PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS AND SALT IMPACTED SOIL BY RECRETOHALOPHYTES AND HALOCONDUCTION

PHYTOREMEDIATION DES HYDROCARBURES DE PETROLE ET DU SOL IMPACTE PAR LES SELS PAR RECRETOHALOPHYTES ET HALOCONDUCTION

A Thesis Submitted to the Division of Graduate Studies of the Royal Military College of Canada by

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ABSTRACT

Two environmental contaminants that are found at many oil and gas extraction sites are petroleum hydrocarbons (PHCs) and salts. Conventional remediation technologies for PHC- and salt-contaminated soils include *ex situ* treatment methods like excavation and disposal, as well as *in situ* treatments such as leaching and the addition of soil amendments. These traditional remediation technologies are expensive, require multiple applications, and could potentially have a negative effect on sensitive ecosystems. A passive solution to remediating PHCs and salts individually that has been shown to work in the past with varying degrees of success is phytoremediation. PHCs are degraded by microorganisms in the root zone (rhizodegradation), while salt tolerant plants, known as halophytes extract salts from the soil. Some halophytes, known as recretohalophytes, have specialized salt glands embedded in their leaf tissue that excrete excess salts onto their leaf surfaces. Under the appropriate conditions, these salts can mobilize into the air by wind, effectively diluting them over a large area, in a process known as haloconduction. Two native recretohalophytes *Distlichils spicata* and *Spartina pectinata* were assessed for their ability to simultaneously degrade PHCs and disperse salts. Soils impacted by high levels of potassium chloride (KCl) were spiked to a total petroleum hydrocarbon (TPH) level of ~10,000 mg/kg. TPH levels significantly decreased to around ~4,000 mg/kg in the first three months of the 22 month experiment. There was no significant difference between the planted and non-planted pots indicating that the TPH decrease was not attributable to the plants. The PHCs did not interfere with the salt excretion capabilities of *S. pectinata,* which significantly ($p<0.05$) reduced the salt levels by ~93%. At optimal humidity conditions (55-65%), the salt crystals of *S. pectinata* measured 31 $\pm$ 24 µm, which is significantly smaller than *D. spicata* crystals measured at 49 $\pm$ 22 µm ($p<0.05$). *S. pectinata* also excreted significantly more salt crystals per unit area of plant surface (60 crystals $\pm$ 41 per 1 mm^2) than *D. spicata* (27 crystals $\pm$ 16 per 1 mm^2). A wind tunnel apparatus was designed and constructed to mobilize and collect excreted salt once it had blown off the plants. The salt was collected from within the apparatus by swabbing the inside at 30 cm increments, washing the moistened cheesecloth, and rinsing any remaining salt off of the plant. A mean total of 34 ± 21 mg of salt (Cl^-) was collected for *D. spicata* and 189 ± 54 mg for *S. pectinata,* which is significantly more total salt than *D. spicata* ($p<0.05$). Combining meteorological data with the results determined in this thesis, it is now possible, for the first time, to model the dispersion of salt at a field site planted with *D. spicata* and *S. pectinata.* These findings contribute to the theory of haloconduction and the implementation of recretohalophytes for phytoremediation of PHCs and salts.

RÉSUMÉ

Les sels et les 'hydrocarbures pétroliers (HCP) sont deux contaminant environnementaux présents aux sites d'extraction de pétrole et de gaz. Les technologies classiques d'assainissement des sols contaminés par les hydrocarbures pétroliers et les sels incluent les méthodes de traitement *ex situ*, comme l'excavation, ainsi que les traitements *in situ* comme le lessivage et les amendements au sol. Ces technologies d'assainissement traditionnelle sont coûteuses, nécessitent plusieurs applications et on le potentielle d'avoir un effet négatif sur les écosystèmes sensibles. La phytoremédiation est une méthode passive pour assainir les HCP et les sels, qui a été démontré dans la littérature, individuellement, avec plusieurs degrés de succès. Les HCP sont dégradés par les micro-organismes présents dans la zone racinaire (rhizodégradation), tandis que les plantes tolérantes au sel, appelées halophytes, extraient les sels du sol. Certains halophytes, connus par le nom recretohalophytes, ont des glandes à sel spécialisées incorporées dans le tissu de la feuille qui excrètent les sels qui sont en excès, sur la surface de la feuille. Alors que les conditions sont appropriées, le vent peut mobiliser ces granules de sel dans l'air, ce qui peut les diluer efficacement sur une grande surface, selon un processus appelé haloconduction. Deux récrétohalophytes indigènes, *Distlichils spicata* et *Spartina pectinata*, ont été évalués pour leur capacité à dégrader simultanément les HCP et dispersé les sels. Les sols affectés par des niveaux élevés de chlorure de potassium (KCl) ont été dopés à une concentration d'hydrocarbures de pétrole totaux (TPH) d'environ 10 000 mg / kg. Les taux de TPH ont diminué de manière significative jusqu'à environ 4 000 mg / kg au cours des trois premiers mois des 22 mois de l'expérience. Il n'y avait pas de différence significative entre les pots plantés et non plantés ce qui indique que la diminution de la TPH n'était pas attribuable aux plantes. Les PHC n'ont pas affecté la capacité d'excrétion de sel de *S. pectinata*, ce qui réduisait considérablement ($p < 0,05$) les niveaux de sel d'environ 93%. Lorsque les conditions d'humidité étaient optimales (55-65%), les cristaux de sel de *S. pectinata* mesuraient 31 + 24 µm, ce qui est plus petit que les cristaux de *D. spicata* mesurés à 49 + 22 µm ($p < 0,05$). *S. pectinata* a également excrété significativement plus de cristaux de sel par unité de surface de la feuille ? (60 cristaux + 41 par 1 mm2) que *D. spicata* (27 cristaux + 16 par 1 mm2). Une soufflerie a été conçu et construit pour mobiliser le sel excrété par les plantes. Le sel a été recueilli à l'intérieur de l'appareil en tamponnant l'intérieur par incréments de 30 cm, en lavant l'étamine humidifiée et en rinçant le sel restant sur la plante. La moyenne de sel recueilli en total étaient 34 ± 21 mg (Cl-) pour *D. spicata* et 189 ± 54 pour *S. pectinata*, ce qui représente significativement plus de sel que *D. spicata* ($p < 0,05$). En combinant les données météorologiques avec les résultats déterminés dans cette thèse, il est maintenant possible, pour la première fois, de modéliser la dispersion de sel sur un site planté de *D. spicata* et de *S. pectinata*. Ces résultats contribuent à la théorie d'haloconduction et à la mise en œuvre de l'application des recretohalophytes pour la phytoremédiation des HCP et des sels.

CO-AUTORSHIP STATEMENT

The student's contribution to the thesis manuscript are as follow:

- Primary researcher responsible for the successful design, implementation and completion of all laboratory and greenhouse experiments conducted at the Royal Military College of Canada (RMC, Kingston, ON)
- Assisted with analytical work at both the Analytical Service Unit at Queen's University (Kingston, ON) and the Phytotechnologies Laboratory (RMC)
- Primary author on all three research papers. Chapter 3 has been submitted for publication in the Journal of Environmental Quality.

Chapter 3: Morris, L.C. Rutter, A., Zeeb, B.A. Phytoremediation of Petroleum Hydrocarbons and Salt.

Chapter 4: Morris, L.C. Rutter, A., Yun, K., Zeeb, B.A. Characterization of Excreted Salt from the Recretohalophytes *Distichlis spicata* and *Spartina pectinata.*

- This paper is co-authored by former MSc student, Kassandra Yun, as well as both of my supervisors. Ms. Yun's contribution was to carry out the initial observations on the optimal time to collect excreted salts from both plants. I completed this work with the full characterization of salt crystals using environmental scanning electron microscopy (ESEM). I also carried out all of the follow-up work on optimizing salt excretion, did the statistical analyses, and wrote the manuscript.

Chapter 5: Morris, L.C. Rutter, A., Zeeb, B.A. Mobilization and Collection of Excreted Salt Particles from *Distichlis spicata* and *Spartina pectinata* in a Wind Tunnel.

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LIST OF ABBREVIATIONS

APCD	Air pollution control device
ASU	Analytical Services Unit
ANOVA	One way analysis of variance
BC	Basal collecting cells
BTEX	Benzene, toluene, ethylbenzene, and xylenes
CCME	Canadian council of the ministers for the environment
CSEM	Conventional Scanning Electron Microscopy
CKD	Cement kiln dust
CRM	Certified reference material
DDW	Double deionized water
DI	Deionized
EC_e	Electrical conductivity of saturated past extract
$EC_{1:5}$	Electrical conductivity of 1:5 soil to water extract
EDS	Electron dispersive spectroscopy
ESEM	Environmental Scanning Electron Microscopy
DRO	Diesel range organics
GRO	Gasoline range organics
IC	Ion chromatography
NSERC	Natural Sciences and Engineering Research Council of Canada
ORO	Oil range organics
PHC	Petroleum hydrocarbons
RMC	Royal Military College of Canada
SAR	Sodium adsorption ration
SEC	Secretory Cells
SEM	Scanning electron microscopy
SI	Salt impacted
SSA	Sea Salt Aerosol
TPH	Total Petroleum Hydrocarbons
USEPA	United States Environmental Protection Agency

1 INTRODUCTION

Soil salinization is a growing environmental concern. At high concentrations, the ions found in salts (e.g. sodium (Na^+) and chloride (Cl^-)) are toxic to plants and other organisms (Munns and Tester 2008). For example, these salt ions can interact with proteins and cause them to fold improperly and become non-functional (Tavakkoli *et al.,* 2010). Soil salinization can occur through natural and anthropogenic mechanisms. Natural mechanisms include the weathering of minerals, as well as the deposition of sea-salt aerosols (SAA) on soils in coastal regions (Soares *et al.,* 2016). Many anthropogenic activities such as land clearing and improper agricultural practices can cause soil salinization (Rietz and Haynes, 2003: Schmer *et al.,* 2012: Seilspepour *et al.,* 2009). Other anthropogenic activities such as road salt application, industrial spills, and the landfilling of industrial waste materials such as cement kiln dust (CKD) can also result in cases of soil salinity (McSorely *et al.,* 2016a & b). Soil salinization can also occur in crude oil extraction, as the main waste by volume in oil extraction is groundwater brine. This groundwater brine can contain many undissolved solids and large quantities of salts, such as sodium chloride (NaCl) (Carty *et al*., 1997).

Crude oil extraction also contaminates soils with organic compounds known as petroleum hydrocarbons (PHCs). Petroleum hydrocarbons are a complex mix of thousands of chemicals some of which are carcinogenic and classified as persistent organic pollutants (POPs) (Kamath *et al.,* 2004; Kirchmann and Wasiyhun, 1998). Individual PHCs can have various effects on soils, but in general, they coat soil particles and decrease the level of oxygen (O_2) available in the soil. This in turn can have a negative effect on the well-being of marine and land ecosystems, as well as human health (Kirchmann and Wasiyhun, 1998).

Remediating soils contaminated with a mix of salts and PHCs is challenging because of the multifaceted physical and chemical effects that exist between the contaminants and soil (Cook *et al.,* 2002). Conventional soil remediation methods for these pollutants include the application of chemical amendments, soil washing/leaching, excavation, and disposal. These methods require frequent application and monitoring which can increase the costs associate with remediating a site. A passive, green, and low-cost remediation technology that can remediate both salts and petroleum hydrocarbons is phytoremediation (Carty *et al.,* 1997). Phytoremediation is an ecological engineering technique that uses plant-based mechanisms to remediate contaminated soil, sediment, and/or water (Anjum *et al*., 2012: Raskin *et al.,* 1997). Some plants can degrade PHCs in their root zone through a process known as rhizodegradation (Gkorezis *et al.,* 2016). The rhizosphere is the most active biological zone of the soil extends 1-5 mm away from the roots and can contain many microorganisms that consume hydrocarbons as vital energy (Das and Chandran, 2011). Grasses are effective for the rhizodegradation of PHCs due to their complex root systems that provide many microorganisms with nutritious root exudates to facilitate the breakdown of the PHCs in soil (ibid).

Halophytes are salt tolerant plants that possess various mechanisms that allow them to survive the stresses imposed by saline environments (Flowers *et al*., 1986). Halophytes have previously been used for the phytoremediation of salinized soils (Leake *et al.,* 2002; Lymbery *et al.,* 2013; Yuan *et al*., 2016). The majority of salt impacted soils exist in arid climates such as India, Africa, the Middle East, and Australia (Ding *et al.,* 2010) and limited research has been conducted on the phytoremediation of salinized soils in colder climates such as Canada (McSorely *et al.,* 2016a & b: Yun *et al.,* 2019). Three mechanisms exist whereby halophytes are able to survive in salt impacted environments; exclusion, accumulation, and excretion (Flowers and Colmer, 2015). In the third mechanism, plants excrete excess salts in a saline solution through specialized salt glands on their leaf surfaces. These salt excreting plants are referred to as 'recretohalophytes' (Ding *et al.,* 2010).

Yensen and Biel (2008) suggested that the salts excreted on the leaves and stems of recretohalophytes can be dispersed by the wind and hence effectively dilute salt levels over a large area. This process of diluting high salt concentrations by recretohalophytes is referred to as 'haloconduction' (Yensen and Biel, 2008). The first step in this process is the movement of salts from the soil to the leaf surfaces. This determines how much salt is available for dispersal. The amount of excreted salt is determined by a number of factors such as species level differences of uptake and excretion, solute concentration, and time of year (Flowers and Colmer, 2015).

McSorely *et al.,* (2016a &b) and Yun *et al.,* (subm) demonstrated that halophytes can be used to re-vegetate and phytoextract Cl^- from a cement kiln dust (CKD)-contaminated site in Canada. CKD is highly saline due to large amounts of potassium chloride (KCl) in this waste product of the cement manufacturing industry. McSorely *et al.,* (2016b) highlighted the need for careful selection of halophytic species for phytoextraction as ions of concern, plant biomass, and salt tolerance mechanisms can all impact phytoextraction efficiency and consequently long-term remediation success. They found that *Spartina pectinata* (a recretohalophyte) was an efficient species for Cl^- extraction, as it does not require harvesting at the end of the growing season. Yun *et al.,* (subm) researched the theory of haloconduction at ground level by collecting the excreted salts from established field plots of *S. pectinata* and another native recretohalophyte, *Distichlis spicata* using cheesecloth mounts at varying distances. Although the use of vascular plants (phytoremediation) has been applied to PHCs and salts individually, to date, no one has researched the use of recretohalophytes to simultaneously remediate these two contaminants.

Following this brief introduction, chapter two is a comprehensive literature review of; i) PHC contamination, ii) soil salinity, iii) the effects of these salt and PHCs on plants, iv) phytotechnologies using halophytes, v) the theory of haloconduction, vi) as well as previous studies of recretohalophyte salt collection research. Chapter three details an experiment conducted to investigate for the first time, the ability of *D. spicata* and *S. pectinata* to simultaneously remediate PHCs and salt. In chapter four, the excreted salt particles on the leaf surfaces of *D. spicat*a and *S. pectinata* were investigated and characterized using environmental scanning electron microscopy (ESEM), focusing on the relative sizes and densities of salt particles. In chapter five, the design and construction of a Plexiglass wind tunnel, to carry out salt dispersal experiments in a controlled setting, is described. Following significant method development, a series of experiments were conducted using the constructed wind tunnel. Finally, a summary of the major findings and conclusions from this thesis research is discussed in chapter 6. Raw data and quality assurance/control are included in the Appendices A-C. This Master's of Science (MSc) thesis contributes to the theory of haloconduction and is the first study to: i) consider the simultaneous remediation of PHCs and salts using recretohalophytes, ii) characterize the excreted salt particles of *S. pectinata* and *D. spicata*, and iii) mobilize and collect the excreted salts of *S. pectinata* and *D. spicata* in a controlled laboratory setting.

2 LITERATURE REVIEW

2.1 COMMON SOIL CONTAMINANTS

2.1.1 Common Environmental Contaminants

Two contaminants that are often found at oil and gas extraction sites are hydrocarbons and salts, which are released into the environment by the spillage of crude oil and produced groundwater brine, respectively (Arocena and Rutherford, 2005; Carty *et al.,* 1997). Groundwater brine can contain many undissolved solids and high levels of salts like sodium chloride (NaCl). Contaminants like crude oil and salts negatively affect soils and the organisms living in them (Atlas, 1981; Carty *et al.,* 1997; Cook *et al.,* 2002). To understand how these two contaminants (crude oil and salts) will disperse and affect the natural environment, it is important to understand each contaminant separately and how they interact with soils.

2.2 PETROLEUM HYDROCARBONS IN SOIL

2.2.1 Petroleum Hydrocarbon Contamination

Crude oil contains contaminants known as petroleum hydrocarbons (PHCs) (Gkorezis *et al.,* 2016). PHCs can contaminate soil and water during the exploration stages of industrial crude oil drilling as well as in the refining and transporting of petroleum products (Qixing *et* al., 2011). PHCs can also infiltrate the soil profile, often from leaking pipelines, tanks, and transfer lines during the transportation of oil, gas, and petroleum products. These contaminants are a combination of thousands of organic compounds (comprised of carbon and hydrogen atoms) arranged in varying structural configurations with diverse physical and chemical characteristics (Gkorezis *et al*., 2016: Kirchmann and Wasiyhun, 1998). PHCs usually consist of alkanes (linear or branched), cycloalkanes, aromatic hydrocarbons, or more complex chemicals like asphaltenes (Qixing *et al.,* 2011). Some PHCs are also persistent organic pollutants (POPs), which can accumulate in soil and water and in so doing affect the well-being of marine and land ecosystems, as well as human health (Kirchmann and Wasiyhun, 1998). There are hundreds of chemical compounds in crude oil and petroleum products, thus it is difficult and not practical to measure each compound separately (Kamath *et al.,* 2004). Lighter end compounds tend to be more mobile due to greater solubility, greater volatility, and lower organic partitioning coefficients (Gkorezis *et al.,* 2016). Larger PHCs affect the physical structure of soil by coating soil aggregates and bind to soil components due to their hydrophobic nature and thus are difficult to remove or degrade (Das and Chandran, 2011).

2.2.2 Total Petroleum Hydrocarbon (TPH) and Fractions

The Canadian Council of the Ministers for the Environment (CCME) defines PHCs into four fractions (Fraction 1-4). Each fraction contains many individual chemicals, but the length of their longest linear hydrocarbon chain determines their designation to one of the four fractions. Fraction 1 (F1) PHCs are the smaller and more volatile hydrocarbons ($nC_{6\text{-}10}$). Fraction 2 (F2) have slightly longer hydrocarbon chains ($nC_{10\text{-}16}$). Fractions 3 ($nC_{16\text{-}34}$), and 4 ($nC_{34\text{-}50}$) are longer chain hydrocarbons that are more difficult to degrade in soil, and are thus found more commonly in weathered soil (Phillips *et al*., 1996). Total petroleum

hydrocarbons (TPH) is a term used to quantify the sum of the fractions found in PHC-impacted soil (Kamath *et al.,* 2004).

2.2.2.1 Gasoline Range Organics (GROS)

GROs (nC_{6-12}) are the most volatile PHCs which include short chain alkanes and mono-aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX compounds) (Figure 2-1) (Kamath *et al.,* 2004: Weishaar *et al.,* 2009). Benzene is acutely toxic and a recognized mutagen and carcinogen which can bioconcentrate and bioaccumulate in the food chain (Kamath *et al*., 2004: Weishaar *et al.,* 2009). The remaining compounds (toluene, ethylbenzene, and xylenes) are common solvents and are not recognized as carcinogenic, but are considered volatile organic compounds (VOCs). Most GROs are detectable within the CCME PHC Fraction 1.

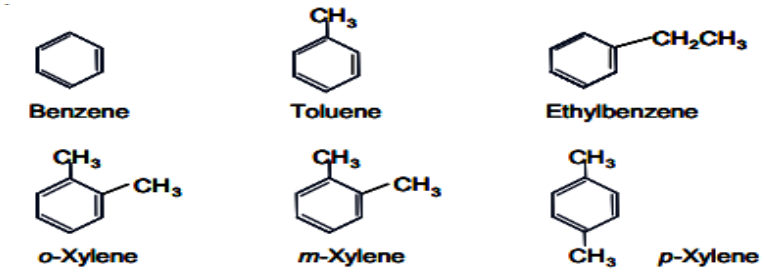

Figure 2-1 Examples of common Gasoline Range Organics (GROs) also known as the BTEX compounds; Benzene, Toluene, Ethylbenzene, and Xylenes (adapted from Kamath *et al*., 2004).

2.2.2.2 Diesel Range Organics (DROs)

DROs include longer chain alkanes (nC_{10-28}) and hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAHs) (Heitkamp *et al.,* 1989; Hunt *et al.,* 2018; Kamath *et al*., 2004). PAHs are organic compounds containing only carbon and hydrogen that can be found in crude oil and tar deposits (Kamath *et al*., 2004) (Figure 2-2). PAH compounds have a higher toxicity and are found in the air, water, and soil, and can persist in the environment for months or years. Some of the more common PAHs are benzo(a)pyrene, phenanthrene, and naphthalene. Multiple ring PAHs can disperse widely while dissolved in water, and have a strong affinity for organic carbon, and settle in organic sediments in rivers, lakes, and other watersheds (Kanaly *et al.,* 1997). Diesel fuel itself mainly consists of alkanes and aromatics with most n-alkanes in the nC_{13-20} range. DROs are detectable mostly within the CCME PHC Fraction 2 range (nC_{10-16}).

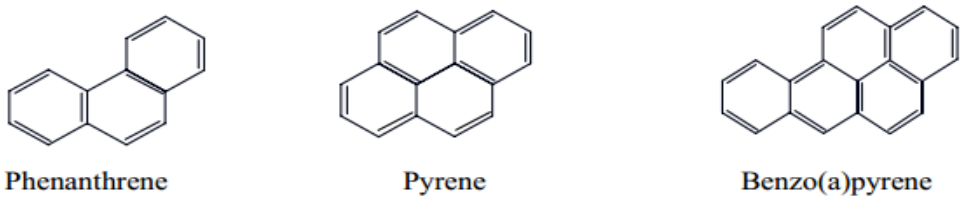

Figure 2-2 Examples of common Diesel Range Organics (DRO) known as polycyclicaromatic hydrocarbons (PAHs) (adapted from Kamath *et al*., 2004).

2.2.2.3 Fuel and Lubricating Oil Range Organics (ORO)

Fuel oils and lubricating oils (nC_{20-50}) have stronger intermolecular forces, higher boiling points, and are more hydrophobic, all of which cause strong adsorption to soil particles. The fuel oil and lubricating oil fractions generally fall within the CCME PHC Fraction 3 (nC_{16-34}) and Fraction 4 (nC_{34-50}).

2.3 FATE OF PHCs IN THE ENVIRONMENT

Generally, the movement of PHCs in soil is dependent on physical factors like soil texture, moisture content, and the specific chemical properties of the individual PHCs (Atlas, 1981: Brady and Weil, 2013; Carty *et al.,* 1997; Gkorezis *et al.,* 2016). Once PHCs have infiltrated the soil, they move by gravitational and/or capillary forces and are transported with soil water just like other nutrients and contaminants (Brady and Weil, 2013). These hydrocarbons will either be distributed into aqueous solution or adsorbed to soil mineral surfaces (Schwarzenbach, 2003). The movement of PHCs in soil is also dependent on the chemical factors of the individual contaminant and soil conditions. The physical factors of soil play an important role in sorption, while the chemical properties of the PHC primarily determine the degradation processes (Kamath *et al.,* 2004).

2.3.1. Physical Factors

Physical factors that affect sorption and transport of PHCs in the soil profile include the specific compound solubility, as well as soil permeability, type, moisture, and temperature (Kanaly *et al.,* 1997: Kirchmann and Waysiyhun, 1998: Carty *et al.,* 1997).

2.3.1.1 Solubility of the Compounds

At very low concentrations, some PHCs are soluble in water but most spills occur in quantities that exceed the PHC solubility capacity of water (Atlas, 1981). When PHC concentrations are high (>4-5% by weight), infiltration and retention of water are reduced (Carty *et al.,* 1997). In saturated conditions, the polar surface of the clay particles of soil create relatively strong dipole interactions with water molecules, and organic compounds must compete with water for binding sites to the clays (Schwarzebach, 2003). Smaller hydrophilic PHCs (F1s) are degraded more easily, whereas the more hydrophobic compounds in the CCME PHC fraction 4 (F4s) and PAHs adsorb more strongly to the organic matter in the soil (Atlas, 1981: Carty

et al., 1997). At weathered sites, the residual oil contamination normally consists of non-ionic, non-volatile, and largely non-polar hydrocarbons (ibid).

2.3.1.2 Soil Moisture and Temperature

Theoretically, all hydrocarbons can be biodegraded over time in soil or aqueous solution, but the amount of time is dependent on multiple factors (Kamath *et al.,* 2004: Kanaly *et al.,* 1997). Microorganisms can decrease hydrocarbons over time in a process known as biodegradation. Soil moisture is essential to biodegradation as the majority of microorganisms live in the water film surrounding soil particles (Schwarzenbach *et al.*, 2003). Soil moisture content in the range of 50-80% is optimal for biodegradation. Higher soil temperatures result in higher microbial metabolic activity, and thus higher rates of biodegradation. Biodegradation of organic compounds generally ceases at 0 °C with optimal biodegradation rates reported in the range of 20 to 25 °C (Carty *et al.,* 1997).

2.3.1.3 Soil Type

Soil texture largely determines how fast and how far PHCs will migrate into a soil profile (Cook *et al.,* 2002). Petroleum hydrocarbons disperse widely in soils with larger pore spaces such as sand and gravel. The soil type also impacts the degree of adsorption of contaminants and nutrients, and all of these factors vary on a site-to-site basis (Brady and Weil, 2013; Schwarzenbach *et al.,* 2003). The permeability of PHCs through the soil profile is impacted by the soil organic matter content (Wild *et al.,* 2005). Increased organic matter content in soil promotes stronger adsorption of larger PHCs in the CCME PHC Fraction 4 and many PAHs, which reduces their abundance in the aqueous phase for transport. With fewer PHCs being transported in the soil water there is a decrease in their bioavailability for degradation by microorganisms (Das and Chandran, 2011).

