TREATMENT OF SALINE WASTEWATERS AND THE REMEDIATION OF SALINIZED SOILS USING NATIVE HALOPHYTIC PLANTS

LA TRAITEMENT D'EAUX USÉES SALINES ET L'ASSAINISSEMENT DES SOLS SALINISÉS AVEC DES PLANTES INDIGÈNES HALOPHYTIQUES

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ABSTRACT

Amélie Anne Steeves Litalien. MSc., Environmental Science. Royal Military College of Canada. October, 2019. Treatment of Saline Wastewaters and the Remediation of Salinized Soils using Native Halophytic Plants. Supervisors: Dr. Barbara Zeeb and Dr. Allison Rutter.

Soil salinization is a pressing issue worldwide affecting native ecosystems and croplands. As climate change can hasten the process of soil salinization, there is a growing need for sustainable methods to manage soil salinity. Some soils become saline due to natural processes but many are the result of anthropogenic activities. For example, leachate from a cement kiln dust (CKD) landfill containing concentrated amounts of potassium chloride has led to the salinization of a wetland region in Bath, ON, the study site in this thesis. Thus, the use of the accumulator halophyte *Salicornia maritima* was first investigated for its ability to survive and extract salts when watered with CKD leachate containing high concentrations of potassium chloride. It was determined that *S. maritima* could survive when watered with leachate up to 2X the average chloride concentration of chloride while accumulating up to 25% of its dry biomass as chloride. Halophytic accumulator plants are suitable in regions where frequent harvesting is feasible, however, recretohalophytes offer the ability to passively phytoextract salts by haloconduction, making the remediation of soils at more remote sites possible. Four recretohalophytes, *Atriplex canescens*, *Armeria maritima*, *Spartina pectinata,* and *Distichlis spicata* were evaluated in a greenhouse setting to determine their salt excretion capacities. *A. maritima, S. pectinata,* and *D. spicata* were deemed suitable for haloconduction, however *A. maritima* would be best suited in highly saline soils (≥4000 μg/g) since below this concetration, it relied primarily on salt accumulation. *S. pectinata* achieved the most consistent and had the highest salt excretions even when grown in soil below 4000 μg Cl^-/g. As remediation via haloconduction is still a novel procedure, the first model to quantify and visualize salt extraction from a site via haloconduction was created next. A simple estimation of salt emission from *S. pectinata* was generated based on greenhouse and wind tunnel studies. It was combined with aerial dispersal modeling using AERMOD to visualize salt dispersal from the study site located in Bath, ON. The model demonstrated that if the site, a 1000 m^2 wetland affected by potassium chloride leachate with average soil chloride concentrations of 4000 μg/g, was planted entirely with *S. pectinata,* site remediation could be achieved in approximately 2-4 years without negatively impacting the surrounding environment as deposition rates remain below background levels. These were the first studies to evaluate *S. maritima*'s ability to extract potassium chloride from leachate, and develop a framework to assess recretohalophytes' ability to phytoremediate soil. The findings of this thesis demonstrate that phytotechnologies can be effective tools for soil salinity management and that haloconduction can provide rapid site remediation without negatively impacting the surrounding environment.

Key Terms: Soil Salinization, Phytoremediation, Recretohalophytes, Haloconduction

RÉSUMÉ

Amélie Anne Steeves Litalien. MSc., Sciences de l'environnement. Collège Militaire Royal du Canada. Octobre, 2019. Enquête sur la traitement d'eaux usées salines et l'assainissement de sols salinisés avec des plantes indigènes halophytiques. Directrices : Dr. Barbara Zeeb and Dr. Allison Rutter.

La salinisation du sol est un problème global qui affecte plusieurs écosystèmes et terres agricoles. Puisque le changement climatique peut accélérer le problème, il existe un besoin pour des méthodes durables qui aident à gérer la salinité des sols. Les sols peuvent devenir salins à cause de phénomène naturel, mais plusieurs sont le résultat d'activités anthropiques. Par exemple, une zone humide située à Bath, ON a été salinisée par du lixiviate d'un entrepôt de poussière de four à ciment qui contient des concentrations hautes en chlorure de potassium. La survie et la capacité d'extraire les sels de l'halophyte accumulateur *Salicornia maritima*, ont donc été étudiées pendant que les plantes ont été arrosées avec ce lixiviat. *S. maritima* a pu survivre lorsqu'elle était arrosée avec du lixivicate contenant jusqu'à 2X le taux moyen de concentration de chlorure et a accumulé jusqu'à 25% de sa biomasse sèche en forme de chlorure. Les halophyte accumulateurs sont surtout utiles là où les récoltes fréquentes sont possibles. Cependant, les récrétohalophytes offrent la capacité de phytoextraire les sels passivement par l'haloconduction, ce qui permet l'assainissement des sols aux sites isolés. Quatre récrétohalophytes, *Atriplex canescens*, *Armeria maritima, Spartina pectinata* et *Distichlis spicata* ont été évalués en serre afin de déterminer leurs capacités d'excrétion de sel. *A. maritima, S. pectinata,* et *D. spicata* seraient utiles pour l'haloconduction, mais *A. maritima* serait seulement appropriée là où la salinité du sol est très élevée; quand elle a poussé dans un sol avec moins de 4000 µg/g chlorure, elle a plutôt sur accumulé du sel dans ses tissus. Cependant, le taux d'excrétion de chlorure de *S. pectinata* a augmenté graduellement alors que la concentration de chlorure dans le sol a augmenté. Cette espèce était la plus consistante et avait l'excrétion la plus élevée lorsqu'elle était cultivée dans du sol avec moins de 4 000 µg Cl^-/ g. L'assainissement des sols par l'haloconduction est encore une procédure nouvelle, donc le premier modèle permettant la quantification et la visualisation de l'extraction de sel par l'haloconduction, a été créé. Ce modèle numérique simple d'assainissement par *S. pectinata* était basé sur des études en serre et en soufflerie. Il a été combiné avec un modèle de dispersion aérienne AERMOD, pour visualiser la dispersion du sel du site d'étude situé à Bath, ON. Le modèle a démontré que si le site était entièrement planté de *S. pectinata*, il pourrait être assaini en 2 à 4 ans sans impact négatif sur le milieu environnant. Ce sont les premières études à évaluer la capacité de *S. maritima* à extraire le chlorure de potassium des lixiviats et à produire un modèle permettant d'évaluer la phytorémédiation du sol par des récrétohalophytes. Les résultats de cette thèse démontrent que les phytotechnologies peuvent être des outils efficaces pour la gestion de la salinité des sols et que l'haloconduction peut permettre une restauration rapide de sites sans impacts négatifs sur l'environnement.

Mots-clés : Assainissement du sol salinisé, Récrétohalophytes, Haloconduction

CO-AUTHORSHIP STATEMENT

The student was the primary researcher and primary author for all the chapters of this thesis. The student was responsible for the design, implementation and analyses described in chapters 3, 4, and 5. William D. Raymond, a co-op student in the Zeeb lab in the Winter of 2019, made significant contributions in the design and implementation of the wind tunnel studies outlined in chapter 5 and is listed as a co-author for that chapter. Dr. Barbara A. Zeeb, and Dr. Allison Rutter provided supervision throughout the projects and provided edits and feedback on all chapters of this thesis.

Chapter 3: Evaluating the phytoremediation potential of the accumulator halophyte *Salicornia maritima* for the treatment of saline leachate.

Chapter 4: Evaluating the impact of soil chloride concentration and salt type on the excretions of four recretohalophytes with different excretion mechanisms.

Chapter 5: Development of a Model for the Dispersal of Salts from Recretohalophytes

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LIST OF ABBREVIATION

AERMET	AERMOD meteorological processor
AERMOD	Aerial dispersal model
ANOVA	Analysis of variance
ASU	Analytical Services Unit
ATP	Adenosine tri-phosphate
CALPUFF	Puff air dispersion model
CAM	Crassulacean acid metabolism
CAN	Canada
CFM	cubic feet per minute = 0.0283 m^3/min
CKD	Cement kiln dust
CRM	Certified reference material
DDI	Double-de-ionized water
DI	De-ionized water
DW	Dry weight
EC	Electrical conductivity
ECCC	Environment and Climate Change Canada
FAO	Food and Agriculture Organization of the United Nations
GAM	Generalized additive model
HiVol	High volume air sampler
HPLC	High throughput liquid chromatography
IC	Ion chromatography
MECP	Ministry of Environment, Conservation, and Parks (Ontario)
MEGAN	Model of emissions of gasses and aerosols from nature
NAVCAN	Navigation Canada
NRC	Natural Resources Canada
ON	Ontario
PC	Principle component
ppm	parts per million
QA/QC	Quality assurance/Quality control
QC	Quebec
R^2	Coefficient of determination
RH	Relative humidity
RMC	Royal Military College of Canada
rpm	Rotations per minute
SAR	Sodium absorption ratio
SD	Standard deviation
SICS	Soil improving cropping systems
STaMPS	Simulator of the timing and magnitude of pollen season
TDS	Total dissolved solids
TFV	Threshold friction velocity
US	United States
US EPA	United States Environmental Protection Agency
USDA	United States department of Agriculture
VOC	Volatile organic compounds
WW	Wet weight

1 INTRODUCTION

Throughout the world, an increasing number of soils are becoming saline, in a process known as soil salinization. Saline soils occur naturally, often in regions where saline bodies of water have dried up, where coastal breezes carry salts inland, and in areas with saline groundwater (Matternicht & Zinck, 2008). Simultaneously, harmful anthropogenic activities such as unsustainable agriculture and oil extraction, are driving the rates of soil salinization to concerning levels (Mahajan & Tuteja, 2015). Soil salinization results in significant damage to agricultural lands worldwide and threatens our ability to produce sufficient crops. Abiotic stresses are the number one cause of crop losses worldwide and high soil salinity is among the major contributing factors (He et al., 2018).

While low concentrations of salts are relatively benign, high soil salinity presents a major challenge to most plant life as well as to many detritivores and soil microbes (East et al., 2017; He et al., 2018; Kefford et al., 2011;). Soil salinization can impact nutrient cycling and carbon storage within soils (Baldwin et al., 2006, Setia et al., 2013). As soils become increasingly saline, organisms have greater difficulty surviving, which can further reduce soil quality, effectively creating a positive feedback loop. This process can be further exacerbated by climate change as temperatures and hydrological cycles shift. Thus, a major threat of soil salinization in many regions is desertification (Amezketa, 2006). Historically, soil salinization and resulting agricultural failure has been associated with the collapse of several societies, thus rising soil salinity must be addressed earnestly (Jacobsen & Adams, 1958; Shahid et al., 2018).

There is growing attention on the subject of soil and freshwater salinization, as the leaching of salts into freshwater bodies can have a significant impact on freshwater species. Freshwater fishes and invertebrates can suffer significant stress due to osmotic shifts which may result in depression of species richness and diversity (Dowse et al., 2017; Herbert et al., 2015; Kefford et al., 2011). Osmotic stress also impacts soil microbes and invertebrates. One of the most visually obvious impacts of soil salinization is the loss of flora that occurs at many salinized sites. High soil salinity presents a particular challenge to plant life as it can induce a drought like state by shifting the osmotic gradient in the soil (Deinlein et al., 2014; Flowers et al., 2015). Many of the ions responsible for soil salinization, including sodium and chloride can induce ion toxicity within plant cells (Bromham et al., 2013; Marschner, 2012). Ninety-eight percent of plant species are considered salt sensitive including most common crop species (Flowers et al., 2008).

However, not all species struggle to survive in saline environments. Halophytes are plants adapted to cope with salinity stress to the extent that they can grow as well in saline soil as non halophytic plants do in arable soil (Flower et al., 2015). These types of plants can employ several methods to manage both drought stress and ion toxicity, including: i) metabolic changes such as the production of protective proteins and prevention of the entry of ions into the roots, ii) the sequestration of harmful ions in the central vacuole of cells, or even iii) the excretion of salts through specialized glands on the leaf surface. While not all of these mechanisms may be used by one plant species, they all allow the plant to manage harmful salts.

Conventional strategies for soil salinity management generally involve a more careful use of irrigation and fertilizers, and treatment often relies on the leaching of salts with large amounts of high quality fresh water (Cuevas et al., 2019). As this approach is not possible in all areas due to a lack of resources or the presence of a high water table, the use of halophytic plant species for the extraction of salts from soil is of growing interest. This method falls into the category of phytoremediation, or the use of plants to extract, stabilize or degrade harmful compounds in the soil. Halophytes can be used to remove salts from the soil without disturbing the soil ecosystem (Jesus et al., 2015). Plants that accumulate salts within the central

vacuoles of their cells can be grown, and their above-ground tissues harvested. This concentrates the salt into a manageable and transportable volume that can also be repurposed (Yun et al., 2019a). Plants that excrete salts can be used to dilute salts by dispersing them into the air column.

Many different plant species have been investigated for their ability to extract salts from soil (Jesus et al., 2015; Krishnapillai & Ranjan, 2005; McSorley et al., 2016). These investigations have been relatively successful but largely hinge on the economics of the situation. Hasanuzzaman (2014) has proposed lists of species that could be repurposed as fodder, for medicinal use or biofuel, etc. While most authors have focused on the treatment of salinized soil, a few have focused on the filtration of brine wastes with semiaquatic halophytes (Farzi et al., 2017). These could be used to treat the waste water from aquaculture systems, mine wastes, runoff etc. The vast majority of these studies focus on sodium chloride and major nutrients such as phosphorus and nitrogen which could contribute to algal blooms (Webb et al., 2012). Few studies have examined the use of plants to treat other saline wastes that could impact soil salinity such as potassium chloride or sulphate salts.

The use of recretohalophytes for remediation purposes is relatively new. Many authors have examined recretohalophytes from a biological perspective and have investigated the role that salt secretion can play in salinity tolerance, but not as a mechanism for soil remediation (Dassanyake & Larkin, 2017). Preliminary work by Sargeant et al. (2008) and McSorley et al. (2016) suggested that recretohalophytes may have exceptional salt extraction capacities and that they may be useful in site remediation. The theory of haloconduction proposed by Yensen and Biel (2008) suggests that salts excreted by recretohalophytes could be transferred into the air column by wind action and dispersed over large distances, effectively diluting the salts. Yun et al. (2019b) demonstrated that emission of salt particles from recretohalophytes is likely possible. However, due to the complexity of the system and site-specific nature of application, it may be difficult to determine when the use of recretohalophytes is appropriate simply based on extraction capacities. As suggested by Litalien & Zeeb (2020), local modelling and aerosol monitoring is likely necessary to confirm applicability at a site specific level.

This Master's thesis contains the first study to investigate the use of a semi-aquatic accumulator halophyte for the treatment of potassium chloride (KCl) rich leachate, as well as the first model for the quantification and prediction of salt dispersion for remediation via recretohalophytes and haloconduction. Following this introduction, chapter two includes the published review entitled "Curing the earth: A review of anthropogenic sources of soil salinization and plant-based strategies for sustainable mitigation" to provide background on the topic of soil salinization and remediation via halophytes. In the subsequent three chapters, remediation of saline leachate by an accumulator halophyte, and remediation of salinized soil by recretohalophytes is explored. In order to address both topics, a wetland field site at a cement plant in Bath, ON was used as it is affected by leachate from cement kiln dust, a by-product of cement manufacturing rich in potassium chloride. The third chapter examines the leachate itself by quantifying the chloride input rates into the site from the leachate drainage pipe. Chapter three also includes a greenhouse experiment using leachate collected from the site, to assess if the native accumulator halophyte *Salicornia maritima* could be used in a remediation design for the extraction of chloride from saline wastes with KCl. The site is also used as a case study for the implementation of recretohalophytes and model development. The fourth chapter examines the excretion capacities of four Canadian recretohalophytic species selected based on their gland type when grown in greenhouse conditions in soil collected from the Bath, ON field site. Excretion rates are examined and the context under which these plants could be applied is explored. Chapter five then discusses the development of a model to quantify the amount of chloride that could be extracted and dispersed by the recretohalophyte *Spartina pectinata* on site by haloconduction. This model provides the framework to assess whether haloconduction may be

suitable at any given field site. Finally, chapter six synthesizes the major findings of this thesis and suggests future areas of research and applications.

2 LITERATURE REVIEW

Curing the earth: A review of anthropogenic soil salinization and plant-based strategies for sustainable mitigation.

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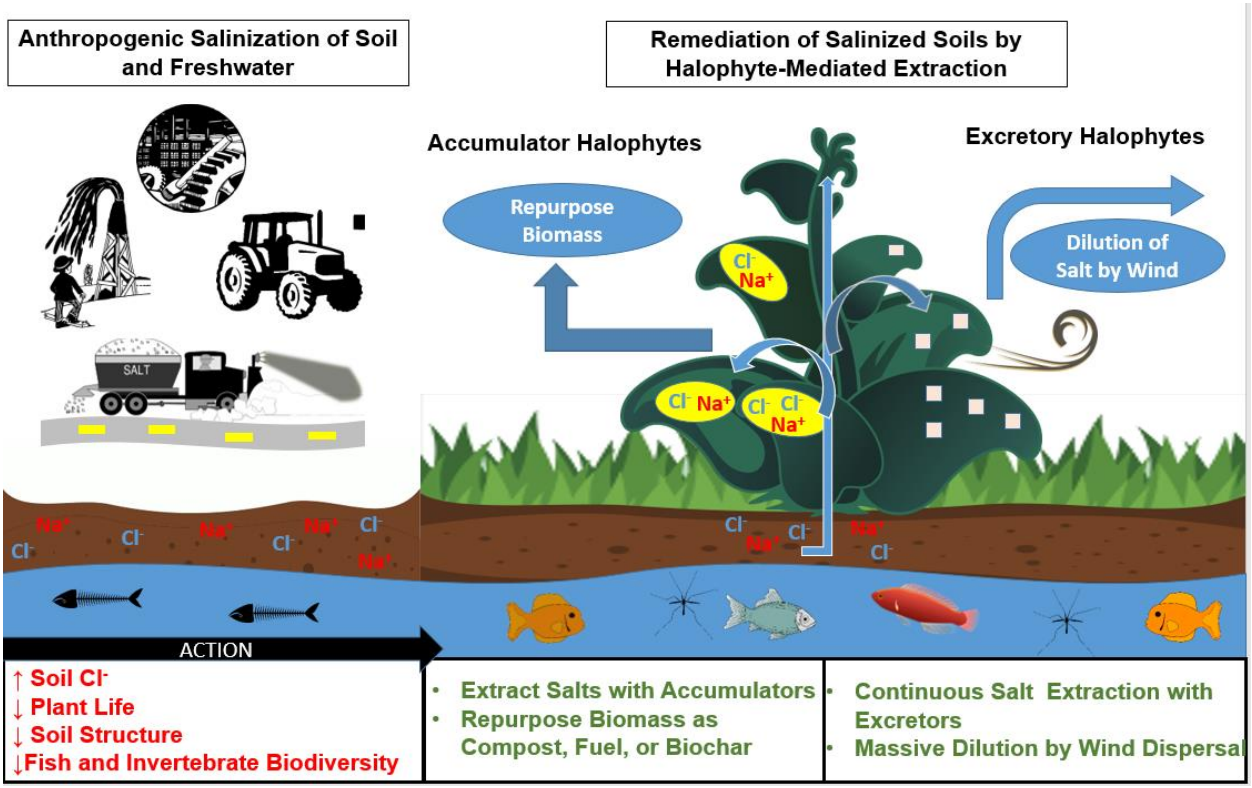
2-1 HIGHLIGHTS

- Sources of anthropogenic soil salinization include unsustainable agricultural practices, road salting, industrial wastes, and petroleum extraction
- Soil salinization has a negative impact on soil structure and quality which can in turn hinder plant growth and induce a drought-like state or ion toxicity
- Halophytic plants that sequester or secrete salts may be used to extract salts from soil
- While the use of accumulator halophytes requires harvesting of biomass and economical disposal or repurposing, excretory halophytes rely on wind dispersal of salts to dilute them below background levels allowing for passive remediation

2-2 ABSTRACT

At low concentrations salts are relatively benign, but anthropogenic activities can drive concentrations to levels that impact soil quality, microbial, plant, and animal life. Soil and freshwater salinization are growing issues worldwide that are difficult to manage with conventional treatments. In this review, salt tolerant plants known as halophytes are evaluated for their potential to phytoremediate salinized soils and prevent leaching of salts into surface and ground water. While most plants are sensitive to high concentrations of salt in their growth media, halophytic plants have developed mechanisms to tolerate and thrive in these environments. Some plants exclude salts at the roots, others sequester salts in their central vacuole, while others secrete salts through specialized salt glands on their leaf surfaces. The extraction of salts from soil by both plants that sequester or secrete salts are reviewed as well as implementation strategies that could drive economic feasibility. Further, phytoremediation of salinized soils is considered in the context of a changing climate.

2-3 GRAPHICAL ABSTRACT



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KEYWORDS

Soil Salinization, Remediation, Sodium Adsorption Ratio, Halophyte, Recretohalophyte

2-4 INTRODUCTION

The impacts of soil and freshwater salinization are widespread and substantial. As a growing number of agricultural lands and ecosystems are affected, greater focus is being allocated to this issue, particularly within the scientific community. There has been a 5-fold increase in publications on this subject since 2004 (Sup. Figure A-1). Salt stress is one of the most important factors contributing to crop losses world-wide. Based on soil surveys conducted between 1970 and 1980, the Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 6.5% of the world's arable and marginal soils are either saline or sodic (Table 2-1) (FAO, 2016). Nelson & Mereida (2001) estimated that 12 million hectares of irrigated agricultural land may no longer be in use as a result of soil salinization. While some countries such as Australia have conducted recent soil assessments, modern data on global soil salinization is limited. However, it is estimated that more than 50% of current croplands may be lost within the 21st century to soil salinization (Mahajan & Tuteja, 2005).

Table 2-1: Global incidence of saline and sodic soils worldwide (1970-1980) (FAO, 2016).

Continent	Area (per million hectares)				
	Total Area	Saline Soils	%	Sodic Soils	%
Africa	1899.1	38.7	2.0	33.5	1.8
Central, Pacific Asia and Australia	3107.2	195.1	6.3	248.6	8.0
Europe	2010.8	6.7	0.3	72.7	3.6
Latin America	2038.6	60.5	3.0	50.9	2.5
Near East	1801.9	91.5	5.1	14.1	0.8
North America	1923.7	4.6	0.2	14.5	0.8
Total	12781.3	397.1	3.1	434.3	3.4

While soil salinization can reduce crop productivity, intensive agricultural practices can themselves be a source of rising soil salinity. Modifying practices to reduce the risk or rate of salinization can improve outcomes, but managing salinized agricultural lands once established, is difficult and often results in land abandonment (Cuevas et al., 2019). Other industries such as oil extraction and cement manufacturing, as well as road maintenance by salting are also associated with soil salinization. Proper disposal of wastes can reduce soil salinization. However, historically salts have not been considered hazardous wastes and disposal sites can be sources of concentrated salts (Golder Associates, 2013). In the case of road salting, the implication of public health and safety combined with a lack of other viable options means that the environmental risks are legally overruled by the safety hazards (The Municipal Act, 2001 (Section 44(1))). Thus there is a need for reliable methods to manage soil salinity in a diversity of locations and climates.

High soil salinity poses a threat not only to agriculture, but also to the function of many ecosystems. Most plants cannot tolerate high concentrations of salts in their growth media and fail to complete a life cycle in these conditions. Plant matter is a source of nutrients to many riparian communities. Decreased plant matter, as well as leaching of salts into freshwater systems, can decrease the diversity and richness of detritivores, macroinvertebrates, and fish (Kefford et al., 2011; Dowse et al., 2017; East et al., 2017). As a result, nutrient cycling can be impacted. Due to significant losses of plant growth, soil organic carbon stocks decrease dramatically in salinized soils. On average, saline soils have lost 3.47 tonnes of soil organic carbon per hectare since they became saline. This has implications not only for measures of land fertility, but is also relevant to global climate change as soil organic carbon acts as a carbon sink (Setia et al., 2013).

Halophytic plants, which make up less than 2% of all terrestrial flora, have adapted to survive in saline conditions (Flowers et al., 2008). Adaptation mechanisms of particular interest include the sequestration of salts inside the central vacuole of cells, and excretion of salts through specialized glands at the leaf surface. Plants that can sequester or excrete salts may be utilized for long-term reclamation of salt impacted sites.

This review examines phytoremediation options as a means of addressing soil salinization. Impacts of soil salinization on plants are examined in detail to provide context for a discussion regarding tolerance mechanisms employed by plants to survive in saline soils. Possible implementation strategies for halophyte-driven phytoremediation are explored while considering the life cycle of the remediation system. The integration of plants into remediation design can provide low cost salt extraction while minimizing soil degradation. The benefits of incorporating appropriate plants can also extend beyond initial remediation plans. For example, the generation of useable by-products include biochar, fodder, and compost. There are also meaningful advantages for the integration of plants into remediation strategies such as mitigating climate change through carbon storage in plant matter and the opportunity to create habitat for native species.

2-5 SOIL SALINIZATION

Soil salinization is the accumulation of salts in soil to the extent that it limits plant growth. It is an issue of growing importance affecting more than 10 million square kilometers of land worldwide (Rengasamy, 2006; Metternicht & Zinck, 2003). Soil salinity is attributable to the presence of inorganic solutes, primarily alkali and alkali earth metals such as sodium and calcium, and associated anions: chloride, sulfate, and carbonate (Sparks et al., 1996). Sodic soils refer to those soils that are particularly rich in sodium compared to the calcium and magnesium content (Bresler et al., 1982).

In 1954, the United States Salinity Laboratory established classification guidelines for saline and sodic soils that remain the standard today (Scianna, 2002). Soil salinity is approximated by the electrical conductivity of a saturated paste (EC_e) measured in decisiemens per meter (dS/m), as it translates to the number of ions dissolved in the soil water (Rhoades, 1982). The sodium adsorption ratio (SAR) is calculated from cation concentrations in the soil water to determine the relative concentration of sodium to calcium and magnesium (Eq 2-1). Together, the EC_e and SAR

determine whether a soil is sodic, saline, or saline-sodic (Table 2-2). While low concentrations of salts are relatively benign, high concentrations can have detrimental effects on soil quality and pose a challenge to the growth of many plant species. According to the standard set by the Ministry of Environment Conservation and Parks (MECP), background EC_e should not exceed 0.57 and the SAR should not exceed 2.4. Remediation standards for EC_e and SAR are 1.4 and 12, respectively when the site is >30 m from a body of water, and 0.7 and 5, respectively when the site is within 30 m of a body of water (MECP, 2017) (Table 2).

Eq. 2-1 $$SAR = \frac{[Na^+]}{\left\{\frac{[Ca^{2+}]+[Mg^{2+}]}{2}\right\}^{1/2}}$$

Table 2-2: Classification of saline and sodic soils (US, 1954; MECP, 2017).

Classification	ECe (dS/m)	SAR
Saline	>4	<13
Saline-Sodic	>4	>13
Sodic	<4	>13
MECP Guidelines		
Background (upper limit)	0.57	2.4
Site Condition Standard	1.4	12
Site Condition Standard within 30 m of water	0.7	5

2-5.1 Sources of Soil Salinization

Soil salinization can occur in many areas as a result of both natural and anthropogenic processes (Ding et al., 2011). Natural means include the deposition of weathered minerals with high salt content, sea breeze deposition, and the capillary rise of saline groundwater in regions with a low water table (Matternicht & Zinck, 2008). Anthropogenic sources include unsustainable agricultural practices, road salt application, and industrial activities that hasten or cause an increase in soil salinity (Rengasamy, 2006).

2-5.1.1 Anthropogenic Source of Soil Salinization: Agriculture

While modern irrigation has allowed for increased plant productivity, the methods with which it is applied can lead to the buildup of salts in agricultural soils. In many arid regions, irrigation waters are applied in quantities such that they only reach the root zone and leach no further. Small amount of salts in the water can build up over time and lead to high soil salinity (Greene et al., 2016). Conversely, excessive irrigation can lead to water table rise and consequently capillary rise of saline groundwater. The extensive use of chemical fertilizers can also contribute to agricultural soil salinization (Endo et al., 2011). Soil salinization is a contributing factor in crop failure and is thus an issue for global food security.

2-5.1.2 Anthropogenic Source of Soil Salinization: Road Salt

Salt, predominantly sodium chloride (NaCl), is applied to roadways to reduce the freezing point of water and help prevent automobile collisions and injuries. However, the application of road

salts in cold climates is associated with soil and freshwater salinization (Chernousenko et al., 2003; Daley et al., 2009). Throughout the winter, salts applied to roads can migrate to roadside soil and accumulate on the frozen ground or snow pack. As temperatures rise, snow melts which may carry away the salts to nearby water bodies. Church & Friesz (1993) reported that up to 55% of applied road salts end up in surface water bodies. The salt that doesn't run off can accumulate in the soil once it has thawed. However, salts are highly soluble and may eventually leach into groundwater. This is particularly a concern with chloride as it doesn't associate as readily with soil particles as cations. Robinson et al. (2017) reported that salts can continue leaching from roadside soils for 2.5-5 months following deposition, depending on soil texture and type. Demand for road salt has been increasing since the 1940s which has important implication for terrestrial life and aquatic health (Daley et al., 2009).

2-5.1.3 Anthropogenic Source of Soil Salinization: Oil Extraction

Most oil deposits originally formed in marine environments and some saline water remains intermixed with the oil (Akinwumi, 2014). Extracted crude oil contains small droplets of saline water that is removed by desalting to improve the quality of the oil (Al-Haddabi & Ahmed, 2007). The saline water is often recycled for extraction, but eventually requires disposal. Mineral salt content of crude oil can be as high as 200 000 ppm (Mohamed et al., 2003). The high salinity of resulting extraction brine and saline tailings can present challenges to site remediation (McFarland et al., 1987; Leung et al., 2003).

2-5.1.4 Other Potential Anthropogenic Source of Soil Salinization

Other major sources of saline wastes include industrial wastes from mining activities, paper and pulp production, power generation, cement manufacturing, and steel production (Morudu, 2009; Muller et al, 2009). Aquaculture, food production, and textiles can also produce large quantities of saline wastes. While many of these industries have processes in place to manage saline waste production, there is also the potential for environmental release which could result in soil or freshwater salinization.

2-6 IMPACTS OF SOIL SALINITY

2-6.1 Soil Structure

Sodic soils are usually hard with a cloddy structure as a result of sodium's interaction with soil particles (Seelig, 2000). Monovalent cations are attracted to a single negatively charged clay particle within soils, but repel one-another. In contrast, divalent cations can interact with two clay particles, pulling them together. Soil colloid dispersion occurs as a result of repulsive forces in soils dominated by Na^+ and K^+ (Figure 2-1A). When both +1 and +2 cations are present, clay particles in the soil both disperse and flocculate which supports soil pore stability and improves air and water movement through the soil (Figure 1-B). Soil dominated by Ca^{2+} and Mg^{2+} however, tend towards flocculation (Figure 2-1C). Since sodic soils are dominated by +1 charged cations, clay particles are particularly dispersed which reduces pore size and can lead to slaking, eliminating macropores and reducing water infiltration (Sparks et al., 1996; Seelig, 2000). Hence,

high sodium and potassium content in soils has meaningful impacts on soil-water relations leading to erosion, low field capacity, and loss of plant life.

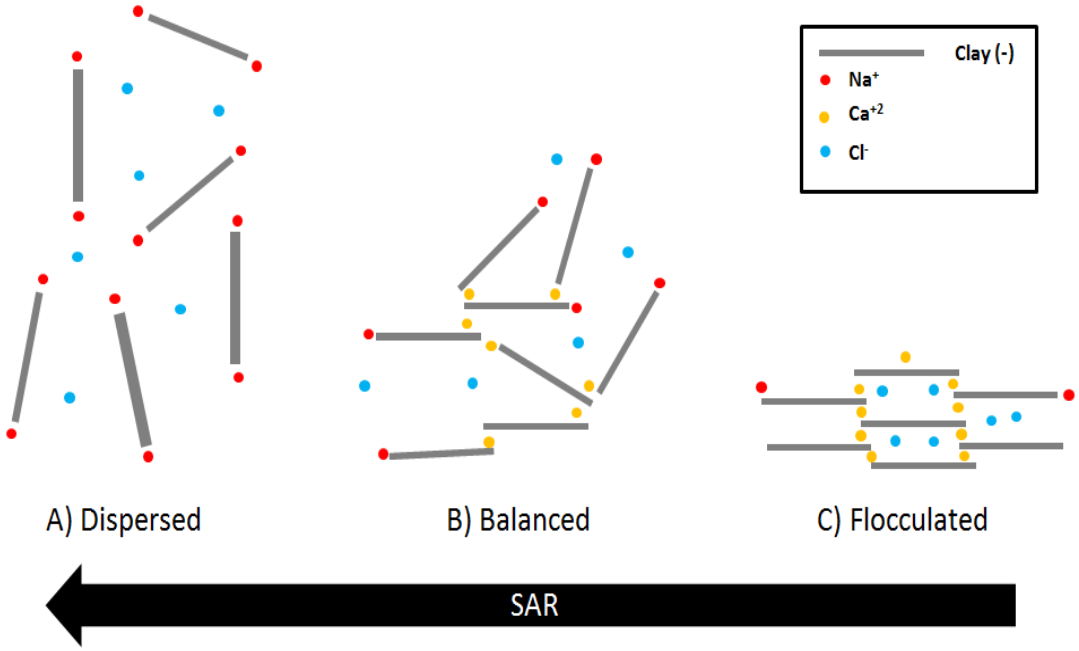

Figure 2-1: Cation interactions with soil colloids (Modified from Rengasamy et al., 1984) (A) Clay dispersion in soils where sodium makes up greater than 15% of all soil cations. (B) Soil aggregates created by a balance of mono and divalent cations (C) Clay flocculation in soils dominated by divalent cations such as calcium.

2-6.2 Impacts on Plants

The disperse nature of sodic soils hinders root penetration. The loss of soil pores also reduces root access to water and oxygen (Rengasamy et al., 1984). While purely saline soils may not impart the same soil structural challenges, osmotic stress and ion toxicity can inhibit seed germination, stunt growth and kill plants (Orlovsky et al., 2016; Bromham et al., 2013).

2-6.2.1 Osmotic Stress

Salt and water concentrations within different tissues are manipulated to maintain proper cellular function (Deinlein et al., 2014). Water travels through root cells until it reaches the xylem. Once in the xylem, the water is carried upwards by the negative pressure of transpiration at the leaf surface and capillary action (Campbell & Reece, 2008). The first step in water uptake relies on an osmotic gradient. Water moves into the plant as a result of the higher osmotic potential inside the root compared to the surrounding soil. In saline soil this osmotic gradient is reduced or reversed and water does not move into the roots as readily (Deinlein et al., 2014). The inability to take up water and even to draw water out of roots induces a state of drought, hindering osmoregulation.

At a cellular level, this can lead to proteins misfolding as many require a hydration shell to fold and function properly (Flowers et al., 2015). At a whole plant level, the inability to access water is also an issue as it is necessary for the maintenance of structural integrity. Under optimal conditions, the vacuole of a plant cell is filled with water. Swelling of the organelle applies positive pressure against the cell wall, a force known as turgor pressure (Campbell & Reece, 2008). When water levels within the plant decrease, fluid is lost from the vacuole and the plant wilts. Such drought-like conditions induce signaling pathways that cause plants to accumulate salts in order to readjust the osmotic gradient and draw water inwards (Deinlein et al., 2014). High intracellular concentration of incompatible solutes such as salts are an issue as they can interact poorly with proteins and their accumulation can lead to ion toxicity.

