



Insights into spatiotemporal distributions of trace elements in kelp (*Saccharina japonica*) and seawater of the western Yellow Sea, northern China

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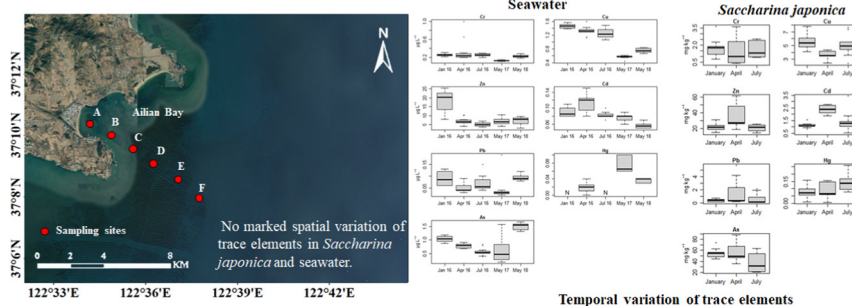
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HIGHLIGHTS

- Most trace elements in *S. japonica* showed no marked spatial or seasonal variations.
- *S. japonica* is a good biomarker for As, Cd, and Pb in seawater.
- The holdfast and meristem are good biomarkers for Cr, Cu, Cd, Pb, and As.
- A risk assessment showed that blade edges are relatively safe for human consumption.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 31 August 2020

Received in revised form 25 January 2021

Accepted 27 January 2021

Available online 3 February 2021

Editor: Filip M.G. Tack

Keywords:

Trace elements

Saccharina japonica

Ailian Bay

Bioremediation capacities

Food safety

ABSTRACT

China is the largest kelp (*Saccharina japonica*) producing country in the world. Kelp consumption is a tradition in many Asian countries. However, *S. japonica* accumulates trace elements that exhibit potential health risks upon ingestion. Here, we selected six sampling sites, from the shelf to offshore waters, in a maricultural area of Ailian Bay in Rongcheng City, northern China and investigated the spatiotemporal distributions of trace elements in kelp and seawater from 2016 to 2018. We then evaluated the bioremediation potential and food safety of *S. japonica*. Most trace elements in *S. japonica* and seawater demonstrated no marked spatial variations. The trace element concentrations in the tested kelp did not exceed the food safety standards, indicating that it is safe for human consumption. Moreover, *S. japonica* has a high bioremediation capability for trace elements, and the kelp in the coastal waters of Rongcheng (with annual yield of 1.7×10^4 t DW) removed 28.60 kg Cr, 165.51 kg Cu, 682.54 kg Zn, 34.00 kg Cd, 52.53 kg Pb, 3.60 kg Hg, and 1305.53 kg As from the seawater per year. Our results will be useful for evaluating the bioremediation potential of *S. japonica* and for food safety management.

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1. Introduction

Seaweed consumption of is a tradition in many Asian countries (McHugh, 2003; Mouritsen et al., 2013). Many seaweeds are rich in fiber, minerals, and trace elements (Holdt and Kraan, 2011), and most

possess bioactivities beneficial to humans. For example, polyphenol from seaweed, as a functional component of the human diet, may prevent chronic diseases (Miranda et al., 2016, 2018; Roohinejad et al., 2017). However, many seaweed species possess multiple trace element-binding sites (Güven et al., 1995; Kuyucak and Volesky, 1989; Reddy and Prasad, 1990). For example, kelp has a great ability to absorb and retain trace elements from the surrounding seawater.

Seaweed readily accumulates nutrients and trace elements from the surrounding environment. Therefore, they have been used extensively as bioindicators of seawater pollution, and they also play important roles in bioremediation techniques owing to their good sorption properties (Giusti, 2001; Sawidis et al., 2002; Huerta-Díaz et al., 2007; Miedico et al., 2017; Afiah et al., 2019; Roleda and Hurd, 2019; Roleda et al., 2019). Seaweed concentrates trace elements to levels many times greater than those in the surrounding environment, and brown seaweed has a relatively high trace element bioaccumulation capacity (Stengel et al., 2005; Li et al., 2011; Miedico et al., 2017).

China is one of the largest coastal countries in the world. At present, the coastal and estuarine environments of China are greatly influenced by increasing trace element-related pollution because of the elevated levels of trace element discharges from various sources, such as industrial and domestic sewage plants, mining, smelting, waste recycling, and aquaculture (Gao et al., 2014; Wang et al., 2020; Zhou et al., 2021). The elevated levels of trace element contamination in China's coastal environment have led to concerns regarding potential health risks associated with seaweed consumption (Chen et al., 2018). Chromium (Cr), Copper (Cu), Zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) are non-degradable pollutants, and the trace elements entering the food chain eventually have adverse effects in humans owing to their consumption of seaweed. In particular, As, Cd, Pb, and Hg are considered highly toxic, even at trace levels, affecting the nervous system and accumulating in the fatty tissues and internal organs of the human body, potentially increasing the risk of developing cancer. Noticeably, As is present in different forms that have different toxicity levels. Although inorganic As is much more toxic than organic As, organic As is genotoxic and a known human carcinogen associated with cancer in particular (Rose et al., 2007). Therefore, these are important trace elements that significantly affect public health (Tchounwou et al., 2012).

Trace elements accumulate in seaweed and its consumption might negatively impact consumer health (Roleda et al., 2019). Of particular interests are the temporal and spatial variations in contaminants, such as trace elements in seaweed, because they may provide information on optimal harvesting periods and location sites. This study aimed at quantifying temporal (both seasonal and inter-annual), and spatial variations in trace element (Cr, Cu, Zn, Cd, Pb, Hg, and total-As) concentrations in the different tissues of the kelp *S. japonica* and its surrounding seawater across a 3-year period. In addition, we assessed trace element bioaccumulations in cultivated *S. japonica*. This study was carried out in Ailian Bay, which is a typical intensive aquaculture area in Rongcheng City, Shandong Peninsula, northern China. The bioremediation potential of the *S. japonica* was evaluated and the potential risks of kelp consumption to consumers were investigated, providing useful information for food safety management.

2. Materials and methods

2.1. Study area and water and *Saccharina japonica* sampling

Ailian Bay (37°08'–37°12' N, 122°32'–122°38'E) is a coastal embayment with an area of 5.56 km² in Rongcheng City, Shandong Peninsula, northern China (Fig. 1). The Ailian Bay has a maximum depth of 14 m and 8 km of coastline (Li et al., 2018). This bay has been used for kelp aquaculture for more than 50 years, and currently, more than 80% of the bay is being used for the long-term culturing of *S. japonica* (Fig. 2).

