Can plants be used to remove Na\(^+\) and Cl\(^-\) from nutrient solution in greenhouse production?

by

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ABSTRACT

CAN PLANTS BE USED TO REMOVE NA⁺ AND CL⁻ FROM NUTRIENT SOLUTION IN GREENHOUSE PRODUCTION?

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The potential of using halophytic wetland plants for the removal Na⁺ and Cl⁻ from recirculated greenhouse nutrient solution was assessed. Eight species were screened for their Na⁺ and Cl⁻ removal capacity and it was determined that Schoenoplectus tabernaemontani and Typha latifolia were the best candidates. A second experiment examined the effect of harvesting and harvesting frequency on plants and their capacity to uptake Na⁺ and Cl⁻. Juncus torreyi and T. latifolia responded positively to harvesting and their Na⁺ and Cl⁻ uptake and biomass production were increased when harvested once or twice throughout the growing season, but harvesting had no effect on S. tabernaemontani. The effect of solution NaCl concentration was investigated and both S. tabernaemontani and T. latifolia, accumulated more Na⁺ and Cl⁻ at higher concentrations. Overall, the plants removed Na⁺ and Cl⁻, but the rates were too low to make it an effective Na⁺ and Cl⁻ management option for the treatment of recirculated greenhouse nutrient solution.
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1.0. Introduction

The greenhouse, nursery, and floriculture sector in Ontario makes up ~30% of the Canadian greenhouse industry (Statistics Canada, 2011). With such a large portion of the industry in Ontario, provincial regulating authorities are focused on reducing the environmental impact of this sector. Organizations such as The Ontario Greenhouse Alliance (TOGA) are working with government regulators and greenhouse growers to find ways to improve the sustainability of the industry. A long-term goal of TOGA and the greenhouse sector is to recirculate and reuse 100% of their water by collecting and treating all the water used in daily operations. However, there are currently limits on how long nutrient solution can be recycled because the quality of the nutrient solution deteriorates each time it is reused.

The accumulation of contaminants, such as Na\(^+\) and Cl\(^-\), in recycled nutrient solution is one of the main factors that limits continued reuse. These ions accumulate when growers capture the runoff from fertigation events and reuse it for future fertigation. Na\(^+\) and Cl\(^-\) can be damaging to greenhouse crops at relatively low concentrations (Robbins, 2010). It is difficult to prevent Na\(^+\) and Cl\(^-\) from entering the nutrient solution. The source of the water used to make the nutrient solution (groundwater, surface water, etc.), the quality of fertilizers, and if the water is treated or diluted before reuse all impact the amount of Na\(^+\) and Cl\(^-\) that will be present in the solution (Robbins, 2010).

The accumulation of Na\(^+\) and Cl\(^-\) in greenhouse irrigation water is often unavoidable. Therefore, it is necessary to provide growers with the ability to remove these ions from the
solution. In the absence of water treatment, the constant accumulation of these limiters often forces growers to discharge the water into the environment, rather than reuse it. Several technologies are capable of removing Na\textsuperscript{+} and Cl\textsuperscript{-} from solution but they are often too expensive and energy intensive (Gagnon et al., 2010; Miller, 2003) for the average greenhouse operation to efficiently utilize. The costs of these technologies per m\textsuperscript{3} of water treated ranges from $0.78–$1.33CAD for reverse osmosis and up to $10 for thermal desalination (Miller, 2003). Consequently, apart from environmental discharge (which is not in line with the industry’s goal of 100% reuse), the only other option is for greenhouses to dilute the collected irrigation water with higher quality water such as rainwater or purchased municipal water, although these sources may contain low levels of Na\textsuperscript{+} and Cl\textsuperscript{-} as well (Flood 1996). Dilution is only a short-term solution, however, because the ions are still present and the concentrations will eventually increase, as the water is recycled. Therefore, greenhouses need to be offered an economical and effective treatment technology so they are able to recycle 100% of their collected nutrient solution.
2.0. Literature Review

2.1. $\text{Na}^+$ and $\text{Cl}^-$ accumulation in recycled greenhouse nutrient solution

Many salts are naturally found in water. Therefore, the source of the water used for the nutrient solution will impact ion levels. When groundwater is used for irrigation there may be salts present in the water due to the location and geology of the aquifer (Flood, 1996). Municipal water also varies in quality depending on where it was taken from and the type of treatment it underwent before reaching the consumer. It is important to know the quality and contents of the water to control the amount of $\text{Na}^+$ and $\text{Cl}^-$ in the irrigation nutrient solution. However, growers often have very limited options when it comes to selecting the water used for their nutrient solutions.

The concentration of these ions can be increased by evapotranspiration. Water may be lost to the atmosphere but the ions remain in the growing media or in solution. The amount of evapotranspiration that will occur and the resulting increase in ion concentrations will depend on the irrigation methods and the plant species.

The addition of fertilizers to irrigation water can also introduce unwanted salts. Essential plant nutrients cannot be added to the water in their pure forms and therefore growers add compounds that contain the desired forms of the element. The irrigated crop or growing substrate may not utilize these extra components and they will remain in the water as it is collected and recycled. When more fertilizer containing $\text{Na}^+$ and $\text{Cl}^-$ is added to the collected
nutrient solution the concentration of these ions continues to increase (Robbins, 2010). For example, fertilizer salts such as KCl add necessary K$^+$ to the water but the plants do not take up large amounts of Cl$^-$ and it remains in the solution or accumulates in the growing media. This problem is compounded when over-fertilization and over-watering occur (Robbins, 2010). Therefore, it is important that growers supply their crops with the optimum ratio and amount of nutrients in order to prevent the introduction of excess ions into the irrigation solution.

2.2. The negative effects of Na$^+$ and Cl$^-$

The presence of Na$^+$ and Cl$^-$ in irrigation water can negatively affect crops depending on the plant species and the concentrations of the ions. Most greenhouse grown crops are sensitive to these ions and irrigation guidelines recommend that only very low levels be allowed in irrigation water. Na$^+$ concentrations should be kept below 2.2 mmol·L$^{-1}$ and Cl$^-$ below 3.9 mmol·L$^{-1}$ (Flood, 1996; Robbins, 2010, Stanghellini et al., 2005). If the concentrations levels become too high the crop will be damaged.

The presence of Na$^+$ in the nutrient solution can cause Ca$^{2+}$ and Mg$^{2+}$ deficiencies. Na$^+$ interferes with these two essential plant nutrients and reduces the amount available for plant uptake (Munns, 2002; Robbins, 2010). Na$^+$ can displace adsorbed Ca$^{2+}$ and Mg$^{2+}$ in the soil matrix causing them to be leached out. When determining if water is suitable for irrigation, the interaction between Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ can be quantified by calculating the sodium adsorption ratio (SAR). The SAR is related to the concentrations of the three ions and
describes the potential occurrence of Na\(^+\) induced Ca\(^{2+}\) and Mg\(^{2+}\) deficiencies (Robbins, 2010).

High concentrations of Na\(^+\) and Cl\(^-\) can also affect the osmotic relationship between the plant roots and the soil solution. In order for root cells to absorb water they must have a higher solute concentration than the external solution. This creates an osmotic gradient which allows external solution to enter the root cells (Munns, 2002). Therefore, when the concentration of Na\(^+\) and Cl\(^-\) becomes too high, the plants will be unable to take up sufficient amounts of water or they will be forced to accumulate the ions in their tissues to maintain the osmotic gradient (Munns, 2002).

If a plant attempts to maintain the osmotic gradient by accumulating Na\(^+\) and Cl\(^-\), most species will experience toxicity. Plants can uptake some Na\(^+\) and Cl\(^-\), however, salt injury can occur if plants uptake too much Na\(^+\) and Cl\(^-\) and are unable to compartmentalize it in the vacuoles. The additional ions may accumulate in the cytoplasm and interfere with enzyme activity. Many enzymes are activated by K\(^+\) within the cytoplasm and Na\(^+\) is able to compete for these binding sites causing enzyme malfunction (Tester and Davenport, 2003). Na\(^+\) also interferes with the role K\(^+\) plays in protein synthesis. The interference by Na\(^+\) causes further damage to plant cells (Tester and Davenport, 2003). The ions also may enter the cell wall and cause it to dehydrate by once again altering the osmotic relationship of the cell (Munns, 2002).
When salt sensitive species uptake salt and do not excrete it or compartmentalize it in the vacuoles, the effects will first be noticeable in the oldest leaves which will begin to die. Unless new leaves can be produced quickly, the entire plant will eventually die (Munns, 2002). Few species are able to tolerate the presence of high levels of salt in irrigation water and the majority of plants produced in greenhouses are sensitive to Na\(^+\) and Cl\(^-\); therefore, it is important to manage the levels of these ions in the irrigation water (Robbins, 2010).

### 2.3. Constructed wetlands

A potential solution to this problem is to use constructed treatment wetlands (CW) specifically designed to remove Na\(^+\) and Cl\(^-\) from greenhouse irrigation wastewater. CWs are a low-cost technology, as low as $0.001/m\(^3\) of water treated, and they also have low installation and operational costs. CWs are currently used to treat other facets of greenhouse nutrient solution runoff such as organic material, various forms of N, and suspended solids (Tanner, 1996; Vymazal, 2010). Any improvements to their performance, such as the capacity to remove additional pollutants, will be beneficial in order to expand their use in the industry.

