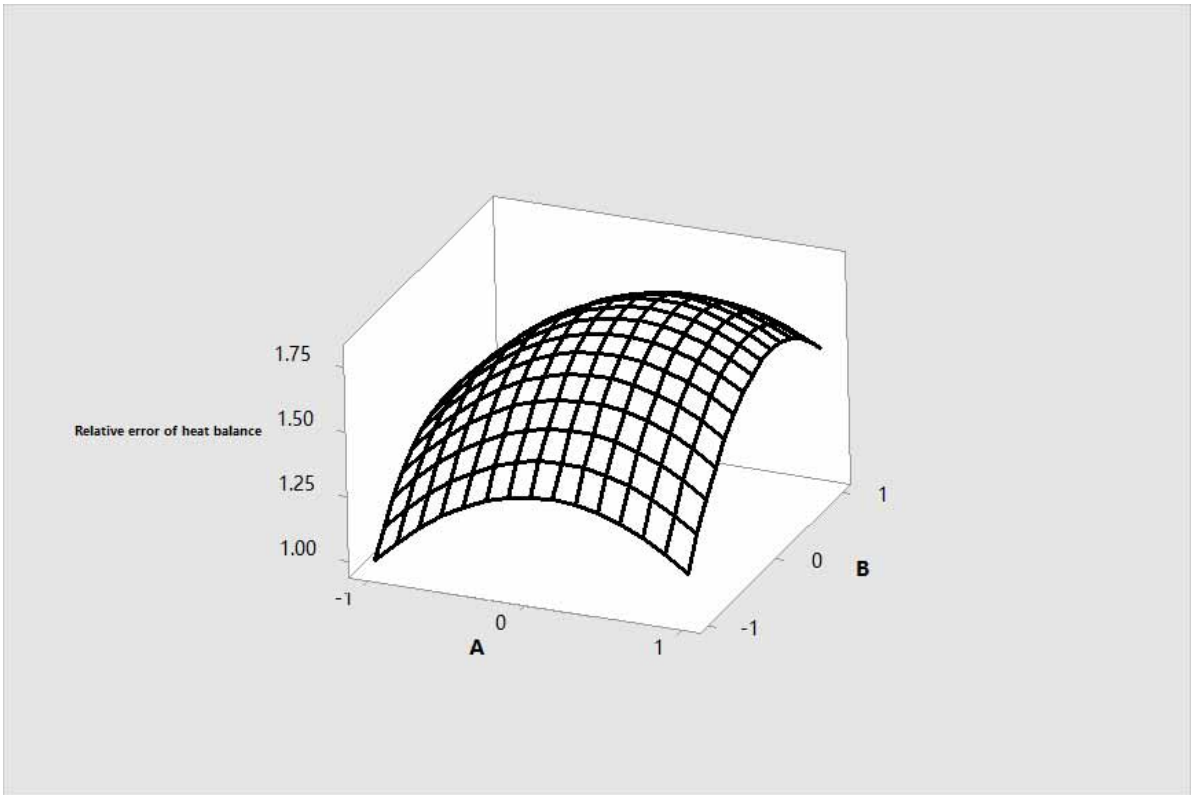


FIGURE 6
Surface Plot of Relative error of heat balance vs B, A

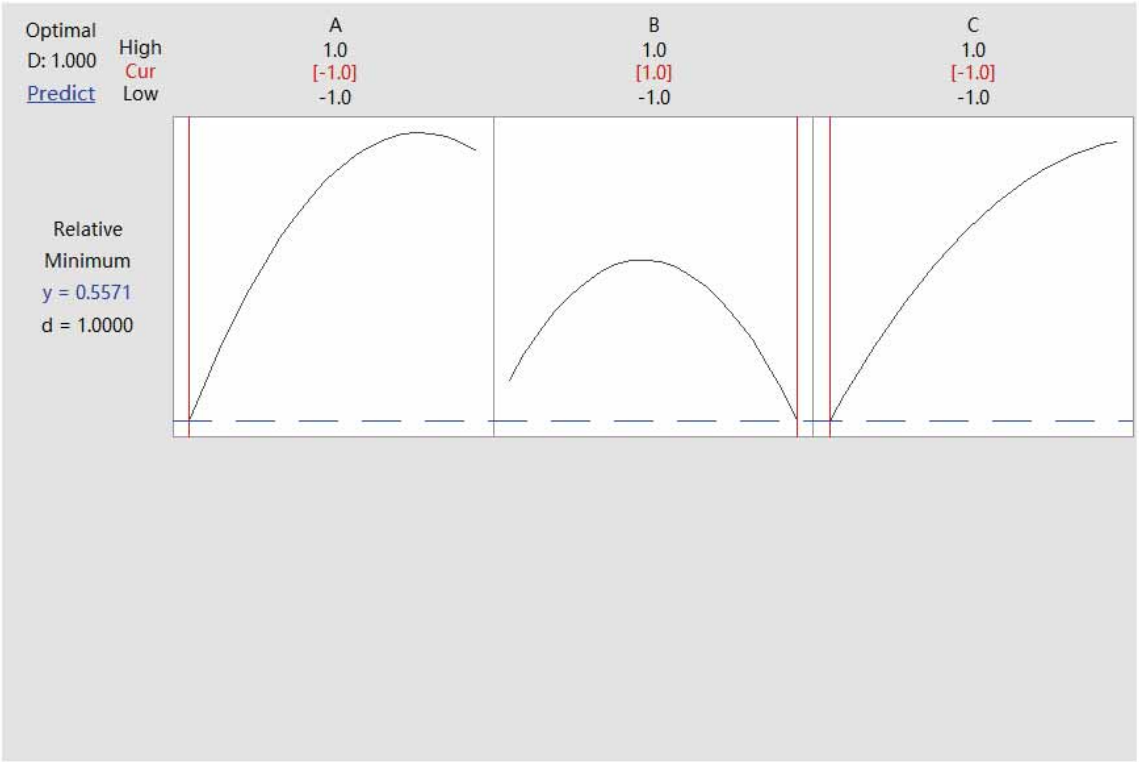


FIGURE 7
Optimization Plot

For Fig.7, the relative error of heat balance is 0.5571 and its absolute value is < 5%, which means experimental data is reasonable.

The relative error of heat balance is 0.5571 when heat transfer area (A) is 0.1 m^2, corrugation depth (B) is 2 mm, thickness of the plate (C) is 0.4 mm.

CONCLUSION

Based on water type heat transfer, this paper studies the performance of water type heat transfer and resistance properties of plate heat exchanger of R410A. The method and model for the simulation calculation of refrigeration system are given. Based on the experiment, the relationship between the heat transfer area, corrugation depth, the thickness of the plate and the relative error of the heat balance (η) is found through the change of the operating parameters.

In this paper, heat transfer experiments were performed on water plate heat exchangers, and the experimental errors were all within the allowable range of the project. The response surface method is used to provide a reference for the design calculation of the plate heat exchanger.

REFERENCES

[1] Wang, Z.Z., Zhao, Z.N. (1993) Analysis of performance of steam condensation heat transfer and pressure in plate condensers. Heat Transfer Eng. 14(4), 32–41.

[2] Wang, L., Christensen, R., Suden, B. (2000) An experimental investigation of steam condensation in plate heat exchangers. Int. J. Heat Exchanger. 1(2), 125–150.

[3] Yan, Y.Y., Lin, T.F. (1999) Evaporation heat transfer and pressure drop of refrigerant R-134a in a plate heat exchanger. J. Heat Transfer – Trans. ASME. 121(1), 118–127.

[4] Yan, Y.Y., Lio, H.C., Lin, T.F., (1999) Condensation heat transfer and pressure drop of refrigerant R-134a in a plate heat exchanger. Int. J. Heat Mass Transfer. 42(6), 993–1006.

[5] Kuo, W.S., Lie, Y.M., Hsieh, Y.Y., Lin, T.F. (2005) Condensation heat transfer and pressure drop of refrigerant R-410A flow in a vertical plate heat exchanger. Int. J. Heat Mass Transfer. 48(25), 5205–5220.

[6] Jokar, A., Eckels, S.J., Hosni, M.H., Gielda, T.P. (2004) Condensation heat transfer and pressure drop of brazed plate heat exchangers using refrigerant R134a, J. Enhanced Heat Transfer. 11(2), 161–182.

[7] Jin, S., Pega Hrnjak, P. (2017) Effect of end plates on heat transfer of plate heat exchanger. International Journal of Heat and Mass Transfer. 108(Part A), 740-748.

[8] Talal, M., Abou Elmaaty, A.E., Kabeel, M. Mahgoub. (2017) Corrugated plate heat exchanger review. Renewable and Sustainable Energy Reviews. 70, 852-860.

[9] Václav Dvořák, Tomáš Vít. (2017) CAE methods for plate heat exchanger design. Energy Procedia. 134, 234-243.

[10] Raja, B.D., Jhala, R.L., Patel, V. (2018) Thermal-hydraulic optimization of plate heat exchanger: A multi-objective approach. International Journal of Thermal Sciences. 124, 522-535.

[11] Xu, K., Smith, R., Zhang, N. (2017) Design and optimization of plate heat exchanger networks. Computer Aided Chemical Engineering. 40, 1819-1824.

[12] Kumar, B., Soni, A., Singh, S.N. (2018) Effect of geometrical parameters on the performance of chevron type plate heat exchanger. Experimental Thermal and Fluid Science. 91, 126-133.

[13] Imran, M., Pambudi, N.A., Farooq. M. (2017) Thermal and hydraulic optimization of plate heat exchanger using multi objective genetic algorithm. Case Studies in Thermal Engineering. 10, 570-578.

[14] Yuan Z., Wu, L., Yuan, Z., Li, H. (2017) Shape optimization of welded plate heat exchangers based on grey correlation theory. Applied Thermal Engineering. 123, 761-769.

[15] Hu, S., Ma, X., Zhou, W. (2017) Condensation heat transfer of ethanol-water vapor in a plate heat exchanger. Applied Thermal Engineering. 113, 1047-1055

Received: **14.05.2018**
Accepted: **08.10.2018**

CORRESPONDING AUTHOR

Jinping Li
Western China Energy & Environment Research Center,
Lanzhou University of Technology,
Lanzhou, 730050 – China

e-mail: 2598824660@qq.com

© by PSP Volume 27 – No. 12B/2018 pages 9768-9774 Fresenius Environmental Bulletin

POLLUTION CHARACTERISTICS OF ATMOSPHERIC PARTICULATES ON EXPRESSWAY FOREST BELTS IN URUMQI

Aliya Baidourela[1,*], Qian Sun[1], Li Liu[1], Kahaer Zhayimu[2], Jing Zhen[1]

[1]College of Forestry and Horticulture, Xinjiang Agricultural University, Urumqi 830052, China
[2]College of Resources & Environmental Science, Xinjiang University, Urumqi 830046, China

ABSTRACT

Particulate matter (PM), especially PM_{10} and $PM_{2.5}$, are hazardous to animal, plant, and human health. With the further opening and development of Urumqi City, environmental problems, especially the pollution of $PM_{2.5}$ in the atmosphere, have received more attention. The road forest belt, an important part of the urban forest, reduces wind and noise, and has a noticeable effect on particulate matter such as $PM_{2.5}$.

In this paper, the main expressway, Waihuan road and Hetan road forest belt in Urumqi are taken as research objects. The variation law of $PM_{2.5}$ and PM_{10} from the road to the forest belt is studied by setting monitoring points at different widths of road shelter forest. $PM_{2.5}$ and PM_{10} concentrations were measured by an air quality detector, and air parameters were measured by an automatic weather instrument. The data were analysed by using Statistical Product and Service Solutions software.

$PM_{2.5}$ and PM_{10} concentrations were the highest at the People's Park observation points, and the $PM_{2.5}$ and PM_{10} were lowest at the observation points in the Martyrs' Cemetery. The concentrations at the observation points on the Kashi Road overpass were second. There were two peaks in $PM_{2.5}$ and PM_{10} concentrations at the Kashi Road overpass due to diurnal variation, while there were no notable peaks at the Martyrs' Cemetery. At the three study sites, $PM_{2.5}$ and PM_{10} concentrations first decreased with increasing distance from the expressway, and then plateaued.

There was a negative correlation between $PM_{2.5}$ and PM_{10} concentrations and forest width. PM concentrations were more stable when the forest width exceeded 10 m. The PM concentration in the forest are influenced by expressway traffic and its internal environmental situation; high-traffic pavements or dense buildings will affect the spread of PM.

KEYWORDS:
Particulate matter, fast road forest belt, pollution characteristics, Urumqi

INTRODUCTION

As the main type of air pollutants, particulate pollutants cause serious damage to animals, plants and human health, especially the fine particles such as PM_{10} and $PM_{2.5}$ are the most serious. The main sources of atmospheric PM include industrial and motor vehicle emissions, coal and biomass combustion, dust, secondary particles produced by oxidation of NOX, SO_2, VOCS ,*etc* .PM emissions from motor vehicles account for approximately 20% of atmospheric PM pollution [1][2]. However, since NO_X and VOCs emitted by motor vehicles are important precursors of atmospheric photochemical reactions, and also precursors of major secondary constituents of urban particulate matter and secondary organic aerosol SOA, their substantial contribution to particulate matter concentration will be even larger [3][4][5]. With the gradual implementation of national western development policy and the rapid development of the national economy, the living standards of the people in the western region have gradually increased. At the end of 2016, the number of motor vehicle in Urumqi, Xinjiang, was 943,100 [6]. Pollution from motor vehicle emissions has become an important component of air pollution in Urumqi [7][8].

According to the communication of the state of the environment in Xinjiang, the capital city, Urumqi, 12.0% and 3.6% of days experience poor environmental air quality and severe pollution, respectively. The annual average concentrations of inhalable PM, fine PM, and NO_2 exceed the national secondary standard [9]. It has been demonstrated that PM pollutants in Urumqi are mainly composed of fine particles with a particle size of less than 2.5μm [10]. With the further opening and development of Urumqi, environmental problems, especially the pollution of $PM_{2.5}$ in the atmospheric environment, have received more attention. Accurate selection of greening tree species with strong ability to absorb particulate pollutants and rational allocation of urban expressways Greening is of great significance in improving the living environment of oasis cities in arid regions.

As tree species in the city, green tree species play an important role in purifying the urban environment by reducing the concentration of particulate pollutants in the atmosphere and purging sand and dust [11]. Green plants retain and absorb $PM_{2.5}$ and other particulate pollutants owing to their foliar characteristics and canopy structure. Road shelterbelts are an important part of urban forests, and not only reduce wind and noise, but also block $PM_{2.5}$ and other PM. Research indicates that the roadside belt can optimize road air quality and reduce the concentration of PM after forest belts [12][13].

Currently, studies on PM pollution have focused on variations in particle concentration, size characteristics, sources, and influencing factors [14][15]. Research on reducing airborne PM pollution in forests has focused on large particle dust, such as total suspended particulate matter (TSP) and PM_{10}, and few studies have been conducted on reducing $PM_{2.5}$. The existing studies are mostly limited to research on green tree species in humid areas and their resistance to industrial dust, and some have studied the physiological and ecological responses of urban forests in humid areas [16][17][18]. Little is known about the relationship between landscaping tree species and $PM_{2.5}$ in oasis cities with more wind and sand, less precipitation, and severe air pollution. There have been almost no studies on the impact of PM in the expressway belts of Urumqi City.

In this paper, the main expressway, Waihuan road, and river beach roadway in Urumqi were taken as research objects. Variations in $PM_{2.5}$ and PM_{10} from road diffusion to forest belt were studied by setting monitoring points at different locations within the road shelterbelts. The minimum width required to block particles was determined to provide a theoretical basis for optimization in the Urumqi greening program for management.

STUDY AREA AND RESEARCH METHODS

Study area. The observation points were located in southern (Martyrs' Cemetery), central (People's Park), and northern Urumqi City (Kashi Road overpass) along the forest expressway belt, with an average width of 45m. Urumqi endures a typical moderate temperate arid continental climate, with a dry climate and an annual average temperature of 6.4 ℃, average annual rainfall of 236 mm, and annual evaporation of 2266 mm. The four seasons are not very distinct; spring and autumn are segmented, while summer and winter continue for a longer time period. In Urumqi City, the southern cement and fertilizer plants, and the northern cement and steel plants are key sources of pollution, and vegetation coverage is not high; the per-capita green area is only 5.36 m^2 [19]. The three observation points in the forested expressway belt included *Fraxinus sogdiana, Ulmus densa, Ulmus pumila L.*, *Populus alba* and common herbs. The stand density was 625 plants hm^{-2}, the average trunk diameter at breast height was 14.6 cm, the average tree height was 11.3 m, and the canopy density was approximately 50%.

In August 2017, the weather conditions in Urumqi were good during the observation period, and there was no strong precipitation greater than 15mm/h. The highest precipitation was 1.7mm/s on August 17, and the monthly average wind speed was 2.08m/s. The highest wind speed is 5.6m/s on August 10th, the monthly average temperature is 22.31°C, and the monthly average humidity is 42.51%.

Research methods. To determine particle concentrations, a BR-HOl-1210 air quality detector was used to measure PM_1, $PM_{2.5}$, and PM_{10}, with a concentration range of 0-600μg m^{-3}. Particle concentration included the concentrations of several particle types and their masses. In this study, mass concentration was used with a unit of μg m^{-3}. The average particle concentration in Urumqi during the survey was obtained from the Xinjiang Environmental Quality Information Release Platform of the Environmental Protection Agency of Xinjiang Uygur Autonomous Region at http://www.xjepb.gov.cn/. Air temperature and humidity were measured by a Kestrel 4500 automatic weather monitor. The measurement range is 29.0–70.0 °C, and the relative humidity measurement range is 5.0% -95.0%.

In this study, we identified *Fraxinus sogdiana*, *Ulmus pumila L.*, and *Salicaceae* willow at the three observation sites from 1 to 15 August 2017, a total of 15 days. According to the morning and evening rush hour of Urumqi City and the habits of residents in the observation points of the People's Park, the daily observation time is 9:00-20:00, and 6 points (0m, 3m, 10m, 15m, 25m, 30m), each observation point is equipped with a BR-HOl-1210 air detector for observation, once every 1h. The concentration of PM_{10} and $PM_{2.5}$ and their mass concentrations were recorded at a height of approximately 1.5 m, simultaneous temperature and relative humidity monitoring was conducted with a meteorological instrument.

Data processing. Statistical Product and Service Solutions (SPSS) software was used to compare the average particle concentrations and conduct linear regression and Pearson rank correlation coefficient analysis.

RESULTS AND ANALYSIS

Daily variations of PM_{10} and $PM_{2.5}$ in the city expressway forest belt. Between the Martyrs' Cemetery, People's Park, and Kashi Road interchange, the average concentrations of $PM_{2.5}$ and PM_{10} in the forest belts varied (Figures 1 and 2). The daily variation of $PM_{2.5}$ concentration also varied between the

different observation points. There were diurnal two peaks in $PM_{2.5}$ at the observation points of the Kashi Road overpass, ranging from 37μg m^{-3} at 10:00-11:00 and 30μg m^{-3} at 16:00-17:00. There was only one peak at the People's Park observation point; 60μg m^{-3} at 14:00-15:00. There was no notable peak at the Martyrs' Cemetery observation point. The highest concentration and the time of the peak $PM_{2.5}$ concentration also differed between observation sites. The lowest concentration recorded in People's Park was 18μg m^{-3} at 10:00, that recorded at the Martyrs' Cemetery was10 μg m^{-3} at 16:00, and that at Kashi overpass was 19μg m^{-3} at 20:00.

The variation of PM_{10} concentration at the three observation sites followed a similar trend to that of $PM_{2.5}$. There were two peaks at the Kashi overpass; 68μg m^{-3} at 11:00 and 57μg m^{-3} at 17:00, and only one at People's Park; 92μg m^{-3} at 14:00. There was no notable peak in the Martyrs' Cemetery. The peak recorded in the People's Park was the highest among the three observation points. The lowest PM_{10} concentration at the People's Park was 42μg m^{-3} at 10:00, that at the Martyrs' Cemetery was 31μg m^{-3} at 16:00, and that at the overpass was 39μg m^{-3} at 13:00.

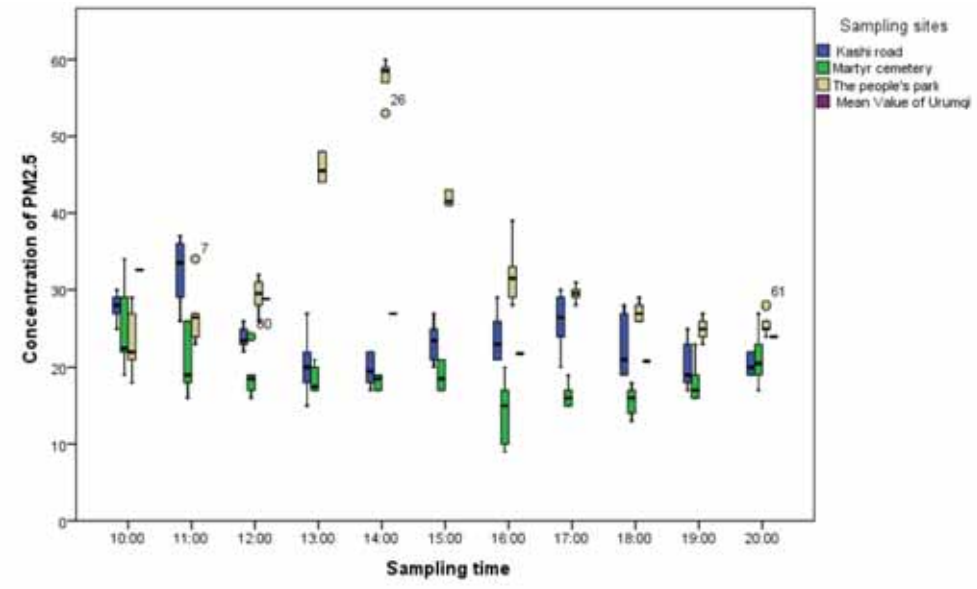


FIGURE 1
Daily variation of $PM_{2.5}$ concentration

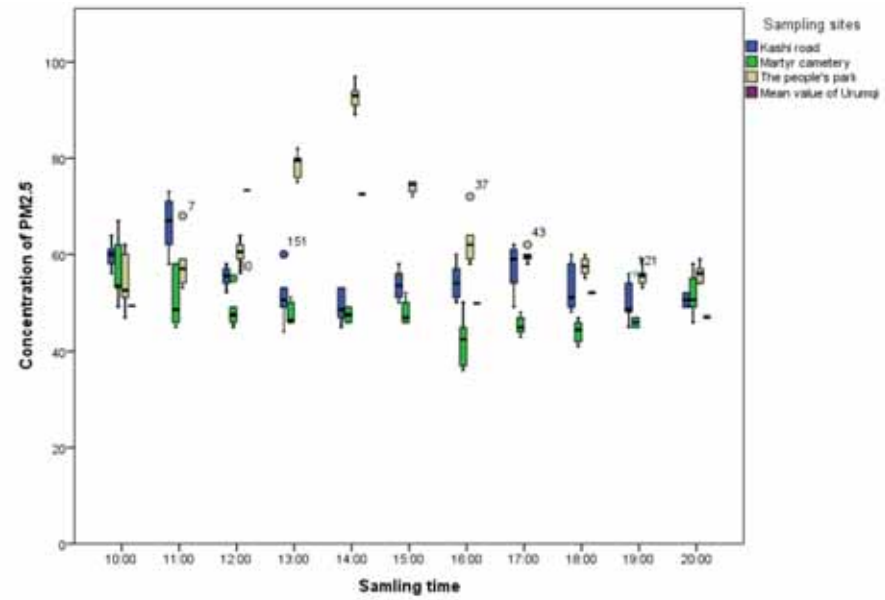


FIGURE 2
Daily variation of PM_{10} concentration

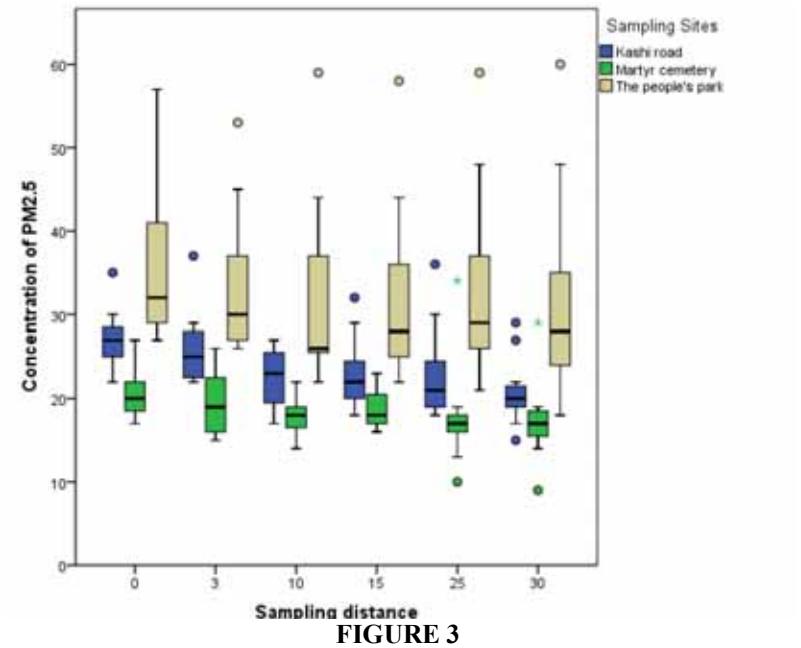


FIGURE 3
Variation of $PM_{2.5}$ concentration at different locations of the road belt

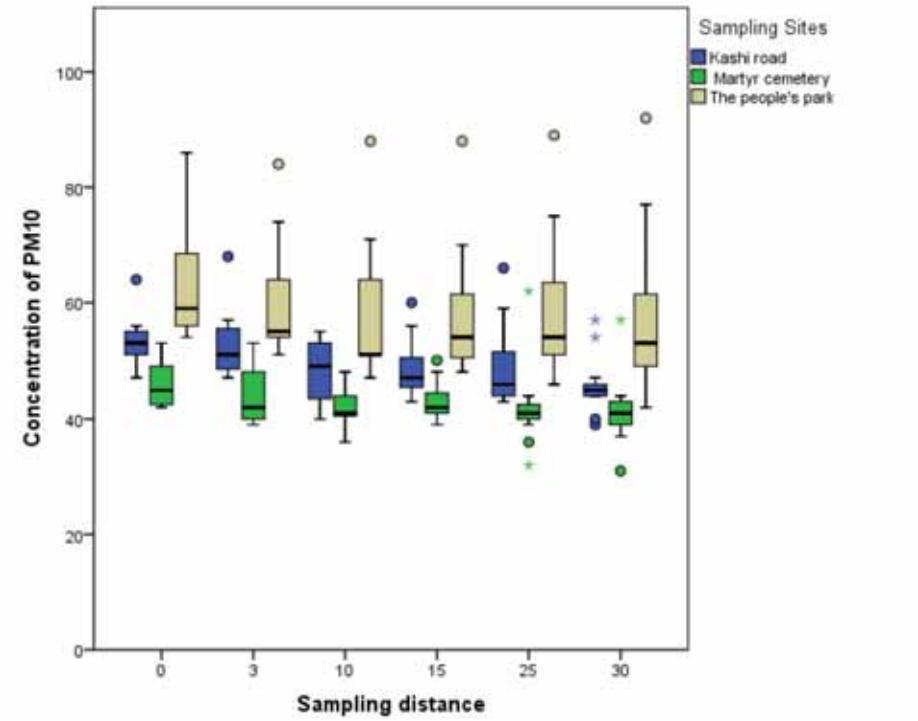


FIGURE 4
Variation of PM_{10} concentration at different locations of the road belt

Variations in PM_{10} and $PM_{2.5}$ at different points of the city expressway. There was a relationship between the concentration of PM in the urban expressway and the distance between expressways. The changes in the concentration of PM in the forest zone at different distances from the three observation points are shown in Figures 3 and 4. The concentrations of $PM_{2.5}$ at the three observation sites decreased initially with increasing distance from the road, and then plateaued. The average $PM_{2.5}$ concentration at the Martyrs' Cemetery was the lowest, and the highest was recorded at the People's Park. Changes in PM_{10} concentration with distance from the road were similar to those of $PM_{2.5}$.

During the same time period, the $PM_{2.5}$ and PM_{10} concentrations were the highest at the observation points of the People's Park, and the lowest at the observation sites of the Martyrs' Cemetery. The concentrations at the Kashi Road overpass were between these. Each sample of $PM_{2.5}$ and PM_{10} outdoor concentrations contained 6 observation points, and

measurements were captured 11 times, giving a total of 198 measurements according to the distance from the freeway (0 to 30 m). The average concentrations of $PM_{2.5}$ at 0, 3, 10, 15, 25, and 30 m from the freeway were 28, 26, 24, 25, 25, and 23μg m^{-3}, respectively, and the average concentrations of PM_{10} at these points were 54, 52, 50, 50, 50, and 48μg m^{-3}, respectively. The correlations and regressions between $PM_{2.5}$ and PM_{10} concentrations and forest width are shown in Table 1. There was a negative correlation between $PM_{2.5}$ and PM_{10} concentrations at different observation sites and the width of forest belts, and they could be returned to their corresponding linear equations. The distance coefficients were all approximately 1, and the constant items all exceeded 30, indicating that the forest belt width affected the PM concentration. The magnitude is smaller.

The table also shows that there is a difference between the $PM_{2.5}$ and PM_{10} concentrations at 0, 3, and 10 m from the expressway, especially at different times, and between $PM_{2.5}$ and PM_{10} concentrations at 0 and 3 m at different observation points.

From the contents of Figures 3 and 4 and Tables 1 and 2, we can determine that the smallest forest width to reduce PM is approximately 3 m and the optimal width is 10 m. This is because the particle concentration decreases most rapidly within the first 0-3 m of the forest belt. Beyond 10 m, there is no notable change in PM concentration. At the three times selected by all the observation points, the PM concentrations at 0 and 3 m were significantly different. According to the letters in the table, there is no significant difference in PM between 10 and 30 m. The regression equation of the x coefficient indicates that, the smaller the distance coefficient, the smaller the impact of distance on PM. The effect of broad forest belts on the reduction of expressway PM reaches a certain value and does not change after that. The concentration of PM 30 m from the road is not affected by vehicle exhaust emissions.

TABLE 1
Regression analysis between $PM_{2.5}$ and PM_{10} concentration and the width of the road belt

		Formula	R^2	Pearson
Kashi road	$PM_{2.5}$	Y=-0.910x+39.229	0.757	-0.787**
	PM_{10}	Y=-0.963x+61.327	0.640	-0.700**
The people' s park	$PM_{2.5}$	Y=-0.967x+41.097	0.750	-0.766**
	PM_{10}	Y=-1.045x+62.623	0.616	-0.768**
Martyr cemetery	$PM_{2.5}$	Y=-1.028x+43.798	0.747	-0.876**
	PM_{10}	Y=-1.145x+64.759	0.626	-0.691**

**Bilateral correlation was significant at the 0.01 level

TABLE 2
General variation trends of $PM_{2.5}$ and PM_{10}

Sampling sites	Pollutants	Time	The distance from city expressway						Remarks
			0m	3m	10m	15m	25m	30m	
The people's park	$PM_{2.5}$	10:00	32.667±2.52a	28.0±1.73b	24.667±2.31bc	22.334±3.21bc	26.334±5.03bc	23.667±3.51c	F<1
		14:00	48.667±4.26	46.667±5.67	48.0±3.28	47.667±4.72	50.0±3.62	49.667±2.28	
		20:00	29.0±1.0a	26.667±1.15b	25.333±0.57b	25.333±0.58b	25.433±1.53b	24.667±2.08b	
	PM_{10}	10:00	58.667±1.53a	55.0±1.0b	47.667±1.54c	48.667±1.54c	47.667±3.05c	42.667±2.61d	F<1
		14:00	72.667±2.52	73.0±2.64	71.0±2.0	71.0±4.58	72.333±2.51	71.444±2.77	
		20:00	55.333±0.57a	52.333±1.53b	50.667±0.57bc	51.667±0.58bc	50.333±2.31bc	49.667±2.08c	
Martyr cemetery	$PM_{2.5}$	10:00	23.0±3.61	35.333±2.08	21.333±3.05	22.667±5.03	21.0±2.35	22.389±2.31	F<1
		14:00	21.333±1.53a	19.333±0.58b	18.667±1.53bc	19.0±2.0b	16.333±1.15c	17.0±1.0b	
		20:00	23.0±3.0a	18.667±2.05b	18.333±1.15b	19.0±3.61b	16.667±0.58b	18.667±1.52b	
	PM_{10}	10:00	49.333±4.73	52.333±3.056	46.667±3.13	46.333±2.08	51.333±3.71	49.0±3.54	F<1
		14:00	46.0±1.73a	42.667±2.08b	41.0±2.0b	42.333±2.05b	40.333±1.15b	42.333±1.05b	
		20:00	53.667±1.15a	46.667±2.51b	45.0±1.58b	52.333±2.97a	44.0±3.61b	41.333±1.30b	
Kashi road	$PM_{2.5}$	10:00	37.333±1.52	31.333±1.08	30.0±1.73	27.0±1.64	26.0±1.81	26.667±1.16	F<1
		14:00	25.334±1.08a	22.667±1.06b	21.333±1.93b	21.0±0.89b	19.667±1.15bc	18.0±1.23c	
		20:00	25.667±1.02a	21.333±0.57b	19.667±1.51b	19.0±1.0b	19.0±0.10b	18.333±1.05b	
	PM_{10}	10:00	73.667±1.59a	63.667±1.78b	56.667±2.21bc	54.333±1.51c	51.667±1.52c	48.667±1.08c	
		14:00	72.667±1.08a	66.0±1.0b	60.33±1.52c	56.0±1.0d	53.667±1.64de	53.0±0.57e	
		20:00	73.0±1.46a	56.33±1.05b	57.33±1.52c	54.33±1.53c	54.33±1.05c	55.55±1.61c	

a, b, c, d, and e indicate the difference between particulate concentrations $P<0.05$

DISCUSSION

Differences in the concentration of particulate matter at different observation points. During the same time period, the $PM_{2.5}$ and PM_{10} concentrations were highest at the People's Park observation points, the lowest concentrations were recorded at the Martyrs' Cemetery observation points, and values between these were recorded at the Kashi Road overpass. These three observation points are located at the southern, northern, and central parts of the river bank in Urumqi. These areas experience different average daily traffic volumes. During the observation period, the average hourly traffic volume at these three locations was 5940 at the People's Park, 4800 at the Martyr's Cemetery, and 5700 at the Kashi Road overpass. A greater traffic volume increases exhaust emissions. Within the same forest belt and the car flow, areas with more forest will have improved PM concentrations. The greening situation near the three observation sites may be the key factor affecting the concentration of PM in the forest belt. The forest near the Martyrs' Cemetery, surrounding the Hetan Expressway, is in good condition. Both sides of the road are afforested, with a combination of different trees, shrubs, and herbaceous plants and the tree species are in good growth condition. The characteristics of the fast lane at the area where only one side of the road has a green belt and the other side contains the business district and high-rise buildings are not conducive to the proliferation of particulate matter.

There was a certain negative correlation between the concentration of $PM_{2.5}$ and PM_{10}in the forest belt and the width of the forest belt. There was a significant difference between the $PM_{2.5}$ and PM_{10} concentrations at 0 and 3 m from the road, while the $PM_{2.5}$ values did not change significantly between 10 and 30 m. It can be said that the width of the fast forest belt should be at least 3m, 10m is the best, and the concentration of the particles is more stable than 10m. Particle concentration in the forest belts is not only affected by the expressway's traffic flow, but also the internal environment. If the inside of the forest belt contains a large sidewalk or forest annexe, the buildings will prevent the diffusion and dilution of PM. Another important factor affecting PM is meteorological conditions, especially air movement, which has a relatively large influence [20][21]. In this paper, the effect of air flow on the concentration of $PM_{2.5}$ and other particles will not be considered.

Differences in the concentration of particulate matter at different time. According to the diurnal $PM_{2.5}$ and PM_{10} concentration variations, there is a peak in PM in the People's Park and two peaks at the Kashi Road overpass, however, there is no notable peak in the Martyrs' Cemetery. This may be due to people's travel habits; traffic flow may be larger at rush hour, resulting in higher PM concentrations. During this study, the highest $PM_{2.5}$ value was recorded at the People's Park monitoring station, which was 60μg m^{-3}. Kelei et al. concluded that the average concentration of $PM_{2.5}$ in Urumqi was between 48.36 and 200.00μg m^{-3}, and the PM concentration was lower in the summer and autumn than it was in the spring and winter. The differences in $PM_{2.5}$ concentrations at different times are related to its major chemical components, which were determined at different time periods so that the types of vegetation that could easily absorb the corresponding chemical substances can be selected [22].

CONCLUSION

1) There is a negative correlation between $PM_{2.5}$ and PM_{10} concentrations and forest belt width at the same observation point.

2) The forest belt width should be at least 3 m. A width of 10 m is optimal, and a width exceeding 10 m would reduce PM concentrations in a more stable manner.

3) The concentration of particulate matter in the forest belt is not only affected by the expressway traffic flow, but also the environmental conditions inside the forest belt.

ACKNOWLEDGEMENTS

This study was supported by National Science foundation of China (NSFC), guarantee No.: 31600572, Postdoctoral foundation of China, guarantee No.: 185789 and Natural Science Foundation of Xinjiang Uyghur Autonomous Region (2017D01B15)

REFERENCES

[1] Liu, X.H., Yu, X.X., Zhang, Z.M, Liu, M.M., Ruan, S.Q.C. (2014) Pollution characteristics of atmospheric particulates in forest belts and their relationship with meteorological conditions. Chinese Journal of Ecology. 33(07), 1715-1721. [in Chinese].

[2] Wu, P. (2017) Study on monitoring method of vehicle emission on urban road net work. Master thesis of Nanjing University of Science and Technology. [in Chinese].

[3] Ying, B., Cai, J.P., Chen, L.P., Shen Z.M., Zou, X.D. (2007) Effects of vegetation status in urban green spaces on particles removal in canyon street atmosphere. Acta Ecologica Ainica. 27(11), 4590 －4595. [in Chinese].

[4] Hu, M., Tang, Q., Peng, J.F., Wang, E.Y., Wang, S.L. (2011) Study on Characterization source apportionment of atmospheric particulate matter in China. Environment and sustainable development. 36(5), 15-19. [in Chinese].
[5] Huang, D.S., Zhang, S.Q. (2013) Health benefits evaluation for PM2.5 pollution control in Beijing-Tianjin-Hebei region of China. China environmental science. 33(1), 166-174 [in Chinese].
[6] Urumqi statistics bureau (2016) Statistical bulletin of the national economic and social development of Urumqi in 2016. http://tjj.urumqi.gov.cn/tzgg/312834.htm [in Chinese].
[7] Ma, W., Li, Y.H., Hou, X.G. (2016) Characteristics of atmospheric NO2 vertical column densities in heating period of Urumqi. Ecology and Environmental Sciences. 25(08), 1351-1355. [in Chinese].
[8] Shi, H.L. (2012) Analysis of Air Pollution Status and Major Pollutants Sources in Urumqi. Master thesis of Xinjiang Agricultural University. [in Chinese].
[9] Environmental protection bureau of Xinjiang Uygur Autonomous Region (2016) Environmental status bulletin of Xinxiang Uygur autonomous region in 2016. [in Chinese].
[10] Zhang, K.L. (2014) Spatial and Temporal Changes of PM2.5 in Urumqi. Master thesis of Xinjiang agricultural university. [in Chinese].
[11] Wang, H.X., Wang, Y.H., Yang, J., Xie, B.Z., Shi, H. (2015) Multi-Scale Comparisons of Particulate Matter and Its Size Fractions Deposited on Leaf Surfaces of Major Greening Tree Species. Scientia Silvae Sinicae. 51(7), 9-19. [in Chinese].
[12] Zhang, Z.D., Xi, B.Y., Cao, Z.G. (2014) Exploration of a quantitative methodology to characterize the retention of PM2.5 and other atmospheric particulate matter by plant leaves: Taking *Populus tomentosa* as an example. Chinese Journal of Applied Ecology. 25(8), 2238-2242. [in Chinese].
[13] Gayle, S.W., Lin, M.Y., Andrey, K., Baldauf, R.W., Isacov, V., Faircloth, J. Jackson, L.E. (2012) Field investigation of roadside vegetative and structural barrier impact on near-road ultra-fine particle concentrations under a variety of wind conditions. Science of the Total Environment. 419, 7-15.
[14] Thurston, G.D., Ito, K., Lall, R. (2011) A source apportionment of U.S. Fine particulate matter air pollution. Atmospheric Environment. 45, 3924 - 3936.
[15] Mayire, R., Xiaokaiti, Y., Kang, H., Xu, T., Ji, Y. (2016) The distribution features of the airborne fine particulate matter PM2.5 ingredients and source analysis in Urumqi. Journal of Xinjiang Medical University. 39(05), 635-639. [in Chinese].
[16] Aliya, B., Yumiti, H., Tayierjiang, A. Kailibunuer, N. (2015) Maximum Dust Retention of Main Greening Trees in Arid Land Oasis Cities, Northwest China. Scientia Silvae Sinicae. 51(03), 57-64. [in Chinese]
[17] Wang, Y.R., Zhang, X.X., Zhao, J.Y., Jiang, Q.O. (2016) Temporal and Spatial Distribution of PM2.5 and Its Relationship with Vegetation Coverage in Beijing during the Period of 2013-2014. Ecology and Environmental Sciences. 25(01), 103-111. [in Chinese].
[18] Li, D.M. (2016) The multi-scale analysis of the dust retention ability of common green tree species in Beijing. Master thesis of Forestry University of Beijing. [in Chinese].
[19] Zhang, K.L. (2014) Spatial and Temporal Changes of PM2.5 in Urumqi. Master thesis of Xinjiang agricultural university. [in Chinese].
[20] Han, X., Wei, W.S., Liu, M.Z., Hong, W., Lu, H., Zhang, T.W. (2013) The influences of airflow on the concentration of PM10，PM2.5 and PM1.0 in Urumqi Xinjiang China. [in Chinese].
[21] Zhang, K.M. (2016) Studies on the Influence of Faehn on Air Pollution over Urumqi in Winter. Master thesis of Lanzhou University. [in Chinese].
[22] Zhao, C.X., Wang, Y.J. (2013) Interactions between fine particulate matter PM 2.5 and vegetation: A review. Chinese Journal of Ecology. 32(8), 2203-2210. [in Chinese]

Received: **14.05.2018**
Accepted: **17.10.2018**

CORRESPONDING AUTHOR

Aliya Baidourela
College of Forestry and Horticulture,
Xinjiang Agricultural University,
Urumqi 830052 – China

e-mail: aliya_12@163.com

© by PSP Volume 27 – No. 12B/2018 pages 9775-9781 Fresenius Environmental Bulletin

PERSISTENT ORGANOCHLORINE PESTICIDES RESIDUES IN FOODSTUFF OF ANIMAL ORIGIN FROM SOUTHERN GOVERNORATES OF JORDAN IN 2016 AND 2017

Tawfiq M Al-Antary[1], Mahmoud A Alawi[2,*], Mohammad Abu Othman[3], Nizar Haddad[4]

[1]School of Agriculture, The University of Jordan, Amman, 11942, Jordan
[2]School of Science, The University of Jordan, Amman, 11942, Jordan
[3]Royal Scientific Society, Amman, Jordan
[4]Ministry of Environment, Amman, 11942, Jordan

ABSTRACT

This investigation was conducted to evaluate the persistent organochlorine pesticides residues in foodstuff of animal origin in southern governorates of Jordan in 2016 and 2017. Ministry of environment of Jordan asked the Royal Scientific Society to monitor pesticides in samples of animal products under the supervision of an official scientific committee. Results of milk products showed that there were three samples out of 35-contaminated with pesticides residues but less than the Maximum Residue Limits (MRL). Results of table egg samples showed that there were five out of 40 samples contaminated with residues but less than the MRL. Results of meat and poultry analysis showed that there were fourteen samples out of 65 contaminated with residues but less than the MRL except one imported meat sample was more than the MRL. However, the contaminated samples were contained dieldrin, endrin, heptachlor, *p,p'*-DDE, *p,p'*-DDT and *o,p'*-DDE. Conclusions and recommendations were stated to minimize the misuse of pesticides and residues in animal products to protect human beings.

KEYWORDS:
Persistent, organochlorines, residues, monitoring, animal products, Jordan

INTRODUCTION

These days, pesticides are commonly used in Jordan and globally for the purpose of reducing pests number or plants, livestock and public health aims, by spraying different types of pesticides to control fungi, weeds, and insects of agricultural crops, victors of human and animal diseases as malaria, dengue fever, elephant disease etc. [1 – 3]. The misuse of pesticides on agricultural pests, and vectors of animal and human diseases cause pesticides residues particularly organochlorines which are able to accumulate in the fat tissues when transporting through various food chains [1, 3, 4]. Due to their unpolarity and persistency, they degrade slowly in the environment components and have wide spread accumulation in food chain [1, 3, 5, 6]. These previous reasons push Ministry of Environment to ask Royal Scientific Society to carry out regular studies, aiming to monitor organochlorine residues in animal product samples under the supervision of Technical Scientific Committee.

However, Ministry of Agriculture of Jordan has banned the use of organochlorines since early eighties of the last century, but unfortunately, previous studies showed residues of these pesticides in different components of the environment [7 – 13]. The contaminated components reported in animal products [7, 14] were previously studied in Jordan [9] and in other countries [14 – 15].

It is the aim of this study to monitor persistent organochlorine pesticides in local and imported foodstuff of animal origin from the southern governorates of Jordan in 2016 and 2017. In addition, occurred residues of these persistent organic pollutants (POPs) will be subjected to the Maximum Residue Limits (MRL) to find out which sample containing more or less to protect citizens and human beings.

MATERIALS AND METHODS

The (AOAC) international analytical method [12, 16] was adopted for extracting the residues of chlorinated pesticides from the animal products.

Glassware and tools. A Soxhlet extractor, separating funnels with Teflon stopcocks, chromatography columns (I.d. = 20 mm, L = 40 – 50 cm) with Teflon-stopcocks, round bottom flasks and volumetric flasks were used. All parts of glassware were washed thoroughly with water, soap, water, distilled water, acetone and finally hexane, dried in a drying oven at 120ºC, and kept tightly closed after cooling to room temperature. Each glassware piece was washed several times with acetone prior to use [13].

Standards, solvents, chemicals and gases. A certified standard mixture containing all the targeted

chlorinated pesticides (purity 99.5–99.9%, 1000 ppm) purchased from Ehrenstorfer (Augusburg, Germany) was diluted to 0.1 ppm with n-hexane containing 0.1 ppm isodrin as internal standard [13]. Petroleum ether (40–60 ºC), dichloromethane, acetone, n-hexane, methanol, acetonitrile and diethyl ether of pesticide residue grade were purchased from Riedel-deHaën (Hannover, Germany) and used as solvents without further purification. Double-distilled water was prepared for use. Anhydrous sodium sulfate (grade ''for residue analysis'') was heated at 550 ºC for 2 h and kept in a closed container. Florisil of ''pesticide residues grade'' (60–100 mesh) was also heated at 550 ºC for 12 h, kept in a closed container and heated once again at 130 ºC for 1 h prior to use. Quartz wool was extracted with petroleum ether. Helium used as carrier gas was of 99.999% purity (grade 5). Argon/methane (95:5% v/v) was used as make-up gas purity of 99.9% [13].

Gas chromatography. A Bruker GC-456 gas chromatograph, equipped with two capillary columns and a ^{63}Ni-electron capture detector (ECD), was used. The first column was a DB-1701 (30 m x 0.32 mm, film thickness 0.25 µm, moderately polar). The second column was a HP-5 (30 m x 0.25 mm, film thickness 0.25 µm, non-polar). Carrier gas (He) of 99.999% purity was used at a flow rate of 1.1 ml/min. The make-up gas (Ar–CH_4) was used at a flow-rate of 25 ml/min. Injector temperature was 280 ºC, while detector temperature was 300 ºC. The column temperature program was as follows: initial temperature 150 ºC (5 min), 150–220 ºC (13 C/min), 220ºC (20 min), 220–250 ºC (20 ºC /min) and 250 ºC (10 min). HP-3395 integrator operated at a chart speed of 5 mm/min was used. Injection volume was 1 µl and split ratio was 1:10 [13].

Extraction methods. Extraction of fat from meat products and eggs. A meat sample of 2–3 kg was cleaned from the bones and then minced with a meat mixer. For eggs, the yolks of 30 eggs were mixed well. A 30-g aliquot of the homogenous meat sample (15 g of the homogenized egg yolks) were weighed and mixed with 50 g anhydrous sodium sulfate [13]. The sample was placed in a thimble and extracted for 6 h in a Soxhlet apparatus using 250 ml petroleum ether. The extract was rotary-evaporated in a pre-weighed round bottom flask (12 mbar) nearly to dryness at 30 ºC. The residue was left in desiccator for half an hour and then the fat residue was weighed to obtain % fat in the sample [13].

Extraction and determination of fat content from milk products. Milk sample with less than 3 g fat was placed in a 250-ml separating funnel with 15 ml petroleum ether and 30 ml of acetonitrile saturated with petroleum ether and then mixed thoroughly for 3 min. The organic layer was separated and gathered in a 1-L separating funnel [13]. Two portions each of 30 ml of acetonitrile saturated with petroleum were added to the aqueous layer in the 250-ml funnel, shaken well and then added to the 1L funnel. Then, 600 ml distilled water, 40 ml saturated sodium chloride solution and 100 ml petroleum ether were added and mixed thoroughly. The aqueous layer was separately placed in another separating funnel with 100 ml petroleum ether, mixed thoroughly and then, the aqueous layer was discarded. The remained organic layer was added to the 1-L separating funnel. The pooled organic extracts were passed through anhydrous sodium sulfate and then evaporated using the rotary evaporator. At this stage, the % fat can be calculated [13].

Clean-up. The fat residues (meat, eggs and milk) were dissolved in petroleum ether and passed through an activated Florisil column for clean-up using petroleum ether/dichloromethane (80/20, v/v) as an eluent [13]. The eluate was collected in a 500-ml round bottom flask and evaporated to dryness at 30 ºC and 12 mbar. The residues were dissolved in 2 ml n-hexane containing 0.1 µg isodrin/ml (internal standard). This final solution was ready for GC injection [13].

Recoveries and quality control. For the evaluation of the extraction efficiency, blank samples were spiked with standard solution mixture of the studied pesticides, and the spiked samples were analyzed using the above-mentioned procedure. Table 1 shows the calculated percent recoveries and the detection limits.

TABLE 1
Limits of detection (DL) and % recoveries of the studied OCP compounds

OCP compound	DL (ppm)	% Recovery
Aldrin	0.005	99.0
Dieldrin	0.005	87.8
Endrin	0.005	79.9
α-Endosulfan	0.005	94.5
β-Endosulfan	0.005	99.2
HCB	0.005	79.3
α-HCH	0.005	94.8
β-HCH	0.005	91.9
γ-HCH	0.005	94.1
Heptachlor	0.005	99.0
o,p'-DDE	0.005	94.8
o,p'-DDD	0.005	86.9
o,p'-DDT	0.005	94.7
p,p'-DDE	0.005	98.7
p,p'-DDD	0.005	90.3
p,p'-DDT	0.005	90.3

Quality control on samples was done through the cooperation with the pesticides analytical laboratory in the Environment Research Center of the Royal Scientific Society and with the Ministry of Agriculture, to ensure the accuracy of the analysis.

Sampling. In this study, a total number of 140 samples of foodstuff of animal origin were gathered

in the time between September 2016 and September 2017 from the cities Ma'an, Karak, Tafila, Aqaba and Ghor Al-Safi. The foodstuff samples were divided into three groups. Group 1 contained fresh milk products, group 2 contained chicken meat and eggs, and group 3 contained red meat. Table 2 shows the details and Figure 1 shows the sampling location map.

TABLE 2
Number of animal products samples gathered between 05.09.2016 and 05.09.2017 distributed on the middle governorates

Food Type		Governorate				
		Ma'an	Karak	Tafila	Aqaba	Ghor Al-Safi
Eggs	Baladi	3	3	5	3	3
	Farms	5	5	4	4	5
Milk	Yoghurt	2	2	2	2	3
Products	Labaneh	2	2	3	3	2
	White Chees	2	2	2	3	2
Red Meat	Local	4	2	4	3	4
	Imported/ Cooled	3	3	3	2	3
	Imported/ Frozen	5	5	4	4	5
Poultry	Local	1	1	2	1	1
	Imported	1	1	1	1	2
Sum		28	26	30	26	30

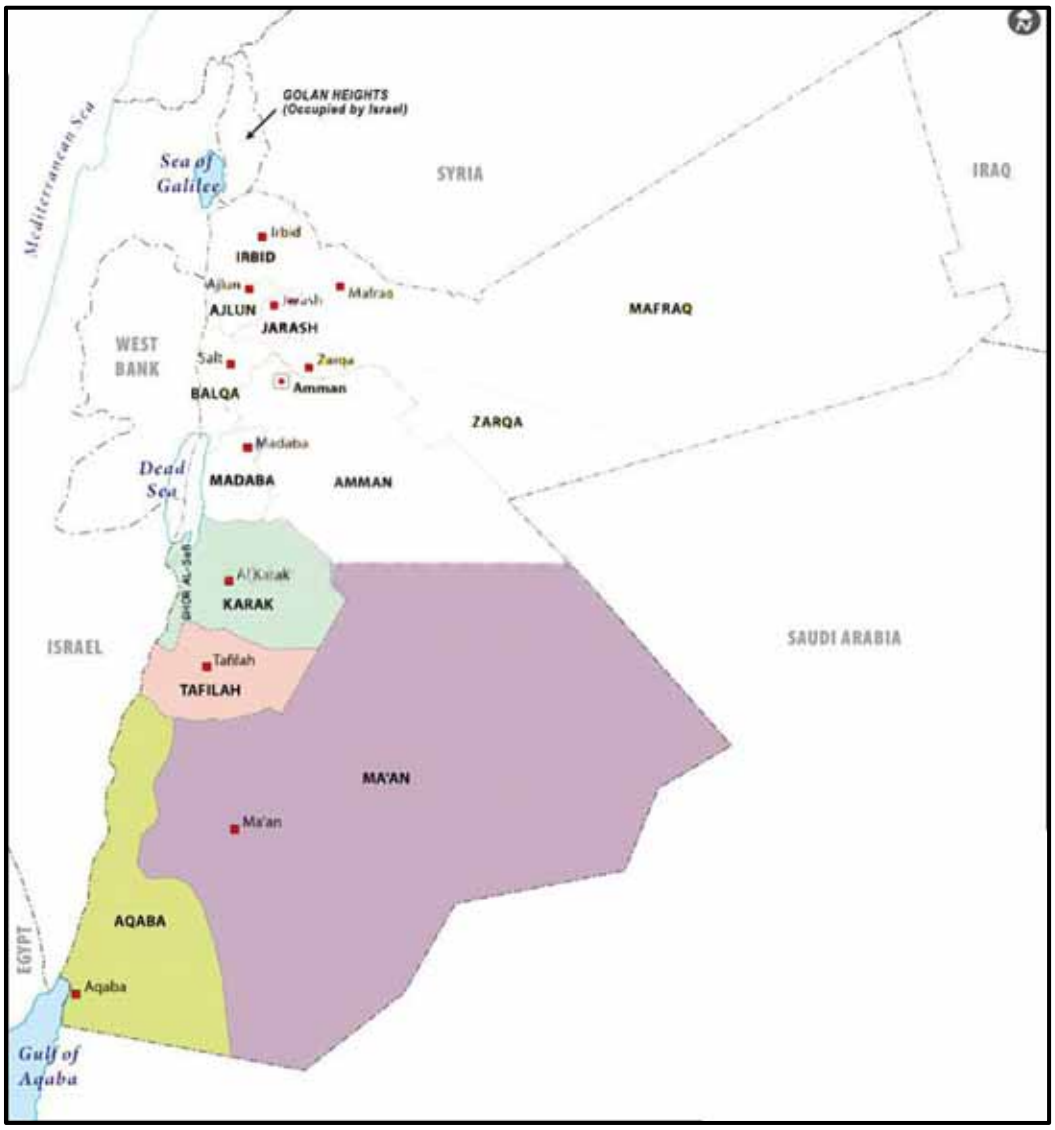


FIGURE 1
Jordan map with sampling locations (colored districts)

RESULTS

Milk product samples. Thirty four samples from the milk products were analysed. The results in Tables 3 and 7 showed that there were three samples contained pesticide residues, but less than the MRL (Table 3). There were no samples contained residues more than the MRL (Table 3).

Table egg samples. Forty samples from the table eggs (23 farmers eggs and 17 Balady eggs) were analysed. The results in Tables 5 and 8 showed that there were five samples from the table eggs contained pesticide residues, but less than the MRL (Table 5).

Meat and Poultry samples. Sixty six samples of meat and poultry products were analyses. 65.2% of the samples were imported. The results in Tables 6 and 9 showed that there were 14 samples contained pesticides residues, but less than the MRL (Table 6). There were one sample contained residues more than the MRL (Tables 6 and 9).

TABLE 3
Number of milk products samples contained OCPs residues collected from September 2016 to September 2017

Milk product	Total analysed samples	No. of samples contained residues	
		< MRL	> MRL
Youghurt	11	1	0
Labaneh	12	1	0
White Chees	12	1	0

TABLE 4
Maximum residue limits in foodstuff of animal origin according to Codex alimentarius (Pesticide Residues in Food, http://www.codexalimentarius.net/mrl

Pesticide	Animal products			
	Eggs*	Red meat*	Milk products*	Poultry*
			F**	
Aldrin + dieldrin	0.1	0.2	0.006	-
DDT***	0.1	5	0.02	-
Endrin	0.2	0.1	0.0008	1.0
Heptachlor	0.05	0.2	0.006	0 .2
γ-HCH = Lindane	0.01	0.1	0.01	0.7
(α + β) -HCH	0.1	-	-	-

*Pesticide concentration as mg/kg fat
** Determination of MRL for milk and milk products according to Codex Alimentarius depends on the amount soluble in fat and expressed on the whole sample. If fat content is less than 2%, the MRL is multiplied with a constant of 0.5, but if fat content is more than 2%, the MRL is multiplied with a constant of 25.
***DDT is the sum of *o,p'*-, and *p,p'*- of DDT + DDE + DDD

TABLE 5
Number of table eggs samples contained OCPs residues collected between September 2016 and September 2017.

Type of table eggs	Total analysed samples	Number of samples contained residues	
		< MRL	> MRL
Farmer eggs	23	4	0
Balady eggs	17	1	0

TABLE 6
Number of meat samples and poultry samples contained OCPs residues collected between Sep. 2016 and Sep. 2017

Animal product	Total samples analysed	No. of samples contained residues	
		< MRL	> MRL
Local red meat	17	6	0
Cooled imported Red meat	14	5	0
Frozen imported Red meat	23	3	1
Local poultry	6	0	0
Imported poultry	6	0	0

TABLE 7
Concentration (mg/kg fat) of found OCPs in milk product samples collected between Sep. 2016 / Sep. 2017.

Milk product↓	Diel-drin	Hepta-chlor	α-HCH	β-HCH	γ-HCH	*o,p'*-DDE	*p,p'*-DDD	*p,p'*-DDE	*p,p'*-DDT
Youghurt	-	0.01	-	-	-	-	-	-	-
Labaneh	-	-	-	-	-	-	-	0.01	-
Imported White chees	-	-	-	-	-	-	-	0.01	-

TABLE 8
Concentration (mg/kg fat) of OCPs in table egg samples collected between Sep. 2016 and Sep. 2017.

Sample type ↓	Diel-drin	Hepta-chlor	α-HCH	β-HCH	γ-HCH	*o,p'*-DDE	*p,p'*-DDD	*p,p'*-DDE	*p,p'*-DDT
Farm egg/ Aqaba	-*	0.01	-	-	-	0.02	-	-	-
Farm egg/ South Ghore	-	-	-	-	-	0.03	-	-	-
Farm egg/ Karak	-	-	-	-	-	-	-	0.02	-
Farm egg/ Tafila	-	-	-	-	-	-	-	0.01	-
Balady egg/ Tafila	-	-	-	-	-	-	-	0.01	-

* (-) means under detection limit

TABLE 9
Concentration (mg/kg fat) of OCPs in local and imported red meat samples collected between Sep. 2016 and Sep. 2017.

Sample type ↓	Diel-drin	Hepta-chlor	Endrin	α-HCH	β-HCH	γ-HCH	*o,p'*-DDE	*p,p'*-DDD	*p,p'*-DDE	*p,p'*-DDT
Fresh local goat/Ma'an	-*	0.01	-	-	-	-	-	-	-	-
Imported cooled veal/Aqaba	-	-	-	-	-	-	-	-	0.03	-
Balady lamb/Aqaba	-	0.01	-	-	-	-	-	-	-	-
Imported fro-zen lamb/Aq-aba	0.25	-	-	-	-	-	-	-	-	-
Imported, cooled veal/ S. Ghore	-	-	-	-	-	-	-	-	0.08	-
Imported fro-zen veal/Aq-aba	-	-	0.02	-	-	-	-	-	0.06	-
Local fresh veal/Aqaba		0.02	-	-	-	-	-	-	-	-
Fresh local veal/Aqaba	-	-	0.02	-	-	-	-	-	-	-
Imported cooled veal/ Ma'an	0.05	-	0.03	-	-	-	-	-	-	-
Fresh local veal/Ma'an	0.06	-	-	-	-	-	-	-	-	-
Fresh local veal/Ma'an	-	-	0.03	-	-	-	-	-	-	-
Imported cooled veal/Tafila	-	-	-	-	-	-	-	-	0.02	-
Imported fro-zen veal/ Tafila	-	-	-	-	-	-	-	-	0.09	-

*(-) means under detection limit

DISCUSSION

140 local and imported foodstuff samples of animal origin were analyzed. Twenty samples (14.29%) contained persistent organochlorine pesticides residues, but less than the FAO/WHO MRL which adopted by Jordan [18]. The contaminated samples were contained dieldrin, endrin, heptachlor, *p,p'*-DDE, and *o,p'*-DDE. There were one sample out of the 140 (0.7%) contained residues more than the Codex Alimentarius MRL (Table 4). However, 1.43% of the samples contained *o,p'*-DDE, 7.14% contained *p,p'*-DDE, 2.86% contained heptachlor, 2.86% contained endrin, and 2.14% contained dieldrin. Those samples contained more than one organochlorine pesticide represented 0.71% of the total analyzed samples.

The monitoring study in Jordan in 2013 and 2014 (13) showed that DDT group (DDT, DDE and DDD) was present in higher percentage of 74.7% indicating that concentration for the same group in the current study has been declined. The HCH and DDT groups were also reported in previous studies in Jordan by Al-Antary et al. [7, 9].

The percentage of local samples containing persistent organochlorine compounds was 14.44%; luckily all samples were not with residues more than the MRL (13 out of 90). On the contrary, 8 imported samples contained residues, but less than the MRL except one sample contained residues more than the MRL. In the 2013/2014 study in Jordan [13] local samples contained residues more than the imported one indicating the continuous decline of the residues in the local study in Jordan [9], 6.7% of the analyzed milk product samples contained organochlorine pesticides less than the MRL.

Cummings et al. [19] reported pesticide residues in poultry meat more than the MRL. Decker [20] from USA found β-HCH in his monitoring of meat and milk samples. Several authors [21] reported that endrin residues from the persistent cyclodien group of insecticides were found in eggs and poultry tissues. Cummings et al., [19] stated that the bodies of many persons contaminated with residues of organochloroine compounds due taking them through meat and poultry.

CONCLUSION

The results of milk products showed that there were three samples out of 35 contaminated with organochlorines, but less than the MRL. Results of table eggs showed 5 samples out of 40 were contaminated with residues, but less than the MRL. Results of meat and poultry analysis showed that14 samples out of 65 were contaminated, but less than the MRL. There was one imported frozen lamb sample collected from Aqaba contained pesticide residues more than the MRL. However, the contaminated samples were contaminated with dieldrin, endrin, heptachlor, *p,p'*-DDE, *p,p'*-DDT and *o,p'*-DDE. Relying on the present study, it is recommended to continue monitoring residues of organochlorines in local and imported commodities of foodstuff of animal origin to protect citizens. In addition, extension programs should be carried out to public about misuse of pesticides and hazards of pesticide residues in human, animal and other components of the environment.

ACKNOWLEDGEMENTS

This work was conducted with the support of the University of Jordan, Royal Scientific Society, Ministry of Environment and the Jordanian Environment Society, to whom we thank.

The authors report no declaration of interest.

REFERENCES

[1] Al-Antary, T.M. (1996) Pesticides and Toxicology. Al-Quds Al-Maftoha University publications, Amman, Jordan. 443p.

[2] Rekha, S.N. and Naik, R.P. (2006) Pesticide residues in organic and conventional food- risk analysis. Chem Health Saf. 13, 12 – 19.

[3] Al-Momany, A. and Al-Antary, T.M. (2008) Pests of home and garden. 2nd edition. Publications of the University of Jordan, Amman, Jordan. 518p.

[4] Hodgson, A. (2003) The high cost of pesticide poisoning in northern Ghana. Pestic News. 62(3), 4 – 8.

[5] Gill, J.P.S. (2000) Studies on pesticide residues in milk and milk products and its public health significance. Ph.D. Dissertation, Pujab Agricultural University, Ludhiana, India.

[6] Aulakh, R.S., Gill, J.P.S., Bedi, G.S., Sharma, J.K., Joia, B.S., and Ockerman, H.W. (2006) Organochlorine pesticide residues in poultryfeed, chicken muscle and eggs at a poultry farm in Punjab, India. J. Food Sci Agric. 86, 741 – 744.

[7] Al-Antary, T.M., Alawi, M., Stetiyah, H., and Oqlah, K. (2012) Comparison study of chlorinated pesticides in animal products within fourteen years in Jordan. Fresen. Environ. Bull. 21, 117 – 122.

[8] Al-Antary, T.M., Alawi, M., Estityah, H. and Al-Oqlah, K. (2013) Comparative study for chlorinated pesticides in human breast milk from Jordan between 2004 and 2008. Fresen. Environ. Bull. 22, 1387 – 1393.

[9] Al-Antary, T.M., Alawi, M., Estityah, H., Haddad, N. and Al-Antary, E. (2016) Chlorinated pesticide residues in human breast milk collected from southern Jordan in 2012/2013. Toxin Rev. 34, 190 – 194.

[10] Al-Antary, T.M., Alawi, M., Estityah, H., Haddad, N. (2017) Organochlorine pesticides residues in human breast milk from the middle governorates in Jordan in 2013/2014. Bull Environ Contam Toxicol. 99, 89 – 92.
[11] Alawi, M., Al-Antary, T.M., Estityah, H., and Al-Oqlah, K. (2012) Comparison study of pesticide residues in agricultural crops in Jordan for seven studies between 1993 and 2008. Fresen. Environ. Bull. 21, 927 – 934.
[12] Alawi, M., Al-Antary, T.M., Estityah, H., Al-Aqqad, S., and Al-Oqlah, K. (2013) Comparative study of chlorinated pesticides in mother milk from Jordan in four studies between 1993 and 2003. Fresen. Environ. Bull. 22, 279 – 285.
[13] Alawi, M., Al-Antary, T.M., Estityah, H., Al-Aqqad, S. and Haddad, N. (2015) Chlorinated pesticides residues in human breast milk from southern Jordan. Comparative study between 2002 and 2012. Fresen. Environ. Bull. 24, 4512 – 4518.
[14] Alawi, M., Al-Antary, T.M., Estityah, H., Haddad, N. (2017) Evaluation of chlorinated pesticides residues in foodstuff of animal origin from middle districts of Jordan in 2013 and 2014. Toxin Reviews. 2, 90 – 100.
[15] Darko, G., and Acquaah, S. (2007) Levels of organochlorine pesticides residues in meat. Int J Environ Sci Tech. 4(4), 521 – 524.
[16] Saxena, M.C., and Siddiqui, M.J. (1980) Pesticides solutions in India: Organochlorine pesticidex in milk of women, buffalo and goat. J. of Dairy Science. 65, 430 – 434.
[17] Association of Official Analytical Chemists (AOAC) (2005) Official methods 970.52: Organochlorine and organophosphorous pesticides residues. Arlington, Va: AOAC.
[18] Pesticides residues in Food and feed (2013) Codex Alimentarius Pesticides residues in food. Available at: www.CodexAlimentarius.net/pestrex/data. Accessed on November 20, 2016.
[19] Cummings, J., Eidelman, M., Turner, V., Reed, D., Zee, K. and Cook, R. (1967) Residues in poultry tissues from low level feeding of five chlorinated hydrocarbon insecticides to hen. J. Assoc Off Agric Chemists. 50, 418 – 425.
[20] Decker, G.C. (1959) Significance of pesticide residues in milk and meat, J. Agric Food Chem. 10(7), 681 – 683.
[21] Terriere, I.C., Arscott, G.H., and Kiigenagi, V.C. (1950) Content of eggs and body tissue of poultry receiving endrin daily diet. J. Agric. Food Chem. 7, 502 – 504.

Received: **21.05.2018**
Accepted: **29.10.2018**

CORRESPONDING AUTHOR

Mahmoud A Alawi
School of Science,
The University of Jordan,
Amman, 11942 – Jordan

e-mail: alawima@ju.edu.jo

© by PSP Volume 27 – No. 12B/2018 pages 9782-9791 Fresenius Environmental Bulletin

THE BIOPHYSICAL STRUSTURE OF ROADSIDE GREEN SPACES: THE IMPACT ON ECOLOGICAL CONDITIONS IN THE URBAN ENVIRONMENT

Nadezda Stojanovic[1,*], Nevena Vasiljevic[1], Milorad Veselinovic[2], Boris Radic[1], Dejan Skocajic[1], Nevenka Galecic[1], Mirjana Tesic[1], Aleksandar Lisica[1]

[1]Department of Landscape Architecture and Horticulture, Faculty of Forestry, University of Belgrade, Kneza Višeslava no. 1, 11000 Belgrade, Serbia
[2]Institute of Forestry, Belgrade, Serbia

ABSTRACT

Automobile traffic, which is considered one of the permanent major sources of various types of pollution in the urban environment, gives a special contribution to urban ecological problems. The establishment of roadside green spaces can greatly reduce the negative ecological consequences that urban traffic produces. In the process of planning and management of urban green spaces, information on the types of biophysical structures of the green spaces and their characteristics in relation to the degree of modification of unfavorable ecological factors are of great importance. This paper investigates the impact of the type of biophysical structure of green roadside spaces in the area of Belgrade on the ecological factors with the highest impact on people's quality of life in the city, including air temperature, air humidity, the urban noise level and wind speed. The results and conclusions of this paper are part of a research of the adaptive design, which provides guidelines for the planning of urban landscape development in the conditions of unpredictable climate change.

KEYWORDS:
ecological impact, adaptive design, biophysical structure, urban green spaces, green infrastructure.

INTRODUCTION

Automobile traffic, which is one of the permanent and major sources of various types of pollution of the urban environment, largely contributes to urban ecological problems. Large areas under asphalt, occupied by urban roads cause overheating of the city, an increase in the urban heat island effect and a decrease in humidity in the immediate vicinity [1, 2]. Intensive car traffic causes an increase in pollutants and urban noise levels, while changes in the configuration of the terrain caused by the routing of urban roads can also affect wind speed [3].

Green spaces are elements of the city structure that directly affect the ecological quality of the city and contribute to the mitigation of ecological problems. The presence roadside greenery in cities has an impact on the reduction of the negative effects of heating, positively affecting the microclimate conditions of the environment, by creating the effect of cooling and increasing the overall urban comfort [4-15]. Green spaces, as well as different forms of greenery influence the movement of the air with their presence, producing the effect of wind intensity reduction, not only within their structure, but also at a certain distance from them [16-18]. The arrangement of green spaces, as well as other elements of the urban landscape structure, is of key importance in the protection against urban noise [19-27].

Therefore, green spaces serve as necessary elements along road corridors, since it has been proven that trees reduce pollutants from the air by absorbing pollutant particles from the atmosphere [28].

In the conditions of climate change, which are unpredictable, scientific research within the framework of adaptive planning (design) of urban landscapes and the application of landscape – environmental principles in the form of green infrastructure are very important [29]. Adaptive design provides an alternative scientific and professional strategic approach in which plans and policies are developed in a context of uncertainty and incomplete knowledge [30]. In this respect, it is important to investigate which elements of green infrastructure, i.e. which types of biophysical structure of green spaces that differ in the spatial arrangement and artificial elements, are the most effective in modifying unfavorable ecological factors [31, 12, 32, 33].

This paper presents a study of the impact of urban roadside green spaces along the main roads in the area of Belgrade classified according to the type of biophysical structure to those ecological factors that affect the quality of people's lives in the city the most, including air temperature, air humidity, the urban noise level and wind speed. The starting assumption is that the environmental impact of roadside green spaces on the investigated ecological

factors depends on the type of biophysical structure to which the green spaces belong. The results of this research can serve as the basis for the creation of guidelines and recommendations for the esatblishment and landscaping of urban roadside green spaces as part of road belt landscaping, in order to improve their ecological functions. In a wider sense, the results of this paper represent a contribution to the research of adaptive planning and design of urban landscapes in the conditions of climate change.

MATERIALS AND METHODS

This study of the impact of the type of biophysical structure of roadside green spaces is based on the monitoring and measurement of the ecological factors of air temperature, air humidity, the urban noise level and wind speed in the sample roadside green spaces along the main roads in the city of Belgrade.

A total of 15 major roads were registered in the area of the city of Belgrade (according to the boundaries of the Master Plan of Belgrade 2021) including, Zrenjaninski road, Višnjička Street, Jurija Gagarina Street, Partizanski road, Bulevar kralja Aleksandra, Rakovički road, Patrijarha Dimitrija, Bulevar JNA , Ibarska magistral road, Bulevar Nikola Tesla, Bulevar Mihajla Pupina, Tošin bunar, Pančevački road, Savska magistral road, Highway E–75, Batajnički road and Cara Dušana Street). A total of 37 sample green spaces that were subjected to research were identified along these roads (Figure 1).

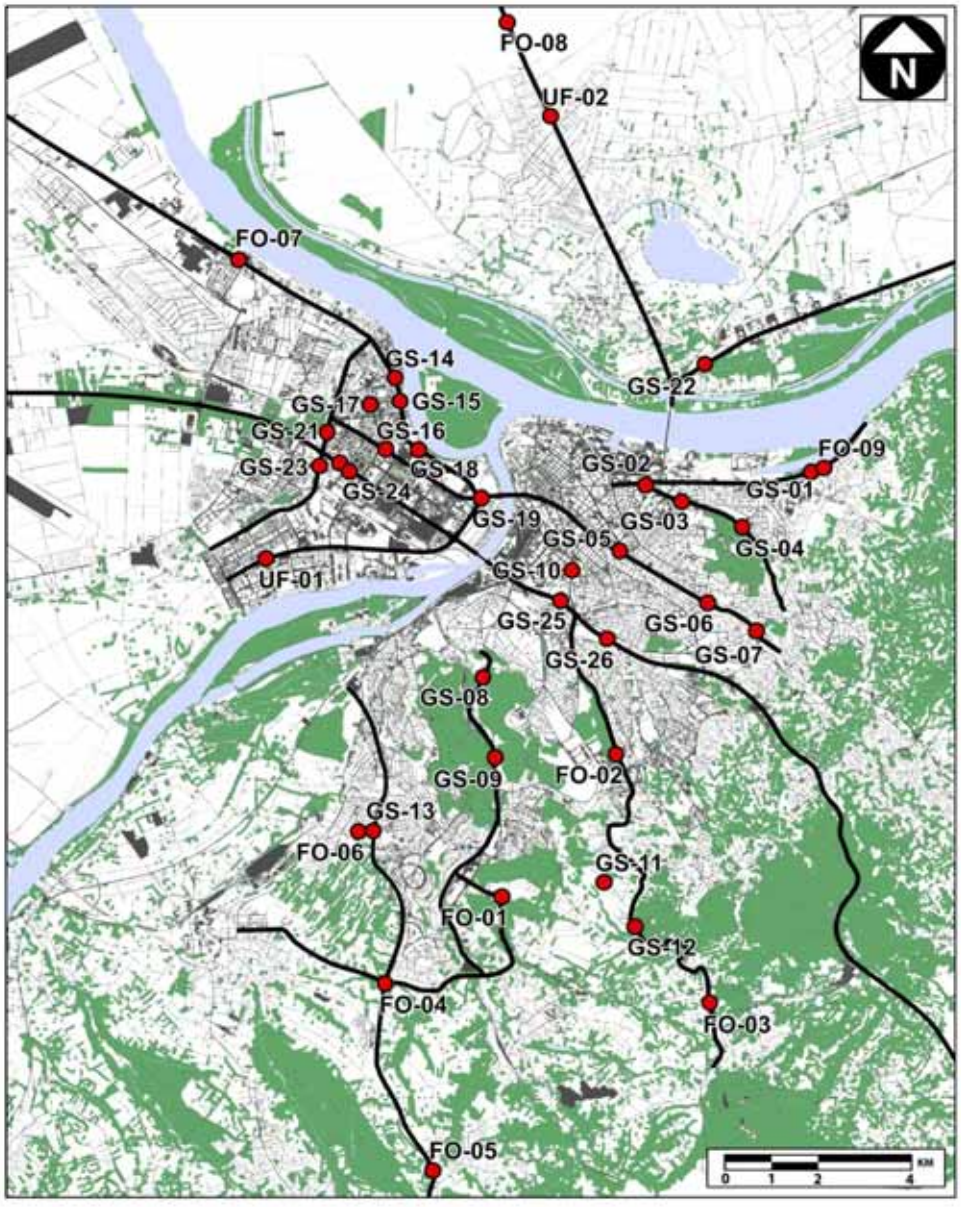
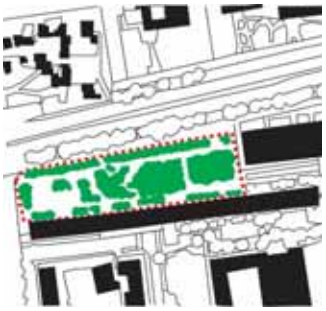
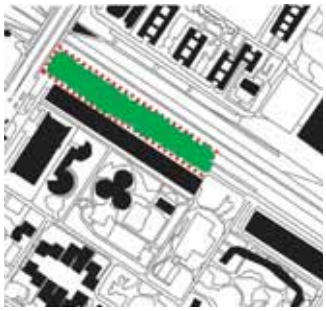
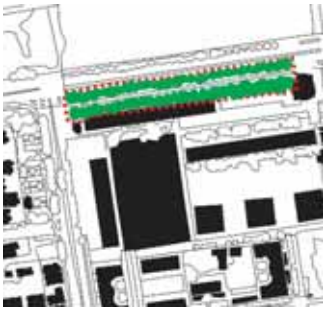


FIGURE 1
A Belgrade city map and the sample green spaces used in this experiment

TABLE 1
Key to determining the type of biophysical structure of a green space

Type 1 – Mosaic arrangement	Type 2 – Dense canopy	Type 3 – Linear arrangement
		

The sample green spaces are distinguished on the basis of the following criteria: (1) spatial – each green space with its position relative to the road is a kind of a protective belt, which extends along the road and is located between the road and the nearby residential buildings, (2) accessibility and availability in the field – green spaces where the required measurements of ecological factors could have been measured over a long period of time were selected.

The biophysical structure of a green space represents the spatial arrangement of its structural elements (biotope of the group of trees and shrubs). Three spatially different types of arrangement of the elements are defined (Table 1), including the *mosaic arrangement* (the elements of the green space are mosaically arranged), the *dense canopy* type (the green space is 90–100% covered by tree and shrub crowns) and the *linear* biophysical structure (when the elements of the biophysical structure of the green space are linearly distributed).

The research of the impact of the type of biophysical structure in the sample green spaces on the investigated environmental factors was carried out over a two–year period. In each of the 37 sample green spaces, measurements were carried out during the vegetation period (in spring, summer and autumn), every year in three series of two consecutive measurements, and the control measurements were performed in a single series of three consecutive measurements. The digital meteorological station DT–8820–CEM, UK was used to measure air temperature, air humidity and the urban noise level (operating range for measurement: for air temperature – from –20°C to 750°C, with a resolution of 0.1°C; for air humidity – from 25% to 95% RH, with a resolution of 0.1% RH, for noise intensity levels – from 35 to 130dB, with a resolution of 0.1dB). Wind speed was measured with a digital wind speed meter AM 4220 – LUTRON, Taiwan (operating range from 0.9 to 35m/s, with a resolution of 0.1m/s). The reading of the measured values was performed in two positions: in front of a green space (side towards the road) and behind it. All measurements were performed in the afternoon hours, on workdays, at each measuring point at an operational height of 130cm. In order to determine the impact of the type of biophysical structure of green spaces on ecological factors, a series of control measurements of air temperature and air humidity were made at the same distance in the direction of the measuring points, in close proximity, but in an open space without established plantings. When measuring air temperature and humidity, the instruments were placed under a shield. The impact of wind on the urban noise level was neutralized with a microphone shield.

The impact of the type of biophysical structure of an investigated green space was obtained as the difference between the mean values measured in front of and behind the investigated green space and the mean values of the difference in comparison to the control measurement. For the ecological factors urban noise level and wind speed, the impact of a green space represents the mean value of the difference in the values of these factors measured in front of and behind the investigated green spaces.

The research was carried out in order to: 1) determine the level of impact of roadside green spaces classified according to the type of biophysical structure on ecological conditions in the urban environment and 2) to determine the significance of the difference in the level of impact of different types of biophysical structures to which these roadside green spaces belong on the ecological factors.

IBM SPSS Statistics 21 and Microsoft Excel 2010 were used for data analysis and graphical presentation of the research results. Parameter statistics was applied. By means of a single–factor analysis of variance (ANOVA), the determined mean differences in the impact of green spaces depending on the type of biophysical structure of the investigated green spaces were tested. The *Levene* test was used to test the homogeneity of variance. The *Tukey HSD* test was used to obtain statically significant differences in the values of ecological factors.

TABLE 2
Statistical parameters for the mean values of the investigated ecological factors for different types of green spaces depending on the arrangement of biophysical structures

Ecological factor	Type of biophysical factor	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Min.	Max.
Air temperature (°C)	Type 1	1.1327	0.57060	0.02542	1.0828	1.1827	0.10	3.20
	Type 2	1.1157	0.62089	0.02987	1.0570	1.1745	0.10	3.40
	Type 3	1.2667	0.69132	0.03474	1.1984	1.3350	0.10	4.70
	Σ	1.1670	0.62766	0.01720	1.1333	1.2008	0.10	4.70
Air humidity (%)	Type 1	1.7262	0.84483	0.03763	1.6523	1.8001	0.20	4.80
	Type 2	1.8813	0.95810	0.04610	1.7906	1.9719	0.10	5.10
	Type 3	2.1687	1.05851	0.05319	2.0641	2.2733	0.40	6.20
	Σ	1.9080	0.96564	0.02646	1.8561	1.9599	0.10	6.20
Urban noise level (dB)	Type 1	15.6883	4.22856	0.18836	15.3182	16.0584	5.40	29.80
	Type 2	15.2130	4.57912	0.22031	14.7799	15.6460	4.20	27.80
	Type 3	16.6288	5.42792	0.27276	16.0925	17.1650	4.00	29.90
	Σ	15.8137	4.75516	0.13029	15.5581	16.0693	4.00	29.90
Wind speed (m/s)	Type 1	0.8871	0.77814	0.03466	0.8190	0.9552	0.10	4.90
	Type 2	0.8838	0.80582	0.03877	0.8076	0.9600	0.10	6.20
	Type 3	0.7985	0.60109	0.03021	0.7391	0.8579	0.10	4.00
	Σ	0.8597	0.74021	0.02028	0.8199	0.8995	0.10	6.20

Type 1 – mosaic arrangement of structures; Type 2 – dense canopy; Type 3 – linear arrangement of structures

The research of the impact of the type of biophysical structure of the investigated green spaces on air temperature, air humidity, the urban noise level and the wind speed were performed by a comparative analysis of the independent variable: the type of biophysical structure (three types): mosaic biophysical structure, dense canopy and linear biophysical structure) and the dependent variable: the impact of the green space, i.e. the difference in the values of the investigated ecological factors in front and behind the green spaces in the case of the urban noise level and wind speed, and the differences in air temperature and air humidity values in front of and behind the green spaces reduced by the mean value obtained in the control measurement.

RESULTS AND DISCUSSION

The sample green spaces that are the subject of this research (37 of them) are classified into three categories according to the type of biophysical structure: the mosaic structure (type 1), the dense canopy structure (type 2) and the linear structure (type 3). A total of 14 sample spaces belong to the group with a mosaic structure, 12 of them are in the group of spaces with a dense canopy structure and 11 of them belong to the group with a linear biophysical structure.

Table 2. shows the statistical parameters for the mean values of the investigated ecological factors depending on the arrangement of the elements of the biophysical structure in the investigated green spaces.

When the ecological factor air temperature is concerned, the mean difference in air temperature values is pretty uniform among the different types of biophysical structures arrangement. The largest mean difference was observed in the green spaces with a linear type of biophysical structures arrangement (1.27 ± 0.69°C), a slightly lower mean difference was recorded in the green spaces with a mosaic type of arrangement (1.13 ± 0.57°C), while in the green spaces with a dense canopy, it amounted to 1.11 ± 0.62°C.

The largest mean difference in air humidity was recorded in the green spaces belonging to the group with a linear arrangement of biophysical structures (2.17 ± 1.06%), a somewhat smaller difference in the green spaces with a dense canopy (1.88 ± 0.96%), while the smallest mean difference in air humidity characterized the green spaces with a mosaic arrangement of biophysical structures (1.73 ± 0.84%).

Through an analysis of the recorded urban noise level values, the largest mean difference was recorded in the green spaces with a linear type of biophysical structures and it amounted to 16.63 ± 5.43dB. A somewhat lower value was found in the green spaces with a a mosaic arrangement (15.69 ± 4.23dB), and the lowest mean difference characterized the green spaces a with a dense canopy (15.21 ± 4.58dB).

Unlike in the case of the ecological factors of air temperature, air humidity and urban noise level, in the case of the ecological factor wind speed, the highest impact of the green spaces with a mosaic arrangement of biophysical structures was found, and it amounted to 0.89 ± 0.78m/s. A slightly lower reduction of wind speeds was achieved by the green

spaces with a dense canopy (0.88 ± 0.81m/s), while the smallest modification of the environmental factor of wind speed was recorded in the green spaces with a linear arrangement of biophysical structures (0.80 ± 0.74m/s).

An analysis of the impact of the type of arrangement of biophysical structures on the size and intensity of ecological factors has shown that green spaces that have a linear arrangement of biophysical structures modify the ecological factors the most, and these factors are air temperature, air humidity and urban noise levels. The green spaces that have a mosaic arrangement of biophysical structures affect wind speed modification the most.

On the one hand, linearly distributed biophysical structures in green spaces behave as obstacles for modifying the explored ecological factors, while on the other, due to the linear arrangement, they provide easier movement of air, which facilitates their cooling. Hagler et al. (2012) [34] also emphasize that live barriers containing trees and shrubs are defined as efficient mechanisms for modifying ecological factors, and their effect is particularly evident in the reduction of the urban noise level. A dense canopy of structures blocks free air movement, which to a certain extent reduces the process of ecological factors modification.

The effect of obstacles that basically have linearly arranged biophysical structures in the modification of air temperature and humidity values is particularly increased when the obstacles (linear structures) consist of densely arranged trees and shrubs (or plants of the second storey). In the case of air temperature modification, that was recorded in the following green spaces: *GS–22*, where the mean difference in the value of this ecological factor is 1.5°C, as well as in green surface *GS–29* (1.3°C). In addition, in the case of air humidity, higher values of the modification of this ecological factor were obtained in the green spaces where the linearly arranged structures consist of trees and shrubs.

The positive effect of linear biophysical structures is particularly manifested in the reduction of the urban noise level. In the green spaces with a linear biophysical structure, higher values of noise reduction were recorded. In green space *GS–09* the mean reduction in urban noise was by 19.7dB, in green space *GS–12*, the amount of urban noise reduction amounted to 18.2dB, in *GS–29* (18.5dB), in *GS–20* (17.7dB) etc. Baldauf et al. (2008) [35] also emphasize that live obstacles from trees and shrubs (linear structures) greatly reduce city noise levels, especially those that come from traffic, by blocking sound waves with their specific structure.

The statistical significance of the established differences in the reduction of the investigated ecological factors was confirmed by a single–factor analysis of variance (Table 3).

TABLE 3
One–factorial analysis of variance between the differences in the mean values of the investigated ecological factors for the investigated types of green spaces depending on the biophysical structure

		Sum of Squares	df	Mean Square	F	Sig.
Air temperature	Between Groups	7.445	2	3.723	9.571	0.000
	Within Groups	516.918	1329	0.389		
	Total	524.363	1331			
Air humidity	Between Groups	27.326	2	13.663	14.960	0.000
	Within Groups	1213.778	1329	0.913		
	Total	1241.104	1331			
Urban noise level	Between Groups	324.108	2	162.054	7.234	0.001
	Within Groups	29771.810	1329	22.402		
	Total	30095.919	1331			
Wind speed	Between Groups	3.793	2	1.897	3.475	0.031
	Within Groups	725.472	1329	0.546		
	Total	729.265	1331			

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
Air temperature	*Welch*	15.730	2	98.695	0.000
	Brown-Forsythe	12.739	2	303.822	0.000
Air humidity	*Welch*	49.276	2	105.676	0.000
	Brown-Forsythe	23.093	2	458.429	0.000
Urban noise level	*Welch*	14.186	2	97.841	0.000
	Brown-Forsythe	8.938	2	318.207	0.000

a. asymptopic F distribution

TABLE 4
Statistical parameters of the Tukey test – differences in the impact of the green spaces types depending on the biophysical structure

Ecological factor	Arrangement of biophysical structures (I)	Arrangement of biophysical structures (J)	Mean Difference (I–J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Air temperature	Type 1	Type 2	0.01700	0.04096	0.909	–0.0791	0.1131
		Type 3	–0.13393*	0.04195	0.004	–0.2324	–0.0355
	Type 2	Type 1	–0.01700	0.04096	0.909	–0.1131	0.0791
		Type 3	–0.15093*	0.04346	0.002	–0.2529	–0.0489
	Type 3	Type 1	0.13393*	0.04195	0.004	0.0355	0.2324
		Type 2	0.15093*	0.04346	0.002	0.0489	0.2529
Air humidity	Type 1	Type 2	0.15506*	0.06223	0.034	–0.3011	–0.0090
		Type 3	–0.44250*	0.06374	0.000	–0.5920	–0.2930
	Type 2	Type 1	0.15506*	0.06223	0.034	0.0090	0.3011
		Type 3	–0.28744*	0.06603	0.000	–0.4424	–0.1325
	Type 3	Type 1	0.44250*	0.06374	0.000	0.2930	0.5920
		Type 2	0.28744*	0.06603	0.000	0.1325	0.4424
Urban noise level	Type 1	Type 2	0.47533	0.30979	0.275	–0.2515	1.2022
		Type 3	–0.94049*	0.31728	0.009	–1.6849	–0.1960
	Type 2	Type 1	–0.47533	0.30979	0.275	–1.2022	0.2515
		Type 3	–1.41582*	0.32871	0.000	–2.1871	–0.6446
	Type 3	Type 1	0.94049*	0.31728	0.009	0.1960	1.6849
		Type 2	1.41582*	0.32871	0.000	0.6446	2.1871

*. The mean difference is significant at the 0.05 level.
Type 1 – mosaic arrangement of structures; Type 2 – dense canopy; Type 3 – linear arrangement of structures

The single–factor analysis of variance (Table 3) revealed the F–value of the ecological factor air temperature of 6.380 (*Welch*), with a statistical significance of 0.02 and 7.087 (*Brown-Forsythe*), and it can be concluded that there are statistically significant differences in the values of air temperature in the three different types of green spaces depending on the arrangement of biophysical structures in them, at the level of statistical significance of 0.01. Using *the Tukey HSD test* (Table 4), it was established that the differences in air temperature are significant at the level of 0.01 between the green spaces with a mosaic arrangement of biophysical structures and the green spaces with linearly arranged biophysical structures (Sig. = 0.004; p <0.01), as well as between the green spaces with a dense canopy of biophysical structures and those with a linear arrangement (Sig. = 0.002; p <0.01). The analysis confirmed that there are no statistically significant differences in the reduction of the ecological factor of air temperature between the green spaces with mosaically arranged biophysical structures and the green spaces with a dense canopy (Sig. = 0.909; p> 0.05).

The results of the single–factor variance analysis showed that for the ecological factor of air humidity *the Statistic* amounted to 23.051 (*Welch test*) and 23.703 (*Brown-Forsythe test*). Therefore, it can be concluded that there are statistically significant differences in values of air humidity in different the types of green spaces depending on the biophysical structure at the level of 0.01. By applying the *Tukey HSD* test statistically significant differences were found between all types of green spaces. In the case of green spaces with a mosaic and linear arrangement of biophysical structures (Sig.=0.000; p<0.01) and between the green spaces with a dense canopy biophysical structure and structures with a linear arrangement the statistical significance is at the level of 0.01(Sig.=0.000; p<0.01), while in the case of green spaces with mosaically arranged biophysical structures and the green spaces with a dense canopy statistical significance is at the level of 0.05, i.e. (Sig.=0.034; p <0.05).

By analyzing the results of a single–factor analysis of variance for the ecological factor of urban noise level, it was established that the statistics value (*Statistic*) amounted to 8.200 (*Welch test*) and 9.284 (*Brown-Forsythe test*), and it can be concluded that there are statistically significant differences in the values of the difference in the urban noise level between different types of green spaces depending on their biophysical structure. The *Tukey HSD* test confirmed that there are statistically significant differences between different types of green spaces depending on their biophysical structure arrangement at the level of 0.01. Statistically significant differences in the reduction of the ecological factor of urban noise are recorded between the green spaces with mosaically and linearly arranged biophysical structures (Sig.=0.009; p<0.01), as well as between the green spaces with a dense canopy and the ones with linearly arranged biophysical structures (Sig.=0.000; p<0.01), while there are no statistically significant differences between the green spaces a mosaic arrangement and the green spaces with a dense canopy (Sig.=0.275;

p> 0.05).

When the wind speed factor is concerned, the *statistic* value is 2.403 (*Welch test*) and 1.977 (*Brown-Forsythe test*), with the significance level of 0.091 (*Welch test*) and 0.139 (*Brown-Forsythe test*), and it can be concluded that for this ecological factor, there are no statistically significant differences between the three different types of green spaces depending on their biophysical structure.

Wind is a highly variable ecological factor compared to air temperature, air humidity or the urban noise level [36, 37]. The investigated green spaces were established as green spaces for protection against the negative impacts of roads rather than as typical windbreaks. Therefore, the situation in the field, which often occurs, is that wind blows at different angles relative to the investigated green spaces. Renterghem et al. 2012 [27] also emphasize that the reduction of wind intensity is the most pronounced when green spaces are perpendicular to the direction of the wind, and that wind speed reduction by green structures is lower if the wind does not blow perpendicularly to the obstacle. These facts can also serve as an explanation for the absence of statistically significant differences in the wind speed reduction between the explored green spaces.

The recommendation of this research is that biophysical structures in green spaces should be placed linearly in order to reduce air temperature and humidity, as well as the urban noise level. The best effects are obtained when linear biophysical structures (barriers) consist of densely distributed trees and shrubs. This recommendation is confirmed by the study Cook (1980) [38], which showed that the best reduction effects, especially of urban noise, are obtained by planting trees and bushes in the form of dense linear belts. In the case of the ecological factor wind speed, the arrangement of biophysical structures does not significantly affect its reduction.

CONCLUSIONS

The uncertain future as a result of climate change requires specific research in the field of urban landscape planning. Temperature reduction, an increase in air humidity and a decrease in the urban noise level, whose major source is traffic, are some of the imperatives of improving the environmental quality in modern cities. Urban landscape planning and design are based on the experimental knowledge that is monitored and checked in different conditions. The adaptive design of green spaces along urban roads as one of the major existing sources of pollution in the city requires familiarity with the most optimal spatial arrangement of biophysical structures, as well as with the environmental form of plant material that can reduce negative impacts. In this research, 37 roadside green spaces in the territory of the city of Belgrade, along 15 main roads, were classified into three types of biophysical structures (mosaic, dense canopy and linear structure). The research investigated the impact of those types of biophysical structure on four ecological factors (air temperature, air humidity, urban noise level and wind speed). The results of the research are the following findings:

– During the growing season, green spaces with a linear arrangement of biophysical structures modify air temperature, air humidity and the urban noise level the most, while green spaces with a mosaic arrangement of biophysical structures affect wind speed modification the most.

– It was established that during the growing season the modification of air temperature and humidity values is significantly increased in green spaces with a linear arrangement of biophysical structures consisting of densely distributed trees and shrubs (or, in general, plants of the second storey).

– The positive effect of linear biophysical structures is especially manifested in the reduction of the city noise level. In green spaces with a linear biophysical structure, which have an obstacle function, higher noise reduction values were recorded.

– This research has shown that there are statistically significant differences in the investigated ecological factors of air temperature, air humidity and the factor of urban noise level, while in the case of the ecological factor of wind speed, statistically significant differences were not found between the three different types of biophysical structure, i.e. the arrangement of biophysical structures did not significantly influence wind speed modification.

– In the case of the ecological factor of air temperature, statistically significant difference at the 0.01 level was found between the green spaces with a mosaic arrangement of biophysical structures and the green spaces with linearly arranged biophysical structures, as well as between the green spaces with a dense canopy of biophysical structures and those with a linear arrangement. There are no statistically significant differences in the reduction of this ecological factor between the green spaces with mosaically arranged biophysical structures and the green spaces with a dense canopy.

– The differences in the values of reduction of the ecological factor air humidity are statistically significant between the green spaces with a mosaic and linear arrangement of biophysical structures and the green spaces with a dense canopy of biophysical structures and linearly arranged biophysical structures, at the level of 0.01, while the difference between the green spaces with a mosaic arrangement of biophysical structures and the ones with a dense canopy reached the level of statistical significance of 0.05.

– The differences in the urban noise level are statistically significant at the level of 0.01 between

the green spaces with mosaically and linearly arranged biophysical structures, as well as between the green spaces with a dense canopy and the ones with linearly arranged biophysical structures. However, between the green spaces with a mosaically arranged structure and the green spaces with a dense canopy, there are no statistically significant differences.

The search for optimal models of urban landscape development in the conditions of climate change is an imperative of future scientific and practical research. Green infrastructure, as a coherent network of open green spaces in the city, is one of the most optimal models for the development of resilient cities. Adaptive design of urban roadside green spaces, as elements of green infrastructure, requires constant experimenting and monitoring. In the uncertain conditions of climate change, such research is a necessary prerequisite as the basis for formulating the guidelines for sustainable urban landscape planning.

ACKNOWLEDGEMENTS

This paper was realized as a part of the project "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the period 2011–2018.

REFERENCES

[1] Alcoforado, M., Andrade, H. (2008) Global warming and urban heat island. In: Marzluff, J.M., Shulenberger, E., Endlicher, W., Alberti, M., Bradley, G., Ryan, C., Simon, U., Zum Brunnen, C. (eds.) Urban Ecology. Springer. 249–62.

[2] Stojanović, N. (2016) Ecological and aestetic function of green spaces along the main routes in Belgrade. Doctoral Dissertation. University of Belgrade, Faculty of Forestry, Belgrade.

[3] Taha, H. (1997) Urban climates and heat islands: albedo, evapotranspiration, and anthropogenic heat. Energy and Buildings. 25, 99–103.

[4] Taha, H., Akbari, H., Rosenfeld, A. (1988) Vegetation canopy microclimate: a field project in Davis, California. Berkley, CA: Lawrence Berkley in Davis, Laboratory Report–24593.

[5] Parker, J. (1989) The impact of vegetation on air conditioning consumption, Controlling summer heat island. In: Akbari, H., Garbesi, K., Martien, P. (eds.) Proceedings of the workshop on: saving energy and reducing atmospheric pollution by controlling summer heat island. Barkley, California: University of California. 42–52.

[6] Givoni, B. (1991) Impact of planted areas on urban environmental quality. Atmospheric Environment. 25B, 289–299.

[7] Ken-Ichi, K. (1991) Evaporative cooling effects in hot and humid urban spaces. Proceedings of Architecture and Urban Space, Ninth International PLEA Conference, Seville, Spain, 631–636.

[8] Gao, W. (1993) Thermal effects of open space with a green area on urban environment. Journal of Architecture, Planning and Environmental Engineering. 448, 15-27.

[9] Chen, Y., Wong, H. (2006) Thermal benefits of city parks. Energy and Building. 38, 105–120.

[10] Chang, R., Huang, L., Chang, D. (2007) A preliminary study on the local cool–island intensity of Taipei city parks. Landscape and Urban Planning. 80(4), 386–395.

[11] Yu, C., Hien, N. (2006) Thermal benefits of city parks. Energy and Buildings. 38, 105–120.

[12] Potchter, O., Cohen, P., Bitan, A. (2006) Climatic behavior of various urban parks during hot and humid summer in the Mediterranean city of Tel Aviv, Israel. International Journal of Climatology. 26(12), 695–711.

[13] Zoulia, I., Santamouris, M., Dimoudi, A. (2009) Monitoring the effect of urban green areas on the heat island in Athens. Environmental Monitoring and Assessment. 156, 275–92.

[14] Georgi, N. Dimitriou, D. (2010) The contribution of urban green spaces to the improvement of environment in cities: case Study of Chania, Greece. Building and Environment. 45, 1401–1414.

[15] Edward, N., Chen, L., Wang, Y., Yuan, C. (2012) A study on the cooling effects of greening in a high–density city: An experience from Hong Kong. Building and Environment. 47, 256–271.

[16] Rosenberg, J. (1974) Microclimate: The biological environment. Wiley, New York.

[17] Gandemer, J. (1981) The aerodynamic characteristics of windbreaks, resulting in empirical design rules. Journal of Wind Engineering and Aerodynamics. 7, 15–36.

[18] Bitog, J., Lee, B., Shin, M., Hong, S, Hwang, S., Mostafa, E., Pang, Z. (2011) A wind tunnel study on aerodynamic porosity and windbreak drag. Forest Science and Technology. 7(1), 8–16.

[19] Aylor, D. (1972) Noise reduction by vegetation and ground. Journal of the Acoustical Society of America. 51(1), 197–205.
[20] Mecklenburg, R. (1976) Landscape Plants to Control Noise. Ph.D. thesis. Michigan State University.
[21] Dimoudi, A., Nikolopoulou, M. (2003) Vegetation in the urban environment: microclimatic analysis and benefits. Energy and Building. 35(1), 69–76.
[22] Babisch, W., Beule, B., Schust, M., Kersten, N., Ising, H. (2005) Traffic noise and risk of myocardial infarction. Epidemiology. 16, 33–40.
[23] Fang, C., Ling, D. (2005) Guidance for noise reduction provided by tree belts. Landscape and Urban Planning. 71, 29–34.
[24] Stansfeld, S., Berglund, B., Clark, C., Lopez-Barrio, I., Fisher, P., Ohrstrom, E., Haines, M., Head, J., Hygge, S., van Kamp, I., Berry, F., On behalf of the RANCH study team, (2005) Aircraft and road traffic noise and children's cognition and health: a cross–national study. Lancet. 365, 1942–1949.
[25] Bluhm, G., Berglind, N., Nordling, E., Rosenlund, M. (2007) Road traffic noise and hypertension. Occupational and Environmental Medicine. 64, 122–126.
[26] Gidlöf-Gunnarsson, A., Öhrström, E. (2011) Noise and well–being in urban residential environments: The potential role of perceived availability to nearby green areas. Landscape and Urban Planing. 83, 115–126.
[27] Renterghem, T., Botteldooren, D., Verheyen, K. (2012) Road traffic noise shielding by vegetation belts of limited depth. Journal of Sound and Vibration. 331, 2404–2425.
[28] Nowak, D.J., Crane, D.E., Stevens, J.C. (2006) Air pollution removal by urban trees and shrubs in the United States. Urban Forestry and Urban Greening. 4, 115–123.
[29] Vasiljević, N., Radić, B., Šljukić, B., Ristić, R. (2016) Landscape planning and green infrastructure in Serbia: From national to Belgrade city planning. In: Valanszki, I., Jombach, S., Filep-Kovacs, K., Fabos, J.G., Ryan, R.L., Lindhult, M.S., Kollanyi, L. (eds.) Proceedings of 5th Fabos Conference on Landscape and Greenway Planning – Landscapes and Greenways of Resilience. Szent István University, University of Massachusetts Amherst, Budapest (Hungary) 389–397.
[30] Ahern, J. (2013) Urban landscape sustainability and resilience: the promise and challenges of integrating ecology with urban planning and design. Landscape Ecology. 28(6), 1203–1212.
[31] Sproken-Smith, R.A., Oke, T. (1998) The thermal regime of urban parks in two cities with different summer climates. International Journal of Remote Sensing. 19(11), 2085–104.
[32] Upmanis, H., Chen, D. (1999) Influence of geographical factors and meteorological variables on nocturnal urban–park temperature differences–a case study of summer 1995 in Göteborg, Sweden. Climate Research. 13,125–39.
[33] Shashua-Bar, L., Hoffman, E. (2000) Vegetation as a climatic component in the design of an urban street: an empirical model for predicting the cooling effect of urban green areas with trees. Energy and Buildings. 31, 221–235.
[34] Hagler, G., Ming-Yeng, L., Khlystovob, A., Baldauf, R., Isakov, V., Faircloth, J., Jackson, L. (2012) Field investigation of roadside vegetative and structural barrier impact on near–road ultrafine particle concentrations under a variety of wind conditions. Science of the Total Environment. 419, 7–15.
[35] Baldauf, R., Thoma, E., Khlystov, A., Isakov, V., Bowker, G., Long, T., Snow, R. (2008) Impacts of noise barriers on near–road air quality. Atmospheric Environment. 42, 7502–7507.
[36] Stojanović, N., Anastasijević, V., Mešiček, M., Miljković, S., Lisica, A. (2016) Effects of Residental Green Spaces On City Noise Reduction. Journal Ecologica XXIII (2016), No. 83, ISSN 0354–3285, UDC: 628.517.2:712.2.3, 577–583.
[37] Stojanović N. (2016) Ecological and aestetic function of green spaces along the main routes in Belgrade place. Doctoral dissertation. Faculty of Forestry, University of Belgrade.
[38] Cook, D.I. (1980) Trees, solid barriers, and combinations: Alternatives for noise control, In: Proceedings, National Urban Forestry Conference. November 13–16, 1978, Washington D.C. Syracuse, NY: State University of New York, College of Environmental Science and Forestry, ESF Publication. 80–003, 330–339.

Received: **04.06.2018**
Accepted: **11.10.2018**

CORRESPONDING AUTHOR

Nadezda Stojanovic
University of Belgrade,
Faculty of Forestry,
Department of Landscape Architecture and
Horticulture, Kneza Višeslava no. 1
11 000, Belgrade – Serbia

e-mail: nadezda.stojanovic@sfb.bg.ac.rs

© by PSP Volume 27 – No. 12B/2018 pages 9792-9798 Fresenius Environmental Bulletin

THE REMOVAL OPTIMIZATION OF REACTIVE BRILLIANT RED X-3B FROM AQUEOUS SOLUTIONS BY USING MODIFIED PEANUT HULL BIOCHAR BASED ON THE RESPONSE SURFACE METHODOLOGY

Di Zhang, Siqi Lv, Baiwen Jiang*, Shi Peng Liang, Shuqin Li

Laboratory of Environmental Remediation, College of Resources and Environmental Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China

ABSTRACT

Biochar with a porous structure, obtained from plant residues, is widely used for waste water treatment. In this work, the removal of Reactive Brilliant Red X-3B from aqueous solutions by using peanut hull biochar (400°C and 600°C) was investigated in a batch experiment. The effects of various parameters such as temperature, adsorption time, dose of biochar and initial concentration of Red X-3B were investigated. Then, the response surface methodology (RSM), based on Box–Behnken design, was employed to obtain the optimum adsorption conditions. The results showed that a maximum red X-3b removal of 85.9% was achieved at optimum conditions: a pH of 3.0, an initial red X-3b concentration of 29.99 mg/L, a temperature of 317.63 K and an adsorbent dosage of 9.0 g/L. The results indicate that peanut hull biochar could be used as an effective and economical adsorbent in the removal of Reactive Brilliant Red X-3B from aqueous solutions.

KEYWORDS:
Peanut hull biochar, Adsorption, Reactive Brilliant Red X-3B, Response surface methodology, Endothermic

INTRODUCTION

High surface area biochar generated from agricultural residue, a cost-effective substitute for activated carbon, was recently identified as a supersorbent for neutral pollutants [1-3]. Synthetic dye wastewater cannot be efficiently treated by traditional methods such as photocatalytic degradation, Fenton degradation, electrochemical degradation, biodegradation and persulfate oxidation [4-6]. Adsorption is an economical yet effective technology for removing dyes from water [7]. Activated carbon (AC) is the most widely used adsorbent for dye removal because of its high degree of porosity and high surface area [8-9]. However, the process of AC production requires carbonization of biomass and subsequent activation which makes the use of AC in wastewater treatment rather expensive [10-12]. Biochar (BC), the carbonaceous product of pyrolyzing biomass without activation has been identified as a good and less expensive substitute for AC [13-14]. The dye adsorption performance of BCs produced from agricultural residues has been summarized by many researchers [15-18]. Over the past few years, Elkady, M. F et al. reported that immobilized eggshell with a polymer mixture of alginate and polyvinyl alcohol was used as a biocomposite adsorbent (ESC) for the adsorption of C.I. Remazol Reactive Red 198 in aqueous solution [19]. Jianhua Huang et al. studied the removal of water-soluble Reactive Red MF-3B from aqueous media by sonication-surfactant-modified attapulgite clay in a batch system. These results show that surfactant-modified clay could be a good adsorbent for treating Reactive Red MF-3B-contaminated waters [20].

In this paper, the effectiveness of a modified peanut hull biochar on removal of Reactive Brilliant Red X-3B was evaluated at various solution pH and salt concentrations. The Box-Behnken experimental design and response surface methodology, which includes four factors (temperature, initial reactive brilliant red X-3B concentration, contact time, and adsorbent dosage), was used to optimize the adsorption conditions. Our objective was to test modified peanut hull biochar as a cost effective substitute for AC for dye removal from wastewaters.

MATERIALS AND METHODS

Materials. Modified peanut hull biochar (MPB) was prepared according to Han et al. method Gao et al. [21]. Ten g of dried peanut hulls were soaked briefly in 80 ml of a 2-M solution of $ZnCl_2$. After 0.5 h mixing by magnetic stirring, hydrolysis and Zn^{2+} precipitation were accelerated by 'ageing' at 70 °C for 0.5 h. The pre-treated biomass was then separated from the solution and subjected to pyrolysis in an oxygen-free furnace under 200 ml min^{-1} N_2 atmosphere. The temperatures used were 400 °C and 600 °C at a heating rate of 5 °C min^{-1}. Then, the samples were held at peak temperature for 1 h. After

cooling, biochar particles were rinsed several times with deionized water and then dried at 70 °C for 1 h. The resulting powder was sieved through a 100-mesh stainless steel sieve. Reactive Brilliant Red X-3B dye (2,7-Naphthalenedisulfonic acid, 5-[(4,6-dichloro-1, 3,5-triazin -2-yl) amino] -4-hydroxy -3 -(phenylazo), disodium salt) was purchased from Jiangsu Zhenyang Dyestuff Technology Co., Ltd. All other reagents used for experimental studies were of analytical grade. A stock solution was prepared by dissolving 1.0 g of Red X-3B dye in 1 L deionized water. The experimental solution was then diluted with deionized water to obtain desired concentrations.

Batch adsorption. The 0.1 g and 0.3 g MPB (400 °C and 600 °C) were placed respectively in 100 ml of dye solution in 250-ml capped glass flasks and agitated at 150 rpm. The samples were taken from the flasks at fixed time intervals and analysed for dye concentrations. Every sample was measured in triplicate and averaged. All sorption isotherm experiments were conducted in 50-mL polypropylene conical tubes (FALCON, Japan), each of which contained 40.0 mg MPB (400°C and 600°C) and 50 mL of a solution of dye at a concentration ranging from 50 mg/L to 500 mg/L (pH = 7.0, adjusted by 0.01 M HCl and NaOH solutions without a pH buffer). All tubes were incubated in a shaker incubator at 25 °C and 150 rpm for 24 h to reach sorption equilibrium. Then, 4 mL of the supernatant was sampled and passed through a GF/B glass membrane (0.45 μm, Whatman, Inc., United States) for subsequent analysis of the dye concentration remaining in the liquid phase. The concentrations of dye in filtrates were determined using a UV–Vis spectrometer (UV-2401PC, Shi-madzu Co., Japan) at a k_{max} of 534 nm.

Box-Behnken experimental design. A response surface methodology (RSM) is a statistical and mathematical technique useful for improving and optimizing processes. In this study, the percent Red X-3B removal was statistically modeled and designed by RSM, and the four factors at 3 levels (– 1, 0, + 1) Box-Behnken design as shown in Table 1 was used for the optimization experiments. RSM optimization experiments were repeated three times, each time with a run of 27 sets, because a total of 27 experiments were necessary to estimate the model coefficients of the polynomial equation. The experimental Box-Behnken design, analysis of variance (ANOVA) and 3D response surface were calculated by using the software-Design Expert 7.1.2-(Stat-Ease, Inc., Minneapolis, USA).

Characterization of activated biochar. The specific surface area and pore size distribution of the materials were evaluated from the nitrogen adsorption–desorption isotherm collected at 77 K in an Autosorb-IQ3 porosimeter. The Brunauer–Emmett–Teller (BET) theory were used to determine specific surface area and pore size distribution.

Data statistics and processing. The amount of dye adsorbed, qt (mg/ g), was calculated using the following equation:

$$q_t = (C_0 - C_t) \times V/W \quad (1)$$

where C_0 and C_t (in mg/ L) denote initial concentration and concentration of the dye after time t, respectively; W is the mass of the adsorbent used (g); and V is the volume of the adsorbate solution (L). The percentage removal of the dye was calculated using the following equation:

$$q_t = (C_0 - C_t) \times V/W \quad (2)$$

To determine the rate controlling step in the adsorption of dyes by the biochars, the experimental data of Red X-3B adsorption on 400°C and 600°C biochar for different dye concentrations were examined using pseudo first order kinetic, pseudo second order kinetic and intra-particle diffusion models. The basic rate equations of these models are given as follows

The pseudo first-order model.

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (3)$$

where q_t and q_e are the amount adsorbed at time t and at equilibrium, respectively, and K_1 is the rate constant of the pseudo-first order sorption process. The integrated rate law, after applying the initial conditions of q_t= 0 at t= 0 is:

$$\ln(q_e - q_t) = \ln q_e - k_1$$

$$\text{or } q_t = q_e\,(1 - \exp(-k_1 t))$$

The pseudo second order model.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

where q_t and q_e are the amount of Red X-3B adsorbed onto the adsorbents at equilibrium at time t (mg g^{-1}), K_2 is the sorption rate constant (g mg^{-1} h^{-1}).

TABLE 1
The level of variables chosen for the test

Factors and its codes	levels		
	-1	0	+1
pH(A)/-	3	6.5	10
Initial concentration of red X-3B (B)/(mg/L)	4	17	30
Temperature(C)/°C	298	308	318
Biochar 600 °C dosage（D）/(g/L)	1.0	5	9.0

TABLE 2
Characteristics of the surface properties of peanut hull biochar at 450 °C and 600°C

Surface properties	450°C	600°C
BET surface area(m^2/g)	577.83	801.03
T-Plot External surface area(m^2/g)	60.09	51.22
T-Plot Micropore surface area(m^2/g)	517.75	749.81
Pore volume (mL/g)	0.46	0.48
T-Plot Micropore volume (mL/g)	0.26	0.38
Average pore diameter (4V/A by BET, nm)	3.19	2.39
Surface carboxyls (mmol g^{-1})	0.22	0.12
Surface lactones (mmol g^{-1})	0.14	0.06
Surface phenolic hydroxyls (mmol g^{-1})	0.06	0.02

TABLE 3
The kinetic parameters for the adsorption of red X-3B on peanut hull biochar under activated 450 °C and 600°C

Temp.	Biochar dosage (g/L)	$q_{e,cal}$ (mg/g)	The pseudo first-order model			The pseudo second order model			Intra-particle diffusion model		
			$q_{e,exp}$ (mg/g)	k_1 h^{-1}	R^2	$q_{e,exp}$ (mg/g)	k_2 (g $mg^{-1}h^{-1}$)	R^2	C (mg/g)	k_{id} (g $mg^{-1}h^{-1}$)	R^2
400	1	3.75	3.89	0.203	0.905	4.55	0.056	0.960	0.16	0.839	0.900
	3	1.69	1.60	0.292	0.894	1.90	0.181	0.987	0.22	0.343	0.930
600	1	4.82	4.86	0.178	0.904	5.64	0.043	0.966	0.21	1.042	0.943
	3	1.96	1.87	0.286	0.947	2.22	0.149	0.996	0.22	0.409	0.935

The intraparticle diffusion model. An empirically found functional relationship, common to most adsorption processes, is that the uptake varies almost proportionally with t 1/2, the Weber–Morris plot, rather than with the contact time t

$$q_t = k_{id} t^{1/2} \quad (5)$$

where k_{id} is the intra-particle diffusion rate constant. According to Eq. (5), a plot of q_t versus t1/2 should be a straight line with a slope k_{id} and intercept C when adsorption mechanism follows the intra-particle diffusion process. Values of the intercept give an idea about the thickness of boundary layer, i.e., larger the intercept the greater is the boundary layer effect. The curve fitting was conducted by OriginPro 9.0 software.

RESULTS AND DISCUSSION

Pore and surface properties of peanut hull biochar Table 2 shows the BET specific surface area, external surface area, pore area, average pore size and pore volume of biochars prepared under different active temperatures. There is a clear improvement in BET surface area with a high temperature treatment. Moreover, the large pore volume (0.48 mL/g) and small average pore diameter (2.39 nm) for the sample treated at 600 °C suggest that narrow and deep pores are formed. The Boehm titration indicates that the amount of acidic groups on BC treated at 400 °C was about two times that on BC treated at 600 °C. This difference is likely an indication of the lower degree of carbonization of BC.

Adsorption kinetics and parameter fitting. To determine the rate controlling step in the adsorption of dyes by the biochars, the experimental data of red X-3B adsorption on biochar treated at 400 °C and 600 °C for different dye concentrations were examined using pseudo first order kinetic, pseudo second order kinetic and intra-particle diffusion models. The basic rate equations of these models are given in section 2. Table 3 indicates that the pseudo first order model does not provide a good description of the kinetics of adsorption owing to the very low regression coefficient (R^2) values. Moreover, it is evident that the intraparticle diffusion model is better than pseudo first order kinetic model, yet the R^2 values for a majority of the data are less than 0.95. The parameters k_{id} and C are listed in Table 3. An increase in parameter C is observed with an increase in initial adsorbent dosage, which signifies the increase in thickness and effect of the boundary layer. This finding shows that although intra-particle diffusion may play a role, it may not be the rate-determining step. This is also validated by the fact that adsorption equilibrium is reached within 120 min, while the diffusion mechanism is usually significant only in the initial few minutes [22]. Table 3 shows that the pseudo second order model fits the experimental data very well ($R^2 > 0.95$).

These findings indicate that red X-3B adsorption on the activated biochar follows the chemisorption mechanism. It is clear from Table 3 that the rate constant, k_2, increases with increases in the initial adsorbent dosage, signifying the shift in rate limiting step from kinetics to mass transfer at high initial concentrations of the dye (>100 mg/ L). The best analysis of kinetic data by the pseudo-second order model

suggests that the rate-controlling step is chemisorption involving valence forces through the exchange or sharing of electrons between the adsorbate molecules and the surface functional groups of adsorbent. Pseudo second-order kinetics are shown to be valid for adsorption of a number of dyes on bio sorbents such as biochar, active carbon, corn residues and so on [23-24].

RSM optimization of red X-3B removal. The results of the RSM optimization are depicted in Table 1. Moreover, statistical analysis about the model of removal of red X-3B by peanut hull waste can be seen in Tables 4 and 5. As the results in Table 4 show, the model F-value of 57.97 shows that the model is significant. There was only a 0.01% chance that the F-value was out of design due to noise. Moreover, the values of "Prob > F" less than 0.0001 and "lack of fit" of 0.0527 indicate that the model terms are significant. In this case, B, D, D2 are significant model terms. Further statistical analyses with standard deviation and mean are shown in Table 5. The "Predicted R-Squared" of 0.9830 is in reasonable agreement with the "Adjusted R-Squared" of 0.9661. In addition, "Adequate Precision" measures the signal to noise ratio with a desirable ratio of greater than 4. In this study, the ratio of 26.426 indicates an adequate signal. Therefore, the model could be used to navigate the design space.

Finally, the three-dimensional (3D) response surface plots (Figure 1) show the effects of parameter's interaction when removing red X-3B with peanut hull biochar as the sorbent. The three-dimensional response surface plot in Fig. 1(a) shows the removal mass of red X-3B versus pH with initial biochar dosage. When the contact time is kept constant, the removal of red X-3B from the solution increases along with increase in the pH. In the range of red X-3B concentrations in the experiment, the red X-3B adsorption capacity initially rose from 1.21 to 3.57 mg/g. Thereafter, under the same temperature, it can be seen from Fig. 1(b) that the adsorption capacity increases as the initial pH value is increased from 1 to 9. Overall, for the removal of red X-3B, the effect of contact time is weaker, whereas the effect of the initial red X-3B concentration is larger. Similarly, the 3D surface plot in Fig. 1(c) shows the effect of the interaction of initial red X-3B concentration and temperature on red X-3B removal. When the initial red X-3B concentration was 4 mg/ L with the temperature increasing, the adsorption capacity red X-3B increased from 48.1% to 51.0%. On the whole, the variation trend of initial red X-3B removal with the initial red X-3B concentration and temperature increases and then decreased slightly. Finally, the 3D response surface plot in Fig. 1(d) reveals the influence of the interaction between temperature and pH on red X-3B removal. The plot clearly shows that in the lower temperature range, the adsorption rate increases with an increase in the adsorbent amount, and then, it gradually decreases. Our data reveals interactions between temperature and adsorbent dose.

TABLE 4
Variance analysis of Box-Behnken experimental design

Source	Sum of squares	df	Mean square	F value	*p*-value Prob>F	significance analysis
model	123.8	14	8.84	57.97	< 0.0001	significant
A	0.24	1	0.24	1.60	0.2269	
B	7.73	1	7.33	50.66	< 0.0001	
C	0.10	1	0.10	0.67	0.4257	
D	101.56	1	101.56	665.71	< 0.0001	
AB	0.027	1	0.027	0.18	0.6791	
AC	0.070	1	0.070	0.47	0.5085	
AD	0.065	1	0.065	0.46	0.5244	
BC	0.060	1	0.060	0.39	0.5406	
BD	0.87	1	0.87	5.73	0.0312	
CD	0.034	1	0.034	0.22	0.6431	
A^2	0.44	1	0.44	2.85	0.1133	
B^2	0.38	1	0.38	2.47	0.1385	
C^2	2.811E-005	1	2.811E-005	1.843E-004	0.9664	
D^2	11.16	1	11.16	73.14	< 0.0001	
Residual	2.14	14	0.15			
Lack of Fit	2.00	10	0.20	5.79	0.0527	Not significant
Pure Error	0.14	4	0.035			
Cor Total	125.94	28				

TABLE 5
Statistical analysis for the red X-3B removal by response surface model fitting

parameter	value	parameter	value
Std. Dev.	0.39	R-Squared	0.9830
Mean	3.30	Adj R-Squared	0.9661
C.V. %	11.84	Pred R-Squared	0.9069
PRESS	11.72	Adeq Precision	26.426

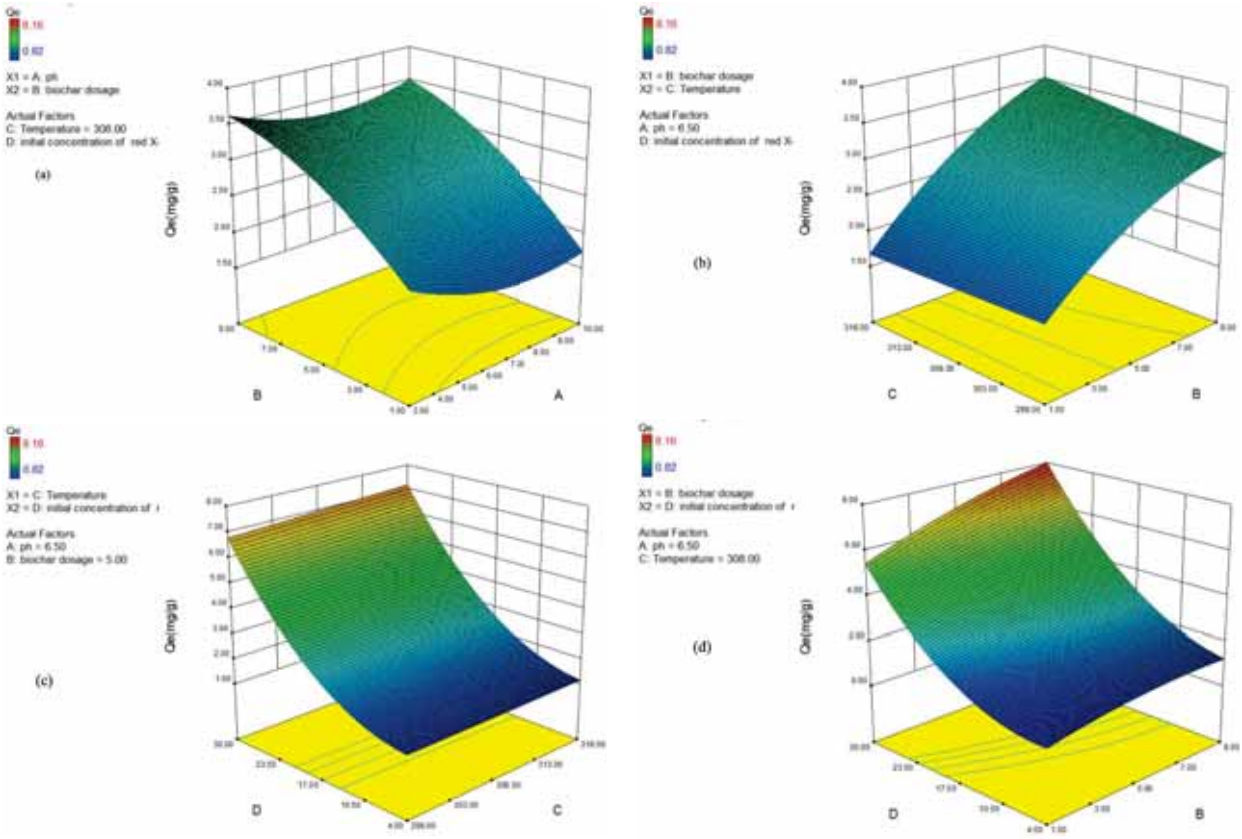


FIGURE 1
The 3D response surface plots showing the effects of interaction. R1: adsorption quantity(mg/g); A: pH; B: Biochar dosage(g/L); C: Temperature; D: initial concentration of red X-3B.(a)Model graph of the interaction A and B variable (b) Model graph of the interaction B and C variable (c) Model graph of the interaction C and D variable (d) Model graph of the interaction B and D variable

Validation of the model. To determine the accuracy of the model and to verify the optimization results, validation experiments were carried out using the optimized conditions, i.e., a pH value of 3.0, an initial red X-3b concentration of 29.99 mg/L, a temperature of 317.63 K and an adsorbent dosage of 9.0 g/L. The experimental red X-3b adsorption capacity under optimal conditions was found to be 85.9%, which was well matched with the predicted values of 8.71 mg/g, with a deviation of only 1.39%. Therefore, the correctness and practicability of the model were further supported by our experimental results.

