

Plant Species for the Removal of Na⁺ and Cl⁻ from Greenhouse Nutrient Solution

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Abstract. Certain ions such as Na⁺ and Cl⁻ can accumulate in recirculating greenhouse nutrient solutions and can reach levels that are damaging to crops. An option for the treatment of this problem is phytodesalinization with Na⁺ and Cl⁻ hyperaccumulating plants that could be added to existing water treatment technologies such as constructed wetlands (CWs). Two microcosm experiments were conducted to evaluate eight plant species including *Atriplex prostrata* L. (triangle orache), *Distichlis spicata* (L.) Greene (salt grass), *Juncus torreyi* Coville. (Torrey's rush), *Phragmites australis* (Cav.) Trin. ex Steud. (common reed), *Spartina alterniflora* Loisel. (smooth cordgrass), *Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla (softstem bulrush), *Typha angustifolia* L. (narrow leaf cattail), and *Typha latifolia* L. (broad leaf cattail) for their Na⁺ and Cl⁻ accumulation potential. An initial (indoor) experiment determined that *J. torreyi*, *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia* were the best candidates for phytodesalinization because they had the highest Na⁺ and Cl⁻ tissue contents after exposure to Na⁺ and Cl⁻-rich nutrient solutions. A second (outdoor) experiment quantified the Na⁺ and Cl⁻ ion uptake (grams of each ion accumulated per m² of microcosm). *J. torreyi*, *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia* accumulated 5.8, 3.9, 8.3, and 9.2 g·m⁻² of Na⁺ and 25.7, 18.2, 31.6, and 27.2 g·m⁻² of Cl⁻, respectively. Of the eight species, *T. latifolia* and *S. tabernaemontani* showed the greatest potential to accumulate Na⁺ and Cl⁻ in a CW environment, whereas *S. alterniflora*, *D. spicata*, and *P. australis* showed the least potential.

Greenhouses use large amounts of water in their operations (Robbins, 2010). Capturing and recycling irrigation runoff is one way that commercial greenhouses can conserve water while reducing fertilizer inputs and minimizing their environmental footprint. One of the difficulties in reusing nutrient solutions is the gradual accumulation of certain ions, especially Na⁺ and Cl⁻, which have a range of sources and at high concentrations can be damaging to greenhouse crops. These ions are often present in the water in low concentrations and they are also components of some fertilizer compounds added to the nutrient solution, for example KCl or NaNO₃ (Robbins, 2010). Greenhouse crops do not commonly remove Na⁺ and Cl⁻ so they leach from the substrate

or remain in solution and their concentrations increase as the water is captured and recycled after irrigation. Both Na⁺ and Cl⁻ can damage greenhouse crops when present at even relatively low concentrations (Stanghellini et al., 2005) so as ion concentrations accumulate above a species-specific threshold, recycled nutrient solutions become unusable. Therefore, for greenhouses to adopt sustainable water management practices, Na⁺ and Cl⁻ need to be managed at concentrations below these threshold levels.

Current treatment options for removing Na⁺ and Cl⁻ from recirculated greenhouse water (i.e., reverse osmosis and ultrafiltration) are often too expensive and impractical for the average grower (Gagnon et al., 2010). Greenhouse growers are therefore often forced to manage their water by discharging portions of it directly into the environment. One viable option for onsite wastewater treatment is the use of CWs, which are a technology already being used by greenhouse growers in various applications (Gagnon et al., 2010; Prystay and Lo, 2001; Seo et al., 2008; Vymazal, 2009). CWs are built to provide a favorable environment for the beneficial biological, chemical, and physical processes that occur in natural wetlands. They have a history of success in removing organics,

forms of nitrogen, and suspended solids from a variety of different wastewaters (Tanner, 1996; Vymazal, 2010). Limited research has been published on the use of CWs for Na⁺ and Cl⁻; however, removal of these ions has been found when plants capable of hyperaccumulating Na⁺ and Cl⁻ are included in these water treatment systems (Lymbery et al., 2006; Morteau et al., 2009; Nilratnisakorn et al., 2009; Shelef et al., 2012). This form of phytoremediation, known as phytodesalinization, could increase the Na⁺ and Cl⁻-removing capacities of CWs.