CWs are planted with emergent wetland plant species in order to enhance water treatment. In most cases the plants themselves are not the major source for the treatment capabilities of CWs, but rather microbial communities play the most important role (Brix, 1997). However, plants are able to improve water treatment by providing oxygen to the root zone, which enhances nutrient cycling and organic matter decomposition. Plants also uptake nutrients and
their roots provide attachment sites for microbial communities (Brix, 1997; Vymazal, 2010). The desalinization capacity of most wetland plants has not been quantified because CWs are not often designed for Na\(^+\) and Cl\(^-\) removal. However, there are plant species that are capable of hyper-accumulating Na\(^+\) and Cl\(^-\). Plants able to tolerate salinity are known as halophytes. In order to survive in saline conditions halophytic plants utilize several mechanisms such as ion exclusion, secretion and compartmentalization of ions in their vacuoles (Manousaki and Kalogerakis, 2011). The addition of halophytic plants, specifically those that accumulate Na\(^+\) and Cl\(^-\), into CWs may be option for improving the quality of recycled greenhouse nutrient solution. Quantifying the amount of Na\(^+\) and Cl\(^-\) that could be removed in a CW environment would be useful in determining the feasibility of using CWs with halophytic plants for treating greenhouse nutrient solution.

**2.3.1. CWs for Na\(^+\) and Cl\(^-\) removal**

While CWs have been used successfully for many applications associated with pollutant removal, little work has been conducted on their ability to remove Na\(^+\) and Cl\(^-\). Based on the information available in the literature it has been shown that, when CWs are able to remove Na\(^+\) and Cl\(^-\), halophytic plants have been primarily responsible for the positive results. In Australia, Lymbery et al. (2006) examined the feasibility of using a CW for the treatment of saline aquaculture effluent that was high in N, P and NaCl. The CW was able to reduce the NaCl load by 44–53% when planted with the facultative halophyte *Juncus kraussii*. However, the NaCl removed by the plants was largely offset by water lost to evapotranspiration.
In two related studies, Nilratnisakorn et al. (2007) and (2009) found that CWs treating synthetic reactive dye wastewater were able to reduce Na⁺ by up to 44% when planted with the halophyte, *Typha angustifolia*. While initial removal was attributed to plant uptake, removal was compromised after three weeks due to toxicity problems associated with exposure to the synthetic dye.

Morteau et al. (2009) assessed the Cl⁻ accumulating ability of three halophytes (*Typha latifolia*, *Atriplex patula*, and *Spergularia canadensis*) when looking for candidates to remove deicing salts from runoff before it enters the watershed. The plants were grown in sand and exposed to NaCl concentrations of 0, 0.25, 2.5, and 25 mmol·L⁻¹. Morteau et al. (2009) reported that both *T. latifolia* and *A. patula* have potential for being used in CW to remove salt from wastewater. Both plants can accumulate relatively high levels of Cl⁻ in their tissue, 63 mg·g⁻¹ of dry weight (DW) for *T. latifolia* and 44 mg·g⁻¹ DW for *A. patula*.

In many of these cases the plant species were not chosen specifically for their ability to accumulate salt, but simply for their ability to tolerate it. Also, these systems were not designed with the primary objective of removing Na⁺ and Cl⁻ and their removal was simply measured along with the suite of primary parameters that the systems were designed to treat. With a design and plant choice more specifically targeted at salinity reduction, the potential for CWs to remove Na⁺ and Cl⁻ could be increased.
2.4. *Halophytic plants for Na\(^+\) and Cl\(^-\) removal*

Certain halophytic plants show potential for salt removal from wastewater. These plants are able to tolerate salinity by accumulating excess ions and are excellent candidates for phytodesalination. The following eight plant species were chosen for inclusion in this research due to their ability to accumulate salt, thrive in a wetland environment, and survive the climate of Ontario. These plants were evaluated in the first experimental chapter.

*Atriplex prostrata* (triangle orach) is a fleshy, annual herb that can grow either erect or prostrate to heights or lengths of 1 m. It is often found in the saline conditions of brackish coastal environments such as marshes or tidal flats (Tiner, 2009); however, it is a facultative halophyte and able to establish itself in non-saline environments as well. Like many halophytes, it survives in saline environments by accumulating ions in its tissue in order to maintain an osmotic potential (Wang et al., 1997). Wang et al. (1997) examined the effect of salinity on the growth of *A. prostrata* and found that when *A. prostrata* was exposed to a solution containing 86 mmol·L\(^{-1}\) of NaCl it was able to accumulate a significant amount of Na\(^+\) and Cl\(^-\). The Na\(^+\) and Cl\(^-\) content of the aerial tissues were 42.3 and 147.1 mg·g\(^{-1}\) DW, respectively.

*Distichlis spicata* (saltgrass) is halophytic perennial grass that can grow up to 40 cm tall and it forms dense mats through spreading rhizomes. It is not an obligate wetland species but the majority of the populations are found in brackish marshes near coastal environments (Tiner, 2009). In some areas such as Australia, *D. spicata* is grown on saline soil and used as a food
crop for livestock (Sargeant et al., 2008). Sargeant et al. (2008) conducted an eight-year comparative field study that examined the ability of *D. spicata* to improve soil quality in waterlogged saline soils found in Australia. They concluded that *D. spicata* was able to decrease the electroconductivity (EC) of saline soil along with providing other benefits. The decrease in EC is likely due to the increased hydraulic conductivity of the soil provided by the root system of the plant. However, there is evidence that it can remove ions such as Na\(^+\) and Cl\(^-\) from soils. Wu et al. (1997) examined the ion secretion mechanisms of *D. spicata* when exposed to a variety of ions. They reported that it is able to accumulate Na\(^+\) and Cl\(^-\). However, the main mechanism *D. spicata* employs to avoid damage from excess ions is through excretion on the leaf surface (Wu et al., 1997) and these ions could easily reenter the system.

*Juncus torreyi* (Torrey’s rush) is a facultative wetland species that is widespread throughout North America. It is a perennial rush and can grow up to 1 m tall in dense patches. It spreads predominantly through rhizomes but it also has globular flowering heads that produce seed. Similar species from the *Juncaceae* family have been used in constructed wetland research to desalinize water with some success (Lymberry et al., 2006). Zingelwa and Wooldridge (2009) examined the possibility of using a CW planted with *Juncus acutus* to reduce chemical oxygen demand (COD) and Na\(^+\) levels in winery wastewater. They found that *J. acutus* was capable of removing Na\(^+\), up to 767 mg\(\cdot\)m\(^2\) of CW. Therefore, the related species *J. torreyi*, which is more commonly found in Ontario, may be able to remove Na\(^+\) and Cl\(^-\) from recycled greenhouse nutrient solution.
Phragmites australis (common reed) is a rapidly growing perennial grass that is found throughout North America and the world (Tiner, 2009). P. australis spreads quickly and produces large amounts of biomass both above and below ground (Tanner 1996). It can grow as tall as 4 m in dense, monoculture stands (Tiner, 2009). Due to its rapid growth and ease of propagation P. australis 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 it is considered invasive; therefore, its use may be restricted in some areas (Tanner 1996). Gorai et al. (2010) report that P. australis was tolerant to saline and hypoxic conditions similar to those that would be found in a CW treating saline wastewater. When P. australis accumulated Na\(^+\) and Cl\(^-\) ions in its roots and aerial tissues, the aerial tissues contained ~10 and ~11 mg·g\(^{-1}\) DW of Na\(^+\) and Cl\(^-\) respectively and the roots ~23 and ~17 mg·g\(^{-1}\) DW of Na\(^+\) and Cl\(^-\) when the solution NaCl concentration was 50 mmol·L\(^{-1}\). P. australis may still be able to accumulate some Na\(^+\) and Cl\(^-\) at lower NaCl concentrations simply due to its very rapid biomass production. However, a large portion of the ions will likely be found in the below ground biomass (Gorai et al., 2010) making it impractical to harvest the biomass to transport the accumulated ions offsite.

Schoenoplectus tabernaemontani (softstem bulrush) is an obligate wetland plant of the Cyperaceae family and can grow up 3 m tall and forms dense colonies through rhizome production. It is commonly found throughout North America in a variety of habitats ranging from fresh and brackish tidal marshes to roadside ditches (Tiner, 2009). S. tabernaemontani is commonly used in constructed wetlands for the treatment of many different types of
wastewater. It has been shown to enhance N removal, reduce organic loads and increase beneficial microbial activity through interactions in the rhizosphere (Tanner, 1996). It was chosen for this study because it is commonly used in CW and has the potential to remove Na\(^+\) and Cl\(^-\).

*Spartina alterniflora* (smooth cordgrass) is an erect perennial grass found in saline marshes. It is an obligate wetland species and will vary in size depending on the regularity of flooding its habitat. When found in regularly flooded marshes, *S. alterniflora* can grow up to 2 m tall but when found in irregularly flooded areas it will typically be much smaller in size, ~50 cm tall (Tiner, 2009). *S. alterniflora* has a wide range and can be found along most coastlines in North America, from the Canadian Maritimes to the American south (Tiner, 2009). *S. alterniflora* is able to survive in very saline conditions by ion accumulation and secretion through the leaves. At salt concentrations less than 170 mmol·L\(^{-1}\), ion accumulation exceeds ion secretion but at concentrations higher than 690 mmol·L\(^{-1}\), ion secretion is the survival mechanism more commonly facilitated (Bradley and Morris, 1991). Bradley and Morris (1991) found that *S. alterniflora* can accumulate up to 47 mg Cl\(^-\) g\(^{-1}\) of DW and 11.5 mg Na\(^+\) g\(^{-1}\) of DW when grown in a solution with 170 mmol·L\(^{-1}\) NaCl.