CONCLUSIONS

In conclusion, the feasibility of red X-3b removal from an aqueous solution with peanut hull biochar as a sorbent is confirmed. In particular, the optimization of red X-3b removal was carried out by employing the response surface methodology with Box-Behnken experimental design. The results show that a maximum red X-3b removal of 85.9% is achieved at optimum conditions: a pH value of 3.0, an initial red X-3b concentration of 29.99 mg/L, a temperature of 317.63 K and an adsorbent dosage of 9.0 g/L. The close agreement of the experimental value with the predicted value confirmed the applicability of the RSM to optimize the above process parameters involved in biosorption using peanut hull biochar. All of the results support the practical application of peanut hull waste in the treatment of dye wastewater.

ACKNOWLEDGEMENTS

This work was supported by the National Key Technology R&D Program (2017YFD0300503) and the Applied Basic Research Programs of Science and Technology Commission Foundation of Heilongjiang Province (No.GC13B111).

REFERENCES

[1] Shen, B., Chen, J., Yue, S. and Li, G. (2015) A comparative study of modified cotton biochar and activated carbon based catalysts in low temperature SCR. Fuel. 156, 47-53.

[2] Bian, R., Joseph, S., Cui, L., Pan, G., Li, L., Liu, X., Marjo, C. (2014) A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. Journal of hazardous materials. 272(4), 121-128.
[3] Qian, K., Kumar, A., Zhang, H., Bellmer, D., Huhnke, R. (2015) Recent advances in utilization of biochar. Renewable and Sustainable Energy Reviews. 42(1), 1055-1064.
[4] Martínez-Huitle, C.A., Brillas, E. (2009) Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods: A general review. Applied Catalysis B Environmental. 87(3–4),105-145.
[5] Khan, M.I., Zafar S., Buzdar, A.R., Azhar, M.F., Hassan, W.A., Aziz, A. (2018) Use of Citrus sinensis leaves as a bioadsorbent for removal of congo red dye from aqueous solution. Fresen. Environ. Bull. 27, 4679-4688.
[6] Matzek, L.W. and Carter, K.E. (2016) Activated persulfate for organic chemical degradation: a review. Chemosphere. 151(2), 178-188.
[7] Khan, M.I., Zafar, S., Azhar, M.F., Buzdar, A.R., Hassan, W., Aziz, A., Khraisheh, M. (2018) Leaves powder of syzgium as an adsorbent for removal of congo red dye from aqueous solution. Fresen. Environ. Bull. 27, 3342-3350.
[8] Yagub, M.T., Sen, T.K., Afroze, S., Ang, H.M. (2014) Dye and its removal from aqueous solution by adsorption: a review. Advances in colloid and interface science. 209(7), 172-184.
[9] Nekouei, F., Nekouei, S., Tyagi, I. and Gupta, V.K. (2015) Kinetic, thermodynamic and isotherm studies for acid blue 129 removal from liquids using copper oxide nanoparticle-modified activated carbon as a novel adsorbent. Journal of Molecular Liquids. 201(2), 124-133.
[10] Cha, J.S., Park, S.H., Jung, S.C., Ryu, C., Jeon, J.K., Shin, M.C., Park, Y.K. (2016) Production and utilization of biochar: a review. Journal of Industrial and Engineering Chemistry. 40(67), 1-15.
[11] Kyziol-Komosinska, J., Rosik-Dulewska, C., Dzieniszewska, A., Pajak, M. (2018) Low – moor peats as biosorbents for removal of anionic dyes from water. Fresen. Environ. Bull. 27, 6-20.
[12] Mohan, D., Sarswat, A., Ok, Y.S. and Pittman Jr., C.U. (2014) Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent–a critical review. Bioresource Technology. 160(5), 191-202.
[13] Huggins, T.M., Haeger, A., Biffinger, J.C. and Ren, Z.J. (2016) Granular biochar compared with activated carbon for wastewater treatment and resource recovery. Water Research. 94(1), 225-232.
[14] Thompson, K.A., Shimabuku, K.K., Kearns, J.P., Knappe, D.R., Summers, R.S. and Cook, S.M. (2016) Environmental comparison of biochar and activated carbon for tertiary wastewater treatment. Environmental science and Technology. 50(20), 11253-11262.
[15] Tan, X.F., Liu, S.B., Liu, Y.G., Gu, Y.L., Zeng, G.M., Hu, X.J. and Jiang, L.H. (2016) Biochar as potential sustainable precursors for activated carbon production: Multiple applications in environmental protection and energy storage. Bioresource Technology. 227(7), 359-372.
[16] Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Ok, Y.S. (2014) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere. 99(3), 19-33.
[17] Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monedero, M.A. and Sonoki, T. (2014) Physical and chemical characterization of biochars derived from different agricultural residues. Biogeosciences. 11(23), 6613-6621.
[18] Erkurt, F.E., Balci, B., Turan, E.S. (2018) Fixed bed study for the adsorptive removal of reactive textile dye with eucalyptus bark/magnetite composite. Fresen. Environ. Bull. 27, 215-224.
[19] Alkan, S., Kul, A.R., Yagiz, A. (2017) Adsorption of textile dyes from aqueous solutions by using kiwifruit (Ordu) peel. Fresen. Environ. Bull. 26, 7045-7053.
[20] Nadaroglu, H., Kalkan, E., Celebi, N. (2014) Equilibrium, kinetic and thermodynamic studies on adsorption of Reactive Black 5 dye by laccase modified-red mud from aqueous solutions. Fresen. Environ. Bull. 23, 70-83.
[21] Safa, Y. and Bhatti, H.N. (2011) Kinetic and thermodynamic modeling for the removal of Direct Red-31 and Direct Orange-26 dyes from aqueous solutions by rice husk. Desalination. 272, 313-322.
[22] Mahmoud, M.E., Nabil, G.M., El-Mallah, N.M., Bassiouny, H.I., Kumar, S. and Abdel-Fattah, T. M. (2016) Kinetics, isotherm, and thermodynamic studies of the adsorption of reactive red 195 A dye from water by modified Switchgrass Biochar adsorbent. Journal of Industrial and Engineering Chemistry. 37(3), 156-167.
[23] Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y. and Yang, Z. (2015) Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere. 125(12), 70-85.

Received: **08.06.2018**
Accepted: **21.10.2018**

CORRESPONDING AUTHOR

Baiwen Jiang
Key Laboratory of Black Soil Protection,
College of Resources and Environment,
Northeast Agriculture University, Harbin,
Heilongjiang 150030 – P.R. China

e-mail: jbwneau@163.com

© by PSP Volume 27 – No. 12B/2018 pages 9799-9811 Fresenius Environmental Bulletin

ESTIMATION OF DROUGHT RISKS USING ARCHIMEDEAN COPULAS IN THE KARASU RIVER, TURKEY

Okan Mert Katipoglu*, Ibrahim Can

Atatürk University, Faculty of Engineering, Department of Civil Engineering, 25100, Erzurum, Turkey

ABSTRACT

In this article, the copula based bivariate drought severity and duration frequence analysis is employed in order to calculate recurrence intervals. Drought duration and severity is acquired by using the runs analysis. for each threshold level (q= 0.5 m^3/s; q =0.3 m^3/s; q= 0.1 m^3/s), Drought severity and duration are presumed to be univariate gamma and exponential distributions, respectively. The Drought return period varies depending on the selected marginal distributions and copula functions. So that the best copula class should be determined. Among probable copulas, Gumbel copula has been found most suitable for drought analysis. The best copula class is determined thereby comparing Bayesian Information Criterion and Akaike Information Criterion of duration and severity of drought. Recurrence intervals for the dry terms lengths of historic and synthetic series are calculated using the selected copula class for each threshold level (q= 0.5, q =0.3, q= 0.1 (m^3/s)). Using these recurrence intervals, drought risks are obtained. Drought frequency analysis provides a detailed view point by considering duration and severity when managing water resource, assessing drought occurrence risks.

KEYWORDS:
Drought, Runs analysis, Copula, Return period, Frequency analysis, Risk management.

INTRODUCTION

Water requirement is an indisputable fact for all living creatures. Drought is a recurring climate phenomenon which is stated as a lack of prolonged precipitation according to the specific reference level. It can last a long time, affect an extensive area, and have destructive effects on agricultural crops, water resources, environment and human being. Because of these effects, drought disaster also damages national and world economy [1, 2]. In order to avoid and manage this disaster, probabilistic theories, stochastic process methods and drought analyses should be applied. Drought frequency analysis plays a key role in the estimation of drought threats, the drought preparation phase, and the drought risk management [3-5].

Modelling dependence structure of hydrologic variables is attracting notice in hydrology and water resources management, where uncertainty and risk analysis has importance in making a decision. An unsuitable model can give rise to an incorrect evaluation of risks [6]. To estimate drought risks, copula-based frequency analysis is used. Copulas are functions that link univariate distribution functions to construct multivariate distribution functions. Copulas can model the dependence structure among random variables, independent of the marginal distributions. They are used for modelling of such random variables stated above and they have been used mostly in various fields of science. Recently, copulas have been used in water resources. For instance, De Michele and Salvadori [7] reviewed modelling of rainfall depth and duration by copulas. De Michele, Salvadori [8] pointed out that flood peak and flood volume may be modelled by copula function. Szolgay, Gaál [9] analysed the bivariate relationship between flood peaks. Shiau and Modarres [10] applied copulas to construct a drought duration-severity-frequency relationship. Lee, Modarres [11] employed copula functions to determine bivariate drought duration and severity frequency analysis. Examining the dependence of two variables, they decided Frank and Gumbel copula functions are more suitable than Clayton function for drought frequency analysis. Fernández and Salas [12] described recurrence intervals and risks related to droughts. Zhang, Xiao [13] analysed return periods to evaluate drought risks. Chung and Salas [14] highlighted the description of return period and estimated the risks of destruction of hydraulic structures.

Hazards of flood and drought can be the most destructive natural disasters that have had a great impact on all living creatures Earth [15]. To overcome this issue, this study suggested copula based bivariate frequency analysis of dry term lengths. In order to dry term lengths, runs analysis is applied. Each drought phenomenon is characterised by drought duration and severity that are fitted first employing probability distributions separately. In order to model the joint distribution of drought duration and severity, copulas are then combined with univariate marginal distributions [16, 17].

Frequency analysis methods are used to estimate return periods. Frequency analysis is defined as a method expressing occurrence probability of expected potential events in future by a series of observation and matching. Frequency analysis outputs should be evaluated in accordance with various acceptances. The verification of frequency analysis results depends on conventional independence acceptances and stability of observations. These acceptances are essential to use distribution fitting techniques. In order to quantify severity and frequency of hydrologic events such as floods and droughts, the return periods should be determined. Risk identification and risk assessment can be examined by using the return periods.

Crisis management is an approach including precautions, before and after droughts. If prevention is insufficient, droughts may give rise to disasters. In order to avoid this situation, occurrence risks of droughts should be timely estimated. Risk management contains precautions before droughts occur. Also, it contains methods which are long-term drought preparation, building drought monitoring centres, risk identification, risk classification, risk assessment, risk mitigation and water utilisation in an effective way [18].

The main objectives of this study are, (1) to determine risk of hydrological droughts by copula based bivariate frequency analysis of dry term lengths of the the Karasu River of Euphrates River valley, in Turkey; (2) to model dependence structures of drought characteristics by using copula-based bivariate frequency analysis; (3) to select the best copula classes by comparing (AIC) and (FPE) of duration and severity of droughts; (4) to calculate return periods of probable droughts of historic and synthetic series may occur in future ; (5) to estimate risks of probable droughts of historical and synthetic series by using streamflow data from the Karasu River; (6) assessment and mitigation of drought risks.

STUDY AREA AND DATA

Description of the study area. The Karasu River is located in the Upper Euphrates River Basin in Eastern Turkey. The location of the Karasu River is shown in Fig. 1. The Karasu River, with a drainage basin area of 2886km^2, is elevated at 1675m above sea level. When the Karasu River is reviewed as climatological, it is snowy between November and March, and rainy in other months. A low-flow regime occurs in winter term because of the effect of this climate and high flows arise in spring term due to melting snow. Upper Euphrates River Basin is occurred by combining the Karasu River and the Murat River. Readers are referred to Ünal et al. (2003) for the climatic features of the basin.

Description of data. For this study, streamflow data recorded at gauging station No. 2154 for the period 1969 to 2009 was obtained from the General Directorate of Electrical Power Resources Survey and Development Administration (shortened as EIE). It contains 480 monthly observations covering 40 years. The streamflow data were available in water years, referring to the period between 1 October of one year and 30 September of the next year. Table 1 indicates basic statistics and information about stations.

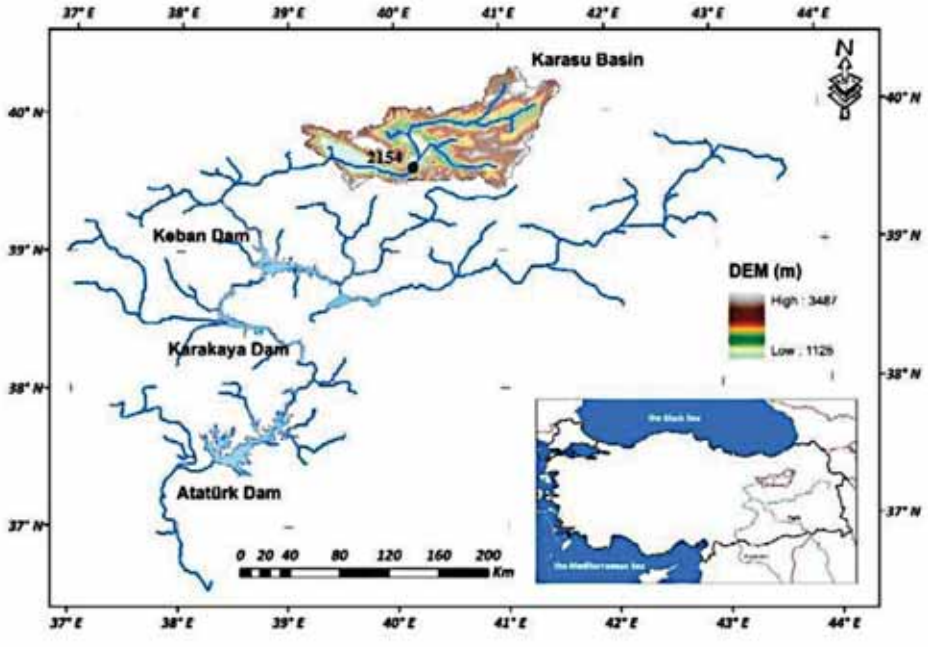


FIGURE 1
Location map of study area.

TABLE 1
Summary of statistics for the monthly flows of the Karasu River of Euphrates River valley

Months	Oct.	Nov.	Dec.	Jan.	Fab.	Mar.	Apr.	May	June	July	Aug.	Sept. Sep.
Meanflow (m^3/ s)	7.069	9.025	7.757	7.130	7.711	17.746	58.908	71.355	26.536	7.902	5.308	5.586
Standard deviation (m^3/ s)	2.574	3.211	1.963	1.686	1.918	10.512	19.948	27.229	15.109	3.905	2.667	3.196
Coefficient of skewness (Cs)	1.647	1.209	1.909	0.964	0.698	1.965	0.334	0.818	0.645	1.253	1.084	1.892
Median of flows (m^3/ s)	6.211	7.992	7.617	6.638	7.328	15.160	56.640	69.300	25.150	7.088	4.480	4.397

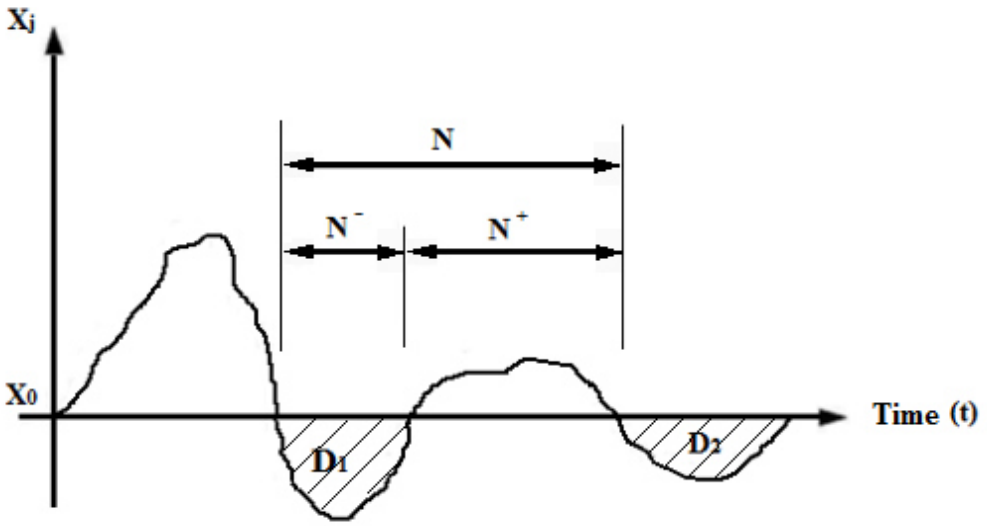


FIGURE 2
Definition of droughts

METHODOLOGY

Threshold selection. Through the flow duration curves, flow rates above the exceedance probabilities of 5%, 25%, 50%, 75%, 95% can be selected as the critical section level. While the flows rates above the exceedance probability of 5% play an important role in studying high flows, those below the exceedance probability of 95% are important for characterizing low flows. Other exceedance probabilities are the percentage values that can be considered important in water resources planning and operation policies. Under the seasonal analyses, the examination of all these percentages have made it possible to obtain more meaningful results in terms of comparison. Determining the dates of the flow values is important in terms of observing the distributions of flows throughout the year [19]. This study used the average annual flows, and threshold levels are obtained by using the flow duration curve.

Runs analysis. Runs Analysis is a statistical method for specific threshold level is used to determine drought and watery terms by evaluating observed historical values. This method for various periods determines run lengths, run sums and run magnitudes by using monthly and annual flows [20].

Yevjevich [20] proposed the runs analysis to determine the drought parameters and to examine their statistical properties (duration, severity, and intensity). The most basic element for deriving these parameters is the truncation or the threshold level; which may be a constant or function of time. A run is defined as a portion of time series of the drought variable X_j, in which all values are either below or above the selected threshold value X_0. Accordingly, it is called either a negative run or a positive run (see Fig. 2).

D_j, is defined as the sum of the j^{th} drought while X_0 is defined as the sum of the severity under the threshold level.

$$D_j = \sum_{i=1}^{m} X_0 - X_j \qquad (1)$$

The drought intensity (Ij) is the average flow deficit per adopted unit of time. N_j^-= Number of drought readings.

$$I_j = \frac{D_j}{N_j^-} \qquad (2)$$

In the runs analysis, the frequency distributions and parameters of random variables such as

run length, run sum and run intensity are examined. The results obtained are used in the planning of water supply, irrigation, hydropower generation, waste dilution, ecology regulation studies in dry periods. The run length is especially used in irrigation projects in which the duration of drought is important and the run sum is used in projects of water supply, hydropower and waste water. The maximum run length at the time of observation (longest dry period) is important in the Run analysis. The longest (or with the greatest total deficit) dry period is when water resources systems will operate on the most demanding conditions. The values corresponding to the various return intervals can be determined by frequency analysis for the maximum run length (run sum, run intensity) within a certain period of time [21].

Maximum dry term lengths of historic series are given in Table 2. Maximum drought durations of historic series for q= 0.5 threshold level occurred in 1975 and lasted for 18 months, as well as a drought in 1982 and severity of drought was 102.72 (m^3 month / s). Maximum drought durations of historic series for q= 0.3 threshold level occurred in 1983 and lasted for 7 months. Maximum drought durations of historic series for q= 0.1 threshold level occurred in 1989 and lasted for 5 months, as well as a drought in 1983 and severity of drought was 20.45 (m^3 month / s).

As a result, the longest droughts may not be the severest ones and the severest droughts may not be the longest ones when comparing drought durations and severities.

Univariate distributions. As Sklar's theorem needs the continuity of marginal distributions, the continuous distribution is required in this study. Two continuous distributions employed largely in drought analysis are exponential and gamma distributions. For instance, the exponential distribution is chosen for fitting drought duration [22]. The gamma distribution has usually been employed to define drought severity [23, 24].

TABLE 2
Maximum dry term lengths of historic series of the monthly flows

For q= 0.5 threshold level			
Year	Time interval	Duration (Month)	Severity (m^3 month / s)
1975	October -March	18*	48.758
1982	August - October	15	102.72*
For q= 0.3 threshold level			
Year	Time interval	Duration (Month)	Severity (m^3 month / s)
1983	November - May	7*	55.833*
For q= 0.1 threshold level			
Year	Time interval	Duration (Month)	Severity (m^3 month / s)
1989	June- October	5*	13.149
1983	April - May	2	20.455*

Note : '*' symbol indicates maximum droughts

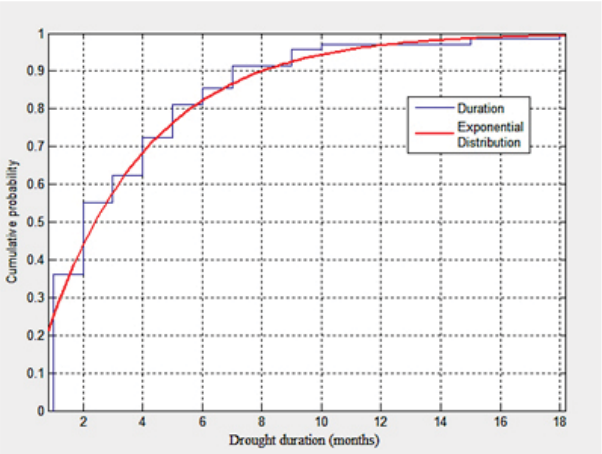


FIGURE 3
Fitting distribution to drought characteristics (a) Applied Exponantial distribution for drought duration and (b) Applied Gamma distribution for drought severity

According to copula theorem, bivariate distribution functions are functions of univariate marginal distributions. Therefore, firstly univariate cumulative distribution functions are fitted to drought severity and duration. Commonly, it is known that drought severity and duration fitted gamma and exponential distributions for hydro-geometric data respectively [24, 25]. Gamma and exponential distributions, for each threshold level (q= 0.5 m^3/s; q =0.3 m^3/s; q= 0.1 m^3/s), are assumed to represent drought severity and duration, respectively [26].

$$f_S(s) = \frac{s^{\alpha-1}}{\beta^{\alpha}\Gamma(\alpha)} e^{\frac{-s}{\beta}}, \quad s > 0 \quad (3)$$

$$f_D(d) = \frac{1}{\lambda} e^{\frac{-d}{\lambda}}, \quad d > 0 \quad (4)$$

where α and β are gamma distribution parameters (α denotes shape of distribution, β denotes scale of distribution) and λ is the parameter of the exponential distribution, which is acquired from the observed data.

TABLE 3
Parameters and %95 confidence intervals for q=0.5

	Parameters	%95 confidence intervals
Duration	λ =3.478	[2.784 - 4.470] *
Severity	α=0.608	[0.459 - 0.805] *
	β =27.113	[17.946 - 40.965] *

These parameters of drought characteristics are estimated by the maximum likelihood method and are illustrated in Table 3. These fitted distributions are illustrated in Fig. 3.a. and Fig. 3.b. which indicate sufficient accordance with the observed drought data. The Kolmogorov-Smirnov (K-S) goodness-of-fit test is employed to determine whether the supposed models can be used to represent the observed data. The results show that the hypothesis of supposed gamma and exponential distributions to model drought severity and duration for each threshold level (q= 0.5 m^3/s ; q =0.3 m^3/s; q= 0.1 m^3/s), respectively, cannot be rejected [10].

Copula concept and properties. The copula functions are useful tools for modeling the dependency structure between random variables and creating the common distribution function. Copula is a function that associates marginal distributions to form a joint distribution [27]. Determining the dependency structure, that is, the joint distribution using copula functions, can be done independently of the choice of the marginal distributions. For this reason, the copula is a realistic and less restrictive tool for modeling dependence [28].

Copulas are used in many areas, primarily modeling financial data. These areas where the copulas are used;

- Investigation of multivariate dependence mechanisms,
- Obtaining new multivariate distributions,
- Stochastic modeling,
- Developing new nonparametric dependency measures,
- Representation of multivariate distribution families.

Copula function. A copula is a multivariate distribution function that is uniform over a range of variably marginal distributions [0, 1]. X_1, X_2, ..., X_n indicate the random variables of F (x_1, x_2, ..., x_n) are the combined cumulative distribution function. The marginal distribution functions of these variables are defined as F_1 (x_1), F_2 (x_2), ..., F_n (x_n). In this case; the capula function can be written as in Eqs. 5.

$$C(F_1(x_1), F_2(x_2), \dots, F_n(x_n)) = F(x_1, x_2, \dots, x_n) \quad (5)$$

Archimedean copula family. Archimedean copula family has a very important place in literature due to some mathematical properties that facilitate the rendering of multidimensional distributions in one dimension [29]. For example, while the Gaussian copula tail dependence modeling and Student t asymmetric tail dependency modeling is insufficient, the Archimedean copula class contains quite useful models for modeling upper and lower tail dependencies.

Archimedean copulas are named as the Archimedean because of its axiomatic characteristic, and in the Ling [30] study, Archimedean copula were first used for these copula functions. Archimedean copula class consists of Gumbel, Ali-Mikhail-Haq, Clayton, Frank and Hougaard copula functions [31-33].

Some families of the Archimedean class are very useful in revealing dependencies in different structures. Archimedean copulas are preferred in practice because these can be associated with Spearman rho and Kendall's tau correlation coefficients in practice. An example of these families is the Gumbel, Clayton and Frank copulas. In the study, these copulas were used because of the properties mentioned above.

Selection the best copula family. The authors should know characterization of the copula, before selection of copula. For example, tail dependance is a significant characterisation. Tail dependance is defined as dependance relation of extreme-values in case of bivariate existence. This dependance impacts on the behaviour of extreme-values of joint distribution. Univariate and bivariate frequency analyses mainly purpose to analyse frequencies of extreme events. We can put forward that upper tail of copula is dependent, when variables of marginal distributions in upper tail are dependent with one another. On the contrary, we can put forward that upper tail of copula is independent, when variables

of marginal distributions in upper tail aren't dependent with one another [34].

The Akaike Information Criterion (AIC) and The Bayesian Information Criterion [35] were used for determining the appropriate probability distribution [36].

Bivariate return periods. The return period can be denoted as the average passed time or average interarrival time between critical cases [21, 37, 38]. Drought is bivariable events and is expressed by the duration and severity of drought, so while determining the frequency of drought, the joint and conditional characteristics of the period have a great deal of involvement in the account. The return period is formulated for two cases of drought risk corresponding to drought events with [D ≥ d and S ≥ s] and drought events with [D ≥ d or S ≥ s]. The copula-based joint return period of drought can be written as follows [24]:

$$T_{and} = \frac{E(L)}{P(D \geq d \text{ and } S \geq s)} = \frac{E(L)}{1 - F_D(d) - F_S(s) + F_{DS}(d,s)} = \frac{E(L)}{1 - F_D(d) - F_S(s) + C(F_D(d), F_S(s))} \quad (6)$$

$$T_{or} = \frac{E(L)}{P(D \geq d \text{ or } S \geq s)} = \frac{E(L)}{1 - F_{DS}(d,s)} = \frac{E(L)}{1 - C(F_D(d), F_S(s))} \quad (7)$$

where T_{and} indicates the joint return period for the probability of exceeding both of drought duration and severity [D ≥ d and S ≥ s], T_{or} indicates the joint return period for the probability of exceeding either one of the drought duration and severity [D ≥ d or S ≥ s] and E(L) is the expected drought interarrival time. The interarrival time is defined as the time between consecutive arrivals [11].

The estimation of drought risks. Drought is a great disaster that causes many environmental and social damages. The consequences such as hunger, collective deaths, serious health problems, increased unemployment, reduced income in agriculture and desertification, which will cause drought in social and economic terms, show that drought is a very damaging natural disaster. Efforts to determine the risk of drought are needed to prevent drought damages. Temporarily arid climate conditions, even though some years may not appear in our region, adverse climate changes in the face of drought in the world and our country, and increased water demands, require risk management on the ground to take into account the drought risk [39].

The risk in a specific field and time interval is potential losses such as human life and financial damage which are occurrences by hazardous situations as well as it may express as hazard and damage occurrence in consequence of mathematical computings. Risks formula, related to return period, is presented below.

$$R = 1 - (1 - \frac{1}{T})^N \quad (8)$$

R: Risk
T: Return period, yearly
N: Year

A number of management and planning processes are needed to solve drought problems and prevent the effects of drought. All of these processes are considered within the concept of drought planning. Risk management concept in drought management and planning is essential. Information obtained using risk assessments contribute to risk management reveal naturel hazards occurrence and guide for precaution [40].

RESULTS

Kendall's τ and parameter estimation. Kendall's τ is applied in order to measure relationship between variables and detect the dependence structure [41].

The simplest (non-parametric) method, For Archimedean copulas, to estimate the parameter θ is by the way a concordance measurement – Kendall's τ – which is a rank correlation coefficient, expressed to measure the orderings of two measured quantities. According to the research by [27], the association between parameter θ and Kendall's τ contains the three Archimedean classes. Especially, a closed-form definition can be obtained from Clayton and Gumbel families (look at [42]).

TABLE 4
Mann Kendall τ values and θ parameters of drought duration and severity for Archimedean copula classes

		θ parameters of copula classes		
Threshold levels	τ	Clayton	Gumbel	Frank
0.5	0.902	18.472	10.236	39.226
0.3	0.868	13.196	7.598	28.647
0.1	0.677	4.189	3.094	10.425

Mann Kendall τ values and θ parameters of each of Archimedean copula classes of drought duration and severity are calculated given in table 4. Drought characteristics in each threshold level indicate a vital dependance according to Mann Kendall τ values.

Goodness-of-fit tests for copulas. AIC and BIC reveal goodness-of-fit measures between fitted copula and empirical joint distribution. [6, 43]. Empirical copula defines empirical distributions of transformed data and employs to determine the best accurate value of BIC.

Monthly flows of historic series, for each threshold level, are subjected to runs analysis. Drought severity and duration are obtained by this

analysis. AIC and BIC values of droughts, for all Archimedean Copula classes, are determined and represented in table 5.

TABLE 5
AIC and BIC values for q=0.5, 0.3, 0.1 threshold level

q=0.5 level	threshold	**Gumbel**	Frank	Clayton
AIC		**-27.897**	-26.642	-17.402
BIC		**-1552.21**	-193.869	-134.484
q=0.3 level	threshold	Gumbel	**Frank**	Clayton
AIC		-7.884	**-14.1903**	-6.726
BIC		-103.99	**-129.571**	-98.997
q=0.1 level	threshold	**Gumbel**	Frank	Clayton
AIC		**-2.998**	-2.202	0.682
BIC		**-135.63**	-41.766	-35.037

Drought severity and duration values of historic series, for q= 0.5 threshold level (median), and produced 10 000 random drought pairs are represented in Fig. 4. The most appropriate copula for historic series is shown Gumbel copula with regards to matching the dependence structure of drought variables. The random drought pairs are produced by Gumbel copula (exhibited using grey '*' marker) are correspond with the drought characteristics of historic series (exhibited using blue 'o' marker). Furthermore, the upper tail of random drought pairs in Clayton and Frank copula does not perform similar trends with the drought characteristics of historic series. Table 5 also shows most appropriate, had minimum AIC and BIC value, by comparing AIC and BIC values of three potential copula. The most appropriate copula for q= 0.3 and q= 0.1 threshold is shown respectively Frank and Gumbel copula in Fig 5. and Fig. 6.

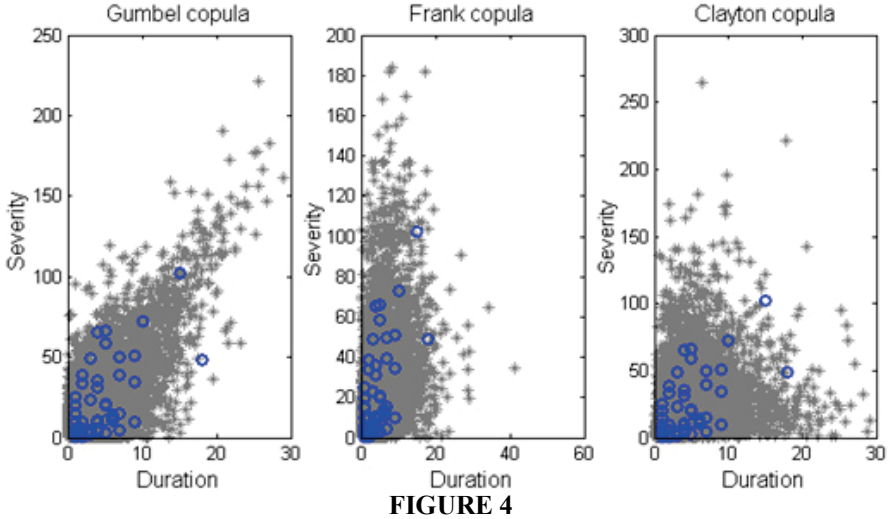


FIGURE 4
Comparison of concordance between drought characteristics of historic series and 10 000 simulated random drought pairs according to tree potential copulas for q=0.5 threshold level (median)

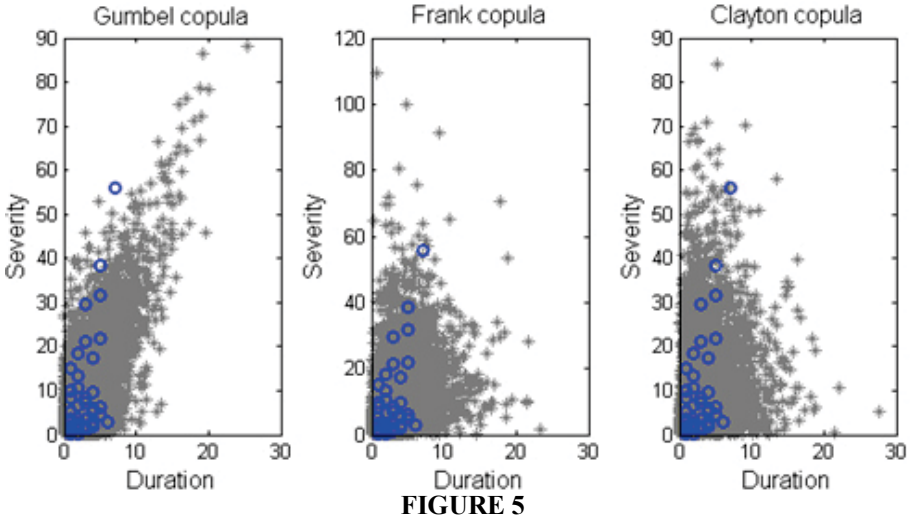


FIGURE 5
Comparison of concordance between drought characteristics of historic series and 10 000 simulated random drought pairs according to tree potential copulas for q=0.3 threshold level

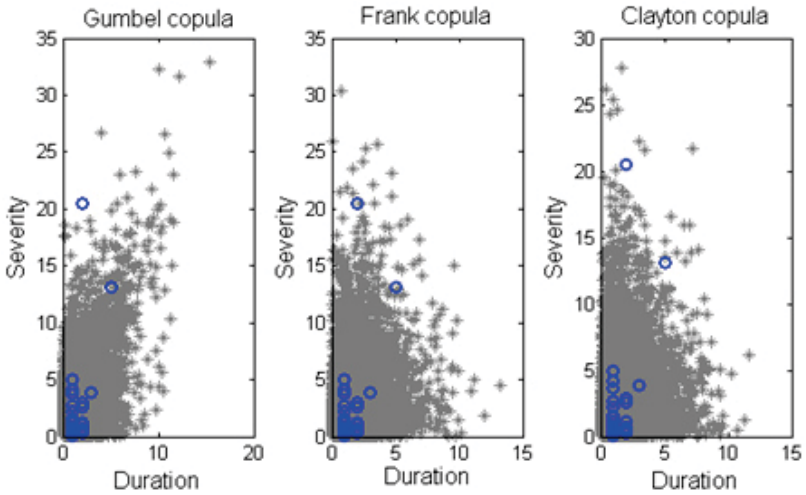


FIGURE 6

Comparison of concordance between drought characteristics of historic series and 10 000 simulated random drought pairs according to tree potential copulas for q=0.1 threshold level

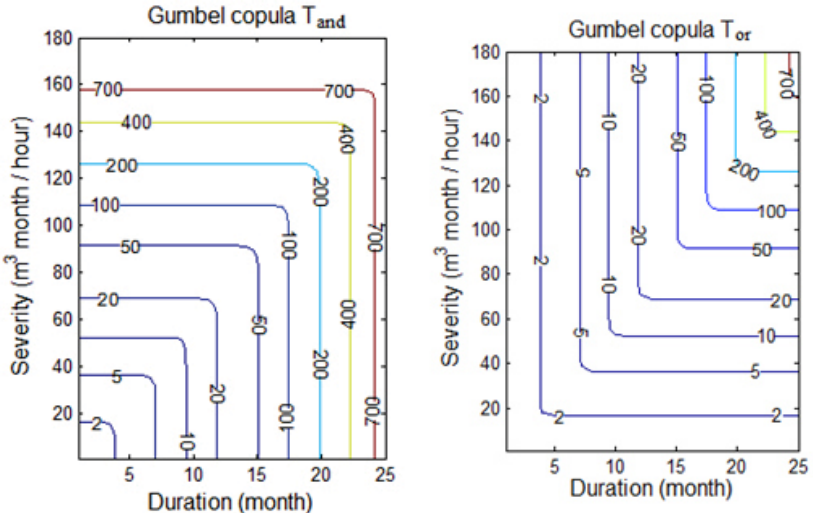


FIGURE 7

Bivariate return period of drought characteristics of historical series for q= 0.5 threshold level are estimated by way of Gumbel copula

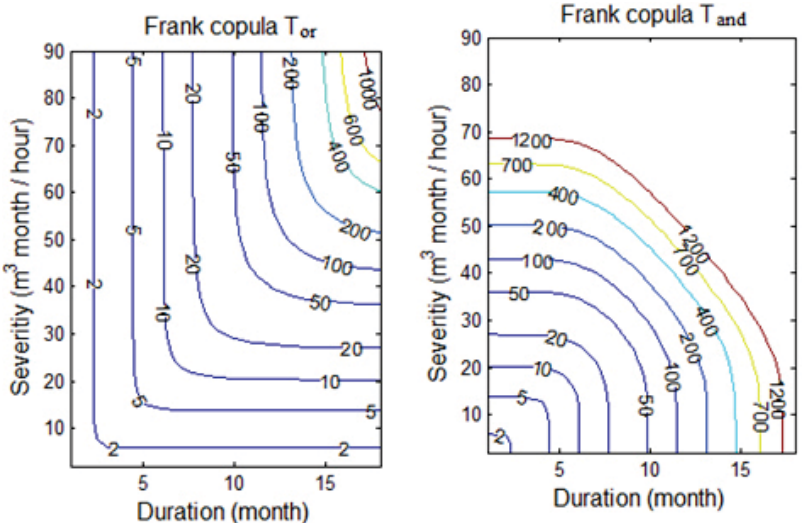


FIGURE 8

Bivariate return period of drought characteristics of historical series for q= 0.3 threshold level are estimated by way of Frank copula

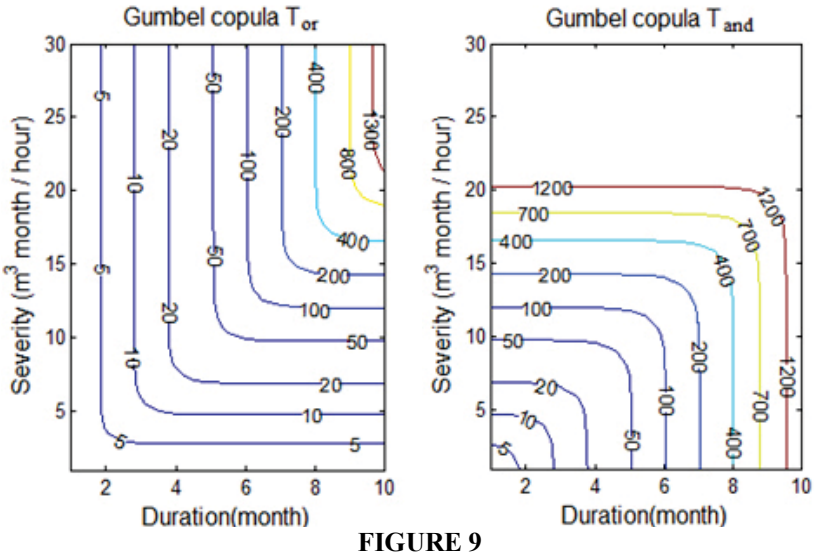


FIGURE 9
Bivariate return period of drought characteristics of historical series for q= 0.1 threshold level are estimated by way of Gumbel copula

TABLE 6
Calculated E(L) values of the monthly flows for each of threshold levels

Threshold levels	q=0.5	q=0.3	q=0.1
E(L) values (Year)	0.660	0.738	1.349

TABLE 7
The return periods of historical and synthetic series of maximum and average droughts for q= 0.5 threshold level

	q= 0.5 threshold level			
	Maximum duration (Month)	Maximum severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	18*	102.72*	116.97	78.44
Synthetic series	27*	195.8*	3030.6	1551.4
	Average duration (Month)	Average severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	3.478	16.479	2.026	1.761
Synthetic series	3.582	18.916	2.255	1.836
	q= 0.3 threshold level			
	Maximum duration (Month)	Maximum severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	7*	55.83*	453.03	14.47
Synthetic series	18*	91.49*	791 000	1385.3
	Average duration (Month)	Average severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	2.344	6.748	2.289	1.947
Synthetic series	2.88	10.355	3.444	2.488
	q= 0.1 threshold level			
	Maximum duration (Month)	Maximum severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	5*	20.455*	1272.3	46.573
Synthetic series	8*	39.376*	328 090	389.955
	Average duration (Month)	Average severity (m^3 month / s)	T_{and} (Year)	T_{or} (Year)
Historic series	1.412	2.203	4.768	3.217
Synthetic series	1.92	4.285	9.181	5.007

Note : '*' symbol indicates maximum droughts

Return period of drought duration and severity. Concept of return period defines as number of trials occurred between a certain critical event and firstly encountered extreme event [44, 45]. Return period can be applied when measuring the risk of failure R of a hydraulic structure designed to withstand a given T-year drought phenomenon during a design life of L years. Such risk of failure is mostly mentioned as the probability that one or more droughts larger than the T-year drought take place during the life of the project [12].

Table 6 presents, for various threshold levels, interarrival time between consecutive droughts of monthly flows. Fig 7, 8, 9 explains return period using Archimedean copulas for each threshold level. The contour lines of T_{or} return period which occurs in case either certain drought severity or duration are exceeded and T_{or} return period which occurs in case both certain drought severity and duration are simultaneously exceeded. The iso-line of joint return periods, calculated using eqs. 5, are formed by overlapping of same return periods of various durations and severities.

Table 7 presents the return periods of historical and synthetic series of maximum and average droughts for all threshold levels by obtaining copula-based bivariate frequency analysis. T_{or} bivariate return period can be calculated in case of exceeding of maximum drought duration or of historical series and T_{and} bivariate return period can be calculated in case of exceeding of maximum drought duration and severity of historical series. The bivariate return periods of historical and synthetic series can similarly be calculated. These calculated return periods of drought duration and severity may be beneficial to plan and manage water resources systems in terms of risk assessment [6]. Maximum values and occurrence risks of synthetic series and historic series droughts are calculated using eqs. 8 and indicated in Appendix.

CONCLUSIONS

In this study, the droughts are characterized by duration and severity defined by the threshold level method. Best fitting marginal distributions are selected for drought duration and severity. The joint Cumulative Distribution Function of the two variables is established using the best fitting copula. The Bayesian information criterion and Akaike information criterion is used to select the best copula from among probable copulas to compute the joint probabilities. Recurrence intervals are calculated using the selected copula classes. The drought risk prediction is based on joint probabilities and return periods, which give significant knowledge for water resources appraisal and the primary conclusions are as follows:

(1) The most appropriate copula for historic series is shown Gumbel copula in terms of matching the dependence structure of drought variables. The random drought pairs are produced by Gumbel copula are correspond with the drought characteristics of historic series. Furthermore, the upper tail of random drought pairs in Clayton and Frank copula does not perform similar trends with the drought characteristics of historic series. However, Gumbel copula is the most appropriate copula, had minimum AIC and BIC value, by comparing AIC and BIC values of three potential copula.

(2) The return periods of synthetic series of maximum droughts by obtaining copula-based bivariate frequency analysis. T_{or} bivariate return period (78.44 years) can be calculated in case of exceeding of maximum drought duration or severity of synthetic series and T_{and} bivariate return period (116.97 years) can be calculated in case of exceeding of maximum drought duration and severity of synthetic series. The risks corresponding to these bivariate return period for 50 years are R_{and} (%34.90) and R_{or} (%47.35).

(3) The drought frequency analysis method proposed in this study is the teaching tool for drought risk management for practical purposes.

(4) Determination of the risk of drought is necessary for reducing the negative effects of drought, tending to alternative water resources and management of water resources.

The results of this study will contribute to predictions of probable droughts, drought disaster mitigation, water resources management and operations, strategy planning, risk management and further studies aiming to decrease the effect of droughts on community and environment. Estimation of droughts will also enable determining the risks and taking precaution for possible drought disasters. So that damage can be minimized. Otherwise, drought might turn into crisis.

ACKNOWLEDGEMENTS

The authors would like to thank the anonymous referees for their detailed reviews of the first submission which have led to a significantly improved paper. In this research, MATLAB 2014 was used for data analysis, downloaded from the official webpage of Ataturk University. This article has been obtained from the postgraduate thesis advocated on 28.08.2015.

APPENDIX. DROUGHT RISKS

TABLE A1

Maximum values and occurrence risks of historic series droughts for all threshold levels

Threshold level	Duration	Severity	Year	T_{and}	T_{or}	R_{and} (%)	R_{or} (%)
q=0.5	18*	102.72*	5	116.97	78.44	4.20	6.21
	18*	102.72*	10	116.97	78.44	8.23	12.04
	18*	102.72*	20	116.97	78.44	15.78	22.63
	18*	102.72*	50	116.97	78.44	34.90	47.35
q=0.3	7*	55.83*	5	453.03	14.47	1.10	30.10
	7*	55.83*	10	453.03	14.47	2.17	51.14
	7*	55.83*	20	453.03	14.47	4.32	76.12
	7*	55.83*	50	453.03	14.47	10.46	97.21
q=0.1	5*	20.45*	5	1272.3	46.57	0.39	10.28
	5*	20.45*	10	1272.3	46.57	0.78	19.51
	5*	20.45*	20	1272.3	46.57	1.56	35.22
	5*	20.45*	50	1272.3	46.57	3.86	66.22

Note: * indicates maximum drought values

TABLE A2

Maximum values and occurrence risks of synthetic series droughts for all threshold levels

Threshold level	Duration	Severity	Year	T_{and}	T_{or}	R_{and} (%)	R_{or} (%)
q=0.5	27*	195.83*	5	3030.6	1551.4	0.16	0.32
	27*	195.83*	10	3030.6	1551.4	0.33	0.64
	27*	195.83*	20	3030.6	1551.4	0.66	1.28
	27*	195.83*	50	3030.6	1551.4	1.64	3.17
q=0.3	18*	91.49*	5	791000	1385.3	0.001	0.36
	18*	91.49*	10	791000	1385.3	0.001	0.72
	18*	91.49*	20	791000	1385.3	0.003	1.43
	18*	91.49*	50	791000	1385.3	0.006	3.55
q=0.1	8*	39.37*	5	328090	389.95	0.002	1.28
	8*	39.37*	10	328090	389.95	0.003	2.54
	8*	39.37*	20	328090	389.95	0.006	5.01
	8*	39.37*	50	328090	389.95	0.015	12.05

Note: * indicates maximum drought values

REFERENCES

[1] Wilhite, D.A. (2000) Drought as a natural hazard: concepts and definitions. Drought Mitigation Center Faculty Publications. 69.

[2] Yoo, J., Kim, D., Kim, H., Kim, T-W. (2014) Application of copula functions to construct confidence intervals of bivariate drought frequency curve. Journal of Hydro-environ-ment Research. 11, 113-122.

[3] Reddy, S.S., Shruthi, K., Reddy, V.S., Raghu, G., Suryanarayana, P., Giridharan, N.V., Reddy, G.B. (2014) Altered ubiquitin-protea-some system leads to neuronal cell death in a spontaneous obese rat model. Biochim Biophys Acta. 1840, 2924-34.

[4] Sen, Z. (1976) Wet and dry periods of annual flow series. Journal of the Hydraulics Division. 10, 1503-14.

[5] Zhang, Q, Chen, Y.D., Chen, X., Li, J. (2011) Copula-based analysis of hydrological extremes and implications of hydrological behaviors in the Pearl River basin, China. J Hydrol Eng. 16, 598-607.

[6] Ganguli, P., Reddy, M.J. (2012) Risk Assessment of Droughts in Gujarat Using Bivariate Copulas. Water Resour Manag. 26, 3301-27.

[7] De Michele, C., Salvadori, G.A (2003) Generalized Pareto intensity-duration model of storm rainfall exploiting 2-Copulas. J Geophys Res-Atmos. 108.

[8] De Michele, C., Salvadori, G., Canossi, M., Petaccia, A., Rosso, R. (2005) Bivariate statistical approach to check adequacy of dam spillway. J Hydrol Eng. 10, 50-7.

[9] Szolgay, J., Gaál, L., Bacigál, T., Kohnová, S., Hlavčová, K., Výleta, R., Parajka, j., Blöschl, G. (2016) A regional comparative analysis of empirical and theoretical flood peak-volume relationships. Journal of Hydrology and Hydromechanics. 64, 367-81.

[10] Shiau, J.T., Modarres, R. (2009) Copula-based drought severity-duration-frequency analysis in Iran. Meteorol App. 16, 481-9.

[11] Lee, T., Modarres, R., Ouarda, T.B.M.J. (2013) Data-based analysis of bivariate copula tail dependence for drought duration and severity. Hydrol Process. 27, 1454-63.

[12] Fernández, B., Salas, J.D. (1999) Return period and risk of hydrologic events. I: mathematical formulation. J Hydrol Eng. 4, 297-307.

[13] Zhang, Q., Xiao, M., Singh, V.P., Chen, X. (2012) Copula-based risk evaluation of hydrological droughts in the East River basin, China. Stoch Env Res Risk. A. 27, 1397-406.
[14] Chung, C.-H., Salas, J.D. (2000) Drought occurrence probabilities and risks of dependent hydrologic processes. J Hydrol Eng. 5, 259-68.
[15] Zhou, Y., Ma, Z., Wang, L. (2002) Chaotic dynamics of the flood series in the Huaihe River Basin for the last 500 years. J Hydrol. 258, 100-10.
[16] Kim, T.-W., Valdés, J.B., Yoo, C. (2006) Nonparametric approach for bivariate drought characterization using Palmer drought index. J Hydrol Eng. 11, 134-43.
[17] Kwak, J.W., Kim, Y.S., Lee, J.S., Kim, H.S. (2012) Analysis of Drought Characteristics Using Copula Theory. World Environmental and Water Resources Congress. 1762-71.
[18] AghaKouchak, A. (2015) A multivariate approach for persistence-based drought prediction: Application to the 2010–2011 East Africa drought. J Hydrol. 526, 127-35.
[19] Dracup, J.A., Lee, K.S., Paulson, E.G. (1980) On the definition of droughts. Water resources research. 16, 297-302.
[20] Yevjevich, V.M. (1967) An objective approach to definitions and investigations of continental hydrologic droughts. Hydrology papers (Colorado State University). No 23.
[21] Shiau, J.-T., Shen, H.W. (2001) Recurrence analysis of hydrologic droughts of differing severity. Journal of Water Resources Planning and Management. 127, 30-40.
[22] Zelenhasić, E., Salvai, A. (1987) A method of streamflow drought analysis. Water Resour Res. 23, 156-68.
[23] Chen, L., Singh, V.P., Guo, S.L., Mishra, A.K, Guo, J. (2013) Drought Analysis Using Copulas. J Hydrol Eng. 18, 797-808.
[24] Shiau, J.T. (2006) Fitting drought duration and severity with two-dimensional copulas. Water Resour Manag. 20, 795-815.
[25] Wong, G., Lambert, M.F., Metcalfe, A.V. (2008) Trivariate copulas for characterisation of droughts. Anziam Journal. 49, 306-23.
[26] Masud, M.B., Khaliq, M.N., Wheater, H.S. (2015) Analysis of meteorological droughts for the Saskatchewan River Basin using univariate and bivariate approaches. J Hydrol. 522, 452-66.
[27] Nelsen, R. (2006) An introduction to copulas, ser. Lecture Notes in Statistics New York: Springer.
[28] Sklar, M. (1959) Fonctions de repartition an dimensions et leurs marges. Publ Inst Statist Univ Paris. 8, 229-31.
[29] Genest, C., MacKay, J. (1986) The joy of copulas: Bivariate distributions with uniform marginals. The American Statistician. 40, 280-283.
[30] Ling C-H. (1965) Representation of associative functions. Pub Math. Debrecen. 12, 189-212.
[31] Gumbel, E.J. (1960) Bivariate exponential distributions. Journal of the American Statistical Association. 55, 698-707.
[32] Clayton, D.G. (1978) A model for association in bivariate life tables and its application in epidemiological studies of familial tendency in chronic disease incidence. Biometrika. 65, 141-51.
[33] Frank, M.J. (1979) On the simultaneous associativity of F (x, y) and x+y− F (x, y). Aequationes mathematicae. 19, 194-226.
[34] Zhang, Q., Li, J.F., Singh, V.P. (2012) Application of Archimedean copulas in the analysis of the precipitation extremes: effects of precipitation changes. Theor Appl Climatol. 107, 255-64.
[35] Ben Aissia, M.A., Chebana, F., Ouarda, T.B.M.J., Roy, L., Desrochers, G., Chartier, I., Robichaud, É. (2012) Multivariate analysis of flood characteristics in a climate change context of the watershed of the Baskatong reservoir, Province of Quebec, Canada. Hydrol Proces. 26, 130-42.
[36] Zhang, L., Singh, V.P. (2007) Bivariate rainfall frequency distributions using Archimedean copulas. Journal of Hydrology. 332, 93-109.
[37] Lloyd, E. (1970) Return periods in the presence of persistence. Journal of Hydrology. 10, 291-8.
[38] Loaiciga, H.A., Mariño, M.A. (1991) Recurrence interval of geophysical events. Journal of Water Resources Planning and Management. 117, 367-82.
[39] Kadıoğlu, M. (2001) Kuraklık Kıranı. Cumhuriyet Bilim Teknik Dergisi. 17-24.
[40] Swaney, J.A. (1996) Comparative risk analysis: Limitations and opportunities. Journal of Economic Issues. 30, 463-73.
[41] Mirakbari, M., Ganji, A., Fallah, S. (2010) Regional bivariate frequency analysis of meteorological droughts. J Hydrol Eng. 15, 985-1000.
[42] Kao, S.C., Govindaraju, R.S. (2007) A bivariate frequency analysis of extreme rainfall with implications for design. Journal of Geophysical Research: Atmospheres. 112.
[43] Karmakar, S., Simonovic, S.P. (2007) Flood frequency analysis using copula with mixed marginal distributions: Department of Civil and Environmental Engineering, The University of Western Ontario.
[44] Benjamin, J., Cornell, C. (1970) Probability, statistics and decision theory for civil engineers. McGraw-Hill, New York.

[45] Serinaldi, F. (2009) Copula-based mixed models for bivariate rainfall data: an empirical study in regression perspective. Stoch Env Res Risk A. 23, 677-93.

Received: **10.06.2018**
Accepted: **12.10.2018**

CORRESPONDING AUTHOR

Okan Mert Katipoglu
Atatürk University,
Faculty of Engineering,
Department of Civil Engineering,
25100, Erzurum – Turkey

e-mail: okan.katipoglu@atauni.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9812-9815 Fresenius Environmental Bulletin

EVALUATION OF HEAVY METAL CONCENTRATIONS IN THE XYLEM SAP OF TURKISH PINE (*PINUS BRUTIA* TEN.) AND HONEYDEW OF *MARCHALINA HELLENICA*, GENNADIUS (HEMIPTERA: MARCHALINIDAE), COLLECTED IN WESTERN ANATOLIA, TURKEY

Rahsan Ivgin Tunca[1,*], Okan Ozgul[1], Murat Yabanli[2], Idris Sener[2]

[1]Mugla Sitki Kocman University, Department of Plant and Animal Breeding, Ula Ali Kocman Vocational School, Mugla, Turkey
[2]Mugla Sitki Kocman University, Department of Aquatic Science, Faculty of Fisheries, Mugla, Turkey

ABSTRACT

The aim of this study was to investigate the Cr, Mn, Cu, Zn, Cd, and Pb concentrations in samples of the sap from the Turkish pine, *Pinus brutia* Ten., and the honeydew from the insect, *Marchalina hellenica* (Gennadius), both of which are components of the food chain in the production of pine honey, that were collected at different locations in western Anatolia, Muğla province, Turkey. Heavy metal concentrations in the samples were determined using microwave-induced wet combustion and mineralization, followed by inductively coupled plasma mass spectroscopy. The results showed that the Cr, Mn, Cu, Zn, Cd, and Pb concentrations were low in both the sap and honeydew samples. This study represents the first reference on heavy metal concentrations in the sap from *P. brutia* and the honeydew from *M. hellenica* within the region.

KEYWORDS:
Honeydew, Turkish pine xylem sap, *Marchalina hellenica, Pinus brutia* Ten., Heavy metals.

INTRODUCTION

The effects of human activities, such as agriculture, urbanization, industrialization, and transportation, as well as the effects of climate change on vegetation can rapidly manifest. Heavy metals, which can be found in different concentrations in soil, water resources, and air, cause pollution when they exceed certain concentrations [1-4]. Because of high accumulations of heavy metals, there is a possibility of contamination in the soil-to-plant continuum [5]; therefore, it is important to regularly monitor and determine how and to what extent plants and the animals in contact with these plants are affected by these factors. Fifty-four percent of the forested areas in Turkey comprise coniferous forests and, within this proportion, Turkish pine (*Pinus brutia* Ten.) forests are the most widely distributed with 5.9 Mha or 27% of the total forested areas [6]. In Turkey, the insect *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae), which feeds on pine sap, is distributed in the south Marmara, Aegean, and west Mediterranean regions, which are affected by the Mediterranean climate [7]. Seventy-five percent of the pine honey production areas in Turkey are in Muğla Province in southwestern Turkey, and only a very low percentage is in the Marmara region [6]. The data used in this study were obtained from Muğla Province, which represents a total forested area of 830854.7 ha, 66.41% (538494 ha) of which is Turkish pine forests. Of the Turkish pine forests, 66305.1 ha (12.31%) host *M. hellenica*, and this is where the pine honey is produced [8]. Ninety percent of all pine honey consumed worldwide is produced in Turkey, with the remaining 10% being produced in Greece. To a great extent, the honey exported from Turkey is produced from the pines growing in Muğla Province, but thousands of beekeepers who settled in various regions of Turkey also produce pine honey, which emphasizes the economic importance of beekeeping in Muğla. Thus, the region is of particular importance in terms of both Turkish pine and the pest insect that produces the honeydew; however, the potential tourism industry in the area is intense. This potential industry could threatened the continued existence of all forested areas, and the impacts from the contaminants that result can affect the environment. Plant sap actively transports elements from soils contaminated with heavy metals. *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae) is a common scale insect species in Turkish pine forests, mainly in the Aegean region, that feeds on pine sap. The insect has been recorded in Turkey, Greece, Italy, and islands in the eastern Mediterranean [9]. The honeydew produced by *M. hellenica* is collected by honeybees to produce pine honey [10]. When *M. hellenica* feeds on the xylem sap of the Turkish pine, there is a high probability that some elements, such as Cr, Mn, Cu, Zn, Cd, and Pb that are present

in the sap could be transported into the insect's secretions. Because the honeybee makes its honey using the *M. hellenica* secretions, there is also a potential risk that these elements could reach the final consumer. In general, several studies have focused on honey production but not enough information is available on the other stages of the food chain. The aim of this study was to fill this gap in the literature by focusing on determining Cr, Mn, Cu, Zn, Cd, and Pb concentrations in different components of the pine honey production process, from the xylem sap to the last step of production using the honeydew.

MATERIALS AND METHODS

The materials examined in this study comprise the xylem sap from the Turkish pine and the honeydew secreted by the insect that feeds on it. The samples were collected from different stations near the road and in the Turkish pine forests that host the insect throughout Muğla province. After collecting the sap and honeydew into sterile eppendorf tubes, the samples were taken to the laboratory and kept at 4°C until analyses. The microwave wet digestion technique was used to dissolve the honeydew and xylem sap samples. A 200-mg sample was placed into a polytetraflouroethylene (PTFE) microwave container, after which 10 mL nitric acid (65%, suprapur, Merck, Germany) was added to the container, and the container was placed into the microwave (200°C, 15 min ramp time, 20 min waiting time, 20 min cooling time). The volume of the dissolved sample was increased to 50 mL using ultrapure water. These liquid samples were then placed into the Agilent 7700x Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent Technologies, Inc., Santa Clara, CA, USA) under the conditions given in Table 1. To draw a calibration curve, 1000 µL AccuTrace Mes-21-1 multi-element calibration solution was mixed for the Cr, Mn, Cu, Zn, Cd, and Pb elements in the tubes. Calibration solutions were prepared from the mixed stock solution at five different concentrations (5, 10, 50, 100, and 200 µg L^{-1}), were inserted into the ICP-MS instrument, and the calibration curve was drawn. The results of the analyses are given as mg kg^{-1} wet weight. To determine the correlations between the metal concentrations in honeydew and Turkish pine sap, the Mann-Whitney U Test was conducted using SPSS v 20 (IBM Corp., Armonk, NY, USA). Statistical significance was determined through the 0.05 alpha level. $P < 0.05$ was determined to be a statistically significant difference between the groups.

TABLE 1
ICP-MS instrument conditions.

Radiofrequency power	1550 W
RF matching	2.1 V
Sample depth	8 mm
Carrier gas	0.95 l/min
Dilution gas	0 l/min
S/C temperature	2°C
Nebulizer type	MicroMist

RESULTS AND DISCUSSION

No previous studies on the presence of heavy metals in the *M. hellenica* honeydew have been conducted in the region; therefore, this study represents the first reference. Plants carry heavy metals from the roots to the shoots by transporting them in the xylem and phloem. Primary transport in the xylem, retranslocation in the phloem, and transfer from the xylem into the phloem are important processes for the distribution of an element within a plant [11]. Plants accumulate heavy metals in their roots and shoots in concentrations higher than those in the soil [12]. Because high concentrations of heavy metals can be found in the various honeys obtained from bee hives, particularly those next to highways and near steel plants [13], honey can be used as an environmental marker [14]. The levels of Cr, Mn, Cu, Zn Cd, and Pb concentration found in our results from Turkish pine xylem sap and honeydew are summarized in Table 2. According to the results, no statistically significant differences in heavy metal concentrations were found between honeydew and pine sap ($p > 0.05$).

TABLE 2
Mean heavy metal concentrations in the samples (mg kg^{-1} ± s.d.).

	Cr	Mn	Cu	Zn	Cd	Pb
Honeydew (n = 18)	0.022 ± 0.005	0.039 ± 0.032	0.047 ± 0.019	0.334 ± 0.02	0.001 ± 0.000	0.012 ± 0.002
Turkish pine sap (n = 34)	0.048 ± 0.031	0.069 ± 0.053	0.042 ± 0.034	0.317 ± 0.081	0.001 ± 0.000	0.015 ± 0.006

Note: s.d.= standard deviation.

Turkish pine bark can also be used to monitor heavy metal contamination [15]. In a previous study conducted in Izmir on Turkish pine bark, 5.0–20.0 mg kg^{-1} Cr, 75–375 mg kg^{-1} Mn, 2.25–15.63 mg kg^{-1} Cd, and 50–375 mg kg^{-1} Pb were detected [16]. In another study, it was reported that the bark of the Turkish pine effectively accumulates Pb [17]. Sawidis et al. [18] detected 2.2–8.1 mg kg^{-1} Cu, 13.1–29.7 mg kg^{-1} Zn, and <1.5–7.3 mg kg^{-1} Pb in Turkish pine leaves. No previous studies on Turkish pine sap were found in the literature. In our study, 0.048 mg kg^{-1} Cr, 0.069 mg kg^{-1} Mn, 0.042 mg kg^{-1} Cu, 0.317 mg kg^{-1} Zn, 0.001 mg kg^{-1} Cd, and 0.015 mg kg^{-1} Pb were detected in Turkish pine sap.

Although there have been no studies on honeydew, there are studies on honeydew honey. In a study conducted in Poland on honeydew honey (n = 2), 4.31 mg kg^{-1} Zn, 0.027 mg kg^{-1} Cd, and 0.037 mg kg^{-1} Pb were detected [13]. In the honeydew honey from Italy, 1.70 mg kg^{-1} Mn, 1.87 mg kg^{-1} Zn, 4.40 mg kg^{-1} Cu, 0.0027 mg kg^{-1} Cd, and 0.09 mg kg^{-1} Pb were detected [19]. In a study by Uren et al. [20] on honeydew honey in Turkey, the results showed 0.752 mg kg^{-1} Mn, 1.05 mg kg^{-1} Cu, and 0.011 mg kg^{-1} Cd. In current study, 0.33 mg kg^{-1} Zn, 0.001 mg kg^{-1} Cd, and 0.012 mg kg^{-1} Pb was found in the honeydew.

The tolerable upper intake levels of heavy metals specified by the World Health Organization and Institute of Medicine, Food and Nutrition Board are as follows: Cr: 250 µg day^{-1}, Mn: 11 mg day^{-1}, Cu: 10 mg day^{-1}, Zinc: 40 mg day^{-1}, and Pb: 25 µg kg^{-1} $week^{-1}$ [21, 22]. According to these values, the heavy metal concentrations found in the samples of plant sap and honeydew in our study do not pose a significant risk for human health.

CONCLUSIONS

The heavy metal concentrations determined in the Turkish pine sap and honeydew samples were found to be lower than the tolerable upper intake levels. No statistically significant differences were found in heavy metal concentrations between the Turkish pine sap and honeydew samples. This study reports the first reference on the evaluation of Cr, Mn, Cu, Zn, Cd, and Pb concentrations in Turkish pine sap and honeydew.

REFERENCES

[1] Michalas, F., Glavac, V., Parlar, H. (1992) The detection of aluminium complexes in forest soil solutions and beech xylem saps. Fresenius Journal of Analytical Chemistry. 343(3), 308-312.

[2] Zarkovic, B., Blagojevic, S. (2007) Influence of long-term fertilization on the cadmium content of calcareous chernozem soil. Fresen. Environ. Bull. 16, 295-298.

[3] Kabala, C., Chodak, T., Szerszen, L., Karczewska, A., Szopka, K., Fratczak, U. (2009) Factors influencing the concentration of heavy metals in soils of allotment gardens in the city of Wroclaw, Poland. Fresen. Environ. Bull. 18, 1118-1124.

[4] Papazoglou, E.G. (2009) Heavy metal allocation in giant reed plants irrigated with metalliferous water. Fresen. Environ. Bull. 18, 166-174.

[5] Khan, Z.I., Ahmad, K., Parveen, R., Bashir, H., Mehmood, N. (2017) High metal accumulation in bitter gourd irrigated with sewage water can cause health hazards. Fresen. Environ. Bull. 26, 4332-4337.

[6] Anonymous (2015) Turkey Forest Presence. General Directorate of Forestry, available at: http://www.ogm.gov.tr/ekutuphane/Yayinlar/T%C3%BCrkiye%20Orman%20Varl%C4%B1%C4%9F%C4%B1-2015.pdf .

[7] Gurkan, B. and Bosgelmez, A. (1989) Bioecology and population dynamics of the pine scale *Marchalina hellenica* (Gennadius). PhD thesis. Hacettepe University, Institute of Science and Technology, Ankara. 87p.

[8] Ozdemir, I., Avci, M. and Korkmaz, M. (2014) Basralı kızılçam ormanları. Arıcının Sesi. 6(11), 22-23.

[9] Bacandritsos, N. (2004) Establishment and honeydew honey production of *Marchalina hellenica* (Coccoidea Margarodidae) on Fir Tree (Abiescephalonica). Bulletin of Insectology. 57(2), 127–130.

[10] Ulgenturk, S., Szentkirályi, F., Uygun, N., Fent, M., Gaimari, S.D., Civelek, H. and Ayhan, B. (2013) Predators of *Marchalina hellenica* (Hemiptera: Marchalinidae) on pineforests in Turkey. Phytoparasitica. 41(5), 529-537.

[11] Marschner, H. (1995) Mineral nutrition of higher plants. 2nd ed. Academic Press, London.

[12] Raskin, I., Kumar, P.N., Dushenkov, S. and Salt, D.E. (1994) Bioconcentration of heavy metals by plants. Current Opinion in Biotechnology. 5(3), 285-290.

[13] PrzybyłOwski, P. and Wilczyńska, A. (2001) Honey as an environmental marker. Food Chemistry. 74(3), 289-291.

[14] Jones, K.C. (1987) Honey as an indicator of heavy metal contamination. Water, Air and Soil Pollution. 33(1-2), 179-189.

[15] Dogan, Y., Durkan, N. and Baslar, S. (2007) Trace element pollution biomonitoring using the bark of *Pinus brutia* (Turkish red pine) in the Western Anatolian part of Turkey. Trace elements and Electrolytes. 24(3), 146.

[16] Turkan, I., Henden, E., Çelik, U. and Kivilcim, S. (1995) Comparison of moss and bark samples as biomonitors of heavy metals in a highly industrialized area in Izmir, Turkey. Science of the Total Environment. 166(1), 61-67.
[17] Gundogdu, A., Ozdes, D., Duran, C., Bulut, V. N., Soylak, M. and Senturk, H.B. (2009) Biosorption of Pb (II) ions from aqueous solution by pine bark (*Pinus brutia* Ten.). Chemical Engineering Journal. 153(1), 62-69.
[18] Sawidis, T., Marnasidis, A., Zachariadis, G. and Stratis, J. (1995) A study of air pollution with heavy metals in Thessaloniki city (Greece) using trees as biological indicators. Archives of Environmental Contamination and Toxicology. 28(1), 118-124.
[19] Pisani, A., Protano, G. and Riccobono, F. (2008) Minor and trace elements in different honey types produced in Siena County (Italy). Food Chemistry. 107(4), 1553-1560.
[20] Uren, A., Serifoglu, A. and Sarikahya, Y. (1998) Distribution of elements in honeys and effect of a thermo electric power plant on the element contents. Food Chemistry. 61, 185–190.
[21] WHO (World Health Organization) (1996) Trace elements in human nutrition and health, (A Report of a re-evaluation of the role of trace elements in human health and nutrition). Geneva, Switzerland.
[22] Institute of Medicine, Food and Nutrition Board (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington D.C.

Received: **11.06.2018**
Accepted: **13.11.2018**

CORRESPONDING AUTHOR

Rahsan Ivgin Tunca
Mugla Sitki Kocman University,
Department of Plant and Animal Breeding,
Ula Ali Kocman Vocational School,
Mugla – Turkey

e-mail: rivgin@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9816-9827 Fresenius Environmental Bulletin

CHANGES IN ANTIOXIDANT ENZYME ACTIVITIES AND BERRY COMPOSITIONS OF WINE GRAPES (MERLOT) SUBJECTED TO WATER STRESS WITH DRIP IRRIGATION

Zhaoquan He[1,2,3], Tonghui Zhang[1,3,*], Xinping Liu[1,3]

[1]Northwest Institute of Eco-Environment and Resources, CAS, Lanzhou, China
[2]University of Chinese Academy of Sciences, Beijing, China
[3]Naiman Desertification Research Station, Northwest Institute of Eco-Environment and Resources, CAS, Chinese Academy of Sciences, Tongliao, Inner Mongolia Autonomous Region, China

ABSTRACT

Water is not only a limiting factor, but also one of the most important mediators of grape berry compositions in semi-arid land. Differences in superoxide dismutase (SOD), catalase (CAT), proline (Pro), malondialdehyde (MDA), and wine grape berry composition were investigated under water stress with drip irrigation. Merlot vines were proposed to the three levels of irrigation threshold (70-75%, 60-65% and 50-55% of field capacity (FC)) at the stage of germination, flowering and enlargement. The sweet taste and the favorable aromatic flavor were improved significantly from the germination to the enlargement stage to which slight water stress (60-65% of FC) was applied, wherein an optimal berry composition was achieved at the flowering stage which was mostly responsible for a positive relationship with high SOD activity. Such as, sugar-acid ratio, total sugar, tannins, total phenols, and anthocyanins of berries increased by 183.85%, 47.79%,66.67%, 100%, 57.52% in comparison to normal irrigation threshold (70-75% of FC), respectively. When Merlot vines were stressed at 50-55% of FC from germination to flowering stage, berry composition, diameter and cluster mass were higher than in normal irrigation (70-75% of FC), resulted from the linear positively correlations between berry diameter, dry matter and Pro content, activities of SOD, CAT observed.

KEYWORDS:
Water stress, Merlot, Antioxidant enzyme activities, Berry composition and appearance.

INTRODUCTION

Drought stress, being defined as the absence of adequate soil moisture, is a major limiting factor for plants to grow normally, and seed germination, seedling, vegetative and flowering of the plant all are sensitive to water stress [1], therefore, the efficiency of applied water use of plants is of vital importance. The by-products accumulation of normal cell metabolism, viz. reactive oxygen species (ROS), resulting in lipid peroxidation (in terms of MDA content), membrane injury, and enzyme inactivation through oxidative damage, such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen (O^-), is a biochemical change of plants subjected to water stress [2, 3]. ROS is scavenged by the principal antioxidant system of plants, including antioxidant enzymes, such as, SOD, CAT [4], which were broadly used for characterizing drought resistance, wherein, SOD is a primary defense line because it detoxify O_2^- into H_2O_2 [5]. Drought increased osmotic solutes, such as the soluble sugars and the Pro, have been observed previously [6-8]. The Pro could be affected by leaf age and position, as well as maintained the membrane integrity through reducing oxidation of membrane lipids [7]. Thereby, the maintenance of turgor via osmotic adjustment is used as a mechanism of drought tolerance.

Grapevine (*Vitis vinifera*) is regarded as the most vital fruit crop worldwide. Wines with high concentration, intense color, soft tannins, fruitiness, and low acidity are produced numerously. Grape berry contains abundant secondary metabolites which affect wine quality through determining its color, aroma, and flavor [9], such as, organic acids favorably affecting human metabolism, sugars, and flavonoids [10]. Generally, at veraison, grape leaf growth has practically ended and sugar starts to accumulate quickly in berries, and is time for treating berries with auxin prior to veraison [11]. Anthocyanins, as the predominant plant pigments and a class of flavonoids, are responsible for grape colors ranging from red to violet [12], wine color, mouthfeel and astringency, as does tannin content and composition. Besides, phenolic compounds are vital in red wine color and taste, as well as are rich in grape juice [13, 14].

Water is the main challenge to grape ripening in dry lands with irregular rainfall distributions, and remarkably affects grape berry compositions [15, 16], determines berry size, increasing with berry solute content [17]. Though grapevine is a greatly

FIGURE 1
Location of the experimental site in China.

drought-tolerant species, with the capability of surviving even under severe water stress [18], it is vital to define the effect of regulatory irrigation on antioxidant enzyme activities, berry composition [15]. There have been several studies in identifying the response of grape berry compositions to water stress [19, 20], but the best stage to which applying water stress and the optimal irrigation threshold for modifying berry compositions were no general consensus.

The various physiological and biochemical changes are the indispensable factors of plants to respond to water stress [21]. Present investigation was to visualize the response of plants to water stress imposed at specific phenological phase in physiological and biochemical indicators for establishing the sensitive phenological phase and the drought resistance indices. Accordingly, it is greatly imperative to analyze the physiological and biochemical variations. Understanding the effect of applying different irrigations to grapevines on antioxidant enzyme activities and berry composition is of great significance to the growers of vine grapes. Only with such knowledge can the irrigation strategy most likely to guarantee the berry quality be chosen. The main objective of the present research was to reveal the response mechanism of grapevines in terms of enzymatic activities of grape berry, berry compositions and appearances to controlled drip irrigation. Ultimately, providing scientific irrigation regulation on the optimal phenological phase for water restrictions, and potentially benefiting the quality of wine grapes.

MATERIALS AND METHODS

Study site. Field tests was conducted at the experimental station in Wuwei (38°34'N, 104°05'E, 1600 m above mean sea level), northwestern China (Figure 1), with a mean annual average rainfall of 158.6mm, an average annual temperature of 7.9℃, an evaporating capacity of 2130 mm, and is described as a continental arid climate, with annual sunshine of 2860 h, and mean annual frost-free period of 154 d. The soil is of loam texture, has 36% FC, and 1.35g cm^{-1} of soil volume weight within 0-100 cm soil layer. The chemical properties were as follows: pH, 8.01; organic matter, 1.38%; available nitrogen, 98.86 mg kg^{-1}; rapidly available phosphorus,53.41mg kg^{-1}; rapidly available potassium, 298.09 mg kg^{-1}.

Experimental layout. Our field trial was conducted in 2016, lasted from April to October. The growth stages were designated as the following, based on the development characteristics of Merlot vines, including the budding stage (May 6th-May 18th), the germination stage (May 19th-June 6th), the flowering stage (June 7th-June 18th), the enlargement stage (June 19th-August 18th), and the ripening stage (August 19th-September 27th). Merlot, as a variety of wine grapes, which was 4 years old, with cold and alkaline resistance, which was suitable for growing in various loamy soils. The experiment had an east-west orientation and grapevines were aligned north-south. The grapevine frame was composed of concrete columns with height of 1.5 m and three wire ropes, of which spacing was 0.5 m, 0.55 m and 0.45 m from top to bottom. The region between every two concrete columns was regarded as a plot, with size of 4.0 m by 0.8 m, row space of 2 m and spacing of 0.6 m between plants. A buffer channel of 1.0 m width was provided on the neighborhood of experimental fields to avoid effects of other soils.

Seven treatments were conducted in a completely randomized design with four replications every treatment, with a total of 28 plots. Each plot was consisted of 1 row of 10 vines, of which 6 trees in centre row were regarded as the observation trees, and the others as buffers. Two irrigation water levels were applied to the stage of germination, flowering, and enlargement separately, viz. 60-65 % of FC was regarded as the moderate water stress and 50-55% FC was regarded as the severe water stress. In addition, 70-75% of FC was served as control (well watered) throughout the growing season. A tube was proposed to conduct irrigation. It was composed of a built-in drip irrigation line of 20mm diameter by which each row was laid, with a drop spacing of 15cm and a drop flow of 0.6 L h^{-1}. Water was applied

TABLE 1
Experimental design of Merlot vines in responses to water stress during the specific phenological stage.

Treatment	SWC threshold throughout the growth stages (percentage of FC)			
	Germination	Flowering	Enlargement	Ripening
T1	60-65	70-75	70-75	70-75
T2	70-75	60-65	70-75	70-75
T3	70-75	70-75	60-65	70-75
T4	50-55	70-75	70-75	70-75
T5	70-75	50-55	70-75	70-75
T6	70-75	70-75	50-55	70-75
CK	70-75	70-75	70-75	70-75

SWC: soil water content. FC: field capacity.

with one pressure-compensated emitter per plant in one drip line per row. Groundwater for irrigation was measured continuously by the flowmeters, with a water-supplied pressure of 0.2MPa. In this experimental year, grapevines were drip-irrigated using groundwater throughout the growing season (early May-end September). To avoid the unnecessary evaporation, all drip irrigation lines were shallowly buried beneath the soils, and drip holes were examined at 10d intervals to prevent dripping from impurity. Irrigation was started once soil water content was below the water level determined as above in each treatment, the irrigation quota was 270m^3 ha^{-1}. Details were shown in Table 1.

Soil and berry analysis. The soil water content (SWC) in 0-20cm, 20-40cm, 40-60cm, 60-80cm, 80-100cm depth was measured using weighing method from the germination to the maturity stage. The soil was sampled at 7d intervals with three repetitions each treatment, soil samples were weighed and then dried in an oven at 105℃for constant weight. The weight of the dry sample was determined gravimetrically, and the soil water content was calculated and expressed as mass percentages. Three grapevines were selected every plot when berries generated, the longitudinal diameter and the transverse diameter of three grapes from each grapevine were recorded on the 5th, 10th, 30th, 50th, 70th day since the enlargement stage, ultimately, all longitudinal and transverse diameters were averaged as the berry size. The ten leaves and three stems were selected randomly every plot on the10th, 20th, 30th, 40th, 50th, 70th, and 100th day since the germination stage, and were oven-dried at 80℃ immediately for constant weight, then dry leaf and stem was averaged as the leaf and stem mass. Weighing fresh nine grapes (FW) with normal appearance and no external defects each plot, and were selected randomly and picked into aluminum box on the10th, 20th, 30th, 40th, 50th, 70th, and 100th day of the enlargement stage, dry weight (DW) of grapes was obtained by oven dry at 85℃, and the dry matter were attained finally by averaging dry matter each plot, which can be calculated as following:

$$\text{Dry matter (\%)} = DW / FW \cdot 100 \quad (1)$$

When grapes were harvested, it could be regarded as the cluster mass via weighing three clusters of berries, selected randomly every plot, with full berries, fewer pests and diseases, as well as averaging clusters of three plots in each treatment.

Enzymatic activities. They were measured on the 10th, 30th, 50th, 70th, 90th day of the enlargement stage, based on the mixture of nine fresh leaves expanded entirely put into liquid nitrogen tank for freeze. SOD activity was measured according to the methods of Beauchamp and Fridovich [22]; CAT activity was determined in accordance with the method of Beers and Sizer [23]; Pro content was determined in accordance with the method of Bates et al [6]. MDA content was determined according to the methods of Heath [24].

Grape berry analysis. Nine berries each treatment with normal appearance were selected randomly and stored when grapes were harvested. Total sugar (g L^{-1}), was measured by the Fibonacci reagent titration method (GB/T15038-2006 method), Titratable acid (g L^{-1}) by acid base titration method (GB/T15038-2006 method), and tannins (g kg^{-1}), Phenols (g kg^{-1}), as well as Anthocyanins (mg kg^{-1}) by the method of spectrophotometer.

Crop management. Except the irrigation scheduling, other agronomic measures, including pruning, pest and weed control, as well as harrowing, were executed uniformly following the local practices.

Statistical analysis. Statistically significant differences among the various treatments with regard to soil water content, physiological indexes, growth, and fruit quality were calculated by one-way analysis of variance (ANOVA) using SPSS 18.0 software, and Duncan's multiple range test was selected to determine the differences between means ($P<0.05$ or 0.01). The graphs were drawn by Sigmaplot 12.5 software.

RESULTS

SWC changes from the germination to maturation. The SWC of T2 at 0-100 cm was invariably lower than that of other treatments at the

enlargement stage, and decreased by 31.25% (P<0.05) on average compared with that of the flowering stage, finally recovered at the ripening stage.

The SWC of T5, at 20cm, 40cm, 60cm, 80cm and 100cm of soil layer, increased respectively by 22.25%, 24.73%, 72.52%, 37.32%, 28.29% in comparison with T2 at the enlargement stage, with statistically significant differences (P<0.01). At the ripening stage, SWC in T5 observably reduced and was significantly lower than that in T2. SWC of T4 continued to reduce throughout the growing season (Figure 2).

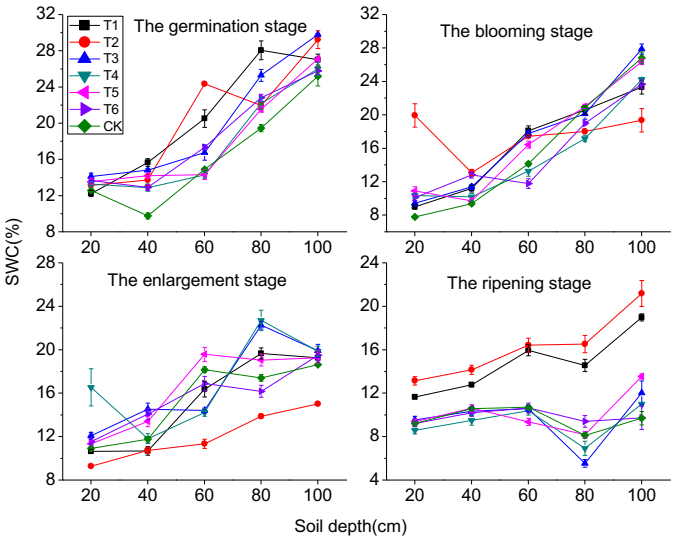


FIGURE 2

The responses of SWC to water stress applied at the stage of germination, flowering, and enlargement.

T1: slight water stress (60-65% of FC) was imposed to grapevines at the germination stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. T2: slight water stress (60-65% of FC) was imposed to grapevines at the flowering stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. T3: slight water stress (60-65% of FC) was imposed to grapevines at the enlargement stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. T4: severe water stress (50-55% of FC) was imposed to grapevines at the germination stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. T5: severe water stress (50-55% of FC) was imposed to grapevines at the flowering stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. T6: severe water stress (50-55% of FC) was imposed to grapevines at the enlargement stage, the normal irrigation threshold (70-75% of FC) was determined to other growth stages. CK: the normal irrigation threshold (70-75% of FC) was imposed to grapevines across the growing season. FC: field capacity.The bar represents the standard errors among treatments on the same day (the same below).

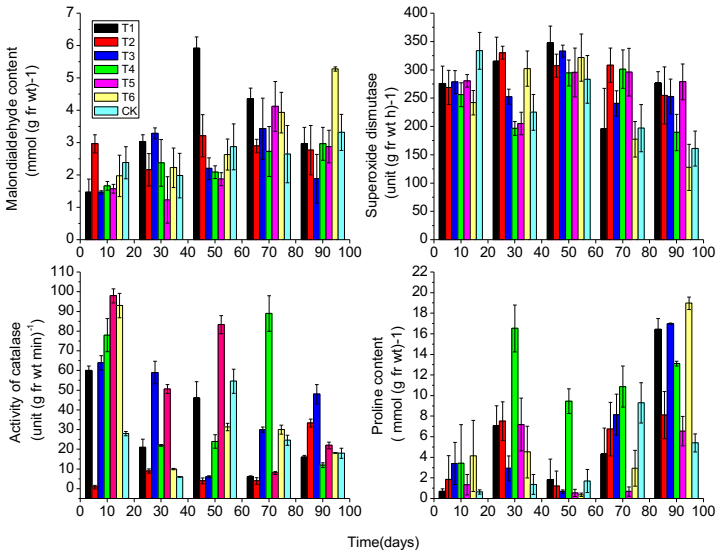


FIGURE 3

The responses of enzymatic activities to water stress applied at stage of germination, flowering, and enlargement.

Enzymatic activity changes since the enlargement stage. In terms of T2, MDA content began to decline slowly from the 50th day, while the Pro content increased sharply from 1.203μmol (g fr wt)$^{-1}$ to 8.112 μmol (g fr wt)$^{-1}$ at the 90th day. The average activity of SOD was at a stable high level of 303.75 unit (g fr wt h)$^{-1}$ from the10th to 70th day, and was higher than that in other treatments.

The Pro content in T4 increased continuously since the 50th day, and was higher than that in CK by 142.25 % on the 90th day, with extremely significant differences (P<0.01). SOD activity was high and stable from the 50th to the 70th day, and CAT activity reached maximum at the 70th day among all treatments.

In T5, the MDA content increased significantly from the 50th day, reaching a peak of 4.12 μmol (g fr wt)$^{-1}$ on the 70th day, which increased by 56.06% compared with CK (P<0.01). The SOD activity was at a high level of 278.99-296.08 unit (g fr wt h)$^{-1}$ since the 50th day (Figure 3.).

The leaf mass and the stem mass in T4 were significantly greater than in CK prior to the 50th day. The leaf mass and the stem mass in T5 grew rapidly since the 50th day, and were significantly larger than that of other treatments from the 70th to the 100th day. In addition, the leaf mass and the stem mass of T5 were higher than that in CK by 25.42% (P<0.01) and 6.78% at average from the 70th to the 100th day (Figure 4).

The longitudinal diameter and the transverse diameter in T5 were greater than CK from the 5th to 70th day. On the 70th day, the longitudinal diameter of T4, T5 was higher than in CK by 2.74%, 4.48%, respectively, but significant difference was not found (Table 2).

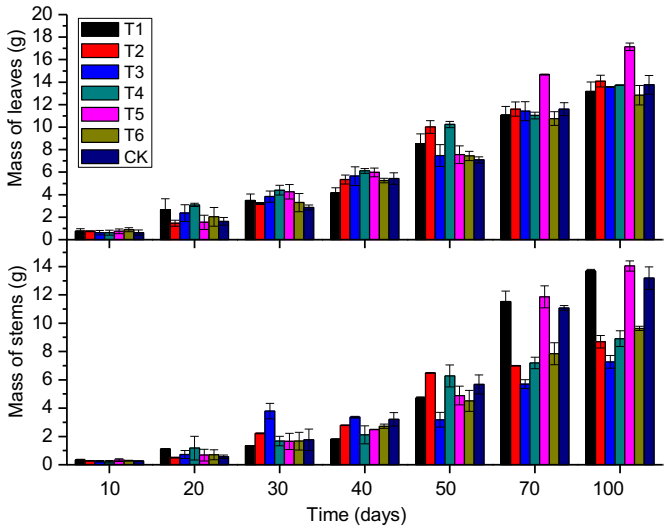


FIGURE 4
The responses of mass of leaves and stems to water stress applied at stage of germination, flowering, and enlargement.

TABLE 2
Effects of water stress applied at germination, flowering, and enlargement stage on longitudinal diameter (a) and transverse diameter (b).

Treatment(a)	5d	10d	30d	50d	70d
T1	4.96±0.18^{a}	6.54±0.11^{a}	10.10±0.24^{a}	10.58±0.70^{a}	11.13±0.54^{a}
T2	4.96±0.48^{a}	6.45±0.62^{a}	10.07±0.81^{a}	10.47±0.67^{a}	11.11±0.51^{a}
T3	4.79±0.73^{a}	6.17±0.96^{b}	9.64±0.24^{a}	10.61±0.38^{a}	11.29±0.43^{a}
T4	5.32±0.43^{a}	7.24±0.22^{a}	9.87±0.74^{a}	10.44±0.90^{a}	11.23±0.53^{a}
T5	5.18±0.55^{a}	6.95±0.91^{a}	10.03±0.48^{a}	10.92±0.25^{a}	11.42±0.64^{a}
T6	5.14±0.37^{a}	6.70±0.29^{a}	9.50±0.27^{a}	10.49±0.18^{a}	11.50±0.63^{a}
CK	5.08±0.28^{a}	6.79±0.30^{a}	10.11±0.76^{a}	10.59±0.48^{a}	10.93±0.25^{a}

Treatment(b)	5d	10d	30d	50d	70d
T1	4.73±0.21^{a}	6.07±0.05^{a}	9.67±0.89^{a}	10.30±0.53^{a}	11.06±0.80^{a}
T2	4.64±0.43^{a}	6.02±0.56^{a}	9.52±0.85^{a}	10.10±0.59^{a}	10.95±0.68^{a}
T3	4.44±0.55^{a}	5.72±0.63^{a}	9.47±0.42^{a}	10.26±0.11^{a}	11.01±0.89^{a}
T4	4.45±0.87^{a}	5.88±0.01^{a}	9.82±0.41^{a}	10.38±0.67^{a}	11.12±0.34^{a}
T5	5.04±0.54^{a}	6.61±0.76^{a}	9.80±0.39^{a}	10.66±0.38^{a}	11.25±0.81^{a}
T6	4.76±0.24^{a}	6.24±0.36^{a}	9.23±0.28^{a}	10.26±0.23^{a}	11.44±0.51^{a}
CK	4.85±0.22^{a}	6.33±0.26^{a}	9.94±0.45^{a}	10.26±0.39^{a}	11.01±0.19^{a}

Values are presented as mean (±S.E.) of three replicates and different letters within lines indicate significant differences (P< 0.05).

TABLE 3

Effects of water stress applied at the germination, flowering, and enlargement stage on berry compositions (a) and appearances (b). Values are the means±SE (n=9) and different letters within lines indicate significant differences (*P<0.05*).

Treatment (a)	Titratable acid (g L^{-1})	Total sugar (g L^{-1})	Tannins (g kg^{-1})	Total Phenols (g kg^{-1})	Anthocyanins (mg kg^{-1})
T1	1.28±0.011^{c}	37.45±0.003^{a}	0.03±0.012^{a}	0.04±0.012^{a}	71.34±1.017^{c}
T2	1.89±0.023^{c}	35.81±0.944^{a}	0.05±0.015^{a}	0.06±0.016^{a}	165.79±1.016^{b}
T3	2.93±0.106^{b}	34.14±1.643^{a}	0.02±0.004^{a}	0.04±0.005^{a}	296.51±1.199^{a}
T4	1.77±0.452^{c}	28.14±0.750^{b}	0.02±0.009^{a}	0.03±0.010^{a}	144.66±2.195^{b}
T5	1.36±0.233^{c}	35.28±0.506^{a}	0.03±0.007^{a}	0.04±0.007^{a}	126.82±2.752^{b}
T6	4.19±0.125^{a}	34.25±1.932^{a}	0.04±0.001^{a}	0.05±0.002^{a}	243.81±1.985^{a}
CK	3.63±0.106^{ab}	24.23±1.199^{b}	0.03±0.006^{a}	0.03±0.007^{a}	105.25±1.980^{b}

Treatment (b)	Longitudinal diameter (mm)	Transverse diameter(mm)	Dry matter (%)	Cluster mass (g)
T1	9.14±0.46^{a}	8.78±0.41^{a}	14.89±0.50^{a}	167.93±1.58^{a}
T2	8.95±0.65^{a}	8.54±0.56^{a}	15.15±0.73^{a}	136.71±0.49^{a}
T3	8.96±0.56^{a}	8.61±0.46^{a}	14.57±0.25^{a}	137.00±3.97^{a}
T4	9.42±0.19^{a}	8.73±0.67^{a}	15.64±0.65^{a}	145.49±0.70^{a}
T5	9.35±0.52^{a}	9.06±0.54^{a}	15.65±0.46^{a}	127.44±1.41^{a}
T6	9.14±0.29^{a}	8.74±0.29^{a}	16.30±0.96^{a}	106.96±1.89^{b}
CK	9.21±0.42^{a}	8.87±0.30^{a}	15.34±0.35^{a}	140.22±2.69^{a}

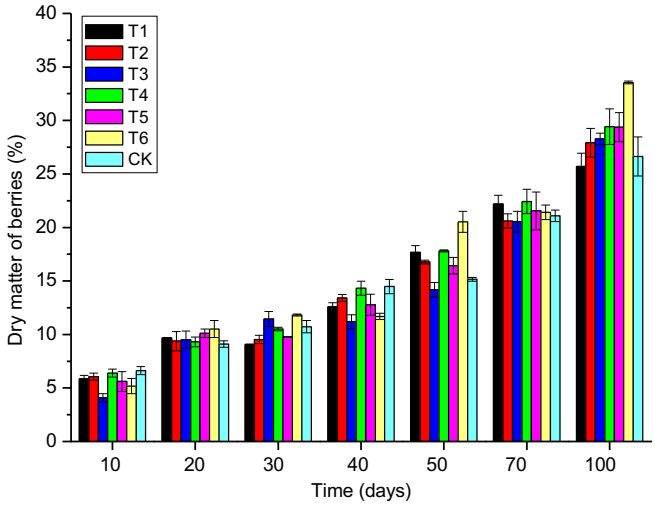


FIGURE 5

The responses of dry matter to water stress applied at stage of germination, flowering, and enlargement.

The transverse diameter was higher in T4 than in CK since the 50th day, the highest berry increment of T4, T5 on the 50th day was found, and increased by 1.17%, 3.90% respectively in comparison to CK.

Since the 50th day, dry matter in T4, T5 and T6 significantly increased, and from the 50th to 100th day, the differences were significant (P<0.01) between T4, T5, T6 and CK, because dry matter in T4, T5 and T6 increased averagely by 10.76%, 7.10% and 20.02% compared with CK (Figure 5).

Changes of grape berry compositions and appearances at the maturity stage. Sugar-acid ratio of T6 was 8.17, which increased by 22.49% compared with CK, but was inferior to that in other treatments, led to acidity accumulations in berries. Tannins, phenols, and anthocyanins increased by 33.33%, 66.67% and 131.65% respectively in comparison with CK, and a remarkable difference was found (P<0.01), which was beneficial to the accumulation of aromatic flavor in berries. Berry appearances (berry size, dry matter, ear mass), were not higher than that of CK.

The phenols and anthocyanins in T4, T5 were higher, and the berry size and dry matter of T4, T5 were higher than that of CK (P<0.05). The sugar-acid ratio of T1, T2, T3 was up to 29.26, 18.95 and 11.65 respectively, sweet taste of fruit was improved. Wherein, tannins, total phenols and anthocyanins were also higher in T2 than in T6, resulting in a favorable fruit flavor. Berry appearances of T1, T2, T3 was lower than that of CK, but there was not significant difference (P>0.05) (Table 3).

TABLE 4
The correlation analysis of berry compositions (total sugar, tannins, anthocyanins) with antioxidant enzyme activities (SOD, CAT, Pro, MDA) after water stress was applied at the germination and the flowering stage.

Treatment	Berry compositions	SOD	CAT	Pro	MDA
	Total sugar	0.291	-0.896*	-0.440	-0.697
T2	Tannins	0.313	-0.906**	-0.419	-0.714
	Anthocyanins	0.294	-0.897*	-0.437	-0.700
	Total sugar	-0.942**	-0.101	0.204	0.447
T4	Tannins	0.953**	0.134	-0.171	-0.416
	Anthocyanins	0.942**	0.101	-0.204	-0.447
	Total sugar	0.943**	0.170	0.920**	-0.688
T5	Tannins	-0.547	-0.734	-0.492	0.987**
	Anthocyanins	0.649	-0.858*	0.697	0.429

SOD: superoxide dismutase. CAT: catalase. Pro: proline. MDA: malondialdehyde (the same below). *, **Significant at the 5 and 1% levels of probability, respectively.

DISCUSSION AND CONCLUSIONS

Water stress is a vital environmental factor affecting plants growth [25]. The accumulation of ROS is one of the biochemical responses of plants to water stress [26, 27], the antioxidant enzymes and osmoregulation were exhibited to be a defense mechanism for protecting plants and improving growth through scavenging ROS [28, 29].

Effects of water stress on wine grape berry compositions. The physiological and biochemical adaptations depended on severity of stress and the stage at which stress occurring [30, 31]. After slight water stress applied at the flowering stage, soil water content of 0-100 cm significantly reduced (Figure 2). Pro, was considered to be positively correlated with osmotic stress tolerance [32], and SOD activity became higher and stable in grape berries, which prevented the cell membrane peroxidation timely. Therefore, there was a decline in MDA content. The berry compositions (sugar, tannins, anthocyanins) was positively related with SOD activity, and was negatively related with MDA content (Table 4), in consequence, leading to sugar, tannins and anthocyanins content in grape berries increased along with the increasing activity of SOD and the decline of MDA content. Consistent with above findings, it was confirmed in previous studies that anthocyanins concentration was increasing effectively under water stress of early season [33], and higher total phenols appeared in berries under water stress at pre-veraison [34], with a strong correlation with antioxidant activity (R^2=0.98) [35]. Sweet taste and fragrant flavor of berries were achieved by balancing of the total soluble sugar, titratable acidity, and total phenols [36], as well as berry composition were enhanced [37].

SWC decreased continuously when severe water stress occurred at the germination stage (Figure 2). Since the 50th day of the enlargement stage, Pro content increased significantly, with a capacity of timely adjusting the osmotic potential, enhancing water retention in cells; in the light of CAT activity fluctuated greatly and stabilized SOD activity, which weakened the protection of cell membranes, MDA gradually increased to restrain the synthesis of antioxidant enzymes scavenging sufficient ROS, this was not conducive to the integrity of cells (Figure 3) [38, 39]. At the ripening stage, the negative linear correlations between SOD and sugar affected the sugar accumulation in grape berries. Whereas, tannins and anthocyanins were positively related to SOD, CAT, and were negatively related to the Pro, MDA, hence, the aromatic flavor of grape berries would be weakened by the rise of Pro and MDA contents.

SWC of the enlargement stage after severe water stress of the flowering stage was strikingly higher than that of slight water stress, then decreased significantly at the ripening stage (Figure 2), it was shown that a transient "re-watering compensation" effect occurred after severe water stress of the flowering stage. The contents of Pro, MDA and SOD activity in grape berries after applying severe water stress in the flowering stage was lower than that of slight water stress, resulted from the high SWC of the enlargement stage after severe water stress of the flowering stage (Figure 3). Such an observation was in parallel to earlier study that the antioxidant activities increased under water stress [40]. Though anthocyanins synthesis pathway was affected by water stress at pre-veraison [41], the anthocyanins accumulation in grape berries commenced at veraison and continued throughout berry ripening [42, 43]. The sugar with regulatory function because of metabolic effect and osmotic stress [44, 45], and anthocyanins accumulations along with the increase of SOD activity in grape berries, due to being closer to the positive correlations with the activity of SOD (Table 4), therefore, the preferable berry composition was obtained. Certainly, there was another perspective that water stress at post-veraison inducing conveniently the degree of water stress in terms of berry phenolic compositions and improving slightly berry anthocyanin concentrations [19, 34], and sugars existed in almost

whole stage of the plant, involved in seeding germination, photosynthesis, berry development and senescence [46], the combination of soluble sugars, organic acids and their ratios largely determined the taste of plants [47], because lower sugar contents would translate into a higher acid content [48].

Effects of water stress on wine grapes in berry appearances. The phenological stages of vegetative and reproductive growth at which water stress is applied obviously influenced grape berry growth and quality [49], plant responses of morphological, physiological, biochemical indicators to water stress were reflected in all plant organs, including stems, leaves, and fruits [50]. In our study, the increased tendency of high activity of SOD and high Pro content from the 10th to the 90th day of the enlargement stage after severe water stress occurred at the germination stage (Figure 3), as well as the linear rising tendencies between the berry size and dry matter (Figure 6), led to the berry appearance enhanced with the increase of SOD activity and Pro content. Hence, Pro was regarded as a key signal of osmoregulation defenses though reducing cell osmotic potential and maintaining leaf cell turgor, resulting in growth improvement [51, 52]. When severe water stress was applied at the flowering stage, a high level of SOD and CAT activity, as well as the linear positively correlations between berry size and dry matter were in favor of the berry appearance (Figure 6) [53]. However, there was no consensus concerning the relationships between berry size and wine quality. It has been demonstrated that the wine with deeper color was produced by smaller grapes [54], and the higher phenolic contents in smaller berries skin [55, 17], encouraging vegetative development and attaining larger berries [56]. Conversely, the indistinctive differences occurred between wines and smaller berries [57]. Our findings were not in agreement with earlier conclusions that severe water stress would be detrimental to berry quality due to a poor canopy development and reduced leaf assimilation rate, ultimately, leading to an inadequate vines capacity to ripen the berries [58], this may be related mostly to the variety of plants and the variance of water stress.

The berry compositions with sweet taste and favorable aromatic flavor could be achieved when grapevines were subjected to slight water stress (60-65% of FC) at the stage of germination, flowering, and enlargement, respectively. Nevertheless, berry appearance was not enhanced. Berry compositions and appearances were improved, including higher sugar acid ratio, total phenols, anthocyanins, as well as the increment of berry diameter, dry matter, and ear mass, after applying severe water stress (50-55% of FC) to the stage of germination, flowering, respectively.

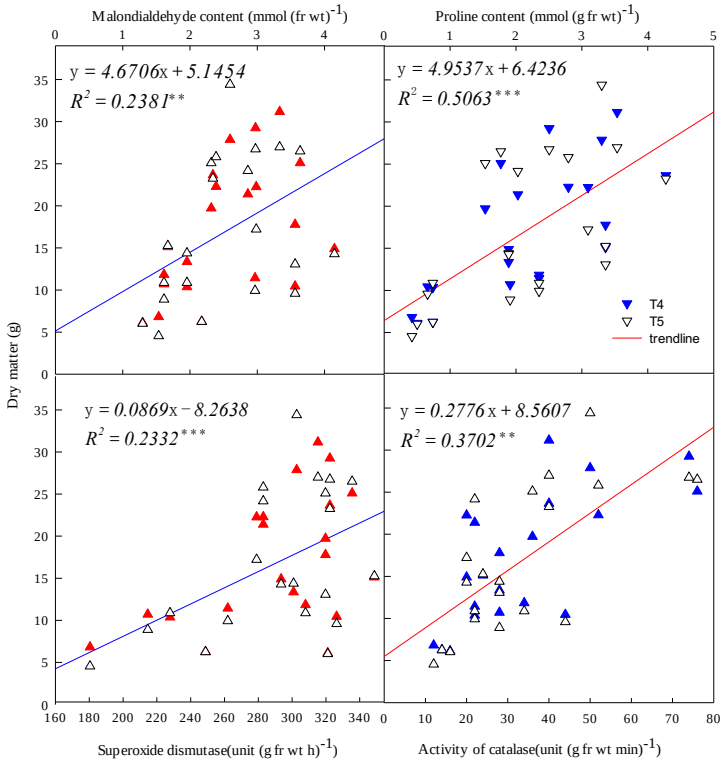


FIGURE 6A

The regression curves between dry matter and antioxidant enzyme activities (SOD, CAT, Pro and MDA) after severe water stress (50–55% of FC) was applied at the germination and the flowering stage.

The solid lines represent the best-fit linear regression for the grape berries. The P-values are denoted as *$P<0.05$, **$P<0.01$, ***$P<0.001$.

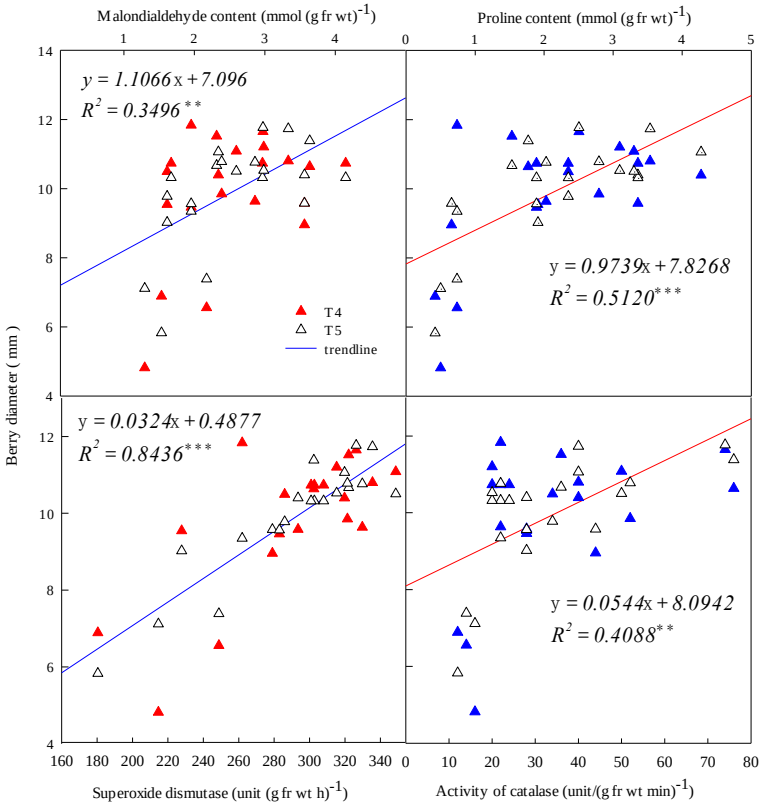


FIGURE 6B

The relationships between berry diameter and antioxidant enzyme activities (SOD, CAT, Pro and MDA) after severe water stress (50-55% of FC) was applied at the germination and the flowering stage.

The solid lines represent the best-fit linear regression for grape berries. The *P*-values are denoted as $^{*}P<0.05$, $^{**}P<0.05$, $^{***}P<0.001$.

As a consequence, it was suggested that in regions where water availability was limited, Merlot vines would be irrigated at the germination, flowering, and enlargement stage with 60-65% of FC to maximize the berry compositions, and irrigated at 50-55% of FC to enhance simultaneously berry compositions and appearances.

ACKNOWLEGEMENTS

The present study was financially supported by the national nature science funds of China (41371053). These authors contributed equally to this work. We are very grateful to the anonymous reviewers and editors for their valuable comments on the manuscript.

REFERENCES

[1] Sy, A., Grouzis, M., Danthu, P. (2001) Seed germination of seven Sahelian legume species. Journal of Arid Environments. 49(4), 875-882.

[2] Scandalios, J.G. (1993) Oxygen stress and superoxide dismutases. Plant Physiology. 101(1), 7-12.

[3] Dionisio-Sese, M.L., Tobita, S. (1998) Antioxidant responses of rice seedlings to salinity stress. Plant Science. 135(1), 1-9.

[4] Burke, J.J., Mahan, J.R. (1991) Environmental Regulation of Cellular Protection Systems. In: Gausman, H.W. (eds.) Plant Biochemical Regulators. New York, Marcel Dekker, 47-58.

[5] Fatima, R.A., Ahmad, M. (2005) Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater. Science of the Total Environment. 346(1), 256-273.

[6] Bates, L.S., Waldren, R.P., Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. Plant and Soil. 39(1), 205-207.

[7] Bandeoğlu, E., Eyidoğan, F., Yücel, M., Öktem, H.A. (2004) Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regulation. 42(1), 69-77.

[8] Gunes, A., Inal, A., Adak, M.S., Bagci, E.G., Cicek, N. (2008) Effect of drought stress implemented at pre- or post-anthesis stage on some physiological parameters as screening criteria in chickpea cultivars. Russian Journal of Plant Physiology. 55(1), 59-67.

[9] Lund, S.T., Bohlmann, J. (2006) The molecular basis for wine grape quality: a volatile subject. Science. 311(5762), 804-805.

[10] Holton, T.A., Cornish, E.C. (1995) Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell. 7(7), 1071-1083.
[11] Böttcher, C., Harvey, K., Forde, C.G., Boss, P.K., Davies, C. (2011) Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. Australian Journal of Grape and Wine Research. 17(1), 1-8.
[12] Van Tunen, A.J., Mol, J.N.M. (1991) Control of flavonoid synthesis and manipulation of flower colour. In: Grierson, D. (ed.) Developmental Regulation of Plant Gene Expression. Plant Biotechnology Series. Vol: 2, Springer, Dordrecht, 94-130.
[13] Arnold, R.A., Noble, A.C., Singleton, V.L. (1980) Bitterness and astringency of phenolic fractions in wine. Journal of Agricultural and Food Chemistry. 28(3), 675-678.
[14] Francesca, F., Zambito, Y., Di, C.G., D'Onofrio, C., Fausto, C., Balbarini, A. (2012) Red grape skin and seeds polyphenols: Evidence of their protective effects on endothelial progenitor cells and improvement of their intestinal absorption. European Journal of Pharmaceutics and Biopharmaceutics. 80(1), 176-184.
[15] Esteban, M.A., Villanueva, M.J., Lissarrague, J.R. (2001) Effect of irrigation on changes in the anthocyanin composition of the skin of cv tempranillo (*Vitis vinifera* L.) grape berries during ripening. Journal of the Science of Food and Agriculture. 81(4), 409-420.
[16] Intrigliolo, D.S., Castel, J.R. (2009) Response of Vitis vinifera cv. 'Tempranillo' to partial root-zone drying in the field: water relations, growth, yield and fruit and wine quality. Agricultural Water Management. 96(2), 282-292.
[17] Roby, G., Harbertson, J.F., Adams, D.A., Matthews, M.A. (2010) Berry size and vine water deficits as factors in wine grape composition: anthocyanins and tannins. Australian Journal of Grape and Wine Research. 10(2), 100-107.
[18] Bosnjak, D. (1999) Irrigation of agricultural crops. Soil Science. 152, 137.
[19] Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A. (2002) Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of Vitis vinifera cv. Shiraz. Am J. Enol. Viticult. 53(53), 261-267.
[20] Girona, J., Marsal, J., Mata, M., Campo, J.D., Basile, B. (2010) Phenological sensitivity of berry growth and composition of tempranillo grapevines (*Vitis vinifera* L.) to water stress. Australian Journal of Grape and Wine Research. 15(3), 268-277.
[21] Nguyen, T.T., Klueva, N., Chamareck, V., Aarti, A., Magpantay, G., Millena, A.C. (2004) Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Molecular Genetics and Genomics Mgg. 272(1), 35-46.
[22] Beauchamp, C., Fridovich, I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 44(1), 276-287.
[23] Beers, R.F., Sizer, I.W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry. 195(1), 133-140.
[24] Heath, R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125(1), 189-198.
[25] Ren, J., Dai, W., Xuan, Z., Yao, Y., Korpelainen, H., Li, C. (2007) The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. Forest Ecology and Management. 239(1), 112-119.
[26] Smirnoff, N. (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist. 125(1), 27-58.
[27] Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 7(9), 405-410.
[28] Morgan, J.M. (1984) Osmoregulation and water stress in higher plants. Annual Review of Plant Biology. 35(1), 299-319.
[29] Liu, C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y. (2011) Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. Environmental and Experimental Botany. 71(2), 174-183.
[30] Vadez, V., Berger, J., Warkentin, T., Asseng, S., Ratnakumar, P., Rao, K.P.C. (2012) Adaptation of grain legumes to climate change: A review. Agronomy for Sustainable Development. 32(1), 31-44.
[31] Mishra, B.K., Srivastava, J.P., Lal, J.P., Sheshshayee, M.S. (2016) Physiological and biochemical adaptations in lentil genotypes under drought stress. Russian Journal of Plant Physiology. 63(5), 695-708.
[32] Ashraf, M., Foolad, M.R. (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 59(2), 206-216.
[33] Matthews, M.A., Anderson, M.M. (1988) Fruit ripening in Vitis vinifera L.: Responses to seasonal water deficits. American Journal of Enology and Viticulture. 39, 313-320.

[34] Santesteban, L.G., Miranda, C., Royo, J.B. (2011) Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in Vitis vinifera L. cv. 'Tempranillo'. Agricultural Water Management. 98(7), 1171-1179.
[35] Kapusta, I., Cebulak, T., Oszmiański, J. (2017) Characterization of polish wines produced from the interspecific hybrid grapes grown in southeast Poland. European Food Research and Technology. 11-15.
[36] Pereira, G.E., Gaudillere, J.P., Leeuwen, C.V., Hilbert, G., Maucourt, M., Deborde, C. (2006) 1 H NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. Analytica Chimica Acta. 563(1), 346-352.
[37] Bielorai, H. (1982) The effect of partial wetting of the root zone on yield and water use efficiency in a drip-and sprinkler-irrigated mature grapefruit grove. Irrigation Science. 3(2), 89-100.
[38] Fan, X.W., Li, F.M., Song, L., Xiong, Y.C., An, L.Z., Jia, Y. (2009) Defense strategy of old and modern spring wheat varieties during soil drying. Physiol Plant. 136(3), 310-323.
[39] Boldaji, S.A.H., Sajedi, R.H., Fahimi, H., Saadatmand, S. (2012) Water availability effects on antioxidant enzyme activities, lipid peroxidation, and reducing sugar contents of alfalfa (Medicago sativa L.). Acta Physiologiae Plantarum. 34(3), 1177-1186.
[40] Hojati, M., Modarressanavy, S.A.M., Karimi, M., Ghanati, F. (2011) Responses of growth and antioxidant systems in Carthamus tinctorius L. under water deficit stress. Acta Physiologiae Plantarum. 33(1), 105-112.
[41] Castellarin, S.D., Matthews, M.A., Gaspero, G.D., Gambetta, G.A. (2007) Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta. 227(1), 101-112.
[42] Coombe, B.G. (1992) Research on development and ripening of the grape berry. Am. J. Enol. Viticult. 43(1), 101-110.
[43] Boss, P.K., Davies, C., Robinson, S.P. (1996) Analysis of the expression of anthocyanin pathway genes in developing Vitis vinifera L. cv shiraz grape berries and the implications for pathway regulation. Plant Physiology. 111(4), 1059-1066.
[44] Rolland, F., Baenagonzalez, E., Sheen, J. (2006) Sugar sensing and signaling in plants: Conserved and novel mechanisms. Annual Review of Plant Biology. 57(1), 675-709.
[45] Solfanelli, C., Poggi, A., Loreti, E., Alpi, A. Perata, P. (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. Plant Physiology. 140(2), 637-646.
[46] Weiss, D. (2010) Regulation of flower pigmentation and growth: Multiple signaling pathways control anthocyanin synthesis in expanding petals. Physiologia Plantarum. 110(2), 152-157.
[47] Adela, K. (2008) Flavor quality of fruits and vegetables. Journal of the Science of Food and Agriculture. 88(11), 1863-1868.
[48] Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2006) Handbook of Enology. Vol.2. The Chemistry of Wine, Stabilization and Treatments. 2nd ed. Wiley and Sons, New York.
[49] Gonzálezaltozano, P., Castel, J.R. (2000) Regulated deficit irrigation in 'Clementina de Nules' citrus trees. II: Vegetative growth. Journal of Pomology and Horticultural Science. 75(4), 388-392.
[50] Cochard, H., Coll, L., Le, R.X., Améglio, T. (2002) Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. Plant Physiology. 128(1), 282-290.
[51] White, D.A., Turner, N.C., Galbraith, J.H. (2000) Leaf water relations and stomatal behavior of four allopatric Eucalyptus species planted in Mediterranean southwestern Australia. Tree Physiology. 20(17), 1157-1165.
[52] Porcel, R., Ruiz-Lozano, J.M. (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany. 55(403),1743-1750.
[53] Chartzoulakis, K., Michelakis, N., Stefanoudaki, E. (1999) Water use, growth, yield and fruit quality of 'Bonanza' oranges under different soil water regimes. Advances in Horticultural Science. 13(1), 6-11.
[54] Gil, M., Pascual, O., Gómez-Alonso, S., García-Romero, E., Hermosín-Gutiérrez, I., Zamora, F. (2015) Influence of berry size on red wine colour and composition. Australian Journal of Grape and Wine Research. 21(2), 200-212.
[55] Kennedy, J.A., Matthews, M.A., Waterhouse, A.L. (2002) Effect of maturity and vine water status on grape skin and wine flavonoids. American Journal of Enology and Viticulture. 53(53), 268-274.
[56] Jackson, D.I., Lombard, P.B. (1993) Environmental and management practices affecting grape composition and wine quality - A review. American Journal of Enology and Viticulture. 44(4), 409-430.
[57] Walker, R.R., Blackmore, D.H., Clingeleffer, P.R., Kerridge, G.H., Rühl, E.H., Nicholas, P.R. (2010) Shiraz berry size in relation to seed number and implications for juice and wine composition. Australian Journal of Grape and Wine Research. 11(1), 2-8.

[58] Romero, P., Fernández-Fernández, J.I., Martinez-Cutillas, A. (2010) Physiological thresholds for efficient regulated deficit-irrigation management in wine grapes grown under semi-arid conditions. American Journal of Enology and Viticulture. 61(3), 300-312.

Received: **15.06.2018**
Accepted: **13.10.2018**

CORRESPONDING AUTHOR

Tonghui Zhang
Northwest Institute of Eco-Environment
and Resources,
Chinese Academy of Sciences,
Lanzhou – China

e-mail:zhangth@lzb.ac.cn

© by PSP Volume 27 – No. 12B/2018 pages 9828-9839 Fresenius Environmental Bulletin

THE PHYSIOLOGICAL-MORPHOLOGICAL (LEAF-LEVEL) RESPONSES OF SOYBEAN (*GLYCINE MAX* L. MERR.) TO THREE REGIMES OF DEFICIT IRRIGATION AND FERTILIZER COUPLING IN HORQIN SANDY LAND, NORTHEASTERN CHINA

Zhaoquan He[1,2,3], Tonghui Zhang[1,3,*], Xueyong Zhao[1,3], Xinping Liu[1,3]

[1]Northwest Institute of Eco-Environment and Resources, CAS, Lanzhou 730000, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Naiman Desertification Research Station, Northwest Institute of Eco-Environment and Resources, CAS, Chinese Academy of Sciences, Lanzhou 730000, China

ABSTRACT

In regions with sandy soil texture and water scarcity, it is little to know the coupling effect of deficit irrigation with fertilization on soybean growth. The three combined effects of deficit irrigation (DI) and nitrogen fertilization (NF) on the leaf-level physiological and morphological parameters were identified. Irrigation was started once soil water content (SWC) was below 60% (T1), 50% (T2), 40% (T3), or 70% (CK) of field capacity (FC), respectively; meanwhile, 54 kg N ha^{-1} (T1), 67.5 kg N ha^{-1} (T2), or 81 kg N ha^{-1} (T3) was assigned at the beginning of the blooming stage. The results showed that the net photosynthetic rate (P_N) and leaf water use efficiency (LWUE) was highest in T3. The leaf area (LA), plant height (SH), and leaf fresh mass (LFM) of T1, T3 increased by 32.69%, 16.05%, 29.10%, 14.93%, 62.49%, 71.43%, respectively, in comparison to CK. Relative water content (RWC, including leaf and pod) was lower in T1, T3 than in CK significantly, which was negative linear relationship with leaf water potential (ψ_w), and decreased continuously across growth stage. Ultimately, the average pod dry mass (PDM) in T3, T1 was up to 1.93g, 1.85g, CK just was 0.26g. Our results suggested that combination of 324mm irrigation and 54 kg N ha^{-1} optimally elevated VG, and 81mm water combining with 81 kg N ha^{-1} coupling most favoured RG and dry matter accumulation (DM) in comparison to single 405mm irrigation, maximizing the use efficiency of water, nitrogen and increasing vegetative growth (VG) and reproductive growth (RG).

KEYWORDS:
Gas exchange, Leaf morphology, Leaf water status, Nitrogen, Water stress.

INTRODUCTION

Due to its high protein, numerous mineral elements, and physiologically active substances that are beneficial to human health, soybean (*Glycine max* L. Merr.) is used as a vital source of quality protein and edible oil [1]. Soybean is popularly cultivated in the Horqin Sandy Land, located in the semiarid area of southeast Inner Mongolia, which was once severely desertified [2]. In this region, the frequency and amount of rainfall is considered to be a primary limitation to plants growth, owing to its volatility. As a result, irrigation is greatly dependent on groundwater. But, excessive irrigation depletes groundwater reserve. The measurement of photosynthesis can be used as an index of metabolic response to water stress [3], when leaf gas exchange activity is limited due to stomatal or nonstomatal limitations [4]. Another response is manifested in growth development limitations *via* inhibition of cellular expansion needed for, leaf area development and main stem elongation [5], as well as in reduction in yield potential [6]. An effective irrigation regime conserving limited water could be achieved via a DI strategy, i.e., one that applies less water than required by plants for increasing growth and ensuring stability in yields [7]. Reducing irrigation during drought tolerance periods to stabilize growth and yield is the primary objective of DI [8]. Therefore, it was an urgent necessity to confirm the effect of DI on plant physiological parameters. In previous studies, resistance to abiotic conditions was mainly expressed in stomatal morphology and dynamics [9]. Although the reduction in stomatal conductance occurred at 90 days after the plants were subjected to WS with 50% FC, the plants remained alive [10]. Taking into account the intensity and duration of WS, a remarkable decrease was also observed in ψ_w and LRWC [11]. Furthermore, the decrease in photosynthetic efficiency, stomatal and nonstomatal limitations, and inhibition of photochemical processes were strongly

induced by abiotic stress [12]. The decrease in photosynthesis was proportionally higher than the decreases in transpiration. Mycorrhizal plants had higher stomatal conductance and lower C_i than non-mycorrhizal plants under low temperature stress, and the endogenous mycorrhizal was found widely in soybean [13].