Certain plant species accumulate and store salt ions in their vacuoles to maintain a proper osmotic gradient and survive in saline environments (Manousaki and Kalogerakis, 2011; Munns, 2002). Plants capable of surviving in saline environments are known as halophytes. Adding halophytic plants that accumulate Na⁺ and Cl⁻ into CWs could increase the removal of these ions from recycled greenhouse nutrient solutions.

The objectives of this study were to:

- Determine the plant tissue dry matter content of Na⁺ and Cl⁻ from eight plant species grown in CW microcosms under controlled-environmental conditions (Expt. 1); and
- Quantify the mass of Na⁺ and Cl⁻ removed by the top performing species (from the first experiment) in outdoor CW microcosms using a simulated greenhouse nutrient solution that contained typical concentrations of Na⁺ and Cl⁻ (Expt. 2).

Materials and Methods

Expt. 1: Controlled environment

Plant material and treatments. A microcosm experiment was conducted in a research greenhouse at the University of Guelph, Ontario, Canada, from 15 Oct. to 16 Nov. 2013. The greenhouse was set at an 18-h light/6-h dark period using high-pressure sodium lamps to supplement natural sunlight, which resulted in an average photosynthetic photon flux at canopy level that was no less than 397 ± 34 μmol·m⁻²·s⁻¹. The temperature was maintained at 20 to 25 °C during the light period and 18.9 °C during the dark period. The relative humidity was maintained between 60% and 80% throughout the experiment.

D. spicata, *J. torreyi*, *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia* were sourced from local nurseries and *P. australis* was sustainably harvested from natural populations near Guelph, Ontario, Canada. *A. prostrata* and *S. alterniflora* were sustainably harvested from natural shoreline populations near New Glasgow, Nova Scotia, Canada. Wild-harvested plant material was potted in a peatmoss potting mix in 10-cm pots, irrigated as needed, and acclimated in the research greenhouse environment for 4 weeks. Plant material from local nurseries was acclimated in the research environment for at least 3 weeks.

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The microcosms consisted of 9-L plastic containers ($0.374 \times 0.241 \times 0.140$ m). A 6.35-mm bulkhead fitting was installed at the bottom of each container to act as a drain for replacing the nutrient solution. They were filled with 6000 cm³ of washed 5- to 10-mm granite stone rinsed with deionized water. The microcosms had a pore volume of ≈ 2 L when planted. The pore volume was determined by adding known volumes of water to the microcosms until the water level was just below the surface of the gravel.

After the acclimatization period, two plants from each species were planted into a microcosm and there were four replicate microcosms for each species, giving a total of 32 microcosms. At the start of the experiment, all the microcosms had a similar amount of plant material based on visual estimations. The plants were given 4 weeks to establish in the microcosms before the start of the trial.

The nutrient solution was designed to simulate captured greenhouse irrigation runoff that would be considered unfit for reuse as a result of excess Na⁺ and Cl⁻ (Stanghellini et al., 2005). The solution was prepared by adding reagent grade NaCl to a nutrient solution, prepared with deionized water and a 20N-8P-20K granular water-soluble fertilizer (All Purpose High Nitrate; PlantProducts®, Brampton, Ontario, Canada). The resulting solution had a NO₃-N concentration of 10.86 mmol·L⁻¹ and Na⁺ and Cl⁻ concentrations of 7.98 and 7.80 mmol·L⁻¹, respectively. The molar concentration of Na⁺ was slightly higher than Cl⁻ because of the Na₂MoO₄ (0.015%) in the fertilizer mix.

Plants were grown in the microcosms, filled with 2 L of nutrient solution, for 5 weeks. Each week the nutrient solution was drained, the microcosms flushed with deionized water, and then refilled with fresh nutrient solution. This was done to keep the root zone concentrations constant. Fresh nutrient solution was added as needed to maintain a relatively constant volume within the microcosms (once a week).