*Typha angustifolia* (narrow leaf cattail) is an emergent wetland plant of the *Typhaceae* family. Its habitat ranges from brackish tidal marshes to inland freshwater wetlands. It is found growing throughout North America but it is particularly abundant in coastal areas. It is a hardy, fast-growing perennial herb that can reach heights of 1.5 m (Tiner, 2009). Research by
Nilranisakorn et al. (2007) and (2009) has shown that *T. angustifolia* is capable of reducing sodium levels in synthetic dye wastewater. *T. angustifolia* is an excellent candidate for use in CWs because of its ability to thrive in saline conditions, its large and rapid biomass production, and its ability to accumulate ions (Chandra and Yadev, 2011). *T. angustifolia* also has been shown to increase the treatment efficiencies of CWs in other applications (Klomjek and Nitisoravut, 2005).

*Typha latifolia* (broad leaf cattail) is a common freshwater wetland species found throughout North America and the world in various climates. It is a rapidly-growing perennial herb reaching heights of up to 3 m. It grows in many different waterlogged habitats, ranging from ponds and ditches to tidal fresh marshes and inland wetlands (Tiner, 2009). *T. latifolia* has been included in CWs since their inception (Reed and Brown 1992). *T. latifolia* is well known for its ability to enhance wastewater treatment by increasing microbial activity, rhizofiltration and nutrient uptake (Stottmeister et al. 2003). Few studies have examined the salt accumulating ability of *T. latifolia*. A study conducted by Morteau et al. (2009) showed that *T. latifolia* is a good candidate for Cl\textsuperscript{−} removal from wastewater. *T. latifolia* was also chosen for its large and rapid biomass production. The ion accumulating ability of *T. latifolia* paired with its large biomass production gives it the potential to remove a significant amount of Na\textsuperscript{+} and Cl\textsuperscript{−} from the recycled nutrient solution.
2.4.1. Biomass harvesting and ion uptake

The harvesting of plant biomass from CWs is not a common practice as it can be labour intensive and, if the litter is left, it can provide organic carbon for microbial populations and can insulate the surface of the wetland in winter months (Vymazal 2010). Also, plant uptake and storage is not crucial for the treatment of the commonly targeted pollutants such as N, organic material, and suspended solids in CWs (Kim and Geary 2001; Vymazal et al. 2010). However, for the removal of Na\(^+\) and Cl\(^-\) from the system, harvesting will be necessary to measure the ions stored in the plant biomass. Therefore, it is important to experimentally assess how harvesting affects Na\(^+\) and Cl\(^-\) accumulation as it may be beneficial to perform multiple harvests throughout the season to increase plant performance.

Vymazal et al. (2010) examined the effect of harvesting on the accumulation of 13 trace elements by the wetland plant *Phalaris arundinacea*. The results showed that biomass production was not affected by harvesting, whether once or twice during the growing season. However, while the uptake of the different elements was affected, there was no clear relationship from element to element. Vymazal et al. (2010) did not include Na\(^+\) and Cl\(^-\) in this experiment so it is unknown what effect harvesting will have on their removal through plant uptake.
2.4.2. The effect of NaCl concentration on uptake

Halophytic plants make osmotic adjustments to survive in saline conditions and as concentrations increase they will be required to accumulate more Na\(^+\) and Cl\(^-\) ions (Munns, 2002). At higher concentrations, the tissue contents of the plants will likely be higher (Glenn and Brown, 1999; Keiffer and Ungar, 1997), suggesting that removal of Na\(^+\) and Cl\(^-\) from solution could be enhanced if solutions were allowed to become more concentrated before treatment. Also, the Na\(^+\) and Cl\(^-\) concentrations at which nutrient solutions require treatment will vary depending on the sensitivity of the crop grown at a particular greenhouse. Therefore, it will be useful to determine how plants perform in nutrient solutions with different NaCl concentrations. However, even if the total removal of the ions increases at different concentrations, the final concentration of the solution is usually the factor that determines reusability, and not the total ion load of the treated water.

For example, Shelef et al. (2012) assessed B. indica’s capacity to phytodesalinize saline irrigation water in arid regions in Israel. They found that B. indica removed the largest percent of Na\(^+\) in medium and low salinities (~2 mmol L\(^{-1}\)) but the total accumulation by B. indica was the largest at the higher salinities. However, Morteau et al. (2009) found that T. latifolia Cl\(^-\) tissue contents were similar when grown in solutions solutions with NaCl concentrations ranging from ~0.2 mmol·L\(^{-1}\) to ~20 mmol·L\(^{-1}\).
2.5. Objectives

In order to assess the feasibility of using plants for the removal of Na$^+$ and Cl$^-$ from recycled greenhouse nutrient solution, three experiments were conducted. The objectives of these experiments were to:

1. Select plant species with the potential to remove Na$^+$ and Cl$^-$ from solution in a CW setting by comparing their tissue contents and uptake capacities when exposed to NaCl.
2. Determine whether periodic harvesting of plant aboveground biomass affects their capacity to uptake Na$^+$ and Cl$^-$ in a CW environment.
3. Determine plant Na$^+$ and Cl$^-$ uptake capacities under different NaCl solution concentrations.
3.0. Plant species for the removal of Na\(^+\) and Cl\(^-\) from greenhouse nutrient solution

3.1. Introduction

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 NaNO\(_3\) (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, in order 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 constructed wetlands (CW), 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 $\text{Na}^+$ and $\text{Cl}^-$; however, removal of these ions has been found when plants capable of hyper-accumulating $\text{Na}^+$ and $\text{Cl}^-$ 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 $\text{Na}^+$ and $\text{Cl}^-$ 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 $\text{Na}^+$ and $\text{Cl}^-$ into CWs could increase the removal of these ions from recycled greenhouse nutrient solutions.

The objectives of this chapter were to:

- Determine the plant tissue dry matter content of $\text{Na}^+$ and $\text{Cl}^-$ from eight plant species grown in CW microcosms under controlled-environmental conditions (Exp 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\(^-\) (Exp 2).

### 3.2. Materials and Methods

Experiment 1: Controlled environment

*Plant material and treatments.* A microcosm experiment was conducted in a research greenhouse at the University of Guelph, ON, Canada from 15 Oct. to 16 Nov. 2013. The greenhouse was set at 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, ON, Canada. *A. prostrata* and *S. alterniflora* were sustainably harvested from natural shoreline populations near New Glasgow, NS, Canada. Wild-harvested plant material was potted in a peat moss potting mix in 10 cm pots, irrigated as needed, and acclimated in the research greenhouse environment for four weeks. Plant material from local nurseries was acclimated in the research environment for at least three weeks.
The microcosms consisted of 9 L plastic containers (0.374 × 0.241 × 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 cm$^3$ of washed 5-10 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 four 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 due to 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, ON). The resulting solution had a NO$_3$-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 Na$_2$MoO$_4$ (0.015%) in the fertilizer mix.
Plants were grown in the microcosms, filled with 2 L of nutrient solution, for five 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 ICP-OES (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, ON, Canada.

Experiment 2: Outdoors

**Plant material and treatments.** The experiment was conducted outdoors adjacent to the Edmund C. Bovey building at the University of Guelph, ON, 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 Exp. 1).

*J. torreyi, S. tabernaemontani, T. angustifolia,* and *T. latifolia* were planted in the microcosms, two similarly-sized plants per microcosm, three weeks before the start of the
experiment to allow them to acclimatize. The experiment was setup 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, 2014a).

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 aboveground 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. Rootzone water levels and Na\(^+\) and Cl\(^-\) concentrations were maintained using the same methodology as Exp. 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 Exp. 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 m\(^2\) 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 \((\text{L} \times \text{mg} \cdot \text{L}^{-1})\). The total mass accumulated by the plant material in each microcosm was also calculated by multiplying the tissue content \((\text{mg} \cdot \text{g}^{-1})\) to the total DW \((\text{g})\). 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 (v. 9.0, SAS Institute Inc., 1999; Cary, NC). For Exp. 1, an analysis of variance (ANOVA) and a Tukey’s test were used to compare the differences in mean ion content among species. For Exp. 2, differences among mean plant tissue ion content, dry weight, total mass of ions accumulated per m\(^2\), 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 \((\alpha = 0.05)\).

3.3. Results and Discussion

Results from Exp. 1 identified *T. latifolia*, *S. tabernaemontani*, *J. torrey*, and *T. angustifolia* as having the greatest desalinization potential based on their tissue contents. These four species were evaluated in Exp. 2 and removed between 4–12% and 10–26% of the Na\(^+\) and Cl\(^-\) ions applied with the nutrient solution (Table 3.1).
Table 3.1. Dry weight (g), tissue concentration (mg·g⁻¹) of Na⁺ and Cl⁻ accumulated per unit growing area (g·m⁻²), and percent (%) of ions removed from the nutrient solution by each of four plant species grown for five weeks in outdoor constructed wetland microcosms, fed with a simulated greenhouse nutrient solution with added NaCl.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry Weight (g)</th>
<th>Na⁺ content (mg·g⁻¹)</th>
<th>Cl⁻ content (mg·g⁻¹)</th>
<th>Na⁺ accum (g·m⁻²)</th>
<th>Cl⁻ accum (g·m⁻²)</th>
<th>% Na⁺ removed (x)</th>
<th>% Cl⁻ removed (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. latifolia</td>
<td>29.2a(y)</td>
<td>8.23a</td>
<td>24.4ab</td>
<td>9.2a</td>
<td>27.2a</td>
<td>12.0a</td>
<td>23.4a</td>
</tr>
<tr>
<td>S. tabernaemontani</td>
<td>28.8a</td>
<td>5.87b</td>
<td>27.2a</td>
<td>3.9b</td>
<td>18.2a</td>
<td>8.7ab</td>
<td>26.4a</td>
</tr>
<tr>
<td>J. torreyi</td>
<td>23.1a</td>
<td>4.43c</td>
<td>20.1b</td>
<td>5.8ab</td>
<td>25.7a</td>
<td>5.4bc</td>
<td>16.0b</td>
</tr>
<tr>
<td>T. angustifolia</td>
<td>11.3b</td>
<td>7.30a</td>
<td>27.9a</td>
<td>8.3ab</td>
<td>31.6a</td>
<td>4.2c</td>
<td>10.7b</td>
</tr>
</tbody>
</table>

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

\(y\) Mean values (n = 3) followed by the same letter are not significantly different at \(P < 0.05\).