2-6.2.2 Ion Toxicity

The dominant ion in sodic soils is sodium. High levels of soil sodium can lead to potassium deficiencies (Bromham et al., 2013). At a cellular level, sodium is a less desirable cation as compared to potassium, but due to their similarity, many K^+ transporters cannot distinguish between the two ions, leading to high sodium and little potassium uptake (Pardo & Quintero, 2002). Several enzymes are sensitive to high sodium concentrations as these ions interact with the protein and reduce functionality (Flowers et al., 2015). Toxicity symptoms may first appear as scorching of leaf tips, then later, leaf bronzing and necrosis of leaf tips and margins (Bernstein, 1975).

While studies of soil salinity generally focus on soil cations, the most common anion in saline soils, chloride, is toxic to plants at high concentrations (Deilein et al., 2014). Chloride is a micronutrient important for photosynthesis, acting as a cofactor in chlorophyll. Chloride is also a counter ion in the maintenance of turgor pressure (Marschner, 2012). At high concentrations within leaves, chloride can interfere with photosynthesis leading to chlorosis and leaf burn; eventually necrosis may occur followed by leaf drop (White & Broadley, 2001). Chloride toxicity is not uncommon in saline soils (White & Broadley, 2001). Since chloride is negatively charged, it does not adsorb to negatively charged soil particles as cations do, but is instead found in the soil water phase (White & Broadley, 2001). Thus, chloride movement is primarily driven by water fluxes, meaning that it can move freely with soil water and can be readily taken up by plants. When Cl^- concentrations in soil are low, active transport dominates Cl^- influx into the roots. In saline soils, Cl^- transport into root cells is primarily passive (White & Broadley, 2001). Plant roots have anion channels which allow chloride to passively move into root cells (Zhang et al., 2004). This occurs primarily when the membrane potential has become positive. For example, when a large amount of Na^+ has been taken up by the roots, Cl^- acts as a counter ion (Teakle & Tyerman, 2010). Chloride can also passively enter the roots if concentrations outside the root are very high compared to internal concentrations (Teakle & Tyerman, 2010). Once in the roots, chloride moves into the xylem and is transported throughout the plant. While chloride levels within plant tissues are typically below 0.1-5.8 mg/g dry weight (DW), toxicity symptoms are observed at concentrations above 4-7 mg/g DW and 15-50 mg/g DW for salt-sensitive and salt-tolerant plants respectively (White & Broadley, 2001).

2-6.2.3 Alkalinity Stress

Soils that are saline, saline-sodic, or sodic, are also typically alkaline. The MECP (2011) reports that sodic soils commonly have a pH above 8.5. While the optimal pH for plants varies, the typical range is between 5.5 and 7.0 (Crouse, 2017). Alkaline soils tend to induce nutrient deficiencies as a result of the redox potential of several major nutrients. For example, above pH 8, iron is oxidized and inaccessible to plant roots (Husson, 2013). Some plants can cope with alkaline environments by secreting organic acids into the root zone which helps to solubilize nutrients (Husson, 2013). Plants adapted to salt stress are often also adapted to alkaline soils (Bromham et al., 2013).

2-6.3 Nutrient cycling

High soil salinity can have significant impacts on nutrient cycling, as changes may be observed in all stages of the food chain from primary production to decomposition. A decrease in plant productivity associated with increased soil salinity could shift the source of organic matter from plant based tissues to halophilic algae (East et al., 2017). Changes in microbial and macroinvertebrate composition and performance could in turn impact the quality of detritus and nutrient recycling (Entrekin et al., 2018). Tyree et al (2016) found that the rate of leaf decomposition by *Lirceus* sp. and *Tipula abdominalis* was impacted by sodium chloride at concentrations relevant to road salt application rates. Hart et al. (1990) found that macroinvertebrates in freshwater ecosystems are particularly sensitive to salt concentrations above 100 mg/L. However, the complexity of the system can make long term changes difficult to predict.

2-6.4 Impacts on Freshwater Biota

Runoff and leaching of salts from salinized soils also poses a threat to neighboring freshwater ecosystems. Osmoregulation in freshwater species such as fishes and macroinvertebrates is an energy demanding process. Dowse et al. (2017) observed increase mortality rates in mayfly nymphs exposed to small increases in salinity. Mortality was not attributable to a breakdown of this species' strong osmoregulatory capacity, but may instead reflect increased stress and energy consumption associated with osmoregulation. Field studies compliment these findings where Ephemeroptera, Trichoptera and Plecoptera species richness and abundance decreased even at low salinities (Kefford et al., 2011; Dowse et al., 2017). Further, freshwater salinization has been associated with a decrease in fish diversity and simplification of food webs (East et al., 2017).

2-7 SALINITY TOLERANCE AMONG PLANTS

There is a large diversity of salt tolerance among plants. Glycophytes are salt-sensitive and cannot complete a life cycle above 200 mM NaCl in their growth media (Flowers et al., 2015). Xerophytes are drought tolerant and many can tolerate, but have stunted growth, at high salinity (Medina et al., 1988). Halophytes are salt tolerant and some even require salt for optimal growth. When grown in saline environments, halophytes have comparable growth rates to glycophytes under non-saline conditions. Salt-tolerant plants make up less than 2% of all terrestrial flora, existing in a diversity of saline habitats from coastal regions and salt marshes to drylands and salt

flats (Flowers & Colmer, 2008). There are also halophytic plants that are not naturally found in salt-enriched regions, but nonetheless are tolerant of high levels of salt, such as the freshwater marsh species *Spartina pectinata* (Flowers & Colmer, 2008). Salt tolerance is not limited to one family, but is found across several clades. Grasses, trees, and herbs alike have adapted to salt stress (Hasanuzzaman et al., 2014). Significant differences in tolerance exist between eudicots and monocots. Optimal growth conditions for halophytic eudicots ranges from 50-250 mM NaCl, but is less than 50 mM NaCl for monocots (Flowers & Colmer, 2008). A decrease in biomass has been observed in some halophytic eudicots under low salt conditions, suggesting that some species may be obligate halophytes. Obligate halophytes are more common among eudicots than monocots which may be associated with the way that salts are managed between these classes (Flowers & Colmer, 2008). According to Hasanuzzaman et al. (2014) many species of Chenopodiceae are obligate halophytes. In order to overcome the difficulties of surviving in saline environments, plants employ several adaptive mechanisms. These include: i) the exclusion of salts at the roots, ii) osmotic adjustment within cells, iii) sequestration of salts within the vacuole, and iv) excretion of salts through specialized glands on leaf surfaces.

2-7.1 Metabolic Regulation

Xerophytes capable of surviving in saline environments can do so as a result of their adaptations to drought stress (Medina et al., 1988). Crassulacean acid metabolism (CAM) is employed such that stomata are only opened at night when water loss by evapotranspiration is lowest (Winter & Holtum, 2014). Water loss is limited, thereby reducing dependence on soil water. Reduced reliance on soil water also means that these plants take up less salt in their roots than glycophytic plants. For example, many cacti that generally don't tolerate salt can survive in saline areas because they take up very little water until the spring flush when salt concentrations are low (Medina et al., 1988). However, other xerophytes may actually accumulate large amounts of salts to help them take up water in areas where soil water is limited (Ma et al., 2016). Species such as *Mesembryanthemum crystallinum* may therefore qualify as xerophytes, but also participate in salt sequestration within cells (Flowers & Colmer, 2008).

2-7.2 Exclusion

Many halophytes are capable of avoiding salt stress by excluding salts from their roots. Exclusion prevents the accumulation of ions to toxic levels within root cells. Some plants that participate in salt exclusion have specialized ion transporters in their roots which favour the uptake of potassium over sodium significantly more than in salt-sensitive species (Rus et al., 2001). While glycophytes may inadvertently take up large amounts of sodium in place of potassium, the excluder halophytes do not face this issue (Maathius & Amtmann, 1999). This simultaneously reduces sodium toxicity as well as potassium deficiency. In contrast, other species rely on ion exporters to remove salts from their roots. *Eutrema salsugineum* also known as *Thelungiella halophila*, is a model halophyte and an excluder; 80% of the sodium taken up into its roots is immediately effluxed (Wang et al., 2006). This efflux capacity may be attributed to root ion antiporters. Qiao et al. (2007) identified a root Na^+/H^+ antiporter in a halophytic grass, *Agropyron elongatum*, that plays a crucial role in salt tolerance. It appears that chloride exclusion occurs primarily by the removal of chloride from root cells after it has already entered the roots (Teakle

& Tyremans, 2010). This efflux is stimulated by increasing internal concentrations of chloride and can reach up to 90% of influx rates in some halophytes (Britto et al., 2004). A reduction in xylem loading of chloride may also play a role in salt tolerance (Moya et al., 2003). While excluders can prevent ion toxicity by preventing salts from entering their roots, they must also rely on other mechanisms to adjust their osmotic potential.

2-7.3 Osmotic Adjustment

Low water content or high salt content within cells is highly problematic for plants as it can prevent the proper functioning of proteins. As a result, plants may produce compatible solutes (osmoprotectants); - molecules that are osmotically active and encourage water movement into roots without interfering with protein function. Osmoprotectants also prevent damage caused by the production of reactive oxygen species that occur under drought conditions and protect cell membranes (Singh et al., 2015). These solutes may include: glycinebetaine, proline, inositol, pinitol, sorbitol, mannitol, and other sugar alcohols (Flowers et al., 2008). Ishitani et al. (1996) found that when *M. crystallinum* is grown in soil with 400 mM salt, proline increased 20-30X and inositol and pinitol increased 80X. Solutes used for osmotic adjustment vary between species. For example, Poacea species primarily accumulate sugars but obligate halophytes in the Chenopodiceae family still rely on salts (Albert et al., 2000).

2-7.4 Sequestration

Another mechanism by which plants can reduce cytoplasmic ion concentrations below toxic levels is sequestration of ions in the central vacuoles of their cells. The vacuole is an organelle of importance in plant tissues as it plays a role in the storage of nutrients, the sequestration of toxins, and the maintenance of turgor pressure (Campbell & Reece, 2008). Halophytes often have larger vacuoles than glycophytes allowing them to sequester larger amounts of salts (Hajibadgheri et al., 1984). Different species have varying capacities for storage of salts within their vacuoles, however it has been estimated that 500 mM Cl^- would be maximal for most plants (Cram, 1973). Vacuolar Cl^- concentrations of the halophyte *Suaeda maritima* have been recorded up to 465 mM with similar concentrations of Na^+ (Hajibagheri & Flowers, 1989). This same species of plant has been seen to accumulate up to 2000 ug/g DW Cl^- and up to 2500 ug/g DW Na^+ in its above-ground tissues (Morteau, 2016).

Transport of cations into the vacuole is an energy demanding process mediated by two active transport mechanisms within the vacuolar membrane, the tonoplast (Teackle & Tyerman, 2010). Proton pumps (H^+ ATPase) are used to generate an electrochemical potential that provides the necessary energy (Barkla et al., 1995). Sodium transport into the vacuole is mediated by a secondary active Na^+/H^+ antiporter (Barkla et al., 1995). Potassium is actively transported by a proton-pumping inorganic pyrophosphatase (Davies et al., 1992). Chloride is loaded into the vacuole via a vacuolar chloride channel that is activated by phosphorylation (Martinoia et al., 2000). Ayala et al. (1996) determined that the salinity of the growth media was a signal for the activation of vacuolar ion transporters in *Salicornia biglovii*. Accumulation of chloride within the vacuole is primarily driven by its role as a counter ion to sodium and potassium (Teakle & Tyerman, 2010). Chloride is preferentially sequestered in epidermal cells as compared to

mesophyll cells which are more important for photosynthesis (Teakle & Tyerman, 2010). Fricke et al. (1996) found that epidermal cells had three times as much chloride as mesophyll cells when grown in saline soil. In studies using *M. crystallinum* it was determined that the ion transporters were activated in leaf cells, but suppressed in root cells (Barkla et al., 2002). Salt accumulation occurs more readily in older leaves that are less sensitive to salt. With increasing salt concentrations in soil, plants may drop these older salt-enriched leaves (Teakle & Tyerman, 2010).

Ion sequestration does not rely on transport into the vacuole alone, but also on retention. Plants need to maintain low sodium to potassium ratios within most tissues in order to prevent toxicity symptoms (Kamel & Sabah, 2015). Since cell membranes such as the tonoplast have a greater permeability to potassium as compared to sodium, the membrane plays a role in the maintenance of Na^+/K^+ ratios (Britto & Kronzucker, 2006). Sodium/potassium ratios are much higher in the vacuole as compared to the cytoplasm (Hajbagheri et al., 1988). These differences in sodium: potassium ratios are accentuated in *Chenopodium* and minimized in grasses. Sodium:potassium ratios vary significantly between species, but improved ion retention is associated with a high ratio of phospholipids to proteins within the tonoplast (Leach et al., 1990). The ability for accumulator halophytes to retain sodium within their vacuoles means that Na^+ may be more reliable for the maintenance of turgor pressure than potassium.

2-7.5 Secretion

As opposed to storing salts within cells, some plants instead secrete them through specialized glands in the shoot epidermis. These secretors, or recretohalophytes, exist within the Asterid, Caryophyllales, Rosid, and Poaceae clades (Breckle, 1990; Santos et al., 2016). Though true salt glands are rare, many resemble structures found in non-recretohalophytic plants (Dassanyake & Larkin, 2017). There are four different types of salt glands that are distributed throughout families within the above clades, with the most basic gland being the salt bladder found exclusively in the Azioaceae and Amaranthaceae (Dassanyake & Larkin, 2017). The second, known as multicellular glands are diverse and widely distributed throughout recretohalophytic dicots such as the Plumbaginaceae, Acanthaceae, and Tamaricaceae. The third and fourth types are found solely in monocot grasses; - bi-cellular glands are found in Chloridoid grasses and unicellular glands are found in Porteresia species (Figure 2-2).

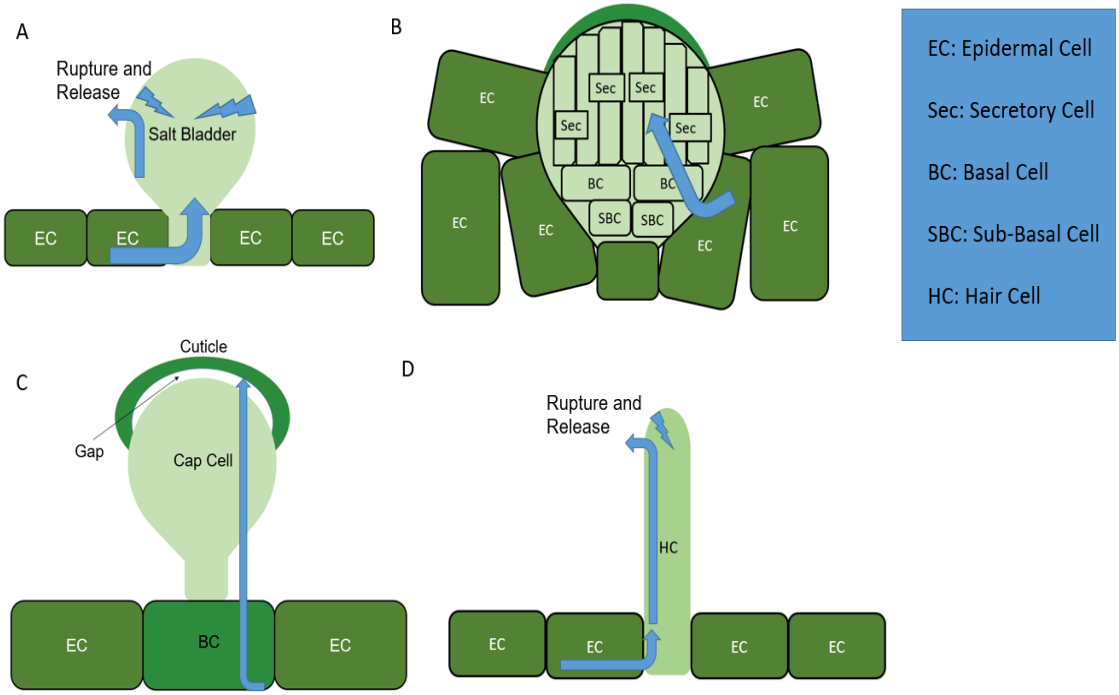

Figure 2-2: (A) Salt bladder (B) Common model of a Multicellular gland (C) Bi cellular gland (D) Unicellular gland. Salt glands are embedded in the epidermis, above mesophyll cells (Modified from Dassanyake & Larkin, 2017). Blue arrows indicate salt movement through cell structures.

2-7.5.1 Salt Bladders

Salt bladders are comprised of a swollen epidermal cell with a very large vacuole. Plants with salt bladders sequester salts within the vacuole of the bladder cell in a similar way to accumulators (Figure 2-2A). However, the bladders may rupture once maximal salt content has been reached, or if the leaf's surface is physically disturbed by touch, rain etc. leaving behind salt deposits on the leaf surface (Dassannayake & Larkin, 2017). These structures are commonly found in eudicotic *Chenopodium* species such as *Chenopodium quinoa* (quinoa), *Chenopodium album* (lamb's quarter), and *Atriplex canescens* (salt bush).

2-7.5.2 Multicellular Glands

Multicellular glands vary significantly between species, suggesting that the trait has evolved independently several times throughout history. Common structures in multicellular glands include basal collecting cells and distal secretory cells which are connected together by one or two stalk cells (Dassannayake & Larkin, 2017). The basal collecting cells create an osmotic gradient through which salt can be moved from mesophyll cells into secretory cells, preventing the backward movement of ions (Figure 2-2B). Mesophyll cells are connected to basal cells and/or sub-basal cells via plasmodesmata allowing salt to flow into the collecting cells. Salts are then actively transported from the basal cells into the secretory cells (Campbell et al., 1974). The interior surfaces of secretory cells have many internal projections which increases the surface area between the secretory and the basal cells. Ion transporters, as well as vesicles carry salts from the basal cells to the secretory cells (Barhoumie et al., 2007). The secretory cells are

covered by a cuticle punctuated with pores which allows the salt solution to flow outwards (Feng et al., 2015). These types of glands are found in genera such as *Limonium*, *Armeria*, and *Glaux*.

2-7.5.3 Bi-cellular

Bicellular salt glands are differentiated trichomes known as microhairs and are comprised of a basal collecting cell and a cap cell (Céccoli et al., 2015). They resemble papillae but are larger and comprised of two cells instead of one (Figure 2-2C). The basal cell is connected to the cap cell by multiple plasmodesmata. Both the cap and basal cells are filled with many small vacuoles which store salts. It is theorized that these vacuoles can fuse with the plasmalemma of the cap cell and release their contents into the space above the cap cell (Wahit, 2003). The cap cell is coated with a cuticle punctuated with pores. There is a gap between the cap cell and the cuticle which creates a cavity in which saline solution can accumulate (Campbell et al., 1974). As the fluid accumulates, it is secreted through the pores of the cuticle. Salt glands are found on both sides of leaf blades, lamellas, and other leaf structures, often between rows of stomata (Céccoli et al., 2015). While salt glands are usually found individually, in Zoysieae such as *Spartina*, they are found in groups of two or three (Marcum et al., 1998). Bicellular glands are only found in grasses of the Chloridoidea family with notable genera including *Spartina* and *Distichlis* (Dassanayake & Larkin, 2017).

2-7.5.4 Unicellular

The species within the genera *Porteresia* have unicellular salt glands comprised of a hair cell with a large vacuole and few organelles (Sengupta & Majumder, 2010). As salts accumulate within the hair, the tip may rupture, releasing the salts (Figure 2-2D) (Segupta & Majumder, 2010). Salt transport into the hair is similar to bi-cellular glands, but release of the salts mimics that of salt bladders (Dassannayake & Larkin, 2017).

2-8 REMEDIATION OF SALINIZED SOILS

2-8.1 Conventional Strategies

The simplest method for the remediation of salt accumulation in soils is leaching, whereby large amounts of fresh water are applied to the soil to wash away the soluble salts (Ravindran et al., 2007). This may be feasible in areas with a low water table where freshwater is readily available and abundant. However, regions impacted by soil salinization are often arid or semi-arid and thus lack sufficient water access. Another physical decontamination method is deep tillage where the surface soil enriched in salts is mixed with soil from deep in the profile, effectively diluting the salt concentration in the upper portion of the soil profile (Proving & Pitt, 2017). While this may support improved plant growth, tilling is highly disruptive and is associated with increased rates of erosion (Gov Sask, 2018). Deep tilling can also increase evaporation rates thus amplifying the salinity issue (Gov Sask, 2018). Chemical amendments may also be applied to improve soil quality. For example, the application of gypsum ($CaSO_4$) can increase the calcium content in the soil and improve the soil structure (Gupta & Abrol, 1990).

2-8.2 Soil Improving Cropping Systems and Phytoremediation of Salt-Impacted Soils

Soil salinity management can be accomplished via soil improving cropping systems (SICS) (Cuevas et al., 2019). These systems draw upon conventional strategies such as leaching with high quality water but also integrate crops that are salt tolerant to improve soil quality. While SICS focuses mainly on agricultural land, plant based remediation strategies using halophytes can be applied to a variety of circumstances to manage saline soils.

Phytoremediation is the use of plant species to extract, stabilize, or breakdown harmful compounds within soil. It is a cost-effective technology that has been applied to metals, organic contaminants, and even salt (Campos et al., 2008; Jesus et al., 2015; Mahar et al., 2016). Phytoremediation by halophytic plants can reduce costs to land owners while limiting disturbance to the soil and associated ecosystems.

Accumulators halophytes and recretohalophytes could be used in phytoextraction applications and accumulators have been used successfully for the reclamation of saline and sodic soils (Jesus et al., 2015). Halophytic species can also improve soil quality in other ways than simply removing salt. Studies using *Leptochloa fusca* (L.) showed that it increased salt leaching which reduced soil salinity and sodicity. This grass also reduced the pH of the soil by releasing CO_2 from its roots and solubilizing $CaCO_3$ (Akhter et al., 2003). Halophytes, like all plants, also increase soil organic matter and sequester soil carbon.

2-9 PHYTOEXTRACTION USING ACCUMULATOR HALOPHYTES

2-9.1 Salt Accumulators of Interest

Accumulators have been used throughout the world to remediate salt-impacted sites (Table 2-3). Many of these species are native to drylands or maritime regions, though some are widely distributed and weedy.

Table 2-3: Chloride extraction potentials of notable halophytes documented in the literature.

Species	Chloride extraction (Kg/ha)	Author (modified from)
Atriplex nummularia Lindl.	300	Silva et al. (2016)
Atriplex patula	490	Krishnapillai & Ranjan (2005)
Chenopodium album	345	Hamidov et al. (2007)
Phragmites australis	650	McSorley et al. (2015)
Salicornia europaea	720	Morteau et al. (2016)
Salicornia maritima	306	Ravindran et al. (2007)

Accumulators preferring an arid ecotype include *Atriplex* species. For example, *Atriplex nummularia Lindl*. was studied for its ability to phytoextract sodium chloride in a semi-arid region of Brazil (Silva et al., 2016). In one season, these plants were able to accumulate on average, 300 kg Cl^-/ha, with similar values for Na^+ (Silva et al., 2016). The Canadian species *A. patula* has also been studied for its effectiveness of remediating oil-extraction brine. When grown in soil containing 17 000 mg Cl^-/kg soil, 0.049 kg/m^2 was accumulated in the aerial parts of the

plant within one growing season which translates to approximately 490 Kg Cl^-/ha (Krishnapillai & Ranjan, 2005).

Another accumulator, *Salicornia* is a succulent halophytic plant with species native to coastal regions of Europe, Africa, Southern Asia, and North America (Gunning, 2016). *Salicornia* species have remarkable salt tolerance, and can grow when irrigated with seawater (Gunning, 2016). *Salicornia europaea* has been shown to accumulate up to 139 g of Na/kg dry weight, and up to 180 g of Cl/kg dry weight (Morteau, 2016). Farzi et al. (2017) found that when watered with a brine of EC 2-10 dS/m, a *Salicornia* biofilter could reduce salinity of leachate water by 30% (Farzi et al., 2017). Similarly, Ravindran et al. (2007) found that *Sueada maritima*, commonly known as herbaceous seepweed and native to the eastern coast of North America, could accumulate 504 kg of NaCl/ha in a four month growing season. Other *Sueada* species have also been of interest for salt remediation as some have been shown to contain up to 10% salt by weight (Chaudhri et al., 1964). *Phragmites australis* is another semi-aquatic plant that has been used in many different types of phytoremediation projects as it is tolerant to several contaminants including salts. With an estimated yearly uptake capacity of up to 65 g Cl^-/m^2 per year, *Phragmites* may be a suitable option for salt remediation, especially in areas affected by other contaminants such as heavy metals and organic pollutants (McSorley et al., 2015; Cicero-Fernández et al., 2016).

A common weedy halophyte native to North America and other parts of the world is *Chenopodium album*, commonly known as lamb's quarter. Hamidov et al. (2007) conducted field trials of salt removal in Uzbequistan using *C. album* and they found that the plant had both high biomass and a large uptake capacity with 3.25 tonnes of biomass produced per hectare and nearly 20% of that biomass accounted for by salt content (570 kg of NaCl/ha).

2-9.2 Management of Accumulator Biomass

In order to remediate soils using salt accumulating halophytes, the biomass produced must be disposed of in a sustainable and economical fashion at the end of a growing season. Methods of disposal may include the use of the plant matter as fodder, repurposing as compost, production of biofuel, or use in other goods. Hasanuzzaman et al. (2014) compiled a list of halophytic accumulators that may be suitable as animal fodder or even for medicinal purposes and consumable crops (Table 2-4).

Table 2-4: Edible halophytes (Modified from Hasanuzzaman et al., 2014).

Plant Species	Salt Tolerance Limit
Aster tripolium	300 mM
Atriplex hortensis	>250 mM
Batis maritima	200 mM
Cochlearia officinalis	100 mM
Crambe maritima	100 mM
Crithmum maritima	>100 mM
Diplotaxis tennifolia	150 mM
Inula coronipus	400 mM
Mesemyranthemum crystallimum	400 mM

Plantago coronopus	250 mM
Portulaca oleracea	<140 mM
Salicornia sp.	> 500 mM
Sarcocornia sp.	> 500 mM
Tetragonia tetragonioides	174 mM

Composting is a suitable method for volume reduction and repurposing. Moisture and heat found in compost piles stimulates the bacterial and fungal break down of plant structural compounds (Epstein, 2017). Salts are released from the plant tissue, allowing them to be leached out and captured, and the compost used as fertilizer. While composting is simple, it requires a large space and considerable time. It is suitable for the disposal of herbaceous halophytes but is less efficient in the breakdown of plants with woody parts (Yun et al., 2019a).

Other disposal methods that allow for the production of usable by-products include combustion or the generation of biogas from the fermentation of plant matter. Here, the plant matter is decomposed by anaerobic bacteria which produce methane in an oxygen-free environment. The resulting gases can be used as natural gas or can be burned for the production of electricity (Lishawa et al., 2015). Severely salinized soils and similar marginal lands are optimal for the production of plant based fuels as they do not encroach on agricultural lands. Halophytes that produce a large biomass such as *Phragmites australis* could be suitable candidates for green fuel production. Vaičekonytė et al. (2013) determined that biofuel pellets produced from *Phragmites* reeds harvested in Montreal (QC) contained 16.9 kJ/g which is comparable to the energy found in brown coal or corn stover (Sup. Table A-1). Up to 2 kg/m^2 of *Phragmites* can be produced per season, thus energy generation could be as high as 338 GJ/hectare a year.

Similarly, accumulator biomass can be used to produce soil amendments like black carbon or biochar via pyrolysis (Weber & Quicker, 2018). The addition of biochar to soils increases soil organic matter (SOM) which plays an important role in structure, water holding capacity and nutrient retention (McSorley, 2015; Busscher, et al., 2010; Kelly, et al., 2014). Biochars may also prove useful in the management of salt-impacted soils. The amendment can improve seed germination and bind toxic ions, thus reducing the impact of salts on plants (McSorley, 2015). Thus, accumulator biomass can be recycled back to the site to further improve soil quality.

2-10 PHYTOEXTRACTION USING RECRETOHALOPHYTES

While there is a fairly large body of research on the phytoextraction potential of many accumulator halophytes, studies on excretors are limited. Sargeant et al. (2008) studied the impacts of *Distichlis spicata* on soil salinity and structure over time. While they did not directly evaluate salt extraction, they did see a reduction in soil salinity through time within the top 10 cm of soil, showing that recretohalophytes do not simply re-contaminate the soil with the salt they excrete. In theory, the use of excretors has a significant advantage over accumulators as there is potential that excreted salt may be dispersed through wind action, thus removing the need for plant harvesting. The theory of haloconduction was proposed by Yensen and Biel (2008) and has been under investigation by Yun et al. (2019b), and Morris et al. (subm.). Recretohalophytes present not only a phytoextraction option with minimal labour costs, but may also allow for more

rapid site remediation. McSorley et al. (2016) determined, based on plant rinses, that the recretohalophyte *Spartina pectinata* could remove significantly more salt from the soil in a growing season as compared to the accumulator *P. australis* when both internal and excreted salts were considered. Thus, recretohalophytes may prove superior to accumulators for salt phytoextraction.

The basis for the remediation potential of recretohalophytes is the atmospheric dispersal of secreted salts over large distances (Figure 2-3). Salts are taken up by plant roots and secreted onto the leaf surface as a concentrated saline fluid. As the water from the secretion evaporates, salt crystals form and can be mobilized by the wind as it blows on the plants and cause them to flutter (Figure 2-4). Once in the air column, Gaussian plume models suggest that the space between particles in the air column will increase with increasing distance from the source effectively becoming more dilute the longer the particles remain in the air and travel further. Particles leave the air column by dry or wet deposition (rain) given the appropriate meteorological conditions.

Wind tunnel experiments conducted by Morris (subm.) suggest that with sufficient wind speeds, up to 90% of excreted salts may be dispersed from the plant and into the air. While this phytotechnology may be useful under specific circumstances, in order to determine its remediation potential at any given site, site-specific factors would need to be considered. Based on methods developed for modelling other natural aerosolized products such as plant pollen, three components are essential for site-specific analysis: i) available aerosol pool, ii) emission factors, and iii) aerial dispersal based on meteorological and topographic data (Zhang et al., 2014).

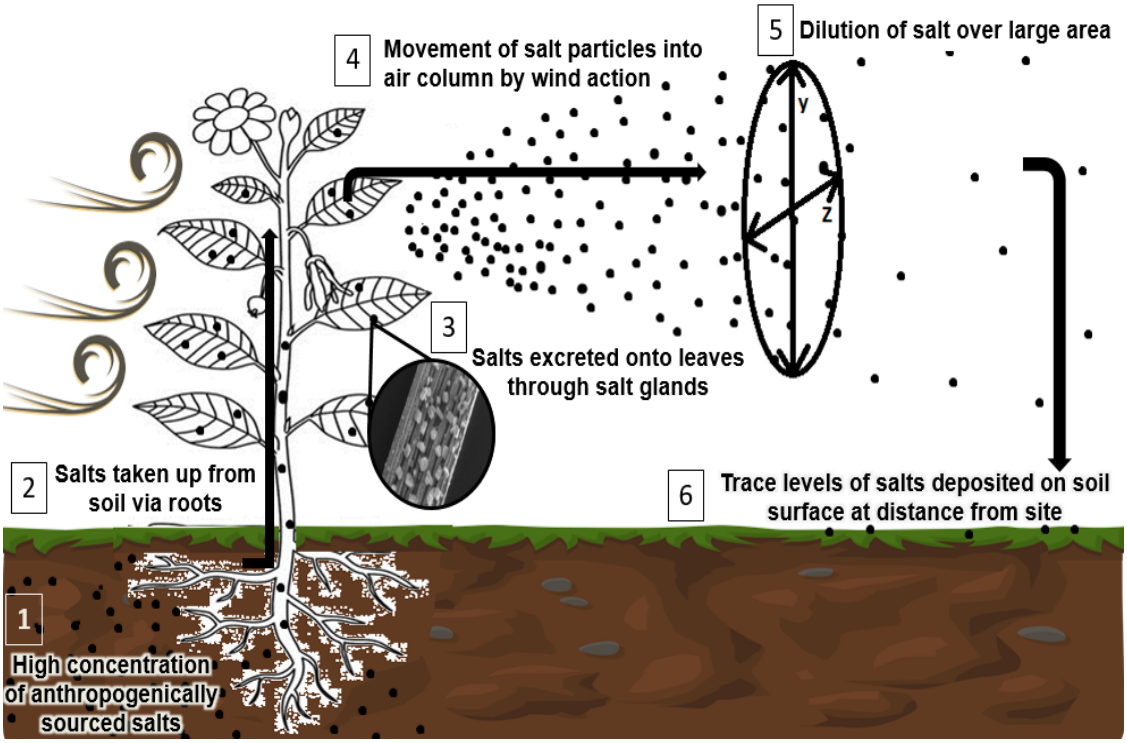

Figure 2-3: Gaussian wind driven salt dispersal of recretohalophytes

Figure 2-4: Salt excretions of *S. pectinata* under optimal conditions with no disturbances (reprinted from McSorley et al. 2016 with authors permission)

2-10.1 Available Salt Pool

While salt removal by recretohalophytes may be superior to that of salt-accumulating plants, the rate at which salts are excreted from leaf tissues is highly variable. These excretion rates are dependent on a number of factors, including species level differences, concentrations of ions in the growth medium, and the nature of the ions in the growth medium. Other factors may also come into play such as diurnal changes like those observed by Ramadan (1998) in *Reaumuria hirtella*, where peak excretion occurred mid-day.

Differences in secretion abilities and rates exist between species. Yun et al. (2019b) determined that *Spartina pectinata* excretes significantly more chloride (22 mg Cl-/g DW) as compared to *Distichlis spicata* (13 mg/g DW) when grown under identical conditions (6 mg Cl^-/g soil). When Rozema & Gude (1981) compared the excretion rates of four recretohalophytes: *Spartina anglica, Limonium vulgare, Armeria maritima, and Glaux maritima,* they found that excretion rates varied in the aforementioned order, with *S. anglica* having the highest excretion rates and *G. maritima* having the lowest (Table 2-5). The authors observed similar trends in chloride excretion between species.

It is critical to note that data presentation may play a role in observed differences in excretion rates between species. For instance, Rozema et al. (1981) found that when excretion rates were reported in concentration per unit of leaf area, *Spartina* excreted significantly more salt, but when results were reported in salt concentration per dry plant mass, *Limonium* had the highest excretion rates. Thus careful consideration should be taken to report results in the manner that most suits application.

Table 2-5: Excretion rates of recretohalophytes exposed to 200 mM salt solutions.

	Number of glands per leaf	Excretion rate of K+ µM/cm^2/6 days	Excretion rate of Na+ µM/cm^2/6 days	Author
Spartina anglica	1200	0.5	2.2	(Rozema et al., 1981)
Limonium vulgare	3000	1.1	1.0	(Rozema et al., 1981)
Armeria maritima	550	0.6	0.1	(Rozema et al., 1981)
Glaux maritima	800	0.3	0.3	(Rozema et al., 1981)
Limonium bicolor	4000	--	100 µg/cm^2	(Leng et al, 2017)

There are also significant differences in excretion rates depending on the salt to which the plant is exposed. Rozema & Gude (1981), found that for most species, salt glands released less potassium than sodium when grown in respective media of equal molarity. This is logical as potassium is a macronutrient and beneficial to the plant, whereas sodium in not (Marschner, 2012). The opposite was observed in *Armeria maritima*, where potassium excretion (0.5 µm/cm^2/6 days) was five times higher than sodium secretions (0.1 µM/cm^2/6 days) when grown under the same conditions. Ding et al. (2008) noted that salt gland diameter was larger in plants grown in media containing NaCl than those observed in plants growing in KCl enriched media. Ding et al. (2008) also observed higher salt excretion rates in plants growing in NaCl rather than KCl. Similar observations have been made with mangrove trees. Mukherjhee (2012) noted that these trees selectively secrete sodium and/or potassium to maintain internal Na/K ratios. Mukherjhee (2012) and McSorley (2016) observed other ions in the excreted solution such as sulphate, and fluoride, however these were in significantly lower quantities. While secreted ions vary largely in conjunction with the soil ions, secretion of divalent cations Mg^{2+} and Ca^{2+} is not favoured by transporters (Céccoli et al., 2015). Rozema & Gude also examined excretion of K^+ and Na^+ from plants grown in media containing both salts. They found that plants exposed to 0.1M NaCl + 0.1M KCl excreted less of each salt when compared to the treatment condition containing 0.1M

NaCl or 0.1M KCl. This demonstrates that the nature of the salts in a growth solution will impact excretion rates.

