



Evaluation of two hybrid poplar clones as constructed wetland plant species for treating saline water high in boron and selenium, or waters only high in boron

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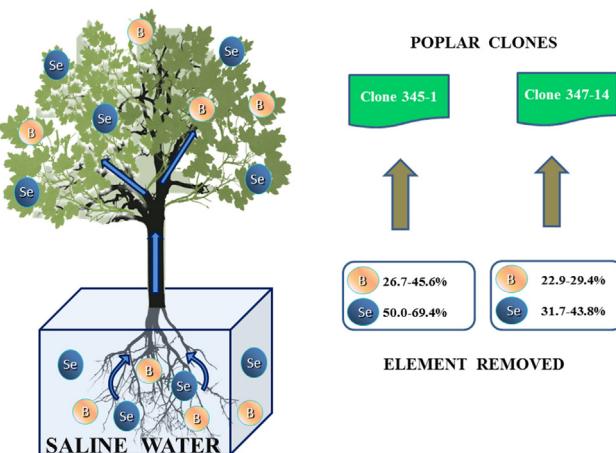
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HIGHLIGHTS

- Two hybrid poplar clones were identified as salt and boron tolerant under wetland conditions.
- Comparable boron and selenium removal percentages were obtained in mesocosms with both poplar clones.
- Both poplar clones accumulated large amounts of boron and selenium in tissues.
- Both poplar clones were effective for treating saline water high in boron and selenium.

GRAPHICAL ABSTRACT



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ABSTRACT

Wetland mesocosms were constructed to assess two hybrid poplar clones (*Populus trichocarpa* × *P. deltoides* × *P. nigra* '345-1' and '347-14') for treating saline water high in boron (B) and selenium (Se), and a hydroponic experiment was performed to test the B tolerance and B accumulation in both clones. In the mesocosm experiment, clone 345-1 exhibited no toxic symptoms at an EC of 10 mS cm⁻¹, while clone 347-14 showed slight toxic symptoms at 7.5 mS cm⁻¹. The removal percentages of B, Se, sodium (Na), and chloride (Cl) ranged from 26.7–45.6%, 50–69.4%, 18.4–24.0%, and 15.8–23.2%, respectively, by clone 345-1, and from 22.9–29.4%, 31.7–43.8%, 16.5–24.2%, and 14.9–23.9%, respectively, by clone 347-1. In the hydroponic experiment, B toxic symptoms were observed at treatments of 150 and 200 mg B L⁻¹ for clones 345-1 and 347-14, respectively. The greatest leaf B concentrations of 3699 and 1913 mg kg⁻¹ were found in clone 345-1 and clone 347-14, respectively. The translocation factor (TF) of clone 347-14 was less than clone 345-1. Clone 345-1 only showed significantly greater ($P < 0.05$) B removal percentages than clone 347-14 when B treatment was <20 mg B L⁻¹. In conclusion, both tested poplar clones competitively accumulated and removed B and Se in constructed wetlands.

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1. Introduction

As a global ecological environment problem, salinization threatens over 20% of the world's agricultural land [1]. Saline farmland becomes a source of saline wastewater when excessive irrigation or rainfall occurs. Agricultural effluent originating from such soils carries not only soluble salts but also other potential inorganic contaminants e.g., naturally-occurring trace elements mobilized from farmland activities. One agricultural region experiencing this salinization problem in association with soluble trace elements is located in the westside of central California. Soils in this area are mainly derived from Cretaceous shale rocks that contain naturally high levels of salts and other trace elements like boron (B) and selenium (Se). Due to the natural salinization and intensive irrigation practices on such salty soils, drainage effluent produced in this area contains high levels of soluble salts, B and Se. For example, a high salinity level (EC) of 15.2 mS cm^{-1} with B and Se concentrations at 14.5 mg L^{-1} and 1.18 mg L^{-1} , respectively, was detected in drainage waters originating in the western San Joaquin Valley (SJV) of Central California [2]. Salts and excessive trace elements in drainage water can bioaccumulate and negatively impact a variety of aquatic organisms by interfering with their cellular internal ionic pressure [3]. Moreover, this consequence can further change the communities of plants, microbes, and aquatic animals in an ecosystem by decreasing species diversity or replacing halo-sensitive species with halophilic species [3]. Though essential to biological systems, Se can pose an ecotoxic risk to aquatic and wildlife ecosystems if its level exceeds the U.S. EPA surface water criterion of $5 \mu\text{g Se L}^{-1}$, while B at $2.5\text{--}8.0 \text{ mg B L}^{-1}$ can both be toxic for many aquatic plants [4], invertebrates [5], and a variety of crops [6]. Therefore, it is necessary to explore effective and affordable strategies for controlling the quality of agricultural effluent in the westside of central California, and thereby protecting the safety of local ecosystem.

Constructed wetlands (CWs) provide a good alternative for treating wastewaters with high loads and unstable concentrations. CWs have been used and tested at different scales for treating various types of saline effluents originating from metallurgical industrial with major contaminants of BOD, COD, nitrogen (N), phosphorous (P) and metals [7], and tannery wastewaters containing COD, BOD, N, and P [8]. Similarly, there are also some successful attempts to use CWs for B [9–11], and Se removal [12–14], respectively. In regards to using CWs for the treatment of wastewater containing high salts, B and Se, however, successful attempts are rare, due to both salt and B toxicities experienced by the plants [1,15]. Additionally, the interaction between salts and B sometimes cause complicated ecophysiological responses of plants and affect their ability to absorb elements [16]. Even though challengeable, CWs offer the possibility for treating the saline drainage effluent high in B and Se, provided that the wetland plant exhibits salt and B tolerance, and possesses superior growth and the ability to accumulate elements (B, Se, Na, Cl) into shoots.

Poplars (*Populus* spp.), which produce high biomass and have a deep root system, are promising species for phytoremediation strategies because of their genetic diversity and easier manipulation through hybridization and selective breeding [17–19]. Previous studies reported promising B accumulation abilities of some poplar clones [20,21]. Earlier research by Bañuelos et al. [22] first evaluated eight different poplar clones for salt and B tolerance and later Bañuelos et al. [17] identified two salt and B tolerant poplar clones (*Populus trichocarpa* × *P. deltoides* × *P. nigra* '345-1'; '347-14') from more than thirty clones tested. Bañuelos et al. [17] demonstrated their salt and B tolerance in a six year field trial in which these two clones were irrigated with poor quality water (e.g., salinity ranging from 10 to 30 mS cm^{-1} and B at 10 mg L^{-1}). Consequently, we hypothesized the following for these two poplar clones: 1) they can survive and grow in wetlands containing saline