Measurements. At the end of the trial, all the above-ground biomass was harvested from each microcosm. The harvested plant material was washed with deionized water and dried in an oven at 65 °C. Once the material reached constant weight, the dry weight (DW) was recorded. Tissue Na⁺ concentration (% DW) was determined by ashing the sample, dissolving the ash in HCl, and then analyzing the solution by inductively coupled plasma atomic emission spectroscopy (AOAC 985.01). The tissue Cl⁻ concentration (% DW) was determined using an electrochemical titration with standardized AgNO₃ (AOAC 969.01). Samples were analyzed by SGS Agri-Food Laboratories in Guelph, Ontario, Canada.

Expt. 2: Outdoors

Plant material and treatments. The experiment was conducted outdoors adjacent to the Edmund C. Bovey building at the University of Guelph, Ontario, Canada (lat. 43°53' N;

long. 80°23' W). The microcosm containers from the first experiment were cleaned and fresh, washed pea stone was added (as described for Expt. 1).

J. torreyi, *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia* were planted in the microcosms, two similarly sized plants per microcosm, 3 weeks before the start of the experiment to allow them to acclimatize. The experiment was set up as a randomized complete block design with three replicates of each species for a total of 12 microcosms.

The experiment was conducted from 25 June to 30 July 2013. The average high temperature was 25.2 °C with a maximum of 32.2 °C and the average low was 14.6 °C with a minimum of 6.7 °C. Total precipitation for this period was 128 mm (Environment Canada, 2014).

Each microcosm was planted with two plants from the assigned species and each microcosm started with a similar amount of plant material. At the beginning of the experiment, all above-ground plant material was removed. It was assumed that all Na⁺ and Cl⁻ that accumulated in the plant tissue from this point forward would come from within the wetland microcosm and the added nutrient solution. Root zone water levels and Na⁺ and Cl⁻ concentrations were maintained using the same methodology as Expt. 1.

At the end of the experiment, the area of growth was measured at the base of the plant at the surface of each microcosm to determine the footprint of each microcosm. The above-ground biomass from each microcosm was harvested, dried, and analyzed for Na⁺ and Cl⁻ tissue contents using the same methodology as Expt. 1. The Na⁺ and Cl⁻ tissue concentrations of each treatment were multiplied by the respective DW to give the total mass of ion accumulated. The total mass of ions accumulated was divided by the area of growth to give the total mass of ion (g) accumulated per m² of CW microcosm.

The total volume of nutrient solution added into each microcosm was recorded and the total mass of Na⁺ and Cl⁻ added to each microcosm was calculated (L × mg·L⁻¹). The total mass accumulated by the plant material in each microcosm was also calculated by multiplying the tissue content (mg·g⁻¹) to the total DW (g). The percent removal by each microcosm was determined by dividing the total mass of ions added by the mass of ions removed.

Statistical analysis. All data were analyzed using SAS (Version 9.0, SAS Institute Inc., Cary, NC). For Expt. 1, an analysis of variance (ANOVA) and a Tukey's test were used to compare the differences in mean ion content among species. For Expt. 2, differences among mean plant tissue ion content, dry weight, total mass of ions accumulated per m², and the percent of ions removed were analyzed among species using an ANOVA and a Tukey's test. Correlation analysis was used to identify what factor had a greater influence on total ion accumulation, biomass production, or tissue content. All data were evaluated using a significance level of $\alpha = 0.05$.

Results from Expt. 1 identified *T. latifolia*, *S. tabernaemontani*, *J. torreyi*, and *T. angustifolia* as having the greatest desalination potential based on their tissue contents. These four species were evaluated in Expt. 2 and removed between 4% to 12% and 10% to 26% of the Na⁺ and Cl⁻ ions applied with the nutrient solution (Table 1).