Plant and leaf age also play a role in determining excretion rates. Leng et al. (2017) found that salt gland density increased with leaf age, while salt excretion per gland plateaued once leaves reached 150 mm^2 in area. While secretion rates increased with leaf age, this is likely due to increasing leaf size. While older leaves may excrete larger quantities of salt, Agarie et al. (2007) noted that younger leaves may rely on excretion for maintenance of internal salt concentrations.

Another factor that may affect excretion rates is the concentration of ions in the growth media. There is little consensus in the literature as to the impacts of increasing media salinity. Mishra & Das (2003) observed that salt secretion rates correlate positively with increasing salt concentrations in the soil, as did Ball et al. (1988). However, Sobrado et al. (2001) observed that under hypersaline conditions excretion rates followed a sigmoidal curve. To add further complexity, Rozema & Gude, (1981) did not observe any change in excretion rates with increasing media concentration, but did see increasing internal salt concentrations. The diversity of these findings could be associated with species-specific differences in maximum excretion rates and the associated media concentrations. For instance, those studies that showed a positive correlation between excretion rates and concentrations of salt in the growth medium may not have increased the media concentration sufficiently to reach a plateau in that particular species. Those who observed no change in excretion may have only been using media concentrations above the plateau. It is difficult to make strong comparisons as most authors have used different species of recretohalophytes. While Ramadan (1998) observed that *Reaumuria hirtella* had increasingly higher excretion rates even beyond 320 mM solutions of NaCl, studies observed that many species including those in the *Spartina* and *Distichlis* genera have maximal secretion rates when grown in soils with 150-200 mM of NaCl (Liphschitz & Waisel, 1974).

Thus, at any given site, the pool of salt available for aerial dispersion would be a factor of the plant species used, the soil salt concentrations at the site, and the age of the plants used, or by proxy, the time of year. But, there are still many unknowns with regard to excretion rates even at the mechanistic level. Few studies have examined the excretion potential of halophytes with salt bladders. While they do not actively excrete salts onto their leaf surfaces, bursting of the salt bladders may result in a similar release of salts which may be comparable to excretions of those species with true salt glands. Diurnal changes in salt excretion have been observed and associated with variable daily transpiration rates, however less is known with regards to the impacts of temperature and humidity on active salt excretion (Ramadan, 1998).

2-10.2 Particle suspension

While there has been significant research into the excretions of recretohalophytes, the prospect of these salts becoming airborne is relatively novel, but not unlike the emission of other plant derived compounds. Studies on the emission of pollen and fungal spores may therefore provide insight into modelling the flux of salt particles from the leaf surfaces into the air. Unlike pollen, recretohalophytes excrete salts throughout the season, and based on observations by Mukherjhee et al. (2012) and Oi et al. (2012), do so continuously. Once salts have been excreted onto a leaf's surface, this 'salt pool' may be available for aerial dispersal by wind action (Figure 2-3).

However, it is unlikely that all salts excreted by a plant would be immediately transferred into the air. Instead, relative humidity, temperature, wind speed, particle size as well as other unknown factors play a role in particle suspension. Studies on the emission of similar natural products such as pollen have used these parameters to determine variable emission factors. However, Zhang et al (2013) stated that these emission factors are often the greatest source of error in a model.

2-10.2.1 Aerosol Emission

Like the emission of particles from waste piles, the salt excreted by halophytes would be subject to frictional forces between itself and the leaf. Friction velocity is a measure of the shear stress that wind applies to an erodible surface (Stunder et al., 1985). Thus, threshold friction velocity (TFV) is the velocity necessary to overcome the frictional force between a particle and a surface, or the wind speed at which particles will detach from a surface and become airborne (Li et al., 2010). Threshold friction velocity is often used to evaluate erosion of soils but it can also be applied to other situations (Ravi et al., 2005). In the case of haloconduction, this would be the wind speed required to overcome the friction between salt particles and the epidermis of a leaf. When wind speeds exceed the TFV, particles will experience a horizontal flux. If there is an impact between particles or updraft, a vertical flux may also be generated (Figure 2-5). Furthermore, studies have demonstrated that the wind speed necessary to move particles on the surface of a leaf are lower than those required to move the same particle on a flat surface, given the turbulence of wind around leaves and resulting movements of the leaves themselves (Jones & Harrison, 2004).

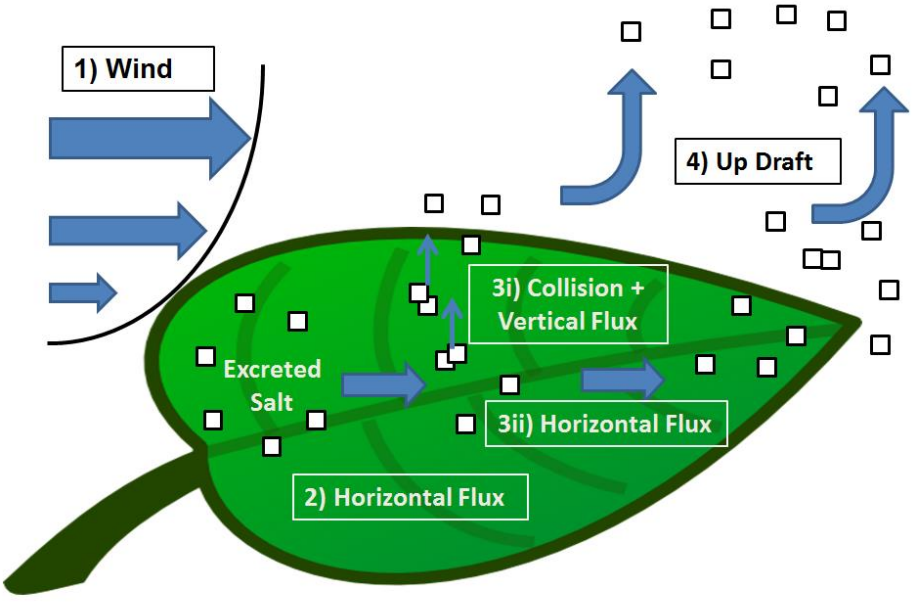

Figure 2-5: Emission of particles from a flat surface (Modified from Laurent et al., 2004)

Factors that determine the threshold friction velocity of a material include particle density and size, as well as factors such as moisture which would contribute to the frictional force between the particle and a surface (Sharratt et al., 2013). Morris et al. (in press) observed that the salt crystals found on recretohalophyte leaves lose their shape and form globular liquid droplets when relative humidity exceeds 70%. The TFV of the liquid droplet would greatly exceed that of the solid crystal given that liquids are subjected to shear stress, while solids are only impeded by friction between the object and the surface (White, 2011). The friction coefficient of salt also increases with increasing relative humidity (Gotoh et al., 1986). Furthermore, research on soil erosion has shown that TFV increases with relative humidity above 65% (Ravi et al., 2005). Consequently, several studies examining the emission of fungal spores from leaf surfaces have demonstrated that particle emission is positively correlated with temperature and negatively correlated with humidity, both of which impact the static frictional force (Leach et al., 1975; Jones & Harrison, 2004).

Size also impacts particle emission, Chamberlain et al. (1975) determined that particles 30-100 µm in size become airborne most readily, whereas those lighter tend to bond more strongly to surfaces and those heavier require a greater force to move them. Morris et al. (in press) determined that *Spartina pectinata* and *Distichlis spicata* salt excretions crystalized to mean cubic lengths of 20 µm and 50 µm, respectively. This being said, Aylor et al. (1981), found that fungal spores (1-10 µm) dispersed in clumps. Under optimal conditions and minimal wind, some recretohalophytes have been observed to form low density salt aggregates resembling spun sugar (Figure 2-4) (McSorley et al., 2016).

Due to the heterogeneity of surfaces and thus friction coefficients, the threshold friction velocity of a substance is generally determined experimentally using a wind tunnel (Stunder et al., 1985). A test surface is placed within a wind tunnel and the wind speed is slowly increased. A laser particle analyzer is then used to detect the wind speed at which particles begin to move into the air (Ligotke, 1989). Several studies examining the release of fungal spores from plant leaves have demonstrated that the minimal wind speed for emission is between 0.5 m/s and 2.0 m/s at the leaf level. Aylor et al. (1981) found that the threshold friction velocity for the release of powdery mildew (*Erysiphe graminis*) (25 µm) from barley was 0.5-1.0 m/s whereas Geagea et al. (1997) found that yellow (*Puccinia striiformis*) and brown rust (*Puccinia recondita f.sp. tritici*) (25 µm) had a threshold friction velocity of 1.3 and 1.8 respectively with maximal release at 2.3 and 2.8 m/s respectively. It should be noted that Gagea et al. (1997) did not allow for leaf fluttering in their experiment. They hypothesized that the differences in threshold friction velocity between species is associated with biological factors impacting the bonding of the spores and the leaf surface. While salts excreted from plant leaves may not be influenced by these same biological forces it might be hypothesized that the threshold friction velocities would fall within a similar range for particles of similar size.

2-10.3 Aerial Dispersal of Particulates

Plants are a source of many particulates and aerosols found within the air column. Matthius-Maser et al. (2000) found that the proportion of biologically derived particles accounted for 28%, 22%, and 10% of all airborne particles in remote continental regions, populated continental regions, and remote maritime regions, respectively. Once airborne, particles and aerosols may

disperse a distance depending on the nature of the emission, the height at which it has been released, meteorological factors, and regional topography. Several models exist to describe the aerial dispersal of particulates with ranges of complexity and specificity. Aerial dispersal models include Gaussian plume atmospheric modeling systems such as AERMOD and Lagrangian trajectory models like CALPUFF which may be used to illustrate the regional dispersal of airborne particulates (US EPA, 2018). Specific models such as MEGAN (Model of Emissions of Gases and Aerosols from Nature) and STaMPS (Simulator of the Timing and Magnitude of Pollen Season) can also be applied to study the regional of global dispersal of plant derived compounds such as VOCs and pollen dispersal, respectively (Guenther et al., 2012; Zhang et al., 2014). While none of these models have been applied to the theory of haloconduction, they may be useful in evaluating the dispersal of salts from recretohalophytes.

2-10.4 Ethical Considerations

While salts are hazardous at high concentrations, low levels are benign and even necessary nutrients for some species. The use of recretohalophytes hinges on the ability to disperse the salts over a very large region, thus eliminating the impacts of high soil salinity. Hence, recretohalophytes should only be implemented as a remediation tool in regions where aerial transport models demonstrate that deposition rates do not greatly exceed background levels. To complement models, regular monitoring could also be implemented.

2-10.4.1 Airborne Salt Monitoring

Standard methods for monitoring salt content in the air come from corrosion studies of sea breezes. One such method, known as the dry plate method, utilizes a fine mesh fabric stretched over a frame. As wind blows through the fabric, salt particles are deposited (Baboian, 2005). The fabric can then be soaked in double-deionized water and the concentration of dissolved salts can be determined. This method has been modified and used for the study of salt dispersal via recretohalophytes by Yun et al. (2019b) and Morris (subm.). The wet candle method is another technique that can be applied. The apparatus is constructed of a tube wrapped in fabric that is kept continually moist with ultra-pure water. As wind blows past the cloth tube, the moisture in the cloth encourages the deposition of salt particles from the air (Baboian, 2005). A variety of volumetric aerosol samplers also exist. These air samplers collect a given volume of air from the surrounding environment and use varying sizes of filters to collect particulate matter. Williams et al. (2000) used a high volume aerosol sampler and a dichotomous aerosol sampler to study the dispersal of road salts over long distances. They determined that aerosolized salt particles were able to be transported to distances over 50 m from the application site. High volume air samplers have also been applied to study airborne sea-salt particles in coastal environments (Murayama et al., 1999).

2-11 CONCLUSION: STRATEGIES FOR IMPLEMENTING SALT PHYTOEXTRACTION

Salt phytoextraction has been demonstrated throughout the literature with high success using a variety of native species. However, there are often significant differences between regions and

even sites within the same area so, careful selection of plant species is necessary to maximize extraction.

1) Select plants based on the extraction method most suitable to the project

Remediation via accumulator halophytes relies on plant uptake and storage of salts within above-ground tissue followed by harvest and disposal of biomass. It may be most suitable for sites where frequent harvesting can be incorporated into standard procedures with minimal re-engineering. For example, this could include the collection of roadside clippings for the remediation of road salts. Phytoextraction is not however suitable in all regions, such as those with a high saline water table, as plants may actually drive salt closer to the surface by capillary action.

While still in the theoretical phase, haloconduction has the potential to be a valuable tool in remediating salt impacted soils particularly in remote regions. This technique relies on the dispersal of salts over a large area effectively diluting the concentrations such that they are no longer hazardous and may even act as nutrients. To ensure appropriate application, regional site modelling would be appropriate, combined with sufficient monitoring to ensure effective implementation. Haloconduction has the potential to be incorporated into long term plans as a form of assisted natural attenuation.

2) Select species based on botanical geography and ecotype

Ideally, selected species would be native to the region which would reduce the potential of spreading invasive species and also reduce the risk of crop failure as these plants would be adapted to the climate. A useful tool in selecting species is the eHalophe halophyte database created by the University of Sussex (2017). Here, users can create a list of halophytic species based on environmental characteristics such as drought prevalence or soil saturation.

3) Select species with largest extraction capacity

Several authors have curated lists of halophytic plant species and their recorded extraction capacities (e.g.: Hasanuzzaman, 2014; Morteau, 2016).

4) Promote optimal growth by integrating supplementary tools as necessary such as halophilic bacteria or mycorrhizae.

For example, Shah et al. (2017) demonstrated that bacterial species, *Oceanobacillus kapialis*, could increase phytoextraction capacity under saline conditions.

5) Repurpose accumulator biomass for economic benefit

The use of accumulators may be ideal where the plant biomass is repurposed for economic benefit such as composting or pyrolyzing the material. It is also possible that water leached from the plant material could be used for road salt applications. Many accumulator halophytes are also edible and could be added to crop rotations allowing farmers to treat salinized soil while generating produce or fodder.

The effective implementation of phytoextraction has the potential to improve soil quality by simultaneously removing salts, enhancing soil structure, and sequestering carbon. While the use of phytoremediation is not yet the standard for the treatment of salinized soils, improved implementation strategies will bring about more economical options.

3 Evaluating the phytoremediation potential of the accumulator halophyte *Salicornia maritima* for the treatment of saline leachate

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(Formatted for submission to the Journal of Environmental Pollution)

3-1 HIGHTLIGHTS

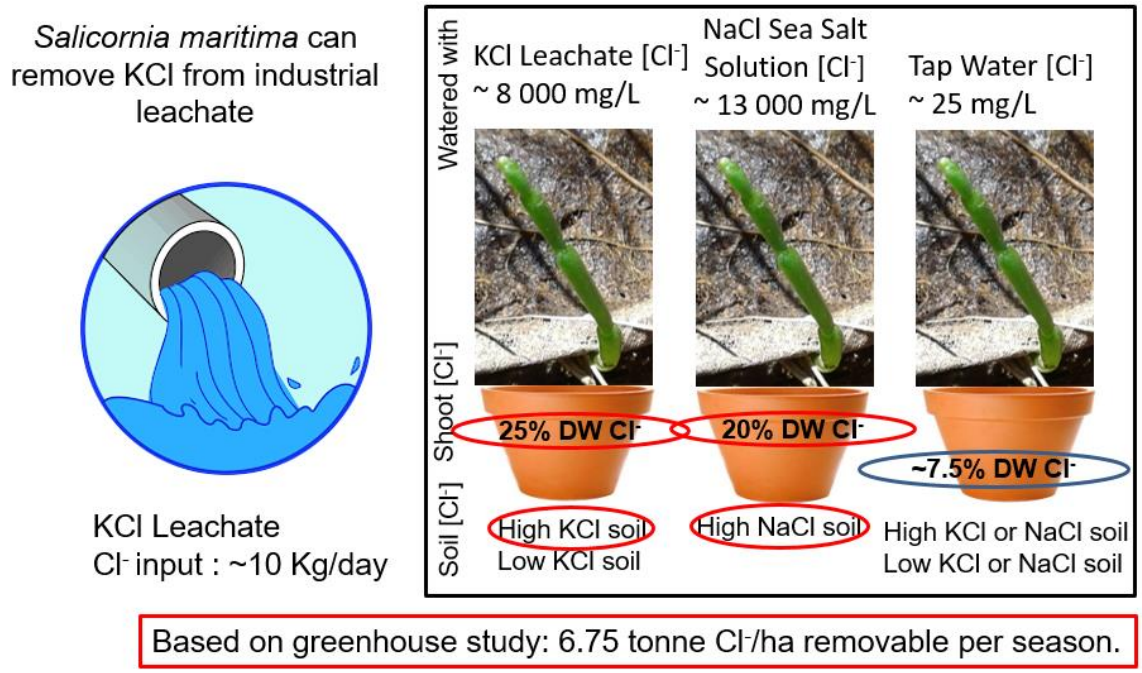
- *Salicornia maritima* demonstrated exceptional tolerance to high KCl leachate
- *S. maritima* accumulated up to 25% of its dry biomass as chloride
- Chloride extraction rates of more than 6.75 tonnes/ha are achievable within a single season with multiple harvests

3-2 ABSTRACT

Saline wastewater can pose a threat to many freshwater ecosystems, negatively impacting soil flora and benthic invertebrates, as well as fishes. Halophytes that have evolved to tolerate high salinity semi-aquatic environments may be useful in treating such wastes. *Salicornia maritima* is a plant native to Canada with high salinity tolerance. This study examines the survivability and extraction capacity of *S. maritima* when exposed to potassium chloride(KCl)-rich leachate from a cement kiln dust landfill. Leachate concentrations ranged from 1000 – 9000 mg Cl^-/L and daily input rates were typically 10 kg Cl^-/ day. Seedlings grown in high KCl soil (4 mg Cl^-/g) and watered with the KCl leachate grew just as well as those grown in similar sodium chloride (NaCl) watering conditions and those grown in background conditions. Chloride uptake was highest among plants watered with KCl leachate and was up to 250 mg/g DW of chloride in other words nearly 25% of the plant's dry weight. *S. maritima* is an ideal candidate for phytoextraction as it can sequester salts within its tissues and the herbaceous nature of the plant makes biomass management by composting feasible. *S. maritima* has the potential to remove upwards of 6.75 tonnes Cl^-/ha in a season and is thus a suitable candidate for integration into remediation designs for CKD leachate impacted sites.

Keywords: Leachate, Soil Salinization, Phytoremediation, *Salicornia*

3-3 GRAPHICAL ABSTRACT



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3-4 INTRODUCTION

Briny by-products are generated in a number of industries from aquaculture to oil extraction. Some authors have suggested that constructed wetlands utilizing semi-aquatic halophytes should be incorporated into treatment designs (Vymazal et al., 2010; Tilley et al., 2002). Halophytes with a marshy ecotype, and both high salt tolerance and accumulation ability are the best candidates. Such plants would not only be able to survive the harsh conditions imposed by the brine, but also remove salts from the growth media and thus act in a remediation capacity. *Salicornia* species including *S. maritima,* a Canadian species that is genetically identical to *S. europaea* common in Europe and the United Kingdom, is among those species that may be suitable for constructed wetland applications (Kadereit et al., 2012).

Salicornia species have remarkable salt tolerance, and can grow when irrigated with seawater (Gunning, 2016). *Salicornia europaea* has been shown to accumulate up to 139 g of Na/kg dry weight, and up to 180 g of Cl/kg dry weight (Morteau, 2016). Farzi et al. (2017) used *S. europaea* as a biofilter to determine if it could remove salt from irrigation water. They found that when watered with a brine with an electrical conductivity (EC) of 2-10 dS/m, *Salicornia* could reduce salinity of leachate water by 30% (Farzi et al., 2017). Edible *Salicornia* species (including *S. europaea* and *S. maritima*) are consumed in several cultures and are a subject of growing interest in areas with little access to fresh water. It has been proposed that *S. maritima* could be used as a filter for aquaculture effluent, both removing salt and providing a harvestable crop (Diaz et al., 2013).

Accumulator species like *Salicornia* store salts within their central vacuoles to maintain low sodium to potassium ratios within their tissues and prevent toxicity symptoms (Kamel & Sabah, 2015). Ion sequestration relies both on the transport of ions into the vacuole as well as their retention. High salt concentrations in growth media was found to be a signal for ion transport into the vacuole in *S. biglovii* (Ayala et al., 1996). Cell membranes such as the tonoplast have a greater permeability to potassium compared to sodium, so the membrane itself plays a role in the maintenance of Na^+/K^+ ratios (Britto & Kronzucker, 2006). The ability for accumulator halophytes to retain sodium within their vacuoles means that Na^+ may be more reliable for the maintenance of turgor pressure than potassium. It is believed that *Salicornia* uses sodium as an osmoregulator, sequestering it within its vacuoles to promote water retention and support turgor pressure (Lv et al., 2012). It is unknown if *Salicornia* species tolerate KCl as efficiently as NaCl as this salt can sometimes be inhibitory for related species such as those in the Chenopodiaceae sub-family.

Cement kiln dust (CKD) is a by-product of cement manufacturing that is rich in KCl. Many existing CKD landfills, designed prior to the US EPA's requirements for management established in 1995, were designed without liners. In these situations, ground water interacts with and dissolves salts in the CKD, carrying them to regions with a high water table (US EPA, 1999). At a cement plant in Bath, ON, KCl leachate has been funneled to a stream outflow point. The purpose of this study is to examine if *Salicornia* could be incorporated into a remediation design to remove KCl from the leachate and prevent further salt contamination into the surrounding environment.

3-5 METHODS

3-5.1 Site Description

The site that motivated the study is a salinized wetland in Bath, ON (44.180564, -76.801349) impacted by cement kiln dust leachate. Cement kiln dust contains concentrated amounts of KCl and when it interacts with rain or groundwater it produces saline leachate. Leachate has been contaminating the 1 000 m^2 region (Bath site) (Figure 3-1).

Figure 3-1: Bath site with a close up of the leachate outflow point in the right panel. Red arrows indicate the direction of flow of the leachate. The red circle indicates the location where the High KCl soil was collected, the blue circle indicates the approximate location that the Low KCl soil was collected.

3-5.2 Leachate Characterization

A sample of leachate was collected every week from the end of April through October, 2018 and analyzed for chloride content via ion chromatography (IC). Chloride is the ion tracked throughout the following experiments as it is the primary ion of concern at the site, given that potassium is a plant macronutrient. At the time of collection, the flow rate was measured using the bucket method, measuring the time required to fill a bucket of known volume (Trimmer, 1994). 20 L of leachate were collected June 24 2018 for use in greenhouse pot experiments using *Salicornia maritima*.

3-5.3 Soil Preparation

'High KCl' soil was collected near the outlet of the leachate (44.180518, -76.801608) and was determined to have a soil chloride concentration of 4 mg/g. 'Low KCl' soil was collected ~50 m beyond the 'High KCl' collection site (44.180564, -76.801349) and was determined to have a soil chloride concentration of 0.15 mg/g (Figure 3-1). Soil was also collected from an unsalted roadside in Frontenac provincial park (44.508335, -76.553574) hereto referred to as 'No Salt' as it contained 0.017 mg/g chloride. A 'Low NaCl' soil treatment was generated by spiking the 'No Salt' soil to 1 mg/g chloride. Each soil was homogenized using the two-dimensional Japanese slab-cake method (Gy, 1992). Although there are no guidelines for chloride in soil per se, the

Ministry of Environment, Conservation, and Parks in Ontario set guidelines for background upper limit of soil electrical conductivity at 0.57 dS/m; the High KCl, Low KCl, Low NaCl, and No Salt soils are approximately 10X, 2X, 2X, and 0.7X this limit respectively (Ont. Reg. 153-04, 2017).

3-5.4 Pot Experiments

Approximately 1-inch-tall *Salicornia maritima* plants (~0.1g dry weight) were collected from an estuary near Point Prim, PE (46.086850, -62.916932) on June 12 2018 and transported to the Royal Military College of Canada (RMC) for experimentation under greenhouse conditions (25 ± 3 ^{O}C, 50 ± 10 % Relative Humidity (RH)). On June 18 2018, seedlings were transplanted into 4 inch pots containing either 'high KCl' (500 g), 'low KCl' (500 g), 'No Salt' (300 g), or 'low NaCl' (300 g) soils. The soil textures from the two sites (Bath vs Frontenac) were different which resulted in different masses required to fill the pots (500 vs 300 g). The 'low NaCl' condition was included to control for growth in the event that *S. maritima* required NaCl to grow normally as has been reported by other authors (Gunning, 2016). Pots containing equal proportions (500 g each) of sand and soil from the Bath site ('high KCl' or 'low KCl') were also included as the highly clayey nature of the soil from the Bath site was anticipated to pose a challenge to the growth of *S. maritima*. Plants were then watered (30 mL/day) with one of three solutions: i) tap water containing 25 mg Cl^-/L, ii) leachate collected from the site with ~7000 mg Cl^-/L, or iii) Instant Ocean® Sea Salt solution of ~13 000 mg Cl^-/L (Figure 3-2). The sea salt solution was included as control for the natural conditions under which *S. maritima* grows. An unplanted version of each treatment condition was included, and all treatment conditions were completed in triplicate. The plants began to flower August 1, 2018 and were preparing to seed August 13 2018, at which point they were harvested. At this time, soil samples were collected by taking a 2 cm diameter full depth soil core from three locations within each pot.

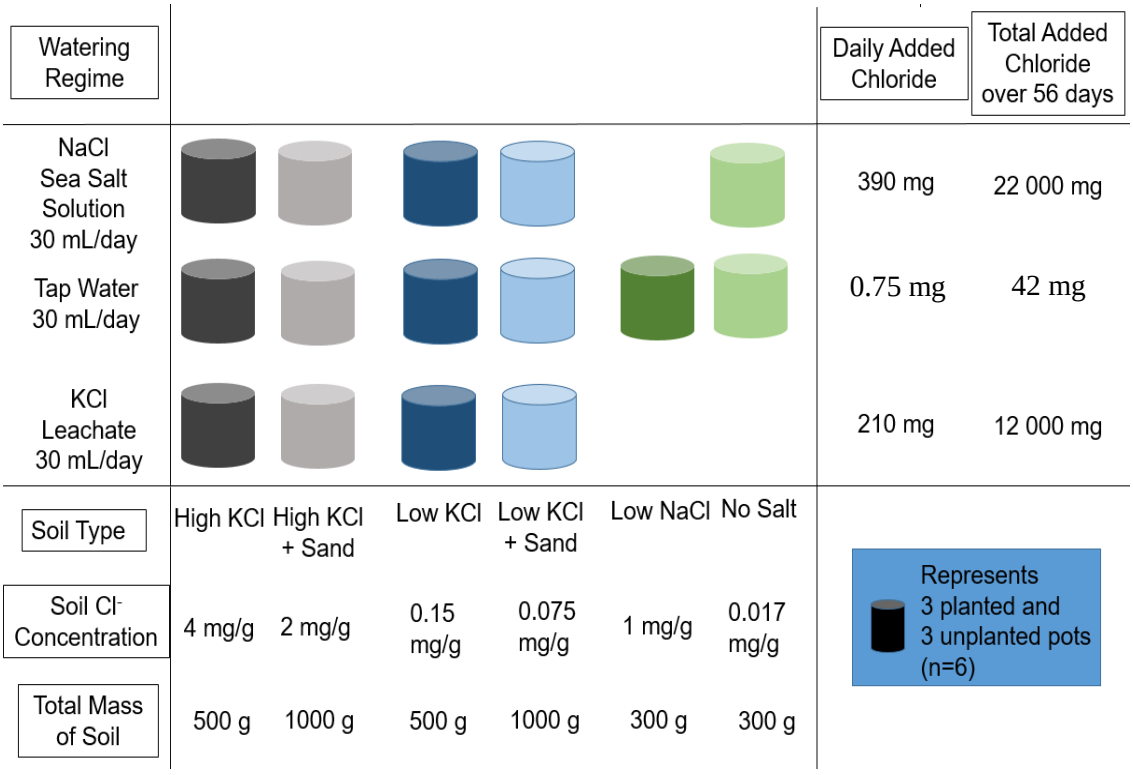

Figure 3-2: Soil and watering conditions for S. maritima greenhouse experiment conducted between June-August 2018

3-5.5 Sample Analysis

Plant shoots were washed, air dried for 24 hrs and weighed to determined the wet weight (WW). The samples were then oven dried at 70 ^{O}C for 24 hrs and weighed for dry weight (DW). The tissue samples were ground (Thomas Wiley Mini Cutting Mill, Model 3383-L10) and a 0.1 g subsample was shaken with double de-ionized (DDI) water on a horizontal shaker plate for 1 hr at 300 rpm to extract the chloride, then the extract was syringe filtered with a 0.45 µm cellulose acetate filter (Munktell, Model 752509). Prior to analysis, the electrical conductivity (Fisher Scientific, Traceable Conductivity, Resistivity, and TDS Meter) was measured and samples were diluted to at or below 150 µS/cm. Analysis by ion chromatography with a Dionex HPLC (High Performance Liquid Chromatography) system (ICS 3000) was performed using an AG4A-SC guard column and an AS4A-SC analytical column. The column flow rate was set to 2.0 mL/min. A carbonate/biocarbonate eluent was prepared by diluting 10 mL of a 100x concentrate of 1.8 mM carbonate and 1.7 mM bicarbonate solution into a 1 L volumetric flask with DDI. Anions were detected using a conductivity detector (US EPA, 1993; Rice et al., 2012).

A 5 g subsample of oven dried (70 ^{O}C for 24 hrs) soil was mixed with DDI water to extract the soil chloride for analysis by IC. The solution was mixed on a horizontal shaker plate for 1 hr at 300 rpm before filtration through filter paper (Fisher P8). Necessary dilutions and analyses were then performed as per the plant tissue. All analyses were conducted at the Analytical Services Unit (ASU) at Queen's University in Kingston, ON.

3-5.6 Quality Assurance & Quality Control

For each batch of samples (30) analyzed by IC, one Environment Canada certified reference material (CRM), Cranberry-05, was included along with a method blank and a calibration check standard (ECCC, 2019). For every 10 samples, a duplicate was included. For all analyses, Cranberry-05 was within 10% of the target. All blanks were less than the detection limit (0.05) and the calibration check standard was within 10% of the target. All duplicates were within 10% of each other.

3-5.7 Data Analysis

Statistical analyses were performed using R Studio version 3.3.3 'Another Canoe'. All data was first tested for normality using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variances using Bartlett's test ($p > 0.05$). The data was log transformed to meet the assumption of normality and was then analyzed via two-way ANOVA followed by Tukey's post-Hoc test.

3-6 RESULTS AND DISCUSSION

3-6.1 Chloride input rates at the Bath site are very high due to leachate

Over the course of the 2018 growing season, chloride input rates were variable but generally correlated with dry and rainy periods (Figure 3-3). Chloride input rates generally fell between ~100 - 400 mg/s or 8.6 – 34.5 kg/day; the most commonly recorded input rate was ~10 kg/day. Thus over the course of a single growing season, 2 700 ± 1 400 kg of chloride are added to the site. Given that the study site is approximately 1 000 m^2, the daily input rate is approximately 1.4 mg/cm^2. In the greenhouse experiment, 4" pots were used with the highest leachate watering rates providing 200 mg/day or approximately 2.5 mg/cm^2, thus the chloride input rates were ~2 times the normal input rate on site.

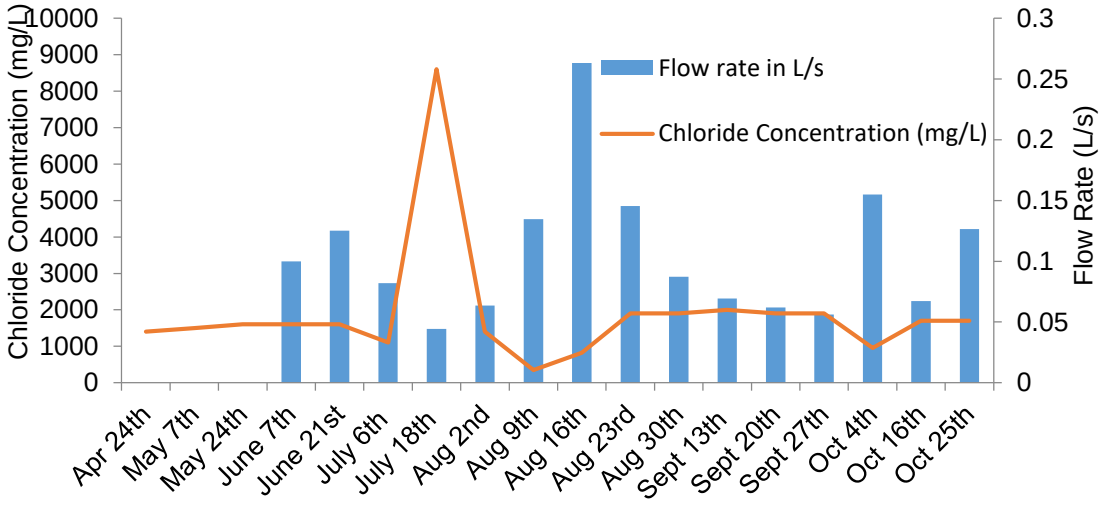

Figure 3-3: Flow rate (red line) and chloride concentration (blue bars) of leachate at the site in Bath, ON over the course of the 2018 growing season. Flow rates were not recorded until June 7th, 2018.

3-6.2 *S. maritima* shows good survivability when exposed to high KCl conditions

When grown under conditions with the highest amount of potassium chloride (high KCl soil + leachate), the *S. maritima* plants grew as well as those grown in high NaCl conditions and low salt (NaCl and KCl) conditions (Figure 3-4). This demonstrates that *S. maritima* can survive when exposed to high concentration of KCl, as daily input rates were significantly above (2X) the average at the Bath site. None of the plants showed significant differences in dry weight, but the plants grown in 'low NaCl' soil and watered with tap water had a significantly higher wet weight than those plants grown in 'high KCl' soil and watered with leachate. Those plants growing in the lowest salt conditions ('low NaCl' soil with tap water) appeared stunted in growth and had fewer branches; one plant did not survive for the duration of the experiment (Figure 3-4F). Soil texture did not have a significant impact on *S. maritima* as there was no difference in growth or survivability between the plants grown in Bath soil vs those in Bath soil with additional sand.