Although the existence of water-soluble fertilizer accounted for promotion of plant growth, highlighting the indispensable effects of fertilization on drought tolerance in beans [14], the response of soybean to nitrogen fertilization remains controversial. Although biological N_2 fixation is sufficient to generally meet the requirement of soybean, frequent additions of NF show a remarkable yield response [15]. The P_N was strongly influenced by various rates of the supplemental NF application [16], and was increased on average by 14-70% on NF application with 90-150 kg N ha^{-1} [17]. Additionally, application of high NF led to dramatically (24%) higher P_N than under low NF application [18], but the damage to photosynthesis of soybean generated by high NF application was similar to nitrogen deficiency through reduced biosynthesis and functioning in the major photosynthetic components [19]. Limitation of nitrogen generated lower CO_2 assimilation and reduction in photosynthesis [20], which was responsible for DM [21]. Meanwhile, leaf senescence, which affects the seed yield of soybean, has not been economically acceptable [22, 23], was mostly attributed to NF accumulated in the vegetative organs and redistributed to the seeds from the seeding to grain-filling stages of soybean [24], because its use efficiency was lower in China (30%) than in the rest of the world (40-50%) [25]. NF application in China has exceeded 30 million tonnes since 2013, and the input has surpassed 200 kg ha^{-1} for farming regions. Even if enormous amounts of NF were required to obtain high protein seed in soybean for high yield, the balance between VG and RG growth against excessive vegetative development would be shifted by excess NF, ultimately delaying plant maturity [26]. Thus, application of reasonable amounts of NF in agricultural fields is suggested strongly [27].

The stages from branching to grain-filling in soybean require more water, while the blooming stage is determined as the peak phase for assimilating nitrogen. Although the response of soybean growth and physiological processes to either NF or DI have received considerable coverage in the scientific literature, few studies have examined whether three given extreme coupling of DI and NF, including the slight deficit irrigation(LDI) plus the lowest nitrogen fertilization(LNF); the moderate deficit irrigation (MDI) plus the moderate nitrogen fertilization (MNF); the severe deficit irrigation (SDI) plus the highest nitrogen fertilization (HNF)), in comparison to normal irrigation, would be in favour of VG and RG of soybean. Therefore, the objectives of our study were to: (1) explore whether VG and RG of soybean could be promoted for maximizing the use efficiency of water and nitrogen by the physiological-morphological (leaf-level) responses; and (2) determine the optimal regime of DI and NF coupling to achieve the goal of saving water and increasing production in the Horqin Sandy Land.

MATERIALS AND METHODS

Experimental site. This study was carried out at the Naiman Desertification Research Station of the Chinese Academy of Sciences (42°58′N, 120°43′E; 360 m a.s.l.) in May-October 2017, located in the eastern part of Inner Mongolia, China. Naiman, as a remarkable desertification-threatened area with gently undulating dunes in the southwest of Horqin Sandy Land, has a continental semiarid monsoon climate. The experimental site, with sandy soil texture sensitive to wind erosion, has a mean annual precipitation of approximately 360 mm, an annual mean evaporation of around 1,950 mm, and an annual mean temperature of 6.4°C, of which minimum monthly average temperature of -13.5°C occurred in January and the maximum of 23.8°C in July. In addition, there was a northwest orientation of wind in winter and a southwest to south direction in summer and autumn, with an average annual wind velocity ranging from 3.2 to 4.1 m s^{-1}. Soil organic carbon content, pH (1:2.5 water), and electrical conductivity (1:5 water) at 0-30cm depth before planting were 2.48g kg^{-1}, 9.23, 62.73 μS cm^{-1}, respectively. The field capacity was 12.77%. The geographical location of experimental site was shown in Figure 1.

Experimental layout. The experiment was arranged as a randomized split-plot design with four treatments, each treatment accommodated three plots, which were T1, T2, T3 and CK respectively (Figure 2a). Combinative application of DI and NF was performed. 70% of FC was regarded as an irrigation threshold maintaining plants normal growth, and plants were in regulated deficit irrigation once irrigation threshold was below 70% of FC. Therefore, four treatments with three replications were used for the trial, T1 was the combination of LDI (the highest irrigation amount) and LNF, T2 was the combination of MDI and MNF, and T3 was the combination of SDI (the lowest irrigation amount) and HNF. In addition, CK was designed as normal irrigation with no NF application. The specific schemes concerning DI and NF application were in following: T1, irrigated when SRWC (soil relative water content, similarly hereinafter) was below 60% of FC, and 54 kg N ha^{-1} was applied at the beginning of the blooming stage. T2, irrigated when SRWC was below 50% of FC, and 67.5 kg N ha^{-1} was applied at the beginning of the blooming stage. T3, irrigated when SRWC was below 40% of FC, and 81 kg N

FIGURE 1
Location of Naiman desertification research station in China.

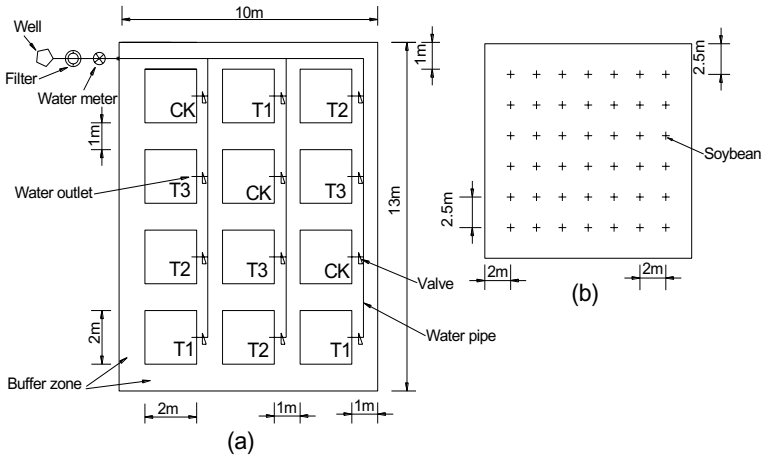


FIGURE 2
(a) The schematic diagram of plot layout;
(b) The enlarged view of plot, + presents sampling points.

ha^{-1} was applied at the beginning of the blooming stage. CK, irrigated when SRWC was below 70% of FC, and no NF was applied.

The irrigation system was composed of well, filter, water meter, water pipe and the valves. Water was applied with one pressure-compensated emitter, Groundwater for irrigation was measured continuously by the flowmeters, with a water-supplied pressure of 0.2MPa. All treatments received fully uniform irrigation at the seeding phase. DI started from the branching stage and the irrigation quota was 270 m^3 ha^{-1}, so each plot ($4m^2$) was irrigated by 27mm, and each treatment (three plots) was irrigated by 81mm totally when SRWC of every treatment was below each deficit irrigation threshold. Urea (containing 46% of N) was used as the NF and was applied in spade slits to avoid loss over the surface, and was sprinkled near the roots of soybean to assure it was fully absorbed by plants. FC and soil bulk density were measured before seeding, where FC was 12.77% and soil bulk density was 1.66 g cm^{-3}. Irrigation date, amount, and accumulation were presented in Table 1, in other growth periods of soybean, SRWC of all treatments were above the irrigation threshold, and need not to be irrigated.

Plant management. The size of every plot was 2m×2m, and the total experimental area was $130m^2$ (13m×10m). Soybean (*Glycine max* L. Merr.) cv 'Mengdou 33' was chosen as the tested cultivar and is widely planted in the experimental area. The experimental site had an east-west orientation and grapes were aligned north-south. Soybean density was 30 seeds m^{-2}, and seedlings were thinned to the final density of 21 seeds m^{-2}. Each plot consisted of seven rows of planted soybeans and six plants every row, the with row space of 2 m and spacing of 2.5 m between soybeans of each row (Figure 2b). The border rows of a plot were avoided in the sampling, to maximise accuracy. The spacing of 1m between each plot was provided for minimizing water movement among treatments, and a buffer channel of 1.0 m width was provided on the neighborhood of experimental fields to avoid edge effects. The field was plowed, harrowed in preparation for 6 days before sowing. Seeds were hand-planted on 27 April and harvested on 5 September in 2016, and weeds were removed manually from the inter-row spaces. Except the DI and NF, other agronomic measures, including pest control, potassium and phosphorous fertilization, were applied uniformly for each treatment as the local experiences.

TABLE 1
Irrigation date, irrigation amounts and irrigation accumulations each treatment.

Treatments	Irrigation threshold	Irrigation amounts(mm)										Irrigation
		June 25	Irrigation amount	July 3	Irrigation amount	July 10	Irrigation amount	July 17	Irrigation amount	July 30	Irrigation amount	Accumulations (mm)
T1	60% of FC	−	81	−	81	−	81	−	81	+	×	324
T2	50% of FC	+	×	+	×	−	81	−	81	+	×	162
T3	40% of FC	+	×	+	×	+	×	−	81	+	×	81
CK	70% of FC	−	81	−	81	−	81	−	81	−	81	405

Note: + refers to SRWC was above the irrigation threshold, and need not to be irrigated. − refers to SRWC was below the irrigation threshold, and need to be irrigated. × refers to no irrigation.

Measurement of soil water content (SWC). The SWC at 10, 20, 40, 60, and 80 cm depths of each plot were measured manually by the oven-dry method from branching to podding stages, and at added observation intervals for 7 days before and after irrigation. The specific sampling method was as follows: two points were selected randomly in each plot, soil in each point was sampled at 0-20 cm, 20-40 cm, 40-60 cm, and 60-80 cm. The soil layers were packed into aluminium specimen boxes, and put into the oven at 105°C for 12 h until constant weight was reached, and then the aluminium specimen box and dry soil weight were weighed. The SWC was calculated ultimately using the following formula:

SWC = (wet soil weight - dry soil weight) / dry soil weight ×100%

SRWC= SWC/FC×100%

Physiological (leaf-level) and morphological parameters. The gas exchange-related parameters of leaves (P_N; g_s; C_i; E) were measured simultaneously in the blooming to grain-filling stages at seven-day intervals for six random leaves per plot. On a sunny morning from 09:00 to 10:00 h, the fully expanded youngest leaves, totally exposed to sun, were selected and a portable photosynthesis system (LI-6400, Li-COR Inc., USA) was used for measurements. Instantaneous water use efficiency (LWUE) was calculated as P_N/E.

The rate at which the chamber pressure increased, for measurement of ψ_w, was too high, thereby influencing data accuracy. Therefore, measurement of increasing repetition times for each treatment (in the present study, each treatment was measured 18 times) minimized the error. The six youngest fully-expanded leaves (fourth to fifth node from the top of main stem) per plot were taken every two days during the grain-filling stage, wrapped immediately with aluminium foil, and placed in small polyethylene bags during cutting. The bags were then sealed and placed in dark containers with ice. using a pressure chamber at a pressurization rate of 0.05 MPa s^{-1} to determine ψ_w, the leaf petiole was sealed in the pressure chamber, the pressure reading was immediately recorded when the xylem sap appeared on the cut surface when the chamber was gradually pressurized.

Three soybeans growing well were tagged randomly in each plot for measuring LA of nine expanded fresh leaves and measuring SH from the ground surface. Leaf length and width were measured on the same day using a tape, at an interval of seven days. The regression of LA was following:

LA = k × leaf length × leaf width

Where, k is the shape coefficient, determined as 0.75.

Ultimately, the mean LA and SH from three repeated plots of every treatment was regarded as the LA and SH of four treatments, separately.

During the podding stage, every two days ten fully expanded fresh leaves and ten fresh pods from each plot were randomly chosen and were removed carefully from the petioles (pods were removed from stems) to minimize solute leakage on the cut surface, the entire leaf and pod had to be packed respectively into the valve bag immediately and weighed as the total leaf fresh mass (LFM) and pod fresh mass (PFM) of each plot. Thereafter, a water-containing plastic tube was used to place the fresh leaf and pod in a closed container, in which the air was saturated by keeping wet tissue paper around the inner wall to maintain high relative humidity. The turgid weight was measured after 24 h and dry weight were recorded as the total leaf dry mass (LDM) and pod dry mass (PDM) each plot after drying for 24 h in an oven at 80°C until constant weight. LRWC and PRWC were calculated by the following equation:

RWC (%) = (FM–DM) / (Turgid weight–DM) × 100.

Meanwhile, five fully expanded fresh leaves from each plot were taken from petioles (reserved petioles length 4-6 cm) at random, packed into the dark containers with ice for measuring ψ_w.

Statistical analysis. Data were expressed as means of the three replicates with standard errors, using SPSS software. The statistical differences

among treatments were evaluated by a one-way ANOVA and by the least significant difference (LSD) at the 5% probability level. The standard deviation (SD) among gas exchange-related parameters of treatments were analysed by Tukey's test ($p < 0.05$).

RESULTS

Soil water content. SWC lowered a minimum in the blooming stage, and increased rapidly from blooming to podding stages in the 0 to 40 cm and 0 to 80 cm soil layers (Figure 3*A*, 3*B*). The recovery timing of T3 lagged behind other treatments by approximately one week. SWC peaked at podding stage, in the subsequent stage, the indistinctive difference was confirmed between T3 and T1, T2. The average fluctuation levels of T1, T2, T3, and CK in the 0 to 40 cm soil layer were greater than those of the 0 to 80 cm soil layer (Figure 1*A,* 1*B*). SWC of CK realized the largest in the podding stage, and was significantly different compared to T3 ($p<0.05$).

Physiological (leaf-level) parameters. In stages from blooming to grain-filling, P_N and LWUE obviously increased in all treatments, while C_i and E fell significantly. The variation in P_N with T3 > T1 > T2 > CK was explored, and significant differences existed between T3 and T1, T2, as well as CK, respectively. The g_s of T1, T2 increased firstly from blooming to podding stage, then decreased obviously, which was the opposite of T3. However, the g_s in CK showed a successive decline. From blooming to grain-filling stage, all gas exchange-related parameters were greater in T1, T2, and T3 than in CK, which revealed a significant difference ($p<0.05$) compared to CK (Table 2).

During the blooming and podding stage, a remarkable difference ($p<0.05$) in P_N, g_s, and E was observed between T1, T2, T3 and CK, respectively. At the podding stage, the difference in g_s between T1 and T3 was significant ($p<0.05$). P_N, g_s and E in T3 were maximal at the grain-filling stage, and exhibited a significant extremely difference ($p<0.01$) in comparison to other treatments (Table 2).

RWC were smaller in T1, T2, and T3 than in CK, and had an extremely remarkable difference ($p<0.01$) compared to CK in LRWC (Figure 4*A*). A decrease was demonstrated in RWC of T1, T2, and T3, but there was no significant difference among them in the grain-filling stage (Figure 4). However, the extent of the decrease in LRWC was significantly higher than that of PRWC. The LRWC of CK significantly increased across the grain-filling stage, but PRWC decreased steadily (Figure 4*B*).

The ψ_w increased continuously at the grain-filling stage, yet the differences were not significant ($p>0.05$) among treatments (Figure 5). The decline in LRWC and PRWC of T1, T2, and T3 presented as above. Meanwhile, ψ_w and LRWC exhibited a positive closely linear correlation in T1, T2, and T3 (Figure 6), but the inversely proportional relationships was found between ψ_w and LRWC in CK.

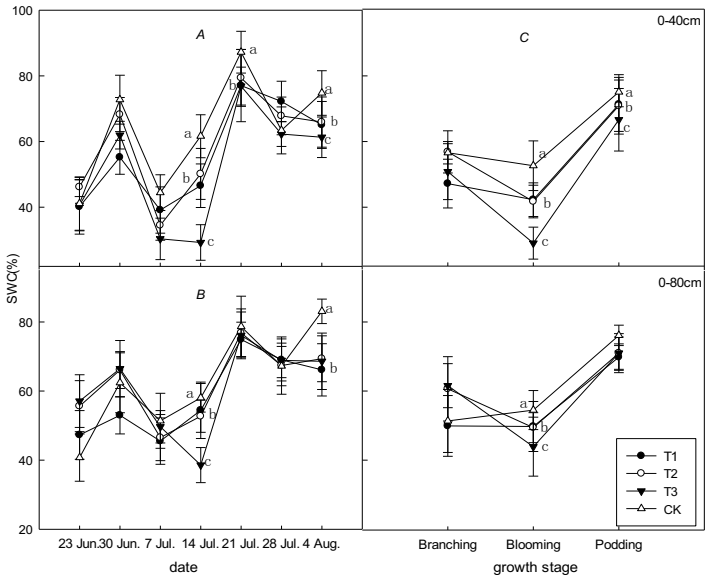


FIGURE 3
Mean SWC variations under DI and NF coupling at a depth of 0 to 40 cm (*A*), 0 to 80 cm (*B*) and from branching to podding stages (*C*).

The error bar represents the standard error.
T1: soybean was stressed at 60% of FC throughout the growing season, and 54 kg N ha^{-1} was applied at the beginning of the blooming stage.
T2: soybean was stressed at 50% of FC throughout the growing season, and 67.5 kg N ha^{-1} was applied at the beginning of the blooming stage.
T3: soybean was stressed at 40% of FC throughout the growing season, and 81 kg N ha^{-1} was applied at the beginning of the blooming stage.
CK: soybean was irrigated at 70% of FC throughout the growing season, and without NF application. DI: deficit irrigation. NF: nitrogen fertilization (the same below). SWC: soil water content.

TABLE 2
Effects of DI and NF coupling on gas exchange-related parameters in the blooming, podding, and grain filling stage.

Growth phases	Treatments	P_N (μmol m^{-2} s^{-1})	g_s (mmol(H_2O) m^{-2} s^{-1})	C_i (μmol(CO_2) mol^{-1})	E (mmol(H_2O) $m^{-2}s^{-1}$)	LWUE (mol(CO_2) mol(H_2O)$^{-1}$)
Blooming	T1	10.46 ± 3.79^{b}	0.71 ± 0.09^{a}	317.59 ± 13.31^{a}	12.96 ± 0.69^{a}	0.81 ± 0.26^{a}
	T2	9.23 ± 3.73^{b}	0.73 ± 0.12^{a}	322.25 ± 15.80^{a}	12.97 ± 0.53^{a}	0.71 ± 0.29^{b}
	T3	11.68 ± 3.15^{a}	0.77 ± 0.07^{a}	314.92 ± 9.60^{ab}	13.14 ± 0.81^{a}	0.89 ± 0.23^{a}
	CK	6.24 ± 3.01^{c}	0.48 ± 0.17^{b}	325.14 ± 8.42^{a}	10.63 ± 3.16^{b}	0.59 ± 0.11^{c}
Podding	T1	16.31 ± 5.65^{b}	1.56 ± 0.69^{a}	297.48 ± 35.91^{a}	9.51 ± 1.20^{a}	1.69 ± 4.67^{b}
	T2	15.11 ± 6.08^{b}	1.06 ± 1.99^{a}	294.41 ± 34.79^{a}	9.03 ± 2.53^{a}	1.66 ± 4.69^{b}
	T3	17.49 ± 4.12^{a}	0.53 ± 0.10^{b}	279.35 ± 12.26^{b}	9.67 ± 1.54^{a}	1.80 ± 4.55^{a}
	CK	11.48 ± 6.43^{c}	0.32 ± 0.22^{c}	276.55 ± 29.76^{c}	6.97 ± 3.40^{b}	1.61 ± 4.91^{b}
Grain - filling	T1	20.06 ± 4.35^{b}	0.45 ± 0.27^{b}	290.15 ± 51.98^{b}	7.96 ± 1.98^{b}	2.51 ± 0.61^{b}
	T2	18.84 ± 5.03^{c}	0.45 ± 0.33^{b}	274.26 ± 60.71^{c}	7.55 ± 2.85^{b}	2.50 ± 0.66^{b}
	T3	22.40 ± 4.22^{a}	0.66 ± 0.36^{a}	306.46 ± 51.38^{a}	9.96 ± 1.44^{a}	2.29 ± 0.65^{bc}
	CK	16.95 ± 8.04^{d}	0.26 ± 0.25^{c}	220.21 ± 40.74^{d}	5.25 ± 2.80^{c}	$3.18 \pm 0.53a$

C_i: intercellular CO_2 concentration. DI: deficit irrigation. E: the transpiration rate. g_s: stomatal conductance. LWUE: leaf water use efficiency. P_N: net photosynthetic rate (the same below).
Data are means ± SD (n=9) based on tukey's test (p<0.05). Difference between data of each column is denoted by the letter.

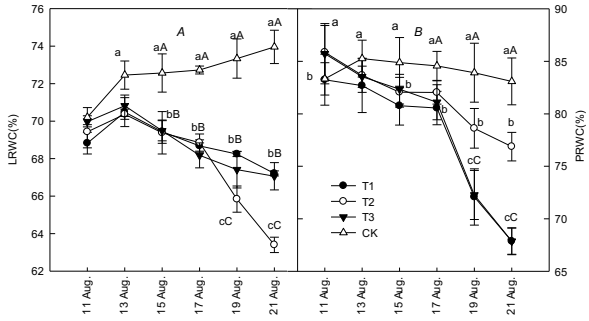


FIGURE 4
The effect of DI and NF coupling on LRWC (*A*) and PRWC (*B*) of soybean.
Error bars represent the standard error of means.
LRWC: leaf relative water content.
PRWC: pod relative water content.

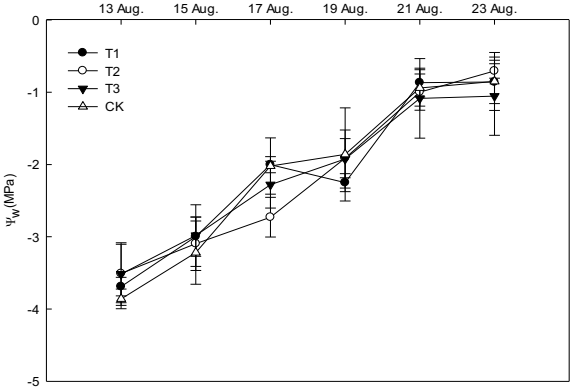


FIGURE 5
The effect of DI and NF coupling on the ψw of soybean.
Error bars represents the standard error of means.
ψ_w: leaf water potential.

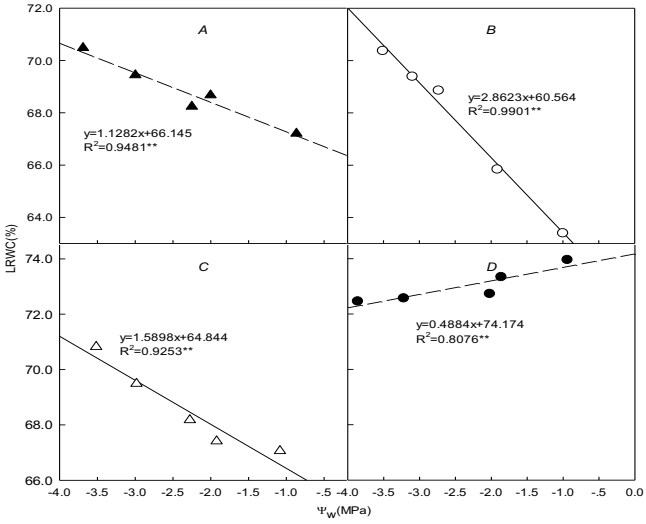


FIGURE 6
The relationships between ψw and LRWC of soybean under DI and NF coupling.
The (*A*), (*B*), (*C*)and (*D*) represents respectively the T1, T2, T3, and CK.
The P-values are denoted as $^{**}P < 0.01$.

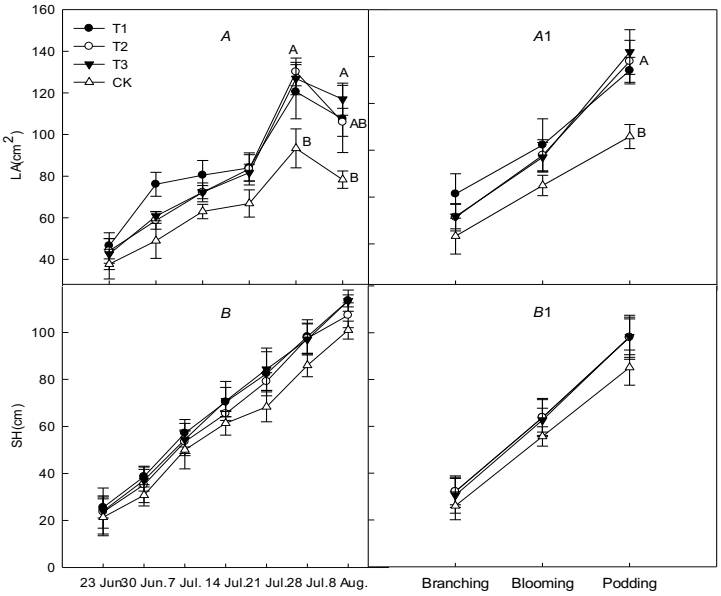


FIGURE 7

The effects of DI and NF coupling on LA (*A*) and SH (*B*) of soybean.

Error bars represent the standard error of means.
The (*A*1) and (*B*1) represents separately the LA and SH variation in stage of branching to podding.
The bar represents the standard error.
LA: leaf area. SH: plant height.

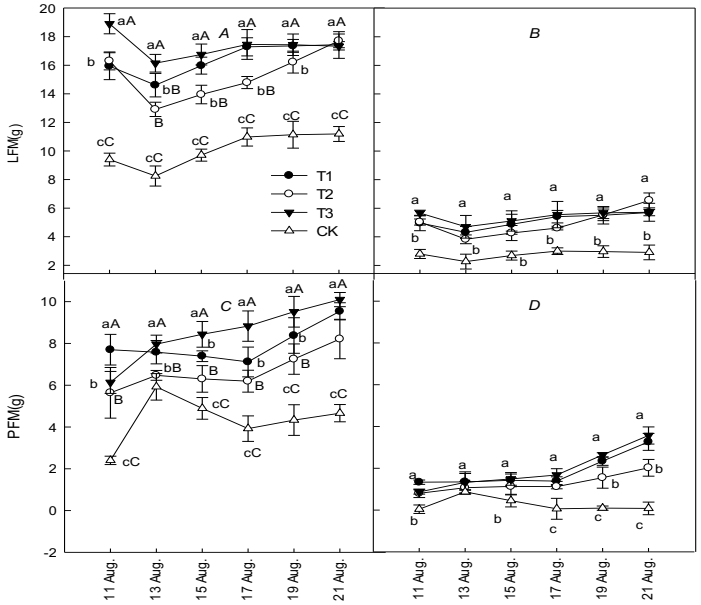


FIGURE 8

The effect of DI and NF coupling on LFM (*A*), LDM (*B*), PFM (*C*) and PDM (*D*) of soybean at the grain-filling stage.

Error bars represent the standard error of means.
LDM: leaf dry mass. LFM: leaf fresh mass.
PDM: pod dry mass. PFM: pod fresh mass.

Morphological parameters. Taken together, average SH and LA increased continuously by high growth rate until the podding stage (Figure 7). From branching to podding stages, the average SH and LA was in line with T1>T3>T2>CK (Figure 7*A*1,7*B*1). Differences among T1, T2, and T3 were unremarkable. In contrast, the extremely significant differences (p<0.01) appeared between T1, T2, T3 and CK, which continued to increase across growth phase. Although the LA and the SH of T3 were lower than that of T1, they increased rapidly by 58.37% and 56.90%, respectively, and were ultimately higher than that in other treatments after the flowering stage. On average, the LA and the SH of T1, T3 increased by 32.69%, 16.05%, 29.10%, 14.93%, respectively in comparison to CK.

LFM, PFM, LDM, and PDM at the grain-filling stage increased steadily, corresponding with T3 > T1 > T2 > CK (Figure 8). Highly significant differences (p<0.01) were found between T1, T2, T3 and CK, while T3 and T2 were significantly different (p<0.05). The average LFM of T3 was higher than that of CK, T1, and T2, by 71.43% (p<0.01), 7.01%, and 13.16% (p<0.05), respectively (Figure 6*A*). Meanwhile, average PFM in T3, T1 increased by 95.24%, 82.65%, respectively, compared to CK (Figure 8*C*).

DISCUSSION AND CONCLUSIONS

Water stress is regarded as one of most vital abiotic indices limiting photosynthesis, thereby affecting plant distribution and yield [28], and soybean is considered to be highly susceptible to water shortage. In addition, plant physiological, morphological parameters and growth are remarkably affected by either the application or efficiency associated with mineral nutrients, wherein nitrogen requirements remain the highest for plants [29], primarily soybean.

Effects of the three extreme DI and NF couplings on gas exchange-related parameters in soybean. Compared with NI (70% of FC) without NF, photosynthesis was improved in the DI and NF coupling, which was attributed to both stomatal and non-stomatal limitations (Table 2). When HNF (81 kg N ha^{-1}) was applied at the blooming stage in SDI (40% of FC), photosynthesis in soybean improved significantly [30] and reached a maximal peak, compared to MDI and LDI. However, during the stage from flowering to grain-filling, photosynthesis and LWUE increased, C_i and E continuously declined.

Nitrogen primarily exists in leaves. There is generally a close relationship between nitrogen and photosynthesis [31]. The P_N decreased with the reduction in NF amount applied [32], which was directly associated with stomatal opening and closure [33]. NF supply in WS would enhance drought resistance in plants by reducing stomatal density and E [34]. Based on SDI (40% of FC) with HNF level (81 kg N ha^{-1}), photosynthetic capacity was influenced by non-stomatal factors, because reduction in stomatal conductance, C_i, and E were contrary to P_N (Table 2). The decrease in internal CO_2 availability and photosynthesis in WS with no NF was responsible for the reduction in stomatal conductance [35]. Hence, NF was determined as a non-stomatal factor improving P_N, resulting from the lowest SWC and maximal NF application in the current phase. This conclusion was expressed as the strongest photosynthetic capacity at the SDI level (40% of FC) with HNF (81 kg N ha^{-1}) application, and was in accordance with the study that photosynthesis can be maximised by optimal distribution of nitrogen [36]. However, elevated CO_2 was also found to enhance leaf photosynthesis in soybean [37], and the reason for the reduction in P_N under WS was the decreased CO_2 supply through the stomata to the mesophyll cells [38]. Moreover, under LDI (60% of FC), MDI (50% of FC) from blooming to grain-filling stage, the invariable decline in C_i, E, and the non-significant differences in SWC between the two conditions, resulted in the increase in photosynthesis, which was caused by the nonstomatal factor, e.g. NF (Figure 3, Table 2), and previous studies showed that nitrogen deficiency decreases photosynthesis [39]. As shown above, optimal availability of fertilization is highly correlated with the specific stage that demands nutrients [40].

Effects of the three extreme DI and NF coupling on VG and RG in soybean. Previous research has suggested that abiotic or biotic constraints strongly determine the growth response to nitrogen in soybean, wherein high P_N and abundant accumulation of nitrogen in seeds are necessary to maximize VG. WS, as a vital abiotic element, critically affected growth and DM [41] by earlier blooming [42], flower or pod abortion, and reduction in seed number and mass [43]. What's more, leaf area index and biomass of plants are affected significantly by temporary water and nitrogen shortage [44]. In our study, the DI and NF coupling was observably beneficial to morphological parameters, VG, RG, and DM in soybean. SH, LA, LM, and PM were prominently higher than that of NI (70% of FC) (Figure 7, 8). VG of soybean was maximal under LDI (60% of FC) with LNF (54 kg N ha^{-1}) application, which appeared to be an insignificant difference compared with that of MDI and SDI. Under SDI (40% of FC) with HNF (81 kg N ha^{-1}) application, RG and DM were maximal (Figure 3). Hence, in contrast with the conventional water management methods in fields, the present study achieved growth and development of soybean and higher use efficiency of water and nitrogen, corresponding with the result that much lower amounts of water and nitrogen were required for VG and RG [45]. Mycorrhizal plants often show significantly higher LRWC in NI than in DI (Figure 2). On one hand, soybean growth required adequate water after NI; on the other hand, loss of water in leaves reduced because g_s decreased, leading to a decrease in E from blooming to grain filling stage. Due to the water consumption in VG and RG stages, LRWC reduced gradually under DI, resulting in a decrease of leaf transpiration. The magnitude of the decrease in LRWC was significantly higher than that in PRWC. In addition, the RWC and ψ_w were used as the direct measures of the plant water status [46], wherein ψ_w was a well-established variable measuring productivity and the plant water content for irrigation scheduling [47]. This was consistent with our study that there were significant linear correlations between LRWC and ψ_w, indicating that LRWC was closely associated with ψ_w, and ψ_w increased with the

decrease in LRWC (Figure 6) under DI. The increase of ψ_w across the growth stages was mainly attributed to SWC increasing from blooming stage (Figure 3), but the decline of LRWC was responsible for NF application, which enhanced P_N and E (Table 2), as well as VG and RG (Figure 7, 8), which consumed large quantities of water, eventually leading to a great improvement in WUE. This was inconsistent with a previous conclusion that ψ_w was decreased by NF supply and was sensitive to stomatal change [48]. The ψ_w represented an increasing characteristic in NI level (70% of FC) and was proportional to changes in LRWC. This could likely due to lower water consumption with high LRWC, resulting from low P_N, E (Table 2) and slow VG, RG (Figure 7,8). Under DI, the roots produced chemical signals, such as increased abscisic acid concentration and pH of xylem sap, which transported to the leaf through the transpiration stream, regulating stomatal opening and leaf growth [49]. Although VG and RG mainly depend on the photosynthetic capacity, there have been numerous correlations between photosynthesis, VG and RG [50]. In present study, enhancement of leaf photosynthetic capacity led to higher DM of soybean, owing to improvement in P_N and new branch contributed to nitrogen [51], and LWUE increased observably, which was advantageous to the VG and RG of soybean. The SH and LA were increasingly higher in the DI and NF combination than in NI (70% of FC), because growth-promoted efficiency increased across the growth stages.

Photosynthesis is an essential physiological process for DM [52]. Noteworthy interaction effects of water and fertilization on photosynthesis have been demonstrated [53]. LA is associated with leaf growth, which would increase by improved photosynthesis, resulting from the application of NF [54]. Averagely, LA increased linearly across the growth stages, and presented maximal growth rate from blooming to podding stage (Figure 7*A*1), but LA reduced from the podding stage (Figure 7*A*), which was likely due to the transformation from VG to RG. Average SH and LA were less in SDI (40% of FC) than in LDI (60% of FC), which may be mainly attributed to the postponement of SWC recovery at the blooming stage, but they increased rapidly beginning from the late blooming stage, resulting from the nitrogen maximum in SDI (40% of FC) from blooming to podding stages, confirming the crucial role of NF for VG (Figure 7). The LM and PM also significantly increased after combined application of DI and NF. However, they decreased when soybean was exposed to DI alone at the blooming stage [55]. During the entire growth cycle after irrigation on 17 July, no remarkable differences in SWC were observed among the three levels of DI, but NF and photosynthesis under SDI (40% of FC) were greater than that in LDI (60% of FC), which provided nutrients for VG and RG of soybean. Ultimately, LFM, LDM, PFM, and PDM of soybean under SDI (40% of FC) were higher than that under LDI (60% of FC) at the grain-filling stage, and at the beginning stage of maturity. The percentage of DM in soybean reached higher than 30% [56]. Certainly, senescence, insect damage, and freeze injury can also influence LDM, as well as the nitrogen mobilization and water availability. However, the percentage of DM during different growth stages was beyond the scope of this study. This needs to be elucidated in future studies.

In summary, improvement in gas exchange-related parameters (P_N, g_s, C_i, and E) of soybean could be achieved, induced by the DI and NF coupling. The maximal P_N occurred when HNF (81 kg N ha^{-1}) was applied during the blooming stage in SDI (40% of FC). Emergence of higher VG and RG were also attributed to the interactive effects of DI and NF, in comparison to single NI. Among those, the greatest VG could be responsible to LDI (60% of FC) with LNF (54 kg N ha^{-1}). When soybean was exposed to SDI (40% of FC), and adding the highest NF amount (81 kg N ha^{-1}) at the beginning of the blooming stage in the scope of deficit irrigation (SRWC was below 70% of FC), maximal RG and DM were attained, whereas LRWC and PRWC were lower in DI than in NI (70% of FC). These results suggested that from the perspective of water-saving, highest reproductive growth of soybean could be also attained by increasing deficit level and adding more nitrogen fertilizer in the blooming stage. Certainly, maximizing the use efficiency of nitrogen fertilizer via irrigating highest water is capable of attaining the peak of vegetative growth.

ACKNOWLEDGEMENTS

We are grateful to all the members of Naiman Desertification Research Station, Chinese Academy of Sciences, for their help in field work. The financial support provided by the National Natural Science Foundation of China (41371053). We are also grateful to other anonymous reviewers for their valuable comments on the manuscript.

REFERENCES

[1] Rosenthal, A., Pyle, D.L., Niranjan, K., Gilmour, S., Trinca, L. (2001) Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. Enzyme Microb Technol. 28, 499-509.

[2] Zhao, H.L., Zhao, X.Y., Zhou, R.L., Zhang, T.H., Drake, S. (2005) Desertification processes due to heavy grazing in sandy rangeland, Inner Mongolia. J. Arid. Environ. 62, 309-319.

[3] Pieters, A.J., El, S.S. (2005) Effects of drought during grain filling on PS II activity in rice. Plant Physiol. 162, 903-911.

[4] Chastain, D.R., Snider, J.L., Collins, G.D., Perry, C.D., Whitaker, J., Byrd, S.A. (2014) Water deficit in field-grown *Gossypium hirsutum* primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. Plant Physiol. 171, 1576-1585.

[5] Lokhande, S., Reddy, K.R. (2014) Reproductive and fiber quality responses of upland cotton to moisture deficiency. Agron. 106, 1060-1069.

[6] Rhine, M.D., Stevens, G., Shannon, G., Wrather, A., Sleper, D. (2010) Yield and nutritional responses to waterlogging of soybean cultivars. Irrig. Sci. 28, 135-142.

[7] Farré, I., Faci, J.M. (2006) Comparative response of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench.) to deficit irrigation in a Mediterranean environment. Agric. Water Manag. 83, 135-143.

[8] Geerts, S., Raes, D., Garcia, M., Miranda, R., Cusicanqui, J.A., Taboada, C., Mendoza, J., Huanca, R., Mamani, A., Condori, O., Mamani, J., Morales, B., Osco, V., Steduto, P. (2009) Simulating yield response of quinoa to water availability with AquaCrop. Agron. 101, 499-508.

[9] Hetherington, A.M., Woodward, F.I. (2003) The role of stomata in sensing and driving environmental change. Nature. 424, 901-908.

[10] Hessini, K., Ghandour, M., Albouchi, A., Soltani, A., Werner, K.H., Abdelly, C. (2008) Biomass production, photosynthesis, and leaf water relations of *Spartina alterniflora* under moderate water stress. J. Plant Res. 121, 311-318.

[11] Chakhchar, A., Lamaoui, M., Wahbi, S., Ferradous, A., Mousadik, A.E., Ibnsouda-Koraichi, S. (2015) Leaf water status, osmoregulation and secondary metabolism as a model for depicting drought tolerance in *Argania spinosa*. Acta Physiol. Plant. 37, 80.

[12] Allakhverdiev, S.I., Nishiyama, Y., Miyairi, S., Yamamoto, H., Inagaki, N., Kanesaki, Y. (2002) Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis*. Plant Physiol. 130, 1443-1453.

[13] Zhu, X.C., Song, F.B., Xu, H.W. (2010) Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil. 331, 129-137.

[14] Shimshi, D. (2010) The effect of nitrogen supply on some indices of plant-water relations of beans (*Phaseolus vulgaris* L.). New Phytol. 69, 413-424.

[15] Thies, J.E., Singleton, P.W., Bohlool, B.B. (1995) Phenology, growth, and yield of field-grown soybean and bush bean as a function of varying modes of N nutrition. Soil Biol. Biochem. 27, 575-583.

[16] Hirasawa, T., Ozawa, S., Taylaran, R., Ookawa, T. (2010) Varietal differences in photosynthetic rates in rice plants, with special reference to the nitrogen content of leaves. Plant Prod. Sci. 13, 53-57.

[17] Janusauskaite, D., Feiziene, D., Feiza, V. (2017) Nitrogen-induced variations in leaf gas exchange of spring triticale under field conditions. Acta Physiol. Plant. 39, 193.

[18] Cabrerabosquet, L., Albrizio, R., Araus, J.L., Nogués, S. (2009) Photosynthetic capacity of field-grown durum wheat under different N availabilities: A comparative study from leaf to canopy. Environ. Exp. Bot. 67,145-152.

[19] ŽIvčák, M., Olšovská, K., Slamka, P., Galambošová, J., Rataj, V., Shao, H.B. (2014) Application of chlorophyll fluorescence performance indices to assess the wheat photosynthetic functions influenced by nitrogen deficiency. Plant Soil Environ. 60, 210-215.

[20] Zong, Y.Z., Shangguan, Z.P. (2014) Nitrogen deifciency limited the improvement of photosynthesis in maize by elevated CO_2 under drought. Integr. Agric. 13, 73-81.

[21] Mastalerczuk, G., Borawska-Jarmułowicz, B., Kalaji, H.M., Dąbrowski, P., Paderewski, J. (2017) Gas-exchange parameters and morphological features of festulolium (*Festulolium* braunii K. Richert A. Camus) in response to nitrogen dosage. Photosynthetica. 55, 20-30.

[22] Gan, Y., Stulen, I., Keulen, H.V., Kuiper, P.J.C. (2004) Low concentrations of nitrate and ammonium stimulate nodulation and N_2 fixation while inhibiting specific nodulation (nodule DW g^{-1} root dry weight) and specific N_2 fixation (N_2 fixed g^{-1} root dry weight) in soybean. Plant Soil. 258, 281-292.

[23] Barker, D.W., Sawyer, J.E. (2005) Nitrogen application to soybean at early reproductive development. Agron. 97, 615-619.

[24] Donnison, I.S., Gay, A.P., Thomas, H., Edwards, K.J., Edwards, D., James, C.L., Thomas, A.M., Ougham, H.J. (2007) Modification of nitrogen remobilization, grain fill and leaf senescence in maize (*Zea mays*) by transposon insertional mutagenesis in a protease gene. New Phytol. 173, 481-494.

[25] Huang, Y., Tang, Y.H. (2010) An estimate of greenhouse gas (N_2O and CO_2) mitigation potential under various scenarios of nitrogen use efficiency in Chinese croplands. Global Change Biol. 16, 2958-2970.

[26] Hocking, P.J., Randall, P.J., Pinkerton, A. (1987) Sulphur nutrition of sunflower (*Helianthus annuus*) as affected by nitrogen supply. Effects on vegetative growth, development of yield components and seed yield and quality. Field Crop. Res. 16, 157-175.

[27] Ashworth, A.J., Taylor, A.M., Reed, D.L., Allen, F.L., Keyser, P.D., Tyler, D.D. (2015) Environmental impact assessment of regional switchgrass feedstock production comparing nitrogen input scenarios and legume-intercropping systems. J. Clean. Prod. 87, 227-234.
[28] Hossinzadeh, S.R., Salimi, A., Ganjeali, A., Ahmadpour, R. (2014) Effects of foliar application of methanol on photosynthetic characteristics, chlorophyll fluorescence and chlorophyll content of chickpea (*Cicer arietinum L.*) under ought stress. J. Urology. 181, 558-558.
[29] Cai, Z., Cai, C., Qi, X., Yao, T. (2004) Effects of fertilization on the growth, photosynthetic characteristics and yield of Coffea arabica. Appl. Ecol. 15, 1561-1564. (In Chinese).
[30] Cechin, I., Tdef, F. (2004) Effect of nitrogen supply on growth and photosynthesis of sunflower plants grown in the greenhouse. Plant Sci. 166, 1379-1385.
[31] Hikosaka, K. (2014) Optimal nitrogen distribution within a leaf canopy under direct and diffuse light. Plant Cell Environ. 37, 2077-2085.
[32] Cruz, J.L., Mosquim, P.R., Pelacani, C.R., Araujo, W.L., Damatta, F.M. (2003) Carbon partitioning and assimilation as affected by nitrogen deficiency in cassava. Photosynthetica. 41, 201-207.
[33] Karimi, G., Ghorbanli, M., Heidari, H., Nejad, R.A.K., Assareh, M.H. (2005) The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrata*. Biol. Plantarum. 49, 301-304.
[34] Tesha, A.J., Kumar, D. (1978) Effect of fertilizer nitrogen on drought resistance in *Coffea arabica* L. Agric. Sci. 90, 625-631.
[35] Plauborg, F., Abrahamsen, P., Gjettermann, B., Mollerup, M., Iversen, B.V., Liu, F.L. (2010) Modelling of root ABA synthesis, stomatal conductance, transpiration and potato production under water saving irrigation regimes. Agric. Water Manage. 98, 425-439.
[36] Connor, D.J., Sadras, V.O., Hall, A.J. (1995) Canopy nitrogen distribution and the photosynthetic performance of sunflower crops during grain filling - A quantitative analysis. Oecologia. 101, 274-281.
[37] Mitchell, R.A.C., Black, C.R., Burkart, S., Burke, J.I., Donnelly, A., de, Temmerman, L., Fangmeier, A., Mulholland, B.J., Theobald, J.C., Oijen, M. (1999) Photosynthetic responses in spring wheat grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. Eur. J. Agron. 10, 205-214.
[38] Hirasawa, T., Iida, Y., Ishihara, K. (1989) Dominant factors in reduction of photosynthetic rate affected by air humidity and leaf water potential in rice plants. Jpn. J. Crop Sci. 58, 383-389.
[39] Li, G., Wan, S., Zhou, J., Yang, Z.Y., Pei, Q. (2010) Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. Ind. Crops and Prod. 31, 13-19.
[40] Lobell, D.B. (2007) The cost of uncertainty for nitrogen fertilizer management: A sensitivity analysis. Field Plant. Res. 100, 210-217.
[41] Rampino, P., Pataleo, S., Gerardi, C., Mita, G., Perrotta, C. (2006) Drought stress response in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environ. 29, 2143-2152.
[42] Desclaux, D., Roumet, P. (1996) Impact of drought stress on the phenology of two soybean (*Glycine max* L. Merr.) cultivars. Field Plants Res. 46, 61-70.
[43] Kokubun, M., Shimada, S., Takahashi, M. (2001) Flower abortion caused by preanthesis water deficit is not attributed to impairment of pollen in soybean. Crop Sci. 41, 1517-1521.
[44] Savić, S., Stikić, R., Radović, B.V., Bogičević, B., Jovanović, Z. (2008) Comparative effects of regulated deficit irrigation (RDI) and partial root-zone drying (PRD) on growth and cell wall peroxidase activity in tomato fruits. Sci. Hortic. 117, 15-20.
[45] Sun, Y., Hu, K.L., Fan, Z.B., Wei, Y.P., Lin, S., Wang, J.G. (2013) Simulating the fate of nitrogen and optimizing water and nitrogen management of greenhouse tomato in North China using the EU-Rotate N model. Agric. Water Manag. 128, 72-84.
[46] Jones, H.G. (2007) Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. Exp. Bot. 58, 119-130.
[47] Chastain, D.R., Snider, J.L., Choinski, J.S., Collins, G.D., Perry, C.D., Whitaker, J. (2016) Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. Plant Physiol. 199, 18-28.
[48] Saneoka, H., Moghaieb, R.E.A., Premachandra, G.S., Fujita, K. (2004) Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris huds*. Environ. Exp. Bot. 52, 131-138.
[49] Thompson, D.S., Wilkinson, S., Bacon, M.A., Davies, W.J. (1997) Multiple signals and mechanisms that regulate leaf growth and stomatal behaviour during water deficit. Physiol. Plant. 100, 303-313.
[50] Silva, E.A., Damatta, F.M., Ducatti, C., Regazzi, A.J., Barros, R.S. (2004) Seasonal changes in vegetative growth and photosynthesis of Arabica coffee trees. Field Crops Res. 89, 349-357.

[51] Nazareno, R.B., Oliveira, C.A.D.S., Sanzonowicz, C., Sampaio, J.B.R., Silva, J.C.P.D., Guerra, A.F. (2003) Crescimento inicial do cafeeiro Rubi em resposta a doses de nitrogênio, fósforo e potássio e a regimes hídricos. (Initial growth of Rubi coffee plant in response to nitrogen, phosphorus and potassium and water regimes.) Pesqui. Agropecu. Bras. 38, 903-910.
[52] Qiu, Z., Wang, L., Zhou, Q. (2013) Effects of bisphenol A on growth, photosynthesis and chlorophyll fluorescence in above-ground organs of soybean seedlings. Chemosphere. 90, 74-80.
[53] Cai, C.T., Cai, Z.Q., Yao, T.Q., Qi, X. (2007) Vegetative growth and photosynthesis in coffee plants under different watering and fertilization managements in Yunnan, SW China. Photosynthetica. 45, 455-461.
[54] Peng, Y., Li, C., Fritschi, F.B. (2014) Diurnal dynamics of maize leaf photosynthesis and carbohydrate concentrations in response to differential N availability. Environ. Exp. Bot. 99, 18-27.
[55] Abayomi, Y.A. (2008) Comparative growth and grain yield responses of early and late soybean maturity groups to induced soil moisture stress at different growth stages. World J. Agric. Sci. 4, 71-78.
[56] Kawamoto, H., Touno, E., Uchino, H., Uozumi, S. (2013) Comparison of fermentation quality and ruminal degradability between two different harvest timings of forage soybean (Glycine max L. Merr.) ensiled with the corn-silage system. Grassl. Sci. 59, 120-123.

Received: **17.06.2018**
Accepted: **12.10.2018**

CORRESPONDING AUTHOR

Tonghui Zhang
Northwest Institute of Eco-Environment
and Resources,
Chinese Academy of Sciences,
Lanzhou – China

e-mail: zhangth@lzb.ac.cn

© by PSP Volume 27 – No. 12B/2018 pages 9840-9850 Fresenius Environmental Bulletin

ARTIFICIAL NEURAL NETWORKS AS NEW ALTERNATIVE METHOD TO ESTIMATING SOME POPULATION PARAMETERS OF TIGRIS LOACH (*OXYNOEMACHEILUS TIGRIS* (HECKEL, 1843)) IN THE KARASU RIVER, TURKEY

Ebru Ifakat Ozcan*, Osman Serdar

Munzur University, Faculty of Fisheries, Tunceli, Turkey

ABSTRACT

In this study, a new method (Artificial Neural Networks) to estimating some population parameters of Tigris loach (*Oxynoemacheilus tigris* (Heckel, 1843)) collected between 2014 and 2015 in 14 different stations from Karasu River (East Anatolia, Turkey). The total length and weight of the species ranged from 62 to105 mm and from 1.7 to 10.9 g for females and 65 to 100 mm and from 2.4 to 9.8 g for males, respectively. The new maximum length for *O. tigris* was found to be 105 mm in this study. The length-weight relation of *O. tigris* was carried out with artificial neural networks (ANNs) and length-weight relationships (LWRs). The results obtained were compared. Length-weight relationships of *O. tigris* were found as $W=0.0049*L^{3.29}$, $R^2=0.95$ for all individuals. According to these values, the growth type of this species was positive allometric (b>3) for all individuals. Regression analysis showed that fish length has high significant correlation with weight ($R=0.97$, $R^2=0.95$, $F_{1,185}=31930.3$, $P<0.001$). According to the comparison of the results obtained with MAPE (%), ANNs provide better results than the LWRs. The results of this study could give useful insight that ANNs can be evaluated as an alternative for length-weight relation estimation. The condition factor values (min-max) were observed as 0.680-1.239 for females and 0.699-1.074 for males. No studies have been done before on this species population parameters.

KEYWORDS:
Artificial neural networks, *Oxynoemacheilus tigris*, an alternative method, length-weight relation, Karasu River

INTRODUCTION

Nemacheilid loaches of the genus *Oxynoemacheilus* are common fishes all over the Middle East. There are 58 available species-group names and 41 species are recognized here as valid [1]. *Oxynoemacheilus tigris*, the Tigris loach, is a species of stone loach from the genus *Oxynoemacheilus* [2]. This critically endangered species is endemic to the Queiq River in Turkey where it occurs ins a short stretch of stream between two reservoirs. It formerly occurred in Syria but it has been local extinction from the Syrian portion of the Queiq. This species is threatened by water abstraction and the increased frequency of droughts caused by climate change, most of the Queiq has already been desiccated. It is, however, abundant in the area it is known from where it can be found in reaches of gravel or mud substrate with moderately fast flowing to near standing water [3]. Also *Oxynoemacheilus tigris* was recorded from Kapozik Kadur Hakkari, upper Tigris drainage [4].

ANNs have been used in differ branches of aquatic science and biology more than other sciences [5]. ANNs models estimate the distributions of demersal fish species [6], estimating the water quality and monthly biologicial oxygen demand [7], forecasting streamflow data [8], determining some morphologicial characterictics of crayfish [9], estimating the existences of small fish in a river [10], estimating aquatic macro-invertebrate varieties [11], predicting population dynamics of aquatic insects [12], estimating and spatially mapping freshwater fish and assemblies of decapods [13] and forecasting population dynamics of fish species [14, 15]. Many authors reported that ANNs gave better results than other methods [5, 15-18]. LWRs methods used in many scientific studies may be insufficient for scientific work [6]. ANNs is an alternative method for other methods in non-linear situations for predict modeling [13].

This study is the first record ANNs as a new and alternative approach to predicting basic biologi-

cal characteristics for *O. tigris* in Karasu River. Predicted and observed values are compared by Mean Absolute Percent Error (%).

MATERIALS AND METHODS

Study area. The study area, which is in the tributary of Karasu River (Yeşildağ stream (40º08'13"N 41º25'49"E), Yeşildere stream (40º08'21"N 41º24'25"E), Köşk stream (40º05'45"N 41º24'48"E), Ağasuyu stream (39º59'35'N 41º08' 56"E), Sincan stream (39º59'40"N 41º07'21"E), Çiğdemli stream (39º58'18"N 41º01'23"E), Han stream (39º56'53"N 40º46'08"E), Taşağıl stream (39º57'44"N 40º34'40"E), Karataş stream (39º56' 13"N 40º07'51"E), Büyükgözenin stream (39º56' 39"N 40º15'03"E), Deliçay stream (39º38'08"N 39º20'18"E), Karnı stream (39º40'24"N 39º13'34"E), Eriç stream (39º30'36"N 38º53'14"E) and Kırık stream (39º29'23"N 38º44'37"E)) (Figure 1).

Sampling/Data collection. Fish samples were transported to the laboratory and fixed with 5% formaldehyde. Fish samples were measured for total length, (TL, in mm) fork length (FL, in mm) and standard length (SL, mm) and total weight (W, in g) and sexes determined by macroscopic observation of gonads; sex ratios were checked with a chi-square test as to whether the ratio differed from 1:1.

Length-weight relation (LWR). The total length-weight relationship was calculated using the equation: $W = a*L^b$, where W is weight (W), L is total length (TL), a is the intercept, and b is the slope. The degree of association between the variables was computed by the determination coefficient, R^2 [19].

Length-length relation (LLR). Length-length relationships were calculated using linear regression analysis. LLRs were measured as FL=a+bSL, SL=a+bTL and TL=a+bFL equations in all individuals [20].

Condition Factor (CF). The condition factor values of fish are obtained with this formula:
$CF = (W / TL^b).100$
Where W is total weight; TL is total length and b is the coefficient of allometric of relationship [21].

All data were formed with statistic analyses using the Excel 2013 and IBM SPSS package version 24 for Windows.

Artificial Neural Networks (ANNs). Artificial neural networks (ANNs) are biologically inspired computer programs designed to adapt the way the human brain processes information. ANNs add their information by determining the patterns and relationships in data and learn (or are trained) through experiment, not from coding. An ANN is consist of hundreds of single units, artificial neurons or processing elements (PE), related to factors (weights), which create the neural structure and are organized in layers. The power of neural computations turns up linking neurons in a network. The behavior of a neural network is designated by the transfer process of its neurons, by the learning rule, and by the architecture itself. The weights are the regulatable parameters and, in that sense, a neural network is a parameterized system. The sum of the weights of inputs composes the activation of neurons. The activation signal is taken place transfer function to generate a single output of the neuron. Transmission process introduces non-linearity to the network. Throughout the course of the training, the connections are optimized until you reach the minimum and the best accuracy of the error. After the network is trained and tested, new data for output can be displayed [22].

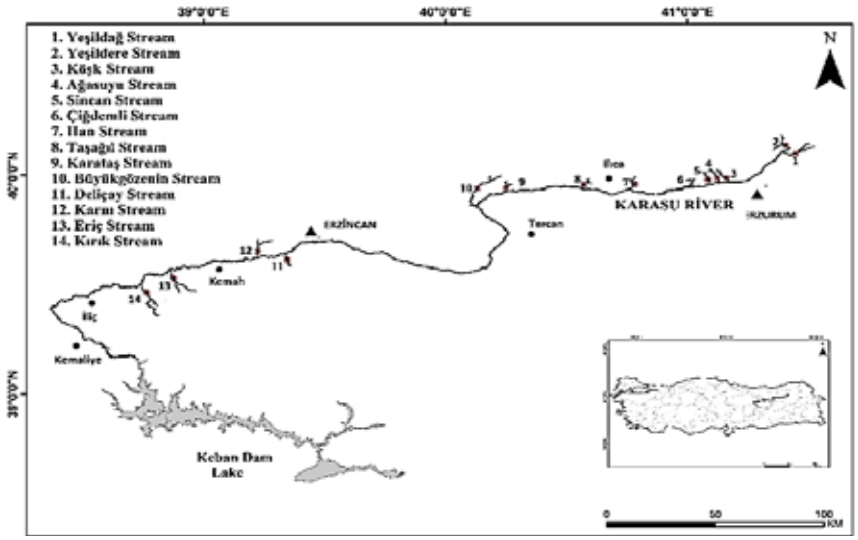


FIGURE 1
Sampling stations of the East Anatolia (Karasu River).

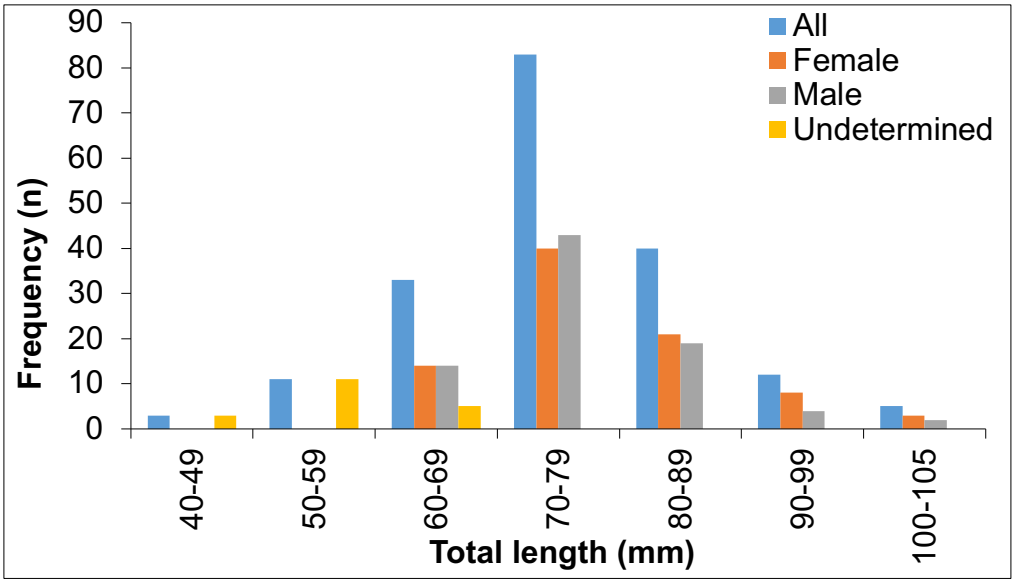


FIGURE 2
The total length–frequency distribution by sex of *O. tigris* in the Karasu River

This model is an interrelatedness group of artificial neurons that process information in parallel. Usually, an ANN is an adaptive system that changes its structure based on external or internal information that flows through the network during the learning phase. Briefly summarized, neural networks are nonlinear, statistical, data-modeling tools used for modeling complex relationships between inputs and outputs, or to find patterns in data [23]. Basically, there are 3 different layers in a neural network:

1. Input Layer (All the inputs are fed in the model through this layer)
2. Hidden Layers (There can be more than one hidden layers which are used for processing the inputs received from the input layers)
3. Output Layer (The data after processing is made available at the output layer) [24].

The mathematical equation of the neuron model is seen in equation.

$$y(k) = F\left(\sum_{i=0}^{m} wi(k).xi(k) + b\right) \text{[25].}$$

- $yi(k)$ is output value in discrete time k,
- F is a transfer function,
- $wi(k)$ is weight value in discrete time k where i goes from 0 to m,
- $xi(k)$ is input value in discrete time k where i goes from 0 to m,
- b is bias

MAPE was used to compare ANNs and other methods. The smaller the MAPE values, the closer are the predicted values to the actual values [17]. MAPE is as follows equality:

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{ei}{Yi}\right| x100 \ldots\ldots\ldots\ldots$$

Yi=the actual observation value, *ei*=the difference between the actual value and the prediction value, *n*=the number of total observations.

Neural Network Toolbox of MATLAB (Ver R2016a) was used for ANNs calculations. Samples (187 *O. tigris*) were caught between 2014 and 2015 by electroshocker from Karasu River. Processes in MATLAB are made up of three parts. These are "training", "testing", and "validation". They were used randomly: 70% in training, 15% in testing, and 15% in the validation [18].

RESULTS

A total of 187 individuals of *O. tigris* (86 female, 82 male and 19 undetermined) were collected during the study. The female/male ratio was found to be 1 to 1.049. Females ranged from 62 to 105 mm in total length (TL) and 1.7 to 10.9 g in weight (W). Males ranged from 65 to 100 mm in TL and 2.4 to 9.8 g in W. The difference in the lengths of females and males were not statistically important (Student t-test, p>0.05). The total length–frequency distribution by sex is given in Figure 2.

The length and weight measurements, number of individuals (n), regression parameters a and b of the LWRs, 95% confidence intervals of b, coefficients of determination (R^2) and condition factor (CF) of the *O. tigris* were given in Table 1. Length-weight relationships of *O. tigris* were found as $W=0.00007*L^{3.03}$, $R^2=0.93$, SE of b=0.0125 and 95 % confidence intervals of b=3.019-3.363, t-test P<0.05 for undetermined; $W=0.0069*L^{3.13}$, $R^2=0.92$, SE of b=0.0057 and 95 % confidence intervals of b=2.934-3.195, t-test P<0.05 for females; $W=0.0077*L^{3.09}$, $R^2=0.91$ SE of b=0.0055 and 95 %

confidence intervals of b=2.881-3.104, t-test P<0.05 for males and $W=0.0049*L^{3.29}$, $R^2=0.95$ SE of b=0.0040 and 95 % confidence intervals of b=3.166-3.504, t-test P<0.05 for all individuals (Figure 3). According to these values, the growth type of this species was positive allometric (b>3) for all individuals and isometric growth (b=3) for undetermined, females and males. Regression analysis showed that fish length has high significant correlation with weight (R=0.97, $R^2=0.95$, $F_{1,185}= 31930.3$, P <0.001) and it is possible to say that 95% increase in weight was due to length increase. When the t-test results were used for the significance condition of the regression coefficients t= 56.510, P <0.01, it was identified that fish-length data could be used in highly reliable to estimate fish-weight.

The length-length relationships between total length, fork length, and standard length and also the estimated parameters of the length-length relationship and the coefficient of determination R^2 of *O. tigris* are presented in Figure 4 and Table 2. All LLRs were highly significant with $R^2>0.94$.

TABLE 1
Length-weight relationships for *O. tigris* in the Karasu River

Sex	N	Length range (mm)	Weight Range (g)	a	b	95% CI of b	R^2	CF
Undeterm.	19	41-60	0.5-1.8	0.00007	3.03	3.019-3.363	0.93	0.773
Female	86	62-105	1.7-10.9	0.00690	3.13	2.934-3.195	0.92	0.901
Male	82	65-100	2.4-9.8	0.00770	3.09	2.881-3.104	0.91	0.916
All	187	41-105	0.5-10.9	0.00490	3.29	3.166-3.504	0.95	0.895

(N: number of individuals, a: intercept, b: slope, CI: confidence limits, R^2: coefficient of determination, CF: Condition factor)

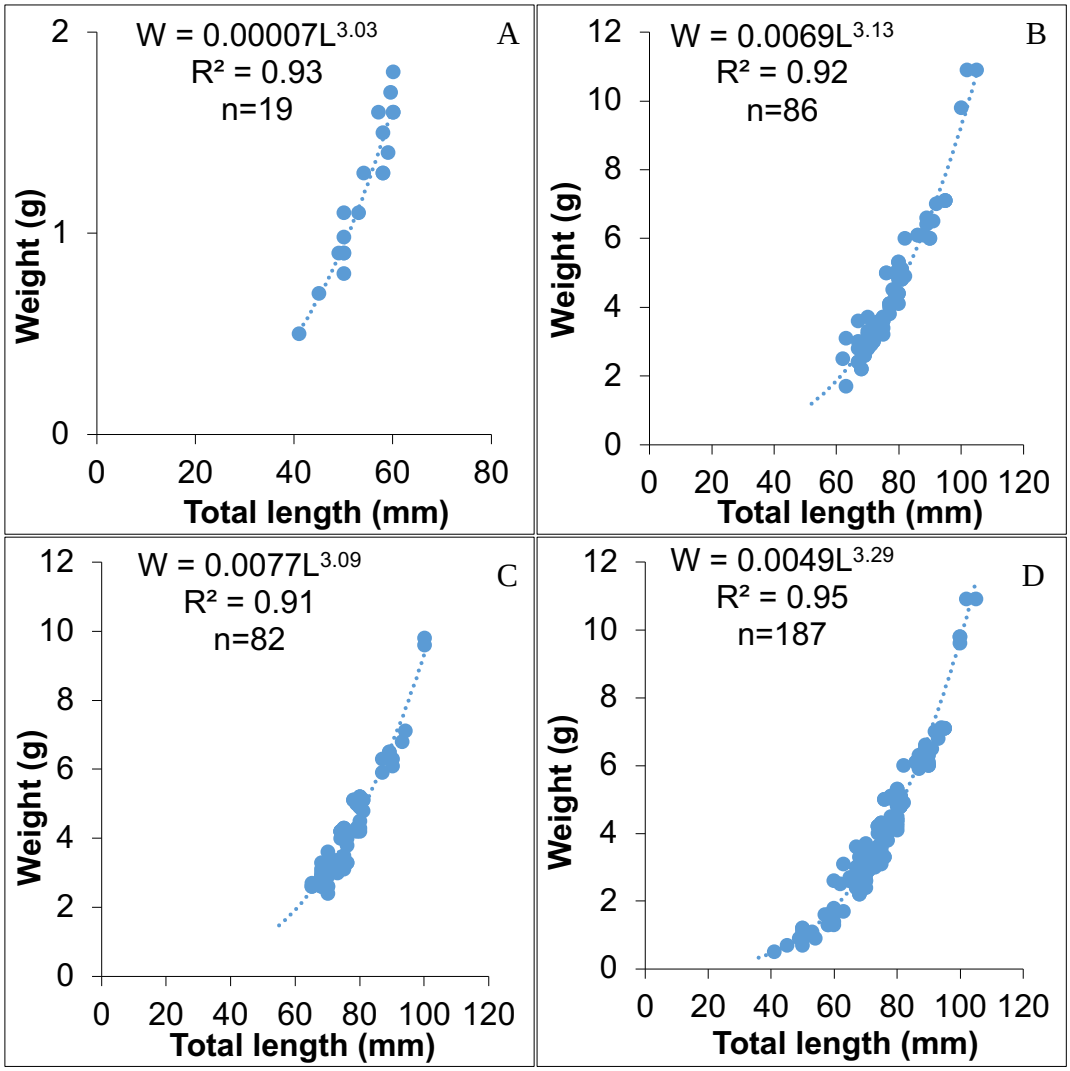


FIGURE 3
Length-weight relationships of *O. tigris* for undetermined (A), female (B), male (C) and all individuals (D) in the Karasu River

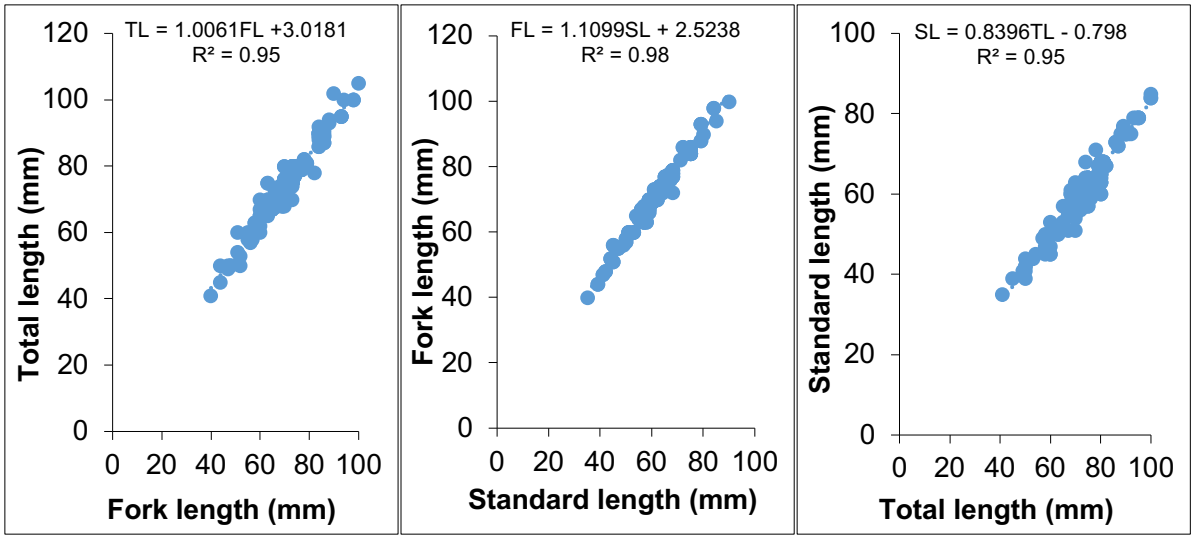


FIGURE 4
Length–length relationships for *O. tigris* in the Karasu River

TABLE 2
Length–length relationships between total length, fork length, and standard length of *O. tigris* in the Karasu River.

Sex	Equation	a	b	R^2
Female	TL= a + bFL	3.978	0.9915	0.93
	FL= a + bSL	3.433	1.0983	0.97
	SL= a + bTL	-0.589	0.8383	0.93
Male	TL= a + bFL	6.613	0.9610	0.91
	FL= a + bSL	5.242	1.0647	0.96
	SL= a + bTL	-2.149	0.8559	0.90
All	TL= a + bFL	3.018	1.0061	0.95
	FL= a + bSL	2.523	1.1099	0.98
	SL= a + bTL	-0.798	0.8396	0.95

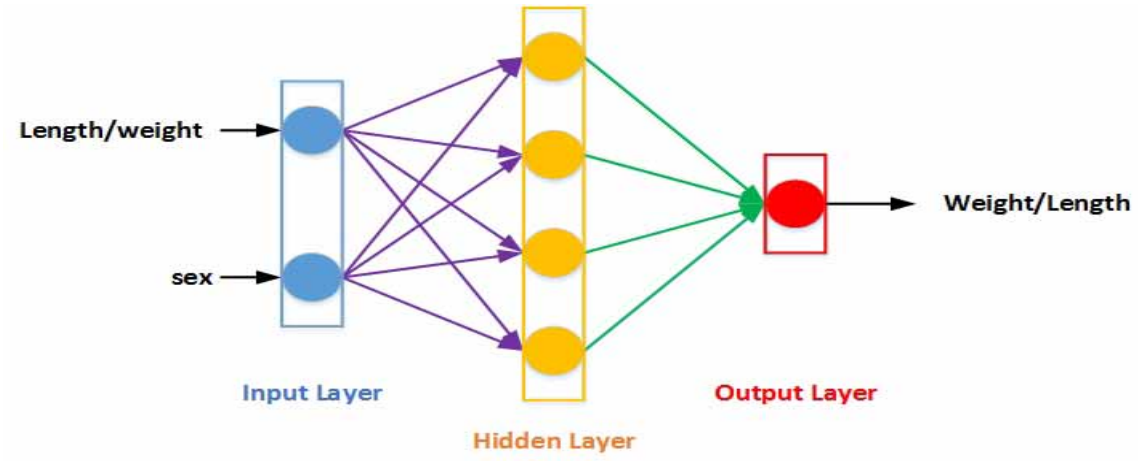


FIGURE 5
Representation an ANN formed of 2 input layers, a hidden layer and an output layer to be estimated

A multilayer feed-forward neural network was used during ANN calculations. A simple drawing of an ANN formed of 2 input layers (length/weight, sex), a hidden layer and an output layer (weight/length) is seen in Figure 5.

According to ANNs, the fit of the actual and predicted values of *O. tigris* are given in the graphs (Figure 6). The values obtained by training, validation and testing are more compatible than in LWRs.

Actual values, ANNs and LWRs data of *O. tigris* are shown in Table 3. All values were classified by sex and length groups. Table 3 obtained by comparison with ANNs and LWRs of *O. tigris*. The

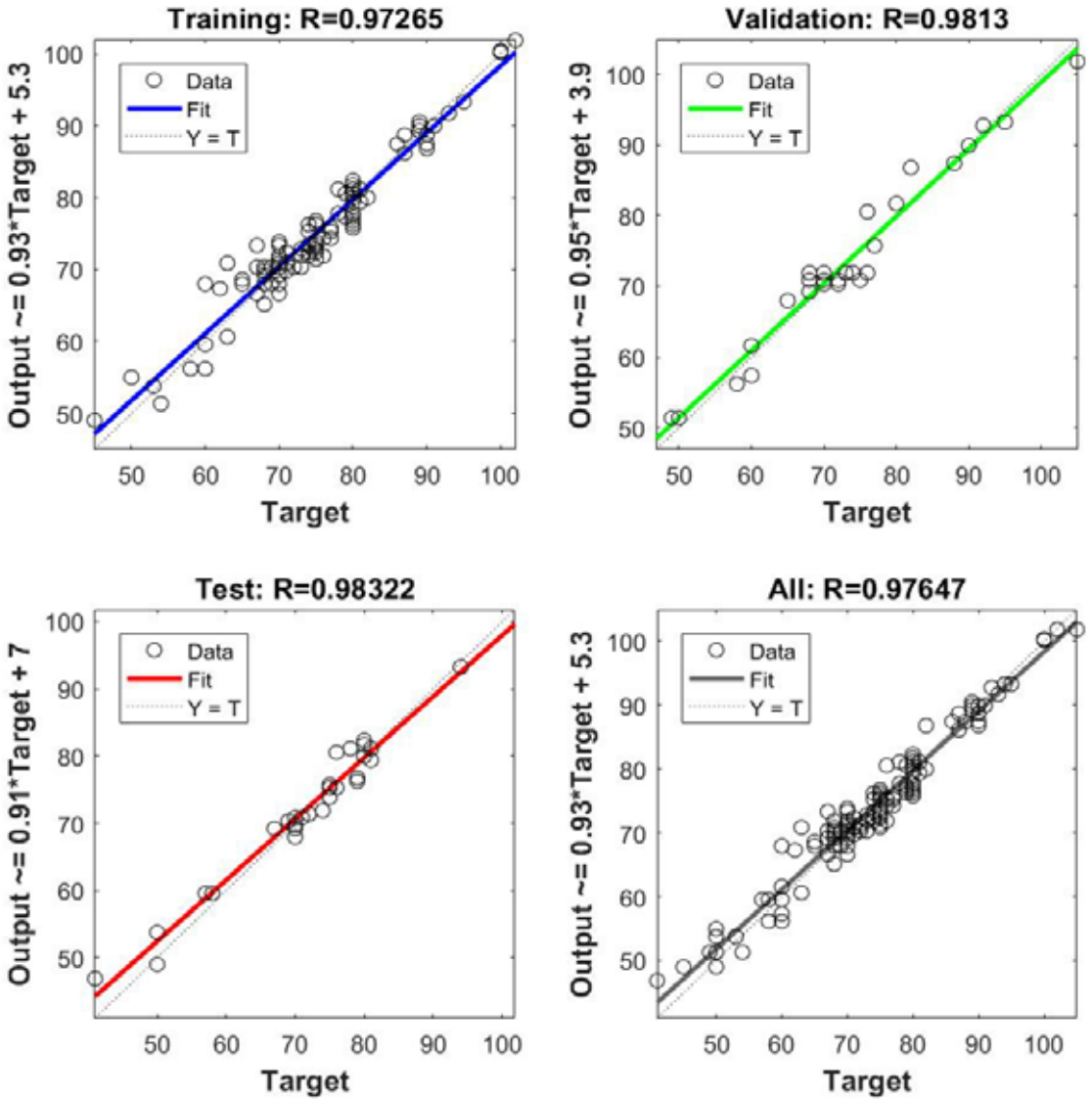


FIGURE 6
The relationship between artificial neural networks for *O. tigris* length-weight relation

TABLE 3
Comparison of real and calculated values by sex for *O. tigris* ANNs and LWRs

Length groups (mm)	Sex	Real Data		ANNs		MAPE(%)		LWRs		MAPE (%)	
		L	W	L	W	L	W	L	W	L	W
40-49	Undeterm.	45.03	0.75	46.10	0.79	2.376	5.333	44.92	0.64	0.244	14.66
50-59	Undeterm.	53.56	1.23	53.63	1.21	0.131	1.626	52.56	1.09	1.867	11.38
60-69	Undeterm.	60.03	1.76	60.55	1.85	1.032	5.114	59.20	1.53	1.382	13.06
	Female	66.67	2.69	67.84	2.65	1.754	1.486	67.31	2.43	0.959	9.665
	Male	67.52	2.90	69.46	2.76	2.873	4.827	69.22	2.20	2.517	24.13
	All	66.02	2.63	67.43	2.58	2.135	1.901	66.89	2.47	1.317	6.083
70-79	Female	73.91	3.62	73.37	3.63	0.730	0.276	74.12	2.92	0.284	19.33
	Male	73.76	3.66	73.58	3.58	0.244	2.185	74.39	3.10	0.854	15.30
	All	73.82	3.67	73.48	3.61	0.460	1.634	74.26	3.21	0.596	12.53
80-89	Female	82.21	5.38	82.24	5.22	0.036	2.973	83.26	4.96	1.277	7.806
	Male	81.90	5.20	81.84	5.14	0.073	1.153	82.97	5.02	1.306	3.461
	All	82.03	5.22	82.05	5.18	0.024	0.766	83.11	5.04	1.316	3.448
90-99	Female	92.34	6.74	90.77	6.67	1.700	0.451	89.61	6.19	2.956	8.160
	Male	91.88	6.63	90.29	6.57	1.730	0.905	89.28	6.49	2.839	2.112
	All	92.11	6.65	90.61	6.64	1.628	0.150	89.50	6.76	2.833	1.654
100-105	Female	102.36	10.51	101.36	10.47	0.977	0.381	103.02	9.98	0.684	5.042
	Male	100.06	9.74	100.28	9.74	0.200	0.000	100.49	8.42	0.400	13.55
	All	101.45	10.22	100.93	10.17	0.493	0.489	102.01	9.75	0.592	4.598
Average MAPE (%)						**1.033**	**1.758**			1.345	9.776

values we obtained with ANNs and LWRs were calculated one by one. It was determined that the ANNs MAPE (%) values were better than MAPE values calculated in length-weight relation for *O. tigris*. In particular, the values obtained with ANNs in the later length groups are much closer to the actual data than LWRs (Table 3).

Best validation performance and validation checks of artificial neural networks were given for length-weight of *O. tigris* in Figure 7 and Figure 8. The optimum epoch is 9 for *O. tigris*, there is no benefit to the system of increasing epoch after that.

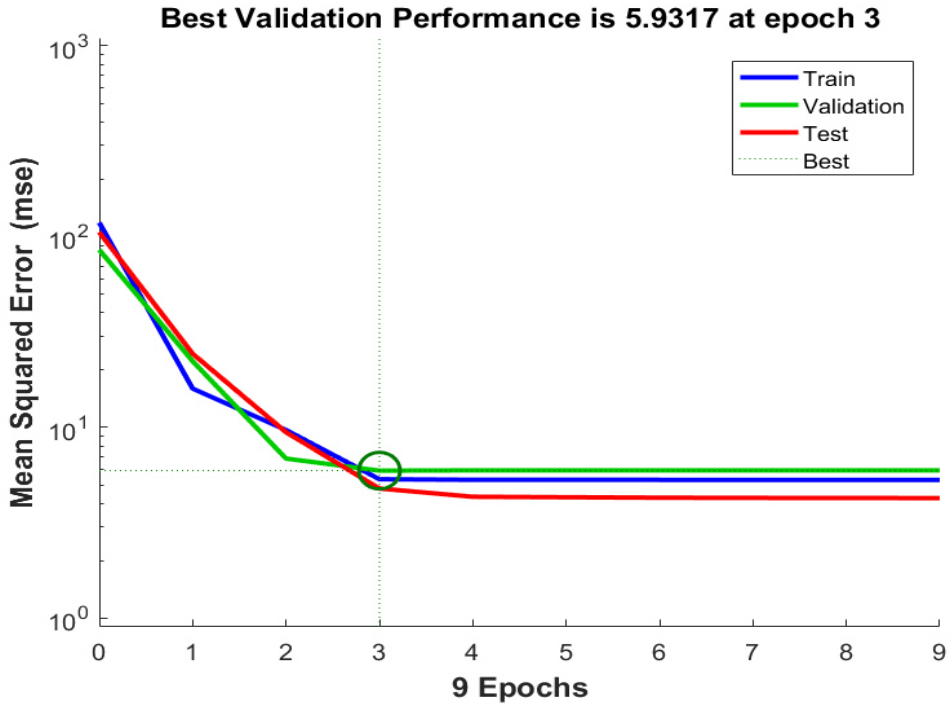


FIGURE 7

Best validation performance of artificial neural networks for *O. tigris* length-weight

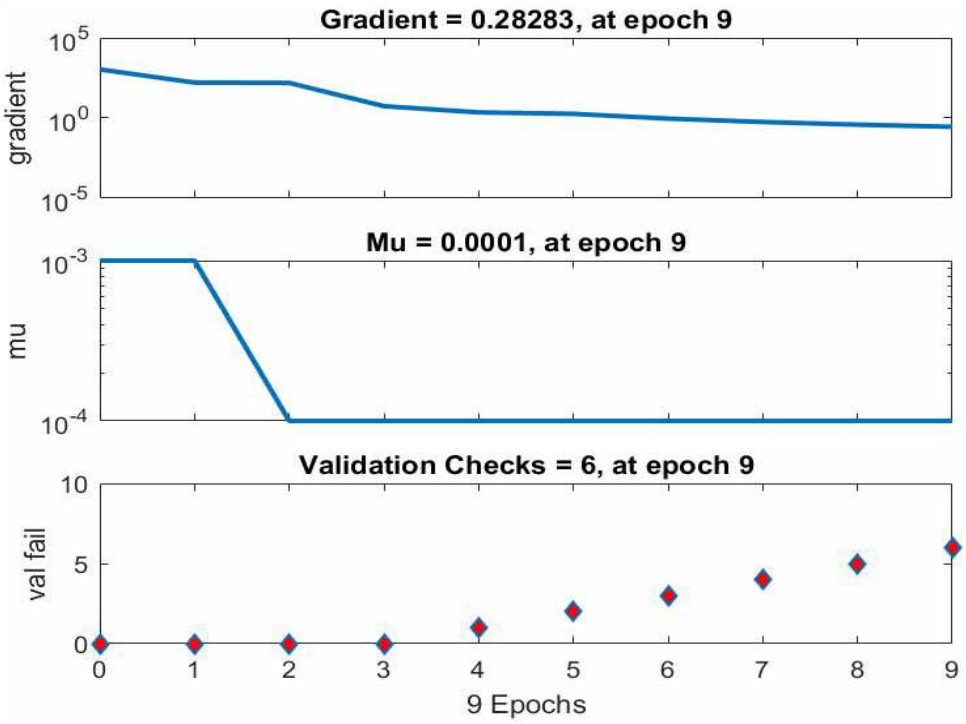


FIGURE 8

Validation checks of artificial neural networks for *O. tigris* length-weight

TABLE 4
Comparison of total length-weight relationships for 11 species of the Nemacheilidae family from other studies

Species	N	Lmax (mm)	a	b	R^2	References
Paracobitis tigris (Heckel, 1843)	84	84	0.0061	3.119	0.94	Birecikligil and Cicek [26] (Euphrates and Orontes rivers)
O. hamwii (Krupp and Schneider, 1991)	28	88	0.0021	3.520	0.95	Ozcan and Altun [31] (Gölbaşı Lake, Hatay)
O. brandtii (Kessler, 1877)	325	78	0.009	2.970	0.96	Golzarianpour et al. [32] (Iran)
O. kermanshahensis (Banarescu and Nalbant, 1966)	38	84	0.015	2.890	0.97	Golzarianpour et al. [32] (Iran)
O. farsicus (Nalbant and Bianco, 1998)	35	71	0.012	2.880	0.94	Golzarianpour et al. [32] (Iran)
O. angorae (Steindachner, 1897)	21	76	0.008	3.010	0.98	Golzarianpour et al. [32] (Iran)
O. angorae (Steindachner, 1897)	127	86	0.008	3.102	0.94	Birecikligil et al. [33] (Kızılırmak River Basin)
O. angorae (Steindachner, 1897)	30	73	0.006	3.237	0.88	Gaygusuz et al. [34] (Balıklı Stream)
O. angorae (Steindachner, 1897)	24	83	0.0062	3.228	0.99	Erk'akan et al. [35] (Söğütözü Creek)
O. samanticus (Banarescu and Nalbant 1978)	40	86	0.0085	2.919	0.92	Erk'akan et al. [35] (Karabogaz Creek)
O. mesudae (Erk'akan, 2012)	14	89	0.0161	2.628	0.95	Erk'akan et al. [35] (Küfü Creek, Çivril)
O. evreni (Erk'akan *et al*., 2007)	27	94	0.0128	2.788	0.92	Erk'akan et al. [35] (Çayir Creek, Andirin)
O. simavicus (Balik and Banarescu, 1978)	17	71	0.0044	3.261	0.95	Erk'akan et al. [35] (Karacalti Creek. Kepsut)
O. angorae (Steindachner, 1897)	44	8	0.011	2.810	0.96	Hasankhani et al. [36] (Sirwan River)
O. kiabii (Golzarianpour, Abdoli and Freyhof, 2011)	205	71	0.0149	2.900	0.89	Jamali et al. [37] (Iran)
O. theophilii (Stoumboudi, Kottelat and Barbieri, 2006)	17	105	0.007	3.070	0.96	Innal et al. [38] (Cüneyt Creek)
O. angorae (Steindachner, 1897)	103	9.8	0.0099	2.929	0.96	Yazıcıoğlu and Yazıcı [39] (Kılıçözü Stream, Kızılırmak)

DISCUSSION

The present study provides the first information on length–weight, length-length relationships and condition factor for *O. tigris* from Karasu River. Nalbant and Bianco [4] examined 4 samples between 56-73 mm from Kapozik Kadur Hakkari, upper Tigris drainage. In their study of Birecikligil and Cicek [26], they found 84 samples and maximum length of 84 mm for *Paracobitis tigris* in tributaries of Euphrates and Orontes rivers in Gaziantep (southeastern Anatolia, Turkey). In our study, the maximum length was 105 mm for *O. tigris* from Karasu River.

The LWRs were highly significant; all species were determined between the length and weight very strong positive relationship in Karasu River (R^2>0.95). The b values of all individuals were determined as 3.29 and 95 % confidence intervals of b=3.166-3.504 for *O. tigris.* The growth of this species was positive allometric. Length–weight relationships may show temporal or spatial variations due to their size range, reproductive activities and stage or environmental factors such as water temperature, food quality and availability, diseases, and competition [27]. A few studies are available in the literature on the biological characteristics of *Oxynoemacheilus tigris*. Birecikligil and Cicek [26] were determined descriptive statistics and length-weight relationship parameters for *P. tigris* caught in tributaries of Euphrates and Orontes rivers in Gaziantep (Table 4). Koyun et al. [28] were found the seasonal prevalence of *Allocreadium isoporum* in *Oxynoemacheilus tigris* from Murat River, Eastern Anatolia. Kılıc et al. [29] were determined karyotype analysis in *Orthrias tigris*, living in the Kura-Aras river basin and Yılmaz et al. [30] an electrophoretic taxonomic study on sarcoplasmic proteins of *Orthrias tigris* habitated in Kars stream.

There have been previous studies on the length-weight relationships of other species of the Nemachelidae family are shown in Table 4 and these values were compatible with our work. These high values of R^2 show that the length of relationships is a linear observed range of values. Regression analyses are shown that fish length has a highly significant correlation with weight ($P<0.001$) for *O. tigris*. Furthermore, when the t-test results were used for the importance of regression coefficients ($P<0.01$), it was identified that total length data could be used in highly reliable to estimate weight.

Length-length relationships are generally used for population parameters of fish species [40-43]. LLRs were significant ($p<0.001$) for *O. tigris* with all R^2 values greater than 0.94. Birecikligil and Cicek [26] were found R^2 value as 0.993 for TL-FL and 0.974 for TL-SL of *P. tigris* caught in tributaries of Euphrates and Orontes rivers in Gaziantep. The present study provides length-length relationship parameters for the first time for *O. tigris* from Karasu River.

Condition factor of *O. tigris* was determined as average 0.901 for females, 0.916 for males and 0.895 for all individuals. There are no data available on the condition factor of *O. tigris*. Birecikligil et al. [33] and Yazıcıoğlu and Yazıcı [39] stated that average condition factors of *O. angorae* were 0.940 and 0.863. These results are compatible with our work.

ANNs are an important model for predict in fisheries. Especially in recent years, ANNs has been used more in physical and chemical sciences in biology and water ecology. ANNs is required for future predictions along with other methods. However, in the majority of these studies it is seen that the ANNs results are better than the results of other conventional methods [44].

In this study, the results obtained with LWRs were compared with results of ANNs using MAPE (%), the initial information on the length-weight relation of *O. tigris* in the Karasu River, (14 different stations) Turkey. According to this comparison, ANNs provided very good results compared to other methods. Thus, ANNs can be used as an alternative and reliable method in fisheries for length-weight relation. The results obtained from this work are very important. Because *O. tigris* assessed as Critically Endangered in the IUCN Red List of Threatened Species [3]. Fisheries managers should consider the creation of freshwater protected areas with regional fisheries organizations. This work will be guided in the future to management and conservation current population of *O. tigris*.

CONCLUSIONS

The results of this study indicated high proximity between the measured and predicted data. The values obtained with Artificial Neural Networks are much closer to their real values. For this reason, in this study it can be concluded that ANNs model applied made a more effective and reliable than LWR.

ACKNOWLEDGEMENTS

Author thank Prof. Dr. Ahmet Bedri OZER for helping Artificial Neural Networks (ANNs); (MATLAB; Ver R2016a) calculations. A part of this study has been accepted for oral presentation at the International Multidisciplinary Congress of Eurasia in 2017 (IV. IMCOFE 2017, Rome, Italy).

The author has proclaimed that no competing interests present.

REFERENCES

[1] Freyhof, J., Erk'akan, F., Ozeren, C. and Perdices, A. (2011) An overview of the western Palaearctic loach genus *Oxynoemacheilus* (Teleostei: Nemacheilidae). Ichthyology Explore Freshwaters. 22(4), 301-312.

[2] Kottelat, M. (2012) Conspectus Cobitidum: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobitoidei). The Raffles Bulletin of Zoology. Supplement. 26, 1–199.

[3] Freyhof, J. (2014) *Oxynoemacheilus tigris*. The IUCN Red List of Threatened Species 2014:e.T19431830A19849456.http://dx.doi.org/10.2305/IUCN.UK.1.RLTS.T19431830A19849456.en. Downloaded on 09 June 2018.

[4] Nalbant, T.T. and Bianco, P.G. (1998) The loaches of Iran and adjacent regions with description of six new species (Cobitoidea). Italia Journal of Zoology. 65, 109-123.

[5] Tureli Bilen, C., Kokcu, P. and Ibrikci, T. (2011) Application of artificial neural networks (ANNs) for weight predictions of blue crabs (*Callinectes sapidus* Rathbun, 1896) using predictor variables. Mediterranean Marine Science. 12(2), 439–446.

[6] Maravelias, C.D., Haralabous, J. and Papaconstantinou, C. (2003) Predicting demersal fish species distributions in the Mediterranean Sea using artificial neural networks. Marine Ecology. 255, 249- 258.

[7] Ucun Ozel, H., Gemici, B.T., Ozel, H.B. and Gemici, E. (2017) Determination of water quality and estimation of monthly biological oxygen demand (bod) using by different artificial neural networks models in the Bartın river. Fresen. Environ. Bull. 26, 5465-5476.

[8] Yarar, A., Onucyildiz, M. and Copty, N.K. (2017) A combined wavelet and neural network model for forecasting streamflow data. Fresen. Environ. Bull. 25, 5217-5225.

[9] Benzer, S. and Benzer, R. (2015) Determine some morphological characteristics of crayfish (*Astacus leptodactylus* Eschscholtz, 1823) with tradional methods and artificial neural networks in Dikilitas pond, Ankara, Turkey. Fresen. Environ. Bull. 24, 3727 – 3735.

[10] Mastrorillo, S., Lek, S., Dauba, F. and Belaud, A. (1997) The use of artificial neural networks to predict the presence of small-bodied fish in river. Freshwater Biology. 38, 237-246.

[11] Park, Y.S., Verdonschot, P.F.M., Chon, T.S. and Lek, S. (2003) Patterning and predicting aquatic macro invertabrate diversities using artificial neural network. Water Research. 37, 1749-1758.

[12] Obach, M., Wagner, R., Werner, H. and Schmidt, H.H. (2001) Modelling population dynamics of aquatic insects ith artificial neural networks. Ecology Modelling. 146, 207-217.

[13] Joy, K.M. and Death, R.G. (2004) Predictive modelling and spatial mapping of freshwater fish and decapod assemblages using GIS and neural Networks. Freshwater Biology. 49, 1306-1052.

[14] Benzer, R. (2015) Population dynamics forecasting using artificial neural networks. Fresen. Environ. Bull. 24, 460-466.

[15] Benzer, S. and Benzer, R. (2016) Evaluation of growth in pike (*Esox lucius* L., 1758) using traditional methods and artificial neural networks. Applied Ecology Environment Research. 14(2), 543–554.

[16] Benzer, S., Karasu Benli, C. and Benzer, R. (2015) The comparison of growth with length–weight relation and artificial neural networks of crayfish, *Astacus leptodactylus*, in Mogan Lake. Journal of Black Sea Mediterranean Environment. 21(2), 208–223.

[17] Benzer, S., Benzer, R., and Günal, A.Ç. (2017) Artificial neural networks approach in length-weight relation of crayfish (*Astacus leptodactylus* Eschscholtz, 1823) in Eğirdir Lake, Isparta, Turkey. Journal of Coastal Life Medicine. 5(8), 330-335.

[18] Benzer, S. and Benzer, R. (2018) New Perspectives for Predicting Growth Properties of Crayfish (*Astacus leptodactylus* Eschscholtz, 1823) in Uluabat Lake. Pakistan Journal of Zoology. 50(1), 35-45.

[19] King, M. (1995) Fisheries biology, assessment and management. Fishing News Books, Blackwell Science, Oxford, UK.

[20] Zar, J.H. (1999) Biostatistical Analysis. 4th ed. Prentice-Hall, New Jersey.

[21] Le Cren, C.D. (1951) The length-weight relationship and seasonal cycle in gonad weight and condition in the Perch (*Perca fluviatilis*). Journal of Animal Ecology. 20, 201-219.

[22] Agatonovic-Kustrin, S. and Beresford, R. (2000) Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. Journal of Pharmaceutical and Biomedical Analysis. 22(5), 717-727.

[23] Cabreira, A.G., Tripode, M. and Madirolas, A. (2009) Artificial neural networks for fish-species identification. – ICES Journal of Marine Science. 66, 1119–1129

[24] URL, 1. (2018) https://becoming human. ai/artificial-neuron-networks-basics-introduction-to-neural-networks-3082f1dcca8c.

[25] Krenker, A., Bešter, J. and Kos, A. (2011) Introduction to the Artificial Neural Networks. In: Suzuki, K. (ed.) Artificial Neural Networks – Methodological Advances and Biomedical Applications. IntechOpen..

[26] Birecikligil, S. and Cicek, E. (2011) Length-weight relationships for 16 freshwater fishes caught in tributaries of Euphrates and Orontes rivers in Gaziantep (southeastern Anatolia, Turkey). Journal of Applied Ichthyology. 27, 1131–1132.

[27] Wootton, R.J. (1990) Ecology of Teleost Fishes. Chapman and Hall, London.

[28] Koyun, M., Ulupınar, M., Mart, A. and Tepe, Y. (2016) Seasonal prevalence of Allocreadium isoporum (Loos, 1894) (Digenea: Allecreadiidae) in *Oxynoemacheilus tigris* (Osteichthyes: Balitoridae) (Steindachner, 1897) from Murat River, Eastern Anatolia, Turkey. Biharean Biologist. 10(1), 1-3.

[29] Kılıc, B., Gül, S., Ozkan, O., Kaya, T. O., Nur, G. and Aksu, P. (2011) Karyotype Analysis in *Orthrias tigris* (Heckel, 1843), Living in the KuraAras River Basin. AKU J. Sci. 11, 43-49.

[30] Yılmaz, M., Ildes, E., Alaş, A. and Koc, E. (2008) An Electrophoretic Taxonomic Study on Sarcoplasmic Proteins of Some Balithorid and Cobitid Fishes Habitated in Kars Stream, Turkey. Kafkas Üniversitesi Fen Bilimleri Dergisi. 1(2), 69-73.

[31] Ozcan, G. and Altun, A. (2016) Length–weight and length-length relationships for four freshwater fish species from Gölbaşı Lake (Hatay), Turkey. Journal of Applied Ichthyology. 32, 1350–1352.

[32] Golzarianpour, K., Abdoli, A., Kiabi, B.H. (2011) Length-weight relationships for nine nemacheilian loaches (Teleostei: Nemacheilidae) from Iran. Journal of Applied Ichthyology. 27, 1411-1412.

[33] Birecikligil, S., Cicek, E., Oztürk, S., Secer, B., Celepoğlu, Y. (2016) Length-length, length-weight relationship and condition factor of fishes in Nevşehir Province, Kızılırmak River Basin (Turkey). Acta Biologica Turcica. 29(3), 72-77.

[34] Gaygusuz, O., Aydın, H., Emiroğlu, O., Top, N., Dorak, Z., Gürsoy Gaygusuz, Ç., Başkurt, S. and Tarkan, A.S. (2013) Length–weight relationships of freshwater fishes from the western part of Anatolia, Turkey. Journal of Applied Ichthyology. 29, 285– 287.
[35] Erk'akan, F., Innal, D. and Ozdemir, F. (2014) Length–weight relationships for some endemic stone and spine loach species in Anatolia. Journal of Applied Ichthyology. 30(1), 244-245.
[36] Hasankhani, M., Keivany, Y., Daliri, M., Pouladi, M. and Soofiani, N.M. (2014) Length–weight and length–length relationships of four species (*Barbus lacerta* Heckel, 1843), *Oxynoemacheilus angorae* (Steindachner, 1897), *Squalius lepidus* (Heckel, 1843) and *Pseudorasbora parva* (Temminck & Schlegel, 1846) from the Sirwan River (western Iran). Journal of Applied Ichthyology. 30(1), 206-207.
[37] Jamali, H., Patimar, R., Farhadi, M., Doost, A. and Daraei, V. (2014) Length-weight relationships for five nemacheilian loaches (Teleostei: Nemacheilidae) from Iran. Journal of Applied Ichthyology. 31(1), 244-245.
[38] Innal, D., Ozdemir, F. and Doğangil, B. (2015) Length-weight relationships of *Oxynoemacheilus theophilii* (Teleostei: Nemacheilidae) from Turkey. International Journal of Fisheries and Aquatic Studies. 2(4), 249-250.
[39] Yazıcıoğlu, O. and Yazıcı, R. (2016) The length-weight, length-length relationship and condition factor of angora loach, *Oxynoemacheilus angorae* (Steindachner, 1897) inhabiting Kılıçözü Stream in Kızılırmak River Basin (Central Anatolia-Turkey). Turkish Journal of Agriculture - Food Science and Technology. 4(12), 1165-1168.
[40] Ozpicak, M., Saygın, S. and Polat, N. (2017) The length-weight and length-length relationships of bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) from Samsun, middle Black Sea region. NESciences. 2(3), 28-36.
[41] Serdar, O., Ozcan, E.I. and Aydın, R. (2017) Length-weight and length-length relationships of *Alburnus mossulensis* and *Acanthobrama marmid* (Heckel, 1843) in the Karasu River (Turkey). Yunus Research Bulletin. 2, 171-176.
[42] Ozcan, E.I. and Serdar, O. (2018) Length-weight and length-length relationships of red-spotted trout (*Salmo trutta macrostigma* (Dumeril, 1858)) in Karasu River (East Anatolia, Turkey). Ecolojical Life Sciences. 13(1), 27-31.
[43] Basusta, A., Basusta, N., Ozer, E.I., Girgin, H. and Aslan, E. (2013) Some population parameters of the lessepsian suez puffer (*Lagocephalus suezensis*) from Iskenderun Bay, Northeastern Mediterranean, Turkey. Pakistan Journal of Zoology. 45(6), 1779-1782.
[44] Brosse, S., Guegan, J., Tourenq, J. and Lek, S. (1999) The use of artificial neural networks to assess fish abundance and spatial occupancy in the littoral zone of a Mesotrophic lake. Ecology Modelling. 120, 299-311.

Received: **18.06.2018**
Accepted: **27.09.2018**

CORRESPONDING AUTHOR

Ebru Ifakat Ozcan
Munzur University,
Faculty of Fisheries,
Tunceli – Turkey

e-mail: ebruozer@munzur.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9851-9858 Fresenius Environmental Bulletin

THE HEAVY METAL ASSESSMENT OF HARSIT STREAM (GIRESUN, TURKEY) USING MULTIVARIATE STATISTICAL TECHNIQUES

Cengiz Mutlu[1,*], Buse Eraslan-Akkan[1], Bulent Verep[2]

[1]Giresun University, Department of Biology, Faculty of Science and Arts, Giresun, Turkey
[2]Recep Tayyip Erdogan University, Faculty of Fisheries, Rize, Turkey

ABSTRACT

The aim of this research was to determine the degree of heavy metal contamination of Harşit Stream in northeastern Turkey. Heavy metals such as Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in water were analyzed using ICP-MS and the result compared with national and international Standard for Drinking Water Quality. The water samples were collected from 7 different sites between June 2014 and May 2015. Statistical analysis of data was carried out using SPSS statistical package programs. Descriptive statistical analysis including One-way ANOVA, significance (0.01 and 0.05) was done. Important differences in the mean values were tested with Duncan's multiple range test. Moreover, the multivariate statistical techniques (hierarchical cluster analysis (HCA), principal component analysis (PCA)), the Pearson correlation were applied to the heavy metal variables.

In water samples, according to analysis results, the following findings were obtained for the concentration ranges of the metals: Al: 4.922-1078.906, Cr: 7.141-74.900, Mn: 0.525-18.102, Fe: 4.188-7.855, Co: 3.262-7.878, Ni: 5.832-44.923, Cu: 0.873-20.649, Zn: 10.367-362.901, Cd: 1.724-19.427 and Pb: 2.570-6.259 µg/L were found. The pollution load index between the heavy metals in the stream produced the following output: Zn > Al > Cr > Cu> Fe > Mn > Co > Pb > Cd > Ni for summer, Al > Zn > Cr > Ni> Cu > Mn > Fe > Co > Pb > Cd for autumn, Al > Zn > Cr > Ni> Fe > Co > Pb > Cu > Mn > Cd for winter, Al > Zn > Cr > Ni> Fe > Co > Mn > Cu > Pb > Cd for spring. Moreover, the distribution of heavy metals between stations was not statistically significant ($p<0.05$, $p<0.01$). Consequently, it can be concluded that the concentrations of some heavy metals in water from Harşit stream are higher than the WHO, EPA and Canada standards.

KEYWORDS:
Harşit Stream, Giresun, Heavy Metal, Water Quality

INTRODUCTION

Today, the threat of pollution from freshwater resources with very large reserves has led to increased work on water quality and pollution due to increased water demand. Physical, chemical and biological factors are used to determine pollution in rivers. The most important purpose of monitoring water quality from physical and chemical factors; determine the factors affecting water quality by detecting changes in pollution sources and hence pollution levels [1-3]. Heavy metals occur as natural constituents of the earth crust and are persistent environmental contaminants since they cannot be degraded or destroyed. They are very important and dangerous toxic environmental pollutants for environment. Sources of metal contamination affecting aquatic ecosystems can be expressed as industries, waste water plants, farming, mining, anthropic accidents, urban and rural areas, navigation traffic and electronic wastes very generally [4-6].

Most important issue for these metals can be incorporated into food chains and concentrated in aquatic organisms to a level that affects their physiological state. Of the effective pollutants are the heavy metals which have drastic environmental impact on all organisms. For a long time and today, heavy metal contamination has become a serious environmental problem. Major sources of toxic metals arising from human activities are domestic and industrial wastewaters and their associated solid wastes. Heavy metals is an important environmental pollutant that is known to have systemic effects on biota in the aquatic environment that surrounds it by the development of technology, and which is also known to have carcinogenic effects to life [7].

Therefore, in this study, Harşit Stream, the longest fresh water source in Giresun province, which has the highest level of surface water volume in Black Sea Water Basin, aims to monitor the heavy metal pollution status of the water mass for one year by physicochemical methods and to determine the changes on the stations basis. In addition, proposals will be made in order to ensure

that the stream basin risks and carry the risk of public health in terms of heavy metal pollution, so that necessary legal restrictions and regulations can be made immediately.

MATERIALS AND METHODS

Study area and stations. Giresun province, which is located in the Eastern Black Sea Region of the Black Sea Region, is between 37º 50 'and 39º 12' E longitudes and 40º 07 'and 41º 08' N latitudes. It has been surrounded by Trabzon and Gümüşhane in the east, Ordu in the west, Sivas and Erzincan in the south, and the Black Sea in the north. The province covers an area of 6.934 km² with 8.5% of the country's land and 8.5% of the country's population. It has a sea coast length of 121 km and has a large volume of river discharge from its 6 different points in the Black Sea. It is known that the chemical substances originating from the agricultural activities that lasted in the settlement area where the agricultural activities are very important in the economy are transmitted to the rivers in various ways [8]. In addition, the trout operations above the production capacity established on stream basins pollute the aquatic environment directly or indirectly.

In this study, it was aimed to reveal the existing physicochemical water quality of Harşit Stream which is discharged from the province of Giresun into the Black Sea. Harşit Stream originates from the Vavuk Plateau in the Gümüşhane province border, enters the provincial lands near Günyüzü and discharges into the sea to the east of Tirebolu. The length within the provincial borders is 50 km and it is the longest river with a total length of 160 km. There are Doğankent I and II hydroelectric power plants on Harşit Brook. The discharge amount of stream is 232 m³ / sec. Harşit Stream has 178 hm³ annual fresh water potential and is the most important fresh water source for the province. In this study, the collection of water and sediment samples from 7 different points was carried out from the Günyüzü site where the Harşit Stream had entered the province border to the point where it was discharged to the Black Sea (Figure 1). Some information about stations is given below including the environmental risk factors:

1st station: The place that Günyüzü region of first entering point of Harşit stream to Giresun city. 2nd station: It represents the region under intensive press of both dam lake and town's waste water and contains the exit of Doğankent town. 3rd station: This station points the region has agricultural efforts, after trout farms and effected by Doğankent HPP.4th station: It represents the region has low level water generally and transfering water to the storage before Aslancık HPP. 5th station: It counterparts the location under the effects of power plant after Aslancık HPP those were sand and gravel production area.6th station: the location that contains many sand-gravel-production areas and domestic waste and gurbage dumping ground. 7th station: It covers the point of Harşit stream connected to the sea and affected by the waste of domestic, industrial and agricultural activity, intensively. The approximate coordinates and elevation values of the operation points are as shown in Table 1.

Methods of water sample analysis. The water samples collected from 7 different stations for 12 months were filtered through 0.45 µm Whatman GF/C type membrane filters and placed in polyethylene bottles. Both Nansen and polyethylene bottles

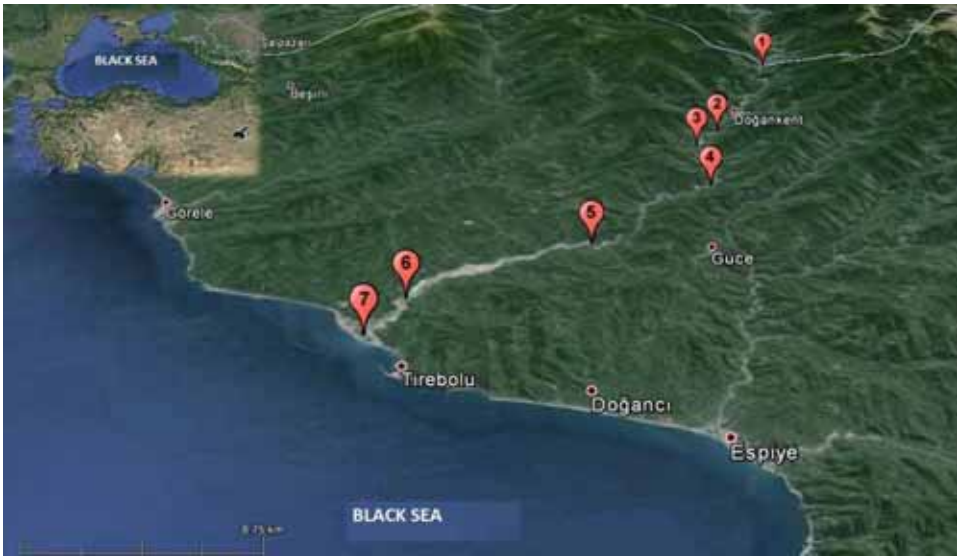


FIGURE 1
Study area

TABLE 1
The location of operation points

Station No	Longitude and Latitude	Altitudes (m)
1	40°45'46.95"K 38°57'37.49"D	369
2	40°49'30.48"K 38°54'29.77"D	166
3	40°50'29.18"K 38°54'5.39"D	145
4	40°51'35.07"K 38°51'35.89"D	93
5	40°55'15.48"K 38°50'56.39"D	52
6	40°59'16.56"K 38°51'36.08"D	36
7	41°00'30.37"K 38°50'46.91"D	6

were rinsed at least three times with media water [9]. Each liter of water was brought to the laboratory in ice-protected containers after 10 mL of 0.1 N HNO_3 was added to reduce the pH to below 2, and stored at + 4 ° C until analysis in ICP-MS system [10]. At the time of the analysis, at least 3 repetitive readings of each sample were performed in compliance with the standards, resulting in ppb level results [11].

The aim of this research which was done in Harşit Stream (Turkey) was to determine some heavy metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in water. The water samples were collected from 7 different sites between June 2014 and May 2015. The samples were collected in 0.5 liter pre-cleaned (with 50% HNO_3 and then thrice with deionized water) polyethylene bottles and acidified with 5 ml concentration HNO_3 per liter of wastewater for the analysis of heavy metals. After collection the samples were placed in coolers with ice bags while being transported to the laboratory and kept at about 4°C until being analyzed. The metal concentrations in the water samples were analyzed using the ICP-MS. The results of the metal contaminant values were evaluated according to the national and international guidelines [11-14]. Statistical analysis of data was carried out using SPSS statistical package programs. Descriptive statistical analysis including One-way ANOVA, significance (0.01 and 0.05) was done. Important differences in the mean values were tested with Duncan's multiple range test. Also, multivariate statistical analysis of the overall water quality variables was performed through principal component and hierarchical cluster analysis (PCA-HCA) techniques [15].

RESULTS AND DISCUSSIONS

In water samples, according to analysis results, the following findings were obtained for the concentration ranges of the metals: Al: 4.922-1078.906, Cr: 7.141-74.900, Mn: 0.525-18.102, Fe: 4.188-7.855, Co: 3.262-7.878, Ni: 5.832-44.923, Cu: 0.873-20.649, Zn: 10.367-362.901, Cd: 1.724-19.427 and Pb: 2.570-6.259 ppb were found (Table 2). Moreover, the distribution of heavy metals between stations was not statistically significant (Table 2). Also, the metal variables had indicated the absence of positive and good correlation (Table 3). Consequently, it can be concluded that the concentrations of some heavy metals in water from Harşit stream are higher than the WHO, EPA and Canada standards (Table 4).

Seasonal variation of the average heavy metal level in the surface water of Harşit river in terms of Al content; autumn> winter> summer> spring, in terms of Cr concentration; autumn> summer> spring> winter, in terms of Mn concentration; autumn> summer> spring> winter, in terms of Fe content; autumn> winter> winter, in terms of Co concentration, autumn> summer> spring> winter, Ni concentration; autumn> spring> winter> summer, in terms of Cu contents; autumn> summer> spring> winter, Zn concentration; autumn> summer> spring> winter, Cd value; autumn> winter> spring, Pb concentration; autumn> summer> spring> winter have been determined (Table 2). It has also been determined that seasonal differences between heavy metal concentrations are important for all metals except Cd (Table 2).

The Al values of the samples are in the range of 4.922-1078.906 ppb and mean values according to the seasons have been found as 76.339 (± 8.250) in the summer, 299.162 (± 65.637) in the autumn, 98.663 (± 25.833) in the winter and 70.793 (± 8.064) ppb in the spring, respectively.The Cr values vary between 7.141-74.900 ppb and mean values according to the seasons have been determined as 46.650 (± 2.609) in the summer, 59.346 (± 2.137) in the autumn, 13.647 (± 0.837) in the winter and 22.139 (± 1.657) ppb in the spring, respectively.In terms of Mn content, the water samples vary in the range of 0.525-18.102 ppb and these mean values according to the seasons have been recorded as 5.267 (± 0.720) in the summer, 7.909 (± 0.701) in the autumn, 1.823 (± 0.279) in winter and 3.173 (±

0.581) ppb in the spring, respectively.While the Fe values were in the range of 4.188-7.855 ppb, the mean values according to the seasons have been calculated as 5.565 (± 0.158) in the summer, 5.664 (± 0.097) in the autumn, 4.460 (± 0.065) in the winter and 4.514 (± 0.049) in the spring, respectively. Co values are in the range of 3.262-7.878 ppb and the mean values according to the seasons have been found to be 4.528 (± 0.185) in the summer, 4.814 (± 0.057) in the autumn, 3.475 (± 0.058) in winter and 3.601 (± 0.042) ppb in the spring, respectively (Table 2).

The Ni contents vary between 5.832-44.923 ppb and the mean values according to the seasons have been found to be 27.213 (± 1.666) in the summer, 31.677 (± 0.670) in the autumn, 7.355 (± 0.253) in winter and 9.427 (± 0.597) ppb in the spring, respectively.Cu values ranged from 0.873 to 20.649 ppb and the mean values according to the seasons have been calculated as 14.383 (± 0.970) in the summer, 15.460 (± 0.481) in the autumn, 2.099 (± 0.244) in winter and 2.766 (± 0.199) in the spring, respectively. Zn contents vary between 10.367-362.901 ppb and the mean values according to the seasons have been determined as 204.369 (± 19.475) in the summer, 245.299 (± 8.861) in the autumn, 41.877 (± 3.930) in the winter and 60.646 (± 6.813) ppb in the spring, respectively. The contents of Cd vary between 1.724-19.427 ppb and mean values according to the seasons have been determined as 1.982 (± 0.030) in the summer, 2.807 (± 0.831) in the autumn, 1.802 (± 0.054) in winter and 1.743 (± 0.004) ppb in the spring, respectively. Pb values are in the range of 2.570-6.259 ppb and mean values according to the seasons have been found to be 3.946 (± 1.158) in the summer, 4.080 (± 0.110) in the autumn, 2.836 (± 0.066) in the winter and 2.893 (± 0.049) ppb in the spring, respectively (Table 2).

TABLE 2
Heavy metal analysis of water samples (ppb)

Heavy Metal	Season	Stations							Mean
		1	2	3	4	5	6	7	
Al	Summer	55.907	110.752	87.284	73.661	69.653	77.162	59.954	76.339^{a}
	Autumn	148.904	432.487	433.784	233.296	456.212	258.248	131.205	299.162^{ab}
	Winter	49.747	69.347	90.642	98.983	89.697	137.655	154.571	98.663^{a}
	Spring	24.643	72.157	91.434	82.407	60.451	105.061	59.401	70.793^{a}
Cr	Summer	38.196	40.191	43.750	46.446	54.145	52.454	51.368	46.650^{c}
	Autumn	68.042	53.916	50.807	51.640	59.538	62.939	68.537	59.346^{d}
	Winter	16.746	15.461	10.798	9.530	12.708	16.023	14.265	13.647^{a}
	Spring	21.313	21.925	17.667	17.554	18.757	29.095	28.662	22.139^{b}
Mn	Summer	2.464	4.294	4.692	5.521	6.114	8.173	5.612	5.267^{b}
	Autumn	6.414	6.858	6.990	6.208	7.877	9.166	11.850	7.909^{c}
	Winter	1.959	1.832	1.390	1.027	1.507	1.686	3.359	1.823^{a}
	Spring	2.376	1.930	2.196	1.462	1.930	7.161	5.158	3.173^{ab}
Fe	Summer	5.336	5.478	5.502	5.938	5.309	5.201	6.189	5.565^{b}
	Autumn	5.267	5.460	6.353	5.511	5.821	5.833	5.400	5.664^{b}
	Winter	4.674	4.268	4.346	4.431	4.382	4.526	4.586	4.459^{a}
	Spring	4.367	4.512	4.531	4.476	4.488	4.762	4.464	4.514^{a}
Co	Summer	3.993	4.136	4.341	4.474	4.636	4.359	5.759	4.528^{b}
	Autumn	4.976	4.617	4.690	4.670	4.869	4.915	4.960	4.814^{b}
	Winter	3.913	3.438	3.367	3.320	3.444	3.452	3.389	3.475^{a}
	Spring	3.572	3.581	3.505	3.497	3.554	3.801	3.695	3.601^{a}
Ni	Summer	20.915	28.317	26.122	26.498	28.496	26.782	33.360	1.666^{b}
	Autumn	32.939	31.328	31.948	30.179	31.238	31.928	32.182	31.677^{c}
	Winter	8.959	7.440	6.413	6.147	7.933	7.735	6.858	7.355^{a}
	Spring	8.836	8.864	8.079	8.258	8.807	12.190	10.959	9.427^{a}
Cu	Summer	10.434	12.679	16.228	14.730	14.671	15.907	16.030	14.383^{b}
	Autumn	15.378	14.651	15.234	12.300	15.784	17.012	17.863	15.460^{b}
	Winter	2.902	1.315	1.191	1.257	1.659	2.989	3.381	2.099^{a}
	Spring	2.751	2.564	2.193	2.319	2.439	3.685	3.408	2.765^{a}
Zn	Summer	173.337	157.228	137.182	170.353	206.309	258.181	327.994	204.369^{b}
	Autumn	219.863	238.004	210.671	239.284	269.498	248.779	290.993	245.299^{b}
	Winter	41.136	32.283	29.296	29.170	45.346	53.432	62.479	41.877^{a}
	Spring	51.461	41.743	44.389	47.462	55.864	90.177	93.426	60.646^{a}
Cd	Summer	1.892	1.929	1.957	1.975	1.980	1.981	2.159	1.982
	Autumn	1.979	1.969	7.800	1.969	1.966	1.995	1.966	2.806
	Winter	2.142	1.738	1.734	1.735	1.737	1.748	1.781	1.802
	Spring	1.744	1.739	1.734	1.731	1.734	1.762	1.758	1.743
Pb	Summer	3.709	3.862	3.921	4.338	3.696	3.510	4.584	3.946^{b}
	Autumn	3.656	3.831	4.708	3.899	4.235	4.477	3.758	4.080^{b}
	Winter	3.052	2.632	2.723	2.811	2.762	2.903	2.969	2.836^{a}
	Spring	2.754	2.881	2.899	2.879	2.868	3.142	2.825	2.893^{a}

The similarity between the different letters in the same column is statistically significant ($p<0.05$).

TABLE 3
The correlation coefficient matrix of heavy metals

		Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Water	**Al**	1									
	Cr	0.210	1								
	Mn	0.425**	0.704**	1							
	Fe	0.316**	0.723**	0.498**	1						
	Co	0.176	0.808**	0.573**	0.863**	1					
	Ni	0.260*	0.952**	0.641**	0.820**	0.847**	1				
	Cu	0.316**	0.912**	0.672**	0.825**	0.834**	0.945**	1			
	Zn	0.255*	0.881**	0.601**	0.752**	0.814**	0.897**	0.867**	1		
	Cd	0.467**	0.090	0.194	0.236*	0.117	0.144	0.159	0.128	1	
	Pb	0.311**	0.724**	0.504**	0.993**	0.864**	0.817**	0.822**	0.741**	0.224*	1

**: p<0.01, *: p<0.05

TABLE 4
The comparison of international standards for drinking water quality

Parameter	WHO	USEPA	USA	China	Canada
Cd	3 µg/L	5 µg/L	5 µg/L	5 µg/L	5 µg/L
Cr	50µg/L	50 µg/L	100 µg/L	50 µg/L (Cr^6)	50 µg/L
Hg	6 µg/L	1 µg/L	2 µg/L	0.05 µg/L	1 µg/L
Ni	70 µg/L	20 µg/L	100 µg/L		
Cu	2000 µg/L	2000 µg/L	1300 µg/L	1000 µg/L	
Pb	10 µg/L	10 µg/L	15 µg/L	10 µg/L	10 µg/L
Mn	400 µg/L	50 µg/L			
Na	200mg/L	200mg/L			
Zn	3000 µg/L				

Heavy metal pollution is more common in aquatic environments than in air and soil environments. In particular, it may be in the form of accumulation in the water, dissolution in water, or direct sediment accumulation. Toxic metal compounds can reach the surface waters (sea, lake, pond, dam, etc.) with river, rain and snow waters and can also penetrate into ground water as well as trace amounts. For this reason, in case of metal pollution, the groundwater, which is not only surface waters but also as drinking water sources, has a pollution parameter feature that should not be ignored as it may contain toxic metals [16].

The highest metal concentrations in heavy metal monitoring in 32 different surface water areas of Konya closed water basin are at ppb level; (Al, 23.798, Cr: 75.563, Mn: 184.63, Fe: 108.773, Co: 1.738, Ni: 24.430, Cu: 12.143, Zn: 153.017, Cd: 0.185 and Pb: under limit, have been reported [17]. In a study carried out in the Lower Gediz Basin, metal concentrations are at ppb level; Pb: 27.0 ± 0.8% Nif Brook, Cr: 48.9 ± 0.9% Muradiye Bridge, Cd: 12.1 ± 0.6% Istanbul Bridge, Cu: 90.2 ± 0.4% Muradiye Bridge, Ni, Fe and Zn respectively were 309.8 ± 0.7%, 914.1 ± 0.3%, 208.3 ± 0.5% L in Karacay station as the highest values. In addition, the investigators have determined that the study area has fourth class water quality characteristics [18]. In a study carried out at Gürleyik Brook Source, metal concentrations were reported as ppb; Cr, 0.056, Mn: 0.03, Ni: 0.004, Cu: 0.04, Pb: 0.012 and Al, Fe, Zn and Cd were found to be below the limit [19].

The metal level in the Dicle River; Cr: 48.58, Ni: 17.32, As: 12.32, Pb: 22.03, Cu: 4.52, Zn: 3.62, Cd and Mn were determined under the limit [20]. Concentrations in international metal monitoring studies are at ppm level in Nakkavagu River (India); Cr: 46.8, Pb: 13.8 and As: 116 [21], Guadalquivir River (Spain) at ppb level; Zn: 1.58, Cu: 2.64, Pb: 178.23, Cd: 14.82 and Ni: 2.31 [22], Elqui River (Chile) ppm; Cu: 6082, Cr: 26, Pb: 147, Cd: 28, As: 1705 and Hg: 3 [23], Tiiaozi River (China) at ppb level; Zn: 80.58, Cu: 44.33, Pb: 9.56, Cd: 0.15, Mn: 177.21 and Ni: 34.23 [24]. As the ppb of the metal levels in the Ganga River (India); It is determined that the composition is in the range of Cr: 41.8-70.16, Mn: 40.62-68.83, Fe: 83.17-117.7, Ni: 31.28-61.11, Cu: 19.42-43.72, Zn: 31.73-71.37, Cd: 11.41-39.24, Pb: 80.55-134.8 [25].