T. latifolia exhibited a high potential for phytodesalination of simulated greenhouse nutrient solution in both experiments. Tissue contents were 4.2 mg Na⁺ (Fig. 1) and 27.0 mg Cl⁻ (Fig. 2) per gram of DW in Expt. 1. Although the *T. latifolia* plants in this experiment accumulated less Cl⁻ than observed by Morteau et al. (2009), they still demonstrated the potential to uptake larger amounts of Na⁺ and Cl⁻ relative to the other species in this experiment (Figs. 1 and 2). In Expt. 2, *T. latifolia* had the greatest Na⁺ and Cl⁻ tissue contents, 8.23 and 24.4 mg·g⁻¹, respectively (Table 1). *T. latifolia* also removed the greatest percentage of Na⁺ and Cl⁻ ions, 12.0% of the total Na⁺ and 23.4% of the total Cl⁻ (similar to *S. tabernaemontani*, 26.4%), from the nutrient solution (Table 1). These results agree well with Morteau et al. (2009) who found *T. latifolia* was able to accumulate up to 63 mg Cl⁻ per gram of DW when grown in similar NaCl concentrations to these trials. They concluded that *T. latifolia* would be a good candidate for the treatment of runoff from road deicing salts. *T. latifolia* is commonly used in CWs because of its hardiness and ability to tolerate many different environmental conditions (Vymazal, 2011); however, it is rarely considered for phytodesalination. From our results, *T. latifolia* demonstrated sufficient performance to recommend for inclusion in CWs.

S. tabernaemontani exhibited phytodesalination ability only slightly lower than that of *T. latifolia*. The Na⁺ content of *S. tabernaemontani* was comparable to the other top performing species in Expt. 1 (3.0 mg·g⁻¹; Fig. 1) but had the highest Cl⁻ content (34.8 mg·g⁻¹; Fig. 2) of any of the tested species. In Expt. 2, *S. tabernaemontani* accumulated relatively large amounts of Na⁺ and Cl⁻ (3.9 and 18.2 g·m⁻², respectively; Table 1) and produced among the greatest biomass of all species; however, Na⁺ accumulation was less than that of *T. latifolia* and *T. angustifolia*. *S. tabernaemontani* is already commonly used in CWs and is known to enhance the treatment of other various pollutants (Tanner, 1996). It is not commonly found in saline environments, although it can withstand moderately saline conditions (Tiner, 2009). Therefore, *S. tabernaemontani* may be a useful addition to CWs for treating greenhouse nutrient solutions.

J. torreyi performed similarly to *T. latifolia* and *S. tabernaemontani* in the categories of Na⁺ and Cl⁻ accumulation; however, the percent removal of Na⁺ and Cl⁻ was lower (Table 1). Ion contents in the foliar dry matter were relatively high in Expt. 1 at 3.9 mg·g⁻¹ Na⁺ (Fig. 1) and 23.8 mg·g⁻¹ Cl⁻

Table 1. Dry weight (g), tissue concentration ($\text{mg}\cdot\text{g}^{-1}$) of Na^+ and Cl^- accumulated per unit growing area ($\text{g}\cdot\text{m}^{-2}$), and percent (%) of ions removed from the nutrient solution by each of four plant species grown for 5 weeks in outdoor constructed wetland microcosms fed with a simulated greenhouse nutrient solution with added NaCl.

Species	Dry wt (g)	Na^+ content ($\text{mg}\cdot\text{g}^{-1}$)	Cl^- content ($\text{mg}\cdot\text{g}^{-1}$)	Na^+ accumulated ($\text{g}\cdot\text{m}^{-2}$)	Cl^- accumulated ($\text{g}\cdot\text{m}^{-2}$)	Percent Na^+ removed ²	Percent Cl^- removed
<i>T. latifolia</i>	29.2 a ^y	8.23 a	24.4 ab	9.2 a	27.2 a	12.0 a	23.4 a
<i>S. tabernaemontani</i>	28.8 a	5.87 b	27.2 a	3.9 b	18.2 a	8.7 ab	26.4 a
<i>J. torreyi</i>	23.1 a	4.43 c	20.1 b	5.8 ab	25.7 a	5.4 bc	16.0 b
<i>T. angustifolia</i>	11.3 b	7.30 a	27.9 a	8.3 ab	31.6 a	4.2 c	10.7 b

²Calculated as total mass (g) of ion accumulated in plant tissue/total ion mass (g) input into the microcosm \times 100%.