*T. latifolia* exhibited a high potential for phytodesalinization of simulated greenhouse nutrient solution in both experiments. Tissue contents were 4.2 mg Na⁺ (Fig. 3.1) and 27.0 mg Cl⁻ (Fig. 3.2) per gram of DW in Exp. 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 (Fig. 3.1 and 3.2). In Exp. 2, *T. latifolia* had the greatest Na⁺ and Cl⁻ tissue contents, 8.23 and 24.4 mg·g⁻¹, respectively (Table 3.1). *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 3.1). These results agree well with Morteau et al. (2009) who found *T. latifolia* was able to accumulate up to 63 mg Cl⁻ per g 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 due to its hardiness and ability to tolerate many different environmental conditions (Vymazal, 2011); however, it is rarely considered for phytodesalinization. From our results, *T. latifolia* demonstrated sufficient performance to recommend for inclusion in CWs.

*S. tabernaemontani* exhibited phytodesalinization ability only slightly lower than that of *T. latifolia*. The Na\(^+\) content of *S. tabernaemontani* was comparable to the other top performing species in Exp. 1 (3.0 mg·g\(^{-1}\); Fig. 3.1), but had the highest Cl\(^-\) content (34.8 mg·g\(^{-1}\); Fig. 3.2) of any of the tested species. In Exp. 2, *S. tabernaemontani* accumulated relatively large amounts of Na\(^+\) and Cl\(^-\) (3.9 and 18.2 g·m\(^{-2}\), respectively; Table 3.1) 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.
Fig. 3.1. Tissue Na\(^+\) content (mg Na\(^+\) / g of foliar dry weight) of eight plant species after five weeks growing in 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. 3.2.** Cl⁻ tissue content (mg Cl⁻ / g of foliar dry weight) of eight plant species after five 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 $P < 0.05$.

*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 3.1). Ion contents in the foliar dry matter were relatively high in Exp. 1, at 3.9 mg·g⁻¹ Na⁺ (Fig. 3.1) and 23.8 mg·g⁻¹ Cl⁻ (Fig. 3.2) and therefore it was included in Exp. 2. Its performance was comparable to *T. latifolia* and *S. tabernaemontani* as it accumulated 5.8 g·m⁻² Na⁺ and 25.7 g·m⁻² Cl⁻ (Table 3.1) but the percent removal of Na⁺ and Cl⁻ was lower than those of *T. latifolia* and *S. tabernaemontani* (Table 3.1). Related species in the *Juncaceae* family have
been evaluated for their phytodesalinization 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 Exp. 1 the tissue contents of *T. angustifolia* reached 3.8 mg·g\(^{-1}\) Na\(^+\) (Fig. 3.1) and 30.5 mg·g\(^{-1}\) Cl\(^-\) (Fig. 3.2). In Exp. 2 *T. angustifolia* removed 8.3 g·m\(^{-2}\) of Na\(^+\) and 31.6 g·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 3.1). *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 Exp. 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 g·m\(^{-2}\). This is comparable to estimated peak stand production of 1445 g·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). While 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 Exp. 2.

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

*A. prostrata* had the highest Na⁺ content of the eight species in Exp. 1 at 11.3 mg·g⁻¹ (Fig. 3.1) but its Cl⁻ content was relatively low, 16.0 mg·g⁻¹ (Fig. 3.2). *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 mmol·L⁻¹ NaCl, *A. prostrata* tissue accumulated ~42 and 137 mg of Na⁺ and Cl⁻ respectively, per g 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 Exp. 1 even though 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 to the other species in this experiment. The Cl\(^-\) content was especially low (9.75 mg·g\(^-1\)) while the Na\(^+\) content (2.20 mg·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 mmol·L\(^-1\) while excretion exceeds secretion at higher salinities. While secretion is less desirable in CW scenarios, since the removed salts could easily reenter the water during precipitation events, this mechanism likely has minimal role in CW applications for greenhouse wastewater treatment as the solution concentrations are in the order of 100× lower. The same study reported that *S. alterniflora* accumulated up to 11.5 mg Na\(^+\) g\(^-1\) and 47.0 mg Cl\(^-\) g\(^-1\) when exposed to NaCl concentrations of 171.1 mmol·L\(^-1\). Again, this salinity is much 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 to 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 (EC) of saline soils in Australia (Sargeant et al.,
2008). However, Wu et al. (1997) reported that even though *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. While 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 g of DW. *P. australis* grows rapidly and produces large amounts of biomass in short periods of time (Tanner, 1996). It is largely due to 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 approximately 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. Due to 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 and B). However, Cl\(^-\) accumulation was affected by biomass production (Fig. 3.3A and B) much more than it was affected by Cl\(^-\) tissue content (Fig. 3.3A). This suggests that plant selection for Na\(^+\) accumulation should exhibit an affinity for both biomass production and ion accumulation while 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 Exp. 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 Exp. 1, the aboveground plant biomass was removed at the start of Exp. 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 useful to investigate the effect and timing of harvesting on the phytodesalinization potential of these plant species.
Fig. 3.3. 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. torrey, and T. angustifolia after five weeks in constructed wetland microcosms with simulated greenhouse nutrient solution with added NaCl. Pearson correlation coefficients ($r$) presented in parentheses for each ion.
3.4. Conclusions

*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 five-week greenhouse and outdoor bench experiments. In the outdoor experiment, the aboveground 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 australisis* 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 over a longer period of time.
4.0. Does harvest frequency of wetland plants enhance the removal of Na$^+$ and Cl$^-$ ions from a greenhouse nutrient solution?

4.1. Introduction

Greenhouse crop production can require large amounts of water and nutrients (Robbins, 2010), which is costly and can be unsustainable. In order to conserve water and fertilizer, growers capture and re-use excess nutrient solution. However, when water is continually recycled, ions such as Na$^+$ and Cl$^-$ tend to accumulate because they are not absorbed from the solution by most plants and these ions are often prevalent in the source water and the nutrient solution (Flood, 1996; Robbins, 2010). Both Na$^+$ and Cl$^-$ can damage plants, even at relatively low concentrations (Munns, 2002; Stanghellini et al., 2005), and it is therefore recommended that Na$^+$ concentrations should be maintained < 2.2 mmol·L$^{-1}$ and Cl$^-$ concentrations be < 3.9 mmol·L$^{-1}$ (Robbins, 2010). Therefore, to continue re-using nutrient solution, growers either discharge waste nutrient solution when a ‘threshold concentration’ has been reached, or install a water treatment technology system (Flood, 1996; Robbins, 2010). Many treatment options (i.e., reverse osmosis) are expensive and often produce a concentrated wastewater solution that requires further treatment (Gagnon et al., 2010).

Constructed wetlands (CW) are water treatment systems designed to mimic the beneficial processes that occur in natural wetlands and are an economically-viable option for wastewater treatment for many applications, including greenhouse nutrient solution treatment (Prystay and Lo, 2001; Gagnon et al., 2010). Currently, CWs are used by greenhouse
operations to manage organic material, total suspended solids (TSS), and nutrient levels (e.g. N and P) in greenhouse wastewater (Tanner, 1996; Prystay and Lo, 2001; Gagnon et al., 2010; Vymazal, 2010). CWs are rarely considered an option for Na\(^+\) and Cl\(^-\) removal, but research suggests CWs may prove to be a useful and economically-viable phytoremediation option if plants that hyper-accumulate Na\(^+\) and Cl\(^-\) are included in CW systems (Morteau et al., 2009; Shelef et al., 2012). Phytoremediation to remove Na\(^+\) and Cl\(^-\) is known as phytodesalinization.

When using CWs for phytodesalinization, the aerial portions of the plants need to be periodically removed to prevent the ions from reentering the system. Harvesting plant biomass from CWs is not a common practice as it can be labor intensive and plant uptake and storage is not crucial for the treatment of the commonly targeted pollutants in CWs (Kim and Geary, 2001; Vymazal et al., 2010). The frequency and timing of harvesting may increase the efficiency of ion removal from CWs (Jinadasa et al., 2008), as has been shown for some elements using *Phalaris arundinacea* plants (Vymazal et al., 2010). Chapter 3 identified *Juncus torreyi* (Torrey’s rush), *Schoenoplectus tabernaemontani* (softstem bulrush), and *Typha latifolia* (broad leaf cattail), as capable Na\(^-\) and Cl\(^-\) accumulating plants and suggests that harvesting could increase ion uptake due to the observed rapid growth after harvesting events. However, further research is needed to determine the effect of multiple harvests on the phytodesalinization capacity of plants in CW systems.
The objective of this chapter was to determine how the frequency of harvesting *J. torreyi*, *S. tabernaemontani*, and *T. latifolia* affects Na\(^+\) and Cl\(^-\) accumulation from a simulated greenhouse nutrient solution in a CW microcosm.