The metal concentrations in the Serbia River network, including the 14 most important surface water sources, such as the Danube River in Europe, have been determined at ppb level; Zn: 1-122.70, Cu: 1-93, Cr: 0.9-8.60, Pb: 0.045-10.20, Cd: 0.04-2.10, Ni: 1-163.90, As: 0.2-14.4 and Hg: 0.1-2.1 [26]. In our work, the concentration of the metal in the total 84 water samples collected from the surface of the Harşit Stream has been found at the ppb level of change interval; Al: 4.922-1078.906, Cr: 7.141-74.900, Mn: 0.525-18.102, Fe: 4.188-7.855, Co: 3.262-7.878, Ni: 5.832-44.923, Cu: 0.873-20.649, Zn: 10.367-362.901, 1.724-19.427 and Pb: 2.570-

6.259.

When we compare the literature with our present findings, it is concluded that the concentration of heavy metal in surface water of Harşit Stream is very high. It has been determined that the international standards for the values obtained during the study have exceed sometimes and the reference ranges of Cr content for WHO, EPA, China and Canada and Cd contents for WHO, EPA, USA, China and Canada have been exceeded throughout the year (Table 2 and 3). According to SWQR [12], Pb and Cu values are; Class I Quality, Ni and Zn values; Class II Quality in summer and autumn seasons, Class I Quality in other seasons, Cr values; in St.1 and St.7 were found to be in Class II quality in winter and summer, respectively, and Class I Quality in other seasons, but very close to Class II Quality in winter and summer seasons.

In addition to all these results, two main sources of pollution were determined according to the findings obtained by applying multivariate statistical techniques in Harşit Stream (Table 5, Figure 2). The first PC, explaining 66.73 % of the total variance has strong positive loadings on Cr, Fe, Co, Ni, Cu, Zn and Pb and moderate positive loading on Mn. This factor named as "inorganic pollutant factor" and can be based on sand-gravel enterprises wastes, soil erosion and seasonal changes. The second factor, accounting for 13.91 % of the total variance and named as "agricultural factor" has a strong positive loading on Al and Cd. agricultural runoff and precipitation are the natural source of these variables in this area [27]. Moreover, the HCA classifies the seven sampling stations into two major clusters (Figure 3). The first cluster corresponds to station 6 and 7. This station is located at the estuarine area, and receives its pollution mainly from sand-gravel enterprises, garbage disposal wastes, and extensive agricultural run-off, domestic and industrial waste. The second cluster corresponds to Stations 2, 5, 4, 1, and 3. These sampling stations are situated on upper regions of the Harşit Stream.

Consequently, all of our analysis indicate that the heavy metals concentrations need to be monitored regularly. Otherwise, these pollutants can be hazardous for aquatic organisms in the Harşit Stream that empty into the Black Sea.

TABLE 5
Varimax rotated factor matrix for the whole data set

Variable	PC 1	PC 2
Eigenvalues	6,673	1,391
Percentage of variance	66,730	13,913
Accumulative %	66,730	80,643
	Factor loadings (varimax normalized)	
Al	0,184	**0,843**
Cr	**0,936**	0,048
Mn	0,659	0,340
Fe	**0,880**	0,211
Co	**0,924**	0,043
Ni	**0,961**	0,099
Cu	**0,945**	0,153
Zn	**0,912**	0,087
Cd	0,040	**0,845**
Pb	**0,880**	0,203

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization
a. Rotation converged in 3 iterations.
The factor loadings were classified according to loading values as; "strong (>0.75)," "moderate (0.75-0.50)," and "weak (0.50-0.30)"

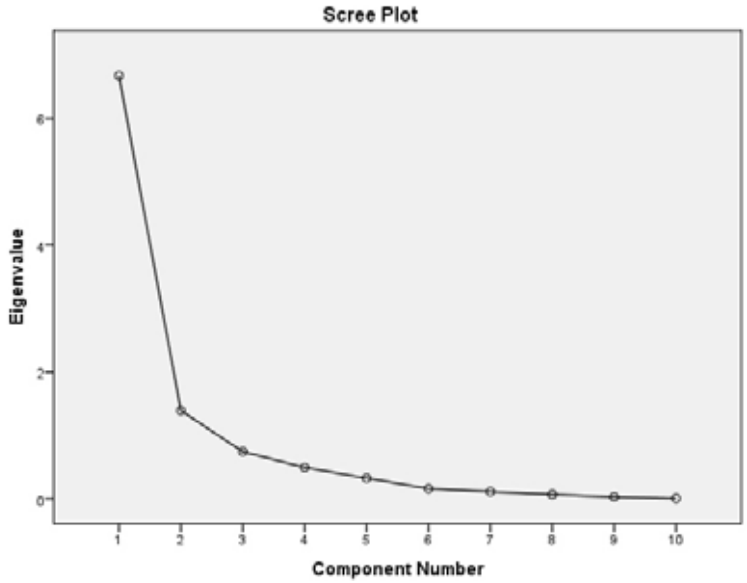


FIGURE 2
Scree-plot for the principal component model of the monitoring data

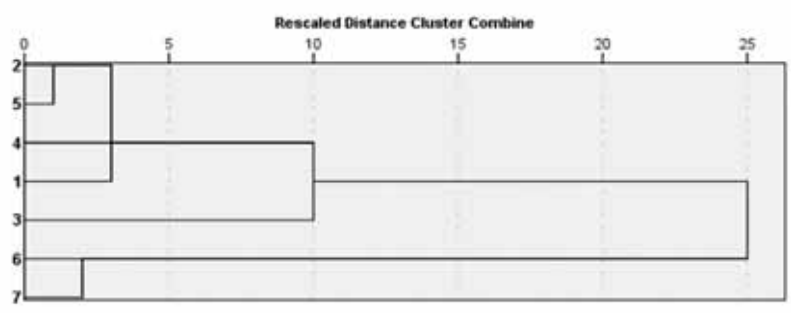


FIGURE 3
Dendogram (using Ward Method) shows clusters of variables

ACKNOWLEDGEMENTS

We thank Giresun University, Project no FEN-BAP-C-250414-02 (Ph.D. Thesis), for the partial financial support. This article has been presented as oral presentation "Determination of Heavy Metal Concentration in Water Samples Collected from Harşit Stream, Giresun-Turkey" in the IBCESS conference of Giresun University in 2016.

REFERENCES

[1] Özbay, Ö., Göksu, M.Z.L. and Alp, M.T. (2011) Investigation of Some Physico-Chemical Parameters of the Water During the Low-Flow and the High-Flow Periods in the River Basins (Berdan Çayı, Tarsus-Mersin). Fırat Univ. Journal of Science. 23(1), 31-39.

[2] Akkan, T. (2017) An Assessment of Linear Alkylbenzene Sulfonate (LAS) Pollution in Harsit Stream, Giresun, Turkey. Fresen. Environ. Bull. 26, 3217- 3221.

[3] Polat, N. and Akkan, T. (2016) Assessment of Heavy Metal and Detergent Pollution in Giresun Coastal Zone, Turkey. Fresen. Environ. Bull. 25, 2884-2890.

[4] Gheorghe, S., Stoica, C., Vasile, G.G., Nita-Lazar, M., Stanescu, E. and Lucaciu, I.E. (2017) Metal Toxic Effects in Aquatic Ecosystems: Modulators of Water Quality. Chapter 4, Water Quality Book. Intech Open, 59-89.

[5] Driscoll, C.T., Yan, C., Schofiel, L., Munson, R. and Holsapple. J. (1994) The mercury cycle and fish in the adirondak lakes. Environ. Sci. Technol. 28, 136-143.

[6] Uncumusaoglu, A.A., Sengul, U. and Akkan, T. (2016) Environmental Contamination of Heavy Metals in The Yaglidere Stream (Giresun), Southeastern Black Sea. Fresen. Environ. Bull. 25, 5492-5498.

[7] Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. (2012) Heavy Metal Toxicity and the Environment. In: Luch, A. (eds.) Molecular, Clinical and Environmental Toxicology. Experientia Supplementum. vol 101. Springer, Basel.

[8] Akkan, T., Kaya, A. and Dinçer, S. (2013) Antibiotic Levels and heavy metal resistance in gram-negative bacteria isolated from seawater, Iskenderun Organized Industrial Zone. Journal of Applied Biological Sciences 7(1), 10-14, 2013.

[9] Alam, M.G.M., Tanaka, A., Stagnitti, F., Allinson, G. and Maekawa, T. (2001) Observations on the Effects of Caged Carp Culture on Water and Sediment Metal Concentrations in Lake Kasumigaura, Japan. Ecotoxicoloyg and Environmental Safety. 48, 107-115.

[10] Thomas, R. (2004) Practical Guide to ICP-MS, Scientific Solutions, Gaithersburg, Maryland, USA, 324p.

[11] APHA. AWWA. WEF (1985) Standart Methods for the Examination of Water and Wastewater. 16. Ed. APHA. Washington. DC.

[12] SWQR (2016) Surface Water Quality Regulations, TR Official Gazette Nr. 29797, Ankara.

[13] Anonymous, (2008) Environmental Quality Standards for trace metals in the aquatic environment. Science Report – SC030194, using science to create a better place, Environment Agency, UK, 177pp.

[14] Sarojam, P. (2012) Analysis of Trace Metals in Drinking Water with the Optima 7000 DV ICP-OES. Application Note.

[15] Uncumusaoğlu A. and Akkan, T., (2017). Assessment of Water Quality of Yağlidere Stream (Turkey) Using Multivariate Statistical Techniquess. Polish Journal of Environmental Studies. 26(4), 1715-1723.

[16] Gunduz, T. (1994) Environmental Problems. Bilge Press, Ankara, (in Turkish).

[17] Uçar, Ş. (2011) Investigation of Heavy Metal Pollution of Surface Water Sources of Konya Basin. Selcuk University, The Graduate School of Natural And Applied Science. Master Thesis. Konya, 118p.

[18] Öner, Ö. and Çelik, A. (2011) Investigation of Some Pollution Parameters in Water and Sediment Samples Collected From theLower Gediz River Basin, Ekoloji. 20(78), 48-52.
[19] Köse, E., Tokatlı, C. and Çiçek, A. (2013) Some Macro and Micro Element Concentrations in Source of Gürleyik Stream (Eskişehir) Away From Anthropogenic Effects. Research Journal of Biological Sciences. 6(1), 40-43.
[20] Varol, M., Gökot, B. and Bekleyen, A. (2010) Assesment of Water Pollution in the Tigris River in Diyarbakır, Turkey. Water Practice and Technology. 5(1), 1-13.
[21] Krishna, A.K., Satyanarayanan, M. and Govil, P.K. (2009) Assessment of Heavy Metal Pollution in Water Using Multivariate Statistical Techniques in an Industrial Area: Accase Study from Patancheru, Medak District, Andhra Pradesh, India. J Hazard Mater. 167, 366–373.
[22] Mendiguchía, C., Moreno, C. and García-Vargas, M. (2007) Evaluation of Natural and Anthropogenic Influences on the Guadalquivir River (Spain) by Dissolved Heavy Metals and Nutrients. Chemosphere. 69, 1509-1517.
[23] Pizarro, J., Vergara, P.M., Rodríguez, J.A. and Valenzuela, A.M. (2010) Heavy Metals in Northern Chilean Rivers: Spatial Variation and Temporal Trends. J Hazard Mater. 181, 747-754.
[24] Dong, D., Liu, X., Guo, Z., Hua, X., Su, Y. and Liang, D. (2015) Seasonal and Spatial Variations of Heavy Metal Pollution in Water and Sediments of China's Tiaozi River. Polish Journal of Environmental Studies. 24(6), 2371-2379.
[25] Pandey, M., Pandey, A.K., Mishra, A. and Tripathi, B.D. (2015) Assessment of Metal Species in River Ganga Sediment at Varanasi, India Using Sequential Extraction Procedure and SEM–EDS. Chemosphere. 134, 466-474.
[26] Dević, G., Sakan, S. and Đorđević, D. (2016) Assessment of the Environmental Significance of Nutrients and Heavy Metal Pollution in the River Network of Serbia. Environ Sci Pollut Res. 23, 282-297.
[27] Norville, W. (2005) Spatial distribution of heavy metals in sediments from the Gulf of Paria,Trinidad. Rev. biol. trop [online]. 53(l.1), 33-40. ISSN 0034-7744.

Received: **19.06.2018**
Accepted: **21.10.2018**

CORRESPONDING AUTHOR

Cengiz Mutlu
Giresun University,
Department of Biology,
Faculty of Science and Arts,
Giresun – Turkey

e-mail: cengizhappy@hotmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9859-9867 Fresenius Environmental Bulletin

THE IMPACT OF POTASSIUM SULPHATE APPLICATION ON *PHASEOLUS VULGARIS* PLANTS GROWN UNDER SALT STRESS

Ceknas Erdinc[1], Ferit Sonmez[2], Aytekin Ekincialp[3,*], Suat Sensoy[4]

[1]Van Yuzuncu Yil University, Faculty of Agriculture, Department of Agricultural Biotechnology, Van, Turkey
[2]Department of Seed Science and Technology, Faculty of Agriculture and Natural Sciences, Abant Izzet Baysal University, Bolu, Turkey
[3]Van Yuzuncu Yil University Baskale Vocational High School Van, Turkey
[4]Van YuzuncuYil University, Faculty of Agriculture, Department of Horticulture, Van, Turkey

ABSTRACT

The present study aimed to investigate the effects of potassium sulphate on certain bean genotypes after K_2SO_4 application based on the analysis of plant growth parameters and macro-micro nutrient element content. The study material included 1 bean genotype (Gevaş) and 3 bean cultivars (Akman-98, Sugar and Önceler) obtained in Lake Van Basin of Turkey. The plants were grown under controlled conditions at 23 ± 2 ºC temperature and 8000 lux light intensity 12 hours light and 12 hours dark photoperiods, with 4 replicates and 8 plants per replicate and randomized lots design. The plants were grown under stress-free conditions until they reached the 3-leaf stage, after which they were exposed to a constant 20 mM salt stress. 500, 1000 and 2000 mg kg^{-1} K_2SO_4 was mixed to the growth medium before seeding except the control plants. Study findings demonstrated the statistical significance of potassium sulphate application especially that of the 1000 mg kg^{-1} and 2000 mg/kg-1 potassium sulphate doses, based on several parameters and its effects on the reduction of salt stress were observed. The variations between the genotypes were observed and it was determined that the genotype Gevaş and cv. Önceler exhibited higher tolerance. Despite the other parameters, the nutrient element parameters such as K/Na and Ca/Na content and plant development parameters such as shoot dry matter and root dry matter content were effective in determination of the positive effects of potassium sulphate.

KEYWORDS:
Bean, NaCl, Stress, Nutrition, K_2SO_4

INTRODUCTION

All plants are exposed to various stress factors during their life cycles. One of the most important environmental stress factors that adversely affects plant yield is salinity [1-3]. Similar to most other environmental stress factors, salt stress effects photosynthesis, leading to water deficiency (stomatal closure), ion toxicity and K deficiency, resulting in oxidative stress [4, 5]. Salinity may be more acute in arid and semi-arid regions with low precipitation, high evapotranspiration and poor water and soil management conditions [6-9]. The accumulation of soluble salts in the root medium leads to osmotic stress, revealing the problems related to the intake and consumption of essential nutrients, which in turn adversely affect various enzyme activities and plant metabolism [10, 11]. Interactions between salts and mineral nutrients could also result in significant food imbalances and deficiencies [12]. Ionic imbalance is caused by excessive Na^+ buildup in cells and decreases the intake of other mineral nutrients such as K^+, Ca^{2+} and Mn^{2+}. High sodium potassium content induced by high sodium ion accumulation inactivates the enzymes and affects metabolic processes in plants [13, 14]. Increased intracellular Na^+ levels by preventing excessive Na, Cl^- and K^+ intake and altering enzyme activity and leading to cellular metabolic alteration through deteriorating and dividing the cellular K^+ intake affects the stomatal opening, and thus reduces the growth ability of the plant. Both K^+ and Ca^{2+} are nutrients that are required to maintain the integrity and operation of cell membranes. [8].

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes cultivated for human consumption, with 23 million ha of global cultivation area [15, 16]. Turkey, despite the absence of the gene centers bean has a rich genetic diversity [17, 18]. Many previous studies have revealed that bean genotypes show a wide variation in their consumption characteristics and morphologically [19]. Common Bean (*Phaseolus vulgaris* L.) is known as a salt sensitive plant species [20] with being one of the most important Fabaceae vegetables produced in the developing countries in the Middle East, especially for human nutrition. [21]. In salt-tolerant species, Na^+ and Cl^- ions are stored in vacuole, so that physiological reactions in the cytoplasm can continue without being affected. In susceptible plants such as beans, however, these ions are pre-

vented from being stored in vacuoles, and elevated Na^+ and Cl^- levels in the cytoplasm halt the enzyme activity [22]. In the present study, conducted with the premise that common bean is a globally important vegetable species and to overcome the obstacles to its cultivation in marginal areas, two salt tolerant and two salt sensitive bean genotypes as determined in previous studies were analyzed and assessed after application of different K doses using morphological and in physiological parameters.

MATERIALS AND METHODS

Plant material. In the present study, two salt tolerant (Gevaş, cv. Önceler) and two salt sensitive (cv. Akman-98, cv. Şeker) bean genotypes as determined in previous studies were used.

NaCl and K_2SO_4 Applications. During the set up phase of the experiment, the seeds were sown in 3 liter pots filled with 2:1 peat-perlite soil mixture and the seeds were covered with vermiculite. The study was conducted under controlled conditions at 23 ± 2 ºC temperature and 8000 lux light intensity 12 hours light and 12 hours dark photoperiods, with 4 replicates and 8 plants per replicate and randomized lots design. All plants until the salt stress formation stage and the control plants after the application of salt were irrigated with Hoagland nutrient solution [23]. The plants were grown under stress-free conditions until reaching the 3-leaf stage, after which they were exposed to a constant 20 mM salt stress. 500, 1000 and 2000 mg kg^{-1} K_2SO_4 was applied to the growth medium before seeding except the control plants.

Determination of Shoot Nutrient Contents. Plant samples were dried at 65 ºC for 48 hours and fired at 550 ºC. The obtained ash was dissolved in 3.3% HCl and N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu content were read with atomic absorption device at Van Yüzüncü Yıl University Research Center laboratory (ICE-3000 SERIES) [24]. Phosphor readings were conducted with a spectrophotometer (Thermo G10S Uv-Vis) [25].

Growth analysis. The morphological parameters of shoot and root length, shoot diameter, leaf number, shoot-root fresh and dry weights, shoot-root dry matter ratio were determined at the end of the experiment.

Statistical Analysis. The study data were assessed with analysis of variance based on completely randomized experimental design and $P < 0.05$ significance level. The statistically significant means were grouped with Duncan Multiple Comparison Test [26] in data analysis. Data analysis was conducted using SAS software.

RESULTS AND DISCUSSION

Effect of potassium on nutrition content. The percentage changes, in addition to the macronutrient element data obtained to determine the effects of K_2SO_4 application on genotypes that were exposed to constant salt application were also calculated (Table 1). As a result of K_2SO_4 application to the genotypes exposed to constant 20 mM salt application, it was determined that the values obtained for N element were statistically significant among genotypes and K_2SO_4 applications. Based on N content, the highest impact of potassium was observed with 1000 mg kg^{-1} dose and with the genotype Gevaş with a 9.30% rate of change. For the phosphorus element, it was found that the differences between applications were statistically significant; however the differences between genotypes were not significant. For P content, the highest effect of potassium was obtained with the 2000 mg kg^{-1} dose, and only cv. Akman and cv. Önceler exhibited the highest rate of change and the highest positive rate of change, respectively when compared to the control. The K element content of plants demonstrated that there were statistically significant differences between applications and genotypes, and the highest positive change was observed with 1000 mg kg^{-1} potassium sulphate dose. In general, it was observed that there were increases in K element content when compared to the control.

There is a continuous race between the Na^+ and K^+ intake in the plant [27, 28]. In several studies, it was reported that high K^+ content increases plant's salt tolerance and increased salinity leads to the lack of potassium element [29, 30]. It was suggested that the increase in K_2SO_4 dose led to an increase in potassium content, which in turn minimized the impact of salt stress on the genotypes. Na increase in soil solution content lead to Ca^+, K^+ and Mg^+ deficiency in plants. Furthermore, Na^+ ion accumulates in the apoplast in cellular wall, destroys the functional structure of the cell wall and adversely affects the cellular Ca^+ balance [31]. Several researchers reported that when Ca^+ content is sufficient, Na^+ ion toxicity is reduced [32, 33]. K and some other macro elements, especially Ca, are known for their alleviating effects on the adverse effects of salinity in plants. In particular, Ca has a positive effect due to its regulatory effects on Na ions that enter the same membrane binding sites in the plant and protects the cell membrane against the toxic effects of salinity [34-36]. Examination of the genotypes Ca content in the present study revealed no statistical significance between the applications. Increases in Ca accumulation were observed at

1000 and 2000 mg kg^{-1} K_2SO_4 doses. On the contrary, negative changes were observed mostly in 2000 mg kg^{-1} dose application. No statistically significant differences were found between the genotypes and the highest positive rate of change (21.85%) was observed with 1000 mg kg^{-1} K_2SO_4 dose application to cv. Akman. In several studies, it was reported that the Na content increased with the increased Na concentration in root sections of plants under salt stress, while cation content such as Ca, K and Mg decreased [37-39]. Thus, salt tolerance could be achieved by reducing the Na^+ and Cl^- ion intake and by increasing the K^+ ion intake in the green sections [40]. In the present study, it was observed that in potassium treated plants; there were significant reductions in Na concentrations and positive increases in Ca, K and Mg content. Although there were no statistically significant differences between applications or genotypes based on Mg content, the highest mean value increase, albeit very slight, was observed with of 2000 mg kg^{-1} K_2SO_4. Although there was no statistically significant difference between genotypes, analysis of the percentage changes compared to the control demonstrated that there were positive changes in the genotype Gevaş (App.1), cv. Akman (App.3), and cv. Şeker (App.1-3).

TABLE 1
Macro nutrition contents of bean genoytypes with/without potassium sulphate (K_2SO_4) application according to 20 mM NaCl

Applications								
				N (%)				
Genotypes	Control	App.1	Change (%)	App.2	Change (%)	App.3	Change (%)	Mean
cv. Akman	4.30±0.40	4.06±0.10	-5.58	3.97±0.16	-7.67	4.52±0.07	5.12	4.17^{B}±0.29
Gevaş	5.16±0.30	4.60±0.08	-10.85	5.64±1.00	9.30	4.99±0.45	-3.29	5.13^{A}±0.67
cv. Önceler	4.62±0.21	4.19±0.40	-9.31	4.44±0.24	-3.90	4.24±0.62	-8.23	4.38^{B}±0.40
cv. Şeker	4.55±0.20	4.01±0.27	-11.87	4.40±0.33	-3.30	4.36±0.35	-4.18	4.33^{B}±0.33
Mean	4.62^{A}±0.40	4.19^{B}±0.31		4.61^{A}±0.81		4.53^{A}±0.51		
				P (mg kg^{-1})				
cv. Akman	5118.67±495.50	5862.00±744.57	14.52	5436.25±502.07	6.20	6054.25±563.18	18.28	5651.07^{A}±639.48
Gevaş	6247.00±825.51	5687.67±701.33	-8.95	5339.00±326.93	-14.53	6178.25±845.84	-1.10	5874.67^{A}±740.22
Önceler	5903.25±613.62	5105.00±491.05	-13.52	5724.00±155.56	-3.04	6099.50±919.83	3.32	5707.94^{A}±667.58
Şeker	5985.75±1053.54	5187.50±1015.43	-13.34	5751.75±702.57	-3.91	5926.00±520.23	-1.00	5698.53^{A}±844.58
Mean	5860.00^{AB}±815.62	5445.40^{B}±758.92		5562.75^{AB}±457.64		6073.73^{A}±670.33		
				K (%)				
cv. Akman	8.03±0.81	7.61±0.58	-5.23	8.72±0.49	8.59	8.71±0.31	8.47	8.24^{A}±0.72
Gevaş	6.93±0.61	8.43±1.40	21.65	8.47±0.45	22.22	8.18±0.36	18.04	8.00^{A}±0.98
cv. Önceler	6.54±1.39	7.15±0.58	9.33	8.00±0.30	22.32	7.61±1.05	16.36	7.33^{B}±1.00
cv. Şeker	7.22±0.21	7.76±0.63	7.48	8.94±0.85	23.82	8.82±0.51	22.16	8.18^{A}±0.92
Mean	7.18^{C}±0.96	7.74^{B}±0.91		8.53^{A}±0.62		8.31^{A}±0.77		
				Ca (%)				
cv. Akman	5.72±0.84	6.24±0.89	9.09	6.97±1.76	21.85	6.19±1.16	8.22	6.28^{A}±1.18
Gevaş	6.19±0.83	6.48±1.32	4.68	5.80±0.48	-6.30	5.60±0.86	-9.53	6.02^{A}±0.90
cv. Önceler	6.23±1.19	5.99±0.86	-3.85	6.55±1.06	5.14	5.90±0.81	-5.30	6.17^{A}±0.92
cv. Şeker	6.27±1.03	6.16±1.14	-1.75	5.92±0.58	-5.58	6.20±1.01	-1.12	6.14^{A}±0.87
Mean	6.10^{A}±0.91	6.22^{A}±0.97		6.31^{A}±1.09		5.97^{A}±0.90		
				Mg (%)				
cv. Akman	2.22±0.08	2.16±0.30	-2.70	2.21±0.37	-0.45	2.34±0.71	5.41	2.23^{A}±0.39
Gevaş	2.30±0.39	2.71±0.56	17.83	2.00±0.18	-13.04	2.09±0.34	-9.13	2.27^{A}±0.45
cv. Önceler	2.43±0.57	1.82±0.13	-25.10	2.42±0.55	-0.41	2.32±0.43	-4.53	2.24^{A}±0.48
cv. Şeker	2.02±0.40	2.27±0.50	12.38	2.18±0.41	7.92	2.38±0.59	17.82	2.21^{A}±0.45
Mean	2.24^{A}±0.39	2.24^{A}±0.49		2.20^{A}±0.39		2.28^{A}±0.49		

Mean values with different lower-case letters among columns are significantly different at $p< 0.05$.
Mean values with different capital letters among rows are significantly different at $p< 0.05$ level.
Control: 0 mg kg^{-1} K_2SO_4 , 20 Mm NaCl, App. 1: 500 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 2: 1000 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App.3: 2000 mg kg^{-1} K_2SO_4, 20 Mm NaCl

Micro-nutrient element data and their changes compared to the control are presented in Table 2. Although there were no significant statistical differences between the data obtained with various applications based on Fe content, there were significant differences between the genotypes. In the Fe content, there were positive increases in all cultivars except for the genotype Gevaş based on percentage change when compared to the control group. There were no statistically significant differences between the applications based on Mn content and significant differences were determined between genotypes. Although there was no statistical significance between potassium sulphate applications based on Na content and genotypes, it was observed that 20 mM salt application had positive effects in plants in potassium sulphate application mean values and decreases in Na content, and the highest decrease was observed with App. 3. Consistent with the present study findings, Kaya and Higgs [41] found that Na content increased significantly in plant leaves and roots in the control group due to the impact of NaCl and that the adverse effect of the salt on the KNO_3 administered plants demonstrated significant decreases when compared to the control group in a study where KNO_3 was administered against salt stress in pepper and explained this with a partial dilution effect due to the increase in dry matter accumulation. When compared to the control, cv. Akman exhibited a positive percentage increase in App. 2 and App. 3, and cv. Önceler exhibited positive increase in App. 2. In other genotypes, percentage changes were negative, and it was determined that the highest decrease was in the genotype Gevaş (42.15%). There were no significant differences between the applications in Zn and Cu elements; however differences between genotypes were statistically significant. The highest positive percentage change was determined in cv. Önceler (41.78%) based on the Zn content data when compared to the control and the highest positive increase between K_2SO_4 applications was observed with the 2000 mg kg^{-1} dose. The sole positive change was observed in cv. Önceler based on the Cu content, and the highest percentage change (27.21%) was determined in App. 2, where 2000 mg kg^{-1} K_2SO_4 was administered.

TABLE 2
Micro nutrition contents of bean genotypes with/without potassium sulphate (K_2SO_4) application according to 20 mM NaCl

Applications								
Genotypes	Control	App.1	Change (%)	App.2	Change (%)	App.3	Change (%)	Mean
				Fe (mg kg^{-1})				
cv. Akman	130.17±16.22	143.74±24.06	10.42	132.86±10.27	2.07	149.32±24.32	14.71	139.61^{B}±19.36
Gevaş	183.41±29.46	167.89±38.89	-8.46	170.50±33.37	-7.04	154.94±49.97	-15.52	169.27^{AB}±35.95
cv. Önceler	159.42±86.53	181.93±36.15	14.12	186.02±29.43	16.69	169.42±42.34	6.27	174.19^{A}±48.07
cv. Şeker	177.77±60.42	205.45±48.99	15.57	182.86±30.57	2.86	178.99±42.93	0.69	186.27^{A}±43.59
Mean	164.86^{A}±54.98	175.21^{A}±41.27		168.06^{A}±32.87		163.16^{A}±38.62		
				Mn (mg kg^{-1})				
cv. Akman	79.23±19.11	71.22±16.93	-10.11	77.89±12.60	-1.69	79.48±10.28	0.32	76.96^{A}±13.98
Gevaş	57.27±14.01	75.82±35.84	32.39	71.64±13.09	25.09	58.28±7.39	1.76	65.75^{B}±20.29
cv. Önceler	49.75±2.73	50.95±6.40	2.41	50.42±6.87	1.35	51.52±6.90	3.56	50.66^{C}±5.39
cv. Şeker	72.81±10.48	76.25±13.02	4.72	70.36±9.53	-3.36	72.19±16.42	-0.85	72.90^{AB}±11.52
Mean	64.76^{A}±16.85	68.56^{A}±21.70		67.58^{A}±14.39		65.37^{A}±15.02		
				Na (%)				
cv. Akman	2.57±0.81	2.46±0.33	-4.28	3.03±1.35	17.90	2.70±1.97	5.06	2.70^{A}±1.20
Gevaş	3.25±1.30	3.00±0.87	-7.69	1.88±0.57	-42.15	2.40±0.83	-26.15	2.63^{A}±1.00
cv. Önceler	3.15±1.58	3.06±0.73	-2.86	3.41±1.58	8.25	2.10±1.24	-33.33	2.93^{A}±1.30
cv. Şeker	2.64±1.19	2.40±1.12	-9.09	2.35±1.33	-10.98	2.26±2.05	-14.39	2.42^{A}±1.32
Mean	2.90^{A}±1.16	2.75^{A}±0.81		2.69^{A}±1.28		2.36^{A}±1.45		
				Zn (mg kg^{-1})				
cv. Akman	22.30±4.66	22.72±3.58	1.88	21.18±2.42	-5.02	18.77±5.47	-15.83	21.24^{A}±4.07
Gevaş	24.79±6.40	27.11±9.53	9.36	20.31±2.55	-18.07	23.04±2.96	-7.06	24.05^{A}±6.10
cv. Önceler	14.17±4.21	18.82±0.53	32.82	17.94±2.06	26.61	20.09±3.50	41.78	17.75^{B}±3.48
cv. Şeker	22.03±3.44	20.86±4.05	-5.31	20.14±6.20	-8.58	21.36±1.52	-3.04	21.11^{A}±3.76
Mean	20.82^{A}±5.95	22.48^{A}±5.93		19.86^{A}±3.60		20.81^{A}±3.65		
				Cu (mg kg^{-1})				
cv. Akman	16.14±4.37	15.68±2.58	-2.85	14.59±2.73	-9.60	15.30±1.59	-5.20	15.43^{B}±2.74
Gevaş	21.36±6.34	17.42±4.17	-18.45	18.01±0.74	-15.68	17.86±2.56	-16.39	18.66^{A}±3.95
cv. Önceler	12.90±2.84	13.69±1.82	6.12	13.79±1.74	6.90	16.41±1.84	27.21	14.20^{B}±2.33
cv. Şeker	16.41±2.79	14.58±3.23	-11.15	15.37±4.20	-6.34	15.30±2.23	-6.76	15.41^{B}±2.94
Mean	16.70^{A}±4.98	15.34^{A}±3.10		15.44^{A}±2.90		16.22^{A}±2.16		

Mean values with different lower-case letters among columns are significantly different at $p< 0.05$.
Mean values with different capital letters among rows are significantly different at $p< 0.05$ level.
App. 0:**:** 0 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 1: 500 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 2: 1000 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App.3: 2000 mg kg^{-1} K_2SO_4, 20 Mm NaCl

TABLE 3
K/Na and Ca/Na contents of bean genotypes with/without potassium sulphate (K_2SO_4) application according to 20 mM NaCl

Applications				K/Na ratio				
Genotypes	Control	App.1	Change (%)	App.2	Change (%)	App.3	Change (%)	Mean
cv. Akman	3.43±1.42	3.21±0.72	-6.41	3.29±1.25	-4.08	5.55±3.33	61.81	3.80^{A}±1.88
Gevaş	2.42±0.99	3.20±1.81	32.23	4.92±1.86	103.31	3.67±1.05	51.65	3.55^{A}±1.63
cv. Önceler	3.07±2.62	2.41±0.42	-21.50	2.81±1.42	-8.47	4.57±2.39	48.86	3.21^{A}±1.92
cv. Şeker	3.09±1.09	3.75±1.50	21.36	4.48±2.28	44.98	6.01±3.31	94.50	4.32^{A}±2.28
Mean	3.00^{B}±1.53	3.14^{B}±1.25		3.83^{AB}±1.75		4.91^{A}±2.51		
				Ca/Na ratio				
cv. Akman	2.43±0.89	2.52±0.40	3.70	2.47±0.51	1.65	3.19±1.77	31.28	2.66^{A}±1.02
Gevaş	2.13±0.78	2.24±0.43	5.16	3.35±1.22	57.28	2.45±0.50	15.02	2.54^{A}±0.87
cv. Önceler	2.87±2.36	1.99±0.17	-30.66	2.21±0.94	-23.00	3.39±1.31	18.12	2.61^{A}±1.40
cv. Şeker	2.62±0.86	3.17±2.04	20.99	2.98±1.42	13.74	3.97±1.98	51.53	3.20^{A}±1.57
Mean	2.51^{A}±1.27	2.47^{A}±1.09		2.74^{A}±1.04		3.25^{A}±1.46		

Mean values with different lower-case letters among columns are significantly different at $p< 0.05$.
Mean values with different capital letters among rows are significantly different at $p< 0.05$ level.
App. 0:: 0 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 1: 500 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 2: 1000 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App.3: 2000 mg kg^{-1} K_2SO_4, 20 Mm NaCl

Na accumulation and lack of element K are essential features in plant salt stress. Thus, although K/Na ratio is considered as an accurate parameter to evaluate salt tolerance in plants, selection of genotypes with high K/Na ratio is an important strategy to minimize growth problems in saline soil [42-44]. The K/Na and Ca/Na ratios obtained in the present study are presented in Table 3. It was determined that the differences between applications were statistically significant based on the K/Na ratio, and application mean K/Na ratios increased with the increase in K dose in each application. There was no statistically significant difference between the genotypes, while the highest positive change (103.31%) was obtained with 1000 mg kg^{-1} K_2SO_4 applied to the genotype Gevaş. The reduction in Ca/Na ratio under saline conditions leads to deterioration of membrane permeability, which in turn increases the severity of toxicity through further intake of other salts, primarily the Na [45-47]. In the present study, although there were no significant differences between the applications and genotypes based on the Ca/Na ratio, it was observed that mean Ca/Na ratios were higher in App. 2 and App. 3. The highest positive change (57.28%) in Ca/Na ratio percentage change when compared to the control was observed with the genotype Gevaş, and positive changes were observed in all cultivars except the 500 and 1000 mg/kg^{-1} K_2SO_4 applications to cv. Önceler. The study findings emphasized the significance of preservative effect of K element in alleviation of the adverse effects of salt stress on plants.

Plant growth. Plant growth parameter data and percentage changes are presented in Tables 4 and 5. Previous studies reported that ground and underground biomass production decreased parallel with the increase in salinity in the irrigation water in bean plants [22, 48]. Thus, root and shoot growth is used as selection criteria in saline media [22, 49]. In the present study, the differences between the applications were statistically significant based on the plant shoot length data; however it was not possible to determine the effect of potassium on the averages obtained with the applications. It was determined that Genotypes x Potassium interaction was significant and the differences among the genotypes were statistically significant, the highest value (17.89 cm) was observed in the genotype Gevaş 2000 mg kg^{-1} potassium application among the genotypes based on Genotypes x Potassium interaction. The lowest interaction was observed in cv. Şeker that was exposed to 500 mg kg^{-1} potassium sulphate dose (7.75 cm). The differences between the plant shoot diameters were statistically significant between genotypes and K_2SO_4 applications; however, the analysis of percentage changes in genotypes compared to the control demonstrated that the overall impact of potassium was not positive. Only cv. Önceler exhibited a positive change with 2000 mg kg^{-1} potassium sulphate dose. There were no statistically significant differences were obtained between plant fresh weight values, positive changes were observed in cv. Akman and the genotype Gevaş for 1000 and 2000 mg kg^{-1} potassium sulphate doses, in the genotype Gevaş and cv. Şeker cultivar for 2000 mg kg^{-1} potassium sulphate dose, and the highest change was observed in the

genotype Gevaş for 2000 mg kg^{-1} potassium sulphate dose with 22.43%. Statistically significant differences were found between genotypes based on shoot dry weight and the highest positive change was determined in cv. Akman exposed to 1000 mg kg^{-1} K_2SO_4. Several studies indicated that as the amount of salt applied to the soil increases, the growth of the plants, and hence the amount of dry matter (root and stem) decreases [50, 51]. In the present study, positive changes were observed in percentage changes in dry matter content when compared to the control, and in particular, increases were observed more clearly in 1000 mg kg^{-1} potassium sulphate application. It was determined that cv. Önceler exhibited an increase in all potassium sulphate doses and the highest increase was observed with 1000 mg kg^{-1} K_2SO_4.

The highest increases in genotypes leaf number were observed with 1000 and 2000 mg kg^{-1} K_2SO_4 applications. It was found that the values obtained with K_2SO_4 applications were statistically significant, and the highest leaf number was obtained with 1000 mg kg^{-1} K_2SO_4, the cultivars with the highest leaf number were cv. Akman (31.89%) and cv. Şeker (23.08%). It was determined in the study that there were statistically significant differences between genotypes and K_2SO_4 applications based on the plant root length values. The highest positive percentage change was determined in the genotype Gevaş exposed to 1000 and 2000 mg kg^{-1} K_2SO_4 (30.48% and 19.39%, respectively). Based on root fresh weight, there was a statistical significance as well. The most positive effect on plant root fresh weight was observed with 1000 mg kg^{-1} K_2SO_4 application. Although root dry weight data were not statistically significant, there were increases in several genotypes when compared to the control. It was determined that there were statistically significant differences between genotypes and Genotypes x Potassium interactions based on the

TABLE 4
Growth characteristics of bean genotypes with/without potassium sulphate (K_2SO_4) application according to 20 mM NaCl

Applications								
Shoot length (cm)								
Genotypes	Control	App.1	Change (%)	App.2	Change (%)	App.3	Change (%)	Mean
cv.Akman	12.87 ± 0.61^{bc}	9.88 ± 2.66^{cd}	-23.23	12.44 ± 1.00^{cd}	-3.34	10.56 ± 1.88^{cd}	-17.95	$11.44^{B}\pm2.01$
Gevaş	16.81 ± 3.36^{ab}	9.92 ± 2.20^{cd}	-40.99	12.00 ± 5.35^{cd}	-28.61	17.89 ± 2.67^{a}	6.42	$14.15^{A}\pm4.69$
cv.Önceler	11.88 ± 2.17^{cd}	10.75 ± 0.96^{cd}	-9.51	9.50 ± 3.54^{cd}	-20.03	10.46 ± 0.85^{cd}	-11.95	$10.81^{B}\pm1.76$
cv.Şeker	11.81 ± 2.19^{cd}	7.75 ± 0.96^{d}	-34.38	10.50 ± 1.91^{cd}	-11.09	10.10 ± 3.55^{cd}	-14.48	$10.04^{B}\pm2.59$
Mean	$13.34^{A}\pm2.95$	$9.57^{C}\pm2.01$		$11.34^{BC}\pm3.15$		$12.25^{AB}\pm4.02$		
Shoot diameter (mm)								
cv.Akman	4.17±0.31	3.74±0.56	-10.31	3.75±0.63	-10.07	3.93±0.56	-5.76	$3.90^{B}\pm0.51$
Gevaş	5.10±0.29	4.46±0.09	-12.55	4.55±0.95	-10.78	4.42±0.37	-13.33	$4.63^{A}\pm0.55$
cv.Önceler	4.73±0.54	4.55±0.64	-3.81	4.26±0.23	-9.94	4.89±0.68	3.38	$4.65^{A}\pm0.57$
cv.Şeker	5.06±0.39	3.89±0.45	-23.12	4.37±0.78	-13.64	4.28±0.28	-15.42	$4.40^{A}\pm0.63$
Mean	$4.76^{A}\pm0.52$	$4.16^{B}\pm0.56$		$4.23^{B}\pm0.74$		$4.38^{AB}\pm0.57$		
Shoot fresh weight (g)								
cv.Akman	3.46±0.92	3.39±0.80	-2.02	3.47±0.44	0.29	2.78±0.41	-19.65	$3.27^{A}\pm0.68$
Gevaş	3.21±0.36	2.44±0.37	-23.99	3.38±1.49	5.30	3.93±0.60	22.43	$3.24^{A}\pm0.94$
cv.Önceler	3.75±0.48	3.19±0.57	-14.93	2.65±0.07	-29.33	3.44±0.75	-8.27	$3.34^{A}\pm0.63$
cv.Şeker	3.83±0.97	2.43±0.69	-36.55	2.85±0.99	-25.59	3.96±2.56	3.39	$3.27^{A}\pm1.49$
Mean	$3.56^{A}\pm0.70$	$2.86^{A}\pm0.72$		$3.15^{A}\pm0.95$		$3.53^{A}\pm1.33$		
Shoot dry weight (g)								
cv.Akman	0.37±0.09	0.36±0.10	-2.70	0.45±0.07	21.62	0.31±0.08	-16.22	$0.38^{B}\pm0.09$
Gevaş	0.40±0.03	0.35±0.09	-12.50	0.45±0.22	12.50	0.41±0.15	2.50	$0.40^{B}\pm0.13$
cv.Önceler	0.52±0.07	0.55±0.13	5.77	0.50±…	-3.85	0.56±0.20	7.69	$0.54^{A}\pm0.13$
cv.Şeker	0.39±0.10	0.28±0.07	-28.21	0.33±0.05	-15.38	0.31±0.08	-20.51	$0.33^{B}\pm0.08$
Mean	$0.42^{A}\pm0.09$	$0.39^{A}\pm0.14$		$0.41^{A}\pm0.13$		$0.40^{A}\pm0.17$		
Shoot dry matter (%)								
cv.Akman	10.80±0.41	10.61±0.73	-1.76	12.94±1.76	19.81	10.49±1.83	-2.87	$11.26^{B}\pm1.55$
Gevaş	12.45±1.24	14.05±1.81	12.85	13.35±5.12	7.23	10.12±3.16	-18.71	$12.49^{B}\pm3.25$
cv.Önceler	14.14±2.91	17.48±4.33	23.62	18.52±…	30.98	16.63±5.91	17.61	$16.27^{A}\pm4.24$
cv.Şeker	10.29±1.13	8.71±0.69	-15.35	12.34±3.85	19.92	9.29±3.52	-9.72	$10.36^{B}\pm2.93$
Mean	$11.92^{A}\pm2.17$	$13.28^{A}\pm4.03$		$13.31^{A}\pm3.70$		$11.71^{A}\pm4.73$		
Leaf number								
cv.Akman	7.40±1.12	7.38±1.11	-0.27	9.76±2.22	31.89	7.19±1.11	-2.84	$7.93^{A}\pm1.71$
Gevaş	7.76±0.76	5.50±1.23	-29.12	7.75±3.40	-0.13	9.07±1.02	16.88	$7.52^{A}\pm2.17$
cv.Önceler	6.38±2.21	6.75±2.06	5.80	7.00±1.41	9.72	6.88±0.16	7.84	$6.71^{A}\pm1.53$
cv.Şeker	6.50±1.91	5.33±1.89	-18.00	8.00±0.82	23.08	7.38±2.50	13.54	$6.80^{A}\pm1.97$
Mean	$7.01^{AB}\pm1.56$	$6.24^{B}\pm1.70$		$8.29^{A}\pm2.27$		$7.63^{A}\pm1.57$		

Mean values with different lower-case letters among columns are significantly different at $p< 0.05$.
Mean values with different capital letters among rows are significantly different at $p< 0.05$ level.
App. 0:: 0 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 1: 500 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 2: 1000 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App.3: 2000 mg kg^{-1} K_2SO_4, 20 Mm NaCl

TABLE 5
Root growth characteristics of bean genotypes with/without potassium sulphate (K_2SO_4) application according to 20 mM NaCl

Applications				Root length (cm)				
Genotypes	Control	App.1	Change (%)	App.2	Change (%)	App.3	Change (%)	Mean
cv. Akman	15.11±1.10	14.50±1.48	-4.04	16.13±2.78	6.75	14.06±2.20	-6.95	14.95^{AB}±1.96
Gevaş	15.52±3.66	11.75±1.52	-24.29	20.25±2.87	30.48	18.53±2.85	19.39	16.51^{A}±4.19
cv. Önceler	14.00±2.16	14.13±2.78	0.93	15.00±0.00	7.14	14.17±1.60	1.21	14.23^{B}±1.89
cv. Şeker	15.17±4.75	15.21±0.63	0.26	16.50±2.38	8.77	14.75±5.87	-2.77	15.41^{AB}±3.61
Mean	14.95^{B}±2.95	13.90^{B}±2.08		17.25^{A}±3.02		15.38^{AB}±3.69		
				Root fresh weight (g)				
cv. Akman	1.46±0.27	2.06±0.60	41.10	2.07±0.19	41.78	1.53±0.29	4.79	1.78^{AB}±0.45
Gevaş	1.46±0.52	1.76±0.30	20.55	1.73±0.97	18.49	1.62±0.20	10.96	1.64^{B}±0.53
cv. Önceler	1.43±0.54	1.89±0.23	32.17	1.75±0.21	22.38	1.21±0.09	-15.38	1.54^{B}±0.41
cv. Şeker	1.98±0.59	2.15±0.55	8.59	2.33±0.74	17.68	1.63±0.40	-17.68	2.02^{A}±0.59
Mean	1.58^{B}±0.50	1.96^{A}±0.43		2.00^{A}±0.65		1.50^{B}±0.30		
				Root dry weight (g)				
cv. Akman	0.18±0.02	0.26±0.09	44.44	0.22±0.02	22.22	0.13±0.06	-27.78	0.20^{A}±0.07
Gevaş	0.19±0.07	0.20±0.05	5.26	0.16±0.06	-15.79	0.19±0.03	0.00	0.19^{A}±0.05
cv. Önceler	0.14±0.03	0.19±0.04	35.71	0.19±…	35.71	0.22±0.11	57.14	0.18^{A}±0.07
cv. Şeker	0.18±0.06	0.17±0.04	-5.56	0.21±0.07	16.67	0.17±0.10	-5.56	0.18^{A}±0.07
Mean	0.17^{A}±0.05	0.21^{A}±0.06		0.20^{A}±0.05		0.18^{A}±0.08		
				Root dry matter (%)				
cv. Akman	$12.49±1.04^{b}$	$12.48±2.25^{b}$	-0.08	$10.80±1.05^{b}$	-13.53	$8.35±4.49^{b}$	-33.15	11.21^{AB}±2.68
Gevaş	$13.34±1.48^{ab}$	$11.39±1.60^{b}$	-14.62	$10.39±2.52^{b}$	-22.11	$11.74±1.09^{b}$	-11.99	11.72^{AB}±1.91
cv. Önceler	$10.19±2.01^{b}$	$10.23±0.82^{b}$	0.39	$10.00±0.00^{b}$	-1.86	$18.06±8.17^{a}$	77.23	12.61^{A}±5.68
cv. Şeker	$9.23±2.19^{b}$	$9.79±1.43^{b}$	6.07	$9.20±1.08^{b}$	-0.33	$10.21±3.39^{b}$	10.62	9.59^{B}±2.06
Mean	11.31^{A}±2.32	11.05^{A}±1.80		10.12^{A}±1.62		12.34^{A}±5.84		

Mean values with different lower-case letters among columns are significantly different at $p< 0.05$.
Mean values with different capital letters among rows are significantly different at $p< 0.05$ level.
App. 0:: 0 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 1: 500 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App. 2: 1000 mg kg^{-1} K_2SO_4, 20 Mm NaCl, App.3: 2000 mg kg^{-1} K_2SO_4, 20 Mm NaCl

root dry matter ratio, and the highest root dry matter ration was observed in 2000 mg kg^{-1} doses. The cv. Önceler (18.06%). Similar to the current study findings, Hemida et al. [21] demonstrated that both Potassium humite (KH) and alpha-tocopherol (TOC) applications significantly increased the shoot length, leaf number and plant dry weight parameters in bean plants grown in saline soil when compared to the control.

CONCLUSION

- K_2SO_4 applications had positive impact on stress conditions in plants exposed to constant 20 mM salt.
- It was determined that there were increases in nutritional element content, especially in K/Na and Ca/Na ratios, in potassium administered plants when compared to the control group. There were clear decreases in plant Na content as the administered potassium dose increased. In these parameters that are determined as significant indicators of salt stress, it was observed that the highest impact was observed with 2000 mg kg^{-1} potassium sulphate dose.
- There were variations in plant growth parameters and it was determined that potassium sulphate application had a positive impact on shoot dry matter and root dry matter content, which are considered as the most effective indicators of salt stress.
- Although there were differences based on K_2SO_4 doses, it was determined that 1000 and 2000 mg kg^{-1} potassium sulphate doses were the most effective.
- Positive effects of potassium on parameters such as leaf number, root length, root fresh weight and root dry weight were determined.
- The most prominent genotypes based on all parameters were cv. Önceler and the genotype Gevaş which is native to in the region.

REFERENCES

[1] Majeed, A., Nisar, M.F. and Hussain, K. (2010) Effect of saline culture on the concentration of Na+, K+ and Cl- in *Agrostis tolonifera*. Curr. Res. J. Biol. Sci. 2(1), 76-82
[2] Cekic, F.O. and Yilmaz, S. (2018) Combined Stress Induced Gaba-Mediated Stress Signaling in Bean Plants. Fresen. Environ. Bull. 27, 4104-4111.
[3] Ozdemir, C., Hamurcu, M., Akyol, Y., Kocabas, O., Ozdemir, A. and Bozdag, B. (2017) Effect of Nitric Oxide on The Anatomy of Two Triticum Aestivum L. Genotypes Under Salinity Stress and Statistical Evaluation. Advances in Food Sciences. 39, 14-21.

[4] Cakmak, I. (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. Journal of Plant Nutrition and Soil Science. 168(4), 521-530.
[5] Yasar, F., Uzal, O. and Yasar, O. (2016) Antioxidant enzyme activities and lipidperoxidation amount of pea varieties (Pisum Sativum Sp. Arvense L.) under salt stress. Fresen. Environ. Bull. 25, 37-42.
[6] Azevedo Neto, A.D., Prisco, J.T., Eneas-Filho, J., Abreu, C.E.B. and Filho, E.G. (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56, 87-94.
[7] Bulut, F. and Akinci, S. (2010) The effect of salinity on growth and nutrient composition in broad bean (*Vicia faba* L.) seedlings. Fresen. Environ. Bull. 19, 2901-2910.
[8] Nawaz, K., Hussain, K., Majeed, A., Khan, F., Afghan, S. and Ali, K. (2010) Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. African Journal of Biotechnology. 9, 5475-5480.
[9] Arslan, M. (2016) Evaluating of Farmyard Manure Applications on Germination, Seedling Growth and Certain Hormone Contents of Silage Maize (*Zea Mays* L.) Under Salt (NaCI) Stress. AFS-Advances in Food Sciences. 147.
[10] Munns, R. (2002) Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239-250.
[11] Lacerda, C.F., Cambraia, J., Cano, M.A.O., Ruiz, H.A. and Prisco, J.T. (2003) Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ. Exp. Bot. 49, 107-120.
[12] McCue, K.F. and Hanson, A.D. (1992) Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. Plant molecular biology.18(1), 1-11.
[13] Karimi, G., Ghorbanli, M., Heidari, H., Khavarinejad, R.A. and Assareh, M.H. (2005) The effects of NaCl on growth, water relations, osmolytes and ion content in Kochia prostrate. Biol. Plant. 49, 301-304.
[14] Dadnia, R.D. and Mojaddam, M. (2017) Role of Saline Water on Proline, Betaine and Some Chemical Markers in Wheat (Triticum Aestivum) Genotypes. Advances in Food Sciences. 39, 57-62.
[15] Broughton, W.J., Hernandez, G., Blair, M.W., Beebe, S.E., Gepts, P. and Vander Leyden, J. (2003) Beans (*Phaseolus* spp.) – Model food legumes. Plant and Soil. 252, 55–128.
[16] Dawood, M.G., Abdelhamid, M.T. and Schmidhalter, U. (2014) Potassium fertiliser enhances the salt-tolerance of common bean (*Phaseolus vulgaris* L.). The Journal of Horticultural Science and Biotechnology. 89(2), 185-192.
[17] Kabay, T., and Şensoy, S. (2016) Enzyme, Chlorophyl and Ion Changes in Some Common Bean Genotypes by Drought Stress. Van Yuzuncü Yıl University Journal of Agricultural Sciences. 26(3), 380-395.
[18] Fidan, E., and Ekincialp, A. (2017) Investigation of Responses of Some Bean (*Phaseolus vulgaris* L.) Genotypes to Different Levels of Salt Stress. Van Yuzuncü Yıl University Journal of Agricultural Sciences. 27(4), 558-568.
[19] Sözen, Ö. (2006) Collecting and describing of local bean (*Phaseolus vulgaris* L.) populations of Artvin province and determination of its morphologic variability. Ondokuz Mayıs University, Institute of Natural and Applied Sciences, Samsun.
[20] Karaca, U. and Uyanoz, R. (2016) Growth, mineral acquisition and ion uptake response of common bean (*Phaseolus vulgaris* L.) to inoculation with Rhizobium under different salt. Fresen. Environ. Bull. 25, 14-22.
[21] Hemida, K.A., Eloufey, A.Z., Seif El-Yazal, M.A. and Rady, M.M. (2017) Integrated effect of potassium humate and α-tocopherol applications on soil characteristics and performance of *Phaseolus vulgaris* plants grown on a saline soil. Archives of Agronomy and Soil Science. 63(11), 1556-1571.
[22] Elkoca, E., Kantar, F.and Güvenç, İ. (2003) The Effects of Different NaCl Concentrations on Germination and Seedling Growth of Dry Bean (*Phaseolus vulgaris* L.) Genotypes. Atatürk University Journal of The Faculty of Agriculture. 34(1), 1-8.
[23] Aktas, H., Dasgan, H.Y., Yetisir, H., Sari, N., Koc, S., Ekici, B., Solmaz, I., Unlu, H.and Aloni, B. (2009) Variations in the response of different lines and hybrids of melon (*Cucumis melo* var. *cantaloupensis*) under salt stress. American-Eurasian J. Agric. and Environ. Sci. 5, 485-493.
[24] Kacar, B. (1984) Plant Nutrition Application Guide Ankara University Agricultural Faculty Publications 214. Ankara. 140p. (in Turkish).
[25] Olsen, S.R. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture: Washington.
[26] Düzgüneş O., Kesici T., Kavuncu O., Gürbüz F. (1987) Research and Trial Methods. Ankara University, Agricultural Faculty Publications, 1021, 381, Ankara, (in Turkish).

[27] Tester, M. and Davenport, R. (2003) Na+ tolerance and Na+ transport in higher plants. Annals of Botany. 91, 503-527. Trends Biotechnol. 8, 358-362.
[28] Yılmaz, E., Tuna, A.L. and Bürün, B. (2011) Tolerance strategies developed by plants to the effects of salt stress. C.B.U. Journal of Science. 7(1), 47–66.
[29] Hagin, J., Olsen, S.R. and Shaviv, A. (1990) Review of interaction of ammonium nitrate and potassium nutrition of crops. Journal of Plant Nutrition. 13, 1211-1226.
[30] Naido, G. (1994) Growth water and ion relations in the coastal halophytes *Trilochin bulbosa* and *T. striata*. Environ Expl. Bot. 34, 419-426.
[31] Çulha, Ş. and Çakırlar, H. (2011) The effect of salinity on plants and salt tolerance mechanisms. AKU J. Sci. 11, 11-34.
[32] Grattan, S.R. (1993) How plants responds to salts. In: Hanson. B., Grattan. S.R. and Fulton. (eds.) Agricultural salinity and drainage. A. University of California Irrigation Program. University of California. Davis. 3-5.
[33] Gomez, J.M., Hernandez, J.A., Jimenez, A., Del Rio, L.A. and Sevilla, F. (1999) Differential response of antioxidative enzymes of chloroplast and mitochondria to long term NaCl stress of pea plants. Free Radic. Res. 31, 11–18.
[34] Busch, D.S. (1995) Calcium regulation in plant cell and his role in signalling. Annual Review in Plant Physiology. 46, 95-102.
[35] Ehret, D.L., Redmann, R.E., Harvey, B.L. and Cipywnyk, A. (1990) Salinity-induced calcium deficiencies in wheat and barley. Plant and soil. 128(2), 143-151.
[36] Yakıt, S. and Tuna, A.L. (2006) The effects of Ca, Mg and K on stress parameters in corn plant (*Zea mays* L.) under salt stress. Mediterranean Agricultural Sciences. 19, 59-67. (in Turkish).
[37] Essa, T.A. (2002) Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. Journal of Agronomy and Crop Science. 188(2), 86-93.
[38] De Lacerda, C.F., Cambraia, J., Oliva, M.A. and Ruiz, H.A. (2002) Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. Environmental and Experimental Botany. 54, 69–76.
[39] Kıpçak, S. and Erdinç, Ç. (2016) Determination of Salt Tolerance Levels of Common Bean (*Phaseolus Vulgaris* L.) Genotypes Grown in Van Lake Basin. Van Yuzuncü Yıl University Journal of Agricultural Sciences. 26, 421-429.
[40] Gorham, J., Jones, W.R.G. and McDonnell, E. (1985) Some mechanisms of salt tolerance in crop plants. Plant and Soil. 8, 15-40.
[41] Kaya, C. and Higgs, D. (2003) Supplementary potassium nitrate improves salt tolerance in bell pepper plants. Journal of plant nutrition. 26(7), 1367-1382.
[42] Deal, K.R., Goyal, S. and Dvorak, J. (1999) Arm location of Lophopyrum elongatum genes affecting K+/Na+ selectivity under salt stress. Euphytica. 108(3), 193-198.
[43] Santa-María, G.E. and Epstein, E. (2001) Potassium/sodium selectivity in wheat and the amphiploid cross wheat X Lophopyrum elongatum. Plant Science. 160(3), 523-534.
[44] Ashraf, M. and Harris, P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.
[45] Davenport, R.J., Reid, R.J. and Smith, F.A. (1997) Sodium-calcium interaction in two wheat species differing in salinity tolerance. Physiologia Plantarum. 99, 323-327.
[46] Kreij, C. (1999) Production, blossom-end wet and uptake of sweet pepper as affect by sodium, cation ration and EC of the nutrition solution. Graterbauwissenschaff. 64(4), 158-164.
[47] Villora, G., Moreno, A., Pulgar, G. and Romero, L. (2000) Yield improvement in zucchini under salt stress. determining micronutrient balance. Scientia Horticulture. 86, 175-183.
[48] Neumann, P.M., Volkenburgh, E.V. and Cleland, R.E. (1988) Stress inhibits bean leaf expansion by reducing turgor, not wall extensibility. Plant Physiol. 88, 233-237.
[49] Ashraf, M. (1994) Breeding for salinity tolerance in plants. Critical Reviews in Plant Sciences. 13(1), 17-42.
[50] Aydın, A., Turan, M. and Sezen, Y. (2000) Effect of sodium salts on growth and nutrient uptake of spinach (*Spinacia olerecea L*) and beans (*Phaseolus vulgaris* L). International Symposium on Desertification. 13-17 June, Konya-Turkey, 525-530.
[51] Romero, R., Aranda, T., Soria, T. and Cuarteo, J. (2001) Tomato plant water relationships under saline growth conditions. Plant Science. 160(2), 265–272.

Received: **28.06.2018**
Accepted: **27.09.2018**

CORRESPONDING AUTHOR

Aytekin Ekincialp
Van Yuzuncu Yil University
Baskale Vocational High School
Van – Turkey

e-mail: aytekincialp@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9868-9879 Fresenius Environmental Bulletin

CORRELATION BETWEEN LANDSCAPE PROPERTIES OF MUSEUM GARDENS AND VISITOR SATISFACTION

Emine Tarakci-Eren, Tugba Duzenli*, Elif Merve Alpak

Department of Landscape Architecture, Karadeniz Technical University, Trabzon, Turkey

ABSTRACT

The influence of museums on the image and prestige of the city, the region or the country they are located is significant. This study aimed to examine the physical characteristics of the museum gardens in Trabzon, Turkey and to determine the views of users about these gardens. Five museums were included in the study: Haghia Sophia Museum, Atatürk Pavilion Museum, Turkish Education History and Technology Museum, Trabzon Metropolitan Municipality City Museum, Ministry of Culture and Tourism Trabzon Museum. In the first stage of the present study, the museums were classified based on their urban location, relationships with their immediate environment, accessibility, area plastics, activity spaces, climatic factors, furniture, water elements, plant and wildlife assets, scenery, presence of a garden, safety status, comfort and convenience. At the end of the phase, the gardens were compared based on the scores they received, and thus the positive and negative aspects of each museum garden were identified. In the second and final stage, a survey was conducted with 275 domestic and foreign visitors that utilized museum gardens to determine the satisfaction with the gardens and preference levels, in other words perceptual properties were determined. At this stage, visitors were asked whether they were satisfied with the museum garden, their purpose for using the garden, the garden features that caused them to prefer the garden, and which elements were effective in using the garden. The deficiencies identified in the study were the lack of open green spaces, comfort, furniture, water elements, plant material, in other words, ignorance of landscaping criteria.

KEYWORDS:
Museum gardens, scorecard, landscape features, user satisfaction, Trabzon

INTRODUCTION

Museums are significant building blocks of the social and cultural urban life and could be defined as the most basic centers of cultural-national identity policies [1,2]. Although museums have a wide area of activities, they are especially important for the global cultural and artistic market. Museums have an impact on several fields such as exhibitions, auctions, art history, education, collection management, art organizations, etc. The types of museums include general museums, which contain various collections such as natural history, science and technology, artistic products and antique relics, and dedicated museums specialized in only one subject. Art museums and art galleries usually exhibit paintings, sculptures, graphics and decorative art works [3].

Museums play a leading role in transforming the traces of different ages in history into the cultural education of the future generations [4-9]. Thus, the past is valued in the present and becomes more meaningful. Because historical marks are only valuable when blended with experience. While the museums offer works about the history of a society, the cities they are located in becomes a center of attraction. Both domestic and foreign audience who are curious about and want to get to know the products of a different culture are informed through museums and the experiences the museums provide [10-13].

The abovementioned functions of the museums are very important. However, these functions alone are not sufficient to attract visitors to the museums, or attract the visitors only once for historical, artistic, etc. purposes. On the other hand, gardens are one of the most significant elements that support the identity and integrity of the museums. As in other environments, a physical and social environment should be created in the museums that includes several tools which guide the land, location of the building, its form, comfort features and the visitors and that includes the methods of promoting the interest and participation [14]. Thus, museum gardens could meet the needs for open space and become meaningful spaces where individuals could visit several times. Therefore, museums should be designed with their surroundings with a visitor-oriented planning [15-19]. To provide user satisfaction, the deficiencies of these institutions that would support the image and prestige should be identified and removed.

Several settlements with historical wealth have several museums where artworks are preserved and exhibited. In countries with historical

wealth, the estates that served several purposes in the past are usually restored and utilized since they could not serve the same purpose today. It is only possible for these estates to survive only when they acquire a new function. The best examples of old estates with new functions where outdoor spaces are also used extensively in Turkey are Topkapı Palace Museum, Dolmabahçe Palace Museum, and Haghia Sophia Museum. Many historical buildings like the abovementioned have been transformed into museums. The main examples are Atatürk Pavilion Museum and the Haghia Sophia museum in Trabzon.

Thus, the museums are a nostalgic connection with the past for each nation, while they could also be utilized as spaces where individuals' needs for an open space could be satisfied. Therefore, in the present study, gardens of certain museums in the Trabzon province were analyzed based on landscaping criteria (urban location, relationships with immediate environment, accessibility, exhibition systems, indoor and outdoor presentation panels, area plastics, activity spaces, furniture, water elements, vegetal landscape elements, scenery, garden planning and design, safety, comfort and convenience). After the current status was identified, a survey study was conducted with domestic and international visitors to determine their satisfaction and preference levels about the museum gardens.

MATERIALS AND METHODOLOGY

A total of 5 museums were included in the study. These are Haghia Sophia Museum, Atatürk Pavilion Museum, Turkish Education History and Technology Museum, Trabzon Metropolitan Municipality City Museum, Ministry of Culture and Tourism Trabzon Museum (Table 1, Figure 1).

Application. Many methods could be used for evaluation of outdoor spaces. These methods are often based on observations and personal assessments, which results in criticism and poor reliability of the findings. The reliability could be established by arriving at conclusions through concrete evidence. One of the approaches that could accomplish reliability is to provide a numerical basis for all values and criteria that contribute to the space by scoring field observations and measurements [20]. The scoring and evaluation method was applied for the gardens of 5 museums located in the city of Trabzon. In the evaluation of museum gardens, 62 design criteria (urban location, relations with immediate environment, accessibility, area plastics, activity spaces, climatic factors, furniture, water elements, presence of plants and wildlife, scenery, presence of a garden, its design, safety status, comfort and convenience) were established with a literature review and utilized. As is the case for all outdoor spaces, it has become a necessity to conduct assessment studies on museum outdoors.

The attention paid to the design of museum buildings should also be paid to the design of the exterior spaces in the museums. Thus, the evaluation of museum gardens, similar to all spaces, is important for determination of how the gardens meet the required conditions, and their shortcoming. Thus, both the values of the gardens in their own design concept and, in comparison, the comparative values between the institutions could be presented. Therefore, the determined evaluation method was constructed as a multi-parameter tabulation system which was based on the museum outdoor characteristics and design principles. Each criterion was evaluated with the observations conducted by the author in every area within the museum site plan and both the staff and visitors were interviewed. For each feature included in the scorecard, the researchers assigned values between 0 and 3 based on the conducted examinations, observations and interviews. The features included in the scorecard are assigned "0" points if the related feature is not present in the museum garden, '1' point if its presence is little, '2,' if its presence is intermediary and '3' points were assigned if its presence is plenty and a total success score was determined for each museum garden based on the determined three most significant design features for this museum garden and the museum gardens were compared based on these success scores. For a total of 62 design criteria, success rates were calculated by dividing the total scores of each museum garden by the maximum score (62x3 = 186) available. Success rates were determined as follows: 0-30% failed, 30-45% inadequate, 45-60% partially successful, 60-85% successful and 85-100% very successful.

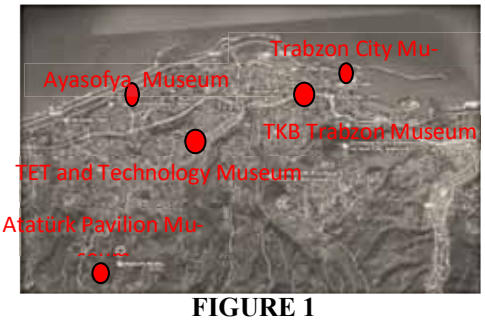


FIGURE 1
Locations of museum gardens in Trabzon urban center

When the users have no benefit or if they could not achieve expected benefits, then no matter how successful the garden design, it could be argued that the garden has failed. Thus, in the second stage, views of users about museum gardens were inquired. A questionnaire that included 5 questions was applied to a total of 275 users, 55 in each museum. The questionnaires were made on a one-to-one basis with the visitors wishing to participate in

each museum. In the first question the participants were asked whether they were satisfied with the museum garden on a 5-point scale(5 = yes, 1 = no), in other questions, they were asked their purposes in using the garden (taking the air, walking, sitting-waiting, chatting, acquiring information, resting, other), the reasons behind their preference for the garden (view, comfort, safety, transportation, none), and which elements were effective in the use of the garden (plants, water, furniture, none). With this method, it would be possible to evaluate the perception of users about the museum gardens.

FINDINGS AND DISCUSSION

Findings about the Scoring Method. Findings related to the general features of the museums are presented in Tables 2 and 3 and then the data were entered in the scorecard based on these findings.

Percentages that were calculated based on the results of the scoring method used in the evaluation of museum gardens are given in Table 3. 0-30% success rate was considered as failure, 30-45% as inadequate, 45-60% as partially successful, 60-85% as successful and 85-100% was considered as very successful.

TABLE 1
Study field

Study Field				
Trabzon Hagia Sophia Museum	Trabzon Atatürk Pavilion Museum	TEH and Technology Museum	Trabzon Municipal City Museum	Ministry of Culture and Tourism Trabzon Museum
				
The name of Hagia Sophia, which is a monastery-church built between 1250 and 1260 by Emperor Manuel I. Komnenos (1238-1263) of the Eastern Rome and Trebizond Empire in 1204. Hagia Sophia refers to "Sacred Wisdom," the second element of holy trinity. The original monastery church building was used as a church after the Ottoman occupation in 1461 led by Fatih Sultan Mehmed and then transformed into a mosque in 1584 with the addition of a minbar and a muezzin lodge by an order by the Sultan and by anotable named Kürd Ali Bey. It has served as a museum and a hospital until recent times. Today, it serves the worshippers as a mosque and a museum.	Trabzon Atatürk Mansion is located in a small cedar forest in Soğuksu district. It was built in the beginning of the 20th century and became government property after 1923. During his visit to Trabzon in 1934 and 1937, Ataturk stayed in this mansion. After his death, the mansion was decorated by Trabzon Municipality with the artifacts of the time and established as the "Atatürk Pavilion Museum"	The Turkish Education History and Technology Museum is located on Yavuz Selim Boulevard in Trabzon Center Ortahisar Neighborhood. This museum was established with the approval of the governor on February 17, 2006, to include all available exhibits that demonstrate the historical development of the education system and the educational process in the Turkish education system under one roof. The museum building was donated by Sevinç Baykal in 2005.	Trabzon Metropolitan Municipality City Museum opened to public on February 24, 2017. The city museum is located on Uzunsokak in Trabzon city center. Before serving as a city museum, the building served as the Mahmut Gologlu Cultural Center and as the central bank between 1963-1994. This three-floor building features historical artifacts, geographical signs as well as images related to the history of the city of Trabzon.	The historical mansion, which was organized as Trabzon Museum, was built as the residence of banker Kostaki Teophylaktos in Trabzon center on Zeytinlik Street in early 1900s (1898-1913). It is known that the architects of the mansion were Italian and several material used for the construction of the mansion were imported from Italy. In 1917, the mansion was purchased by Namıkoğlu family when Kostaki Teophylaktos bankrupted. It served as military headquarters, government building, inspectors buildings and girls' vocational school. Since 1987, it started serving as a museum, and since 1988 it was established as Trabzon museum. The museum features archaeological artifacts, mausoleums and ethnographic artifacts.

TABLE 2
General characteristics of museum gardens

Trabzon Hagia Sophia Museum	Trabzon Atatürk Pavilion Museum	Trabzon Education History and Technology Museum	Trabzon City Museum	Ministry of Tourism and Culture Trabzon Museum
• It is located 4.5 km from the city square. • A stadium, hospitals and fairgrounds are located in the immediate environment of the museum. • It was built on an approximately 1 ha land, it has a large garden. • Mass-space analysis demonstrated that it constituted mostly open spaces. On the open space, an entrance and security unit, circulation elements, furniture, plant landscaping elements are present. Furthermore, there is a tea saloon and a café run by the museum. • It was observed that there were gravel, dirt and natural stone paved walking paths in the garden. There are long and short lighting elements. Circultaion elements include stairs. There are no facilities for disabled or special users. • There are sitting units and garbage bins. • There is a cascade pool, however there was no water in the pool. • The museum is surrounded by walls and the south garden wall is covered with red clinker. • Certain historical artifacts are on display in the garden. • There are informative billboards about the museum. • The museum faces north. Seaview is prominent in the location. • Recently conducted urban transformation project highlighted the museum, demolishing the surrounding buildings which were unsuitable for the plan.	• It is located 6 km travel distance from the Trabzon city square. • Government hall, hospital, school and a park are located in the immediate vicinity of the museum. • Atatürk Pavilion Museum is built on an approximately 7 ha forest land with a large garden. • There are an entrance and security unit, a formal garden, furniture, plants in the museum garden and there is as tea saloon in the back garden. • The museum has a vista view of the city of Trabzon.	• It is located 3 km travel distance from the Trabzon city square. • A mosque, a recreation area, school and a theatre are located in the immediate vicinity of the museum. • It consists of a small building and a garden. • There is no security unit in the entrance. It is accessed only through a door. It is necessary to make an appointment to visit the museum. • The garden is not suitable for users with disabilities because the garden paths are too narrow and include steep stairs. No ramps are available. • In the garden sitting units and walls in different heights are available. • It was determined that there were significant elevation differences in the garden topographically in different areas. • The museum is located by Yavuz Selim Boulevard that was constructed as asecond alternative route in the city within the context of the urban transformation project, however the elevation difference between the boulevard and the museum is 6 m. In other words, building-garden-road connection is established by steep stairs.	• It is located 1.5 km travel distance from the Trabzon city square. • Businesses, public offices, banks, a square, school and a hospital are located in the immediate vicinity of the museum. • The museum does not have a garden with boundaries. The museum is located on the most active transportation axis in Trabzon center and it is separated from the road only by a 4 m wide sidewalk. • When the area between the building front and the road is examined with respect to landscape planning, a name panel for the museum, a high lighting unit, a garbage bin and two woody plants were observed. • There are no other landscaping components, elements or planning. • Security staff are employed, however there is no security unit.	• It is located 500 m travel distance from the Trabzon city square. • Businesses, public offices, banks, a square, school and a hospital are located in the immediate vicinity of the museum. • It was built on an approximately 2000 m^2 land. • Landscaping is under the influence of neoclassism movement. • In the garden, right at the entrance of the mansion, a Tyke (custodian goddess of cities) sculpture is visible. • Furthermore, there are several lighting units, walls, stairs, sitting units, garbage bins, entrance doors, garage, cascade pool (without water), column footings, plant elements are present in the garden. • An analysis of the furniture would demonstrate that the garbage bins, lighting and sitting unites were made of plastic material and are not suitable for the historical building. • The garden has three entrance doors. The first is the main entrance which is utilized for controlled entrance to the museum. Another is reserved for the parking lot. The final entrance is for pedestrians but is not currently used.

TABLE 3
Analysis of museum gardens based on plant material

Trabzon Hagia Sophia Museum	Trabzon Atatürk Pavilion Museum	Trabzon Education History and Technology Museum	Trabzon City Museum	Ministry of Tourism and Culture Trabzon Museum
• It is located 4.5 km from the city square. • A stadium, hospitals and fairgrounds are located in the immediate environment of the museum. • It was built on an approximately 1 ha land, it has a large garden. • Mass-space analysis demonstrated that it constituted mostly open spaces. On the open space, an entrance and security unit, circulation elements, furniture, plant landscaping elements are present. Furthermore, there is a tea saloon and a café run by the museum. • It was observed that there were gravel, dirt and natural stone paved walking paths in the garden. There are long and short lighting elements. Circultaion elements include stairs. There are no facilities for disabled or special users. • There are sitting units and garbage bins. • There is a cascade pool, however there was no water in the pool. • The museum is surrounded by walls and the south garden wall is covered with red clinker. • Certain historical artifacts are on display in the garden. • There are informative billboards about the museum. • The museum faces north. Seaview is prominent in the location. • Recently conducted urban transformation project highlighted the museum, demolishing the surrounding buildings which were unsuitable for the plan.	• It is located 6 km travel distance from the Trabzon city square. • Government hall, hospital, school and a park are located in the immediate vicinity of the museum. • Atatürk Pavilion Museum is built on an approximately 7 ha forest land with a large garden. • There are an entrance and security unit, a formal garden, furniture, plants in the museum garden and there is as tea saloon in the back garden. • The museum has a vista view of the city of Trabzon.	• It is located 3 km travel distance from the Trabzon city square. • A mosque, a recreation area, school and a theatre are located in the immediate vicinity of the museum. • It consists of a small building and a garden. • There is no security unit in the entrance. It is accessed only through a door. It is necessary to make an appointment to visit the museum. • The garden is not suitable for users with disabilities because the garden paths are too narrow and include steep stairs. No ramps are available. • In the garden sitting units and walls in different heights are available. • It was determined that there were significant elevation differences in the garden topographically in different areas. • The museum is located by Yavuz Selim Boulevard that was constructed as asecond alternative route in the city within the context of the urban transformation project, however the elevation difference between the boulevard and the museum is 6 m. In other words, building-garden-road connection is established by steep stairs.	• It is located 1.5 km travel distance from the Trabzon city square. • Businesses, public offices, banks, a square, school and a hospital are located in the immediate vicinity of the museum. • The museum does not have a garden with boundaries. The museum is located on the most active transportation axis in Trabzon center and it is separated from the road only by a 4 m wide sidewalk. • When the area between the building front and the road is examined with respect to landscape planning, a name panel for the museum, a high lighting unit, a garbage bin and two woody plants were observed. • There are no other landscaping components, elements or planning. • Security staff are employed, however there is no security unit.	• It is located 500 m travel distance from the Trabzon city square. • Businesses, public offices, banks, a square, school and a hospital are located in the immediate vicinity of the museum. • It was built on an approximately 2000 m^2 land. • Landscaping is under the influence of neo-classism movement. • In the garden, right at the entrance of the mansion, a Tyke (custodian goddess of cities) sculpture is visible. • Furthermore, there are several lighting units, walls, stairs, sitting units, garbage bins, entrance doors, garage, cascade pool (without water), column footings, plant elements are present in the garden. • An analysis of the furniture would demonstrate that the garbage bins, lighting and sitting unites were made of plastic material and are not suitable for the historical building. • The garden has three entrance doors. The first is the main entrance which is utilized for controlled entrance to the museum. Another is reserved for the parking lot. The final entrance is for pedestrians but is not currently used.

Based on the scoring table and all criteria, the Hagia Sophia Museum received the highest score with 130 points and the success rate for this museum was calculated as 71%. Accordingly, it was considered as successful. If Ataturk is a mansion, it has a total score of 125 and the success rate is 67%. Atatürk Pavilion scored 125 points and the success rate for this museum was 67%. Atatürk Pavillion was also considered successful. Turkish Education History and Technology and Trabzon Metropolitan Municipality City Museums failed because they had a success rate of 0-30% based on the final classification. The Ministry of Tourism and Culture Trabzon Museum received 103 points and 55% success rate. It was considered partially successful (Figure 2, Table 4).

FIGURE 2
Museum garden scorecard

TABLE 4
Museum and garden evaluation scorecard

SCORECARD FOR TRABZON MUSEUM GARDENS		TASM	TAPM	TEHTM	TCM	TCMM
Location	1. Is the museum located outside the city limits and is the location is easy to? visit	2	1	1	3	3
	2. Is the museum close to the urban center?	2	1	1	3	3
	3. When its urban location and immediate vicinity is examined, is there any negative aspects?	2	2	1	1	2
Transportation Accessibility	4. Can all user groups (disabled, children, elderly) access the museum easily?	2	1	0	2	2
	5. Does the garden have vehicle access?	3	3	1	1	3
	6. Is the museum located on a dense transportation network?	2	1	0	1	3
	7. Are other public spaces easily accessible from the museum?	2	1	1	2	3
	8. Is there an entrance unit in the museum garden?	3	3	1	3	1
	9. Is there a parking lot in the museum garden?	3	0	0	2	0
	10. Is there a clear transportation axis that connects the garden to the museum building?	3	3	1	2	0
	11. Are the activity areas in the garden easily accessible?	1	1	0	1	0
	12. Are there promenade paths in the garden?	3	2	0	1	0
	13. Are the promenade paths in the garden suitable for all user groups?	1	1	0	1	0
Scenery	14. Does the museum garden have a prominent view?	3	3	0	1	0
	15. Is the museum scenery active all day long?	3	3	0	1	0
Garden	16. Is there a garden in the museum?	3	3	3	2	0
	17. Is the garden size adequate?	3	3	1	2	0
Design	18. Is the museum garden design elaborate?	2	2	0	1	0
	19. Can museum garden design encourage visits by all individuals?	1	1	0	1	0
	20. Are furniture designs in the garden adequate for the historical texture of the museum?	1	1	0	1	0
	21. Do the plant species in the garden promote the prestige of the garden?	2	2	1	2	0
	22. Are the furniture aesthetic?	1	1	0	1	0
Area plastics	23. Are the museum and the garden located on a land with lower slopes topographically?	2	1	0	2	1
	24. Are there fewer or more stairs and ramps in the museum garden?	2	1	0	1	3
Border elements	25. Is there a structural landscape element that borders the museum garden?	3	3	3	3	0
	26. Is there a vegetal landscape element that borders the museum garden?	1	2	0	0	0

Activity Spaces	27.	Are there activity areas in the museum garden?	1	1	1	1	0
	28.	Are there visitor admittance areas in the museum garden?	1	1	0	1	0
	29.	Are there sitting spaces in the museum garden?	2	2	1	2	0
	30.	Are there food-beverage facilities in the museum garden?	1	1	0	0	0
	31.	Are there leisure areas in the museum garden?	2	2	1	1	0
	32.	Is it suitable for crowded groups?	3	2	0	1	0
Climatic Factors	33.	Are garden spaces suitable for seasonal use?	1	1	0	1	0
	34.	Were the activity areas in the garden designed based on climatic factors?	1	1	0	1	0
	35.	Do snow and ice accumulate in the garden during winter?	1	1	1	1	1
Furniture	36.	Is there a garden entrance?	2	2	1	2	0
	37.	Is there a garden wall?	3	3	1	2	0
	38.	Are there sitting units in the garden?	3	3	1	2	0
	39.	Is there a pergola or cover element in the garden?	3	3	0	0	0
	40.	Is there a lighting unit in the garden?	3	3	1	3	1
	41.	Are there shade elements in the garden?	3	2	0	0	0
	42.	Is the garden paved?	3	3	1	3	1
	43.	Are there grass areas in the garden?	3	3	2	3	0
	44.	Is there a plastic object in the garden?	3	3	0	3	0
	45.	Are there introductory and directional boards in the area?	2	2	0	1	0
	46.	Are these exhibition systems in the garden?	0	0	0	0	0
Water Element	47.	Is there a water element in the garden?	2	3	0	2	0
	48.	Is there a moving water element (artifical fall, fountain, etc.) in the garden?	1	2	0	1	0
Plants and Wildlife	49.	Are there plants in the garden?	3	3	3	3	1
	50.	Are there wildlife in the garden (birds, butterflies, etc.)?	3	3	3	3	0
	51.	Are the plants maintained well?	3	3	2	3	0
	52.	Are the plants in harmony with the nature?	3	3	2	3	0
	53.	Are there colorful and fragrant plans?	3	3	3	3	0
	54.	Do the plants demonstrate textural and formal diversity?	3	3	3	3	0
	55.	Are there plants with beautiful fruits and flowers?	3	3	3	3	0
Security, Comfort and Convenience	56.	Is there a security unit at the entrance?	1	3	0	3	0
	57.	Is the pavement adequate for walking comfortably without falling?	2	3	0	1	0
	58.	Are garden pathways, stairs and ramps suitable for all users?	0	0	0	0	0
	59.	Are there spaces suitable for collective use?	3	3	0	1	0
	60.	Furniture sizes are adequate for all user groups?	1	1	0	1	0
	61.	Are the furniture comfortable to use?	1	1	0	1	0
	62.	Is unauthorized entrance to the garden prevented?	1	3	3	3	0
Total (100% success: 186p)			**130**	**125**	**48**	**103**	**28**
Success Rate			**71%**	**67%**	**25%**	**55%**	**15%**

Abbreviations: TASM (Trabzon Hagia Sophia Museum), TAPM (Trabzon Atatürk Pavilion Museum), TEHTM (Turkish Education History and Technology Museum), TCM (Trabzon City Museum), TCMM (Ministry of Tourism and Culture Museum),

Survey Findings. Demographics. The survey was conducted with a total of 275 users, 55 in each museum garden, 138 subjects were male and 137 were female. 143 respondents were locals and 132 were visitors to the city. The vast majority of respondents were between the ages of 18-29 (98) and 30-39 (102) (Table 5). The $\chi 2$ tests conducted for the distribution of demographic characteristics yielded significant results, the findings are presented in Table 5 ($p < 0.01$).

Findings on the satisfaction question. Responses to the question "Are you generally satisfied with the museum garden?" demonstrated that users were most satisfied with Atatürk Pavilion Museum garden (mean 4.25) and Hagia Sophia Museum garden (mean 4.21). Trabzon city museum (mean 3.40) was the third and Trabzon Education History and Technology Museum (mean 1.92) and Ministry of Tourism and Culture Trabzon Museum garden (mean 1.00) were the last in satisfaction ranking. In the graph, distribution of the answers (5 = yes, 1 = no) is presented, and it was clear that the satisfaction with the Atatürk Pavilion Museum and Hagia Sophia Museum gardens was high (Figure 3). This finding was also consistent with the museum gardens scorecard determined in the first study phase. Atatürk Pavilion Museum and Hagia Sophia Museum Gardens, which received the highest rates in all criteria (location, comfort, security, furniture, vegetal elements, water, transportation), scored the highest points in user satisfaction, while Trabzon Education History and Technology Museum and Ministry of Tourism and Culture Trabzon Museum

gardens with the lowest rates in other criteria received the lowest mean scores in user satisfaction. Accordingly, it could be argued that user satisfaction was correlated with to the level of the presence of landscaping criteria in gardens. Correlations between the variables related to the museum satisfaction levels were analyzed with one-way analysis of variance (ANOVA). As a result, it was found that satisfaction level scores for each museum differed within itself and differed within the overall total that included the five museums gardens ($p < 0.01$, $N = 275$). ANOVA results are presented in Table 6.

Purpose of use findings. Responses to the question "For which purposes you use the museum garden?" were assessed with crosstab analysis (Table 7), and it was observed that the museum gardens were used to take fresh air the most by users (mean 85). When the differences in use between the museums were examined, it was observed that the Trabzon Education History and Technology Museum and Ministry of Tourism and Culture Trabzon Museum gardens that received low scores in landscaping criteria received the response “none” frequently, thus these gardens were not utilized. However, it was determined that three museum gardens with high and somewhat high criteria scores were used for taking the air, chatting, and collecting information.

Based on these findings, it could be argued that when the museum gardens are organized with adequate landscaping criteria, the users would use the gardens for useful activities such as taking the air, chatting, collecting information, etc. Active activities in the space provide positive psychological effects by enhancing the pleasure and joy [21], while passive activities provide the same by increasing positive [22]. Thus, museum gardens should include spaces that provide beneficial activities.

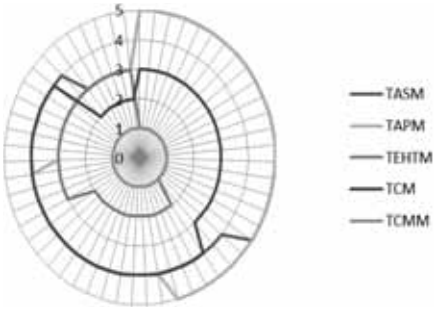


FIGURE 3
Satisfaction distribution between museum gardens

Findings on features that promote preference. Responses to the question "Which features influenced your preference for a garden positively?" were assessed with crosstab analysis (Table 8), and it was observed that the most important reason for user preference was "transportation" (mean 100). However, the highest reason for preference of the TCMM and TEHTM gardens, which received negative landscaping criteria scores, was "none", meaning that the users did not revisit the site again because there was no activity available in these

TABLE 5
Demographics of survey respondents

Demographic Structure		Museums					Total	$\chi 2$	df
		Trabzon Hagia Sophia Museum	Trabzon Atatürk Pavilion Museum	Trabzon Education History and Technology Museum	Trabzon City Museum	Ministry of Tourism and Culture Trabzon Museum			
Gender	Man	27	28	28	28	27	**138**		
	Woman	27	27	27	28	28	**137**	**8.033[b]**	**1**
Age	18-29	20	19	20	20	19	**98**		
	30-39	21	20	21	17	23	**102**		
	40-49	11	13	11	9	9	53	**66.455[a]**	**3**
	50 and over	2	3	2	3	2	12		
User Type	City users	28	29	29	28	29	**143**		
	Users out of city	27	26	26	27	26	132	**123.165[c]**	**2**

TABLE 6
One way Anova test data

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	459,833	4	114,958	244,925	,000
Within Groups	126,727	270	,469		
Total	586,560	274			

TABLE 7
Data on purposes of use of museum gardens

Purposes of Use	Museums					
	TAM	TAPM	TEHTM	TCM	TCMM	Total
Taking the air	23	**26**	22	**14**	**0**	**85**
Sitting-Waiting	8	3	0	10	0	21
Chatting	15	18	10	13	0	56
Collecting information	**9**	8	**0**	10	0	27
Walking	0	**0**	0	0	0	0
None	0	0	23	8	55	86
Total	55	55	55	55	55	275

TABLE 8
Data on preferred features of the museums

Preferred features	Museums					Total
	TAM	TAPM	TEHTM	TCM	TCMM	
View	**23**	**17**	14	**12**	0	66
Comfort	9	12	0	11	0	32
Security	0	8	0	11	1	20
Transportation	**23**	**18**	**19**	**21**	**19**	**100**
None	0	0	**22**	0	**35**	57
Total	55	55	55	55	55	275

TABLE 9
Data on Elements Effective on Museum Garden Use

Elements effective on use	Museums					
	TAM	TAPM	TEHTM	TCM	TCMM	Total
Plants	**22**	**29**	20	17	1	89
Water element	14	0	0	0	0	14
Furniture	19	15	5	0	0	39
None	0	11	**30**	**38**	**54**	**133**
Total	55	55	55	55	55	275

TABLE 10
Analysis of the differences between museum gardens

	t	df	Sd	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Purpose of use	27,290	274	2,031	3,342	3,10	3,58
Preference features	35,222	274	1,498	3,182	3,00	3,36
Effective elements	34,658	275	1,337	2,790	2,63	2,95

museum gardens, which were not actually gardens, and these museum gardens were used only to pass through when visiting the museum itself. As a result, it could be observed that museum garden preference declined as landscape criterion scores dropped, users could not find a feature to prefer these gardens. The museum gardens with favorable landscaping criteria scores were preferred for the views as well as for the transportation facilities. Because visitor needs to feel well oriented to enjoy the experience. In museums, this feeling begins with the view from the entrance to the garden [23-24].

Findings on the Elements Effective on Use. Responses to the question "Which elements were effective on your use of the garden?" were assessed with crosstab analysis (Table 9), and none of the elements was found to be effective in making the users to use the garden (mean 133). However, it was determined that the presence of plants and furniture in Trabzon Hagia Sophia Museum and Trabzon Atatürk Pavilion Museum gardens with high landscaping criteria scores positively affected the usage. In other words, it could be stated that the landscaping elements that would positively affect the usage were quite insufficient in these gardens in general. However, the presence of natural elements

such as water and plants improves satisfaction and use [25-29]. Thus, landscaping elements such as plants, water elements, etc. should be improved in museum gardens.

Finally, to determine whether the differences between purposes of use, preferred features and effective elements on the use of museum gardens were significant, One-Sample T test was conducted with SPSS (v. 23.0) software. The test results demonstrated that museum gardens had a statistically significant effect on the purposes of use, preference features and elements effective on use ($p < 0.01$) (Table 10).

CONCLUSION AND RECOMMENDATIONS

Preserving the vitality of historical buildings as museums for social and cultural sustainability depends on the suitability of these spaces to criteria for contemporary use [30]. Museums and their gardens can be used to the extent that they could meet user requirements and desires that differentiate with their lifestyles. The extent to which museum gardens could meet current requirements can be assessed either during the use or before the preservation efforts. The museum environment could be used only when they communicate with its visitors [31-34].

To preserve the cultural heritage, to pass it on to future generations and to maintain social and cultural sustainability, studies that would reveal and vitalize such values with cultural, social and touristic uses along with their immediate environment should be conducted. To restitute historical buildings and their gardens as museums and to preserve their unique fabric, relief, restitution, restoration and renovation studies should be conducted using scientific and current techniques [35]. Utilization and refunctioning of the museums and gardens that aim to exhibit our cultural values that needs protection with a social awareness and responsibility are important to maintain social and cultural sustainability. It is quite important to increase the preference for museums and gardens to raise a social awareness in the preservation and development processes by local governments [36, 37]. The museums are cultural and educational environments that increase creativity [38]. Museums and their gardens should be designed as pieces of history that enhance the imagination of visitors.

In the past, spaces that were shaped by the cultural structure and natural environment develop parallel to technological and economic advances today. The museums designed in historical buildings should be transformed into spaces that could meet user needs through a series of transformations in urban culture and spaces.

In short, the historical buildings and artifacts, which constitute our cultural heritage, are inherently important in transferring the cultural heritage to future generations and to ensure social and cultural sustainability. However, it was determined by the findings of the present study that the museum gardens in Trabzon scrutinized in the study were not suitable for use by all user groups, the selected furniture were incompatible with the historical structure, there were deficiencies in open green spaces, and in addition, due to the lack of comfort, furniture, and water elements, these spaces do not enable the transfer the cultural form and lifestyles to future generations, and it was further determined that these spaces could not provide user satisfaction.

Especially, the relationships between museum gardens or outdoor spaces and the immediate surroundings and landscaping plans should be revised based on the conservation/use balance. These spaces should be transferred to future generations with landscaping plans that cater domestic and international users. Thus, museum gardens that include adequate furniture-plant-water elements, and that are natural, informative, legible, comfortable, and with comfortable access and transportation should be constructed. The museum authorities should take these issues into consideration and should revise and expand the gardens, or should consider museum gardens as a part of the museum based on the abovementioned criteria.

REFERENCES

[1] Erbay, M. (2011) Planning of Exhibition and Presentation Techniques in Museums. Beta Printing, Istanbul
[2] Goulding, C. (1999) Interpretation and presentation. In: Leask, A., Yeoman, I. (eds.) Heritage Visitor Attractions: An Operations Management Perspective. Cassell, London.
[3] Sancar, C. (2004) Urban Accessories - Culture and Entertainment. In: Karaaslan, Ş.İ. (Ed.) Planning and Design of Urban Areas. Akademi Bookstore Publications, Trabzon, 267-279.
[4] Cleere, H. (1989) Introduction to the rationale of archaeological, heritage management in the modern world. Unwin Hyman, London.
[5] Byrne, D. (1991) Western hegemony in archaeological management. History and Archaeology. 5, 269-76.
[6] Hudson, K. (1987) Museums of influence. Cambridge University Press, Cambridge.
[7] Jenkins, K. (1991) Re-thinking History. Routledge, London.
[8] Simpson, R. (1993) From healing to heritage. Annals of Tourism Research. 120, 164-181.
[9] Thomas, J. (1991) Archaeology and the notion of ideology. In: Baker, F., Thomas, J. (eds.) Writing the Past in the Present. University College, Lampeter.

[10] Bateson, J. (1991) Managing services marketing. 2nd ed. Dryden Press, Forth Worth, TX.
[11] Smith, C. (1989) Museums artefacts and meanings: The new museology. Reaktion Books, London.
[12] Squire, S. (1994) The cultural values of literary tourism. Annals of Tourism Research. 21, 103-20.
[13] Urry, J. (1990) The tourist gaze: Leisure and travel in contemporary societies. Sage, London.
[14] Shostack, G. (1985) Planning the service encounter. In: Czepiel, J., Solomon, R., Surprenant, C. (eds.) The Service Encounter. Lexington Books, Lexington, MA.
[15] Broadhurst, R. (1989) The search for new funds. In: Uzzell, D. (ed.) Heritage Interpretation. vol. 2, Belhaven Press, London.
[16] Hewison, R. (1991) Commerce and culture. In Boylan, P. (ed.) Museums 2000. Routledge, London.
[17] Malcolm-Davies, J. (1990) Shopping for sponsors. Museums Journal. 21.
[18] McManus, P. (1989) What people say and how they think in a science museum. In: Uzzell, D. (ed.) Heritage Interpretation. vol. 1, Belhaven Press, London.
[19] Perot, P. (1993) Funding sponsorship and corporate support. In: Boylan, P. (ed.) Museums 2000. Routledge, London.
[20] Atabeyoğlu, Ö., Bulut, Y. (2007) Evaluation of outdoor quality efficiency of public institutions with scoring method. Turkish Journal of Forestry. Serial A. 1, 92-106.
[21] Crowhurst-Lennard, S.H., Lennard, H.L. (1987) Livable cities. Southampton, NY: Center for Urban Well-Being.
[22] Huang, Shu-Chun, L. (1998) A Study of People's Perception of Waterscapes in Built Environments. Doctor of Philosophy. Texas A & M University, Texas.
[23] Screven, C.G. (1986) Exhibitions and information centers: Some principles and approaches. Curator: The Museum Journal. 29(2), 109-137.
[24] Shackley, M. (1999) Tourism development and environmental protection in southern Sinai. Tourism Management. 20(4), 543-548.
[25] Beer, A.R. (1990) Environmental planning for site development. Routledge, London.
[26] Herzog, T.R. (1989) A Cognitive analysis of preference for urban nature. Journal of Environmental Psychology. 9, 27-43.
[27] Purcell, A.T., Lamb, R.J. (1984) Landscape perception: An examination and empirical investigation of two central issues in the area. Journal of Environmental Management. 19, 31-63.
[28] Wohlwill, J.F. (1983) The concept of nature: A psychologist's view. In: Altman, I., Wohlwill, J.F. (eds.) Human Behaviour and the Environment. New York: Plenum. Behavioural Natural Environment. 6, 5-37.
[29] Ulrich, R.S., Parsons R. (1992) Influences of passive experiences with plants on individual well-being and health. In: Relf, D. (ed.) The Role of Horticulture in Human Well-Being and Social Development. Timber Press, Oregon.
[30] Dikmen, Ç.B., Gültekin, A.B. (2009) Intelligent building concept in architectural design process within scope of sustainable building design. 21. International Congress of Building and Life: Nature, City and Sustainability. Chamber of Architects Branch Office of Bursa.
[31] Delaney, J. (1992) Ritual space in the Canadian museum of civilisation. In: Shields, R. (ed.) Lifestyle Shopping. Routledge, London.
[32] Langman, L. (1992) Neon cages: shopping for subjectivity. In: Shields, R. (ed.) Lifestyle Shopping. Routledge, London.
[33] Shields, R. (ed.) (1992) Lifestyle shopping: The subject of consumption. Routledge, London.
[34] Zukin, S. (1991) Landscapes of power: From Detroit to Disney World. University of California Press, Berkeley, CA.
[35] Kurtar C., Somuncu, M. (2013) Conservation and Sustainability of Urban Cultural Heritage: The Ankara Hamamönü Example. Journal of Ankara Studies. 1(2), 35-47.
[36] Berber, B., Edgü, E. (2016) The effect of social and cultural change of Kütahya on urban architecture. Erciyes University Faculty of Architecture IV. Environment-Design Congress 05-06 May 2016, Kayseri, 363-374.
[37] Dikmen Ç.B., Gültekin, A.B. (2009) Intelligent Building Concept in Architectural Design Process within Scope of Sustainable Building Design. 21. International Congress of Building and Life: Nature, City and Sustainability, Chamber of Architects, Branch Office of Bursa.
[38] Davidson, L., Sibley, P. (2011) Audiences at the "new" museum: Visitor commitment, diversity and leisure at the museum of New Zealand Te Papa Tongarewa. Visitor Studies. 14(2), 176-194.

Received: **03.07.2018**
Accepted: **11.11.2018**

CORRESPONDING AUTHOR

Tugba Duzenli
Department of Landscape Architecture,
Faculty of Forestry,
Karadeniz Technical University,
61080 Trabzon – Turkey

e-mail: tugbaduzenli@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9880-9886 Fresenius Environmental Bulletin

GROWING ENVIRONMENTAL AWARENESS IN TURKEY'S SEAFOOD PURCHASE OPTIONS: THE POSITION OF SUPERMARKETS

Levent Sangun[1], O Inanc Guney[2,*]

[1]Department of Food Processing, Vocational School of Adana, Cukurova University, Adana, Turkey
[2]Vocational School of Adana, Cukurova University, Adana, Turkey

ABSTRACT

This study provides to identify the purchase intentions, preferences and effects of demographic characteristics of the individuals on seafood. Data used in the research was obtained from the face-to-face questionnaire which was applied to randomly selected 407 individuals during May-June 2016 period in the city of Adana, Turkey. For this purpose, logistic regression method and chi-square test were applied on the obtained data and statistically significant differences ($p<0.05$, $p<0.01$) were found between the responses. The results show that factors as age, income, education and structure of the profession effects consumers on the place of purchase and determine the preference of traditional markets (local market or fish market) or supermarkets for seafood products. It is also found that consumers who prefers supermarkets mainly prefer wild fish in fresh form and dominant factors influencing purchasing behavior of seafood are freshness and accessibility for this consumer group ($p< 0.01$). Besides, accessibility, reliability and freshness are the main factors on consumers' preference of supermarkets on seafood purchasing ($p< 0.01$).

KEYWORDS:
Consumer behavior, logistic regression, purchase intention, supermarkets, seafood, environmental awareness.

INTRODUCTION

Depend on the global welfare increases, and health concerns consumers became more selective on food choices and shift to well-qualified healthy ecological food products like seafood. Today consumers prefer more animal based products than before such as meat and fish instead of cereals and pulses [1]. Moreover, consumers desire to use less effort and time for purchase and consumption activities thus they are demanding more service applications than before at retail side. Accordingly, there is a need to determine consumer needs and desires to understand the importance of product specific characteristics and economic factors in developing and executing marketing strategies especially for ecological products [2]. Hence, supermarkets as organized food retailers became more important on many food purchase activities like ecological products including seafood in recent years [3].

Role of supermarkets on purchase behavior. Today, economic growth, market integrations, urbanization, and changing lifestyles are associated with transformations in the food systems of countries. Consequently, supermarkets have an increased role on food purchase activities in modern societies with many advantages such as food safety and quality standards, vertical market integration, and international trade in high-value products [4]. Supermarkets also have the potential to affect dietary choices for better or worse, and it is important to understand how the presence of these stores influences consumer decisions [5]. In Turkey, investments in food retailing sector which has started early 1990's encouraged sales of fresh seafood within these organizations. It is estimated that in domestic seafood market 70% of the products reach to the costumers through traditional market (fish markets, local market places and wholesalers serving hotels and restaurants) and 30% by modern supermarkets in Turkey [6].

Global seafood consumption, consumer behavior and environment. Seafood is an essential component of human diet due to its precious nutritional content and outstanding health benefits. It is an important source of high quality protein, iodine, micronutrients and fatty acids, which provide various health benefits such as reducing cardiovascular diseases and boosting the immune system efficiency and seafood consumption can contribute to the feeding of a growing world population [7, 8, 9, 10].

Average world per capita fish consumption increased from 9.9 kg to 19.2 kg from 1960 to 2012. World fish and aquaculture production reached to 158 million tons and in terms of utilization, 136.2 million tons was for human consumption and 21.7 tons for non-food uses. World fishery market has a dynamic structure, especially on demand side and it is becoming much more complex and stratified, with greater diversification among species and product

forms [11]. Demand increase for seafood over the past years is associated with some factor like, population growth, increased incomes, urbanization, and awareness on healthy food consumption and the strong expansion and diversity of seafood production. It is accepted that, behavior of food and seafood consumption is influenced by many interrelating factors like product attributes (quality, convenience, odor, flavor), personal factors (preference, attitudes, knowledge, perception), and cultural and social environment [12]. Supermarkets as seafood suppliers and distribution channel also has affected the market especially for recent years. While seafood is consumed worldwide, consumption has also always been higher in the developed countries than the developing or least developed countries [3, 5, 11, 13, 14, 15].

Since 1950's fish stocks are decreasing depend on increases on the global fish consumption trend and unfair fishing practices [16, 17]. This situation causes problems on food security and long-term social welfare and effects biodiversity [18, 19].

However, improvement of environmental awareness or misinformation about seafood consumption is also effective. Environmental pollution and pollution of the water world directly affect aquaculture and fisheries production and consumption [20].

In this study, it is aimed to reveal preferences on purchase location as traditional markets or supermarkets due to differences on the demographic features and the situation of supermarkets on seafood purchase behavior of households. In this context, demographic structures and the factors determining consumers' seafood consumptions and purchase patterns were analysed and interpreted.

MATERIALS AND METHODS

Design and Sampling. The survey was applied randomly to the individuals around the shopping malls and supermarkets in Adana province, Turkey on May and June 2016. To investigate seafood consumption patterns and the effects of supermarkets on consumer's attitudes, a face-to-face questionnaire was designed by the authors. The fallowing formula was calculated to determine sample size of the research [21].

$$n = \frac{N * t^2 * p * q}{d^2 * (N-1) + t^2 * p * q} \quad (1)$$

n= Sample size; N= Population size; p= Frequency of occurrence of the event to be investigated (%50); q= Unrecognized frequency of events to be investigated (1-p) (%50); t= t value (1.96); d= Absolute error or precision as mentioned in previous section (Usually 0.05 or 0.10) [22, 23].

The questionnaire was applied to 407 individuals and 347 of them are detected as consuming seafood. Within seafood consumers, 165 are found as making seafood purchase frequently from supermarkets and the analyses. Rest of the consumers are frequently shopping from the other traditional markets like fish markets or local markets.

Theoretical framework. Data used for analysing purchasing behavior and patterns consist from two parts. First part of the consumer survey was focused on determining the factors associated with purchasing behavior and patterns for seafood in the markets. Effective attributes on seafood consumption can qualified as, sensory properties, nutritional value, health related aspects, price, convenience, availability, seasonality, country of origin, obtaining method (wild or farmed) and some product forms [14]. In relation to this, second part was covered by the socio-demographic characteristics of the consumers.

The demographic characteristics of consumers who are in purchasing behavior from supermarkets are evaluated by the percentage-frequency method. In addition, Binary Logistic Regression analysis was applied to determine the effect of these demographic characteristics on purchasing behavior. Logistic regression function is given below;

$Y_1,\ldots,Y_n$ values are statistically independent. Independent variables (X_k) are independent of each other.

$$Y_i \in (0,1), \qquad i = 1,2,\ldots\ldots,n \quad (2)$$

$$P(Y_i=1/X_i)=P_i, \qquad i = 1,2,\ldots\ldots,n \quad (3)$$

$$P_i = \frac{1}{1+e^{-(\beta_0+\beta_1 X_i)}} \quad (4)$$

In equation 4, β coefficient represents the slope and X coefficient represents the independent variable of the sample [24]. When conversion is made,

$$Z_i = \beta_0 + \beta_1 X_i \quad (5)$$

$$P_i = \frac{1}{1+e^{-Z_i}} \text{ is obtained.} \quad (6)$$

In equation 6, P_i expresses the probability that the desired event belongs to the categorical variable. P_i takes value between 0 and 1and Z_i takes values between - ∞ and + ∞. [25, 26].

Hypothetical framework. H_o: Individuals socio-demographic differences have no impact on the seafood purchase location.

H_1: Individuals socio-demographic differences have an impact on the seafood purchase location.

Data analyses. Logistic regression analysis was used to test the relationship between the dependent and the independent variables. Dependent variable in study is analysed as 0: supermarket buyers and 1: buyers from traditional markets. The independent variables in the analyse are composed of gender (1: male, 2: female), age (1: 25<, 2:25-34, 3: 35-44, 4: 45-54, 5: 54>), education (1: only literate; 2: primary-secondary school graduate; 3: high school; 4: graduate; 5: postgraduate), occupation (1: worker; 2: officer; 3: artisan; 4: self-employed; 5: private sector employee; 6: student; 7: house wife; 8: unemployed) and income (1: no income; 2: 1000 TL<; 3: 1001 TL-2000 TL; 3: 2001 TL-3000 TL; 4: 3001 TL-4000 TL; 5: 4000 TL>).

The categorical data obtained from the respondents for the attitudes were tested with chi square test. The results were interpreted according to the significance level of 0.05 and 0.01. The data analyses were performed using SPSS 20.0.

RESULTS AND DISCUSSION

Results of the consumers who uses supermarkets frequently for seafood purchase shows that, monthly seafood consumption of the consumers is 3 kg per household in average and there is no difference in consumption quantity among supermarket users and traditional market users. Frequency and percentage distributions according to the demographic structure of the respondents is mainly consist of men (60.6%) and 80% of them has the degree of high school or higher education, the size of households is usually 4-5 individuals. The important point is 72.1% of the individuals who purchase from supermarkets are under 35 who are consist of young. Supermarket is a prime location on seafood procurement especially among the young consumers [27].

Survey results. The results of univariate logistic regression analysis for the demographic characteristics and place of purchase preference are given in Table 1.

Probability ratio test results indicated that the variables with probability level (p) below 0.25 (p> 0.25) given in Table 1 were selected as candidate variables for the multivariate model [28]. In this case, all variables except gender were taken into multivariate models and the results are given in Table 2.

As it seen in Table 2, when the Exp (odds) values are examined the probability of purchasing seafood from the supermarket than traditional markets increase by 24.2% [(1-0.758) * 100)] as the average age of the consumer's decreases. In addition, the increase in income also increases supermarket usage by 11% [(1-0.889) * 100] compared to traditional markets and as education level increases the likelihood of using supermarkets increases by 27.5% [(1-0.725) * 100]. Research results also shows that comparing with the other professions, low-skilled workers and non-workforce individuals are 1.047 times more likely to use traditional markets than supermarkets in the purchase of seafood products. Depend on the analyses results we reject the H_0 hypothesis of the research.

TABLE 1
Results of logistic regression model

Variable	B	S.E.	Wald	df	P	Exp(B)	95% C.I.for Exp(B)	
							Lower	Upper
Gender	-.062	.221	.080	1	.777	.939	.609	1.448
Age	-.318	.104	9.324	1	.002	.727	.593	.892
Education	-.282	.154	3.325	1	.068	.755	.558	1.021
Profession	.097	.050	3.694	1	.055	1.102	.998	1.216
Income	-.253	.084	9.122	1	.003	.776	.659	.915
Constant			.832					

TABLE 2
Results of logistic regression model

Variable	B	S.E.	Wald	df	P	Exp(B)	95% C.I.for Exp(B)	
							Lower	Upper
Age	-.277	.116	5.715	1	.017*	.758	.604	.951
Education	-.322	.162	3.946	1	.047*	.725	.527	.996
Profession	.046	.056	.672	1	.412	1.047	.938	1.170
Income	-.118	.101	1.376	1	.241	.889	.730	1.082
Constant	1.675	.664	6.356	1	.012*	5.336		

*: p<0.05

Characteristics of consumers who uses supermarkets for seafood purchase. Seafood consumption frequency basis on variety of the respondents was investigated with the research. The Chi-Square test and percentage frequency values for the variety-based consumption among the individuals who purchase seafood from supermarkets are given in Table 3.

When Table 3 is examined, it is found that there is a statistically significant difference between the frequency of consumption of all type of seafood ($p < 0.01$). Within seafood varieties. 86.1% of consumers reported that they always purchase wild fish from the supermarkets ($p< 0.01$). Freshwater fish has secondary preference and the purchase status of other species from the supermarkets (shrimp, crab, mussel, squid, octopus, lobster and clam) is very low. This result show that there is a low demand position and availability problem for shellfish and mollusc species in Turkey.

Purchase behavior of the consumers for different product forms were also investigated with the survey. Table 4 gives Chi-square test results for the frequency of purchasing preferences according to consumption patterns for different product forms. According to this, it was found that there was a statistically difference between consumption of all product forms ($p <0.01$).

According to Table 4, it is found that there is a statistically difference between consumption of all product forms ($p< 0.01$). When the consumption frequencies are examined, it has been determined that the vast majority of consumers (91.5%) are showing seafood buying behavior as fresh form. Quick deterioration situation and short storage life of the seafood products effects consumers and consumers have to minimize their procurement process and this situation effects the purchase frequency and form of the seafood. In this context, supermarkets located in the neighbourhood of the consumers are very important on purchasing activities [1].

Other product forms that are relatively less preferred are frozen and canned products. Purchase preference for other product forms (marinated, smoked, salted, canned, dried and pre-cooked) are quite low according to the results.

It is very important to identify which factors are effective on seafood consumers who purchase seafood from supermarkets to achieve the objective of the study. Chi-Square test for factors influencing the purchase behavior on seafood was given in Table 5.

The results in Table 5 show that, there is a significant effect for all factors ($p <0.01$). The most important factors affecting the purchasing behavior of supermarket customers on seafood are determined as freshness and accessibility. Secondary important factors are seasonality and price of the product. Other factors affecting seafood purchase o are packaging, type of the seafood, size, fishing technique, presentation, origin and wild or aquaculture. [13, 29], also found price and availability as important factors for seafood consumption [12]. Within the scope of the research supermarket customers were asked why they prefer to purchase from supermarkets seafood. The results were given in Table 6.

TABLE 3
Seafood consumption frequencies

Variable	Never f (%f)	Rarely f (%f)	Sometimes f (%f)	Always f (%f)	df	Chi-Square
Marine Fish	4(2.4)	4(2.4)	15(9.1)	142(86.1)	3	330.055**
Freshwater Fish	69(41.8)	18(10.9)	56(33.9)	22(13.3)	3	46.030**
Shrimp	137(83.0)	13(7.9)	15(9.1)	-	2	183.418**
Crab	151(91.5)	8(4.8)	5(3.0)	1(0.6)	3	389.933**
Mussel	106(64.2)	9(5.5)	37(22.4)	13(7.9)	3	146.636**
Squid	129(78.2)	11(6.7)	17(10.3)	8(4.8)	3	249.909**
Octopus	155(93.9)	5(3.0)	5(3.0)	-	2	272.727**
Lobster	153(92.7)	6(3.6)	5(3.0)	1(0.6)	3	403.994**
Clam	159(96.4)	5(3.0)	1(0.6)	-	2	295.127**

**: p<0.01

TABLE 4
Seafood purchase preferences of according to product forms

Variable	Never f (%f)	Rarely f (%f)	Sometimes f (%f)	Always f (%f)	df	Chi-Square
Fresh	1(0.6)	1(0.6)	12(7.3)	151(91.5)	3	391.291**
Frozen	120(72.7)	7(4.2)	29(17.6)	9(5.5)	3	207.630**
Marinated	144(87.3)	17(10.3)	4(2.4)	-	2	217.564**
Smoked	154(93.3)	7(4.2)	4(2.4)	-	2	267.382**
Salted	146(88.5)	9(5.5)	9(5.5)	1(0.6)	3	355.703**
Canned	127(77.0)	10(6.1)	25(15.2)	3(1.8)	3	243.800**
Dried	157(95.2)	5(3.0)	2(1.2)	1(0.6)	3	433.279**
Pre-cooked	150(90.9)	9(5.5)	4(2.4)	2(1.2)	3	382.903**

**: p<0.01

TABLE 5
Factors affecting seafood purchasing behavior

Variable	1 f (%f)	2 f (%f)	3 f (%f)	4 f (%f)	5 f (%f)	df	Chi-Square
Freshness	-	1(0.6)	3(1.8)	12(7.3)	149(90.3)	3	376.939**
Accessibility	6(3.6)	3(1.8)	12(7.3)	32(19.4)	112(67.9)	4	251.879**
Price	9(5.5)	3(1.8)	21(12.7)	49(29.7)	83(50.3)	4	132.606**
Season	10(6.1)	5(3.0)	16(9.7)	49(29.7)	85(51.5)	4	138.242**
Packaging	7(4.2)	7(4.2)	11(6.7)	77(46.7)	63(38.2)	4	141.576**
Fishing Technique	10(6.1)	9(5.5)	50(30.3)	58(35.2)	38(23.0)	4	61.939**
Seafood Type	8(4.8)	3(1.8)	67(40.6)	47(28.5)	40(24.2)	4	88.667**
Presentation	12(7.3)	10(6.1)	42(25.5)	43(26.1)	58(35.2)	4	53.818**
Origin	15(9.1)	6(3.6)	36(21.8)	52(31.5)	56(33.9)	4	59.152**
Size	11(6.7)	23(13.9)	51(30.9)	36(21.8)	44(26.7)	4	31.455**
Wild or Aquaculture	6(3.6)	6(3.6)	11(6.7)	45(27.3)	97(58.8)	4	187.333**

**: $p<0.01$

TABLE 6
Reasons for choosing supermarkets

Variable	1 f (%f)	2 f (%f)	3 f (%f)	4 f (%f)	5 f (%f)	df	Chi-Square
Easy to Access	4(2.5)	3(1.9)	11(6.9)	39(24.4)	103(64.4)	4	223.625**
Reliability	-	2(1.3)	8(5.1)	51(32.3)	97(61.4)	3	147.772**
Access to information	14(8.8)	9(5.7)	11(6.9)	54(34.0)	71(44.7)	4	100.750**
Variety	7(4.4)	9(5.6)	19(11.9)	58(35.2)	67(41.9)	4	63.250**
Freshness	2(1.3)	-	18(11.3)	47(29.4)	93(58.1)	3	119.650**
Price Promotions	18(11.3)	6(3.8)	30(18.8)	53(33.1)	53(33.1)	4	54.938**
Possibility of cooking	13(8.2)	5(3.1)	40(25.2)	51(32.1)	50(31.4)	4	57.824**

**: $p<0.01$

The results in Table 6 show that, although there is a significant effect for all factors ($p <0.01$). It is especially important that consumers prefer to shop seafood at supermarkets because they can access easily, have high reliability and can reach fresh seafood. Other important factors which effect the preference of the supermarket customers are facility to access to the information on seafood, large variety opportunities, price promotions and possibility of cooking facilities.

Seafood costumers which are more associated with large and organized food retailers can benefit from many facilities served by supermarkets like convenience, variety, quality, safety and low prices [3].

Supermarket activities on seafood marketing can cope with many factors which averse consuming seafood as perceived difficulty in purchasing. preparing and cooking and some physical properties like the smell [1, 29].

CONCLUSIONS

Seafood has an essential role due to its valuable nutrient content on nutrition need of the individuals and is a very important component of healthy food consumption. Consumption of seafood is increasing globally and developments in distribution channels and retailing activities have also significant impact on this increase. While the consumption of seafood in Turkey is following a fluctuating trend, it is observed that supermarkets have increased their activities in the area of seafood marketing in recent years. This could affect Turkey's floating structure in the consumption of seafood.

Supermarkets offer a larger variety of all types of products. regardless of the consumer's dietary needs with offering more types of goods, brands, flavour, functionalities and levels of processing. This is expected to increase the dietary diversity of consumers which effects seafood amount and diversity of consumption positively [5, 30, 31]. While traditional markets like fish market is still active on merchandise, supermarkets have taken the market share gradually in selling seafood products due to life pattern change [32].

As a result of the analysis, it is determined that about 45% of seafood consumers make purchases from supermarkets and that these consumers are mainly composed of young people (%71). Logistic regression model also shows that decreasing consumers' age increases the use of supermarkets (24.2%). In terms of seafood purchase. the increase in income and the increase in the level of education are also increasing the tendency towards using supermarkets. [33] also found in their studies about

dairy consumption purchase intention that the education level of the individuals who purchase dairy products from supermarkets is significantly higher than the ones who purchase from the traditional markets (local market or fish market). As a conclusion, on preference of purchase location of seafood some socio-demographic features have impacts and thus we reject H_0 hypothesis.

In addition, it has been determined that those who prefer supermarkets in the purchase of seafood are composed of individuals with skilled occupations.

The results also show that, most of the supermarket customers have fresh sea fish consumption preference in case of seafood from. The most important factors affecting their buying behavior are freshness and accessibility. The consumption of frozen food in Turkey is quite low (150 gr. per capita) and increases in this area will be reflected in the consumption and purchase of seafood. Consumers' preferences for supermarkets on seafood purchase is easy accessibility, reliability and access to fresh products. As well as the availability of price promotions and access to information are other important factors.

From the results of the research it can be concluded that consumers' demographic characteristics as age, education, income and profession has influence on place of purchase for seafood products. In addition, fresh sea fish is the most preferred seafood product of the consumers who uses supermarkets and the factors that affect the seafood purchasing of this consumer group are freshness, accessibility, price and seasonality. Accessibility is the most important factor on preferring supermarkets to purchase seafood products.

ACKNOWLEDGEMENTS

This study was derived from the FBA-2017-8176 project, which was supported by the Scientific Research Projects Unit of the University of Çukurova, Turkey.

REFERENCES

[1] Leek, S., Maddock, S. (2000) Situational determinants of fish consumption. British Food Journal. 102, 18-39.

[2] Akpinar, M.G., Gul, M., Ceylan, R.F. (2018) Pet bottled water market: Consumer response from Turkey. Fresen. Environ. Bull. 27, 2536-2543.

[3] Jensen, H.H. (2006) Changes in seafood consumer preference patterns and associated changes in risk exposure. Marine Pollution Bulletin. 53, 591-598.

[4] Mergenthaler, M., Weinberger, K., Qaim, M. (2009) The food system transformation in developing countries: A disaggregate demand analysis for fruits and vegetables in Vietnam. Food Policy. 34, 426-436.

[5] Rischke, R., Kimenju, S.C., Klasen, S., Qaim, M. (2015) Supermarkets and food consumption patterns: The case of small towns in Kenya. Food Policy. 52, 9-21.

[6] Köse, S., Gökoğlu, N., Moltokay, S., Baygar, T., Özer, N.P., Çolakoğlu, F.A., Meriç, İ., Alçiçek, Z. (2010) The status of aquatic products processing industry in Turkey, problems and solutions. Chamber of Agricultural Engineers of Turkey Technical Congress VII, Ankara, 821-852.

[7] Vanhonacker, F., Pieniak, Z., Verbeke, W. (2010) Fish market segmentation based on consumers' motives. Barriers and risk perception in Belgium. Journal of Food Products Marketing. 16(2), 166-183.

[8] Bemrah, N., Sirot, V., Leblanc, J.C., Volatier, J.L. (2008) Fish and seafood consumption and omega 3 intake in French coastal populations: CALIPSO survey. Public Health Nutrition. 12(5), 599-608.

[9] Carlucci, D., Giuseppe, N., Devitiis, B.D., Viscecchia, R., Biagia, D.D., Rosaria, V., Bimbo, F., Nardone, G. (2015) Consumer purchasing behavior towards fish and seafood products. Patterns and insights from a sample of international studies. Appetite. 84, 212-227.

[10] Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., Nunes, M.L. (2013) Survey into the seafood consumption preferences and patterns in the Portuguese population. Gender and regional variability. Appetite. 64, 20-31.

[11] Food and Agricultural Organization of the United Nations (2014) The state of world fisheries and aquaculture. Opportunities and challenges. Rome, 1-243.

[12] Thong, N.T., Olsen, S.O. (2012) Attitude toward and consumption of fish in Vietnam. Journal of Food Products Marketing. 18(2), 79-95.

[13] Myrland, O., Trondsen, T., Johnston, R.S., Lund, E. (2000) Determinants of seafood consumption in Norway: Lifestyle, revealed preferences and barriers to consumption. Food Quality and Preference. 11, 169-188.

[14] Claret, A., Guerrero, L., Aguirre, E., Rincón, L., Hernández, M.D., Martínez, I., Peleteir, J.B., Grau, A., Rodríguez, C.R. (2012) Consumer preferences for sea fish using conjoint analysis: Exploratory study of the importance of country of origin. obtaining method. Storage conditions and purchasing price. Food Quality and Preference. 26, 259-266.

[15] Timmer, C.P. (2009) Do supermarkets change the food policy agenda? World Development. 11, 1812-1819.

[16] Claret, A., Guerrero, L., Ginés, R., Grau, A., Hernández, M.D., Aguirre, E., Peleteiro, J.B., Fernández-Pato, C., Rodríguez-Rodríguez, C. (2014) Consumer beliefs regarding farmed versus wild fish. Appetite. 79, 25-31.
[17] Risius, A., Janssen, M., Hamm, U. (2017) Consumer preferences for sustainable aquaculture products: Evidence from in-depth interviews, think aloud protocols and choice experiments. Appetite. 113, 246-254.
[18] Jiang, S. (2010) Aquaculture, capture fisheries, and wild fish stocks. Resource and Energy Economics. 32, 65-77.
[19] Smith, S., Varble, S., Secchi, S. (2017) Fish consumers: Environmental attitudes and purchasing behavior. Journal of Food Products Marketing. 23(3), 267-282.
[20] Dikel, S. (2005) Cage fishing. Çukurova University Faculty of Fisheries Adana, 1-203.
[21] Yazıcıoğlu, Y., Erdoğan, S. (2004) SPSS applied scientific research methods. Detay Yayıncılık. Ankara, 448.
[22] Karkacıer, O., Goktolga, Z.G. (2011) A case study investigating farmers' view regarding soil analysis: Estimates using a logit model. J. Agr. Sci. Tech. 13, 467-476.
[23] Gurbuz, İ.B., Manaroz, M., (2018) Local sustainability: Evaluating visitors' level of satisfaction in Cumalıkızık, Turkey. Fresen. Environ. Bull. 27, 3433-3438.
[24] Sayılı, M., Büyükköroğlu, A.M. (2013) Analysis of factors affecting consumers' attitude towards purchasing foodstuffs via e-commerce. Journal of Agricultural Sciences. 18, 246-255.
[25] Baydemir, M.B. (2014) A review on logistic regression analysis. Inonu University Institute of Social Sciences. Isparta.
[26] Uzmay, A., Cinar, G. (2017) Factor affecting to domestic and imported cattle meat consumption probability of consumer in Turkey. J. Agr. Sci. Tec. 19, 501-510.
[27] Smith, S., Varble, S., Secchi, S. (2017) Fish consumers: Environmental attitudes and purchasing behavior. Journal of Food Products Marketing. 23(3), 267-282.
[28] Bircan, H. (2004) Logistic regression analysis: An application on medical data. Journal of Kocaeli University Institute of Social Sciences. 2, 185-208.
[29] Verbeke, W., Vackier, I. (2005) Individual determinants of fish consumption: Application of the theory of planned behavior. Appetite. 44, 67-82.
[30] Asfaw, A. (2008) Does supermarket purchase effect the dietary practices of households? Some empirical evidence from Guatemala. Development Policy Review. 26, 227-243.
[31] Hawkes, C. (2008) Dietary implications of supermarket development: A global perspective. Development Policy Review. 26, 657-692.
[32] Lee, T., Liao, Y.C., Li, J.M. (2011) Marketing strategies of fishery products for supermarkets and farmers' markets in Taiwan. Journal of Food Products Marketing. 17(4), 420-440.
[33] Bozoglu, M., Chung, L., Huang, C.L., Florkowski, W.J., Topuz, B.K. (2015) Consumers' purchase intention toward safety labelled dairy products in the Black Sea Region of Turkey. Journal of Agricultural Sciences. 20, 434-445.