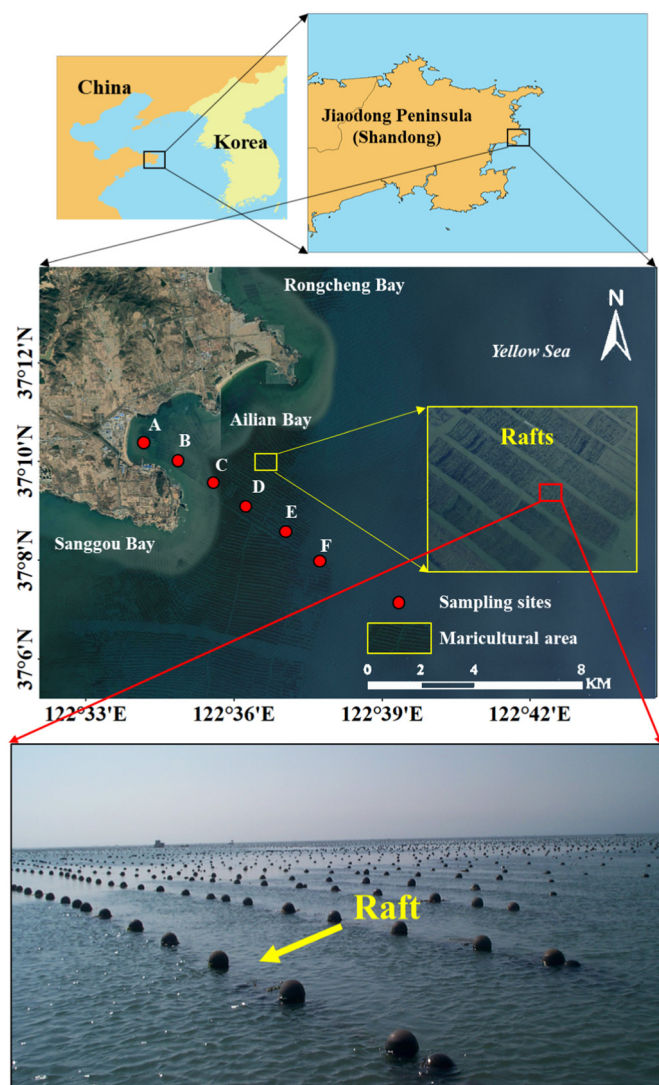


Fig. 1. *Saccharina japonica* sampling sites in Ailian Bay, northern China.

Saccharina japonica is cultivated from November to July of the following year.

We selected six sites in the maricultural area of Ailian Bay, from shelf to offshore waters, to sample *S. japonica* and water (Fig. 1; Table 1). One sample of water at each site was taken on 17 January 2016, and two samples of water at each site were taken on 6 April, 13 July 2016, 20 May 2017, and 20 May 2018. During water sampling, three samples of *S. japonica* at each site were taken, and an additional three samples at each site were taken for whole-body trace element measurement on 20 May 2017. At least 1 kg of wet biomass was harvested per sample, and this biomass contained at least 1–5 adult or 20–50 juvenile kelp sporophytes. Surface water samples were collected using a 2.5-L plastic bucket. Each water sample was filtered through Whatman GF/F glass fiber filters (0.7 μm) in the laboratory within 4 h of sampling. All the water samples were stored at –20 °C prior to analyses. In the laboratory, holdfast, meristem, central fascia, and blade edges were separated from the *S. japonica* samples (Fig. 2D). All the *S. japonica* samples were desalinated with distilled water and then air dried. The dry samples were ground to fine powder, sieved through an 80-mesh screen, and sealed in plastic containers for later analyses. To investigate trace element distributions (except As) in *S. japonica*, samples collected at sites B, D, and F on 20 May 2018 were used. To explore the annual variations

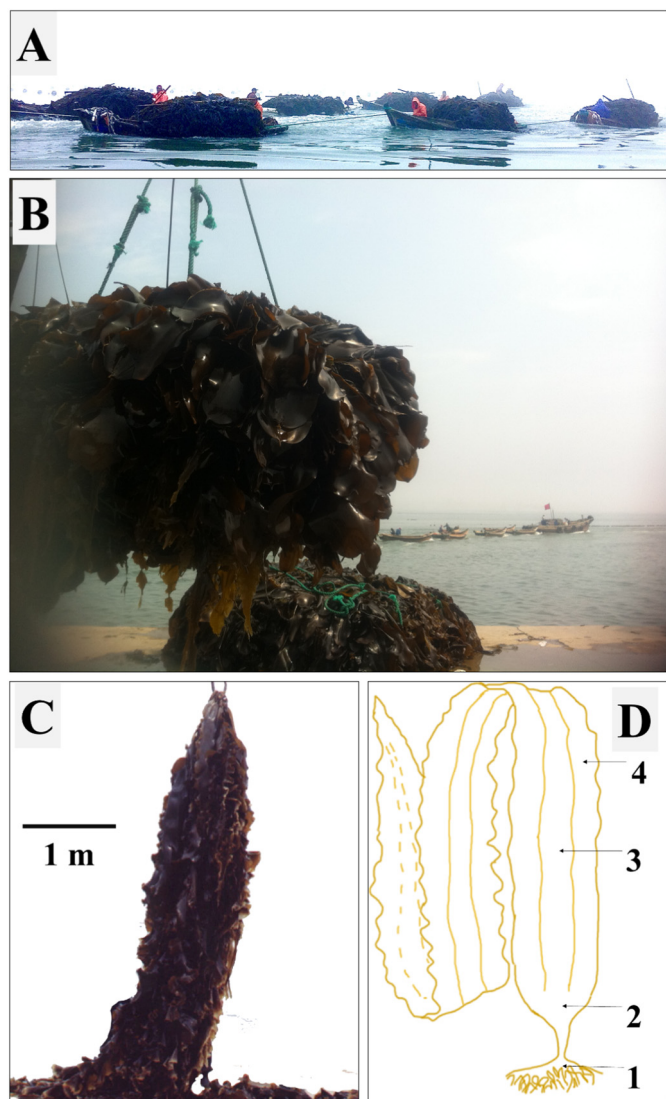


Fig. 2. *Saccharina japonica* and its morphology in Ailian Bay, northern China. *Saccharina japonica* harvesting (A–C), and its morphology (D), including the holdfast (i), meristem (ii), central fascia (iii), and blade edge (iv). Photos were taken by Yi Zhou.

in trace elements in *S. japonica*, samples collected at all sites on 20 May 2017 (except site A) and 20 May 2018 were used. To explore the seasonal variations in trace elements in *S. japonica*, samples collected at sites C, E, and F on 17 January, 6 April, and 13 July 2016 were used.

2.2. Analysis of trace elements

2.2.1. Seawater

In total, 10 mL filtered seawater was diluted with ultrapure water to 100 mL, and the levels of Cd, Cu, Zn, Cr, and Pb were measured using a Thermo Scientific iCAP Qc ICP-MS (Thermo Fisher Scientific Inc., Horsham, UK).

For the Hg analysis, 5 mL potassium peroxodisulfate (50 g L^{-1}) and 2 mL H_2SO_4 (1.84 g mL^{-1}) were added to 100 mL filtered seawater for 24 h. Then, 2 mL hydroxylamine hydrochloride solution (100 g L^{-1}) was added to the digested samples. The digested samples were analyzed using atomic fluorescence spectroscopy (Jitian AFS-930, Beijing Jitian Instrument Corp., Beijing, China).

For the As analysis, 10 mL HCl and 2 mL thiourea-ascorbic acid-reducing agent (3 g ascorbic acid and 5 g thiourea dissolved in 100 mL water) were added to 100 mL filtered seawater. Then, the digested

Table 1

The date, site, and purpose for sampling *Saccharina japonica* and seawater from Ailian Bay, Rongcheng City, northern China.