**4.2. Materials and Methods**

*Wetland microcosms and growing conditions.* An outdoor microcosm experiment was conducted outside the Edmund C. Bovey Building, University of Guelph, Guelph, ON, Canada (lat. 43°31’38” N, long. 80°13’45” W) on a gravel surface in a non-shaded area from 15 May to 18 Sept 2013 (18 weeks). The microcosms were 36 L (0.045 m × 0.026 m × 0.032 m) opaque plastic containers wrapped in white plastic and set on a plywood bench ~20 cm off the ground. Containers were filled with 0.030 m\(^3\) of 5-10 mm washed granite stone and fitted with a 1.27 cm polpropylene bulkhead plumbing fitting in the bottom corner so the nutrient solution could be drained and replaced. The pore volume in the gravel of the planted microcosms were ~8 L, as determined by adding known volumes of water to microcosms until the water level was visible at the surface of the microcosm. Over the 18-week period of the experiment, the average daily air temperature ranged from 11.1 to 23.2°C (Environment Canada, 2014b).

*Plant material.* *J. torreyi*, *S. tabernaemontani*, and *T. latifolia* plants were obtained from an Ontario nursery. The plants were kept outside to acclimate for four weeks, before being planted in the gravel of the microcosms, three weeks prior to the start of the experiment. For each species, the microcosm was planted with similar-sized plant material (based on visual estimations), two plants per microcosm.
**Nutrient solution.** The nutrient solution was prepared using deionized water, reagent grade NaCl, and a 20N-8P₂O₅-20K₂O granular water-soluble fertilizer (All Purpose High Nitrate, PlantProducts®, Brampton, ON). The resulting solution had a NO₃-N concentration of 10.9 mmol·L⁻¹, and Na⁺ and Cl⁻ concentrations of 8.0 and 7.8 mmol·L⁻¹, respectively. The molar concentration of Na⁺ was slightly higher than Cl⁻ because of the Na₂MoO₄ (0.015%) in the fertilizer. The nutrient solution was designed to simulate a discharged recirculated greenhouse fertigation solution with concentrations of Na⁺ and Cl⁻ above desirable levels for use (Stanghellini et al., 2005).

**Experimental protocol and harvesting schedules.** At the beginning of the experiment all aboveground biomass was cut down to the gravel surface of the microcosms and removed so the initial Na⁺ and Cl⁻ accumulation in plant leaves would be zero. Therefore, we assumed all Na⁺ and Cl⁻ accumulated in plant leaves throughout the experiment were from the supplied nutrient solution.

Each microcosm was filled with 8 L of nutrient solution. The microcosms were drained and flushed with deionized water every week and 8 L of fresh nutrient solution was added in order to maintain a constant Na⁺ and Cl⁻ concentration in the root zone.

Each of the three plant species was subject to each of three harvesting schedules (i.e., no harvest, one harvest, and two harvests), resulting in 9 treatments. The experiment was set up as a randomized complete block design with three replicates of each treatment to give a total
of 36 experimental units. The harvest on the one-harvest treatment occurred on 24 July (week 10). The harvests on the two-harvest treatment occurred on 19 June (week 5) and on 21 August (week 14). All experimental units, regardless of treatment, were subject to a final harvest at the end of the experiment, on 18 Sept. 2014 (18 weeks) to allow for a final destructive analysis of the plant material.

At each harvest, shoots were cut down to the surface of the microcosms, washed with deionized water, and stored in paper bags. The harvested material was dried in an oven at 65°C until a constant weight was achieved, and the biomass as dry weight (DW) was measured. Tissue Na⁺ concentration (% DW) was determined by ashing the sample, dissolving the ash in HCl, and then analyzing the solution by ICP-OES using AOAC method 985.01 (Agri-Food Laboratories, Guelph, ON). The tissue Cl⁻ concentration (% DW) was determined using an electrochemical titration with standardized AgNO₃ (AOAC 969.01; Agri-Food Laboratories, Guelph, ON).

The Na⁺ and Cl⁻ tissue contents (mg·g⁻¹) were multiplied by the total biomass (g) produced during each harvesting period to give the total mass of ions accumulated. The mass of Na⁺ and Cl⁻ accumulated during each harvest period were summed to give the total mass accumulated over the 18-week period. Before each harvest, the area of growth, or surface area footprint, was measured at the base of the plant at the surface of the microcosm. The total mass of ions accumulated was divided by the area to give the ion mass accumulated per unit area (g·m⁻²). The area was also used to calculate the biomass produced per m².
Statistical analysis. Mean values of plant tissue ion content, biomass per m², and mass of ions accumulated per m² were calculated and the variance was analyzed using an ANOVA (\( P < 0.05 \)). A Tukey’s test was conducted to compare the means and correlation analysis was used to identify the relationship between total ion accumulation and biomass production. SAS (v. 9.0, SAS Institute Inc., 1999; Cary, NC) was used for all statistical tests.

4.3. Results and Discussion

The three plant species chosen for the current study demonstrated potential to remove Na⁺ and Cl⁻ ions from wastewater, based on the results of Chapter 3. Results of the current study showed that different species responded differently to harvest frequency for tissue Na⁺ and Cl⁻ contents (Table 4.1), shoot biomass (Fig. 4.1), and total Na⁺ and Cl⁻ (g) accumulated per m² of microcosm surface (Fig. 4.2 and 4.3, respectively).

Multiple harvests increased the overall performance of *T. latifolia*. Following two harvests (TL-2), close to 1.5× more biomass was produced (3719 g·m⁻²) than when it was harvested once (2628 g·m⁻²; TL-1), and 3× more than when *T. latifolia* shoots were not harvested (1170 g·m⁻²; TL-0; Fig. 4.1). Harvest frequency had a similar effect on *T. latifolia* Na⁺ and Cl⁻ accumulation per m². When harvested twice, *T. latifolia* accumulated 24.7 g·m⁻² of Na⁺ in total, which was 1.5× > the 16.3 g·m⁻² accumulated by TL-1. When *T. latifolia* was not harvested only 6.9 g·m⁻² of Na⁺ was accumulated, approximately 3× < TL-2 (Fig. 4.2).
Fig. 4.1. Total dry weight biomass (g·m$^{-2}$) produced relative to microcosm surface area for each treatment after 18-weeks in constructed wetland microcosms supplemented with NaCl nutrient solution. TL: *Typha latifolia*; JT: *Juncus torreyi*; ST: *Schoenoplectus tabernaemontani*. The numbers after the species name indicate the number of harvests. Bars (mean ± SE; n = 3) with the same letter are not significantly different at $P < 0.05$. 
Fig. 4.2. Total Na\(^+\) (g·m\(^{-2}\)) accumulated relative to microcosm surface area for each treatment after 18-weeks in constructed wetland microcosms supplemented with NaCl nutrient solution. TL: *Typha latifolia*; JT: *Juncus torreyi*; ST: *Schoenoplectus tabernaemontani*. The numbers after the species name indicate the number of harvests. Bars (mean ± SE; n = 3) with the same letter are not significantly different at \(P < 0.05\).

The number of *T. latifolia* harvests was less important for Cl\(^-\) than Na\(^+\) accumulation. There was no difference \((P < 0.05)\) in total Cl\(^-\) accumulation per m\(^2\) between TL-2 and TL-1 at the final harvest, with an average accumulation of 88.2 g·m\(^{-2}\) when both treatments were considered. However, Cl\(^-\) accumulation per m\(^2\) for TL-0 was lower than TL-2 and TL-1 (i.e., 25.6 g·m\(^{-2}\), 3.5× less) due to the increased biomass production of the harvested treatments (Fig. 4.3).
Fig. 4.3. Total Cl\(^{-}\) (g·m\(^{-2}\)) accumulated relative to microcosm surface area for each treatment after 18-weeks in constructed wetland microcosms supplemented with NaCl nutrient solution. TL: *Typha latifolia*; JT: *Juncus torreyi*; ST: *Schoenoplectus tabernaemontani*. The numbers after the species name indicate the number of harvests. Bars (mean ± SE; n = 3) with the same letter are not significantly different at \(P < 0.05\).

There was a positive correlation between ion uptake and biomass production, \(r = 0.97\) and \(r = 0.97\) for Na\(^+\) and Cl\(^{-}\) respectively. To increase Na\(^+\) and Cl\(^{-}\) uptake by *T. latifolia* it is necessary to increase its biomass production by harvesting *T. latifolia* throughout the growing season. Martin et al. (2003) report that a single harvest of part of the *T. latifolia* shoots (i.e., 70–85% harvested) increased its overall biomass production; however, Jindasa et al. (2008) found that too many harvests (i.e., four within ~175 d) negatively affected the biomass production of the closely related species, *Typha angustifolia*. This research indicated that two harvests can maximize biomass production; however, the timing could possibly be altered to
further improve the output, or even allow for a third harvest. Therefore, further research is needed to evaluate the optimal number of harvests and also the timing of each harvest.

*J. torreyi* responded differently to harvesting frequency than *T. latifolia*. For this species, the one harvest treatment, JT-1, was the top performer for biomass production and Na\(^+\) and Cl\(^-\) uptake. JT-1 produced 4555 g·m\(^{-2}\) biomass whereas the two-harvest treatment, JT-2, and the no harvest treatment, JT-0, only produced 2583 and 2423 g·m\(^{-2}\), respectively, ~1.5× less (Fig. 4.1). Harvesting had less of a pronounced effect on Na\(^+\) accumulation, however, as the three treatments accumulated similar amounts, i.e., 11.9, 12.8 and 7.4 g·m\(^{-2}\) for JT-0, JT-1 and JT-2, respectively.

The Cl\(^-\) uptake by *J. torreyi* showed a correlation to biomass production \((r = 0.98)\). JT-1, the treatment that produced the most biomass, accumulated over twice as much Cl\(^-\) \((111.3 \text{ g·m}^2)\) than JT-2 \((45.5 \text{ g·m}^2)\) and 3× > JT-0 \((35.3 \text{ g·m}^2)\). Therefore, to increase the biomass production and Cl\(^-\) accumulation *J. torreyi* should be harvested only once, in the middle of the growing season.