Received: **06.07.2018**
Accepted: **03.11.2018**

CORRESPONDING AUTHOR

O Inanc Guney
Vocational School of Adana,
Çukurova University,
Adana – Turkey

e-mail: inancguney@gmail.com

© by PSP Volume 27 – No. 12B/2018 pages 9887-9893 Fresenius Environmental Bulletin

THE EFFECT OF GAMMA IRRADIATION AT DIFFERENT DOSES APPLIED TO SAYAR-314 AND ACALPI-1952 COTTON VARIETIES SEEDS ON YIELD, YIELD COMPONENTS AND FIBER TECHNOLOGICAL PROPERTIES IN M_5 GENERATION

Ahmet Yilmaz[1,*], Hasan Haliloglu[1], Vedat Beyyavas[2], Cevher Ilhan Cevheri[2], Osman Copur[1]

[1]Harran University, Agricultural Faculty, Field Crops Department, Sanliurfa, Turkey
[2]Harran University, Sanliurfa Technical Sciences Vocational High School, Park and Garden Plants Department, Sanliurfa, Turkey

ABSTRACT

This study was conducted to determine the effects of different gamma irradiation doses yield, yield components and fiber technological properties on cotton varieties of Acalpi-1952 and Sayar-314. The seeds of each cultivar were irradiated by 100, 200, 300 and 400 gray doses at The Foundation of Türkiye Atomic Energy. M_1, M_2, M_3 and M_4 generations were grown between 2000-2004 cotton growing period and 11 lines coming from Sayar-314 variety selected from M_4 generation and 6 lines selected from Acalpi-1952 variety selected from M_4 generation. Selected all mutant lines and Sayar-314 and Acalpi-1952 were sown as parent varieties. Stoneville-453 and Sayar-314 varieties were growing as check varieties of region. Important agricultural and technological characters such as plant height, number of monopodial branches, number of sympodial branches, number of bolls, boll weight, boll seed cotton weight, 100 seed weight, first picking hand ratio, seed cotton yield, ginning outturn, fiber length, fiber strength and fiber fineness were investigated in M_5 generation. According to results; Positive and negative variations were observed with respect to the control cultivars depending on the irradiation doses.

KEYWORDS:
Cotton breeding, mutation, Cobald-60, yield, yield components

INTRODUCTION

It is an important source of natural fibre for textile industry and edible oil in tropical and subtropical regions of the world. Nowadays the quality criteria such as fiber yield, fiber strength, fiber length, fiber fineness, ginning outturn and uniformity are all important properties of the textile manufacturer, and so they are also important criteria that a cotton breeder must take into consideration. Variety improvement by the conventional methods generally require a long time, too much work and too much money. For this reason, in order to make the improvement in a shorter time, to work with a plan and to acquire new cultivars, the mutation breeding method has started to be used [1, 2].

It was reported that mutagens affected the number of monopodial and sympodial branches in cotton, and that cultivars with higher or lower number of monopodial branches and sympodial branches were obtained as compared to check variety [3].

Some researchers were reported that the coefficients of variations number of bolls, ginning outturn, seed index, number of sympodial branches and plant height increased compared with the control group [4, 5].

According to some researchers determination of gamma irradiation doses effects on cotton, seeds were irradiated different dose of Cobalt-60 and it is recorded that superior plants were selected than control at M_5 progeny for ginning outturn, fiber fineness, fiber length and fiber strength [6, 7].

Some researchers were reported that higher means and variance for boll weight in cotton under mutation. Among the different mutagens employed in cotton mutation population were observed to induce highest mean and significantly superior mutants than control in terms of number of boll per plant [8, 9, 10, 11].

Seed cotton yield was highest recorded in mutation generations. These observations are reasonably in full conformity with those reported some researchers [5, 9, 16] who stated that some lower doses of both chemical and physical mutagen induce positive mutation for increasing seed cotton yield plant.

Muhammed et al. [12] noted that the effect of radiation applications on cotton seeds were obtained positive and negative values on number of branches plant, number of bolls is an important factor for cotton seed yield, cotton mutant lines showed significant different in terms of number of bolls plant.

This study was planned and conducted to in-

vestigate the variations in the resulting M_5 mutant generations of different irradiation doses from Cobalt-60 source applied to seeds of two cotton cultivar seeds.

MATERIALS AND METHODS

Materials. 11 lines coming from Sayar-314 (*Gossypium hirsutum* L.) variety selected from M_4 generation and 6 lines selected from Acalpi-1952 (*Gossypium barbadense* L.) variety, Sayar-314, Stoneville-453 and Acalpi-952 cotton cultivars all constitute the plant material. Sayar-314, Stoneville-453 and Acalpi-952 cultivars were used as check varieites at the study. Additionally, base fertilizer, surface fertilizer, herbicides, insecticides, and tractor tools and equipments constitute the material of the present study.

The research field is plane and nearly plane with heavy texture; and in general it is deep, too much calcareous with clayey texture, and it has such a characteristic that it cracks in summer in dry conditions. The whole profile is calcareous with a pH of 7.4-7.6 and with an organic matter of 0.4-0.009 %, and it has a low salinity, high cation change capacity and it has a clayey texture and low Na content [13].

It is reported that in Sanliurfa Province where the test is carried out between April and November when cotton vegetation prevails and measured in a long period of time, the maximum temperature varies from 30.8 ^oC to 46.5 ^oC, minimum temperature varies from -6 ^oC to 16 ^oC, average temperature varies from 12.9 ^oC to 31.5 ^oC, relative humidity varies form 27 % to 58 %, and total precipitation varies from 0.3 mm to 50.9 mm [14].

Methods. The seeds of Acalpi-1952 and Sayar 314 varieties had counted 4x1000 and then put plastic baggies separately. The baggies that content seeds were irradiated 100, 200, 300 and 400 gray doses with cobalt 60 mutagen in Atom Energy Foundation of Turkey, at 17.04.2000.

The seeds belong every irradiated doses (100, 200, 300 and 400 gray) were sown at separately plots in Harran University Agricultural Faculty experiment fields at 19.04.2000. Each plots consist of 25 rows with 10 m length was established at a 0.7 m row spacing and plants were placed 25 cm apart on beds. The edge rows of every plot were sown control cv. (no radiation). Necessary cultivation process (irrigation, cultivation and fertilization) were apply to experiment.

A total of 17 lines extracted from Sayar-314 and Acalpi-1952 M_4 mutant lines which were selected for the test and as the standard cultivars of the region Sayar-314 and Stoneville-453 cotton cultivars. Acalpi-1952 cotton cultivars obtained from *G. barbadense* L. and *G. hirsutum* L. by crossbreeding were all sown by hand into the test fields at Harran University, Faculty of Agriculture experiment fields on 2 May 2004. The experiment was a randomized block design with three replications. Plots were four rows, 12 m in length and spaced 0.70 m. In this study to make M_5, the necessary maintenance like irrigation, hoeying, weeds and pests was carried out according to convenient. In study, the following observations were made for the yield of randomly selected 20 plants in each plot or for the plots yield. In addition, randomly selected cotton fibers from plots were analysed by SANKO Pure Cotton Company Fiber Laboratory in Gaziantep.

Plant height, number of monopodial branches, number of sympodial branches, number of bolls, boll weight, boll seed weight, 100 seed weight, first picking hand ratio, seed cotton yield, ginning outturn, fiber length, fiber strength and fiber fineness were investigated.

Data were analyzed using analysis of variance statistical programes MSTAT-C [15]. Means were grouped Duncan test at the 0.05 signifance level of probability.

RESULTS

Irradiating M_1, M_2, M_3 and M_4 generations of Sayar-314 and Acalpi-1952 cotton cultivars, 11 lines created from Sayar-314 cultivar and 6 lines created from Acalpi-1952 cultivar and control varieties were tested for yield at M_5 generations and findings obtained were summarized as follows.

Plant Height (cm). It is observed from Table 1 that plant height varies between 84.93 and 108.31 cm, the shortest plant height is from Acalpi-1952 Line-5 (84.93 cm), the longest plant heights are from Acalpi-1952 Line-1, Line-6 and Acalpi-1952 cultivar (108.31, 106.32 and 107.89 cm) respectively, and most of the lines have heights higher than Sayar-314 and Stoneville-453 cultivars which are the standard cultivars of the region. In M_5 generations by Cobalt 60 applications, plant heights in some lines increased, but in some others decreased with respect to control plant. This result is in conformity with the findings of some researchers related with the effect of mutagens on plant height [2, 3, 4, 5].

Number of Monopodial Branches (per plant). It is observed from Table 1, related to the cultivars and lines tested that the number of monopodial branches varies from 1.17 to 2.76 per plant, minimum number of monopodial branches is from Acalpi L_5 (1.17 per plant), maximum number of monopodial branches is from Acalpi-1952 (2.76 per plant), most of the lines have greater number of monopodial branches than Sayar-314 and Stone-

ville-453 cultivars which are the standard cultivars of the region, but still the number of monopodial branches of the standard cultivars and lines remains within normal limits. In general the number of monopodial branches of the lines is greater than that of their control cultivar. This result is in parallel to the findings of some researchers related to this matter [3, 5, 12].

Number of Sympodial Branches (per plant). It is observed from Table 1, related to the cultivars and lines tested that the number of sympodial branches varies from 13.66 to 20.63 per plant, minimum number of sympodial branches is from Sayar-314 L_{11} (13.66 per plant), maximum number of sympodial branches is from Acalpi-1952 L_6 (20.63 per plant), most of the lines have greater number of sympodial branches than Sayar-314 and Stoneville-453 cultivars which are the standard cultivars of the region. This result is in parallel to the findings of some researchers related to this matter [3, 4, 5, 12].

Number of Bolls (per plant). It is observed from Table 1, related to the cultivars and lines tested, that the number of bolls varies from 15.99 to 21.66 per plant, minimum number of bolls is from Sayar-314 L_4 (15.99 per plant), maximum number of bolls is from Acalpi-1952 L_3 (21.66 per plant), most of the lines originated from Sayar-314 have less number of bolls per plant than Sayar-314 ve Stoneville-453 cultivars which are the standard cultivars of the region, but some of the lines originated from Acalpi have greater number of bolls per plant. Results related to the number of bolls obtained by some researchers working in mutation improvement support our findings [3, 4, 5, 9, 10, 11,12, 16, 17].

Boll Weight (g). Boll weight is one of the most important character which is affecting yield directly, and it is desirable to have greater boll weight. It is observed from Table 1, related to the cultivars and lines tested, that the boll weight varies from 5.15 to 7.05 g, minimum boll weight is from Sayar-1952 L_3 (5.15 g), maximum boll weight is from Acalpi-1952 L_3 (7.05 g), most of the lines form higher boll weight than Sayar-314 ve Stoneville-453 cultivars which are the standard cultivars of the region. In terms of boll weight, lines forming the highest values were obtained from 200 gray irradiations, and lines forming the lowest values were obtained from 400 gray irradiation. In general, most of the lines formed higher boll weight values than those of the control cultivars. This result is in conformity with the findings of some researchers related to boll weight [3, 8].

TABLE 1
Plant Height, Number of Monopodial Branches, Number of Sympodial Branches, Number of Bolls, Boll Weight and Boll Seed Cotton Weight Values Observed in M_5 Generation Obtained From Sayar-314 and Acalpi-1952 Cotton Cultivars by Irradiation and Values Obtained from the Control Cultivars, and Groups Formed According to Duncan Test.

Cultivars and Lines	Plant Height (cm)	Number of Monopodial Branches (Per plant)	Number of Sympodial Branches (Per plant)	Number of Bolls (per plant)	Boll Weight (g)	Boll Seed Cotton Weight (g)
Sayar-314 L_1	98.71 b	2.03 a-g	15.44 f-j	16.98 de	5.53 d-f	4.21 c-f
Sayar-314 L_2	92.31 b-d	2.01 d-h	15.82 e-j	17.04 c-e	6.26 a-d	4.52 de
Sayar-314 L_3	106.59 a	2.69 ab	17.43 b-f	17.73 b-e	5.15 f	3.67 f
Sayar-314 L_4	95.19 b-c	1.53 e-h	15.77 e-j	15.99 e	6.43 a-c	4.20 c-f
Sayar-314 L_5	87.51 de	1.25 gh	16.34 c-ı	16.04 e	6.45 a-c	4.55 de
Sayar-314 L_6	87. 89 de	1.66 d-h	16.70 c-ı	18.53 a-e	6.91 a-b	4.78 a-c
Sayar-314 L_7	88.59 c-e	1.76 c-h	14.59 ı-j	16.83 d-e	6.50 a-c	4.51 a-e
Sayar-314 L_8	88.67 c-e	1.38 f-h	16.04 d-ı	17.60 b-e	6.60 a-c	4.68 a-d
Sayar-314 L_9	90.13 c-e	1.20 gh	14. 70 h-j	18.42 a-e	6.78 a-c	4.92 ab
Sayar-314 L_{10}	93.79 b-d	1.35 f-h	16.85 c-h	16.03 e	6.36 a-d	4.65 a-d
Sayar-314 L_{11}	92.11 b-d	1.55 e-h	13.66 j	16.30 de	6.51 a-c	4.62 a-e
Acalpi-1952 L_1	108.31 a	2.29 a-e	19.75 ab	20.81 ab	5.26 e-f	3.68 f
Acalpi-1952 L_2	87.56 de	1.85 b-h	18.19 a-d	19.79 a-d	6.45 a-c	4.34 b-e
Acalpi-1952 L_3	90.14 c-e	1.74 c-h	15.85 e-j	21.66 a	7.05 a	4.98 a
Acalpi-1952 L_4	90.99 c-e	2.14 d-f	15. 01 g-j	18.24 de	6.44 a-c	4.53 a-e
Acalpi-1952 L_5	84.93 e	1.17 h	16.90 c-g	20.73 a-c	5.99 c-f	4.08 d-f
Acalpi-1952 L_6	106.32 a	2.46 a-d	20.63 a	17.16 b-e	6.11 b-e	4.33 b-e
Acalpi-1952	107.89 a	2.76 a	18.51 a-c	19.38 a-e	5.95 c-f	4.02 e-f
Sayar-314	102.73 b	2.57 a-c	17.68 b-e	19.68 a-e	5.54 d-f	4.25 c-f
Stoneville-453	88.61 c-e	1.54 e-h	16.46 c-ı	17.61 b-e	6.35 a-d	4.33 b-e
EMS	12.195	0.180	1.196	3.435	0.187	0.093

EMS: Error Means Square

Seed Cotton Weight per Boll (g). Seed cotton weight per boll is one of the characteristics affecting yield directly, and it is desirable to have greater boll seed cotton weight. It is observed from Table 1, related to the cultivars and lines tested, that the boll seed cotton weight varies from 3.67 to 4.98 g, minimum boll seed cotton weight is from Sayar L_3 and Acalpi-1952 L_1 lines (3.67 and 3.68 g) respectively, maximum boll seed cotton weight is from Acalpi-1952 H_3 (4.98 g), and this value is followed by Acalpi-1952 L_4, Sayar-314 L_6, Sayar-314 L_7, Sayar-314 L_8, Sayar-314 L_9, Sayar-314 L_{10} and Acalpi-1952 L_4 lines, and most of the lines formed higher boll seed cotton weight than Sayar-314 ve Stoneville-453 cultivars which are the standard cultivars of the region. The highest boll seed cotton weight values were obtained from 200 and 300 gray irradiation doses, and in general, most of the lines formed higher boll seed cotton weight values than those of the control cultivars. These results can be said to be in the same direction as those of some researchers [3].

100 Seed Weight (g). It is observed from Table 2, related to the cultivars and lines tested, that 100 seed weight varies from 9.39 to 11.43 g, minimum 100 seed weight is from Sayar-314 cultivar (9.39 g), maximum 100 seed weight is from Acalpi-1952 L_4 (11.43 g), and this value is followed by Sayar-314 L_7. Most of the lines exceeded the control cultivars in terms of 100 seed weight. The highest value in terms of 100 seed weight is from 200 gray irradiation doses, and the lowest value is from 400 gray irradiation doses. On the other hand, the cultivars and lines tested formed normal values in terms of 100 seed weight. While the results such that mutagens formed mutations as obtained by some researchers investigating the effect of mutagen applications upon 100 seed weight [2] support our findings, unlike some researchers [3] Kuşdemir, 1999 expressing that mutagen applications did not affect the 100 seed weight in cotton contradicts our findings. This may arise from the fact that the vegetable materials used by Kuşdemir, 1999 [3], had different genetic structure and the mutagens used had different chemical structure and the environmental conditions were different from ours.

First Picking Hand Ratio (%). Since first picking hand ratio is important from the earlinnes point of view, it is desirable to be high as a percentage. It is observed from Table 2, related to the cultivars and lines tested, that first picking hand ratio varies from 63.70 to 87.82 %, the lowest first picking hand ratio is from Sayar-314 L_3 line (63.70 %), the highest first picking hand ratio is from Sayar-314 L_8 (87.82 %), and this value is followed by Sayar-314 L_7, Sayar-314 L_2 and Acalpi-314 L_3 lines. Some of the lines exceeded the control cultivars in terms of first picking hand ratio (Table 2).

TABLE 2
100 Seed Weight, First Picking Hand Ratio, Seed Cotton Yield, Ginning Outturn, Fiber Length, Fiber Strength and Fiber Fineness Values Observed in M_5 Generation Obtained From Sayar-314 and Acalpi-1952 Cotton Cultivars by Irradiation and Values Obtained from the Control Cultivars, and Groups Formed According to Duncan Test.

Cultivars and Lines	100 Seed Weight (g)	First Picking Hand Ratio (%)	Seed Cotton Yield (kg ha^{-1})	Ginning Outturn (%)	Fiber Length (mm)	Fiber Strength (g/tex)	Fiber Fineness (micronaire)
Sayar-314 L_1	10.60 b-d	84.18ab	3566 h	40.03 a-b	29.77 c-g	28.78 d-f	3.91 d-g
Sayar-314 L_2	10.19 d-g	86.72 a	4226 e-g	39.62 ab	29.48 e-g	28.32 ef	4.06 b-d
Sayar-314 L_3	11.15 ab	63.70 d	2403 ı	30.91 e	31.95 ab	31.23 a-c	3.42 j
Sayar-314 L_4	10.26 c-f	83.52 ac	4281 d-g	39.23 ab	29.17 fg	29.12 c-f	4.32 ab
Sayar-314 L_5	9.62 f-h	85.27 ab	4404 b-f	39.91 ab	28.67 g	29.25 c-e	4.27 a-c
Sayar-314 L_6	9.70 e-h	85.95 ab	4865 ab	38.53 a-c	31.02 a-f	32.73 a	3.64 h-j
Sayar-314 L_7	11.41 a	87.73 a	4246 e-g	37.85 a-d	31.55 a-d	32.82 a	3.70 f-ı
Sayar-314 L_8	10.24 c-g	87.82 a	4148 e-g	37.10 a-d	29.05 g	30.93 a-d	3.41 j
Sayar-314 L_9	9.54 gh	85.27 ab	4783 a-c	39.38 ab	29.67 d-g	28.67 e-f	3.93 d-f
Sayar-314 L_{10}	10.24 c-g	82.10 ab	4301 d-g	40.92 a	30.13 b-g	31.00 a-d	4.28 ab
Sayar-314 L_{11}	10.52 b-d	81.97 ac	4163 e-g	38.47 a-c	28.47 g	28.08 e-f	4.49 a
Acalpi-1952 L_1	10.51 b-d	76.10 c	4028 fg	31.97 e	32.08 ab	33.22 a	3.63 h-j
Acalpi-1952 L_2	9.52 gh	85.85 ab	4712 a-d	40.50 ab	30.17 b-g	31.35 a-c	4.01 c-e
Acalpi-1952 L_3	10.29 c-f	87.12 a	4901 a	39.58 ab	29.77 c-g	29.48 c-e	3.94 d-f
Acalpi-1952 L_4	11.43 a	85.03 ab	4490 a-e	41.05 a	29.10 fg	33.02 a	4.25 a-c
Acalpi-1952 L_5	10.38 c-e	78.32 bc	4034 fg	34.07 de	29.87 c-g	30.10 b-e	3.67 g-ı
Acalpi-1952 L_6	10.09 d-h	64.12 d	3888 gh	36.40 b-d	31.65 a-c	29.73 c-e	3.78 e-h
Acalpi-1952	10.95 a-c	76.12 c	4057 e-g	35.05 c-e	32.48 a	32.07 ab	3.63 h-j
Sayar-314	9.39 g	67.68 d	4346 c-f	39.53 ab	31.40 a-e	26.93 f	3.51 ıj
Stoneville-453	9.53 gh	85.77 ab	4502 a-e	39.68 ab	29.17 fg	29.42 c-e	3.42 j
EMS	0.131	14.915	509.2 42	4.273	0.944	1.279	0.015

EMS: Error Means Square

Seed Cotton Yield (kg ha^{-1}**).** It is observed from Table 2, related to the cultivars and lines tested, that seed cotton yield over decare varies from 2403 to 4901 kg ha^{-1} the lowest total yield over decare is from Sayar-L_3 line (2403 kg ha^{-1}), the highest second hand seed cotton yield is from Acalpi L_3 (4901 kg ha^{-1}), and this value is followed by Sayar L_6 line (4865 kg ha^{-1}). Some of the lines were found to be higher than the control cultivars in terms of yield (Table 2). This result is in parallel to the findings of some researchers [3, 5, 9, 18, 19].

Ginning Outturn (%). Since cotton is raised mainly for its fiber and the harvested seed cotton is evaluated according to its ginning outturn, having high ginning outturn is one of the most important quality characteristics of a cotton cultivar. It is observed from Table 2, related to the cultivars and lines tested, that ginning outturn varies from 30.91 % to 41.05 %, the lowest ginning outturn is from Sayar-314 L_3 line (30.91 %), and the highest ginning outturn is from Acalpi-1952 L_4 line (41.05 %). In some of the lines, ginning outturn was found to be higher than the control cultivars. This result is in parallel to the findings of some researchers [3, 4, 5, 6, 7].

Fiber Length (mm). Having high fiber length, which is one of the most important fiber qualities, is necessary for a strong high quality thread manufacturing. It is observed from Table 2, related to the cultivars and lines tested, that fiber length varies from 28.47 mm to 32.48 mm, the longest fiber length is from Acalpi-1952 cultivar line (32.48 mm), and the shortest fiber length is from Sayar-L_{11} line (28.47 mm). In some lines (Sayar-314 L_3, Sayar-314 L_6, Sayar-314 L_7, Sayar-314 L_{10}, Acalpi-1952 L_1, Acalpi-1952 L_2, Acalpi-1952 L_6), fiber length is over 30 mm, and this value is equivalent to or higher than that of the control cultivars. Thus some researchers [3, 6, 7] reported that mutagens changed the fiber quality characteristics, and that they obtained some new lines of superior quality by selecting the ones which developed in positive direction.

Fiber Strength (g/tex). It is observed from Table 2, related to the cultivars and lines tested, that fiber strength varies between 26.93 g/tex and 33.22 g/tex, the strongest fibers are from Acalpi-1952 L_4 line; and Sayar-314 L_6, Sayar-314 L_7 and Acalpi-1952 L_1 lines are inside the same statistical group. However Sayar-314 L_3, Sayar-314 L_8, Sayar-314 L_{10}, Acalpi-1952 L_2, Acalpi-1952 L_5 lines and Acalpi-1952 cultivar all have strong fibers over 30 g/tex (Table 2). The weakest fibers were encountered in Sayar-314 cultivar (26.93 g/tex). In some lines (Acalpi-1952 L_4, Sayar-314 L_6, Sayar-314 L_7, Acalpi-314 L_1, Sayar-314 L_3, Sayar-314 L_8, Sayar-314 L_{10}, Acalpi-314 L_2 and Acalpi-314 L_5) fiber strength was found to be superior to the control cultivars. Findings related to fiber strength in our study are confirmed by some researchers [3, 19, 20].

Fiber Fineness (micronaire). It is observed from Table 2, related to the cultivars and lines tested, that fiber fineness varies from 4.49 micronaire to 3.41 micronaire, the thicknest fibers are from Sayar-314 L_{11} line (4.49 micronaire), and the thinnest fibers are from Stoneville-453, Sayar-314 L_8, Sayar-314 L_3 lines, and these values are followed by Acalpi-1952 L_5, Acalpi-1952 L_1 ve Sayar-314 L_6 lines. In some lines (Sayar-314 L_8, Sayar-314 L_3, Acalpi-1952 L_5, Acalpi-1952 L_1 and Sayar-314 L_6), the fineness quality of fibers was found to be as high as the control cultivars (Table 2). This result is in parallel to the findings of some researchers [3, 6, 7, 19, 20].

DISCUSSION AND CONCLUSION

Sayar-314 and Stoneville-453 (*G. hirsutum* L.), which are the standard cultivars of the South Eastern Anatolian Region (GAP), have fibres of medium length. These cultivars are not resistant to heat and drought. Acalpi-1952 (*G. barbadense* X *G. hirsutum* L.), which is of *barbadense* group, has long fibers and is resistant to Verticillium, but it is a transient cultivar. According to the results of M_5 generations carried out to obtain a cultivar which has superior characteristics to those cultivars mentioned above; In terms of seed cotton yield, from the lines tested in this study, Acalpi-1952 L_3, Sayar-314 L_6, Sayar-314 L_9, Acalpi-1952 L_2 lines gave as much as or more seed cotton yield than the control cultivars. These lines were also in high ranks in terms of characteristics such as number of sympodial branches, number of bolls, boll weight and boll seed cotton weight, which are related to yield directly or indirectly (Tables 1 and 2). While some lines showed better characteristics in terms of ginning outturn, fiber fineness, fiber strength and fiber length; but not a single line had better characteristics in terms of all yield and quality characteristics in total.

ACKNOWLEDGEMENTS

This research was supported by TÜBİTAK

REFERENCES

[1] Aysal, İ., Kaynak, M.A. Oğlakçi, M. and Çölkesen, M. (1994) A Research on Determination of Effects of Mutation in Population Developed by Cobalt 60 Mutagen in Cotton (*Gossypium hirsutum* L.) under Harran Plain Conditions. Field Crops Congress, University of Aegean, Faculty of Agriculture. Department of Field Crops, Field Crops Science Association, TÜBİTAK (The Scientific Research Council of Turkey) and ÜSİGEM, Vol. II. Plant Improvement Abstractions. Aegean University, College of Agriculture Offset Press, Bornova, İzmir, 150-153.

[2] Yildirim, M.B. and Tugay, M.E. (1977) Evaluation of Artificial Mutations in Barley and Wheat Improvements. Turkish Journal of Plant Science. 4(3), 289.

[3] Kuşdemir, A. (1999) A Study of Effects of Mutation of Cobalt 60 in M_1 and M_2 Generations in Cotton Plant (*Gossypium hirsutum* L.). PhD thesis. University of Cukurova, Graduate school of Natural and Applied Sci. Adana.

[4] Yue, J. and Zou, J. (2012) Study of Radiation Effects on Upland Cotton (*Gossypium hirsutum* L.) Pollen Grain Irradiated by 60 Co-gamma Ray. Journal of Agricultural Science. 4(7), 85.

[5] Khan, S., Muhammad, I.K., Asim, M., Khan, F. and Khan, A.R. (2014) Gamma rays induced variations in some cotton (*Gossypium hirsutum* L.) genotypes and their evaluation in the environment of Bannu. Inter. J. Adv. Res. 2(10), 512-518.

[6] Küçüktaban, F., Çoban, M., Çiçek, S. and Yazici, L. (2016) Effects of Different Gamma Rays (Cobalt 60) Radiation Dose for Fiber Quality Traits on M_2 Generation in Cotton (*G. hirsutum* L.) Variety İpek 607. Journal of Central Research Institute for Field Crops. 25(Special issue-2), 106-111.

[7] Çoban, M., Çiçek, S. and Yazici, L. (2017) Effects of Gamma Rays (Cobalt 60) Radiation Doses on M_4 Population of Cotton (*Gossypium hirsutum* L.) Variety of İpek 607. KSU J. Nat. Sci. 20, 232-235.

[8] Sahood, A., Siddiqui and Malik, M.N.A. (1998) Significant enhancement of genetic variability by promoting positive mutagenesis X hybridisation interaction in *G. hirsutum*, World Cotton Ref. Conference-2 Athens, Greece.

[9] Patil, V.S., Kalpande, H.V., Chinchane, V.N. and Chavan, S.K. (2018) Studies on Genetic Variability for Yield and Yield Contributing Traits through Induced Mutation in Desi Cotton (*Gossypium arboreum* L.). International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7692 Special Issue-6, 1373-1382.

[10] Badigannavar, A.M., Katageri, I.S. and Khadi, B.M. (2000) Effect of gamma rays and EMS on quantitative characters in cotton. DAE BRNS symposium Mumbai, 230-235.

[11] Remya, R.V. (2011) Mutagenesis, screening and evaluation for biotic (jassid, bacterial leaf blight) and abiotic (drought) stresses in cotton *(Gossypium hirsutum* L.*)*. Ph.D. Thesis unpub. University Agricultural Science. Dharwad.

[12] Muhammad, A., Wazir, S.M., Ullah, H., Ullah, R. and Afridi, S. (2015) Performance of Selected Irradiated Cotton Varieties During M_2 Generation. American-Eurasian Journal Agric. and Environ. Sci. 15(2), 154-160.

[13] Dinç, U., Şenol, S., Sayin, M., Kapur, S. and Güzel, N. (1988) South Eastern Anatolian Region Soils (GAT). I. Harran Plain, TÜBİTAK Agriculture and Forestry Group, Guided Research Project Final Report, Project no. TOAG-534, Adana.

[14] Anonymous (2004) Şanlıurfa Meteorological Station Records. https://www.mgm.gov. tr/?il = Sanliurfa,Turkey.

[15] Anonymous (1989) User's guide to MSTAT-C analysis of agronomic research. experiments. Michigan State University, USA.

[16] Muthusamy, A., Vasanth, K. and Jayabalan, N. (2005) Induced high yielding mutants in cotton (*Gossypium hirsutum* L.) Mutation Breeding News Letter and Reviews. 1, 6-8.

[17] Arulbalachandran, D., Mullainathan, L., Velu, S. and Thilagavathi, C. (2010) Genetic variability, heritability and genetic advance of quantitative traits in black gram by effects of mutation in field trail. African Journal of Biotechnology. 9(19), 2731-2735.

[18] Karaevoi, S.Y., Maksudov, Z.Y. and Amirkulov, D. (1981) Induced Mutagenesis in Ecologically Distant Intraspecific Hybrids of Cotton. 1-Yavses Konf. Po Prikl. Radiobiol. Teor. Iprikl. Aspekty Radiats- Biol Technol. 10-12 noyab. Kishinev, Voldavian USSR. 69-70

[19] Kerbabaeva, Z.A., Konoplya, A.S. and Konoplya, S.P. (1984) Qualitative Evaluation of Natural Early Leaf Fall in Isogenic Lines of Cotton. Sel'skokhozyaistvennaya Biologiya, 11, 10, Turkmen Univ. Ashkabad. 31-33.

[20] Mamedov, K., Bazhanova, A.P. and Dzraimaniyazav, B. (1987) Ionizig Radiation and Economicaly Useful Traits in Cotton of the Species *G. barbadense* L. Plant Breeding Abst. 57, 11.

Received: **10.07.2018**
Accepted: **21.11.2018**

CORRESPONDING AUTHOR

Ahmet Yilmaz
Harran University,
Agricultural Faculty,
Field Crops Department
Sanliurfa – Turkey

e-mail: hayilmaz@harran.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9894-9901 Fresenius Environmental Bulletin

INVESTIGATION OF ANTICANCER EFFECT OF ACETIC ACID DERIVATIVES CONTAINING 1,2,4-TRIAZOLE MOIETY AGAINST TWO DIFFERENT CANCER CELL LINES

Akif Evren Parlak*

Firat University, Environmental Protection and Control Program, Keban Vocational School of Higher Education, Elazig, Turkey

ABSTRACT

The purpose of this study is to investigate the anticancer activity of [(4-substituted-5-pyridin-4yl-4H-1,2,4-triazol-3-yl)thio]acetic acid derivatives and to determine their pharmacological potential. In this study, the effects of test compounds on human breast cancer (MCF-7) and murine leukemia cells (L1210) were investigated *in vitro*. In this *in vitro* study, it was determined that test compounds inhibited the proliferation of cancerous cells in different cancer cell lines significantly higher compared to the control group. Test compounds were found to reduce the ratio of live cancer cells in the control group, depending on the dose and time interval in the administration group. As a result, it can be stated that test compounds have anticancer activity in different cancer series in *in vitro* conditions.

KEYWORDS:
Triazole, anticancer, breast cancer, leukemia

INTRODUCTION

Triazoles represent a class of five-membered heterocyclic compounds that are of highly important in the preparation of new triazole derivative drugs due to the same number of carbon and nitrogen atoms and at the same time exhibiting different biological activities in terms of variable structural variations. Synthesis and biological activity studies for 1,2,4-triazole derivatives towards pharmacological targets have been increasing [1, 2]. Finding more efficient new preparation methods for triazole derivatives is important in terms of pharmacological, green chemistry, sustainability and economy. There is a constant need of identifying prototype effective chemicals against new diseases, viruses and bacteria or microorganisms that have resistance to specific agents targeting these microorganisms. The highly variable structural derivatives of triazole group compounds are pharmaceutical agents that are pharmacologically viable and have a high potential in the market since they can exhibit different pharma-cological properties. Cancer is the second most common life-threatening disease that has reached pandemic dimensions and causes most of the deaths in the world [3]. Cancer occurs as a result of abnormal cell growth and spreading to other organs and tissues due to deoxyribonucleic acid (DNA) damage. Malignant tumors and neoplasms are commonly used terms instead of cancer. Cancer cases have been increasing day by day. According to the data of World Health Organization, cancer related deaths accounted to 8.2 million people in 2012 and 8.8 million people in 2015. It is estimated that in 2030 there will be 21 million cancer patients in the world [4-7]. Although cancer treatments vary widely today, re-activation of apoptosis (programmed cell death) in cancerous cells is a commonly used treatment strategy in traditional therapy methods. Accordingly, having apoptotic activity is one of the key molecular properties sought in studies designing new anti-cancer molecules. The goal in cancer treatment is to develop compounds with reduced duration of treatment or no adverse effects that provide more effective treatment by increasing selective toxicity. For this reason, there is a need to search for new alternative agents in cancer treatment and prevention. Investigation of new active pharmaceutical agents will provide new approaches for the development of potential cancer drugs in cancer treatment.

In this study, anticancer activity of four different compounds (4a-d) derived from synthesized [(4-substituted-5-pyridin-4yl-4H-1,2,4-triazol-3-yl)thio]acetic acid was investigated using MCF-7 human breast cancer and L1210 murine leukemia cells obtained from a cell culture bank (ATCC, USA).

EXPERIMENTAL

Chemistry. [(4-substituted-5-pyridin-4yl-4H-1,2,4-triazol-3-yl)thio]acetic acid derivative compounds used in this study have been synthesized previously by Cetin et al. [8].

Synthesis of the test compounds obtained in three presses is briefly summarized; Starting mate-

rials was obtained from fluka or aldrich; i) The treatment of isonicotinohydrazide with isothiocyanates gave thiosemicarbazides (2a-d), respectively, in nearly quantitative yields; ii)On the treatment of thiosemicarbazides (2a-d) with aqueous sodium hydroxide, 1,2,4-triazole derivatives were obtained in 75-85% yields (3a-d); a solution of the 0.01mole triazole (3a-d) and sodium hydroxide (0.01m, 0.4g) in 30mL ethanol was refluxed for 0.5h. To this solution, ethylbromoacetate (0.01m, 1.65g) was added, and the resulting mixture refluxed for 4 hour. After cooling, the solution was poured on ice and the solid mass thus separated recrystallized from suitable solvent (4a-d). In addition, the structures of the synthesized test compounds (4a-d) were confirmed by elemental analyses; IR (Mattson 1000 FTIR spectrometer), ^{1}H-NMR (Varian Mercury 400 MHz FT-NMR spectrometer), ^{13}C-NMR spectra (100MHz ^{13}CNMR spectrometer in DMSO-d6 with TMS as an internal standard) and elemental analysis data (LECO-CHNS-938). These four test compounds are given in table 1 and synthesis and mechanism of test compounds given in figure 1.

BIOLOGICAL METHODS

Anticancer Activity. In determining anticancer activity, MCF-7 human breast cancer and L1210 leukemia cells obtained from a cell culture bank (ATCC, USA) were used.

Cell Culture Treatment. Frozen cells obtained from a cell culture bank (ATCC, USA) were thawed at room temperature and transferred into a 75 ml flask. Previously prepared DC_5 (25 mL) was added to the flask and the flasks were placed in a Nuaire 5% CO_2 - 95% O_2 incubator (Playmouth, MN, USA). On a daily basis, the state of the cells was checked using a Soif brand inverted microscope (Soif Optical Inc., China), and at the end of the third day the DC_5 in the flasks was withdrawn and replaced with fresh DC_5. This process was repeated every three days.

Increasing cells began to form layers on top of each other by covering the base of the flask completely. At the end of the 15th day, the medium in the flasks was withdrawn and replaced with 3 ml of trypsin and placed in an incubator. The flasks were gently shaken every 2-3 minutes to allow the cells to detach from the adhered surface. After all cells were detached from the surface of the flask, 12 mL of DC_5 was added to the flasks and trituration (dissociation by drawing the suspension in and out of the pipette) was carefully carried out so that the cells were homogeneously dispersed into the solution.Cells were counted using a hemocytometer. Cell suspensions were placed in each flask with 5×10^6 cells per flask and DC_5 was added (up to a total volume 25 ml), and all flasks were placed in the incubator. Cell culture and experiments were performed in a sterile Class II Laminair Flow. [9, 10]

TABLE 1
Physical and analytical data of test compound 4a-d.

Code of Compounds	Nomenclature of compounds	Description
4a	Ethyl[(5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetate	Yield: 1.85g, (70%); m.p. >375°C; IR(KBr) ν_{max}/cm^{-1}: 3490-3233, 3120-3000, 2970-2867, 1742, 1610; ^{1}HNMR(D_2O) δ/ppm: 1.07(t, J=7.33, 3H), 3.42(q,J=6.96, 2H), 3.78(s, 2H), 7.78(d, J=6.23, 2H), 8.22 (d, J=5.83, 2H); Anal.calcd. for $C_{11}H_{12}N4O_2S$:C 49.99, H 4.58, N 21.20%; found:C 49.97, H 4.59, N 21.22%.
4b	[(4-Ethyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetic acid	Yield: 2.03g, (77%); m.p. >375°C; IR(KBr) ν_{max}/cm-1: 3558-3100, 3120-2887,1716,1615; ^{1}HNMR(D_2O)δ/ppm: 1.20(t, J=6.96, 3H), 4.05(q, J=7.33, 2H),3.83(s, 2H), 7.58(dd, J=6.23, 1.47, 2H),8.64(d,J=5.87,2H);^{13}CNMR(D_2O) δ/ppm:175.20, 166.87,152.00, 149.89, 135.01, 123.50, 40.70, 38.25, 14.58;Anal.calcd. for $C_{11}H_{12}N_4O_2S$: C 49.99, H 4.58, N 21.20%; found:C 50.02, H 5.01, N 21.20%.
4c	[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetic acid	Yield: 2.59g, (83%); m.p. >375°C; IR(KBr) ν_{max}/cm^{-1}: 3452, 3100-2896, 1728, 1615;^{1}HNMR(DMSOd6) δ/ppm: 3.78(s, 2H), 7.58(dd, J=6.23, 1.47, 2H),7.40-7.44(m, 2H), 7.58-7.60(m, 3H), 8.64(dd,J=5.86, 1.47, 2H); Anal.calcd. for $C_{15}H12N_4O_2S$:C57.68, H 3.87, N 17.94%; found: C 57.69, H 3.90, N 17.92%.
4d	[4-(4-Methylphenyl)-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl]thio}acetic acid	Yield: 2.18g, (67%); m.p. >375°C; IR(KBr) ν_{max}/cm^{-1} 3497-3120, 3100-2966, 1730, 1628; ^{1}HNMR(D_2O) δ/ppm: 2.21(s, 3H), 3.78(s, 2H), 6.18-6.20(m, 2H),6.70(d, J=8.05, 2H), 7.72(d, J=5.86, 1.47, 2H), 8.68(dd, J=6.23, 1.47, 2H); Anal.calcd. for $C_{16}H_{14}N_4O_2S$:C 58.88, H 4.32, N 17.17%; found: C 58.90, H 4.35, N 17.17%.

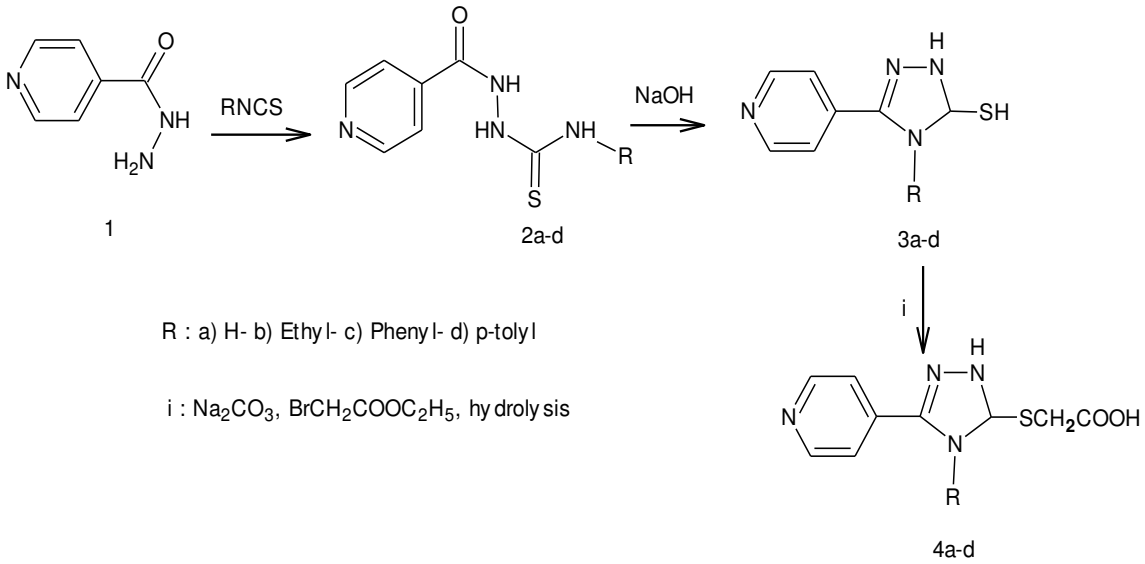


FIGURE 1
Synthesis scheme of test compounds

TABLE 2
The anticancer activity (% cell viability) of test compounds (4a-d) on the L1210 cells.

Groups	24 hour 7,5 µM	24 hour 15 µM	24 hour 30 µM	48 hour 7,5 µM	48 hour 15 µM	48 hour 30 µM
Control	73,33±3,06	73,33±3,06	73,33±3,06	68,31±4,42	68,31±4,42	68,31±4,42
4a	72,24±2,08	70,67±2,07	67,38±3,13 [a]	38,17±2,93 [c]	35,33±7,3 [c]	34,83±1,72 [c]
4b	71,50±3,45	67,44±5,72 [a]	54,50±3,02 [b]	38,67±4,32 [c]	28,1±2,42 [c]	25,50±2,43 [c]
4c	65,00±4,12 [a]	48,66±2,16 [b]	47,76±2,5 [b]	44,67±3,0 [b]	23,33±3,7 [c]	17,84±1,5 [c]
4d	49,24±4,02 [b]	40,33±2,50 [b]	38,99±0,79 [b]	44,00±7,67 [b]	19,3±5,49 [c]	4,34±1,72 [c]

a-c The difference between the groups with different letters on the same line is statistically significant. The data are presented as mean and standard error. a : $p < 0.05$; b : $p < 0.01$; c : $p < 0.001$

Cell Viability. The cells to be studied were detached from the flasks by adding trypsin and the cell suspension was centrifuged for 5 min at 2000 rpm. The trypsin-medium mixture in the tubes was replaced with DC_5, and the cells were allowed to become *single cell suspensions* by trituration. Using a hemocytometer, the cells were counted and the number of cells was adjusted to $1x10^6$/ml for MCF-7 and $1x10^4$/ml for L1210 cells required for the experiments. Preliminary experiments were performed to determine the doses and the up-and-down method was used [9]. It was decided that doses should be 7.5, 15 and 30 µM. One ml of cell suspension was transferred to each test tubes and the agents to be tested were added on the suspension at various concentrations. Same amount of compound solvents was also added to the *vehicle* tubes and all tubes were placed in the incubator. The amount of solvent in the cell suspensions was not more than 1%. Twenty-four hours later, tubes were removed from the incubator and trituration was performed; the cell suspension was mixed with 0.4% tryphan blue at a ratio of 1:1 (v/v) and 100 cells randomly selected on the hemocytometer were counted. Cell viability rate was expressed as a percentage. The same procedure was repeated after 48 hours and the experiment was concluded [11, 12].

STATISTICS

All statistical analyses were performed using the SPSS/PC package program. The data were expressed as mean ± standard error. One - way Anova analysis and Tukey test were performed for anticancer property at the end of experimental studies.

RESULTS AND DISCUSSION

The goal in cancer treatment is to improve existing compounds or to develop new compounds with reduced or no adverse effects that occur during or after treatment, to reduce the duration of the treatment and to provide more effective treatment by increasing selective toxicity. For this reason, there is a need for rapid search for new alternative agents in cancer treatment and prevention. Investigation of new active pharmaceutical agents and improving existing active substances will provide new and unique approaches to potential cancer drugs in preventing breast cancer or treating breast cancer cases and in the complete destruction of cancerous cells.

In recent years, drugs containing an active substance with a triazole ring such as anastrozole, letrozole, vorozole have been used in the treatment

of patients with breast cancer caused by breast and estrogen. Anastrozole, which contains a triazole ring, is also used in the treatment of patients with breast cancer. Recent studies have also noted that these compounds are important in the prevention and treatment of breast cancer [13].

Leukemia is a malignant blood disorder characterized by uncontrolled and abnormal proliferation of white blood cells and is a type of cancer that affects the blood production system (lymphatic system and bone marrow) in the body [14]. Anti-tumor drug studies for leukemia began with the discovery of L. Brockman et al. concluding that 2-formylpyridine thiosemicarbazone was active against leukemia in L1210, L4946 murine cells [15].

In light of the abovementioned information, we planned to investigate the anti-tumor effects of the investigated compounds on breast cancer and leukemia cell series. Obtained findings were presented in tables where experimental group was compared to the control group for each of the parameters. The viable cell count results of the L1210 cells and MCF-7 cells treated with the compounds are given in Tables 2 and Table 3, respectively.

When the results were examined, it was determined that all of our test compounds resulted in significantly higher cell death in cancerous cells in MCF-7 (breast cancer) cells compared to the control. In this study, three different concentrations (7.5 µM, 15 µM, 30 µM) were administered for 24 and 48 hours. According to the results obtained, it can be noted that higher concentrations of triazole compounds were more effective in destroying cancerous cells. Particularly at 48 hours with 30 µM concentration, very few live cells were counted in the MCF cell line. As a matter of fact, no cells were seen in the tubes treated with 4c and 4d test compounds. A 6-hour experiment was conducted for these compounds (4c and 4d) at 30 µM concentration and it was found that cell structure was intact at this time point but all cells were dead (Figure 2).

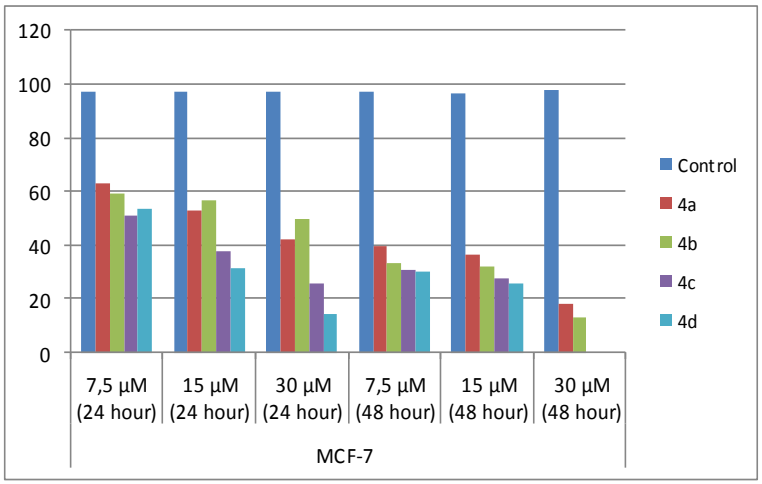


FIGURE 2
Comparison of anticancer activity of test compounds against MCF-7 cell lines at the different concentrations (7,5–15-30 µM) at 24 and 48 hour.

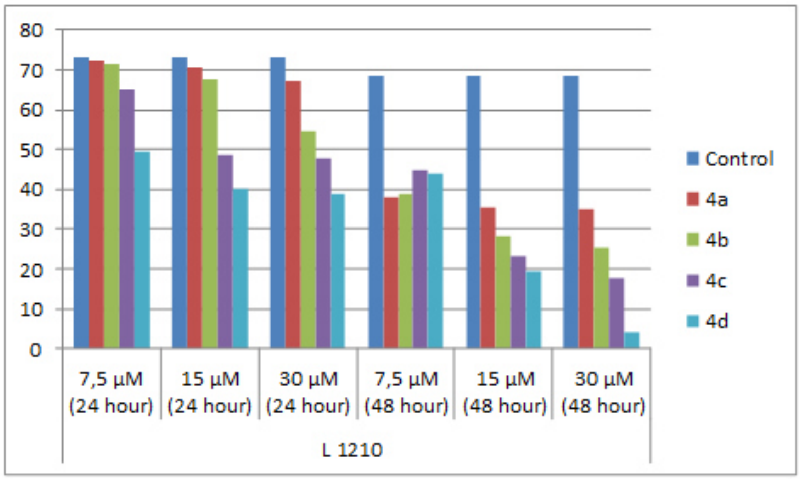


FIGURE 3
Comparison of anticancer activity of test compounds against L1210 cell lines at the different concentrations (7,5–15-30 µM) at 24 and 48 hour.

TABLE 3
The anticancer activity (% cell viability) of test compounds (4a-d) on the MCF-7 cells.

Groups	24 hour 7,5 µM	24 hour 15 µM	24 hour 30 µM	48 hour 7,5 µM	48 hour 15 µM	48 hour 30 µM
Control	97,17±2,14	97,17±2,14	97,17±2,14	97,17±2,14	96,67±1,03	97,67±1,03
4a	62,83±7,93 [a]	53,13±4,99 [b]	42,17±3,22 [b]	39,6±9,54 [b]	36,33±2,73 [b]	18,33±2,5 [c]
4b	59,00±1,16 [b]	56,64±4,05 [b]	50,00±4,89 [b]	33,13±2,89 [c]	31,83±5,12 [c]	13,27±3,9 [c]
4c	50,67±1,13 [b]	37,48±3,26 [c]	25,66±3,66 [c]	30,83±5,42 [c]	27,67±3,05 [c]	*ND
4d	53,50±6,75 [b]	31,17±4,83 [c]	14,13±5,47 [c]	30,00±5,02 [c]	25,66±5,16 [c]	*ND

a-c; The difference between the groups with different letters on the same line is statistically significant. The data are presented as mean and standard error. a : $p < 0.05$; b : $p < 0.01$; c : $p < 0.001$, * (Not Determined)

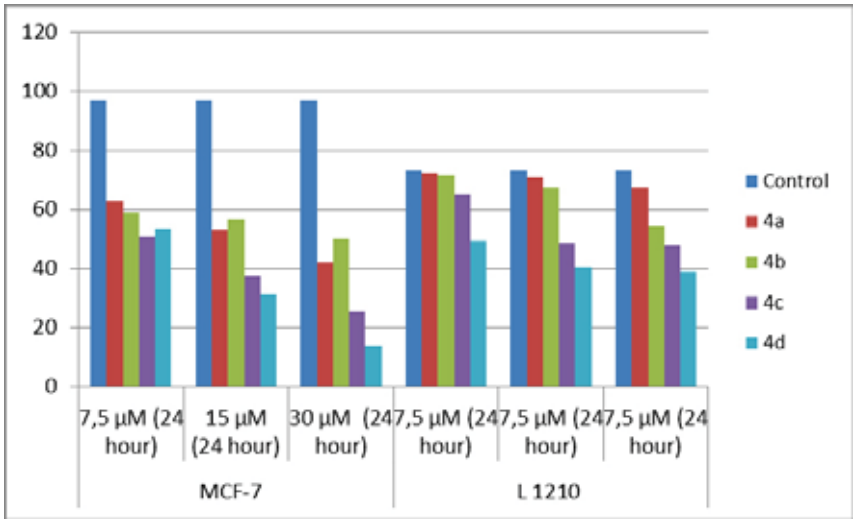


FIGURE 4
Comparison of anticancer activity of test compounds against MCF-7 and L1210 cell lines at the different concentrations (7,5–15-30 µM) at 24 hour.

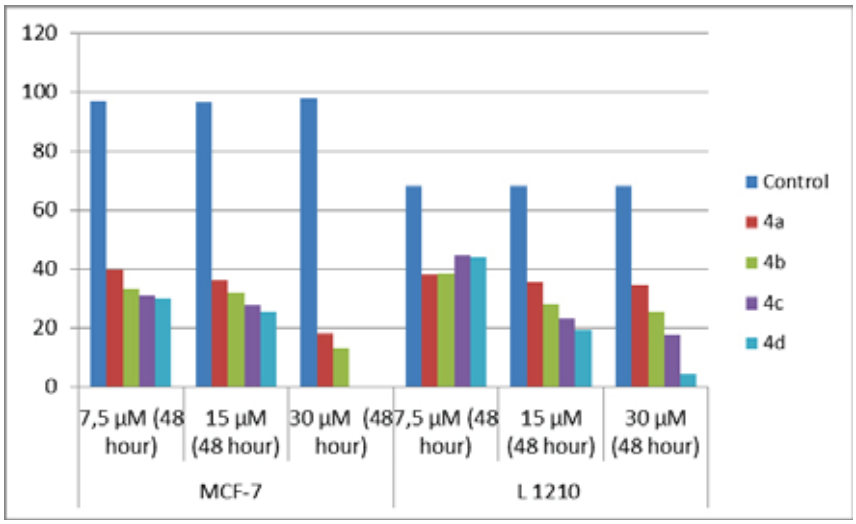


FIGURE 5
Comparison of anticancer activity of test compounds against MCF-7 and L1210 cell lines at the different concentrations (7,5–15-30 µM) at 48 hour.

Similar results were obtained in L1210 (murine leukemia) cells. In this cell line, it was also found that all test compounds resulted in significantly higher cell death in cancerous cells in MCF-7 (breast cancer) cell line compared to the control. Looking at the results, it can be seen that the anticancer activity of the test compounds changed with time and dose. It was determined that as the time and concentration increased, the anticancer effects increased. Highest effect was seen at 48 hours and at the highest concentration (Figure 3).

Test compounds were effective in both cancer cell lines, but it can be said that they were slightly more effective in MCF-7 cells compared to L1210 cancer cells (Figure 4 and 5). As a matter of fact, the lack of cells that could be counted in MCF-7 cell line at 48 hours treated with test compounds 4c and 4d is an indication thereof. Another remarkable result is that apoptotic activity at 48 hours and highest concentration in both cell lines was ranked as 4d > 4c > 4b > 4a. When a general evaluation in terms of structural activity is made, it may be conceivable that this is due to the presence of a biologically active group, such as pridine, in addition to the methylphenyl (p-tolyl), phenyl, ethyl and H group bound to the triazole ring. It is also interesting that although all the test compounds have very similar structures, they exhibit different levels of anticancer effects. It is believed that the compounds having the same structural skeleton but different substituents may have resulted in the occurrence of these different levels of anticancer activity. In particular, the results suggest that our 4c and 4d coded test compounds increase the electron enrichment of the ring, and that the ring with increased electron density has higher activity, which in turn increases anticancer activity. This can be further supported by the fact that the anticancer activities of 4b and 4c, which contain electron donating substituents such as methylphenyl and phenyl, are much higher than other compounds. The effects of test compounds against both cancer cell lines are shown in Figure 4 and 5.

Literature review revealed studies investigating anticancer activities of triazoles with results supporting our study. For example, in one study, some of the synthesized compounds were evaluated in terms of *in vitro* activity against colon cancer (HT-29) and reported to have higher activity than the standard [16]. When other similar studies were investigated, it was found that similar types of heterocyclic compounds with 4,5-dihydro-1H-1,2,4-triazol-5-on ring demonstrated antioxidant and antitumor activity in addition to biological activity studies, which support our findings [17-20]. In another study, triazole derivative compounds were reported to be potent anti-cancer agents using the MTT method, one of the enzymatic test techniques used in cytotoxicity examination of MCF-7, U-87-MG and HCT-116 human cancer cells. Compounds containing a 1,2,4-triazole pyridine ring have been reported to be potent anti-cancer agents as a result of *in vitro* activities [21]. In another study conducted, it has been reported that some 4-arylmethyleneamine-4H-1,2,4-triazoles have significant anti-cancer activity against human cancer cells [22]. In another study, anti-cancer activity of some compounds with 1,2,4 triazole structure was evaluated. These compounds have been reported to exhibit activity against kidney, leukemia, colon, and breast cancer [23]. When results of another study are examined, it can be seen that there are scientific studies including histopathological evaluations in some cancer cells and showing that compounds containing 1,2,4-triazole ring affect the cytotoxicity and proliferation properties against cancer cells, which are consistent with our study. For example, in one study, the clinical administration of 1,2,4-triazole and its derivatives is an important issue, especially in terms of anti-breast cancer. Triazole compounds have been reported to exhibit broad spectrum anticancer effects on MCF-7 breast cancer lines [24]. In another study, 1(-4-methoxyphenyl) -5- (3,4,5-trimethoxyphenyl) -1H-1,2,4-triazole-3-carboxamide compounds showed remarkable anti-inflammatory activity, and histopathological examination revealed a low incidence of gastric ulceration compared with indomethacin [25]. In a different study, some new compounds containing the 1,2,4-triazole and 1,3,4-thiadiazole moiety were evaluated in terms of cytotoxic effect on human colorectal cancer cell line HT-29, breast cancer cells MDA-MB-231, cervical cancer cells and human liver cancer cell line. Some of the triazoles and derivatives used in this study showed high cytotoxicity against HT-29 cells. Biological data reported so far has revealed that the nature and position of the substituents affect the cytotoxicity against cancer cells as well as the proliferative properties of the tested compounds to Lep3 [26].

CONCLUSION

Cancer is the most common disease of our age, and it is very difficult to treat and it results in death. For this reason, new compounds and methods are being developed using different methods, techniques and analyses every day to prevent and treat cancer. The most common tumor type that affects women both in the world and in our country is breast cancer. Leukemia is among the cancer types that cause the most fatalities. Today, radiotherapy and chemotherapy methods used in cancer treatment have serious side effects in patients. Therefore, investigation of new chemotherapeutics with fewer side effects in terms of the methods and agents used in treatment is an important issue in cancer studies [27]. The use of anti-cancer agents that induce apoptosis (programmed cell death), a natural way of killing cells in the physiological process, is a valuable method in the development of specific new chemotherapeutic drugs. With this mechanism, the cancerous cells are killed, and they do not harm the healthy cells in the tissue they are in, and the side effects of the drug are lessened [28].

In our study, *in vitro* anticancer activities of originally synthesized compounds were investigated and compared. In order to evaluate the anticancer capacity of a substance in a more healthy and effective manner, several different cancer cell

lines should be studied. Our study aimed to reveal the anticancer capacities of the compounds more clearly by using 2 different cancer cell lines. As a result of the study, the anticancer effect of the test compounds was demonstrated and we believe that our findings contribute to the studies in this field.

ACKNOWLEDGEMENTS

Thank to Dr. Ahmet Cetin and Metin Koparir for the gift of test compounds.

REFERENCES

[1] Orek, C., Sarac, K., Parlak, A.E., Koparir, P., Turkoglu, S., Koparir, M. (2018) Synthesis, structure investigation, spectral properties and in vitro antioxidant evaluation of new 1-(3-methyl-3-mesityl)-cyclobutyl-2-(5-thiophen-4-ethyl-2h-[1, 2, 4] trıazol-3-ylsulfanyl)-ethanone. Fresen. Environ. Bull. 27, 2992-3005.

[2] Zhang, W., Li, Z., Zhou, M., Wu, F., Hou, X., Luo, H., Liu, H., Han, X., Yan, G., Ding, Z., Li, R. (2014) Synthesis and biological evaluation of 4-(1,2,3-triazol-1-yl)coumarin derivatives as potential antitumor agents. Bioorg. Med. Chem. Lett. 24, 799–807.

[3] Ölgen, S., Bıçak, I., Nebioğlu, D. (2002) Angiogenesis and New Aspects of Cancer Chemotheraphy. J. Fac. Pharm, Ankara. 31(3), 193-214.

[4] WHA70.12: World Health Assembly (2018) Cancer prevention and control in the context of an integrated approach http://www.who.int/en/news-room/fact-sheets/detail/cancer

[5] World Health Organization (2014) WCP World Cancer Report 2014. Lyon: WHO Press

[6] International Agency for Research on Cancer. Launch of GLOBOCAN (2008) http://www.iarc.fr/en/media-centre/iarcnews/2010/GLOBOCAN2008.pdf.

[7] American Cancer Society. (2011) Global Cancer Burden to Nearly Double by 2030. http://www.cancer.org/myacs/newengland/global-cancer-burden-to-double-by-2030

[8] Cetin, A., Cansiz, A., Koparir, M., Kazaz, C. (2006) The Synthesis and Spectral Investigations of New Derivatives Of 1,3,4-Oxadiazole, 1,3,4-Thiadiazole, And 1,2,4-Triazole. OCAIJ. 2(5-6), 140-149.

[9] Offing, O.E., Martelli, S. (1994) Antibacterial Activity of Metal Complexes of Benzil and Benzoin Thiosemicarbazones. IL Farmaco. 49, 513-518.

[10] Ferrari, B.M., Capacchi, S., Pelosi, G., Reffo, G., Tarasconi, P., Albertini, R., Pinelli, S., Lungni, P. (1999) Synthesis, Structural Characterization and Biological Activity of Helicin Thiosemicarbazone Monohydrate and a Copper (II) Complex of Salicylaldehyde Thiosemicarbazone. Inorganica Chimica Acta. 286, 134-141.

[11] Kumammoto, T., Toyooka, K., Nishida, M., Kuwahara, H., Yashimura, Y., Kawada, J., Kubota, S. (1990) Effect of 2,4-Dihydro-3H-1,2,4- Triazole-3 –Thiones and Thiosemicarbazones on lodide Uptake by The Mause Thyroid, The Relationship Between Their Structure and Antithyroid Activity. Chem. Pharm. Bull. 38, 2595-2596.

[12] Sur, B., Chatterjee, S.P., Sur, P., Maity, T., Reychoudhury, S. (1990) Studies on the Antineoplasticity of Schiff Bases Cataining 5-Nitrofuran and Pyrimidine. Oncology. 47, 433-438.

[13] Demirbaş, A. (2004) A convenient synthesis of 3,6-disubstituted-1,4-dihydro-[1,2,4,5]tetrazines and preparation of new acetic acid derivatives containing 5-oxo-4 phenylamino-4,5-dihydro-[1,2,4]triazole. Turkish Journal of Chemistry. 28, 311- 323.

[14] Chen, L., Wilson, D., Jayaram, H.N. And Pankiewicz, K.W. (2007) Dual inhibitors of inosine monophosphate dehydrogenase and histone deacetylases for cancer treatment. J. Med. Chem. 50(26), 6685–6691.

[15] Brockman, R.W., Thomson, J.R., Bell, M.J., Skipper, H. E. (1956) Observations on the antileukemic activity of pyridine-2- carboxaldehyde thiosemicarbazone and thiocarbohydrazone. Cancer Res. 16(2), 167-170.

[16] Hafez, H.N., Alsalamah, S.A., El-Gazzar, A.B.A. (2017) Synthesis of thiophene and N-substituted thieno[3,2-d] pyrimidine derivatives as potent antitumor and antibacterial agents. US National of Medicine National Institutes of Health. 275-292.

[17] İkizler, A., Gümüş, F., Özden, S., Abbasoğlu, U. (1989) Biological activities of some 1,2,4-triazoles and 1,2,4-triazolin-5-ones. Pharmazie. 54, 506.

[18] Yüksek, H., Küçük, M., Alkan, M., Bahçeci, Ş., Kolaylı, S., Ocak, Z., Ocak, U., Şahinbaş, E., Ocak, M. (2006) Synthesis and andioxidant activities of some new 4-(4-hydroxybenzylidenamino)-4,5- dihydro-1H-1,2,4-triazol-5-one derivatives with their acidic properties. Asian J. Chem. 18, 539.

[19] İkizler, A.A., Demirbaş, A., Johansson, C.B., Çelik, C., Serdar, M., Yüksek, H. (1998) Synthesis and biological activity of some 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives. Acta Polon Pharm. 55, 117.

[20] Yüksek, H., Demirbaş, A., Johansson, C.B., Çelik, C., İkizler, A.A. (1997) Synthesis and antibacterial activities of some 4,5-dihydro-1H-1,2,4-triazol-5-ones. Arzneim.- Forch./Drug Res. 47, 405.
[21] Wang, X.-M., Xu, J., Li, Y.P., Li, H., Jiang, C.S., Yang, G.D., Lu, S.M., Zhang, S.Q. (2013) Synthesis and anticancer activity evaluation of a series of [1,2,4] triazolo[1,5-a]pyridinylpyridines in vitro and in vivo. European Journal of Medicinal Chemistry. 67, 243-251.
[22] Bekircan, O., Kahveci, B., Kucuk, M. (2006) Synthesis and Anticancer Evaluation of Some New Unsymmetrical 3,5-Diaryl-4H-1,2,4-Triazole Derivatives. Turk. J. Chem. 30, 29-40.
[23] Lesyk, R., Vladzimirska, O., Holota, S., Zaprutko, L., Gzella, A. (2017) New 5-substituted thiazolo[3,2-b][1,2,4]triazol-6-ones: synthesisand anticancer evaluation. US National of Medicine National Institutes of Health.
[24] Genc, M., Karagoz Genc, Z., Tekin, S., Sandal, S., Sirajuddin, M., Hadda Taibi, B., Sekerci, M. (2016) Design, Synthesis, in vitro Antiproliferative Activity, Binding Modeling of 1,2,4,-Triazoles as New Anti-Breast Cancer Agents. Acta Chim. Slov. 63, 726–737
[25] Abdel-Aziz, M., Beshr, E.A., Abdel-Rahman, I.M., Ozadali, K., Tan O.U., Aly, OM. (2014) 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamides: synthesis, molecular modeling, evaluation of their anti-inflammatory activity and ulcerogenicity. Eur J Med Chem. 22(77), 155-65.
[26] Mavrova, A.T., Wesselinova, D., Tsenov, J.A., Lubenov, L.A. (2014) Synthesis and antiproliferative activity of some new thieno[2,3-d]pyrimidin-4(3H)-ones containing 1,2,4-triazole and 1,3,4-thiadiazole moiety. Eur. J. Med. Chem. 86, 676-83.
[27] Poggi, M.M., Coleman, C.N., Mitchell, J.B. (2001) Sensitizers and Protectors of Radiation and Chemotherapy. Current Problems in Cancer. 25(6), 338-245
[28] Ellis, P.A., Simit, I.E., Carthy, M.C., Salter, D.S. (1997) Preoperative chemothreapy induces apoptosis in early breast cancer. Lancet 349.

Received: **13.07.2018**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Akif Evren Parlak
Fırat University,
Environmental Protection and Control Program,
Keban Vocational School of Higher Education,
Elazig – Turkey

e-mail: akifevren@firat.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9902-9905 Fresenius Environmental Bulletin

IDENTIFICATION AND PREVENTION OF MELOIDOGYNE GRAMINICOLA FROM RICE IN HAINAN

Kai Rui, Xinhu Zhang*, Shunyi Yang, Tingwei Zhang

College of Plant Protection, Gansu Agricultural University; Lanzhou 730070, China

ABSTRACT

In 2015-2017, yellow patches emerged on rice in ten counties. Root knots were observed in the rice roots, and numerous second-stage juveniles (J2) belonging to the genus *Meloidogyne* were isolated from soils around the rice roots. The root-knot nematodes were identified by using mtDNA-SCAR-sequence. The results indicated that the root- knot nematode was *M. graminicola*. Agricultural and chemical control measures are proposed.

KEYWORDS:
Meloidogyne graminicola, rice, identification, prevention

INTRODUCTION

The root-knot nematode *Meloidogyne graminicola* is widely distributed in every rice-producing country in South and Southeast Asia and is considered one of the most important pests affecting Asian rice [1-4]. After second-stage juveniles (J2) hatch from eggs, they invade at the root tips and inject pharyngeal secretions into vascular cells to induce a specialized nematode feeding site called a giant cell [5].

Root-knot nematodes keep these giant cells, which are embedded in galls after J2 root penetration [6], live as a food resource throughout their life cycle. These root galls are typically direct reflection of infection level of nematode and always correlate with susceptibility of plants to nematode [7].

M. graminicola infection causes substantial damage to rice root systems in nurseries and significant yield loss in the field [8-10]. In Bangladesh and Thailand, nematicide application in *M. graminicola*-infested rice fields resulted in yield increases of 16 to 33% [11]. In the Philippines, rice yields following two crops of cowpea and treatment with carbofuran resulted in a 34% increase, and nematicide application in upland rice fields in Indonesia resulted in yield increases of 28 to 87% [12]. Although *M. graminicola* has been identified in most rice-growing areas in southern China, systematic investigation of rice yield loss after nematode infection is lacking.

Many economical practices have been used alone or in combination to manage *M. graminicola* population densities below the damage threshold. For example, crop rotation with non-host plants, flooding and fallowing for several months can effectively decrease populations of *M. graminicola* and reduce yield losses [13].

However, these management practices have a number of drawbacks. The wide host range of *M. graminicola* limits the use of crop rotation, and flooding cannot be applied in water-limited areas. Furthermore, areas left fallow for several months or a crop season can significantly reduce overall output. Popular in many rice planting areas, soil sterilization with chemical nematicides is the most effective management method. Nonetheless, chemical nematicides are expensive, environmentally harmful and pose potential risks to beneficial organisms. Accordingly, searching for resistant or tolerant rice varieties may be an eco-friendly management strategy to manage this nematode.

MATERIALS AND METHODS

Sampling. The populations were randomly selected in ten different counties to cover rice agroecosystems diversity. All rice plants sampled were 25 to 50-days old at the vegetative stage (before panicle initiation). Plants sampled around Sanya were usually direct-seeded while most other plants sampled had been transplanted. At each survey site, the rice root systems of 50 randomly collected plants were carefully scanned for the presence of characteristic galls (with terminal hooks) indicating infection by *M. graminicola*. A representative composite infected root sample was collected, immediately placed in a plastic bag and transported to the laboratory. For each survey site, the number of crop cycles per year, the rice varieties and the plant protection practices were recorded. Samples were labelled and kept at 4°C in a refrigerator. In the laboratory, galls were dissected using a stereomicroscope and were either transferred to a 0.4 M NaCl solution at 4°C until nematode extraction was performed or used to confirm the presence of *M. graminicola* by acid fuchsin staining.

Statistical analyses. Statistical analyses were done using the statistical software R. The stylet lengths were transformed by 1/(stylet length) to obtain a normal distribution. All other requirements

were answered. ANOVAs were performed on the body length, the stylet length and the reproductive factor (Rf). Differences among populations (or locations) for body length, stylet length and Rf were analysed by multiple comparisons using the test of Tukey's Honest Significant Differences (Tukey HSD). A non-parametric Spearman's rank correlation test was performed between the body lengths and stylet lengths. The significance level was considered at 0.05 indicating that the observed result would be highly unlikely under the null hypothesis.

Development of a SCAR marker specific to *M. graminicola*. To develop a Sequence Characterised Amplified Region marker (SCAR), ten populations of *M. graminicola* originating from Hainan was used for details on the SCAR marker development: Specific primers were designed (SCAR-MgFW: 5'-GGGGAAGACATTTAATTGATGATCAAC-3' and SCAR-MgRev: 5'-GGTAC-CGAAACTTAGGGAAAG-3'). Amplifications were performed using the following conditions: 1 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 60°C and 1 min at 68°C followed by a final extension step of 10 min at 68°C. For each nematode population, genomic DNA was purified from aliquots of 1000 eggs by a phenol–chloroform method. Total genomic DNAs (gDNA) from *M. incognita*, *M. javanica* and *M. arenaria* were kindly provided by Kan Zhuo (SCAU, China) . DNA extraction and PCR from single J2 was performed according to the protocol described for ''ITS amplification and sequencing'' with the ''Phusion High-Fidelity DNA Polymerase'' (Thermo Fisher Scientific Inc.) and the following conditions: 98°C for 30 s; 35× (98°C, 10 s; 60°C, 30 s; 72°C, 40 s); 72°C for 10 min.

RESULTS AND DISCUSSION

Characteristics and sampling of survey sites. Rice root-knot nematode disease can infect the entire rice growth period, and it is mainly at the seedling stage of direct seeding rice. The site of the disease was mainly in the roots of rice, and the above-ground parts showed symptoms such as dwarfness, yellowing, and poor growth.

In rice root-knot nematode disease, the second-stage juveniles(J2) of root-knot nematodes invade the rice roots from above the root cap, searching for parasitic sites in the roots and establishing a parasitic relationship with rice, and further develop into adults.

Giant cells formed at the parasitic site under the action of the effector secreted by the esophageal glands of the root-knot nematode, causing the roots of the rice to expand to form nodules and it affects the formation of rice fibrous roots (Fig.1), thereby affecting the water and nutrient absorption of the seedlings. Part of the symptoms are short stature, yellowing, poor growth, decreased tillering and other symptoms (Fig.2).

FIGURE 1
The main symptoms of root-knot nematode in rice roots

FIGURE 2
Field symptoms of rice root-knot nematode

The frequency of occurrence of *M. graminicola* was higher on the bounds of the surveyed fields, near the paths, than in the middle of the fields. The nematodes were most abundant in younger plants AQ (one month after transplantation) than in older ones. In addition, *M. graminicola* was often found in roots of several weed species (e.g., Cyperus iria L.) at the edges of the rice fields as previously described. During the first month after transplantation, infested fields showed symptomatic patches with lower plant density and a delay in rice development. In these patches, the rice root systems showed the typical hook-like root galls caused by *M. graminicola*. These typical galls were detected on all rice developmental stages and in all rice agro-ecosystems surveyed.

SCAR amplification results. SCAR amplification of the DNA sequence of the root-knot nematode of *M. graminicola* was performed. Analysis by 12 g/L agarose gel electrophoresis showed that each individual *M. graminicola* sample produced a DNA strip with a specificity of approximately 500 bp. (Fig.3).

FIGURE 3
Expansion mtDNA electrophoresis

Molecular identification result. The SCAR product was sequenced and the sequencing result showed that the fragment was 531 bp. The alignment of this fragment in the Gen Bank database showed that the homology was 100% with the known gene in the database (GU187309). The nematode can be identified as *M. graminicola*.

In this study, the specific DNA sequence was used to identify the rice root-knot nematode that occurred in all infected rice in Hainan. The pathogenic nematode has caused serious economic losses to some direct-seeded rice growing areas in Hainan Province. This study is also the first report on the direct seeding of the root-knot nematode in Hainan Province.

In 1963, it was first discovered on the *Echinochloa colonum* L. in Louisiana, USA. The study showed that the nematode could parasitize the roots of rice, onion and many monocotyledonous weeds. After infecting rice, it will cause serious production cuts and even destroy the field. Hainan is located in a tropical warm and humid monsoon climate with a large amount of rainfall and an average annual temperature of 22 to 27°C. Crops are often planted continuously for many years, causing serious diseases and particularly suitable for root-knot nematode in the rice growing season. Once the root-knot nematode colonizes the rice growing area, it is difficult to eradicate it.

Prevention. Phytosanitary. The pathogenic nematodes are the potential quarantine subject in China and the phytosanitary work should be strengthened. It is forbidden that diseased seedlings are introduced into the disease-free zone.

Agricultural control. (1) Planting with paddy-upland crops; (2) Planting non-host plants after harvesting rice; (3) Influencing farmland into water and soaking in water for ploughing; (4) Disease-free matrix is used to cultivate disease-free seedlings; (5) Increasing in sick fields Lime application can only protect the new roots and reduce the infection.

Nematicide application. Mainly suitable for application in Hainan, fosthiazate, abamectin, fluopyram and dazomet can be used to cultivate disease-free seedlings.

CONCLUSIONS

We have studied the diversity of *M. graminicola* populations using the data obtained from some selected morphometric traits, host range, reproduction and virulence experiments and developed a DNA marker. We demonstrated here that *M. graminicola* is spread in fact all around Hainan. Characteristic *M. graminicola* inducing-terminal root galls with hook-like shape were found in all rice fields surveyed. Agricultural and chemical control measures are proposed.

REFERENCES

[1] Zhan, L.P., Ding, Z., Peng, D.L., Peng, H., Huang, W.K. (2018) Evaluation of Chinese rice varieties resistant to the root-knot nematode *Meloidogyne graminicola*. Journal of Integrative Agriculture. 17, 621-630.

[2] Kyndt, T., Denil, S., Haegeman, A., Trooskens, G., Bauters, L., Van Criekinge, W., De Meyer, T., Gheysen, G. (2012) Transcriptional reprogramming by root knot and migratory nematode infection in rice. New Phytologist. 196, 887–900.

[3] Ji, H., Gheysen, G., Denil, S., Lindsey, K., Topping, J.F., Nahar, K., Haegeman, A., De Vos Winnok, H., Trooskens, G., Van Criekinge, W., De Meyer, T., Kyndt, T. (2013) Transcriptional analysis through RNA sequencing of giant cells induced by *Meloidogyne graminicola* in rice roots. Journal of Experimental Botany. 64, 3885–3898.

[4] Bellafiore, S., Jougla, C., Chapuis, É., Besnard, G., Thi, X.N. (2015) Intraspecific variability of the facultative meiotic parthenogenetic root-knot nematode (*Meloidogyne graminicola*) from rice fields in Vietnam. Comptes Rendus Biologies. 338, 471-483.

[5] Kyndt, T., Vieira, P., Gheysen, G., De Almeida-Engler, J. (2013) Nematode feeding sites: Unique organs in plant roots. Planta. 238, 807–818.

[6] Gheysen, G., Mitchum, M.G. (2011) How nematodes manipulate plant development pathways for infection. Current Opinion in Plant Biology. 14, 415–421.

[7] Wang, C., Ulloa, M., Mullens, T.R., Yu, J.Z., Roberts, P.A. (2012) QTL analysis for transgressive resistance to root-knot nematode in interspecific cotton (*Gossypium* spp.) progeny derived from susceptible parents (*Gossypium* spp.). Plos One. 7, e34874.
[8] Patil, J., Gaur, H.S. (2014) The effect of root-knot nematode, *Meloidogyne graminicola*, on the quality and vigour of rice seed. Nematology. 16, 555–564.
[9] Xiao, T.J., Chen, F., Gao, C., Zhao, Q.Y., Ran, W. (2013) Bacillus cereus X5 Enhanced Bio-Organic Fertilizers Effectively Control Root-Knot Nematodes (*Meloidogyne* sp.). Pedosphere. 23, 160-168.
[10] Dimkpa, S.O.N., Lahari, Z., Shrestha, R., Douglas, A., Gheysen, G., Price, A. (2016) A genome-wide association study of a global rice panel reveals resistance in Oryza sativa to root-knot nematodes. Journal of Experimental Botany. 67, 1191–1200.
[11] Sharma-Poudyal, D., Pokharel, R., Shrestha, S.M., Khatri-Chhetri, G.B. (2004) Evaluation of common Nepalese rice cultivars against rice root knot nematode. Nepal Agriculture Research Journal. 5, 33–36.
[12] Dutta, T.K., Ganguly, A.K., Gaur, H.S. (2012) Global status of rice root-knot nematode, *Meloidogyne graminicola*. African Journal of Microbiology Research. 6, 6016–6021.
[13] Rahman, M.L. (1990) Effect of different cropping sequences on the root-knot nematode, *Meloidogyne graminicola*, and yield of deepwater rice. Nematologia Mediterranea. 18, 213–217.

Received: **23.07.2018**
Accepted: **20.10.2018**

CORRESPONDING AUTHOR

Xinhu Zhang
College of Plant Protection,
Gansu Agricultural University
Lanzhou 730070 – China

e-mail: ndzxh@gsau.edu.cn

© by PSP Volume 27 – No. 12B/2018 pages 9906-9912 Fresenius Environmental Bulletin

ANTI-QUORUM SENSING ACTIVITY OF 1, 3-DIHYDRO-2H-BENZIMIDAZOL-2-ONE DERIVATIVES

Ebru Onem[1,*], Yasemin Dundar[2], Seyhan Ulusoy[3], Ningur Noyanalpan[2], Gulgun Bosgelmez-Tinaz[4]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Suleyman Demirel, Isparta, Turkey
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Gazi, Ankara, Turkey
[3]Department of Molecular Biology, Faculty of Arts and Sciences, University of Suleyman Demirel, Isparta, Turkey
[4]Department of Basic Pharmacy Science, Faculty of Pharmacy, University of Marmara, Istanbul, Turkey

ABSTRACT

Many Gram-negative bacteria use N-acyl homoserine lactone (AHL) signal molecules to monitor their own population density and coordinate gene regulation in a process called quorum sensing (QS). *Pseudomonas aeruginosa* controls production of virulence factors (elastase, pyocyanin or swarming motility) via QS. The discovery that QS system regulates bacterial virulence has afforded a novel opportunity to control infections without interfering with growth. The aim of this study was to investigate anti-quorum sensing effect of synthesized benzimidazole derivatives which are N-acyl homoserine lactone analogs. All chemicals synthesized as quorum sensing inhibitors were tested against virulence factors -elastase, pyocyanin, swarming motility- in *Pseudomonas aeruginosa* PA01. Firstly 1,3-dihydro-2H-benzimidazol-2-one (U65), 5-methyl-1,3-dihydro-2H-benzimidazol-2-one (U77) and 1,3-diacetyl-1,3-dihydro-2H-benzimidazol-2-one (U92) were screened for anti-quorum sensing activity using a biomonitor strain, Quorum Sensing Selector Strain 1 and *Chromobacterium violaceum* CV026, Chromobacterium violaceum VIR07. All of the synthesized compounds showed anti-QS activity and exhibited significant inhibitory effect on production of elastase, pyocyanin and swarming motility. In conclusion, our results demonstrated that synthesized benzimidazole derivatives inhibited bacterial communication in *Pseudomonas aeruginosa* which is crucial for the infections and further studies of these molecules should be investigated on other bacteria.

KEYWORDS:
Quorum-sensing inhibitors, virulence, benzimidazole, *P.aeruginosa* PA01

INTRODUCTION

Bacteria were thought to be living cells separately for years and sought primarily to find nutrients and multiply. However people's general perception of bacteria has changed with the discovery that bacteria are able to communicate with each other through a mechanism called quorum sensing (QS) system [1]. QS system relies on two major components, a small diffusible signaling molecule which accumulates in a population density-dependent manner and a transcriptional activator protein which in concert with signalling molecule activates the expression of target genes [2]. *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that commonly responsible for severe nosocomial infections in immunosuppressed patients, and also causes some chronic diseases such as cystic fibrosis [3]. Besides high incidence and infection severity, the resistance of *P. aeruginosa* to conventional antimicrobial treatment has increased in recent years [4]. A major contribution to this resistance is provided by beta-lactamase production, efflux systems and modifications of specific target sites or the outer membrane [5].

From the pathogenesis of these infections, QS system is held responsible. QS system regulates production of various virulence factors such as elastase, alkaline protease, piyocyanin, phospholipase and exotoxinA in *P. aeruginosa*. Besides these factors, biofilm formation and swarming motility have also been controlled by this system. Mainly two QS systems in *P. aeruginosa* have been characterized, *las* and *rhl*. The las system composes of a transcriptional regulator LasR, and *N*-(3-oxododecanoyl-homoserine lactone ($3OC_{12}$-HSL) signaling molecule that is synthetized by *lasI* gene. Second system *rhl* is controlled by regulator RhlR and this system components are *N*-butyryl-homoserine lactone (C4-HSL) and autoinducer synthetize gene *rhlI*. A third signal, *Pseudomonas* quinolone signal (PQS), is also intricately involved in the las and rhl quorum-sensing systems [6].

Treatment of infectious diseases with current antibiotics is becoming increasingly difficult due to resistant microorganisms. It is known that QS plays major role in compose of biofilm formation and it is estimated that biofilms account for up to 80% of infectious diseases [7, 8]. Therefore, this is a crucial problem for treatment with antibiotics because of approximately more than a thousand times resistant according to planktonic cells. Besides biofilm, pyocianin is also critical for virulence especially in cystic fibrosis patients because of cell damage in lungs [9].

Pyocyaninis a blue pigment involved in pathogenesis by inducing apoptosis in neutrophils and suppressing cell respiration [10]. Another factor in *Pseudomonas* infections is elastase which causes local tissue damage and, as a result, inflammatory reactions help to spread the infection [11]. Hereby after the discovery that quorum sensing is responsible for the virulence in some bacteria, new researches have focused on blockage of this system [12]. For this aim, besides some plant extract also chemical compounds have been tried in lots of researches. In present study, Some benzimidazole derivatives were synthesized and examined their inhibition activity on some virulence factors such as elastase, pyocyanin, and swarming motility in *P. aeruginosa* PA01 (Figure 1).

MATERIALS AND METHODS

Chemistry. All chemicals which were synthesized as quorum sensing inhibitors (QSIs) and solvents were purchased locally from Merck AG and Aldrich Chemicals. Microwave reactions were carried out at atmospheric pressure in MicroSYNTH Microwave Labstation. Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected. Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectra were recorded on a Perkin-Elmer Spectrum 400 FT-IR and FT-NIR spectrometer with a Universal ATR sampling accessory. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm).

1,3-Dihydro-2H-benzimidazol-2-one (U65) [13]. The mixture of o-phenylenediamine (1.08 g, 0.01 mol) and urea (0.9 g, 0.015 mol) was heated to 140ºC and stirred for 6 min under microwave irradiation (MWI). Reaction mixture was poured onto crushed ice. The precipitated solid product was removed by filtration and dissolved in 10% NaOH and converted to the benzimidazole by acidification with concentrated HCl to give a solid precipitate. The product was collected by suction filtration, washed with water and dried. Yield 1.14 g (85%). mp: 315ºC (305ºC [18], 304–305ºC [14]).

5-Methyl-1,3-dihydro-2H-benzimidazol-2-one (U77) [15, 16]. The mixture of 4-methyl-o-phenylenediamine (1.22g, 0.01 mol) and urea (0.9 g, 0.015 mol) was heated to 140ºC and stirred for 6 min under MWI. Reaction mixture was poured onto crushed ice. The precipitated solid product was removed by filtration and dissolved in 10% NaOH and converted to the benzimidazole by acidification with concentrated HCl to give a solid precipitate. The product was collected by suction filtration, washed with water and dried. Yield 0.74 g (50%). mp: 298 º C (292 º C [20]).

1,3-Diacetyl-1,3-dihydro-2H-benzimidazol-2-one (U92) [17, 18]. The acetyl chloride (0.02 mol) and TEA (0.022 mol) were added to a solution of 1,3-dihydro-2*H*-benzimidazol-2-one (0.01 mol) in 40 mL of dry THF cooled at 4°C. The reaction mixture was heated to 70 º C for 20 min under MWI, added to 200 mL of ice water and stirred for 1 h. The resulting precipitate was filtered, washed with water, dried, and recrystallized from ethanol. Yield 64%. mp: 151ºC (149-151ºC [12]). FTIR-ATR, cm^{-1}: 1764, 1750, 1714 (C=O), ^{1}H-NMR ($CDCl_3$)δ: 8.23 (2H, dd, benzimidazole H4, H7), 7.27 (2H, benzimidazole H5, H6), 2.77 (6H, s, CH_3).

Bacterial strains and growth conditions. For testing anti quorum sensing effects of U65, U77, U92, QSIS1, *Chromobacterium violaceum* CV026 and *Chromobacterium violaceum* VIR07 strains were used. Inhibition effect of benzimidazole derivatives on QS regulated virulence factor production was tested in *P. aeruginosa* PA01 strain. PAO-JP2 was used as negative control.

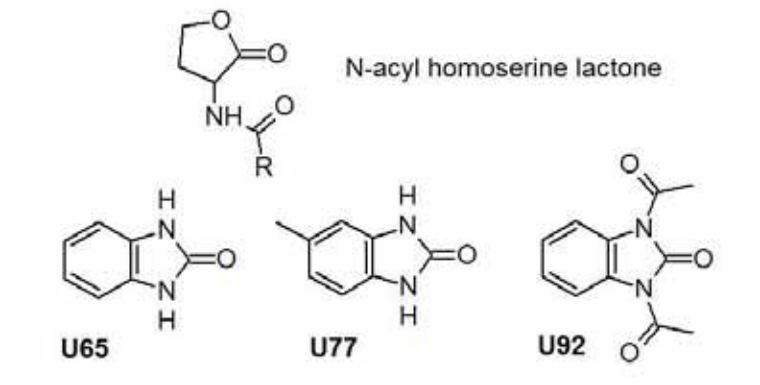


FIGURE 1
Chemical structure of AHL and the synthesized compounds

Anti-quorum sensing activity screening by quorum sensing inhibitor assay. Quorum sensing inhibitor selector 1 (QSIS1) strain was constructed by cloning the luxR QS system from *Vibrio fischeri* in an *E. coli lac*$^+$ strain [19]. This gene encoding the protein phospholiphase A and causing cell death, if it transcribed, is inserted under control of the *luxI* promotor. In presence of non-toxic QSI, *phlA* will not be expressed, and bacteria go on normal growth [20]. For in vitro screening quorum sensing inhibitor (QSI) compounds, developed a system based on recombinant bacteria background giving rise to a blue circle of growth in X-Gal supplemented medium [19].

QSIS1 strain was inoculated to 10 ml BT media. BT media contain 0, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0, 01 mM $FeCl_3$, 10% 10XA, 0,025% thiamine, 0,5% glucose, 0,5% casoamino acid. And incubated 14-16 min a shaker (180 rpm) at 30 °C. Another day BT agar which was containing 0,1 mM $CaCl_2$, 1 mM $MgCl_2$, 0,01 mM $FeCl_3$ and 2% bacto agar was prepared. Temperature of BT agar medium was decreased to 45°C. Then added X-gal (40 mg/ml), IPTG (Isopropyl β-D-1-thiogalactopyranoside) (100 mM), 10XA (100 mg/ml), thiamine (25 µg/ml), ampicillin (100 mg/ml), 0,5% glucose, 0,5% casaminoacid, 3-oxo-C6 HSL (100 nM) and 800 µl QSIS1 strain were added overnight culture. Glass pastor pipette was used to make small wells on the medium. QSIs (U65, U77 and U92) was dissolved dimethylsulphoxide (DMSO; Merck) and added these wells 50µl. Each petri dishes contained extra well for a known QSI as a positive control. After one hour on the room temperature, plates put incubator at 30°C for overnight. Blue circular ring around small well showed us QSI presences [20].

***Chromobacterium violaceum* 026-*Chromobacterium violaceum* VIR07 pigment production assay.** To detection of QS inhibition *Chromobacterium violaceum* can be used also. *Chromobacterium violaceum* a Gram-negative bacterium and produces the characteristic purple pigment violacein. In *C. violaceum* 026, violacein is inducible by adding AHL which have N-acyl side chains from C4 to C8 in length and *C. violaceum* VIR07 is inducible by with *N*-acyl side chains from C10 to C14 in length [21].

C. violaceum 026 and *C. violaceum* VIR07 strains were cultured in Luria-Bertani (LB) medium at 30 °C for 16-18 h. 5 ml soft agar (0,5 % agar) containing 5 µl OHHL (3-oxo-C6 HSL) and OdDHL)-(3-oxo-C12-HSL) was prepared for *C. violaceum* 026 and *C. violaceum* VIR07 respectively. After adding 100µl bacterial culture, this mixture was poured onto the agar medium. Small wells were made on the medium by using glass pastor pipette. Finally 50 µl of QSIs added each well and incubated 37°C about 16-18 h.

Elastase experiment. Elastase activity was measured using the Elastin Congo Red (ECR; Sigma) assay [22]. Cells were grown in LB broth supplemented with test compounds (final concentration 3 mM, 1,5 mM, 0,75mM) at 37ºC for 16 h, centrifugation at 15000 rpm at 4ºC for 10 min. Then 0.5 mL supernatant was added to 1 mL of assay buffer (30 mM Tris buffer, pH 7.2) containing 10 mg of ECR. The mixture was incubated at 37 ºC for 6 h. Insoluble ECR was removed by centrifugation and the absorption of the supernatant was measured at 495 nm. LB medium was used as a negative control.

Pyocyanin experiment. Pyocyanin assay was performed to detect the inhibitory effect of U65, U77 and U92 on pyocyanin production. *P. aeruginosa* PA01 and clinical isolates were grown in 10 ml LB broth medium with test compounds (final concentration 3 mM, 1,5 mM, 0,75mM) and incubated 37°C for 16-18 h. To maximize production, pyocyanin was extracted with 5 ml of chloroform and shaken for 30 seconds. After adding chloroform two phases were formed and 2 ml of bottom phase were taken another test tubes. Then 1 ml HCl water was added and shaking for about 30 seconds. A pink phase which was formed at the top of the tubes was measured at 520 nm [23].

Swarming motility assay. Swarming medium which was contained 8g nutrient broth 1^{-1}, 5 g bacto agar l-1 and 0,5% glucose and 1,5 mM concentration of QSIs were prepared. After poured swarm medium 5 µl each supernatant of bacterial cultures were added middle of the medium. Let plates air dry about 15 min at the room temperature and all plates incubated overnight at 37°C. The ability to swarm was assessed by the distance of swarming from the central inoculation site [24].

Statistically Evaluation. In order to determine the differences, ANOVA (analyses of variance) was performed. JMP 8.0 statistical package program was used in the statistical analyses.

RESULTS

QSIS1 and CV026, VIR07 assay. All of three derivatives (U65, U77, U92) showed anti quorum sensing activity according to QSIS1 assay (compose of blue circle showed antiQS activity Figure 2). Patulin was used for positive control which is reported before as QSI [25]. And also chromobacterium assay indicated same results (inhibition of purple pigment production Figure 3).

To confirm the ability of anti-quorum sensing effect of synthesized derivatives U65, U77, U92; we investigated inhibition effect of these derivatives on some virulence factors (pyocyanin, elastase, swarming motility) which are regulated by QS system. For

FIGURE 2
QSI activity of derivatives on QSIS1 strain C: (patulin-control)

FIGURE 3
QSI activity of derivatives on *C. violaceum* 026, C: (PeA-control)

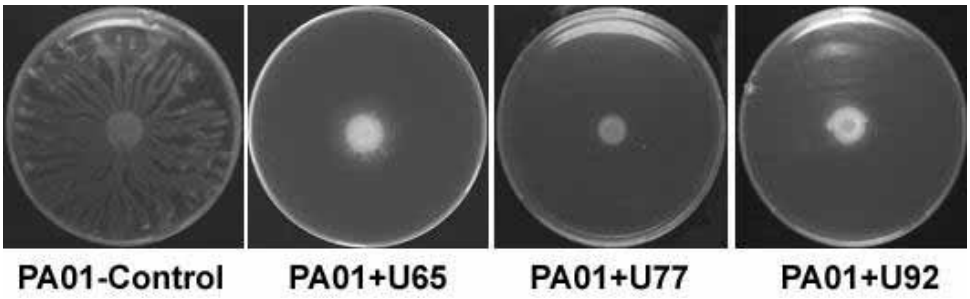


FIGURE 4
Inhibition effects of U65, U77, U92 derivatives on swarming motility on *P. aeruginosa* PA01.

this purpose, we used references strain *P. aeruginosa* PA01. The synthetic molecules which were used in this study are similar to natural homoserine lactone molecule and bioisoster of 2(3H)-benzoxazole and 5-Chloro-2(3H)-benzoxazole [26]. To determine the inhibitory effect of synthetic AHL analogs all tests were carried out presence and absence of benzimidazole derivatives. And benzimidazole derivatives were used at three different (3 mM, 1,5 mM and 0,75 mM) concentrations which have no antibacterial effect on *P. aeruginosa* PA01 growth. The inhibition effect of benzimidazole derivatives on the production of pyocyanin pigment was tested and three derivatives showed significant reductions on production of pyocyanin pigment on *P. aeruginosa* PA01 at 3mM concentration. Inhibition rates were close and the most inhibitory effect was observed in U65 (69%). Other results for U77, U92 respectively 61% and 50% have seen. Similar inhibition effect in lower dose of U65 and U77 were detected and we found that inhibition on production of pyocyanin at 0,75 mM concentration and these results were statistically significant. Another virulence factor we examined was elastase and results showed that U65, U77, U92 inhibited production of elastase (from 19% to ~44%) at all tested concentrations. And the lowest concentration (0,75 mM) of derivatives resulted in significant reduction. As a result of our swarming motility experiments, the greatest inhibitions have observed at 1, 5 mM concentration on *P. aeruginosa* PA01 (Figure 4). The highest inhibitory effect was seen in U77 with 80 %. Other derivatives U65 and U92 showed same inhibition rate (74%) on swarming motility.

DISCUSSION

Different strategies are developed to interfere with QS system. This is achieved by inhibition of synthesis of autoinducers, degrading autoinducers, inhibition of AHL signal dissemination or inhibition of AHL signals reception [3]. Some of the natural or synthetic QS inhibitors show excellent activity to inhibit bacterial pathogenicity and in the literature many researches can be obtained for this subject. Varieties of studies have focused on blocking the receptor protein with the natural or synthetic AHL analogs [27]. So far; many synthetic molecules which have inhibited quorum sensing system have been reported. It was previously reported that synthetic homoserine lactone derived

sulfonylureas and N-phenylacetanoyl-Lhomoserine lactones could respectively inhibit Lux quorum sensing system [28]. And also N-acyl cyclopentylamides which were synthesized as AHL analogs inhibit quorum sensing system not only lux system but also las system in *P. aeruginosa*. In previous study 2(3H)-benzoxazole and its derivatives were synthesized and had anti quorum sensing activity [26]. Subsequently, in this study we synthesized acyl homoserine lacton analogs and anti-quorum sensing effect of these molecules was examined. Non-antibacterial concentrations were used for anti-quorum sensing experiment. And we found that synthesized three molecules (1,3-Diacetyl-1,3-dihydro-2H-benzimidazol-2-one (U65), 5-Methyl-1,3-dihydro-2H-benzimidazol-2-one (U77), 1,3-Dihydro-2H-benzimidazol-2-one (U92) showed inhibitory effect on the production of virulence factors (pyocyanin, elastase, and swarming motility) in *P. aeruginosa* PA01 with different rate. In this study, synthesized benzimidazole derivatives which have inhibitory effect on the quorum sensing system have not been reported previously.

P. aeruginosa infection is multifactorial and contains a large number of virulence factors. Pyocyanin and elastase are crucial virulence factors in *P. aeruginosa* infections. We therefore examined the effect of molecules on elastase and pyocyanin production. Many studies have been carried out on the inhibition of pyocyanin with synthetic molecules and synthesized molecules which was as an autoinducer analog 3-oxo-C12- (2aminocyclohexanone) inhibited *lasI* and *rhlI* reporters as a result production of pyocyanin and elastase on PA01 [29]. Our molecules U65, U77 also showed inhibition effect on production of elastase and pyocyanin by 45%- 37% and 69%, 61% respectively. While U92 had no significant inhibition on elastase, but it showed significant inhibitory effect on pyocyanin production.

The variance analysis of the data detected from the effects of the synthesized molecules on PA01 was made and the results were obtained in Figure 5. According to results, all derivatives (U65, U77, U92) showed inhibition effect on virulence factors and the results were statistically significant ($p<0.01$). When all these results were evaluated, it was interpreted that the U65 showed the most effective inhibition on all virulence factors.

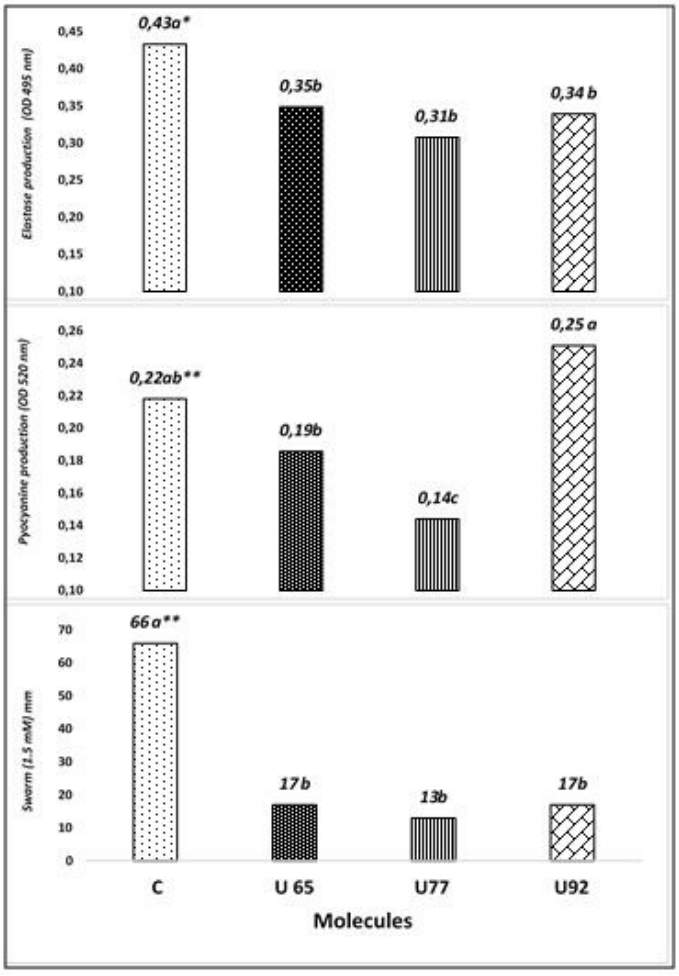


FIGURE 5
The inhibition effects of derivatives on virulence factors in *P. aeruginosa* PA01.

* Means within chemicals with the same letter are not significantly different by LSD's Multiple Range Test at $p<0.05$.
** Means within chemicals with the same letter are not significantly different by LSD's Multiple Range Test at $p<0.01$.

In conclusion this study focuses on design and synthesis of new anti-quorum sensing agents. In accordance with this purpose acyl homoserine lacton analogs were synthesized with various ring structures (1,3-Diacetyl-1,3-dihydro-2H-benzimidazol-2-one (U65), 5-Methyl-1,3-dihydro-2H-benzimidazol-2-one (U77), 1,3-Dihydro-2H-benzimidazol-2-one (U92)) and examined their inhibitory effect on QS. As a result of the experiment we found that all three molecules showed inhibitory effect on virulence factors (pyocyanin, elastase, swarming motility) in *P. aeruginosa* PA01. Inhibition effect of these benzimidazole derivatives on QS hasn't been reported previously. New approaches and drugs should be developed and studied to overcome antibacterial resistance in the treatment of infectious diseases. Strategy to be targeted should be blocking communication between bacteria that is one of the most important research topics that have recently been promising and overreaching in the fight against microbial infections.

REFERENCES

[1] Henke, J.M. and Bassler, B.L. (2004) Bacterial social engagements. Trends in Cell Biology. 14, 648-656.

[2] Lade, H., Paul, D. and Kweon, J.H. (2014) Quorum quenching mediated approaches for control of membrane biofouling. Int. J. Biol. Sci. 10, 550-565.

[3] Khalifa, A.B.H., Moissenet, D., Thien, H.V. and Khedher, M. (2011) Virulence factors in *Pseudomonas aeruginosa*: mechanisms and modes of regulation. Ann. Biol. Clin. 69, 393-403.

[4] Obritsch, M.D., Fish, D.N., MacLaren, R. and Jung, R. (2004) National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob Agents Chemother. 48, 4606-4610.

[5] Djordjevic, Z.M., Folic, M.M. and Jankovic, S.M. (2017) Previous antibiotic exposure and antimicrobial resistance patterns of *Acinetobacter spp*. and *Pseudomonas aeruginosa* isolated from patients with nosocomial infections. Balkan Med. J. 34, 527-533.

[6] Pesci, E.C., Milbank, J.B.J., Pearson, J.P., McKnight, S., Kende, A.S., Greenberg, E.P. and Iglewski, B.H. (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc Natl Acad. 96, 29-34.

[7] More, P.G., Karale, N.N., Lawanda, A.S., Rajmane, S.V., Pawar, S.V. and Patil, R.H. (2013) A 4-(o-methoxyphenyl)-2-aminothiazole: an anti-quorum sensing compound. Medicinal Chemistry Research. 22, 4183-4191.

[8] Ishida, T., Ikeda, T., Takiguchi, N., Kuroda, A., Ohtake, H. and Kato, J. (2007) Inhibition of Quorum Sensing in *Pseudomonas aeruginosa* by N-Acyl Cyclopentylamides. Appl Environ Microbiol. 73, 3183-3188.

[9] Denning, G.M., Wollenweber, L.A., Railsback, M.A., Cox, C.D., Stoll, L.L. and Britigan, B.E. (1998) Pseudomonas pyocyanin increases interleukin-8 expression by human airway epithelial cells. Infect. Immun. 66, 5777-5784.

[10] Fuqua, C., Parsek, M.R. and Greenberg, E.P. (2001) Regulation of gene expression by cell to-cell communication: acyl-homoserine lactone quorum sensing. Annual Review of Genetic. 35, 439-468.

[11] De Kievit, T.R. and Iglewski, B.H. (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun. 68, 4839-4849.

[12] Hauser, A.R., Mecsas, J. and Moir, D.T. (2016) Beyond Antibiotics: New therapeutic approaches for bacterial infections. Clinical Infection Diseases. 63, 89-95.

[13] Kym, P. (1907) Some condensations with urea and urea as a means of forming ammonia derivatives. Journal fur Praktische Chemie (Leipzig). 75, 323-27.

[14] Kidwai, M., Saxena, S. and Mohan, R. (2005) A new method for the synthesis of heterocycles from o-phenylenediamine. Journal of the Korean Chemical Society. 49, 288-291.

[15] Harvey, I.W., McFarlane, M.D., Moody, D.J. and Smith, D.M. (1988) o-Nitroaniline derivatives. Part 9. Benzimidazole N-oxides unsubstituted at N-1 and C-2. Journal of the Chemical Society Perkin Transactions 1. 3, 681-689.

[16] Khajavi, M.S., Hajihadi, M. and Naderi, R. (1996) Synthesis of heterocyclic compounds from o-substituted anilines under microwave irradiation. Journal of Chemical Research, Synopses. 2, 92-93.

[17] Heller, G., Buchwaldt, A., Fuchs, R., Kleinicke, W. and Kloss, J. (1925) Tautomeric phenomena in heterocyclic compounds. Journal fur Praktische Chemie (Leipzig). 111, 1-22.

[18] Harrison, D. and Smith, A.C.B. (1961) Acyl derivatives of 2-oxobenzimidazoline. Journal of the Chemical Society. 1961, 4827-4830.

[19] Bjarnsholt, T., Gennip, M.V., Jakobsen, T.H., Christensen, L.D., Jensen, P.Ø. and Givskov, M. (2010) In vitro screens for quorum sensing inhibitors and in vivo confirmation of their effect. Nature Publishing Group. 5, 282-293.

[20] Rasmussen, T.B., Bjarnsholt, T., Skindersoe, M.E., Hentzer, M., Kristoffersen, P., Köte, M., Nielsen, J., Eberl, L. and Givskov M. (2005a) Screening for Quorum Sensing Inhibitors (QSI) by use of a Novel Genetic System, the QSI Selector. Journal of Bacteriology. 187, 1799-1814.

[21] McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R. and Camara, M. (1997) Quorum sensing *and Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology. 143, 3703-3711.
[22] Ohman, D.E., Cryz, S.J. and Iglewski, B.H. (1980) Isolation and characterization of a *P. aeruginosa* PA01 mutant that produces altered elastase. J. Bacteriol. 142, 836-842.
[23] Essar, D.W., Eberly, L. and Hadero, A. (1990) Crawford I. Identification and characterization of genes for a second anthranilate synthase in *P. aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. Journal of Bacteriology. 172, 884-900.
[24] Rashid, M.H. and Kornberg, A. (2000) Inorganic polyphosphate is needed for swimming, swarming and twitching motilities of *P. aeruginosa*. Proc. Natl. Acad. Sci. 97, 4885-4890.
[25] Rasmussen, T.B., Skindersoe, M.E., Bjarnsholt, T., Phipps, R.K., Christensen, K.B., Jensen, P.O., Andersen, J.B., Koch, B., Larsen, T.O., Hentzer, M., Eberl, L., Hoiby, N. and Givskov, M. (2005b) Identity and effects of quorum sensing inhibitors produced by *Penicillum species*. Microbiolog. 151, 1325-1340.
[26] Miandji, A.M., Ulusoy, S., Dündar, Y., Özgen, S., Onurdağ, F.K., Boşgelmez-Tınaz, G. and Noyanalpan, N. (2012) Synthesis and Biological Activities of Some 1,3-Benzoxazol-2(3H)-One Derivatives as Anti-Quorum Sensing Agents. Arzneimittelforschung Drug. Res. 62, 330-334.
[27] Kline, T., Bowman, J, Iglewski, B.H., De Kievit, T., Kakai, Y. and Passador, L. (1999) Novel synthetic analogs of the Pseudomonas autoinducer. Bioorg. Med. Chem. Lett. 9, 3447-3452.
[28] Friedland, I., Gallagher, G., King, T. and Woods, G.L. (2004) Antimicrobial susceptibility patterns in *Pseudomonas aeruginosa*: Data from a multicenter intensive care unit surveillance study (iss) in the United States. J Chemother. 16, 437-441.
[29] Smith, R.S. and Iglewski, B.H. (2003) *P. aeruginosa* quorum–sensing systems and virulence. Current Opinion in Microbiology. 6, 56-60.

Received: **23.07.2018**
Accepted: **22.09.2018**

CORRESPONDING AUTHOR

Ebru Onem
Department of Pharmaceutical Microbiology,
Faculty of Pharmacy,
University of Suleyman Demirel,
Isparta – Turkey

e-mail: ebruonem@sdu.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9913-9921 Fresenius Environmental Bulletin

THE RELATIONSHIP PRODUCTION BEHAVIOURS AND PERCEPTION OF GENETIC RESOURCE CONSERVATION OF THE FARMERS PRODUCING DRY BEAN LANDRACES IN TURKEY

Mustafa Kan*

Kirsehir Ahi Evran University, Agricultural Faculty, Department of Agricultural Economics, Kirsehir, Turkey

ABSTRACT

Turkey to be an important gene center for many plants and animals in the world, carry Turkey to a significant position both in terms of biodiversity and genetic resources. However, technological advances, developments in the use of inputs in agriculture, growing population and nutritional needs have confronted manufacturers with the fact that their production resources could be used more efficiently. The producers are planning their production with the level of consciousness and behavior in production, and also with genetic resource-landraces perception, which affects the sustainability of genetic resources-landraces. This study reveals the relationship between genetic resource-landraces perception and production behaviors of dry bean landrace (DBL) producers as a result of the survey conducted with 140 DBL producers from 27 districts of a total of 8 provinces located in Middle Kızılırmak Valley in Turkey. Genetic Resource-Landrace Perception (GRLP) and Production Behavior (PB) indexes of the producers were established by factor analysis. As a result, even though the economic value is high, landraces are being produced more by old school producers, which are defined as "conservative". Moreover, it has been determined that these producers are higher in genetic resource-landraces perception than that defined as “innovative”. While the state is making policy regarding the protection of genetic resources and the sustainability of landraces, innovative and prominent individuals need to be taken more into consideration when determining the target population. Although the aforementioned individuals represent an innovative and environmentally conscious high-level, they are composed of individuals with low genetic resource- landrace perception and are the groups that trigger more erosion of genetic resources with more commercial-oriented thinking.

KEYWORDS:
Natural Resources and Environment, Middle Kızılırmak Valley, Dry Bean Landraces, Genetic Resources, Production Behaviour, Turkey

INTRODUCTION

Rapid developments in agriculture, particularly in the second half of the 20th century, have brought about significant increases in productivity. Progress in plant breeding, as well as this intensification in agriculture, has begun to cause the introduction of high yielding new varieties into the market and the production of lower-yielding landraces to decline. The period named as a green revolution characterized by the use of technological and chemical inputs, also has a separate prescription for plant rehabilitation, especially the achievements achieved in wheat and paddy have marked this period. When evaluated in terms of meeting the growing food needs of the population, the results of this period have led most industrial countries to achieve sustainable food surplus in the second half of the twentieth century and the threat of hunger has ceased to exist [1].

When the ecological, economic and social cost of this process, which is perceived as modernization, is understood to be rather heavy, one of the most important problems experienced emerged as an erosion of genetic resources. Genetic erosion is a process involving the replacement of traditional, indigenous and landraces with genetically uniform, highly efficient modern varieties. Insufficient knowledge about climate change intensive agriculture, rapid development processes and urbanization, the destructive effect of modern agriculture on habitat, especially the scientific, social, cultural and economic importance of plant germplasm are the main drivers of this process [2]. Especially with the green revolution, it is reported that in many countries modern varieties have taken the place of most of the landraces [3, 4, 5]. Looking at the positive direction of this process, although the increase in yield seems to be important, the contraction of the gene pool and the loss of biodiversity constitute an environmental problem.

Turkey in the world of agriculture, is the gene center of many cultivated plants. In terms of origin of the cultured plants in the world, a total of eight gene centers were determined including Turkey (Near East and Mediterranean) [6]. Mediterranean and Near Eastern Centers of diversity and origin centers that was announced by the Vavilov (1994) also

coincides with Turkey [7]. Turkey is one of the world's richest countries in terms of plant genetic resources. In addition, according to J. Harlan there are 5 micro-gene centers with more than 100 species shows widespread variations in Turkey, which is primary and secondary gene center of many plants [7]. In our micro gene centers; varieties and form richness are observed in feed plants of barley, rye, oat, rape, lentil, chickpea, faba bean, kidney bean, vetch, sainfoin and leguminous crops [8].

Despite the lack of advantages, Turkey is an important diversity area for many vegetable species. Kidney bean, which is one of them, is one of the most important plant species for Turkey in terms of direct use in human nutrition and nutrients it contains. Beans have arrived in Turkey in the 17th century and is a legume crops that can be grown by aiming to have both dry or fresh in almost every part of Turkey and showed wide variation [9]. This legume grain contains about 22-24% protein, mineral matter and vitamin-rich contents, being an important agriculture product for human nutrition as referring "both meat and bread" [10].

South-Eastern Anatolia and Samsun-Tokat-Amasya micro gene centers are centers of genetic diversity for beans although they are not the gene center [11]. In Turkey, there are many studies on the subject of beans as the genetic resources in terms of collection, assessment and using in breeding [12]. Turkey on the fertile lands of Anatolia, thanks to the fact that Anatolian lands have hosted many civilizations and thanks to the ecological diversity that it has, like on that in almost all plant species, that also led beans to the emergence of variations in many years. materials that enter the country from different sources have been cultivated for many years in the regions they are located and different kinds of bean landraces have been formed. Locally grown bean plants contain a large number of different genetic material features [13]. Although there are improved bean varieties in the world and in Turkey, many producers are still producing and marketing local bean genotypes [14]. Therefore, in different regions of Turkey it is still possible to find bean landraces genotypes. In particular, the Middle Kızılırmak Basin, which is close to the Samsun-Tokat-Amasya micro gene centers, is an important center for the richness of dry bean landraces.

One of the effective ways to increase grain yield and quality is the use of genetic resources in breeding [14]. Thus, the sustainability of genetic resources is ensured as well as preventing genetic erosion. Although ex-situ method used to protect genetic resources is one of the important methods, in-situ conservation [15] and usage of them in as economic development tool [16] provide significant advantages in terms of sustainability. There are studies that suggest that there is a relationship between protecting genetic resources and biodiversity and ensuring sustainability, and people's environmental awareness [17, 18, 19, 20, 21]. For this reason, activities to improve environmental awareness in society can contribute to the conservation of biodiversity, and will also have a positive impact on the conservation and sustainability of genetic resources. In addition, according to TurkStat statistics in Turkey, negative pressure of 42.12% shrinkage in the field of dry bean cultivation between 2004-2017 [22] on both production and genetic resources makes it important to determine the factors that can be effective in farmers' production decisions.

With this study, in the Middle Kızılırmak Basin which is an important gene center for dry bean landraces (DBL), it is intended to reveal the general characteristics of producers dealing with dry bean landraces production and the perceptions of genetic resources / landraces. Thus, the conclusions have been drawn about what are the production decisions of DBL producers about genetic resources / landraces and what might be the factors that affect this perception. The basis of this work is both the original field data and the results of similar studies on the subject. Therefore, the generated synthesis is one of the important studies in the field with the identification of factors that may be effective in ensuring the protection of genetic resources and sustainability in Turkey. In particular, measures for the protection of genetic resources in agriculture policy in Turkey, considering that constitutes one of the most important items in agenda, the results of the study are also important in terms of policy makers.

MATERIALS AND METHODS

Data and Study Area. The main material of the study consists of the data obtained through a questionnaire survey with 140 DBL producers from a total of 8 villages (Ankara, Aksaray, Çankırı, Kayseri, Kırıkkale, Kırşehir, Nevşehir, Sivas) in the Middle Kızılırmak Basin within the scope of the "Middle Kızılırmak Valley Morphological and Molecular Characterization of Dried Bean Landraces and Determination of Genotypes Resistant to Root Nematode and Socio-Economic Characteristics of Producers" Project supported by the R & D Projects Program of the General Directorate of Agricultural Research and Policy (TAGEM) of the Ministry of Agriculture and Forestry (MoAF) (Figure 1).

The lack of a specific database on the producers engaged in the production of landraces in Turkey, makes it difficult to sample for the work to be done with DBL producers. In such studies, preliminary interviews with relevant experts and local residents prior to the study are indicative of the areas in which the study will be conducted, and interviews with producers meeting these criteria can be conducted in these areas. A similar approach on this issue has been applied in the study conducted in Turkey with wheat landraces by [5]. The database of the study of dry

FIGURE 1
The Map of Survey Area

bean and soil samples from the producers of DBL breeding in 2016 was used to determine the producers to collect data in the middle Kızılırmak Basin where the work was carried out. Producers included in the survey conducted in the region in 2016 formed the sample set of the survey works conducted in 2017. Negotiations with Provincial / District Agriculture and Forestry Directorates experts, Agricultural Chambers and local people have been effective in identifying DBL producers. As a result of interviews and surveys conducted in the region, a total of 140 producers were reached (78.21%) from the manufacturers that were sampled in 2016 and face-to-face questionnaires were completed.

In the study, the personal characteristics of the DBL producers were questioned using a 5-point scale (5 = Strongly agree / 1 = Strongly agree) to measure GRLP. In this context, 12 variables to determine the personal characteristics of the producers, and 18 variables to determine the genetic resources / local population perceptions were assigned. A reliability test was applied to the questionnaire in order to show whether the collected Likert scaled data reflects a measured likelihood. To test the reliability of the generated scale, the Cronbach Alpha coefficient was examined in the reliability analyzes most commonly used. The Cronbach Alpha coefficient is 0.60 and above, indicating that the developed scale is reliable [23, 24].

Due to the excess of variables, index values were created by using Factor Analysis Method for personal characteristics and GRLP variables. Factor analysis is one of the multivariate analysis techniques commonly used in various fields, particularly in the social sciences. Factor analysis aims to find a small number of new unrelated variables by combining the variables associated with each other in a (p) variable event. This analysis is applied to reduce the number of variables if there are too many variables and to easily interpret them [25]. Kaiser-Meyer-Olkin (KMO) and Bartlett tests were performed to evaluate whether the data set is appropriate in factor analysis [24]. The value found in the KMO test gives information about the suitability of the data set for factor analysis and if the calculated value is less than 0.50, it can not be accepted, 0.50 weak, 0.60 medium, 0.70 good, 0.80 very good, 0.90 excellent [26]. The Bartlett Test of Sphericity is used to test whether the correlation matrix is a unit matrix with all diagonal terms 1 and non-diagonal terms 0. This test requires that the data come from multiple normal distributions [25]. The Varimax rotation technique has been utilized in the rotation processes for better interpretation of the factors [23]. The variables used in factor analysis are presented in Table 1.

In addition, chi-square independence tests were performed in order to obtain information on whether the independent variables were independent of each other or not, and the results were interpreted according to the chi-square dependence coefficients in the study [27]. In the analysis of continuous variables, Variance Analysis was used to determine whether there was a statistically significant difference between groups with more than 2 levels. In the case of significant difference in statistics, Duncan Analysis of Multiple Comparison Methods was applied in order to show which group the difference originated from [28].

RESULTS AND DISCUSSION

Along with the Green Revolution, both the use of chemicals and the development in the breeding process have led to a dramatic increase in the productivity of producers. Many studies indicate that landraces are not used by many producers, especially

large producers, especially those following developments, due to reasons such as poor yield, and that these productions remain in restricted areas [15]. When looking at the factors affecting the use of landraces; the demographic, social and economic factors of the producers as well as the geographical structure of the place where the production takes place affects. Both studies conducted both in Turkey in the international arena, it is stated that the probability of reaching such landraces increases under difficult geographical conditions in rural areas remote from the main centers [15]. For this reason, some demographic and geographical variables belonging to the producers of DBL production in the Middle Kızılırmak Valley where the study is conducted are given in Table 2. Table 2 shows that DBL producers are over 50 years old, education is low (have more than 85% primary and below education levels) and household width is about 4 people. In particular, the low number of households can create a negative production pressure for products based on human labor, such as dried beans. In recent years, the production of the dry beans has decreased due to the fact that the dry bean is based on human labor during the harvest and the production of chickpeas, which are suitable for machine-harvesting, is increasing.

Another variable that is examined under the demographic characteristics is the social security situation of the producers. As seen in Table 2, only 37.14% of producers make farming as a profession. The rest of the group operates in other occupational groups (workers, civil servants, trades, etc.) besides farming. When the geographical characteristics of the producers' locations are examined, it is determined that the producers live at an average altitude of 1,155 m (Table 2). If we call the geographical area for 1,200 m and above of the Central Anatolia Region as mountainous area [29], it is determined that 42.14% of the producers live in these mountainous areas above 1,200 m. Another important factor in deciding landraces production is the distance from the main settlements. According to the results of the research, producers indicate that DBL productions are usually made in areas remote from the main centers [15] along with the average distance of DBL producers to the nearest district center İs calculated as 16.27 km and the distance to the nearest provincial center is 71.96 km (Table 2).

TABLE 1
Variables for Factor Analyses

	Production Behaviours (PB) of Dry Bean Landrace (DBL) Producers		Genetic Resources-Landrace Perception (GRLP) of DBL Producers
1	Be the first to adopt new technologies.	1	Landraces have been replaced by commercial varieties in many cases
2	Consult with the people aroun, search and then innovate.	2	The productivity of landraces is decreasing day by day
3	Investigate the development of all kinds of agriculture and animal husbandry.	3	In the disappearance of landraces in the region, too many trade-type inflows to the market/region are effective
4	Take care to participate in all kinds of activities related to agriculture and animal husbandry.	4	Year by year produced landraces began to change color, type and shape.
5	Also give information to others about the techniques learned or practiced.	5	No longer there are landraces seeds as before.
6	The manufacturers around, imitate what practiced.	6	Trying to plant the most productive variety instead of many kinds of bread.
7	First see the result of tried techniques in production then apply..	7	Landraces are more adaptive to the region than the developed varieties.
8	There are producers that taken as examples and followed their applications.	8	Farmer no longer has landraces.
9	Investigate where is the mistake when could not get the results that wanted in production.	9	It was difficult to reach the landraces seed.
10	Do not need to consult anyone other than myself.	10	There is tenderness in our village for the protection of landraces
11	Not afraid to try something new.	11	Do not think it will be too much trouble if landraces disappear
12	Consider whether applications are harmful to nature and then apply according to that	12	We lost the seeds of landraces. Planted seeds are not landraces.
		13	There is very special genes in landraces.
		14	These varieties must be state-protected. The farmer has nothing to do.
		15	The landraces that used to produce have disappeared. Do not think no one can find their seed anywhere.
		16	These varieties are produced only for home needs. Do not make money.
		17	Producing only landraces is not a good economic strategy. Must be produced with commercial varieties.
		18	As a farmer we are responsible for the protection of landraces.