Sampling date (y/m/d)	Site	Replication of sample at each site		Sampling purpose
		Water	<i>S. japonica</i>	
2016/1/17	A, B, C, D, E, F	1	3	(1) To explore trace elements of whole bodies; three samples collected at all sites on 20 May 2017 (except site A) and 20 May 2018 were used. (2) To investigate trace element distributions (except As) in <i>S. japonica</i> ; samples collected at sites B, D, and F on 20 May 2018 were used. (3) To explore the annual variations in trace element in <i>S. japonica</i> ; samples collected at all sites on 20 May 2017 (except site A) and 20 May 2018 were used. (4) To explore seasonal variations in trace elements in <i>S. japonica</i> ; samples collected at sites C, E, and F on 17 January, 6 April, and 13 July 2016 were used.
2016/4/6		2	3	
2016/7/13		2	3	
2017/5/20		2	6	
2018/5/20		2	3	

samples were analyzed using atomic fluorescence spectroscopy (Jitian AFS-930, Beijing Jitian Instrument Corp.).

The detection limits were $6.00 \times 10^{-3} \mu\text{g L}^{-1}$ Cr, $1.10 \times 10^{-2} \mu\text{g L}^{-1}$ Cu, $1.11 \times 10^{-1} \mu\text{g L}^{-1}$ Zn, $2.00 \times 10^{-3} \mu\text{g L}^{-1}$ Cd, $1.00 \times 10^{-3} \mu\text{g L}^{-1}$ Pb, $1.00 \times 10^{-3} \mu\text{g L}^{-1}$ Hg, and $1.00 \times 10^{-2} \mu\text{g L}^{-1}$ As.

2.2.2. *Saccharina japonica*

A mixed acid of 5 mL HNO_3 and 1 mL HClO_4 was used to digest 0.1 g [dry weight (DW)] of *S. japonica* samples at 140–220 °C. The digested samples were dissolved with 1 mL HNO_3 (1:1, V/V), and then, 25 mL ultrapure water was used to dilute the dissolved samples. The levels of Cd, Cu, Zn, Cr, and Pb in samples were detected using Thermo Scientific iCAP Qc ICP-MS (Thermo Fisher Scientific Inc.).

For the Hg analysis, 10 mL HNO_3 and 1 mL HClO_4 (GR) were added to 0.1 g (DW) of samples for digestion at 140–160 °C. Samples were dissolved in 5 mL HCl and diluted to 50 mL using 1% oxalic acid solution. Then, 2 mL supernatant was taken to determine the Hg level using atomic fluorescence spectroscopy (Jitian AFS-930, Beijing Jitian Instrument Corp.).

For the As analysis, 10 mL HNO_3 and 1 mL HClO_4 were used to digest 0.2 g (DW) of samples at 160 °C for 1 h. Samples were treated with 5 mL thiourea-ascorbic acid-reducing agent (3 g ascorbic acid and 5 g thiourea dissolved in 100 mL water) and dissolved in 25 mL diluted H_2SO_4 (H_2SO_4 :water = 1:9, V:V). Then, the residual was diluted to 50 mL using ultrapure water and held overnight. In total, 2 mL supernatant was taken to determine the As level using atomic fluorescence spectroscopy (Jitian AFS-930, Beijing Jitian Instrument Corp.).

The detection limits of the different elements were $6.00 \times 10^{-6} \mu\text{g g}^{-1}$ Cr, $1.10 \times 10^{-5} \mu\text{g g}^{-1}$ Cu, $1.11 \times 10^{-4} \mu\text{g g}^{-1}$ Zn, $2.00 \times 10^{-6} \mu\text{g g}^{-1}$ Cd, $1.00 \times 10^{-6} \mu\text{g g}^{-1}$ Pb, $1.00 \times 10^{-6} \mu\text{g g}^{-1}$ Hg, and $1.00 \times 10^{-5} \mu\text{g g}^{-1}$ As. All the detection limits were based on a 98% confidence level (three standard deviations).

2.2.3. Quality control

All the chemicals used in present study were guaranteed reagent grade. All the laboratory ware were immersed in 10% HNO_3 for 72 h, rinsed with ultrapure water three times, and dried at 60 °C for 24 h before use. *Saccharina japonica* was selected as the certified reference material for plant analysis. The recovery rates of all the elements were 90.8%–104.1%, and the determination accuracy was less than 3% the

relative standard deviations. Replicate samples and reagent blanks were treated in the same way. The results showed that there was no contamination during the analysis, and the relative standard deviations of all the samples were less than 5%.

2.3. Data handling and analysis

Results are presented as the mean \pm SD. For the *S. japonica* analysis, trace element concentrations are provided on a dry-weight basis. Because the homogeneity of variance was not significantly different ($P < 0.05$), spatiotemporal variations in trace element concentrations (seawater and *S. japonica* from different sampling sites, *S. japonica* from different seasons, and different tissues of *S. japonica*) were statistically analyzed using Scheirer-Ray-Hare tests. Two-sided *t*-tests were used to identify specific treatment differences. Homogeneity of variance was tested using Levene's test. R v.4.0.2 for Windows 8.1 was used for the data analyses. A statistical significance level of 0.05 was used.

The bioconcentration factors (BCFs) of elements in the kelp tissue were calculated using the concentrations of trace elements in seawater. The BCF is an indicator of an organism's ability to take up and accumulate trace elements present in the environment, thus the BCFs were calculated for assessment of bioremediation capacity of *S. japonica*. BCFs were determined using the following equation:

$$BCF = C_{\text{plant tissues}}/C_{\text{seawater}}$$

where $C_{\text{plant tissues}}$ ($\mu\text{g g}^{-1}$ DW) and C_{seawater} ($\mu\text{g L}^{-1}$) represent the trace elements in the plant tissues of *S. japonica* and seawater, respectively (Lin et al., 2016).

2.4. Health risk assessment

GB3097-1997 provided by SEPBC PRC and SOB PRC (2004) was used for the assessment of seawater quality regarding trace element concentrations, and Level I values of the different elements in GB3097-1997 were $50 \mu\text{g L}^{-1}$ Cr, $5 \mu\text{g L}^{-1}$ Cu, $20 \mu\text{g L}^{-1}$ Zn, $1 \mu\text{g L}^{-1}$ Cd, $1 \mu\text{g L}^{-1}$ Pb, $0.05 \mu\text{g L}^{-1}$ Hg, and $20 \mu\text{g L}^{-1}$ As (SEPBC PRC and SOB PRC, 2004). GB 2762-2017 (NHFPC PRC, 2017), NY 5073-2006 (MOA PRC, 2006), and LEX-FAOC171002 (FAO, 2013) were used for assessment of *S. japonica* quality regarding trace element concentrations, and the food safety standards of the different elements were 50 mg kg^{-1} Cu, 1 mg kg^{-1} Pb (based on fresh algae with a 85% water content), 0.5 mg kg^{-1} Hg (methyl mercury), and 1.5 mg kg^{-1} (inorganic As) (NHFPC PRC, 2017; MOA PRC, 2006; FAO, 2013).