In general, *S. tabernaemontani* performed poorly in all the categories regardless of harvesting frequency. There was no difference between the harvesting treatments (i.e., ST-0, ST-1 and ST-2) for biomass production as well as Na\(^+\) and Cl\(^-\) uptake, indicating that harvesting does not influence *S. tabernaemontani*’s phytodesalinization capacity. Kim and Geary (2001) examined the influence of harvesting on a related species, *Schoenoplectus mucronatus*, and
its capacity to uptake P from solution. They found that the no-harvest treatments performed better as *S. mucronatus* produced more biomass and accumulated more P.

Biomass production is an important factor when evaluating the phytoremediation potential of plants, regardless of the targeted pollutant. Therefore, increasing biomass production through harvesting can increase the phytoremediation potential of these plant species for Na\(^+\) and Cl\(^-\) and possibly other pollutants. Overall, *T. latifolia* and *J. torreyi* produced more biomass than *S. tabernaemontani* and harvesting frequency influenced the biomass production of *T. latifolia* and *J. torreyi*, but not *S. tabernaemontani*. For each species the no-harvest treatment produced the lowest amount of biomass (Fig. 4.1). The treatments that produced the greatest amount of biomass were: JT-1 4555 g·m\(^{-2}\) and TL-2 3719 g·m\(^{-2}\). Greater biomass production increased total Cl\(^-\) accumulation and that can be more of a factor for total Cl\(^-\) removal than the Cl\(^-\) tissue content (Morteau et al., 2009) and increased biomass production can also lead to increased Na\(^+\) accumulation (Shelef et al., 2012).

Vymazal et al. (2010) conducted a study in which the wetland plant, *Phalaris arundinacea*, was harvested either once or twice in the growing season. The effect of harvesting on biomass production and the accumulation of 13 trace elements was examined (Na\(^+\) and Cl\(^-\) were not included). Contrary to our results, they found that the plants still produced similar amounts of biomass, regardless of harvesting frequency, and there was no clear relationship between ion accumulation and harvesting frequency as each element responded differently. This is likely due to the differences between the plant species as it was clear from our results that species response to harvesting varies.
Among all three species, *T. latifolia* and *J. torreyi* treatments had the greatest Na\(^+\) accumulation, while all three harvesting frequencies of *S. tabernaemontani* accumulated the least Na\(^+\). *T. latifolia* also had the highest Na\(^+\) tissue contents, regardless of harvesting frequency, followed by *J. torreyi* and *S. tabernaemontani* (Table 4.1). These results indicate that species selection is important when considering options for Na\(^+\) removal from wastewater.

**Table 4.1.** Tissue Na\(^+\) and Cl\(^-\) contents of three wetland plant species subject to different harvesting frequencies after 18 weeks of growth in constructed wetland microcosms with NaCl-supplemented nutrient solution.

<table>
<thead>
<tr>
<th>Trt Wetland Plant Species</th>
<th>Number of Harvests</th>
<th>Na(^+) (mg·g(^{-1}))</th>
<th>Cl(^-) (mg·g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>JT-0 <em>J. torreyi</em></td>
<td>0</td>
<td>5.0b(^e)</td>
<td>14.7e</td>
</tr>
<tr>
<td>JT-1 <em>J. torreyi</em></td>
<td>1</td>
<td>2.8c</td>
<td>24.8bc</td>
</tr>
<tr>
<td>JT-2 <em>J. torreyi</em></td>
<td>2</td>
<td>2.8c</td>
<td>19.7d</td>
</tr>
<tr>
<td>ST-0 <em>S. tabernaemontani</em></td>
<td>0</td>
<td>1.6c</td>
<td>21.8cd</td>
</tr>
<tr>
<td>ST-1 <em>S. tabernaemontani</em></td>
<td>1</td>
<td>1.4c</td>
<td>27.7ab</td>
</tr>
<tr>
<td>ST-2 <em>S. tabernaemontani</em></td>
<td>2</td>
<td>1.5c</td>
<td>24.6bcd</td>
</tr>
<tr>
<td>TL-0 <em>T. latifolia</em></td>
<td>0</td>
<td>5.9ab</td>
<td>21.8cd</td>
</tr>
<tr>
<td>TL-1 <em>T. latifolia</em></td>
<td>1</td>
<td>6.7a</td>
<td>31.0a</td>
</tr>
<tr>
<td>TL-2 <em>T. latifolia</em></td>
<td>2</td>
<td>6.4ab</td>
<td>26.2abc</td>
</tr>
</tbody>
</table>

\(^e\)Mean values (n = 3) followed by the same letter in a column are not significantly different at \(P < 0.05\).

Both species selection and harvesting frequency were important for Cl\(^-\) accumulation since the least Cl\(^-\) accumulation occurred for the no-harvest treatments and all *S. tabernaemontani* treatments (Fig. 4.3). *S. tabernaemontani* was generally outperformed by the other species.
with the same harvest schedules, implying that *S. tabernaemontani* is not as suited for Cl\(^-\) removal as the other species. There was no difference between the top two Cl\(^-\) accumulating treatments, JT-1 (111.1 g·m\(^{-2}\)) and TL-2 (94.8 g·m\(^{-2}\)), indicating that either treatment could be a viable option for the phytoremediation of Cl\(^-\).

### 4.4. Conclusions

In general, harvesting resulted in increased biomass production in CW systems, in turn resulting in greater Na\(^+\) and Cl\(^-\) accumulation. Species selection was an important factor for Na\(^+\) accumulation, and *T. latifolia* proved to be the best species. Two harvests increased the Na\(^+\) accumulation of *T. latifolia*, compared to a single and no-harvest. *J. torreyi* and *T. latifolia* accumulated the highest levels of Cl\(^-\), following one and two harvests, respectively. Harvesting frequency also increased biomass production for *J. torreyi* and *T. latifolia* but had no effect on *S. tabernaemontani*. However, ion removal efficiency of all treatments was low relative to the total amount of ions applied to the microcosms and further research is needed to develop methods to increase the efficiency of ion uptake by harvested plants in CWs to remediate greenhouse nutrient solution runoff.
5.0. The effect of different NaCl solution concentrations on the phytodesalinization potential of two wetland plant species

5.1. Introduction

The accumulation of Na$^+$ and Cl$^-$ ions in recycled greenhouse nutrient solution limits the ability of a grower to continually reuse collected nutrient solution. These ions are present in greenhouse nutrient solutions because they are often found in the source water and nutrient fertilizers. Na$^+$ and Cl$^-$ accumulate in recycled nutrient solutions because most plants do not uptake much from solution. Eventually the ion concentrations will reach levels that can damage the crops and the nutrient solution will need be discharged or treated. Salt tolerant plants capable of storing Na$^+$ and Cl$^-$ in their tissues may be able to remove the excess ions from the nutrient solution (Chapter 3 and 4; Shelef et al., 2012; Freedman et al., 2014), a process known as phytodesalinization. These plants could be added to constructed treatment wetlands (CW), a technology that utilizes the beneficial processes found in natural wetlands, and has been adopted by the greenhouse industry for the treatment of excess nutrients (N and P), suspended solids, and organic material (Tanner, 1996; Prystay and Lo, 2001; Gagnon et al., 2010; Vymazal, 2010). Research suggests that the addition of hyper-accumulating plants to constructed wetlands could enhance the Na$^+$ and Cl$^-$ removal capacity of these systems (Lymbery et al., 2006; Morteau et al., 2009; Nilratnisakorn et al., 2009; Shelef et al., 2012).

When using phytodesalinization as a treatment option the Na$^+$ and Cl$^-$ concentration of the solution needs to be considered, as it will affect uptake. Also the Na$^+$ and Cl$^-$ tolerance of
different greenhouse crops varies so the levels at which the nutrient solutions will require treatment will also vary. Therefore, it is useful to examine how different Na\(^+\) and Cl\(^-\) concentrations affect the phytodesalinization capacity of halophytic plants.

Halophytic plants make osmotic adjustments to survive in the presence of Na\(^+\) and Cl\(^-\) and as concentrations increase they will be required to accumulate more Na\(^+\) and Cl\(^-\) ions (Munns, 2002; Tester and Davenport, 2003). At higher concentrations the tissue contents of the plants will likely be higher and the total amount of Na\(^+\) and Cl\(^-\) removed could increase. Shelef et al. (2012) assessed *B. indica*’s capacity to phytodesalinize saline irrigation water in arid regions in Israel. They found that *B. indica* removed the largest percent of Na\(^+\) in medium and low salinities (~2 mmol·L\(^-1\)) but the total accumulation by *B. indica* was highest at the higher salinities. Morteau et al. (2009) found that *T. latifolia* Cl\(^-\) tissue contents increased when grown in solutions with increasing NaCl concentrations ranging from ~0.2 mmol·L\(^-1\) to ~20 mmol·L\(^-1\). Similarly, other studies in the literature report that increasing NaCl concentrations result in higher tissue contents of both Na\(^+\) and Cl\(^-\) in the plants (Glenn et al., 1999; Keiffer and Ungar, 1997; Morteau et al., 2009; Shelef et al., 2012), meaning a greater amount would be removed from solution. However, when treating greenhouse nutrient solution for reuse it is often the final concentration of the ions in the solution that is important, and not the total load. Therefore the final concentration of the solution also needs to be considered along with the total amount of Na\(^+\) and Cl\(^-\) removed.