2.2.3 Chemical Factors

Two important chemical factors in the soil that affect microorganism health, and thus the rate of biodegradation, are oxygen and soil pH (Kanaly *et al.,* 1997; Kirchmann and Wasiyhun, 1998).

2.2.3.1 Oxygen

The rate of aerobic biodegradation is typically limited by of the amount of oxygen that can penetrate the soil. A large fraction of the microbial population within soil depends on oxygen as the terminal electron acceptor in metabolism (cellular respiration) (Schwarzenbach *et al.,* 2003).

2.2.3.2 Soil pH

Soil pH is an indicator of hydrogen ion activity in the soil. A pH in the range of 5 to 9 is generally acceptable for biodegradation, with a pH of 6.5-8.5 considered optimal for biodegradation efficiency. Soil pH also affects the availability of nutrients as the solubility of phosphorous, an important nutrient in biological systems, is maximized at a pH value of 6-7. Crude oil contamination increases the soil pH and reduces the

available phosphorus for use by microorganisms involved in biodegradation (Kanaly *et al.,* 1997; Kirchmann and Wasiyhun, 1998; Schwarzenbach *et al.,* 2003).

2.4 PHC EFFECTS ON PLANTS

2.4.1 Physiological Response of Plants to PHCs

Petroleum spills can cause damage to plants by impeding important soil functions, which in turn disrupt certain plant functions (Kirchmann and Wasiyhun, 1998). At higher levels, PHC contamination at oilfield sites is often sufficient to impede the growth of plants, prevent seed germination, and upset the balance of soil microbial communities (Carty *et al.,* 1997: Gkorezis *et al.,* 2016: Hunt *et al.,* 2018). For example, the effective porosity of soil can be reduced by PHC residues surrounding soil particles. This subsequently limits air and water movement to plant roots (Carty *et al.,* 1997).

The initial physiological response of plants to environmental contaminants includes the uptake and subsequent translocation and accumulation of the contaminant in the plant organs (roots and shoots) (Wild *et al.,* 2005; Gkorezis *et al.,* 2016). The rates of these processes are dependent on the individual compound's concentration, lipophilicity, solubility, and volatility (Gkorezis *et al.,* 2016; Hunt *et al.,* 2018; Wild *et al.,* 2005). In order for a PHC to be taken up into the plant, it must first penetrate a number of plant tissues including the cuticle and the cell wall (Hunt *et al.,* 2018). The uptake and translocation pathways for PHCs in plants are not yet fully understood, but there is expected to be limited PHC uptake by plants.

2.4.2 Issues in Determining PHC Fate in Plants

The CCME has developed remedial guidelines and a risk assessment framework for both ecological and human exposure to PHCs (Hunt *et al.,* 2018). One of the assumptions used in the derivation of these guidelines is that plants are unable to take up PHCs from contaminated soil and therefore subsequent exposure at higher trophic levels in not a concern (Hunt *et al.,* 2018). The time between the plants being initially exposed to PHCs and then measured in plant tissues, may cause PHCs in the plant to be reduced below detectable levels by degradation within the plant. Once inside a plant, hydrocarbons, particularly n-alkanes (C_{10-22}) can be degraded rapidly in plant tissues. The PHCs are initially oxidized to the corresponding alcohol, then to fatty acids, and eventually to CO_2 and H_2O (Balasubramaniyam, 2015: Hunt *et al.,* 2018).

Currently, there is no standardized method specific for plant tissue PHC analysis. The CCME states that the preferred method to recover PHCs from soils for analysis is solvent extraction with acetone/hexane, followed by quantification via gas chromatography with a flame ionization detector (GC-FID). Although the CCME method was developed for soil PHC analysis, some studies have adopted the GC-FID method to analyze PHCs in plant tissues (Hunt *et al.,* 2018).

2.5 REMEDIATION OF PETROLEUM HYDROCARBONS

There are many methods for remediating PHC affected soils including, but not limited to, excavation and disposal, chemical treatments, and bioremediation (Cook *et al.,* 2002; Schwab *et al.,* 1999). Excavation and disposal is a common remediation approach, but normally no single technology can remediate an entire site efficiently and as such the remediation of PHC-impacted sites often requires a multidisciplinary approach to reach acceptable levels. The CCME provides criteria for acceptable TPH and fraction levels for different soil types and land uses (Table 2-1). Furthermore, the exposure pathway and the specific compounds (F2-F4) are important components in determining acceptable levels at various sites (CCME, 2008).

Table 2-1 Canadian Council of the Ministers for the Environment (CCME, 2008) petroleum hydrocarbon guidelines for various land uses, exposure pathways, and soils. All values are reported in mg/kg unless otherwise stated.

		FINE-GRAINED SOILS				COARSE-GRAINED SOILS			
Land Use	**Exposure Pathway**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
Agricultural	Direct Contact w/ Soil (DC)	12000	6800	15000	21000	12000	6800	15000	21000
	Vapour Inhalation - basement (VI)	710	3600	NA	NA	40	190	NA	NA
	Vapour Inhalation - slab-on-grade (VI)	610	3100	NA	NA	30	150	NA	NA
	Ecological Soil Contact (ESC)	210	150	1300	5600	210	150	300	2800
	Protection of Potable GW (GW-P)	170	230	NA	NA	240	320	NA	NA
	Protection of GW for Aquatic Life (GW-A)	RES	RES	NA	NA	970	380	NA	NA
	Protection of GW for Livestock (GW-L)	4200	10000	NA	NA	5300	14000	NA	NA
	Management Level	800	1000	3500	10000	700	1000	2500	10000
	Governing Objective	***170***	***150***	***1300***	***5600***	***30***	***150***	***300***	***2800***
	Governing Pathway	***GW-P***	***ESC***	***ESC***	***ESC***	***VI***	***VI***	***ESC***	***ESC***
Residential	Direct Contact w/ Soil (DC)	12000	6800	15000	21000	12000	6800	15000	21000
	Vapour Inhalation - basement (VI)	710	3600	NA	NA	40	190	NA	NA
	Vapour Inhalation - slab-on-grade (VI)	610	3100	NA	NA	30	150	NA	NA
	Ecological Soil Contact (ESC)	210	150	1300	5600	210	150	300	2800
	Protection of Potable GW (GW-P)	170	230	NA	NA	240	320	NA	NA
	Protection of GW for Aquatic Life (GW-A)	RES	RES	NA	NA	970	380	NA	NA
	Management Level	800	1000	3500	10000	700	1000	2500	10000

	Governing Objective	***170***	***150***	***1300***	***5600***	***30***	***150***	***300***	***2800***
	Governing Pathway	***GW-P***	***ESC***	***ESC***	***ESC***	***VI***	***VI***	***ESC***	***ESC***
Commercial	Direct Contact w/ Soil (DC)	19000	10000	23000	RES	19000	10000	23000	RES
	Vapour Inhalation (VI)	4600	23000	NA	NA	320	1700	NA	NA
	Ecological Soil Contact (ESC)	320	260	2500	6600	320	260	1700	3300
	Protection of Potable GW (GW-P)	170	230	NA	NA	240	320	NA	NA
	Protection of GW for Aquatic Life (GW-A)	RES	RES	NA	NA	970	380	NA	NA
	Offsite Migration (OM)	NA	NA	19000	RES	NA	NA	4300	RES
	Management Level	800	1000	5000	10000	700	1000	3500	10000
	Governing Objective	***170***	***230***	***2500***	***6600***	***240***	***260***	***1700***	***3300***
	Governing Pathway	***GW-P***	***GW-P***	***ESC***	***ESC***	***GW-P***	***ESC***	***ESC***	***ESC***
Industrial	Direct Contact w/ Soil (DC)	RES	RES	RES	RES	RES	RES	RES	RES
	Vapour Inhalation (VI)	4600	23000	NA	NA	320	1700	NA	NA
	Ecological Soil Contact (ESC)	320	260	2500	6600	320	260	1700	3300
	Protection of Potable GW (GW-P)	170	230	NA	NA	240	320	NA	NA
	Protection of GW for Aquatic Life (GW-A)	RES	RES	NA	NA	970	380	NA	NA
	Offsite Migration (OM)	NA	NA	19000	RES	NA	NA	4300	RES
	Management Level	800	1000	5000	10000	700	1000	3500	10000
	Governing Objective	***170***	***230***	***2500***	***6600***	***240***	***260***	***1700***	***3300***
	Governing Pathway	***GW-P***	***GW-P***	***ESC***	***ESC***	***GW-P***	***ESC***	***ESC***	***ESC***

2.5.1 Excavation and Disposal

This process, also known as dig and dump, involves digging out contaminated soil from the affected site and taking it to a landfill where it is not considered a hazard to human and ecological health. Alternatively, the soil can be taken to a treatment facility where it could potentially be treated and/or incinerated. The main disadvantages of this remediation method are the high costs of transporting the contaminated soil to its final destination and the fact that the soil is moved, but not necessarily remediated (Gkorezis *et al.,* 2016; Hunt *et al.,* 2018; Kirchmann *et al.,* 1998).

2.5.2 Bioremediation

Bioremediation is becoming a more common remediation technique due to its versatility and relatively low cost (Das and Chandran, 2011; Kanaly *et al.,* 1997; Kirchmann and Wasiyhun, 1998). Many microorganisms can transform PHCs to non-hazardous compounds. When conducting *in situ* bioremediation at a field site, certain conditions may be modified to ensure optimal bacterial growth, soil moisture, pH, and mineral nutrients (Atlas *et al.,* 1981, Carty *et al.,* 1997).

2.5.2.1 Microorganisms and Enzymes

Microorganisms use PHCs as an energy source, by degrading the compounds into smaller parts with the help of enzymes (Kanaly *et al.,* 1997). Without these enzymes, natural chemical degradation can take years to occur because the activation energy necessary to trigger the redox reaction is too great (Gkorezis *et al.,* 2016). Enzymes accelerate redox reactions by lowering the required activation energy (Das and Chandran, 2011). The organic matter content of soil is often proportional to the abundance of microorganisms and enzymes available to biodegrade the PHCs (Carty *et al.,* 1997: Kanaly *et al.,* 1997).

2.5.2.2 Electron acceptors

Biodegradation is often described as an electron transfer process (Atlas, 1981; Das and Chandran, 2011; Kanaly *et al.,* 1997). When PHCs are degraded, electrons are released and taken up by electron acceptors that differ depending on whether it is aerobic or anaerobic conditions. The most important electron acceptor is oxygen, which works under aerobic conditions. Nitrate, sulphate and Iron (III) are some of the electron acceptors working under anaerobic conditions. In the biological degradation process of alkanes (compounds found in PHCs), first the terminal methyl group of the alkane is oxidized, the resulting alcohol is oxidized to the corresponding aldehyde, and further to carboxylic acid (Atlas, 1981; Das and Chandran, 2011; Kanaly *et al.,* 1997).

2.6 SOIL SALINITY

2.6.1 Classification and Guidelines

Soil salinization can occur due to many different natural and anthropogenic factors. Natural factors include the weathering of high salt content minerals, airborne salt depositions from wind and rain, in addition to the movement of salts through the ground water table to surface soil (Barrett-Lennard, 2002; Brady and Weil, 2013). Anthropogenic factors include road salt application, industrial spills, poor irrigation practices, land clearing, and improper landfilling of industrial waste material (Rietz and Haynes, 2003; Schmer *et al.,* 2012; Seilspepour *et al.,* 2009).

Salt buildup can result in three types of soils classified as: i) saline, ii) saline-sodic, and iii) sodic (Rietz and Haynes, 2003). Sodic soils have a high sodium (Na^+) content that negatively affects seed germination (ibid). The classification of saline or sodic is determined through many methods including analyzing the soil pH, soil electrical conductivity (EC), the exchangeable sodium percentage (ESP), and sodium adsorption ratio (SAR) (Rietz and Haynes 2003: Seilsepour *et al*., 2009). The most commonly accepted and easiest method for measuring soil pH in saline and sodic soils is analysis of the saturated paste pH, followed by analysis of saturation paste extract pH. Saline soils often have a pH of >8 (Mavi *et al.,* 2012).

Electrical conductivity (EC) is one of the most popular salinity measurements methods available due to its simplicity and low cost (Lundmark and Olofsson, 2007). EC is the ability of a material to conduct an electrical current and is expressed in units of miliSiemens per meter (mS/m) or deciSiemens per meter (dS/m). There are many methods for measuring soil EC however, the standard procedure is to analyze the saturated soil paste extract (EC_e). In this method, soil samples are saturated, equilibrated 2 hours, and extracted by vacuum through filter paper.

The exchangeable sodium percentage (ESP) is a measure of the relative amount of sodium ions present on the soil surface, expressed as a percentage of the total cation exchange capacity (CEC). Soil CEC are often determined using laborious and time-consuming methods and as a result new methods have been developed for a more economical and simple soil salinity index (Seilsepour *et al*., 2009). The sodium adsorption ratio (SAR) concept was developed as an alternative approximation to ESP measurement when used for soil extracts, and to predict soil ESP equilibrium from irrigation water quality (Seilsepour *et al*., 2009).

SAR is an adjusted ratio of sodium ions (Na^+) relative to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions. It is a measure of the saturated soil solution and not the soil itself, and represented by the following equation:

Equation 2-1 $$SAR = [Na^+]/(0.5[Ca^{2+}] + 0.5[Mg^{2+}])^{1/2}$$

Saline soils are generally defined as having a high EC_e and a low SAR whereas saline-sodic soils have both a high EC_e and SAR (Table 2-2). Sodic soils have a low EC_e and high SAR due to the dominance of sodium

on the exchange complex, and are considered the most extreme cases of soil salinization (Seilsepour *et al.,* 2009).

Table 2-2 Classification of soil salinity and accompanying MOE guidelines for EC_e and SAR (MOE, 2011).

Classification	**ECe (dS/m)**	**SAR**	**pH**
Saline	>4	<13	<8.5
Saline-Sodic	>4	>13	<8.5
Sodic	<4	>13	>8.5
MOE Guideline			
Background (upper limit)	0.57	2.4	-----
Site Condition Standard	1.4	12	-----
Site Condition Standard within 30 m of water	0.7	5	-----

2.6.2. Soil Chloride

Chlorine occurs in aqueous solution as the monovalent chloride anion (Cl^-), and is typically the dominant anion of concern in salinized soil (Tavakkoli *et al*., 2010). In the literature, many studies on soil salinity have focused on Cl^- because of its simplicity, its non-reactive nature, and because it is easily soluble and transported throughout the soil profile (Lundmark and Olofsson, 2007). Chloride concentration in the soil is more important in reducing growth and yield in many plant species, especially agricultural crops, than other ions associated with salinity such as such as Na^+ and K^+ (Tavakkoli *et* al., 2010). Excess Cl^- can be toxic to plants at high concentrations, with critical concentrations for toxicity estimated to be 4–7 mg/g for Cl^- sensitive species and 15–50 mg/g for Cl^- tolerant species (Tavakkoli *et al*., 2010; White and Broadley, 2001). Chloride is also an essential micronutrient that does not have toxic effects in plants at low concentrations (Hawkesford *et al.,* 2012). In plants, Cl^- helps regulate enzyme activities in the cytoplasm, is a co-factor in photosynthesis, acts as a counter anion to stabilize membrane potential, and is involved in turgor and pH regulation (Tavakkoli *et al*., 2010: White and Broadley, 2001). Water fluxes determine the movement of Cl^- in the soil profile (White and Broadley, 2001). In field conditions, when evapotranspiration exceeds rainfall, there is an upward movement of Cl^- in the soil profile. Heavy rainfall saturates soil aggregate pore spaces with water, such that less evapotranspiration occurs and there is a downward movement of Cl^- in the soil profile (ibid). Physical irregularities in soil structure such as the presence of aggregates or cracks also affect Cl^- movement (Barrett-Lennard, 2002).

2.6.3 Factors Affecting the Severity of Soil Salinity

2.6.3.1 Relative Presence of Cations

Positively charged cations are attracted and adsorbed to the negatively charged functional groups of clay particles and organic colloids in the soil matrix (Sparks, 2003). Soil colloids are the most active portion of

the soil and physical and chemical properties of soils (Brady and Weil, 2013). The strength of adsorption between a cation and colloid is dependent on the charge of the ion and its hydration state and follows to order of.

$$Al^{3+} > Sr^{2+} > Ca^{2+} > Mg^{2+} > Cs^{+} > K^{+} > Na^{+} > H^{+}$$

Sodic soils have a high SAR and thus high concentrations of Na^{+}. Sodium ions are large which results in soil dispersion, and during this dispersion, the soil particles swell and expand causing soil pores to become narrow (Sparks, 2003). Saline soils have high concentrations of Ca^{2+}, which are larger than Na^{+} and thus adsorb to soil particles more strongly (Brady and Weil, 2013). These larger Ca^{2+} ions create larger pore spaces which promote soil particle aggregation and flocculation (groupings of particle aggregates) (Brady and Weil, 2013). As the SAR increases, the rate of flocculation increases and the soil aggregate structure deteriorates (McBride, 1994). The limits or eliminates the movement of air and water through the soil profile and can inhibit the ability of plant roots to penetrate the soil (Eckhard *et al*., 2012).

2.6.3.2 Presence of Calcium Carbonate and Effects on Soil pH

The pH of calcareous soils (7.5-8.5) is determined by the presence of $CaCO_3$ (pH of the soil solution increases with $CaCO_3$), which plays an important role in the carbonate-bicarbonate buffer system (McBride, 1994). The pH of the soil solution not only affects the solubility of nutrients in soils, but it also affects the ability of plants to take up nutrients. Anion uptake is inhibited when the pH of the external medium increases (Eckhard *et al*., 2012). The CO_2 in soils results in soil alkalinity accumulating in the form of carbonate (CO_3^{2-}) or bicarbonate (HCO_3^{-}) salts, which are available for the Na^{+} ions in sodic soils to form highly soluble compounds (McBride, 1994). As the concentration of CO_3^{2-} and HCO_3^{-} increases, $CaCO_3$ precipitates and the pH rises to the limit of solubility for the sodium carbonates. The Na^{+} (as well as K^{+}) carbonates dissolve readily in water to form solutions with very high pH (McBride, 1994). The concentration of free calcium carbonate ($CaCO_3$) in the upper soil horizon varies from a few percent to 95% (Eckhard *et al.,* 2012).

2.6.3.3 Waterlogging

Waterlogged soils have excessive water and thus low oxygen levels. Total depletion of oxygen (hypoxia) and absence of oxygen (anoxia) can occur when oxygen is no longer available as a terminal electron acceptor (Greenway *et al.*, 2006). When free oxygen is no longer available, the microorganisms start using terminal electron receptors other than O_2. This causes a sequence of redox reactions that take place as follows: nitrate (NO_3^{-}) reduction to nitrite (NO_2^{-}) (denitrification); manganese oxides (mainly Mn^{4+}) reduction to manganese (Mn^{2+}); iron (III) (Fe^{3+}) reduction to iron (II) (Fe^{2+}); sulphate (SO_4^{2-}) reduction to (H_2S); and carbon dioxide (CO_2) reduction to methane (CH_4) (Chapin *et al*., 2002). Low oxygen concentrations are often accompanied by high CO_2 concentrations (Greenway *et al.*, 2006) and so this may lead to a decrease in soil pH.

Waterlogging results in plant-stress which affects growth, productivity, and species distribution (Eckhard *et al*., 2012). The severity of negative effects depends on the plant species and its developmental stage, as well as soil properties and soil temperature. Aerobic respiration and ATP-dependent biosynthetic processes are decreased to save oxygen, and are eventually replaced by anaerobic respiration, which is a less efficient form of metabolism that reduces ATP formation, decreases cytosolic pH, leads to an increase in toxic fermentation products, and inhibits nutrient uptake. Under saline conditions, waterlogging causes increased sodium and chloride shoot concentrations and rapid leaf senescence (Barrett-Lennard, 2002). At low O_2 concentrations, the selectivity of K^+/Na^+ uptake by roots decreases in favour of Na^+, which reduces the transport of K^+ to the shoots, enhancing Na^+ transport to the shoots (Smethurst *et al*., 2005). The Na^+, as well as Cl^- concentrations in plant tissues also increases with an increase in temperature (West and Taylor, 1980).

2.6.4 Cement Kiln Dust

Cement kiln dust (CKD) is a waste by-product of the cement manufacturing process and is made up of fine, dry particulate matter (Kunal *et al.,* 2012). It is a stable, non-combustible, non-explosive, highly alkaline, solid powder (McSorely *et al.,* 2016a & b: Yun *et al.,* 2019). CKD is primarily comprised of calcite ($CaCo_3$), potassium sulphate (K_2SO_4), calcium sulphate ($CaSO_4$), quartz (SiO_2), aluminum oxide (Al_2O_3), iron oxide (Fe_2O_3), magnesium oxide (MgO), and potassium chloride (KCl) (Golder Associates, 2013). In accordance with the Cement Sustainability Manufacturing Program, 26 North American based companies have adopted a 60% voluntary target to reduce landfilling of CKD (from a 1990 baseline) by the year 2020 (Adaska and Taubert, 2008). Although there have been efforts to sustainably produce and manage CKD, considerable amounts are still landfilled which poses a risk to the environment. To date, standards and regulations for how CKD should be managed are still under review. At sites where CKD is landfilled, chloride is often the ion of concern as Cl^- is highly mobile and has the potential to impact ground and surface waters beyond contaminated area boundaries (McSorely *et al.,* 2016 a &b: White and Broadley, 2001).

2.7 SALINITY TOLERANCE IN PLANTS

2.7.1 Effects of Plant Growth

Most plants do not have adaptations to cope with salinity stress (Gupta and Huang, 2014). In the first stages of salinity stress, plant growth and metabolism are suppressed (Briskin and Bloom, 2010). Pores on the leaf surface (stomata) are responsible for gas exchange, and during salinity stress their activity is reduced (Gupta and Huang, 2014). This results in a reduction in plant photosynthetic activities which suppresses overall plant growth. As salinity stress continues, injury to the foliage of the plant can occur in the form of chlorosis and necrosis (Briskin and Bloom, 2010). Salinity stress can also have adverse impacts on plant reproduction and development by delaying or preventing the germination of seeds (Brady and Weil, 2013; Eckhard *et al*., 2012; Munns and Tester, 2008).

2.7.2 Osmoregulation

Water crosses the plant membranes by osmosis (Eckhard *et al.,* 2012). The influx of water into plant cells maintains the plant's turgor pressure (Gupta and Huang, 2014). In order to maintain water uptake through the roots to prevent wilting, plants must have an osmotic potential lower (more negative) than the surrounding soil (Brady and Weil, 2013). Plants can lower their internal potentials to maintain water uptake by: i) increasing the solute concentration in tissues by dehydration, and/or ii) increasing the solute concentration in tissues by the uptake and accumulation of solutes from the external water medium (Ceccoli *et al.,* 2015: Eckhard *et al.,* 2012). In salt-impacted soils, it is difficult for plants to take up water as the external solute concentration is high and the osmotic potential is low (Munns and Tester, 2008). This promotes the movement of water out of the plant resulting in plants having to use additional energy to make osmotic adjustments, and this creates osmotic stress (Eckhard *et al.,* 2012: Munns and Tester, 2008).

2.7.3 Ion Uptake and Transport: Sodium and Chloride

Sodium (Na^+) and Cl^- are usually the dominant ions in salt impacted soils and both are toxic to plants when accumulated in the cytoplasm at high concentrations (Eckhard *et al*., 2012). In salt impacted soils, Na^+ and Cl^- will accumulate in the leaf tissues over time which inhibits the plants' normal adaptations to acquire nutrients, and ultimately results in cytotoxicity (Briskin and Bloom, 2010). Higher concentrations of salt can lead to dehydration, causing protein denaturation and membrane destabilization (Ceccoli *et al.,* 2015). Solutes like Na^+ and Cl^- move from the soil into plant roots through the apoplasm and/or the symplasm (White and Broadley, 2001). The apoplastic pathway for ions is through the free space between root cells where ions are available for selective uptake into the cells. In salinized soils, the ions in the apoplast are restricted from entry into the xylem. The symplastic pathway allows ions to be taken up directly into the root cells through plasmodesmata. Plasmodesmata are channels that enable transport and communication directly between cells that can constrict or dilate to allow the passage of solutes. Ions are transported in this intracellular way to the xylem where they are distributed to the rest of the plant. These pathways may be used separately or simultaneously and have different transport rates. There are also channels for passive uptake that exist in the plasma membrane, which allow passive influx of Cl^- into plant cells occurring over short periods of time if the Cl^- levels in the substrate increase slightly, however, Na^+ is always transported through the plasma membrane passively (Eckhard *et al*, 2012; Teakle and Tyerman, 2010).

2.7.4 Other Factors Affecting Uptake and Regulation

2.7.4.1 Ion Competition:

2.7.4.1.1 Na^+/K^+

Sodium ions have similar physicochemical properties to those of potassium ions (K^+) and therefore compete with K^+ for their use in various plant functions. Mimicry sodium ions can bind to high-affinity potassium transporters and/or by travelling into the cytoplasm passively through non-selective cation channels (Brady and Weil, 2013; Eckhard *et al*., 2012). In addition to Na^+/K^+ competition, Na^+ displaces Ca^{2+}, reducing cytosolic Ca^{2+} concentrations responsible for activating Na^+ detoxification, thus blocking the mechanism

for its own detoxification (Radin *et al*., 2012). Potassium is an essential plant macronutrient involved in many plant physiological functions such as the maintenance of osmotic pressure and plant turgor, growth and photosynthesis, translocation of nutrients, stomatal opening, and the activation of many enzymatic reactions (Brady and Weil, 2013; Hawkesford *et al*., 2012). Furthermore, membrane depolarization is caused by the presence of Na^+ and can result in reduced levels of K^+ to be taken up by plants. This results in potassium deficiency in conjunction with cytotoxicity in the plant (Eckhard *et al*., 2012).