According to the consumption of unprocessed *S. japonica* biomass, potential health risk assessment was conducted by estimating the exposure dose, the targeted hazard quotient (THQ), and the hazard index (HI) (see Roleda et al., 2019).

The exposure doses for each trace element were estimated as:

$$\text{Exposure dose} = \frac{c_i * d_{dw}}{bw} \quad (1)$$

where c_i is the concentration of the element i in dry *S. japonica* biomass (mg (kg dw)^{-1}) collected on 20 May 2017 from Ailian Bay, d_{dw} is the quantity of dry *S. japonica* biomass ingested daily (5.2 g), and bw is the mean body weight for Chinese women (62.2 kg) and men (73.5 kg), respectively. Regarding *S. japonica* consumption, we used average ingestion data from the FAO available for China (5.2 g; Chen et al., 2018). Bodyweight data were obtained from www.worlddata.info (Worlddata.info, 2018). In the present study, As was the total As, and the proportion (%) of inorganic As (i-As) in total As (t-As) was computed from empirical data presented in Almela et al. (2006): i-As was about 1.72% of t-As in different kelp species (Laminariales).

The THQ_{*i*} for each trace element i was estimated as:

$$THQ_i = \frac{\text{Exposure dose}_i}{RfD_i} \quad (2)$$

where the RfD_i is the recommended reference dose for chemicals provided by US EPA (US EPA, 2007).

The sum of all THQs for each trace element was referred to as the HI. When HI < 1, no health risk is expected to occur; If HI \geq 1, there is moderate or high risk for adverse human effects (Chen et al., 2018; Roleda et al., 2019).

3. Results

3.1. Trace elements in seawater

Although there were no significant differences in trace element concentrations among seawater samples taken from different sites (Table 2; $P > 0.05$), there were significant differences among sampling dates (Fig. 3, Table 2; $P < 0.001$). Therefore, trace element concentrations in seawater demonstrated marked seasonal variations (Table 2; $P < 0.001$). Trace element concentrations in seawater followed the following order: [Zn] > [Cu]; [As] > [Cr] > [Cd]; [Pb] > [Hg] (Fig. 3), and the Zn concentration was greater than those of other trace elements by approximately one or two orders of magnitude (Fig. 3). In addition, the Zn concentration in winter (17 January 2016) was significant greater than in other seasons in 2016 ($P < 0.05$). A similar trend was found for Pb (Fig. 3). The trace element concentrations at all the sampling times (except Hg in May 2017) were all well below the maximum permissible limits (Level I) of the Water Quality Standards for Seawater (GB3097-1997).

3.2. Trace elements in different tissues and the whole body of *S. japonica*

The Cr, Cu, and Cd concentrations differed significantly among the different tissues of *S. japonica* (Table 3; $P < 0.05$), while those of Zn, Cd, and Hg showed significant differences among sites (Table 3; $P < 0.05$). The Cr concentrations of the holdfast were significant greater than those of the other tissues (Fig. 4; $P < 0.05$). In addition, Zn and Cu were the most abundant elements in both seawater and *S. japonica* (Fig. 4).

The BCFs of different kelp tissues are shown in Fig. 5. The BCF values were < 11,000 for Zn, Hg, and Cr (except in the holdfast, having a score of approximately 16,600), and the values were > 20,000 for Cd and Pb. Most of the trace elements concentrations (Fig. 4) and BCFs (Fig. 5) of the central fascia were middle level compared with the other tissues.

Trace element contents in the whole bodies of the *S. japonica* collected on 20 May 2017 are shown in Fig. 6. For all the samples, the mean values of the elements, in descending order, were as follows: As ($77.027 \pm 43.237 \text{ mg kg}^{-1}$) > Zn ($40.269 \pm 22.779 \text{ mg kg}^{-1}$) > Cu ($9.77 \pm 5.49 \text{ mg kg}^{-1}$) > Pb ($3.098 \pm 1.725 \text{ mg kg}^{-1}$) > Cd ($2.002 \pm 1.118 \text{ mg kg}^{-1}$) > Cr ($1.692 \pm 0.942 \text{ mg kg}^{-1}$) > Hg ($0.216 \pm 0.123 \text{ mg kg}^{-1}$).

Table 2

Two-way analysis of variance of sampling date and site on the trace element concentrations in seawater ($\mu\text{g L}^{-1}$) from Ailian Bay, Rongcheng City, northern China.

Variable	P value						
	Cr	Cu	Zn	Cd	Pb	Hg	As
Date	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Site	0.577	0.933	0.342	0.992	0.818	0.926	0.794
Date \times site	0.996	0.999	0.897	0.994	0.925	0.999	0.976

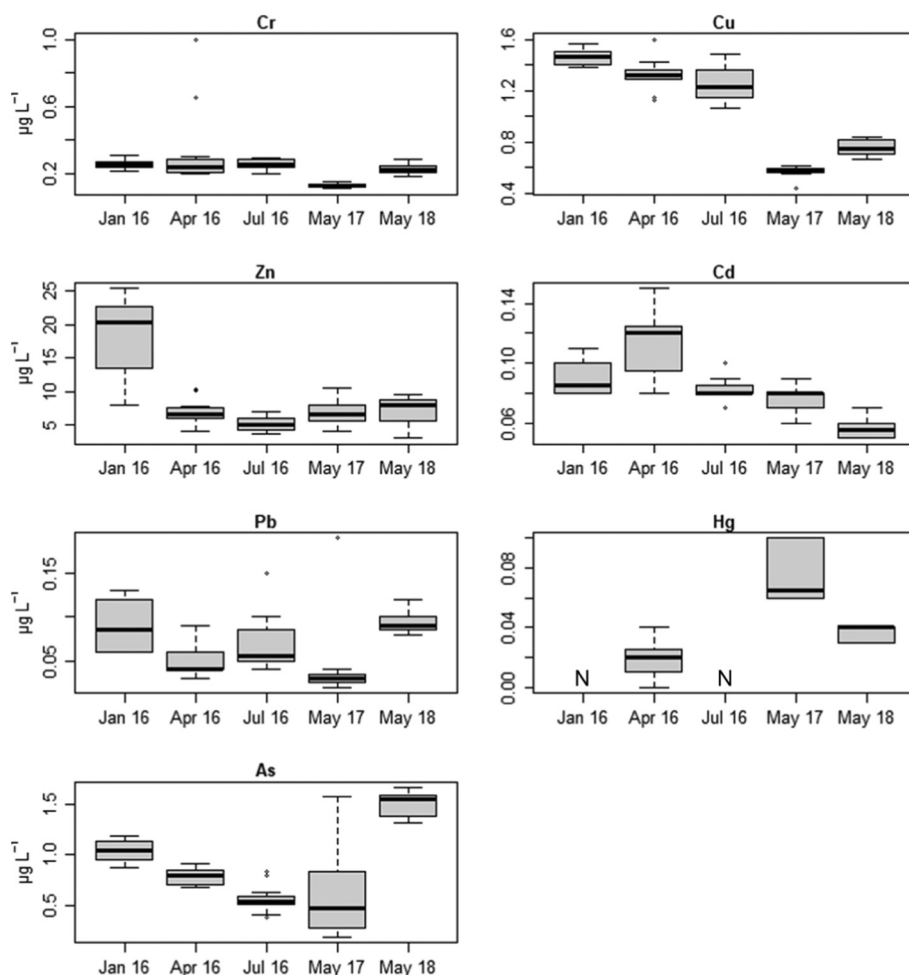


Fig. 3. Trace element concentrations in seawater ($\mu\text{g L}^{-1}$) across all the sites in Ailian Bay, Rongcheng City, northern China. Boxes represent lower and upper quartiles with medians (line) inside the boxes ($n = 3$). N indicates not detected.