The objective of this chapter was to evaluate the effect of increasing NaCl concentrations on the Na\(^+\) and Cl\(^-\) uptake of two wetland plant species, *S. tabernaemontani* and *T. latifolia*.
Two factors were examined: the total Na\(^+\) and Cl\(^-\) removed from solution by the plants and the final Na\(^+\) and Cl\(^-\) concentrations after exposure to the plants.

### 5.2. Materials and Methods

*Experimental design.* A hydroponic experiment was conducted in a research greenhouse at the University of Guelph from 14 May 2014 to 2 July 2014. The temperature was controlled to remain below 25 °C and the relative humidity was maintained between 60% and 80% throughout the experiment. The experiment included two plant species, *S. tabernaemontani* and *T. latifolia*, and four nutrient solutions with increasing NaCl concentrations. It was a RCBD with five blocks for a total of 40 experimental units. The four nutrient solution NaCl concentrations were: 2, 4, 8, and 16 mmol·L\(^{-1}\). The different NaCl concentrations were chosen based on what levels would be considered unfit for reuse due to the ranging sensitivities of various greenhouse crops (Stanghellini et al., 2005). The nutrient solutions were made by mixing deionized water, reagent grade NaCl, and a 20N–8P\(_2\)O\(_5\)–20K\(_2\)O granular water-soluble fertilizer (All Purpose High Nitrate, PlantProducts®, Brampton, ON).

A hydroponic system was used to provide more control of the solution and to allow for more accurate measurements of volume and ion concentrations. The hydroponic units consisted 2 L plastic buckets filled with nutrient solution. The plant material was floated on the surface of the nutrient solution by placing it in Styrofoam discs with a 5 cm hole cut in the middle, containing a small plastic basket. The plastic basket sat just below the bottom of the disc to allow the roots to be completely submerged in the nutrient solution.
The plant material was sourced from a local nursery and given four weeks to acclimate to the greenhouse conditions. The plant material was then divided into single shoots, with approximately the same-sized root ball, and these were used for each experimental unit. The roots were rinsed and the plants were placed in the hydroponic system, one plant per treatment, and irrigated with regular greenhouse feeding solution for two weeks before the start of the experiment.

*Experimental protocol and measurements.* At the start of the experiment the initial fresh weight (FW) of the plant material was measured so that as the experiment progressed the change in biomass could be monitored. The water content of each plant species was calculated by drying plant samples of similar size to the plants used in the experiment. This was done so that the FW could be converted into dry weight (DW).

Every two weeks the nutrient solution was changed and fresh nutrient solution added. Each time this occurred the remaining volume of solution in each bucket was measured, a sample of the solution was taken for Na\(^+\) and Cl\(^-\) analysis, and the FW of the plant material was measured. A sample of the fresh nutrient solution was also analyzed for Na\(^+\) and Cl\(^-\) concentration each time the solution was changed. The amount of Na\(^+\) and Cl\(^-\) removed from solution by each experimental unit was calculated using this equation:

\[
\text{Mass of ion removed} = (\text{initial conc.} \times \text{initial volume}) - (\text{final conc.} \times \text{final volume})
\]
The amount of ion removed was then divided by the DW of the plant to give the removal per g of DW. This metric allowed for a better comparison between the different sized species included in this trial.

The Na\(^+\) and Cl\(^-\) concentrations were determined using ions selective electrodes, Thermo Scientific ROSS® Sure-Flow® Sodium Combination ISE (Thermo Fisher Scientific, Beverly, MA.) and Chloride ionplus® Sure-Flow® Solid State Combination ISE (Thermo Fisher Scientific, Beverly, MA.) connected to a Thermo Orion 290Aplus meter (Orion Research Inc., Boston, MA).

**Statistical Analysis.** Mean values of Na\(^+\) and Cl\(^-\) removed by the plant species were calculated and the variance was analyzed with an ANOVA \((P < 0.05)\) using SAS (v. 9.0, SAS Institute Inc., Cary, NC). Regression analyses were used to identify the relationship between ion uptake and NaCl solution concentration using GraphPad Prism (v. 5.03; GraphPad Software Inc., La Jolla, CA).

**5.3. Results and Discussion**

*T. latifolia* and *S. tabernaemontani* removed more Na\(^+\) as the solution NaCl concentration increased. The *T. latifolia* and *S. tabernaemontani* grown in 2 mmol·L\(^{-1}\) both removed an average of 3.6 mg·g\(^{-1}\) of DW after seven weeks, and they both removed 10.7 and 10.8 mg·g\(^{-1}\) of DW, respectively, when grown in 4 mmol·L\(^{-1}\) (Fig. 5.1A and 5.1B). However, the *S. tabernaemontani* grown in 8 and 16 mmol·L\(^{-1}\) removed \(~2\times\) more Na\(^+\) than *T. latifolia*. This
contradicts our previous research (Chapter 4), which found *T. latifolia* was able to accumulate more Na\(^+\) than *S. tabernaemontani*. However, this may be due to the different growing conditions of these two experiments. In Chapter 4 the plants were grown in microcosms containing gravel but in this experiment the plants were grown hydroponically, and *T. latifolia* did not perform as well in this environment. The biomass production of *T. latifolia* in this experiment was lower than in microcosms (Chapter 4) and lower biomass production has been shown to result in less Na\(^+\) accumulation (Freedman et al., 2014; Chapter 4).
Fig. 5.1. Cumulative $\text{Na}^+$ accumulated (mg $\text{Na}^+$ / g of foliar dry weight) by $S$. tabernaemontani (A) and $T$. latifolia (B) after seven weeks in 2 L hydroponic systems with nutrient solutions containing different NaCl concentrations, 2, 4, 8, and 16 mmol·L$^{-1}$. Data are means ± SE ($n = 5$).

$T$. latifolia and $S$. tabernaemontani also removed more $\text{Cl}^-$ as the concentration of NaCl in solution increased. After seven weeks of growth in the lowest concentration of 2 mmol·L$^{-1}$,
the plant species removed statistically similar amounts ($P < 0.05$) of Cl $-7.7$ and $10.4 \text{ mg} \cdot \text{g}^{-1}$ of DW (Fig. 5.2A and 5.2B). In the higher concentrations, 4, 8, and 16 mmol·L$^{-1}$, $S.\tabernaemontani$ removed more Cl$-$ (29.4, 47.7, and 105.4 mg·g$^{-1}$ of DW, respectively) than $T.\latifolia$ (16.2, 27.6, and 53.5 mg·g$^{-1}$ of DW, respectively). The amounts removed by $S.\tabernaemontani$ at each concentration were $\approx 2 \times$ the amount of Cl$-$ removed by $T.\latifolia$ (Fig. 5.2A and 5.2B). This agrees with the results in Chapter 3 in which it was found that $S.\tabernaemontani$ removed more Cl$-$ from solution than $T.\latifolia$. Morteau et al. (2009) examined $T.\latifolia$’s Cl$-$ uptake capacity and found that it was able to accumulate more Cl$-$ than the $T.\latifolia$ plants in our experiment, with tissue contents of 63 mg·g$^{-1}$ of DW. This could again be due to the different growing conditions as Morteau et al. (2009) used microcosms rather than a hydroponic system.
Fig. 5.2. Cumulative Cl\(^{-}\) accumulated (mg Cl\(^{-}\) / g of foliar dry weight) by *S. tabernaemontani* (A) and *T. latifolia* (B) after seven weeks in 2 L hydroponic systems with nutrient solutions containing different NaCl concentrations, 2, 4, 8, and 16 mmol·L\(^{-1}\). Data are means ± SE (n = 5).
When considering the Na\(^+\) and Cl\(^-\) uptake of each species a clear trend was observed. The amount of Na\(^+\) and Cl\(^-\) removed after seven weeks was directly related to the concentration of NaCl in solution. For *S. tabernaemontani* the amount of Na\(^+\) removed when grown in NaCl concentrations of 2 mmol·L\(^{-1}\) was 3.6 mg·g\(^{-1}\) of DW, and at 4 mmol·L\(^{-1}\) it was 10.8 mg·g\(^{-1}\) of DW, 3.0× more. At 8 mmol·L\(^{-1}\) the removal was 2.6× more than at 4 mmol·L\(^{-1}\), and at 16 the removal was 3.4× more than it was at 8 mmol·L\(^{-1}\). Similarly, the amount of Na\(^+\) removed by *T. latifolia* at 4 mmol·L\(^{-1}\) was 2.9× more than at 2 mmol·L\(^{-1}\), at 8 mmol·L\(^{-1}\) the uptake was 1.6× more than at 4 mmol·L\(^{-1}\), and at 16 mmol·L\(^{-1}\) it was 2.6× more than at 8 mmol·L\(^{-1}\). The general trend is that the Na\(^+\) uptake was approximately 2.5–3.0× greater as the solution NaCl concentration doubled. Regression analysis revealed that the Na\(^+\) uptake by both species was linearly related to the NaCl solution concentration, for *S. tabernaemontani*: \(y = -15.6 + 0.15x, R^2 = 0.9721, P = 0.0140\); for *T. latifolia*: \(y = -2.5 + 0.35x, R^2 = 0.9842, P = 0.0079\).