2.7.4.1.2 Cl^-/NO_3^-

Anion competition occurs between chloride (Cl^-) and nitrate (NO_3^-), an important source of nitrogen for plant life (Hawkesford *et al.,* 2012). Competition for transport across the tonoplast occurs, affecting (i) accumulation in vacuoles, (ii) cytoplasmic concentrations and uptake, and (iii) several anion channels and proton-coupled symporters in the plasma membranes of root cells (White and Broadley, 2001). In saline soils, this Cl^-/NO_3^- competition may also impair nitrogen uptake by plants (Gupta and Huang, 2014).

2.7.4.2 Charge Balance

Charge balance is critical as it directly relates to pH regulation in the plant (Rietz and Haynes, 2003). Ions are ultimately taken up from the soil by different transport proteins in order to maintain charge balance (White, 2012). Plants have a preferred cytosolic pH range of 7.3-7.6. Excessive cation uptake results in an increase in cytosolic pH whereas anion uptake decreases cytosolic pH. Plants maintain this pH by transporting protons across the plasma membrane or tonoplast and by forming and removing carboxylic acids, which consume protons (Rietz and Haynes, 2003: White, 2012).

2.7.4.3 Calcium

Calcium is a macronutrient in plants found largely in the apoplasm and plays a role in cell structure and is a regulatory component of macromolecules (Hawkesford *et al*., 2012). Calcium can be removed from its binding site in the cell membrane and exchanged with Na^+ in salt impacted soils, exacerbating the salinity toxicity in plants (White, 2012). In response to salinity and osmotic stress, cytosolic calcium concentrations quickly increase and are responsible for signaling cellular responses (Eckhard *et al.,* 2012). Rhizosphere Ca^{2+} concentration influences the selectivity of ion uptake and accumulation of K^+ and Na^+. In certain cases, extracellular Ca^{2+} has been show to inhibit Na^+ influx through voltage-insensitive cation-channels in favour of K^+ uptake, also limiting K^+ efflux, and therefore helps to maintain high K^+/Na^+ ratios in saline soils, and hence salt tolerance (Munns and Tester, 2008).

2.7.5 Indirect Effects of Salinity on Plants: Reduced Microbial Activity

Under saline conditions, the low osmotic potential of the soil solution affects the ability of microbes to access water (Mavi *et al.,* 2012). Salinity has been found to decrease the size and increase the stress on microbial communities (Rietz and Haynes, 2003; Setia *et al.,* 2011). A negative exponential relationship has been found to exist between EC and microbial biomass indicating that small increases in salinity can dramatically alter microbial communities (Rietz and Haynes, 2003). Reduced microbial activity can indirectly affect plant growth by decreasing nutrient cycling, organic matter degradation, and soil respiration (Mavi *et al.,* 2012; Setia *et al.,* 2011).

2.8 REMEDIATION OF SALINIZED SOILS

2.8.1 Chemical Amendments

Conventional remediation strategies include the addition of amendments and/or the application of low salinity water to leach/flush salts from the soil profile (Barrett-Lennard, 2002: Cook *et al.,* 2002). Chemical treatments can remove or exchange with the Na^+ and Cl^- ions in the soil. Calcium in the form of gypsum ($CaSO_4$) is the most common amendment used to correct saline-sodic or sodic soils because it provides a slightly more soluble source of calcium (Grattan and Grieve, 1998). Increasing calcium (Ca^{2+}) availability can enhance the leaching of ions such as Na^+ (Rietz and Haynes, 2003: Cook *et al*., 2002). Sulfuric acid (H_2SO_4) is also used as a chemical amendment because it is able to solubilize calcium carbonate (Grattan and Grieve, 1998).

2.8.2 Leaching Applications

Leaching can reduce the salt levels in soil with the application of low-salt water to the soil surface. The low-salt water will dissolve the surface salts, then move them through the soil profile to below the root zone (Brady and Weil, 2013). Leaching applications only work well on saline soils that have good structure and internal drainage. Salinized soil with poor drainage or low capacity for leaching exacerbates the salinity stress in plants (Sparks, 2003). Soils with poor drainage easily become waterlogged and water has difficulty moving downward through the soil profile and oxygen is no longer present as an electron acceptor for physiological plant functions (Lundmark and Olofsson, 2007). The drawback associated with these leaching and surfactant amendments is that they often require regular application.

2.9 REMEDIATION OF SOILS CONTAMINATED WITH PHCs AND SALTs

Remediating soil mixed with PHCs and salts is a particularly difficult challenge due to the complex interactions between the contaminants and soil (Carty *et al.,* 1997). The presence of excess salts such as NaCl can harm the microorganisms responsible for bioremediation (Gkorezis *et al.,* 2016). As well, the addition of salts to PHC contaminated soil may amplify the challenges involved in remediating PHC-affected soil by reducing the solubility and bioavailability of organic compounds for remediation (Schwarzenbach, 2003). Effective remediation strategies for soils co-contaminated with salts and PHCs are

integrated technologies that are typically applied to remediate each contaminant, such as soil washing. PHCs can be washed by separating fine grained soil containing the strongly adsorbed hydrocarbons in soil/water slurry (Gkorezis *et al.,* 2016). Salts can be flushed from the soil profile using low-salinity water. Additionally, there are many combinations of surfactants that can be applied to flush residual PHCs and salts from the soil depending on the site conditions. Often these washing and leaching remediation techniques simply move the contamination from the soil to the ground water below (Gkorezis *et al.,* 2016). Due to the high costs associated with the invasive removing, transporting, treating, and incinerating PHC-contaminated soils and the complications associated with various soil washing techniques, a passive, low-cost remediation technology would be more ideal. One remediation approach that has already been shown to work for both PHC-contaminated and salt-impacted soils individually is phytoremediation (Carty *et al.,* 1997, Das and Chandran, 2011: Gkorezis *et al.,* 2016).

2.9.1 Phytotechnologies

Phytotechnologies are a group of plant-based technologies where vascular plants are used to solve science and/or engineering problems. Phytoremediation is a specific phytotechnology that uses a vascular plant's natural ability to exclude, accumulate, immobilize, metabolize, or degrade contaminants present in soil, sediment, and/or water (Anjum *et al.,* 2012). In phytoextraction, a type of phytoremediation, contaminants are taken up by plants, and then translocated to above ground plant tissues where it is generally sequestered. The plant material is then harvested and transported to a facility where it can be treated (often incinerated or composted) (Glick and Stearns*,* 2011). Phytoremediation has been proven effective for the cleanup of large, lightly contaminated soils, sludges, and waters and is especially effective in areas where the contaminant is relatively shallow and the area is so large that traditional clean-up methods are not economically feasible. Although there are many benefits associated with phytoremediation, there are also some drawbacks. For example, when the concentration of contaminants is too high, plants may not grow as some contaminants can be toxic (Raskin *et al.*, 1997). The contaminants must be located primarily near the surface of the soil or the roots will not be able to access the contaminants in the soil. Other limitations to phytoremediation include: seasonal and climate dependencies of plants, contaminants may leech into ground water or bioaccumulate in animals, and mixed contaminants (inorganic/organic) may require multiple types of plants and methods (Glick and Stearns*,* 2011). For all these reasons, the implementation of phytoremediation requires decisions made on a site-by-site basis.

2.9.2 Phytoremediation of PHCs

In order to survive and thrive in PHC contaminated environments, plants must exhibit a tolerance to one or more components of petroleum mixtures, high competiveness, fast growth, and the ability to produce and secrete hydrocarbon degrading enzymes (Gkorezis *et al*., 2016). Plants may be influenced positively by the presence of bacteria that are able to: synthesize plant hormones, such as, indole-3-acetic acid, gibberellins, and cytokinins; suppress ethylene production, fix nitrogen, and mobilize nutrients such as phosphorus and other minerals important to plant growth and development (Hardoim *et al.,* 2008; Gkorezis *et al*., 2016; Glick and Stearns, 2011).

2.9.2.1 Rhizodegradation

Living plants have very intricate root systems that bring microbes, nutrients, and contaminants into contact with each other. The rhizosphere is the zone of soil under the direct influence of roots and extends 1-5 mm from the root surface (Gkorezis *et al.,* 2016). Plant roots excrete a variety of organic exudates, into the rhizosphere which can increase nutrients available to microorganisms. Due to the presence of nutrient rich root exudates, microbial populations are 5-100 times more active in the rhizosphere than in the bulk soil (Maqbool *et al.,* 2012). Plants and microorganisms can degrade PHCs independently, however, their interaction stimulates microbial communities in the rhizosphere to degrade PHCs using catabolic enzymes in a process known as rhizodegradation (Maqbool *et al.,* 2012). Contaminants generally exhibit a treatable concentration range, above which the contaminant prevents or slows metabolic activity. Conversely, if the contaminant levels are too low, plants and/or microorganisms may not be physiologically capable of reducing contaminant concentrations to very low levels. This is because the uptake and metabolism of the contaminant stops at low concentrations, even when environmental conditions are optimal. Low contaminant concentrations also may cause microbes capable of degrading the contaminant to switch to alternative and more available substrates (Heitkamp, 1989: Maqbool *et al.,* 2012).

2.9.3 Phytoremediation of Salinized Soils

2.9.3.1 Halophytes

Halophytes are salt tolerant plants that have physiological mechanisms that allow them to thrive in saline soil, but they make up only ~2% of the world's terrestrial flora (Barhoumi *et al.,* 2008; Flowers and Colmer, 2015; Yensen and Biel, 2008). Halophytes can inhabit near ocean shore shallows (e.g. *Avicennia*, mangroves), coastal salt marshes, inland salt lakes, and saline desserts (Flowers and Colmer, 2015). Physiologically, halophytes are able to tolerate and grow in saline conditions by reducing the concentration of ions like Cl^- in their xylem such that the quantity of salt delivered to the leaves is decreased (Ceccoli *et al.,* 2015: Flowers and Colmer, 2015). Salt tolerant plants that grow better under high salinities are known as euhalophytes and these plants are very efficient at regulating Na^+ and Cl^- at high salt concentrations (Flowers and Colmer, 2015: Grattan and Grieve, 1998). Halophytes of all kinds can control ionic balance at the foliar and root levels through various mechanisms and these mechanisms can vary by species (Yensen and Biel, 2008). Halophytes are remarkable due to their ability to live in conditions that are typically lethal to other plants species and thus can play an important role in environmental remediation and agricultural sustainability (Flowers and Colmer, 2015: Yensen and Biel, 2008). McSorely *et al.,* (2016b) proposed that emphasis should be placed on classifying and understanding the salt tolerance mechanisms (exclusion, accumulation, and excretion) of halophytes, as these mechanisms can impact phytoextraction potential.

2.9.3.2 Exclusion Mechanism

Exclusion occurs when halophytes restrict the uptake of ions associated with soil salinization and leave them to accumulate in the soil (Yensen and Biel, 2006). In some exclusion mechanisms, roots may accumulate high concentrations of ions, but the transport to above ground tissues is restricted (Flowers and Colmer, 2015). Re-translocation is another process that may occur, where ions transported into above

ground tissues are then transported back to the roots (Dassanayake and Larkin, 2017). Excluder halophyte species are not suitable for the remediation of salinized soil because they cause salts to accumulate in the rhizosphere which increases the salt levels in the soil over time (Yensen and Biel, 2006).

2.9.3.3 Accumulation Mechanism

In saline soil, salt ions are brought up into plant tissue through the roots and compartmentalized in the vacuoles of plant cells. By compartmentalizing the excess ions, accumulator halophytes can maintain the uptake of water. Increasing evidence demonstrates the roles of a salt overly sensitive stress-signaling pathway in ion homeostasis and salt tolerance. This signaling pathway consists of three proteins, which facilitate long distance transport of Na^+ from root to shoot (Gupta *et a*l., 2014). Overexpression of these proteins grants salt tolerance in plants by signaling vacuolar functions to transport and store excess Na^+ ions as they travel from root to shoot (Gupta *et al*., 2014). Accumulator halophytes have developed an efficient method to keep the ion concentration in the cytoplasm at a low level by regulating the uptake of Na^+ and Cl^- while maintaining cytoplasmic K^+ and Mg^{2+} concentrations at levels required for activation of essential enzyme activities (Flowers and Colmer, 2015). Membranes along with their associated components play an integral role in maintaining ion concentration within the cytosol during the period of stress by regulating ion uptake and transport. Maintaining cellular Na^+/K^+ homeostasis is pivotal for plant survival in saline environments (Gupta *et al*., 2014).

2.9.3.4 Excretion Mechanism

Some halophytic plants have specialized excretory organs on their leaf and stem tissues that excrete excess salt and are referred to as recretohalophytes, with ~370 species worldwide (Yuan *et al*., 2016). Most recretohalophytes are found in dry arid, desert, seawater, and inland saline ecosystems where natural soil salt levels tend to be higher (Flowers and Colmer, 2015). Recretohalophytes act as conductors of salts from the soil to shoots by moving salts ions through their vascular system (Ceccoli *et al.,* 2015). The excretory organs of these plants are salt glands and salt bladders adapted for dealing with ionic homeostasis in the cells (Ceccoli *et al.,* 2015: Yuan *et al.,* 2016). The main function of the salt glands and bladders is to excrete excess salt ions that invade the plant. These mechanisms of excretion vary between plants and are not yet fully understood (Yensen and Biel, 2006). The main ions excreted by salt glands include Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- (Ding *et al.* 2010; Naz *et al.,* 2009; Oi *et al.,* 2014). The rate of excretion varies based on the plant species, the substrate concentration, temperature, humidity, salt gland density, and leaf size (Yuan *et al*., 2016). Excretory (recretohalophytes) halophytes have the potential to reduce salt levels in the soil without the need for harvesting at the end of a growing season (McSorely *et al*., 2016b; Yensen and Biel, 2008).

2.10 SALT EXCRETION MECHANISMS OF RECRETOHALOPHYTES

2.10.1 Salt Glands

There are many uncertainties that still exist surrounding the true processes that take place in excreting excess salt ions (Dassanayake and Larkin, 2017; Yuan *et al.,* 2016; Ceccoli *et al.,* 2015; Semenova *et al.,*

2010). Salt secreting structures known as hydathodes directly secrete salt ions onto leaf surfaces (Yuan *et al*., 2016: Dassanayake and Larkin, 2017). There are four different types of hydathodes: i) salt bladders, ii) multicellular glands, iii) bicellular hairs, and iv) unicellular hairs (Dassanayake and Larkin, 2017) (Figure 2-3). Although salt glands in different species possess varying characteristics, their common characteristics are a thickened cuticle surrounding the salt gland, many plasmodesmata, a large number of developed mitochondria, and no chloroplasts (Ding *et al.,* 2010: Yuan *et al.,* 2016). An ion can be rapidly transported from a mesophyll cell into a salt gland and secreted out of the gland with a force that is generated by mitochondrial activity and is transported in vesicles, eventually being excreted through pores (Feng *et al.,* 2014: Yuan *et al.,* 2016).

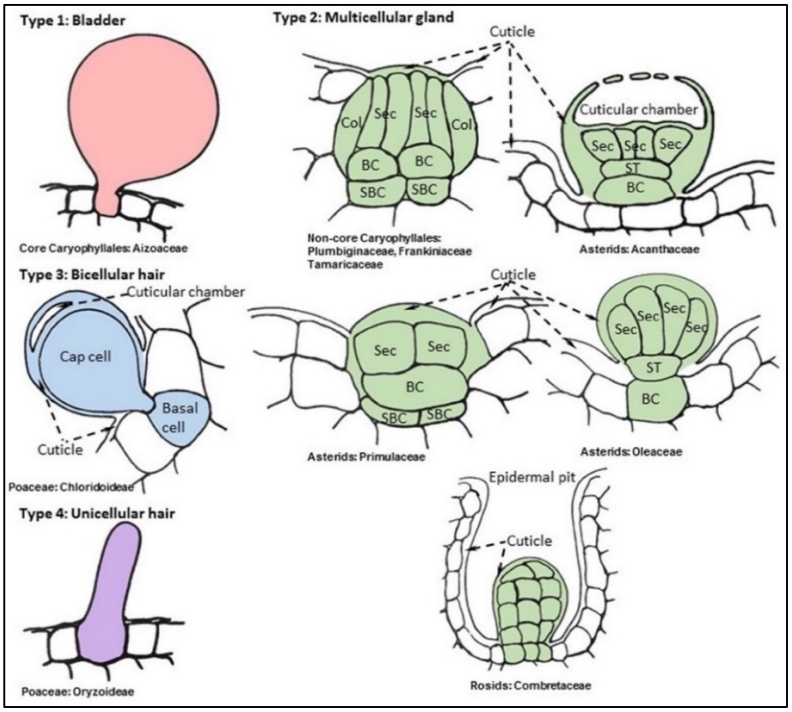

Figure 2-3 Four categories of salt gland structures found in excretory halophytes. Collecting cell (Col), secretory cell (Sec), basal collecting cell (BC), sub-basal collecting cell (SBC), and stalk cell (ST) (from Dassanayake and Larkin, 2017).

2.10.1.1 Salt Bladder

Plants use salt bladders to sequester excess salt ions away from active cells by depositing large quantities of salt in external, trichome-like balloons, known as epidermal bladder cells (EBCs) (Shabala *et al*., 2014) (Figure 2-3). These EBCs behave and look like inverted vacuoles and can sequester 1000-fold more Na^+ compared to 'traditional' leaf cell vacuoles (Dassanayake and Larkin, 2017; Shabala *et al.*, 2014). When the salt bladders swell to their capacity, they rupture and deposit salt in the form of an aqueous solution on the epidermal surface (Dassanayake and Larkin, 2017). EBCs also can carry out active metabolism related to energy generation, UV protection, and stress signaling. EBCs occur most commonly in the Chenopod and Caryophyllales orders which are found mostly in salty dry habits and salty marshlands (Dassanayake and Larkin, 2017: Shabala *et al.,* 2014).

2.10.1.2 Multicellular gland

Multicellular salt glands have various cells known as basal collecting cells (BC) and distal secretory cells (Sec). These cells are in a cuticle-lined structure that is slightly sunken into the epidermis of the plant (Dassanayake and Larkin, 2017). The collecting cells (Col) create a salt efflux gradient to collect salt from neighbouring mesophyll cells and transport them to secretory cells (Faraday and Thomson, 1986). The salt is actively transported through the symplast from the collecting cells into the secretory cells, and then the salt solution is deposited outside the cell via pores in the cuticle (Dassanayake and Larkin, 2017). The sub-basal collecting cell (SBC) channel the flow of salt through the secretory cells and percent leakage back into the neighboring tissues via the apoplast (Tan *et al.,* 2013). In some species, there is a cuticle chamber above the secretory cells to store the secreted salts (Dassanayake and Larkin, 2017). Multicellular glands are found commonly in the Caryophyllales order (ibid).

2.10.1.3 Unicellular hair

Unicellular hairs lack organelles and appear to be completely filled with vacuoles (Dassanayake and Larkin, 2017). It is possible for the plant to adjust the number of salt hairs per cm^2 of leaf with increasing salt concentration (Sengupta and Majumder, 2009). These hairs are structurally simple trichomes without any distinct basal and cap cells, and are the source of salt secretion with adjacent four stomatal guard cells, which are also hairs. *P. coarctata* has finger-shaped and uniformly arranged upper (adaxial) surface hairs, and peg-like hairs arranged in groups on the lower (abaxial) surface hairs (Sengupta and Majumder, 2009). At high salt concentrations, the upper surface hairs do not rupture and excrete visible salt on the plant surface. The lower surface hairs swell, rupture, and collapse in high salt concentrations, and then regrow when there is less salt present in the soil substrate (ibid).

2.10.1.4 Bicellular Hair

Bicellular salt glands (hairs) have collecting compartments known as a basal cell and cap cell. The basal cell is embedded in the epidermis and the cap cell protrudes from the leaf surface (Barhoumi *et al*., 2008; Ceccoli *et al*., 2015: Dassanayake and Larkin, 2017). Semenova *et al.,* (2010) proposed that first, the salt solution is transported through the shoot tissues to the salt glands via the apoplast. Next, it is accumulated in the vacuoles of collecting cells and flows to the extracellular channels of the basal cell. The energy required for this is provided by many, densely packed mitochondria (Ceccoli *et al*., 2015). The salt solution is mechanically pumped into the apoplastic space of the cap cell, then accumulates in the collection chamber, and is finally excreted on the leaf surface through the pores in the cuticle (Figure 2-4). Under the appropriate conditions, the saline solution crystallizes above the cuticle on the plant surface as water evaporates from the salt solution (Shabala *et al.,* 2014). The driving force to move salt from the vacuole of the collecting cell, through the basal cell, and to the cap cell, occurs from impulses of mechanical compression-expansion or pulsation of the extracellular channels of the plasma membrane (Semenov *et al*., 2010).

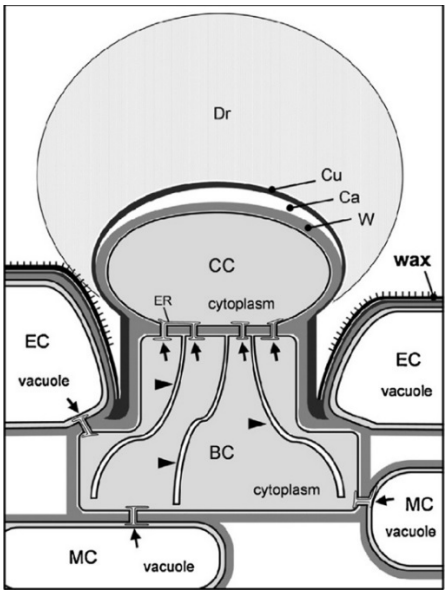

Figure 2-4 Schematic of a bicellular hair. Basal cell (BC), cavity (Ca), cap cell (CC), cuticle (Cu), droplet (Dr), epidermal cell (EC), endoplasmic reticulum (ER), mesophyll cell (MC), cell wall (W) (from Oi *et al*., 2014).

2.10.2 Recretohalophytes Native to Ontario

Two recretohalophytes that are native to Ontario are *Distichlis spicata* (seashore or inland grass) and *Spartina pectinata* (prairie cord grass). Both are members of the Poacea family and Chloridoideae sub family (Eppely, 2006; Kim *et al.,* 2012). *D. spicata* and *S. pectinata* are extremely salt tolerant C4 halophytic grasses that exist in salt affected areas such as coastal grasslands, bogs, and salt flats. Both grasses have bi-cellular salt glands on their leaves that excrete salts on the leaf surfaces (Ceccoli *et al.,* 2015: Kim *et al.,* 2012).

2.10.2.1 Distichlis spicata

D. spicata has a hearty root system that forms a sod and has previously been investigated in the phytoremediation of some salt impacted sites (Leake *et al.,* 2002; Lymbery *et al.,* 2013; Yuan *et al*., 2016). Its efficacy in removing nutrients has been monitored under varying salinities. It was determined that *D. spicata* performed with higher efficiency at lower salinities (3,000 mg L^{-1}), but maintained excretion capabilities at higher salinities (15,000 mg L^{-1}) (Lymbery *et al.,* 2013). An eight-year study was conducted in Western Australia and it was observed that a *D. spicata* plot improved aggregate stability with no significant accumulation of salt in the root zone of the grass. This suggested that the salts excreted by these plants were being blown away and not falling directly back onto the soil below (Yensen and Biel, 2008). Hence, *D. spicata* has the potential to be an effective conductor of salt from the soil to the air. Furthermore, *D. spicata* is a euhalophyte meaning it has the greatest salt tolerance of all halophytic plants. It has the advantage of maintaining low-salt levels in the shoot tissues, making it edible for animals, while also tolerating very saline conditions (Yensen and Biel, 2006).

2.10.2.2 Spartina pectinata

The use of *S. pectinata* to remediate Cl^- from a salt-impacted site has been explored at an industrial landfill in Bath, Ontario (McSorely *et al.,* 2016b). Initially in this study, the salt accumulation potential of *S. pectinata* was compared to a known accumulator halophyte, *Phragmities australis*. It was determined that *S. pectinata* is far less efficient at accumulating Cl^- than *P. australis,* however, when considering the excretion potential, *S. pectinata* was shown to have the potential to remove 58% more Cl^- than *P. australis* (McSorely *et al.,* 2016b).

2.11 HALOCONDUCTION

In 2008, Yensen and Biel proposed the theory of haloconduction which states that salts excreted on the leaf surfaces of recretohalophytes can be mobilized into air and redistributed across large areas (Figure 2-5). This method of salt transport by wind is similar to that which occurs in coastal regions, where 80% of inland salt arrives from wind currents carrying microscopic salt that originated from the ocean. Under certain conditions, over 50% of excreted salt may become airborne and under optimal conditions recretohalophytes such as *Distichlis* have the potential to disperse 5-50 tons of salt/ha/year (Yensen and Biel, 2008). Research is required to determine how much salt is excreted by recretohalophytes and the distances that these salts can travel (Yun *et al.,* subm). Many salt ions are nutrients that are essential to organisms and for this reason, salt is benign as an environment contaminant unless at unless at high concentrations. The excretion and subsequent dispersion of salt by wind could, in fact, be beneficial in providing nutrients to other plant and animal organisms in the area, while reducing the total salt contamination level in soil at a contaminated site (McSorely *et al*., 2016b).