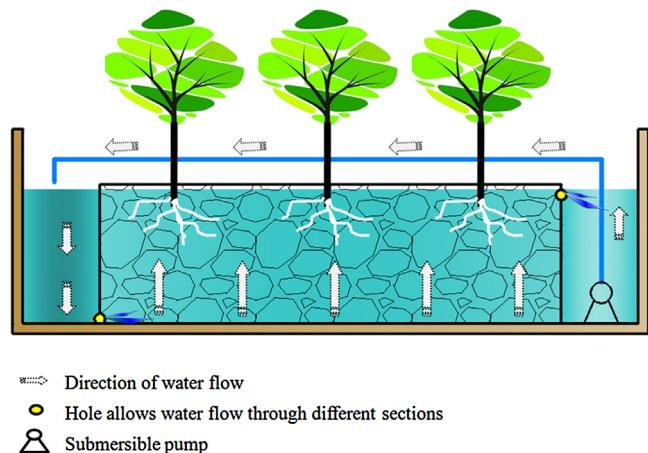


Fig. 1. Schematic diagram of the mesocosm wetlands.

water high in Se and B; 2) they might be promising wetland plants for treating agricultural drainage effluent or poor quality waters in the westside of central California.

The goal of the present work was to evaluate the above two hybrid poplar clones for their suitability as constructed wetland plant species for the phytoremediation of B and Se in saline waters. The specific objectives were to: 1) test the salt tolerance and growing of the poplar clones in mesocosm wetlands containing saline water high in B and Se; 2) evaluate the effectiveness of these poplar clones for removing selected elements in mesocosm wetlands and measure the distribution of elements (e.g., B, Se, Na, Cl) in their tissues; and 3) determine hydroponically the tolerance of poplars to high levels of B, and their ability to accumulate and remove B from solution. The results obtained from these experiments will provide a realistic reference for subsequent testing in pilot wetlands under field conditions.

2. Materials and methods

2.1. Materials

Two experiments were carried out at USDA, San Joaquin Valley (SJV) Agricultural Research Center in Parlier (SJVARC), CA under greenhouse growing conditions in 2015. Hardwood cuttings (approximately 25 cm in length and 2 cm in diameter) collected from two hybrid Populus clones, (*Populus trichocarpa* × *P. deltoides*) × *P. nigra* '345-1' and '347-14', were used in this study. These poplar clones were already identified to be salt and B tolerant [17]. Although there is no specific study to test their Se tolerance, these poplar clones have been growing well in soils from westside of central California, which contain high levels of Se (water extractable concentrations of 0.2 mg Se L^{-1}). Cuttings were initially collected in 2014 from 10 year old poplar trees growing in a non-saline soil (Hanford sandy loam) in SJV and were stored at 4°C until required. In Experiment 1, mesocosms were made from six 0.096 m^3 plastic tanks with the dimensions of 80 cm length × 40 cm width × 30 cm height. Submersible pumps (Sunterra 104506; Peoria, IL USA; 30 gal h^{-1}) and rubber tubing (with 9 mm inner diameter and 1 mm outer diameter) were set up to circulate water (approximate $3.0 \times 10^{-5} \text{ m}^3 \text{ s}^{-1}$) from the outlet back to the inlet of mesocosms (Fig. 1). Gravel with diameter of $2\text{--}3 \text{ cm}$ was used as substrate and added to a depth of about 12 cm in all mesocosms. In Experiment 2, a hydroponic system was constructed with 2.5 L round plastic pots (with 13 cm and 18 cm in bottom and top diameters and 15 cm in depth) and a piece of floating styrofoam (1.5 cm in thickness, for attaching

Table 1

The salinity, boron and selenium levels in three trials of Experiment 1.

	EC (mS cm^{-1})	Boron (mg L^{-1})	Selenium (mg L^{-1})
Trial 1	4.5	5	0.25
Trial 2	7.5	10	0.5
Trial 3	10	20	1.0

the poplar cuttings). Each mesocosm and hydroponic system was aerated by an aerator pipe at a rate of $5.6 \text{ L air min}^{-1}$. Both experiments were conducted in an environment-controlled greenhouse with $28/22^\circ\text{C}$ day/night temperatures, 40–50% relative humidity of ambient air, 16 h photoperiod, and minimum and maximum light intensity (photosynthetic photon flux) of 200 and $450 \mu\text{E m}^{-2} \text{ s}^{-1}$. All chemical reagents used in this study were purchased from Fisher Chemical, LLC., US.

2.2. Mesocosm experiment (experiment 1)

Sixteen 25 cm *Populus* hardwood cuttings of each respective clone were evenly planted in a mesocosm containing 19 L of 1/8 strength Hoagland's solution by vertically inserting the cuttings into gravel layer. There were three replicated mesocosms for each individual clone for each respective treatment (described below). Approximately, 4 L of 1/8 strength Hoagland solution were added weekly into each mesocosm to maintain nutrient levels. During this period, the cuttings developed roots, branches and leaves. The experiment consisted of three different trials with increases of salinity, B and Se in wetland solutions (See Table 1) and was initiated after 30 days of plant growth. Synthetic influent water representing typical agricultural effluent in the westside of the SJV was prepared with the following: salinity (NaCl), B (H_3BO_3), Se (Na_2SeO_4), and 1/8 strength Hoagland solution, and the pH was adjusted to 6.5. Each mesocosm was thoroughly rinsed by deionized water before they were filled with the influent water. The hydraulic retention time (HRT) of each trial was 10 days. During the operation, deionized water was added daily to each mesocosm to make up for evaporation losses and to maintain water volume at 19 L, followed by the method described in previous study [12]. Water samples were collected from the outlet section of each mesocosm on Days 0 (initial day of influent water), 1, 2, 4, 6, 8, and 10 of each trial, and stored in refrigerator in preparation for analyses of B, Se, Na, Cl, and EC. Plant samples (top and bottom half leaves, top and bottom half stems, and roots) were collected at the end of the third trial from each cutting. The top leaves and stems were defined as the top 50% (from midpoint) of each branch, while those plant parts below the 50% midpoint were defined as bottom leaves and stems. Dead leaves (bottom half) were collected immediately when they fell, stored, and processed as described below. Tissue samples from each respective part were then mixed together from each mesocosm to form a composite sample. Fresh biomass samples were washed, dried for 3 days at 65°C , weighed, ground to a fine powder in a UDY cyclone mill equipped with a 1 mm mesh screen, and analyzed for B, Se, Na and Cl, as described later.