^yMean values ($n = 3$) followed by the same letter are not significantly different at $P < 0.05$.

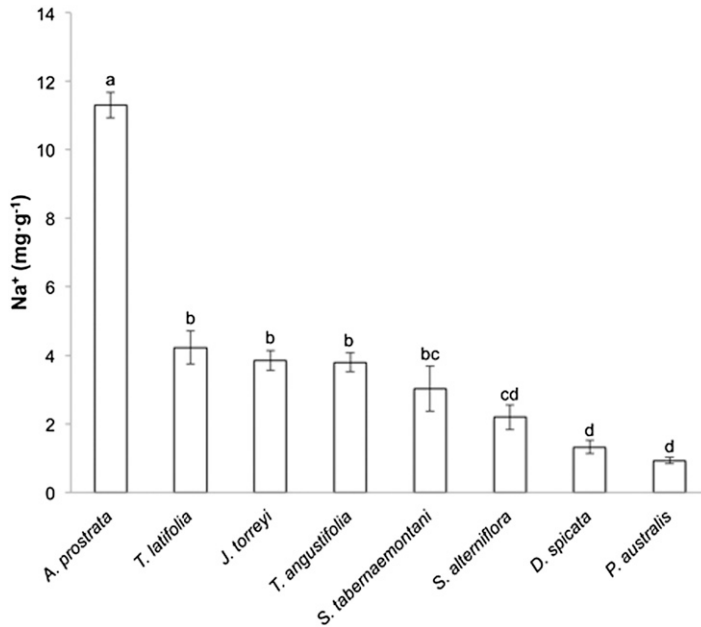


Fig. 1. Tissue Na^+ content ($\text{mg Na}^+/\text{g}$ of foliar dry weight) of eight plant species after 5 weeks growing in constructed wetland microcosms with simulated greenhouse nutrient solution with added NaCl. Data are means \pm SE ($n = 4$). Bars bearing the same letter are not significantly different at $P < 0.05$.

(Fig. 2) and therefore it was included in Expt. 2. Its performance was comparable to *T. latifolia* and *S. tabernaemontani* because it accumulated $5.8 \text{ g}\cdot\text{m}^{-2} \text{ Na}^+$ and $25.7 \text{ g}\cdot\text{m}^{-2} \text{ Cl}^-$ (Table 1) but the percent removal of Na^+ and Cl^- was lower than those of *T. latifolia* and *S. tabernaemontani* (Table 1). Related species in the *Juncaceae* family have been evaluated for their phytodesalination abilities in constructed wetlands with some reported success. LyMBERY et al. (2006) found that wetland mesocosms planted with *Juncus kraussii* were able to reduce NaCl loads by up to 54%; however, they did not specifically investigate the NaCl-removing mechanisms, so the actual role that the plants played is unknown. ZINGELWA and WOOLDRIDGE (2009) reported that *Juncus acutus* was capable of removing up to 7.67 g of Na^+ per m^2 of CW when treating winery wastewater high in organics and Na^+ .

T. angustifolia performed relatively well as both a Na^+ and Cl^- accumulator. In Expt. 1 the tissue contents of *T. angustifolia* reached $3.8 \text{ mg}\cdot\text{g}^{-1} \text{ Na}^+$ (Fig. 1) and $30.5 \text{ mg}\cdot\text{g}^{-1} \text{ Cl}^-$ (Fig. 2). In Expt. 2 *T. angustifolia* removed $8.3 \text{ g}\cdot\text{m}^{-2}$ of Na^+ and $31.6 \text{ g}\cdot\text{m}^{-2}$ of Cl^- , which is comparable to the other species but its percent removals were the lowest of the four

species, 4.2% of Na^+ and 10.7% of Cl^- (Table 1). *T. angustifolia* also produced the least amount of biomass (11.3 g), which was less than half of the aboveground biomass produced by either of the three other species in Expt. 2. However, based on the recorded area of coverage by *T. angustifolia* in this experiment, a biomass production of 11.3 g equates to a potential production of $1128 \text{ g}\cdot\text{m}^{-2}$. This is comparable to estimated peak stand production of $1445 \text{ g}\cdot\text{m}^{-2}$ reported by MASON and BRYANT (1975) and given more time or different conditions, it is possible that *T. angustifolia* could have produced a biomass similar to that of the other species in this experiment.