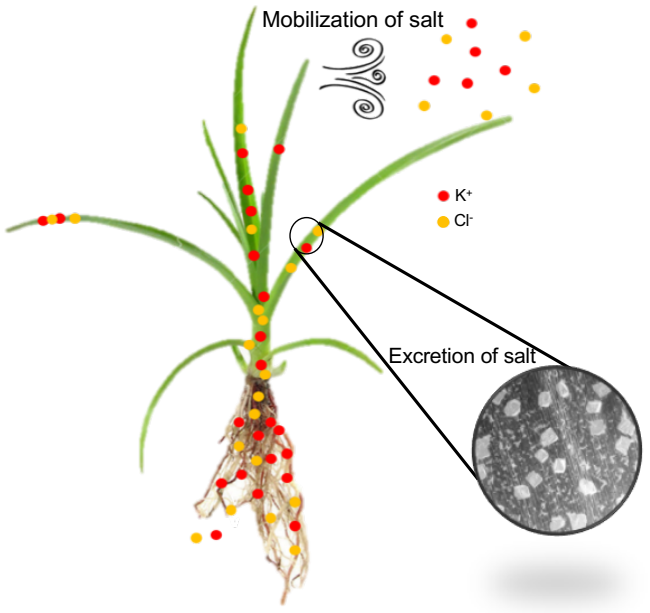

Figure 2-5 Representation of the theory of haloconduction. Salt is phytoextracted from the contaminated soil by a recretohalophyte, transported to the above ground plant tissues and excreted through salt glands onto the surface of the plant. As wind disturbs the salt crystals, they are mobilized off of the plant and transported via the wind away from site (adapted from McSorley *et al*., 2016b).

2.11.1 Windborne Salt Collection Methods

2.11.1.1 Airborne salts

Atmospheric particles can be divided into falling particles (diameter >10 μm) and buoyant particles (diameter <10 μm) (Mocrillo *et al.,* 2000). Smaller buoyant particles may travel hundreds of kilometres in the air without sedimentation. An example of well characterized airborne particles are sea salt aerosols (SSA) (Madry *et al*., 2011: Soares *et al.,* 2016). SAA particles range from 0.1-400 μm in size and disperse hundreds of kilometers before settling (Meira *et a*l., 2008: Morcillo *et al.,* 2000). Sea salts can become airborne in many scenarios but most commonly during the crashing of whitecap ocean water waves. When the waves break, air bubbles burst and droplets of salt water can become suspended in the air (Soares *et al.,* 2016). Although SSA forms very differently from salts excreted by recretohalophytes, we may be able to use characteristics and behaviours from SSAs to inform our work with haloconduction (Yun *et al.,* subm). Salt is hygroscopic meaning its growth and shape changes as a function of relative humidity (Madry *et al.,* 2011). Salt size depends not only on the current ambient relative humidity, but also on the history of relative humidity that the particle has been exposed to and will be exposed to (Madry *et al.,* 2011). The longer a salt particle is airborne the more conditions it is exposed to leading to potential for changes in physical and chemical characteristics.

2.11.1.2 Sea Salt Collection

Several methods have been developed to collect SSAs. Some of the first studies involved in collected windborne salts were to determine its effects on coastal agriculture operations (Lomas and Gat, 1967). Lomas and Gat (1967) investigated the effects of windborne sea salts on a coastal citrus grove in Israel in order to justify the implementation of windbreaks. They developed measuring stations, which consisted of a post with a mounted frame of a double layer of 20 x 20 cm muslin. These stations were left out in the field for a period of 10 days at a height of 1.5 m and orientated perpendicular to the prevailing wind direction until collection (Lomas and Gat, 1967). The ‘wet candle method’ is a standard test method for determining atmospheric chloride deposition rate (Figure 2-6) (ASTM, 1996). The method allows researchers to calculate the amount of salts deposited from the atmosphere on a given area per unit time. Another windbourne collection device are salt vanes. In the salt vane method, pollen filters (Cour-filets) are cut in 20 x 20 cm and placed in a frame of overlapping layers which allows air to pass through while collecting salts in the filters. Once the filters are collected they are washed with deionized (DI) water and analyzed for chloride levels.

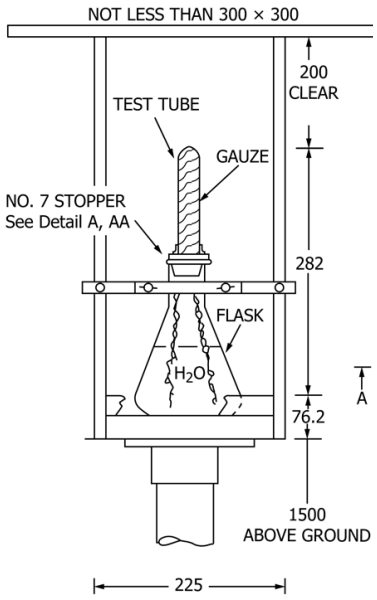

Figure 2-6 A wet candle apparatus (ASTM, 1996).

2.11.2 Salt Modelling

Meira *et al*. (2008) attempted to develop a model that represents marine aerosol behaviour by factoring in distance from the sea and wind speed. Although the factors that affect SSA production, transportation, and deposition differ from those that affect excreted salts by recretohalophyte, the established models may be a useful tool for predicting haloconduction. Multiple models can be used in conjunction with each other to recreate the state of the atmosphere for a given time period as accurately as possible. Overall, the use of multiple reliable models paired with *in situ* measurements of excreted and dispersed salts could be promising for use in the phytoremediation of salinized soils via haloconduction. These models require specific input parameters related to the excreted salt crystals such as salt crystal diameter, number of salts per unit area, and the behavior of the salts under varying conditions (temperature and humidity).

2.12 RECRETOHALOPHYTE RESEARCH

The salt secretion activity of the bicellular salt glands of *D. spicata* and *S. pectinata* can be observed with the naked eye (Figure 2-7). Studies have been conducted on recretohalohpytes such as *Limonium bicolor* and *Atriplex canescens,* by brushing the salt bladders from the leaves and analyzing the solution. These methods do not: i) provide direct evidence of salt excretion by a single gland, ii) provide the behaviour of the salts under varying conditions, or iii) make it possible to quantify the amount of salt particles per unit area on the leaf surface (McSorely *et al.,* 2016b: Yuan *et al.,* 2016). There are methods that have been developed for determining the composition of these excreted salts including x-ray fluorescence. X-ray fluorescence and energy dispersive x-ray spectroscopy (EDS) have shown that the recretohalophyte glands secrete ions such as Ca^{2+}, Mg^{3+}, Na^{+}, and Cl^{-} (Yuan *et al.,* 2016). Scanning electron microscopy (SEM) has been recognized as the most precise and accurate means to observe salt secretion, however, the use of SEM

and X-ray microanalysis in the evaluation of salt secretion is affected by a complicated sample preparation procedure (Yuan *et al.,* 2016).

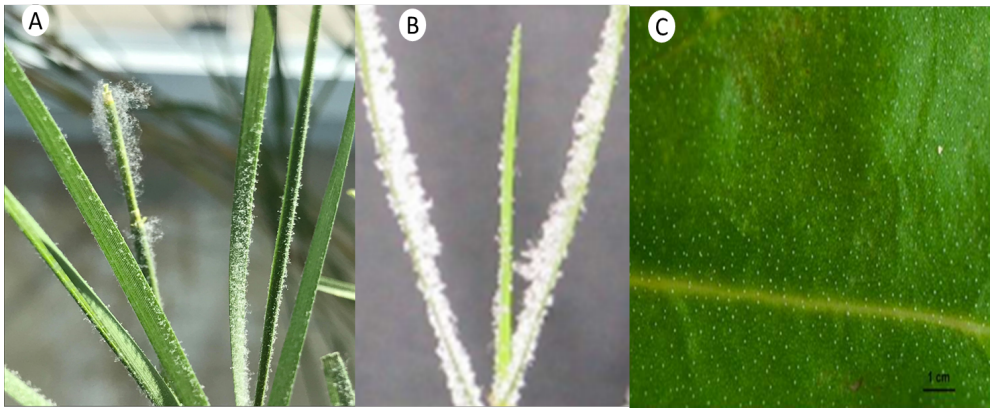

Figure 2-7 (A) Salt excretion on the stem and leaves of *Spartina pectinata* (photo taken by L. Morris in the RMC Greenhouse). (B) The leaf blades of *D. spicata* exhibiting salt crystals on their leaf surfaces under conditions that prevented the slightest air flow. This photograph was reproduced from Semenova *et al.,* (2010). (C) Leaves of *Limonium bicolor* (adapted from Feng *et al.,* 2014).

2.12.1 Scanning Electron Microscopy (SEM)

Conventional scanning electron microscopy (CSEM) requires samples to be completely dry and vacuum stable, and conductive (Stabentheiner *et al.,* 2010). Living cells and tissues such as a leaf sample, are considered 'wet' and require steps to preserve and stabilize their structure (Muscariello *et al.,* 2005). It is difficult to image samples of plants in CSEM so most scanning electron microscopes have a setting that allows for a gaseous environment in the specimen chamber, referred to as environmental scanning electron microscopy (ESEM) (Kolb and Müller, 2004). ESEM has become an essential tool in studying plant surfaces and has profoundly influenced our understanding and knowledge of plants (Stabentheiner *et al.,* 2010).

2.12.1.1 Scanning Electron Microscopy Imaging of Distichlis spicata and Spartina pectinata

Extensive images of the salt glands and excreted particles of *S. pectinata* and *D. spicata* are not yet found in the literature, however the ultrastructure of the salt glands of *S. pectinata* and *D. spicata* can be compared to those of *Aeluropus littoralis* (Figure 2-8) (Barhoumi *et al.,* 2008). McSorely *et al.,* (2016b) visually analyzed the excreted salts on *S. pectinata* plants of similar size using SEM. Furthermore, their work was the first to show clear evidence of the composition of the excreted salt particles on *S. pectinata* by EDS. The salt contained mostly Cl^- and K^+ which were the same principal salt ions in their study soil substrate.

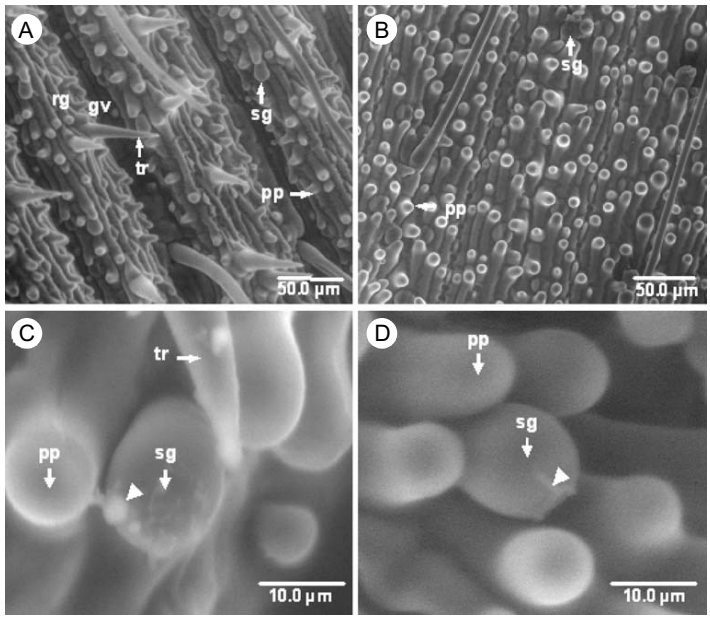

Figure 2-8 Scanning electron micrographs displaying the salt glands (sg), trichome (tr), grooves (gv), and papillae (pp) of *Aeluropus* littoralis plants on abaxial (a, c) and adaxial (b, d) surfaces Note that the ultrastructure of *A. littoralis* is comparable to that of *D. spicata* and *S. pectinata* (Barhoumi *et al*., 2008).

3 PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS AND SALT

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3.1 ABSTRACT

The soils at, and surrounding, many oil and gas extractions sites are co-contaminated with petroleum hydrocarbons (PHCs) and salts. These sites are challenging to remediate and a low-cost, passive, and long-term remediation technique that could be used at some of these sites is phytoremediation. Some salt tolerant plants, known as recretohalophytes, have specialized salt glands on their leaf surfaces that excrete excess salts. Under the appropriate conditions, these salts can mobilize into the air by wind in a process known as haloconduction. This study investigated the ability of two recretohalophyte grasses, *Distichlis spicata* and *Spartina pectinata*, to rhizodegrade PHCs in salt impacted soil while simultaneously reducing salt levels. The two species were planted into potassium chloride (KCl) salt-impacted soil and background (control) soil from a salinized research site on the property of the Lafarge Plant in Bath, Ontario. Both types of soil were spiked with a 1% diesel and oil lubricant solution to contaminate the soil with PHCs to a TPH level of ~10,000 mg/kg. TPH decreased significantly over the course of the 22-month experiment; however, results were similar in the planted and non-planted controls indicating that the decrease in PHCs was not attributable to the enhanced microbial activity of rhizosphere. The salt excretion and haloconduction by *D. spicata* and *S. pectinata* contributed to a Cl^- reduction in the non-spiked salt impacted soils in this study, however, only *S. pectinata* was significantly different ($p<0.05$) from the non-planted controls. As well, *S. pectinata* contributed to a significant Cl^- reduction in the PHC-spiked salt impacted soil when compared to the non-planted controls. The mechanisms involved in the excretion of salt by recretohalophytes are still not fully understood, and it is not well established in the literature how PHCs may interfere with the salt excretion abilities of these plants. This study indicates that low levels of PHCs (1% of soil) did not affect the excretion capabilities of *S. pectinata*. Salt remediation using recretohalophytes is a promising new technology for soils co-contaminated with salt and hydrocarbons at oil and gas extraction sites.

3.2 INTRODUCTION

Two of the most common soil contaminants found at oil and gas extractions sites are petroleum hydrocarbons (PHCs) and salts which are generated from spilled crude oil and produced water brine, respectively (Carty *et al.,* 1997). PHCs are composed of thousands of structurally different compounds, with varying degrees of toxicity to the environment and human health (Kamanth *et al.,* 2004; Gkorezis *et al.,* 2016). Once brought to the surface, PHCs can be further transported by water or air, and/or adsorb to soil particles causing the soil to degrade and interfering with soil ecosystems. The primary waste by volume from oil extraction is groundwater brine brought to the surface during petroleum extraction; it can contain many different pollutants including large quantities of salts (Carty *et al*., 1997). When brought to the surface, this brine salinizes the soil. Remediating soils mixed with PHCs and salt is challenging due to the very different natures of the contaminants and the complex interactions between these contaminants and the soil.

Phytoremediation is the use of green plants and their associated microorganisms to stabilize or reduce contamination in soil, sludge, sediment, surface water, or ground water. It has been well established in the literature that many plant species have the ability to degrade PHCs in the soil rhizosphere (Heitkamp and Cerniglia, 1989: Singh and Jain, 2004).

Halophytes are salt tolerant plants that have different physiological mechanisms that allow them to thrive in saline soil, but they make up only ~2% of the world's terrestrial flora (Flowers and Colmer, 2015: Barhoumi *et al.,* 2008). Some halophytes (termed 'recretohalophytes') tolerate saline soil by an excretion mechanism in which excess salt ions are excreted through specialized salt glands onto their leaf surfaces (Yuan *et al.,* 2016: Ding *et al.,* 2010).

Yensen and Biel (2008) postulated the theory of haloconduction which states that a certain percentage of the salts excreted by recretohalophytes can be mobilized into the air by wind. They theorized that wind could continuously mobilize these excreted salts and move them away from a contaminated site to areas of lower salt concentration without the need for plant harvesting. This mechanism of salt remediation has only very recently been proven in the literature (Yun *et al*., subm.). A concern with this remediation approach is that salts may re-contaminate adjacent soil, rather than be carried away and deposited elsewhere (Burke *et al.,* 2000; Weis *et al.,* 2002; Weis and Weis, 2004). However, depending on meteorological conditions, some salt particles have the ability to travel long distances and furthermore can be micronutrients at lower concentrations.

Despite the ubiquitous nature of soils co-contaminated with hydrocarbons and salts, to date, no one has attempted to investigate the simultaneous remediation of petroleum hydrocarbons via rhizodegradation and salts via haloconduction. This study investigates the ability of two known recretohalophytic grasses, *Distichlis spicata* and *Spartina pectinata*, to simultaneously phytoremediate PHCs and salts from contaminated soils.

3.3 MATERIALS AND METHODS

3.3.1 Plant Selection

The salt grasses *Distichlis spicata* and *Spartina pectinata* are native to Ontario and were selected for this study based on their potential to extract Cl^- from salinized soil as reported by McSorely *et al*. (2016a &b) and Yun *et al.* (subm). Both are members of the Poacea family and Chloridoideae subfamily. *D. spicata* is commonly known as seashore salt grass, inland salt grass, and desert salt grass. It is an extremely salt tolerant, C4 halophytic grass that thrives areas such as coastal grasslands and salt flats. It has a hearty root system that forms a sod (Hansen *et al.,* 1976). *S. pectinata* is commonly known as prairie cord grass. This plant is found in a variety of habitats and environments such as wet prairies, marshes, along bogs and ponds. *S. pectinata* has tough roots and woody rhizomes that penetrate deep into the soil and their sturdy hollow stems can grow as tall as three metres (Kim *et al.,* 2012).

3.3.2 Plant Acquisition and Maintenance

3.3.2.1 Spartina pectinata

Plugs of *S. pectinata* seedlings (eight weeks old, 15 cm in height) were obtained from Norview Gardens Ltd. in Norwich, Ontario. They were transplanted (one plant per pot), into the study soil and grown to a height of ~40-50 cm with >15 shoots at the RMC greenhouse facility. A further nine *S. pectinata* plants measuring approximately 45 cm in height were obtained from the research site at the Lafarge Cement Plant in Bath, Ontario, and transplanted and maintained over several months at the same facility.

3.3.2.2 Distichlis spicata

Five kilograms of *D. spicata* seeds were donated to RMC by BrettYoung, a seed production and distribution company based out of Calmar, Alberta. The seeds were germinated by submerging seeds in a petri dish of tap water and leaving them for 7-10 days, and then transplanted into potting soil in seedling trays. Once they were approximately five inches in height (~60 days), they were transplanted into soils from the Lafarge field site and maintained over several months in the greenhouse facility at RMC (McSorely *et al.*, 2016: Yun *et al*., subm.).

3.3.3 Bulk Soil Collection from Field Site

The soil for this experiment was obtained from a salinized study site located at the Lafarge Cement manufacturing plant property in Bath, Ontario (79º48' Long 79º48' Lat). Cement kiln dust (CKD) was landfilled on the Lafarge property from 1973 to 2003 at a rate of ~30,000 tons per year in two approved, but unlined, landfill cells, with a total area of 27.9 ha. To the east of the landfill cells there is a lower elevation marshland known as the 'cliff site' (Yun *et al*., 2019). CKD has very high levels of potassium chloride (KCl) and the cliff site receives drainage from the CKD landfill, resulting in a marshland that has very high levels of chloride [Cl^-] (bulk soil mean 3850 ± 1610 μg/g; n=41) (McSorely *et al*., 2016a & b). Cl^- is the anion of concern at this site because excess soil Cl^- can have adverse effects on plant growth, soil quality, and microbial activity. The site has high levels of K^+ as well; however, it is not an ion of concern

as K^+ is essential for photosynthesis, protein synthesis, and other cellar processes. In addition, K^+ has a high permeability across cell membranes and can be removed by plants, especially when luxury quantities of the nutrient exist. The Cl^- concentrations were greatest at the southeast corner of the site and this soil was collected, dried, and homogenized for this experiment (mean 7987± 2942 µg/g; n=18). Background soil (referred to as 'control' soil) was collected ~120 m east of the site. Despite having a much lower Cl^- concentration (mean Cl^- 809 ± 233 µg/g; n=18), this 'control' soil still has elevated Cl^- levels compared to background soils in Canada.

3.3.4 PHC- Spiking Procedure

The control and salt-impacted soils used in each pot were individually spiked with a diesel and lubricating oil solution to contaminate the soil with PHCs to a TPH level of approximately 10,000 mg/kg. The diesel fuel was obtained from a Petro Canada gas station and the oil lubricant was Castrol GTX. The soils were spiked by adding 5.25 g of the oil-lubricant and 5.25 g of diesel fuel to 25 mL of methanol (MeOH) and then adding this mixture to 525 g of soil on an aluminum pie-plate. Organic compounds (such as PHCs) are often added to soil or sediment using water-soluble solvents such as methanol or acetone to ensure they are soluble and remain in solution during mixing (Northcott and Jones, 2000). Soils were homogenized for 3 minutes immediately after spiking. In the homogenization process, the original 525g pile of soil was quartered by random scooping using a scoopula. Each of the four piles were manually mixed and re-combined into a central pile by scooping from the four piles in an alternating manner (Gy, 1999). 425 g was used for the non-planted controls and the plugs of *D. spicata* and *S. pectinata,* which were all prepared the following day to allow for the total evaporation of MeOH from the soil substrate. An initial soil sample of each pot (100 g) was taken at the same time.

3.3.5 Experimental Design

The two plant species were planted into control and potassium chloride (KCl) salt impacted soil from the Lafarge Plant in Bath, Ontario. Each of the four treatments (control soil; control + PHC-spike; salt-impacted soil; salt-impacted + PHC-spike,) was run in triplicate (Figure 3-1).

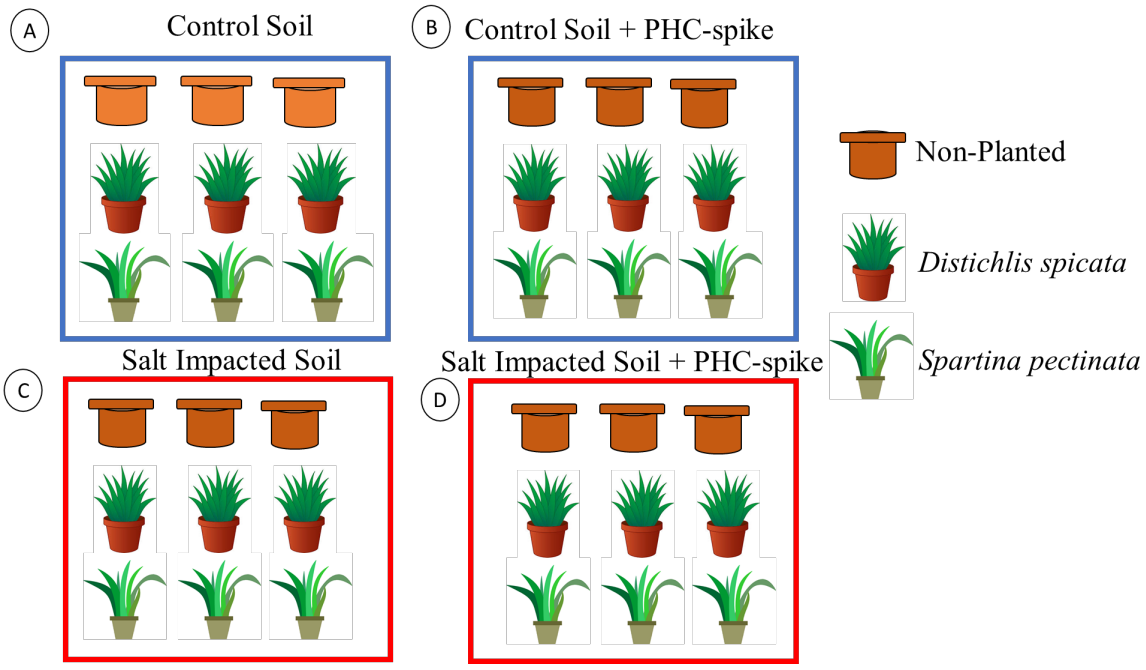

Figure 3-1 Experimental design for the simultaneous phytoremediation of petroleum hydrocarbons (PHCs) and soil chloride (Cl^-). Four soil treatments with *Distichlis spicata* and *Spartina pectinata* were undertaken as follows: A) = control soil, B) = control soil spiked with PHCs, C) = salt-impacted soil, and D) = salt-impacted soil spiked with PHCs.

3.3.6 Plant Maintenance for Salt Excretion

This experiment was conducted for 22 months in the RMC greenhouse. All treatments and non-planted controls were watered with ~25 mL of tap water per day. In an effort to maximize the Cl^- remediation, the excess salts being excreted by the recretohalophytes were mobilized by wind generated from fans placed in front of the experiment space (Figure 3-2). The fans blew at a very gentle wind speed of 0.3 m/s. As the plants excreted salt, the wind blew that salt off the plants to within a ventilated enclosure (Figure 3-2). The non-planted controls were placed in front of the vegetated planters as seen in Figure 3-2. Positions of the plants on the table were rotated every two weeks.

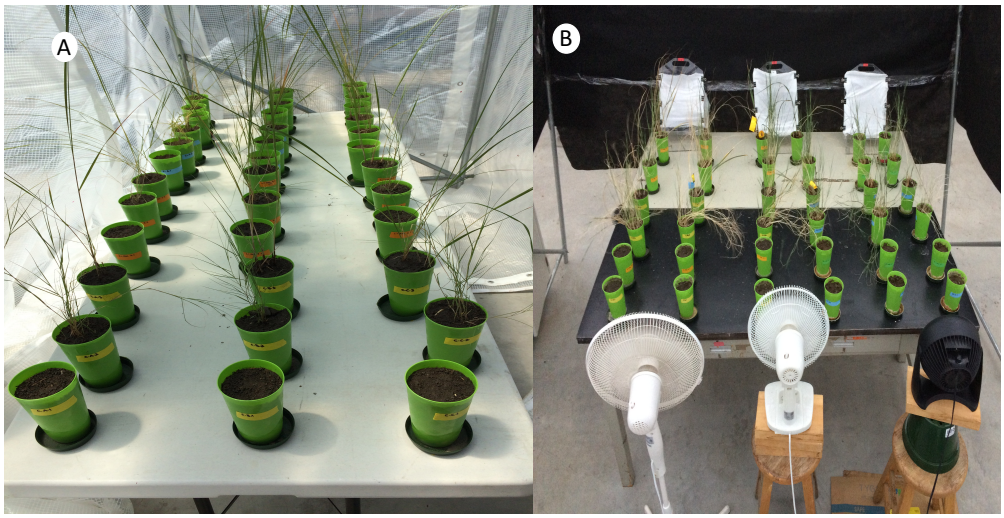

Figure 3-2 Arrangement of the experimental planters in a ventilated enclosure (A) and in a partitioned section of the greenhouse (B) at the Royal Military College of Canada. The excreted salts were mobilized by wind generated from fans placed in front of the experiment space (B). The non-planted controls were placed in front of the vegetated planters (B) in an effort to minimize the deposition of airborne salts into the control soil.