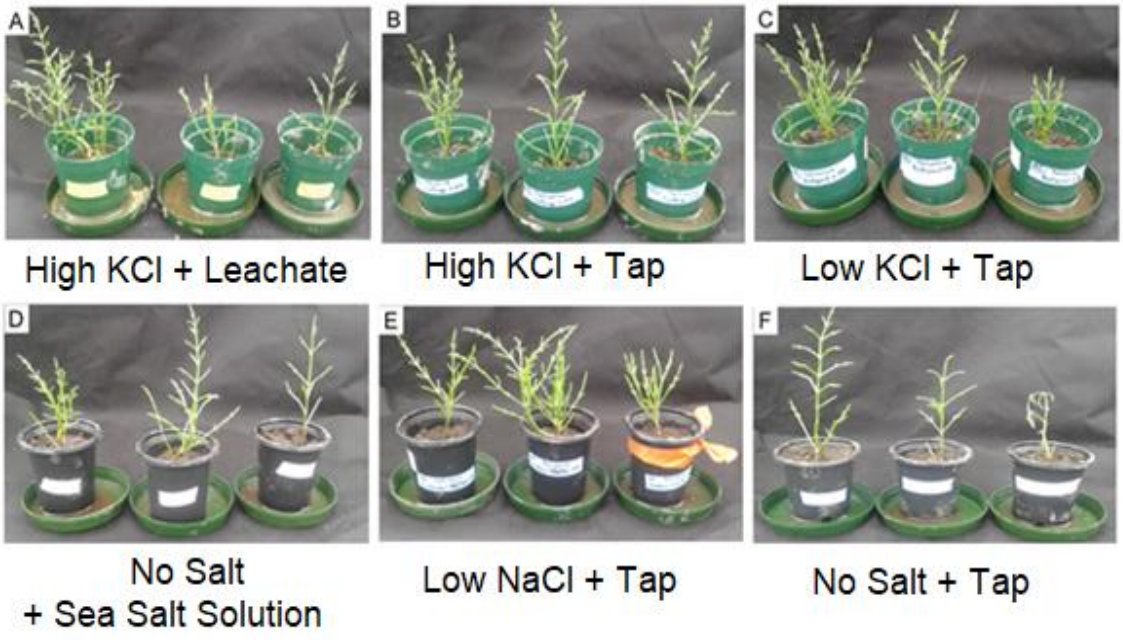

Figure 3-4: Salicornia maritima growing in high KCl soil + leachate (A), high KCl soil + tap water (B), low KCl soil + tap water (C), no salt soil + sea salt solution (D), low NaCl soil + tap water (E), no salt soil + tap water (F).

3-6.3 *S. maritima* has a large chloride uptake capacity

Under the highest potassium chloride exposure conditions (high KCl soil+ KCl leachate), *S. maritima* accumulated nearly 225 mg Cl^- per plant, making up to 25% of the total dry weight. Similarly, those plants watered with sea water (NaCl) contained on average 20% Cl^- by dry weight. Plants growing in high KCl soil and watered with KCl leachate contained significantly

higher concentrations of chloride than those grown in low KCl soil or No salt soil and watered with tap water (Figure 3-5). There were no significant differences between chloride uptake in plants growing with KCl compared to those growing with similar exposure to NaCl. Under the lowest chloride conditions, the plants contained 7.5% chloride suggesting that these plants took up large quantities of salt when they were young and still growing in saline estuary conditions (Figure 3-5B). It is possible that this NaCl could have contributed to the plants' survival in high KCl conditions. However, Barcia-Piedras et al. (2019) found that a close relative of *S. maritima*, *Arthrocnemum macrostachyum*, had a decreased desalinization capacity when pre-exposed to saline conditions, due to a reduction in biomass. Thus, the chloride uptake capacity of *S. maritima* could be even higher than what was observed, without prior exposure to NaCl. Diaz et al. (2013) demonstrated *Salicornia*'s exceptional chloride uptake when exposed to NaCl, where *S. biglovii* exposed to ~2300 mg Cl^-/L accumulated 170 mg Cl^-/g DW. However, this is the first time that removal of chloride from KCl brines has been demonstrated.

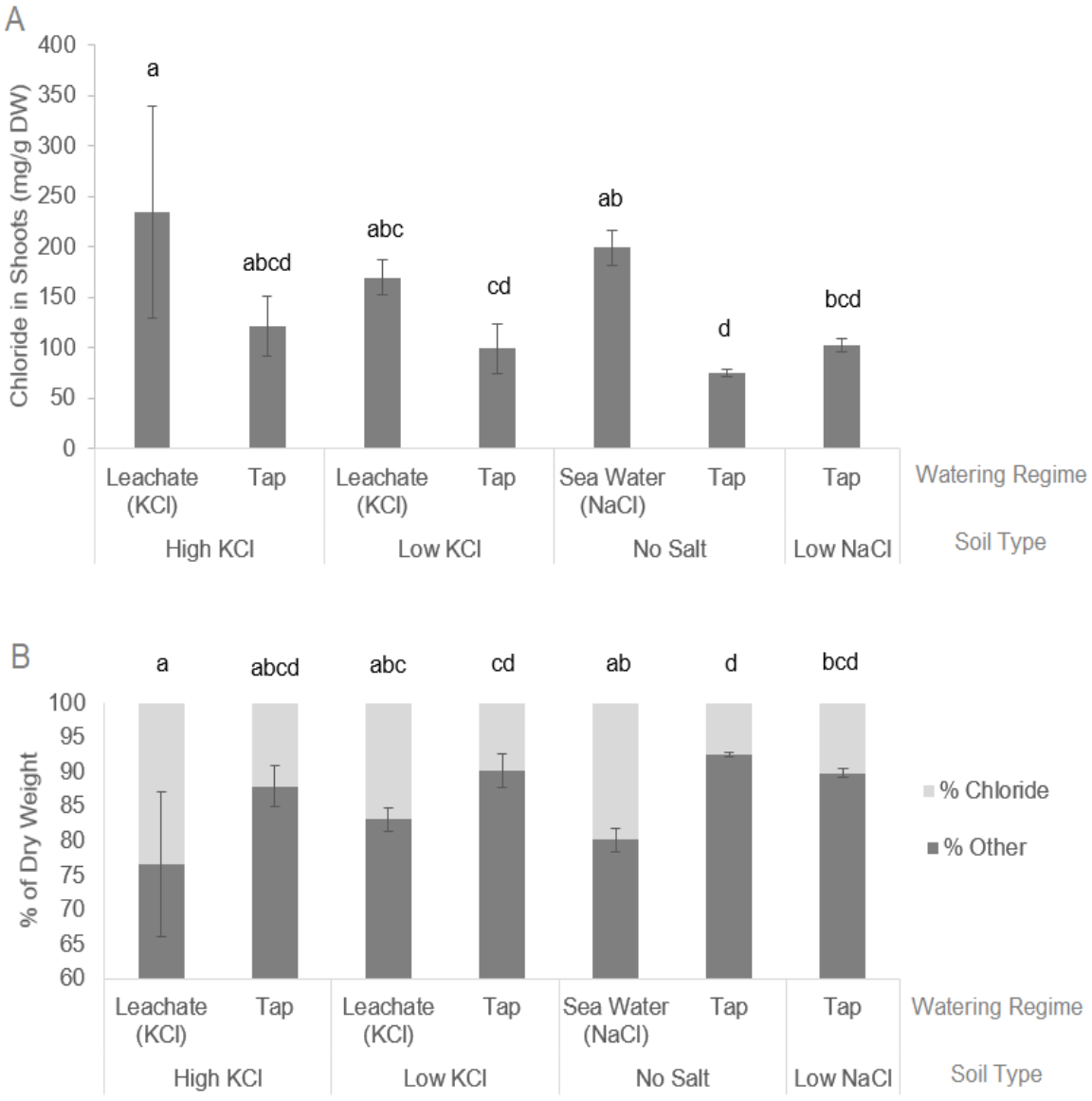

Figure 3-5: A) Chloride accumulated within S. maritima above ground tissue in mg/g dry weight. B) Proportion of S. maritima dry weight comprised of chloride. Lowercase letters indicate significant differences. For example, the treatment of low KCl soil + Leachate (abc) is not significantly different from the high KCl soil + leachate treatment (a) but is significantly different from the no salt+ tap treatment (d).

3-6.4 Changes in soil concentration

The measured final soil chloride concertation of the planted high KCl treatment + KCl treatment was significantly lower than the soil chloride expected, based on the sum of the initial soil chloride concentration and the amount added via watering (Figure 3-6). However, the amount of chloride taken up by the plants was not sufficient to explain the observed difference. The

calculated final soil chloride concentration, accounting for the amount of chloride taken into the plant, was also significantly higher than the measured final soil chloride concentration. The planted pots of the high KCl treatment had lower soil chloride than the unplanted pots, but the difference was not significant. Thus, leaching of salts likely occurred.

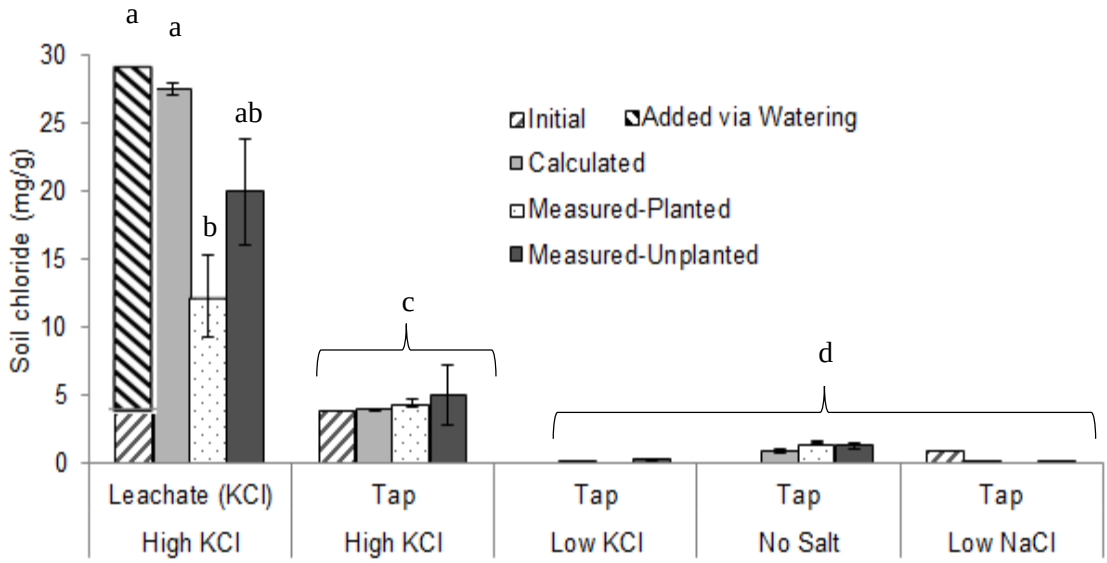

Figure 3-6: Soil chloride in planted and unplanted pots at the end of the 10-week experimental period compared to the initial soil concentration + added chloride inputs, and the calculated final soil chloride concentration based on chloride accumulation within shoot tissues. Lowercase letters indicate significant differences. The stacked hatched bars represent the sum of the initial soil concentration and the chloride added via watering, making the total height of the hatched bar the expected final soil concentration.

The rate of chloride inputs at the Bath site are very high, and likely cannot be matched by the chloride removal capacity of plants on site. Thus, chloride will continue to accumulate in the soil or leach into surrounding areas and fresh water bodies. However, soil watered with leachate contained up to four times as much as was initially in the soil (Figure 3-6). This demonstrates that the soil found on site has a high chloride retention ability. Engineered methods are likely necessary to manage the salts entering the Bath site and could incorporate *S. maritima*. Once leachate has stopped entering the site, long-term site remediation is likely possible using halophytic species including *S. maritima*, given the exceptional uptake capacity.

It has been reported that *Salicornia* may produce up to 27 tonnes DW/ha in an 8 month growing season (Gunning, 2016). Given the size of the study site (0.1 ha), and an extraction rate of 250 mg/g, it is estimated that up to 675 Kg of chloride could be removed in an 8 month growing season. These extraction rates are comparable to those of the invasive *Phragmites australis* currently growing onsite which has an extraction ability of 650 Kg chloride per growing season (McSorley, 2015). However, while their total extraction is similar, *S. maritima* is native and produces half the biomass of *P. australis*. The smaller amount of biomass and herbaceous nature of the plant would make disposal and composting of harvested shoots much easier and more feasible to manage. *Salicornia* species have been cultivated throughout the world as ‘cut and

come again' crops, so multiple harvests are possible, further increasing extraction capacity (Venture & Sagi, 2013).

3-7 CONCLUSION

While chloride input rates to the Bath site likely approach 2 000 Kg/season which exceeds *S. maritima's* chloride extraction capacity of 675 Kg/season, this plant may still be suitable for the treatment of smaller volumes of leachate. *S. maritima* demonstrates exceptional survivability and chloride uptake capacity when exposed to elevated concentrations of either sodium or potassium chloride. This herbaceous halophyte is thus a good candidate for the extraction of chloride from brines.

4 Evaluating the impact of soil chloride concentration and salt type on the excretions of four recretohalophytes with different excretion mechanisms.

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4-1 HIGHLIGHTS

- *Armeria maritima*, *Spartina pectinata*, and *Distichlis spicata* are suitable species for remediation via haloconduction
- *A. maritima* had the highest total extraction capacity at high soil chloride
- *S. pectinata* had the most consistent excretion capacity and is the most suitable for remediation of soils with lower soil chloride

4-2 ABSTRACT

Four native Canadian recretohalophytic species: *Atriplex canescens*, *Armeria maritima*, *Spartina pectinata*, and *Distichlis spicata* were examined to determine their relative uptake and excretion of chloride in the context of phytoremediation. Adult plants were grown in soils contaminated with either sodium chloride or potassium chloride at various concentrations, then manually washed to collect the excreted salts. *A. canescens* which has salt bladders, was found to have negligible excretions, suggesting that these structures release minimal amounts of salt onto the leaf's surface. *S. pectinata* and *D. spicata* had increasing chloride excretions with increasing soil chloride. *A. maritima* showed minimal excretion until a threshold soil salinity was reached. This species shifted from a reliance on internal sequestration to secretion at higher soil salinity. The salt used in the media did not impact these trends, but *D. spicata* excreted significantly more chloride under sodium chloride conditions. While all four species studied were able to translocate significant amount of salt to their shoots, only *S. pectinata*, *D. spicata*, and *A. maritima* are suitable candidates for remediation by haloconduction. Among these, *A. maritima* showed the greatest potential and significantly reduced the soil chloride concentration by up to 60% in the highest concentration treatment (4 mg/g).

ACKNOWLEDGEMENTS

This research was supported by Lafarge Canada Inc. as well as Zeeb & Rutter's Collaborative Research and Development Grant (CRDPJ 504080-16).

4-3 INTRODUCTION

While most plants cannot tolerate saline environments, halophytes overcome salinity stress by employing one or more adaptive mechanisms including: i) the exclusion of salts at the roots, ii) sequestration of salts within the vacuole, and iii) excretion of salts through specialized glands on their leaf surfaces (Deinlein et al., 2014). Plants that utilize the third mode of salt tolerance are known as recretohalophytes. There are four different types of salt glands: salt bladders, multicellular glands, bi-cellular glands, and uni-cellular glands (Dassanyake & Larkin, 2017; Litalien & Zeeb, 2020).

While many recretohalophytic species exist throughout the world (such as tropical mangroves), only 12 are native to Canada (USDA, 2019). Of these, two have salt bladders (*Atriplex canescens* and *Chenopodium album),* four have multicellular glands (*Glaux maritima* and *Armeria maritima, Limonium vulgare* and *Limonium carolianium)* and six have bi-cellular glands (*Buchloe dactyloides, Bouteloua curtipendula, Bouteloua gracilis, Distichlis spicata*, *Spartina pectinata,* and *Spartina gracilis)*. Salt bladders are not uncommon among species within the *Atriplex* genus however, *A. canescens* carries a much higher density of salt glands on its leaves than other *Atriplex* species. No Canadian species with unicellular hairs have been identified (USDA, 2019).

While all recretohalophytes share the ability to mobilize salt from the soil onto their leaf surfaces, the rate at which salts are excreted from leaf tissues is highly variable (Leng et al., 2017; Mukherjhee, 2012; Rozema et al., 1981). There are significant differences in excretion mechanisms which may play a role in excretion capacity in addition to species level differences (Dassanyake & Larkin, 2017). Furthermore, excretion rates are likely linked to salt uptake capacity and therefore salt concentrations in the soil, however there are a number of conceivable relationships possible (Mishra & Das, 2003; Sobrado et al., 2001; Rozema et al., 1981). While some salts present challenges to plant growth through osmotic stress, some ions are more toxic than others at the cellular level which may impact the rate at which they are excreted (Marschner, 2012; Rozema et al., 1981).

Recretohalophytes are interesting from a biological standpoint, but may also be useful in the remediation of salinized soils. Soil salinization is a growing issue worldwide attributable to the accumulation of inorganic salts within soils via natural and anthropogenic processes (Litalien & Zeeb, 2020). Halophytic plants can be used sustainably to extract salts from soil, through a process known as phytoremediation. Most authors implementing phytoremediation to treat salinized soils have focused on plants that accumulate salts within their above-ground tissues (Morteau, 2016; Hasanuzzaman, 2014; Jesus et al., 2015). There is however, growing interest in the use of recretohalophytes as they may provide shorter remediation timeframes than accumulator plants. While the use of recretohalophytes in soil remediation is still relatively novel, McSorley et al. (2016) demonstrated that excretion from recretohalophytes is significant

and may have the potential to translocate large amounts of salt out of the soil in a single season. Furthermore, Yun et al. (2019b) demonstrated that at least some of these salts are dispersible through the air through a process known as haloconduction.

Recretohalophytes have been studied by several authors, but very few have assessed their remediation potential (Yun et al., 2019b; McSorley et al., 2016; Sargeant et al., 2014). Most of the plants studied to date were grown in inert media rather than soil which may not accurately represent remediation conditions, and changes in media concentration have not been studied. Furthermore, no studies have evaluated the release of salt from salt bladders to determine if the amount is significant. Finally, few of the previously studied species are relevant to the Canadian context as most are non-natives. This study seeks to: i) determine differences in excretion between four Canadian recretohalophytic species (*Atriplex canescens*, *Armeria maritima*, *Spartina pectinata*, and *Distichlis spicata)*, ii) quantify the influence of the salt type and concentration used in the growth media, and iii) evaluate their efficacy in the context of phytoremediation.

4-4 METHODS

4-4.1 Plant Preparation

One plant species with multicellular glands, *Armeria maritima*, one with salt bladders, *Atriplex canescens*, and two species with bi-cellular glands, *Spartina pectinata* and *Distichlis spicata*, were selected to provide comparisons both between salt gland types and within a salt gland type.

A. canescens seeds were acquired from Sheffield's Seed Co. (Four-Wing Saltbush Lot #13768) and grown to 10 cm tall plants under greenhouse conditions (25 ± 3 ^{O}C, 50 ± 20 % humidity) from February to June 2018. During the same period, *D. spicata* plants were grown from seed (Brett-Young Seeds, Inland Saltgrass, Lot #DIST12, Calmar, AB) to 20 cm tall plants under greenhouse conditions. *S. pectinata* seedlings were procured from Norview Gardens and grown under greenhouse conditions from April to June, 2018 to obtain 20 cm tall plants. Adult *A. maritima* Splendens plants (10 cm tall) were acquired from a Loblaws garden centre in June 2018 and allowed to acclimate to greenhouse conditions for two weeks.

4-4.2 KCl Soil Preparation

Soil with high concentrations of potassium chloride (KCl) was collected at a cement kiln dust (CKD) impacted site in Bath, ON. Clean control soil was collected 20 m north of the site just beyond a stream that runs behind the field site (McSorley, 2016). Each soil type was dried and homogenized using the two-dimensional Japanese slab-cake method (Gy, 1992). Subsamples of each homogenized soil were collected and analyzed via ion chromatography as described below. The chloride concentrations in the soil from the CKD site and the control region were determined to be 4050 μg/g and 140 μg/g respectively. In order to create a rage of soil concentrations (n=5), the control and chloride contaminated soils were mixed and further homogenized (Table 4-1). 150 g of each of the five soils was added to 4 inch pots.

4-4.3 NaCl Soil Preparation

In order to generate the sodium chloride spiked soils, uncontaminated soil was removed from an un-salted roadside in Frontenac Provincial Park in ON, CAN (44.508335, -76.553574). The soil was air-dried for one week and homogenized using the two-dimensional Japanese Slab Cake method and spiked to produce four soil conditions (Table 1). To spike the soil, 300 g of dry soil was placed on a clean pie plate and mixed for three minutes with a solution consisting of the appropriate amount of NaCl dissolved in 80 mL deionized water. The soil was placed in four-inch plastic pots.

Table 4-1: Potassium and sodium chloride soil treatment conditions

Treatment (KCl)	Ratio of Contaminated Soil to Control Soil		Approximate [Cl^-] (µg/g)
100%	1:0		4 000
75%	3:1		3 000
50%	1:1		2 000
25%	1:3		1 000
0%	0:1		150
Treatment (NaCl)	NaCl added (g)	[Na^+] (µg/g)	[Cl^-] (µg/g)
High	2.286	3000	4620
Medium	1.524	2000	3080
Low	0.762	1000	1540
Control	0	50	17 (background level)

4-4.4 Experimental Set-Up

Adult native recretohalophytic plants *Atriplex canescens*, *Armeria maritima*, *Spartina pectinata*, and *Distichlis spicata* were transferred into 4 inch pots containing each of the 9 treatment soils (5 KCl, 4 NaCl) above and grown in the RMC greenhouse from June to August, 2018. Three unplanted pots were also included for each treatment and watered to control for chloride leaching through the soil. Each treatment was run in triplicate for a total of 27 pots per treatment and 135 pots total. Throughout this period, temperatures were maintained between 23 and 27 ^{O}C and relative humidity between 40 and 60%; plants were watered every other day with ~30 mL of tap water, which was determined to contain 25 mg/L Cl^-.

Every two weeks post-transfer, the shoot portion of the plants were washed with ultrapure water by tilting the pots and placing the shoots in a 4L zip lock bag before spraying with water and gently massaging within the bag (Yun et al., 2019b). Sufficient water was used to fully submerge the shoots. At the end of the 10 week growth period, the plants were washed a final time before harvesting the shoot tissue. At this time, soil samples were also collected by taking a 2 cm diameter full depth soil core from three locations within the pot.

4-4.5 Sample Analysis

The volume of water used to conduct plant washes was measured for each sample and a ~10 mL subsample was used for analysis. The subsample was syringe filtered with a 0.45 µm cellulose acetate filter (Munktell, Model 752509). Prior to analysis, the electrical conductivity (Fisher Scientific, Traceable Conductivity, Resistivity, and TDS Meter) was measured and samples were diluted to at or below 150 µS/cm. Analysis by ion chromatography with a Dionex HPLC (High Performance Liquid Chromatography) system (ICS 3000) was performed using an AG4A-SC guard column and an AS4A-SC analytical column. The column flow rate was set to 2.0 mL/min. A carbonate/biocarbonate eluent was prepared by diluting 10 mL of a 100x concentrate of 1.8 mM carbonate and 1.7 mM bicarbonate solution into a 1 L volumetric flask with DDW. Anions were detected using a conductivity detector (Yun et al., 2019b; US EPA, 1993; Rice et al., 2012). Tissue samples were oven dried at 70 ^{O}C for 24 hrs and weighed for dry weight (DW). The tissue samples were ground (Thomas Wiley Mini Cutting Mill, Model 3383-L10) and a 0.1 g subsample was shaken with double de-ionized (DDI) water on a horizontal shaker plate for 1 hr at 300 rpm to extract the chloride, then filtered and analyzed by IC as above. A 5 g subsample of oven dried (70 ^{O}C for 24 hrs) soil was mixed with DDI water to extract the soil chloride for analysis by IC. The solution was mixed on a horizontal shaker plate for 1 hr at 300 rpm before filtration through filter paper (Fisher P8). Necessary dilutions and analyses were then performed as per the method used for the plant rinse samples. All analyses were conducted at the Analytical Services Unit (ASU) at Queen's University.

4-4.6 Quality Assurance & Quality Control

For each batch of samples (30) analyzed by IC, one Environment Canada certified reference material (CRM), Cranberry-05, was included along with a method blank and a calibration check standard. For every 10 samples, a duplicate was included. For all analyses, Cranberry-05 was within 10% of the target. All blanks were less than the detection limit (0.05) and the calibration check standard was within 10% of the target. All duplicates were within 10% of each other.

4-4.7 Data Analysis

Statistical analyses were performed using R Studio version 3.3.3 'Another Canoe'. All data was first tested for normality using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variances using Bartlett's test ($p > 0.05$). The data was log transformed in order to meet the assumptions and was then analyzed via two-way ANOVA followed by Tukey's post-Hoc test.

4-5 RESULTS & DISCUSSION

Shoot length and dry weight did not vary significantly within species despite being grown in soil with different concentrations of chloride and type of salt (Sup. Figure C-1), indicating that none of the soil chloride concentration used had an impact on plant biomass production. Similarly, wet weights were not significantly different, except *A. maritima* plants grown in the highest concentration of NaCl soil (4600 µg Cl^{-}/g) had a significantly lower wet weight than those grown in control soil. *A. maritima* plants grown in NaCl soil also had a lower mean wet weight than those grown in soil containing KCl. All of the plants appeared healthy across soil chloride

concentration except *A. martima* at the highest salt concentration (4000+ μg Cl^-/g), where plants had many chlorotic leaves and appeared stressed (Figure 4-1H). Despite this, they continued to produce new leaves throughout the experiment.

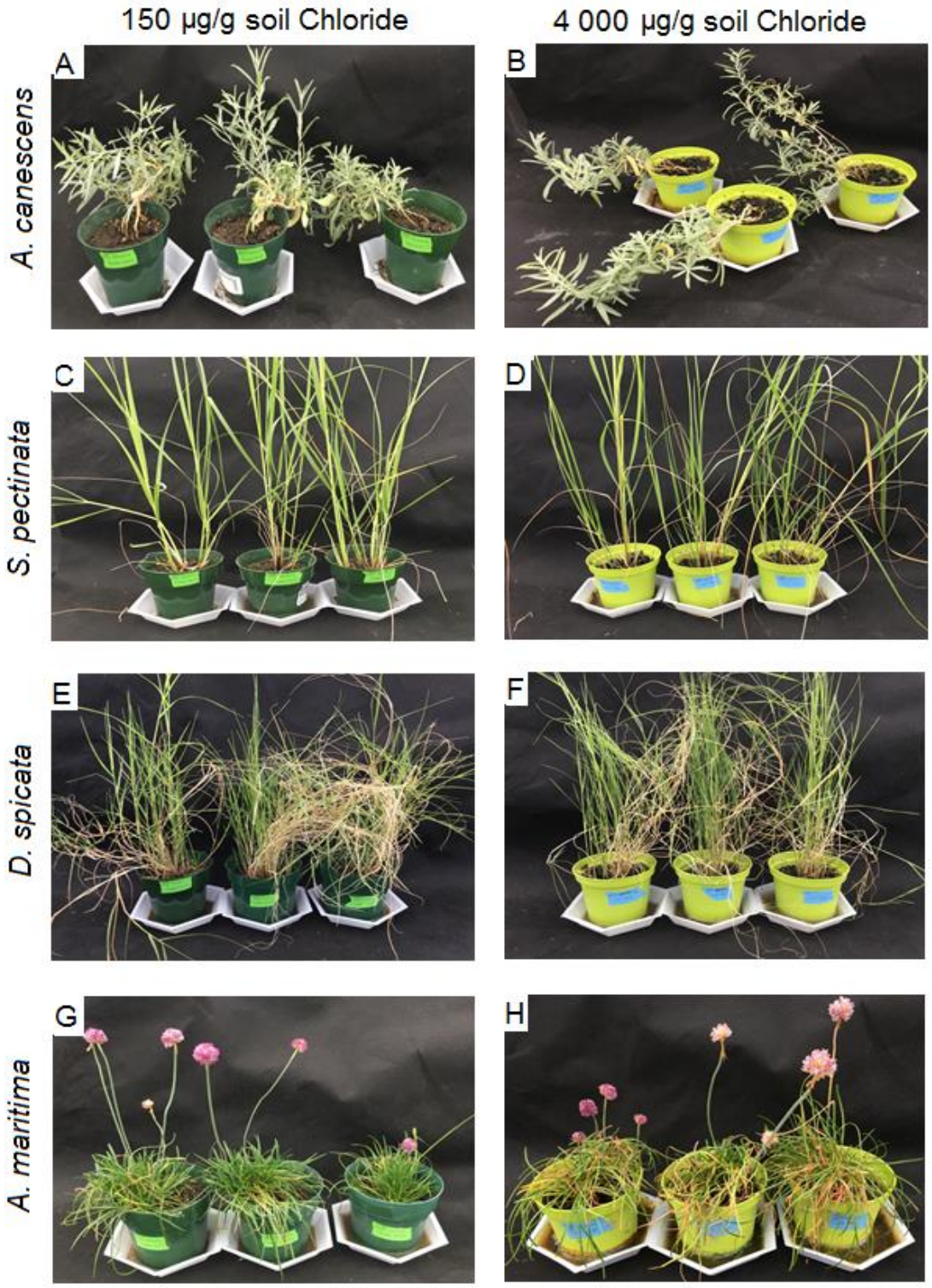
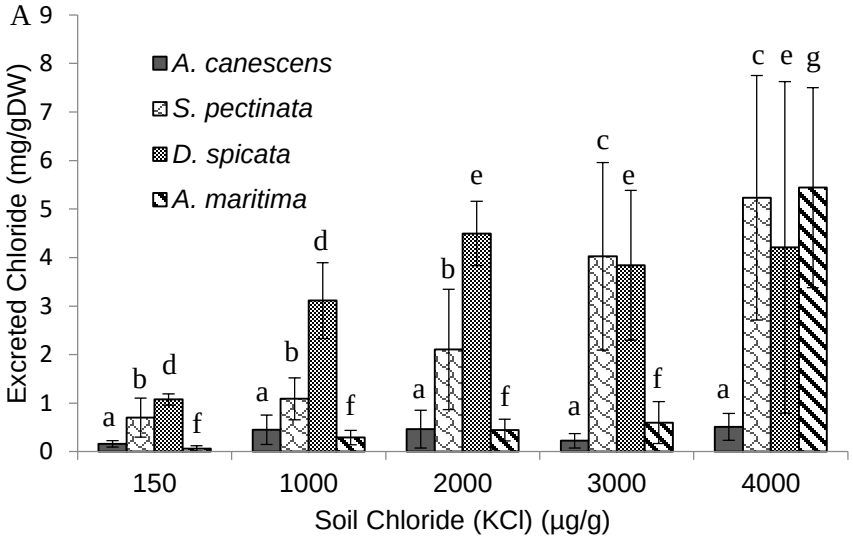


Figure 4-1: Canadian recretohalophytes grown in the lowest (150 µg/g) and highest (4000 µg/g) concentration of KCl soil are shown at the end of the 10 week experimental period.

4-5.1 Impact of species differences on excretion and accumulation

4-5.1.1 Excretion

Species specific trends were observed when comparing the excretion rates within each species at different soil chloride concentrations (Figure 4-2 A & B). Excretion by *A. canescens* was consistenly minimal throughout the experiment. *D. spicata* and *S. pectinata* both show a positive relationship between soil chloride and chloride excretion. By 3000 µg/g soil chloride, excretion rates for *D. spicata* and *S. pectinata* were significantly higher than the control (150 g/g soil chloride). *A. maritima* showed low excretion rates, that were not significantly different from the control (150 µg/g soil chloride) until the 4 000 µg/g soil chloride condition, where excretion rates were significantly higher. Many researchers have observed different trends in excretion with changing soil salinity which highlights the species specific nature of the relationship (Mishra & Das, 2003; Sobrado et al., 2001; Rozema & Gude, 1981).



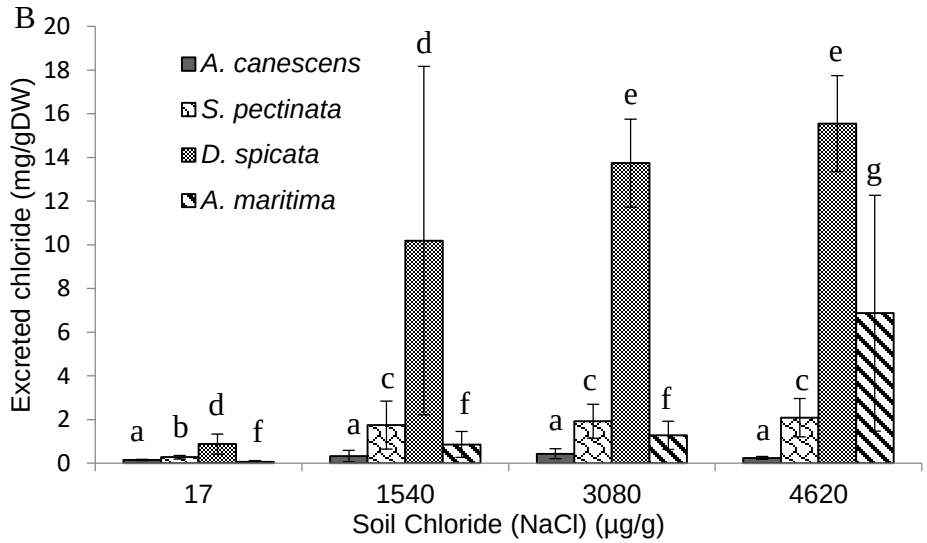


Figure 4-2: Mean chloride (n=3 ± standard deviation) concentration (mg/g shoot DW) excreted by four Canadian recretohalophytes in a two-week period. A) shows plants grown in soil with KCl, while B) shows plants grown in soil with NaCl. Significant differences in excretion within species, as compared to excretion when grown in the control soil, are indicated by lowercase letters.

4-5.1.2 Accumulation

When grown in control soil ([Cl^-]= 150 µg/g) all the species accumulated similar amounts of chloride into their above ground tissues on the basis of dry weight. When exposed to the highest concentration of potassium chloride however, both *A. martima* and *A. canescens* accumulated significantly more chloride into their shoots than *S. pectinata* and *D. spicata* (Table 4-2).

Table 4-2: Chloride accumulated in above-ground shoot tissue of four Canadian recretohalophytes at the end of the 10-week treatment period when grown in control soil, and the soil with the highest chloride concentration (KCl) (n=3 ± standard deviation).

Species	Grown in soil with 150 µg/g Cl^-	Grown in soil with 4000 µg/g Cl^-
	Chloride Accumulated in Shoots (mg/g DW)	
A. canescens	11.2 ± 0.9	28.5 ± 5.6
S. pectinata	10.2 ± 3.2	19.4 ± 3.2
D. spicata	6.71 ± 5.6	10.0 ± 4.0
A. maritima	17.8 ± 1.6	40.6 ± 11

4-5.1.3 Excretion vs Accumulation

All four species accumulated significantly more chloride than they excreted over the course of the 10-week experiment, however *S. pectinata, D. spicata, and A. maritima* all excreted 40-50%

of the chloride taken into their tissues (Figure 4-3A). Rozema and Gude (1981), found similar results in that *Spartina anglica*, *Limonium vulgare*, *Glaux maritima* and *Armeria maritima* were able to secrete up to 60, 33, 20 and 4% of the absorbed sodium respectively. When comparing the proportion of excreted chloride with increasing soil chloride concentrations, *A. maritima* excreted less than 10% of the absorbed chloride until 4000 µg/g Cl^- soil where this species shifted and excreted a significantly higher proportion (50%) of chloride than at any other soil chloride concentration. (Figure 4-3B).