T. angustifolia is often found in coastal marshes but it can survive in a variety of conditions (TINER, 2009). *T. angustifolia* has been shown to be useful for the phytoremediation of solutions containing various heavy metal ions (CHANDRA and YADAV, 2011), and other research focusing on the performance of *T. angustifolia* in CWs treating synthetic dye wastewater reported that it was able to reduce Na^+ levels in the wastewater (NILRATNISAKORN et al., 2007, 2009). Although our first microcosm experiment indicated that *T. angustifolia* has the potential to accumulate Na^+ and Cl^- from solution in a wetland environment, it

was outperformed by the other three species in Expt. 2.

The following four species were not included in Expt. 2.

A. prostrata had the highest Na^+ content of the eight species in Expt. 1 at $11.3 \text{ mg}\cdot\text{g}^{-1}$ (Fig. 1) but its Cl^- content was relatively low, $16.0 \text{ mg}\cdot\text{g}^{-1}$ (Fig. 2). *A. prostrata* is naturally found in brackish environments in saline coastal marshes (TINER, 2009) and therefore it was included in this study. WANG et al. (1997) examined the effect of salinity on the growth of *A. prostrata* and reported that when grown in solutions with $85.6 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$, *A. prostrata* tissue accumulated ≈ 42 and 137 mg of Na^+ and Cl^- , respectively, per gram DW. The NaCl concentration used by Wang et al. (1997) was much higher than the one used in this study but the *A. prostrata*'s Na^+ tissue content was relatively high in Expt. 1 although NaCl concentration in solution was lower. However, its Cl^- accumulation was lower than other species suggesting that *A. prostrata* may not be suitable for Cl^- removal at lower concentrations. Also, *A. prostrata* is not a perennial species or native to Ontario so the uptake of ions would need to be very high to justify the cost of replanting every year in a CW.

S. alterniflora had Na^+ and Cl^- tissue contents that were low compared with the other species in this experiment. The Cl^- content was especially low ($9.75 \text{ mg}\cdot\text{g}^{-1}$), whereas the Na^+ content ($2.20 \text{ mg}\cdot\text{g}^{-1}$) was comparable to the better performing species. *S. alterniflora* is commonly found in coastal marshes and facilitates two mechanisms to manage the high osmotic potential resulting from the NaCl in these saline conditions: ion accumulation and secretion. BRADLEY and MORRIS (1991) determined that the relative impact of these mechanisms varies according to the salinity of the water. Ion accumulation is prevalent at solution NaCl concentrations less than $171.1 \text{ mmol}\cdot\text{L}^{-1}$, whereas excretion exceeds secretion at higher salinities. Although secretion is less desirable in CW scenarios, because the removed salts could easily re-enter the water during precipitation events, this mechanism likely has a minimal role in CW applications for greenhouse wastewater treatment because the solution concentrations are in the order of $100 \times$ lower. The same study reported that *S. alterniflora* accumulated up to $11.5 \text{ mg Na}^+/\text{g}$ and $47.0 \text{ mg Cl}^-/\text{g}$ when exposed to NaCl concentrations of $171.1 \text{ mmol}\cdot\text{L}^{-1}$. Again, this salinity is much