3.3.7 Sample Collection and Preparation

The soil in each planter was sampled five times during the experiment (at time 0, 3 months, 6 months, 1 year, and 22 months). During each sampling event, ~20 g of soil was carefully removed from the pots from just below the surface soil (~1-3 cm) and near the root-zone (depth ~5-10 cm) and placed into glass test tubes and kept refrigerated or frozen until analysis.

3.3.8 Soil Sample Analysis

Samples were analyzed at the Queen's University Analytical Services Unit (ASU) for CCME PHC fractions F1-F4 and chloride (Cl^-). The Canada Wide Standard for Petroleum Hydrocarbons in Soil was established by the Canadian Council for Ministers of the Environment (CCME) in 2008, and prescribes analysis of PHCs in four specific fractions (F1: C6 to C10; F2: >C10 to C16; F3: >C16 to C34 and F4: C34+). For example, F1 includes all extractable hydrocarbons between the linear straight chain hydrocarbons nC_6 (hexane) and nC_{10} (decane).

3.3.8.1 Petroleum Hydrocarbon (PHC) Analysis

Soil samples were analyzed according to the Reference Method for Canada-Wide standard for Petroleum Hydrocarbons in Soil-Tier 1 Method by the Canadian Council of Ministers of the Environment (CCME, 2008). For the F1 fraction, 5 g of the sample was extracted into 10 mL of methanol and the extract analyzed by SPME (solid phase microextraction) GC-FID (gas chromatography with flame ionization detection) using an Agilent 7890B and an Agilent 80 GC Sampler (PAL autosampler). For fractions F2-F4, a sample of ~5 g was extracted three times in 1:1 hexane: acetone and sonicated for 20 min. Toluene was added to the combined extracts. The extract was concentrated by rotoevaporation and applied to a silica extraction column. Extracts were analyzed using an Agilent 6890 equipped with an FID and a SPB-1 fused silica capillary column. The results were expressed as milligrams of TPH and fractions per kilogram of dry weight soil (Table 3-1). Detection limits for each fraction were 10 mg/kg.(CCME, 2008).

Table 3-1 PHC levels Fractions and TPH (sum of fractions) for the 'Initial' 1% PHC-spiked soils sample (control and salt-impacted soil (SI)). TPH levels ~10,000 mg/kg.

Plant Type	F1 (mg/kg)	F2 (mg/kg)	F3 (mg/kg)	F4 (mg/kg)	TPH (mg/kg)
Non-planted (Control soil + spike)	12	3590	6700	244	10530
D. spicata (Control soil + spike)	14	3270	6300	230	9820
S. pectinata (Control soil + spike)	13	3260	5980	207	9450
Non-planted (SI soil + spike)	10	3220	6300	212	9710
D. spicata (SI soil + spike)	10	3600	6730	202	10530
S. pectinata (SI soil + spike)	11	3130	6010	207	9380
Mean	**12 ± 1.5**	**3340 ± 198**	**6330 ± 323**	**217 ± 16**	**9900 ± 514**

3.3.8.2 Soil chloride (Cl^-)

Chloride analysis was performed by extracting 5 g of soil with 25 mL of water and shaking on a horizontal shaker for 1 hour then filtering through Whatman No.42 filter paper. Filtered samples were analyzed by ion chromatography with a Dionex HPLC system (ICS 3000), using an AGU4A-SC guard column and an AS4A-SC analytical column.

3.3.9 Quality Assurance and Quality Control

3.3.9.1 Petroleum Hydrocarbons (TPH) analysis

Each batch (20 samples) of PHC soil samples were extracted and processed with one blank, one analytical spike, one matrix spike, and one analytical duplicate. Standards containing nC6, nC10, and toluene were run for F1 analysis. The F1s (nC6 to nC10) response factors were within 30% of response for toluene, which is within the acceptable range as defined by CCME. The F2s (nC10 to nC16) and nC34 response factors were within 10% of average. The C50 response factors were within the acceptable 30% of the average response factor of the nC10, nC16, and nC34 hydrocarbons. All analytical blanks were less than 10 mg/kg (below detection limit). The mean difference between the control standard and the control standard target was less than 20%; all mean relative standard deviation (%RSD) between the sample duplicates were less than 25%. Raw data can be found in Appendix A.

3.3.9.2 Soil chloride (Cl^-)

For all samples, one method blank and one Environment Canada certified reference material (CRM) Cranberry-05 were included for each batch of samples that were analyzed by ICP-OES. One analytical duplicate was also completed for every 10 samples that were analyzed. The mean relative standard deviation (% RSD) for the duplicate samples were all <1%. All of the blanks were less than detection limits and the quality control standard was within 5% of the target. The Environment Canada CRM Cranberry-05 was within 10% of the certified value for all analyses.

3.3.10 Statistical Analysis

Statistical analysis of the data was performed using XLSTAT statistical software for Microsoft Excel 2018. A two-factor analysis of variance (ANOVA) was performed to compare non-planted vs planted in each soil-treatment type for the TPH, fractions, and Cl^- levels using a significance level of $\alpha=0.05$, followed by a post hoc Tukey comparison.

3.4 RESULTS AND DISCUSSION

3.4.1 Plant Growth

Plant health in this experiment was evaluated based on visual observations of the plant's ability to excrete salt. The plants excreted salt continuously for the first 18 months of the 22-month experiment. Throughout the 22 months, some of the shoots turned brown and died yet there were still green shoots growing in the same pot. The dead material was trimmed away to promote new growth throughout the experiment. This cycle of plant growth and trimming away dead material continued for 18 of the 22-month experiment. At 18 months, the planters started to become root-bound and their ability to excrete was noticeably affected. By 20 months, all of the plant shoots of both species were nearly completely dead and brown. Figure 3-3 shows an example of a *S. pectinata* in PHC-spiked soil with dead material and healthy excreting shoots in the same planter.

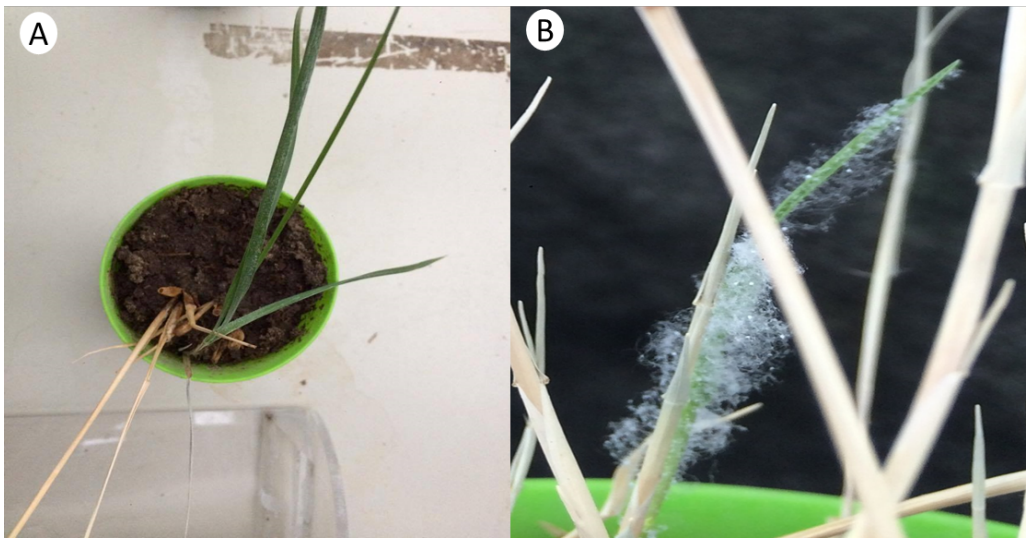

Figure 3-3 An example of dead shoots and healthy excreting shoots of a PHC-spiked A) *S. pectinata* and B) *D. spicata* plant grown in PHC-spiked soil. The dead material was trimmed away to promote new growth throughout the experiment.

3.4.2 Total Petroleum Hydrocarbons (PHC)

The initial TPH levels were 9900 mg/kg and significantly decreased by 63% and 64% over the course of the 22-month experiment to 3500 ± 700 and 3700 ± 700, respectively in control and salt impacted soils. TPH levels decreased significantly ($p<0.05$) in the first three months of the experiment and remained

unchanged for the remainder of the 22-months. There was no significant difference between the planted and non-planted controls indicating that the PHC decrease in the soil was not attributable to the enhanced microbial activity of the rhizosphere. Figures 3-4, 3-5, 3-6, and 3-7 show CCME F2 and F3 levels over the course of the experiment. While the overall results correspond with the total TPH results, it is interesting to note that the F2 fraction in the salt impacted soils was not significantly reduced until the six month sampling. Hence, while PHC remediation of the F2 fraction via volatilization and degradation by soil microbes was evident by six months, it is possible that the high salt content slowed this process down. This process is not yet fully understood, but the presence of salts may interfere with PHC degradation in the plant rhizosphere.

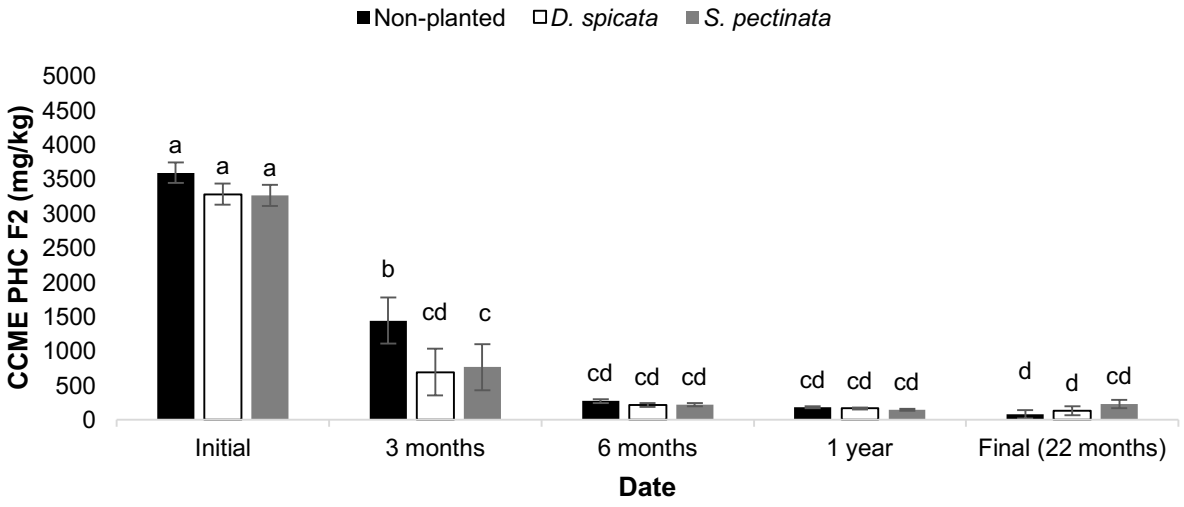

Figure 3-4 Fraction 2 (F2) levels (mg/kg) in control (background) soil spiked with diesel and oil lubricant. Significant differences are represented by lower case letters ($p<0.05$). There was a significant difference in F2 levels at 3 months between the non-planted controls and the plants indicating that this initial decrease was attributable to enhanced microbial effect of the rhizosphere.

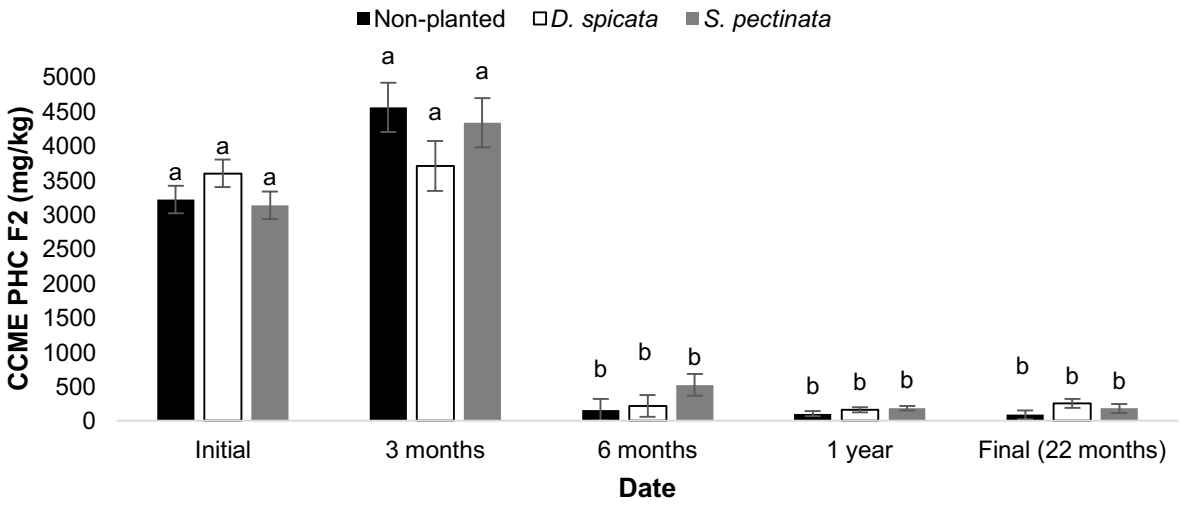

Figure 3-5 Fraction 2 (F2) levels (mg/kg) in KCl salt impacted (SI) soil spiked with diesel and oil lubricant. Significant differences are represented by lower case letters ($p<0.05$). There was a significant decrease in F2 levels at 6 months, however, there was no significant differences between the non-planted controls and the plants.

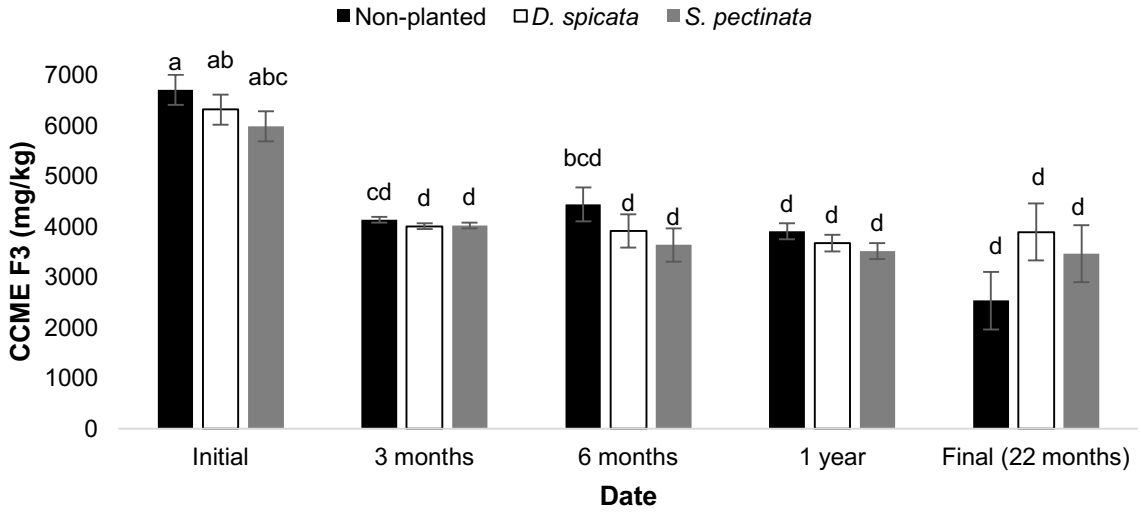

Figure 3-6 Fraction (F3) levels in control (background) soil spiked with diesel and oil lubricant. Significant differences represented by lower case letters ($p<0.05$). There was a significant decrease in F3 levels at 3 months, however, there was no significant differences between the non-planted controls and the plants.

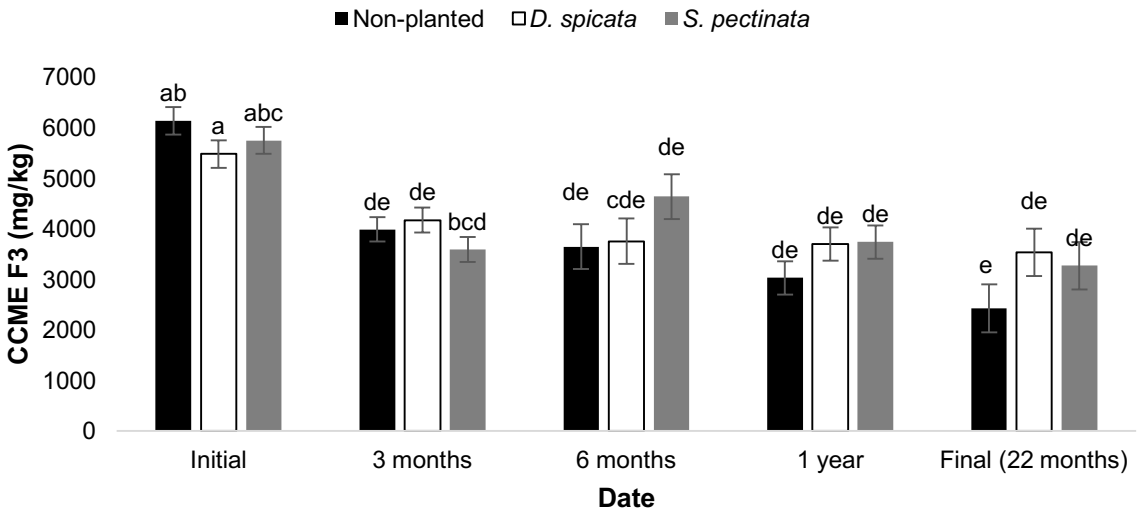

Figure 3-7 Fraction 3 (F3) levels (mg/kg) in KCl salt impacted (SI) soil spiked with diesel and oil lubricant. Significant differences represented by lower case letters ($p<0.05$). There was a significant decrease in F3 levels at 3 months, however, there was no significant differences between the non-planted controls and the plants.

3.4.3 Soil Chloride (Cl^-) levels

3.4.3.1 Control Soils

The initial mean chloride concentrations [Cl^-] of the control soils were 809 µg/g ± 233 µg/g; n=18. In the both control soils (non spiked and PHC-spiked soil), the Cl^- levels actually increased in the non-planted planters during the 22-month experiment. The figures representing this trend can be found in Appendix A. This increase is most likely the result of some of the excreted salt particles that became airborne and settled in the non-planted controls. The Cl^- levels reduced in the vegetated planters with *D. spicata* and *S. pectinata*, however, this decrease was not significantly different from the initial Cl^- levels.

3.4.3.2 Salt Impacted Soils

The initial mean [Cl^-] of the salt-impacted soils (both non-spiked and PHC-spiked) was 7987 ± 2942 µg/g; n=18. Some similar patterns emerged in both soil types during the course of the 22-month experiment. In the non-spiked soils, there was an approximate 23%, 85%, and 92% reduction in [Cl^-] in the non-planted, *D. spicata,* and *S. pectinata* planters, respectively. The [Cl^-] in the PHC spiked soils decreased by 35%, 75%, and 96% in the non-planted, *D. spicata,* and *S. pectinata* planters, respectively. In the non-spiked soil, both *D. spicata and S. pectinata* significantly reduced the [Cl^-] ($p<0.05$) during the 22-month experiment, however, only in the *S. pectinata* planted soils was the final [Cl^-] significantly different from the non-planted controls (Figure 3-8). In both soil types (non-spiked and PHC-spiked), *S. pectinata* significantly reduced the [Cl^-] ($p<0.05$) indicating that the Cl^- extraction and excretion capabilities of *S. pectinata* were not affected by the PHCs (Figures 3-8).

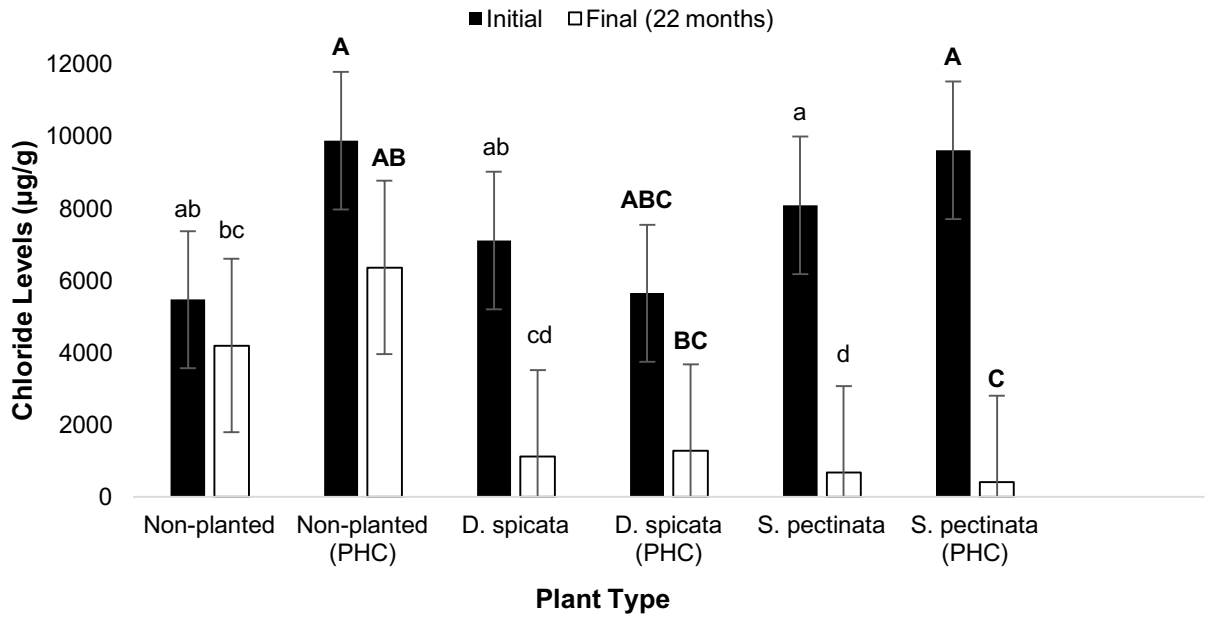

Figure 3-8 Chloride levels in both types of salt impacted soil (non-spiked and spiked). The soils spiked with petroleum hydrocarbons (PHCs) are labelled as such. Significant differences ($p<0.05$) in the non-spiked soils are represented by the lowercase letters, and by bold uppercase letters for the PHC spiked soils.

3.5 CONCLUSION

Both TPH and chloride levels were significantly reduced over the course of the experiment. There was no significant difference between the planted and non-planted controls indicating that TPH decrease was not attributable to the enhanced PHC degradation properties of the rhizosphere. F2 remediation was delayed in the salt impacted soils, indicating that the higher salt levels had an initial effect, possibly on hydrocarbon degraders in the soil. The plants remained healthy and actively excreted salt for 18 of the 22-month experiment. The salt excretion and haloconduction by *D. spicata* and *S. pectinata* contributed to a Cl^- reduction in the non-spiked salt impacted soils in this study, however, only *S. pectinata* was significantly different ($p<0.05$) from the non-planted controls. *S. pectinata* also contributed to a significant Cl^- reduction in the PHC-spiked salt impacted soil when compared to the non-planted controls. The Cl^- levels in the control soils were unchanged in the pots planted with *D. spicata* and *S. pectinata.* Sometimes, when salt levels are too low, recretohalophytes are unable to excrete any 'excess' salts; this appears to be the case for *D spicata* in this study. McSorely *et al.,* (2016a & b) showed that *S. pectinata*, was 160% more effective at removing soil Cl^- than other halophytes, which accumulate Cl^- in their plant biomass. Recretohalophytes like *S. pectinata* and *D. spicata,* have the potential to mobilize many tons of salt per hectare of land per annum via haloconduction (McSorely *et al.,* 2016b; Yensen and Biel, 2008; Yun *et al.,* subm). The rate of uptake and excretion of excess salt by recretohalophytes is dependent on several factors like weather conditions and humidity, but also by the conditions of the substrate in which they are planted. These findings demonstrate that a PHC spike of 1% does not interfere with the excretion capabilities of *D. spicata* or *S. pectinata*. Future studies will focus on similar experiments with sodium chloride. This is the first study to show that PHC levels of 1% do not inhibit the ability of *S. pectintata* to remediate Cl^- through excretion and haloconduction. Phytoremediation using *D. spicata* and *S. pectinata* may be a useful new technology for the remediation of salts from PHC impacted soils at oil or gas extraction sites.

4 CHARACTERIZATION OF EXCRETED SALT FROM THE RECRETOHALOPHYTES *DISTICHLIS SPICATA* AND *SPARTINA PECTINATA*

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4.1 ABSTRACT

Recretohalophytes are salt tolerant plants that excrete excess salts through specialized glands on their leaf surfaces. They have the potential to be applied as a phytotechnology to remediate salt impacted soils by removing the salt from the soil and allowing it to be mobilized on the wind (haloconducted) and hence dispersed over a wide area where the salt ions will act as nutrients rather than pollutants. This study is the first to characterize the excreted salts of two recretohalophytic grasses, *Spartina pectinata* and *Distichlis spicata* using scanning electron microscopy (SEM). At above optimal conditions for salt excretion (i.e. >65% humidity and >26 °C), salt appeared on the stem and leaf surfaces as a sap-like excretion that could not be easily mobilized. The mean diameter of the salt crystals excreted by *S. pectinata* (31 $\pm$ 24 µm) are significantly smaller than those excreted by *D. spicata* (49 $\pm$ 22 µm) ($p<0.05$). *S. pectinata* excreted significantly more salt crystals per unit area of plant surface (60 crystals $\pm$ 41 per 1 mm^2) than *D. spicata* (27 crystals $\pm$ 16 per 1 mm^2). These salt crystal characteristics can now be used to assist with determining the optimal species for haloconduction, and in particulate dispersal modelling systems to help determine the fate of the excreted salts once they become airborne by wind.