TABLE 2
Some Statistical Data Belonging to DBLP and Their Settlement

Demographic Variables	Minimum	Maximum	Mean
Farmer Age	28.00	84.00	54.09
The Number of Household Member (Woman)	1.00	6.00	2.15
The Number of Household Member (Man)	1.00	5.00	2.02
The Number of Household Member (Total)	2.00	11.00	4.17
Education Level (%)			
Illiterate		0.71	
Literate		4.29	
Primary School		82.14	
Secondary School		9.29	
Vocational High School		1.43	
University		2.14	
Social Security (%)			
No Social Security		5.00	
Government Retirement Fund		4.29	
Social Security Authority (Worker)		29.29	
Social Security Organization (artisans and the self-employed)		17.14	
Social Security Organization (Farmers)		37.14	
Other		7.14	
Geographical Variables	**Minimum**	**Maximum**	**Mean**
Altitude (m)	625.00	1691.00	1155.25
Altitude (%)			
Highland (<1200 m)		42.14	
Lowland (>=1200 m)		57.86	
Distance to District Center (km)	0.00	55.00	16.27
Distance to City Center (km)	16.00	200.00	71.96

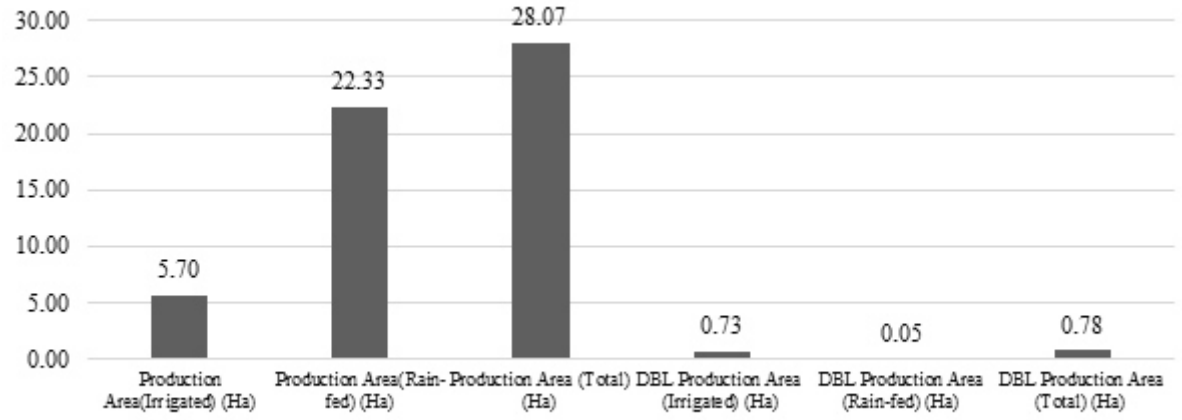


FIGURE 2
Agricultural Statistics Belonging to The Dry Bean Landrace (DBL) Producers

Some agricultural statistics of DBL producers are given in Figure 2. Dry bean production is mostly based on a watery production system, and in some regions it has been determined that the production is made as dry. The total amount of DBL producers processed in 2016 is 28.07 ha, of which 79.57% is composed of dry agricultural land. 2.79% of total production land is devoted to DBL production and 37.86% of this production is made in very small land like garden type and 62.14% of the production is made in field type land greater than 0.1 hectare.

The main purpose of the study is to demonstrate the production behavior and genetic resource / landraces perception of DBL producers and indicate the change in dry bean landraces production according to determined indices. For this reason, 2 different data collection tools were used in the research. In the first part, a scale of 12 items developed by the researchers was used to determine the production behavior of DBL producers. The KMO coefficient of the 12-item scale was 0.827; Bartlett Test X^2 = 531.45; $p < 0.01$ and factor analysis was deemed appropriate. As a result of the factor analysis, it was determined that the scale, on which item analysis and

Varimax rotation operation carried out, was collected in three dimensions. The four-factor scale, that eigenvalue is greater than 1, with 12 items accounts for 56.59% of the variance. The Cronbach Alfa reliability coefficient of the dimension which is related with the DBL producers' production behavior was calculated as 0.69 (Table 3).

The second dimension of the scale contains 18 items. This dimension identifies the safety perception of the producers for landraces and genetic resource perception. The KMO coefficient of the scale consisting of 20 items is 0.775; Bartlett Test X^2 = 965.03; $p < 0.01$ and factor analysis was deemed appropriate. As a result of the factor analysis, it was determined that the scale, on which item analysis and Varimax rotation operation carried out, was collected in four dimensions. The four-factor scale, that eigenvalue is greater than 1, with 20 items accounts for 62.44% of the variance. The Cronbach Alfa reliability coefficient of the landraces and genetic resource perception dimension was calculated as 0.69. Production behavior and genetic resources / landraces and environmental perception indexes were established by gathering the reduced factors obtained in the first and second dimensions (Table 3).

In the production behavior index, the fact that the index value goes from negative to positive suggests that the producers are more innovative, use more technology, take more risks, follow information and technology, and are environment-conscious producers in their practice. The index value created in this context was used both as a continuous variable and divided into quartiles to group DBL producers as traditionalists, middle traders, middle innovators and innovators. In addition, in the calculation of descriptive statistics for producers, producers classified as traditionalists and innovators according to median value.

In the genetic resource / landaces and environmental perception index, as the index value goes from positive to negative indicates that the producers think, that landraces are lost, that these seeds are difficult to find; and that the producers who produce landraces do not produce it as an economic gain but rather produce with the aim of meeting household needs. Manufacturers with high index value believe that a production strategy based only on landraces will not be economically feasible and that responsibility for the protection of landraces and genetic resources is much more on the state. In addition, these producers have a strong belief that the disappearance of landraces will not be a problem and that no special genes will be found in these varieties.

When Table 4 is examined, it is seen that the producers dealing with DBL production in the research area are divided into quartiles according to the production behavior index and variance analysis and descriptive statistical results are given accordingly. Firstly, it is seen that GRLP index values have statistically meaningful difference according to production behavior. Both Table 4 and Figure 3 are examined; it is seen that the group identified as "Conservative", which has a negative GRLP index value, has higher GRLP. It has been found that innovative producers, who are environmentally responsible and able to be leaders in the society, mostly do not adopt a production strategy based on local populations, often do not have enough knowledge about the value of landraces as genetic resources. Also these producers think these varieties are lost and the disappearance of such varieties does not constitute a major problem. These producers believe that the protection of genetic resources and is a more state policy.

The studies on environmental perception in Turkey are mostly about energy consumption, economic growth, development and climate change interactions. Many studies have been conducted within the framework of the Environmental Kuznet Hypothesis. The Environmental Kuznets Hypothesis implies that growth will not ultimately have a negative impact on the environment, on the contrary, growth will affect the environment positively. In Turkey, some studies [30, 31, 32, 33] on this subject suggest that along with the growth the default Environmental Kuznets Hypothesis has not taken place and continuation of environmental pollution.

TABLE 3
Kaiser-Mayer-Olkin (KMO) and Bartlett Test Results

Dry Bean Landrace Producers' Behavior Data Set		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.827
Bartlett's Test of Sphericity	Approx. Chi-Square	531.45
	df	66
	Sig.	0.000
Total Variance		56.59
Cronbach Alpha		0.69
The Producers' Genetic Resource/Landraces and Environement Perception Data Set		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.775
Bartlett's Test of Sphericity	Approx. Chi-Square	965.03
	df	153
	Sig.	0.000
Total Variance		62.44
Cronbach Alpha		0.69

TABLE 4
Descriptive Statistics and Variance Analyse by DBL Producers' Behaviors

Variables	Production Behavior Index Quartiles of DBLP Producers					F Value/Kruskall Wallis (K-W) Chi Square
	Conservative	Mid-Conservative	Mid-Innovative	Innovative	Average	
The Index of Genetic Resource-Landrace Perception	-0.63 ***a***	0.02 ***ab***	-0.19 ***ab***	0.80 ***b***	0.00	2.60*
Age (Year)	59.51 ***a***	54.80 ***ab***	50.57 ***b***	51.46 ***b***	54.09	5.38***
Education (Year)	5.51 ***a***	5.66 ***ab***	7.09 ***b***	6.57 ***ab***	6.21	(K-W) 8.51**
Distance to District Center (Km)	17.17	15.71	14.49	17.71	16.27	(K-W) 1.11
Altitude (m)	1213.97 ***a***	1197.29 ***a***	1064.91 ***b***	1144.83 ***ab***	1155.25	2.65*
Total DBL Production Area (Ha)	0.35	0.44	1.16	1.18	0.78	(K-W) 4.47
Private Production Area (Ha)	10.16	9.91	14.96	16.00	12.76	(K-W) 6.73*
Irrigated Production Area (Ha)	3.83 ***a***	2.11 ***a***	5.98 ***ab***	10.88 ***b***	5.70	(K-W) 22.54***
Share of DBL Prod. Area in Irrigated Prod. Area (%)	9.18	20.49	19.36	10.89	13.72	
Total Production Area (Inc. Rented and Sharecropping) (Ha)	21.24 ***a***	21.21 ***a***	38.91 ***b***	30.92 ***ab***	28.07	(K-W) 10.35**

* Statistically significant at 90% confidence level, ** Statistically significant at 95% confidence level, *** Statistically significant at 99% confidence level

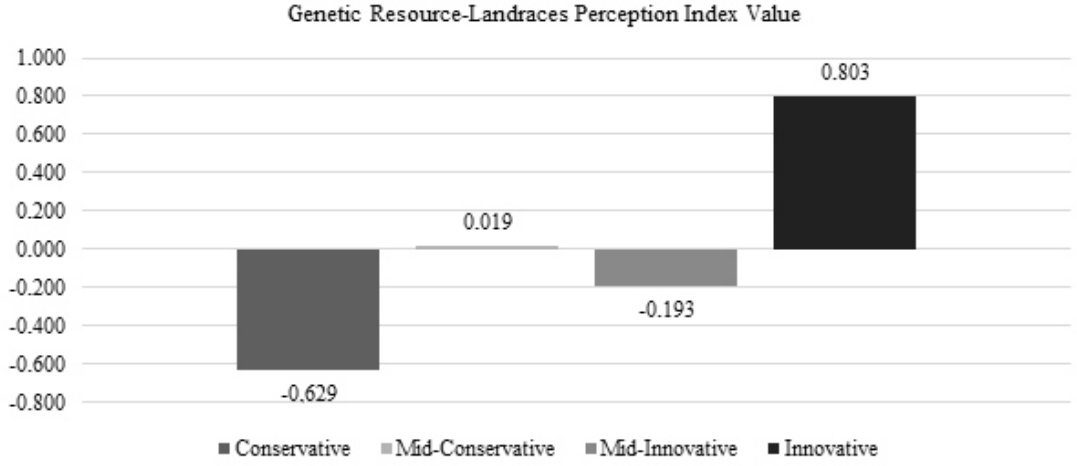


FIGURE 3
Genetic Resources-Landraces Perception Index Value by Production Behaviors of DBLP

The mass of high GRLP index value producer qualified as conservative, mostly represents high-age, low-educated, living in high-altitude areas in terms of the geographical location they live in and producing in smaller areas (Table 4). The group that gives more importance to the production of landraces and conservation of genetic resources, shows similarities with the studies of [34] and [35] who work in wheat landraces in Turkey.

CONCLUSION

Turkey is one of the rare countries in the world in terms of genetic diversity, with each passing day landraces disappear and places with high productivity varieties. Many factors affect the disappearance of local populations, especially the genetic resource and landraces perception of producers, as well as innovation and environmental considerations in production behavior. With this study, the relationship between genetic resources-landrace perception and production behavior of farmers growing the local population in Turkey has been demonstrated through DBL producers. As a result, it is seen that manufacturers with high genetic resource-landrace perception, who are still seeing landraces as an economic asset in Turkey and think that landraces must be protected and maintained by both the state and the producers, are the part of the traditional structure which is defined as conservative. Contrary to what is known, although innovative groups seem to be highly sensitive to the environment, they think that genetic resources do not create economic value.

Within the framework of the Environmental Kuznets Hypothesis studies in Turkey is reviewed based on genetic resources; it is seen that Turkey still do not evaluate the terminology of development and growth along with environmental protection, conservation of genetic resources, sustainable use of local potential and economic value creation. It is necessary that the group defined as Innovative should be guided to have more willing and pioneering attitudes towards the protection of genetic resources and existing values, and policies should be designed accordingly. In ensuring the sustainability of landraces during policy development, targeting the group identified as innovative will ensure that it has a more effective policy implementation outcome.

ACKNOWLEDGEMENTS

The data for the study was compiled from "Yerel Kuru Fasulye Popülasyonları Yetiştiren İşletmelerin Sosyo-Ekonomik Özellikleri-Socio-Economical Characterizations of Farmers Producing Dry Bean Landrace Population" Work Package of the Project "Orta Kızılırmak Vadisi Yerel Kuru Fasulye Popülâsyonlarının Morfolojik ve Moleküler Karakterizasyonu ile Kök Lezyon Nematoduna Karşı Dayanıklı Genotiplerin Belirlenmesi-Morphological and Molecular Characterization of Dry Bean Landraces in the Middle Kızılırmak Valley and Determination of Resistant Genotypes against Root Nematode" supported by the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM / 16 / AR-GE / 55) in Turkey.

REFERENCES

[1] IFPRI (2002) Sustainable Food Security for All by 2020. Proceedings of an International Conference, September 4-6, Bonn, Germany. International Food Policy Research Institute.

[2] Sharma, S., Upadhyaya, H., Roorkiwa, M., Varshney, R., Gowda, C. (2013) Chickpea. In: Singh, M., Upadhyaya, H., Bisht, I. (Eds.) Genetic and Genomic Resources of Grain Legume Improvement. Elsevier Inc. 81-111.

[3] Scholten, M., Maxted, N., Ford-Lloyd, B. (2006) UK National Inventory of Plant Genetic Resources for Food and Agriculture. Birmingham: School of Biosciences, University of Birmingham.

[4] Van de Wouw, M., Kik, C., Van Hintum, T., Van Treuren, R., Visser, B. (2009) Genetic erosion in crops: concept, research results and challenges. Plant Genetic Resources: Characterization and Utilization. 8(1), 1-15.

[5] Kan, M., Küçükçongar, M., Keser, M., Morgounov, A., Muminjanov, H., Özdemir, F., Qualset, C. (2015) Wheat Landraces in Farmers' Fields in Turkey: National Survey. Collection, and Conservation, 2009-2014. Rome: FAO.

[6] Kalkınma Bakanlığı (Ministry of Development) (2001) Eighth Five Year Development Plan, Plant Production Specialized Commission Report. Sub-Commission Report of Industrial Plants (in Turkish). Ankara. Available at: www3.kalkinma.gov.tr/DocObjects/Download/3033/oik656.pdf.

[7] Demir, İ. (1990) General Plant Breeding. E.Ü. Ziraat Fakültesi Yayınları No 496, E.Ü.Z. F. Ofset Atelyesi İzmir, 366p. (in Turkish).

[8] Altındal, D., Akgün, İ. (2015) Plant genetic resources and the status of cereals (in Turkish). Journal of Adnan Menderes University Agricultural Faculty. 12(1), 147-153.

[9] Sozen, O., Bozoglu, H. (2013) Bean bio-diversity in Artvin province. Journal of Field Crops, 22(1), 1-5.

[10] Sozen, O., Karadavut, U., Ozcelik, H., Bozoglu, H., Akcura, M. (2018) Genotype x environment interaction of some dry bean (*Phaseolus vulgaris* L.) genotypes. Legume Research. 41(2), 189-195.

[11] Şehirali, S., Özgen, M., Karagöz, A., Sürek, M., Adak, S., Güvenç, İ., Tan, A., Burak, M., Kaymak, H.Ç., Kenar, D. (2005) Conservation and use of plant genetic resources. TMMOB Ziraat Mühendisleri Odası VI. Teknik Kongresi. Cilt 1. Kozan Ofset, Ankara. 253-273. (in Turkish).

[12] Sozen, O., Bozoglu, H. (2013) Morphological variability of colored dry bean (*Phaseolus vulgaris* L.) germplasm from Artvin province. Greener Journal of Agricultural Science. 3(10), 697-701.

[13] Karataş, A., Büyükdinç, D., İpek, A., Yağcıoğlu, M., Sönmez, K., Ellialtıoğlu, Ş. (2017) An Overview to Morphological and Molecular Characterization Studies of Beans in Turkey. Turkish Journal of Scientific Review. 10(1), 16-27. (in Turkish).

[14] Toklu, F., Coyne, C., Asikli, S., Aydin, O., Karaköy, T., Özkan, H. (2016) Evaluation of Common Bean (*Phaseolus Vulgaris* L.) Collection for Agromorphological and Seed Mineral Concentrations. Fresen. Environ. Bull. 25, 1143-1152.

[15] Kan, M., Küçükçongar, M., Mourgounov, A., Keser, M., Özdemir, F., Muminjanov, H., Qualset, C. (2016) Wheat landraces production on farm level in Turkey: Who is growing in where? Pak. J. Agri. Sci. 53(1), 159-169.

[16] Kan, M., Kan, A., Gülçubuk, B., Peker, K., (2016) The Importance of Local Products in Regional Development Dynamics in Turkey (in Turkish). In: Peker, A.E. (Ed.) Regional Development. Çanakkale, Türkiye, 231-270.
[17] Falk, J. (1983) Field trips: A look at environmental effects on learning. Journal of Biological Education. 17, 137-142.
[18] Armstrong, J., Impara, J. (1991) The impact of an environmental education program on knowledge and attitude. The Journal of Environmental Education. 22, 36-40.
[19] Dilbirliği, E. (2007) Assesment of Strategies on Sustainable Use of Plant Biodiversity and Genetic Resources (in Turkish). Ankara: Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Peyzaj Mimarlığı Anabilim Dalı. M.Sc. dissertation (Unpublished).
[20] Uyanık, M., Kara, Ş., Gürbüz, B. (2012) Importance of biodiversity in terms of sustainable development. Türk Bilimsel Derlemeler Dergisi. 5(2), 125-127. (in Turkish).
[21] Sausa, E., Quintino, V., Palhas, J., Rodrigues, A., Teixeira, J. (2016) Can environmental education actions change public attitudes? an example using the pond habitat and associated biodiversity. PLoS One. 11(5), 1-13.
[22] Turkish Statistical Institute (TURKSTAT). (2018) Crop Production Statistics (2014-2017). Turkey. Retrieved June 23, 2018, from https://biruni.tuik.gov.tr/medas/?kn=92&locale=en.
[23] Özdamar, K. (1999) Statistical Data Analysis with Package Programs 2 (Multivariate Analysis). Eskişehir, Türkiye: Kaan Kitabevi. (in Turkish).
[24] Akgül, A., Çevik, O. (2005) Statistical Analysis Techniques in Business Management Applications in SPSS. Ankara, Türkiye: Mustafa Kitabevi. (in Turkish).
[25] Hair, F., Anderson, R., Tahtam, R., Black, W. (1992) Multivariate Data Analysis. Macmillan Publishing Company.
[26] Sharma, S. (1996) Applied Multivariate Techniques. New York: John Wiley Sonc Inc.
[27] Çömlekçi, N. (2001) Scientific Research Method and Statistical Significance Tests. Eskişehir: Bilim Teknik Yayınevi. (in Turkish).
[28] Kesici, T., Kocabaş, Z. (2007) Biostatistics (in Turkish). Ankara Üniversitesi Eczacılık Fakültesi Yayın No: 94, Ankara
[29] Oğuz, C., Ergun, H., Kan, M., Kan, A., Demiröz, E., Küçükçongar, M. (2016) The Poverty Phenomenon and Its Effect on Migration in Agriculture; Case Study of Konya. In: Efe, R., Cürebal, İ., Nyussupova, G., Atasoy, E. (eds.) Recent Researches in Interdisciplinary Sciences Sofia: St. Kliment Ohridski University Press. 29-52.
[30] Başar, S., Temurlenk M.S. (2007) Adapted Environmental Kuznets Curve: A case study on Turkey. Atatürk Üniversitesi İktisadi ve İdari Bilimler Dergisi. 21(1), 1-12. (in Turkish).
[31] Akbostancı, E., Türüt-Aşık, S., Tunç, G.İ. (2009) The relationship between income and environment in Turkey: Is there an Environmental Kuznets Curve? Energy Policy. 37(3), 861-867.
[32] Özturk, I., Acaravci, A. (2010) CO_2 emissions, energy consumption and economic growth in Turkey. Renewable and Sustainable Energy Reviews. 14(9), 3220-3225.
[33] Kocak, E. (2014) The validity of the Environmental Kuznets Curve hypothesis in Turkey: ARDL Bounds Test approach. İşletme ve İktisat Çalışmaları Dergisi. 2(3), 62-73. (in Turkish).
[34] Meng, E.C.H. (1997) Land Allocation Decisions and *In Situ* Conservation of Crop Genetic Resources: The Case of Wheat Landraces in Turkey. Ph.D. dissertation. University of California, Davis, California.
[35] Kruzic, T.J., Meng, E. (2006) Wheat Landrace Cultivation in Turkey: Household Land-use Determinants and Implications for On-Farm Conservation of Crop Genetic Resources. International Association of Agricultural Economists Conference. August 12-16, 2006, Gold Coast, Australia.

Received: **02.08.2018**
Accepted: **12.10.2018**

CORRESPONDING AUTHOR

Mustafa Kan
Kırşehir Ahi Evran University,
Agricultural Faculty,
Department of Agricultural Economics,
Kirsehir – Turkey

e-mail: mustafa.kan@ahievran.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9922-9930 Fresenius Environmental Bulletin

TREATMENT OF SIMULATED WASTEWATER SPIKED WITH PHENOLS USING TiO_2/GO/SiO_2 UNDER ENVIRONMENTAL CONDITIONS

Waed Alahmad[1,*], Hamed Alshammari[2], Obaid Aldosari[3], Rani Alsomadi[4]

[1] Chemistry Department, Faculty of Science, Ha'il University, P.O. Box 2440, 81451 Ha'il, Saudi Arabia
[2] Department of Chemistry, College of Science and Human Studies at Hautat Sudair, Majmaah University, Majmaah 11952, Saudi Arabia
[3] Chemistry Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University, Alkharj, 11942, Saudi Arabia
[4] Physics Department, Faculty of Science, Ha'il University, P.O. Box 2440, 81451, Hail, Saudi Arabia

ABSTRACT

The degradation of bisphenol A, 4-chlorophenol and phenol were investigated, using synthesized photocatalysts, were it prepared by sonication and a sol-gel methodology. The catalyst indicated as I; TiO_2: GO, II; TiO_2: GO: SiO_2 (50%) and III; TiO_2: GO: SiO_2 (75%). The irradiation interval was seven hours at solar radiation energy 6.35- 5.00 kWh/m^2/day. Degradation measurement conducted by valid HPLC method. The degradation activity was in order catalyst I > III > II catalyst. TiO_2 modified with GO and SiO_2 for enhancing surface area, increasing Vis light absorption, reuse, and handling. The modification was confirmed by X-ray diffraction, FTIR, scanning electron microscope and X-ray photoelectron spectroscopy analysis. Catalyst I was 87% degradation, the lowest degradation 55% for II. The prepared catalyst is more easily handled in the degradation process than TiO_2 anatase, due to their packing and crystallinity, making the filtration and reuse simpler.

KEYWORDS:
Bisphenol A., 4-Chlorophenol, Aquatic system, Solar irradiation, Photodegradation, Wastewater.

INTRODUCTION

Phenol and its derivatives are among the numerous organic pollutants discharged into the environment, poses a serious threat to the ecosystems, due to solubility in water, toxicity, endocrine disrupting abilities and carcinogenic behavior. Phenols released to the environment through some industrial wastewater including petroleum refining, paper manufacturing, coal, and others [1-4]. Degradation of phenols takes place by several methods; including adsorption, biological treatment, liquid membrane, ion exchanges and advanced oxidation processes (AOPs) [3]. Heterogeneous photo-catalytic oxidation, one of the AOPs, considered to be an energy efficient method when it joins with sunlight, and as green technology, where complete mineralization of organic contaminants take place [1-4].

Photocatalytic process via semiconductors is regarded as appropriate treatment methodology for the removal of organic pollutants. TiO_2 is the most used semiconductor photocatalyst, due to its high stability, low environmental effect, strong oxidization power, and availability with low price [5, 6].

The photocatalytic process started when the catalyst is irradiated by light (UV or Vis), reaching or exceeding its bandgap energy (the energy difference between the valence band and the conduction band), the next step hydroxyl radicals formed, with its high oxidation ability the degradation process is carried out. However, as scientific research there are some limitations have been observed for TiO_2, the large bandgap which lies in the UV range limits the use in solar light, the recombination (***e-h*** disappearance), and the poor selection for the pollutant [7, 8].

The photocatalytic performance of TiO_2 is currently improved by conventional technologies, including doped (metal ions or nonmetallic elements), and coupling with other semiconductor enhancing the surface area. Graphene and its derivatives are a good candidate for the improvement of TiO_2 activity with its remarkable properties [7-10].

These properties include large surface area and high electronic conductivity which enhancing both charge transportation and charge separation. Also, the hydroxyl groups at the edges functioned as hydrophilic groups (favorable reactive sites for guest materials). Furthermore, the addition of graphene or graphene oxide into TiO_2 quashes the recombination of the photogenerated *e-h*, while the carbon in the co-doped with Ti enhances broad light absorption [7, 8, 11].

The work is aimed to study the proper molar ratios of Ti, GO and Si as oxides in achieving a photocatalyst with the optimized advantages of the three oxide elements. Phenols herein are taken as an example of an aquatic pollutant, where its degradation measured on HPLC. Also, the degradation was under environmental conditions.

EXPERIMENTAL

Materials and Chemicals. Graphene oxide (GO: 15-20 sheets with 4-10% edge-oxidized) from Sigma-Aldrich. Titanium oxide anatase (TiO_2), Tetraethyl orthosilicate (TEOS), Phenol, Bisphenol A, 4-Chlorophenol, Methanol, Acetonitrile, Ethanol, Acetonitrile and *o*-phosphoric acid (H_3PO_4), all HPLC grade, PS/DVB 1000 mg/6ml, RP C18 HPLC column, were purchased from Sigma-Aldrich. Pressure batch ultrasonic reactor (UIP, Hielscher Ultrasonics). UltiMate 3000 HPLC Systems from thermo fisher, with chromeleon CDS software.

TABLE 1
Weight/weight % for the three prepared catalyzes I, II, III from the total weight of the catalyst

Catalyst	GO %	Ti %	Si %
I	1.96	98	0
II	1.29	45.5	50
III	0.966	24.15	74.9

Synthesis of catalyst. First, 1 mg GO dispersed in 20 ml Ethanol by sonication (sonicating with a probe sonicator for 20 min impulse mode with 2s on and 5s off at 35% amplitude) until it reaches a saturated concentration. TiO_2 anatase with TEOS is added with ratios (Table 1). Then 60 ml DW added (an immediate condensation take place), then slow stirring at 70℃ overnight, then dry at 150℃, calcination at 500 C for four h.

Characterization of materials. Phase identification of the catalysts was conducted using X-ray diffraction spectrum (XRD). X-ray photoelectron spectroscopy (XPS). Scanning electron microscopy with field emission (SEM-EDS; JSM-7800F, JEOL, USA). Fourier transform infrared spectrophotometry (FTIR; Perkin-Elmer 843) the measurement range was from 500–4000 cm^{-1}, with a 4 cm^{-1} resolution and 0.475 cm^{-1}/s scanning speed, the applied technique was attenuated total reflectance (ATR) with smart i-TR.

HPLC Method validation and conditions. Preparation of standard calibration graphs for the three standards phenol, bisphenol A, and 4-chlorophenol, was according to the valid analytical procedure. Different concentrations of phenols were used to establish the calibration curve. They were linear with the concentration range of the phenols target. The standard mixture was prepared in 1000ppm concentration and stored at 4°C for one month. The chromatographic identification and quantification were performed for a group of phenols; 4-Chlorophenol, Bisphenol A, and Phenol, table 2 give the HPLC conditions.

Sample preparation and extraction. 100 ml DW was spiked with a mixture of the phenols (with the desired concentration). Table 3 shows the calibration range for each, prior extraction the samples were acidified to pH= 2 with 0.1M H_3PO_4, then extracted on the SPE columns (conditioning; 3 ml acetonitrile, 3 ml methanol, 3 ml DW pH=2), elution in 3 ml methanol.

Procedure and Analysis. For establishing adsorption equilibrium the spiked samples were placed in the dark for 30 min, then the degradation under sunlight toke place for 7 hours, the temperature ranged from 30-35℃. At an interval of 1h, an aliquot of 10ml was collected, extracted then the degradation was measured on HPLC. Catalyst loading was 0.1, 0.2 and 0.3 g / 100 ml (to evaluate the impact of catalyst dosage on photocatalytic efficiency), experiments were performed at initial phenol concentration from 50 ppm for 4-Chlorophenol, Bisphenol A and 5 ppm for Phenol. The pH= 6 for the solutions.

TABLE 2
The determination of phenolic compounds conditions on HPLC.

Column	C18 (4.6 mm× 250 mm) , particle size 5 µm diameter, injection volume 10 µl
Mobile phase	A: Water with (0.1% o-phosphoric acid) B: Acetonitrile with (0.1% o-phosphoric acid)
Detection	280 nm, DAD

TABLE 3
Calibration data for Bisphenol, 4-Chlorophenol, and Phenol, retention times (t_r), regression, calibration range, detection limits and recovery.

Compound	Regression equation	t_R (min)	Calibration range	LOD	Spike level	Recovery %	RSD (n=3, %)
Bisphenol A	y = 0.315x - 0.0166 R^2 = 0.9993	7.798	50, 25, 10, 5, 1 ppm	0.1 ppm	25 5	87% 98%	1.6 5.7
4-Chlorophenol	y = 0.2841x + 0.0366 R^2 = 0.9998	8.467	50, 25, 10, 5, 1 ppm	0.1 ppm	25 5	92% 83%	1.7 5.5
Phenol	y = 0.2015x + 0.0141 R^2 = 0.9981	8.999	5, 2.5, 1, 0.5, 0.1 ppm	0.05 ppm	2.5 0.5	86% 92%	2.7 3.7

Degradation measurements. Degradation efficiency of the phenols was measured according to Eq. 1, where C*o* initial concentration, C*i* sample concentration:

$$Degradation\ efficiency = (Co\text{-}Ci/Co)\times 100\% \quad \text{Eq. 1}$$

For the catalysts I, II, and III the degradation efficiency took in dark conditions and under sunlight exposure, as a comparison commercial TiO_2 anatase was also investigated. The photodegradation of phenol was studied using a batch of 200 ml beakers with spiked samples, slow stirring and under direct sunlight. The concentrations were calculated by a calibration curve. Solar irradiance directly (south-west 22.5° from the south) in September and October is 6.35- 5.00 kWh/m^2/day onto a horizontal surface [12].

RESULTS AND DISCUSSION

Characteristics of the Catalyst. The XRD patterns of TiO_2 anatase and the catalyst I, II and III are shown in Fig.1. The results show that the major phase of the photocatalyst is anatase phase. The pattern indices the phases of (101), (004), (200), (105), (211), and (204) reflection of anatase TiO_2 characteristic peaks at 2θ of 25.3◦, 37.8◦, 48.0◦, 53.9◦, 55.1◦, and 62.7◦, respectively. Silicon is introduced to the catalyst in the liquid phase (TEOS) there is no characteristic peak for Si found, could be for the high crystallinity of anatase and the high intensity of the peaks. Even Though in EDS the Si and C are found [7, 13-15].

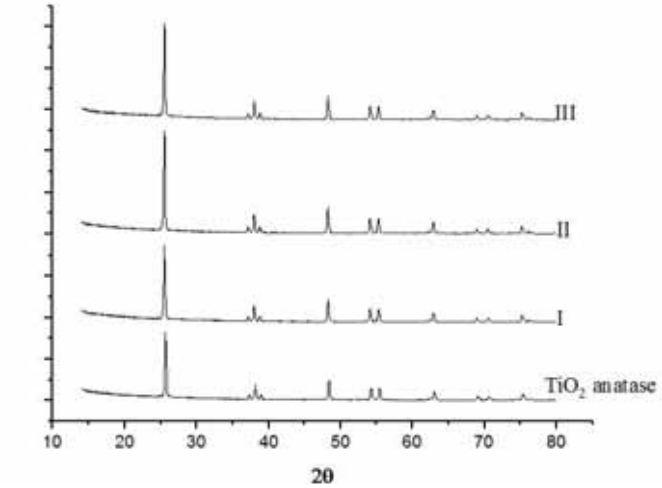


FIGURE 1
XRD pattern for TiO_2 anatase, prepared catalyst I, II, III.

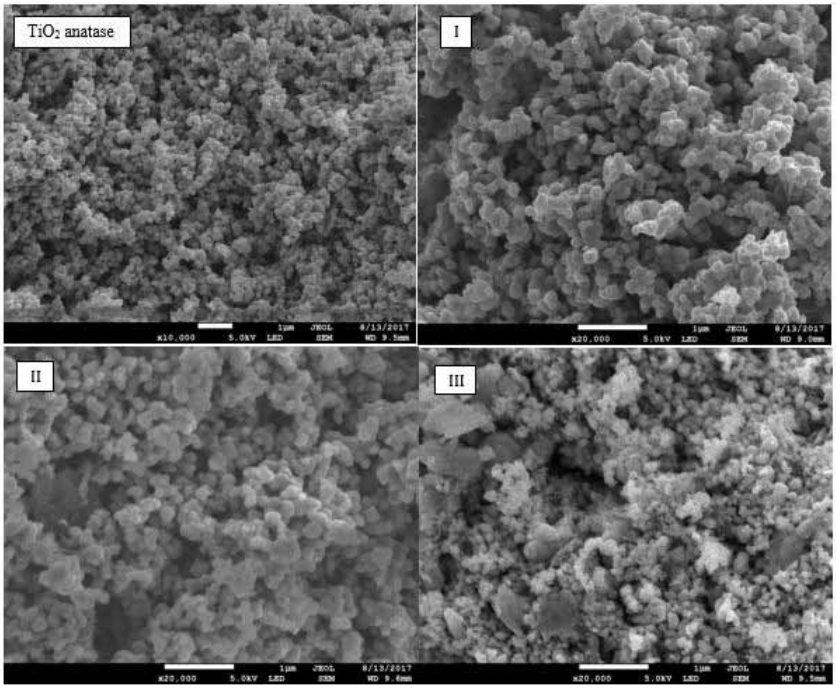


FIGURE 2
SEM image for the catalyst I, II, III and TiO_2 anatase.

Morphology characterization. The morphology of the catalyst I, II, and III was investigated by the SEM in Fig.1. The particles of the catalyst tended to agglomerate [5]. The morphology of the three catalyzes vary between TiO_2 anatase shape and GO as the amount of the SiO_2 increase, TiO_2 anatase has regular spherical morphology particles while the GO has sheet-like morphology [7].

The structure of GO sheet is seen in Fig. 2 and 3. I and II morphology are more containing to TiO_2 anatase spherical shape. Meanwhile, the catalyst III have more morphology of GO (Fig.2 and 3).

Fig.2 shows the EDS element mapping of Ti, Si, C, and O, confirming the formation of TiO_2, SiO_2 and GO. The atomic % of Ti, Si, and C in the EDS analysis close in ratio to the mole ratios in table 1. The EDS analysis results fit into the SEM images.

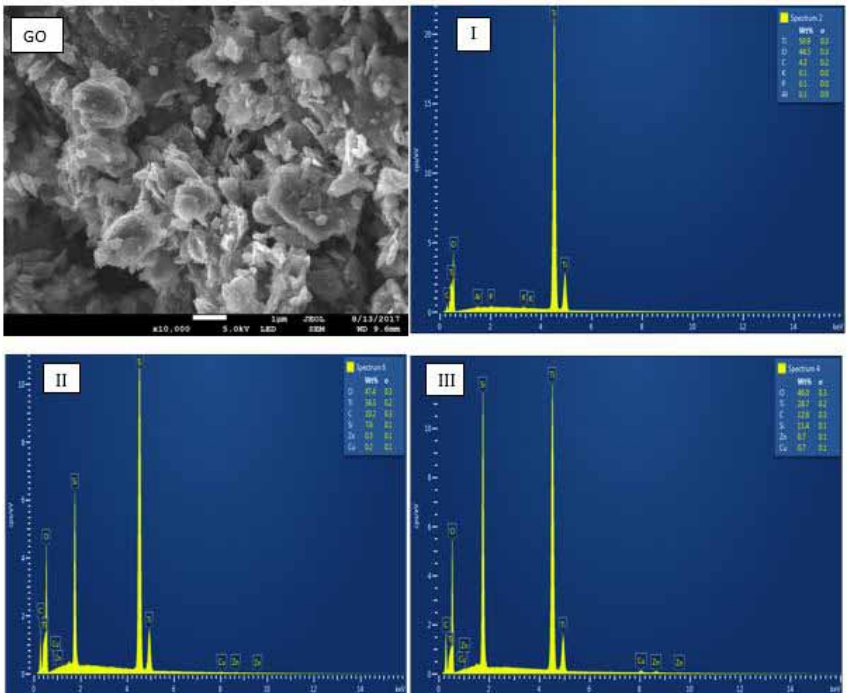


FIGURE 3
EDS for the catalyst I, II, III and GO SEM image.

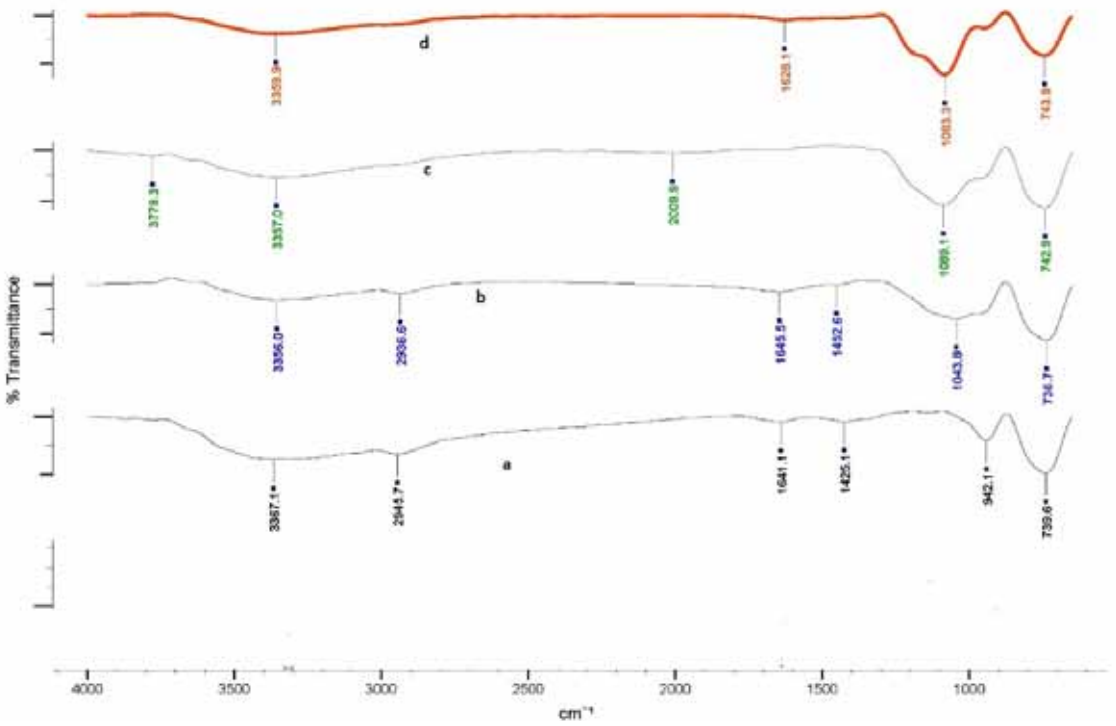


FIGURE 4
FTIR spectrum for a) TiO_2 anatase b) I c) II d) III

FTIR Analysis. FTIR analysis used to investigate the type of functional groups presented in the structure of catalyst, the peak at 736 -742 cm^{-1} assigned to Ti-O. The peak at 942 cm^{-1} corresponding to Ti-O-Ti bond is found in TiO_2 anatase (Fig. 4, a) in which the intensity decreases by adding GO and SiO_2 [6, 15].

There is a small broad peak around 950 cm^{-1} regions indicating the Ti-O-Si bonds. The absorption peak at 1083 cm^{-1} is assigned to symmetric and asymmetric O-Si stretching vibrations (Fig.4, b, c). After adding SiO_2 to the catalyst, a broad peak (1100-1300 cm^{-1}) appears which represents the breathing vibrations of -O- (epoxy groups) in our case it is found at 1220 cm^{-1}, and the stretching vibration of C-O groups at 1050 cm^{-1}. The stretching and breathing absorption for Si-O-C is located in this area as well. [16, 17] The broad peak at around 3356 cm^{-1} is assigned to the stretching mode characteristic of physical sorption water on oxide supports. Additionally, the peak at 740 cm^{-1} related to Ti-O-C vibration indicating the GO nanocomposites emerging to the TiO_2 bulk. GO, and Si is capable of binding to the TiO_2 surface via interaction of hydroxyl group of TiO_2 [16, 17].

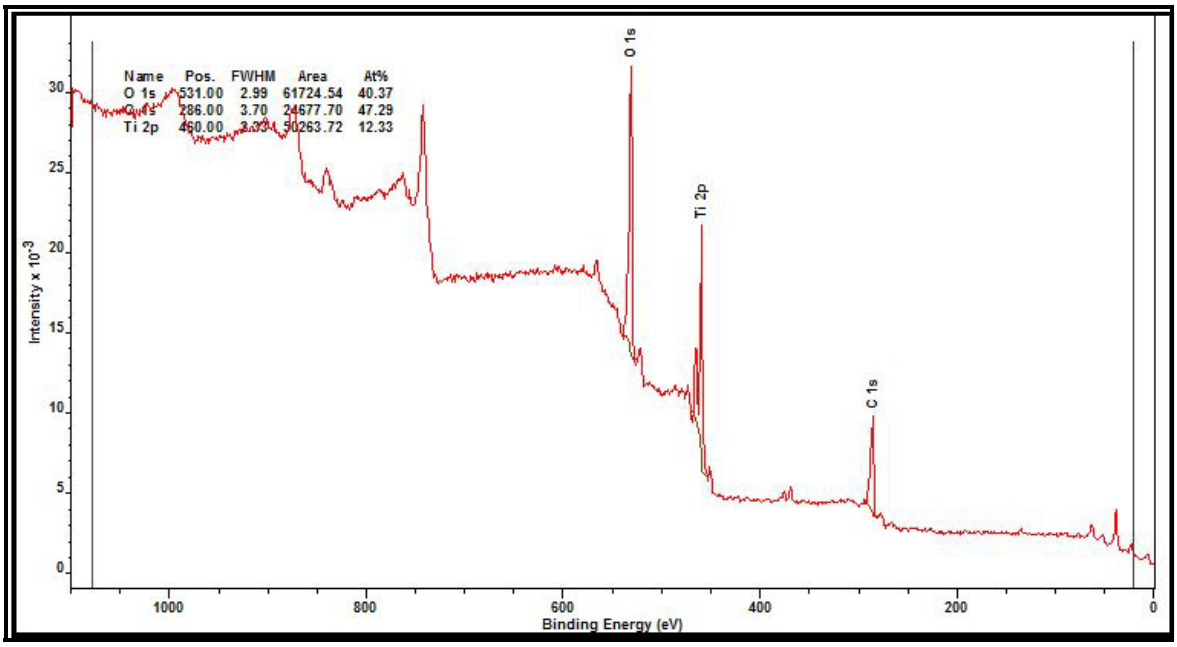


FIGURE 5
XPS spectrum of catalyst I, with peak analysis, binding energy, and atomic %.

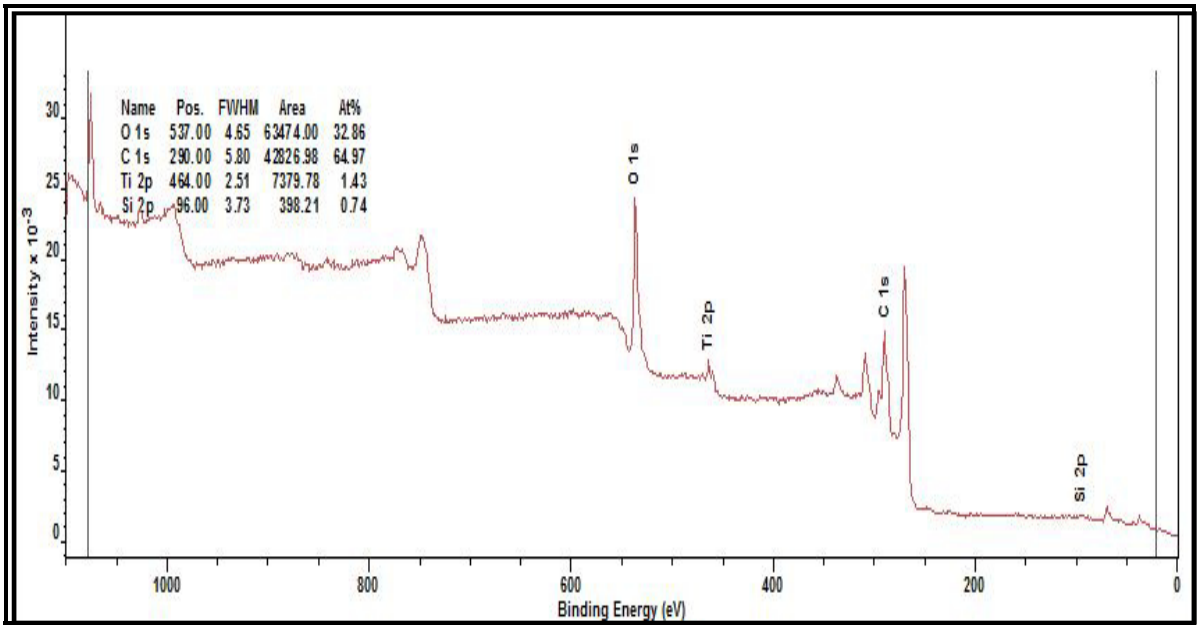


FIGURE 6
XPS spectrum of catalyst II, with peak analysis, binding energy, and atomic %.

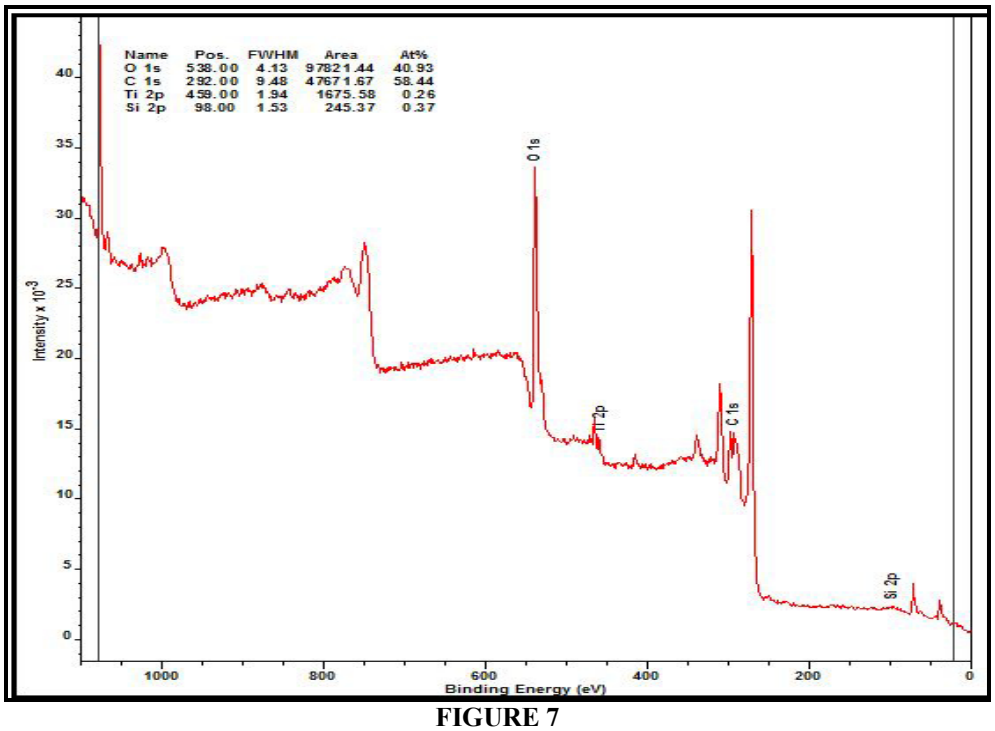


FIGURE 7
XPS spectrum of catalyst III, with peak analysis, binding energy, and atomic %.

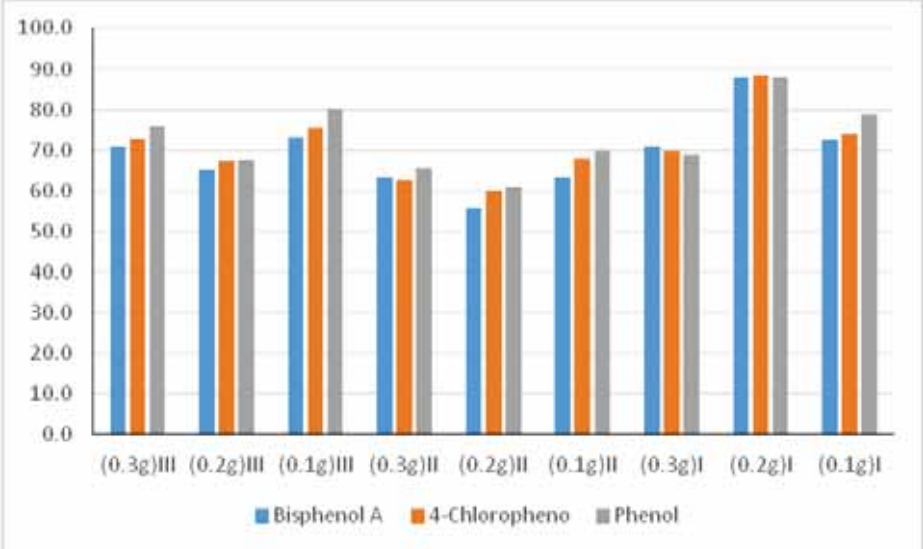


FIGURE 8
The Degradation % of bisphenol A, 4-chlorophenol, and phenol with (0.1, 0.2, 0.3 loads in the 100 ml spiked sample).

XPS analysis. The XPS spectra of wide-scan for the nanocomposites catalysts I, II and III are shown in Fig. 5, 6 and 7. The spectrum shows the signals of O ^{1}s at 531 eV, C ^{1}s at 286 eV, Ti ^{2}p at 454 eV and Si ^{2}p at 96 eV [18, 19]. The presence of the Ti ^{2}p signal in the survey spectrum validates the introduction of GO onto TiO_2. The signals at 284 eV and 286 eV represent the Ti-C and Ti-O-C bonding, the high-resolution of the C^{1}s signal indicate that the connection occurs through a covalent bond between GO and TiO_2 resulting in a uniform distribution of GO nanosheets over the TiO_2 nanoparticles [20, 21].

Photocatalytic Degradation of phenols, HPLC analysis. Phenols degradation was examined under the following conditions: 5-6 pH and 7 hours sunlight exposure. A comparison of the phenols degradation % of Catalyst I, II and III is illustrated in Table 2.

Fig. 8 represented the degradation % for the group of phenols under sunlight with the three prepared catalysts and compared to TiO_2 anatase. As shown, catalyst I was the most degradable %, followed by III, then catalyst II was the least degradable %. Catalyst I (TiO_2: GO) at 0.2 g amount load gave a high degradation %. Cruz et al. studied the

degradation of pesticides over GO-TiO_2 composite giving a higher photocatalytic activity compared to TiO_2 P25 [22-24].

Another study conducted by Lee and Yang also proved that the GO-TiO_2 composite gave a high removal of heavy metals from water matrix [25]. Furthermore, degradation of 99% of MB within 6 min over rGO-Fe_3O_4-TiO_2 nanocomposite was determined by Banerjee et al [26].

Catalyst III (TiO_2: GO: SiO_2) gave an excellent degradation % (65-77%) in the third position, SEM image indicated that the GO sheets spread more in the anatase as the % of SiO_2 increased. Catalyst II was the lowest among all even though the % of composition was almost 50% for both TiO_2 and SiO_2.

Experimentally, all samples were degraded at pH 5-6. First, the natural water pH lies between 8-5; giving the opportunity for no more farther modification of the pH (wastewater treatment or water treatment). Secondly, it was found in several studies that the highest degradation of phenols lies at pH 5-6, farther more the highest activity for TiO_2 as photocatalyst occurs at pH 5-6 [21-25]. The pH plays a central role in photocatalyst activity affecting the ionic states of the catalyst surface, the adsorption (interaction between the catalyst and the pollutant) either promoted or inhibited according to the ionic state [21]. The adsorption of phenol is favored at pH 5-6 due to the formation of H-bonding between phenols eventually increasing the photocatalytic activity [22, 23].

TiO_2 band gap lies in the UV (<385 nm) range, solar spectrum constitutes only about 3–5% of UV radiation and 50% are Vis light. Practically, this feature actively restricts the use of solar light as a light source for the photocatalysis purposes [25, 26].

Hydroxyl radicals control the photo-oxidation process using TiO_2 under UV light. Meanwhile, under visible light irradiation, other reactive species are formed ($O_2^{\bullet -}$ and $^1O_2$), which play a principal role in the photocatalytic process. Adding the GO to the TiO_2 creates new features for the catalyst; GO hindering the electron-hole recombination by accepting the photo-excited electrons, where electrons transfer from TiO_2 to GO (charge separation) [23]. The explanation of these features comes from the Ti–O–C bond, where it makes two band energy gaps (double absorption edges) [25-27].

Mechanism of Phenols photodegradation

$$PhOH\ or\ RPhOH \rightarrow\rightarrow\rightarrow OHPhOH\ (Para\ \&\ Ortho) + Dimers + OPhO$$

Photocatalytic initiation step by e- & h+ generation

$$HOOCCH2CH2COOH \rightarrow\rightarrow\rightarrow CO2 + H2O$$

Mineralization step for the phenols in aquatic sample

CONCLUSION

TiO_2 is conceders as a unique photocatalyst, the removal of its limitations was by various methods, and one of them is the modification by adding other oxides to its bulk. Herein the addition of GO and SiO_2 were used, I; TiO_2: GO, II; TiO_2: GO: SiO_2 (50%) and III; TiO_2: GO: SiO_2 (75%). The outcome of the study gave more easily used catalyst than bare TiO_2 as it is very powder meanwhile the prepared catalyst was highly impacted and crystalline. Three types of phenols were taken as an example of water pollutants; their degradation ranged from 55% - 90%.

An optimized condition for the photocatalytic process is needed to reach the highest removal of any pollutant. The removal of the phenols (Bisphenol A, 4-chlorophenol, and phenol) was in a very good ratio but the catalyst farther modification needed, if we take in mind an application in the wastewater treatment plants. Fixation of the catalyst on the surface or membranes can achieve a better use. The degradation takes place under sunlight at pH 5-6 with several loading, the highest degradation % I>III>II. Silicon insertion to the TiO_2: GO composite enhances the GO spreading in the titanium matrix, where SEM image shows that as Si% increased the GO sheet was more noticeable. As a result, the degradation of phenols % increased.

The unique bond of Ti-O-C as a photocatalyst provided an opportunity to consider the sunlight as a source of photoinitiation process. Absorb higher solar spectra, promote efficient charge transfer, and minimize recombination, high photonic efficiency, self-photo charge ability, and regeneration. However, reusing and uniform illumination of that is the ultimate goal to reach in synthesizing a photocatalyst. Photocatalysis has emerged as a promising clean, advanced oxidation technology, which could address the ever-increasing global concerns for environmental pollution reduction based on the utilization of solar and UV energy.

ACKNOWLEDGEMENTS

This work was supported by grants from University of Hail UOH, (Project ID: SC150302).

The author declares that there is no conflict of interest regarding the publication of this paper.

REFERENCES

[1] Renuka, M.K. and Gayathri, V. (2018) UV/ Solar light induced photocatalytic degradation of phenols and dyes by Fe (PS-BBP) Cl_3. Journal of Photochemistry and Photobiology A. 353, 477-487.

[2] Reszczynska, J., Esteban, D.A., Gazda, M. and Zaleska, A. (2014) Pr-doped TiO_2. The effect of metal content on photocatalytic activity. Physicochemical Problems of Mineral Processing. 50(2), 515-524.

[3] Nickheslat, A., Amin, M., Izanloo, H., Fatehizadeh, A. and Mousavi, S. (2013) Phenol Photocatalytic Degradation by Advanced Oxidation Process under Ultraviolet Radiation Using Titanium Dioxide. Journal of Environmental and Public Health. 2013, 1-10.

[4] Udom, I., Myers, P., Ram, M., Hepp, A., Archibong, E., Stefanakos, E. and Goswami, D. (2014) Optimization of Photocatalytic Degradation of Phenol Using Simple Photocatalytic Reactor. American Journal of Analytical Chemistry. 5(11), 743-750.

[5] Laia, C., Wanga, M., Zenga, G., Liua, Y., Huanga, D., Zhanga, C., Wanga, R., Xua, P., Chenga, M., Huanga, C., Wua, H. and Qin, L. (2016) Synthesis of surface molecular imprinted TiO_2/graphene photocatalyst and its highly efficient photocatalytic degradation of target pollutant under visible light irradiation. Applied Surface Science. 390, 368-376.

[6] Xu, H., Ding, M., Liu, S., Li, Y., Shen, Z. and Wang, K. (2017) Preparation and characterization of novel polysulphone hybrid ultrafiltration membranes blended with N-doped GO/TiO_2 nanocomposites. Polymer. 117, 198-207.

[7] Rahimi, R., Zargari, S., Yousefi, A., Yaghoubi Berijani, M., Ghaffarinejad, A. and Morsali, A. (2015) Visible light photocatalytic disinfection of E.coli with TiO_2–graphene nanocomposite sensitized with tetrakis (4-carboxyphenyl) porphyrin. Applied Surface Science. 355, 1098-1106.

[8] Cole, M., Lindvall, N. and Yurgens, A. (2012) Noncatalytic chemical vapor deposition of graphene on high-temperature substrates for transparent electrodes. Applied Physics Letters. 100(2), 022102-022110.

[9] Štengl, V., Henych, J., Vomáčka, P. and Slušná, M. (2013) Doping of TiO_2-GO and TiO_2-rGO with Noble Metals: Synthesis, Characterization and Photocatalytic Performance for Azo Dye Discoloration. Photochemistry and Photobiology. 89(5), 1038- 1046.

[10] Štengl, V., Bakardjieva, S., Grygar, T., Bludská, J. and Kormunda, M. (2013) TiO_2-graphene oxide nanocomposite as advanced photocatalytic materials. Chemistry Central Journal. 7(1), 41-52.

[11] Alizadeha, B., Delnavaza, M. and Shakeri, A. (2018) Removal of Cd (II) and phenol using novel cross-linked magnetic EDTA/ chitosan/TiO_2 nanocomposite. Carbohydrate Polymers. 181, 675-683.

[12] Solar Irradiance Calculator (2018) *Solar Electricity Handbook*, [Online]. Available: http://www.solarelectricityhandbook.com/solar-irradiance.html. [Accessed: 03- Feb- 2018].

[13] Mohamed, M., Eid, S. and El-Etre, A. (2017) Methanol photo-oxidation at graphene and carbon nanotubes modified TiO_2 nanosheets electrocatalysts. Journal of Photochemistry and Photobiology A. 338, 37-48.

[14] Kim, S., Ali, I. and Kim, J. (2017) Phenol degradation using an anodized graphene-doped TiO_2 nanotube composite under visible light. Applied Surface Science. (In press).

[15] Yaseen, M., Shah, Z., Veses, R., Dias, S., Lima, E., Reis, G., Vaghetti, J., Alencar, W. and Mehmood, K. (2017) Photocatalytic Studies of TiO_2/SiO_2 Nanocomposite Xerogels. Journal of Analytical and Bioanalytical Techniques. 8, 384-388.

[16] Sun, S., Ding, H. and Zhou, H. (2017) Preparation of TiO_2-coated barite composite pigments by the hydrophobic aggregation method and their structure and properties. Scientific Reports. 7, 1-12.

[17] Morsy, F.A., El-Sherbiny, S., Samir, M. and Fouad, O.A. (2016) Application of nanostructured titanium dioxide pigments in paper coating: a comparison between prepared and commercially available ones. Journal of Coatings Technology and Research. 13, 306-312.

[18] Haubner, K., Murawski, J., Olk, P., Eng, L., Ziegler, C., Adolphi, B. and Jaehne, E. (2010) The Route to Functional Graphene Oxide. Chemical Physics and physical Chemistry. 11(10), 2131-2141.

[19] Vasilyeva, M., Rudnev, V., Zvereva, A., Ustinov, A., Arefieva, O., Kuryavyi, V. and Zverev, G. (2018) FeOx, SiO2, TiO2/Ti composites prepared using plasma electrolytic oxidation as photo-Fenton-like catalysts for phenol degradation. Journal of Photochemistry and Photobiology A. 356, 38-45.

[20] Li, Z., Qi, M., Tu, C., Wang, W., Chen, J., and Wang, A. (2017) Highly efficient removal of chlorotetracycline from aqueous solution using graphene oxide/TiO2 composite: Properties and mechanism. Applied Surface Science. 425, 765-775.

[21] Choquette-Labbé, M., Shewa, W., Lalman, J. and Shanmugam, S. (2014) Photocatalytic Degradation of Phenol and Phenol Derivatives Using a Nano-TiO_2 Catalyst: Integrating Quantitative and Qualitative Factors Using Response Surface Methodology. Water. 6(12), 1785-1806.

[22] Dougna, A., Gombert, B., Kodom, T., Djaneye-Boundjou, G., Boukari, S., Leitner, N. and Bawa, L. (2015) Photocatalytic removal of phenol using titanium dioxide deposited on different substrates: Effect of inorganic oxidants. Journal of Photochemistry and Photobiology A. 305, 67-77.
[23] Umar, M. and Abdul Aziz, H. (2013) Photocatalytic Degradation of Organic Pollutants in Water. Publisher InTech Open Access Publisher. 195-208.
[24] Cruz, M., Gomez, C., Duran-Valle, C., Pastrana-Martínez, L., Faria, J., Silva, A., Faraldos, M. and Bahamonde, A. (2017) Bare TiO_2 and graphene oxide TiO2 photocatalysts on the degradation of selected pesticides and influence of the water matrix. Applied Surface Science. 416, 1013-1021.
[25] Lee, Y. and Yang, J. (2012) Self-assembled flower-like TiO2 on exfoliated graphite oxide for heavy metal removal. journal of Industrial and Engineering Chemistry. 18(3), 1178-1185.
[26] Banerjee, S., Benjwal, P., Singh, M., and Kar, K. (2018) Graphene oxide (rGO)-metal oxide (TiO2/Fe3O4) based nanocomposites for the removal of methylene blue. Applied Surface Science. 439, 560- 568.
[27] Umrao, S., Abraham, S., Theil, F., Pandey, S., Ciobota, V., Shukla, P., Rupp, C., Chakraborty, S., Ahuja, R., Popp, J., Dietzek, B. and Srivastava, A. (2014) A possible mechanism for the emergence of an additional band gap due to a Ti–O–C bond in the TiO_2–graphene hybrid system for enhanced photodegradation of methylene blue under visible light. RSC Advances. 4(104), 59890-59901.

Received: **03.08.2018**
Accepted: **15.10.2018**

CORRESPONDING AUTHOR

Waed Alahmad
Chemistry Department,
Faculty of Science,
Ha'il University,
P.O.Box 2440,
81451 Ha'il – Saudi Arabia

e-mail: w.alahmad@uoh.edu.sa

© by PSP Volume 27 – No. 12B/2018 pages 9931-9935 Fresenius Environmental Bulletin

PERSISTENT ORGANIC PESTICIDE RESIDUES IN HUMAN MILK SAMPLES FROM SOUTHERN GOVERNORATES OF JORDAN IN 2016/2017

Tawfiq M Al-Antary[1], Mahmoud A Alawi[2,*], Mohammad Abu Othman[3], Nizar Haddad[4]

[1]Department of Plant Protection, School of Agriculture, The University of Jordan, Amman-11942, Jordan
[2]Department of Chemistry, School of Science, The University of Jordan, Amman-11942, Jordan
[3]Royal Scientific Society, Amman-11942, Jordan
[4]Ministry of Environment, Amman-11942, Jordan

ABSTRACT

One hundred and twenty samples of human breast milk were collected from five governorates located south of Jordan in 2016 and 2017. This study was conducted to monitor pesticides residues of organochlorines. These organochlorines persistent pollutants have appeared in the analyzed mother milk samples in low concentrations despite the Ministry of Agriculture of Jordan have banned their uses since forty years ago. However, the results indicated that 18.33% of the analyzed samples contained *p,p'*-DDE, 2.5% *p,p'*-DDT, 1.67% *o,p'*-DDE, 3.33% dieldrin and 0.83% heptachlor. The highest concentration was 3.0 mg/kg fat milk of *p,p'*-DDE in samples of Aqaba, but the highest percent of positive samples was 27.7% in Tafila. The analyzed samples did not contain endrin, HCB, *α*-HCH, *β*-HCH, *γ*-HCH, α-endosulfan, *o,p'*-DDD, *o,p'*-DDT and *p,p'*-DDD. There were a decrease in average of HCH's in the analyzed samples collected from the five districts compared to previous study, but slightly increase in DDT and cyclodiens groups. It is recommended to continue monitoring of pesticide residues in human milk and other environmental components in Jordan.

KEYWORDS:
Human milk, Persistent pesticides, Residues, Monitoring, Jordan.

INTRODUCTION

Orgnochlorine pesticides are occurred in most environmental components due to their persistence and being highly soluble in lipids. They accumulate gradually in fatty organs in the biological systems through the food chains introduced in the environment [1-5]. They redistributed and accumulate in tissues with high fat content such as adipose tissues, the liver, kidney, brain and breasts [2, 3, 6, 7]. New born and infants might exposed to these persistent organochlorine pollutants (POPs) through placenta and breast feeding [5, 8 – 10]. Parental exposure to these POPs might induce lower birth weight, neurodevelopment delay, disturbance of thyroid hormone status [3, 5, 8, 11 – 13].

Unfortunately some of these POPs are still in use particularly DDT in some Asian and African countries to control Anopheles mosquito to reduce malaria cases [2, 3, 14]. In the last fifteen years, it was observed that cancer cases have been increased noticeably due to several reasons including the use of these POPs [5, 9, 10, 15]. Therefore in Jordan, the Ministry of Environment has asked the Royal Scientific Society to monitor the occurrence of organochlorine residues in breast milk of delivered women in Jordan.

It is the aim of this study to monitor organochlorine residues particularly DDT and its metabolites, HCHs, HCB and cyclodiens members in human milk of delivered women in five governorates of southern Jordan in 2016 and 2017, to minimize the misuse of pesticides and to develop regulations and registration of organochlorines.

MATERIALS AND METHODS

Sampling Locations. One hundred and twenty women breast milk samples were collected in 2016 and 2017 from the five southern governorates of Jordan. The number of samples collected from each governorate was proportional to the population density [3, 16]. These were 24 samples from Ma'an, 30 samples from Karak, 18 samples from Tafila, 24 samples from Aqaba and 24 samples from southern Jordan Valley. In these five districts lives about 9.4% of the total population of Jordan. Also, within these districts, lies part of the Jordan Valley with the highest agricultural activities and use of pesticides.

Sampling. In cooperation with the Ministry of Health/ "Mother and Child" centres, 25 – 30 mL of mother's milk were taken from each volunteer woman and placed in a 50-mL well cleaned and dry glass bottle, transferred in cooling boxes to the

laboratory, and stored at -20ºC until analysis. Data concerning age, body weight, delivery number, last delivery date, fatty food intake, exposure to pesticides and sampling date for each lady were gathered in a prepared questionnaire [2, 16].

Standards, Solvents, Chemicals and Gases. The used solvents acetone, dichloromethane, petroleum ether (40 – 60 ºC) was all of the p.a. quality, whereas n-hexane used was of GC-quality. Standards of the individual chlorinated pesticides were of purity between 99.5% - 99.9% and purchased from Dr. Ehrenstorfer GmbH (Augsburg/ Germany). Anhydrous sodium sulphate (p.a. quality) was heated at 550ºC for 2 h. Florisil® (p.a. quality, 60 – 100 mesh) was heated overnight at 550 ºC, mixed with distilled water to give 3% (w/w), mixed well and kept for 12 h in a closed container prior to use. Helium (99.99% purity) and make-up gas argon/methane (95% + 5%; 99.9% purity) were used. Elution mixture was petroleum ether + dichloromethane) 80: 20 %, v/v) [2, 16].

Extraction, Clean-up And Determination. Extraction, cleanup and determination were carried out according to DFG-method [17], with the following details: Glassware was dried at 110ºC after washing with soap, water, distilled water, acetone and n-hexane. Twenty-five grams Florisil were added to chromatography column (50 x 2 cm with Teflon stopcock) containing 100 mL petroleum ether. 10 g milk sample were mixed with 25 g Florisil (3% water), added to the column, and the excess solvent was collected in a 500-mL round bottom flask. The column was eluted with 300 mL of the elution mixture. The eluates were evaporated nearly to dryness using rotary evaporator at 35 ºC and 12 mbar. The remaining solvent was evaporated using a stream of nitrogen gas. The residues were dissolved in 2 mL n-hexane containing 0.3 µg/mL aldrin as internal standard (I.S.) and 2 µL of this final extract were injected onto the GC column [2,16].

The GC used was PU-304 instrument, equipped with ^{63}Ni-electron capture detector, a split injector and a SPB- 608 capillary column (L=30m, I.D. = 0.25 mm, film thickness = 0.25 µm). The GC was used under the following conditions: injector (250ºC), detector (300ºC), column temperature program: 150ºC (5 min), 150 - 220ºC (10 ºC/min), 220 ºC (20 min), 220 – 290 ºC (20 ºC/min), and 290 ºC (10 min). Carrier gas (He): 1.1 mL/min, make-up gas: 40 mL/min, and split ratio (1:50). For the confirmation of the results, a SPB-5 capillary column (L = 30 m, I.D. = 0.25 mm, film thickness = 0.25 µm) was used [2,16].

Determination of Fat Content. The fat content (%) was determined according to Al-Tarawneh et al. [15] and al-Antary et al. [16]. Ten g of each milk sample were weighed and mixed thoroughly in a separating funnel with 2 mL of 25% ammonia, 25 mL diethyl ether and 25 mL petroleum ether (40 – 60 ºC). The organic solvents layer was separated and the previous extraction steps were repeated twice. The pooled organic extracts were filtered through anhydrous sodium sulphate layer into a weighed round bottom flask. The solvents were evaporated at 30 ºC and 200 mbar. The round bottomed flask with the residues was placed overnight in a desiccator. The round bottom flask with residues was reweighed, and from the weight difference, the % fat content was calculated.

Evaluation of the Extraction Method. Extraction and clean-up methods were evaluated by spiking blank samples with known concentrations of each of the studied chlorinated pesticides, and each of these samples was extracted and cleaned-up according to the above mentioned method. The experimentally found concentration was related to the theoretically added concentration, in order to calculate the % recovery. The detection limit for each compound was calculated as signal to noise ($S/N \geq 3$) from the chromatogram of the standard mixture of the sixteen studied pesticides after diluting several times. Each solution was injected twice. The detection limits were 0.005 ppm for all studied compounds and the % recoveries ranged between 80.5% and 99.5%.

RESULTS AND DISCUSSION

Table 1 shows average and range of residue concentrations of the chlorinated pesticides in human milk samples collected from the southern governorates Ma'an, Karak, Tafila, Aqaba and the southern Jordan valley in 2016 and 2017.

The samples collected from Ma'an contained dieldrin and *p,p'*-DDE. Their concentrations were 0.3 and 0.25 mg/kg milk fat and the % of positive samples were 8.3% and 16.7%, respectively.

Table 1 shows the average and range of residues of the chlorinated pesticides in mother milk samples collected from Karak governorates in 2016 and 2017, which are dieldrin, *p,p'*-DDE and *p,p'*-DDT. Their concentrations were 0.06, 0.74 and 0.08 mg/kg milk fat and the % of positive samples were 3.3, 10.0 and 3.3% respectively.

Table 1 shows average and range of concentrations of organochlorine pollutants found in mother milk samples collected from Tafila governorate in 2016 and 2017. The samples contained only *p,p'*-DDE. Its concentration was 0.33 mg/kg milk fat and the % of positive samples was 27.7%.

TABLE 1

Number (N) and % of positive samples (N%), average and range of chlorinated pesticide residues of human milk samples in (mg/kg milk fat) collected from Ma'an, Karak, Tafila, Aqaba and southern Jordan Valley governorates in 2016 and 2017

District	Total analyzed samples	Found pesticide	N	N%	Average residue	Range Min.	Max.
Ma'an	24	Diedrin	2	8.3	0.30	0.14	0.46
		p,p'-DDE	4	16.7	0.25	0.02	0.70
Karak		Diedrin	1	3.3	0.06	0.06	0.06
	30	*p,p'*-DDE	3	10.0	0.74	0.59	0.91
		p,p'-DDT	1	3.3	0.08	0.08	0.08
Tafila	18	*p,p'*-DDE	5	27.7	0.33	0.11	0.77
Aqaba	24	Heptachlor	1	4.2	0.03	0.03	0.03
		p,p'-DDE	6	25.0	0.87	0.01	3.00
S. Jordan		Aldrin	1	4.2	0.12	0.12	0.12
valley	24	*o,p'*-DDE	2	8.3	0.49	0.03	0.94
		p,p'-DDE	4	16.6	0.27	0.16	0.49
		p,p'-DDT	2	8.3	0.28	0.03	0.52

Table 1 shows the average and range of residues of the chlorinated pesticides in mother milk samples collected from Aqaba governorates in 2016 and 2017. The samples contained heptachlor and *p,p'*-DDE. Their concentrations were 0.03 mg/kg and 0.87 mg/kg milk fat and the % positive samples were 4.2 and 25%, respectively.

Table 1 shows the average and range of residues of the chlorinated pesticides in mother milk samples collected from southern Jordan valley district in 2016 and 2017. The samples contained aldrin, *o,p'*-DDE, *p,p'*-DDE and *p,p'*-DDT. Their concentrations were 0.12, 0.49, 0.27 and 0.28 mg/kg milk fat and the % of positive samples were 4.2, 8.3, 16.6 and 8.3% respectively.

It is observable that all studied locations contained *p,p'*-DDE, the main metabolite of DDT and in high repeatability ranged from 10.0 and 27.7%. Al-Antary et al., [2] reported that the found *p,p'*-DDE was ranged between 20 and 60% in milk samples collected from middle governorates of Jordan in 2013 and 2014. The highest residue concentration of *p,p'*-DDE was found in Tafila but still lower than the concentration found in a similar study of Al-Antary et al. [2]. This is an indication that DDT has been rarely used illegally since its banning in the early eighties of the previous century. DDE is an important metabolite for the parent *p,p'*-DDT. The ratio between DDT and DDE is commonly used as a good indicator of DDT exposure history, where a high DDE/DDT ratio value (> 5) suggests recent exposure [6, 18, 19]. However, organochlorine pesticides are among POPs that cause concern in human health particularly infants [2, 3, 7, 20]. They are able to pass from the pregnant to embryos and through the mother milk to new born babies [2,3, 6, 7]. Organochlorine pesticides including DDT members, HCH, HCB and cyclodiens particularly dieldrin and endrin are persistent in the environment. They are able to reach human beings through the food chain and accumulate in fatty tissues in the human and animals for long periods. The infants might feed on the contaminated mother milk [2, 3, 4, 7, 9, 21, and 22].

The results indicated that 18.33% of the analyzed samples contained *p,p'*-DDE, 2.5% *p,p'*-DDT, 1.67% *o,p'*-DDE, 3.33% dieldrin and 0.83% heptachlor. The highest concentration was 3.0 mg/kg milk fat of *p,p'*-DDE in samples of Aqaba, but the highest % of positive samples was 27.7% in Tafila samples. The analyzed samples did not contain endrin, HCB, α-HCH, β-HCH, γ-HCH, α-endosulfan, β-endosulfan, *o,p'*-DDD, *o*,p'-DDT and *p,p'*-DDD. Despite Jordan has banned the use of all organochlorines since 40 years ago [1], but it is still occurring in low residue concentration in the collected mother breast milk. Several workers in the world did obtain similar trend for pesticides residues in their countries: Dillon et al. [23] in Quebec/Canada, Stuetz et al., [13] in Thailand, Sanghi et al.,[24] in India, Tadevosyan et al., [25] in Armenia, Ennanceur et al., [26] in Tunesia, Al-Targi et al.,[8] in Lybia, Mortrza et al., [27] in Iran, Rahmawati [28] in Indonesia, Bakirci et al., [29] in Turkey, Solomon and Weiss [30] in Germany, Al-Antary et al. [2, 3, 7, 9] and Alawi et al. [4] in Jordan.

In conclusion, there was a decrease in average of HCHs in the analyzed samples collected from the five southern districts in Jordan compared to the study of Alawi, 2012 [9]. There was a slight increase in averages of DDT and cyclodiens group in the analyzed samples compared with the study of Alawi, 2012 [10]. It is recommended to continue monitoring of pesticides residues in mother breast milk and other environmental components to overcome the misuse of pesticide, to build data base of pesticides residues information and to develop regulations for the registration of use of pesticides in Jordan.

ACKNOWLEDGEMENTS

The authors would like to thank the University of Jordan, Ministry of Environment, Royal Scientific Society and the Jordanian Environment Society for financing and help to conduct this study.

Compliance with ethical standards. Informed Consent. Informed consent was obtained from all individual participants included in this study. The protocol of this research project has been approved by a constituted Ethics Committee of the institutions within which the work has been conducted.

REFERENCES

[1] Al-Antary, T.M. (1996) Pesticides and Toxicology. Al-Quds Al Maftoha University Publications, Amman, Jordan. 643p.

[2] Al-Antary, T.M., Alawi, M., Estityah, H. and Haddad, N. (2017) Organochlorine pesticides residues in human breast milk from the middle governorates in Jordan in 2013/2014. Bull Environ. Contam. Toxicol. 99, 89 – 92.

[3] Al-Antary, T.M., Alawi, M., Said, M. and Haddad, N. (2018) Monitoring of pesticide residues in agricultural crops in southern governorates of Jordan in 2011/2012. Fresen. Environ. Bull. 27, 2418 – 2426.

[4] Alawi, M., Al-Antary, T.M., Estityah, H., Al-Aqqad, S. and Haddad, N. (2015) Chlorinated pesticides residues in human breast milk from southern Jordan: Comparative study between 2008 and 2012. Fresen. Environ. Bull. 24, 4512 – 4518.

[5] Alawi, M., Al-Antary, T.M., Estityah, H. and Haddad, N. (2017) Evaluation of chlorinated pesticides residues in foodstuff of animal origin from middle districts of Jordan in 2013 – 2014. Toxin Reviews. 36(2), 94 – 100.

[6] Du, J., Gridnevam Z., Gaym M., Lai, C., Trengove, R., Hartman, P. and Geddes, D. (2016) Longitudinal study of pesticide residue levels in human milk from Western Australia durin 12 months of lactation: Exposure assessment for infants. Scientific Report. 6, 1 – 27.

[7] Al-Antary, T.M., Al-Dabbas, M. and Shaderma, A. (2015) Evaluation of three treatments on carbsulfan removal in tomato juice. Fresen. Environ. Bull. 24, 733 – 739.

[8] Al-Targi, Z., Abou Al Ela, R., and Al-Dressi, A. (2011) Organochlorine pesticide residues in human milk in El-Gabal Al-Akhdar. International Conference on Life Science and Technology IPCBEE. 3, 146 – 149.

[9] Alawi, M., Al-Antary T.M., Hussein, E. and Al-Oqlah, K. (2012) Comparison study of pesticides residues in agricultural crops in Jordan in seven studies between 1993 and 2008. Fresen. Environ. Bull. 21, 927 – 934.

[10] Alawi, M., Al-Antary, T.M., Estityah, H., Al-Aqqad, S. and Al-Oqlah, K. (2013) Comparative study for chlorinated pesticides in mother milk from Jordan in four studies between 1993 and 2003. Fresen. Environ. Bull. 22, 279 - 285.

[11] Yu, H., Zhou, Z., Zhang, X., and wang, D. (2003) Levels of organochlorine Pesticides in Beijing human milk. Bull Environ Contam Toxicol. 70, 193 – 197.

[12] Ribas-Fito, N., Cardo, E., Sala, M., Eulalia de Muga, M., Mazon, C., Virdu, A., Kogevinas, M., Grimalt, J. and Sunyer, J. (2003) Breast feeding, exposure to organochlorine compounds, and neurodevelopment in infants. Pediatrics. 111e, 580 585.

[13] Stuetz, T., Prapamontol, T., Erhardt, J., Classen, H. (2001) Organochlorine pesticides residues in human milk of a huong hill tribe living in Northern Thailand. Sc. Total Environ. 273, 53 – 60.

[14] Nair, A., Mandapati, R., Dureja, P., and Pillai, M. (1996) DDT and HCH load in mothers and their infants in Delhi; India. Bull Environ Conam Toxicol. 56, 58 – 64.

[15] Al-Tarawneh, M., Khatib. S., and Arqub, K. (2010) Cancer incidence in Jordan. East Mediter Health J. 16(8), 837 – 845.

[16] Al-Antary, T.M., Alawi, M., Estityah, H. and Al-Oqlah K. (2013) Comparative study for chlorinated pesticides in mother milk from Jordan in four studies in mother milk from Jordan in four studies between 2005 and 2008. Fresen. Environ. Bull. 22, 1387 – 1393.

[17] Their, H.P., Zeumer, H (1987) Manual of Pesticides Residues Analysis. DFG, Wiley, Weinheim

[18] Zhou, J., Zing, X., Zhing, K., Zhu, X., Ma, L., Xu, Q., Zhang, X., Yu, Y., Sheng, G., and Fu, J. (2012) Musks and organochlorine pesticides in breast milk from Shanghai, China: Levels, temporal trends and exposure assessment. Ecotoxicology and Environmental Safety. 84(10), 325 – 333.

[19] Mishra, K. and Sharm, R. (2011) Assessment of organochlorine pesticides in human milk and risk exposure to infants from North-East India. Science of the Total Environment. 409, 4939 - 4949.

[20] Bouchard, M., Chevier, J., Harley, K., Kogut, K., Vedar, M., Calderon, M., Trujimo, C., Johnson, C., Bradmon, A., Barr, D., Eskanazi, B. (2011) Prenatal exposure to organphosphate pesticides and IQ in 7-Year-oldchildren. Environmental Health Perspectives. 119, 1189–1195.

[21] Heifetz, E., and Taylor, S. (1989) Mother's milk or mother's poison? Pesticides in breast milk. J. Pesticides Reform. 9(3), 15 – 17.
[22] Polder, A., Oldlant, J., Trachev, A., Foreid, S., Saninova, T., and Skaare, G. (2003) Geographic variation of chlorinated pesticides, toxaphens and PCss in human milk from sub-arctic and arctic locations in Russia. Sci Total Environ. 306, 179 – 195.
[23] Dillon, J., Martinm G., and O'brinem H, (1981) Pesticide residues in human milk. Food Cosmet Toxicol. 19, 437 – 442.
[24] Sanghi, R., Pillia, M., Jayalekshmi, T., and Nair, A. (2003) Organochlorine and organophosphorous pesticides residues in breast milk from Bhopal, Madhya Pradesh. Toxicology. 22, 73 – 76.
[25] Tadevosyam, A., Reynolds, S., Killey, K., Fourtes, L., Mairapetyam, A., Tadevosyam, M. and Beglaryan, S. (2007) Organochlorine pesticides residues in breast milk in Armenia. J Pre-clinical Clin Res. 1(1), 84 – 88.
[26] Ennaceur, N., Gandouiva, M. and Driss, M. (2007) Organochlorine pesticides residues in human milk of mother's living in Northern Tunesia. Bull Environ Contam Toxicol. 78(5), 325 – 329.
[27] Mortaza, Z., Mousavi, S., Baghestani, M., and Aitio, A. (2017). An assessment of agriculture pesticide use in Iran, 2012 – 2014. J Environ Health Sc Eng. 15, 1-10.
[28] Rahmawati, S., Margana, g., Yoneda, M., and Oginawati, K. (2013) Organochlorine pesticide residue in Catfish (*Clarias sp.*) collected from local fish cultivation at Citarum watershed, west Java province, Indonesia. Procedia Environmental Sciences. 17, 3 – 10.
[29] Bakirici, G., Acay, D., Bakirici, F., and Otles, S. (2014) Pesticide residues in Fruits and vegetables from the Aegean region, Turkey. Food Chemistry. 160(10), 379-392.
[30] Solomon, G., and Weiss, P. (2002) Chemical contaminants in breast milk: time trends and regional variability. Environ Health Perspect. 110(6), A339 – A347

Received: **14.08.2018**
Accepted: **11.11.2018**

CORRESPONDING AUTHOR

Mahmoud A Alawi
Department of Chemistry,
School of Science,
The University of Jordan,
Amman, 11942 – Jordan

e-mail: alawima@ju.edu.jo

© by PSP Volume 27 – No. 12B/2018 pages 9936-9941 Fresenius Environmental Bulletin

ENVIRONMENTAL QUALITY ASSESSMENT AND EVOLUTION ANALYSIS OF WATER QUALITY IN MEIZHOU BAY, CHINA

Xiaofeng Guo*, Zhouhua Guo

Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, Fujian Province, China

ABSTRACT

This study, based on the water quality monitoring data of Meizhou Bay from 2005 to 2016, conducted an environmental quality assessment of the water pollution condition by adopting the comprehensive evaluation and the eutrophication evaluation. The results of the comprehensive evaluation from 2005 to 2016 showed that the water quality index(*WQI*) was between 0.40 and 1.04, and the water quality in Meizhou Bay was clean for all years except for 2016, during which the bay was lightly polluted. The results of the eutrophication index (*EI*) from 2005 to 2016 showed that the *EI* was between 0.02 and 0.34, indicating that the water in Meizhou Bay was oligotrophic. From the perspective of the environmental quality evolution of this marine area, both of the quality evaluation indexes, i.e., *WQI* and *EI*, showed a steady upward trend, which should be addressed by the relevant authorities.

KEYWORDS:
Water quality, Environmental quality assessment, Evolution analysis, Meizhou Bay

INTRODUCTION

Meizhou Bay is located in the central coastal region of Fujian Province and is an important port on the southeast coast of China (see Fig. 1). Surrounded by the mainland on three sides, with Meizhou Island at the bay mouth, Meizhou Bay is a semiclosed narrow bay extending far inland and characterized by ice-free and silt-free deep water, vast ports and low sediment concentrations [1]. In recent years, according to the regional development plan, the Meizhou Bay rim region will become a new state-level petrochemical base on the southeast coast of China [2]; however, the wastewater produced during the production process of the petrochemical industry features high organic pollutant concentrations, high flow rates, complex compositions and high quantities of hazardous substances, exerting excessive pressure on the marine environment therein [3, 4]. In addition, oil films of varying thickness can be formed on the water surface by certain amounts of petroleum and damage the reaeration process of water, thus affecting the water quality, the animals, and the plants living in the water [5, 6]. Therefore, the environmental assessment of water quality in Meizhou Bay is of great significance.

In recent years, some studies have been carried out on the distribution patterns, sources and pollution situation of pollutants in the seawater of Meizhou Bay [7-9]; however, these studies generally had a limited timespan and lacked researches on long-term changes in water quality . This study, with marine monitoring data of Meizhou Bay from 2005 to 2016, conducted an assessment of the seawater environmental quality and eutrophication level of the bay by adopting the comprehensive evaluation index method and analyzed seawater quality trends to provide a scientific basis for environmental protection work in this marine area.

MATERIALS AND METHODS

Monitoring Items and Analytical Approaches. In this study, data were taken from the monitoring data of Meizhou Bay from 2005 to 2016 by the Third Institute of Oceanography, State Oceanic Administration, and the collection, preprocessing, storage, transportation, and analysis of seawater samples were conducted in strict accordance with the relevant provisions of the *Specifications for Oceanographic Survey* [10] and the *Specification for Marine Monitoring* [11]. According to the marine environmental characteristics of Meizhou Bay and characteristic pollutants of the petrochemical industry, the following 8 monitoring factors were included in the statistics: dissolved oxygen(DO), chemical oxygen demand(COD), dissolved inorganic nitrogen(DIN), active phosphates, petroleum, Cd, Hg, and As. Table 1 shows the statistical results of various monitoring indicators of water quality in Meizhou Bay from 2005 to 2016.

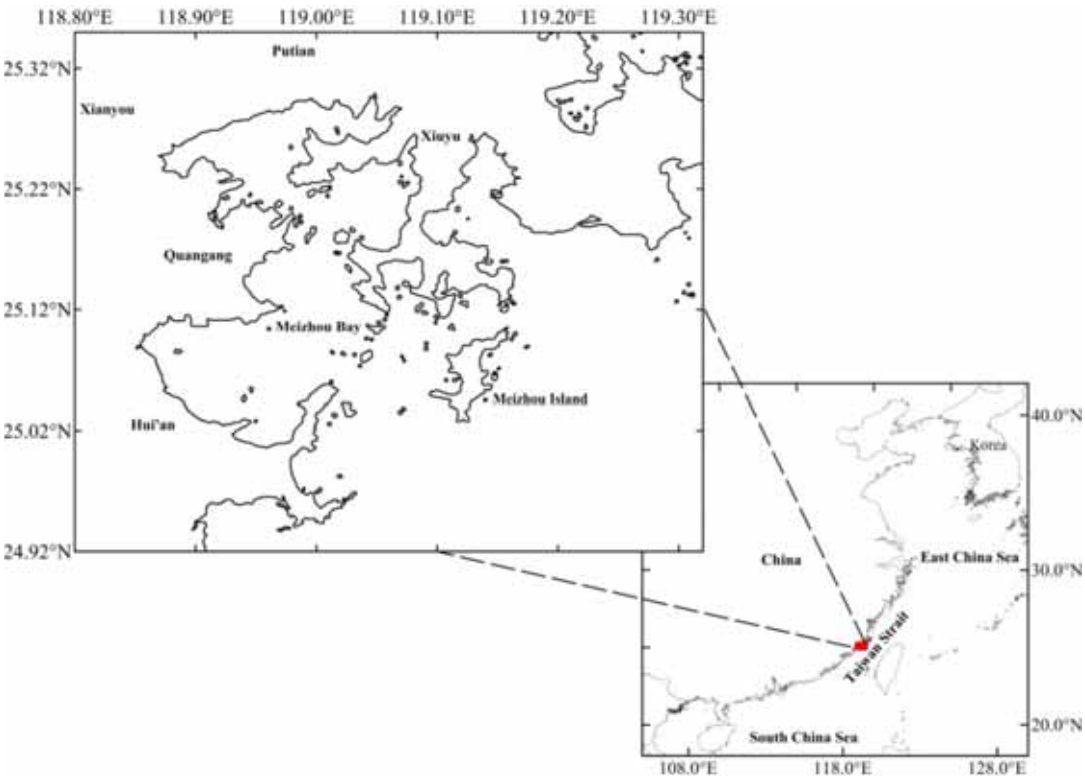


FIGURE 1
Geographical location of Meizhou Bay

TABLE 1
Statistics of 2005-2016 Pollutant Contents in the Water of Meizhou Bay

Year	DO ($mg \cdot g^{-1}$)	COD ($mg \cdot g^{-1}$)	DIN ($mg \cdot g^{-1}$)	Active phosphates ($mg \cdot g^{-1}$)	Petroleum ($\mu g \cdot g^{-1}$)	Cd ($\mu g \cdot g^{-1}$)	Hg ($\mu g \cdot g^{-1}$)	As ($\mu g \cdot g^{-1}$)
2005	6.78	0.45	0.181	0.023	8.9	0.027	0.007	0.78
2006	7.62	0.66	0.159	0.006	9.6	0.013	0.030	1.7
2007	7.45	0.60	0.156	0.010	9.7	0.012	0.028	1.9
2009	7.42	0.54	0.155	0.020	10.0	0.016	0.031	1.5
2011	7.26	0.62	0.172	0.035	10.0	0.018	0.036	1.2
2013	7.16	0.70	0.256	0.036	10.3	0.015	0.038	1.6
2015	7.18	0.71	0.249	0.040	10.9	0.020	0.042	1.4
2016	6.90	0.79	0.280	0.042	11.2	0.017	0.042	1.5

Evaluation Methods. The single-factor index method was adopted to evaluate the equivalent influences of various contamination factors on the environment, and the comprehensive index method was adopted to evaluate the overall environmental quality status and eutrophication level of the water [12]

Single-factor Index Method. The single-factor index method formula is:

$$P_i = C_i / C_s$$

where P_i is the single-factor evaluation index; C_i is the measured value of pollutant *I*; C_s is the evaluation standard of pollutant *i*, which adopts the category II standard value of the *Sea Water Quality Standard*. When P_i > 1, the water quality exceeds the prescribed water quality standard.

The single-factor evaluation index of DO is:

$$P = \frac{|DO_f - DO|}{DO_f - DO_s} \left(DO \geq DO_s\right) ,$$

$$P = 10 - 9 \times \frac{DO}{DO_s} \left(DO < DO_s\right),$$

$$DO_f = \frac{468}{31.6 + T}$$

where *P* is the single-factor index of DO; DO_f is the

saturated dissolved oxygen concentration; T is the water temperature, 19.1℃; DO is the measured concentration; and DO_s is the evaluation standard of DO.

Comprehensive Evaluation Index Method. In the comprehensive evaluation, the Nemerow index method using the sum of squares of the average and maximum values of the subindex, which considers the influences of both the average and maximum subindexes, was adopted:

$$WQI = \sqrt{\frac{P_{\max}^2 + P_{avg}^2}{2}}; P_{avg} = \frac{1}{n}\sum_{i=1}^{n} P_i ;$$

where water quality index(WQI) is the Nemerow index, $P_{\max}$ is the maximum single-factor index of various pollutants, and P_{avg} is the average value of a single-factor index of various pollutants. Table 2 shows the pollution grading standard of the Nemerow water quality index [13-14].

Eutrophication Index Evaluation Method. The eutrophication index (EI) equation, which was forwarded by the Japanese scholar Okaichi Tomotoshi in 1972 [15], is shown below:

$$EI = \frac{COD \times DIN \times DIP}{4500} \times 10^6$$

where COD, DIN, and DIP are the monitored values (mg/L) of chemical oxygen demand, inorganic nitrogen, and inorganic phosphorus, respectively. The equation was introduced to China by Jingzhong Zhou in 1983 and has been widely applied to the evaluation of the eutrophication status of offshore areas in China by many researchers. The EI evaluation grades are: oligotrophic, $EI < 0.5$; mesotrophic, $0.5 \le EI < 0.75$; moderately eutrophic, $0.75 \le EI < 1$; eutrophic, $1 \le EI < 3$; hypereutrophic, $EI \ge 3$ [16-17].

Bin Chen [18] et al., based on the research results of scholars studying Meizhou Bay and Xiamen western sea area in Fujian province [19, 20], optimized the above formula as:

$$EI = \frac{COD \times DIN \times DIP}{COD' \times DIN' \times DIP'}$$

where COD', DIN' and DIP' are the coastal eutrophication threshold values of the chemical oxygen demand, inorganic nitrogen, and inorganic phosphorus, respectively. According to many research findings, COD' is 1~3 mg/L, DIN' is 0.2~0.3 mg/L, and DIP' is 0.01~0.02 mg/L. In this study, based on the characteristics of the Fujian offshore area, the following values were taken as the thresholds of single index of eutrophication for Meizhou Bay: COD' 3 mg/L, DIN' 0.3 mg/L, DIP' 0.03 mg/L.

RESULTS AND DISCUSSION

Results of Water Quality Pollution Evaluation. Table 3 shows the results of seawater quality evaluation in Meizhou Bay. According to the single-factor index results, the water quality indexes of DO, COD, inorganic nitrogen, petroleum, Cd, Hg, and As were all below 1, indicating that the annual average concentrations of these indexes met the category II standards set forth in the *Sea Water Quality Standard*. The single-factor index of inorganic phosphorus was below 1 from 2005 to 2009 but was above 1, i.e., 1.17-1.40, from 2011 to 2016, thus failing to meet the category II standards therein. According to the comprehensive evaluation index results, the WQI was between 0.40 and 0.99 from 2005 to 2015, indicating that the seawater in Meizhou Bay was clean. However, the WQI in 2016 was 1.04, indicating the water in this area was lightly polluted.

According to the evaluation results, from 2005 to 2016, the EI was between 0.02 and 0.34, showing that the water in Meizhou Bay was oligotrophic, which agreed with the research results of Yuanmin Sun et al. [21].

TABLE 2
Pollution Grading Standard of the Nemerow Water Quality Index

WQI	<1	1~2	2~3	3~5	>5
Water quality grade	Clean	Lightly polluted	Polluted	Heavily polluted	Severely polluted

TABLE 3
2005-2016 Meizhou Bay Area Water Quality Evaluation Indexes

Year	Single-factor index								P_{avg}	WQI	EI
	DO	COD	DIN	DIP	Petroleum	Cd	Hg	As			
2005	0.58	0.15	0.60	0.77	0.18	0.005	0.04	0.03	0.29	0.58	0.07
2006	0.38	0.22	0.53	0.20	0.19	0.003	0.15	0.06	0.22	0.40	0.02
2007	0.42	0.20	0.52	0.33	0.19	0.002	0.14	0.06	0.23	0.40	0.03
2009	0.43	0.18	0.52	0.67	0.20	0.003	0.16	0.05	0.27	0.51	0.06
2011	0.47	0.21	0.57	1.17	0.20	0.004	0.18	0.04	0.35	0.86	0.14
2013	0.49	0.23	0.85	1.20	0.21	0.003	0.19	0.05	0.40	0.90	0.24
2015	0.48	0.24	0.83	1.33	0.22	0.004	0.21	0.05	0.42	0.99	0.26
2016	0.55	0.26	0.93	1.40	0.22	0.003	0.21	0.05	0.45	1.04	0.34

FIGURE 2
Changes in the water quality evaluation index values in Meizhou Bay

Analysis of the Evolution Trend of Seawater Environmental Quality in Meizhou Bay. The changing trends of *WQI* and *EI* for the water quality of Meizhou Bay from 2005 to 2016, as shown in Fig. 2, were in reasonable agreement with each other. The diagram in Fig. 2 shows that, from 2005, the *WQI* decreased in 2006 and 2007 and began to steadily increase in 2009, indicating that the water quality in Meizhou Bay deteriorated over time; furthermore, in 2016, the *WQI* was 1.04, showing the water was lightly polluted. With regard to the changing trend in *EI*, after a decline in 2006, the *EI* began to rise gradually in 2007 and reached the highest value of 0.34 in 2016; although still oligotrophic, the water eutrophication level was on the rise.

Over the past decade (2005 to 2016), despite the increasing amount of pollutants discarded in Meizhou Bay due to human activities, the water quality in Meizhou Bay was generally clean. Because of the strong tidal action in Meizhou Bay, frequent water exchange occurs across the bay. According to the estimate of Hao Liu et al., derived by using a 3D baroclinic primitive equation ocean model, the half exchange period of water in Meizhou Bay was 5d, and the 80% exchange period was 15d [22]; thus, sound water exchange conditions could contribute to the relatively stable pollutant concentration in the water of Meizhou Bay.

Factors Influencing Water Quality in Meizhou Bay. Industrial & Agricultural Wastewater Pollution. As a result of the heavy use of fertilizers and pesticides in agricultural production, massive amount of fertilizers are drained into Meizhou Bay with farm drainage and surface runoff. In addition, according to the regional development plan, by 2030, the discharges of the main pollutants from industrial enterprises in Meizhou Bay region, namely, COD, total nitrogen and petroleum, will reach 29602 t/a, 7337 t/a and 504 t/a, respectively, and impose a higher pressure on the water quality environment in Meizhou Bay [23].

Marine Engineering & Construction. In recent years, Meizhou Bay witnessed the rapid development of harbor-oriented industries and has established a new upsurge in sea reclamation. Sea reclamation and other marine engineering and construction projects reduce the bay area, impair the water exchange capacity and lower the marine environmental capacity, leading to changes in sediment deposition and pollutant transport patterns and intensifying the cumulative pollution in the bay.

Mariculture Pollution. Meizhou Bay has a vast tidal-flat area and large-scale mariculture industry; however, due to a lack of scientific planning, the intensive aquaculture distribution, excessive feeding and other problems exist in some bay areas; thus, unconsumed feed, the faeces of cultured organisms, as well as the medicines and chemicals used in aquaculture are discharged into the seawater, causing water quality degradation.

CONCLUSIONS

(1) From 2005 to 2016, the annual average concentrations of DO, COD, inorganic nitrogen, petroleum, Cd, Hg and As in the water of Meizhou Bay all met the category II standards set forth in the *Sea Water Quality Standard*, but the average

concentration of inorganic phosphorus from 2011 to 2016 exceeded the category II standard threshold. According to the *WQI* evaluation results, from 2005 to 2016, the *WQI* fell between 0.40 and 1.04, and the water quality in Meizhou Bay was clean in all years except for 2016, during which the water was lightly polluted. According to the *EI* evaluation results, from 2005 to 2016, the *EI* was between 0.02 and 0.34, indicating that the water in Meizhou Bay was oligotrophic.

(2) From the perspective of the water quality changes, both the *WQI* and *EI* of the water in Meizhou Bay showed a steady upward trend that should arouse the attention of relevant authorities, who should carry out comprehensive improvement in this area.

(3) There are various reasons for the gradual water degradation in Meizhou Bay, including industrial and agricultural wastewater discharge, changes in hydrodynamic conditions resulting from marine engineering and construction, and mariculture pollution.

REFERENCES

[1] Yu, J. (1988) Collection of Land Planning of Meizhou Bay Area. Fuzhou: Fujian Maps Press.

[2] Fujian Provincial People's Government. (2007) Development Plan of Petrochemical Base in Meizhou Bay of Fujian (2006-2020). Fuzhou.

[3] Lu, F., Gao, Z., Jia, Y. and Yang, D. (2010) Environmental Situation and Assessment of Jinzhou 9-3 Oilfield Sea Areas. Acta Oceanologica Sinica. 32(1), 161-169.

[4] Xu, J., Xu, S., Li, C., Liu, Y. and Lin, L. (2010) Winter Eco-environmental Quality Assessment of Petrochemical Sewage Waters in Daya Bay. Journal of Agro-environment Science. 32(1), 161-169.

[5] Yang, B. and Dai, B. (2009) Exploration on the industrial policy and pollutant emission characteristics of petrochemical industry. Chemical Industry and Engineering Progress. 28(Suppl), 505-5-7

[6] Li, Y., Zheng, X. and Sun, J. (2012) Distributions and Ecological Assessment of Heavy Metals of Chengdao Oilfield Sea Areas. Marine Environmental Science. 31(2), 190-194.

[7] Xie, L. (2012) Analysis and Evaluation of Heavy Metals in Sea Water, Sediments and Aquatic Organisms from Meizhou Bay. Straits Science. 7, 33-37.

[8] Wang, X., Tian, C. and Zheng, S. (2008) Research on the Distributed Characteristics of Petroleum Hydrocarbon in Surface Seawater of Meizhou Bay. Journal of Huaqiao University (Natural Science). 29(2), 241-244.

[9] Cai, Z., Wang, X. and Zheng, S. (2008) Seasonal Variation and Assessment of Water Quality in Meizhou Bay. Marine Environmental Science. 27(2), 161-164.

[10] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China. (2007) GB12763-2007 Specifications for Oceanographic Survey. Beijing: Standards Press of China.

[11] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China. (2007) GB17378-2007 Specification for Marine Monitoring. Beijing: Standards Press of China.

[12] Han, B. and Wang, B. (2006) Comments on Two Methods for Comprehensive Assessment of Ecological Environment Quality in Estuarine and Coastal Waters. Advances in Marine Science. 2, 43-47.

[13] Lu, F., Gao, Z., Jia, Y. and Yang, D. (2010) Environmental Situation and Assessment of Jinzhou 9-3 Oilfield Sea Areas. Acta Oceanologica Sinica. 32(1), 161-169.

[14] Li, J., Feng, M. and Yu, R. (2001) Assessment on the Situation of Water Quality in Liaodong Bay Shallow Waters. Marine Environmental Science. 20(3), 42-45.

[15] Okaichi, T. (1972) The Pollution of Shallow Sea and the Occurrence of Red Tide, Mechanism of the Occurrence of Red Tide in Inner Bay. Japan Aquatic Resources Protection Association. 1972, 58-76.

[16] Wang, X., Li, K. and Shi, X. (2006) Marine environmental capacity of pollutants in Jiaozhou Bay. Beijing: Science Press.

[17] Wu, H., Chen, K., Chen, Z., Chen, Q., Qiu, Y., Wu, J. and Zhang, J. (2012) Evaluation for the ecological quality status of coastal waters in East China Sea using fuzzy integrated assessment method . Marine Pollution Bulletin. 64(3), 546-555.

[18] Chen, B., Wang, J., Tang, J. and Wen, S. (2002) Prediction to Trend of Nutrient Status in Meizhou Bay, Fujian. Journal of Oceanography in Taiwan Strait. 21(3), 322-327.

[19] Zhang, S., Yang, Q., Qiu, H. and Lin, Q. (1994) Red Tide and Preventive and Control Measures. Beijing: China Ocean Press.

[20] Lin, Y., Chen, X., Zhuang, D., Tang, S. and Lin, R. (1992) A Preliminary Study on Red Tide Caused by Eutrophication in Enclosure Ecosystems. Oceanologia et Limnologia Sinica. 23(3), 312~316.

[21] Sun, Y., Chen, B., Ma, Z. and Yu, W. (2010) Assessment and Cause Analysis of Eutrophication in the Adjacent Sea Areas of South Subtropical Islands. Marine Science Bulletin. 29(5), 572-576.
[22] Liu, H., Pan, W., Luo, Z. and Shi, X. (2009) Numerical study on the tidal regime in Meizhou Bay. Journal of Oceanography in Taiwan Strait. 28(1), 118-122.
[23] Fujian Academy of Environmental Science. (2011) Environmental Impact Statement of Development Plan of Meizhou Bay Rim Region. Fuzhou.

Received: **16.09.2018**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Xiaofeng Guo
Third Institute of Oceanography,
State Oceanic Administration,
Xiamen 361005, Fujian Province – P R. China

e-mail: guoxiaofeng@tio.org.cn

© by PSP Volume 27 – No. 12B/2018 pages 9942-9948 Fresenius Environmental Bulletin

EFFECT OF VERMICOMPOST APPLICATION ON SOME PLANT CHARACTERISTICS IN LETTUCE (*LACTUCA SATIVA* L.)

Turgay Kabay[1,2,*], Yekbun Alp[2], Suat Sensoy[2,3]

[1]Ercis Vocational School, Van Yuzuncu Yil University, Van, Turkey
[2]Horticultural Sciences, Institute of Natural and Applied Sciences, Van Yuzuncu Yil University, Van, Turkey
[3]Department of Horticulture, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Turkey

ABSTRACT

Vermicompost, an organic material, has a positive effect on plant growth and can be applied to all areas where organic farming is conducted. This study was carried out as a pot experiment in greenhouse conditions in order to determine the effects of vermicompost application on plant growth in lettuce cultivation. Lettuce seeds were sown in the vials containing 2: 1 mixture of peat and perlite. After the cotyledon leaves emerged, the seedlings were transferred into the 2 liters pots. Then, the control plants grown in peat (1/2 volume) + perlite (1/2 volume) media were irrigated with Hoagland nutrient solution until the end of the experiment, whereas the vermicompost applied plants grown in peat (1/3 volume)+perlite (1/3 volume) + vermicompost (1/3 volume) media were irrigated with only tap water. Twenty-seven days from the planting, the differences in seedling fresh and dry weights, shoot weights, stem diameters, root fresh and dry weights, leaf numbers, leaf temperatures, leaf areas, chlorophyll contents, total phenolic contents, total antioxidant values and antioxidative enzymes were determined. Considering these parameters, it can be said that vermicompost application has positive effects on lettuce growth.

KEYWORDS:
Compost, *Lactuca sativa* L., Vermicompost.

INTRODUCTION

Lettuce is a vegetable plentifully produced and consumed in the world. It has been adversely affected by excessive chemical fertilization [1] For this reason, the use of organic fertilizers and composts is of great benefit to producers and researchers without harming human health. Organic farming methods have become the most important issue for producers and researchers. Besides composts and organic materials that will not harm human health, vermicompost that have become widespread recently are an important cultivation place for organic farming.

The vermiculture called vermicompost is the process of culturing earth worms for different purposes [2, 3, 4]. It has been emphasized that vermicompost has a significant potential in greenhouse environment, open field and horticultural plants in the studies to determine the effects of vermicompost application on soil and plant growth [5, 6, 7, 8, 9, 10]. Many recent studies have shown that vermicompost is preferred instead of chemical fertilizers due to its organic structure [11, 12, 12, 13, 14]. Tomato and lettuce were also reported that in comparison with cattle manure and vermicompost and it was determined that vermicompost better than cattle manure due to the effects of vermicompost on plant growth [15]. In a study made for evaluating the total development of lettuce leaves in relation to nutrition, three different types of fertilization were carried out with 2 organic and 1 inorganic fertilizers; as a result of vermicompost application, Mg, Fe, Zn and Cu had the highest values, whereas Na had the lowest value. [16].

In the study conducted with the LC-54 and LC-2063 genotypes, vermicompost was found to be beneficial between 40% and 60% on seed of *Linum usitassimum* L., and emphasized that the LC-54 genotype showed better efficiency and performance than the LC-2063 genotype [17]. It has been stated that when 1.5 tons of vermicompost is applied in the soil of vegetable production, the physical structure of soil has changed positively and organic carbon, N, P, K, Ca, Zn and Mn amounts have increased [18].

In a study conducted on tomato grown in aquaculture; vermicompost, peat and pearlite increased root weight and dry weight, root volume, number of fruits and average photosynthesis ratio compared to controls, indicating that vermicompost was found to be effective in improving plant growth [19]. The positive effects of organic fertilizer and vermicompost applications on different cultivated plants were observed by many different researchers [10, 20, 21, 22, 23, 24, 25, 26, 27, 39, 40, 41, 42, 43].

If organic materials do not harm human health, are used and spread in grown products, it will be great benefit to producers and researchers. In this study, we aimed to determine the effects of vermicompost application on plant growth in lettuce cultivation.

MATERIALS AND METHODS

This study was conduct to determine the effects of vermicompost application on growth and development of lettuce plant, cv. Yedikule. Lettuce seeds were sown in the vials containing a 2: 1 mixture of peat and pearlite. There were two growing media applications, with and without vermicompost. After the first true leaves were emerged, the seedlings were transferred to 2 liters pots consisting of either vermicompost (1/3 volume) + peat (1/3 volume) + perlite (1/3 volume) or peat (1/2 volume) + perlite (1/2 volume) to determine the effects of vermicompost application on plant growth and development. The later application was considered as a control application. All plant groups were irrigated equally and by the amount of water that they have needed. However, the control plants were irrigated with Hoagland nutrient solution until the end of the experiment; whereas the vermicompost applied plants were irrigated with only tap water. Besides, Hoagland nutrient solution was prepared to be 5 grams of nutrient solution per liter.

The present study was conducted according to a completely randomized experimental design with three replications, each having two pots each having a plant. The study was carried out in under greenhouse conditions. The measurements and analyzes made at the end of the study are given below:

Determination of fresh and dry weights (g). All harvested plants were weighed on a precision scale and divided by the number of plants to determine seedling weights. Then, the same samples were waited at room temperature a day and then dried at 65 ºC for 48 hours, then the stem and root dry weights were weighed on a precision scale [28, 29, 30].

Determination of shoot height and diameter. Shoot height in lettuce plants were measured in cm/meters (± 0.5). The stem diameter was measured in mm (± 0.1) with the aid of the digital display compass [28, 31].

Determination of the total leaf numbers. Number of leaves in each lettuce plant were counted [28, 31].

Determination of leaf surface temperature (˚C). The leaf surface temperatures were measured with a handheld infrared thermometer.

Determination of leaf area (cm^2). The area of collected leaves was determined in terms of cm^2 using a planimeter.

Determination of chlorophyll content. The 0.25 g samples from the leaves were homogenized in 80 % acetone in a dimly lit place without direct light and the samples were filtered, then the extract was completed with 25 ml of acetone. Prepared samples were read at 663 and 645 nm wavelengths for determine chlorophyll a, chlorophyll b and total chlorophyll and the same sample were read at 470 nm wavelength to determine carotenoid content [30, 32, 33, 34, 35].

Chlorophyll a (mg g^{-1}) = (12,7 * 663 nm) – (2.69 * 645 nm) * V / W*1000

Chlorophyll b (mg g^{-1}) = (22,91 * 645 nm) – (4.68 * 663 nm) * V / W*1000

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoid (µg ml^{-1}) = ((1000 * 470 nm) – (3.27 * Chlorophyll a) – (104 * Chlorophyll b)) / 229 [32].

Determination of total phenolic content and total antioxidant activity. 5 g of sample taken from lettuce leaves, 25 ml of methanol was added and homogenized for 2 minutes with a homogenizer at medium speed, and then exposed to dark conditions at room temperature for 30 minutes. The samples were filtered on filter paper and put into the Eppendorf tubes and stored at -80 °C until analysis.

The total phenolic content was determined by spectrophotometer by Folin-Ciocaltaeu calorimetric method [36]. The absorbance values of the solutions were read spectrophotometrically at 725 nm wavelength and the total phenolic content expressed as mg gallic acid equivalent (GAE) / kg^{-1} fresh weight (FW). Ferric Reducing. Antioxidant Power (FRAP) (Iron (III) reduction antioxidant power) method was used to determine antioxidant activity [37]. The readings were read at 593 nm in the absorbance spectrophotometer and the antioxidant activity values were given as the Trolox equivalent (TE) / mg^{-1}.

Antioxidative enzyme analyzes. The frozen 1 g leaf sample (third leaf from the bottom of the plants) was homogenized with a mixture of 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA and 1 mM ascorbic acid (pH: 7.5), then the samples were homogenized at 4 ºC for 30 minutes at 18000 rpm. The ascorbate peroxidase (APX) activity was determined immediately in the homogenate with this prepared. Catalase (CAT) 1 g of frozen leaf sample was homogenized with 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA mixture (pH: 7.5), and the homogenate was centrifuged at 18000 rpm for 30 minutes at 4 ºC for the determination of CAT and superoxide dismutase (SOD) activity. CAT activity was detected in a portion of the homogenate and the rest of the extract was stored at -20 °C for SOD determination [30, 35, 38].

Catalase (CAT) activity. Catalase activity was determined by monitoring the disappearance of $H_2O_2^-$ at a wavelength of 240 nm. 0.05 M phosphate

buffer (KH_2PO_4), 1.5 mM H_2O_2 mixture (pH: 7.0) was used as the reaction solution. 2.5 ml of reaction solution and 0.2 ml of plant extract were mixed. In spectrophotometer, 0 and 60 second readings were taken at 240 nm wavelength. The reaction was started by the addition of 0.1 ml enzyme extract. The evaluation was made taking into account the change in absorbance within 1 minute [30, 35, 38].

Superoxide dismutase (SOD) activity. It was determined the inhibition of nitroblue tetrazolium (NBT) by at a wavelength of 560 nm. As the reaction solution, a mixture of 50 mM Na-phosphate buffer (Na_2HPO_4 x $H_2O_2^-$), 0.1 mM Na-EDTA, 33 µM NBT, 5 µM riboflavin, 13 mM methionine (pH: 7.0) were used. 2.5 ml of reaction solution was mixed with 0.1 or 0.2 ml of plant extract. The reaction was achieved at 25 ºC with 75 µmol m-2 s-1 (40 W) for 10 minutes under light. The control solution was left in the dark condition for the same period without enzyme. Control and reaction solution read at 560 nm. As SOD activity unit, 50% of NBT was determined as reductive activity [30, 35, 38].

Ascorbate peroxidase (APX) activity. Ascorbate peroxidase activity was measured at 290 nm depending on the ascorbic acid reducing $H_2O_2^-$. As the reaction solution, 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM H_2O_2 mixture (pH: 7.0) were used. 3 ml of reaction solution and 0.1 ml of plant extract were mixed. The 0. and 60 second readings were taken at 290 nm wavelength in the spectrophotometer. The reaction was started by the addition of 0.1 ml enzyme extract. The evaluation was made taking into account the change in absorbance within 1 minute [30, 35, 38].

Statistical analysis. The statistical analysis of the data obtained the study containing vermicompost or not was subjected to independent T test according to the completely randomized experimental design with three replications.

RESULTS AND DISCUSSION

In recent years, vermicompost, which has been used in all fields of vegetable production, is seen in the studies that increase efficiency and quality of production. In the present study, the some parameters of lettuce plant applied with vermicompost were also ameliorated. The seedling fresh weight of lettuce plant was 45.233 g in the control application, whereas the seedling fresh weight in the application having vermicompost was 56.483 g. Thus, there was a 24.871 % increase in vermicompost application (Table 1). The dry weight of plants applied with vermicompost was increased by 21.622 % (Table 1). There was an increase of 7.275 % in the root fresh weights of lettuce plants applied with vermicompost, and the root dry weight increased by 33.705 % compared to control application (Table 1). There was an increase in the shoot height and stem diameter of the plants subjected to vermicompost application compared to control application 2.778% and 21.099 %, respectively (Table 1). The total leaf number increased by 25.290 % and the leaf area increased by 9.742 % in the plants subjected to vermicompost application (Table 1). The leaf surface temperature was 19.167 °C in the control application, but it was 18.500 °C in the application having vermicompost (Table 1).

The study of vermicompost and NPK applications on growth and yield of mustards; plant weights, leaf number of plants, number of branches, fresh weights of plant, dry weights of plant and total seed yields were increased. [25]. Tomato and lettuce are also reported to give better results in vermicompost in a study comparing cattle manure with vermicompost [15]. It is stated that the vermicompost applications give better results in the study for evaluating the total development of the leaf sizes in relation to the feeding in lettuces (*Lactuca sativa* L.) [16]. Application of 50 kg sulfur and 4.0 tones vermicompost / ha in garlic (*Allium sativum* L.) , vermicompost and sulfur application tests significantly increased plant height, number of leaves per plant, head neck thickness increased by 25.7% compared to fresh weight control plants [24]. The parameters such as plant height, number of leaves, number of branches, root diameter, higher fruit yield, fruit weight and fruit diameter were positively affected in the growth of the pepper (*Capsicum chinense*) of organic and inorganic fertilizers [26]. It was stated that vermicompost and nitroxin inoculation in different levels of rosemary (*Rosmarinus officinalis* L.) plant has important influence on morphological and physiological characteristics such as plant heights, fresh and dry weights, root dry weights, chlorophyll a, total chlorophylls and leaf flavonoids [23]. In a study conducted on tomato grown in aquaculture; vermicompost, peat and pearlite increased root weight and dry weight, root volume, number of fruits and average photosynthesis ratio compared to controls [19]. Vermicompost applications were increased that number of leaves, root heights, leaf areas, leaf dry weights, root lengths, number of roots, total number of fruit/vegetables, total yield, chlorophyll content, fruit juice pH value, fruit juice, micro and macro TSS nutrients, nutrient content, carbohydrate (%) and protein (%) content and the quality of fruits and seeds in the plant [10]

TABLE 1
Effects of vermicompost application on plant and root fresh and dry weights shoot height and stem diameter, leaf number, leaf temperature and leaf area of lettuce (*Lactuca sativa* L.*)*.

Traits	Control Application (Peat + Pearlite + Hoagland solution)	Vermicompost application (Vermicompost + Peat + Pearlite)	Change (%)
Seedlings Fresh Weight (g*)	45.233	56.483	24.871
Seedlings Dry Weight (g)	3.020	3.673	21.622
Root Fresh Weight (g)	4.687	5.028	7.275
Root Dry Weight (g)	0.267	0.357	33.708
Shoot Height (cm)	27.000	27.750	2.778
Stem Diameter (mm*)	6.322	7.668	21.099
Leaf Number*	14.500	18.167	25.290
Leaf Area (cm^2)	124.200	136.300	9.742
Leaf temperature (^0C)	19.167	18.500	-3.480

* $P < 0.05$

There was a significant difference between applications of chlorophyll content in lettuce plants. In the vermicomposted application, while chlorophyll a was increased by 13.160 % and chlorophyll b was decreased by 6.306 %. For vermicomposted application, the total chlorophyll increased by 7.661 % (Table 2). In carotenoid content, there was a 50.079 % increase in the vermicomposted application (Table 2). Total antioxidant and total phenol were higher in the vermicomposted application. In the vermicompost applications total antioxidant increased by 36.898 %, and total phenol increased by 67.388 % (Table 2).

There were also changes in antioxidative enzyme contents in lettuce plant. In the vermicomposted application, the content of catalase (CAT) decreased by 38.376 %, the content of superoxide dismutase (SOD) decreased by 34.943 and the content of ascorbate peroxidase (APX) decreased by 48.558 (Table 2).

It has been emphasized that vermicompost has a significant potential in greenhouse environment, open field and horticultural plants in studies to determine the effects of vermicompost application on soil and plant growth [5, 6, 7, 8, 9, 10].

In the study conducted with the LC-54 and LC-2063 genotypes, vermicompost was found to be beneficial between 40% and 60% on seed of *Linum usitassimum* L., and emphasized that the LC-54 genotype showed better efficiency and performance than the LC-2063 genotype [17]. It has been stated that when 1.5 tons of vermicompost is applied in the soil of vegetable production, the physical structure of soil changes positively and organic carbon, N, P, K, Ca, Zn, Mn amounts increase [18].

In a study applying chemical fertilizer and vermicompost on pea (*Pisum sativum* cv. *Bonneville*), it is indicated that the best effect was observed vermicompost application in terms of the effects on soil microbial diversity and root nodulation and AMF colonization. [22]. Vermicompost applied in Chinese cabbage; the contents of phosphorus, potassium, magnesium, calcium, manganese, barium and molybdenum indicate that the nutrients increase bioavailability and give the best result for plant growth and improved antioxidant activity [21]. In the drought-affected bean, while grain yields, yields, protein yields, leaf relative water contents, membrane stability, protein and proline percentage were affected negatively due to drought however when used vermicompost, in combination with cattle manure + vermicompost and horse manure + vermicompost improved these parameters and the best results were obtained with the applications of cattle manure + vermicompost and horse manure + vermicompost [20]. In a study of the production of geranium (*Pelargonium zonale* L.) and calendula (*Calendula officinalis* L.); green waste compost (GWC) and green waste vermicompost (GWV) were applied and green waste vermicompost (GWV) has been reported to more increase growth and flowering compared to green waste compost (GWC) [27].

TABLE 2
Determination of changes in the content of chlorophyll, carotenoid, total antioxidant capacity (TAC), total phenolic content (TPC), catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) in lettuce (*Lactuca sativa* L.*)* by vermicompost.

Traits	Peat+perlite	Peat+perlit+ vermicompost	Change (%)
Chlorophyll a (mg g^{-1})	12.941	14.644	13.160
Chlorophyll b (mg g^{-1})	5.090	4.769	-6.306
Carotenoid (µg ml^{-1})	1.907	2.862	50.079
Total Chlorophyll (mg g^{-1})	18.027	19.408	7.661
TAC (TE / mg^{-1} *)	7.743	10.600	36.898
TPC (GAE / kg^{-1} FW*)	10.671	17.862	67.388
CAT* (mmol/g^{-1} FW*)	0.000271	0.000167	-38.376
SOD (unit/g^{-1} FW)	149.479	97.246	-34.943
APX (mmol/g^{-1} FW)	0.208	0.107	-48.558

*** =P < 0.05,** (**FW=** Fresh Weight**, GAE**= Gallic Acid Equivalent, **TE**= Trolox Equivalent).

CONCLUSION

In the present study conducted to determine the effects of vermicompost application on plant growth in lettuce, the vermicompost was found to give better results in several parameters. In the plants grown with vermicompost + seedling fresh and dry weights, shoot height, stem diameter, root fresh and dry weights, total leaf number, leaf area, chlorophyll a, carotenoid, total chlorophyll content, total phenolic and total antioxidant content were increased, but chlorophyll b content, leaf temperature, CAT, SOD and APX contents were decreased. At the end of our study, we believe that vermicompost gives better results than control Hoagland nutrient solution and it will be allow producers to produce with less cost.

REFERENCES

[1] Sensoy, S., Abak, K., Dasgan, H.Y. (1996) Effect of equivalent amounts of mineral and organic fertilizer applications on nitrate accumulation, yield and quality in lettuce. *GAP I.* Vegetable Agriculture Symposium. 7-10 may. (in Turkish).

[2] Ersahin, Y, (2007) Acquiring vermicompost products and their application alternatives through agricultural production. Journal of Agricultural Faculty of Gaziosmanpasa University, JAFAG. 24(2), 99-107. (in Turkish).