3.3. Annual variations in trace elements in *S. japonica*

There were no significant differences in the Cr, Cu, Zn, and Hg concentrations within the central fascia of *S. japonica* between May 2017 and 2018 ($P > 0.05$). The Pb in 2018 was more than two-fold that in 2017 ($P < 0.05$; Fig. 7). In addition, the Cr and Zn concentrations demonstrated marked spatial variations (Table 4; $P < 0.05$).

3.4. Seasonal variations in trace element in *S. japonica*

There were no significant differences in the Cr, Pb, and As concentrations within the central fascia of *S. japonica* among sampling dates in 2016 (Fig. 8; $P > 0.05$). Although some of the trace element concentrations (Cu, Zn, Cd, and Hg) varied significantly among sampling times (Table 5; $P < 0.05$), no consistent trend was observed in these variations

(Fig. 8). In addition, the Cr and Pb concentrations demonstrated marked spatial variations (Table 5; $P < 0.05$).

3.5. Bioconcentration factors (BCFs) of the *S. japonica*

Most of central fascia at different sampling times shared a similar trend in BCFs, with the values decreasing in the following order: As (~67,000) > Cd (~19,000) > Pb (~17,000) > Cr (~6100) > Cu (~5900) > Zn (~3900) > Hg (<3900) (Fig. 9). Although the Cd concentrations were one order of magnitude smaller than those of Zn (Fig. 8), the BCF for Cd was significant greater than that for Zn (Fig. 9; $P < 0.05$). The BCF of the whole bodies of the *S. japonica* in Ailian Bay on 20 May 2017 had a similar decreasing trend for the different trace elements (Fig. 10).

3.6. Health risk assessment

The mean levels of trace elements investigated in *S. japonica* were below the maximums recommended by NHFPC PRC (2017), MOA PRC (2006), and the FAO (2013). The THQ for a single element was less than 1.00 (Table 6). The HIs were 0.747 (women) and 0.633 (men) for average exposure, and 1.614 (women) and 1.366 (men) for maximum exposure (Table 6). It is indicated that there is low health risk for those trace elements by intake of *S. japonica*. However, in terms of the maximum exposure level, they may be a potential adverse effect on human health.

Table 3

Two-way analysis of variance of tissue and site on the trace element concentrations in *Saccharina japonica* (mg kg^{-1} DW) collected at B, D and F sites on 20 May 2018 in Ailian Bay, northern China.

Variable	P value					
	Cr	Cu	Zn	Cd	Pb	Hg
Tissue	<0.001	<0.001	0.17	0.014	0.123	0.625
Site	0.566	0.633	0.009	0.001	0.209	<0.001
Tissue × site	0.885	0.776	0.204	0.599	0.038	0.603

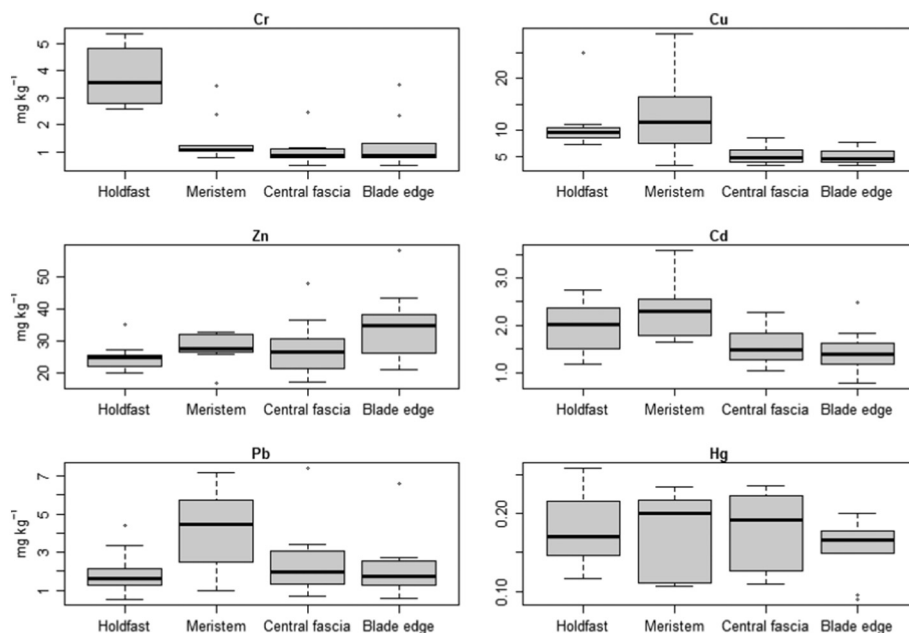


Fig. 4. Trace element concentrations in different tissues of the *Saccharina japonica* (mg kg^{-1} dry weight) collected at sites B, D and F on 20 May 2018 in Ailian Bay, northern China. Boxes represent lower and upper quartiles with medians (line) inside the boxes ($n = 3$).

4. Discussion

4.1. Bioremediation capacities of the cultivated *S. japonica*

Macroalgae concentrate nutrients and trace elements to levels many times greater than those found in the surrounding waters, and the mass culturing of seaweed is an economic and efficient way to address the environmental and health problems found in the estuaries and coastal waters that result from anthropogenic activities (Roleda and Hurd, 2019; Miedico et al., 2017). The biofiltering capacity of kelp to eliminate trace elements was correlated with its growth rate. The trace element (especially Zn and Pb) concentrations in the seawater were comparative high during the winter sampling (January 2016) when the kelp was small in size with a slow growth rate, but the concentrations decreased during the spring (April 2016) and summer (July 2016) when the fronds had grown (Fig. 3). This was consistent with our previous study in which the bioremediation capacities of *Sargassum* spp. increased along with thalli growth (Yu et al., 2016).

Bioremediation capacities of seaweed vary among the different trace elements. Bioremediation capacities for different trace elements are dependent on the selective binding of heavy trace elements by algal

polysaccharides, such as alginic acid, carrageenan types, and agar (Güven et al., 1995). In our study, *S. japonica* contained a high As concentration ($77.027 \text{ mg kg}^{-1}$), owing to its ability to concentrate the As derived from the seawater (Norman et al., 1987). In the present study, Pb and Cd also had high BCF values (approximately 3×10^4), which could be explained by their high binding capacities with algal polysaccharides (Güven et al., 1995).