2.3. Hydroponic experiment (experiment 2)

Boron concentrations in some types of wastewater can be much higher than that found in agricultural drainage water in the west-side of central California. Scientists are struggling in finding B tolerant plant species, which are also good B accumulators [15]. Therefore, a hydroponic experiment was conducted to determine both the B accumulation at varied B concentrations and the upper limits of B tolerance for the two poplar clones. Since the Se levels tested in Experiment 1 basically represent the highest Se concentrations found in most types of wastewater [12–14], Se tolerance was

not further tested in hydroponic experiment. One 25 cm poplar cutting was planted into each hydroponic system containing 2 L of 1/4 strength Hoagland solution. A new 1/4 strength Hoagland solution was added weekly to maintain the nutrient levels. After 30 days of plant growth, the following B treatments were respectively introduced: 0.25 (control), 10, 20, 30, 50, 100, 150, and 200 mg B L^{-1} , with three replicates for each B concentration. Boron solutions were prepared with H_3BO_3 and 1/4 strength Hoagland solution, and pH was adjusted to 6.5. Poplars were exposed to the respective B treatments for 14 days. Similarly, deionized water was added daily to each pot to maintain the solution volume at 2 L during the experiment, followed by the method described in previous study [12]. Water samples were collected on Day 0 (initial exposure to respective B treatment) and on Day 14 for the analysis of B. Poplars were harvested on Day 14, and equally divided into top and bottom parts from midpoint, as described in Experiment 1. Leaves were separated into top and bottom, while stems were not separated because of the limited biomass for analysis. Roots were collected. All samples were washed, dried for 3 days at 65°C , weighed, ground to a fine powder (as described in Experiment 1), and prepared for analysis, as described below.

2.4. Samples analysis

Ground tissue samples of 0.5 g were wet acid digested with $\text{HNO}_3\text{-H}_2\text{O}_2\text{-HCl}$, as described by Bañuelos and Akohoue [23]. Water samples (without filtration) were acid-digested with HNO_3 and H_2O_2 , according to EPA method 3010A [24]. Boron, Se and Na concentrations in plant and water samples were analyzed by an inductively coupled plasma optical emission spectrometer (Agilent 7500cx, Santa Clara, USA), according to Agilent manufacture protocol. National Institute of Standard and Technology (NIST) wheat flour (SRM 1567; Se content of $1.1 \pm 0.2 \text{ mg kg}^{-1}$, with a recovery of 94%) was used as an external quality control standard for Se content in plant material. Electrical conductivity (EC) was determined at room temperature using an Orion Model conductivity meter. Chloride was analyzed (after extraction with 2% acetic acid) by potentiometric titration using a Mettler Toledo titrator. The standard protocol described by the Western States Proficiency Testing Program was used for the analysis.

2.5. Statistical analysis

SPSS 19.0 (Inc., USA) statistical package for Windows was used for data analysis. Statistical comparison between means of two groups was performed by Independent Sample t Test, while the statistical comparison of more than two groups was performed by One-way ANOVA, Tukey. Significance was set at the 5% level. All data graphs were prepared by using OriginPro 8.0 package.

3. Results

3.1. Biomass and elements accumulation of poplar clones in mesocosms

During the experiment, clone 345-1 exhibited no visual toxicity symptoms at an EC of 10 mS cm^{-1} , while slight visual symptoms of salt toxicity (chlorotic and necrotic bottom leaves) was observed on clone 347-14 at an EC of 7.5 mS cm^{-1} at the end of Trial 2. No visual B toxicity symptoms (generally described as chlorotic at the margins and tips of leaves) were observed on either clone through the three trials. Though non-significant, the biomass of most tissues (except for dead bottom leaves) in clone 345-1 was greater than biomass measured in clone 347-14 (Fig. 2), however, more dead leaf biomass was collected from clone 347-14 than clone 345-1.

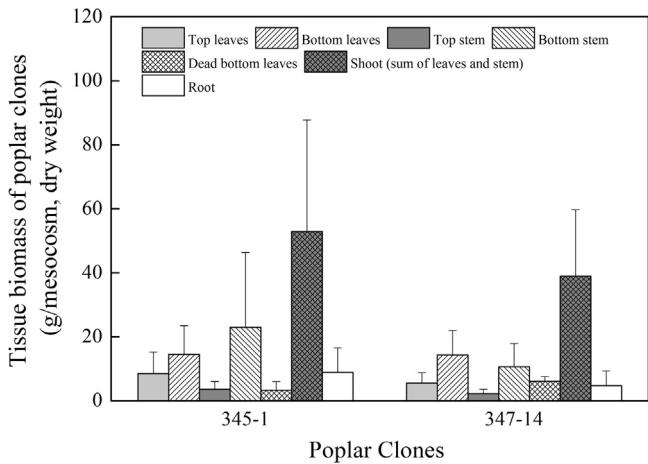


Fig. 2. Tissue biomass of two poplar clones growing in mesocosms after 30 days of salt, B and Se treatment. Values represent the mean of three replicates and error bars represent standard deviation.

The accumulation and partitioning of elements in poplars varied with tested element and poplar clones (Fig. 3). Average B concentrations in tissues of both poplar clones ranged as follows: bottom leaves > top leaves > dead bottom leaves > top stem ≈ bottom stem ≈ root (Fig. 3a). Top, bottom, and dead bottom leaves of clone 345-1 accumulated significantly greater ($P < 0.05$) amount of B than clone 347-14, while no significant differences were observed in stems and roots between two clones. The average tissue Se concentrations for both clones ranged as follows: root > top leaves > bottom leaves > dead bottom leaves ≈ top

stem > bottom stem (Fig. 3b). Comparing the two clones, a greater shoot Se concentration was observed in clone 345-1 than in clone 347-14. In contrast, root Se concentration of clone 347-14 was greater than clone 345-1. Average Na concentrations in tissues of clone 345-1 ranged as follows: bottom leaves > top leaves > dead bottom leaves > roots > top stem > bottom stem, and for clone 347-14 the order was root > dead bottom leaves > top stem ≈ bottom stem > bottom leaves > top leaves (Fig. 3c). Top, bottom, dead bottom leaves and stem of clone 345-1 accumulated more Na than clone 347-14 for each respective part (Significant ($P < 0.05$) for top and bottom leaves). Substantial amount of Na accumulated in roots of both clones, although clone 347-14 accumulated relatively more Na than clone 345-1. Both clones accumulated more Cl in leaves than stem and root tissues (Fig. 3d). Chloride concentrations were generally greater in top and bottom leaves of clone 345-1 than those in 347-14. There were, however, no substantially different Cl concentrations in stems and roots between the two clones.