[3] Karacal, İ. and Tufenkci, S. (2010) New approaches and fertilizer-environment relationship in plant nutrition, Agricultural Engineering VII. Technical Congress, 257-268, 11-15 January, Ankara. (in Turkish).

[4] Tutar, U. (2013) Investigation of antimicrobial activity on some plant pathogens of obtained from the earthworm's vermicompost. Cumhuriyet Science Journal. 34(2), 1-12. (in Turkish).

[5] Atiyeh, R.M., Subler, S., Edwards, C.A., Bachman, G., Metzger, J.D., Shuster, W. (2000a) Effects of vermicomposts and compost on plant growth in horticultural container media and soil. Pedobiologia. 44, 579–590.

[6] Gutie´rrez-Miceli, F.A., Santiago-Boraz, J., Molina, J.A.M., Nafate, C.C., Abdul-Archila, M., Llaven, M.A.O., Rinco´n-Rosales, R., Dendooven. L. (2007) Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (*Lycopersicum esculentum*). Bioresource Technology. 98,2781–2786.

[7] Singh, R., Sharma, R.R., Kumar, S., Gupta, R.K., Patil, R.T. (2008). Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (Fragaria x ananassa Duch.). Bioresource Technology. 99, 8507–8511.

[8] Roy, S., Arunachalam, K., Dutta, B.K., Arunachalam, A. (2010) Effect of organic amendments of soil on growth and productivity of three common crops viz. Zea mays, Phaseolus vulgaris and Abelmoschus esculentus. Applied Soil Ecology. 45(2), 78–84.

[9] Joshi, R., Vig, A.P., Singh, J. (2013) Vermicompost as soil supplement to enhance growth, yield and quality of *Triticum aestivum* L.: a field study. Int J Recycl Org Waste Agric. 2, 2–7.

[10] Joshi, R., Singh, J., Vig, A.P. (2015) Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. Reviews in Environmental Science and Bio/Technology. 14(1), 137-159.

[11] Sinha, R.K., Valani, D. (2009) Agronomic studies of earthworms and vermicompost vis-a`-vis conventional cow dung compost and chemical fertilizers on tomato (*Lycopersicum esculentum*). Vermiculture Project of School of Engineering (Environment), Griffith University, Brisbane, Australia.

[12] Adhikary, S. (2012) Vermicompost, the story of organic gold: a review. Agricultural Science. 3(7), 905–917.

[13] Lim, S.L., Wu, T.Y., Lim, P.N., Shak, K.P. (2015) The use of vermicompost in organic farming: overview, effects on soil and economics. J Sci Food Agr. 95, 1143–1156.

[14] Crowder, D.W., Reganold, J.P. (2015) Financial competitiveness of organic agriculture on a global scale. Proc Natl Acad Sci. 112, 7611–7616.

[15] Atiyeh, R.M., Arancon, N.Q., Edwards, C.A., Metzger, J.D. (2000b) Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. Bioresour Technol. 75, 175–180.

[16] Hernandez, A., Castillo, H., Ojeda, D., Arras, A., Lopez, J. Sanchez, E. (2010) Chilean Journal of Agricultural Research. 70(4), 583-589.

[17] Makkar, C., Singh, J., Parkash, C. (2017) Vermicompost and vermiwash as supplement to improve seedling, plant growth and yield in *Linum usitassimum* L. for organic agriculture. Int J Recycl Org Waste Agricult. 6, 203–218.

[18] Azarmi, R. Giglou, M.T., Talesmikail, R.D., (2008) Influence of vermicompost on soil chemical and physical properties in tomato (*Lycopersicum esculentum*) field. African Journal of Biotechnology. 7(14), 2397-2401.

[19] Haghighi, M, Barzegar, MR, da Silva, JAT. (2016) The effect of municipal solid waste compost, peat, perlite and vermicompost on tomato (*Lycopersicum esculentum* L.) growth and yield in a hydroponic system. Int J Recycl Org Waste Agricult. 5, 231–242.

[20] Mahmoudi, S.V.R., Nasri, M., Azizi, P. (2016) The effect of different type of vermicompost organic fertilizer litter on quantitive, qualitative and biochemical characteristics of green mung bean (*Vigna radiata* L.) in drought stress conditions in Varamin. International Journal of Advanced Biotechnology and Research. 7, 205-215.

[21] Abbey, L., Udenigwe, C., Mohan, A., Anom, E. (2017) Microwave irradiation effects on vermicasts potency, and plant growth and antioxidant activity in seedlings of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). Journal of Radiation Research and Applied Sciences. 10(2), 110-116.

[22] Maji, D., Misra, P., Singh, S., Kalra, A. (2017) Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of *Pisum sativum*. Applied Soil Ecology. 110, 97-108.

[23] Nourbakhsh, F., Chalavi, V., Akbarpour, V. (2017) Effect of Vermicompost and Nitroxin on Vegetative Growth and some Biochemical Properties of Rosemary Herb (*Rosmarinus officinalis L.). Majallah-i ʻUlum-i Bāghbānī*. 30(2), 178-184.

[24] Patidar, M., Shaktawat, R.P.S., Naruka, I.S. (2017) Effect of Sulphur and Vermicompost on Growth, Yield and Quality of Garlic (*Allium sativum* L.). Scientists Joined As Life Member Of Society Of Krishi Vigyan, 54.

[25] [25] Singh, U., David, A. A., Sharma, U., Kumar, V. (2017). Effect of different levels of organic and inorganic nutrient sources on growth and yield of mustard (*Brassica juncea* L.). Crop Research. (0970-4884), 52.

[26] Ganeshnauth, V., Jaikishun, S., Ansari, A.A., Homenauth, O. (2018) The Effect of Vermicompost and Other Fertilizers on the Growth and Productivity of Pepper Plants in Guyana. In Automation in Agriculture-Securing Food Supplies for Future Generations. InTech.

[27] Gong, X., Li, S., Sun, X., Wang, L., Cai, L., Zhang, J., Wei, L. (2018) Green waste compost and vermicompost as peat substitutes in growing media for geranium (Pelargonium zonale L.) and calendula (*Calendula officinalis* L.). Scientia Horticulturae. 236, 186-191.

[28] Kusvuran, Ş. (2010) Relationships between Physiological Mechanisms of Tolerances to Drought and Salinity in Melons. PhD thesis. Institute of Natural and Applied Sciences, Cukurova University, Adana, Turkey. (in Turkish).

[29] Kabay, T (2014) Determination of Drought and High Temperature Tolerant and Sensitive Bean Genotypes in Lake Van Basin. PhD thesis) Faculty of Agriculture, Van Yuzuncu Yil University, Van, Turkey. (in Turkish).

[30] Alp, Y. (2017) The effect of drought stress on early plant development in some native and commercial tomato genotypes. (M. Sc. Thesis). Institute of Natural and Applied Sciences, Van Yuzuncu Yil University, Van, Turkey. (in Turkish).

[31] Alp, Y. and Kabay, T. (2017) The effect of drought stress on plant development in some landraces and commercial tomato genotypes. Yuzuncu Yil University Journal of Agricultural Science. 27(3), 391-399. (in Turkish).

[32] Lichtenthaler, H.K. and Wellburn, A.R. (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions. 11, 591 - 592.

[33] Zengin, F.K. (2007) Effects of some heavy metals on pigment content in bean (*Phaseolus vulgaris* L. cv. *Strike*) seedlings. KSU Journal of Science and Engineering. 10(2), 164-172. (in Turkish).

[34] Amira, M.S., Qados, A. (2011) Effect of salt stress on plant growth and metabolism of bean plant (*Vicia faba* L.). Journal of The Saudi Society of Agricultural Sciences, 10, 7-15.

[35] Kabay, T. and Sensoy, S. (2016) Enzyme, chlorophyl and ıon changes in some common bean genotypes by drought stress. Yuzuncu Yil University Journal of Agricultural Science. 26(3), 380-395. (in Turkish).

[36] Swain, T. and Hillis, W.E. (1959) The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 10(1), 63-68.

[37] Benzie, I.F. and Strain, J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay.Analytical biochemistry. 239(1), 70-76.

[38] Jebara, S., Jebara, M., Limam, F., Aouani, M.E. (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. Journal of Plant Physiology. 162(8), 929-936.

[39] Belliturk, K., Hinisli, N., Adiloglu, A. (2017) The effect of vermicompost, sheep manure, and cow manure on nutrition content of curly lettuce (Lactuca sativa var.). Fresen. Environ. Bull. 26, 1116-1120.

[40] Boran, D., Namli, A., Akca, M.O. (2017) Determination of quality parameters of vermicompost under different thermal techniques. Fresen. Environ. Bull. 26, 5205-5212.

[41] Akbassova, A.D., Sainova, G.A., Aimbetova, I.O., Sunakbaeva, D.K., Akeshova, M.M. (2016) Impact of polyfunctional vermicompost on the productivity of vegetable root crops. Fresen. Environ. Bull. 3755.

[42] Abdimutalip, N., Toychibekova, G., Mehmet, A.O., Oshakbayev, M. (2014) Management of agricultural wastes and soil neutralization by vermicomposting with californian red worms. Fresen. Environ. Bull. 23, 640-644.
[43] Wróbel, S. (2010) Sunflower yields as an indicator of zinc polluted soil detoxification. Fresen. Environ. Bull. 19, 330-334.

Received: **30.08.2018**
Accepted: **27.11.2018**

CORRESPONDING AUTHOR

Turgay Kabay
Ercis Vocational School,
Van Yuzuncu Yil University,
Van, Turkey

e-mail: tkabay@yyu.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9949-9953 Fresenius Environmental Bulletin

STUDY ON THE ADVANCED TREATMENT TECHNOLOGY OF COAL GASIFICATION WASTEWATER

Meichang Zhang*, Baoshan Jia, Feng Zhang

Liaoning Technical University, Fuxin, 123000 China

ABSTRACT

Coal gasification wastewater (CGWW) is major waste stream resulting from a number of activities of the low/medium temperature gasification unit that occurs during the production of natural gas. The resulting effluent contains a broad spectrum of organic and inorganic contaminants and exerts a negative influence on the environment, mainly due to the presence of toxic and refractory compounds. So far, various technologies have been applied for treatment of CGWW. The coagulation and sedimentation combined with ozone oxidation technology are applied for advanced treatment of CGWW. The central composite design model was used to investigate the influence of influencing factors on the treatment effect. After 60 min of treatment, COD can be reduced to 30.6 mg/L and UV_{254} to 0.175. After deep treatment by coagulation-ozone combined technology, the combined removal rates of COD and UV_{254} were 97.6% and 99.0%, respectively, and the effluent indicators reached the first-level discharge standard of Integrated Wastewater Discharge Standard (GB 8978-1996). The treatment strategy is handy in treating CGWW.

KEYWORDS:
CGWW, Pretreatment processes, Advanced processes, COD removal, UV_{254}

INTRODUCTION

Coal plays an important role in meeting global energy demands. Coal chemical industries supply 41% of the global energy demand through coal combustion [1-3]. In addition, coal is an important part of primary energy source in China, accounting for about 65–70% of total primary energy consumption [4]. It is predicted that proportion of coal in energy consumption will be over 50% by 2030 and around 30% by 2050 [5]. Recently, researches about new technologies for the utilization of coal become a hotspot [6]. Coal has been considered as an alternative energy of traditional gas and oil [7] and has been used to produce many valuable chemicals via coal gasification, liquefaction and coking [8]. Among them, coal gasification sees plenty of potentials in most of developing countries. In the next few decades, coal gasification will be an important member in the green and renewable market due to the transition of energy structure.

However, considerable expansion of such business brought a side-effect since a significant increase of amounts of CGWW generated from a number of activities of the low/medium temperature gasification unit. During the coal-to-gas process, condensate water (coming from spray cooling system) and gas washing wastewater were the main contributors to CGWW. Pollutants in CGWW stepped from decomposition of the coal, which mainly contained carbon, hydrogen, oxygen, nitrogen and sulfur. It was worth mentioning that one kind of wastewater, called coking wastewater, had similar compositions to CGWW, while the major difference between them was about their origin. Coking wastewater mainly came from residual ammonia water formed in the process of gas cooling, stream generated from coal gas purification and wastewater produced from refining of oil or crude benzene and recovery of products. Compared with coking wastewater, the CGWW seemed more complex. As the focus inclined to new types of coal chemical industry (coal-to-gas), it was unavoidable to generate CGWW. To this end, the governments worldwide put forward strict standards of CGWW access to environment. With the improvement of imposed requirements and legal penalties for non-compliance [9] (Djukic et al., 2016), wastewater was expected to be a reused and renewable resource to sustain human life (Siegel, 2008). From 1970s to 1990s, the US made unremitting efforts to deal with CGWW in order to alleviate the environmental consequences. Since then, the challenge shifted to China because of its basic energy structure of "rich coal, deficient oil and lean gas". Additionally, other countries, such as India, Korea, etc., are coal producers and suffer the similar environmental pollution [10-12], indicating that it is a world issue.

In the last thirty years, scientists have been continually looking for different ways to treat CGWW. Generally, biological treatment (anaerobic, anoxic and aerobic processes) is widely applied to treat CGWW after some pretreatments, such as coagulation and air flotation, while the biological degradation is always inhibited by refractory and toxic compounds and not capable of producing effluents that comply with the wastewater discharge standard. As

a result, further advanced treatment of CGWW, such as catalytic ozonation, Fenton oxidation, etc., is required to meet the increasing strict standard.

However, most of these methods are either limited by their efficacy or due to cost ineffectiveness. The coagulation/flocculation process can be used as an alternative in pharmaceutical wastewater treatment scenarios due to their feasibility and cost effectiveness [13]. In this process, chemicals addition changes the physical state of dissolved and suspended solids, and promotes the elimination of these solids by precipitation. Coagulation treatment has also been carried out for the reduction of turbidity and removal of color and pathogens and is effective for the removal of organic matter [14-17].

Ozone oxidation transforms the non-biodegradable material into biodegradable form or CO_2 including the removal of taste, color [18], particles, chemical oxygen demand (COD), total organic carbon (TOC) and increase the biodegradability of the wastewater [19].

Therefore, the efficiency of coagulation/flocculation and ozone oxidation processes in reducing the color, turbidity, and chemical oxygen demand of wastewater to meet the existing legislative guidelines, was tested in this comparative study. The investigation was carried out to determine the suitable type of coagulant, coagulant dosage and also to evaluate the influence of pH on the coagulant efficiency because the coagulation mechanism is related to the pH conditions.

MATERIALS AND METHODS

Chemical coagulation. Coagulants, alum and lime, were used to treat the effluent. Optimal pH and dosages of both the coagulants were determined by jar test method. A defined dose (0.5–1.0 g/L) of coagulant (lime/alum) was added to a series of six samples set each of 1L in reaction vessels and stirred at 80 rpm for 5 min to destabilize the pollutants and 40 rpm for 25 min to allow the collision between particles and their aggregation in bigger size. The experiments are performed at pH 6.9 (i.e. the actual pH of the effluent). After coagulation, the samples were allowed to settle down at room temperature.

Oxidation by ozone. Ozonation was carried out in bubble column reactor made of Perspex. The internal diameter of the reactor was 3.4 cm. A JQ-6M PURETECH model ozone generator, with a maximum ozone production capacity at the rate of 1.1 L/min was used. The gas was nourished into the sample using diffuser stones to treat the 500 mL sample of wastewater. The retention time (ozonation time) varied from 10 to 60 min. All experiments were performed at ambient temperature. The treated samples were withdrawn from the reactor at regular time intervals for analyses. The color absorption was measured at 254 nm.

RESULTS AND DISCUSSION

For Fig.1, surface plot of COD removal between ozonation time and pH is present. It can be seen that the pH of solution drops to acidic range with an increase in coagulant dose. To overcome this problem, the effect of pH on alum coagulation was observed. The pH was maintained in the range of 4–10 using acid (1 M H_2SO_4) or base (1 M NaOH) solutions. COD removal was increased from pH 4 to 6. And the maximum removal (64%) was observed at pH 6 which remained unchanged afterwards. Ozonation time is important for COD removal.

Surface plot of COD removal between settling time and pH is shown in Fig.2. It can be seen that COD removal increased with the increase of settling time, but the COD removal decreased after reaching a certain settling time, because the removed material was redissolved into the solution. Therefore, the optimal settling time ranges from 40 min to 60 min.

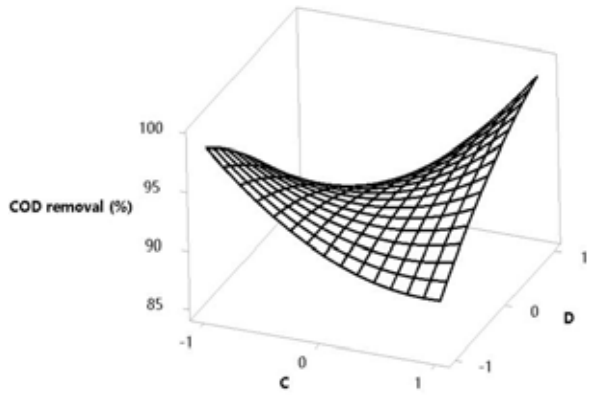


FIGURE 1
Surface Plot of COD removal (%) vs D, C

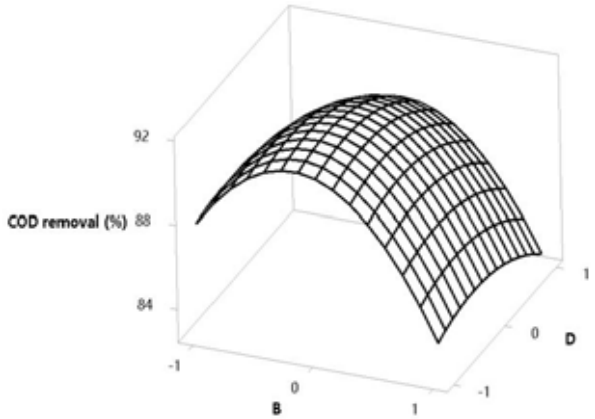


FIGURE 2
Surface Plot of COD removal (%) vs D, B

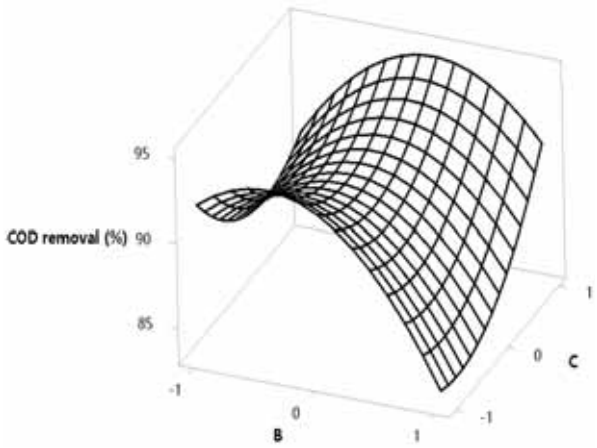


FIGURE 3
Surface Plot of COD removal (%) vs C, B

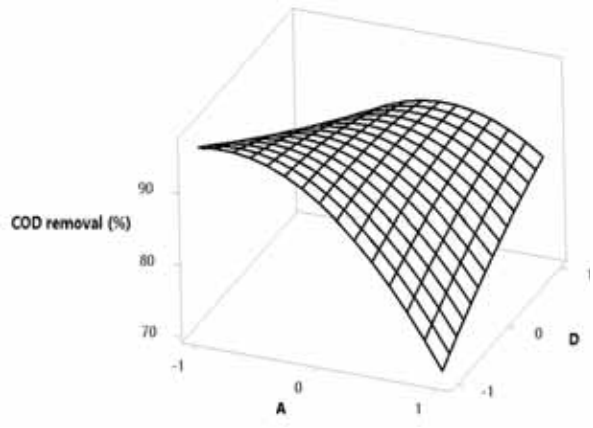


FIGURE 4
Surface Plot of COD removal (%) vs D, A

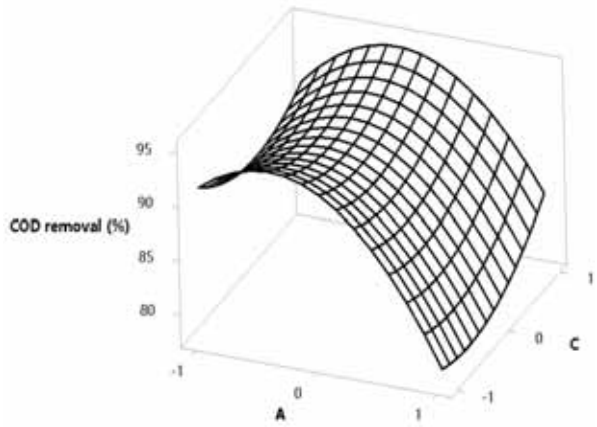


FIGURE 5
Surface Plot of COD removal (%) vs C, A

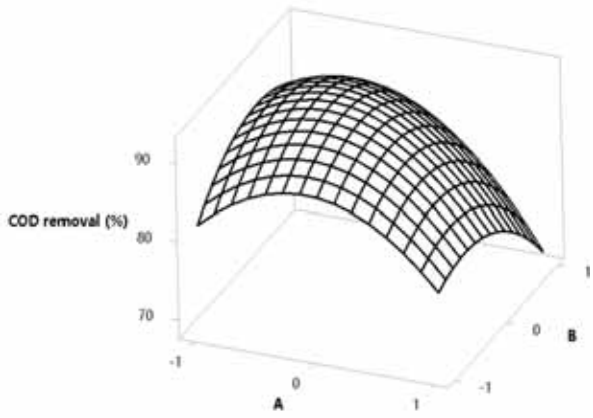


FIGURE 6
Surface Plot of COD removal (%) vs B, A

Surface plot of COD removal between settling time and ozonation time is shown in Fig.3. It can be seen that COD removal increased with the increase of ozonation time. At the initial stage of ozonation, ozone generated hydroxyl radical that react with double bond or functional group of organic compounds present in wastewater causing incomplete oxidation, generating ozone oxidation resistant intermediates resulting in high color removal because the oxidation potential of hydroxyl ion is greater than the molecular ozone. More and more ozone reacts with organic matter in the reaction system, and macromolecules are difficult to degrade organic matter to be gradually oxidized into small molecular organic substances, CO_2 and H_2O. When the reaction time is 60 min, the ozone dosage is 200 mg / L, and the COD removal rate reaches the maximum and tends to be balanced and stable. After ozone oxidation, the appearance of effluent water is clear and odorless. The COD in wastewater can be reduced to 30.6 mg / L, and the removal rate of ozone oxidation COD is as high as 84.7%.

Surface plot of COD removal between coagulant dose and pH is shown in Fig.4. It can be seen that COD removal increases with pH gradually, showing a trend of increasing first and then decreasing. When the pH is 6.5, the COD concentration is reduced from the initial 600 mg / L to 240 mg / L, and the removal rate reaches 60%, so choose 6.5 as the best coagulation reaction pH.

The coagulant dose was gradually increased, and the COD removal rate of the treated wastewater was gradually increased. When the concentration is insufficient, the potential drop of the colloidal particles is small, and it is impossible to effectively collide to form larger particles, and increasing the concentration is advantageous for the formation of particles. When the dosage is 400 mg / L, the removal rate of each indicator is no longer significantly increased. The COD concentration was reduced from the initial 650.0 mg / L to 210 mg / L, and the removal rate was 67.7%. Therefore, from the economic point of view, the optimal dosage was 400 mg/L.

Fig. 5 shows effect of coagulant dose and ozonation time on COD removal. Ozonation showed 13.6% reduction in COD of un-buffered samples after 60 min. The ozonation was carried out at original pH of wastewater (pH 6.9). Ozonation is a pH and composition dependent reaction process. Generally ozonation carried out through direct molecular ozone reaction pathway in acidic pH and radical

chain type reaction pathway in basic pH range. 10% COD was removed within first 10 min and incremental improvement was observed afterwards. It was observed that ozone oxidation is favorable only for buffered samples. The pH of wastewater was not controlled and it was dropped in this type of oxidation.

Fig. 6 shows effect of coagulant dose and settling time on COD removal. It can be seen that coagulant dose was gradually increased, and the COD removal rate of the treated wastewater was gradually increased. When the concentration is insufficient, the potential drop of the colloidal particles is small, and it is impossible to effectively collide to form larger particles, and increasing the concentration is advantageous for the formation of particles.

Ozone oxidation effect of coagulation sediment supernatant. The effluent after coagulation treatment was determined to be COD 210 mg / L, and p H was in the range of 4 to 6. Ozone gas with a gas flow rate of 1 L/min was set in a reaction column containing 500 m L of coagulation sedimentation supernatant, and ozone oxidation treatment was carried out according to the method described in the above test. The wastewater COD and UV_{254} were measured and tested. The result is shown in Fig. 7.

The coal gasification wastewater treated by ozone oxidation needs to adjust the wastewater p H to 6 to 9 with 0.1 mol/L sodium hydroxide.

It can be clearly seen in Fig. 7 that after the coagulation treatment, the supernatant was subjected to O_3 oxidation treatment, and the COD and UV_{254} in the supernatant had obvious removal effects. When the reaction time was 10 min, the UV_{254} removal rate gradually became stable. When the reaction time was 50 min, the ozone dosage was 400 mg / L, and the COD removal rate reached the maximum and became stable and stable. The effluent of the effluent after the oxidation of the water is clear and odorless, the COD in the wastewater can be reduced to 30.6 mg / L; the UV_{254} is reduced from the initial 2. 22 to 0. 175, and the removal rate is 92.1%. After the treatment by the coagulation-ozone combination, the combined removal rates of COD and UV_{254} were 97.6% and 99.0%, respectively.

CONCLUSION

The present study was aimed at treating pharmaceutical industry wastewater employing chemical coagulation and ozonation process. Coagulation was carried out by alum and lime. Parameters like coagulant dose, settling time and pH ranging from 0.5 to 1.0 g/L, 10 to 60 min and 4 to 10 respectively were investigated. After 60 min of treatment, COD can be reduced to 30.6 mg/L and UV254 to 0.175. After deep treatment by coagulation-ozone combined technology, the combined removal rates of COD and UV254 were 97.6% and 99.0%, respectively, and the effluent indicators reached the first-level discharge standard of Integrated Wastewater Discharge Standard (GB 8978-1996). The treatment strategy is handy in treating CGWW.

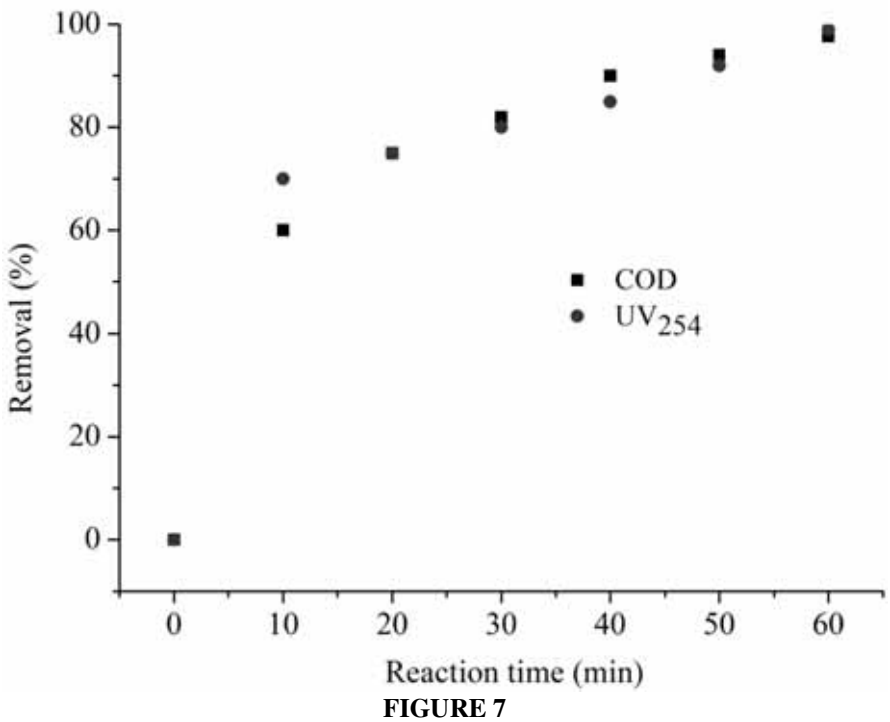


FIGURE 7
Effect of reaction time on the effect of ozone oxidation on COD and UV_{254}

REFERENCES

[1] Wang, Y., Wang, S., Guo, Y. (2013) Evaluation of The Combined Processes of Supercritical Water Oxidation and Gasification for Lurgi Coal Gasification Wastewater Treatment. Fresen. Environ. Bull. 22, 2695-2700.

[2] Xu, P., Xu, H. (2018) Enhanced A(2)/O Process For Treatment Of Heterocyclic And Polycyclic Aromatic Hydrocarbons In Coal Gasification Wastewater. Fresen. Environ. Bull. 27, 5119-5124.

[3] Zhu, H., Han, Y., Xu, C., Han, H., Ma, W. (2018) Overview of the state of the art of processes and technical bottlenecks for coal gasification wastewater treatment. Science of The Total Environment. 637–638, 1108-1126.

[4] Song, M., Wang, J., Zhao, J. (2016) Coal endowment, resource curse, and high coal-consuming industries location: analysis based on large-scale data. Resour. Conserv. Recycl. 129, 333-344.

[5] Chang, S., Zhuo, J., Meng, S., Qiang, S. (2016) Clean coal technologies in China: current status and future perspectives. Engineering. 2, 447-459.

[6] Yang, S., Qian, Y., Liu, Y., Wang, Y., Yang, S. (2017) Modeling, simulation, and techno-economic analysis of Lurgi gasification and BGL gasification for coal-to-SNG. Chem. Eng. Res. Des. 117, 355-368.

[7] Zhou, L., Ren, X., Zhang, X. (2012) Policies for coal-to-liquids industry in China. Chem. Ind. Eng. Prog. 31, 2207-2212.

[8] Zhao, Q., Liu, Y. (2016) State of the art of biological processes for coal gasification waste-water treatment. Biotechnol. Adv. 34, 1064-107.

[9] Djukic, M., Jovanoski, I., Ivanovic, O.M., Lazic, M., Bodroza, D. (2016) Cost-benefit analysis of an infrastructure project and a cost-reflective tariff: a case study for investment in wastewater treatment plant in Serbia. Renew. Sust. Energ. Rev. 59, 1419-1425.

[10] Havelcová, M., Melegy, A., Rapant, S. (2014) Geochemical distribution of polycyclic aromatic hydrocarbons in soils and sediments of El-Tabbin, Egypt. Chemosphere. 95, 63-74.

[11] Kim, Y.M., Park, D., Lee, D.S., Jung, K.A., Park, J.M. (2009) Sudden failure of biological nitrogen and carbon removal in the full-scale pre-denitrification process treating cokes wastewater. Bioresour. Technol. 100, 4340-4347.

[12] Saikia, K., Sarkar, B.C. (2013) Coal exploration modelling using geostatistics in Jharia coalfield, India. Int. J. Coal Geol. 112, 36-52.

[13] Mo, J., Hwang, J.-E., Jegal, J., Kim, J. (2007) Pretreatment of a dyeing wastewater using chemical coagulants. Dyes Pigm. 72(2), 240–245.

[14] Huerta-Fontela, M., Galceran, M.T., Ventura, F. (2011) Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Res. 45(3), 1432–1442.

[15] Le-Minh, N., Khan, S.J., Drewes, J.E., Stuetz, R.M. (2010) Fate of antibiotics during municipal water recycling treatment processes. Water Res. 44(15), 4295–4323.

[16] Matilainen, A., Vepsäläinen, M., Sillanpää, M. (2010) Natural organic matter removal by coagulation during drinking water treatment: a review. Adv. Colloid Interface Sci. 159(2), 189–197.

[17] Verma, A.K., Dash, R.R., Bhunia, P. (2012) A review on chemical coagulation/ flocculation technologies for removal of colour from textile wastewaters. J. Environ. Manage. 93(1), 154–168.

[18] Adams, C.D., Gorg, S. (2002) Effect of pH and gas-phase ozone concentration on the decolorization of common textile dyes. J. Environ. Eng. 128(3), 293–298.

[19] El-Din, M.G., Smith, D.W. (2002) Ozonation of kraft pulp mill effluents: process dynamics. J. Environ. Eng. Sci. 1(1), 45–57.

Received: **31.08.2018**
Accepted: **21.10.2018**

CORRESPONDING AUTHOR

Meichang Zhang
Liaoning Technical University,
Fuxin, 123000 – China

e-mail: 1845400337@qq.com

© by PSP Volume 27 – No. 12B/2018 pages 9954-9961 Fresenius Environmental Bulletin

CURRENT SITUATION IN MEDITERRANEAN GREENHOUSES AND A STRUCTURAL ANALYSIS EXAMPLE (MERSIN PROVINCE)

Burak Saltuk*

Siirt University, Agriculture Faculty, Biosystem Engineering Department, 56100 Siirt, Turkey

ABSTRACT

Greenhouse cultivation is one of the most important income generating branches of agriculture. Nowadays, computer softwares are used for anything as it is being used for planning greenhouses, more robust construction and economical results are obtained this way. Business owners, investing their money in greenhouses, are copying the structural features of existing greenhouses with all wrong calculated parameters and errors. Leaving their valuable cash and future of their investment in the hands of an iron-smith. As a result, the greenhouses which are built without static and strength calculations, more materials are used, or insecure constructions are being applied. When an economic loss occurs depending on structural damage it will unavoidably lead to economic losses for farmers and implicitly for the country. This study, emphasises on the structural analyses of a two-span gothic roofed plastic covered greenhouse, having an area of 900 m^2 located in Mersin province. Structural analysis of the greenhouse was made with SAP2000 program. Mechanical properties of steel used in gothic roofs, plastic covered greenhouse's, theoretical load calculations are made depending on the TS 498 and TS EN 13031-1 Turkish standards.Variable loads on the greenhouse are calculated as distributed loads with classical methods by analyzing the gothic roofed plastic covered greenhouse according to load combinations (wind, plant, fixed) with SAP2000 program.

KEYWORDS:
Greenhouses, Structural Analysis, Mediterranean, SAP2000

INTRODUCTION

A greenhouse is an agricultural structure that can cultivate plants economically during periods, even when the natural environment is not suitable and can provide growth factors needed for plant production and allow mechanization The production of vegetables and fruits in the greenhouse has a broader place in answering the increasing food demand. [1].

No matter how broad agricultural areas are spread, the agriculture sector seems distant from answering the needs of the human population. Therefore about 795 million people are undernourished globally [2].

The increase in crop production is possible with continuity. In the next century, the impact of greenhouse production will increase further, depending on the climatic changes and increasing food demand. However, if greenhouses are built in unsuitable climatic conditions it will eventually lead to loss instead of profit. In recent decades, with the proliferation of plastics all over the world greenhouses are spread all over the world. Site selection is a crucial factor for profitable and sustainable greenhouse cultivation. [3].

A wide variety of greenhouse structural selections are available, from simple plastic houses to very sophisticated glasshouses. To supply high-quality vegetables year-round in the greenhouse packages commonly used for that purpose are with reference to the Mediterranean basin [4]. Decreasing and pollution of natural water resources gradually as a result of global warming forces growers to use marginal quality waters in irrigated agriculture [5]. The intensive cultivation and excessive inorganic fertilizer application in green-house agriculture, causes serious soil-based envi-ronmental problems [6].

Since air temperature and humidity are the two significant parameters affecting thermal comfort significantly, and an evaporative cooling system can handle only sensible load, the conventional evaporative cooling system is suitable for the dry and temperate climate where the humidity is low [7].

Most plants grown in greenhouses are warm-season species, adapted to average temperatures in the range 17–27 °C, with approximate lower and upper limits of 10 and 35 °C. If the average minimum outside temperature is < 10 °C, the greenhouse is likely to require heating, particularly at night. When the average maximum outside temperature is < 27 °C, ventilation will prevent excessive internal temperatures during the day; however, if the average maximum temperature is > 27–28 °C, artificial cooling may be necessary. The maximum

greenhouse temperature should not exceed 30–35 °C for prolonged periods [8]. High temperatures may cause in increased activity of pests along with sunburns, that could cause economic hazards, for example *Tetranychus cinnabarinus*, which is one of the most economically important pests of greenhouse-grown vegetables and ornamentals in the southwestern part of Turkey /Antalya [9]. Therefore, it is concluded that plastic house shading using might potentially reduce insect infestation and improve fruit yield and quality in cucumber during summer time [10].

There is no doubt that operating costs come to the forefront of production. Especially in the plant growing season, more utilization of natural light and heat is essential to decrease the costs. For this, besides the greenhouse construction, the covering material, the position of the greenhouse (orientation), the climate characteristics of the region are also influential. Greenhouses should be built peculiar to climatic characteristics of zones they will be established. For example, in a region with a tropical humid climate, where protection from the rain is the greenhouse's main purpose (prevalence of the umbrella effect), the type of construction preferred may be different from that desirable in a semi-desert or Mediterranean climate region [11].

Speaking of which, in today's world agriculture sector is using plastics intensively (plastic cover materials, chemical fertilizers and pesticide boxes, post-harvest wastes and drip irrigation laterals etc..Through conscious use and disposal of materials, especially like plastics the damages that agricultural wastes will pose on nature, soil and water resources will be minimized [12].

Mediterranean region have considerable advantages to build greenhouses because the nighttime and daytime temperature differences are very low, the number of frosty days are minimal and snowy days are rare. This way greenhouse heating costs can be kept to a minimum during the winter season. But, farmers avoid some of key factors such as greenhouse constructions, lighting, ventilation and heating in the greenhouses to avoid initial investment costs. As a result, production decreases and sometimes due to weak construction materials, greenhouses may collapse [13].

[14], point out that greenhouses are costly agricultural structures and due to this, modern day technology must be projected in greenhouses, in recent years there has been an absolute increase in Turkey's greenhouse area, but at the same time, many problems arise due to the wrong applications of modern technology, such as carrier construction, ventilation, heating, cooling, shading, irrigation, air conditioning and so on, many topics can be counted.

A study [15] showed that changing conditions increased the need for greenhouse farming, but also emphasized the importance of the properties that carrier materials should have in greenhouse constructions. The increase in the income from the greenhouses attracts more people and especially the farmers who carried out their agricultural activities in the form of family businesses. As a result, investments in greenhouses have begun to increase day by day. At this point; for detached greenhouses, various profiles any greenhouse structure is constituted by determining appropriate structure system directed towards medium sized foundations. And for each structure, the amount of steel equipment and structure is defined by graphics. By this way, a significant facility is provided for designer and producer. And furthermore, the applicator can reach the preliminary data about the optimum investment cost of greenhouses.

Unfortunately, the construction materials selected for the construction of these greenhouses are not given enough care regarding types, sections, and properties, support, and installation. Even establishments that provide loans to greenhouses do not take any notice if these greenhouse projects are prepared properly or not, some of them can provide loans based on copy projects various means.

This study proposes a solution, to construct proper greenhouses with efficient material durability with true quantity. So this way economic harms could be eliminated long before they occur.

MATERIALS

The greenhouse sites in Mersin province starts from central parts and longs to the coasts of the western side of Mediterannean part. Tomato, pepper, and cucumber are mainly cultivated. Even though the ecological conditions of these areas are favorable, the quality of the product is often low because of the poor production techniques. Despite these, greenhouse investments are spreading. Mersin greenhouse production has shown much improvement in recent years [16]. The intensity of exports, market shares, climate conditions are all important reasons for this development.

In Mersin Province, plastic covered, spring or autumn production with gothic glazed greenhouses are used. For the analysis with software, considering the continuity of the upper and lower head during the application phase in the modeling, the bars were considered to be rigid bound. The load values found as a result of these calculations are combined, and the SAP2000 program has been used to affect the loads in combinations [17]. The current state of the selected greenhouse was compared with the obtained data and the profile characteristics (width, length, length) and post - analysis profile characteristics of the investigated greenhouse. As a result of the comparisons, it was determined that there is no significant difference between the amount of material used in the greenhouse examined and the

amount of material reached after the analysis made with SAP2000 program. However, it has been observed that the security of the greenhouse has fallen into danger because of the erroneous detection of sections of the carrier systems.

TABLE 1
Greenhouse Presence in Mersin Region and Turkey for (2013-2017) [16]

		Mersin	Turkey
Low tunnel (da)	2013	21925	157737,4
	2014	23017	156720
	2015	26067	161541,1
	2016	24965	169867,3
	2017	30066	191399,1
Glass Greenhouse (da)	2013	6343	80739,4
	2014	6472	80975,7
	2015	5970	79976,9
	2016	6300	80137,1
	2017	13763	85748,9
Plastic Greenhouse (da)	2013	72864,9	278661,3
	2014	75253,4	298651
	2015	73184,4	306073,7
	2016	79421,4	328745,4
	2017	87815,5	355120,9
High tunnel (da)	2013	47640,7	97986,4
	2014	54103,2	107095,4
	2015	57287,5	112673,6
	2016	56483,8	112973,6
	2017	58402,8	119898,7

The open space of the two-span plastic gothic roof is 19.70 m, the distance between the scissors is 6.50 m, and the roof slope angle is $\alpha = 26^{o}$. Greenhouse element lengths are cm and cross-section measurements are mm. When the greenhouse dimension analysis is performed, it is determined that it has 45.00 m length, two spans gothic types, 4.00 m height column, and 90 cm depth on a constant basis. The greenhouse was built in 2011.

METHODS

The research was carried out in three stages, locating of glass and plastic greenhouses in the mentioned area, in-situ examination and office work. Glass and plastic covered greenhouse areas in Mediterranean province, and locations where greenhouses are concentrated especially are appointed with data gathered from provincial and district directorates of agriculture. In this way, the concerned plastic covered, and gothic roofed greenhouse located in Mersin province is chosen for greenhouse structural analysis. Mersin has an essential share regarding greenhouse production in Turkey. Situ measurements have determined the structural elements of the selected greenhouse. Besides, the profiles, types and, sizes of the greenhouses, the conditions (structural features, material properties, roof system, cover material) and their qualifications for cultivation have been determined in the field works. The general principles are applied in the selection of the subdivisions, and greenhouses with different roof and construction characteristics are determined. According to the loads on the platform, the loads on the calculation and projecting of the sections of the tension and compression rods are considered. In the study, the moving loads on the selected greenhouses, simulated with meteorological observation records between 1980 and 2015 taken from the Meteorology Directorate's. Possible [18]. wind velocities and other climatic data in the study area are considered as projecting criteria in the SAP2000 program. The calculation with SAP2000 computer program is explained in detail.
The external loads acting on the bearing systems are calculated according to the materials, the slope of the roof. They are transformed into a uniformly distributed load and altogether applied to the structure. As the external load; wind load, plastic cover load, and plant load were picked. SAP2000 is a static analysis program for both steel and reinforced concrete structures and is a general-purpose structural analysis program used for the analysis and sizing of building system models. The program performs the static analysis of the bearing system according to the finite elements method. The loads specified in Table 2. All operations were performed on the SAP2000 screen with the help of the particular "Graphical User Interface."

According to this;

Fixed loads (H): Core loads (roof weight, plant load)

Moving loads: (HZ): Wind effect; horizontal and lateral wind forces, loads occurring in the installation stages,

The loads applied to the system are added to the system through specific loading situations. These loading states are G, Q, RXP, RXN, RYP, RYN. Shown in Table 2.

FIGURE 1
Steel Level of Stretch Ratio

TABLE 2
Assignment of Loads from Carrier Systems

Snow Load	The coastal areas where the greenhouse is heavily built have not been considered because of the lack of snow [19-20-21].
Wind Load	The highest wind speed measured in Mersin province in February is 34,2 m/s; and taken as $q = \frac{34,2^2}{1600} = 0,74\ kN/m^2 = 74\ kgf/m^2$ [18-20]
Earthquake Load	Earthquake load was not considered in the study because of the small total weight in greenhouse constructions [19].
Cover Load	A load of 250 g / m^2 for 100-micron thick polyethylene (PE) material [19-20]
Plant Load	The plant load for the greenhouse is 15 kg / m^2 [20-21]
Carrier System Weight	ST37 soft structure steel has a unit volume weight of 7.85 t / m^3, a tensile strength of 3.700 kg / cm^2, a yield limit of 2.400 kg / cm^2 and an elasticity modulus of 2.100.000 kg / cm^2. The SAP2000 program calculates the structural weight per unit volume weight according to the cross sections entered [19-20]

• G: Constant load (H):

• Q: Plant load; Plant weights for model greenhouses were taken at avarage15 kg / m^2

• Moving load (Hz):

• RXP: Wind load in + X direction

• RXN: Wind load in the -X direction

• RYP: Wind load in + Y direction

• RYN: Wind load in -Y direction

The SAP2000 program automatically calculates the vertical building loads by taking advantage of the unit volume weight of the materials used. As a result, greenhouse structure is modeled originally regarding carrier system and load distribution. This situation allowed to make a real calculation. These loads, which are defined and added as distributed loads to the elements, are added to the calculations at specific rates by way of combinations. Accordingly, six different loading combinations were created. The program worked according to these load groups. In the event of the most adverse conditions occurring during the analysis, the results are assessed.

C0: G + 0.3Q (constant + 0.3 moving load)

C1: G + Q (constant + moving load)

C2: G + Q + RXP (constant + moving load + + wind load in X direction)

C3: G + Q + RXN (constant + 0.3 moving load + wind load in -X direction)

C4: G + Q + RYP (fixed + moving load + + wind load in Y direction)

C5: G + Q + RYN (constant +0.3 moving load + wind load in -Y direction)

American AISC-ASD89 standarts and Turkish standarts TS 498 and TS EN 13031-1 are the same. These standarts are selected as the steel design directive. In this regulation, the capacity ratio (Demand / Capacity Ratio Limit) is set to 1. This value can be summarized as the ratio of the most negative stress (kN/m^2) to the steel safety stress (kN/m^2) obtained as a result of the combinations. This ratio should be between 0 and 1 (Figure 1). It is seen that the color scale has the highest steel level of stretch ratio with red color and very little tension in gray color. Therefore, it can be said that the section defined in cases where the tensile ratio is closer to 1 or greater than 1 is insufficient when it is 0, no stress occurs, and between 0.5 and 0.7, it is the optimum section stress.

The primary purpose of dissolving the widely used greenhouse samples in the region is to show that by using the SAP2000 structural analysis program, it is possible to study in detail the exceptional points in the conveying system, the complex junction details and the critical regions to which large loads are transferred, and more accurate results can be achieved.

The applied method is explained in the research findings of selected parameters for design, types of sections, loads, methods of calculating load components, programs used, analysis results according to models, load curves for the equations and components used in dimensioning, section effects and stresses. EUROCODE for the sections used for greenhouse construction. The analysis was carried out with the profile (IPN, HEA, T, UPN, L, 2L) sections selected here. With the SAP2000 program, the horizontal and lateral forces at the joints and stresses that can occur in XZ and YZ axes can be easily seen on the screen. The analysis and the dimensioning of the bearing systems are made according to the flow diagram given in the study.

RESULTS AND DISCUSSION

The sections used in the construction were found in the current situation by making measurements in the greenhouse, and the baseline loads were found by using the information in the method section. SAP2000 program analyzed the gothic roofed plastic glazing greenhouse according to load combinations (wind, plant, fixed). The loads on the greenhouse are calculated as the distributed load with the classical method. These values are; taken into consideration as load values in the SAP2000 program. Considering the characteristics of the building; the values required for load analysis are taken from recalculations of earlier literature. It is also assumed that the roof and side ventilation openings of the greenhouse are closed. Considering

the continuity of the upper and lower head during the application phase of the modeling, the bars were rigidly bound (Figure 2).

Modeling was done in SAP2000, and cross-sectional effects were obtained. As a result of the analyzes, the compression, tensile and bending stresses in the steel profile covered with plastic material are shown in SAP2000. (Figure 3)

In the SAP2000 program, the load characteristics of the load combinations were determined by acting on the short and long axis of the greenhouse regardless of the direction of the wind effect. Strain ratios in greenhouse columns are well above the permissible limit. The strain rate can be expressed as the ratio of the most negative stress to the steel safety stress as a result of loading combinations. The desired stretch ratio is between 0 and 1 (Figure 4).

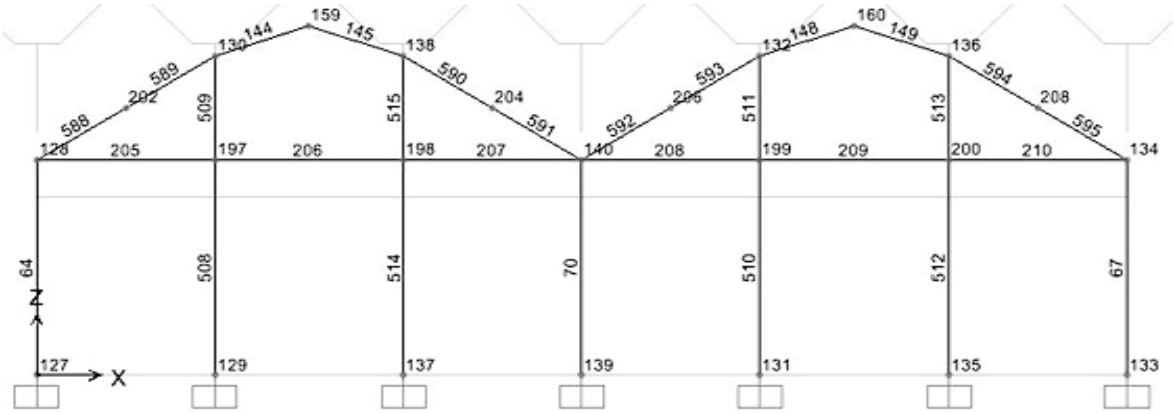


FIGURE 2
The numbering of Gothic Roofed Greenhouse Construction Elements

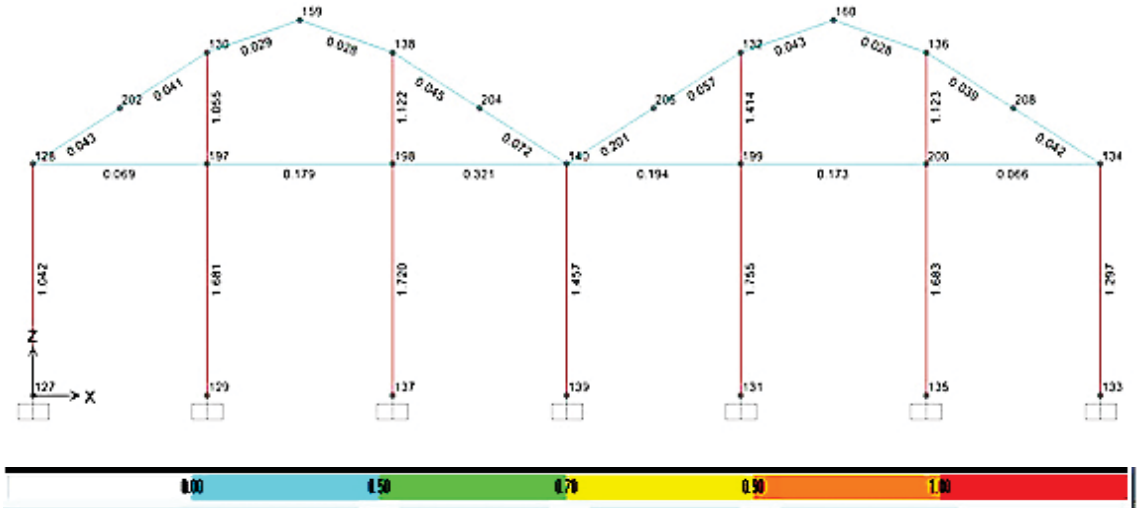


FIGURE 3
SAP2000 Program (RXP Direction) Steel Level of Stretch Ratio

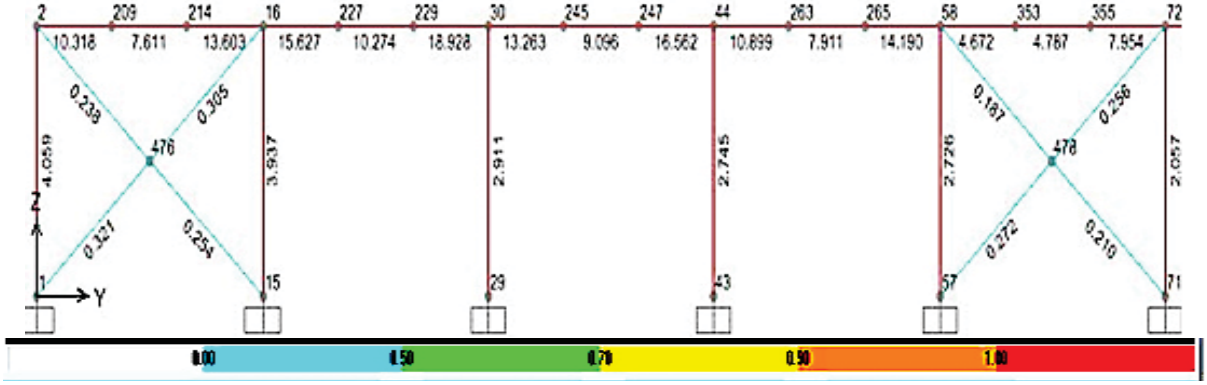


FIGURE 4
SAP2000 Program (RYP Direction) Steel Level of Stretch Ratio

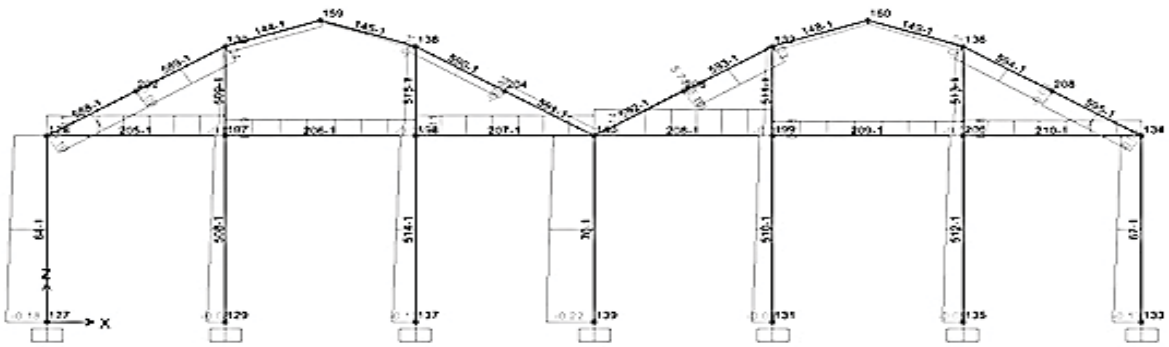


FIGURE 5
Demonstration of Spreading Loads on the Short Axis (XZ) of Gothic Roof Greenhouse in SAP2000 Program

The connections of the columns of the upper and lower heads were made as joints and regulated. When all the structural members were considered rigidly connected, it was observed that the cross sections of the upper and lower head beams were enough. The wind load on the greenhouse is effective both in the short and the long axis. Wind load was determined to be active on the greenhouse, depending on the height of the greenhouse and the length of the windshield area. (Figure 5)

As a result of SAP2000 analysis, a structural element which is insufficient in Figure 4,5,6 is shown. In this element, which is defined as a bifurcated rigid, only a force is generated in the axial direction, and therefore, tension is generated in the axial direction. Fa = 919.118> Fe = 756.694. (K * L / r). In this case, the cross-section can be replaced with the upper sections or the length (L) can be shortened with intermediate members (such as tensioner, diagonal). According to the analysis results obtained with SAP2000; this ratio is seen like 2 or more in the columns. Therefore, it has been determined that the identified sections are insufficient. It was determined that it would be economical to construct 2 ½ "(B76,1X3,25) steel pipes used on rooftops with 2" (B60,3X3,25) steel pipes after analysis. In the present case in the column section, 3 "(B88, 9x4) light series are used. In the present case, colonization needs to be improved. 3 "medium (TS301 / 3) or thick series (TS301 / 4) can be used to provide sufficient strength. Also, if a different profile can be used, 4 "(B114,3x4) light steel tubing must be used in the columns. As a result of the analysis made with SAP2000 program, the top headings of the greenhouse are not changed regarding saving in construction; it is necessary to increase the cross-section of the greenhouse columns. More rigid elements may be preferred over the main bearing elements instead of the pipe profile. HEA or NPU steel profiles. By using crosses in every range, the stiffness of the structure of the structure can be increased. In summary; under specified loads, the specified sections are insufficient.

CONCLUSION

The lack of ready-made greenhouse projects, the lack of knowledge of the necessary structural features and the lack of care during construction make the greenhouses poorly engineered agricultural production structures. Greenhouses are simple structures, making as much use of the existing ecological conditions as possible. This leads to a significant loss of productivity and quality.

To prevent structural damage;

• The use of large cross-section structural construction material reduces both the amount of light entering the greenhouse and the interior columns of the wood or profile used restrict the in-house greenhouse mechanization. To avoid this, the smallest sections that can provide the necessary strength in the plan and the most significant openings that will allow for the mechanization need to be identified.

• Due to easier installation and ease of use of materials in plastic covered greenhouses, bowed roof systems should be preferred.

• According to long years measurements; For Mersin, the highest wind speed was 34.2 m/s with winds swirling west-southwest in February [18]. The long axis should be positioned taking into consideration the prevailing wind direction while planning the runway. Considering the wind load, the sidewall heights in the study area should be at least 2 m and at most 4 m.

• Project selection and construction must be strictly carried out under the supervision of agricultural engineers, for greenhouse construction standards [20-21].

• Applications such as heating, irrigation, fertilization, ventilation, and construction planning, which require high technology and therefore require high installation costs, should be automated and controlled by the computers and current programs.

• Appliances made by public or private companies; must have a plastic or glass covered spring or cradle roof and automation that is up-to-date, conforming to European Union standards and re-

gional climatic conditions. [21-22].

REFERENCES

[1] Baytorun, A.N. (1995) Greenhouses. Cukurova University Faculty of Agriculture Books Publication No.110 Adana, Turkey 1-374.

[2] Anonymous, (2015) FAO Reports the State of Food Insecurity in the World http://www.fao.org/3/a-i4646e.pdf Accessed 03.05.2018 1-62

[3] Zabeltitz, C.V. (2011) Integrated Greenhouse Systems for Mild Climates. Springer-Verlag Berlin Heidelberg https://www.kisa.link/LlZf Accessed 1.11.2018

[4] Castilla, N., Hernandez, J. (2007) Greenhouse Technological Packages For High-Quality Crop Production ISHS Acta Horticulture. 761, 285-297

[5] Aydinsakir, K,. Dinc, S., Buyuktas D., Karaguzel U.O., Ozkan, C.F., Vuran F.A. (2018) Effects of Salinity Levels and Substrates on Yield and Flower Quality of Soilless Cultivated Rose Grown in an Unheated Greenhouse. Fresen. Environ. Bull. 27, 1424-1436

[6] Qui, J., Qian, Y., Fenglin, Z., Lvdan, Z. (2017) Impact of Drip Irrigation and Buried Straw Layer on the Soil Cultivated Environment: A Case Study Under Greenhouse. Fresen. Environ. Bull. 26, 5413-5419.

[7] Costelloe B. and Finn, D. (2003) The indirect evaporative cooling potential in air-water systems in temperate climates. Energy and Buildings. 35(6), 573-591.

[8] Kittas, C., Katsoulas, N., Bartzanas, T., Bakker, S. (2013) Good Agricultural Practices for greenhouse vegetable crops principles for Mediterranean climate areas FAO, Rome. 35-63 http://www.fao.org/3/a-i3284e.pdf.

[9] Topuz, E., Madanlar, N., Erler, F. (2012) Evaluation of Fumigant Toxicity of Mentha Pulegium Essential Oil Against Tetranychus Cinnabarinus under Greenhouse Conditions. Fresen. Environ. Bull. 21, 2739-2745.

[10] Araj, S., Kasrawi, M., Alsmirat, N., Othman, Y.,, Salem, N., Tabieh., M. (2018) Influence of Greenhouse Shading Techniques on Pests Numbers, Leaf-Level Physiology, Fruit Yield and Quality of Cucumber. Fresen. Environ. Bull. 27, 5721-5726.

[11] Montero, J., Teitel, M., Baeza, E., Lopez, J., Kacira, M. (2013) Good Agricultural Practices for greenhouse vegetable crops Principles for Mediterranean climate areas FAO, Rome. 63-92 http://www.fao.org/3/a-i3284e.pdf Accessed 01.05.2018

[12] Boyacı, S. (2018) Environmental problems caused by agricultural wastes resulting from greenhouses and high tunnel cultivation and solution suggestions. Fresen. Environ. Bull. 27, 2510-2517.

[13] Saltuk, B. (2005) A Study on the Structural Analysis and Development of Plastic Greenhouses in Mersin and its Provinces. Çukurova University, Agriculture Faculty. MSc Thesis. Adana Adana, Turkey.

[14] Tekinel, O., Baytorun, A.N. (1990) New Technologies in Greenhousing. 5. Greenhouse Symposium, Ege University Faculty of Agriculture, İzmir, 11-20.

[15] Mandalik, A. (2001) Single Span-Steel Construction Determination of the Preliminary Cost of Structures in the Underground Yildiz Technical University Architecture Program. MSc Thesis. İstanbul, Turkey.

[16] Anonymous (2018) Greenhouse Potential and protective cover type for Turkey Turkish Statistical Institute https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr Accessed 05.11.2018

[17] Çağdaş, S. (2004) Modeling of Applied SAP2000 Building Systems Static and Dynamic Analysis. Turkmen Publishing, İstanbul, Turkey, 593p.

[18] Anonymous (2015) Climate values in Mersin Province between 1980 and 2015. Meteorology Directorate network, Adana.

[19] Anonymous (1997) Turkish Standardization Institute TS 498 Design Loads for Buildings, Account Values of Loads to be Taken into Consideration the Dimensioning of Structural Elements. Turkish Standards Institute. ICS 91.040 Ankara

[20] Anonymous (2003) Turkish Standardization Institute TS EN 13031-1 Greenhouses Design and Construction Part 1:Commercial Production Greenhouses ICS 65.040.030 Ankara

[21] Simsek, E. (2014) Greenhouses Production Systems Unit 2 Anadolu University, Eskisehir, Turkey Publication No:2275 ISBN 978-975-06-0949-7.

[22] Montero, J., Teitel, M., Baeza, E., Lopez, J., Kacira, M. (2013) Cultures protegees en climate Mediterranean. FAO, Rome. Good Agricultural Practices for greenhouse vegetable crops Principles for Mediterranean climate areas Page: 37 http://www.fao.org/3/a-i3284e.pdf.

Received: **03.08.2018**
Accepted: **21.11.2018**

CORRESPONDING AUTHOR

Burak Saltuk
Siirt University
Agriculture Faculty
Biosystem Engineering Department
56100 Siirt – Turkey

e-mail: bsaltuk@siirt.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9962-9971 Fresenius Environmental Bulletin

DETERMINATION OF REACTIONS OF CUMHURIYET-75 AND SELIMIYE-95 WHEAT *(TRITICUMAESTIVUM* L.) VARIETIES SEEDS TO SALT, HEAVY METAL AND LIME TREATMENTS

Nezahat Turfan*

Kastamonu University, Faculty of Arts and Sciences, Department of Biology, Kastamonu, Turkey

ABSTRACT

It is intended to determine the reactions of the summer Cumhuriyet-75 and winter Selimiye-95 varieties to salt, heavy metal and lime treatments in the research. To this end 1,000 kernel weight (1,000 K), β-carotene, glucose, sucrose and starch amount, lipid peroxidation levels (malondialdehyde-MDA) concentration of hydrogen peroxide (H_2O_2), APX, CAT, GPOX and SOD activities were measured in grains of wheat varieties grown in pots with volume of 10 L between the years of 2013-2014 in campus area of the Kastamonu University. 1,000 kernel weight in Cumhuriyet-75 variety was found to be higher in lime ($CaCO_3$) and 75 mM NaCl treatments and β-carotene content was found to be higher in 225 mM NaCl and $FeCl_3$ treatments according to the results of the analysis. Glucose and sucrose content showed increase in $ZnCl_2$ treatment while the amount of sucrose decreased in all treatment groups. The amount of β-carotene was higher in Selimiye-95 variety in all treatment groups while glucose and starch were higher in $FeCl_3$ and 75 mM NaCl treatments and sucrose was higher in other treatment groups except lime. Gupx activity was high in 75 mM NaCl treatments, APX activity was lower in $ZnCl_2$ treatments; CAT activity was lower in 75 mM NaCl treatments and SOD activity was lower in $ZnCl_2$ and 150 mM NaCl treatments. MDA concentration was lower in 150 mM and 75 mM salt treatments and H_2O_2 was lower in all salt concentrations. While 1,000 kernel weight in Selimiye-95 variety decreased in all treatment groups, the amount of β-carotene increased compared to control group. Glucose and starch were higher in $FeCl_3$ and 75 mM NaCl treatment groups while sucrose was higher in other treatment groups except lime. APX activity increased in $ZnCl_2$ treatment while CAT activity increased in other treatments except 75 mM NaCl treatment. GPOX activity is lower in 75 mM NaCl treatment and SOD activity is lower in 150 mM NaCl and ZnCl2 treatments. MDA concentration in Selimiye-95 was lower in 150 mM and 75 mM salt treatments and H_2O_2 was lower in all salt concentrations and higher in all other treatments. As a result, Cumhuriyet-75 variety was found to be mildly tolerant in $FeCl_3$ and 225 mM NaCl treatments and sensitive in $NiCl_2$ and $ZnCl_2$ treatments. Selimiye-95 variety was found to be highly tolerant in 75 mM NaCl and sensitive in $ZnCl_2$ and $CaCO_3$ treatments. When all the data are taken into consideration, it was concluded that the responses of the varieties to the treatments changed according to the type and concentration of stress, and Selimiye-95 variety was tolerant compared to Cumhuriyet-75 75 variety.

KEYWORDS:
Cumhuriyet-75, Selimiye-95, Stress Treatments

INTRODUCTION

Grains are one of the basic nutrition sources of humans. Wheat constitutes the staple food source of approximately 35% of the world's population inasmuch as it is a good source of nutrients and has ease of production, transportation, storage and processing. EU ranks first in wheat production with 135.8 million tons (total of 27 countries) while China and India are in second and third place respectively with 115 and 80.8 million tons according to wheat production in 2010/11 period. Turkey realizes 2.6% of world wheat production with 17.5 million tons [1]. Wheat contains 65-75% starch, 8-15% protein, 1-5% fat, 1.53% sugar, 1-2% ash, 11 to 13% water and other nutrients such as carotenoids, vitamins and minerals [2, 3]. Wheat's grain yield largely depends on the storage capacity determined in the vegetative period as well as the photosynthesis capacity during grain filling period. Inasmuch as the amount and type of assimilates accumulated in wheat grain is affected by photosynthesis in grain filling period, morphological and physiological characteristics of this period and also life efficiency of photosynthetic organs also change [4, 5]. Plants are subjected to more than one stress factor in the habitat they live in at the same time. The conditions in their growth environment are not uniform; some factors required for plant growth and development are wanting while some factors are far from being ideal. Abiotic stress factors such as drought, salinity, heavy metal toxicity and

deterioration of soil structure affects wheat production and lead to losses in yield and quality [6, 7]. It is reported in the studies carried out that wheat grown in conditions containing salts, heavy metals, lime ($CaCO_3$) and drought stress completes the vegetative and generative life cycle thereof faster than normal conditions and accordingly accumulation of assimilates are reduced in the leaves and kernels and the nutrient accumulation in the kernels are affected while production of biomass is reduced [8, 9, 10]. Sure enough, Prasad et al. [11] and Poustini [12] have reported that wheat flowering and grain filling period are very sensitive to drought stress. Makino [4], Gregersen et al. [13] and Guohua et al. [14] have reported that stress factors of the early period of grain filling stimulate senescence of leaves and as a result the chloroplast structure is deteriorated. Damage in chloroplasts leads to increase in catabolic activity such as respiration rate, pigment degradation, protein denaturation in addition to oxidation in membrane lipids. All these occurrences reduce the photosynthetic activity and inhibit the transport of macromolecules from the leaves to developing parts such as flower, seed, root and stem accumulation thereof as well as storage capacity for storage of nutrients and the amount of starch, simple sugars, proteins and etc. in storage organs [15, 16, 17]. Turki et al. [18] have reported that salt stress leads to reduction in grain yield stress and suppression of carbohydrates synthesis due to decrease of photosynthetic capacity and reduction thereof due to decrease of starch accumulation; Öztürk et al. [19] and Erdem [20] have reported that total soluble protein content in wheat exposed to heavy metal toxicity is reduced; Kaut et al. [21] and Fager [22] have reported that quality and yield of grain is very low in soils low in organic matter and sand; Horton [9] and Long et al. [7] have expressed that the salt treatments in tolerant species increase nitrogen metabolism by stimulating protein synthesis. It is intended to research the effects of salt, heavy metal and lime treatments on 1,000 kernel weight (1,000 K), β-carotene, glucose, sucrose and starch amount, lipid peroxidation levels (MDA) concentration of hydrogen peroxide (H_2O_2), APX, CAT, GPOX and SOD activities of the summer Cumhuriyet-75 and winter Selimiye-95 bread wheat genotypes in this study.

MATERIALS AND METHODS

Plant material. Cumhuriyet-75 (bread type) variety registered by the Ege (Agean) Institute of Agricultural Research in 1976 and Selimiye-95 (bread) variety produced by crossbreeding registered Trakya (Thrace) Institute of Agricultural Research in 2009 were used in the study. Cumhuriyet-75 is a summer type spiny wheat which that the biggest grain among the white wheat varieties. Its planting is recommended in coastal zones and rural and basal areas. Selimiye-95 is a winter variety without spine. It is recommended for regions where planting is made in winter.

Planting Operations of Varieties. The study was launched in 3rd week of October. 50 seeds were planted in a pot with three replications for each treatment group and subsequent to germination they were reduced to 20 plants in each pot. The pots have a volume of 10 L in and consist of a mixture in ration of garden turf: peat: sand (1: 1: 1). The treatments were made while the seedlings were at 4-5 leaf stage by treatment of twice a week until the ears became yellow. Salt treatment (75 mM, 150 mM and 225 mM NaCl), heavy metal treatment (0.2 mg/L in the form of $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime treatment (2 mg/L in the form of $CaCO_3$) were solubilized in tap water with ds 0.04 and with freshly prepared solutions each time. After the ears were fully mature they were harvested and dried for a week. The collected grains had measurements of 1,000 kernel weight, water content, β-carotene, lycopene, lipid peroxidation level (malondialdehyde-MDA), hydrogen peroxide (H_2O_2), glycosuric acid and starch amount as well as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPOX) and peroxide dismutase (SOD) activities.

1,000 kernel weights (g) was calculated by weighing separately 100 specimen kernels obtained from 10 ears 4 times and their average was taken and proportioned to 1,000 kernels [23].

Chemical Analyzes. Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) according to method of Lutts et al. [24] while the amount of H_2O_2 quantity was determined according to method of Velikova et al. [25]. Determination of the amount of glucose and sucrose was carried out using anthron separator according to method of Pearson et al. [26].

Determination of the enzyme activity and preparation of the extracts. The extracts were prepared from first three leaves of the plants which were treated by control and stress. Accordingly, nearly 0.5 gram fresh leaf samples were homogenized with 50 mM (pH 7.6) phosphate (P) buffer solution (10 mL) ground in liquid nitrogen and containing 0.1 mM Na-EDTA. The homogenized samples were centrifuged for 15 min at 15000 g and +4 ° C, and then the enzyme activities in the resulting supernatant were determined according to the methods of Çakmak [27]. Catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) activities were measured according to the methods of Bergmeyer [28], Nakano and Asada [29], Chance and Maehley [30] and (Çakmak [31] respectively under nitro blue tetrazolium chloride (NBT) light by 0_2^- reduction.

TABLE 1
Effects of salt (75 mM, 150 mM and 225 mM NaCl), heavy metals (0.2 mg/L $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime (2 mg/L $CaCO_3$) treatments in 1,000 kernel weight, moisture content and β-carotene contents of Cumhuriyet-75 and Selimiye-95 variety seeds.

	Cumhuriyet-75			Selimiye-95		
	Kernel weight (gr)	Moisture (%)	β-karoten mg/g	Kernel weight (gr)	Moisture (%)	β-karoten mg/g
Control	2.64±0.001c*	33.91±0.02b	0.655±0.002e	3.46±0.005f	32.83±0.03a	0.657±0.001a
75 mM	2.89±0.005d	35.34±0.02b	0.582±0.002b	3.33±0.002e	34.58±0.02b	0.706±0.002c
150 mM	2.33±0.002a	36.63±0.04c	0.614±0.002d	2.97±0.002c	35.75±0.01c	0.756±0.002f
225 mM	2.41±0.004b	37.63±0.02c	0.682±0.010f	3.29±0.002d	34.03±0.02b	0.723±0.002e
$FeCl_3$	2.40±0.002b	36.04±0.02c	0.657±0.011e	2.80±0.002b	35.83±0.30c	0.677±0.002b
$NiCl_2$	2.43±0.001b	34.42±0.05b	0.595±0.010c	3.08±0.003c	38.63±0.02d	0.675±0.002b
$ZnCl_2$	2.24±0.001a	39.56±0.01d	0.570±0.013a	2.32±0.001a	40.10±0.01d	0.727±0.002e
$CaCO_3$	3.24±0.001e	32.19±0.01a	0.579±0.010b	3.25±0.001d	42.83±0.010f	0.717±0.002d

Statistical analysis of data. The statistical analysis of the data obtained as a result of the study was conducted according to the ANOVA and Tukey tests at 95% confidence interval by virtue of the SPSS 20 program.

RESULTS

The 1,000 kernel weight of seeds varied depending on the type and concentration of stress treatments (Table 1). In the Cumhuriyet-75 variety 1,000 kernel weight was 22.7 lower in $ZnCl_2$ treatment, while it was 11.67% lower in 150 mM NaCl of 150 mM NaCl treatment and 9.4% lower in $FeCl_3$ treatment compared to control and 7.22% higher in $CaCO_3$ (Table 1). In the Selimiye-95 variety 1,000 kernel weight decreased compared to control in all stress treatments. 1,000 kernel weight has the lowest value in $ZnCl_2$ (33%, $FeCl_3$ (19.2%) and 150 mM NaCl (14.12%) treatments ($p < 0.05$).

The amount of β-carotene in the seeds was found higher in Selimiye-95 variety (Table 1). In the Cumhuriyet-75 variety β-carotene amount decreased compared to control in $ZnCl_2$ (13%), $CaCO_3$ (11.6%) and 75 mM salt (11.1%) treatments while a partial increase was observed in 225 mM salt (4.13%) treatment ($p < 0.05$). In the Selimiye-95 variety, the highest β-carotene was observed in 150 mM salt (15.16%), $ZnCl_2$ (10.72%) and 225 mM salt (10.46%) treatments respectively (Table 1). Moisture content (%) in seeds showed difference according to type, stress type and concentration ($p < 0.05$). The lowest moisture rate in the Cumhuriyet-75 seeds was in $CaCO_3$ and control groups while the highest moisture content was observed in $ZnCl_2$ (18.7%) and 225 mM NaCl (5.11%) treated seeds. Moisture content in Selimiye-95 seeds was higher than control in all treatment groups. The highest moisture content was recorded in $CaCO_3$ (31.41%), $ZnCl_2$ (23.98%) and $NiCl_2$ (19.6%) treatment groups (Table 1). Malondialdehyde (MDA) concentration has increased in the Cumhuriyet-75 variety in stress treatments other than $CaCO_3$ (17:14%) (Table 2). MDA has the highest value especially in the 150 mm (2.85 times more) and 225 mm (2 times) salt and $ZnCl_2$ (67.92%) treatments. heavy metal, 225 mm salt and $CaCO_3$ treatments in the Selimiye-95 variety have increased the amount of MDA compared to control group (Table 2). MDA content is highest in $NiCl_2$ (49.23%), $ZnCl_2$ (43.3%), $FeCl_3$ (41.63%), 225 mM salt (27.44%) and $CaCO_3$ (21%) treatments respectively ($p < 0.05$ Table 2). Hydrogen peroxide (H_2O_2) concentration has decreased in Cumhuriyet-75 variety in all treatment groups while it has decreased only in salt treatment in Selimiye-95 variety decreased compared to control group (Table 2). The lowest H_2O_2, in Cumhuriyet-75 variety has been observed in $CaCO_3$ (60.86%), $FeCl_3$ (%53.56), $NiCl_2$ (7.26%) and $ZnCl_2$ (21:54%) treatments ($p < 0.05$).

In Selimiye-95 variety H_2O_2 decreased with increasing salt concentration. The highest H_2O_2 was determined in $ZnCl_2$ (2.52 times more), $FeCl_3$ (31.2 times more), $NiCl_2$ (98%) and $CaCO_3$ (93%) treatments compared to control groups ($p < 0.05$, Table 2). The amount of glucose increased in the Cumhuriyet-75 variety only in $ZnCl_2$ treatment. Glucose amount was in the lowest value in 150 mM (62%) and 225 mm (34.27%) salt and $NiCl_2$ (27%) treatments compared to control treatments (Table 3). The highest glucose in Selimiye-95 variety was in $FeCl_3$ (97.9%) and 74 mM salt (54.6%) treatments, and the lowest glucose was in 225 mM salt (74.64%), $CaCO_3$ (5.97%), $ZnCl_2$ (50.72%) and $NiCl_2$ (47.22%) treatments ($p < 0.05$). The amount of sucrose decreased in all treatment groups in the Cumhuriyet-75 variety compared to all control groups. Sucrose decreased very much in particular in 75 mM NaCl (4.75 times more), $NiCl_2$ (3.97 times more), $ZnCl_2$ (3.33 times more), 225 mM NaCl (2.7 times more) and $CaCO_3$ (58.66%) treatments (Table 3). In Selimiye-95 variety low sucrose content was found only in $CaCO_3$ (Table 3). Sucrose has the highest value in $NiCl_2$ (37.9%), 225 mM NaCl (36%) and $FeCl_3$ (27%) treatments ($p < 0.05$). Starch content in the varieties showed similar changes to changes in glucose

content. The lowest starch in Cumhuriyet-75 variety compared to control group was detected in 150 mM and 225 mM NaCl, $NiCl_2$, $ZnCl_2$ and $CaCO_3$ treatments. The highest starch in Selimiye-95 variety was in FeCl3 and 75 mM salt treatments respectively, while it has lower value in 225 mM NaCl, $CaCO_3$, $ZnCl_2$, and $NiCl_2$ treatments (Table 3).

Changes in Antioxidant Enzyme Activity. In the Cumhuriyet-75 variety ascorbate peroxidase (APX) activity showed an increase in 150 mM NaCl (2.93 times), 225 mM NaCl (95.2%), 75 mM NaCl (6.3%) and $FeCl_3$ (52.4%) treatments compared to control group while $CaCO_3$ (2.38 times more) and $ZnCl_2$ (40.5%) decreased (Table 8). APX activity only in Selimiye-95 variety $ZnCl_2$ (13.71%) were reduced in treatment. The highest APX was seen in 150 mm and 75 mm salt, $CaCO_3$ and $FeCl_3$ treatments respectively (Table 4). Catalase (CAT) activity decreased in the Cumhuriyet-75 variety in $CaCO_3$ treatment compared to the control group and decreased in Selimiye-95 variety in 75 mM NaCl treatment compared to the control group (Table 4). The highest CAT activity in Cumhuriyet-75 variety was respectively in $ZnCl_2$ (2.67 times more), $FeCl_3$ (89%), 225 mM NaCl (77.62%) and $NiCl_2$ (45.46%) treatments. The highest CAT activity in Selimiye-95 variety compared to control group was determined in 150 mm (2 times) and 225 mM NaCl (92.23%), $NiCl_2$ (86.85%) and $FeCl_3$ (67.93%) treatments (Table 4). Guaiakol peroxidase (GPOX) activity was higher in salt and $FeCl_3$ treatment in the Cumhuriyet-75 variety compared to the control group and was lower in $NiCl_2$, $ZnCl_2$ and $CaCO_3$ treatments (Table 4). GPOX activity in Selimiye-95 variety was the highest in 75 mM NaCl, $NiCl_2$ and $CaCO_3$ treatments (Table 4). Superoxide dismutase (SOD) activity types was lower in all Cumhuriyet-75 varieties compared to control group in all treatment groups and was higher in all treatment groups in the Selimiye-95 variety except $ZnCl_2$ (Table 4).

TABLE 2

Effects of salt (75 mM, 150 mM and 225 mM NaCl), heavy metals (0.2 mg/L $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime (2 mg/L $CaCO_3$) treatments in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) amounts of Cumhuriyet-75 and Selimiye-95 seeds.

	Cumhuriyet-75		Selimiye-95	
	MDA µmol/g	H_2O_2 µmol/g	MDA µmol/g	H_2O_2 µmol/g
Control	31.87±0.06b*	317.71±0.16h	24.49±0.11b	262.76±0.16d
75 mM	35.94±0.04c	300.79±1.10f	20.58±0.13a	255.42±0.25c
150 mM	90.61±0.07h	260.25±0.16e	19.61±0.15a	244.08±0.16b
225 mM	64.65±0.04g	313.34±0.25g	31.20±0.02c	228.45±0.16a
$FeCl_3$	49.62±0.04e	147.56±0.45b	34.68±0.08d	606.98±1.61g
$NiCl_2$	41.56±0.02d	232.92±0.16c	36.54±0.15e	520.50±1.61f
$ZnCl_2$	53.51±0.08f	249.28±0.34d	35.09±0.02d	662.76±1.61h
$CaCO_3$	26.41±0.06a	124.36±0.18a	29.61±0.09c	506.56±1.61e

*Statistically there is a significant difference with confidence level $p < 0.05$

TABLE 3

Effects of salt (75 mM, 150 mM and 225 mM NaCl), heavy metals (0.2 mg/L $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime (2 mg/L $CaCO_3$) treatments on the amounts of glucose, saccharose and starch in the Cumhuriyet-75 and Selimiye-95 variety seeds.

	Cumhuriyet-75			Selimiye-95		
	Glucose mg/g	Sucrose mg/g	Starch mg/g	Glucose mg/g	Sucrose mg/g	Starch mg/g
Control	51.60±0.02e*	22.70±0.02f	36.94±0.02c	4.31±0.02d	14.54±0.12b	31.04±0.02d
75 mM	41.40±0.02d	4.78±0.02a	29.63±0.02b	6.66±0.03e	16.60±0.10b	47.81±0.03e
150 mM	19.40±0.01a	20.51±0.02	13.89±0.01a	3.05±0.01c	16.75±0.02b	21.83±0.02c
225 mM	33.91±0.02b	8.40±0.02c	24.27±0.02b	1.09±0.02a	19.79±0.03c	7.71±0.02a
$FeCl_3$	49.45±0.02e	13.87±0.02e	35.38±0.02c	8.52±0.02f	18.48±0.02c	60.93±0.02f
$NiCl_2$	37.67±0.03c	5.73±0.02b	27.00±0.03b	2.27±0.02b	20.05±0.04c	16.43±0.02b
$ZnCl_2$	53.16±0.01f	6.82±0.06c	38.15±0.01d	2.12±0.01b	15.30±0.04b	15.16±0.01b
$CaCO_3$	41.79±0.01d	9.39±0.01d	29.90±0.01b	1.98±0.01b	11.40±0.03a	14.10±0.01b

TABLE 4
Effects of salt (75 mM, 150 mM and 225 mM NaCl), heavy metals (0.2 mg/L $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime (2 mg/L $CaCO_3$) treatments on the APX, GPOX, CAT and SOD activities in the Cumhuriyet-75 and Selimiye-95 variety seeds.

	APX EU/mg Protein		CAT EU/mg Protein		GPOX EU/mg Protein		SOD EU/mg Protein	
	Cumhuri-yet-75	Selimiye-95	Cumhuri-yet-75	Selimiye-95	Cumhuri-yet-75	Selimiye-95	Cumhuri-yet-75	Selimiye-95
Control	0.009±0.002	0.008±0.001	0.044±0.001	0.032±0.001	0.007±0.001a	0.032±0.002c	121.75±0.02	122.55±0.17b
75 mM	0.014±0.001	0.046±0.001	0.056±0.001	0.020±0.001	0.025±0.006f	0.090±0.001f	110.65±0.07	123.44±0.01c
150 mM	0.050±0.002	0.017±0.001	0.099±0.001	0.065±0.001	0.0413±0.002g	0.020±0.002a	115.44±0.03	122.21±0.03b
225 mM	0.016±0.001	0.016±0.001	0.077±0.001	0.070±0.002	0.009±0.002b	0.030±0.003b	118.60±0.04c	129.61±0.04d
%0.2 $FeCl_3$	0.013±0.001	0.021±0.001	0.083±0.001	0.060 0.001	0.016±0.0001e	0.022±0.004a	115.83±0.06b	124.98±0.01c
%0.2 $NiCl_2$	0.010±0.001	0.015±0.002	0.063±0.001	0.062±0.0001	0.009±0.001b	0.076±0.004e	117.50±0.06c	127.18±0.03d
%0.2 $ZnCl_2$	0.005±0.001	0.006±0.002	0.120±0.001	0.044±0.001	0.010±0.001c	0.032±0.004c	114.87±0.04b	76.28±0.01a
%0.2 $CaCO_3$	0.008±0.001	0.031±0.001	0.041±0.001	0.049±0.001	0.014±0.001d	0.065±0.005d	116.46±0.04c	133.79±0.07e

The lowest SOD activity in the Cumhuriyet-75 variety was determined in 75 mM NaCl treatment. SOD activities have the highest in $CaCO_3$, and 225 mM NaCl and $NiCl_2$ treatments in Selimiye-95 variety (Table 4).

DISCUSSION

A seed is a complex structure composed of organs, tissues and cells shape, chemical content and functions of which differ. Therefore, the morphology chemistry, physiology and function of the seeds change depending on the types, duration severity, of stress factors the plant species they belong to are exposed to as well as the life cycle of the developmental phase at the time when the stress is effective [32]. In this study, the mechanism of resistance to salt, heavy metal and lime treatments of Cumhuriyet-75 and Selimiye-95 wheat varieties have been researched basing on 1,000 kernel weight, β-carotene, proline, protein, lipid peroxidation, hydrogen peroxide, glucose, sucrose and starch amount, ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase enzyme activity changes. Carotenoids are pigments that give yellow-red color to plants. These compounds play an important role in maintaining energy transfer in the reaction center photosynthetic systems and protection of the reaction center in autoxidation. Wheat and wheat products vary in color depending on the type and amount of carotenoids they contain. Additionally, these compounds are found in greater amounts in tolerant [33, 34, 35]. The amount of β-carotene in the study varied depending on the stress type, concentration and genotype. In Cumhuriyet-75 variety, β-carotene was high in 225 mm salt and $FeCl_3$ treatments and low in $ZnCl_2$, and $NiCl_2$ and $CaCO_3$ treatments. In Selimiye-95 variety β-carotene stress values increased in all treatment groups compared to the control groups (Table 1). According to the results of the study, Selimiye-95 variety is more durable than Cumhuriyet-75. Many researchers have reported that pigment values are high in tolerant species and low in sensitive species [36, 37]. Yıldız and Terzi [38] have reported that pigment content is high in salt-tolerant barley varieties and low in susceptible species; Pandey et al. [39] and Van Oijen et al. [40] have expressed that pigment synthesis is suppressed in plants exposed to heavy metals, carotenoids and the anthocyanin contents are decreased and, Kahrizi and Sedghi [41] have found that salt stress does not affect the carotene content in tolerant wheat varieties but has degraded the carotene content in susceptible species; Pallett and Yong [42] have reported that because carotenoids are highly stable compounds they work in increasing tolerance of plants to oxidative stress and that they are more in tolerant species. One of the important features that affect grain yield in grains is the 1,000 kernel weight. 1,000 kernel weights may vary depending on varieties and environmental factors [18, 43, 44]. 1,000 kernel weights is high in Cumhuriyet-75 variety in $CaCO_3$ to 75 mM salt treatments and low in all treatment groups in Selimiye-95 variety (Table 1). Many researchers have found that mineral deficiencies/toxicity and nitrogen and lime amount in the soil or arid conditions and climatic changes [15, 45, 46] affect 1,000 kernel weight; salt stress reduces the accumulation of assimilates and grain weight by inducing senescence in leaves of susceptible species and by increasing the respiratory rate [41, 6,12]; heavy metal treatments reduces the grain weight by inducing oxidative stress and blocking the photosynthetic capacity in sensitive wheat and rice genotypes [19, 47] and it has also been stated that the grain yield has decreased depending on leaf senescence under stress conditions [48, 13, 44]. When wheat grains are dry, metabolic reactions proceed at the slowest rate. However, even under normal conditions grains respire and some physical, chemical and biochemical changes occur as a result of metabolic events in its structure and structure, chemistry and physiology of seeds after impaired after some time [49, 50]. Altan, [51] and Anonymous [52] have reported that the robustness, size and fullness of the seeds depends on the ripeness

degree of the seeds and unripe seeds respire more in storage and warehouse conditions thereby increasing temperature of the medium and consequently the seeds are quickly degraded. Furthermore unripe seeds also provide a suitable environment for bacteria, mold production and insect and microorganism activity [50, 51, 53, 52, 54]. The moisture content of the seeds has increased in both types of stress treatments. The moisture content of Cumhuriyet-75 variety was lowest in $CaCO_3$ treatment and highest in ZnCl and 225 mM NaCl treatments. It was highest in all treatment groups in Selimiye-95 variety compared to control groups. Seed moisture content was at highest level especially in $CaCO_3$ and $ZnCl_2$ treatments (Table 1). According to these results, Cumhuriyet-75 variety seeds lose their water rapidly in lime treatments while Selimiye-95 variety seeds lose their water rapidly in salt treatments. Other treatment groups have increased the moisture content in the seeds. This situation has been thought to result from inhibition of the accumulation of storage substances, such as starch in the seed by long period treatment of stress conditions inhibiting photosynthesis and carbon metabolism [13, 14, 55]. Hoseney [56] and Kent [57] found that the moisture content of wheat is 14% for preventing degradation in seeds and the seeds with moisture content above these values should be brought to the appropriate limits by drying or ventilating to ensure safe storage of moisture contents; Altan [51] and Döven [49] stated that the characteristic structure of grain with inappropriate moisture content is impaired and the moisture in the pile of seeds passes to the air spaces between the particles, causing moisture and temperature to rise in the product , occurrence and increase of microbiological activity and spreading into the pile. Seed quality parameters such as chemical content, liveliness, germination power and speed, stress factors exposed during the developmental stage differ depending on seed harvesting time and seed storage conditions [32, 4, 58, 59]. Researchers report that the deterioration in seed substantially are caused by lipid peroxidation and the effect of free radicals [60, 61, 62]. Aldehydes and other toxic compounds accumulating as a result of lipid peroxidation (malondialdehyde-M) increase that free radical amount in the seed by inducing oxidative stress in addition to disrupting the cellular membrane integrity [63, 64, 65]. Excessive MDA and ROS accumulation accelerate catabolic degradation of macromolecules such as proteins, carbohydrate and enzymes responsible for germination and hinder physiological functions such as germination power and speed as well as seed viability [66, 3, 67]. The amount of MDA in the study is high in salt and heavy metal treatments in the Cumhuriyet-75 variety style and is low in lime treatment. In Selimiye-95 variety, it is low in 75 mM and 150 mM NaCl treatments and high in other treatment groups. H_2O_2 content is low in all treatment groups in Cumhuriyet-75 variety compared to control group and it is low in salt treatments in Selimiye-95 variety while it is quite high in metal and lime treatment (Table 2). The fact that Cumhuriyet-75 variety is sensitive in salt and heavy metal treatments in line with MDA and H_2O_2 data and tolerant in treatment of lime while Selimiye-95 variety was tolerant to salt treatment and sensitive to heavy metal and lime treatments. MDA and H_2O_2 results are consistent with results of studies performed in this area. Sung [62], Jung and Chiu [68] have reported that seed age in soybeans increases lipid peroxidation level and H_2O_2 content and these compounds degrade the seed structure, chemistry and germination ability; Wilson and McDonald [69] have expressed that lipid peroxidation in seeds increases the H_2O_2 content ; Goel et al. [61] have found that MDA content and effect of oxidative stress increases in artificial aging tests on cotton seeds; Kahrizi and Sedghi [41] have reported that salinity reduces the protein content in susceptible wheat varieties but increases the MDA and H_2O_2 content; Dexter [3] has expressed that that drought prevent the accumulation of storage material in the endosperm in durum wheat genotypes and stimulates the accumulation of MDA and ROS; Guohua et al. [14] have expressed that nitrogen content affects the chemical and physiological activity of the seed; Kruger and Reed [35] have stated that environmental factors affect the effect of oxidative stress, enzyme activity and color in the seeds; Waters et al. [5] have found that heavy metal toxicity/deficit at the vegetative growth stage of wheat affects the grain filling and changes the amount of protein, carbohydrate, MDA and H_2O_2 in endosperm; Marshall and Lewis [58] have mentioned that seed storage conditions disrupt the seed structure and chemistry and consequently degrades seed viability and germination power. It is reported in studies on seed carbohydrate reserves that transport and storage capacity of assimilates from sources they are synthesized to organs such as seeds, rhizomes, roots and stems depend on physiological and ecological changes in the vegetative development stage [13, 70, 55]. These compounds are transported to the embryo by breaking down to their monomers by respiration reactions in the seed germination stage and are used as carbon, nitrogen and energy source in the growth and development of the embryo [67, 71, 35]. Glucose is the key substance of cellular respiration and starch synthesis. Sucrose is active in the regulation of respiration, intracellular osmotic balance and germination as well as in the regulation of oxidative stress in stress conditions [72, 73, 17]. In the Cumhuriyet-75 variety, glucose decreased in other treatment groups except Zn treatment compared to control group. High salt concentrations particularly, have adversely affected the amount of glucose. The amount of sucrose was low in all treatment groups. In Selimiye-95 variety glucose was high in $FeCl_3$ and 75 mM salt treatment; sucrose was low only in $CaCO_3$ treatment. Because the starch consists of glucose monomers

[74, 73], starch content was similar to glucose content in both types (Table 3). Glucose, sucrose and starch amount's being low in Cumhuriyet-75 variety in salt application and glucose and starch's being high in Selimiye-95 variety in 75 mM salt application and sucrose's being high in all salt applications indicates that Cumhuriyet-75 variety is sensitive to all salt applications while Selimiye-95 variety is tolerant to all salt applications. Sure enough, Kahrizi and Sedghi [41] found that the amount of gluten and starch in wheat exposed to salt stress decreased in susceptible varieties but increased in tolerant varieties; Pattanagul and Thitisaksakul [16] and Gill and Singh [75] found that the amount of starch and sucrose increased in resistant rice varieties and decreased in susceptible varieties Austin et al. [10] found that salt stress inhibited photosynthetic metabolism in the wheat, inhibiting asimylate transport to the grains and thereby affecting the amount of starch; Makino [4], Raines [15], Gregersen et al. 13] and Guohua et al. [14] reported that stress factors in early period of grain filling stimulated senescence in leaves which resulted in degradation of the chloroplast structure, reduced photosynthetic activity, and thus storage capacity for accumulation of assimilate and nutrients in the seeds decreased. Furthermore, it was reported that sucrose plays a role as a building material and energy source in plant growth and development, and as a signal molecule in the perception of environmental stimuli and in increase of cellular tolerance [72, 73, 70]; and heavy metal toxicity effects photosynthetic capacity and transport of assimilates in plants [76, 77], carbohydrate metabolism [40, 78] as well as the metabolism of respiration [79] and causes MDA accumulation and decreased enzyme activities by inducing oxidative stress [80]. Enzymes such as APX, CAT, GPOX and SOD are antioxidant compounds which have effective roles to maintain the cell's cell cycle, chemical properties, color, shape and morphology. These compounds are in equilibrium under normal conditions but their amounts increase in tolerant varieties under stress conditions and play significant role in the clearing of ROS's caused by oxidative stress in cells [81, 82, 27]. In the Cumhuriyet-75 variety, APX activity is lower in salt, $ZnCl_2$ and $CaCO_3$ treatments, CAT activity is lower in $CaCO_3$ activity and SOD activity is lower in all treatment groups compared to control group and GPOX activity is higher in all treatment groups (150 mM and 225 mM NaCl). In Selimiye-95 variety APX activity is higher in $ZnCl_2$ treatment and CAT activity is higher in 75 mM NaCl and activity and GPOX activity is higher in salt treatment and SOD activity is decreased in $ZnCl_2$, 75 mM and 225 Mm NaCl treatment groups compared to control group. In both varieties, negative relation was seen between $ZnCl_2$ treatment and APX and SOD activities. Furthermore in both varieties, it contributed to increased tolerance in APX salt, $FeCl_3$, $NiCl_2$, CAT 150 mM and 225 mM NaCl and heavy metal treatments (Table 4). Esfendiari et al. [83] found that SOD, CAT, APX and GR activities were in high levels in wheat while Doğan [84] determined that SOD, CAT, APX and GR activities were in high levels in tomato in genotypes with high tolerance to salt stress while they were in low levels in tomato in genotypes susceptible to salt stress; and Muradoğlu et al. [81]; Yaşar et al. [85); Dubey and Pandey [80]; Pandey et al., - [39]; Panda and Khan [86] and Çakmak and Horst [27] have determined that APX, CAT and SOD activities vary depending on the concentration and duration of administration, and that enzyme activity is reduced in susceptible varieties in strawberries, peas, black lentil , spinach, rice and soybeans respectively. In the workplace, Cumhuriyet-75 and Selimiye-95 stress applied to the families of 4-5 Leafs in the beginning and the beginning of the year. It is believed that this situation has the effect of changing the activity.

ACKNOWLEDGEMENTS

This study has been carried out by virtue of the assistance provided through the of KUBAP-01 / 2013-17 project.

REFERENCES

[1] Anonim (2010) Turkis Grain Board, Ankara. Cereals Report.
[2] Aslın, H. (1986) Investigations on morphological, anatomical and some physiological properties of various Triticum (Wheat) species. PhD Thesis. University of Ege Institute of Natural and Applied Sciences.
[3] Dexter, J.E. (2007) Relationships between dough strength, polymeric protein quantity and composition for diverse durum wheat genotypes. Journal of Cereal Science. 45, 140-149.
[4] Makino, A. (2011) Photosynthesis, Grain Yield, and Nitrogen Utilization in Rice and Wheat. Plant Physiology. 155(1), 125-129.
[5] Waters, B.M., Uauy, C., Dubcovsky, J. and Grusak, M.A. (2009) Wheat (Triticum aestivum) proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. Journal of Experimental Botany. 60(15), 4263-4274.
[6] Asgari, H.R., Cornelis, W. and Van Damme, P. (2011) Effect of salinity on wheat (*Triticum aestivum* L.) grain yield, yield components and ion uptake. DESERT. 16,169-175.
[7] Long, S.P., Zhu, X.G., Naidu, S.L. and Ort, D.R. (2006) Can Improvement in Photosynthesis Increase Crop Yields? Plant, Cell and Environment. 29(3), 315-330.

[8] Barneix, A.J. (2007) Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. J. Plant Physiol. 164, 581-590.
[9] Horton, P. (2000) Prospects for Crop Improvement Through the Genetic Manipulation of Photosynthesis: morphological and Biochemical Aspects of Light Capture. Journal of Experimental Botany. 51(2), 475-485.
[10] Austin, R.B., Organ, C.L., Ford, M.A. and Blakwell, R.D. (1980) Contribution to grain yield from pre-anthesis assimilation in tall and dwarf barely phenotypes in two contrasting seasons. Ann. Bot. 45, 309-319.
[11] Prasad, D.A.G., Rahimpouran, S. and Komala, H.P. (2014) Ecotoxicological effects of iron on the activities of antioxidant enzymes in Safflower (*Carthamus tinctorius* L.) seedlings. Int. J. Pure App. Biosci. 2(5), 18-123.
[12] Poustini, K. (2002) Evaluation of the response of 30 wheat cultivars to salinity. Iranian J. Agric. Sci. 3, 57-64.
[13] Gregersen, P.L., Holm, P.B. and Krupinska, K. (2008) Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biology. 10, 37-49.
[14] Guohua, M., Tang, L., Zhang F. and Zhang, J. (2002) Carbonhydrate storage and utilization during grain filling as regulated by nitrogen application in two wheat cultivars. Journal of Plant Nutrition. 25(2), 213-229.
[15] Raines, C.A. (2011) Increasing Photosynthetic Carbon Assimilation in C3 Plants to Improve Crop Yield: Current and Future Strategies. Plant Physiology. 155(1), 36-42.
[16] Pattanagul, W. and Thitisaksakul, M. (2008) Effect of Salinity stress on growth and carbohydrate metabolism in tree rice (Oryza sativa) cultivars in differing salinity tolerance. Indian Journal of Experimental Biology, 46,735-742.
[17] Zeaaman, S.C., Smith, S.M. and Smith, A.M. (2007) The diurnal metabolism of leaf starch. Biochemical Journal. 401, 13-28.
[18] Turki, N., Harrabi, M. and Okuno, K. (2012) Effect of Salinity on Grain Yield and Quality of Wheat and Genetic Relationships among Durum and Common Wheat. Journal of Arid Land Studies. 22(1), 311-314.
[19] Öztürk, L., Erenoğlu, B., Kaya, Y., Altıntaş, Z., Haklı, E., Andi, E. And Yılmaz, Ö. (2011). Investigation of Physiological Mechanisms Affecting the Transport of Zinc to Wheat. Tubitak Project Final Report. No:108T436.
[20] Erdem, H. (2009) Determination and Characterization of The Genotypes with High Seed Zinc Concentration and Resistant to Zinc Deficiency in Triticum Spelta Wheat. PhD Thesis, University of Cukurova Institute of Natural and Applied Sciences Department of Soil Science.
[21] Kaut, A.H.E., Mason, H.E., Navabi, A., Donovan, J.T.O. and Spaner, D. (2008) Organic and Conventional Management of Mixtures of Wheat and Spring Cereals. Agronomy for Sustainable Development. 28(3), 363-371.
[22] Fageria, N.K. (2002) Influence of Micronutrients on Dry Matter Yield and Interaction with Other Nutrients in Annual Crops. Pesq. Agropec., Bras. 37(12), 1765-1772.
[23] Uluöz, M. (1965) Wheat Flour and Bread Analysis Methods. Ege University Faculty of Agriculture Publications. No:57, İzmir.
[24] Lutts, S., Kinet, J.M. and Bouharmont, J. (1996) NaCl-Induced Senescence in Leaves of Rice (*Oryza sativa* L.) Cultivars Differing in Salinity Res istance. Annals of Botany. 78, 389- 398.
[25] Velikova, V., Yordanov I., Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. Plant Sci. 151, 59-66.
[26] Pearson, D., Melon, H.K. and Ronald, S. (1976) Chemical analysis of Food. 8th edition. Churchill Livingstone. 5-63.
[27] Çakmak, I. and Horst W.J. (1991) Effect of Aluminium on lipid peroxidation, Superoxide dismutase, Catalase and Peroxidase activities in root tips of Soybean (*Glycine max*). Physiol. Plant. 83, 463-468.
[28] Bergmeyer, H.U. (1974) Methods of Enzymatic Analysis. New York, Academic Press.
[29] Nakano, Y. and Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Physiologia Plantarum. 115, 393-400.
[30] Chance, B., Maehly, S.K. (1995) Assay of catalase and peroxidase. Methods Enzymol. 2, 764-775.
[31] Çakmak, İ. (2002) Plant nutrition Research: Priorities to Meet Human Needs for Food in Sustainable Ways. Plant and Soil. 247, 3-24.
[32] Palabıyık, B., Pekşen, E. (2013) Effects of Seed Storage Duration on Seed Yield and Yield Related Characteristics of Common Bean Grown in Different Environments. Iğdır Univ. J. Inst. Sci. and Tech. 3(2), 93-10.
[33] Foyer, C.H. and Noctor, G. (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum. 119, 355-364.
[34] Molas, J. (2002) Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni (II) complexes. Environ. Exp. Bot. 47, 115-126.
[35] Kruger, J.E. and Reed, G. (1988) Enzymes and color. In: Pomeranz, Y. (Ed.) Wheat: Chemistry and Technology. 3rd ed. Vol. I. American Association of Cereal Chemists, St. Paul, MN, 441-500.

[36] Demmig-Adams, B. and Adams, W.W. III. (2006) Photoprotection in an Ecological Context: The Remarkable Complexity of Thermal Energy Dissipation. New Phytologist. 172(1), 11-21.
[37] Meloni, D.A., Oliva, M.A., Martinez, C.A. and Cambraia, J. (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental Botany. 49, 69-76.
[38] Yıldız, M. and Terzi, H. (2013) Effect of NaCl Stress on Chlorophyll Biosynthesis, Proline, Lipid Peroxidation and Antioxidative Enzymes in Leaves of Salt-Tolerant and Salt-Sensitive Barley Cultivars. Tarım Bilimleri Dergisi. 19, 79-8880.
[39] Pandey, N., Pathak, G.C., Pandey, D.K. and Pandey, R. (2009) Heavy metals, Co, Ni, Cu, Zn and Cd produce oxidative damage and evoke differential antioxidant responses in spinach. Braz. J. Plant Physiol. 21(2), 103-111.
[40] Van Oijen, T., Van Leeuwe. M.A., Gıeskes, W.W.C. and De Baar, H.J.W. (2004) Effects of iron limitation on photosynthesis and carbohydrate metabolism in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). Eur. J. Phycol. 39, 61-171.
[41] Kahrizi, S. and Sedghi, M. (2013) Effect of Salt Stress on Grain Reserve Composition in Ten Durum Wheat Cultivars. Journal of Stress Physiology and Biochemistry. 9(3), 113-121.
[42] Pallett, K.E. and Young, A.J. (1993) Carotenoids. In: Alscher, R.G., Hess, J.L. (eds.) Antioxidants in higher plants. Boca Raton, FL: CRC Press, 91-110.
[43] Wang, Y., Suo, B., Zhao, T., Qu, X., Yuan, L., Zhao, X., Zhao, H. (2011) Effect of nitric oxide treatment on antioxidant responses and psbA gene expression in two wheat cultivars during grain filling stage under drought stress and rewatering. Acta Physiol. Plant. 33, 1923-1932.
[44] Tiryakioğlu, M., Koç, M. (2007) The yield formation in the current bread wheat (*Triticum aestivum* L.) varieties in Cukurova region: relation of aging elements in first leaves. Turkey VII. Field Crops Congress, Erzurum, 25-27 June.
[45] Yazar, S., Karadoğan, T. (2008) Determination of the Yield and Quality Traits of Some Durum Wheat Genotypes Under the Lowland and Aridland Conditions of Central Anatolian Region. Journal of The Agriculture Faculty of Süleyman Demirel University. 3(2), 32-41.
[46] Johnson, R.C., Witters, RE. and Ciha, A.J. (1981) Daily patterns of apparent photosynthesis and evapotranspiration in a developing winter wheat crop. Agron. J. 73, 414-418.
[47] Maheshwari, R. and Dubey, R. (2009) Nickel-induced oxidative stress and the role of antioxidant defence in rice seedling, Plant Growth Regul. 59, 37-49.
[48] Delibacak, S. and Ongun, A.R. (2016) Influence of Treated Sewage Sludge Applications on Corn and Second Crop Wheat Yield and Some Soil Properties of Sandy Loam Soil. Fresen. Environ. Bull. 25, 43-54.
[49] Döven, S. (1998) TGB. Investigation of Drying, Storage and Transfer Structures of Doğankent Drying and Storage Facilities. Undergraduate thesis. University of Cukurova Agriculture Faculty, Department of Agricultural Machinery. Adana. 47p.
[50] Anonymous (2007) Turkish Grain Board, Ankara. Cereals Report.
[51] Altan, A. (1986) Cereal processing Technology. Publication of Agriculture Faculty of Cukurova University. Lecture Book, No:13, Adana, 107p.
[52] Anonymous (2014) Turkish Grain Board, Cereals report.
[53] Altan, A. (2002) Grain processing technology Unpublished lecture notes. Adana. 150p.
[54] Elgün, A., Ertugay, Z. (2002) Cereal Processing Technology. Publication of Agriculture Faculty of Atatürk University. Forth edition, no:718, Erzurum. 411p.
[55] Dennis, D.T. and Blakeley, S.D. (2000) Carbohydrate metabolism. In: Buchanan, B., Gruissem, W. and Jones, R. (eds.) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologist. Rockville MD, 630-674.
[56] Hoseney, R.C. (1986) Principles of Cereal Science and Technology. American Association of Cereal Chemists, Inc. St. Paul, Minnesota, 378p.
[57] Kent, N.L. (1982) Technology of Cereals. Pergamon Press. Third Edition. U.S.A. 221p.
[58] Marshal, A.H. and Levis, D.N. (2004) Influence of seed storage conditions on seedling emergence, seedling growth and dry matter production of temperate forage grasses. Journal Seed. Sci. Technol. 32, 493-501.
[59] ISTA (1993) International Seed Testing Association. International Rules for Testing Seed. Seed Science and Technology 21. Supplement.
[60] Cornell, H. (2003) The Chemistry and Biochemistry of Wheat. Chapter 3. In: Cauvain, S.P. (Ed.) Bread Making Improving Quality. Woodhead Publishing Lim. and CRC Press LLC, Abington, Cambridge and Boca Raton, 31-66.
[61] Goel, A. and Sheoran, I.S. (2003) Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. Biologia Plantarum. 46, 429-434.
[62] Sung, J.M. (1996) Lipid peroxidation and peroxide scavenging in soybean seeds during ageing. Physiologia Plantarum. 97, 85-89.

[63] Bailly, C. (2004) Active oxygen species and antioxidants in seed biology. Seed Sci Res. 14, 93-107.
[64] Mc Donald, M.B. (1999) Seed deterioration: Physiology, repair and assessment. J. Seed. Sci. Technol. 27, 177-273.
[65] Bernal, I.L. and Leopold, A.C. (1998) The dynamics of seed mortality. Journal of Experimental Botany. 49(326), 1455-1461.
[66] Saha, R.R. and Sultana, W. (2008) Influence of seed aging on growth and yield of soybean. Bangla J Bot. 37, 21-26.
[67] Nkang, A. (2002) Carbohydrate composition during seed development and germination in two sub-tropical tree species (*Erythrina* caffra and *Guilfoylia monostylis*). J. Plant Physiol. 159, 473-483.
[68] Jung, J.M. and Chiu, C.C. (1995) Lipid peroxidation and peroxide scavenging enzymes in naturally aged soybean seed. Plant Science. 110, 45-52.
[69] Wilson, D.O. and McDonald, M.B. (1986) The lipid peroxidation model of seed ageing. Seed Science and Technology. 14, 269-300.
[70] Koch, K.E. (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology. 7, 235-246.
[71] Bewley, J.D. (2001) Seed Germination and Reserve Mobilization. Encyclopedia of Life Sciences. Nature Publishing Group / www.els.net.
[72] Xu, F., Tan, X., Wang, Z. (2010) Effects of Sucrose on Germination and Seedling Development of *Brassica Napus*. International Journal of Biology. 2(1), 150-154.
[73] Hammond, J.P., White, P.J. (2008) Sucrose transport in the phloem. Integrating root responses to phosphorus starvation. Journal Experimental Botany. 59, 109-113.
[74] Sulpice, R., Pyl, E.T. (2009) Starch as a Major Integrator in the Regulation of Plant Growth. Proceedings of the National Academy of Science. 106(25), 10348-10353.
[75] Gill, K.S. and Singh, O.S. (1985) Effect of salinity on carbohydrate metabolism during paddy (Oryza sativa) seed germination under salt stress condition. J. Exp. Biol. 23, 384-386.
[76] Gill, S., Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909-930.
[77] Öncel, I., Keleş, Y., Üstün, A.S. (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution. 107, 315-320.
[78] Greger, M., Lindberg, S. (1986) Effects of Cd+2 and EDTA on young sugar beets (Beta vulgaris) I. Cd+2 uptake and sugar accumulation. Physiol. Plant. 66, 69-74.
[79] Lamoureaux, R.J., Chaney, W.R. (1978) The effect of cadmium on net photosynthesis transpiration and dark respiration of excised silver maple leaves. Physiol Plant. 4(3), 231-236.
[80] Dubey, D., Pandey, A. (2011) Effect of Nickel (Ni) On Chlorophyll, Lipid Peroxidation and Antioxidant Enzymes Activities in Black Gram (Vigna Mungo) Leaves. I.J.S.N. 2(2), 395-40.
[81] Muradoğlu, F., Encu, T., Gündoğdu, M., Canal, S.B. (2016) Influence of Lead Stress on Growth, Antioxidative Enzyme Activities and Ion Change in Root and Leaf of Strawberry. Fresen. Environ. Bull. 25, 623-632.
[82] Zhang, W., Xiu, L., Chen, W. (2016) Remediation Effects of Maize-Straw-Derived Biochar on Rice Grown in Cadmium-Contaminated Soil in North China. Fresen. Environ. Bull. 25, 602-611.
[83] Esfandiari, E., Shekari, F., Esfandiari, M. (2007) The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. Not. Bot. Hort. Agrobot. Cluj. 35(1), 1842-4309.
[84] Doğan M. (2012) The Affect of Nitrogen Application on Salt Stress and Antioxidant Enzyme Activities. Süleyman Demirel University Graduate School of Natural and Applied Sciences. 16(3), 297-306.
[85] Yaşar, F., Uzal, Ö., Yaşar, Ö. (2016) Antioxidant Enzyme Activities and Lipid peroxidation amount of Pea Varieties (*Pisum sativum* sp. arvense l.) under Salt Stress. Fresen. Environ. Bull. 25, 37-42.
[86] Panda, S.K., Khan, M.H. (2003) Antioxidant efficiency in *Oryza sativa* leaves under heavy metal toxicity. J. Plant Biol. 30, 23-29.

Received: **13.05.2017**
Accepted: **25.11.2018**

CORRESPONDING AUTHOR

Nezahat Turfan
Kastamonu University,
Faculty of Arts and Sciences,
Department of Biology,
Kastamonu – Turkey

e-mail: nturfan@kastamonu.edu.tr

© by PSP Volume 27 – No. 12B/2018 pages 9972-9978 Fresenius Environmental Bulletin

GENOTOXICITY ASSESSMENT OF DRINKING WATER FROM DIFFERENT SOURCES USING THE *ALLIUM CEPA* TEST PROCEDURE

Soukaina Ennaceur*

Department of Biology, Faculty of Sciences in Yanbu, Taibah University, Al Madinah Al Mounawra, Kingdom of Saudi Arabia

ABSTRACT

The *Allium cepa* biomarker was applied to assess the cytotoxic and genotoxic effects of drinking water from different sources (tap water, bottled water and distribution network water) in Saudi Arabia. Waters samples were collected randomly from different locations in Yanbu city to evaluate the mitotic index and the chromosome aberrations on root meristematic cells of *Allium cepa*. The study was conducted during the four seasons to estimate possible seasonal influences on DNA damage. A significant statistical decline in the mitotic index (MIs) in root-tip meristematic cells of *Allium cepa* was only observed for tap water samples collected from two regions: Yanbu Al-Balad and Yanbu AL-Sinaiiya. The decreases of the MIs in cells germinated in the local water distribution network samples did not have any statistical significance ($p>0.05$). Root cells germinated in bottled water samples (local and imported brands) exhibited acceptable values of MIs. The study of chromosome abnormalities caused by the exposure to the tested drinking water samples was considered as the frequencies of chromosome aberrations (CAs). The highest inductions of CAs were identified in plants grown in tap water samples and only one of the distribution network water samples, mainly during the summer and spring seasons. Relatively low values of CAs were found for bottled drinking water and water samples from D2 and D3. The predominant chromosome aberrations detected in the present study were: vagrant chromosomes, stickiness and anaphase bridges.

KEYWORDS:
Drinking water, Genotoxicity, Cytotoxicity, *Allium cepa* test, Chromosome aberration, Mitotic index.

INTRODUCTION

The decrease in the quality and availability of drinking water is considered one of many problems caused by mainly global warming in many parts of the world. According to the United Nations reports, Saudi Arabia was classified as a water-scarce nation [1] because of the limitation of renewable water resources. This limitation leads to an over-exploitation of precious groundwater resources until their depletion [2] (Lippman, 2014) and an estimated annual increase of 8.8% in seawater desalination to overcome population needs for potable water [3]. Recently, groundwater resources and seawater desalination have contributed to 40% and 50% of drinking water needs in Saudi Arabia, respectively [4]. These water resources do not always meet the standards for human consumption. There is therefore a need for water treatments to meet international standards. Furthermore, over 50 groundwater treatment plants and 27 seawater desalination plants are located in different regions of the country. In general, the treatment process of raw water (groundwater and seawater) consists of three important steps: pre-treatment of raw water, filtration and water chlorination. The latest step is performed once a week for 1 hour to prevent microbial growth in water pipes. However, it has been reported that the chlorination step could produce mutagenic and/or carcinogenic by-products due to the reaction of the chlorine with the organic matter in water [5]. Several studies have shown that disinfection of drinking water by the chlorination process results in the formation of disinfection by-products with mutagenic and carcinogenic effects. In fact, an association between the mutagenicity of drinking water treated with chlorine and urinary and gastrointestinal tract cancers has been observed [6-8, 15].

Although the government has established quality standards for drinking water to make it safe for consumption, further assessment of the drinking water quality must be conducted in Saudi Arabia to determine the toxic chemicals existing in drinking water and to evaluate their possible mutagenic effects. Diverse studies performed in Saudi Arabia showed that drinking water was contaminated by several chemical compounds such as: pesticides [9], heavy metals [10, 11], fluoride content [12] and radioactive elements [13]. The major sources of water pollution are industrial wastewater, domestic discharges, industrial and oil disposal into the sea, and pesticide use in agriculture. For heavy metals, important quantities of Cu, Zn, and Cd reach drinking water due to the metallic piping used for domestic water

transportation [14]. Based on these findings, it was mandatory to study the mutagenic effects of the chemicals present in drinking water to predict possible risks on humans when consuming infected and/or contaminated drinking water. To the best of our knowledge, this is the first study conducted in Saudi Arabia to assess the cytotoxic and genotoxic effects of different types of drinking water to evaluate human health risks associated with the consumption of these potable waters.

Several investigations have been conducted worldwide to assess the quality of drinking water using genotoxic tests [15-17]. These tests were able to identify the compounds responsible for DNA damages leading to health risks for humans. The *Allium cepa* root anaphase-telophase test is considered favorable to assess genetic disturbances and damages during the mitotic cycle due to the large size of chromosomes and their reduced number (2n=16). The *Allium cepa* test is the best plant bioassay used to evaluate the toxicity of water [6, 18, 19].

The aim of this study was to evaluate the genotoxic effects of drinking water samples on MI and DNA damage by applying the *Allium* anaphase-telophase test.

MATERIALS AND METHODS

Sample collection. The present study was performed over a period of over one year (March 2015-June 2016) to assess the genotoxic and cytotoxic effects of drinking water from different sources under different seasonal conditions.

Tap water samples were collected from 20 houses located in two different regions of Yanbu: ten (T1) from Yanbu Al Balad (city center) and ten (T2) from Yanbu AL-Sinaiiya (industrial area). Before sample collection, water was allowed to flow for about 5 min. The volume of water samples was fixed to 5 L. The water analysis was conducted on the day of collection from March 2015 to July 2015. In some cases, samples were conserved in closed flasks at 5°C to be analyzed at the appropriate time.

Fifteen brands of bottled water were purchased from different local supermarkets and stores in Yanbu. 12 of these brands (B1) are manufactured in Saudi Arabia; the remaining brands (B2) are imported from other countries. For the water analysis, 3 batches of each brand were used. The analysis of bottled water was conducted from October 2015 to December 2015.

Water from three distribution networks (D1, D2 and D3) was collected on a monthly basis from Marchs 2016 to June 2016. Six-liter volume samples were individually collected in cleaned polyethylene plastic bottles and transported in ice boxes to the laboratory in the Department of Biology at the Faculty of Sciences in Yanbu City. The water samples were carefully labeled and stored at 5°C until the analysis process.

Chemical and physical analysis. Once in the laboratory, several physicochemical analyses were carried out. The pH values of the water samples were evaluated using a Hanna instrument 211 Microprocessor pH meter (Switzerland) at 25°C. The temperatures were measured using a regular thermometer during sample collection. Specific conductivity, total organic matter (TOM) and dissolved oxygen (DO) analyses were performed on the water samples at a regional certified laboratory according to standard methods.

***Allium cepa* test procedure.** The *Allium cepa* test procedure was applied according to the method described by Fiskesjö [20] and adopted by Knoll et al., [21] and Fachinetto et al., [22]. Small, uniformly sized and healthy onion bulbs (*Allium cepa* L. 2n=16) were purchased from the local supermarket. The first peels and old roots were carefully removed. Bulbs were then cleaned and placed in distilled water in the dark at room temperature. After 48h the onions were placed on jars containing the tested water samples, at laboratory conditions and left for germination during five days. Roots were fixed in a Carnoy solution (ethanol: acetic acid 3:1) for 24h, transferred to 70% (v/v) ethanol and stored until their analysis. To prepare the slides, roots were hydrolyzed using 1N HCl at 60°C for 5 to 6 min and immediately rinsed with distilled water. Only the meristematic regions were used in the slide analysis. Slide staining was performed using the aceto-orcine solution for 10 min. The roots were squashed by applying a slight pressure on the cover slip and the excess stain was removed with filter paper. To ensure the effectiveness of the assay, distilled water was used as a negative control and 10ppm methyl methane sulfonate (MMS) was used as a positive control.

The slide examination was carried out using a light microscope with 400X. A total of 5000 cells (1000 cells in each root tip) for each water sample were checked to determine the different mitotic stages and to calculate the MI. 800 cells in anaphase or telophase were counted for each water sample to evaluate the chromosome aberrations. Both MIs and CAs were expressed as percentages.

Statistical analysis. The comparison between drinking water samples and controls (distilled water) in term of CA and MI were analyzed using the Man-Whitney test ($p<0.05$ for significance). The evaluation of the association between the different cytogenetic endpoints (CA and MI frequencies) scored in Allium cepa meristematic cells and the seasonal variations were investigated using the Spearman correlation test. All statistical analysis was performed using the SPSS version 12.0.

RESULTS

Physical and chemical analysis. The results of the physical and chemical analyses of the drinking water samples are shown in Table 1. Significant variations in pH values, TOM and DO were observed with seasonal changes. The variations of temperature and specific conductivity were highly significant when comparing values recorded in summer with those registered in winter. In fact, water temperature values varied between 14.56°C (bottled water during winter) and 36.12°C (tap water during summer). The values of the TOM and DO in the water samples might be affected by the high temperature fluctuations in Saudi Arabia.

The recorded pH values varied from 6.10 to 7.26. These values were in accordance with the international norms, especially for bottled water and water of the distribution network. However, some variations were observed for pH values in the tap water samples collected from Yanbu Al Sinaiiya during winter (pH tended to be acidic). This observation might be due to the enrichment of the water with dissolved matter during the rainy season.

The water conductivity is related to the ion concentration. Ions are formed from dissolved salts and inorganic materials such as chlorides, sulfides, carbonate compounds and alkalis [23]. In potable water, the conductivity was set to 50-500 µs/cm [24]. Water conductivity variations with seasonal changes were directly related to the temperature fluctuations. When the water temperature increases, conductivity increases [23].

This process is associated with the increase in ion mobility, salt, and mineral solubility with increasing temperature [25]. The results recorded in the present study were in accordance with the facts described above. In fact, higher conductivity values were detected during summer than in the other seasons for the tap water samples collected from the two studied areas: 406.11µs/cm in water from Yanbu Al Balad and 398.67µs/cm in water from Yanbu Al Sinaiiya.

TABLE 1
Physical and chemical analysis of tap water, bottled water and distribution network water samples collected from Yanbu city.