Because fronds are not in contact with the sediment, the kelp took up and accumulated elements only from the seawater. Our study showed that the seawater was free of trace element contamination in Ailian Bay during all the sampling times, which was mainly because the cultivated kelp worked efficiently as a biofilter in the bay. Because the kelp had high BCFs, it could accumulate large amounts of trace elements from the surrounding seawater throughout the culture period. By multiplying the trace element concentration (Fig. 6) by the total yield of the kelp in the coastal waters of Rongcheng (with an annual yield of approximately $1.7 \times 10^4 \text{ t DW}$), we calculated annual total masses of

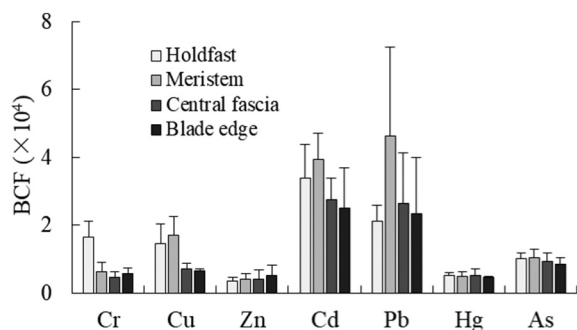


Fig. 5. Trace element bioconcentration factors (BCFs) in different tissues of *Saccharina japonica* sampled from Ailian Bay, northern China, on 20 May 2018. Values are mean \pm SD ($n = 3$).

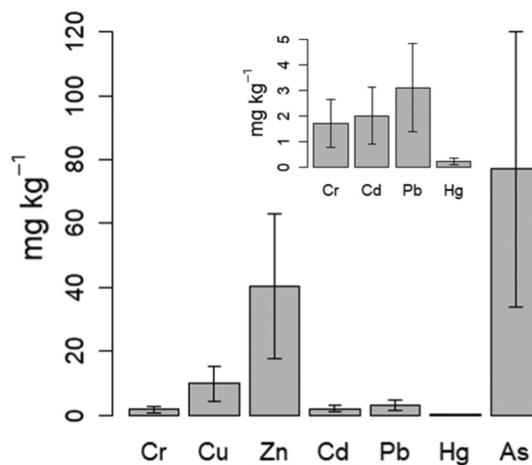


Fig. 6. Trace element contents (mg kg^{-1} dry weight) of whole bodies of *Saccharina japonica* collected on 20 May 2017 from Ailian Bay, northern China. Values are mean \pm SD ($n = 3$).

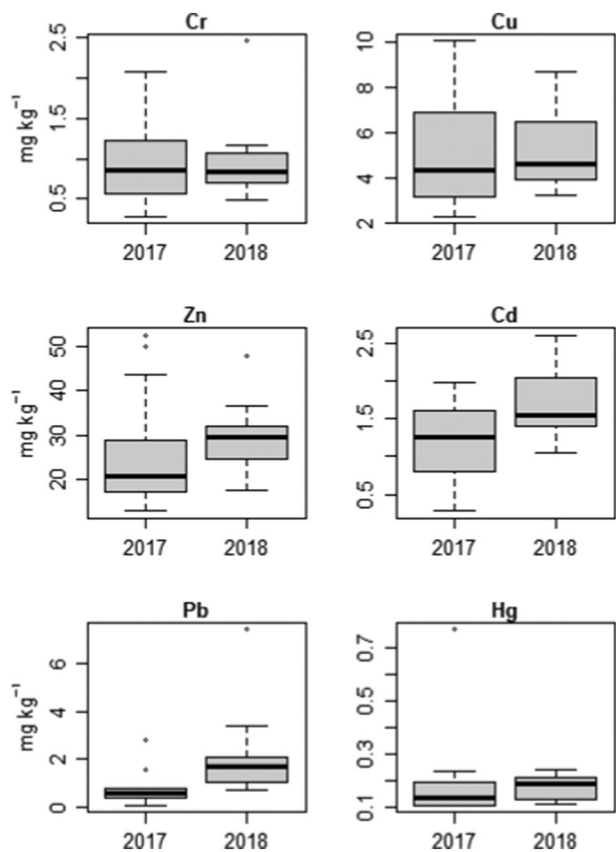


Fig. 7. Trace element concentrations in the central fascia of *Saccharina japonica* (mg kg^{-1} dry weight) at different sampling sites collected on 20 May 2017 and 20 May 2018 in Ailian Bay, northern China. Boxes represent lower and upper quartiles with medians (line) inside the boxes ($n = 3$).

28.60 kg Cr, 165.51 kg Cu, 682.54 kg Zn, 34.00 kg Cd, 52.53 kg Pb, 3.60 kg Hg, and 1305.53 kg As can potentially be removed from the seawater by harvesting the cultivated kelp in Rongcheng City.

Coastal currents play an important role in material transportation, especially sediment transportation (Bian et al., 2013). Many studies found that Lubei Coastal Current can transport the sediments from Bohai Sea via Bohai Strait to Chengshan Cape, and then bring high content of suspended matter through the research area (Fig. 11; Bian et al., 2013). In addition, sediments are important carriers of trace metals. Therefore, coastal current plays an important role in the distribution and diffusion of trace metals in marine environment. For example, Li et al. (2019) reported that the transportation of trace metals in the Bohai Bay is mainly affected by the circulation in the Bohai Bay, which was heavily polluted. In our present study area with low level of urbanization, the heavy industry is underdeveloped with less terrestrial emission, and most of factories along the coast were mainly related to aquaculture. In addition, our results showed that there were no significant differences in trace element concentrations among seawater

Table 4

Two-way analysis of variance of date and site on the trace element concentrations in the central fascia of *Saccharina japonica* (mg kg^{-1} DW) from different sampling sites collected on 20 May 2017 and 20 May 2018 in Ailian Bay, northern China.

Variable	P value					
	Cr	Cu	Zn	Cd	Pb	Hg
Date	0.917	0.613	0.085	0.014	0.001	0.176
Site	0.038	0.241	0.006	0.069	0.245	0.14
Date × site	0.149	0.085	0.166	0.596	0.078	0.09

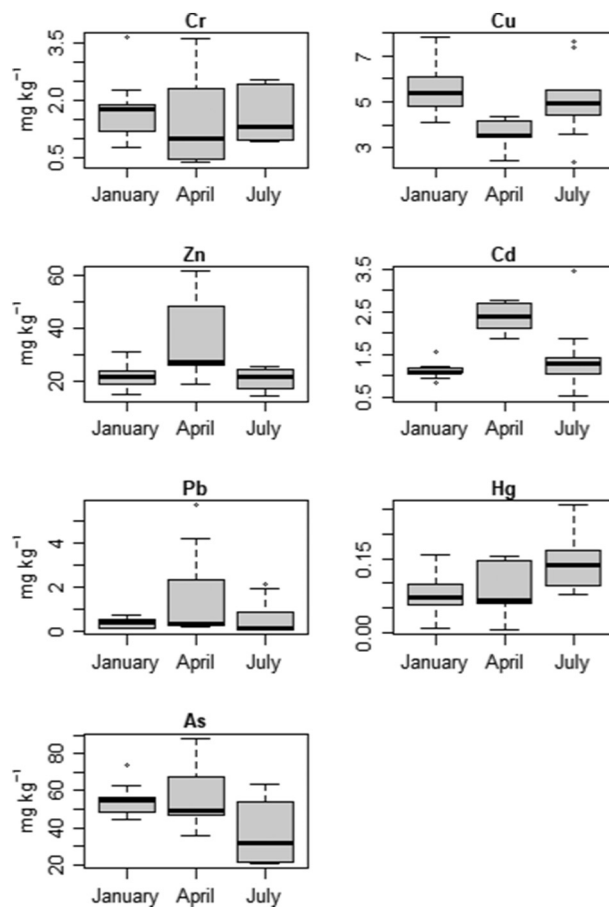


Fig. 8. Trace element concentrations in the central fascia of *Saccharina japonica* (mg kg^{-1} dry weight) collected on 17 January, 6 April, and 13 July 2016 at the C, E, and F sampling sites in Ailian Bay, northern China. Boxes represent lower and upper quartiles with medians (line) inside the boxes ($n = 3$).

samples taken from different sites. Accordingly, the trace metals in Ailian Bay may be transported by Lubei Coastal Current, and possibly derive from the Bohai Sea. However, this hypothesis needs to be further validated.