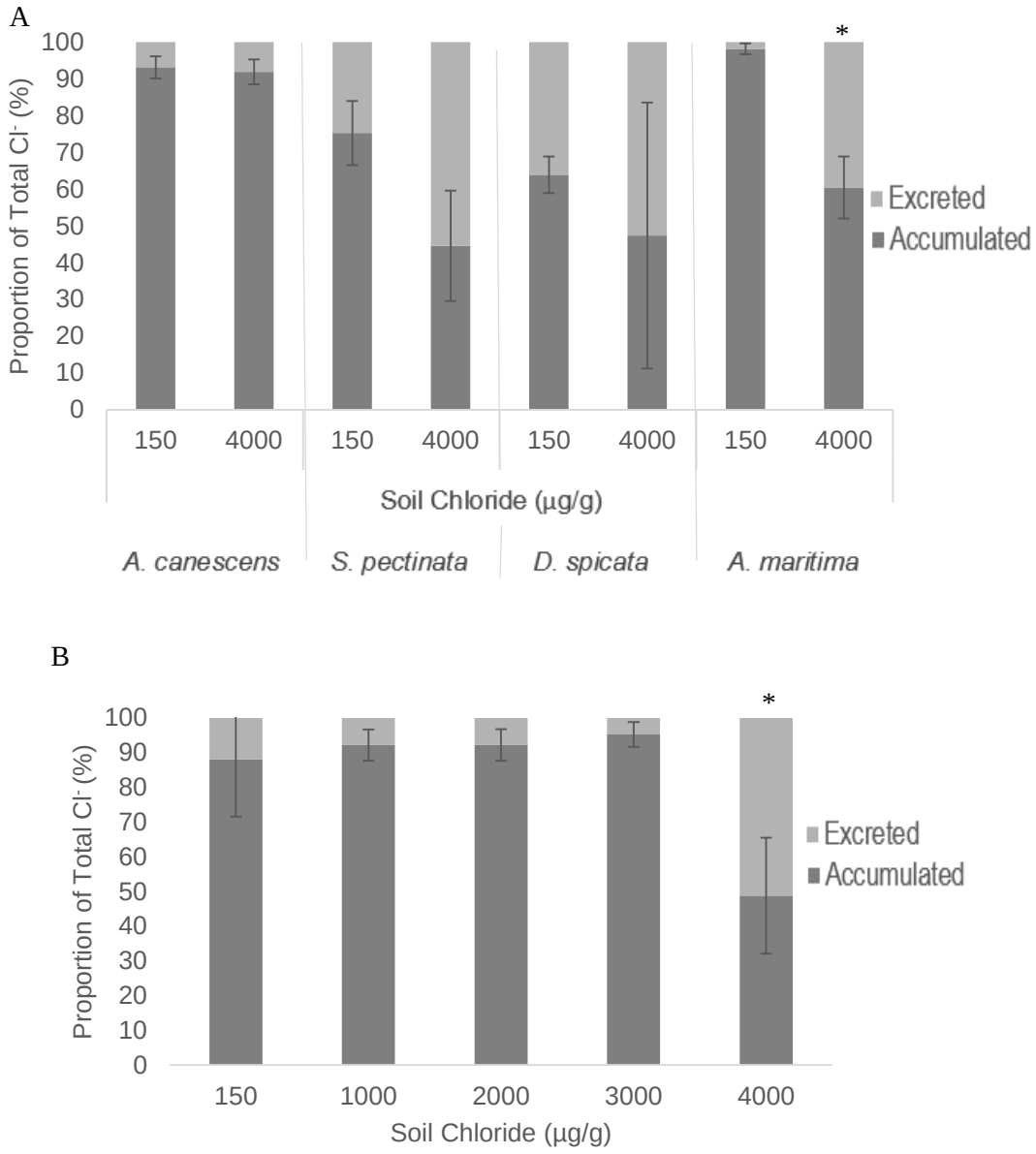


Figure 4-3: The proportion of chloride accumulated vs excreted by each plant species (n=3 ± standard deviation). A) shows all species, B) shows *A. maritima* at all five soil chloride concentrations. * Indicates significance within species.

At the highest concentration of soil chloride, salt excretions were visible on the older leaves of *A. maritima* which appeared stressed (Figure 4-4). This suggests that *A. maritima* may shift from primarily accumulating salts to excreting salts once a threshold of salinity stress has been surpassed. Transcriptome analysis under these conditions could potentially provide valuable information regarding salt translocation in this species.

Figure 4-4: *A. maritima* grown in soil containing 4 000 µg/g chloride in the form of potassium chloride is shown; A) an older leaf and B) young leaves.

4-5.2 Impacts of the salt used in the growth media on excretion

The amount of chloride excreted varied based on whether the plants were grown in soil containing NaCl or KCl. *D. spicata* excreted significantly more chloride when grown in soil containing NaCl ~3000 µg/g chloride (Figure 4-5). This is similar to Rozema & Gude's (1981) finding that *Spartina anglica*, *Limonium vulgare,* and *Glaux maritima* excreted significantly more sodium than potassium and reflects the fact that potassium is a macronutrient and essential to the plant, whereas sodium is not (Marschner, 2012). Mukherjhee (2012) also noted that mangroves selectively secrete sodium and/or potassium to maintain internal Na/K ratios. Since chloride generally acts as a counter ion, higher chloride excretions could be expected from plants grown in sodium chloride containing media (White et al., 2011).

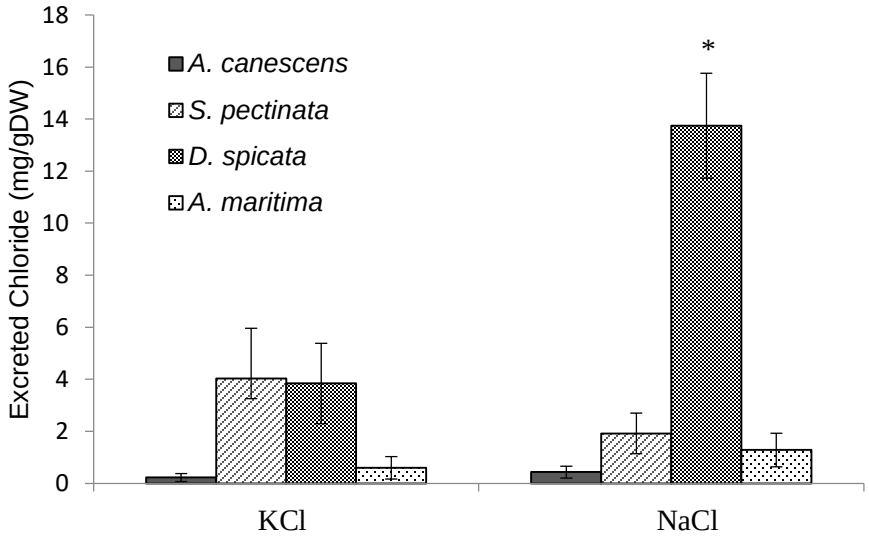


Figure 4-5: Mean chloride (n=3 ± standard deviation) concentration (mg/g DW) excreted by Canadian recretohalophytes in a two-week period when grown in soil containing approximately 3000 µg/g chloride in the form of potassium or sodium chloride. * Indicates significant difference between KCl and NaCl treatment.

4-5.3 Recretohalophytes in the context of remediation

Excretion by *A. canescens* was negligible compared to the other species studied, with less than 1000 µg Cl^-/g DW per 2 weeks. While other studies have shown that *Atriplex* species including *A. canescens* can accumulate significant amounts of salt in their salt bladders these authors used manual brushing to remove the bladders and determine salt concentrations within these organs (Pan et al., 2016; Freitas & Breckle, 1992). By simply washing the leaf surfaces to remove salts found on the undisturbed leaves in this study, the amount of chloride available for haloconduction was quantified and determined to be minimal (Figure 4-2). Thus, despite *A. canescens* having the capacity to translocate large amounts of salts to its salt bladders, it is likely that the majority of the salt remains sequestered in these organs and is not relevant to remediation by haloconduction.

4-5.3.1 Phytoexcration capacities

Based on the combined excretion and accumulation rates measured, it was calculated that over the course of a regular 16 week growing season, 14 ± 0.86, 16 ± 2.1, 17 ± 11, and 34 ± 2.1 g/m^2 of chloride could be removed by *A. canescens*, *S. pectinata*, *D. spicata*, and *A. maritima*, respectively when grown in soil concentrations ~4000 µg Cl^-/g. Despite visual symptoms of stress in older leaves, *A. maritima* continued to produce new healthy biomass while translocating significant amounts of chloride into its shoot tissues. Thus, *A. maritima* may be the most suitable recretohalophyte for the remediation of soils with chloride levels >4 000 µg/g, but *S. pectinata* or *D. spicata* would be more suitable for soils with chloride concentrations between 1 000 and 3 000 µg/g as their excretion rates are considerably higher at lower soil chloride concentrations. Between *S. pectinata* and *D. spicata*, *D. spicata* shows much higher variability in its extraction

rates as demonstrated by the high standard deviation for this species. *S. pectinata* on the other hand shows consistent extraction rates particularly in potassium chloride soils (Table 4-3).

Table 4-3: Measured removal (10 weeks) in 4" pots, n=3 and estimated salt removable by recretohalophytes across a 1 m^2 area in one season (16 weeks) when grown in soil with a high Cl^- concentration (4 mg/g)

		A. canescens[1]	*S. pectinata*[2]	*D. spicata*[3]	*A. maritima*[4]
Accumulated Cl^-	mg/pot	97 ± 0.4	54 ± 1.3	31 ± 1.3	142 ± 1.1
	$(g/m^2)^a$	12.3 ± 0.05	6.90 ± 0.16	4.00 ± 0.16	18.0 ± 0.14
	$(g/m^2)^b$	71.0 ± 1.0	29.0 ± 1.5	10.2 ± 1.4	60.9 ± 0.93
Excreted Cl^-	mg/pot	7.4 ± 4.0	46 ± 9.8	64 ± 52	77 ± 9.8
	$(g/m^2)^a$	1.50 ± 0.8	9.3 ± 2.0	13.0 ± 11	16.0 ± 2.0
	$(g/m^2)^b$	10.2 ± 5.6	63.0 ± 30	30.6 ± 25	65.0 ± 25
Total	mg/pot	98 ± 1.1	63 ± 3.2	44 ± 12	158 ± 3.1
	$(g/m^2)^a$	14.0 ± 0.86	16.0 ± 2.1	17.0 ± 11	34.0 ± 2.1
	$(g/m^2)^b$	81.0 ± 6.6	93.0 ± 32	41.0 ± 26	126 ± 26

Data is presented as mean (n=3) ± standard deviation, a) calculated from the mg/pot and the planting density used in this experiment (Table C-2), b) calculated based of mg/g DW and literature values for biomass, [1](Glen et al., 1999); [2](Helios et al., 2013), [3](USDA, 2018), [4](Schwartz et al., 2001) (Table C-3).

4-5.3.2 Expected vs measured changes in soil media concentration

A significant decrease in soil chloride concentration between planted and unplanted pots was measured with *A. maritima* at the highest soil chloride concentration, where soil chloride dropped from ~ 4 000 µg/g to ~ 1 500 µg/g (Figure 4-6). Based on the amount of chloride removable by excretion and sequestration in above ground tissue, it was calculated that *A. maritima* would be able to reduce the soil chloride concentration from ~ 4 000 µg/g to ~ 2 900 ± 40 µg/g, by the end of the 10-week experiment. The measured change in soil chloride was not significantly different from the expected change in soil chloride. Based on predicted extraction rates, it was expected that *A. canescens* would significantly reduce the soil chloride to approximately 3 400 ± 200 µg/g, the final soil concentrations measured was similar to the expected value but not significantly different from the initial soil concentration. On the contrary, based on the extraction rate of *S. pectinata* and *D. spicata* it was not expected that a significant decrease in soil chloride would be observed (3 600 ± 40 µg/g and 3 800 ± 160 µg/g respectively) over the course of the experiment, which was consistent with experimental results. These species would require a longer period for extraction to see marked decreases in soil chloride. However, *D. spicata* showed consistently high variability in extraction and altered soil chloride.

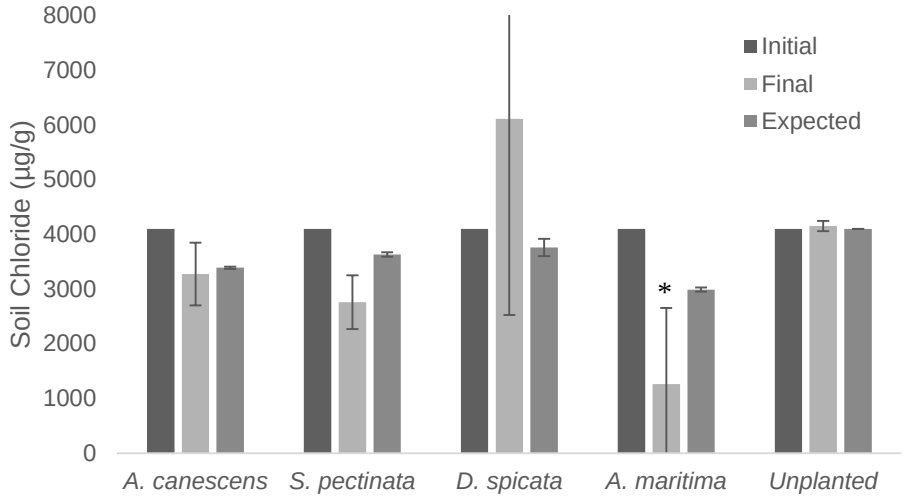


Figure 4-6: Change in soil concentration from the start to end of the 10-week experiment (n=3 ± standard deviation). *Indicates significant difference between the initial and final soil concentration. Note that an additional 50 µg of chloride were added to each pot over the course of the experiment from watering but that this did not represent a significant increase in soil chloride.

While excretion is small relative to accumulation in all species studied, over the course of a growing season, the cumulative amount of salt available for dispersal is significant, especially when plants are grown in soil containing higher concentrations of chloride. While *A. canescens* does not appear to be a candidate plant for remediation via haloconduction, the other three species could be useful in several applications. For instance, *A. maritima* might be an excellent choice in roadside gardens in cities that salt their roads. It is an attractive species with showy pink to white flowers commonly found in ornamental gardens and has demonstrated exceptional salt extraction abilities (USDA, 2019). *S. pectinata* has also been used as an ornamental grass, and has the advantage of being taller which would be more conducive to the dispersal of salt via wind action.

4-6 CONCLUSION

The issue of soil and freshwater salinization is of growing interest in regions where road salting is performed and where salt-rich by-products such as cement kiln dust and oil extraction brine are common. Halophyte-driven remediation methods such as haloconduction may be useful in treating these salinized soils but rely on recretohalophytes who can efficiently translocate salts from the soil onto their leaf surfaces. Based on this study, useful species native to Canada include *A. maritima*, *S. pectinata*, and *D. spicata* but not *A. canescens* which appear to behave more like an accumulator halophyte than a recretohalophyte.

5 Development of a Model for the Dispersal of Salts from Recretohalophytes

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(Formatted for submission to the Journal of Environmental Science and Technology)

5-1 HIGHLIGHTS

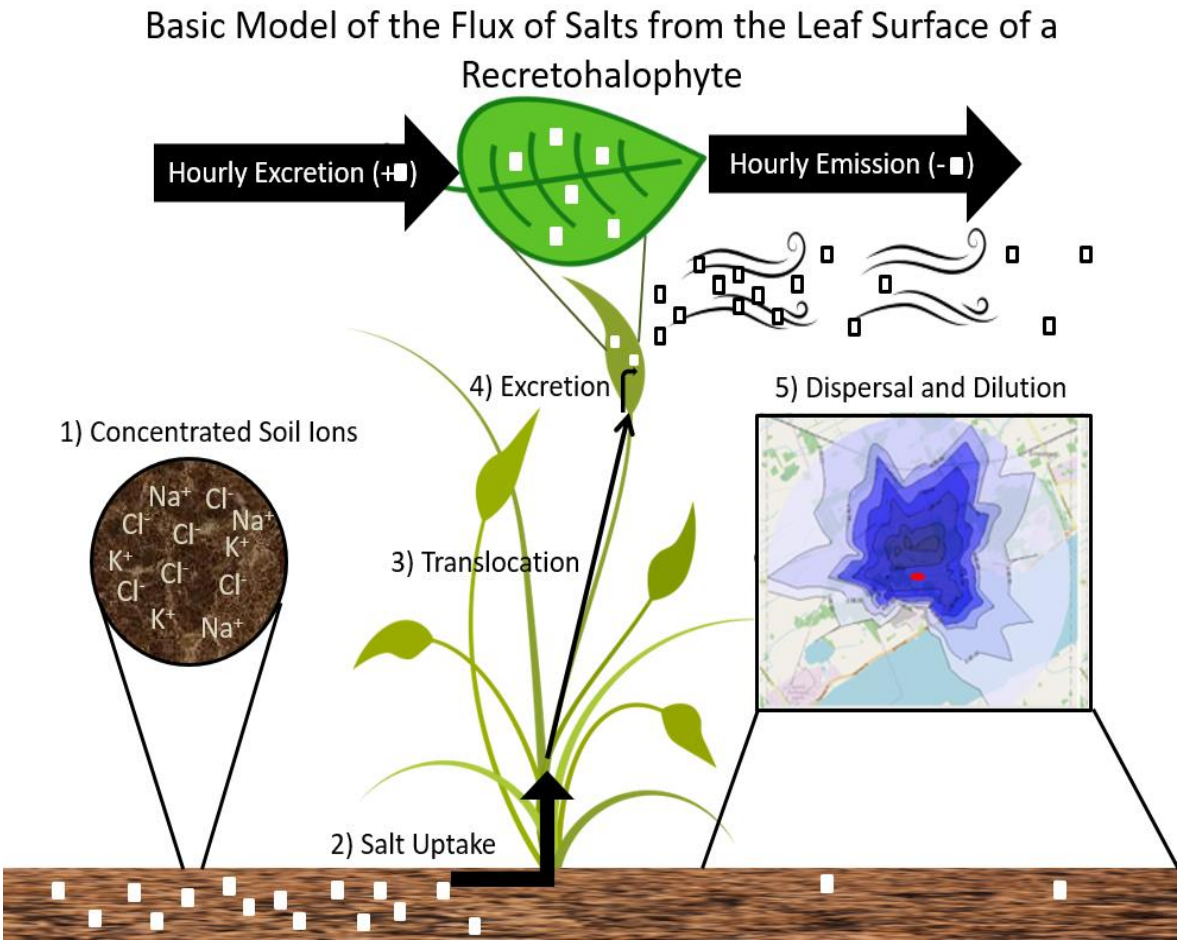
- The first model of haloconduction was developed and aerial dispersal visualized using AERMOD View
- Recretohalophytes have the potential to extract more than 200 kg of chloride per season from a 1000 m^2 site
- Haloconduction does not present significant risk to the surrounding environment as chloride deposition rates remain below background levels
- Aerial chloride monitoring revealed low chloride concentrations on site, but negligible levels off-site

5-2 ABSTRACT

A novel method for the remediation of salinized soils utilises recretohalophytes; - plants that secrete salts onto their leaf surfaces. Wind blows the excreted salts from the leaves, dispersing and diluting them over great distances. In this study the first model was established to estimate the amount of salt that could be transferred from a given field site via haloconduction. Further, the model allows for the determination of the location and concentrations of deposited salts. Greenhouse and wind tunnel experiments were used to determine excretion and salt emission rates of *Spartina pectinata.* Based on this data a theoretical emission profile for *S. pectinata* at a salt-impacted field site in Bath, ON was generated. AERMOD View modelling software was used to visualize the dispersal of the emitted salts. Finally, a field monitoring program was implemented to determine actual chloride deposition rates and airborne concentrations using passive wet candles and a high volume air sampler. Based on this model, approximately 180 kg/year of potassium chloride (KCl) salt could be displaced from the Bath site and deposited over a ~70 km^2 region, while maintaining deposition concentrations well below background levels.

KEYWORDS: Recretohalophytes, Haloconduction, Aerial Dispersal Model, AERMOD, Remediation

5-3 GRAPHICAL ABSTRACT



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5-4 INTRODUCTION

Soil salinization affects many ecosystems worldwide and is becoming a growing issue in agricultural regions due to improper management of fertilizers and irrigation (Nelson & Mereida, 2001; Endo et al., 2011; Cuevas et al., 2019). While salts are benign at low concentrations, high soil salinity reduces soil quality and can cause ion toxicity as well as osmotic stress to plants and soil organisms (Setia et al., 2013; Deinlein et al., 2014; East et al., 2017). There is growing interest in developing sustainable methods for the long term management of soil salinity and salinized soil remediation (Cuevas et al., 2019). One sustainable method is the use of plants and their phytoextraction capacities (Litalien & Zeeb, 2020).

Recretohalophytes are salt tolerant plants that excrete salts on their leaf surfaces. Yun et al. (2019b) were the first to demonstrate that these salts could be dispersed by wind action as proposed by Yensen and Biel (2008) via haloconduction. In this process, salt crystals formed on the leaf surface can be mobilized by the wind as it blows on the plants and causes them to flutter. Once in the air column, Gaussian plume models suggest that the space between particles in the air column will increase with increasing distance from the source due to molecular diffusion (Leelossy et al., 2014). Particles leave the air column by dry deposition or wet deposition (rain) given the appropriate meteorological conditions (Litalien & Zeeb, 2020).

In order to determine the remediation potential of this phytotechnology at any given site, site-specific factors need to be considered. Based on methods developed for modelling other natural aerosolized products such as plant pollen, three components are essential for site-specific analyses: i) available aerosol pool, ii) emission factors, and iii) aerial dispersal based on meteorological and topographic data (Zhang et al., 2014).

There are more than 130 recretohalophytic plant species throughout the world, but only 12 are native to Canada (University of Sussex, 2017; USDA, 2019). Within this short list, ever fewer are likely good candidates for remediation via haloconduction. The best suited plants i) have a high translocation capacity to move salts from the soil onto their leaf surfaces, ii) consistently produce salt excretions, and iii) are tall enough that wind action could actually transfer salts into the air (Litalien & Zeeb, 2020). One species that meets these criteria is *Spartina pectinata* which grows to a height of ~1 m and was studied by both Yun et al. (2019b) and in preliminary studies by Litalien et al. (2019, Chapter 4).

While little is known regarding the emission factors for salt from the leaves of *S. pectinata,* or recretohalophytes in general, studies on the emission of fungal spores from the leaf surfaces of plants using wind tunnels provides a valuable framework to study the phenomena (Aylor et al., 1981; Gagea et al., 1997). Several models exist for the visualization of the atmospheric dispersal of particulates (US EPA, 2018). AERMOD is a standard Gaussian plume atmospheric modeling system used by major regulatory bodies including the US EPA for the monitoring of industrial air pollutant emissions (US EPA, 2018). The objective of this study was to generate the first model of haloconduction by estimating the excretion and emission rates of salts from *S. pectinata*, and visualizing the dispersal of airborne salts using AERMOD.

5-5 METHODS

5-5.1 Study Site and Field Validation

A salinized wetland site in Bath, ON, impacted by cement kiln dust leachate with concentrated amounts of potassium chloride (KCl), was selected to model and validate haloconduction. The site is hereto referred to as the "Bath site" (Figure 5-1A). Five plots 1 m^2 of *Spartina pectinata* were planted on site in 2015 and maintained until the end of the 2019 growing season. A weather station (Davis Instruments, WeatherLink 6.0.3) was installed onsite in April, 2018 to provide surface level meteorological data. It was supported with data from a regulatory weather monitoring station installed at the Bath cement plant, as well as Natural Resources Canada (NRC) historical weather data from the Kingston airport station (NAVCAN Climate ID: 6104149) located ~20 km from the site.

Throughout the 2018 and 2019 field seasons, randomly selected 100 cm^2 sections of *S. pectinata* growing at the field site were washed with ultrapure water by tilting the pots and placing the shoots in a 4 L ziplock bag before spraying with water and gently massaging within the bag as per Yun et al.'s (2019b) method. Sufficient water was used to fully submerge the shoots. The amount of chloride in these solutions represents the amount of chloride found on the plants on the sampling date, given the meteorological conditions.

In order to monitor salts entering the air column 'wet candles' (2018 and 2019) and a high volume air sampler (HiVol) (Tisch Environmental, Model 5012) borrowed from Natural Resources Canada were used on site. A 'wet candle' is an apparatus consisting of a glass vial wrapped in fabric that is kept continually moist by wicking ultra-pure water from a flask below (Figure 5-1B). As wind blows past the cloth tube, the moisture in the cloth encourages the deposition of salt particles from the air (Baboian, 2005). High volume air samplers suction air from the surrounding environment and collect particulates on a filter paper (Figure 5-1C). The volume of air is calculated from the calibrated air flow rate in cubic feet per minute (CFM) and the duration of the run. With this information the concentration of chloride in the air can be calculated. The wet candles were sampled on a bi-weekly basis while the HiVol sampler was run for ~10 hours (~50 CFM) once per week. Three background samples were also collected throughout the 2019 season with the HiVol ~5 km north east of the site (44.200704, 76.795453) to establish background aerial concentration of chloride within the region. Chloride deposition values and concentrations determined for the 2018 and 2019 field season were used to validate the AERMOD output files.

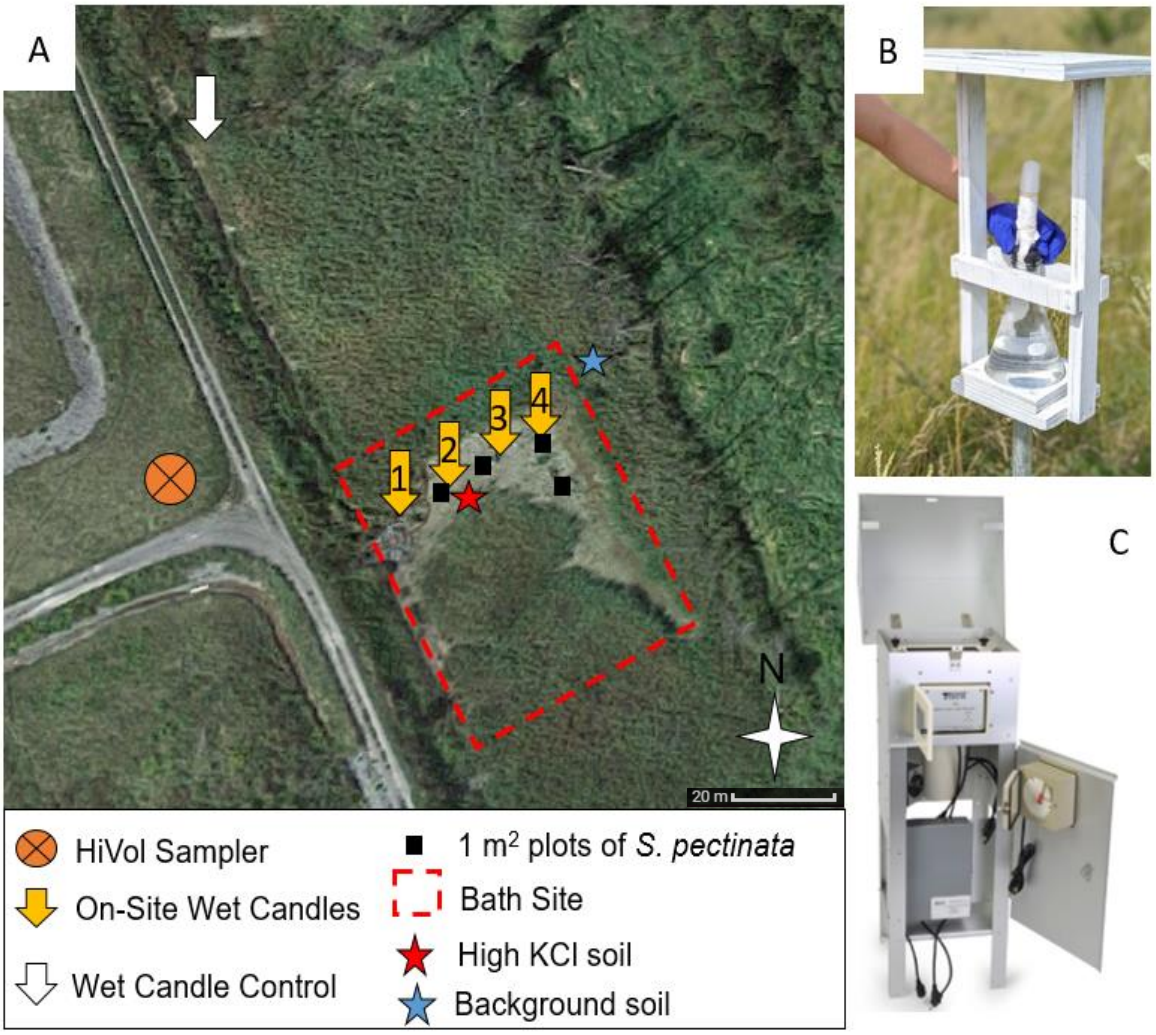


Figure 5-6: The site in Bath, ON showing (A) a close up of the site with the contaminated region delineated in red and the location of the air samplers, soil sources and *S. pectinata* plots indicated (B) wet candle, and (C) high volume air sampler (HiVol).

5-5.2 Part 1: Estimation of Excretion Rates by *Spartina pectinata*

Based on previous experimental data on recretohalophytes, plant size and soil chloride concentrations were assumed to be the greatest factors contributing to varied uptake and excretion rates within a species (Litalien et al., Chapter 4). The relationship between soil chloride concentration, plant size, and excretion rates was determined experimentally via a greenhouse pot study. *S. pectinata* seedlings (sourced from Norview Gardens, Norwich, ON) were grown under greenhouse conditions (25 ± 2 ^{O}C, 40 ± 20 % relative humidity (RH)) for 2 months until ~20 cm tall before being transplanted into 4 inch pots containing one of 5 treatment soils.

High KCl soil collected from the Bath site, near wet candle 2 (Figure 5-1A), and background soil collected just beyond the northern corner of the border of the site, were homogenized using the two-dimensional Japanese slab cake method and mixed in proportions of 1:0, 2:1, 1:1, 1:2, 0:1 of background soil: contaminated soil to produce 5 soils with approximate chloride concentrations of 150, 1000, 2000, 3000, and 4000 µg/g, respectively (Gy et al., 1992). The plants were then grown undisturbed in the RMC greenhouse from June to August 2018 (25 ± 2 ^{O}C, 50 ± 20 % RH). Throughout this period, plants were watered every other day with tap water (25 mg Cl^-/L). Triplicates of each condition were included. After the plants were potted, the shoot portion of the plants were washed with ultrapure water on a bi-weekly basis as per the method used in the field and analyzed for chloride content by ion chromatography.

5-5.2.1 Sample Analyses

The cloth from the wet candles and the filter paper from the HiVol were shaken vigorously for 2 minutes with 300 mL of double de-ionized (DDI) water and then placed in a sonicator bath for 30 minutes to extract the salts. The chloride concentration of the resulting solutions was then determined by ion chromatography as per Yun et al. (2019b)'s method, as were the plant wash solutions. All analyses were conducted at the Analytical Services Unit (ASU) at Queen's University.

5-5.2.2 Quality Assurance & Quality Control

For each batch of samples (30) analyzed by IC, one Environment Canada certified reference material (CRM), Cranberry-05, was included along with a method blank and a calibration check standard (ECCC, 2019). For every 10 samples, a duplicate was included. For all analyses, Cranberry-05 was within 10% of the target. All blanks were less than the detection limit (0.05) and the calibration check standard was within 10% of the target. All duplicates were within 10% of each other.

5-5.2.3 Plant Wash Data Analysis

A multiple non-linear regression was generated from the plant wash data following a generalized additive model (GAM) format (Hastie et al., 1986). Conceptually, the relationship between each of the variables can be added together to generate one smooth function. Excretion is the sum of the impact of plant height (and by proxy age), and soil concentration on excretion. The MATLAB curve fitting tool (MathWorks R2017b) was used to generate a regression for the estimated hourly excretion rate of *Spartina* plants relative to their soil concentration and height.

5-5.3 Part 2: Estimating Emission Factors

After conducting a multiple component analysis of weather data and field plant wash values, collected in 2018 using R Studio version 3.3.3 'Another Canoe', it was determined that high concentrations of salts found on the plants themselves correlated positively with high temperature and high humidity, and correlated negatively with high wind speeds (Sup. Figure E-1). Similar findings were observed when Gagea et al., (1997) studied the emission of fungal spores from plant leaves and determined that temperature mainly played an indirect role by influencing humidity but did not itself impact emission (Jones & Harris, 2004). Thus the model includes the

assumption that the greatest factors contributing to emission were humidity and wind speed. Based on experiments studying the emission of fungal spores from plant leaves and the preliminary work conducted by Morris et al. (subm.) to study the emission of salt particles from plant leaves, wind tunnel trials were conducted to determine the relationship between wind speed and the proportion of salt emitted into the air from the leaf surfaces of *S. pectinata*.

5-5.3.1 Wind Tunnel Trials

Dormant *S. pectinata* plugs were acquired from BambooPlants (Online Nursery) in January of 2019, transplanted into soil collected from the Bath site, and allowed to grow for 4 months (25 ± 2 ^{O}C, 50 ± 20 % RH) before beginning wind tunnel testing. The wind tunnel was designed to provide a testing zone 45 cm tall by 45 cm wide and a maximum wind speed of 4 m/s, based on the principles outlined in Barlow et al.'s (1999) Low-Speed Wind Tunnel Testing (Figures E-2 and E-3). Plants were placed in a covered plant stand for 1 week, before undergoing each test. Each plant (n=3) was tested at 0, 0.5, 2, and 4 m/s. The 0 m/s trial was included to control for losses of salt due to the movement of the plant from the covered plant stand into the wind tunnel and related disturbances. After being exposed to the given wind speed for 1 hour, the plant was washed by wiping each leaf with gauze soaked in de-ionized (DI) water in order to minimize overall disturbance. Following each trial, the testing zone of the wind tunnel was washed thoroughly using DI water to eliminate any residual salts.

5-5.4 Part 3: Estimation of Hourly Salt Emission

5-5.4.1 Estimated Available Salt Pool

At any given point in time, the amount of salt available for dispersal is the sum of the salt excreted by the plant during that hour and the salt that was not dispersed in the previous hour (Eq. 5-1). However, rain can wash salts from the surface of the leaves. While in natural systems, it may require a heavy rainfall to remove all salts, to simplify, it was assumed that it if rain occurs during the given hour, all salts would be removed and the available salt pool drops to 0.

Eq. 5-1

$$Salt\ Pool = \begin{pmatrix} Excretion\ \left({}^{g}/_{m^2}\right) \\ +\ Salt\ Pool\ from\ previous\ hour\ \left({}^{g}/_{m^2}\right) \\ -Salt\ Emitted\ during\ previous\ hour\ \left({}^{g}/_{m^2}\right) \end{pmatrix} \times Wash\ Factor$$

*Wash Factor: (if rain) = 0, (if no rain) =1

5-5.4.2 Hourly Emission Profile

Meteorological data was used to calculate the emission factor based on the average wind speed for that hour (Eq. 5-2). An hourly emission profile was generated by the product of the available salt pool, and the predicted emission factor. A humidity factor was also included given that above 70% humidity Morris et al. (in press) determined that salt crystals of *S. pectintata* form liquid

droplets as a result of their hygroscopic nature. The available chloride pool was used to determine the chloride emission profile. The chloride emission profile was converted into a potassium chloride emission profile using molar ratios as particle dispersion occurs for the whole salt, and particle size distribution data exists only for the salt crystals themselves.

Eq. 5-2

$$Emission = (Salt\ Pool)(Emission\ Factor)(Humidity\ Factor)$$

* Humidity Factor: (if humidity >70%) = 0, (if humidity <70%) = 1.

5-5.5 Part 4: Modelling Dispersal of airborne chloride

AERMOD View (Lakes Environmental, AERMOD View 6.9.1, Version 16216r (regulatory version)) was used to determine the theoretical deposition rate and concentration of potassium chloride in the air over the course of the 2018 and 2019 field seasons. AERMET (meteorological processor) was used to convert on-site weather data into AERMOD-ready surface meteorological files for 2018 and 2019. All five plots of *S. pectinata* were modelled as area sources and assumed to have approximately the same emission rates. The validated model was then used in conjunction with weather data provided by the regulatory group at the Bath cement plant for 2011-2015 to determine long term estimates for site remediation by haloconduction. AERMOD is most accurate at a regional scale of 5 km, so a uniform polar receptor grid was used with a radius of 5 km.