4.2 INTRODUCTION

Salt can impact soil through natural factors, such as weathering of minerals, and from anthropogenic factors, such as the improper landfilling of industrial waste material (Rietz and Haynes, 2003). Regardless of the source of salt, its buildup may result in soils classified as: i) saline, ii) saline-sodic, or iii) sodic (Rietz and Haynes, 2003). Plant health is affected by soil salinization and is expressed by a decrease in leaf surface area, improper gas exchange through the stomata which reduces the amount of absorption of CO_2, and decreased photosynthetic activities (Tavakkoli *et al*., 2010: Gupta *et al*., 2014).

Halophytes have evolved such that they can survive in salinized soil conditions. These plants which make up only ~2% of the world's terrestrial flora (Grattan and Greieve, 1998) have various mechanisms to tolerate saline soil conditions, one of which involves salt excretion. Excretory halophytes, also referred to as 'recretohalophytes' number approximately 370 species worldwide and have specialized glands (i.e. hydathodes) on their leaf tissues that excrete excess salts to maintain a steady metabolic state in salinized soils (Yuan *et al*., 2016).

In 2008, Yensen and Biel proposed the theory of haloconduction which hypothesizes that salt crystals excreted on the leaf surfaces of recretohalophytes may be mobilized into the air and redistributed across large areas. In this proposed process, a percentage of excreted salt that becomes airborne may move to areas of lower salt concentration thereby reducing the salt levels at an impacted site. This theory has not yet been proven in the literature, but it has the potential to be an environmentally-friendly, economical approach for the remediation of salt-contaminated soils, as salt could be continuously removed from the soil without intervention (i.e. plant harvest). The ions in some salts such as potassium chloride (KCl), are macro and micronutrients, respectively that are beneficial to organisms and hence are benign unless at high concentrations (Gupta *et al*., 2014). The excretion and subsequent dispersion of salts (i.e. KCl) by wind could in fact be beneficial by providing nutrients to other plant and animal species in the area, while reducing the total salt contamination level in site soils (McSorely *et al*., 2016a, b). Although salt dispersal from plants has never been documented, it is expected to be similar to the dispersal of sea salt aerosols which is well characterized (Madry *et al*., 2011), and has shown that some of these particles (0.1 to 400 μm in size) disperse hundreds of kilometres before settling (Morcillo *et al.,* 2000: Meira *et a*l., 2008).

McSorley *et al*., (2016a, b) and Yun *et al.*, (subm.) explored the theory of haloconduction through laboratory and field experiments by studying the relative mass of salt excretions on the stem and leaf surfaces of two recretohalophytic grasses, *Distichlis spicata* and *Spartina pectinata*. McSorely *et al*. (2016b) also imaged both plant species at varying magnifications using scanning electron microscopy (SEM) and used electron dispersive spectroscopy (EDS) to confirm that the salt crystals contained high levels of the dominant ions in the saline soil in which they were growing. To date, no information on salt particle size or distribution on plant surfaces has been undertaken. Hence, the goal of this study is to further recretohalophyte research by characterizing the excreted salt particles on the stem and leaf surfaces of *D. spicata* and *S. pectinata*, focusing on their relative size and density. This information will be essential as input parameters for modelling the dispersal of the excreted salts.

4.3 MATERIALS AND METHODS

4.3.1 Soil Description

Greenhouse studies were undertaken at the Royal Military College of Canada (RMC). The soil for these studies was obtained from a salt-contaminated site at the Lafarge Cement Plant in Bath, Ontario (79º48' Long 79º48' Lat) where a cement kiln dust (CKD) landfill caused elevated levels of K^+ and Cl^- to occur in soils (Yun *et al.* 2019). The soil has a silty clay loam texture and is considered saline-sodic, with an electrical conductivity ($EC_{1:5}$) of 2.6-20.5 dS/m (n=10) and a sodium adsorption ratio (SAR) of 15.4 (McSorely *et al*., 2016a). Chloride levels range from 1000 to 7780 µg/g (mean 3850 ± 1610 µg/g; n=41) which is one to two orders of magnitude higher than mean chloride levels in Canadian soils (~100 µg/g). Soil was collected, dried, and homogenized for this experiment (mean 7757 ± 2181 µg/g; n=6). Chloride is the most abundant inorganic anion in plant cells, with elevated concentrations (>20 mg/g dry weight) resulting in toxicity, thereby limiting plant growth, particularly in arid and semi-arid regions, and is hence the anion of concern in most soil salinization studies (Teackle and Tyerman, 2010), including this one.

4.3.2 Plant Acquisition and Maintenance

4.3.2.1 Spartina pectinata

Plugs of *S. pectinata* seedlings (eight weeks old, 15 cm in height) were obtained from Norview Gardens Ltd. in Norwich, Ontario. They were transplanted (one plant per pot), into the study soil (7757 ± 2181 µg/g) and grown to a height of ~40-50 cm with >15 shoots at the RMC greenhouse facility. A further nine *S. pectinata* plants measuring approximately 45 cm in height were obtained from the research site at the Lafarge Cement Plant in Bath, Ontario, and transplanted and maintained over several months at the same facility. Plants were selected for SEM imaging based on visual observations of their salt excretion in any given week.

4.3.2.2 Distichlis spicata

Five kilograms of *D. spicata* seeds were donated to RMC by BrettYoung, a seed production and distribution company based out of Calmar, Alberta. The seeds were germinated by submerging seeds in a petri dish of tap water and leaving them for 7-10 days, and then transplanted into potting soil in seedling trays. Once they were approximately five inches in height (~60 days), they were transplanted into the salt-contaminated soil (7757 ± 2181 µg/g) from the Lafarge field site and maintained over several months in the greenhouse facility at RMC.

4.3.2.3 Maintenance

Prior to SEM imaging, plants were placed on a covered plant stand where temperature and humidity could be controlled (Figure 4-1). SEM imaging was also conducted on plants that remained in the RMC greenhouse where humidity sometimes exceeded 80% and temperatures ranged up to 34 ºC.

Figure 4-1 Covered plant stands. The plants were rinsed, watered, and placed inside the stands for one week prior to imaging by SEM. The stands were encased with plastic wrap to prevent air disturbances. A grow light on a 12-hour cycle was provided to assist with growth and salt excretion. The conditions in the stands were considered optimal with relative humidities of 55-65% and temperatures of 22-26 °C.

4.3.3 Data Acquisition and Analysis

Data was collected from images (micrographs) of the salt crystals taken by scanning electron microscopy (SEM) using a Quanta 250 FEG scanning electron microscope. Only large plants (>15 shoots of ~40-50 cm in height) of *S. pectinata* and *D. spicata*, that had salt visible to the naked eye, were selected for SEM imaging. In the case of *S. pectinata*, these plants included some of those grown from plugs and transplanted into saline soil from the field site, and others harvested directly from the site. In all cases, the plants were carefully removed one at a time from the covered plant stand to avoid disturbing any excreted and accumulated salt on their surfaces, and leaf samples were cut into 1 cm pieces. Environmental scanning electron microscopy (ESEM) was used in order to preserve the crystalline shape of the salt crystals. ESEM allows imaging of biological specimens that are wet and uncoated in a high pressure chamber with an atmosphere of water vapor. ESEM, a gaseous secondary electron detector (GSED), and a cooling stage set to 5 °C, were used. 150 SEM micrographs from ~20 plant specimens (n=10 each species) were individually analyzed using Fiji data software (Schindelin *et al.,* 2012). Each individual salt crystal in each micrograph was measured along the straightest edges obtaining two measurements (length and width) per crystal (Figure 4-2).

Statistical analysis of the data was performed using Excel Stats 2016. The difference between the mean salt crystal diameters (μm) and the mean number of salt crystals per unit area of each plant species was tested using a two-sample t-test with a 95% confidence interval ($\alpha = 0.05$). The distribution of the data (crystal diameter and crystal density per mm^2) was tested using the Shapiro-Wilk normality test. Although the data was skewed and did not follow a normal distribution, the sample sizes were large. Over 2,000 diameter measurements were made and over >1000 crystals counted per plant species. Given this large sample size, a two sample t-test was statistically acceptable to analyze differences between the means in this study.

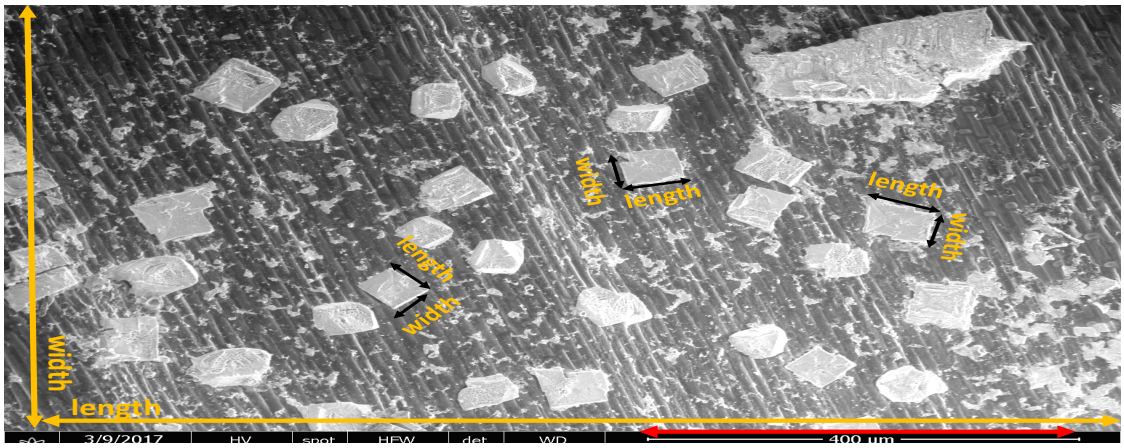

Figure 4-2 An example of a SEM micrograph measured using the line-tool in Fiji by Image J. The micrograph was obtained with a Quanta 250 FEG SEM, under low vacuum mode, using a low field detector (LFD). Three examples are provided, but all crystals were measured in each micrograph (n=33 here).

4.4 RESULTS AND DISCUSSION

4.4.1 Suboptimal vs Optimal conditions

Yensen and Biel (2008) predicted that under certain conditions, over 50% of excreted salt on recretohalophytes such as *D. spicata*, may become airborne, however, these conditions were never specified. Salt particles are more likely to travel longer distances in a crystalline form at lower humidity than as an aqueous-droplet at higher humidity. Prior to characterization of the discreet salt crystals, it was necessary to determine optimal conditions for observation. Plants with noticeable salt excretion, visible to the naked eye, were selected for SEM imaging in this study. The *D. spicata* and *S. pectinata* plants that were selected had >15 shoots and were ~40-50 cm in height, although only small <1 cm cuttings were used for SEM imaging. Preliminary observations of these plants under varying temperatures and humidity clearly indicated that there was a narrow range of optimal conditions. In particular, when temperatures exceeded 26 ºC and humidity exceeded 70%, visual observations of sap-like excretion, rather than discreet salt crystals, were consistently observed on the stem and leaf surfaces (e.g. Figure 4-3d). Clearly in these suboptimal conditions it was impossible to obtain images of discreet salt crystals.

To further define optimal versus sub-optimal conditions, a time lapse video was taken with the SEM (Figure 4-3) facilitating real time visualization of how temperature and humidity affect the size and shape of the excreted crystals. Figure 4-3 depicts two salt crystals (one larger and one smaller) undergoing a loss of crystalline structure as the humidity in the chamber increases from ~55% to >80% and then returns to ~55% humidity. Salt (KCl) is hygroscopic, thus, at higher humidity the salts will attract enough atmospheric water such that the crystals lose shape and become liquid.

From observing this video and 100s of SEM micrographs at varying temperatures and humidity, optimal conditions were determined to be 22-26 ºC and humidity of 55-65%. Plants and SEMs showing salt crystals at these conditions are shown in Figure 4-4.

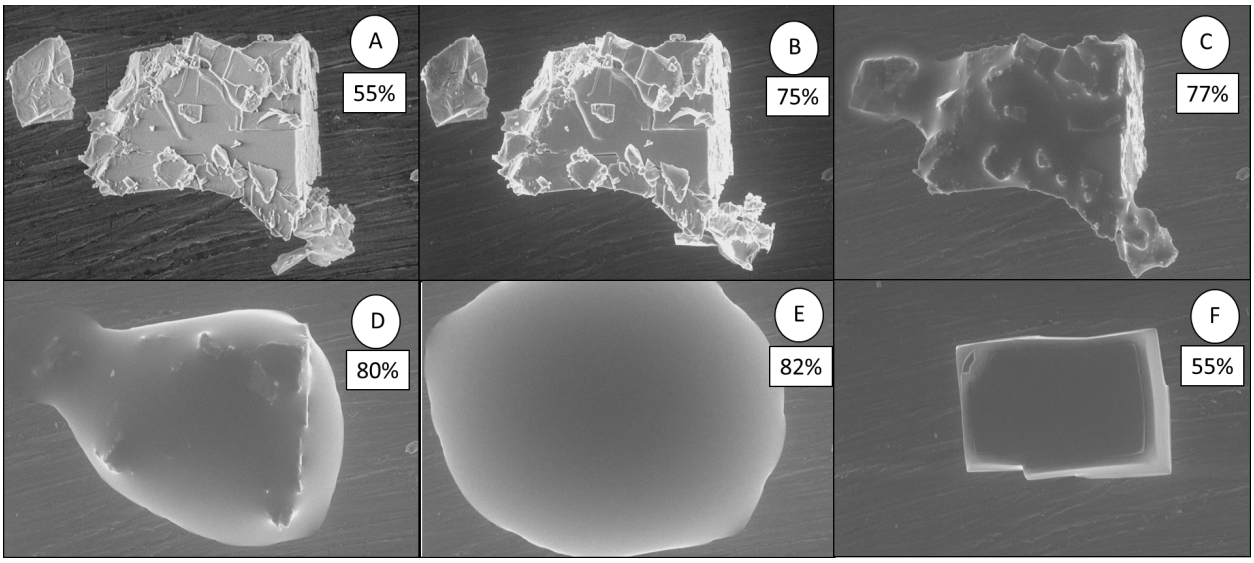

Figure 4-3 A time lapse of a salt crystal imaged by a Quanta 250 FEG scanning electron microscope (SEM), under Environmental scanning electron microscopy (ESEM) with a gaseous secondary electron detector (GSED). The humidity (%) in the SEM chamber was adjusted to various levels beginning at (A) ~55%, then increasing to (B) 75% and (C) 77%, (D) 80%, (E) >82%, and then again decreasing to (D) ~55%.

When the plants were in a covered plant stand within the range of optimal conditions, the crystals were well defined with straight edges that were easily measured with the tools provided in the Fiji software by Image-J (Figure 4-4). Under these conditions, the salts formed a 'cloud-like' structure resembling cotton candy (Figure 4-4A and B) that was easily disturbed with any air moved past the plant and it was not difficult to envision how widespread dispersal of the salts might take place if the plants were exposed to wind.

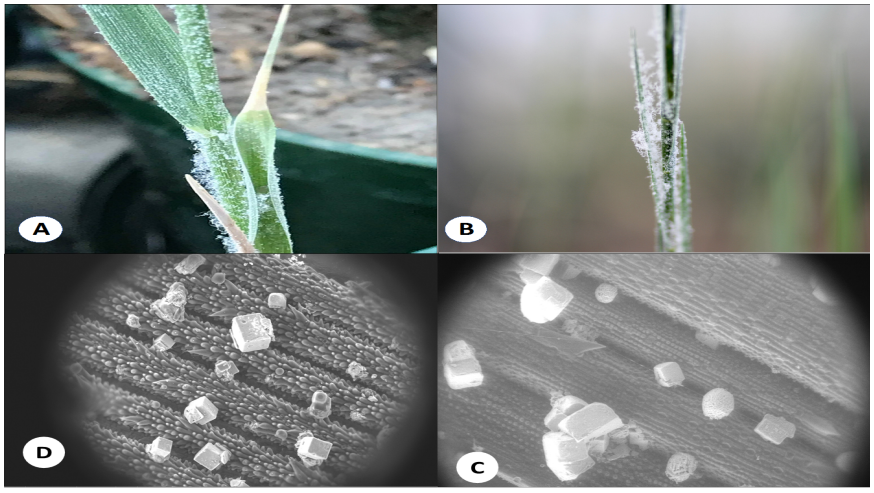

Figure 4-4 Salt excreted and crystalized under optimal conditions (i.e. 22-26 ºC and humidity of 55-65%) on the leaf surface of (A) *S. pectinata* and (B) *D. spicata* growing in the RMC greenhouse. SEM micrographs of (C) *S. pectinata* and (D) *D. spicata* with clear discreet salt crystals.

4.4.2 Particle Size and Density

Using the optimal conditions determined in section 'Suboptimal vs Optimal conditions', a total of 109 SEM images for *D. spicata* and 41 for *S. pectinata* were analyzed, allowing for 2047 and 2556 diameter measurements for *D. spicata* and *S. pectinata* crystals, respectively. There was a large range in the salt crystal diameters for both plant species. For *D. spicata* the crystals ranged from 5-188 μm (median = 45 μm) and for *S. pectinata* they ranged from 1-254 μm (median = 25 μm). The smallest observed crystals may be fragments of larger crystals detached during humidity and temperature fluxes, while the larger crystals are likely formed by the merging and subsequent evaporation of more than one saline droplet excreted by the bicellular hairs on the leaf surfaces. Although there was a large range in the diameters measured, some of these values were outliers. All of the reported outliers occurred at the upper bounds of the data. These observations were not dropped in calculating the mean as they represent how large crystals can be formed during humidity changes. The mean diameter for *S. pectinata* was 31 $\pm$ 24 μm, which was significantly smaller ($p<0.05$) than the mean salt crystal diameter of 49 $\pm$ 22 μm measured on *D. spicata* (Figure 4-5).

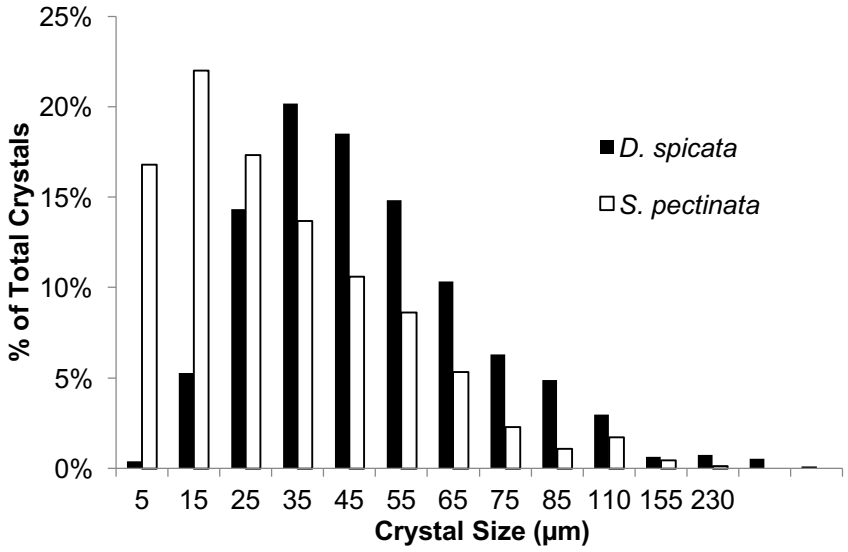

Figure 4-5 Distribution of salt crystal diameters imaged on the leaf surfaces of *Distichlis spicata* and *Spartina pectinata*.

The mean image areas on the SEM micrographs for *D. spicata* and *S. pectinata* were used to extrapolate the number of salt crystals excreted per unit area. The number of salt crystals per 1 mm^2 ranged from 6-132 (median = 23) and 12-129 (median = 44) crystals for *D. spicata* and *S. pectinata,* respectively. *S. pectinata* was observed to excrete significantly more mean salt crystals per unit area (60 $\pm$ 41 crystals per 1 mm^2) than *D. spicata* (27 $\pm$ 16 crystals per 1 mm^2) (Figure 4-6).

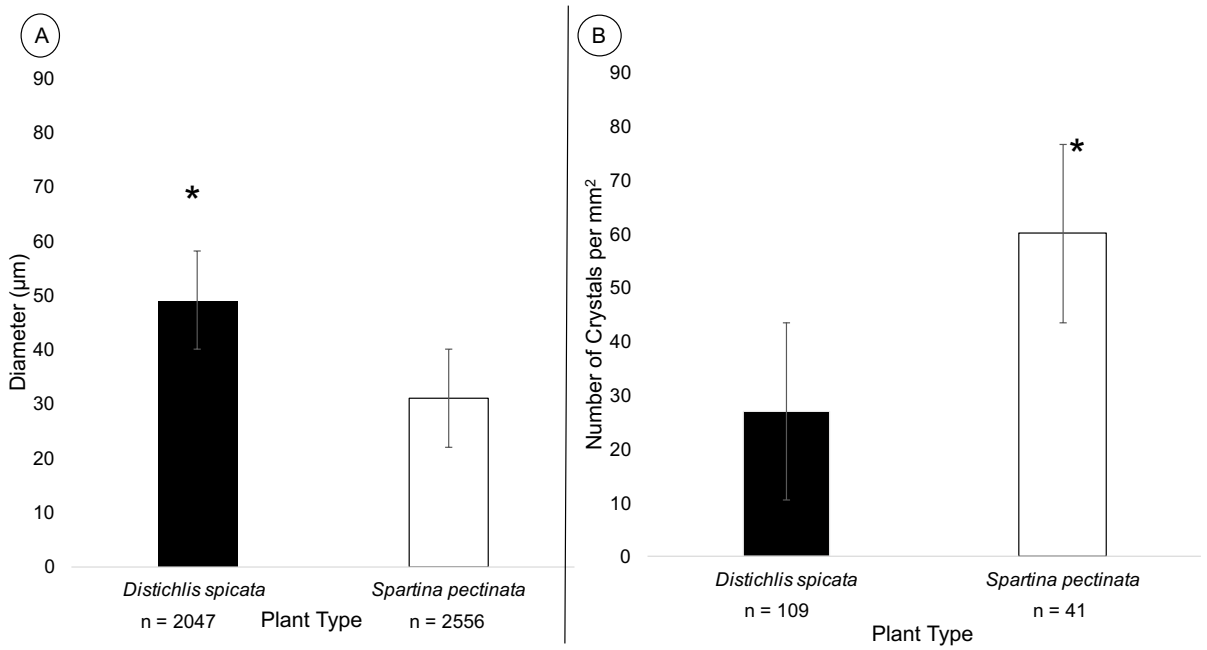

Figure 4-6 A) Mean salt crystal diameters, and B) number of salt crystals per unit area on the leaf surfaces of *Distichlis spicata* and Spartina pectinata. Significant differences are indicated by the asterisk.

4.5 OTHER FACTORS AFFECTING SALT EXCRETION

Several factors may contribute to the rate of salt excretion including concentration and composition of saline ions present in the growth substrate, time of day, temperature, humidity, salt gland density, and leaf size (Ceccoli *et al*., 2015). Oi *et al.,* (2014) researched the salt glands of Rhodes grass and found that, providing that the concentration of salt in the substrate is high enough, the salt glands appear to excrete the salt solution continuously for a long period of time even after the salt is wiped off of the plant surface. This study found that to be also true for *S. pectinata* and *D. spicata.* The bicellular hairs (microhairs) on both *S. pectinata* and *D. spicata,* which excrete the excess salts, occur individually rather than in clustered groups, and it is possible for the number of salt glands (density) to increase in response to salt concentration (Naz *et al*., 2009). The salt (Cl^-) concentrations of the soil used for this experiment (7757 ± 2181 µg/g; n=6) were high and thus these bicellular glands would emerge and continually excrete salt, even after the leaf surfaces had been wiped clean. The salt particles in both droplet and crystalline form can move on the leaf surface and thus it is possible to see excreted droplets and crystals together on the same surfaces at various temperatures and humidity levels.

4.6 CONCLUSION

Under optimal conditions, the salt crystals of *S. pectinata* leaves were both significantly smaller and more densely packed than those on *D. spicata* ($p<0.05$) leaves. We may be able to use characteristics and behaviours from sea salt aerosols (SAA) to further our work with haloconduction as SSAs range in size from 0.1 to 400 μm and the size of the salt crystals measured in this study fall within this range. Kreindenwies *et al.,* (1998) reported that in clean air masses (inland), SSA concentrations vary from 50 to 100 particles per cm^3. In the proper temperature and humidity conditions, smaller salt particles can travel further distances by wind, hence *S. pectinata* is likely to be a better plant for the phytoremediation of salt impacted soils. Furthermore, *S. pectinata* grows taller (up to 2 m) than *D. spicata* and thus interacts with more wind, thereby having the ability to disperse salt over a further distance than *D. spicata* which grows to a maximum height of ~0.5 m. Combining meteorological data with results from airborne salt collection and the input parameters determined in this study, it will now be possible, for the first time, to model the distance and deposition location of salt excreted by *D spicata* and *S. pectinata*. These findings are important for the implementation of recretohalophytes and haloconduction for salt remediation.