3.2. Elements removal by mesocosms

The efficiency of the poplar mesocosms at removing B, Se, and salt is presented in Fig. 4. The concentrations of all tested elements, as well as salinity (EC) level declined over time in water column for all trials. The removal percentage of each respective element varied with poplar clone and influent concentrations. Boron average removal percentages at Trial 1 (5 mg B L⁻¹ treatment) were 26.7% and 29.4% for clone 345-1 and clone 347-14, respectively on Day 10 (Fig. 4a). With increasing influent B concentration (Trial 2 and Trial 3), no difference was observed during Days 2–8, however, the average B removal percentages were 45.6% and 35% with clone 345-1 on Day 10 for Trial 2 and Trial 3, respectively, which

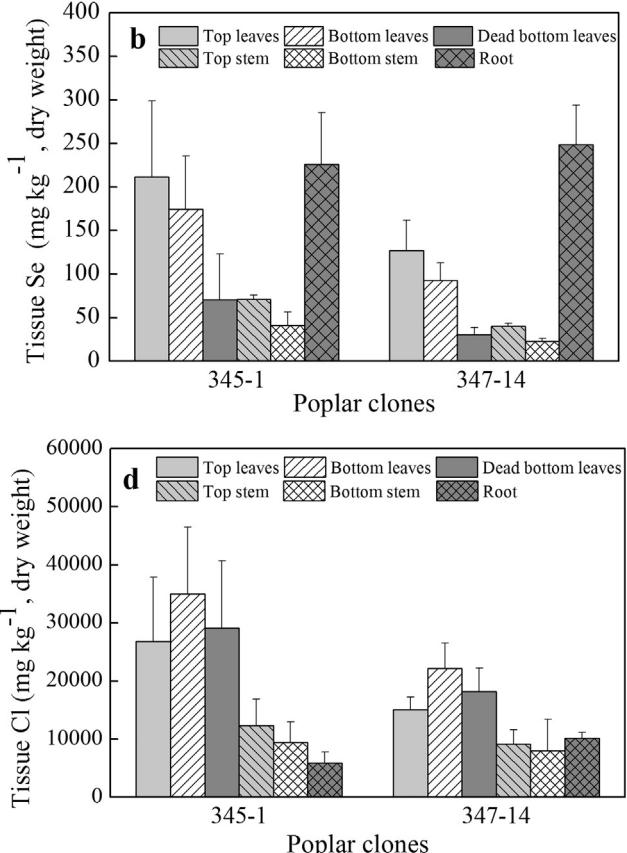
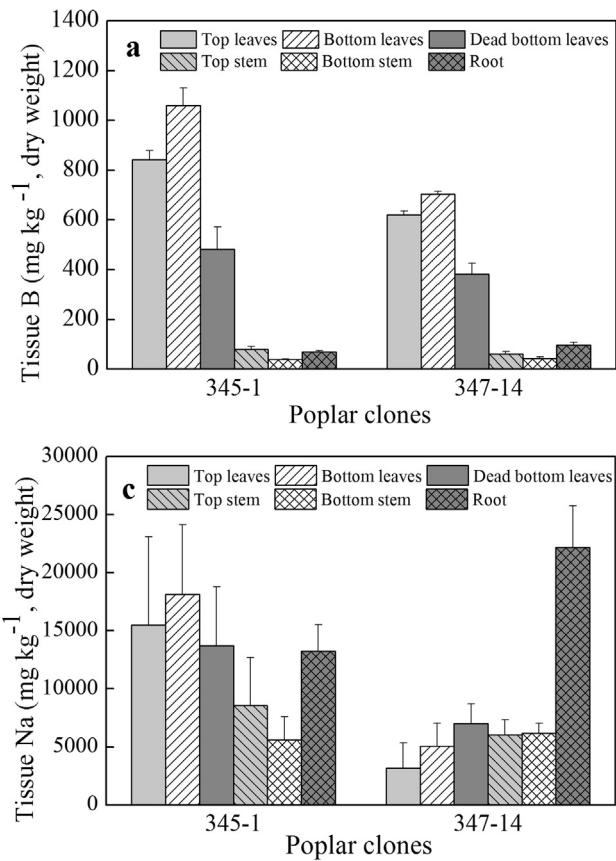


Fig. 3. Concentrations of B, Se, Na and Cl in different tissues of poplar clones growing in mesocosms after 30 days of salt, B and Se treatment. Values represent the mean of three replicates and error bars represent standard deviation.