A similar trend was observed when considering Cl\(^-\) removal. *S. tabernaemontani* removed 2.8× more Cl\(^-\) at 4 mmol·L\(^{-1}\) than it did at 2 mmol·L\(^{-1}\), 1.6× more at 8 mmol·L\(^{-1}\) than at 4 mmol·L\(^{-1}\), and 2.2× more at 16 than at 8 mmol·L\(^{-1}\). *T. latifolia* removed 2.1× more Cl\(^-\) at 4 mmol·L\(^{-1}\) than it did at 2 mmol·L\(^{-1}\), 1.7× more at 8 mmol·L\(^{-1}\) than at 4 mmol·L\(^{-1}\), and 1.9× more at 16 than at 8 mmol·L\(^{-1}\). Regression analysis revealed a similar linear relationship to the NaCl solution concentration, for *S. tabernaemontani*: \(y = -1.3 + 0.15x, R^2 = 0.9928, P = 0.0036\); for *T. latifolia*: \(y = -2.1 + 0.31x, R^2 = 0.9981, P = 0.0009\). This confirms that there is a relationship between the amount of ions accumulated by the plant and the solution NaCl concentration. The trend agrees with other findings in the literature that report that Na\(^+\)
and Cl− tissue contents of plants increase as solution NaCl concentrations increase (Glenn et al., 1999; Keiffer and Ungar, 1997; Kong and Zheng, 2014 Shelef et al., 2012).

The increase in accumulation is likely a result of the plants attempting to maintain an osmotic gradient at increasing NaCl concentrations by absorbing and storing the ions in their vacuoles. This allows plants to offset the solution concentration and continue to uptake water as needed (Tester and Davenport, 2003). This mechanism of survival is what makes these plants candidates for phytodesalinization. However, because the amounts removed by the plants were so closely correlated to the increase in concentration it would not be useful to allow for a solution to become more concentrated in an attempt to increase the phytodesalinization rates of the plants. The ratio of removal to concentration indicates that a similar percentage would be removed regardless of concentration.

An important characteristic to consider when assessing the efficacy of a potential Na+ and Cl− treatment method is the final concentration in solution. However, in all cases, regardless of the initial solution concentration, the final concentration increased (Table 5.1) even though the plants were able to consistently remove Na+ and Cl− at each concentration. When treating greenhouse nutrient solution the concentration is more important than the total Na+ and Cl− load in the water, suggesting phytodesalinization with these plant species may not be the best option.
Table 5.1. Percent change in Na\(^+\) and Cl\(^-\) concentrations and volume after seven weeks in 2 L hydroponic systems containing S. tabernaemontani and T. latifolia. The nutrient solution was supplemented with NaCl.

<table>
<thead>
<tr>
<th>Species</th>
<th>Initial NaCl Conc. (mmol·L(^{-1}))</th>
<th>Change in Na(^+) (%)(^a)</th>
<th>Change in Cl(^-) (%)</th>
<th>Change in Volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. tabernaemontani</td>
<td>2</td>
<td>14a</td>
<td>10a</td>
<td>-12a</td>
</tr>
<tr>
<td>S. tabernaemontani</td>
<td>4</td>
<td>11ab</td>
<td>9a</td>
<td>-13a</td>
</tr>
<tr>
<td>S. tabernaemontani</td>
<td>8</td>
<td>9ab</td>
<td>8a</td>
<td>-11a</td>
</tr>
<tr>
<td>S. tabernaemontani</td>
<td>16</td>
<td>6b</td>
<td>7a</td>
<td>-11a</td>
</tr>
<tr>
<td>T. latifolia</td>
<td>2</td>
<td>7b</td>
<td>6a</td>
<td>-10a</td>
</tr>
<tr>
<td>T. latifolia</td>
<td>4</td>
<td>6b</td>
<td>6a</td>
<td>-10a</td>
</tr>
<tr>
<td>T. latifolia</td>
<td>8</td>
<td>8b</td>
<td>8a</td>
<td>-11a</td>
</tr>
<tr>
<td>T. latifolia</td>
<td>16</td>
<td>8b</td>
<td>9a</td>
<td>-13a</td>
</tr>
</tbody>
</table>

\(^a\)Mean values (n = 5) followed by the same letter in a column are not significantly different at P < 0.05.

5.4. Conclusions

The increase in concentration could be caused by evapotranspiration (ET). The final volumes of the solutions were consistently 10–13% less than the initial volumes (Table 5.1).

Freedman et al. (2014) report similar findings. They assessed the phytodesalinization potential of B. indica and found that it accumulated Na\(^+\) but the final concentration in solution still increased. They therefore recommend managing ET rates by finding the ideal amount of the time to expose the water to the plants. Tilley et al. (2002) found that decreasing ET rates in CWs treating saline aquaculture effluent that were planted with T. latifolia resulted in lower salinities after treatment. Overall, due to the increase in final Na\(^+\) and Cl\(^-\) concentration we conclude that phytodesalinization is not the best option for the treatment of recycled nutrient solution. However, it may be possible to avoid this increase in concentration with proper management of ET.
6.0. General Conclusions

This thesis assessed the viability of using wetland plants to remove Na\(^+\) and Cl\(^-\) from a recycled greenhouse nutrient solution. Three experiments were conducted to examine the different aspects that could affect phytodesalinization. In the first component of the first experiment eight wetland plant species, chosen based on a review of the literature, were grown in CW microcosms with NaCl supplemented nutrient solution. The tissue Na\(^+\) and Cl\(^-\) contents of the plants were determined and it was found that *J. torreyi*, *S. tabernaemontani*, *T. angustifolia*, and *T. latifolia* were the best candidates for phytodesalinization. These four species were included in the second part of this experiment in which the total Na\(^+\) and Cl\(^-\) accumulated was quantified. *T. latifolia* and *S. tabernaemontani* accumulated the most and it was concluded that could be added to CWs to potentially enhance Na\(^+\) and Cl\(^-\) removal. However, we recommended further assessment in a longer-term experiment with larger microcosms.

The second experiment evaluated the effect of harvesting on wetland plants’ capacity to uptake Na\(^+\) and Cl\(^-\) from solution. *J. torreyi*, *S. tabernaemontani*, and *T. latifolia* were planted in CW microcosms outdoors and exposed to a NaCl supplemented nutrient solution for 18 weeks during the summer months of 2013. Three harvesting schedules were assigned, 0, 1, and 2 harvest(s) throughout the growing season. It was determined that both *J. torreyi* and *T. latifolia* responded well to harvesting (1 or 2 times) and an increase in biomass production was observed. This increase in biomass production resulted in more Na\(^+\) and Cl\(^-\) removal. Harvesting had no effect on *S. tabernaemontani’s* biomass production or ion
accumulation. Overall, it was concluded that harvesting is able to increase the Na\textsuperscript{+} and Cl\textsuperscript{−} uptake of \textit{J. torreyi} and \textit{T. latifolia} by increasing their biomass production.

The third and final experiment examined the effect of different NaCl solution concentrations on \textit{S. tabernaemontani} and \textit{T. latifolia}\textquotesingle s phytodesalinization capacity. The plants were grown hydroponically for seven weeks in four NaCl concentrations, 2, 4, 8, and 16 mmol·L\textsuperscript{−1}. Both plant species removed more Na\textsuperscript{+} and Cl\textsuperscript{−} in the higher concentrations and a correlation between uptake and concentration occurred. However, the final solution concentrations were higher after exposure to the plants even though the plants were removing Na\textsuperscript{+} and Cl\textsuperscript{−} which could be caused by the water loss to ET.

The overall conclusions of this thesis research are:

1. The uptake of both Na\textsuperscript{+} and Cl\textsuperscript{−} is related to biomass production and increasing biomass production will increase Na\textsuperscript{+} and Cl\textsuperscript{−} removal.
2. Harvesting, whether once or twice in the growing season, can increase the biomass production of \textit{J. torreyi} and \textit{T. latifolia}.
3. Phytodesalination by the selected plant species is not a viable option for the treatment of recycled greenhouse nutrient solution due to the increase in final concentration.
4. These plants were able to reduce the overall Na\textsuperscript{+} and Cl\textsuperscript{−} load, however, and they should be included in treatment systems in which the water is discharged, rather than reused.
6.1. Looking Forward

To fully satisfy all the questions posed by this thesis further research would be needed. It would be interesting to consider using leaf area index to represent the area of plant growth, rather than the basal area, which was used in this thesis. When considering harvesting plant biomass from a CW it would be useful to have a system to determine when it would be most appropriate to perform the harvests based on plant growth. In our research the harvest schedule was predetermined but with a more in depth look into the plants’ growth rates and responses to harvests the performance could be improved. A system using accumulated temperature, such as degree days, a measurement commonly used in agriculture, could indicate when the plants would be ready for harvest. It would also be useful to examine the distribution of Na\(^+\) and Cl\(^-\) in the foliar tissue of the plant. This information could also help determine the ideal timing and methods for harvesting. The long-term effect of repeated harvests over the years could impact the natural the life cycle of the plant and would need to be considered.

The next step would be to build and assess the performance of a full-sized system. The sizing of CW system could be calculated using the ion uptake of a plant per unit area, as determined by this research, however, many variables need to be considered. Therefore, the most appropriate technique would be to calculate the first order rate constants using the following equation:

\[
k_a = -q \ln \left[ \frac{C_{out} - C^*}{C_{in} - C^*} \right]
\]
where $k_a$ is the first order area-based plug flow rate constant (m yr$^{-1}$), $q$ is the hydraulic loading rate (m yr$^{-1}$), $C_{out}$ is the outlet concentration (mg L$^{-1}$), $C_{in}$ is the inlet concentration (mg L$^{-1}$), and $C^*$ is the background concentration (mg L$^{-1}$; Jamieson et al. 2007). This type of calculation did not fall within the scope of this thesis but it is important to highlight what is required when designing a full scale CW treatment system.
7.0. References


Tiner, R.W. 2009. Field guide to tidal wetland plants of the northeastern United States and neighboring Canada: vegetation of beaches, tidal flats, rocky shores, marshes, swamps, and coastal ponds. University of Massachusetts Press, Amherst, MA.