5-6 RESULTS & DICUSSION

5-6.1 Emission Source

When applying AERMOD View modelling software, gases or particles can be modelled, so in the case of haloconduction, salts were modelled as particles. The particle size distribution for *S. pectinata* was used from Morris et al. (in press) and it was assumed, for simplification, that the particle size distribution found on the leaf surfaces would be the same as that emitted from the leaf. In order to generate an hourly emission profile, chloride excretion rates were based on greenhouse and wind tunnel studies conducted to estimate the rate that excreted chloride is transferred into the air column.

5-6.1.1 Estimation of Excretion Rates by Spartina pectinata

A positive correlation (RMSE=0.008) was observed between chloride excretion and both plant height and soil chloride. This is intuitive as plants take up more chloride when it is available, but then need to dispose of the chloride (Rozema et al., 1981). Larger plants also have a greater surface area over which to excrete salts (Leng et al., 2017). The relationship between plant height and chloride excretion can be described as exponential. The relationship between soil chloride and chloride excretion can be described as sigmoidal as the rate of increase in chloride excretion tapers off after approximately 2 000 µg/g soil chloride (Figure 5-2). When combined in a generalized additive model, excretion rates of *S. pectinata* can be described by Eq 5-3. Based on field observations, *S. pectinata* generally begins to grow in early May and reaches a maximum

height of approximately 100 cm by the beginning of August in Southeastern, ON, CAN. The plants maintain their height until mid September when the seasons begin to change (Sup. Table E-1 & Sup. Figure E-4).

Eq. 5-3

$$Estimated\ Excretion = \frac{(1.5 \times 10^{-3})(Soil\ \text{chloride}\ concentration(\mu g/g)^2)}{1.5 + Soil\ \text{chloride}\ concentration(\mu g/g)^2} \text{X}\ Plant\ Height\ (cm)^2$$

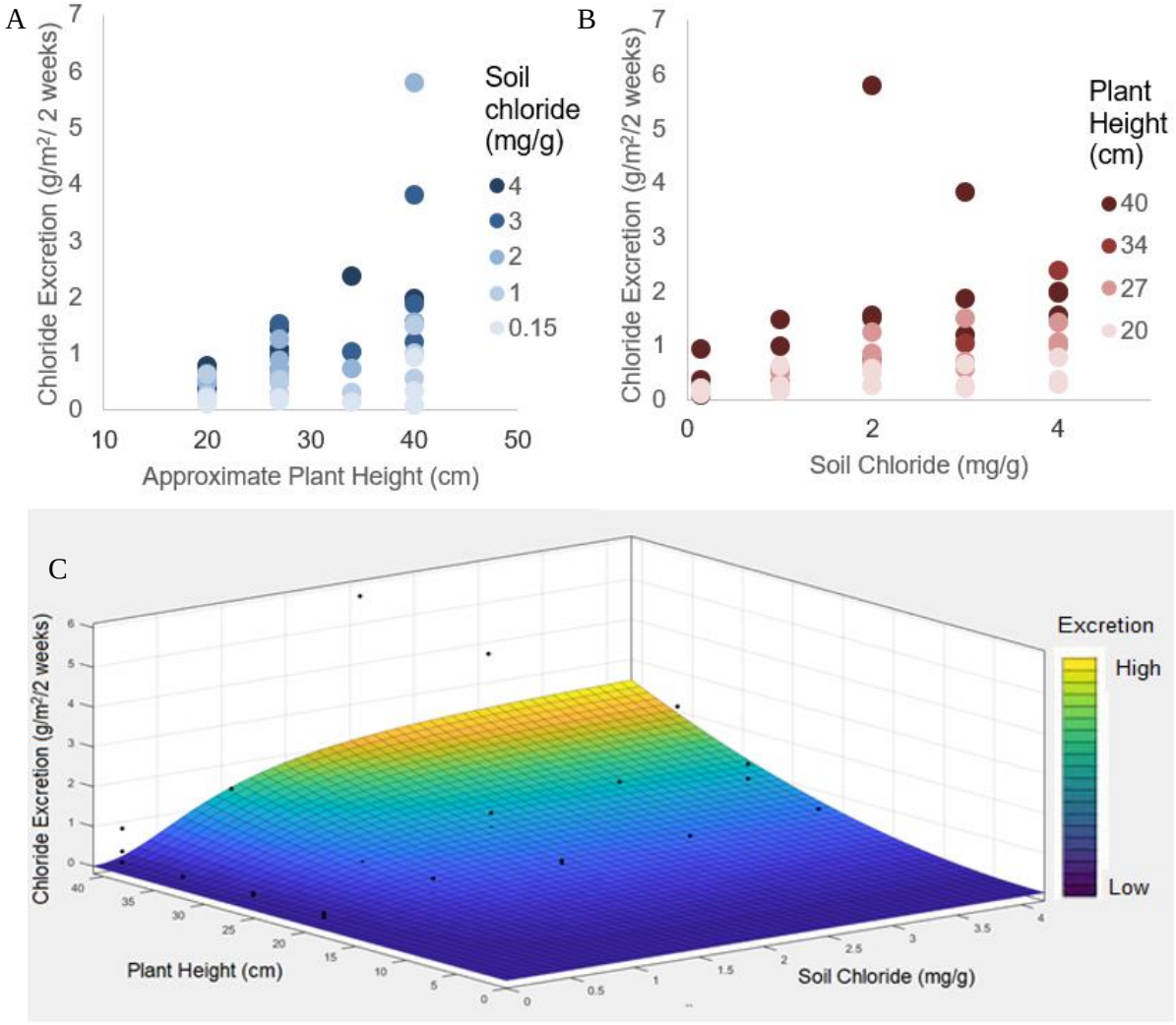


Figure 5-2: Excretion rate of *Spartina pectinata* in relation to a) plant height, and b) soil chloride concentration. c) The 3-way relationship between plant height, excretion, and soil chloride. The colour ramp provides visual contrast and is another representation of the excretion.

5-6.1.2 Particle Emission

Wind at the site blows predominantly from the southwest to the northeast (Figure 5-3). The most common wind speed category observed in 2018 and 2019 was 0.5-2 m/s (23.4% of the time), but gusts reached up to 10 m/s. Up to 50% of the time was considered 'calm' or less than 0.5 m/s (Figure 5-3).

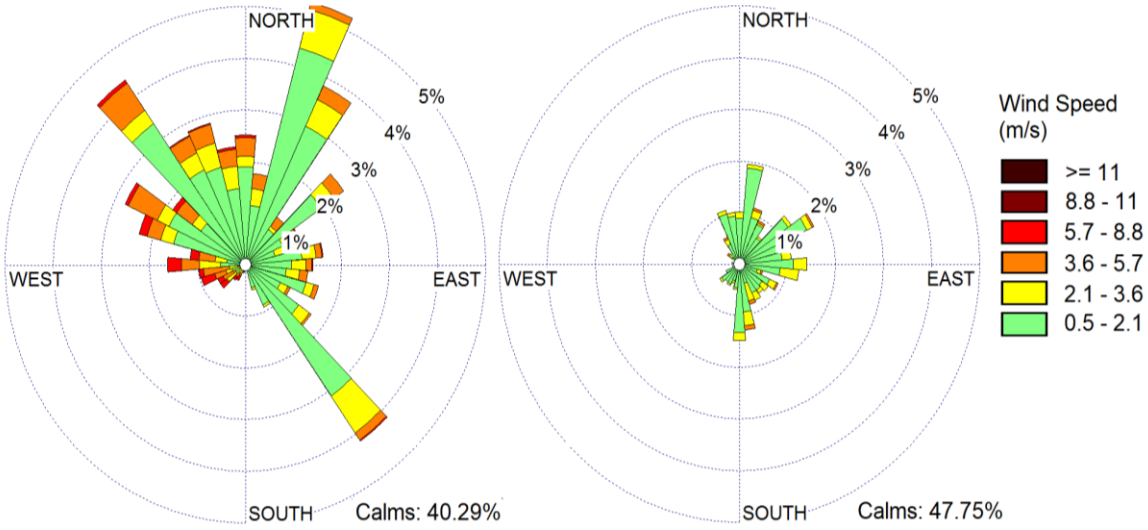


Figure 5-3: Wind flow vectors for the Bath site in A) 2018 and B) 2019 for May through August. The frequency of the wind vector is indicated by the band length while the wind speed is indicated by the colour of the band. The rings are numbered with the frequency that the wind speed occurs. 'Calms' indicates the % of time that wind speeds were below 0.5 m/s.

When exposed to wind speeds below 0.5 m/s, negligible chloride emission was observed so during 'calm' periods, particle emission is unlikely. This is similar to Aylor et al.'s (1981) finding that the minimum wind speed to release powdery mildew (*Erysiphe graminis*) from barley leaves was between 0.5 and 1 m/s. The mean release rate of the total chloride found on the *S. pectinata* plants was 20%, and 30% at 2 m/s and 4 m/s, respectively (Figure 5-4). Thus, the majority of the time that winds are blowing, 20% of the chloride found on the leaf surfaces of *S. pectinata* is likely to be transferred into the air column. Due to the constraints of the wind tunnel, wind speeds above 4 m/s were not performed, however based on the observed wind speeds and emission rates, the relationship between wind speed and chloride emission was deemed approximately logarithmic for the purpose of estimating emission rates. It is possible that higher wind speeds could produce greater than predicted emission rates and thus the estimated emission may be an underrepresentation. For this reason, separate runs in AERMOD were also conducted as if emission were 100% to evaluate if there would be a significant difference (Sup. Figure E-5). Furthermore, wind gusts are also not accounted for as the duration and timing are not represented in the meteorological data and thus difficult to predict. Again, this means that the emission rate calculated here is likely underestimated.

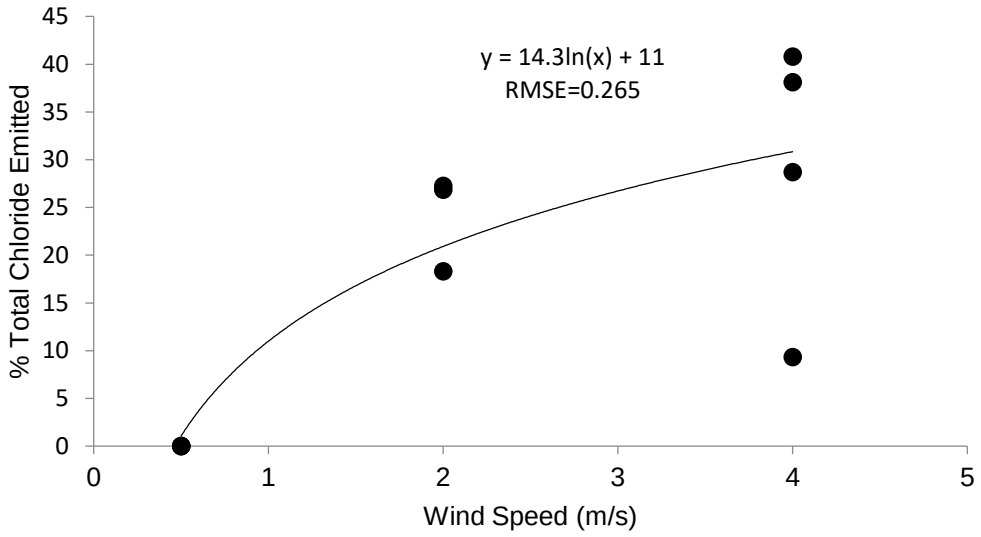


Figure 5-7: Proportion of total chloride emitted from the leaves of S. pectinata with respect to wind speed as determined by wind tunnel trials n=9.

5-6.2 Available Salt Pool and Emission Profiles

Only about 30% of hours within the 2018 and 2019 field season fell within the requirements of no rain and <70% humidity. When the theoretical chloride pool is compared to the actual amount of chloride found on *S. pectinata* plants growing at the field site, the chloride concentration per square meter of plants is on the same order of magnitude. Fluctuations in the amount of chloride found on the plants also aligned with peaks and troughs in the theoretical salt pool (Figure 5-5A). Thus, it is likely that the estimated excretion and emission rates are sufficiently accurate for further modelling using the AERMOD (Figure 5-5B & C).

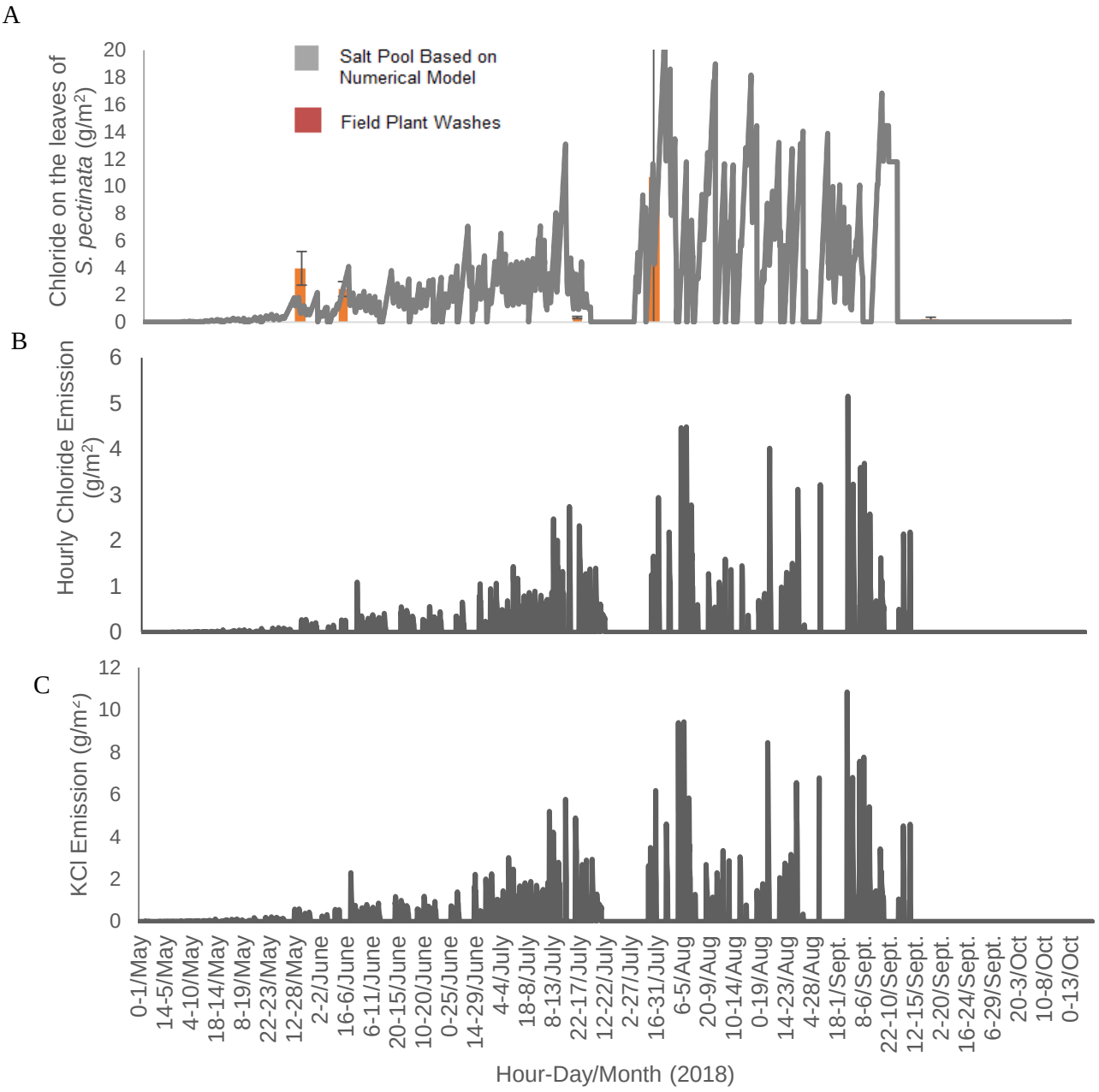


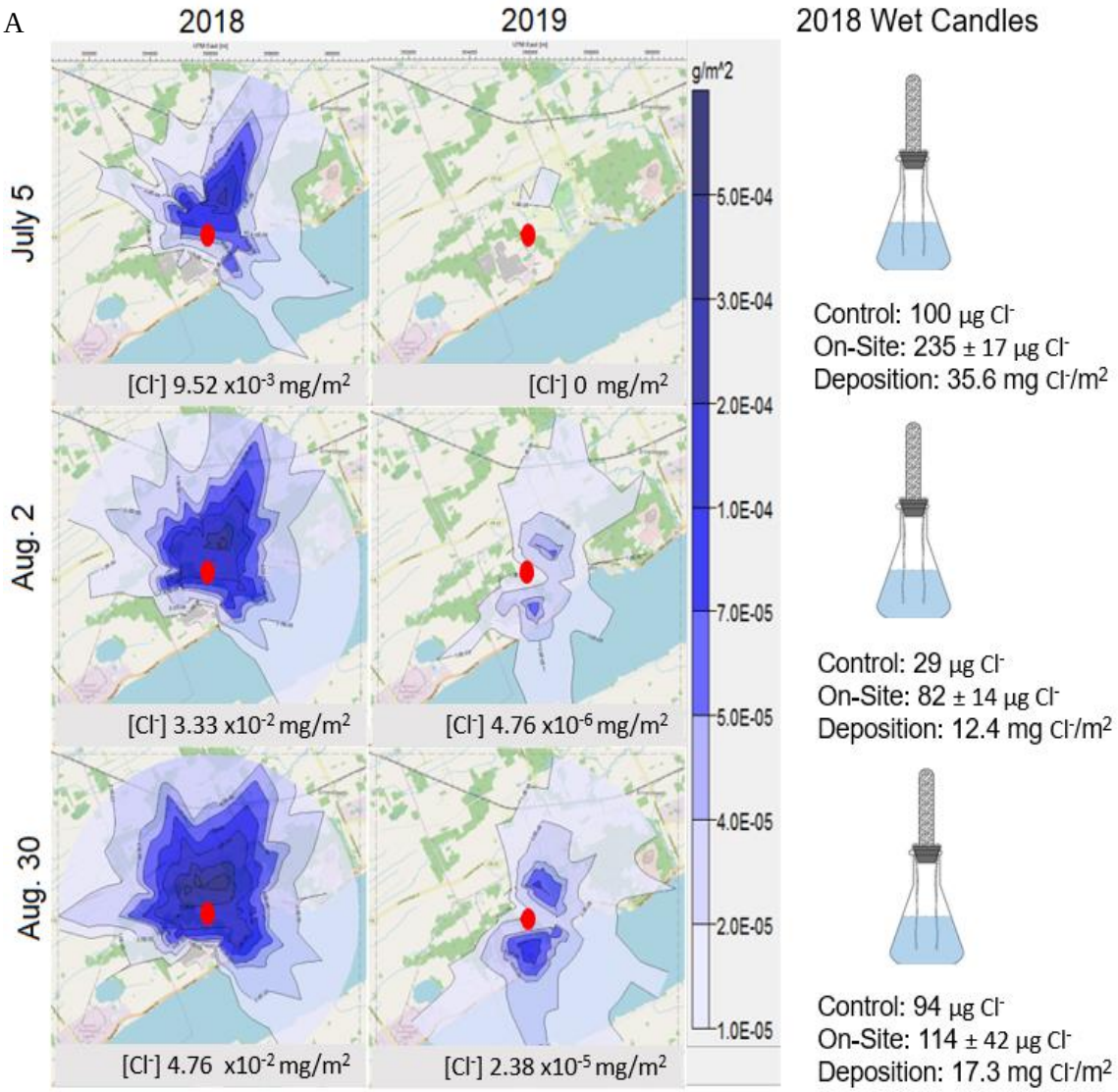
Figure 5-8: A)Calculated salt pool (line in grey) and actual salt pool as represented by the amount of chloride found on *S. pectintata* plants growing at the Bath site (orange bars) at different times throughout the 2018 season. This is representative of the true 'available chloride pool' for that given time point. Note that 100 cm^2 sampling regions were used and converted to m^2 estimates. B) Chloride emission profile calculated from the 2018 available salt pool. C) Potassium chloride emission profile calculated from the chloride emission profile and molar ratios. Dates read as Hour-Day/Month, for example 20-19/May refers to the 20^{th} hour (24 hr clock: 00-23) of the 19^{th} day in May of 2018.

5-6.3 Validation based on aerial concentration and deposition rates for 2018 and 2019

Over the course of a season, deposition does not occur near the site but instead downwind. As the season progresses, airborne salt concentrations steadily increase and deposition increases as well. Stark differences were observed between the 2018 and 2019 projections, highlighting the importance of meteorological factors and their influence on emission and dispersal (Figure 5-6). While in the 2018 season, deposition became quantifiable by June 6th, in 2019, deposition was not significant until July 18th. This highlights the need for a longer timeframe to provide meaningful long term predictions to allow averaging between years.

Compared to the deposition rates predicted by the model for 2018, the wet candles collected significantly more chloride than was expected (Figure 5-6A). This could be due to an underestimation of emission in the model. However, chloride deposition rates could be higher on site due to the close proximity of the wet candles to the recretohalophytes. AERMOD is a regional model and therefore it determines the average concentration within a section of the receptor grid used by the model, and hence the high concentrations found immediately on site are averaged with the nearby lower concentrations within the same area of the grid (Lakes Environmental, 2019; US EPA, 2018). Aerial chloride concentrations measured with the high volume air sampler were also higher than the predicted values, likely for the same reasons. However, the predicted aerial chloride concentrations are very low, and thus probably not distinguishable from background levels, except on site where chloride concentrations would be higher. Another source of chloride that could have impacted the aerial monitoring is dust from soil on site.

A


2018
2019
2018 Wet Candles
July 5
Aug. 2
Aug. 30
g/m^2
5.0E-04
3.0E-04
2.0E-04
1.0E-04
7.0E-05
5.0E-05
4.0E-05
2.0E-05
1.0E-05
[Cl-] 9.52 x10-3 mg/m2
[Cl-] 0 mg/m2
[Cl-] 3.33 x10-2 mg/m2
[Cl-] 4.76 x10-6 mg/m2
[Cl-] 4.76 x10-2 mg/m2
[Cl-] 2.38 x10-5 mg/m2
Control: 100 µg Cl-
On-Site: 235 ± 17 µg Cl-
Deposition: 35.6 mg Cl-/m2
Control: 29 µg Cl-
On-Site: 82 ± 14 µg Cl-
Deposition: 12.4 mg Cl-/m2
Control: 94 µg Cl-
On-Site: 114 ± 42 µg Cl-
Deposition: 17.3 mg Cl-/m2

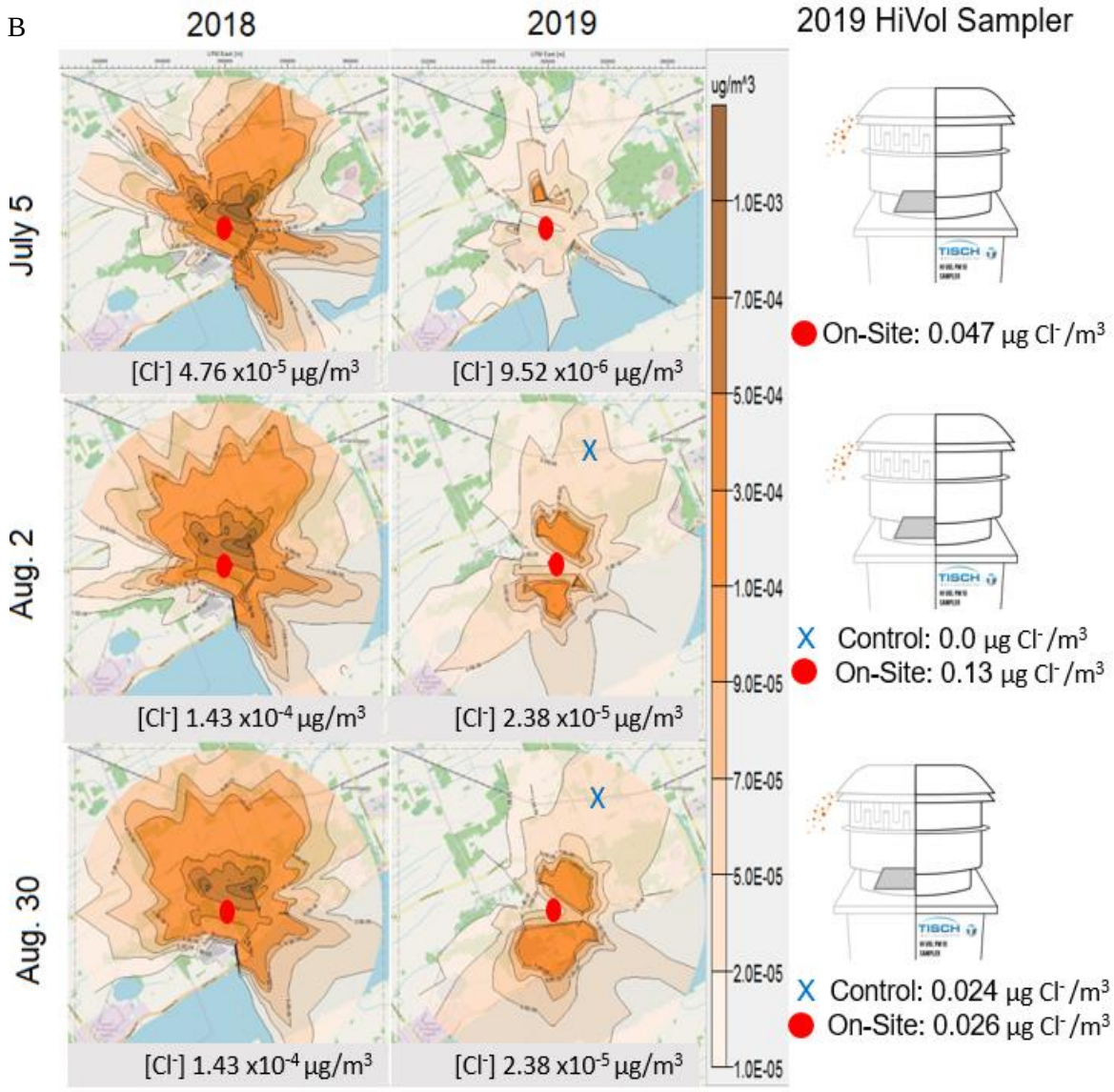


Figure 5-9: AERMOD View generated maps of the theoretical A) deposition (g/m^2) and B) concentration ($\mu g/m^3$) of KCl for the 2018 and 2019 field seasons, given estimates based on the actual *S. pectinata* plots at the Bath site. The red dots represent the location of the Bath site. On-site chloride concentrations calculated for each date, based on molar ratios and the AERMOD output are shown at the bottom of each map. The amount of chloride collected by the wet candles devices (n=4 on site, n=1 control) for each date in 2018 is illustrated in A) along with the deposition rate calculated from these values. Similarly, the concentrations of chloride measured using the high volume air sampler (n=1) in 2019 are shown in B). The blue X indicates the location where the control air samples were collected.

5-6.4 Dispersal: Long term site predictions

If the entirety of the 1000 m^2 plot was planted with *S. pectinata* in 2011, by the end of 2015, approximately 915 kg of KCl could be dispersed over an area of approximately 68 km^2 with an average deposition concentration of 0.0123 g/m^2. Assuming a bulk density of approximately 1.33g/cm^3 and a depth of 1 cm, only about 0. 9 μg/g of potassium chloride would be added over the course of 5 years, with only about 0.44 μg/g of chloride, a concentration well bellow background levels (~20-100 μg/g soil chloride) for the region (Mann et al, 2019). Not only is deposition below background levels, it is also added progressively through time and thus there is the opportunity for plants and other organisms to take up and use these small amounts of KCl. At these low concentrations, salt is not only harmless but would actually act as a macro/micronutrient (Marschner, 2012). An average of 183 kg of KCl could be removed per year without harm to surrounding ecosystems (Figure 5-7). As this may be an underestimate, runs with 10X, 100X, and 1000X emission were also conducted to include a safety factor (Sup. Figure E-6). With emission rates up to 100X those produced from the model, the average deposition concentration would remain within background concentrations. Even at 1000X, yearly input rates would remain within 200 μg/g over 5 years. Thus, the use of recretohalophytes is unlikely to cause harm to the surrounding environment while providing significant benefit in the dispersal and dilution of salts.

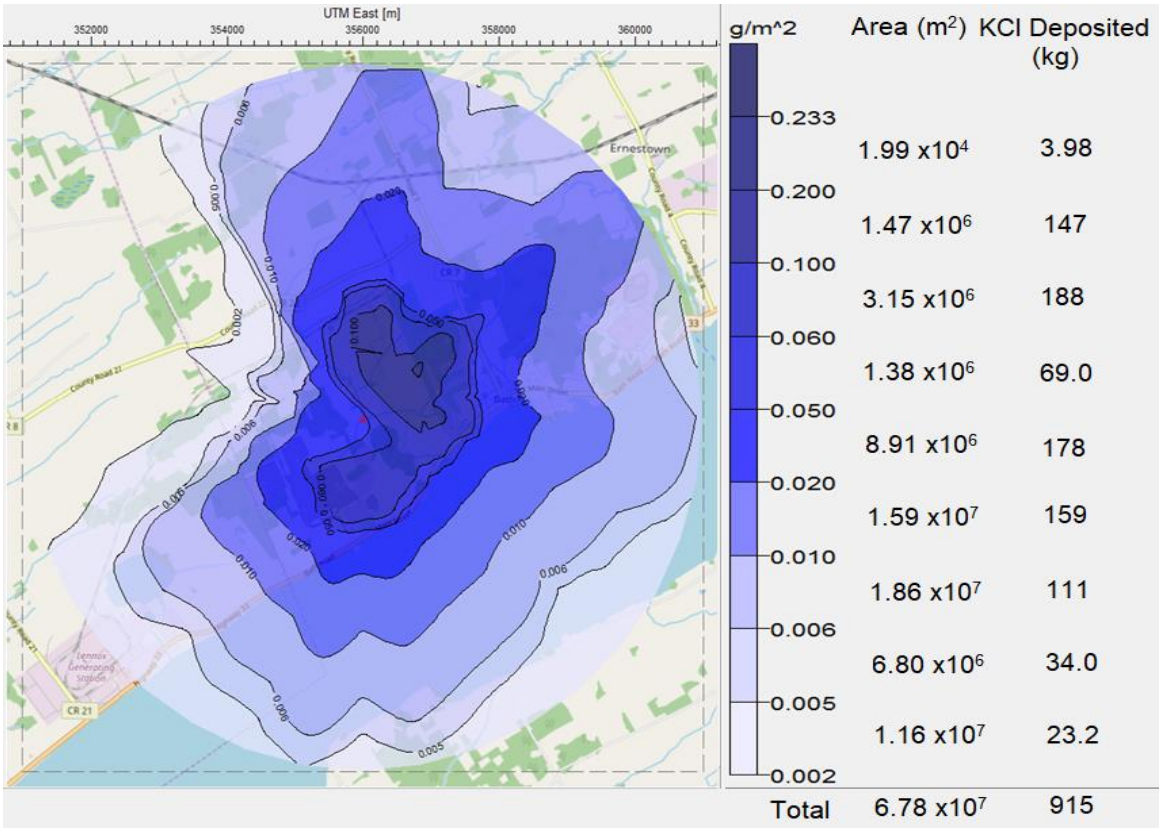


Figure 5-10: Total deposition of potassium chloride at the end of 2015, if the entire 1000 m^2 Bath site was planted with *S. pectintata* in 2011. The deposition map was generated using AERMOD View based on meteorological data from Lafarge Canada.

The model described herein, is the first to allow users to predict the amount of salt that can be phytoextracted by a recretohalophyte and determine where, and at what concentrations, the salts will deposit, effectively ensuring that they are below background. Remediation timeframes can thus be more accurately projected. Based on this model and estimates of the total amount of salts found of site by McSorley et al. (2016), the Bath site could be fully remediated with *S. pectinata* in a ~2-4 year timeframe while posing minimal threat to the surrounding environment. Furthermore, this model only studies the 5 km region surrounding the site, so extraction rates could be even higher as the low concentration plumes likely extend further than presented here. Future studies could involve long distance transport models. This study illustrates that haloconduction is a suitable treatment option in agricultural regions where the land is relatively flat. Future studies could look at recretohalophytic species that prefer a dry ecotype such as *Bouteloua curtipendula* and *Bouteloua gracilis* for use in an agricultural setting as these could potentially be used as cover crops (USDA, 2019). Hence, we propose that with very minimal maintenance costs, recretohalophytes may provide an efficient means of saline soil remediation.

6 CONCLUSION

While salts are benign at low concentration, high soil salinity presents a challenge to plants and soil organisms (Flowers et al., 2008; East et al., 2017). Soil salinity can rise dramatically as a result of both natural and anthropogenic processes and climate change is likely to hasten these impacts (Cuevas et al., 2019; Matternicht & Zinck, 2008; Rengasamy, 2006). A positive feedback loop of decreasing soil quality can occur as salt ions accumulate in soil, the soil structure worsens, which combined with osmotic stress and ion toxicity, reduces plant growth (Deinlein et al., 2014; Orlovsky et al., 2016; Bromham et al., 2013). The reduction in plant cover reduces CO_2 sequestration and organic carbon inputs into soil which further reduces soil quality and contributes to climate change (Setia et al., 2013). In order to manage saline soils and increase plant cover, salt tolerant plants known as halophytes can be used.

While all halophytic plants can survive in saline soils, those that sequester large amounts of salts in their shoots, and those that excrete salts, recretohalophytes, are useful for remediation (Flower & Colmer, 2013; Litalien & Zeeb, 2020). The choice of halophyte type is dependent on the context. Accumulators are useful in regions where frequent harvesting is possible, but recretohalophytes are preferred where a semi-passive remediation strategy is necessary (Litalien & Zeeb, 2020). Remediation using accumulator halophytes is backed by a growing body of research but still lacks in some areas such as the treatment of saline wastewater (Hasanuzzaman, 2014; Morteau, 2016). Accumulator plants require harvesting and appropriate disposal of biomass, but recretohalophytes rely upon wind dispersal to remove salts from soil (Litalien & Zeeb, 2020). While Yun et al. (2019b) and Morris et al. (subm.) completed the proof of concept, still little was known regarding the excretion rates of many Canadian recretohalophytes. There was no framework to accurately quantify the amount of salt that could be removed from a site via haloconduction and determine to where it will be dispersed.

This thesis is the first to use *Salicornia maritima* to treat saline leachate rich in potassium chloride. By growing *S. maritima* plants in greenhouse conditions and watering with cement kiln dust leachate with high amounts of potassium chloride, it was determined that *S. maritima* tolerated extremely high concentrations of this salt while accumulating up to 25% of its dry biomass as chloride. This accumulator species extracted 675 g Cl^-/m^2 in a typical Canadian growing season. Due to its ability to concentrate salts within its tissues, *S. maritima* is an ideal candidate for phytoremediation. In addition, as it is an herbaceous plant with a relatively small biomass, compared to other accumulator plants, which would allow for simpler biomass management.

The use of accumulator plants still requires extensive energy to harvest the biomass. While this may be feasible in some regions, remote locations may benefit from the use of recretohalophytes which can be used passively to remediate soils. Four recretohalophytes native to Canada, *Atriplex canescens, Armeria maritima, Spartina pectinata,* and *Distichlis spicata*, were studied in a greenhouse setting to quantify their salt excretion and compare their salt tolerance, excretion mechanisms, and applicability for phytoremediation. It was determined that *A. maritima, S. pectinata,* and *D. spicata* could all be useful in the context of haloconduction but that *A. canescens* behaves more like an accumulator species. Of the three species, *A. maritima* excretes

the most chloride at concentrations above 4 000 μg Cl^-/g, but *S. pectinata* excretes the most reliably below 4 000 μg Cl^-/g.