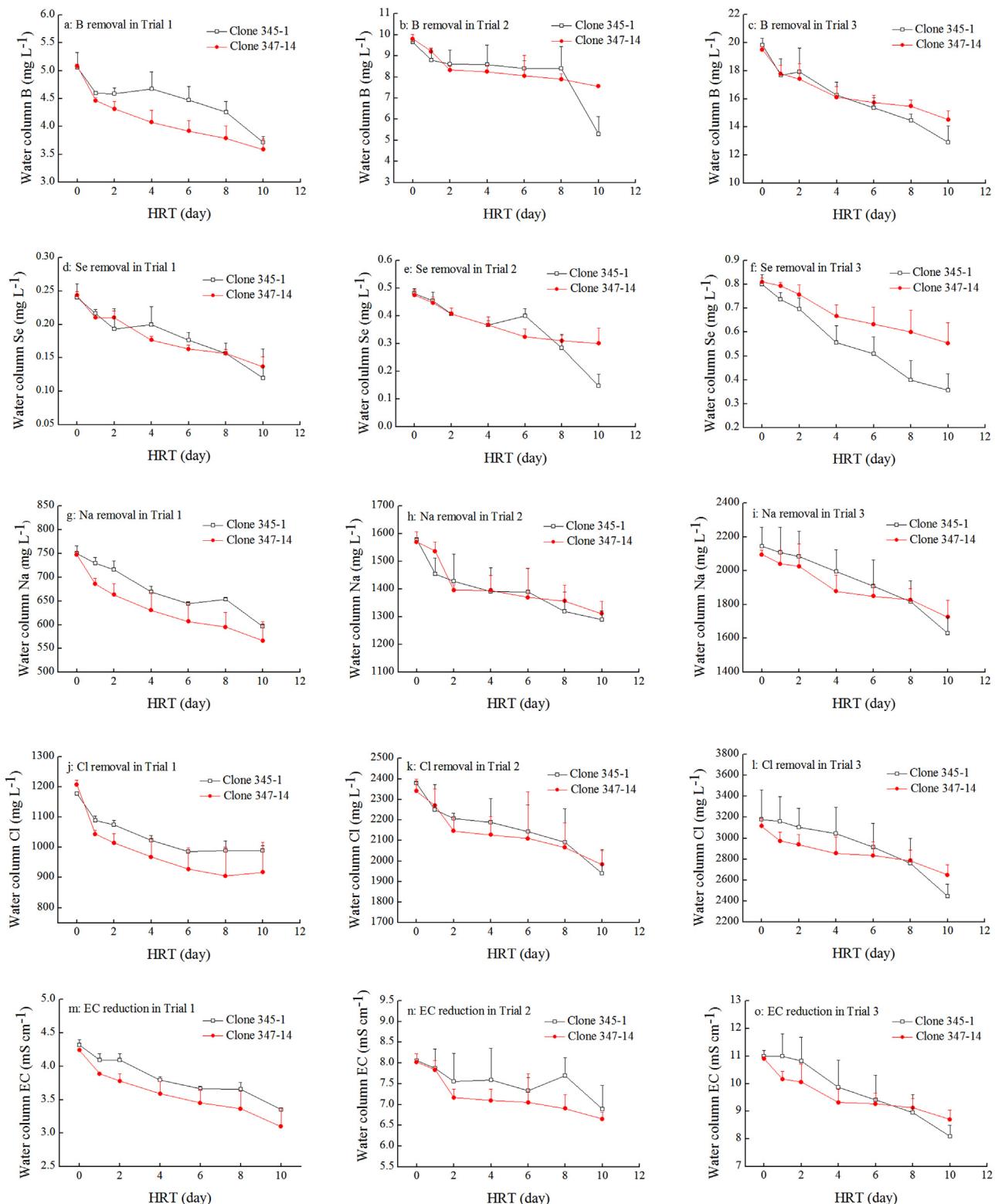


Fig. 4. Changes of B (a–c), Se (d–f), Na (g–i), Cl (j–l) concentrations and EC (m–o) in water column over time for mesocosm experiment. Values represent the mean of three replicates and error bars represent standard deviation.

were substantially greater than 22.9% and 25.5% with clone 347-14 for Trial 2 and Trial 3, respectively (Fig. 4b and c). In regards to Se removal, average removal percentages of Se by mesocosms planted with clones 345-1 and 347-14 were 50% and 43.8%, respectively, on Day 10 of Trial 1 (0.25 mg Se L^{-1} treatment), although there was no significant difference between two clones (Fig. 4d).

With the 0.5 mg Se L^{-1} treatment, average Se removal percentage was 69.4% with clone 345-1 compared to 36.8% with clone 347-14 on Day 10 (Fig. 4e). At high Se treatment (Trial 3, 1.0 mg Se L^{-1}), average Se removal percentage was 55.4% with clone 345-1 compared to 31.7% with clone 347-14 on Day 10 (Fig. 4f). The removal of Na and Cl, as well as the reduction in salinity (EC) level, showed a

similar trend with B removal in mesocosms for both clones. Under low salinity treatment (Trial 1, EC of 4.5 mS cm^{-1}), clone 347-14 overall exhibited both higher removal percentages of tested elements and reduction in salinity than clone 345-1 (Fig. 4g, j, and m). At medium salinity treatment (Trial 2, EC of 7.5 mS cm^{-1}), no substantial differences were observed in removal percentages or reduction in salinity between the two clones (Fig. 4h, k, and n). For high salinity treatment (Trial 3, EC of 10.0 mS cm^{-1}), no substantial differences in removal percentages or reduction in salinity were observed during Days 2–8, however, a greater removal percentage of tested elements and reduction in salinity was observed on Day 10 (Fig. 4i, l and o). Generally, the reduction of salinity was not as efficient as B and Se removal by both clones. The average removal percentages of Na and Cl with clone 345-1 ranged from 18.4–24.0% and 15.8–23.2% on Day 10, respectively, for the three trials, while the removal percentages ranged from 16.5–24.2%, and 14.9–23.9%, respectively, for clone 347-14.

Apart from plant absorption, there are other pathways for element removal in CWs. Lin and Terry [14] discussed the role of Se volatilization in wetland systems. Although this parameter was not measured in this study, its role in Se removal is fully acknowledged in this study. The contribution of plant absorption for elemental removal was estimated based on the tissue biomass data, plant tissue element concentrations, influent water loads and the reduction of water column element concentrations in each trial. Plant absorption during the 30 days operation of Experiment 1 accounted for the following percentages of the total element removed: Clone 345-1 accounted for approximately 11.3% (B), 47.1% (Se), 3.8% (Na), and 4.4% (Cl), respectively, while clone 347-14 accounted for about 10.2% (B), 36.4% (Se), 6.5% (Na), and 3.2% (Cl), respectively.

3.3. Biomass and tissue B concentrations of poplar clones in hydroponics

In the hydroponic experiment, the growth and B accumulation of two poplar clones was evaluated under exposure to different B treatments (ranging from control to 200 mg B L^{-1}) for 14 days. There were no visual B toxicity symptoms (chlorotic leaf margins) observed on clones 345-1 and 347-14 until B concentration was increased to 150 and 200 mg B L^{-1} , respectively, in solution. Although the shoot and root biomass of clone 345-1 showed no statistical differences among different B concentrations in solution, the shoot biomass was greater at treatments of 10–100 mg B L^{-1} compared to both control and 150–200 mg B L^{-1} (Fig. 5). For clone 347-14, the shoot biomass was greater at treatments of 10–150 mg B L^{-1} compared to treatments of control and 200 mg B L^{-1} , respectively. Shoot biomass at treatments 10, 30, and 50 mg B L^{-1} were significantly greater ($P < 0.05$) than 200 mg B L^{-1} , respectively. In general, the root biomass of clone 347-14 decreased with increasing B concentrations (10–200 mg B L^{-1}) in solution. Treatment 10 mg B L^{-1} showed significantly greater ($P < 0.05$) root biomass than those from 100 to 200 mg B L^{-1} treatments. Comparing the two clones, the total biomass of clone 345-1 was generally less than clone 347-14, which likely contributed to varied results in Experiment 1.

Tissue B concentrations increased in both clones with the increasing B concentrations in solution (Fig. 6). Average tissue B concentrations of clone 345-1 ranged as follows: bottom leaves > top leaves > root > stem. For clone 347-14, the range of B accumulation was similar to clone 345-1 when B concentrations in solution were less than 100 mg B L^{-1} . When B concentrations in solution were between 150 and 200 mg B L^{-1} , B accumulation ranged as follows: bottom leaves > root > top leaves > stem. Comparing the two clones, greater concentrations of B were accumulated in both top and bottom leaves of clone 345-1 than clone 347-14 under most B treatment concentrations. The greatest B concentration was 3699 mg kg^{-1} in bottom leaves of clone 345-1, and

1913 mg kg^{-1} in bottom leaves of clone 347-14. No significant difference was observed in B accumulation in stems between two clones. There was no significant difference in B accumulation in roots between both clones. However, roots of clone 347-14 accumulated relatively higher concentrations of B, especially under high B treatments.

Translocation factor (TF) values illustrating the mobility of B from the root to shoot were calculated as the ratio of B concentration in the bottom leaves to that in the root. The bottom leaves represent the largest biomass and greatest B concentration in Experiment 2. Irrespective of B concentrations in solution, TF of clone 347-14 was always less than clone 345-1 (Fig. 6), indicating a better ability of clone 347-14 to restrict the transport of B from root to shoot. The above results might partly explain the better B tolerance of clone 347-14 than clone 345-1, as more B was stored in roots, and not translocated to leaves. Additionally, TF of both clones decreased when B concentrations in solution were greater than 100 mg L^{-1} , especially for clone 347-14.