Summer					
Water resource	pH±SD	T(°C) ±SD	Cond. (µs/cm) ±SD	TOM (ppm) ±SD	DO (mg/L) ±SD
Tap water (T1)	7.26±0.02	36.12±2.70	406.11 ±1.67	260.12±2.78	3.13±2.98
Tap water (T2)	7.14±0.03	35.56±2.18	398.67±2.72	255.81±1.91	3.88±1.60
Bottled water (B1)	7.02±0.01	28.09±0.05	188.09±0.03	120.65±0.02	7.10±0.03
Bottled water (B2)	7.06±0.01	25.44±0.06	260.78±0.02	167.81±0.04	6.67±0.01
Dist. Stat. Water (D1)	7.17±0.03	36.09±2.98	280.37±2.90	179.55±1.52	5.99±109
Dist. Stat. Water (D2)	7.08±0.04	35.11±1.41	258.03±2.13	165.19±2.42	6.10±2.52
Dist. Stat. Water (D3)	7.13±0.02	34.97±3.71	250.65±3.64	160.01±1.56	6,28±1.85
Autumn					
Water resource	pH±SD	T(°C) ±SD	Cond. (µs/cm) ±SD	TOM (ppm) ±SD	DO (mg/L) ±SD
Tap water (T1)	7.36±0.03	30.01±2.98	386.43±3.92	247.82±1.91	4.72±3.13
Tap water (T2)	7.20±0.04	32.67±3.62	374.04 ±2.81	239.13±2.14	4.06±3.32
Bottled water (B1)	7.10±0.01	18.9±0.03	190.00±0.01	122±0.01	7.00±0.02
Bottled water (B2)	7.19±0.01	19.10±0.01	278.03±0.0	178±0.04	7.34±0.03
Dist. Stat. Water (D1)	7.18±0.03	33.67±1.17	263.46±3.29	169.90±2.63	6.10±2.12
Dist. Stat. Water (D2)	7.10±0.03	28.34±2.72	261.14±4.14	167.00±2.25	6.67±1.53
Dist. Stat. Water (D3)	7.19±0.02	31.29±1.19	257.87±3.34	165.22±2.18	6..25±2.65
Winter					
Water resource	pH±SD	T(°C) ±SD	Cond (µs/cm) ±SD	TOM (ppm) ±SD	DO (mg/L) ±SD
Tap water (T1)	6.97±0.07	20.08±3.98	290.65±4.21	186.00±3.90	5.78±2.11
Tap water (T2)	6.10±0.05	24.77±2.29	309.15±4.56	198.20±2.52	5.19±3,84
Bottled water (B1)	7.11±0.01	14.56±0.02	187.89±0.03	120±0.04	7.30±0.01
Bottled water (B2)	7.22±0.01	17.87±0.05	264.98±0.01	170±0.05	7.00±0.01
Dist. Stat. Water (D1)	7.01±0.04	22.45±2.17	197.70±3.16	126.05±3.13	6.81.13±2.22
Dist. Stat. Water (D2)	7.17±0.05	23.68±3.12	204.62±2.88	131.24±2.24	6.73.44±3.42
Dist. Stat. Water (D3)	7.11±0.03	21.87±1.58	201.71±2.30	129.69±3.33	6.98.53±3.13
Spring					
Water resource	pH±SD	T(°C) ±SD	Cond (µs/cm) ±SD	TOM (ppm) ±SD	DO (mg/L) ±SD
Tap water (T1)	7.31±0.02	33.28±2.23	329.91 ±3.82	211.04±2.32	4.56±1.78
Tap water (T2)	7.25±0.04	30.84±2.15	310.56±4.03	199.77±1.93	4.21±2.41
Bottled water (B1)	7.12±0.01	17.39±0.04	179.50±0.04	115±0.02	7.18±0.02
Bottled water (B2)	7.11±0.01	20.97±0.04	257.84±0.05	165±0.03	6.45±0.01
Dist. Stat. Water (D1)	7.07±0.02	28.37±2.62	198.89±2.08	127.32±0.98	6.78.54±1.75
Dist. Stat. Water (D2)	7.11±0.04	30.86±3.23	211.27±4.16	135.37±1.78	6.50.41±1.38
Dist. Stat. Water (D3)	7.19±0.02	33.83±1.48	221.43±2.00	142.00±1.32	6.66.53±0.80

TABLE 2
Calculated Mitotic indexes (MIs) in root-tip meristematic cells exposed to different types of drinking water

Water resource	Summer		Autumn		Winter		Spring	
	CN	MI ± SD	CN	MI ± SD	CN	MI ± SD	CN	MI ± SD
Tap water (T1)	5067	13.66**±0.86	5311	15.37*±0.44	5352	16.52*±0.67	5325	14.77*±1.05
Tap water (T2)	5090	12.74**±0.77	5224	14.09*±0.63	5147	15.37*±1.15	5276	13.71**±0.98
Bottled water (B1)	5182	19.98±0.14	5212	20.55±0.43	5140	20.01±0.93	5152	20.09±0.22
Bottled water (B2)	5125	19.88±0.82	5265	20.12±0.41	5172	20.11±0.56	5167	19.98±0.73
Dist. Stat. Water (D1)	5255	17.63±0.71	5311	17.90±0.66	5171	18.60±1.01	5159	16.54*±0.48
Dist. Stat. Water (D2)	5002	18.33±0.88	5203	19.01±0.94	5011	19.23±0.65	5307	18.67±0.67
Dist. Stat. Water (D3)	5170	18.98±1.15	5012	19.52±1.02	5029	20.54±1.33	5240	18.33±0.64
Distilled water	5391	20.59±0.95	5360	20.59±0.95	5114	20.59±0.95	5271	20.59±0.95
MMS	5001	6.35±0.87	5234	6.35±0.87	5275	6.35±0.87	5171	6.35±0.87

SD: standard deviation, CN: counted cells, *: significant difference ($p<0.05$), **: high significant difference ($p<0.01$)

The TOM is an essential parameter in drinking water quality evaluation. The origin of dissolved solids in water might be natural resources, industrial wastewater, sewage, piping used in water distribution and the chemicals used in water treatment. In the present study, the registered TOM values were correlated with fixed standards. However, relatively higher values were found in tap water samples (average: 257.96 ppm).

Dissolved oxygen is related to the levels of suspended solids in water samples. If suspended solids in water are highly concentrated, the temperature will increase due to heat absorption by the suspended solids and, consequently, warmer water contains less dissolved oxygen than colder water [26]. In the present work, water collected in summer had lower values of DO (3.13 mg/L in tap water) than that collected in winter (6.98 mg/L). For bottled water samples, the DO values were set to around 7 mg/L which is considered as the acceptable level for excellent drinking water.

***Allium cepa* bioassay.** Due to its sensitivity to aquatic environmental pollution, the *Allium cepa* test has recently been recognized as an efficient bioassay applied in cytogenotoxicity studies of several environmental pollutants in different types of water (drinking water, river and lake water, industrial effluents, etc.). The test is based on the evaluation of DNA damages, chromosome aberrations and mitotic cycle disturbance.

In the present research, the measurement of cytotoxicity caused by the exposure to the tested water samples was carried out by calculating the mitotic index (MI) in the root-tip meristematic cells of onion bulbs. The genotoxicity measurement of the water samples was evaluated by the determination of different chromosome aberrations (CA) observed during the anaphase-telophase of the cell division. Table 2 showed the values of the MI calculated in the cells exposed to different water types, in the negative (distilled water) and the positive (MMS) controls. The MI was expressed in terms of divided cells/total number of counted cells.

The highest MI value was recorded in meristematic cells of rootlets germinated in distilled water, and the lowest MI value was detected in cells exposed to MMS solution at 10 ppm. Statistically significant decreases were registered for the MIs calculated for meristematic cells in plants grown for five days in the tap water samples collected from the two studied areas ($p<0.05$). It is clear that such decrease in MI values was much more significant ($p<0.01$) during the summer season (MI average= 13.20%) than during winter (MI average=15.94%). In general, the MIs in cells treated with the distribution network water samples (D1, D2 and D3) decreased when compared with the negative control. The differences had no statistical significance for all seasons ($p>0.05$). Only water samples from D1 showed a significant decrease (MI=16.54 %) during spring. The MIs recorded for root cells germinated in the different brands of the bottled water samples showed no significant variations when compared to the negative control.

The significant decline in MIs registered in the present study indicates cell division retardation in the root-tip meristematic cells and might be due to DNA synthesis inhibition or G2 phase blocking during the cell cycle as explained by Sudhakar et al., [27] (2001). These significant low values of MIs reflect alterations in the cell cycle probably caused by exposure to cytotoxic agents present in the tested water samples. Furthermore, the registered variations registered in the water's chemical composition with the seasonal variations of the conditions, might have direct effects on the cell division and, in turn, on the mitotic index values. Similar results were reported by Abda et al., [15] and by [28-29] Monarca et al. who found significant decreases in cell division over the four seasons with the highest decline being in autumn.

The genotoxic potential of the tested water samples on root-tip meristematic cells of *Allium*

cepa plants was evaluated by considering the following chromosomal aberrations: C-metaphase, delayed anaphase, vagrant chromosomes, stickiness and anaphase bridges (Table 3). The analysis of chromosomal aberrations during the anaphase-telophase stage showed higher values of total abnormalities in cells treated with MMS (positive control) (7.58%). Few abnormalities were observed in cells grown in distilled water (negative control) (3.12%).

The highest values of total abnormalities were induced by tap water samples during summer with significant statistical differences when compared to the negative control ($p<0.05$). The abundant abnormalities detected were delayed anaphase (5.13%), anaphase bridge formations (2.07%) and stickiness (1.11%) for tap water collected in YanbuAl Balad. For water samples collected from Yanbu AL-Sinaiiya, the majority of abnormalities were anaphase delayed, (4.65%) and anaphase bridge formations (1.71%), detected during the summer.

Statistically significant results were also found in the analysis of root cells germinated in the distribution network water samples from station D1 during spring with a total abnormality frequency of 8.58%, delayed anaphase of 4.53%, anaphase bridges of 1.62% and stickiness of 1.14%. The frequencies of CAs in root-tip cells grown on all the brands of the bottled water samples showed no detectable genotoxic effects.

In general, the highest frequencies of chromosome aberration inductions by the tested water samples were observed during summer and spring (with fewer effects). These results could be explained by the critical weather conditions in the studied areas, which led to an increase in chemical concentrations in the water. As a consequence, serious damage could be caused to the DNA of the *Allium cepa* cells treated with the different water types, and where stickiness, vagrant chromosomes and anaphase bridges were the most detectable damages.

Vagrant chromosomes were detected with high frequencies in *Allium cep*a cells grown in tap water samples and some distribution network water samples. This chromosomal abnormality is caused by mitotic spindle dysfunction indicating aneugenic effects of the chemical compounds present in the test water samples [30]. The vagrant chromosomes induced in the root-tip meristematic cells of onion bulbs might cause unequal chromosome numbers in daughter cells and therefore unequal sizes and/or irregular nuclei shapes of daughter cells [31]. Chromosome bridges result from chromosomal breaks and are therefore considered as clastogenic effects of the tested water samples [32, 33].

TABLE 3
Frequencies of chromosome aberrations observed in different water samples using the *Allium cepa* test during the four seasons

	Summer						Autumn					
Water resource	C-metaphase	DA	VC	S	AB	TA (±SD)	C-metaphase	DA	VC	S	AB	TA (±SD)
Tap water (T1)	0.53	5.13	0.74	1.11	2.07	9.58±1.97	0.72	3.24	0.85	1.56	1.19	7.56±0.28
Tap water (T2)	0.66	4.65	0.69	0.74	1.71	8.45±1.29	0.57	3.85	0.99	0.89	1.42	7.72±0.94
Bottled water (B1)	0.44	1.34	0.61	0.68	0.80	3.87±0.32	0.49	1.29	0.68	0.71	0.81	3.98±0.69
Bottled water (B2)	0.55	1.41	0.67	0.76	0.89	4.28±0.92	0.46	1.29	0.62	0.66	0.78	3.81±0.53
Dist. Stat. Water (D1)	0.41	2.58	0.58	0.74	1.01	5.32±0.27	0.79	1.76	0.94	0.62	0.87	4.98±0.47
Dist. Stat. Water (D2)	0.66	2.13	0.50	1.07	0.97	5.33±1.04	0.33	1.75	0.58	0.92	0.75	4.33±0.57
Dist. Stat. Water (D3)	0.54	2.62	0.41	1.33	1.08	5.98±0.70	0.47	2.16	0.68	0.86	0.95	5.12±1.11
Distilled water	0.32	1.13	0.42	0.52	0.73	3.12±0.24	0.32	1.13	0.42	0.52	0.73	3.12±0.24
MMS (10ppm)	0.61	4.17	0.77	0.85	1.18	7.58±0.34	0.61	4.17	0.77	0.85	1.18	7.58±0.34
	Winter						Spring					
Water resource	C-metaphase	DA	VC	S	AB	TA (±SD)	C-metaphase	DA	VC	S	AB	TA (±SD)
Tap water (T1)	0.41	3.38	0.68	1.11	1.27	6.85±0.41	043	4.78	0.61	1.09	1.94	8.85±0.23
Tap water (T2)	0.42	2.44	0.66	0.85	1.01	5.38±0.35	0.55	4.47	0.58	0.85	1.70	8.15±0.58
Bottled water (B1)	0.52	1,39	0.63	0.71	0.81	4.06±0.62	0.47	1.36	0.63	0.70	0.79	3.95±0.32
Bottled water (B2)	0.50	1.24	0.65	0.63	0.72	3.74±1.01	0.48	1.34	0.64	0.73	0.84	4.03±0.62
Dist. Stat. Water (D1)	0.43	1.67	0.63	0.58	0.84	4.37±0.54	0.62	4.53	0.67	1.14	1.62	8.58±1.12
Dist. Stat. Water (D2)	0.58	1.53	0.61	0.61	0.79	4.12±1.29	0.58	2.71	0.87	0.97	1.15	5.74±0.57
Dist. Stat. Water (D3)	0.48	1.66	0.68	0.59	0.71	4.07±0.36	0.39	2.10	0.60	1.06	1.10	5.25±0.73
Distilled water	0.32	1.13	0.42	0.52	0.73	3.12±0.24	0.32	1.13	0.42	0.52	0.73	3.12±0.24
MMS (10ppm)	0.61	4.17	0.77	0.85	1.18	7.58±0.34	0.61	4.17	0.77	0.85	1.18	7.58±0.34

DA: delayed anaphase, VC: vagrant chromosome, S: stickiness, AB: anaphase bridge, TA: total anomalies

To understand the detected cyto-genotoxic effects of the studied water types listed in the present study, an up dated chemical analysis of water samples is necessary to evaluate the contamination levels by several chemical pollutants. Many decades ago, several authors have assessed the presence of environmental contaminants in drinking water from Saudi Arabia. Al Saleh and AL Doush [10] evaluated trace elements in tap water and bottled drinking water collected from Riyadh city. The authors found high concentrations of Cd, Fe, Hg, Ni, and Zn in household drinking water samples with values exceeding the guideline limits fixed by international organisms for drinking water. Authors supposed that the primary sources of these trace elements were industrial effluents. They also clarified that these high levels of contamination might be caused by the corrosive nature of the water distribution pipes and by water storage tanks if not maintained and if improperly installed. The trace elements in the bottled water were found to be within the guideline limits.

Furthermore, Al Saeid et al., [9] (2011) studied the levels of contamination of groundwater samples by organochlorines, organphosphorus, carbamates and pyrethroids. They found that there was a general contamination by the studied contaminants in all the sampling areas, with higher levels of DDT, DDE dimethoate and chloroneb. The authors confirmed that these results are alarming for human health when this contaminated water is used. Another recent study was conducted to assess water treatment plants in Saudi Arabia and the contamination by radionuclides in raw and treated waters [13]. It was demonstrated that raw water samples collected from deep groundwater had a radium activity exceeding the international limits, which is also hazardous to human health.

In conclusion, large an up to dated bio-monitoring studies must be conducted in Saudi Arabia to evaluate the levels of contamination by environmental contaminants of different drinking water types and to assess the water quality in order to reduce human health concerns related to the presence of chemical pollutants in potable water.

ACKNOWLEDGEMENTS

The author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] Samad., N.A., Bruno, V.L. (2013) The urgency of preserving water resources. Environ. News. 21, 3-6.

[2] Lippman, T.W. (2014) Biggest Mideast crisis: growing water scarcity. Business Mirror. May 24. Available at: http://www.ipsnews.net/2014/05/biggest-mideast-crisis-probably-dont-know-enough/. Accessed August 17, 2015.

[3] DeNicola, E., Aburizaiza, O.S., Siddique, A., Khwaja, H., Carpenter, D.O. (2015) Climate Change and Water Scarcity: The Case of Saudi Arabia. Ann. Glob. Health. 81 342– 353.

[4] Al-Zubari, W.K. (2015) Sustainable water resources management in the Gulf Cooperation Council countries. WWAP (United Nations World Water Assessment Programme). Facing the Challenges. Case Studies and Indicators. UNESCO, Paris.

[5] Rook, J. (1974) Formation of haloforms during chlorination of natural waters. Water Treat. Examin. 23, 234–243.

[6] Khallef, M., Liman, R., Konuk, M., Hakk, I.H., Benouareth, D.E., Tabet, M., Abda A. (2015) Genotoxicity of drinking water disinfection by-products (bromoform and chloroform) by using both Allium anaphase-telophase and comet tests. Cytotechnology. 67, 207-213.

[7] Schenck, K.M., Wymer, L.J., Lykins, B.W. Jr., Clark, R.M. (1998) Application of a Finnish mutagenicity model to drinking waters in the U.S. Chemosphere. 3, 451–464.

[8] Tao, X., Zhu, H., Matanoski, G.M. (1999) Mutagenic drinking water and risk of male esophageal cancer: a population-based case-control study. Am. J. Epidemiol. 150, 443–451.

[9] El Saeid, M.H., Al Turki, A.M., Al Wable, M.I., Abdel-Nasser, G. (2011) Evaluation of pesticide residues in Saudi Arabia ground water. Res. J. Environ. Sci. 5, 171-178.

[10] Al-Saleh, I., Al-Doush, I. (1998) Survey of trace elements in household and bottled drinking water samples collected in Riyadh, Saudi Arabia. Sci. Total Environ. 216, 181-192.

[11] Alyet, N.S.M., Ali, R.M., El Badawy, H.A. (2013) Assessment of Drinking Water Quality. Life Sci. J. 10, 1795-1803.

[12] Alabdula Aly, A.I. (1997) Fluoride content in drinking water supplies of Riyadh, Saudi Arabia. Environ. Monit. Assess. 48, 216-272.

[13] Al-Jaseem, Q.K.h., Almasoud, F.I., Ababneh, A.M., Al-Hobaib, A.S. (2016) Radiological assessment of water treatment processes in a water treatment plant in Saudi Arabia: Water and sludge radium content, radon air concentrations and dose rates. Sci. Total. Environ. 563–564, 1030–1036.

[14] Fuge, R., Perkins, W. (1991) Aluminum and heavy metals in portable waters of the north Ceredigion area, mid-Wales. Environ. Geochem. Health. 13, 56-65.

[15] Abda, A., Benouareth, D.E., Tabet, M., Liman, R., Konuk, M., Khallef, M., Taher A. (2015) Mutagenicity and genotoxicity of drinking water in Guelma region, Algeria. Environ. Monit. Assess. 187, 1-15.
[16] Monarca, S., Feretti, D., Zani, C., Rizzoni, M., Casarella, S., Gustavino, B. (2005) Genotoxicity of Drinking Water Disinfectants in Plant Bioassays. Environ. Mol. Mutagen. 46, 96-103.
[17] Smaka-Kincl, V., Stegnar, P., Lovka, M., Toman, M.J. (1996) The evaluation of waste, surface and ground water quality using the Allium test procedure. Mutat. Res. 368, 171-179.
[18] Fiskesjö, G. (1985) The *Allium test* as a standard in environmental monitoring. Hereditas. 102, 99–112.
[19] Tabrez, S., Shakil, S., Urooj, M., Damanhouri, G.A., Abuzenadah, A.M., Ahmad, M. (2011) Genotoxicity testing and biomarker studies on surface waters: an overview of the techniques and their efficacies. J. Environ. Sci. Health Part C Environ. Carcino. Ecotoxicol. Rev. 29, 250–275.
[20] Fiskesjö, G. (1994) The *Allium Test* II: Assessment of chemical's genotoxic potential by recording aberrations in chromosomes and cell divisions in root tips of *Allium cepa* L. Environ. Toxicol. Water Qual. 9, 234-241.
[21] Knoll, M.F., Silva, A.C.F., Tedesco, S.B., Canto-Dorow, T.S. (2006) Effects of Pterocaulonpolystachyum DC. (Asteraceae) on onion (*Allium cepa*) root-tip cells. Genet. Mol. Biol. 29, 539-542.
[22] Fachinetto, J.M., Tedesco, S.B. (2009) Antiproliferative and mutagenic activity of aqueous extracts of Baccharis trimera (Less.) A. P. de Candolle and Baccharisarticulata (Lam.) Pers. (Asteraceae) on the test system of Allium cepa. Rev. Bras. Pl. Med. 11, 360-367.
[23] Miller, R.L., Bradford, W.L., Peters, N.E. (1988) Specific Conductance: Theoretical Considerations and Application to Analytical Quality Control. In U.S. Geological Survey Water-Supply Paper. Retrieved from http://pubs.usgs.gov/wsp/2311/report.pdf.
[24] Lenntech, B.V. (2009) Water treatment and purification Holding B.V 1998-. http://www.lenntech.fr/applications/ultrapure/conductivite/eau-conductivite.htm.
[25] Whipple, K. (2002) Factors Influencing Hydraulic Roughness. In Surface Processes and Landforms. Retrieved from http://ocw.mit.edu/courses/earth-atmospheric-and-planetary-sciences/12-163-surface-processes-and-landscape-evolution-fall-2004/labs/roughnes_handout.pdf.
[26] Hickin, E.J. (Ed.) (1995) River Geomorphology. Chichester: Wiley.
[27] Sudhakar, R., Gowda, N., Venu, G. (2001) Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia. 66, 235-239.
[28] Monarca, S., Richardson, S.D., Feretti, D., Grottolo, M., Thruston, A.D., Zani, C., Navazio G., Ragazzo P., Zerbini I., Alberti A. (2002) Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid. Environ. Toxicol. Chem. 21, 309–318.
[29] Monarca, S., Rizzoni, M., Gustavino, B., Zani, C., Alberti, A., Feretti, D., Zerbini I. (2003) Genotoxicity of surface water treated with different disinfectants using in situ plant tests. Environ. Mol. Mutagen. 41, 353–359.
[30] Leme, D.M., Marin-Morales, M.A. (2009) Allium cepa test in environmental monitoring: a review on its application. Mutat. Res. 682, 71–81.
[31] El-Ghamery, A.A., El-Kholy, M.A., El-Yousser, A. (2003) Evaluation of cytological effects of Zn in relation to germination and root growth of *Nigella sativa* L. and *Triticumaestivum* L. Mutat. Res. 537, 29–41.
[32] Masood, F., Malik, A. (2013) Cytotoxic and genotoxic potential of tannery waste contaminated soils. Sci. Total Environ. 444, 153–160.
[33] Radić, S., Stipanicev, D., Vujcic, V., Rajcic, M.M., Sirac, S., Pevalek-Kozlina, B. (2010) The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Sci. Total Environ. 408, 1228–1233.

Received: **13.07.2018**
Accepted: **16.11.2018**

CORRESPONDING AUTHOR

Soukaina Ennaceur
Department of Biology,
Faculty of Sciences in Yanbu,
Taibah University,
Al Madinah Al Mounawra – Kingdom of Saudi Arabia

e-mail: ennaceurs@yahoo.fr

© by PSP Volume 27 – No. 12B/2018 pages 9979-9988 Fresenius Environmental Bulletin

HYDROMORPHOLOGICAL ASSESSMENT OF SELECTED LOWLAND WATERCOURSES WITH THE USE OF THE MHR METHOD

Piotr Lewandowski[1,*], Krzysztof Gorecki[2]

[1]Poznan University of Life Sciences, Institute of Construction and Geoengineering, ul. Piątkowska 94E, 60-649 Poznan, Poland,
[2]Poznan University of Life Sciences, Department of Entomology and Environmental Protection, ul. Dąbrowskiego 159, 60-594 Poznan, Poland

ABSTRACT

The article takes up the subject of hydromorphological valorization of watercourses. It is one of the most important issues of the Water Framework Directive 2000/60/EC (WFD). The Directive requires an assessment of the ecological status or potential of watercourses. That assessment should be based on the assessment of biological, physicochemical and hydromorphological elements. The latter constitutes a significant difficulty. It includes, among others, the hydrological regime, river continuity and morphological conditions, connection of surface waters to groundwater bodies, flaw rate and assessment of the floodplain area. The final assessments were introduced by the CEN European Standard (EN 15843) developed after the WFD. Thereafter, other formal EU requirements appeared, making this problem more complicated. In Poland, the River Hydromorphological Monitoring (*Polish: Monitoring Hydromorfologiczny Rzek* - MHR) method was developed in 2009 according to the above-mentioned requirements. Basing on a photointerpretation of orthophotomaps, it is a consensus between the scientific accuracy and organizational and financial possibilities of this monitoring method. In this method, four elements are subject to assessment: hydrological regime, river continuity, riverbed morphology and floodplain area. Among others, five lowland watercourses of Wielkopolska were subjected to a survey with the use of this method. They belong to three categories of naturalness constituting six Uniform Parts of Water Bodies (UPWBs). These are small and medium watercourses with different sizes of water catchment area, general length and main watercourse length. The results of the survey were presented and compared to results obtained with other methods - KOKŚ (*the Department of Environmental Protection and Management*) score evaluation method and RHS method. This comparison indicates similar assessments, in spite of the use of different number of assessed parameters and conducting surveys at different times.

KEYWORDS:
Water Framework Directive, River Hydromorphology, MHR Method, Wielkopolska Region

INTRODUCTION

According to the Water Framework Directive 2000/60/EC (WFD), it is necessary to conduct assessments of ecological status or potential of watercourses [1]. This includes the assessment of biological, physicochemical and hydromorphological elements. The hydromorphological assessment includes the assessment of the hydrological regime, river continuity and riverbed morphology. The connection of surface waters to groundwaters, the flaw rate and the river continuum continuity [2] with the presence of barriers and ichthyofauna migration constraints [3] are also of great importance.

A center that initiated (1992) the research on hydromorphology of watercourses in Poland was the Department of Environmental Protection and Management at Agricultural Academy in Poznań. The original methodology was published in 1995 [4-7]. Until 2002, 709 km of waterways in Wielkopolska (Warta and Noteć) [8] and over 650 km of smaller watercourses have been assessed.

Experiences of the team from that center served to develop the River Hydromorphological Monitoring (MHR) method for the Central Inspectorate of Environment Protection in Warsaw [10-15]. It is fully compliant with the WFD. It assumes an assessment of the hydrological regime, river continuity, riverbed morphology and floodplain. A recommendation to assess the latter was introduced by the CEN European Standard (EN 15843) [16].

The MHR method was tested in different regions of the country. In Wielkopolska, five tributaries of the middle course of the Warta River belonging to three categories of naturalness were chosen: Junikowski Stream, Wirynka and Wrześnica are natural watercourses, Meszna - heavily modified, the Ślesiński Canal - an artificial one. Furthermore, the results obtained thanks to the MHR method were compared to the results of other methods.

The aim of the article is to present the results of hydromorphological assessment of five watercourses (six UPWBs) with different categories of naturalness using the MHR methodology. Furthermore, an additional aim is to verify the accuracy of the results obtained with the use of this method. That is why, for the purposes of comparison, the data obtained with the MHR method for the analyzed watercourses were compared to the results obtained thanks to the KOKŚ score evaluation method and the River Habitat Survey (RHS) [17].

AREA AND METHODS

The River Hydromorphological Monitoring (MHR) method developed in 2009 was applied in the hydromorphological survey of selected watercourses [10-15]. Among test watercourses, we chose five sites situated in Wielkopolska (Ślesiński Canal, Junikowski Stream, the Meszna, Wirynka and Września Rivers) (Fig. 1). There were characterized by a differentiated main watercourse length, the level of anthropopressure (among others, regulation of the riverbed and use of the valley) and waters category. They were constituting six UPWBs with a total length of 117.41 km. Their general characteristics is contained in Table 1.

While presenting the research methodology, it is advised to present the general assumptions serving to develop it. This also results from an innovative approach to its preparation. This methodology, apart from the WFD requirement, also takes into consideration the CEN European Standard (EN 15843) requiring, among others, an assessment of the floodplain area [16]. Also, Langhammer was pointing out the significance of the assessment of the watercourse valley [18]. He was highlighting the connections of watercourse modification changing the water flow rate and facilities limiting its continuity (river continuum) and the retention capacity of the valley. In case of water-impounding structures, the possibility upward migration of fish was taken into account in the MHR method. According to Błachuta et al., [3] the impoundment height of 0.4 m in lowland landscape and of 0.7 m in highland and mountainous landscape are crucial for this factor. Experiences from the research carried out in Poland [4-9, 22-23] as well as in Austria, the Czech Republic, the Netherlands, Germany and Slovakia [24-26] were used while developing the method.

Due to the WFD and requirements for reporting of monitoring results to the European Commission in the framework of the Eionet-Water network [27], it is also necessary to apply the INSPIRE directive [28] and to provide metadata; elaboration of a new method of a new watercourse assessment protocol turned out to be necessary. It constitutes the basic document applied in research conducted with the use of MHR methodology. It must include both the inventory and assessment of the status for natural watercourses and of the ecological potential for heavily modified and artificial watercourses.

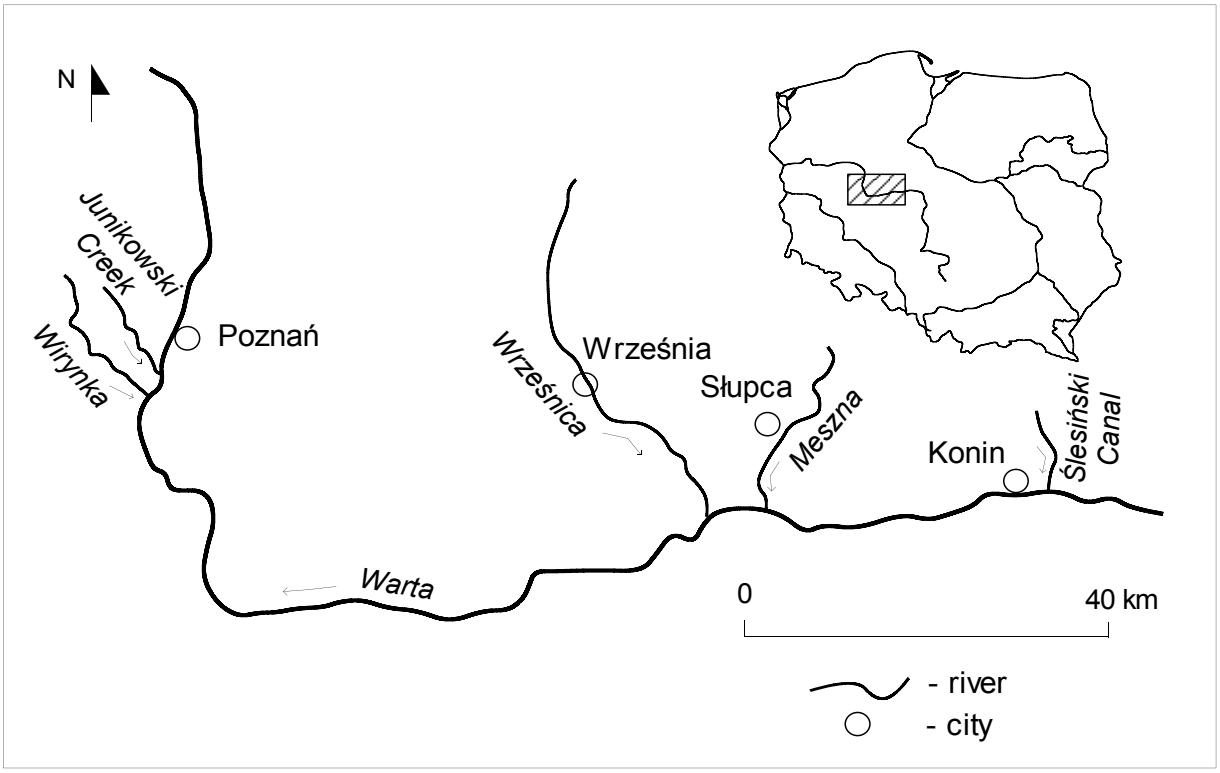


FIGURE 1
Location of investigated watercourses

TABLE 1
Hydrographic characteristics investigated watercourses (own study based on Czarnecka et al., 2005 [19-20] and Rapor, 2005 [21])

No	Name of the UPWB	Code for UPWB	Length of UPWB (km)		Catchment area (km^2)	
			Overall	The main watercourse	The UPWB	Of the watercourse at the point closing the UPWB
1	Junikowski Stream	PLRW60001718576	11.0	11.0	48.91	48.91
2	Wirynka	PLRW600017185729	46.1	18.3	102.34	102.34
3	Wrześnica	PLRW60001718389	111.8	56.61	375.56	375.56
4	Meszna to Bawół Stream	PLRW600023183679	20.3	20.3	41.63	273.34
5	Meszna from the tributary of Babiń to the river mouth	PLRW60002418369	3.4	3.4	15.09	715.71
6	Ślesiński Canal from Pątnowskie Lake to the river mouth	PLRW6000018349	7.8	7.8	49.86	442.65
Total			200.4	117.41	633.39	1685.17*

* without the catchment area at the closing point the UPWB of Meszna river to the Bawół Stream

Several relevant assumptions were adopted while developing the methodology and assessment protocols [10]: 1) the assessment methodology requires additional field and office works; 2) the assessment of hydromorphological status is carried out for each UPWB of all categories of watercourses (but limited to the main watercourse without its tributaries); 3) the basic assessment of the watercourse is carried out in the office based on orthophotomaps, 1 topographic maps (in scale min. 1:10,000), information on the watercourse's administrator and existing documents and publications; 4) the hydromorphological status/potential assessment is carried out on the basis of two models of map-based protocols - for natural and heavily modified watercourses (analogous assessment) and for artificial watercourses; 5) the map-based UPWB assessment is corrected on the basis of field surveys on the basis of an elaborated field protocol; 6) the field surveys comprise, in total, a minimum 10% of the length of map-based surveyed watercourse. The localization of field sections is determined so as to explain doubts and determine the data that cannot be obtained in the office; 7) the protocols include the results of the inventory and calculated values of the Ecological Quality Ratio (EQR) for the natural watercourses or the Ecological Potential Ratio (EPR) for heavily modified and artificial watercourses; 8) the assessment with the use of the protocol has a hierarchic structure in which the basic elements are assessed on the basis of numerous features and these, in turn, on the basis of attributes. The final EQR/EPR assessment is an arithmetic mean of the assessment of elements, the elements - of indices and indices - of attributes; 9) the assessment of the ecological state/potential is composed of 4 hydromorphological elements (hydrological regime, river continuity, riverbed morphology, floodplain valley) determined on the basis of 16 features for natural and heavily modified watercourses, relatively 19 for artificial watercourses that are assessed on the basis of attributes; 10) limit values for all five classes of status and four classes of ecological potential were determined with the use of statistical analysis of the results of the surveys (with a score evaluation) that have been carried out up until now in Poland (about 2,200 km) and experiences of other countries [9, 22-26]. The limit values for the classes determined in this way for specific categories of surface watercourses are presented in Fig. 2 for the results of the analyzed UPWBs. A broad description of the new methodology was presented in numerous publications [10-15].

The current orthophotomaps (resolution of 0.5 m) and topographical maps in scale 1:50,000 and 1:10,000 were used in a map-based assessment of the above-mentioned sites. Also, commonly available aerial and satellite photos were being used (geoportal.gov.pl). The available, archival melioration documentation and Raster Map of Hydrographical Division of Poland [29]. It should be noted that in the inventory of watercourses of the cited map, the UPWB Meszna "from the tributary of Bawół Stream to the river mouth" was mistakenly called "the Meszna River from the inflow from Babiń to the river mouth".

The Hydrological Atlas of Poland (*Atlas Hydrologiczny Polski*) was used in order to assess the "hydrological regime" element [30-31]. Gauging stations taken into account in the map-based assessment exist only in two UPWBs. These include Meszna River from the inflow from Babiń (Bawół Stream) to the river mouth (km 4.0 – Kąty village) and Wrześnica (km 3.6 - Samarzewo). For the other watercourses on which there are no gauging stations, the "River flow" features are not assessed according to the MHR methodology. In the case of Ślesiński Canal constituting an artificial watercourse – according to the methodology, the "Hydrological regime" element was not assessed.

The field surveys conducted in May and in Ju-

ly 2009 on 13 sections were selected on the basis of the map with the total length of 59 km comprising about 50% of the UPWBs main watercourses length. Topographical maps in scale 1:10,000 and more precise as well as GPS were used during this research. A photographic documentation illustrating hydrotechnical facilities, values environmental elements (e.g. beaver lodges and traces of their presence), characteristic morphological elements of the watercourse (e.g. natural river thresholds, large woody debris and eroded escarpments).

RESULTS

The surveyed watercourses (Junikowski Stream, the Wirynka, Wrześnica, Meszna Rivers and the river mouth section of Ślesiński Canal) are direct tributaries of the middle course of the Warta River (Fig. 1, Tab. 1). The total length of the main watercourses subjected to the study amounts to 117.41 km. Taking into consideration the WFD typology based on the water catchment area, the surveyed watercourses belong to small (10-100 km^2) and average ones (100-1000 km^2). The Junikowski Stream (48.91 km^2) was qualified to the first group, the others (Wirynka, Wrześnica, Meszna and Ślesiński Canal) with the water catchment area of 102.34-715.71 km^2 were qualified to the second group.

The highest EQR values among the natural watercourses were reached by Junikowski Stream (0.81) and Wirynka (0.79) (Tab. 2). So high values of the features were resulting mainly from the assessment of the two surveyed elements: the hydrological regime (respectively 0.90 and 0.92) and the river continuity (1.0 each). The Junikowski Stream and Wirynka were characterized by a natural water flow and lack of facilities with the impoundment height above 0.4 m. In the case of the Wrześnica River, a lower EQR value (0.52) was caused by assessment of the river continuity (0.2). This assessment results from the presence of one large water impounding structure (Psary dam) cutting off over 40% of the river length without the possibility of fish migration. The EQR values of riverbed and floodplain valley morphological elements in the case of natural watercourses were oscillating within the same brackets and amounted to respectively 0.61–0.69 and 0.55–0.65. In the final assessment, the Junikowski Stream and Wirynka were qualified to a very good ecological status, whereas the Wrześnica River to a moderate status (Fig. 2).

The Meszna River was classified to heavily modified rivers and divided into two UPWBs; it obtained two very different scores of the ecological potential. The value for the first of them (Meszna River to the Bawół Stream) supplying the "Słupca" retention reservoir in water is considerably lower (0.46) than the one for the water mouth section – the Meszna River from the inflow from Babiń to the river mouth (0.70). Such a large difference in the final assessment results mainly from the assessment of the "River continuity" element. That is caused by a lack of facility cutting off the river continuity on its short 3.4 km-long river mouth section of the second UPWB of this watercourse. The values of other elements ("hydrological regime", "riverbed morphology" and "floodplain valley") are similar to the both UPWBs of the Meszna River. The EPR assessment for "The Meszna River to the Bawół Stream" is not significantly deviated from the EQR values for the Wrześnica River (a natural watercourse). Whereas the assessment of "the Meszna River from the inflow from Babiń to the river mouth" is similar to the assessment of natural watercourses (Junikowski Stream and Wirynka).

TABLE 2
The ecological quality ratios (EQR) and ecological potential ratios (EPR) investigated watercourses

No	Categories of watercourses	Uniform Parts of Surfaces Water (UPWB)	Ecological quality and potential ratios Elements				Total rating
			Hydrological regime	River continuity	Bed morphology	Floodplain valley	
1	Natural watercourses	Junikowski Stream	0.90	1.00	0.67	0.65	0.81
2		Wirynka	0.92	1.00	0.69	0.55	0.79
3		Wrześnica	0.65	0.20	0.61	0.63	0.52
4	Heavily modified watercourses	Meszna to Bawół Stream	0.67	0.20	0.41	0.56	0.46
5		Meszna from the tributary of Babiń to the river mouth	0.74	1.00	0.50	0.56	0.70
6	Artifical watercourse	Ślesiński Canal from the Pątnowskie Lake to the river mouth	0.00	0.20	0.85	0.55	0.40

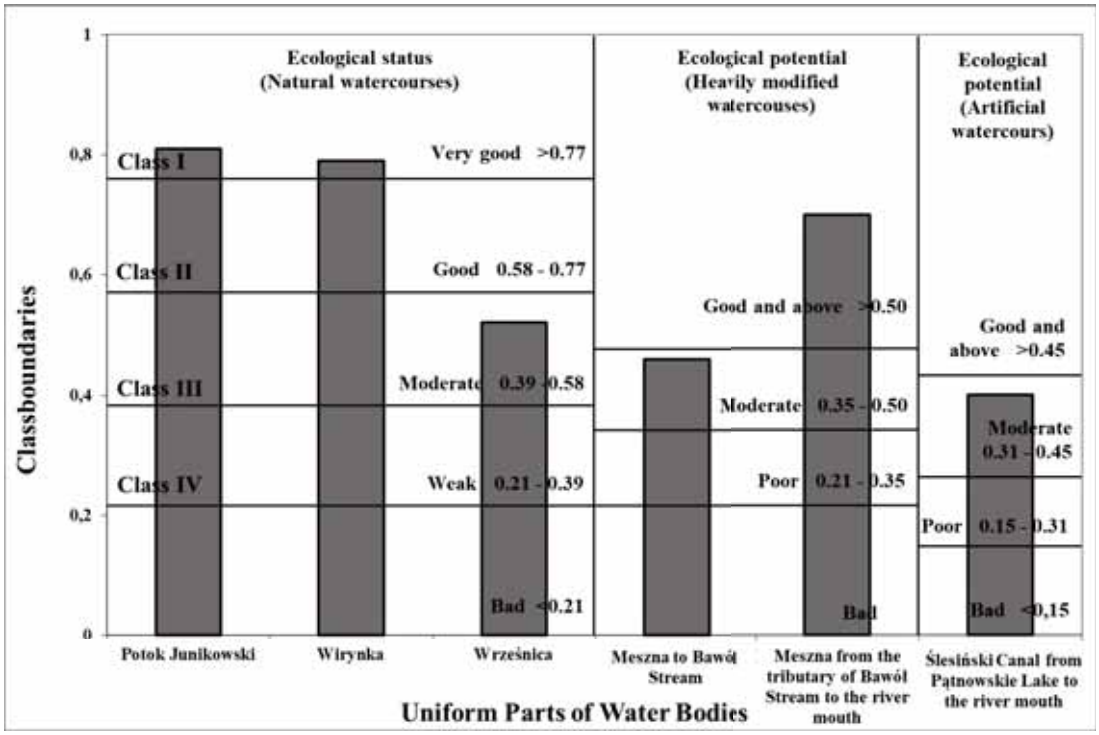


FIGURE 2
Classification status and ecological potential investigated watercourses including the limits for each category of surface watercourses

TABLE 3
Summary of the hydromorphological valorization watercourses by different methods

No	Uniform Part of Water Bodies (UPWB)	The results of valorization using the method		
		MHR	KOKŚ	RHS
1	Junikowski Stream	I	-	-
2	Wirynka	I	-	-
3	Wrześnica	III	III	IV
4	Meszna to Bawół Stream	III		III
5	Meszna from the tributary from Babiń to the river mouth	II	III	III
6	Ślesiński Canal from the Pątnowskie Lake to the river mouth	III	III	-

The Ślesiński Canal is an artificial watercourse constituting a part of the Warta-Gopło Canal qualified to the second waterway class [32]. It has not been used for transporting goods for ages, only for pumping water from the Warta River to the Pątnowskie Lake for the needs of "Pątnów" and "Konin" power stations. Its continuity was assessed very low (0.2) due to two locks (Morzysław – km 0.23 and Pątnów – km 7.58) without fish ladders. The riverbed morphology had a high score (0.85) which results from a significant cross-section, low scope of technical reinforcements, large number of vegetation and a very well-shaped riparian zone. A narrow floodplain valley in which this channel has been dug is characterized by a low surface diversity, significant level of meadows, lack of facilities, embankments and nature protection areas. In such conditions, the EPR for the "Floodplain valley" amounted to 0.55 and its final score for the UPWB Ślesiński Canal from the Pątnowskie Lake to the river mouth to the Warta River reached 0.40. It results mainly from the lack of watercourse continuity. The obtained score for the Ślesiński Canal indicates a moderate (class III) ecological potential (Fig. 2).

For the purposes of comparison, the data obtained with the MHR method were compared (Tab. 3.) to the results obtained thanks to the KOKŚ [4-9] and River Habitat Survey (RHS) [17] methods.

DISCUSSION

Due to the WFD implementation requirements, in the nearest future, the subject of hydromorphological valorization of watercourses will become one of the most important problems to be solved in the EU countries. Each of the member states may develop and implement its own method-

ology in this matter, adjusted, among others, to the geographical conditions. However, due to utilitarian reasons, it should include not only the guidelines of this document, but also the other subsequent and recommended formal regulations. The complexity of the problem and implementation possibilities affect the search for alternative methodological solutions. For the same reason, survey methods based on photointerpretation of aerial photos become more and more important. Some of the first researchers indicating this methodological solution were Germans [33-35]. Works aiming to conduct a hydromorphological assessment of watercourses with the use of methods based on photointerpretation were also started in Poland [4-7, 22]. They brought, among others, a comparison of results of the survey conducted with the use of field and photointerpretation methods for the same watercourses and an overview of their advantages and disadvantages. The comparative survey of photointerpretation methods (LAWA Overview Survey) with field methods were also conducted in the Czech Republic and Germany [36-37]. A very broad overview of worldwide used methods was presented by Belletti et al. [38].

On the basis of the overview of hydromorphological methods existing in Europe (mainly German methods), Schwarz [39] developed a method adjusted to large rivers according to the CEN norm and WFD requirements. The surveys on Drava's and Mur's hydromorphology in Austria and Croatia were conducted with the use of this method in 2005 from a boat and from the land on about 350 km of these rivers. The field surveys were based on experiences acquired from about 2000 km of watercourses from both of these countries. The hydromorphology may also be an element used for watercourse management. An analysis of the very broad literature in the field of the influence of technical maintenance measures on the ecological status of agricultural lowland rivers is contained in a paper written by Bączyk et al. [40]. The paper contains an incredibly broad overview of 203 articles on this subject from 33 European countries. It also has an impact on the durability and consequences of river reconstruction [41].

Sometimes political factors also have an influence on the riverbed morphology and the presence of ecologically valuable river islands affecting the continuity of the ecological corridor. Such an example is the Evros/Meriç River, a border river for Greece and Turkey. It is one of the most militarized rivers in Europe, where the access to the river corridor is strictly defined. This green watercourse line of 218 km in length has never been completely studied in terms of biodiversity in the riparian zones. Its value was assessed recently thanks to satellite photos. Over 219 river islands constitute important natural refuges for biodiversity on the watercourse that enhance the ecological integrity of the corridor and a rich biodiversity [42].

The KOKŚ score evaluation method [4-9], index method [43] and the River Habitat Survey (RHS) [17] should be qualified to a group of methods basing only on field surveys of biological parameters assessment. The first two methods cover the entire length of the watercourse, whereas in the RHS method, it is necessary to spin off representative sections of the river of 500 meters in length composed of 10 partial sections of 50 meters in length. The KOKŚ score evaluation method [4-7], according to Belletti et al. [38], called the "EcomorphEval" and the index method, are Polish methodologies. The first of them served to assess over 2200 km of watercourses located in Wielkopolska, Mazovia, Małopolska, Masuria and Lowe Silesia [9]. Among them, we can find Meszna, Wrześnica and Ślesiński Canal [8-9]. It is based on assessment of 7 or 8 parameters [8]. A much less popular index method assesses: the aquatic zone (of the valley), the ecological and landscape attractiveness of the near-valley zone as well as the floodplain and the above-floodplain zones. The above-mentioned parameters were assessed through assessment systems of relevant criteria and their weights assigned to them. Among others, the Jeziorka and Wkra Rivers [43-45] were assessed with the use of them.

Among the sites analyzed with the use of the British RHS method transposed to Polish conditions, we can find, among others, the Meszna and Wrześnica Rivers. A comparison of the results is presented in Table 3. In the final assessment, the Meszna River from the river mouth of the inflow from Babiń (the Bawół Stream) to the river mouth to the Warta River (two sections subjected to the survey) were qualified to the 3rd category (the mean HQA value of 38 and 16 of the HMS), just like the upper, much longer part of this watercourse (two sections subjected to the survey) that also belongs to class III (the mean HQA value of 36.3 and 16.6 of the HMS). Eleven sections were subjected to a watercourse analysis on the Wrześnica River. In the final result, this river was qualified to class IV (the mean HQA value of 29.1, HMS - 38.7). For the Wrześnica River, only the river mouth section in Samarzewo village did not have characteristics indicating a conducted riverbed or banks regulation in any of its survey profiles of 50 meters in length. It results from regulation, extension and profiling of almost the entire length of the watercourse during World War II. Only the river mouth section of the river meanders naturally. Also, a relatively intensive use of the watercourse valley and the presence of technical devices and bank reinforcements are not without significance. In the case of the Meszna River, its upper part had a worse result in the RHS assessment due to an intensive, artificial profiling of the banks and lower abundance of aquatic flora and buffer strips. It should be

remembered that both watercourses have been significantly modified by construction of artificial water reservoirs in their medium parties, which, in the case of the Meszna River, contributed to a significant change in the riverbed.

Among the analyzed watercourses, Meszna, Wrześnica and Ślesiński Canal were also assessed with the use of the KOKŚ score evaluation method [8-9]. Valorization with the use of the KOKŚ methodology (called "the EcomorphEval" according to Belletti et al. [38]) was conducted on the above-mentioned watercourses in 1995 (Wrześnica), 1996 (Ślesiński Canal) and in 1997 (Meszna). These watercourses obtained the same final score (3rd naturalness category); however, their mean values were different. And thus: The Ślesiński Canal reached the mean of 3.0 points, the UPSW Meszna from the inflow from Babiń (Bawół River) to the river mouth - 2.9, the upper part of this watercourse (UPWB Meszna to Bawół Stream) - 2.96, whereas Wrześnica - 2.77 points. Therefore, it can be stated that the watercourses had quite similar values of the assessment, whereas Wrześnica found itself slightly above the threshold for the 4the category of naturalness that amounts to 2.74. The fact, that Wrześnica as a natural watercourse reached such a low score in the score evaluation method may seem to be controversial. However, it should be noted that this is an UPWB covering the entire length of the watercourse. A shorter UPWB section of the heavily-modified Meszna River from the inflow from Babiń (Bawół Stream) to the river mouth obtained a better result in the final assessment with the use of this method also, because on a shorter length, the probability of the occurrence of a higher number of technical devices affecting the hydromorphological assessment is lower. Similar remarks were made by Górecki and Lewandowski [46] who compared assessments of 709 km of a waterway called the "Great Loop of Wielkopolska" (GLW). The GLW is composed of the two largest rivers of Wielkopolska – Warta and Noteć and the Warta–Gopło channel connecting them. By comparing the assessment results obtained with the use of the KOKŚ score evaluation method (EcomophEval) and MHR, they compared 17 UPWBs (12 natural and 5 artificial ones) composing the above-mentioned waterway.

Undoubtedly, in the final assessment of the analyzed watercourses, determination of threshold values for the EQR/EPR in specific valorization methods is of key importance. The issue of appropriate determination of thresholds between the classes of status or ecological potential was also addressed by Kamp et al. [35] while describing the river hydromorphological monitoring in Germany. The tabular overview of limit values for specific classes for the chosen eight valorization methods and EN 15843 standards [16] were published in a paper written by Ilnicki et al. [13]. It should be stressed that different watercourse valorization methods assume a different scale, although the score within the brackets from 1 to 5 points for five classes specified in the WFD prevails. At the same time, both the index method by Oglęcki and Pawłat [44] and the KOKŚ score evaluation method [4-9] had different approaches to the equivalence of the assessed parameters. In the first case, a complicated weight system was applied, whereas in the second case, they were equivalent. Similar to the KOKŚ (EcomorphEval) method, the valorization conducted with the MHR method does not take into account the weights of specific elements, features and attributes of the analyzed watercourse. At the same time, in the case of heavily-modified watercourses, as was the case for both Meszna's UPWBs, the EPR assessment is similar or better than the assessment of natural watercourses. It justifies the advisability of analogous treatment of both watercourse categories (common map-based protocol), but it also reveals the need to determine limit values for classes of status and of ecological potential in a different way.

CONCLUSION

In the case of the presented survey results for five watercourses constituting six UPWBs (differentiated in terms of their anthropogenic modification), despite the fact of taking into consideration a different number of assessed parameters in the applied methodologies and conducting works at different time (1995-2009), the obtained results are very similar. In the final assessment of the watercourses, the determination of threshold values for the establishment of classes of ecological status/potential in specific valorization methods fulfils the crucial role. However, taking into consideration formal conditions, the MHR is currently the only method in Poland that meets the requirements of the WFD and other formal regulations. For the purposes of practice, the equal treatment (common map-based protocol) of natural and heavily-modified watercourses as well as differentiation of classes' thresholds applied in its assumptions turned out to be justified. This method is a consensus between a scientific accuracy and financial and organizational possibilities of implementation of hydromorphological monitoring in Poland.

REFERENCES

[1] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water Policy (2000) Official Journal of the European Union L 327, 22.12.2000, Document 300L0060.

[2] Vanotte, R.L., Minshall, G.W., Cumminis, K.W., Sedel, J.R. and Cushing, C.E. (1980) River continuum concept. Can. J. Fish. Aquat. Sci. 37(1), 130-137.
[3] Błachuta, J., Jarząbek, A., Kokoszka, R. and Sarna, S. (2006) Weryfikacja wskaźników dla prowadzenia oceny stanu ilościowego i morfologicznego jednolitych części wód powierzchniowych wraz ze zmianą ich wartości progowych dla uściślenia wstępnego wyznaczania silnie zmienionych części wód. Krajowy Zarząd Gospodarki Wodnej (KZGW), Warszawa. 1-72. Available from: http://www.rdw.org.pl/obj.php?act/show/id/9bda43feb844f3b9751136578930ea.
[4] Ilnicki, P. (1995) Ekomorfologiczna waloryzacja cieków wodnych. Wiadomości Melioracyjne i Łąkarskie. 1, 5-7.
[5] Ilnicki, P. and Lewandowski, P. (1995) Ekomorfologiczna waloryzacja wybranych rzek Wielkopolski. Wiadomości Melioracyjne i Łąkarskie. 4, 162-165.
[6] Ilnicki, P. and Lewandowski, P. (1995) Methodische Untersuchungen zur ökomorphologischen Fliessgewässerbewertung. Z. Kulturtech. Landentw. 36(5), 255-259.
[7] Ilnicki, P. and Lewandowski, P. (1995) Ökomorphologische Fliessgewässerbewertung im polnischen Flachland. Z. Kulturtech. Landentw. 36(6), 323-326.
[8] Ilnicki, P. and Lewandowski, P. (1997) Ekomorfologiczna waloryzacja dróg wodnych Wielkopolski. Bogucki Wyd. Nauk., Poznań. 1-128.
[9] Lewandowski, P. (2012) Polish investigations on river hydromorphology. Pol. J. Environ. Stud. 21(4), 957-965.
[10] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2010) Podstawowe uwarunkowania metodyczne oceny stanu ekologicznego cieków wodnych na podstawie elementów hydromorfologicznych. Infrastruktura i Ekolologia Terenów Wiejskich. 9, 41-52.
[11] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2010) Metodyka prowadzenia monitoringu stanu hydromorfologicznego polskich rzek. Infrastruktura i Ekolologia Terenów Wiejskich. 9, 101-111.
[12] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2010) Principles of hydromorphological surveys of polish rivers. J. Water Land Dev. 14, 3-13.
[13] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2010) Ecological quality classes of river hydromorphology in Poland. J. Water Land Dev. 14, 15-27.
[14] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2011) Charakterystyka elementów hydromorfologicznych cieków sztucznych przy użyciu metody MHR. Acta Sci. Pol., Formatio Circumiectus. 10(1), 17-32.
[15] Ilnicki, P., Górecki, K., Grzybowski, M., Krzemińska, A., Lewandowski, P. and Sojka, M. (2011) Badania hydromorfologii cieków nizinnych przy pomocy metody MHR. Woda Środowisko Obszary Wiejskie. 11, 1(33), 97-112.
[16] EN 15843 (2010) Water quality - Guidance standard on determining the degree of modification of river hydromorphology. European Committee for Standarization CEN Brussel.
[17] Szoszkiewicz, K., Zgoła, T., Jusik, S., Hryc-Jusik, B., Dawson, F.H. and Raven, P. (2009) Hydromorfologiczna ocena wód płynących. Podręcznik do badań terenowych według metody River Habitat Survey w warunkach polskich. Bogucki Wydawnictwo Naukowe, Poznań–Warrington. 1-134.
[18] Langhammer, J. (2009) Applicability of hydromorphological monitoring data to locate flood risk reduction measures: Blanice River basin, Czech Republic. Environ. Monit. Assess. 152(1-4), 379-392.
[19] Czarnecka, H. (ed.) (2005) Atlas podziału hydrograficznego Polski. Część 1. Mapy w skali 1:200 000. Część 2. IMGW, Warszawa. 1-115.
[20] Czarnecka, H. (ed.) (2005) Atlas podziału hydrograficznego Polski. Część 2. Charakterystyka zlewni. IMGW, Warszawa. 1-558.
[21] Raport dla obszaru dorzecza Odry z realizacji art. 5 i 6, zał. II, III, IV Ramowej Dyrektywy Wodnej 2000/60/WE. Ministerstwo Środowiska, Warszawa. 1-291. Available 15.04.2018 from: http://www.kzgw.gov.pl/files/raporty/Raport_2005_Odra.pdf.
[22] Ilnicki, P., Lewandowski, P. and Olejnik, M. (2005) Hydromorfologiczna waloryzacja rzek w Polsce - dotychczasowe prace i zamierzenia. Konferencja „Typologia i Warunki Referencyjne Wód Powierzchniowych". IMGW Warszawa. 101-108.
[23] Lewandowski, P. (2005) Porównanie metod waloryzacji cieków wodnych na przykładzie rzeki Warty. Zeszyty Problemowe Postępów Nauk Rolniczych. 507, 333-340.
[24] Ilnicki, P., Lewandowski, P. and Olejnik, M. (2008) Metody hydromorfologicznej oceny rzek stosowane w Europie przed i po ustanowieniu Ramowej Dyrektywy Wodnej. Gospodarka Wodna. 10, 393-397.
[25] Ilnicki, P. and Lewandowski, P. (2009) Hydromorfologiczna ocena rzek wyżynnych i górskich w Austrii. Gospodarka Wodna. 10, 414-418.

[26] Ilnicki, P. and Lewandowski, P. (2010) Wdrażanie oceny stanu hydromorfologicznego rzek w Czechach i Słowacji. Wiadomości Melioracyjne i Łąkarskie. 1, 26-30.
[27] Regulation (EC) No 401/2009 of the European Parliament and of the Council of 23 April 2009 on the European Environment Agency and the European Environment Information and Observation Network (Codified version). Official Journal of the European Union L 126/13, 21.5.2009, Dokument 32009R0401.
[28] Directive 2007/2/EC of the European Parliament and of the Council of 14 March 2007 establishing an Infrastructure for Spatial Information in the European Community (INSPIRE). Official Journal of the European Union L 108/1, 25.4.2007, Dokument 32007L0002.
[29] Rastrowa Mapa Podziału Hydrograficznego Polski (2008) Zakład Hydrografii i Morfologii Koryt Rzecznych Instytutu Meteorologii i Gospodarki Wodnej w Warszawie. IMGW PIB Warszawa.
[30] Stachý, J. (ed.) (1986) Atlas Hydrologiczny Polski. Część I. Wydawnictwa Geologiczne, Warszawa. 1-924.
[31] Stachý, J. (ed.) (1987) Atlas Hydrologiczny Polski. Część II. Wydawnictwa Geologiczne, Warszawa. 1-57.
[32] Arkuszewski, A., Olędzki, R., Siadak, M., Śliwczyński, A., Wierzbicki, T. and Wołoszyn, E. (1985) Morfologia dróg wodnych śródlądowych w Polsce. Wydawnictwo Komunikacji i Łączności, Warszawa. 1-538.
[33] Faber, F. (1989) Die Luftbildauswertung, eine Methode zur ökologischen Analyse von Strukturveränderungen bei Flissgewässern. Schriftenreihe für Landschaftspflege und Naturschutz 31. Bundesforschungsanastalt für Naturschutz und Lanschaftsökologie, Bonn-Bad Godesberg. 1-119.
[34] Gießübel, J. (1993) Erfassung und Bewertung von Fließgewässern durch Luftbildauswertung. Landwirtschaftsverl. Schriftenreihe für Landschaftspflege und Naturschutz 37. Bundesforschungsanastalt für Naturschutz und Lanschaftsökologie. Bonn-Bad Godesberg. 1-77.
[35] Kamp, U., Binder, W. and Hölzl, K. (2007) River habitat monitoring and assessment in Germany. Environ. Monit. Assess. 127(1), 209-226.
[36] Weiß, A., Matoušková, M. and Matschullat, J. (2008) Hydromorphological assessment within the EU – Water Framework Directive – transboundary cooperation and application to different water basins. Hyrobiologia. 603(1), 53-72.
[37] Šípek, V., Matoušková, M. and Dvorak, M. (2010) Comparative analysis of selected hydromorphological assessment methods. Environ. Monit. Assess. 169, 309-319.
[38] Belletti, B., Rinaldi, M., Buijse, A.D., Gurnell, A.M. and Mosselman, E. (2015) A review of assessment methods for river hydromorphology. Environ. Earth Sci. 73(5), 2079-2100.
[39] Schwarz, U. (2008) Hydromorphological inventory and map of the Drava and Mura rivers (IAD pilot study). Large Rivers, 45-59.
[40] Bączyk, A., Wagner, M., Okruszko, T., and Grygoruk, M. (2018) Influence of technical maintenance measures on ecological status of agricultural lowland rivers–Systematic review and implications for river management. Science of The Total Environment. 627, 189-199.
[41] Wyżga, B., Zawiejska, J., and Gurnell, A.M. (2018) Effects and persistence of river restoration measures: Ecological, management and research implications. Science of The Total Environment. 628-629 1098-1100.
[42] Zogaris, S., Markogianni, V., Özeren, S.C., and Dimitriou, E. (2015) Assessment of riparian zone and river island conditions in a transbonduary greenbelt: the Evros/Meriç river (Greece-Turkey). Fresen. Environ. Bull. 24, 269-277.
[43] Oglęcki, P. and Pawłat, H. (2000) The index method of small lowland river environmental evaluation. Annual Warsaw Agricultural University SGGW Land Reclamation. 30, 37–43.
[44] Oglęcki, P. (2006) Ocena hydromorfologiczna rzek nizinnych na przykładzie rzeki Wkry. Infrastruktura i Ekologia Terenów Wiejskich. 4(1), 175-184.
[45] Wasilewicz, M. and Oglęcki, P. (2006) Porównanie wybranych metod oceny stanu ekologicznego rzek na przykładzie badań środkowej Wkry. Infrastruktura i Ekologia Terenów Wiejskich. 4(3), 171-178.
[46] Górecki, K. and Lewandowski, P. (2017) Hydromorfologiczna ocena Wielkiej Pętli Wielkopolski – porównanie metod i wyników. Nauka Przyroda Technologie. 11(1), 5–22.

Received: **29.06.2018**
Accepted: **10.11.2018**

CORRESPONDING AUTHOR

Piotr Lewandowski
Poznań University of Life Sciences
Institute of Construction and Geoengineering
ul. Piątkowska 94E
60-649 Poznań – Poland

e-mail: piotr.lewandowski@mail.up.poznan.pl

© by PSP Volume 27 – No. 12B/2018 pages 9989-9987 Fresenius Environmental Bulletin

THE EVALUATION ON SUSTAINABLE DEVELOPMENT OF REGIONAL WATER RESOURCES IN CHINA BY FUZZY-AHP

Yi Liu[1,*], Die Lu[1], Sabina Shahbazzade[2]

[1]Hangzhou Dianzi University, Management School, Hangzhou, China
[2]University of San Francisco, Computer Science Department, CA, USA

ABSTRACT

The evaluation on region sustainable development of water resources involves many regional factors such as the society, economy, resource and environment. This paper puts forward the sustainable development evaluation of regional water resources based on Fuzzy-Analytic Hierarchy Process, which uses triangular fuzzy number to express the experts' judgment information and strengthens the scientific nature of experts decision-making. The experiment result shows that FAHP method can analyses and evaluate the sustainable utilization of regional water resources more accurately, which can provide effective decision-making for the local government of China.

KEYWORDS:
Regional Water Resources, Sustainability Evaluation, Fuzzy Logic, Analytical Hierarchy Process

INTRODUCTION

With the development of China economy, the contradiction problem between supply and demand of water resources become more prominent, which restrict the Chinese society development. According to the sustainable development principles, the limited water resources should be rationally allocated in time and space through technology and management in order to meet the water demand of major water user without affecting the ecological environment. Furthermore, the society, economy, resources and environment of the region will be developed stability and sustainable [1]. The evaluation on sustainable utilization of regional water resources is the core issue of the regional sustainable development strategy. The development level of regional water resources sustainable utilization be determined by comprehensive analysis of water resources management. Therefore, many scholars devoted to research the evaluation on the water resources optimal allocation.

The coordinate of human and nature development is the key part and base for regional water resources sustainable development, that is to say, the development of society, economy, resources and environment should be coordinated. Therefore, the evaluation index of sustainable utilization of water resources should embody this leading idea [2]. Loucks [3] comprehensively evaluated the sustainability of water resources according to the reliability, resilience, vulnerability and fairness. Sandoval-Solis [4] improved the content structure of sustainability index by added two performance indicators of mean square deviation and the maximum water rate, and proposed the sustainable indicators for different water resource systems, which can analyze the sustainability of water resource under different management schemes. Gasco [5] used the input-output model to evaluate the sustainable development of water resources. Chinese scholars Jin Juliang and Wei Yiming [6] used genetic algorithms to evaluate the sustainable development of water resources from the aspect of society, economy, resources and environment. He Guohua, Wang Ni [7] established the fuzzy comprehensive evaluation model of water resources allocation with entropy weight, which can make a comprehensive analysis on the allocation of region water resources, in order to provide reference for the rational development and utilization on the region water resources. Song Yifan, Guo Zhongxiao [8] constructed the harmony evaluation system of water resources in ErLianHaoTe city from three aspects, such as the total intake water amount, water use efficiency and the protection of ecological environment. This evaluation system reflected the harmony degree between water resources and social economy in ErLianHaoTe City, which have reference significance for the construction of water-saving society of drought areas in northern China. Liao Huchang [9] applied the Malmquist index method to the dynamic analyze the water resources utilization efficiency from the data of the 12 western provinces, which expanding the production scale to optimize the industrial structure is the only way to improve the efficiency of water resources.

Water resources evaluation involves multiple stakeholder subjects, and it has many fuzzy factors, such as multiple index, multiple attribute, multiple level and multiple stage. The Fuzzy Sets Theory is

proposed by L.A. Zadeh in his paper in 1965 by introducing the "membership function" and using the accurate mathematical methods to describe the fuzzy concept [10], Since Van Laarhoven applied fuzzy logic theory to Analytic Hierarchy Process (AHP) method, a group of scholars devoted themselves to the study of fuzzy AHP [11]. Fuzzy-AHP not only be used in the evaluation problem with comprehensive subjective factors, but also in the evaluation problem with comprehensive objective factors. The Fuzzy-Analytic Hierarchy Process have better evaluation result for the more structure level and complex the objective problem [12].

This paper constructs the water resources evaluation index system including natural reserves, economic development, social conditions and ecological environment of water resources. Considering the fuzzy and uncertainty of the evaluation index, this paper put forward the Fuzzy-AHP method to evaluate the sustainable development of water resources in Zhejiang Province. Fuzzy Logic theory quantified the fuzzy information of expert decision preference, avoided information loss in decision-making process as well as improved the quality and reliability of decision making, which can provide reasonable references and suggestions for sustainable development of water resources in Zhejiang province.

DESCRIPTION OF SUSTAINABLE DEVELOPMENT EVALUATION ON REGIONAL WATER RESOURCES.

The water resources system is the core and main subject of region sustainable development, which be composed of water resources, society, economy and ecological environment. This paper construct the sustainable development evaluation index system of the regional water resources arranged from the target layer, the criterion layer, the subsystem and the index layer respectively.

(1)Target layer: The sustainable development coefficient is S. As the comprehensive indicator , the target level measure the comprehensive level and ability of the water resources system to support the sustainable development of the whole society, which is composed of the development coefficient, coordination coefficient and fairness coefficient at the standard level.

(2)Criterion layer: It is composed of three criterion: the development coefficient (A), the coordination coefficient (B) and the fairness coefficient (C). The development coefficient reflects the comprehensive development degree of the whole region, The coordination coefficient reflects the coordination degree of the society, economic and ecological environment subsystem in region with water resource subsystem, The fairness coefficient reflects the development fairness degree of sub-regions resources usage.

(3)Index layer: The coefficient index layer is composed of society, economy, ecological environment and water resources development indicator. The society indicators reflect the growth rate of population and the degree of urbanization in the social development. The economy indicators reflect the GDP per capita and the proportion of third industry. The ecological environment development indicators reflect the percentage of forest coverage, the amount of Chemical Oxygen Demand(COD) into the river and the sewage treatment rate. The water resources development indicators reflect the amount of water supply, the quota of agricultural water usage and the quota of industrial water usage.

This paper establish the regional water resource sustainable development evaluation index system from the water resources situation, economic development, social conditions and ecological environment of Zhejiang province. According to the scientific and objectivity and systematic and operability principles, this paper establish 12 second-level indicators including the surface water resources supply, per capita grain yield, per capita water volume and sewage treatment rate [13]. The specific index system is shown in Figure 1 and Table 1.

TABLE 1
The hierarchical evaluation structure of regional water resource sustainable development

	Dimension	Criteria	Index meaning
Water resources sustainable development	Water Resource	Surface water supply/10^8m^3	Surface water supply
		Groundwater supply/10^8m^3	Ground water supply
		Annual precipitation/mm	Reflects the local precipitation/year
		Industrial water consumption/10^8m^3 /total industrial output value	Total industrial water consumption
	Economy	Per capita grain output/ton	Total grain output/total population
		Third industry water consumption/10^8m^3	The third industry water consumption
		Per capita water resources/10^4m^3	Total water resources/total population
	Society	GDP growth rate/%	(Current year GNP－Last year GNP)/ Last year GNP*100%
		Per capita GDP/Million	Total GDP/Total population
		Waste water treatment rate/%	Sewage treatment/Sewage output *100%
	Ecology	Vegetation plant coverage/%	Built-up area vegetation plant area/ Total built-up area*100%
		Per capita green area/m^2	Total area of green space/total population

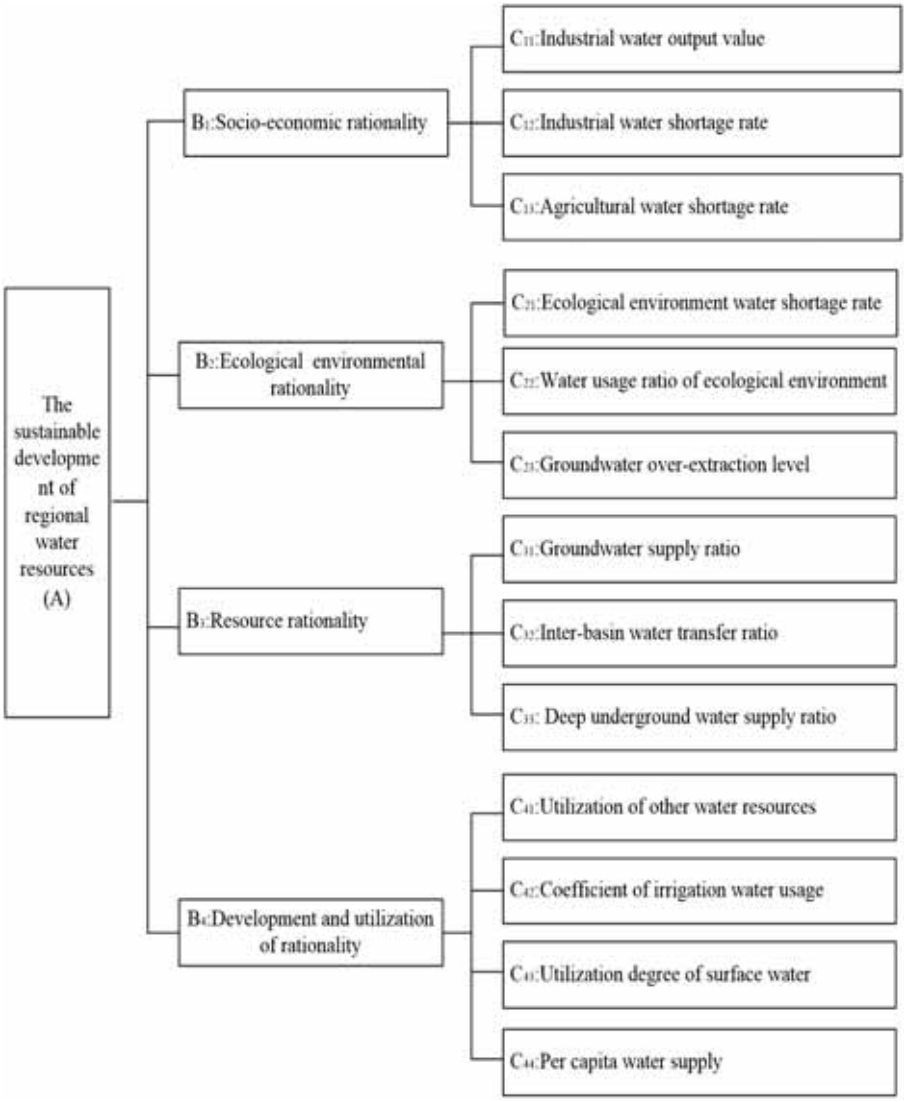


FIGURE l
The regional water resource sustainable development evaluation system

METHOD METHODOLOGY

The analytical hierarchy process method is a multi-objective decision method combined qualitative analysis and quantitative analysis, which the calculate and rank the relative importance of each program so that the complicated problems can be organized hierarchically. While the fuzzy set theory can solve the human decision-making problem by taken the fuzziness factors into account. The Fuzzy AHP method is used to synthesize the experience views of different experts, so as to form a final weight of the index system. The decision-makers always apply the fuzzy AHP evaluation model to evaluate the important issues in order to reduce subjective judgment factors. Based on the traditional analytic hierarchy process (AHP),This paper introduces the triangular fuzzy number theory to form the fuzzy analytic hierarchy process (FAHP)[14]. The mathematical expression of the fuzzy number is shown in formula (1),the corresponding graphical meaning is shown in Figure 2 [15].

$$\tilde{M} = \begin{cases} 0, x < l \\ (x-l)/(m-l), l \le x \le m \\ (u-x)/(u-m), m \le x \le u \\ 0, x > u \end{cases} \quad (1)$$

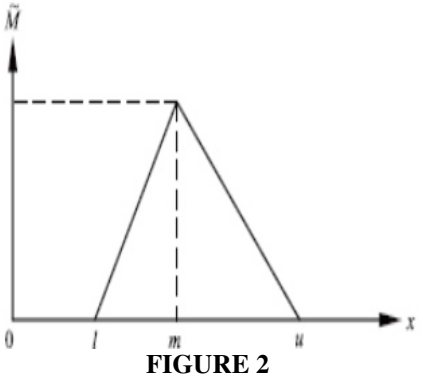


FIGURE 2
Triangular fuzzy number $\tilde{M} = (l, m, u)$

TABLE 2
Triangular fuzzy number of evaluation variables

evaluation variables	Triangular fuzzy numbers	Reciprocal triangular fuzzy numbers
Absolutely more important	(8，9，9)	(1/9，1/9，1/8)
Strongly more important	(6，7，8)	(1/8，1/7，1/6)
Obviously more important	(4，5，6)	(1/6，1/5，1/4)
Slightly more important	(2，3，4)	(1/4，1/3，1/2)
Equally important	(1，1，2)	(1/2，1，1)

The index weight of triangular fuzzy number can be obtained by the optimistic index method, the weight vector of the degree of fuzzy number is obtained by formula (2)

$$I(\tilde{M}_i)=\frac{1}{2}\alpha(m_i+u_i)+\frac{1}{2}(1-\alpha)(l_i+m_i)=$$

$$\frac{1}{2}[\alpha u_i+m_i+(1-\alpha)l_i](i=1,2,3,...,n) \quad (2)$$

In the formula (2) I is the number of evaluation indicator, α is an optimistic index, the range of variations is within the[0,1]. If decision makers express pessimism, then value of α is close to 0, else decision makers express optimistic and value of α is close to 1. In this paper, the value of α set at 0.5. The normalized weight vector $W=(w_1,w_2,....,w_i)^T$ is obtained by formula (3).

$$w_i=I(\tilde{M}_i)/\sum_{k=1}^{m}I(\tilde{M}_i) \quad \text{k=1,2,…m} \quad (3)$$

The expert assessment answers can be divided into absolutely, strongly, obviously, slightly and equally important levels, as shown in Table 2.The evaluation of experts are transformed into triangular fuzzy number matrices according to the fuzzy number transformation rules shown in Table 2, and then the triangular fuzzy number matrices is verified by consistency check. RI is average random consistency index, which value is 0,0, 0.58,0.90,1.12,1.24, 1.32, 1.41,1.45 (n=1,2,…,9). CI is detected consistency values. CR represents the ratio of CI to RI. Evaluation results can be accepted, when the CR<0.1.

Select the evaluation index and construct the hierarchical structure model. Research the evaluation problem and construct the hierarchical structure model, divided the interrelated indexes into three levels: target layer A, criterion layer B and measure layer C according to the different attributes, which the indexes in the same level are subordinate to the upper level and upper level indexes dominate the hierarchy and are affected by them. The target layer of the AHP is the final goal of the problem, the standard layer is the criterion affecting the realization of the goal, the indicator layer is a measure to achieve the goal.

According to the each index relationship of the hierarchical structure model, the first-level index (criterion layer) constitutes the comprehensive evaluation factors set, which can construct the fuzzy comprehensive evaluation factor set and sub-factor set.

The A is the first-level indicators, A={B_1,B_2,B_3,…,B_i}.B_i is sub-factor set of second level, B_i ={C_1, C_2, C_3,…, C_{ik}} (k=1,2,3,…,n) ,k is the index number of the second level.

Determine the factor weight sets of each level in the analytic hierarchy process.

$$W=\{w_1,w_2,\cdots,w_n\}$$

$$w_i=\{w_{i1},w_{i2},\cdots,w_{ik}\}(i=1,2,3,...,n) \quad (4)$$

Construct the evaluation set V, which is the levels set of the evaluation object by the evaluator , V={V_1,V_2,V_3,…,V_m} and m is the number of evaluation grades, usually 4 to 9.

Obtaining the fuzzy comprehensive first level evaluation from the evaluation Matrix of relevant experts. obtain the comprehensive evaluation indicators vector B_i according to the formula：

$B_i=W_i\cdot R_i$ (i=1, 2, 3,…, n) (5)

$$R=\begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1m} \\ r_{21} & r_{22} & \cdots & r_{2m} \\ \vdots & \vdots & & \vdots \\ r_{n1} & r_{n2} & \cdots & r_{nm} \end{bmatrix}$$

Obtaining fuzzy comprehensive Second-level evaluation. After get the comprehensive evaluation vector B_i, The total objective evaluation Matrix $B_i=(B_1, B_2, B_3,\ldots, B_n)^T$ can be formed. the total target evaluation vector S can be obtained according to the following formula:

$S=W\cdot B$ (6)

According to the principle of maximum degree membership function, the total objective evaluation vector S determines the evaluation level corresponding to the target layer.

SUSTAINABLE DEVELOPMENT EVALUATION OF ZHEJIANG PROVINCE WATER RESOURCES BY THE FAHP

The Zhejiang Province is one of the economy developed areas where locate in the center of the Yangtze River Delta in the Eastern China, which the map of Zhejiang Province is shown on Figure 3. The rivers are crisscrossed in the province and can be divided into six hydrological regions as follows: Hang-Jia-Hu district of Northern Zhejiang, Xiao-Shao-Ning district of Eastern Zhejiang, Qiantang river district, Taizhou district, Wenzhou district and Oujiang River middle and upper reaches area. In addition to surface freshwater resources such as rivers and lakes, Zhejiang Province also has about 22.11/10^8m^3 of groundwater resources, which the recoverable capacity is about 4.84/10^8m^3, accounting for 21.89%. In 2017, the water supply index reached the design guarantee rate of urban and rural domestic water and important industrial water use, and reached 95%. The guarantee rate of the industrial water usage is 90%, the water consumption of agriculture irrigation is 75-90%, the water consumption of the environment is 80% [16].The spatial distribution of water resources in Zhejiang Province is uneven. The middle and lower reaches of the Qiantang river are densely populated and economically developed. The cultivated land accounts for nearly half of the province's total area, while the amount of water resources accounts for only 1/5 of the province's total. The cultivated land of the Oujiang River middle and lower reaches is only one fourth of the province, and the amount of water resources accounts for nearly half of the province, the details show Table 3.

The regional water resources evaluation system has two levels index indicators such as water resources, economy, society and ecology respectively. The specific steps of the fuzzy AHP are as follows:

The Factor Domain U and Comment Level V of Evaluation Target, The fuzzy set $U = \{u_1, u_2, ..., u_n\}$ is the evaluation index set for the n target object, $V = \{v_1, v_2, ..., v_m\}$ is the evaluation grades set of m factors. If the evaluation level is 5 grades, It can be expressed as $V = \{v_1, v_2, ..., v_5\}$, and each level can be correspond to the fuzzy subset.

This paper select the sustainability development of water resources in the Zhejiang as the decision-making problem. The u_1 represents Hang-Jia-Hu district of Northern Zhejiang, u_2 represent Xiao-Shao-Ning district of Eastern Zhejiang, u_3 represent Qiantang river district, u_4 represent Taizhou district, u_5 represent Wenzhou district, u_6 represent Oujiang River middle and upper reaches area. Five experts participate in the decision-making of regional water resources, indicators system as shown in Table 4.

FIGURE 3
Zhejiang Province Map

Determining Evaluation Criteria And Evaluation Matrix. According to the <<2015 Zhejiang Statistical Yearbook>>, <<China water resources bulletin>> and other related materials, This paper classify the sustainability development of water resources into five grades (low, regular, median, good and excellent) by fuzzy-AHP. The classification of each rating index is shown in Table 4.

TABLE 3
Water Resource in Zhejiang Province from 2010 to 2015

year	per-capital production water	per-capital ecological water	per-capital living water	per-capital water resources	total resource /10^8m^3	water total population /10^4people	ecological water /10^8 m^3	domestic water /10^8 m^3	production water /10^8 m^3
2010	1.618	0.066	0.200	1.885	855.23	5.120	20.54	62.04	501.22
2011	1.902	0.024	0.207	2.133	931.35	5.180	7.55	64.94	595.94
2012	3.191	0.080	0.204	3.475	1397.61	5.442	26.33	67.29	1050.59
2013	1.328	0.014	0.208	1.550	744.21	5.463	4.56	68.80	438.88
2014	3.476	0.014	0.220	3.710	1444.79	5.477	4.51	73.08	1151.80
2015	1.887	0.016	0.224	2.126	930.90	5.498	5.17	74.43	627.57

TABLE 4
Evaluation of water resources development indicators of the grading standards

	Low-V1	Regular-V2	Median-V3	Good-V4	Excellent-V5
Surface water supply/10^4m^3	>1000	>800～≤100	>600～≤800	>400～≤600	≤400
Groundwater supply/10^4m^3	>9000	>700～≤900	>500～≤700	>300～≤500	≤300
Annual precipitation/mm	<250	>250～≤500	>500～≤700	>700～≤800	>800
Water consumption of industrial output /10^4m^3	>4	>3～≤4	>2～≤3	>1～≤2	≤1
Per capita grain output/t	<0.2	>0.2～≤0.4	>0.4～≤0.6	>0.6～≤0.75	>0.75
Water consumption of the third industry/10^4m^3	<2×10^4	>2×10^4～≤3×10^4	>3×10^4～≤4×10^4	>4×10^4～≤5×10^4	>5×10^4
Per capita water Resources/m^3	<50	>50～≤75	>75～≤100	>100～≤125	>125
GDP growth Rate/%	<7	>7～≤8	>8～≤9	>9～≤10	>10
Per capita GDP/Million Yuan	<2	>2～≤3	>3～≤5	>5～≤7	>7
Wastewater Treatment Rate/%	<50	>50～≤65	>65～≤80	>80～≤95	>95
Vegetation-Plant Coverage/%	<10	>10～≤30	>30～≤40	>40～≤50	>50
Per capita Green Area/m^3	<7	>7～≤10	>10～≤13	>13～≤16	>16

Calculation fuzzy evaluation matrix by the membership function. The water resources sustainability evaluation matrix of e_k decision maker can be expressed $R_k=(R_1,R_2,\ldots,R_k)^T$,k=1,2,…,S. For the fuzzy evaluation matrix A , attribute evaluation value R_k and expert weight W_k can be obtained by optimistic index method of fuzzy-AHP, expressed as:

$$W=\{w_1,w_2,\cdots,w_n\}\in F(U) \qquad (7)$$

Calculation fuzzy evaluation matrix by the membership function. The water resources sustainability evaluation matrix of e_k decision maker can be expressed $R_k=(R_1,R_2,\ldots,R_k)^T$,k=1,2,…,S. For the fuzzy evaluation matrix A , attribute evaluation value R_k and expert weight W_k can be obtained by optimistic index method of fuzzy-AHP, expressed as:

$$W=\{w_1,w_2,\cdots,w_n\}\in F(U) \qquad (7)$$

Make the fuzzy evaluation on the Xiao-Shao-Ning district of eastern Zhejiang as an example, Show as table 5.

can be

$$B=W\circ R=(w_1,w_2,\cdots,w_m)\circ\begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1m} \\ r_{21} & r_{22} & \cdots & r_{2m} \\ \vdots & \vdots & & \vdots \\ r_{n1} & r_{n2} & \cdots & r_{nm} \end{bmatrix}=(b_1,b_2,\cdots,b_n)$$

TABLE 5
2013-2015 The degree of membership of the indicators corresponding to each grade in Zhejiang Province

year	Criteria	u_1	u_2	u_3	u_4	u_5	u_6
2013	r_{i1}	0	0.340	0	0.521	0.021	0.097
	r_{i2}	0.515	0.160	0.335	0.179	0	0.303
	r_{i3}	0.170	0	0.140	0	0.379	0
	r_{i4}	0	0.205	0	0.211	0.511	0.305
	r_{i5}	0.325	0.195	0.525	0.089	0.089	0.295
2014	r_{i1}	0.075	0.155	0	0.392	0.123	0.127
	r_{i2}	0.525	0.145	0.391	0.108	0	0.373
	r_{i3}	0	0	0.209	0	0.208	0
	r_{i4}	0.215	0.320	0.067	0.233	0.367	0.380
	r_{i5}	0.185	0.380	0.333	0.167	0.192	0.120
2015	r_{i1}	0.055	0.105	0	0.150	0	0.125
	r_{i2}	0.145	0.395	0.133	0.250	0.150	0
	r_{i3}	0	0	0.567	0	0.350	0.175
	r_{i4}	0.040	0.355	0	0.375	0.375	0.345
	r_{i5}	0.760	0.145	0.3	0.225	0.125	0.355

Fuzzy operator. According to the evaluation results of single evaluation factor, the evaluation grade of each criterions is calculated and obtain the fuzzy matrix R .

$$\tilde{R} = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1m} \\ r_{21} & r_{22} & \cdots & r_{2m} \\ \vdots & \vdots & & \vdots \\ r_{n1} & r_{n2} & \cdots & r_{nm} \end{bmatrix}$$

Due to the large number factors in this problem, it is reasonable to choose a weighted average fuzzy operator in order to the fuzzy synthesis results and weight index. The evaluation matrix R and weight set W are determined, matrix multiplication can be calculated according to the average weighted fuzzy operator of $M(\bullet,\oplus)$ .Therefore, the evaluation set B is as follows:

$$B_j = (b_1, b_2, \ldots, b_m) = \sum_{i=1}^{m} w_i r_{ij} (j = 1,2,\ldots,m)$$

$$\sum_{j=1}^{m} r_{ij} (j = 1,2,\ldots,m)$$

Calculate fuzzy evaluation value. After the membership and weight of the evaluation index is determined, it is necessary to determine the fuzzy operator according to the characteristics of the index system. The fuzzy evaluation model of Zhejiang province is made up of three elements (U,V,R) and the evaluation result B can be expressed as:

B=W*R (8)

Based on result R of the secondary index by Fuzzy evaluation and weight W of the first order Index determined by triangular fuzzy number, The fuzzy evaluation of sustainable water resources development of the Xiao-Shao-Ning district in eastern Zhejiang Province is as follows.

$$B = W \times R = (0.213\ \ 0.258\ \ 0.285\ \ 0.244) \begin{bmatrix} 0.758 & 0.242 & 0.000 & 0.000 & 0.000 \\ 0.239 & 0.196 & 0.211 & 0.039 & 0.315 \\ 0.000 & 0.000 & 0.253 & 0.488 & 0.259 \end{bmatrix}$$

$$= (0.223\ \ 0.116\ \ 0.228\ \ 0.223\ \ 0.211)$$

According to the evaluation results, the social subsystem and the ecological environment of Xiao-Shao-Ning district is very discordant with economic development. Therefore, the evaluation grade of the water resources sustainable development in the Xiao-Shao-Ning district is regular. Similarly, the sustainable development level of water resources in other regions of Zhejiang province can be obtained as table 6.

RESULTS ANALYSIS AND COUNTERMEASURES

According to the above analysis results, the water resources utilization of Zhejiang province was not stable due to the differences in water resources, utilization and awareness of water conservation .

The sustainable development indicators of water resources in Hang-Jia-Hu district and the Xiao-Shao-Ning district of eastern Zhejiang province are relatively lower, which the sustainable development state of water resources is at the critical condition. The sustainable development of water resources in the QianTang, Taizhou and Wenzhou areas have reached the harmony standard. The Oujiang middle and upper reaches area reach very harmonious standard because the vegetation coverage rate and the per capita green area are relatively high.

With the guidance of "A total of five water treatment " and "Eight-Eight Strategy" by the Zhejiang province government, the utilization of water resources in Zhejiang has been achieved success in recent years. The policy significances of this research are as following:

First, according to the development goals of the "13th Five-Year Plan", Zhejiang Province should strengthen greater investment in water - saving and water- utility technologies, develop and utilize highly efficient and suitable technologies to improve the water-usage performance level of industrial enterprises, which will provide support for maintaining stable and sustainable development of the economy.

Second, Zhejiang Province is a province with more people with less land. The growth of population has led to an increase in the use of water resources, so that the per capita water resources of Zhejiang province is less than 1,800 m^3. Zhejiang province have 5 billion cubic meters renewable water resources every year, so it is necessary to make full use of the renewable water resources.

Third, Zhejiang Province should also strengthen the management of domestic sewage discharge and emission reduction while reducing the intensity of water consumption. It is necessary for the management department to improve the water resources market management mechanism. Through administrative penalties to ensure that illegal costs are greater than the law-abiding cost, and strengthen the consciousness of government responsibility at same time. Complete market allocation will not only ignore ecological security, but also hinder the sustainable development of economy.

TABLE 6
The sustainable development evaluation of water resources in Zhejiang Province

Region	Low	Regular	Median	Good	Excellent	Grades
Hang-Jia-Hu district	0.223	0.116	0.228	0.223	0.211	regular
Xiao-Shao-Ning district	0.143	0.106	0.287	0.345	0.120	regular
Qiantang river district	0.153	0.104	0.258	0.359	0.125	good
Taizhou district	0.143	0.040	0.463	0.299	0.056	median
Wenzhou district	0.208	0.099	0.268	0.290	0.129	median
Oujiang river district	0.143	0.071	0.239	0.209	0.338	excellent

CONCLUSIONS

The sustainable development evaluation of water resources involves factors such as the regional society economy development, the ecological environment protection and the resources sustainable development. This paper puts forward the Fuzzy AHP method to evaluate the sustainable development of regional water resources of Zhejiang Province according to the expert decision from the aspects of the society, economy, resources and environment. Experiments show that FAHP method can make the accurate evaluation on water resources sustainable utilization, which can provide effective water resources sustainable development decision for Zhejiang Province's government.

ACKNOWLEDGEMENTS

This work is supported by the "Zhijiang Young Social Sciences Scholar" Philosophy and Social Sciences Project of Zhejiang Province at China (Grant No. 16ZJQN018YB).

REFERENCES

[1] Cymes, I. (2018) Use of water quality indices as a tool in water resources management. Fresen Environ Bull. 27, 2777-2784.
[2] Arslan, O. (2015) An optimization model for the management of multi-objective water resources systems with multiple dams in series. Fresen Environ Bull. 24, 3100-3105.
[3] Denli, H.H., Denli, G. O. (2017) The use of soil and water resources at the mediterranean region in Turkey. Fresen Environ Bull. 26, 520-526.
[4] Sandoval-Solis, S., Mc Kinney, D.C., Loucks, D.P. (2010) Sustainability index for water resources planning and management. Journal of Water Resources Planning and Management. 137, 381 -390.
[5] GASC, G., Hermosilia, D., GASC, A. (2005) Application of a physical input-output table to evaluate the development and sustainability of continental water resources in Spain. Environmental Management. 36, 59-72.
[6] Jin, J.L., Wang, M.W., Wei, Y.M. (2006) Group Decision Based Ideal Interval Method for Evaluating Sustainable Water Resources Utilization. Journal of Industrial Engineering and Engineering Management. 20, 78-83.
[7] He, G.H., Wang, N., Xie, J.C. (2016) Establishment and application of fuzzy comprehensive assessment model for harmony of water allocation based on entropy weigh. Journal of Northwest A&F University. 30, 214-220.
[8] Song, Y.F., Guo, Z.X., Xu, X.M. (2016) Evaluation of Harmony Degree between Water Resources and Economic Society Development in Erenhot City. Journal of China Hydrology. 36, 66-70.
[9] Liao, H.C., Dong, Y.M. (2011) Utilization efficiency of water resources in 12 western provinces of China based on the DEA and Malmquist TFP index. Resources Science. 33, 273-279
[10] Zadeh, L.A. (1991) Similarity relations and fuzzy orderings. Information Sciences. 3, 177-200.
[11] Van Laarhoven, P.J.M., Pedrycz, W. (1983) A fuzzy extension of Saaty's priority theory. Fuzzy Sets and Systems. 1, 229-241.
[12] Satty, T.L. (1990) How to make a decision: The Analytical Hierarchy Process. European Journal of Operational Research. 48, 9-26.
[13] Wang, J.G., Zhang, J. (2006) Study on the evaluation index of the harmonious degree of water resources allocation. Annual meeting of the Chinese Society of Systems Engineering, 6, 723-730.
[14] Satty, T.L., Tran, L.T. (2010) Fuzzy judgments and fuzzy sets. International Journal of Strategic Decision Sciences. 1, 23-40.
[15] Ertugrul, I., Tus, A. (2007) Interactive fuzzy linear programming and an application sample at a textile firm. Fuzzy Optimization and Decision Making. 6, 29-49.
[16] Lin, H.F. (2010) An application of fuzzy AHP for evaluating course website quality. Computers & Education. 54, 877-888.

Received: **19.07.2018**
Accepted: **29.09.2018**

CORRESPONDING AUTHOR

Yi Liu
Hangzhou Dianzi University, Management School,
Hangzhou, China

e-mail: liuyi@hdu.edu.cn

© by PSP Volume 27 – No. 12B/2018 pages 9998-10004 Fresenius Environmental Bulletin

INVESTIGATING FINGERPRINT FINDINGS ON IMPROVISED INCENDIARY WEAPONS OBTAINED AT CRIME SCENES IN THE CASES OF THROWING MOLOTOV BOMBS

Yakup Gulekci[1], Kadir Dastan[2,*], Gulten Rayimoglu[3], Fatma Cavus Y[3], Emel Hulya Yukseloglu[3]

[1]Istanbul Provincial Security Directorate, Department of Crime Scene Investigation
[2]Istanbul Yeni Yuzyil University, Faculty of Health Sciences
[3]Istanbul University, Institute of Forensic Sciences

ABSTRACT

Explosions and molotov bomb throwing actions have prominent economic, psychological and social effects on the collective memory since they inflict nonrecoverable wounds. One of the prominent purposes of the crime scene investigation is to investigate the fingerprints in order to identify the identity of the offender. The purpose of this study is to determine the impact of heat on the quality of fingerprints on the findings obtained in crime scene and to create the standards to use as a routine investigation method in criminal laboratories of the law enforcers. Few studies have been published and little is known about this subject. The paper aims to discuss these issue.

In this study, 720 fingerprint samples, which were created according to the conditions that would exist in the crime scenes after an explosion or handmade incendiary weapons incidents, had been examined. “Super Glue” and “SPR” methods were used on metal and glass samples and “Ninhydrin” method were used on paper samples in order to create fingerprints. All the samples were exposed to temperatures varying between 50ºC-300ºC.

When different temperatures were taken into consideration, fingerprints suitable for comparison were obtained under all surface and environmental conditions up to 300ºC.

We are of the opinion that this study which is conducted for the first time in our country would significantly contribute to the identification of attackers and shed light on the incidents, especially in terrorist events.

KEYWORDS:
Fingerprint, Explosion incidents, Molotov bomb incidents, Fingerprint development methods

INTRODUCTION

By virtue of the rapid developments within the scope of the fight against crime and criminals, it is now possible to perform an extensive and sophisticated examination of findings collected at crime scene. Particularly, it is possible to determine the type of the explosive by examining the findings obtained after terrorist actions such as explosion or fire-starting incidents with improvised bombs [1]. In our world, the period of conquering or invading countries by force is now over. Besides espionage or psychological warfare, the use of various groups in the country has come to the fore. Organizations based on a specific ideological structure have established the ground for an effective response in their actions by using Molotov cocktails, which are prepared in a short time in order to carry out their actions within the cities, are easily procured and are not considered as weapons until the last amendment. The impact of these actions on the environment can be understood by determining the magnitude of the demonstration and the duration of the impact, the situation of ecosystem in certain geographic regions, the use of explosion or the use of hand-made incendiaries.

The firebomb known as “molotov cocktail” is prepared by bottling a fair amount of sulphuric acid, a mixture of gasoline and paraffin, and burned with a wick. If some kind of adhesive agent or detergent is added to its content, it threatens the life and property safety and disturbs the public order. As it is prepared in a short time by using easily supplied materials and has a high destructive power, molotov cocktails have been increasingly used in recent terrorist actions. Moreover, the offenders who use molotov cocktails are generally masked, thus, identifying the offenders becomes difficult. Hence, the utmost prominent alternative identification method is the fingerprint evidence [2]. However, the conditions to obtain fingerprints should be evaluated as findings in the crime scene are exposed to heat in the terrorist actions [3]. It is noticed in literature search that most of the studies conducted about the fingerprints aim to develop methods to understand the impacts of fuel types used as liquids in molotov cocktails on fingerprint development, to determine distances and methods of developing fingerprints [4]. In this context, the

purpose of this study is to investigate the contamination of the fingerprints found on the findings at the crime scene with molotov cocktail and the temperature ranges that would make it possible to obtain fingerprints for identification [5].

MATERIAL AND METHODS

Fingerprint samples were taken from six people, three of them were women, between the ages of 26-50, who signed the consent form to use their fingerprints as materials. By considering the factors affecting fingerprint development, three young and three middle-aged individuals were chosen to obtain fingerprints.

Fingerprint development process of study was conducted in laboratories of the Institute of Forensic Sciences, Istanbul University with the contributions of fingerprint development laboratories of Istanbul Provincial Security Directorate, Department of Crime Scene Investigation. Investigation period of this study conducted by using the modelling method and the study lasted nine months.

Preparation of the Samples. Fingerprint samples are obtained from six volunteers who signed Clarified Consent Forms. Fingerprints are placed on various surfaces such as microscope slide for glass, A4 size paper for paper and galvanized sheet metal and pieces of metals for metals. Voluntary individuals, who signed Clarified Consent Forms, touched every surface and waited for 10 seconds. Particular attention is attached so that the period of touching the surfaces would not exceed 10 seconds.

Environmental Conditions. Fingerprints obtained from volunteers are used for developing fingerprints for three months on different surfaces in the environments that we would face at the crime scenes. Thus, we were able to determine how long that a fingerprint would stay visible at a crime scene. In this study, in order to create the environments we would face during a crime scene investigation after an explosion or fire caused by handmade bombs, we created following samples:

· Fingerprint samples only exposed to heat;

· Samples contaminated with the liquid in the molotov cocktail and exposed to heat on the surface, (Encoded names of subjects are: YG-M and FC-M)

· Samples that are dipped into the liquid in the molotov cocktail for 1 minute and then placed on a surface to be exposed to heat. (Encoded names of subjects are: M-YG and M-FC)

By taking into consideration the factors affecting the fingerprints, the fingerprints of two volunteers out of 6 (six), whose fingerprints were regarded as fingerprints having the best quality, were contaminated with the liquid in the molotov cocktail and placed on surfaces after being dipped in the molotov liquid are collected and 10 different tests were performed.

A total of 180 samples is used on three different surfaces and under six different temperatures for each of the 10 tests. Four tests have been performed in three months and a total of 720 samples are prepared. In order to prevent the mixture of the samples and contamination, information about volunteers was marked on the stickers.

Temperature Values. Samples extracted from the test environment were exposed to temperatures of 50ºC, 90ºC, 110ºC, 150ºC, 200ºC, and 300ºC for 3 minutes at each degree.

Fingerprint Development Process:

"Super Glue" and "SPR" method is applied to metal and glass samples and "Ninhydrin" method is applied to paper samples, all of which were exposed to different temperatures, to examine whether fingerprints would develop or not.

Exceptional Case. The development of the fingerprints was inspected on the soot, which covers molotov bottles that are exposed to fire in terrorist acts like explosions or handmade bombs at certain distances. The soot, before the investigation, is cleaned by using 2% of sodium hydroxide (NaOH).

RESULTS

Fingerprint Development Results. Four (4) quarterly (Month Zero, Third month, Sixth month, and Ninth month) tests were performed and 720 samples were exposed to temperatures of 50ºC, 90ºC, 110ºC, 150ºC, 200ºC, and 300ºC for 3 minutes at each temperature. "Super Glue" method is used for metal and glass samples and "Ninhydrin" method is used for paper samples; all the samples have been exposed to different temperatures to investigate whether fingerprints would develop or not [6]. High quality scaled photo shooting, as well as the image intensifying (Photoshop) process, are applied to each sample in order to evaluate if the fingerprints are suitable for the identification.

Results of fingerprint samples of each volunteer on different surfaces are classified according to the classification system used in Turkey and by taking into consideration at least 13 fingerprints characteristics.

-When we evaluate the study in terms of fingerprint development; it is determined that fingerprints on glass surfaces are more appropriate for the comparison than the ones on metal and paper surfaces (Figure 1).

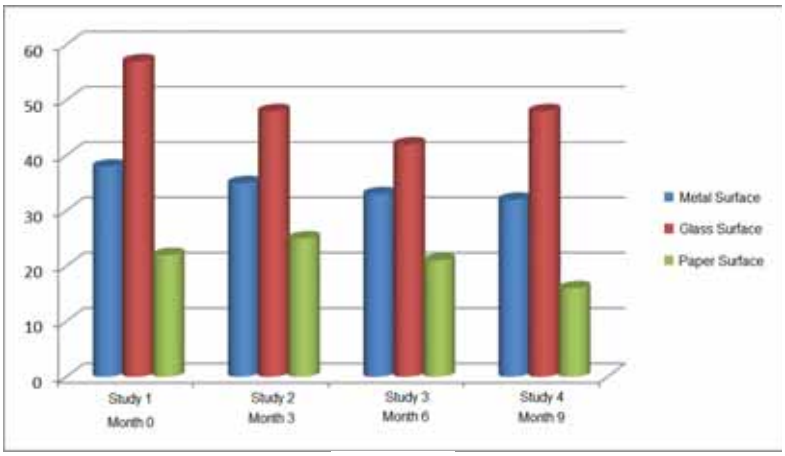


FIGURE 1
Evaluation Of Fingerprints Based On The Type Of The Surface And The Time

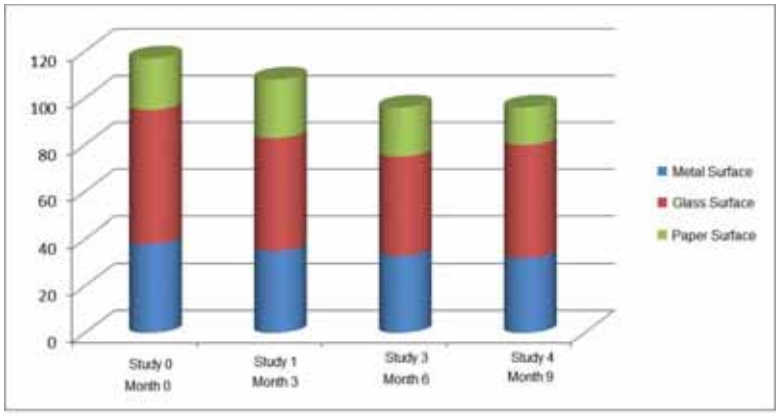


FIGURE 2
Evaluation of fingerprints on different surfaces in terms of comparability and time

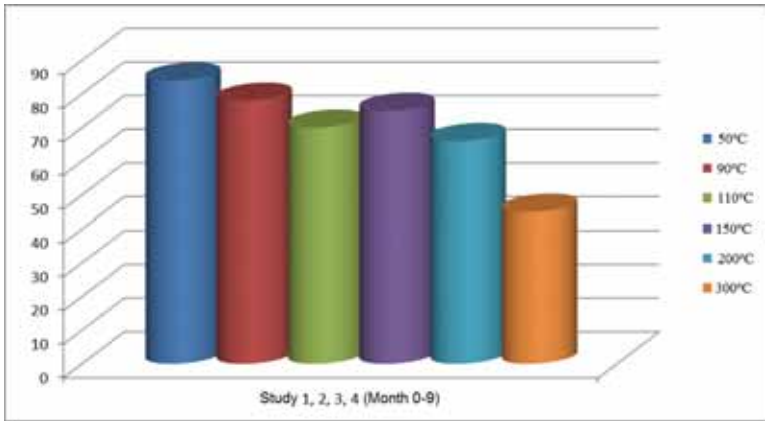


FIGURE 3
Evaluation of identifiable fingerprints in terms of time and temperature ranges

-When the total number of comparable fingerprints is considered; it is observed that the number of comparable fingerprints decreases from month zero to ninth (Figure 2).

-When we take into consideration the temperature parameter, we discovered that comparable fingerprints were obtained up to 300ºC heat. It is determined that the number of comparable fingerprints reduces when the temperature increases [7]. It is obvious that fingerprints exposed only to heat develop more quality prints than the samples exposed to the molotov liquid (Figure 3).

-When environments that we would face during crime scene investigation are evaluated; the impact of the fingerprints found on molotov bottles exposed to fire at certain distances on the development of the fingerprints is investigated. No fingerprint development is discovered on the surfaces in the center of the blast and within the immediate vicinity (Figure 4 and 5).

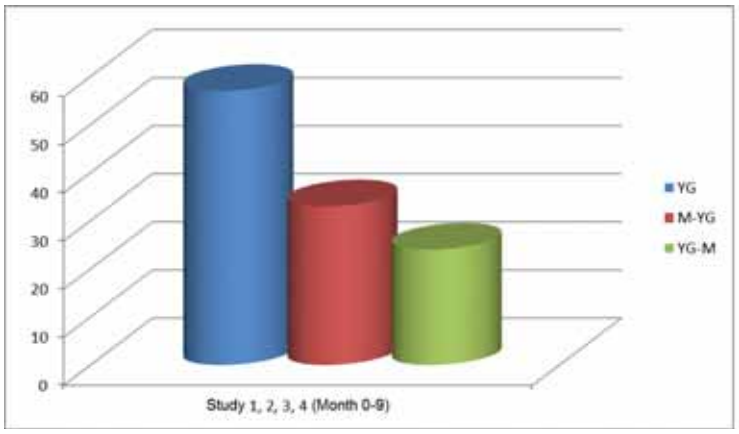


FIGURE 4
Comparison of Volunteer YG's fingerprints in terms of exposure to molotov liquid

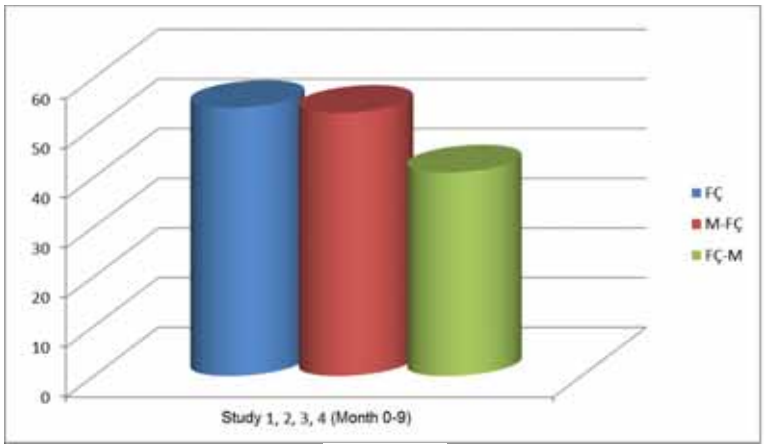
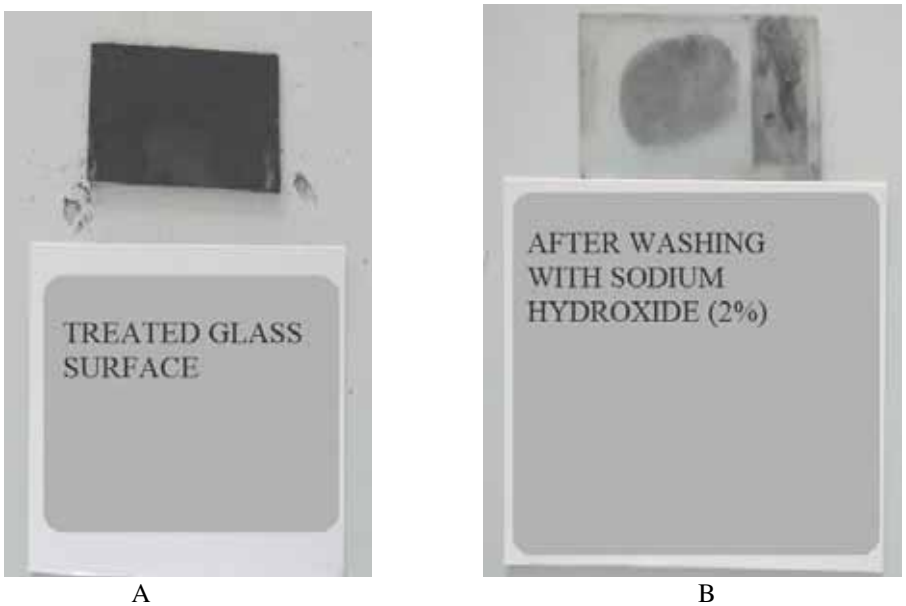


FIGURE 5
Comparison of Volunteer FC's fingerprints in terms of exposure to molotov liquid



A B

FIGURE 6
Cleaning the surface of the soot with sodium hydroxide (2%) and detection of the fingerprint by using foil

-When the soot is cleaned with 2% sodium hydroxide (NaOH); it is observed that the soot on the surface creates a protective layer for fingerprints and protects fingerprints without being deformed at the high temperatures (Figure 6).

DISCUSSION

First, in order to test the samples in a similar environment to the crime scene environment in the study, findings used in terrorist actions such as an explosion or handmade incendiary weapons are taken into consideration and the types of surfaces suitable to place fingerprints are determined [9]. Surfaces used in the study to place fingerprints are selected as glass, metal, and paper as they are commonly used in daily life and easy to supply, and these are the places where molotov liquid and explosive mixtures are placed.

When surfaces are compared, it is observed that best surface to develop identifiable fingerprints is the glass surface, which is followed by metal and paper surfaces respectively.

According to the studies, which confirm that results that are more successful are obtained from the glass surfaces, the porous structure of surface increases the sweat rate. While this provides the precision of papilla lines and it also allows more epithelial cells to release [10].

While it is prevented to press the surfaces for more than 10 seconds when leaving fingerprint samples, volunteers kept waiting for 10 seconds when passing from one surface to another. The main reason of this is that the excreta, released from the pore holes, creates the fingerprints in 10 seconds at the most. While the pressure power unintentionally increases as the time passes, the pressure deteriorates the natural appearance of the fingerprint. This affects negatively the development of comparable fingerprints. Waiting for 10 seconds when passing from one surface to another aims equal distribution of excreta coming through pore holes to every surface.

The previous studies confirm that the number of epithelial cells left at the first contact and excreta coming through pore holes doesn't increase the excreta and the number of cells when the surface is touched more than 10 seconds [11].

When the total number of identifiable fingerprints is analyzed; a decrease is detected in the number of identifiable fingerprints from Zeroth month to Ninth month. If we evaluate the impact of the temperature, we observed that identifiable fingerprints would be obtained up to 300ºC, however, the number of identifiable fingerprints decreases as temperature increases.

The temperature affects adversely the development of fingerprints because the temperature higher than 200ºC vanishes the organic components in fingerprint liquid [12]. Studies confirmed that only inorganic molecules are left in fingerprint component [13].

It is found that fingerprints exposed only to heat develop more quality prints than the samples exposed to the liquid in the molotov cocktail. When results of identifiable fingerprints are taken into consideration; it is discovered that samples which were left on the surface one minute after they were dipped into the the liquid in the molotov cocktail and exposed to heat (namely M-YG and M-FC) develop more identifiable and quality fingerprints than the ones contaminated by the liquid in the molotov cocktail on the surface and exposed to heat (namely YG-M and FC-M).

Even though the variable of temperature exists in every case, the main reason why the fingerprint development changes according to the environmental conditions is the contamination [14]. It is determined in the study that oily layer forms on surfaces, which are dipped in the liquid in the molotov cocktail, and this affects positively the fingerprint development on the surface. However, contamination of the liquid in the molotov cocktail on the surface with a fingerprint has an adverse effect on the permanence of fingerprints.

In the study conducted by Israeli police by using different liquids in the molotov cocktail, it is ascertained that gasoline and diesel fuels form a protective layer on the surface. It is verified that quality results are obtained from fingerprints left on the surfaces contaminated with these fuel types, which are easy to supply [15].

Fingerprint development is a unique field of specialization, which makes latent fingerprints visible and determines the fingerprints, which are suitable for the classification [16]. The main purpose of every method is taking advantage of the adhesive and coloring features of the used chemicals into the sweat and components of the sweat. Selection of the method is completely based on the experience of the implementer. Different characteristics of samples due to surface types (porous - nonporous surfaces) differ according to the method.

The best method is determined by considering the followings;

- Structure of the surface (porous-nonporous, absorbent-nonabsorbent etc.)
- Existence of a contamination, which affects fingerprint (oil, blood etc.)
- External factors (precipitation, temperature, moisture etc.)
- The period that fingerprint remains on the surface.

In our study, out of the samples, which were exposed to different temperatures; “Super Glue (Cyanoacrylate)” and “SPR” method is applied to metal and glass samples while “Ninhydrin” method is applied to paper samples, and we investigated whether fingerprints would develop or not.

In the study conducted by Israeli police to determine which fingerprint development method is more productive in molotov bombing incidents, it is ascertained that best results were obtained from SPR (Small Particle Reagent) method [15]. It is observed, in our preliminary study, that the success

rate of fingerprints obtained via SPR method decreases as fingerprints of the surface become old but the quality of fingerprints obtained via Super Glue (Cyanoacrylate) method doesn't deteriorate in time. In addition, when Super Glue (Cyanoacrylate) method is used after drying surfaces affected by the liquid in the molotov cocktail , Super Glue method created more quality results than the SPR method. Hence, Super Glue (Cyanoacrylate) method is used for glass and metal surfaces in this study. Studies conducted in this field also support our study [17].

When the environments that we would face during crime scene investigation are evaluated; the impact of fingerprints on molotov bottles exposed to fire from certain distances on fingerprint development is investigated and no fingerprint development is detected on surfaces in the center of the blast and within the immediate vicinity. When the soot is cleaned with 2% NaOH; it is ascertained that the soot on the surface creates a protective layer for fingerprints and protects fingerprints without being deformed at higher temperatures.

CONCLUSIONS

Even though it is generally believed that liquids and flammable fuels used to prepare molotov cocktails dissolve oily substances and they would harm fingerprints, it is confirmed that semi-burned carbon particles spreading from the center of fire in crime scenes form a protective layer on surfaces and this layer protects the structure of fingerprints at high temperatures. NaOH is a basic substance used in the detergent industry. It makes fingerprints visible by cleaning unburnt semi-carbon particles in molotov bombing incidents. [18]

It is ascertained that fingerprints would develop for a period of more than 9 months if the fingerprint development is not affected by climatic conditions. In our study, the permanence and quality of fingerprints vary according to age, gender, the effect of climate and environment, the type of food consumed, personal factors along with state of mind (excitement, stress etc.), impact of time (age of fingerprint), pressure power applied on surface, impact of contamination. Hence, the fingerprints of the same person on different surfaces develop differently within different time intervals [19,20]

We believe that this study, which is conducted for the first time in Turkey, would significantly contribute to the identification of the offenders and clarify the incident, especially in terrorist attacks.

ACKNOWLEDGEMENTS

We would like to thank the Istanbul Provincial Security Directorate and the Istanbul University, Scientific Research Projects Unit for supporting this study with project number 56843.

REFERENCES

[1] De Forest, P.R., Lee, H.C., Gaensslen, R.E., (1983) “Forensic Science, An Introduction to Criminalistics”, USA, McGrow-Hill Series, Chapter. 12, pp.330-351.

[2] Lee, H.C. and Gaensslen, R.E., (2013) “Advances in Fingerprint Technology”, 3nd. Ed.,CRS Press, Boca Raton, FL.57-72.

[3] Cole, S.A., (2005) “Morethanzero: Accounting of error in latent fingerprint identification”, The journal of criminal low and Criminolog. 95(3).

[4] Hueske, E. (2009) “Firearms and Fingerprints” 38-40.

[5] Fisher, B.A.J., Block, S. (1998) “Techniques of Crime Scene Investigation”, CRC Press, 5th Edition Boca Raton. 5-26, 34-72.

[6] Lennard, C. (2001) “The Detectionand Enhancement of Latent Fingerprints”, Lyon, 13th Interpol Forensic Science Symposium.

[7] Lennard, C., (2007) “Fingerprint Detection: Current Capabilities”, Australian Journal of Forensic Sciences. 39,2, 55-71.

[8] Margot, P., Lennard, C., (1992) “Manual of Fingerprint Detection Techniques”, Lousanne: Univercity of Lousanne. pp.7-13.

[9] Saferstein, R. (2004) “Criminalsitics: An Introduction to Forensic Science”, Second International Edition, Chapter. 14, 201-225.

[10] Pesaresi, M., Buscemi, L., Alessandrini, F., Cecati, M., Tagliabracci, A. (2003) “Qualitative and quantitative analysis of DNA recovered from fingerprints”, International Congress Series. 1239, 947-51.

[11] Balogh, M.K., Burgera, J., Bender, K., Schneider, P.M., Alta, K.W. (2003) “STR genotyping and mtDNA sequencing of latent fingerprint on paper”, Forensic Science International . 137, 188-95.

[12] Berglund, L.G., Gallagher, R.R., McNall, P.E. (1973) “Simulation of the thermal effects of dissolved materials in humans sweat”, Comput. Biomed. Res. 6, 127-38.

[13] Deans, J. (2006) “Recovery of Fingerprints from Fire Scenes and Associated Evidence”, Science&Justice. 46, 3

[14] Hawthorne Mark, R. (2009) “Fingerprints Analysis and Understanding”, 4-11.

[15] Shelef, R., Levy, A., Rhima, I., Tsaroom, S., Elkayam, R. (2003) “Improving the invisible fingerprints on molotov cocktails”, Israeli General Directorate of Security, Department of Identification and Forensic Sciences.

[16] Sodhi, G.S., Kau, J. (2005) “The forgotten Indian pioneers of fingerprint science Current Science”. 88(1), 185-91.

[17] Gardner Sarah, J., Cordingley Thomas, H., Francis Sean, C. (2016) "An investigation into effective methodologies for latent fingerprint enhancement on items recovered from fire", Science and Justice. 56, 241-246.
[18] Stow, K.M., McGurry, J. (2006) "Therecovery of fingermarks from soot-covered glass fire debris", ScienceJjustice. 46(1).
[19] Cebi, İ. (1999) "Factors Affecting the Comparative Development of Latent Fingerprints", Journal of Police Sciences, Ankara.1(3), 61-72.
[20] Polat, O. (2015) "Notes on Criminology and Criminalistic", Seckin Publishingss, Ankara, pp. 437.

Received: **09.10.2017**
Accepted: **13.10.2018**

CORRESPONDING AUTHOR

Kadir Dastan
Istanbul Yeni Yuzyil University,
Faculty of Health Sciences
Maltepe Mah., Yilanli Ayazma Caddesi, No: 26
P.K.34010 Cevizlibag/Zeytinburnu-Istanbul,Turkey

e-mail: kadirdastan@gmail.com

AUTHOR INDEX

SUBJECT INDEX

FEB – GUIDE FOR AUTHORS

General

FEB accepts original papers, review articles, short communications, research abstracts from the entire sphere of environmental-chemistry,-biology,-microbiology,- technology, -biotechnology and-management, furthermore, about residue analysis/ and ecotoxicology of contaminants.

Acceptance or no acceptance of a contribution will be decided, as in the case of other scientific journals, by a board of reviewers. Papers are processed with the understanding that they have not been published before (except in form of an abstract or as a part of a published lecture, review or thesis);that they are not under consideration for publication elsewhere; that their publication has been approved by all co-authors, if any, as well as-tacitly or explicitly- by the responsible authorities at the institute where the work has been carried out and that, if accepted, it will not be published elsewhere in the same form, in either the same or another language, without the consent of the copyright holders.

Language

Papers must be written in English. Spelling may either follow American (Webster) or British (Oxford) usage but must be consistent. Authors who are less familiar with the English language should seek assistance from proficient colleagues in order to produce manuscripts that are grammatically and linguistically correct.

Size of manuscript

Review articles should not exceed 30 typewritten pages. In addition up to 5 figures may be included.

Original papers must not exceed 14 typewritten pages. In addition up to 5 figures may be included.

Short-Communications should be limited to 4 typewritten pages plus not more than 1 illustration.

Short descriptions of the authors, presentation of their groups and their research activities (with photo) should together not exceed I typewritten page. Short research abstracts should report in a few brief sentences (one-fourth to one page) particularly significant findings. Short articles by relative newcomers to the chemical innovation arena highlight the key elements of their Master and PhD-works in about I page.

Book Reviews are normally written in-house, but suggestions for books to review are welcome.

Preparation of manuscript

Dear authors,

FEB is available both as printed journal and as online journal on the web. You can now e-mail your manuscripts with an attached file. Save both time and money. To avoid any problems handling your text please follow the instructions given below:

When preparing your manuscripts have the formula K/SS (Keep It Simple and Stupid) in mind. Most word processing programs such as MS-Word offer a lot of features. Some of them can do serious harm to our layout. So please do not insert hyperlinks and/or automatic cross-references, tables of contents, references, footnotes, etc.

1. Please use the standard format features of your word processor (such as standard.dot for MS Word).
2. Please do not insert automatisms or secret link-ups between your text and your figures or tables. These features will drive our graphic department sometimes mad.
3. Please only use two fonts for text or tables "Times New Roman" and for graphical presentations "Arial".
4. Stylesheets, text, tables and graphics in shade of grey
5. Turn on the automatic language detection in English (American or British)
6. Please - check your files for viruses before you send them to us!!

Manuscripts should send to:parlar@wzw.tum.de
or:parlar@prt-parlar.de

Thank you very much!

STRUCTURE OF THE MANUSCRIPT

Title page: The first page of the manuscript should contain the following items in the sequence given: A concise title of the paper (no abbreviations). The names of all authors with at least one first name spelled out for every author. The names of Universities with Faculty, City and Country of all authors.

Abstracts: The second page of the manuscript should start with an abstract that summarizes briefly the contents of the paper (except short communications). Its length should not exceed 150-200 words. The abstract should be as informative as possible. An extended repetition of the paper's title is not considered to be an abstract.

Keywords: Below the Summary up to 6 key words have to be provided which will assist indexers in cross-indexing your article.

Introduction: This should define the problem and, if possible, the frame of existing knowledge. Please ensure that people not working in that particular field will be able to understand the intention. The word length of the introduction should be 150 to 300 words.

Materials and methods:
Please be as precise as possible to enable other scientists to repeat the work.

Results: Only material pertinent to the subject must be included. Data must not be repeated in figures and tables.

Acknowledgements: Acknowledgements of financial support, advice or other kind of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organisations should be written in full.

References: Responsibility for the accuracy of references rests with the authors. References are to be limited in number to those absolutely necessary. References should appear in numerical order in brackets and in order of their citation in the text. They should be grouped at the end of the paper in numerical order of appearance. Abbreviated titles of periodicals are to be used according to Chemical or Biological Abstracts, but names of lesser known journals should be typed in full. References should be styled and punctuated according to the following examples:

ORIGINALPAPERS:
l. Author, N.N. and Author, N.N. (Year) Full title of the article. Journal and Volume, first and last page.
BOOK OR PROCEEDING:
2. Author, N.N. and Author, N.N. (Year) Title of the contribution. In: Title of the book or proceeding. Volume (Edition of klitor-s, ed-s) Publisher, City, first and last page

DOCTORAL THESIS:
3. Author, N.N. (Year) Title of the thesis, University and Faculty, City

UNPUBLISHED WORK:
Papers that are unpublished but have been submitted to a journal may be cited with the journal's name followed by "in press". However, this practice is acceptable only if the author has at least received galley proofs of his paper. In all other cases reference must be made to “unpublished work" or "personal communication".

Discussion and Conclusion: This part should interpret the results in reference to the problem outlined in the introduction and of related observations by the author/s or others. Implications for further studies or application may be discussed. A conclusion should be added if results and discussion are combined.

Corresponding author: The name of the corresponding author with complete postal address

Precondition for publishing:
A minimum number of 25 reprints must be ordered and prepaid.
1 - 4 pp.: 200,- EURO + postage/handling
5 - 8 pp.: 250,- EURO + postage/ handling
More than 8pp: 350,- EURO +postage/ handling.
The prices are based upon the number of pages in our journal layout (not on the page numbers of the submitted manuscript).
Postage/ Handling: The current freight rate is Germany 10 €, Europe 18,00 €, International 30 €.
VAT: In certain circumstances (if no VAT registration number exists) we may be obliged to charge 7% VAT on sales to other EU member countries.MESAEP and SECOTOX members get a further discountof 20% (postage/ handling full).