5 MOBILIZATION AND COLLECTION OF EXCRETED SALT PARTICLES FROM *DISTICHLIS SPICATA* AND *SPARTINA PECTINATA* IN A WIND TUNNEL

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5.1 ABSTRACT

Some halophytes tolerate saline soil using a tolerance mechanism known as excretion. These plants are known as recretohalophytes and have specialized salt glands that excrete excess salt onto their leaf tissue. According to the haloconduction theory, a percentage of salt excreted by recretohalophytes may be mobilized into the air and dispersed over a large area. This study investigated the salt dispersal capabilities of two recretohalophytes, *Distichlis spicata* and *Spartina pectinata* under controlled laboratory conditions. A wind tunnel was designed and built out of clear Plexiglass to be able to visualize the effect of wind (supplied by a fan) on the plants. In its final iteration, the wind tunnel included a double layer of cheesecloth to collect salt dispersed to the end of the tunnel. After a 48 wind trial, salt was collected in the layers of cheesecloth, swabbed from the inner tunnel surfaces in 30 cm increments using moistened cotton swabs, and rinsed from the surfaces of the plants. A mean total of 34 ± 21 mg of salt (measured as Cl^- levels) was collected for *D. spicata* and 189 ± 54 mg for *S. pectinata*. Surprisingly, there was significantly more salt rinsed from the plants ($p<0.05$) than retrieved via swabbing the interior surfaces of the tunnel and washing from the layers of cheesecloth. The total amounts of salt retrieved in each of the trials was also significantly lower ($p<0.05$) than the amount of salt rinsed from comparably sized plants, leading to the conclusion that up to 70% of the salt blown from the plants escaped the cheesecloth at the end of the wind tunnel.

5.2 INTRODUCTION

Halophytes have specific mechanisms to tolerate saline soil conditions; - one of these mechanism is excretion. These 'recretohalophytes' of which there are ~370 species worldwide (Yuan *et al*., 2016), have specialized salt glands known as hydathodes on their leaf tissues that excrete excess salt. The haloconduction theory, introduced by Yensen and Biel (2008), postulates that a percentage of the salt excreted by recretohalophytes will be released from the plant into the air by the wind and dispersed over a large area. The consistent uptake, excretion, and mobilization of salt from recretohalophytes will allow the salt to move from areas of high concentration to areas of lower salt concentration. This method of salt transport is similar to that which occurs in coastal regions, where ~80% of inland salt arrives from wind currents carrying microscopic salt particles that originated from the ocean (Yensen and Biel, 2008). Two recretohalophytes that have proven to be successful in salt uptake and excretion are *Spartina pectinata* (prairie cord grass) (Helios *et al*., 2014; McSorley *et al*., 2016a & b) and *Distichlis spicata* (inland salt grass) (Yun *et al*. subm).

Yun *et al*. (subm) and Morris *et al*. (Chap 4 this document) determined that the salt excretion rates of these grasses is maximized in 'optimal conditions' where relative humidity is between 55-65% and temperatures are 22-26 °C. By placing the plants in a covered plant stand for one week the possibility of any physical disturbance that may mobilize the salt into the air was removed (Yun *et al*., subm.; Morris *et al*, Chap 4). Yun *et al.* (subm) conducted 'salt rinsing' experiments on small (<5 shoots of ~15 cm in height), medium (6-15 shoots of ~ 30 cm in height), and large (>15 shoots of ~50 cm in height) plants of *S. pectinata* and *D. spicata* in optimal conditions, and determined that large *S. pectinata* plants excreted significantly more salt than all other plant and size categories ($p<0.05$). In general, for both plant species the larger the plants, the more efficient it is at excreting salt.

To explore the theory of haloconduction, Yun *et al,* (subm) conducted field experiments to quantify and collect excreted salts from *S. pectinata* and *D. spicata* using cheesecloth mounts at varying distances from established field plots. These cheesecloth mounts were adapted from research by Lomas and Gat (1967) who collected airborne salt particles on a coastal orange grove using muslin. Yun *et al.,* (subm) found that their cheesecloth mount design was effective at collecting the airborne salt particles, but found that variability in humidity, wind direction and precipitation affected their results as the cheesecloth mounts were openly exposed to the elements.

The goal of the current study was to design and build a wind tunnel to demonstrate the salt dispersion from *S. pectinata* and *D. spicata* in a controlled laboratory setting. This work began in 2017 with the assistance of an undergraduate student (Brauer 2017). It was continued in 2018 with a second undergraduate student (Arnold 2018) and involved modifying Yun *et al.'s* (subm.) system to capture the dispersed salt in order to quantify it. Since 2017, the design of the Plexiglas wind tunnel has evolved through several iterations with each modification enhancing some design aspect. In its final iteration, the wind tunnel was used to determine the distance of salt dispersal at realistic wind speeds as determined at a local field site.

5.3 MATERIALS AND METHODS

5.3.1 Wind Tunnel

This is the first study to attempt to create an appropriate bench-top wind tunnel for recretohalophyte research and as such there was method development required. The wind tunnel was built out of Plexiglas such that it was possible to see the inside of the tunnel. A fan was used to generate wind which mobilized and dispersed the excreted salt crystals within the wind tunnel apparatus. The cheesecloth method of capturing salt (Yun *et al*., subm) was adapted for collecting the windborne salt in the wind tunnel.

5.3.1.1 First Design Evolution

The first evolution of the Plexiglass wind tunnel devised in 2017 (Brauer 2017) drew air from an open end across the plant to collect salt on three sheets of cheesecloth (Grade 50) located 30 cm behind the plant, and 30 and 90 cm in front of the plant (Figure 5-1). The inner surfaces of the tunnel were swabbed with moistened cotton gauze to collect any salt not captured in one of the three cheesecloth sheets. Of the total salt collected within the apparatus, 65% was from the cheesecloth and the other 35% was swabbed from the panels. It was determined that the three layers of cheesecloth were causing re-circulation of air within the wind tunnel. In addition, the diffusion of air through the cheesecloth sheets resulted in an inconsistent wind speed that ranged from 0.2-1.5 m/s. Yun *et al.,* (subm) reported wind speeds of ~4 m/s in their field study of *D. spicata* and *S. pectinata* and hence the same wind speed was desired for this study.

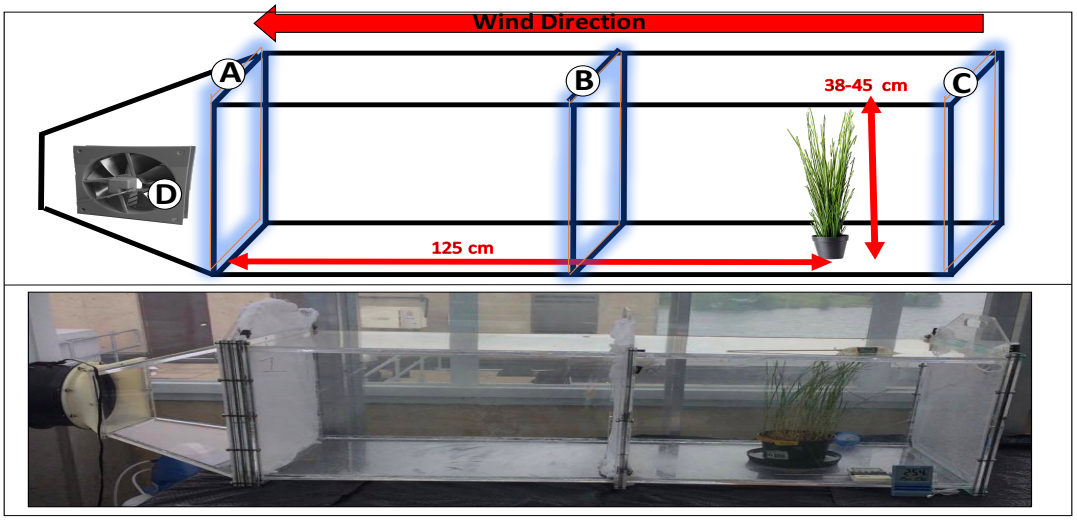

Figure 5-1 Schematic of the first wind tunnel evolution with three cheesecloth collection points (A), (B), (C). The fan (D) drew air through the wind tunnel from the open end of the tunnel at cheesecloth collection point (C).

5.3.1.2 Second Wind Tunnel Evolution

In 2018, the wind tunnel was modified by switching the wind direction, using fewer cheesecloth mounts, using higher grade (grade 80) cheesecloth, and expanding the overall length (Arnold, 2018). The wind direction was altered so that the plant was being exposed to the wind at a speed greater 1.5 m/s. Fewer cheesecloth mounts were used in an effort to reduce the amount of air diffusion and recirculation that occurred in the first evolution. Additionally, a higher grade cheesecloth was used so that the smaller thread spacing could capture more salt. Furthermore, the wind tunnel was expanded to assess the distances that these salt crystals could travel. Ultimately, each of the modifications presented further complications that needed to be addressed.

With this design evolution, the fan was incapable of reaching wind speeds similar to that of field conditions (~4 m/s). This was determined to be due to the fan capabilities and the rectangular geometric design of the wind tunnel. As the fan did not have an adjustable wind speed, a Variac (trademark name of a variable autotransformer) was used to adjust the voltage travelling to the fan, which in turn allowed adjustment of the wind speed. Unfortunately, due to inefficient diffusion and poor external geometry, the wind speed remained below the desired speed of 4 m/s. The inefficient diffusion was caused by the circular fan which was significantly smaller in cross sectional area than the square tunnel. When the airflow leaving the fan attempted to expand across the short length diffuser, it experienced air separation. This resulted in a turbulent flow at the test interface (the plant) and a decrease in airflow velocity throughout the tunnel. The corners of the square test section resulted in an additional disturbance to the flow field.

5.3.1.3 Final Wind Tunnel Evolution

The final design of the wind tunnel was completed in 2019. To compensate for the loss in wind speed within the tunnel, its internal geometry was rounded out using laminate flooring. In addition, a honeycomb condenser made from the same Plexiglas material as the wind tunnel, was used to centralize and straighten the airflow at the beginning of the tunnel. The condenser also allows for the airflow to be forced into small tubes which causes more airflow to be concentrated at the test interface (i.e. at the plant) (Figure 5-2). In this final design evolution, the windspeed was measured at ~4 m/s allowing the salt to be mobilized and collected in the cheesecloth fibers at the far end of the tunnel, 150 cm from the plant.

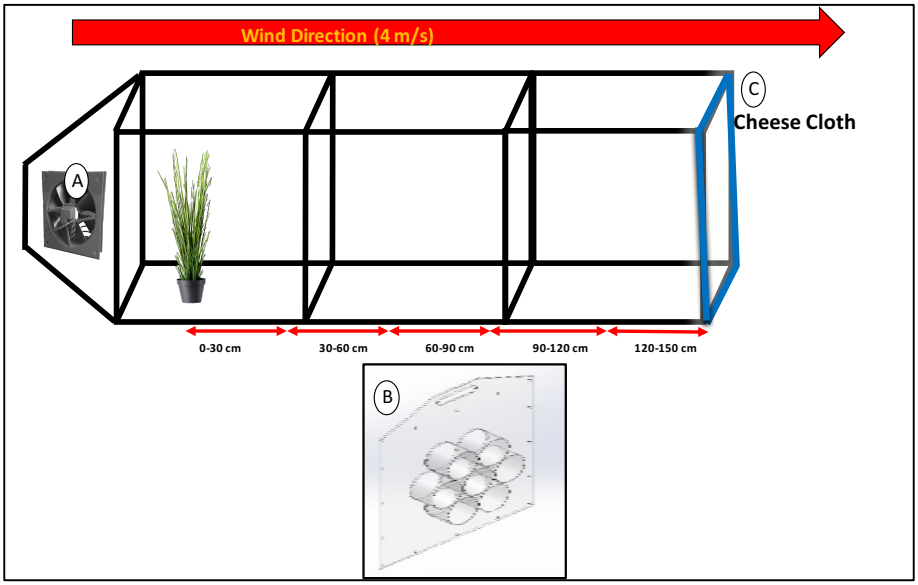

Figure 5-2 Schematic of the wind tunnel used in this experiment A Variac controlled the fan wind speed (A), an acrylic honeycomb design increased the wind speed to ~4.0 m/s fan. Salt was collected in a cheesecloth mounts (C) and swabbed from the inner surfaces in 30 cm increments.

5.3.3 Plant Acquisition and Maintenance

5.3.3.1 Spartina pectinata

Plugs of *S. pectinata* seedlings (eight weeks old, ~15 cm in height) were obtained from Norview Gardens Ltd. in Norwich, Ontario. They were transplanted (one plant per pot), into the study soil (7757 ± 2181 µg/g) and grown to a height of ~40-50 cm with >15 shoots at the RMC greenhouse facility. These plants were maintained over several months at RMC.

5.3.3.2 Distichlis spicata

Five kilograms of *D. spicata* seeds were donated to RMC by BrettYoung, a seed production and distribution company based out of Calmar, Alberta. The seeds were germinated by submerging seeds in a petri dish of tap water and leaving them for 7-10 days, and then transplanted into potting soil in seedling trays. Once they were approximately 12 cm in height (~60 days), they were transplanted into the salt-contaminated soil (7757 ± 2181 µg/g) from the Lafarge field site and maintained over several months in the greenhouse facility at RMC.

5.3.2 Experimental Design

Plants of *D. spicata* and *S. pectinata* with >15 shoots and a height of ~50 cm, were placed into optimal conditions (Morris *et al.,* Chap 4) for 1-week to allow for undisturbed salt excretion. The individual plants

were placed in a plant stand with the sides covered by plastic wrap (to reduce physical disturbances) and grown under a 12-h fluorescent photoperiod for one week, with relative humidity between 55-65 % and temperatures between 22-26 ºC. Plants were carefully removed to avoid disturbing any excreted and accumulated salt and then placed into the wind tunnel apparatus for a 48-h period.

5.3.4 Salt Collection Methods

5.3.4.1 Swabbing

The internal surfaces of the wind tunnel were swabbed using one gauze pad dampened with deionized (DI) water per 30 cm (Figure 5-3). The swabbing was carried out manually while wearing methanol sanitized latex gloves. Once the interior was swabbed, the gauze pads were placed into individual centrifuge tubes with 50 mL of DI water, and then refrigerated. Following this sampling procedure, the inside of the Plexiglas tunnel was sanitized with methanol three times, followed by DI water once, to prepare for the next trial.

5.3.4.2 Cheesecloth

Cheesecloth (80 grade, thread count = 40 x 32 threads/inch) was purchased from Nusso Textiles in Toronto, Ontario, CA. In this study, two 40 x 40 cm cheesecloth layers were mounted at the open end of the tunnel and were moistened with DI water (~25 mL) at the beginning of the trial and after 24-h to assist in catching the salt (Figure 5-3). After 48 hours, the cheesecloth was carefully removed without disturbing the accumulated salt, folding it in on itself and placing it a 1-L Mason jar. 100 mL of DI water was added to the jar. The jar was sealed and then manually shaken for one minute. The rinse water was then poured into a collection vessel and all of the water was manually wrung from the cheesecloth. The cheesecloth was rinsed two more times with 100 mL DI water each time and shaken for 1 minute for a total of 300 mL DI water and three minutes of shaking following the methodology of Yun *et al.,* (subm.).

5.3.4.3 Plant Rinse

Each plant was removed from the tunnel following the trial, and carefully inverted into a Ziploc bag and rinsed with DI water to remove the excreted salt. The volume of water used to rinse each plant was recorded such that the total mass of salt (Cl^-) collected could be calculated. Bags were sealed and stored in a fridge at 4 °C for future analysis.

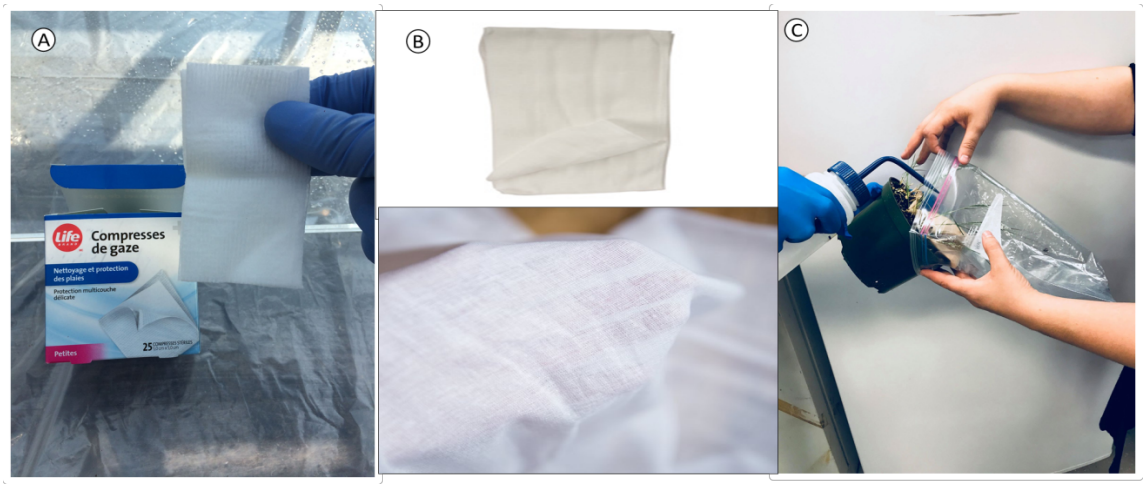

Figure 5-3 Salt was collected from within the wind tunnel by; (A) swabbing the inside at 30 cm increments, (B) capturing it in tightly woven grade 80 cheesecloth that allowed air to pass through while trapping salt in its fibres, and (C) rinsing any remaining salt off of the plant.

5.3.5 Sample Analysis

The soils used in this study were contaminated with high levels of potassium chloride (KCl). The elevated levels of potassium allow the soil to maintain a high K^+/Na^+ ratio (McSorley et al., 2016a and b), resulting in chloride being the main ion of concern. Chloride (Cl^-) analysis was completed at the Analytical Services Unit (ASU) at Queen's University. The total volume of each sample was recorded and a subsample of 10 mL was placed in a glass test tube before being analyzed via ion chromatography (IC) with a Dionex HPLC (High Performance Liquid Chromatography) system (ICS 3000), using an AG4A-SC guard column and an AS4A-SC analytical column. The column flow rate was set to 2.0 mL/min. Prior to analysis conductivity was measured. If the conductivity was measured at >100 µSv, the samples were diluted to the appropriate levels using double deionized water (DDW). A carbonate/biocarbonate eluent was used by diluting 10 mL of a prepared 100x concentrate of 1.8 mM Carbonate and 1.7 mM Bicarbonate solution into a 1 L volumetric flask with (DDW). Anions were separated according to their molecular weight and charge and were then detected using a thermal conductivity detector (TCD) (Rice *et al.,* 2012).

5.3.6 Quality Assurance and Quality Control

One method blank and one Environment Canada certified reference material (CRM) Cranberry-05 were included for each batch of samples that were analyzed by ICP-OES. One analytical duplicate was also completed for every 10 samples that were analyzed. The mean relative standard deviation for the duplicate samples were all <2%. All of the blanks were less than detection limits and the quality control standard was within the 10% of the certified value for all analyses.

5.3.7 Data Analysis

Statistical analysis of the data was performed using Excel Stats 2018. The differences between the mean total amount of salt collected (mg) between *D. spicata* and *S. pectinata* was analyzed using a two-sample t-test with a 95% confidence interval ($\alpha = 0.05$).

5.4 RESULTS AND DISCUSSION

5.4.1 Wind Tunnel Trials

A mean total of 34 ± 21 mg of salt (Cl^-) was collected after the 48-h trial for *D. spicata* and 189 ± 54 mg for *S. pectinata.* Seventy percent of the *D. spicata* salt and 90% of the *S. pectinata* salt was rinsed off of the plants, which was significantly more salt than what was collected from the internal swabbing and cheesecloth rinsing ($p<0.05$) (Table 5-1). For *D. spicata,* 8% of the collected salt was washed out of the cheesecloth and the remaining 22% was swabbed from the internal surfaces of the tunnel. For *S. pectinata*, 1% of the collected salt was washed from the cheesecloth and the remaining 9% was swabbed from the internal surfaces of the tunnel (Figure 5-4).

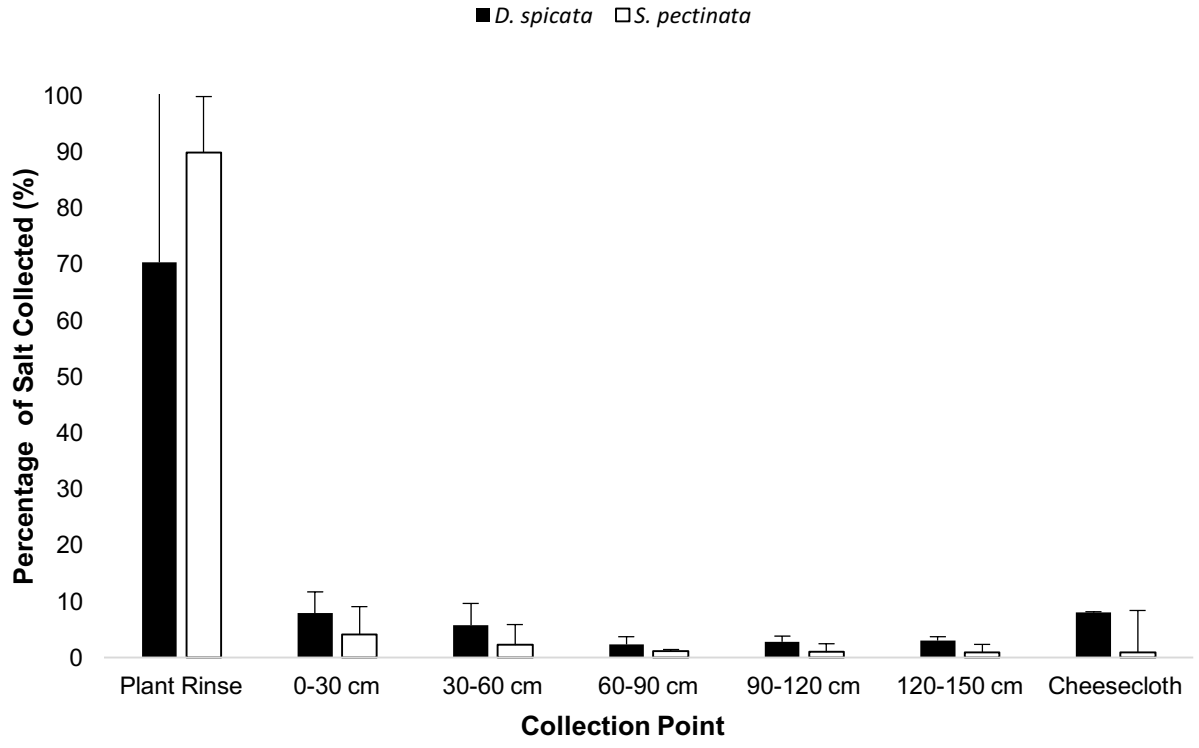

Figure 5-4 Percentages of salt collected in the plant rinse, at several distances along the wind tunnel and in the cheesecloth at the end of the wind tunnel for *D. spicata* and *S. pectinata*.

5.4.2 Salt Escape

5.4.2.1 Salt rinsing

Yun *et al.,* (subm) found that large *S. pectinata* plants on average excreted 589 ± 362 mg salt in a week, while large *D. spicata* plants excreted 143 ± 66 mg. The plants used in this experiment were within Yun et al.'s (subm) parameters for large plants (>15 shoots of ~50 cm), yet the total amount of salt collected from the plant rinse, cheesecloth, and swabbing combined was significantly less than that rinsed by Yun *et al.,* (subm) (Table 5-1).

Table 5-1 A comparison of chloride levels (mg) collected from comparably sized plants from this study (swabbed from wind tunnel surfaces, collected in cheesecloth, and rinsed from the plants) and from the study (rinsed from plants) by Yun *et al.,* (subm).

	Chloride Levels of 'Large' Plants (mg)	
Plant Type	**Wind Tunnel Trials**	**Yun *et al.*, (subm)**
D. spicata	34 ± 21	143 ± 66
S. pectinata	186 ± 54	589 ± 362

Having repeated the experiment multiple times, and having collected on average a total of only 32% of the amount of salt for *D. spicata* and 24% of that for *S. pectinata* in the wind tunnel as was previously collected by Yun *et al.* (subm) using comparably sized plants, it appears that many (or most) of the smaller salt crystals escaped through the two layers of cheesecloth at the end of the wind tunnel. Despite being dampened to prevent salt particle escape, the cheesecloth may have dried to a point where smaller salt particles were able to pass through the fine mesh. The cheesecloth used for the wind tunnel trials was 80 high-grade with 40 x 32 thread counts per square inch, which equates to a thread every 0.625 x 0.780 mm (625 x 780 µm). This thread spacing is larger than the mean salt crystal diameters of *D. spicata* (49 $\pm$ 22 µm) and *S. pectinata* (31 $\pm$ 24 µm) (Morris *et al*., Chap 4). Thus, it is reasonable to deduce that a large percentage of the salt may have been able to escape during the 48-h trial. For future trials, it will be important to use cheesecloth with a tighter weave, and hence smaller mesh size, to prevent any salt from escaping. Furthermore, trials should be less than 48-h to prevent the possibility of the dampened cheesecloth drying out and thus letting the airborne salt to escape through the thread spacing.

5.5 CONCLUSION

This study was the first to use a wind tunnel to investigate the salt dispersal capabilities of two recretohalophytic grasses *D. spicata* and *S. pectinata*. The design of the Plexiglas wind tunnel developed through numerous models with each amendment improving design features. In its final form, the wind tunnel was used to determine the distance of salt dispersal at wind speeds of ~4 m/s. Excreted salts of these recretohalophytes were able to be mobilized into the air via the wind generated from the fan and honeycomb air condenser, collected in cheesecloth and from the tunnel walls, and quantified using ion chromatography. This study marks the first attempt at collecting recretohalophyte salts under ideal conditions to assist with determining their impacts at a local field site. It contributes to the theory of haloconduction which has the potential to be a passive and effective way to remediate salt impacted soils. Future research should consider using a shorter trial length, as well as design modifications that include using more layers of (and higher grade) cheesecloth to capture all of the dispersed salts

6 CONCLUSIONS

Although salts are neither mutagenic nor carcinogenic they can have adverse environmental effects (Tavakkoli *et al.,* 2010). Some salt ions (e.g.: Na^+ and Cl^-) are toxic to plants and microbial communities at high concentrations. There are natural and anthropogenic ways that soils become salinized including the deposition of coastal sea salt aerosols (SAA), and the improper handling of industrial waste (Grattan and Greieve, 1999; Rengasamy, 2006). In crude oil extraction, geological formations may break, releasing the liquid trapped in the pores of the rocks. This liquid can contain many undissolved solids and large quantities of salts such as NaCl (Cook *et al.,* 2002). Some PHCs can be carcinogenic and can accumulate in soil during crude oil extraction. Chemical amendments and e*x situ* excavation and treatment are common ways to remediate PHC-contaminated soil, but these methods are quite invasive. A passive solution to remediating saline soil contaminated with PHCs is phytoremediation (Atlas *et al.,* 1981; Carty *et al.,* 2002).