3.4. Boron removal by poplar clones in hydroponics

Boron concentrations in solutions were reduced at all B treatments by both clones after 14-day exposure (Fig. 7). Under low B treatments (control – 20 mg B L^{-1}), clone 345-1 was most effective and showed significantly greater ($P < 0.05$) B removal percentages than clone 347-14, which was consistent with the results found in both Trial 2 (10 mg B L^{-1}) and Trial 3 (20 mg B L^{-1}) of Experiment 1. No significant difference in B removal percentage was observed between the two poplar clones when B treatments were greater than 20 mg B L^{-1} . When B treatments were greater than 20 mg B L^{-1} , B removal percentage generally decreased for both clones.

4. Discussion

4.1. Tolerance of poplar clones to salt and B

Plants are usually more sensitive to high levels of salt and B [25]. Compared to Se treatments (0.25 – 1.0 mg Se L^{-1}) in this study, Se concentrations were too low to cause toxicity to plants. Therefore, only the tolerance of poplar clones to salinity and B will be discussed. As reported in results, clone 345-1 has a better salt tolerance at an EC of 10.0 mS cm^{-1} than clone 347-14, while clone 347-14 is more tolerant to B than clone 345-1 (based upon visual observation during two experiments). Most plant species experience significant growth reductions at salinity levels greater than 10.0 mS cm^{-1} or at B levels greater than 10 mg B L^{-1} [25]. In addition to visual toxicity symptoms, biomass of poplar clones was also impacted by salt/B stress (Figs. 2 and 5). In Experiment 2, biomass of clone 347-14 was generally greater than clone 345-1 under low B treatments (from control to 20 mg B L^{-1}), which was similar with B treatment levels (5, 10, and 20 mg B L^{-1}) in Experiment 1 (Fig. 5). However, the biomass of clone 347-14 was generally less than clone 345-1 in Experiment 1 (Fig. 2), which was treated by not only B, but also treated to Se and salinity. This observation indirectly indicates that biomass of clone 347-14 was reduced by salinity treatment. The reduction of biomass has been reported as a typical salinity toxicity symptom exhibited by most plants [26]. High salinity can reduce transpiration and plant water uptake [22], and affect the stomatal conductance and the resulting net photosynthesis rates [27] and solute accumulation [27], which inhibit plant growth, and nutrient absorption and utilization by poplars. In our study, clone 345-1 showed no visual toxicity symptoms. Even though this clone accumulated more salt ions in the aboveground biomass compared to clone 347-14, indicating that instead of restricting the absorption

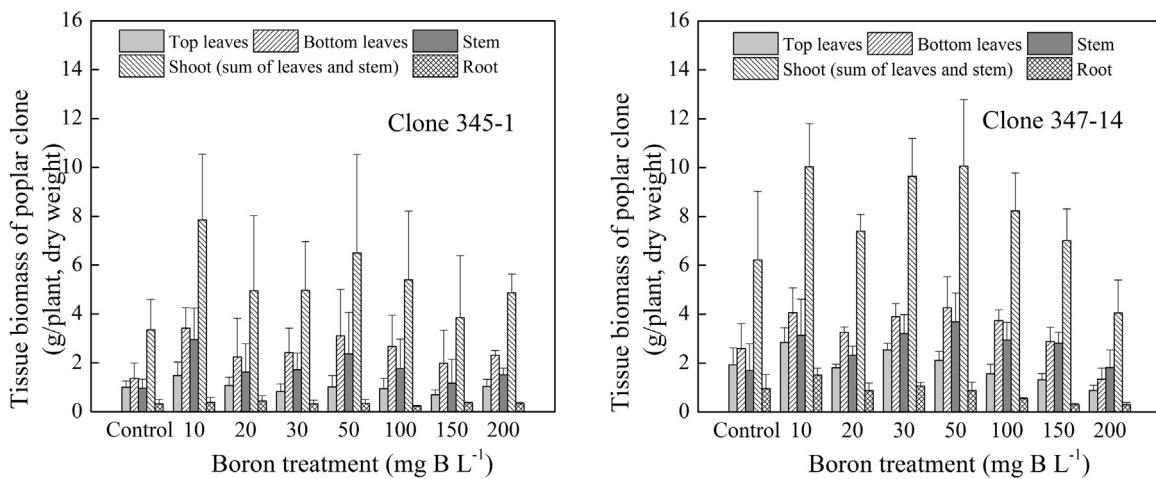


Fig. 5. Tissue biomass of two poplar clones growing in hydroponics after 14 days of B treatment. Values represent the mean of three replicates and error bars represent standard deviation.

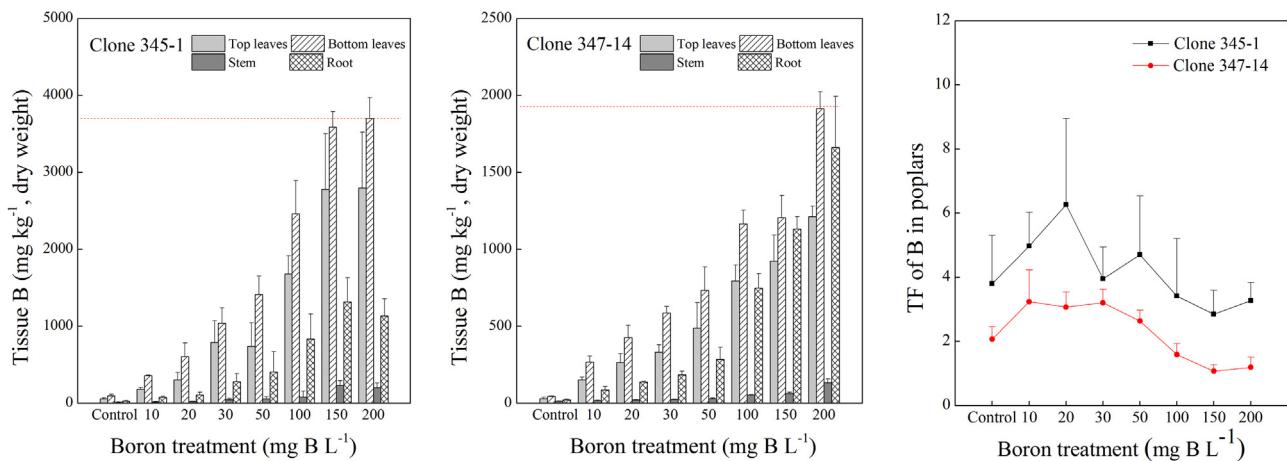


Fig. 6. Tissue B concentrations and translocation factor (TF) of B in two poplar clones after 14-day B treatment. Values represent the mean of three replicates and error bars represent standard deviation.