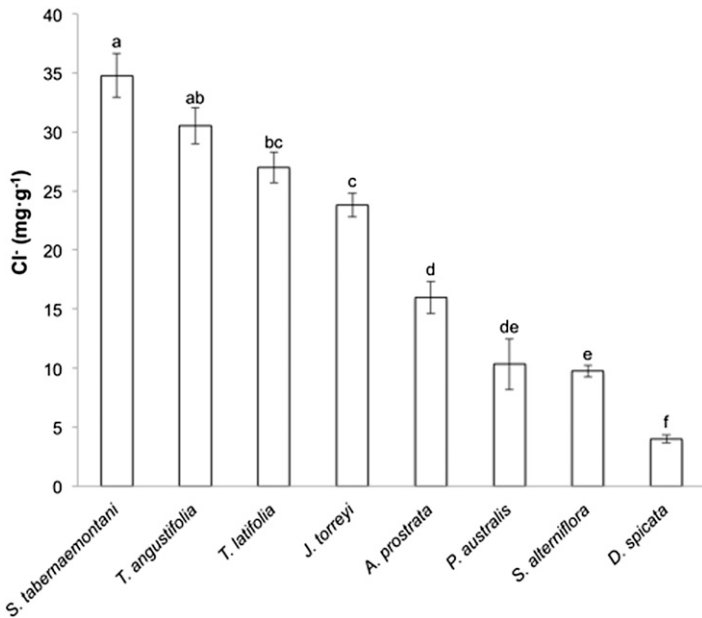


Fig. 2. Cl⁻ tissue content (mg Cl⁻/g of foliar dry weight) of eight plant species after 5 weeks in growing constructed wetland microcosms with simulated greenhouse nutrient solution with added NaCl. Data are means ± SE (n = 4). Bars bearing the same letter are not significantly different at P < 0.05.

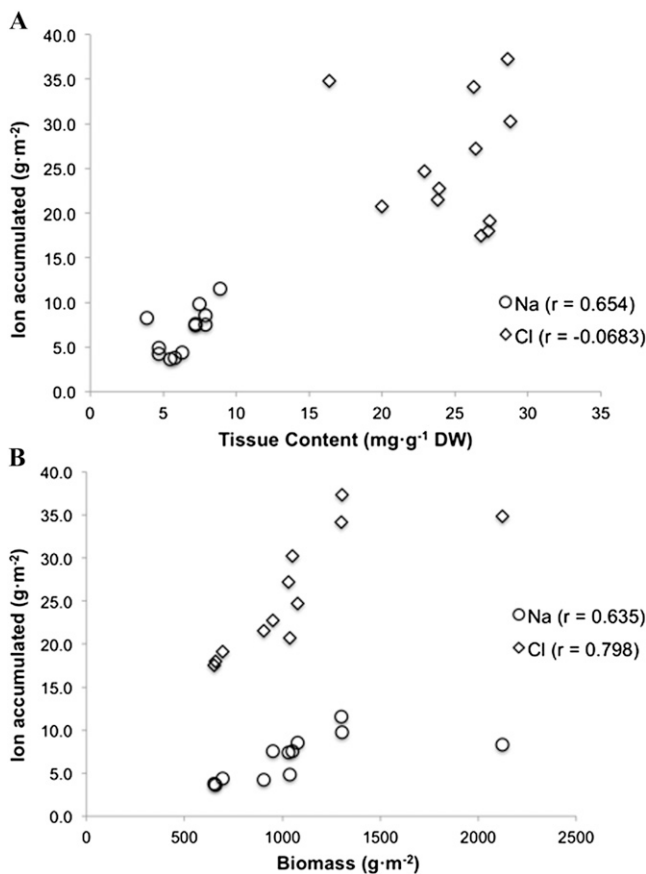


Fig. 3. The effect of Na⁺ and Cl⁻ tissue content (A) and biomass production (B) on the mass of Na⁺ and Cl⁻ accumulated by *T. latifolia*, *S. tabernaemontani*, *J. torreyi*, and *T. angustifolia* after 5 weeks in constructed wetland microcosms with simulated greenhouse nutrient solution with added NaCl. Pearson correlation coefficients (r) presented in parentheses for each ion.

higher than the one used in this experiment and it is clear from the results that *S. alterniflora*'s ability to accumulate Na⁺ and Cl⁻ is affected by the NaCl concentration in solution. Therefore,

in the relatively low salinities of reused greenhouse nutrient solutions, *S. alterniflora* may not be able to accumulate substantial amounts of Na⁺ and Cl⁻.