Having established *S. pectinata* as a suitable candidate for haloconduction, it was necessary to determine how much salt could be transferred into the air column over the course of a season. By determining the relationship between chloride excretion, soil chloride, and the size/age of a plant it was possible to estimate the amount of salt excreted at any point in time over the growing season. Combined with wind tunnel studies that were used to determine the percent of salt that is actually emitted from a plant's leaves, a simple prediction of potassium chloride emission rates over the course of a season was generated. With this emission profile, it was possible to utilize the AERMOD View aerial dispersal model and visualize the transport of salt particles and where they deposit. It was determined based on this model, that a study site impacted by potassium chloride rich cement kiln dust leachate, located in Bath, ON, could be remediated in a timeframe of 2-4 years.

Together, the results presented in this thesis demonstrate the efficacy of phytoextraction for the treatment of saline waste waters and the remediation of salinized soils. The model presented herein also provides the framework to more accurately predict remediation time frames. Future work could involve the *in-situ* integration of *S. maritima* into a biofilter design in order to treat the saline leachate. Furthermore, the remainder of the 12 recretohalophytes native to Canada should be evaluated for their phytoextraction capabilities with sodium chloride and potassium chloride salts. Other salts could also be investigated such as fertilizer salts to determine if phytoextraction via haloconduction might be possible at other types of sites with salinized soils. Finally, further refinement of the rudimentary model for haloconduction could account for progressively declining soil chloride and thus changing emission rates over the course of the remediation timeframe. Longer range models such as CALPUFF could also be used to visualize long range transport of salt crystals. Fine scale adjustments to the model may also increase the accuracy of predictions.

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APPENDIX A Supplemental Materials for Chapter 2 Publication

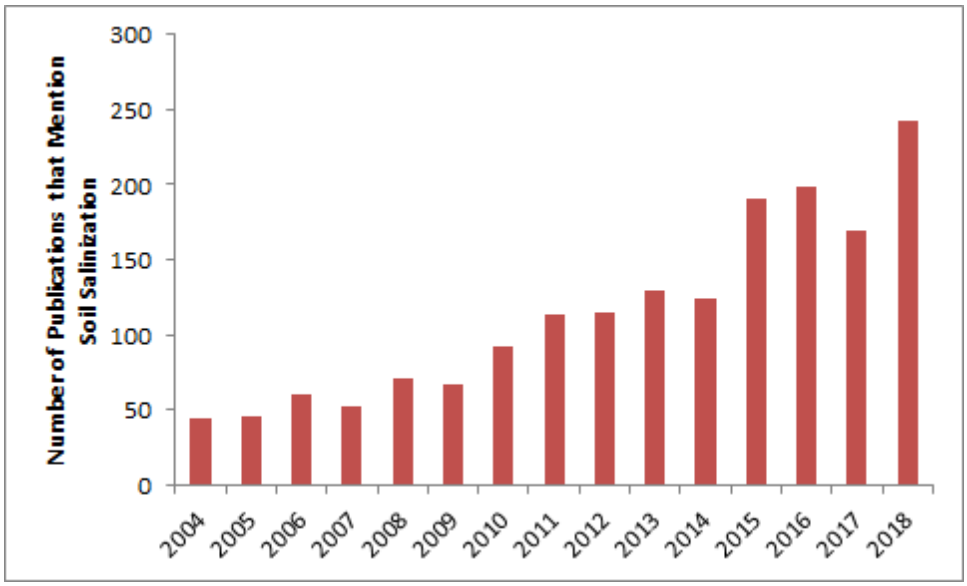

Figure A-1: Number of publications referring to soil salinization through time (Data from Web of Science, Clarivate Analytics, 2019).

Table A-1: Comparison of energy produced from common fuels (Data from Francis &Peters, 1980; Speight, 2011)

Fossil Fuel	Heat Content		Renewable Fuel	Heat Content	
	kJ/g	MJ/tonne		kJ/g	MJ/tonne
Natural Gas	52	52 200	Corn Stover	17	17 400
Gasoline	47	46 500	Wood Chips	18	17 800
Diesel	46	45 700	Biodiesel	37	37 800
Coal	24	23 900	Renewable Diesel	44	43 600
Bituminous Coal	27	27 200			
Brown Coal	17	17 400			

APPENDIX B Supplemental Data for Chapter 3

B-1: Raw Data for Chapter 3

Table B-1: Leachate flow rates and concentrations at the Bath site over the course of the 2018 season

Leachate Collection Date	Leachate Concentration (mg/L)	Flow Rate (L/s)	Input rate (mg/s)
24/04/2018	1400[a]		
07/05/2018	1500[a]		
24/05/2018	1600[a]		
07/06/2018	1580[c]	0.1*	158
21/06/2018	1600[c]	0.13	200
06/07/2018	1100[c]	0.08	90
18/07/2018	8600[c]	0.04	381
02/08/2018	1400[c]	0.06	89
09/08/2018	350[c]	0.13	47
16/08/2018	820[c]	0.26	216
23/08/2018	1900[c]	0.15	276
30/08/2018	1900[c]	0.09	166
13/09/2018	2000[c]	0.07	139
20/09/2018	1900[c]	0.06	118
27/09/2018	1900[c]	0.06	107
04/10/2018	950[c]	0.16	147
16/10/2018	1700[c]	0.07	114
25/10/2018	1700[c]	0.13	215

[a,c] Indicate corresponding QA/QC (Table B-5)

*Flow rates began to be measured on 07/06/2018

Table B-2: *Salicornia maritima* dry weigh wet weight measurements at the end of the 10-week experiment

Soil	Watering Solution	Wet Weight (g)	Dry Weight (g)
High KCl	KCl Leachate	1.95	1.35
High KCl	KCl Leachate	1.80	0.61
High KCl	KCl Leachate	1.32	0.53

High KCl	Tap Water	6.67	0.90
High KCl	Tap Water	6.46	0.84
High KCl	Tap Water	6.42	0.82
Low KCl	KCl Leachate	3.46	1.14
Low KCl	KCl Leachate	4.52	0.86
Low KCl	KCl Leachate	4.01	1.14
Low KCl	NaCl Sea Salt Solution	2.26	0.36
Low KCl	NaCl Sea Salt Solution	3.78	0.85
Low KCl	NaCl Sea Salt Solution	3.22	0.57
Low KCl	Tap Water	7.76	0.92
Low KCl	Tap Water	4.50	0.71
Low KCl	Tap Water	2.74	0.32
High KCl + Sand	KCl Leachate	3.23	0.71
High KCl + Sand	KCl Leachate	3.70	0.91
High KCl + Sand	KCl Leachate	4.65	1.18
High KCl + Sand	Tap Water	5.85	0.67
High KCl + Sand	Tap Water	6.14	0.82
High KCl + Sand	Tap Water	4.40	0.51
Low KCl + Sand	KCl Leachate	3.93	0.79
Low KCl + Sand	KCl Leachate	1.32	0.97
Low KCl + Sand	KCl Leachate	4.84	1.79
Low KCl + Sand	NaCl Sea Salt Solution	1.31	0.22
Low KCl + Sand	NaCl Sea Salt Solution	0.20	0.66
Low KCl + Sand	NaCl Sea Salt Solution	1.09	0.26
Low KCl + Sand	Tap Water	5.64	0.81
Low KCl + Sand	Tap Water	4.62	0.72
Low KCl + Sand	Tap Water	1.04	0.23
No Salt	NaCl Sea Salt Solution	5.53	0.82
No Salt	NaCl Sea Salt Solution	7.50	1.04
No Salt	NaCl Sea Salt Solution	3.99	0.56
Low NaCl	Tap Water	6.45	0.74
Low NaCl	Tap Water	15.87	1.75
Low NaCl	Tap Water	11.73	1.19
No Salt	Tap Water	8.09	0.96
No Salt	Tap Water	5.00	0.60
No Salt	Tap Water	0.33	0.17

Table B-3: *Salicornia maritima* tissue concentration at the end of the 10-week experiment

Soil	Watering	Tissue Concentration (mg/g DW)
High KCl	KCl Leachate	356[d]
High KCl	KCl Leachate	180[d]
High KCl	KCl Leachate	169[d]
High KCl	Tap Water	110[e]
High KCl	Tap Water	99[e]
High KCl	Tap Water	155[e]
Low KCl	KCl Leachate	189[c]
Low KCl	KCl Leachate	160[c]
Low KCl	KCl Leachate	160[c]
Low KCl	Tap Water	71[e]
Low KCl	Tap Water	118[e]
Low KCl	Tap Water	109[e]
No Salt	NaCl Sea Salt Solution	210[d]
No Salt	NaCl Sea Salt Solution	179[d]
No Salt	NaCl Sea Salt Solution	209[d]
Low NaCl	Tap Water	98[d]
Low NaCl	Tap Water	99[d]
Low NaCl	Tap Water	110[d]
No Salt	Tap Water	71[d]
No Salt	Tap Water	79[d]
No Salt	Tap Water	75[d]

[c,d,e] Indicate corresponding QA/QC (Sup. Table B-5)

Table B-4: *S. maritima* initial soil and watering concentrations as well as soil concentration at the end of the 10-week experiment

Soil	Watering	Initial Soil Concentration (µg/g)	Watering Concentration (mg/L)	Final Soil concentration (µg/g)[e]
High KCl	KCl Leachate	4000[f]	8600[c]	8823
High KCl	KCl Leachate	4000[f]	8600[c]	14520
High KCl	KCl Leachate	4000[f]	8600[c]	13300
High KCl	Tap Water	4000[f]	25[h]	4184
High KCl	Tap Water	4000[f]	25[h]	4282
High KCl	Tap Water	4000[f]	25[h]	4725
Low KCl	KCl Leachate	150[f]	8600 [c]	N/A
Low KCl	KCl Leachate	150[f]	8600 [c]	N/A
Low KCl	KCl Leachate	150[f]	8600 [c]	N/A
Low KCl	NaCl Sea Salt Solution	150[f]	1300 [c]	N/A
Low KCl	NaCl Sea Salt Solution	150[f]	1300 [c]	N/A
Low KCl	NaCl Sea Salt Solution	150[f]	1300 [c]	N/A

Low KCl	Tap Water	150[f]	25[h]	102
Low KCl	Tap Water	150[f]	25[h]	104
Low KCl	Tap Water	150[f]	25[h]	126
High KCl + Sand	KCl Leachate	2000[f]	8600[c]	N/A
High KCl + Sand	KCl Leachate	2000[f]	8600[c]	N/A
High KCl + Sand	KCl Leachate	2000[f]	8600[c]	N/A
High KCl + Sand	Tap Water	2000[f]	25[h]	N/A
High KCl + Sand	Tap Water	2000[f]	25[h]	N/A
High KCl + Sand	Tap Water	2000[f]	25[h]	N/A
Low KCl + Sand	KCl Leachate	75[f]	8600[c]	N/A
Low KCl + Sand	KCl Leachate	75[f]	8600[c]	N/A
Low KCl + Sand	KCl Leachate	75[f]	8600[c]	N/A
Low KCl + Sand	NaCl Sea Salt Solution	75[f]	13000[c]	N/A
Low KCl + Sand	NaCl Sea Salt Solution	75[f]	13000[c]	N/A
Low KCl + Sand	NaCl Sea Salt Solution	75[f]	13000[c]	N/A
Low KCl + Sand	Tap Water	75[f]	25[h]	N/A
Low KCl + Sand	Tap Water	75[f]	25[h]	N/A
Low KCl + Sand	Tap Water	75[f]	25[h]	N/A
No Salt	NaCl Sea Salt Solution	17[g]	13000[c]	N/A
No Salt	NaCl Sea Salt Solution	17[g]	13000[c]	N/A
No Salt	NaCl Sea Salt Solution	17[g]	13000[c]	N/A
Low NaCl	Tap Water	1000[g]	25[h]	1513
Low NaCl	Tap Water	1000[g]	25[h]	1421
Low NaCl	Tap Water	1000[g]	25[h]	1629
No Salt	Tap Water	17[g]	25[h]	99
No Salt	Tap Water	17[g]	25[h]	151
No Salt	Tap Water	17[g]	25[h]	87

[c,e,f,g,h] Indicate corresponding QA/QC (Sup. Table B-5)

Table B-5: QA/QC for analyses completed at ASU for Chapter 3

	Blank	Control (mg/L)	Control Target (mg/L)	% of Target	Cranberry-05 (mg/L)	Cranberry-05 Target (mg/L)	% of Target
a (08/06/2018)	<0.05	5.1;5.1	5.0	102; 102	36	35	103
b (23/10/2018)	<0.05; <0.05; <0.05; <0.05	5.4; 5.5; 4.8; 4.9; 4.8	5.0	108; 110; 96; 98; 96	38; 38	35	109
c (18/12/2018)	<0.05; <0.05	5.2; 5.0; 5.1; 5.2	5.0	104; 100; 102;	37; 37	35	106; 106

				104			
d (14/09/2018)	<0.05	5.3	5.0	106	37	35	106
e (10/06/2019)	<0.05; <0.05	4.9(X3); 4.8(X3)	5.0	98; 96	37; 37	36	103; 103
f (17/08/2018)	<0.05	5.2	5.0	104	37	35	106
g	<0.05	5.4	5.0	108	38	35	109

B-2 Calculations for Chapter 3

B-2.1 Leachate Calculations

$$Flow\ Rate\ \left(\frac{L}{s}\right) = \frac{1}{Time\ require\ to\ fill\ 1L\ \left(\frac{s}{L}\right)}$$

$$Chloride\ Input\ Rate\ \left(\frac{mg}{day}\right) = \left(Chloride\ Concentration\ \left(\frac{mg}{L}\right)\right)\left(Flow\ Rate\ \left(\frac{L}{s}\right)\right)\left(86400\ \left(\frac{s}{day}\right)\right)$$

B-2.2 *S. maritima* Tissue Calculations

$$Tissue\ Chloride\ Concentration\ \left(\frac{mg}{g}\right) = \left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)\left(0.01\frac{L}{DW(g)}\right)$$

$$Tissue\ Chloride\ (\%) = \frac{Tissue\ Concentration\ \left(\frac{mg}{g}\right)}{1000\ \left(\frac{mg}{g}\right)}$$

$$Chloride\ accumulated\ \left(\frac{mg}{plant}\right) = \left(Tissue\ concentration\ \left(\frac{mg}{g}\right)\right)\left(Plant\ dry\ weight\ \left(\frac{g}{plant}\right)\right)$$

B-3 Soil chloride concentrations

$$Measured\ Soil\ Chloride\ Concentration\ \left(\frac{mg}{g}\right) = \frac{\left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.025L)}{Mass\ of\ dry\ soil\ used\ (g)}$$

$$Chloride\ added\ via\ watering\ (mg) = \left(Fluid\ concentration\ \left(\frac{mg}{L}\right)\right)\left(0.03\ \left(\frac{L}{day}\right)\right)(56\ day)$$

$$Calculated\ Final\ Soil\ Chloride\ \left(\frac{mg}{g}\right) = \frac{\left(\begin{array}{c}\left(\left(Initial\ soil\ concentration\ \left(\frac{mg}{g}\right)\right)(Amount\ of\ Soil\ (g))\right) \\ +(Amount\ added\ via\ watering\ (mg)) \\ -(Amount\ accumulated\ in\ plant\ (mg))\end{array}\right)}{Amount\ of\ soil\ (g)}$$

B-4 Chloride extraction rates

$$Chloride\ Extracted\ (kg/ha) = \frac{\left(Tissue\ concentration\ \left(\frac{mg}{g\ DW}\right)\right)\left(\frac{tonnes\ DW}{ha}\right)\left(1x10^6\left(\frac{gDW}{tonne\ DW}\right)\right)}{1x10^6\left(\frac{mg\ Cl^-}{kg\ Cl^-}\right)}$$

$$Chloride\ Extracted\ \left(\frac{kg}{ha}\right) = \left(Chloride\ Extracted\ \left(\frac{kg}{ha}\right)\right)(0.1ha)$$

APPENDIX C Supplemental Materials for Chapter 4 Publication

Table C-1: Mean shoot length and wet and dry weights at the end of the 10 week experiemental period.

Species	Salt	Number of Shoots	Shoot length (cm)	Shoot wet weight (g)	Shoot dry weight (g)
A. Canescens	KCl	N/A	25 ± 7	8.7 ± 2.0	3.4 ± 0.9
	NaCl	N/A	24 ± 7	6.2 ± 3.1	2.4 ± 1.1
S. pectinata	KCl	9 ± 3	40 ± 25	5.9 ± 2.6	2.8 ± 1.3
	NaCl	8 ± 5	34 ± 19	5.2 ± 2.5	2.4 ± 1.2
D. spicata	KCl	46 ± 13	21 ± 10	5.8 ± 1.3	2.8 ± 0.8
	NaCl	22 ± 7	20 ± 8	4.3 ± 1.3	2.3 ± 0.7
A. maritima	KCl	N/A	18 ± 5	17.08 ± 4.9*	3.5 ± 1.1
	NaCl	N/A	23 ± 2	12.4 ± 3.8	2.5 ± 0.9

Note: KCl n= 60, NaCl n=48

Shoot length of *A. maritima* refers not to the length of an individual leaf but rather the spread of the mound, between the tips of the longest leaves

*Indicates significant difference between KCl and NaCl treatments

Table C-2: Chloride excretion of Canadian recretohalophytic species grown in soil containing approximately 4000 µg/g chloride as KCl or 4620 µg/g chloride as NaCl

Species	Salt	Chloride Excreted						
		mg Cl^- /pot	g shoot DW/pot	mg Cl^- /g DW	g shoot DW/m^2	g Cl^-/m^2 soil surface area	Leaf surface area cm^2/pot	µg Cl^- /cm^2 leaf surface area
A. canescens	KCl	1.47 ± 0.80	3.4 ± 0.9	0.51 ± 0.28	433 ± 115	0.19 ± 0.10		---
	NaCl	0.73 ± 0.48	2.4 ± 1.1	0.24 ± 0.06	306 ± 140	0.09 ± 0.05		---

S. pectinata	KCl	9.13 ± 1.95	2.8 ± 1.3	5.23 ± 2.52	357 ± 166	1.16 ± 0.25	49 ± 24	220 ± 111
	NaCl	6.17 ± 4.08	2.4 ± 1.2	2.08 ± 0.88	306 ± 153	0.78 ± 0.52		---
D. spicata	KCl	12.8 ± 10.4	2.8 ± 0.8	4.21 ± 3.42	357 ± 102	1.63 ± 1.32	72 ±13	160 ± 135
	NaCl	50.5 ± 9.50	2.3 ± 0.7	15.6 ± 2.20	293 ± 89	6.44 ± 1.21		---
A. maritima	KCl	15.4 ± 1.96	3.5 ± 1.1	5.44 ± 2.06	446 ± 140	1.97 ± 0.25		---
	NaCl	17.0 ± 13.5	2.5 ± 0.9	6.87 ± 5.40	318 ± 114	2.17 ± 1.72		---

*Planting densities of *S. pectinata* and *D. spicata* reflect those observed in field plots, while the plants used in this experiment are shorter than those observed in the field they represent the midpoint in height and thus reflect the average excreted over the course of a season.

*Planting densities of *A. maritima* reflect the gardening tag's recommendation of one plant per four inches

*Planting densities of *A. canescens* reflect its size as a seedling, however these plants can reach up to 2m wide full grown

*Leaf surface area was determined by measuring the length and width of leaves and calculating the area based on the assumption that the leaves were approximately triangular, the stem was also measured assuming it is approximately a cylinder.

Table C-3: Literature biomass values

	Biomass (g DW/m^2)	Source
A. canescens	2 500	Glen et al., 1999
S. pectinata	1510	Helios et al., 2014
D. spicata	908	USDA, 2018
A. maritima	1500	Schwartz et al., 2001

APPENDIX D Supplemental Data for Chapter 4

D-1: Raw Data for Chapter 4

Table D-1: Dry weight, wet weight and tissue concentrations of 4 recretohalophytic species at the end of the 10 week experimental period

Sample Code	Species	Soil Chloride Concentration*	Wet Weight	Dry Weight	Tissue Chloride (mg/g DW)[a]
		KCl Soil			
E1	*A. canescens*	4000	7.47	2.51	28.8
E2	*A. canescens*	4000	8.69	2.76	33.9
E3	*A. canescens*	4000	8.95	3.29	22.7
F1	*A. canescens*	3000	12.76	4.64	
F2	*A. canescens*	3000	8.04	2.61	
F3	*A. canescens*	3000	11.47	4.44	
G1	*A. canescens*	2000	9.90	3.82	
G2	*A. canescens*	2000	11.24	4.64	
G3	*A. canescens*	2000	6.24	2.15	
H1	*A. canescens*	1000	9.84	4.18	
H2	*A. canescens*	1000	7.71	3.27	
H3	*A. canescens*	1000	5.87	2.36	
I1	*A. canescens*	150	8.05	3.90	10.3
I2	*A. canescens*	150	7.64	3.51	11.1
I3	*A. canescens*	150	6.31	2.71	12.2
E4	*S. pectinata*	4000	2.05	0.95	15.9
E5	*S. pectinata*	4000	5.48	2.60	22.2
E6	*S. pectinata*	4000	5.70	2.58	20.1
F4	*S. pectinata*	3000	4.21	2.13	
F5	*S. pectinata*	3000	2.11	0.96	
F6	*S. pectinata*	3000	6.31	2.95	

G4	*S. pectinata*	2000	5.74	2.80	
G5	*S. pectinata*	2000	7.67	3.61	
G6	*S. pectinata*	2000	10.45	5.35	
H4	*S. pectinata*	1000	6.08	2.71	
H5	*S. pectinata*	1000	5.61	2.73	
H6	*S. pectinata*	1000	9.06	4.48	
I4	*S. pectinata*	150	5.74	2.48	7.9
I5	*S. pectinata*	150	2.78	1.22	13.8
I6	*S. pectinata*	150	9.93	5.07	8.7
E7	*D. spicata*	4000	5.73	3.12	5.6
E8	*D. spicata*	4000	5.86	3.09	11.1
E9	*D. spicata*	4000	6.40	2.93	13.4
F7	*D. spicata*	3000	6.08	2.55	
F8	*D. spicata*	3000	6.26	3.04	
F9	*D. spicata*	3000	5.24	2.41	
G7	*D. spicata*	2000	2.84	1.33	
G8	*D. spicata*	2000	7.16	3.67	
G9	*D. spicata*	2000	5.67	2.70	
H7	*D. spicata*	1000	3.80	1.80	
H8	*D. spicata*	1000	8.77	4.52	
H9	*D. spicata*	1000	5.22	2.39	
I7	*D. spicata*	150	6.06	2.90	1.1
I8	*D. spicata*	150	5.57	3.39	6.9
I9	*D. spicata*	150	6.48	2.89	12.2
E13	*A. maritima*	4000	11.73	2.39	37.5
E14	*A. maritima*	4000	12.81	2.64	52.9
E15	*A. maritima*	4000	22.31	4.20	31.4
F13	*A. maritima*	3000	24.04	5.64	23.3
F14	*A. maritima*	3000	19.92	4.53	24.9
F15	*A. maritima*	3000	17.53	3.29	38.1
G13	*A. maritima*	2000	20.59	5.02	26.8
G14	*A. maritima*	2000	17.95	3.28	32.6
G15	*A. maritima*	2000	21.26	4.02	22.8
H13	*A. maritima*	1000	7.45	1.83	23.0
H14	*A. maritima*	1000	19.75	4.76	20.3
H15	*A. maritima*	1000	16.18	3.53	19.2
I13	*A. maritima*	150	8.69	3.18	18.2
I14	*A. maritima*	150	18.05	3.81	16.0
I15	*A. maritima*	150	17.98	1.74	19.2
		Sodium Chloride			
A7	*A. canescens*	1500	6.22	2.23	
A8	*A. canescens*	1500	7.44	2.78	

A9	*A. canescens*	1500	6.38	2.36
B7	*A. canescens*	3000	9.79	3.53
B8	*A. canescens*	3000	3.74	1.30
B9	*A. canescens*	3000	6.01	2.13
C7	*A. canescens*	4500	9.61	4.02
C8	*A. canescens*	4500	1.28	0.98
C9	*A. canescens*	4500	12.14	4.61
D7	*A. canescens*	17	3.63	1.47
D8	*A. canescens*	17	4.94	2.04
D9	*A. canescens*	17	3.57	1.57
A10	*S. pectinata*	1500	2.27	1.09
A11	*S. pectinata*	1500	2.93	1.39
A12	*S. pectinata*	1500	7.98	3.95
B10	*S. pectinata*	3000	5.69	2.57
B11	*S. pectinata*	3000	1.00	0.48
B12	*S. pectinata*	3000	7.58	3.53
C10	*S. pectinata*	4500	5.37	2.17
C11	*S. pectinata*	4500	9.15	4.44
C12	*S. pectinata*	4500	4.60	1.92
D10	*S. pectinata*	17	4.06	1.96
D11	*S. pectinata*	17	4.27	2.04
D12	*S. pectinata*	17	8.04	3.77
A13	*D. spicata*	1500	4.33	2.40
A14	*D. spicata*	1500	3.75	2.00
A15	*D. spicata*	1500	4.16	2.14
B13	*D. spicata*	3000	4.08	1.98
B14	*D. spicata*	3000	4.11	2.14
B15	*D. spicata*	3000	3.80	2.06
C13	*D. spicata*	4500	5.11	2.69
C14	*D. spicata*	4500	6.54	3.66
C15	*D. spicata*	4500	6.85	3.45
D13	*D. spicata*	17	2.18	1.27
D14	*D. spicata*	17	2.97	1.66
D15	*D. spicata*	17	3.66	1.98
A19	*A. maritima*	1500	10.39	1.87
A20	*A. maritima*	1500	12.99	2.77
A21	*A. maritima*	1500	19.00	4.87
B19	*A. maritima*	3000	13.13	3.18
B20	*A. maritima*	3000	10.68	1.87
B21	*A. maritima*	3000	6.83	1.12
C19	*A. maritima*	4500	17.00	2.50
C20	*A. maritima*	4500	15.35	2.41

C21	*A. maritima*	4500	15.74	2.55
D19	*A. maritima*	17	8.13	1.98
D20	*A. maritima*	17	9.45	1.95
D21	*A. maritima*	17	9.78	2.50

[a] Indicate corresponding QA/QC (Table D-4)

*Initial KCl soil chloride concentrations (besides 150, and 4000 µg/g conditions) are estimates based on the proportional mixing of the two soils.

Table D-2: Chloride Excretion of four Canadian recretohalophytic species at the end of a 10-week experimental period

Sample Code	Species	Soil Chloride Concentration	Excreted Chloride (mg/g DW)[b,c,d]
		KCl Soil	
E1	*A. canescens*	4000	0.26
E2	*A. canescens*	4000	0.81
E3	*A. canescens*	4000	0.47
F1	*A. canescens*	3000	0.40
F2	*A. canescens*	3000	0.14
F3	*A. canescens*	3000	0.14
G1	*A. canescens*	2000	0.40
G2	*A. canescens*	2000	0.12
G3	*A. canescens*	2000	0.88
H1	*A. canescens*	1000	0.30
H2	*A. canescens*	1000	0.80
H3	*A. canescens*	1000	0.25
I1	*A. canescens*	150	0.19
I2	*A. canescens*	150	0.21
I3	*A. canescens*	150	0.09
E4	*S. pectinata*	4000	8.06
E5	*S. pectinata*	4000	3.24
E6	*S. pectinata*	4000	4.40
F4	*S. pectinata*	3000	5.60
F5	*S. pectinata*	3000	4.61
F6	*S. pectinata*	3000	1.87
G4	*S. pectinata*	2000	3.53
G5	*S. pectinata*	2000	1.50
G6	*S. pectinata*	2000	1.29
H4	*S. pectinata*	1000	0.82
H5	*S. pectinata*	1000	1.58

H6	*S. pectinata*	1000	0.86
I4	*S. pectinata*	150	0.71
I5	*S. pectinata*	150	1.11
I6	*S. pectinata*	150	0.30
E7	*D. spicata*	4000	6.45
E8	*D. spicata*	4000	0.28
E9	*D. spicata*	4000	5.90
F7	*D. spicata*	3000	3.68
F8	*D. spicata*	3000	5.45
F9	*D. spicata*	3000	2.38
G7	*D. spicata*	2000	5.08
G8	*D. spicata*	2000	4.64
G9	*D. spicata*	2000	3.77
H7	*D. spicata*	1000	3.80
H8	*D. spicata*	1000	3.28
H9	*D. spicata*	1000	2.27
I7	*D. spicata*	150	1.18
I8	*D. spicata*	150	0.95
I9	*D. spicata*	150	1.09
E13	*A. maritima*	4000	7.28
E14	*A. maritima*	4000	5.83
E15	*A. maritima*	4000	3.22
F13	*A. maritima*	3000	0.36
F14	*A. maritima*	3000	0.34
F15	*A. maritima*	3000	1.10
G13	*A. maritima*	2000	0.19
G14	*A. maritima*	2000	0.51
G15	*A. maritima*	2000	0.63
H13	*A. maritima*	1000	0.42
H14	*A. maritima*	1000	0.13
H15	*A. maritima*	1000	0.32
I13	*A. maritima*	150	0.13
I14	*A. maritima*	150	0.04
I15	*A. maritima*	150	0.02
	Sodium Chloride		
A7	*A. canescens*	1500	0.20
A8	*A. canescens*	1500	0.14
A9	*A. canescens*	1500	0.63
B7	*A. canescens*	3000	0.17
B8	*A. canescens*	3000	0.64
B9	*A. canescens*	3000	0.49
C7	*A. canescens*	4500	0.28

C8	*A. canescens*	4500	0.28
C9	*A. canescens*	4500	0.17
D7	*A. canescens*	17	0.12
D8	*A. canescens*	17	0.18
D9	*A. canescens*	17	0.14
A10	*S. pectinata*	1500	2.73
A11	*S. pectinata*	1500	1.94
A12	*S. pectinata*	1500	0.57
B10	*S. pectinata*	3000	2.82
B11	*S. pectinata*	3000	1.45
B12	*S. pectinata*	3000	1.49
C10	*S. pectinata*	4500	2.82
C11	*S. pectinata*	4500	2.32
C12	*S. pectinata*	4500	1.11
D10	*S. pectinata*	17	0.35
D11	*S. pectinata*	17	0.30
D12	*S. pectinata*	17	0.19
A13	*D. spicata*	1500	14.13
A14	*D. spicata*	1500	15.44
A15	*D. spicata*	1500	1.01
B13	*D. spicata*	3000	14.66
B14	*D. spicata*	3000	15.14
B15	*D. spicata*	3000	11.43
C13	*D. spicata*	4500	16.84
C14	*D. spicata*	4500	16.80
C15	*D. spicata*	4500	13.01
D13	*D. spicata*	17	0.67
D14	*D. spicata*	17	1.40
D15	*D. spicata*	17	0.56
A19	*A. maritima*	1500	0.79
A20	*A. maritima*	1500	1.48
A21	*A. maritima*	1500	0.30
B19	*A. maritima*	3000	0.56
B20	*A. maritima*	3000	1.48
B21	*A. maritima*	3000	1.81
C19	*A. maritima*	4500	12.94
C20	*A. maritima*	4500	5.08
C21	*A. maritima*	4500	2.60
D19	*A. maritima*	17	0.04
D20	*A. maritima*	17	0.05
D21	*A. maritima*	17	0.12

[b,c,d] Indicate corresponding QA/QC (Table D-4)

*Initial KCl soil chloride concentrations (besides 150, and 4000 µg/g conditions) are estimates based on the proportional mixing of the two soils.