4.2. Tissue element concentrations and distributions

For all the samples, the mean values of the elements, in descending order, were as follows: $\text{As} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cd} > \text{Cr} > \text{Hg}$. The mean trace elemental content of *S. japonica* (t-As: $77.027 \text{ mg kg}^{-1}$) in this study was lower than those of studies in Japan, which had t-As values of 104 to 116 mg kg^{-1} . However, as a brown species, the t-As content of *S. japonica* was greater than that of red seaweeds (Roleda et al., 2019, 2021). The levels of Pb (3.098 mg kg^{-1}) and Cd (2.002 mg kg^{-1}) in this study were greater than those in Japan, in which Pb was $<0.05 \text{ mg kg}^{-1}$ and Cd was 0.074 to 0.908 mg kg^{-1}

Table 5

Two-way analysis of variance of date and site on the trace element concentrations in the central fascia of the *Saccharina japonica* (mg kg^{-1} DW) collected on 17 January, 6 April, and 13 July 2016 at the C, E, and F sampling sites in Ailian Bay, northern China.

Variable	P value						
	Cr	Cu	Zn	Cd	Pb	Hg	As
Date	0.634	0.004	0.010	0.001	0.431	0.024	0.054
Site	0.049	0.271	0.743	0.613	0.031	0.216	0.144
Date × site	0.771	0.252	0.084	0.773	0.267	0.056	0.015

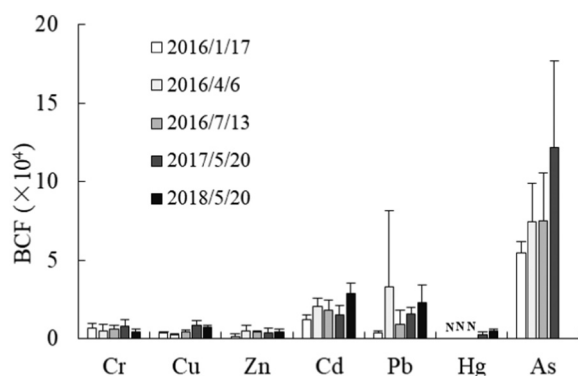


Fig. 9. Temporal variations in trace elements bioconcentration factors (BCFs) of the central fascia of *Saccharina japonica* from Ailian Bay, northern China. N indicates not detected. Values are mean \pm SD ($n = 3$).

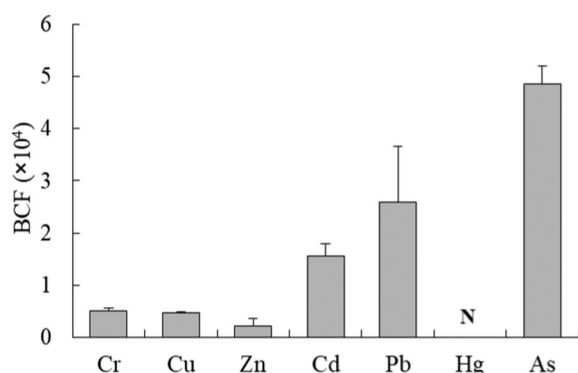


Fig. 10. Trace element bioconcentration factors (BCFs) of whole bodies of *Saccharina japonica* from Ailian Bay, northern China, on 20 May 2017. N indicates not detected. Values are mean \pm SD ($n = 3$).

(Almela et al., 2006). The Cu level (9.77 mg kg^{-1}) was slightly greater than that in Chilean seaweed (7.46 mg kg^{-1}) (Astorga-España et al., 2015). The differences in these trace element contents among countries may be caused by the seaweed species, as well as the diverse growing environments (Larrea-Marín et al., 2010; Akcali and Kucuksezgin, 2011; Chen et al., 2018). In addition, trace elements were tested after air-drying in our study, but many studies have used freeze-drying (Roleda et al., 2019, 2021), which, compared with air-drying, better

Table 6

Estimated exposure of Chinese women and men to the trace elements from consumption of unprocessed seaweeds, including a health risk assessment. The targeted hazard quotient (THQ) and the hazard index (HI) are calculated for health risk assessment. At $\text{HI} < 1.0$, the expected health risk is minimal. Data are means and maximum (in brackets, to quantify high risk). Data used for assessment, are trace element contents (mg kg^{-1} dry weight) of whole bodies of *Saccharina japonica* collected on 20 May 2017 from Ailian Bay, northern China.

	RfD ^a $\mu\text{g (kg day)}^{-1}$	Women		Men	
		Exposure dose ^b $\mu\text{g (kg bw day)}^{-1}$	THQ	Exposure dose ^b $\mu\text{g (kg bw day)}^{-1}$	THQ
Cr	3	0.141(0.320)	0.047(0.107)	0.12(0.270)	0.04(0.090)
Cu	40	0.817(1.708)	0.02(0.043)	0.691(1.445)	0.017(0.036)
Zn	300	3.367(7.318)	0.011(0.024)	2.849(6.193)	0.009(0.021)
Cd	1	0.167(0.359)	0.167(0.359)	0.142(0.304)	0.142(0.304)
Pb	3.6	0.259(0.547)	0.072(0.152)	0.219(0.462)	0.061(0.128)
Hg	0.3	0.018(0.039)	0.06(0.129)	0.015(0.033)	0.051(0.109)
i-As ^c	0.3	6.440(13.967)	0.369(0.801)	5.449(11.819)	0.312(0.678)
		HI _{women}	0.747(1.614)	HI _{men}	0.633(1.366)

^a Recommended reference dose (RfD) according to the US EPA (2007), National Center for Environmental Assessment for chronic oral exposure (per kg bodyweight); for Hg, reference dose for chronic inhalation exposure (per kg air).

^b Based on daily intake of 5.2 g dw (Chen et al., 2018) and an average body weight of Chinese women (62.2 kg) or men (73.5 kg) according to World Data Information (<https://www.worlddata.info>).

^c i-As was calculated based on the percentage of i-As in t-As presented by Almela et al. (2006) where i-As was 1.72% of t-As in different kelp species (Laminariales).

preserves the surface structures (Studer et al., 1981). Our study only tested total As concentrations. Usually organic As is less toxic than inorganic As. Seaweed primarily contains As in the form of arsenosugars, which may be metabolized into a wide range of As compounds. Although these metabolites are cytotoxic in bladder and lung cells, they occurred at only low levels in human urine (Taylor et al., 2017).