D. spicata exhibited low ion accumulation compared with the other species. The Na⁺ tissue content was 1.3 mg·g⁻¹ and the Cl⁻ content was 4.0 mg·g⁻¹. *D. spicata* was chosen for this experiment based on its ability to survive in coastal marshes (Tiner, 2009) and on its noted ability to decrease the electrical conductivity of saline soils in Australia (Sargeant et al., 2008). However, Wu et al. (1997) reported that although *D. spicata* is capable of accumulating salts, it would excrete them through its leaves to avoid damage, although they did not report the tissue ion content at which secretion occurred. Although this mechanism for survival in saline conditions may make *D. spicata* a less ideal candidate for the removal of Na⁺ and Cl⁻ from wastewater in CWs, it was still included in this experiment because it was unknown how high the tissue contents would reach before excretion was facilitated (Wu et al., 1997).

P. australis had low tissue contents for both Na⁺ and Cl⁻ at 0.93 mg and 10.3 mg, respectively, per gram of DW. *P. australis* grows rapidly and produces large amounts of biomass in short periods of time (Tanner, 1996). It is largely the result of this rapid growth that it is one of the most commonly used plants in CWs, especially in Europe (Tanner, 1996). However, *P. australis* is not native to North America and is considered invasive and its use is restricted in some areas (Tanner, 1996). *P. australis* is tolerant of saline conditions and has been reported to accumulate considerable amounts of Na⁺ and Cl⁻ when grown in water with a NaCl concentration of ≈200 mmol·L⁻¹ (Gorai et al., 2010). However, *P. australis* was not successful at accumulating substantial levels of Na⁺ and Cl⁻ in the foliar tissues when grown in the lower concentrations of these ions in our experiment. As a result of its poor performance and status as an invasive weed in Ontario, it was concluded that *P. australis* would not be useful in CWs for removing these ions from recycled greenhouse nutrient solution.

General observations. Correlation analysis revealed both ion tissue content and biomass were equally important factors when considering Na⁺ accumulation (Fig. 3A–B). However, Cl⁻ accumulation was affected by biomass production (Fig. 3B) much more than it was affected by Cl⁻ tissue content (Fig. 3A). This suggests that plant selection for Na⁺ accumulation should exhibit an affinity for both biomass production and ion accumulation, whereas biomass production is the more important parameter when selection for Cl⁻ removal.

Higher Na⁺ tissue contents were observed in the second experiment than the first for *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia*. *S. tabernaemontani* also had a higher Cl⁻ content in the second experiment. This increased Na⁺ and Cl⁻ accumulation could have been caused by the environmental conditions of Expt. 2 in which the plants were exposed to higher wind speeds and lower relative humidity. These

conditions can result in greater transpiration rates, which can affect the uptake of ions (Munns and Termaat, 1986). However, unlike Expt. 1, the above-ground plant biomass was removed at the start of Expt. 2. Therefore, more Na⁺ and Cl⁻ accumulation may occur during early stages of plant growth or during rapid growth periods. All of the tested plant species grew rapidly when cut down, suggesting that regular biomass harvesting may be beneficial for increasing the accumulation of Na⁺ and Cl⁻ in plant tissues. To avoid having the ions reintroduced into the system, harvested plant tissue will need to be removed. Therefore, it would be useful to investigate the effect and timing of harvesting on the phytodesalinization potential of these plant species.

Conclusion

T. latifolia and *S. tabernaemontani* had the greatest potential of all species to remove Na⁺ and Cl⁻ from solution in a CW environment. Both species performed well in the 5-week greenhouse and outdoor bench experiments. In the outdoor experiment, the above-ground portion of *T. latifolia* was able to remove 12.0% of the Na⁺ and 23.4% of the Cl⁻ and *S. tabernaemontani* was able to remove 8.7% and 26.4% of the Na⁺ and Cl⁻ added to the CW microcosms. Our research also showed that *S. alterniflora*, *D. spicata*, and *P. australis* were not suitable for Na⁺ and Cl⁻ removal from greenhouse nutrient solutions in CW conditions. However, further research is needed to confirm these results using large-scale CW studies. In addition, an evaluation of species performance over a longer period of time (e.g., multiple years) in a full-scale system is needed to develop the most efficient CW species compositions.

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