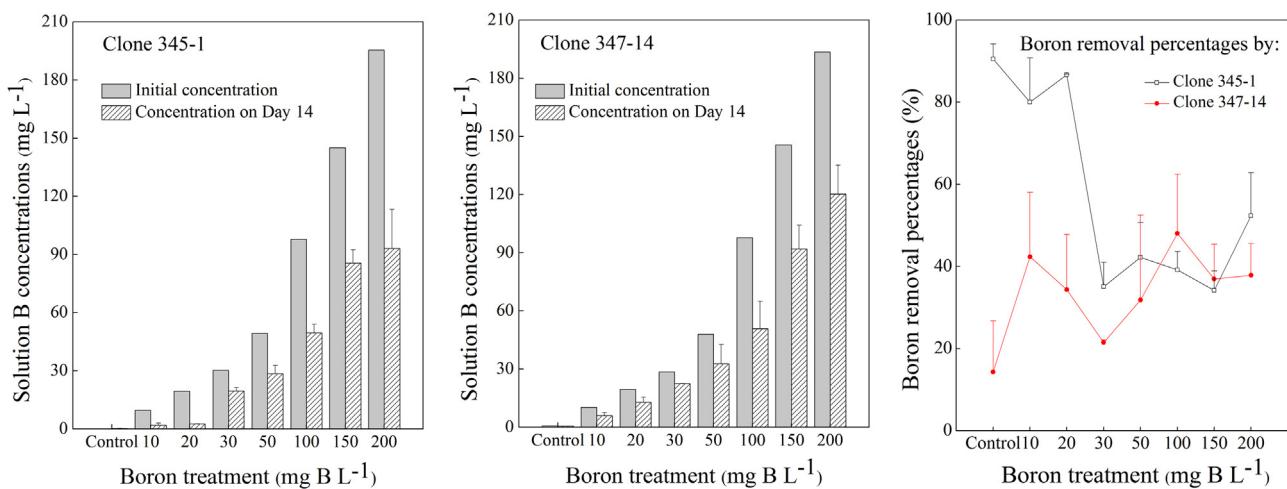


Fig. 7. Changes in B concentrations and B removal percentages after 14-day B treatment for both clones. Values represent the mean of three replicates and error bars represent standard deviation.

of salts into root, clone 345-1 has some internal regulating function to survive under high salinity (EC of 10.0 mS cm^{-1}).

The relatively greater biomass (Fig. 5) and higher B removal percentages (Fig. 7) found in both poplar clones under low B treat-

ment than high B treatment (Experiment 2) indicates that both poplar clones prefer higher B concentrations for growth than those typically found in 1/4 strength Hoagland solution. When B concentration in solutions was increased to $150\text{--}200 \text{ mg L}^{-1}$, however,

the tested clones may survive with one or more of the following potential B tolerance mechanisms: (1) Clone 345-1 restricted the accumulation of B into its root tissue. As shown in Fig. 6, the root B concentration of clone 345-1 at 200 mg B L⁻¹ treatment was less than that at 150 mg B L⁻¹ treatment. This apparent restriction of B into the root might be achieved by some efflux transporter proteins that enable B to be pumped out of cells [28]. However, no similar B restriction into the root was observed in clone 347-14, indicating that this B tolerance mechanism may not exist for clone 347-14. (2) Both clones have some ability to restrict the transport of B from root to shoot when B treatment was increased, as the TF of both clones generally decreased when the B treatment was greater than 50 mg B L⁻¹ (Fig. 6). This B tolerance mechanism was more clearly observed in clone 347-14 than clone 345-1, which might be a result of different genetic features and the related different functioning proteins between two clones [29]. (3) Apart from the above potential mechanisms, both poplar clones might also have some other internal regulating system to survive with high concentrations of B in their tissues, as the B concentration in tissues of both clones are relatively higher than many other species, as discussed below.

4.2. Comparison of the efficiencies of CWs and the elements accumulation in plants with previous studies

The above results indicated that the absorption of the tested elements by both clones played an important role in B and Se removal, while other pathways such as the elemental adsorption onto substrate might be more significant for Na and Cl removal. Therefore, we further compared both the removal efficiencies and the accumulation of B and Se in plant tissues from this study with previous studies. The percentages of B and Se removed by both poplar clones in mesocosms were high compared to other plant species tested in previous studies, although it is difficult to make strictly valid comparisons when the experimental conditions differ widely from study to study (See Table 2). For example, all the CWs listed in Table 2 with a similar or a better B removal percentage than the current study, were constructed with very efficient substrates such as the organic-based medium, and had a longer HRT compared to the current study. Many researchers have demonstrated the effectiveness of organic-based media and sand in improving the B retention in CWs [10,30]. For example, Türker et al. [10] have reported that 60% of B was stored in the filtration medium (mixture of organic-based peat and sand) and only 7% of B was absorbed by plant. In this study, we focused more on the direct role of poplars in element removal from simulated B and Se-laden drainage waters and B waters, and did not include the role of substrate and/or operation parameters of CWs in this removal process. In other words, higher B removal percentage could be achieved provided that the CWs with the tested poplar clones were equipped with an efficient substrate such as organic-based material and with more optimized operation parameters like a longer HRT and a better oxygen supplying condition. Similarly, Se removal percentages obtained in the current study were not the highest compared to previous studies; results were effective, however, there is room for improvement. For example, Huang et al. [12] reduced Se concentrations from 15 µg Se L⁻¹ to <0.1 µg Se L⁻¹ in 72 h in a mesocosm by growing cattails in a substrate of cattail litter overlying sand and peat moss sediment, indicating the potential of improving Se removal percentage by using an efficient substrate.

The competitive B and Se removal percentages obtained in this study could be attributed to the amount of B and Se accumulated by the two poplar clones. Türker et al. [15] have reviewed the concentration of B in more than 40 wetland plants growing in either natural polluted wetlands or CWs. Among all those species reviewed, only floating plants *Lemna minor* and *Lemna gibba* accumulated more than 1000 mg B kg⁻¹ in their tissues: B concentration

in *Lemna minor* biomass was 1168 mg kg⁻¹ after 7-day treatment of 100 mg B L⁻¹ solution in a microcosm system [31]. *Lemna gibba* accumulated 2711 mg B kg⁻¹ dry biomass when the plant was grown in 150 mg B L⁻¹ solution in a microcosm system for 7 days [32]. Based upon the hydroponic experiment in the current study, the average B concentration in bottom leaves of clone 345-1 was in excess of 1000 mg B kg⁻¹ when the B treatment was 30 mg B L⁻¹, while the leaf B concentration was 3699 mg kg⁻¹ when the B treatment was increased to 200 mg B L⁻¹ (Fig. 6). For clone 347-14, the average B concentration in its bottom leaves was also greater than 1000 mg B kg⁻¹ when the B treatment was 100 mg B L⁻¹, and as high as 1913 mg B kg⁻¹ when the B treatment was increased to 200 mg B L⁻¹ (Fig. 6). This observation demonstrates that the tested poplar clones (especially clone 345-1) can accumulate considerable amount of B in their biomass compared to many other wetland plants.