In the current study, the element concentrations varied among kelp tissues, sampling sites and sampling time, and this is common in many seaweeds (Stengel et al., 2005; Roleda et al., 2019; Yu et al., 2016). Moreover, Roleda et al. (2019) compared the levels of trace elements (As, Cd, Hg, and Pb) in *Alaria esculenta*, *Saccharina latissima*, and *Palmaria palmata* and found that they varied among species. Furthermore, Larrea-Marín et al. (2010), and Akcali and Kucuksezgin (2011) emphasized that the element concentrations in kelp varied among the culture regions. However, most trace elements demonstrated no marked spatial variations in our study, but this may be explained by the small study area (Fig. 1). In addition, the Pb of *S. japonica* in 2018 was more than two-fold that in 2017 (Fig. 7), and this difference may have been caused by the diverse growing environments (Larrea-Marín et al., 2010; Akcali and Kucuksezgin, 2011; Chen et al., 2018), which was indicated by the different Pb levels in the seawater in 2017 and 2018 (Fig. 7).

The seaweed's composition may be influenced by various external and internal factors, and previous studies demonstrated that the trace element concentration in seaweed decreases during the growing periods and increases during the winter dormant period (Brown et al., 1999). From May to July, which is the period of active seaweed growth and high biomass, the trace element concentrations in the kelp of Ailian Bay were diluted (Fig. 3), resulting in the significantly reduced trace element concentrations in the central fascia (Fig. 8). The accumulation of elements in seaweed reflects dissolved trace element bioavailabilities (Johnstone et al., 2015). The holdfast and meristem were tissues that readily accumulated most of the trace elements, with the exceptions of Zn and Hg (Fig. 4), and therefore, these tissues may act as good biomarkers of trace elements in seawater. The high BCF values of the central fascia indicated that *S. japonica* is a good biomarker for As, Cd, and Pb. However, when considering food safety, the blade edge of the kelp, which is rich in Zn (a necessary element for humans) but lacks other trace elements, is the first choice for human consumption.

4.3. Seaweed safety: health risk assessment

The consumption of *S. japonica* is generally considered safe. In the present study, the mean levels of trace elements investigated in *S. japonica* were below the maximums recommended by NHFPC PRC (2017), MOA PRC (2006), and the FAO (2013), indicating that it is safe

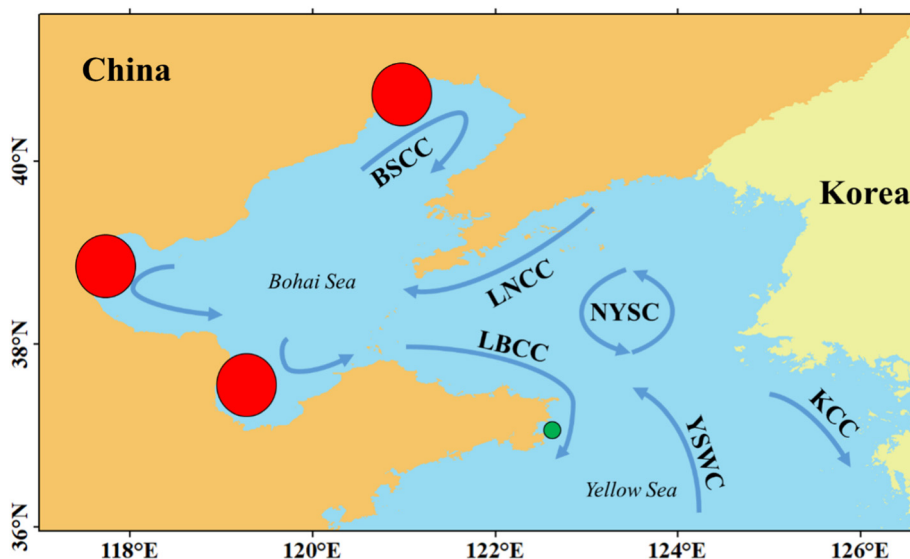


Fig. 11. The ocean circulations in Bohai Sea and North Yellow Sea. These currents are: the Bohai Sea Coastal Current (BSCC), the Yellow Sea Warm Current (YSWC), the Lubei Coastal Current (LBCC), the Korea Coastal Current (KCC), the Liaonan Coastal Current (LNCC), and North Yellow Sea Cold Water Mass (NYSC) (Modified after Zhang et al., 2016). The green circle represent *Saccharina japonica* sampling site in Ailian Bay (●), northern China. The red circle represent the most seriously trace elements contaminated areas of Bohai Sea (●; Modified after Gao et al., 2014).

for consumption and it does not pose a significant health risk to humans. Our results are similar to the finds of several studies conducted in Italy, South Korea and Spain, where total trace elemental intake does not appear to pose any threat to the consumers (Hwang et al., 2010; Desideri et al., 2016; Rubio et al., 2017). In addition, previous studies have reported that deaths and illnesses resulting from eating seaweed were not a result of trace element contamination, but due to the presence of other materials, like prostaglandin E-2 (Cheney, 2016; Hsu et al., 2007) and lethal toxins (Cheney, 2016; Haddock and Cruz, 1991; Yotsu-Yamashita et al., 2004). However, health risks can occur when consumers were in high exposure levels, including that large quantity of seaweeds are consumed at once, and/or seaweeds are consumed over prolonged periods of time (Roleda et al., 2019). Therefore, because of the hazards presented by some trace elements (e.g., heavy metals) to the human body, the continuous surveillance of *S. japonica* is necessary to protect consumer health (Yong et al., 2017). Furthermore, regulations regarding the maximal concentrations of trace elements in *S. japonica* should be established in Asian countries.

5. Conclusion

In the present study, the cultivation of *S. japonica* promotes bioremediation capability for trace elements, and total trace elemental intake from the harvested biomass for human consumption does not appear to pose any threat to the consumers. We found that *S. japonica* had a high bioremediation capability for trace elements. The bioremediation of trace elements by *S. japonica* correlated with its growth rate. *S. japonica* may be a good biomarker for As, Cd, and Pb levels in the seawater. In addition, the holdfast and meristem are good biomarkers for Cr, Cu, Cd, Pb, and As. Overall, trace element concentrations in *S. japonica* were below the food safety standards. Although there is no food safety problem in our study area at present, the continuous surveillance of *S. japonica* is necessary to protect consumer health owing to the hazards posed by some trace elements to the human body. Furthermore, regulations regarding the maximal concentrations of trace elements in seaweed should be established in Asian countries.

CRedit authorship contribution statement

Shaochun Xu: Investigation, Data curation, Writing – original draft, Software, Visualization. **Zonghe Yu:** Writing – original draft, Writing –

review & editing. **Yi Zhou:** Funding acquisition, Writing – review & editing. **Feng Wang:** Investigation. **Shidong Yue:** Investigation. **Xiaomei Zhang:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Yao Liu for assistance in testing the trace element concentrations. This research was supported by the National Key Research and Development Program of China (2019YFD0901301; 2019YFD0900800) and the Taishan Scholars Program (Distinguished Taishan Scholars).

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