Table D-3: Final Soil Chloride Concentration for the 4000 µg/g soil condition

Sample Code	Species	$[Cl^-]$ (mg/g)[e]
E1	*A. canescens*	2 750
E2	*A. canescens*	3 190
E3	*A. canescens*	3 880
E4	*S. pectinata*	3 290
E5	*S. pectinata*	2 310
E6	*S. pectinata*	2 670
E7	*D. spicata*	3 840
E8	*D. spicata*	4 250
E9	*D. spicata*	10 300
E13	*A. maritima*	1 220
E14	*A. maritima*	1 360
E15	*A. maritima*	1 190
E10	Unplanted	2 890
E11	Unplanted	3 920
E12	Unplanted	5 650

[e] Indicate corresponding QA/QC (Table D-4)

Table D-4: Quality Control and Quality Assurance

	Blank	Control	Control Target	% of Target	Cranberry -05	Cranberry -05 Target	% Target
a (22/01/2019)	<0.05	5.2; 5.1; 5.1, 5.2	5.0	104; 102; 102; 104	35, 35	35	100; 100
b (24/08/2018)	<0.05	5.2	5.0	104	38	35	109

c (14/09/2018)	<0.05	5.3	5.0	106	37	35	106
d (23/10/2018)	<0.05 (X4)	5.4; 5.5; 4.8; 4.9; 4.8	5.0	108; 110; 96; 98; 96	38; 38	35	109;109
e (02/11/2018)	<0.05, <0.05	4.9; 5.0; 4.9; 5.1	5.0	98; 100; 98; 102	38	35	109

D-2 Calculations for Chapter 4

D-2.1 Excreted Chloride concentration

$$Excreted\ chloride\ \left(\frac{mg}{pot}\right) = (IC\ concentration\ \left(\frac{mg}{L}\right)\left(Volume\ of\ plant\ wash\ \left(\frac{L}{pot}\right)\right)$$

$$Excretion\ \left({}^{mg\ Cl^-}/_{g\ DW}\right) = \frac{Excretion\ \left({}^{mg Cl^-}/_{pot}\right)}{Shoot\ DW\ \left({}^{g}/_{pot}\right)}$$

$$Excretion\ \left({}^{g\ Cl^-}/_{m^2}\right) = \left(\frac{Excretion\ \left({}^{mg Cl^-}/_{pot}\right)}{78.5\ \left({}^{cm^2}/_{pot}\right)}\right)\left(\frac{10000\left({}^{cm^2}/_{m^2}\right)}{1000\ \left({}^{mg}/_{g}\right)}\right)$$

$$Excretion\ \left({}^{\mu g\ Cl^-}/_{cm^2\ leaf\ surface\ area}\right) = \frac{Excretion\ \left({}^{mg\ Cl^-}/_{pot}\right)}{Leaf\ surface\ area\ \left({}^{cm^2}/_{pot}\right)} * (1000\ \left({}^{\mu g}/_{mg}\right)$$

Excretion based on literature values:

$$Excretion\ \left({}^{mg\ Cl^-}/_{m^2}\right) = \left(Excretion\ \left({}^{mg\ Cl^-}/_{g\ shoot\ DW}\right)\right)\left(Biomass\ \left({}^{g}/_{m^2}\right)\right)$$

$$Total\ Excreted\ chloride\ in\ 10\ weeks = \frac{Excretion\ \left(\frac{mg}{pot}\right)}{2\ weeks}(10\ weeks)$$

D-2.2 Accumulated Chloride concentration

$$Accumulated\ chloride\ \left(\frac{mg}{g}\right) = \frac{\left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.01L)}{Mass\ of\ dry\ tissue\ used\ (g)}$$

$$Accumulated\ chloride\ \left(\frac{mg}{plant}\right) = \left(Tissue\ concentration\ \left(\frac{mg}{g}\right)\right)\left(Dry\ weight\ \left(\frac{g}{plant}\right)\right)$$

$$\text{Accumulated chloride } (g/m^2)_{\text{this experiment}} = \left(\frac{Accumulated\left(mg^{Cl^-}/_{pot}\right)}{78.5\left(cm^2/_{pot}\right)}\right)\left(\frac{10000\left(cm^2/_{m^2}\right)}{1000\left(mg/_g\right)}\right)$$

$$\text{Accumulated chloride } (g/m^2)_{\text{literature values for biomass}} = \left(Accumulated\ \left(mg\ Cl^-/_{g\ shoot\ DW}\right)\right)\left(Biomass\ \left(g/_{m^2}\right)\right)$$

D-2.3 Ratio of Accumulated: Excreted

$$Total\ chloride\ translocated\ into\ the\ plant\ (mg) = accumulated\ \left(\frac{mg}{plant}\right) + total\ excreted\ \left(\frac{mg}{plant}\right)$$

$$Percentage\ excreted = Total\frac{excreted}{total\ translocated}\ (100)$$

$$Percentage\ accumulated = Total\frac{accumulated}{total\ translocated}\ (100)$$

D-2.4 Soil chloride concentration

$$Measured\ soil\ chloride\ concentration = \frac{\left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.025\ L\ DDI)}{mass\ of\ dry\ soil\ used\ (g)}$$

$$Calculated\ final\ soil\ chloride\ concentration\ \left(\frac{mg}{g}\right) = \frac{\left(\left(Initial\ soil\ concentration\ \left(\frac{mg}{g}\right)\right)\left(amount\ of\ soil\ \left(\frac{g}{pot}\right)\right)\right) - total\ translocated\ (mg)}{amount\ of\ soil\ \left(\frac{g}{pot}\right)}$$

APPENDIX E Supplemental Materials for Chapter 5 Publication

E-1 Factors that Impact Emission

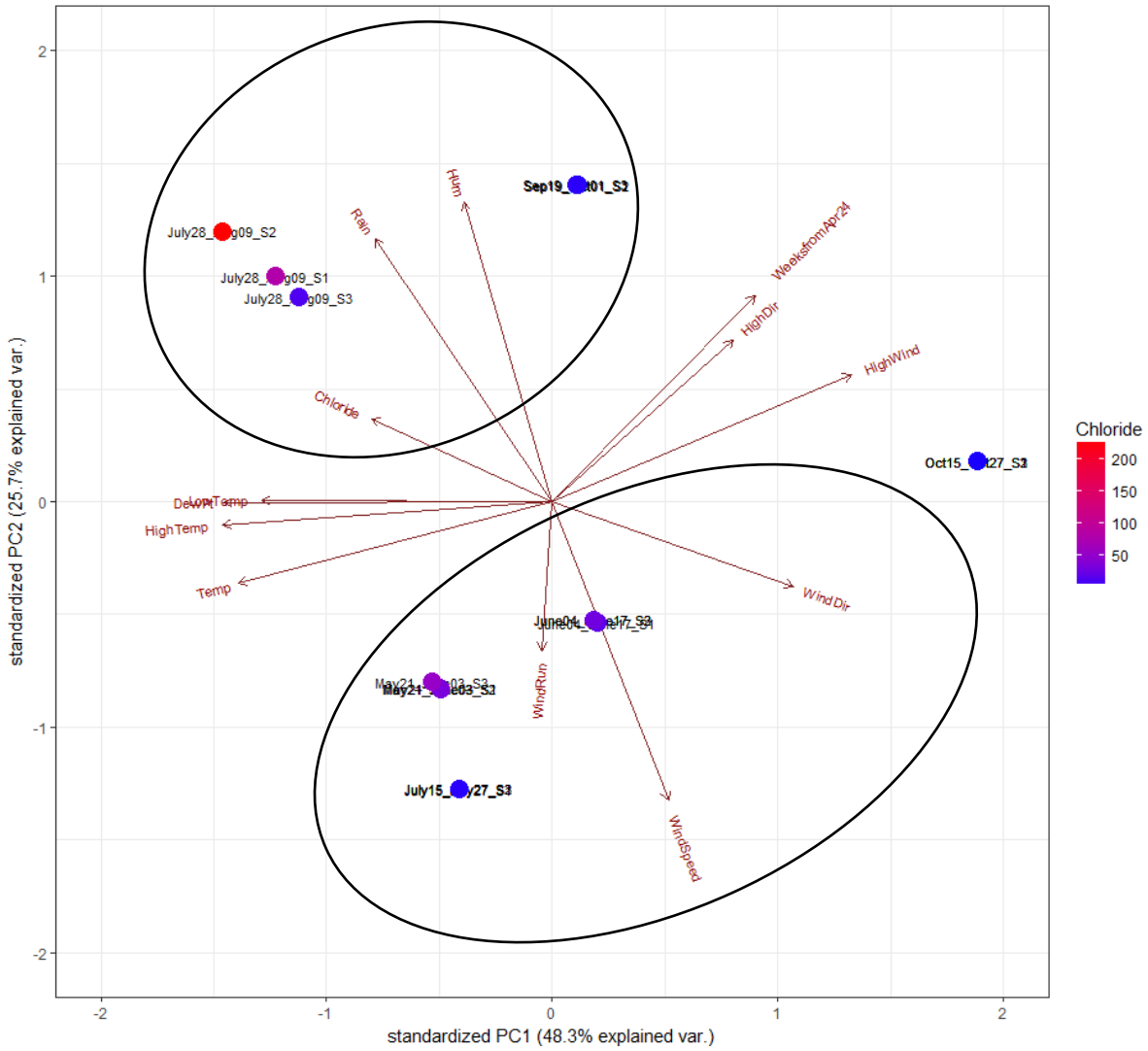

Figure E-1: Principle component analysis of meteorological factors and the amount of chloride found on *S. pectinata* plants grown at the Bath site throughout the 2018 field season. Humidity, rain, high chloride, and samples collected mid season cluster together, and wind speed, and samples collected early in the season cluster together.

E-2 Wind Tunnel Design

The wind tunnel used a Howden blower with a volumetric flow rate of 0.4 m^3/s as the drive system, followed by a 1.8 m long diffuser with a 15^O angle to prevent flow separation. The diffuser attached to a flow conditioner comprised of two metal mesh screens (4.5 cm X 3.2 cm hexagons and 0.16 cm squares) to generate homogenous flow and a honeycomb sheet to remove

turbulence (Sup. Figure E-2). A contraction cone with an area ratio of 6.5 connected the flow conditioner to a 45 cm x 45 cm acrylic testing zone.

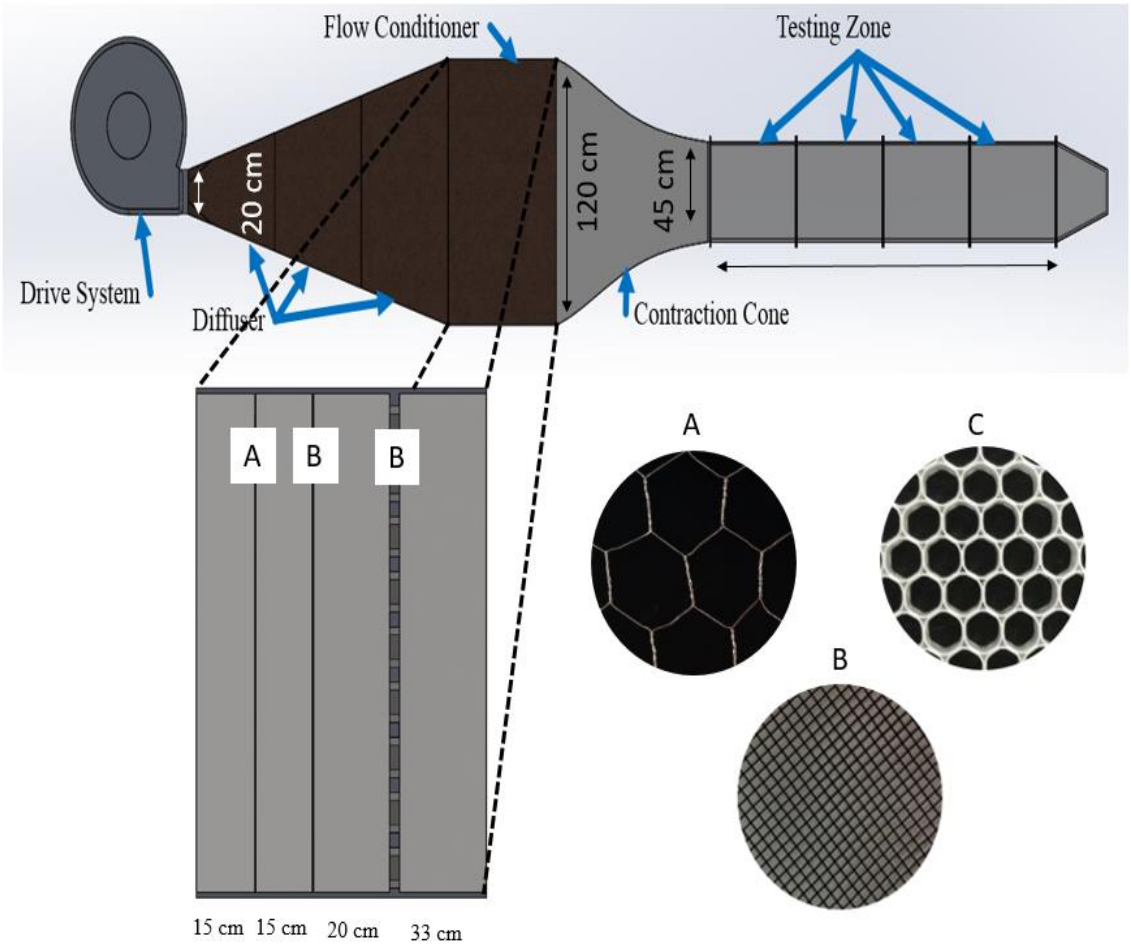

Figure E-2: Wind tunnel design A) 1-inch chicken wire mesh, B) window screening, C) honeycomb.

The wind speed was varied using a 30A Veriac transformer (Staco Energy, Model 3PN15101B) and the resulting wind speed was measured using a hot-wire anemometer (VelociCheck Air Velocity Meter, Model 8330). The homogeneity and consistency of flow speed was determined at 30 cm, 60 cm, and 90 cm from the beginning of the testing zone and found 90 cm was to be the most appropriate distance to place the plant. This method was repeated to confirm homogeneity and consistency of flow for each of the tested wind speeds (~0, 0.5, 1, 1.5, 2, 3, 4 m/s) (Sup. Figure E-3).

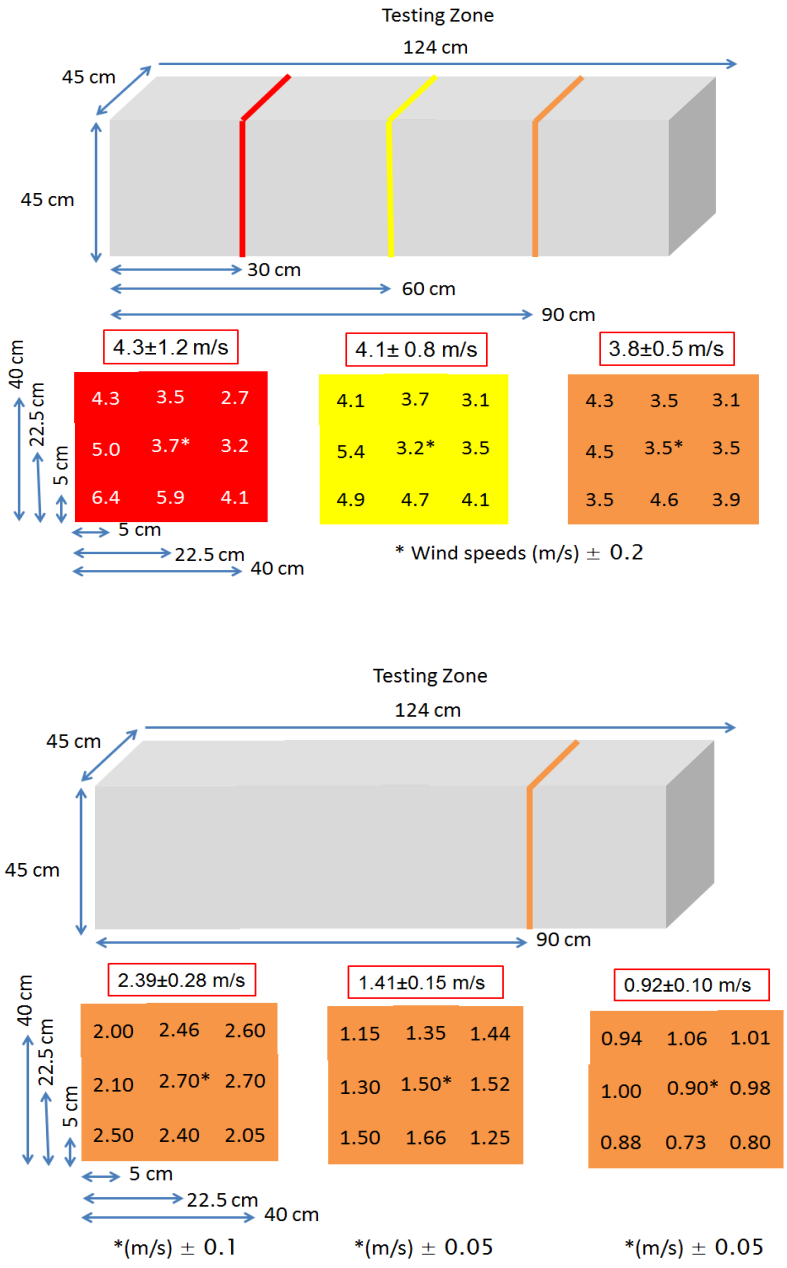

Figure E-3: Wind tunnel validation A) impact of distance on the homogeneity of wind speed at approximately 4 m/s. B) Homogeneity of wind speed at approximately 2.5 m/s, 1.5 m/s and 1 m/s.

Table E-1: Relationship between the time of year (2018, 2019) and the height of *S. pectinata* when grown at the Bath site

Time of Year	Approximate Age (Hours)	Approximate Height (cm)
May 01	0	0
June 01	744	30
Jully 01	1464	80
August 01	2208	100
September 01	2239	100

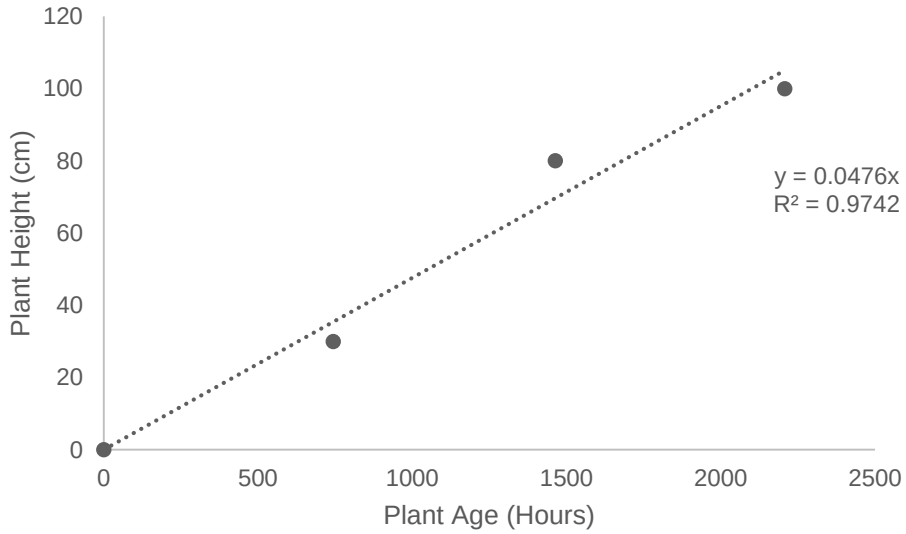

Figure E-4: Relationship between plant age (based May 1st = 0) and plant height.

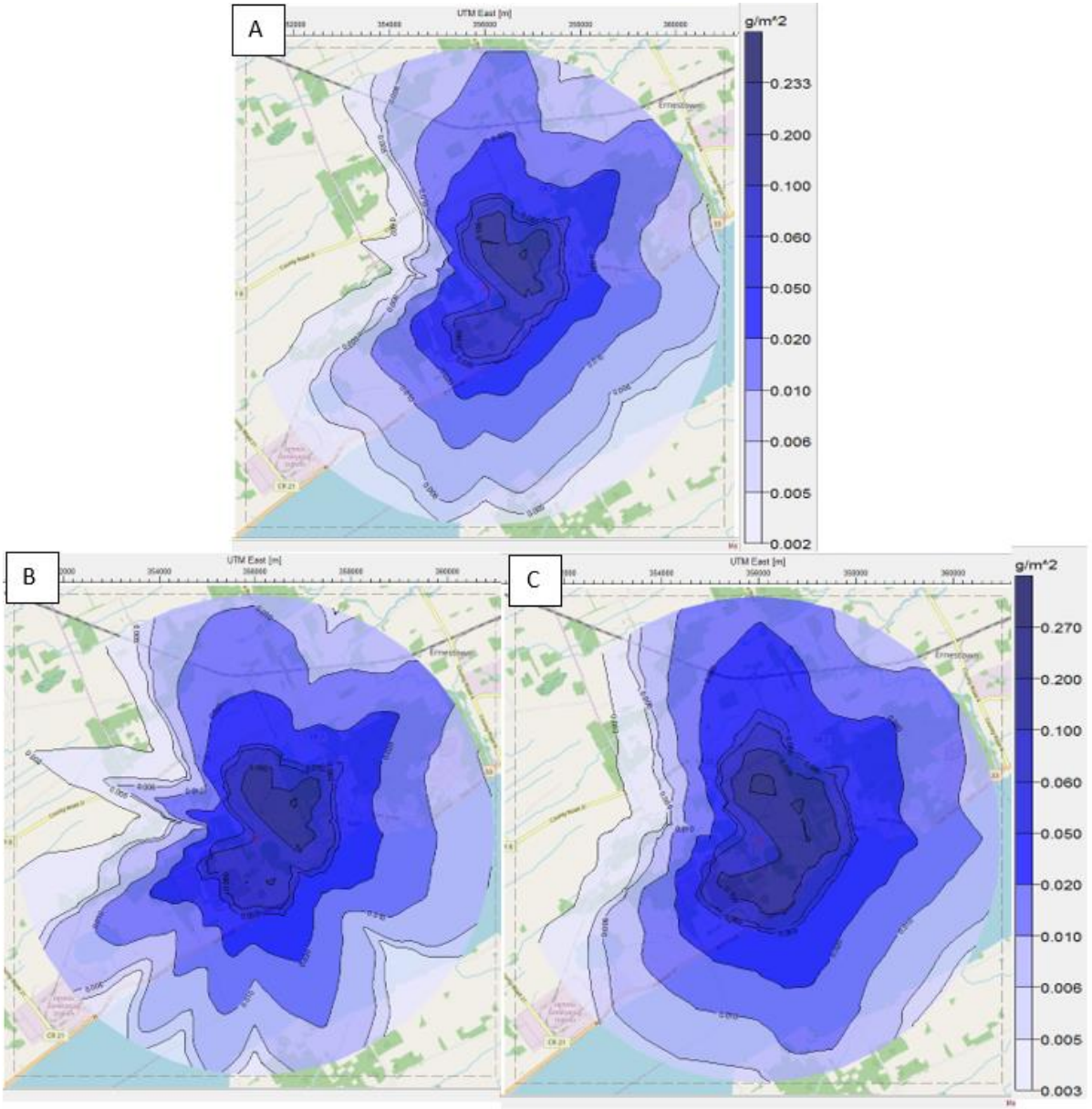

Figure E-5: Total deposition at the end of the 5-year period (2011-2015) if the entire site was planted with *S. pectinata* and emission was A) baseline calculated B) if the emission factor was 100% (humidity and rain still considered) of C) if emission was equal to excretion.

Table E-2: Estimated vs Actual deposition and Concentration data for the 2018 and 2019 field season

Sampling Date	Estimated Deposition Rate (mg/m^2)	Actual Deposition (mg/m^2)	Estimated Aerial Concentration ($\mu g/m^3$)	Actual Aerial Concentration ($\mu g/m^3$)
06/06/2018	0	25.2	4.76×10^{-5}	N/A
06/21/2018	4.76×10^{-3}	46.3	4.76×10^{-5}	N/A
06/07/2018	9.52×10^{-3}	35.6	4.76×10^{-5}	N/A
02/08/2018	3.33×10^{-2}	12.4	1.43×10^{-4}	N/A
16/08/2018	4.76×10^{-2}	17.3	1.43×10^{-4}	N/A
Control				
06/06/2018	0	24.0		
06/21/2018	4.76×10^{-3}	21.2		
06/07/2018	9.52×10^{-3}	15.1		
02/08/2018	3.33×10^{-2}	4.4		
16/08/2018	4.76×10^{-2}	25.8		
04/06/2019	0		9.52×10^{-6}	0.51
12/06/2019	0		9.52×10^{-6}	
21/06/2019	0		9.52×10^{-6}	0.12
28/06/2019	0		9.52×10^{-6}	
04/07/2019	0		9.52×10^{-6}	0.047
18/07/2019	0		9.52×10^{-6}	
25/07/2019	4.76×10^{-6}		2.38×10^{-5}	0.05
01/08/2019	4.76×10^{-6}		2.38×10^{-5}	0.13
10/08/2019	4.76×10^{-6}		9.52×10^{-6}	0.29
15/08/2019	4.76×10^{-6}		2.38×10^{-5}	0
22/08/2019	9.52×10^{-6}		2.38×10^{-5}	0.53
30/08/2019	9.52×10^{-6}		2.38×10^{-5}	0.026
Background Samples				
31/07/2019	4.76×10^{-6}		9.52×10^{-6}	0
13/08/2019	9.52×10^{-6}		9.52×10^{-6}	0
29/08/2019	9.52×10^{-6}		9.52×10^{-6}	0.024

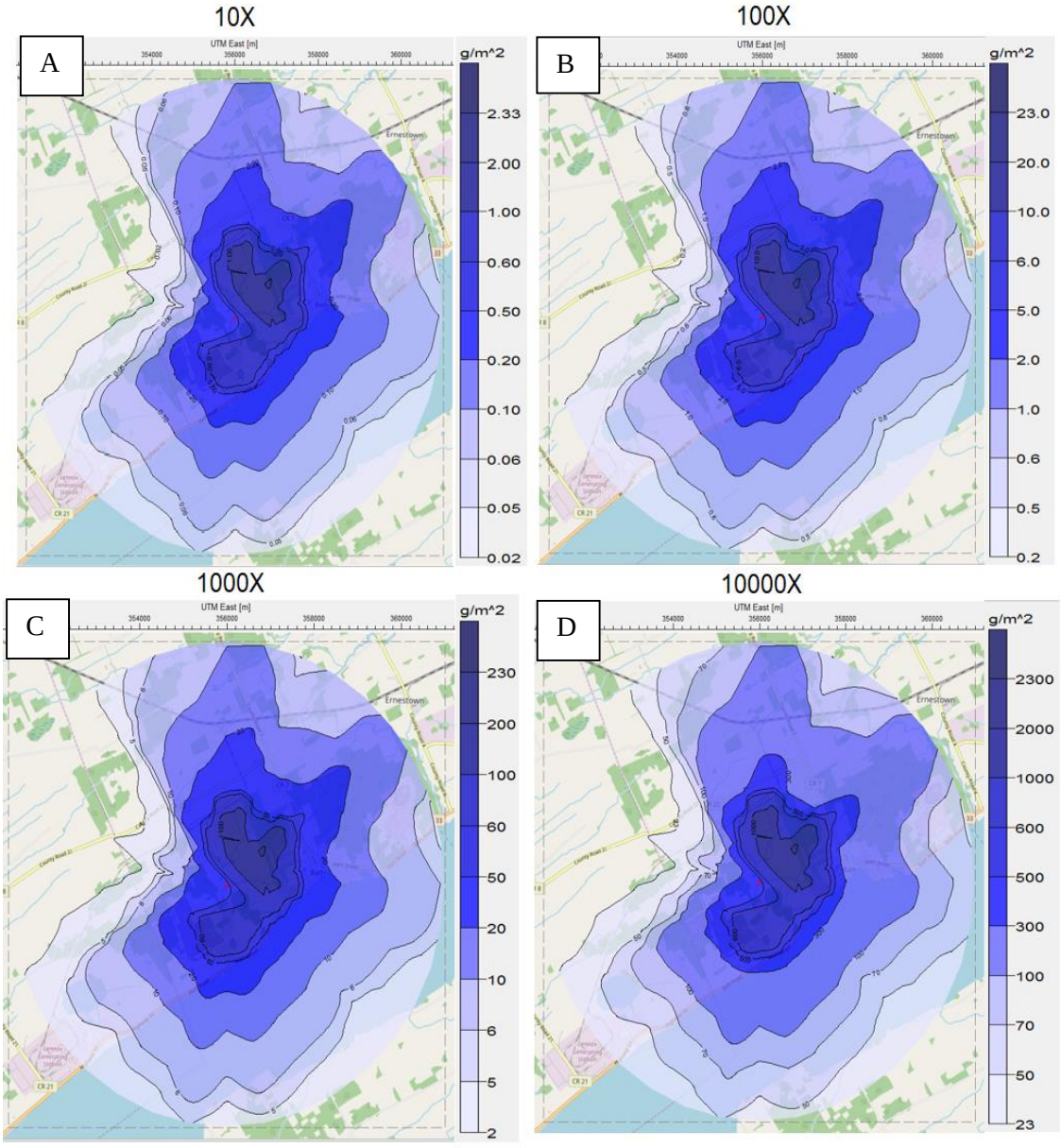

Figure E-6: KCl deposition at the end of the 2011-2015 period if emission was A)10X, B)100X, C)1000X, and D)10 000X that used in the original model.

APPENDIX F Supplemental Data for Chapter 5

F-1: Raw Data for Chapter 5

Table F-1: Chloride Excretion of *S. pectinata*

Sample Name	Soil Chloride Concentration (mg/g)	Approximate Plant Height (cm)	Chloride Excretion (g/m^2)[a,b,c,d]
E4	4	20	0.30
E5	4	20	0.40
E6	4	20	0.80
F4	3	20	0.65
F5	3	20	0.22
F6	3	20	0.27
G4	2	20	0.56
G5	2	20	0.27
G6	2	20	0.49
H4	1	20	0.27
H5	1	20	0.64
H6	1	20	0.17
I4	0.15	20	0.18
I5	0.15	20	0.22
I6	0.15	20	0.11
E4	4	28	0.97
E5	4	28	1.07
E6	4	28	1.44
F4	3	28	1.52
F5	3	28	0.56
F6	3	28	0.70
G4	2	28	1.26
G5	2	28	0.69
G6	2	28	0.88
H4	1	28	0.28
H5	1	28	0.55
H6	1	28	0.49
I4	0.15	28	0.22
I5	0.15	28	0.17
I6	0.15	28	0.19
E4	4	34	2.39
F4	3	34	1.05
G4	2	34	0.73
H4	1	34	0.32
I4	0.15	34	0.15
E4	4	40	1.99

E5	4	40	1.56
E6	4	40	1.96
F4	3	40	3.83
F5	3	40	1.22
F6	3	40	1.88
G4	2	40	5.81
G5	2	40	1.56
G6	2	40	1.50
H4	1	40	1.49
H5	1	40	1.00
H6	1	40	0.56
I4	0.15	40	0.36
I5	0.15	40	0.94
I6	0.15	40	0.09

[a,b,c,d] Indicate corresponding QA/QC (Table F-5)

Table F-2: Wind Tunnel, *S. pectinata* Salt Wash Data

Sample Name	Wind Speed (m/s)					
	0[e,f]	0.5[e]	2[f]		4[f]	
	mg/plant	mg/plant	mg/plant	% emitted	mg/plant	% emitted
5B	2.66[e]	2.65	---	---	---	---
10B	1.75[e]	2.0	---	---	---	---
1000B	1.07[e]	1.04	---	---	---	---
5C	5.26[f]	---	4.30	18.3	3.81	27.6
500A	4.30[f]	---	3.15	26.9	---	---
5000C	0.58[f]	---	---	---	0.41	28.7
1000C	2.07[f]	---	1.50	27.3	1.88	9.33
5000B	1.45[f]	---	---	---	0.86	40.8

[e,f] Indicate corresponding QA/QC (Table F-5)

Table F-3: Field Plant Washes of *S. pectinata* from the Bath site in 2018

	Chloride washed (mg/100cm^2)[g]					
	29/05/2018	07/06/2018	18/07/2018[d]	02/08/2018	22/09/2018	18/10/2018
S1	31.4	18.0	2.54	80.8	1.69	0.09
S2	33.4	27.6	4.03	226	1.14	0.17
S3	53.8	27.5	3.60	12.6	3.88	1.51

[g] Indicate corresponding QA/QC (Table F-5)

Table F-4: Wet Candle Samples

Wet Candle Name	Wet Candle Sampling Date	Total chloride collected (μg)[h,i,]
W1	06/06/2018	50
W2	06/06/2018	45

W3	06/06/2018	35.5
W4	06/06/2018	36
WCC	06/06/2018	39.5
WCB	06/06/2018	16
W1	21/06/2018	230
W2	21/06/2018	350
W3	21/06/2018	340
W4	21/06/2018	300
WCC	21/06/2018	140
WCB	21/06/2018	54
W1	06/07/2018	230
W2	06/07/2018	230
W3	06/07/2018	220
W4	06/07/2018	260
WCC	06/07/2018	100
WCB	06/07/2018	75
W1	02/08/208	95
W2	02/08/208	67
W3	02/08/208	93
W4	02/08/208	73
WCC	02/08/208	29
WCB	02/08/208	19
W1	16/08/2018	72
W2	16/08/2018	170
W3	16/08/2018	120
W4	16/08/2018	94
WCC	16/08/2018	170
WCB	16/08/2018	34

[h,i] Indicate corresponding QA/QC (Table F-5)

Table F-4: High Volume Air sampler calibrated volumes

Start Date	Run Time (hrs)	Measured Volume (CFM/hr)	Calibrated volume (m^3)	Chloride Collected ($\mu g/m^3$)[j]
June 4, 2019	3.77	46	293	0.67
June 12, 2019	10.07	45	713	
June 21, 2019	17.5	45	1295	0.15
June 28, 2019	15.73	45	1295	0.13
July 11, 2019	8.2	48	643	0.12
July 18, 2019	9.4	48	719	0.06
July 31, 2019	15.3	58	779	0.02
Aug. 01, 2019	5.85	55	235	0.32
Aug. 10, 2019	3.27	58	307	0.44
Aug. 14, 2019	12.56	58	1206	0
Aug. 15, 2019	1.94	60	200	0.075
Aug. 22, 2019	6.71	60	677	0.60
Aug. 29, 2019	12.31	58	1218	0.06

Aug. 30, 2019	11.6	60	1164	0.06
Mean ± SD	10 ± 5	53 ± 6	770 ± 400	0.25 ± 0.22

[i] Indicate corresponding QA/QC (Table F-5)

Table F-5: Quality Control and Quality Assurance

	Blank	Control	Control Target	% of Target	Cranberry -05	Cranberry -05 Target	% of Target
a (17/08/2018)	<0.05	5.2	5.0	104	37	35	106
b (24/08/2018)	<0.05	5.2	5.0	104	38	35	109
c (14/09/2018)	<0.05	5.3	5.0	106	37	35	106
d (23/10/2018)	<0.05 (X4)	5.4; 5.5; 4.8; 4.9; 4.8	5.0	108; 110; 96; 98; 96	38; 38	35	109; 109
e (05/04/2019)	<0.05	5.3; 5.5; 5.4; 5.1	5.0	106; 110; 108; 102	35	35	100
f (10/06/2019)	<0.05, <0.05	4.9; 4.9; 4.9; 4.8; 4.8; 4.8	5.0	98; 98; 98; 96; 96; 96	37; 37	36	103; 103
g (18/12/2018)	<0.05	5.2; 5.0; 5.1; 5.2	5.0	104; 100; 102; 104	37; 37	35	106; 106
h (03/06/2018)	<0.05	5.6	5.0	112	36	36	100
i (14/09/2018)	<0.05	5.3	5.0	106	37	35	106
j (11/09/2019)	<0.05; <0.05	4.8; 4.8	5.0	96; 96	35	36	97

F-2 Calculations for Chapter 5

F-2.1 Excretions of S. pectinata

$$Excretion\ \left(g\ Cl^{-}/_{m^2}\right) = \left(\frac{Excretion\ \left(mg\ Cl^{-}/_{pot}\right)}{78.5\ \left(cm^2/_{pot}\right)}\right)\left(\frac{10000\left(cm^2/_{m^2}\right)}{1000\ \left(mg/_{g}\right)}\right)$$

F-2.2 Proportion of chloride emitted

$$Amount\ of\ Excreted\ Chloride\ on\ a\ plant\ (mg) = \left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.03L\ DI\ used\ for\ extracting\ plant\ wash)$$

$$Percentage\ of\ Salt\ Emitted = \frac{\left(Amount\ on\ plant\ at\ X\ \left(\frac{m}{s}\right)\right) - \left(Amount\ on\ plant\ at\ 0\ \left(\frac{m}{s}\right)\right)}{Amount\ on\ plant\ at\ 0\ \left(\frac{m}{s}\right)}\ (100)$$

F-2.3 KCl:Cl molar ratio

KCl: CL = 21:10

$$Amount\ KCl = (2.1)(Amount\ of\ Cl)$$

F-2.4 Chloride Deposition

$$Chloride\ on\ wet\ candle\ (\mu g) = \left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.3\ L\ DI)(1000\ \frac{\mu g}{mg})$$

$$Chloride\ depostion\ \left(\frac{\mu g}{m^2}\right) = \frac{\left(chloride\ on\ wet\ candle\ (\mu g)\right)}{(surface\ area\ of\ wet\ candle\ (cm^2)}(\frac{10\ 000\ cm^2}{m^2})$$

F-2.5 Aerial Chloride concentration

$$Chloride\ on\ HiVol\ filter\ paper\ (\mu g) = \left(IC\ concentration\ \left(\frac{mg}{L}\right)\right)(0.3\ L\ DI)(1000\ \frac{\mu g}{mg})$$

$$Chloride\ depostion\ \left(\frac{\mu g}{m^3}\right) = \frac{\left(chloride\ on\ HiVol\ filter\ paper\ (\mu g)\right)}{(volume\ of\ air\ collected\ (m^3)}$$

F-2.6 Site Remediation

$$Amount\ of\ chloride\ deposited\ in\ an\ area\ (mg) = (Area\ (m^2))(deposition\ concentration\ (\frac{mg}{m^2}))$$

$$Total\ amount\ deposited\ (kg) = \left(\ \Sigma(chloride\ deposited\ (mg))\right)\left(\frac{kg}{1\ x10^6 mg}\right)$$

$$Yearly\ removal\ \left(\frac{kg}{year}\right) = \frac{Total\ amount\ deposited\ (kg)}{number\ of\ years\ in\ model\ timeframe\ (years)}$$

$$Increase\ in\ soil\ chloride\ concentration\ from\ deposition\ \left(\frac{\mu g}{g}\right) = \frac{\left(chloride\ deposited\ \left(\frac{mg}{m^2}\right)\right)\left(1000\ \left(\frac{\mu g}{mg}\right)\right)\left(\frac{m^2}{10\ 000\ cm^2}\right)}{1cm\ deep\ (cm)(bulk\ density\ 1.33\ (\frac{g}{cm^3})}$$

$$Remediation\ timeframe\ (years) = \frac{450^a(kg)}{Yearly\ removal\ (\frac{kg}{year})}$$

[a] Chloride available within the top 10 cm of the site based on McSorley et al. (2016)