For Se accumulation, the tested poplar clones also showed competitive results. In the mesocosm experiment, clone 345-1 accumulated 211 and 174 mg Se kg⁻¹ DW in the top and bottom leaves, respectively, after 10 days exposure respectively to 0.25 mg Se L⁻¹, 0.5 mg Se L⁻¹, and 1.0 mg Se L⁻¹ (a total of 30 days exposure) (Fig. 3b). Clone 347-14 accumulated 127 and 93 mg Se kg⁻¹ DW in the top and bottom leaves, respectively (Fig. 3b). These data are much higher than 25–70 mg Se kg⁻¹ DW measured in seven wetland plant species exposed to 1.44 mg Se L⁻¹ for 64 days [30].

4.3. The potential use of poplars in CWs for saline, B and Se laden water treatment

The results obtained in the present study provide evidence in support of the concept of using the tested poplar clones as wetland plants for the treatment of saline, B and Se laden water commonly found as drainage effluent in the westside of central California. Firstly, both clones can survive in saline water high in B and Se based upon visual observation during both experiments. This result is in agreement with our previous studies that both clones outperformed other clones and can survive and grow well in high salinity, B and Se growing conditions [17]. The two clones showed different salt and B tolerant abilities in this study that can be selectively applied in CWs for practical treatment. For example, clone 345-1 could be used for a salt driven water treatment (e.g., when EC is close to 10 mS cm⁻¹), while clone 347-14 would be better for B driven water treatment (e.g., when B is close to 150 mg B L⁻¹). Secondly, as discussed above, both tested clones exhibited promising Se (in Experiment 1) and B (in both experiments) removal percentages, and can accumulate considerably high concentrations of B and Se in their tissues. This ability allows them to meet the basic and the most important requirement of being a wetland plant for phytoremediation of B and Se. Thirdly, poplars are fast-growing perennial plants with a long life, which enable wetland constructers to save economic and labor costs in using them for CW treatment. The well-developed root system of poplars allows them to have high hydraulic control capacity. When the trees are cut, new stems regrow from a cut tree stump. Therefore, undesirable elements can be easily collected and removed by cutting branches, which includes leaves that have accumulated high concentrations of respective elements. Meanwhile, poplar cuttings or whips can be used for regenerating new trees. Importantly, the large biomass production (average of 10–20 dry Mg ha⁻¹ year⁻¹) by poplars has already been identified as one of their qualified features for phytoremediation [19,22,37], in addition to easy-management of planting and maintaining. The large biomass of poplars also allows them to be used in biomass fuel gasification [38], which can create extra economic value apart from their ecological value.

Table 2

Comparison of B and Se removal by CWs according to literature.

	Type/scale of CWs	Plant species	Influent B/Se con. (mg L ⁻¹)	HRT (d)	Filtration material	Mean B/Se removal (%)	Reference
Boron	Microcosm CWs	Mixture of 14 different species	30–60	12	Mixture of coarse Colma sand and organic-based potting medium	32	[30]
	UF MCW ^a	<i>Typha latifolia</i>	25–125	14	Gravel with a layer of peat moss/sand mixture	4–21.3	[33]
	SF CW ^b	<i>Phragmites australis</i>	0.4–1.7	n.d.	n.d.	–2	[34]
	HF CW ^c	<i>Phragmites Phalaris</i>	0.11	8	Gravel (4–8)	21.8	[11]
	HF CW	<i>Phragmites Phalaris</i>	0.21	10	Crush rock	25.1	[11]
	HF CW	<i>Phragmites australis</i>	0.76	7	Gravel (3–20)	–1.8	[11]
	VF MCW ^d	<i>Phragmites australis</i>	29–32	n.d.	Zeolite, Cocopeat, Crushed limestone	12	[35]
	Poly-culture MCW	Mixture of <i>Typha latifolia</i> and <i>Phragmites australis</i>	165–227	15	Gravel, sand	32	[9]
	Mono-culture MCW	<i>Typha latifolia</i>	9.1–2019	15	Gravel, sand	40	[9]
	Mono-culture MCW	<i>Phragmites australis</i>	6.2–2019	15	Gravel, sand	27	[9]
Selenium	PE MCW ^e	<i>Typha latifolia</i>	6–40	14	Mixture of organic-based peat and sand	67.4	[10]
	SF MCW	Poplar clone 345-1	5–20	10	Gravel	26.7–45.6	Current study
	SF MCW	Poplar clone 347-14	5–20	10	Gravel	22.9–29.4	Current study
	Field SF CW	Mono-culture or a mixture of 8 species	0.019–0.022	7–21	Local natural sediment	21–55	[36]
	Field SF CW	Mixture of <i>Schoenoplectus californicus</i> and <i>Tamarix</i> sp.	0.0022–0.0054	9–18	Local natural sediment	56–70	[13]
	SF MCW	<i>Typha latifolia</i>	0.0125	21	Sand-peat moss	89	[12]
	HF CW	<i>Phragmites Phalaris</i>	0.00125	8	Gravel (4–8)	43.2	[11]
	HF CW	<i>Phragmites Phalaris</i>	0.00096	10	Crush rock	33.4	[11]
	HF CW	<i>Phragmites australis</i>	0.00065	7	Gravel (3–20)	23.1	[11]
	Microcosm CWs	Mixture of 14 different species	1.44	12	Mixture of coarse Colma sand and organic-based potting medium	64	[30]
SF MCW	Poplar clone 345-1	0.25–1.0	10	Gravel	50–69.4	Current study	
	Poplar clone 347-14	0.25–1.0	10	Gravel	31.7–43.5	Current study	

n.d. indicates no data/info.

^a Upward flow mesocosm constructed wetland (CW).^b Surface flow CW.^c Horizontal flow CW.^d Vertical flow microcosm CW.^e Prototype Engineered mesocosm CW.

5. Conclusions

This two parts study investigated the salt and B tolerance, bioaccumulation and removal ability of B and Se, respectively, in two salt and B tolerant poplar clones. Both clones were grown in both simulated agricultural drainage water containing high salt, B and Se (mesocosms) and simulated wastewaters containing high concentrations of B (hydroponics). Both tested poplar clones showed acceptable salt and B tolerance, although each clone showed some differences between each other. Both clones accumulated considerable amount of B and Se in their tissues, and exhibited competitive B and Se removal efficiency in mesocosms compared to previous studies, especially for clone 345-1. The accumulation and distribution of B, Se, Na, and Cl in plant tissues depended on poplar clone and element analyzed. We demonstrated in mesocosms and in hydroponics that both tested poplar clones are potential wetland plants for treating either B and Se contaminated saline water or B contaminated waters.

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