Phytoremediation is a low cost, *in situ*, and sustainable alternative option to remediate environmental contaminants. It has been well established in the literature that some plant species have the ability to decrease PHC levels in the soil through a degradation process known as rhizoremediation (Rietz and Haynes, 2003: Das and Chandran, 2011). Microorganisms in the rhizosphere have catabolic enzymes that breakdown and use the complex PHC compounds as vital energy. From a phytoremediation perspective, it would be extremely beneficial to have a plant that could rhizoremediate PHCs from salty (salinized) soil as these two types of contamination coexist at oil extraction sites. Halophytes are salt tolerant plants that possess various mechanisms that allow them to survive the stresses imposed by saline environments (Flowers and Colmer, 2015). Recretohalophytes tolerate excess salts by excreting them through specialized glands on their leaf and stem surfaces (Schmer *et al*., 2012).

This thesis project investigated the ability of two recretohalophyte grasses, *Distichlis spicata* and *Spartina pectinata*, to simultaneously phytoremediate salts (measured as Cl^-) and PHCs. Cl^- is typically the anion of concern in salinized soil as excess Cl^- can be toxic to plants at high concentrations (Tavakkoli *et al.,* 2010). The effectiveness of PHC-reduction in chapter 3 of this thesis was measured by the change in total petroleum hydrocarbons (TPH). The two species were planted into potassium chloride (KCl) impacted soil and control (background) soil from a salinized research site on the property of the Lafarge Plant in Bath, Ontario. Both soils were spiked with a 1% diesel and 1% oil lubricant solution to contaminate them with PHCs to a TPH level of ~10,000 mg/kg. TPH decreased significantly in the first three months of the experiment, but did not decrease again during the rest of the 22-month experiment. There was no significant difference in TPH levels between the non-planted controls and the recretohalophytes, indicating that this TPH decrease was not attributable to the enhanced microbial effect of the rhizosphere. It is important to note, the F2 fraction in the salt impacted soils was not significantly reduced until the six month sampling. It is possible that the high salt content, initially slowed the degradation process down. In both of the salt impacted soils (non-spiked and PHC-spiked), *S. pectinata* was the only plant which significantly decreased Cl^- levels ($p<0.05$). It was determined that although the two selected recretohalophytes did not contribute to TPH degradation, *S. pectinata* remediated significantly more Cl^- in the salt impacted soils indicating that TPH levels did not negatively affect its salt excretion abilities. The study contributes important new information to recretohalophyte research as the interaction between PHCs and salt excretion capabilities of halophytes is not well established in the scientific literature.

This thesis also analyzed the excreted salt particles of the recretohalophytes to determine the mean diameter and number of crystals per unit area (mm^2). Optimal conditions for salt excretion were determined to be 55-65% relative humidity and temperatures of 22-26 °C. Above these thresholds, salt appeared on the stem

and leaf surfaces as a sap-like excretion (aqueous droplet) that would not be easily mobilized by wind. It was determined that the mean diameter of the salt crystals excreted by *S. pectinata* (31 $\pm$ 24 µm) were significantly smaller than those excreted by *D. spicata* (49 $\pm$ 22 µm) ($p<0.05$). *S. pectinata* also excreted significantly more salt crystals per unit area of plant surface (60 crystals $\pm$ 41 per 1 mm^2) than *D. spicata* (27 crystals $\pm$ 16 per 1 mm^2). Smaller salt particles can travel further distances by wind and therefore *S. pectinata* is likely to be a better plant for the phytoremediation of salt impacted soils. Furthermore, *S. pectinata* grows taller (as tall as 2 m) than *D. spicata* (as tall as 0.5 m) and thus interacts with more wind, thereby having the ability to disperse salt over a further distance than ground level *D. spicata*. These salt crystal characteristics can now be used in a particulate dispersal modelling systems to help determine the fate of the excreted salts once they become airborne by wind.

Finally, this thesis project examined the mobilization of excreted salt crystals under laboratory conditions using a Plexiglas wind tunnel. Several iterations of the design considered variables including: i) pulling vs pushing the air across the plants, ii) salt collection methods, and iii) optimizing wind speeds. In the final design, a generated wind from an open end at 4 m/s and that wind hit the plant and travelled through the tunnel. The open end of the wind tunnel was fitted with a moistened cheesecloth mount adapted from previous recretohalophyte salt collection research. The cheesecloth was a tightly woven grade 80 that allowed air to pass through while trapping salt in its fibers. The salt was collected from within the apparatus three ways; swabbing the inside at 30 cm increments, washing the moistened cheesecloth, and rinsing any remaining salt off of the plant. In these trials, there was significantly more salt ($p<0.05$) (measured as Cl^-) collected in the *S. pectinata* trials compared to *D. spicata.* This study marks the first attempt at collecting recretohalophyte salts under ideal conditions to assist with determining their impacts at a local field site.

Combining meteorological data with the results determined in this thesis, it is now possible, for the first time, to model the dispersion of salt at a field site planted with *D. spicata* and *S. pectinata*. Overall, the results from this thesis demonstrate that phytoremediation by recretohalophytes and haloconduction could be used to sustainably remediate salt-impacted soils. This study suggests that *S. pectinata* would be the more suitable recretohalophyte for the phytoremediation of salt impacted soils by haloconduction as its excretion capabilities were not affected by PHCs. Furthermore, *S. pectinata* excretes smaller and more densely packed salt crystals than *D. spicata*. Finally, *S. pectinata* grows taller than other ground level recretohalophyte grasses, like *D. spicata,* and thus can interact with more wind.

The number of global salt impacted sites has been increasing annually and is resulting in arable lands that are becoming unusable. A decrease in arable lands for food production threatens future food security. The constant uptake, excretion, and subsequent dispersal of salts by recretohalophytes may prove to be a useful form of salt remediation at some salt-impacted sites especially if the sites contain salts such as potassium chloride (KCl) or magnesium chloride (MgCl). Since potassium (K^+) and magnesium (Mg^{2+}) are macronutrients, recretohalophytes could effectively be diluting high salt concentrations through haloconduction, while dispersing fertilizer over a larger areas. These technologies may be utilized for the remediation of salt impacted roadsides, agricultural spaces, and industrial sites. Future recretohalophyte and haloconduction studies should consider using soil substrates with other salts (MgCl, NaCl, etc.) as well as various other recretohalophytic genera such as, *Atriplex, Limonium,* and *Avicennia.*

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8 APPENDICES

Appendix A

Raw Data for Chapter 3: Phytoremediation of Petroleum Hydrocarbons and Salt

Appendix B

Raw Data for Chapter 4: Characterization of Excreted Salt from the Recretohalophytes *Distichlis spicata* and *Spartina pectinata,*

Appendix C

Raw Data for Chapter 5: The Collection of the Excreted Salt Particles of Two Recretohalophytes *Distichlis spicata* and *Spartina pectinata,*

APPENDIX A

Raw Data for Chapter 3

The Rhizodegradation of Petroleum Hydrocarbons (PHCs) by the Recretohalophytes Distichlis spicata and Spartina pectinata.

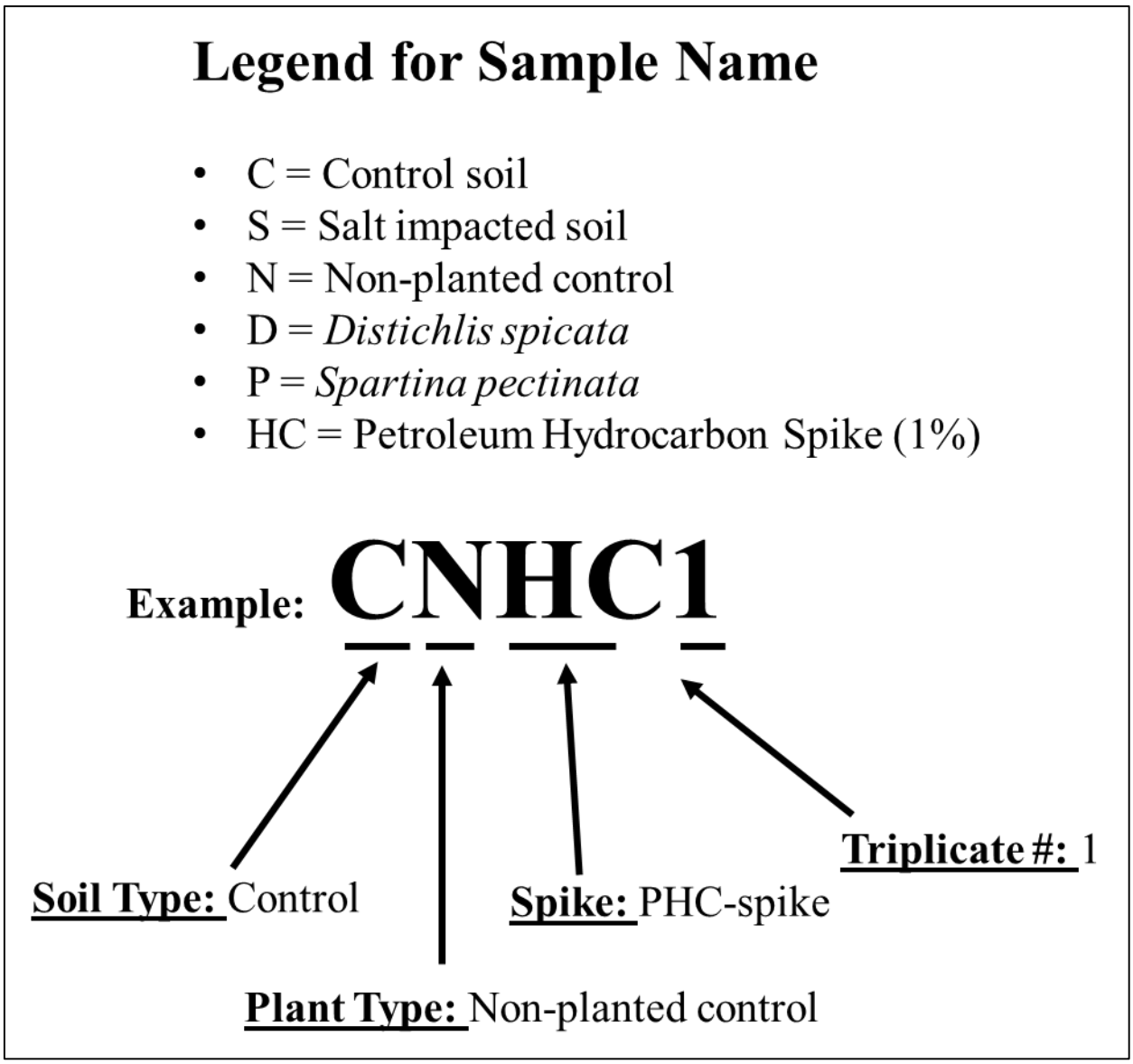

Figure A-1 Legend that describes the process in creating the sample IDs

Table A-1 Fractions and TPH (sum of fractions) for the 'Initial' 1% PHC-spiked soils samples. Quality assurance and quality control data are included at the bottom of the table.

Sample ID	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
CN1	13	3490	6070	226	9800
CN2	10	3600	6730	252	10600
CN3	13	3670	7300	253	11200
CD1	14	2740	5230	187	8170
CD2	15	3500	7010	244	10800
CD3	13	3580	6680	259	10500
CP1	13	2930	5470	190	8600
CP2	12	3110	5740	190	9050
CP3	12	3730	6720	242	10700
Mean	13	3372	6328	227	9936
SD	1	334	683	28	1022
SN1	10	2990	5940	194	9120
SN2	10	3200	6320	199	9720
SN3	10	3460	6640	243	10300
SD1	10	3490	6650	223	10400
SD2	10	3950	6670	176	10800
SD3	10	3350	6870	207	10400
SP1	10	2830	5430	171	8430
SP2	12	3650	6750	256	10700
SP3	10	2920	5860	195	9000
Mean	10	3316	6348	207	9874
SD	1	346	467	27	797
Laboratory QA/QC					
Blank	<10; <10; <10	<10; <10; <10	<10; <10; <10	<10; <10; <10	-
Control	136 ; 144 ; 129		138 ; 144 ; 126 ; 138		-
Control Target	154		158		-
%QC Recovery	88		87		

Table A-2 Fractions and TPH (sum of fractions) for the '3 month' 1% PHC-spiked soil samples. Quality assurance and quality control data are included at the bottom of the table.

Sample ID	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
CN1	10	1850	5020	141	7010
CN2	10	982	3830	90	4900
CN3	10	1480	3540	110	5130
CD1	10	769	4360	138	5270
CD2	10	603	4010	130	4740
CD3	10	690	3630	103	4420
CP1	10	687	3650	113	4450
CP2	10	870	4240	100	5210
CP3	10	725	4160	138	5020
Mean	10	962	4049	118	5128
SD	0	400	439	18	726
SN1	10	1190	2650	87	3930
SN2	10	990	2660	76	3730
SN3	10	2030	3870	109	6010
SD1	10	745	2150	57	2950
SD2	10	1010	3480	109	4600
SD3	10	746	2720	92	3560
SP1	10	642	2570	96	3310
SP2	10	1090	2320	59	3470
SP3	10	1770	4320	134	6220
Mean	10	1135	2971	91	4198
SD	0	446	700	23	1111
Laboratory QA/QC					
Blank	<10 ; <10 ; <10	<10 ; <10 ; <10	<10 ; <10 ; <10	<10 ; <10 ; <10	-
Control	135 ; 129 ; 126		140 ; 162 ; 163 ; 152		-
Control Target	154		158		-
%QC Recovery	84		98		-

Table A-3 Fractions and TPH (sum of fractions) for the '6-month' 1% PHC-spiked soil samples. Quality assurance and quality control data are included at the bottom of the table.

Sample ID	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
CN1	10	306	4470	391	5170
CN2	10	253	4530	284	5070
CN3	10	240	4290	274	4800
CD1	10	327	4860	366	5550
CD2	10	95	3120	210	3430
CD3	10	213	3740	238	4190
CP1	10	156	3150	215	3520
CP2	10	229	3770	286	4290
CP3	10	260	3970	282	4510
Mean	10	231	3989	283	4503
SD	0	67	572	58	684
SN1	10	114	3310	260	3680
SN2	10	221	3740	335	4300
SN3	10	137	3340	195	3670
SD1	10	228	4210	311	4750
SD2	10	247	4320	309	4880
SD3	10	164	3550	249	3960
SP1	10	311	5840	615	6770
SP2	10	220	3740	270	4230
SP3	10	290	4020	281	4590
Mean	10	215	4008	314	4537
SD	0	62	729	113	890
Laboratory QA/QC					
Blank	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	-
Control	135 ; 143 ; 136		143 ; 155 ; 146		-
Control Target	160		154		-
%QC Recovery	86		96		
CP1	-	156	3070	241	3467
CP1-D	-	156	3220	188	3564
%RSD	-	0.00	2.38	12.4	1.4
SP2-	-	185	2890	201	3276
SP2-D	-	255	4580	330	5165
%RSD	-	15.91	22.62	24.3	22.4

Table A-4 Fractions and TPH (sum of fractions) for the '1-year' 1% PHC-spiked soil samples. Quality assurance and quality control data are included at the bottom of the table.

Sample ID	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
CN1	-	157	3320	175	3650
CN2	-	107	3530	237	3870
CN3	-	263	4840	309	5410
CD1	-	130	2960	139	3230
CD2	-	76	3290	234	3600
CD3	-	277	4740	244	5260
CP1	-	154	3260	165	3580
CP2	-	68	3290	211	3570
CP3	-	195	3810	300	4310
Mean	-	159	3671	224	4053
SD	-	71	636	55	738
SN1	-	95	2440	110	2650
SN2	-	107	3290	199	3600
SN3	-	105	3260	207	3570
SD1	-	107	2970	125	3200
SD2	-	205	4560	283	5050
SD3	-	168	4220	316	4700
SP1	-	216	3710	189	4110
SP2	-	130	3470	171	3770
SP3	-	210	3530	255	4000
Mean	-	149	3494	206	3850
SD	-	48	596	65	688
Laboratory QA/QC					
Blank	-	<10	<10	<10	-
Control	-		170		-
Control Target	-		171		-
%QC Recovery	-		99		
SP3	-	126	3130	151	3407
SP3-D	-	134	2790	127	3051
%RSD	-	3.08	5.74	8.6	5.5

Table A-5 Fractions and TPH (sum of fractions) for the '22-month' 1% PHC-spiked soil samples. Quality assurance and quality control data are included at the bottom of the table.

Sample ID	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
CN1	-	306	4470	391	5170
CN2	-	253	4530	284	5070
CN3	-	240	4290	274	4800
CD1	-	327	4860	366	5550
CD2	-	95	3120	210	3430
CD3	-	213	3740	238	4190
CP1	-	156	3150	215	3520
CP2	-	229	3770	286	4290
CP3	-	260	3970	282	4510
Mean	-	231	3989	283	4503
SD	-	67	572	58	684
SN1	-	114	3310	260	3680
SN2	-	221	3740	335	4300
SN3	-	137	3340	195	3670
SD1	-	228	4210	311	4750
SD2	-	247	4320	309	4880
SD3	-	164	3550	249	3960
SP1	-	311	5840	615	6770
SP2	-	220	3740	270	4230
SP3	-	290	4020	281	4590
Mean	-	215	4008	314	4537
SD	-	62	729	113	890
Laboratory QA/QC					
Blank	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	<10 ; <10 ; <10 ; <10	-
Control	135 ; 143 ; 136		143 ; 155 ; 146		-
Control Target	160		154		-
%QC Recovery	86		96		
CP1	-	156	3070	241	3467
CP1-D	-	156	3220	188	3564
%RSD	-	0.00	2.38	12.4	1.4
SP2-	-	185	2890	201	3276
SP2-D	-	255	4580	330	5165
%RSD	-	15.9	22.6	24.3	22.4

Table A-6 Raw data for chloride levels (mg/L) and calculated concentrations (µg/g) in the control soils (non-spiked and PHC spiked). Soils spiked with hydrocarbons have HC in the sample name.

Sample ID	Initial (mg/L)	Initial (µg/g)	Final (mg/L)	Final (µg/g)
CN1	54[a]	1070[a]	300[c]	1430[c]
CN2	27[d]	395[d]	370[c]	1820[c]
CN3	24[d]	431[d]	350[d]	1670[d]
Mean	35	634	340	1640
SD	16	384	36	198
CD1	47[a]	944[a]	140[c]	695[c]
CD2	60[d]	1150[d]	120[c]	593[c]
CD3	90[d]	870[d]	120[d]	596[d]
Mean	66	988	127	628
SD	22	146	11	58
CP1	14[a]	1010[a]	130[c]	642[c]
CP2	49[d]	817[d]	140[c]	695[c]
CP3	120[d]	1110[d]	120[c]	593[c]
Mean	61	976	130	644
SD	54	147	10	51
CNHC1	41[b]	783[b]	300[c]	1480[c]
CNHC2	84[d]	586[d]	290[c]	1370[c]
CNHC3	82[d]	729[d]	290[d]	1430[d]
Mean	69	700	293	1430
SD	25	102	6	54
CDHC1	23[b]	455[b]	120[c]	592[c]
CDHC2	92[d]	955[d]	130[c]	634[c]
CDHC3	150[d]	897[d]	120[d]	592[d]
Mean	88	769	123	606
SD	64	274	6	24
CPHC1	87[a]	876[a]	120[c]	120[c]
CPHC2	32[d]	586[d]	120[c]	120[c]
CPHC3	81[d]	898[d]	110[d]	110[d]
Mean	67	787	117	547
SD	30	174	6	34

[a] Sample from Batch 1 (Analyzed at the ASU 14 February 2017)

[b] Sample from Batch 2 (Analyzed at the ASU 15 February 2017)

[c] Sample from Batch 3 (Analyzed at the ASU 15 November 2018)

[d] Sample from Batch 4 (Analyzed at the ASU 29 March 2019)

Table A-7 Raw data for the Initial and Final chloride levels (mg/L) and calculated concentrations (µg/g) in the salt impacted soils (non-spiked and PHC spiked). Soils spiked with hydrocarbons have HC in the sample name.

Sample ID	Initial (mg/L)	Initial (µg/g)	Final (mg/L)	Final (µg/g)
SN1	252[a]	4970[a]	770[c]	4060[c]
SN2	<0.05[d*]	<0.05[d*]	860[c]	4410[c]
SN3	730[d]	5950[d]	730[d]	4090[d]
Mean	491	5460	787	4190
SD	338	693	67	194
SD1	389[a]	7270[a]	280[c]	1280[c]
SD2	600[d]	7500[d]	120[c]	541[c]
SD3	650[d]	7160[d]	330[d]	1510[d]
Mean	546	7310	243	1110
SD	139	174	110	507
SP1	367[a]	7050[a]	94[c]	436[c]
SP2	430[d]	5770[d]	130[c]	554[c]
SP3	1300[d]	10920[d]	230[d]	1020[c]
Mean	699	7910	151	669
SD	521	2680	70	308
SNHC1	499[b]	9990[b]	1100[c]	5640[c]
SNHC2	370[d]	6620[d]	1400[c]	7150[c]
SNHC3	2600[d]	12990[d]	1200[d]	6260[d]
Mean	1160	9860	1230	6350
SD	1250	3180	153	758
SDHC1	322[b]	6030[b]	430[c]	2005[c]
SDHC2	710[d]	3870[d]	61[c]	285[c]
SDHC3	410[d]	7000[d]	310[d]	1480[d]
Mean	481	5630	267	1270
SD	204	1600	188	912
SPHC1	536[b]	11230[b]	51[c]	244[c]
SPHC2	290[d]	5490[d]	61[c]	287[c]
SPHC3	1900[d]	12060[d]	140[d]	671[d]
Mean	909	9600	84	400
SD	867	3570	49	235

[a] Sample from Batch 1 (Analyzed at the ASU 14 February 2017)

[b] Sample from Batch 2 (Analyzed at the ASU 15 February 2017)

[c] Sample from Batch 3 (Analyzed at the ASU 15 November 2018)

[d] Sample from Batch 4 (Analyzed at the ASU 29 March 2019)

[*] Indicates an outlier that was not used in the calculation of the mean

Table A-8 Quality assurance data (QC and CRM) of soil chloride (Cl^-) analysis by Ion-Chromatography for Chapter 3.

Quality Assurance/Quality Control	All values are (mg/L) unless otherwise stated		
Soil Chloride Batch 1			
Control (mg/L)	4.9 ; 5.1		
Control Target (mg/L)	5		
% of Target	98 ; 102		
Soil Chloride Batch 2			
Control (mg/L)	4.8		
Control Target (mg/L)	5		
% of Target	96		
Soil Chloride Batch 3			
Control (mg/L)	5.4 ; 5.4	Cranberry-05 (ppm)	36
Control Target (mg/L)	5.0	Cranberry-05 Target (ppm)	35
% of Target	108	% of Target	103
Soil Chloride Batch 4			
Control (mg/L)	5.4 ; 5.4	Cranberry-05 (ppm)	36
Control Target (mg/L)	5.0	Cranberry-05 Target (ppm)	35
% of Target	108	% of Target	103

Table A-9 Quality assurance data (duplicates and blank) of soil chloride (Cl^-) analysis by Ion-Chromatography for Chapter 3.

Quality Assurance/Quality Control		All results are in (mg/L) unless otherwise stated	
Soil Chloride Batch 1			
BNHC1-2	544	SP1-3	4756
BNHC1-2-D	544	SP1-3 D	4783
% RSD	0.0	% RSD	0.4
Soil Chloride Batch 2			
SD1-3	2679	SPHC1-3	6045
SD1-3-D	2675	SPHC1-3-D	6060
% RSD	0.1	% RSD	0.2
Soil Chloride Batch 4			
CPHC-2	34	FCN-3	330
CPHC-2-D	31	FCN-3-D	370
% RSD	6.5	% RSD	8.1
FSDHC-3	310		
FSDHC-3-D	310		
% RSD	0.0		

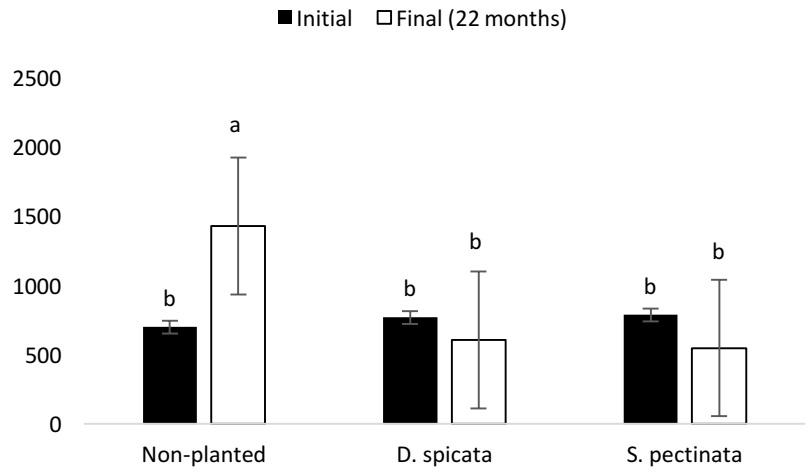

Figure A-2 Chloride levels in control soil. Significant differences represented by lower case letters ($p<0.05$). .

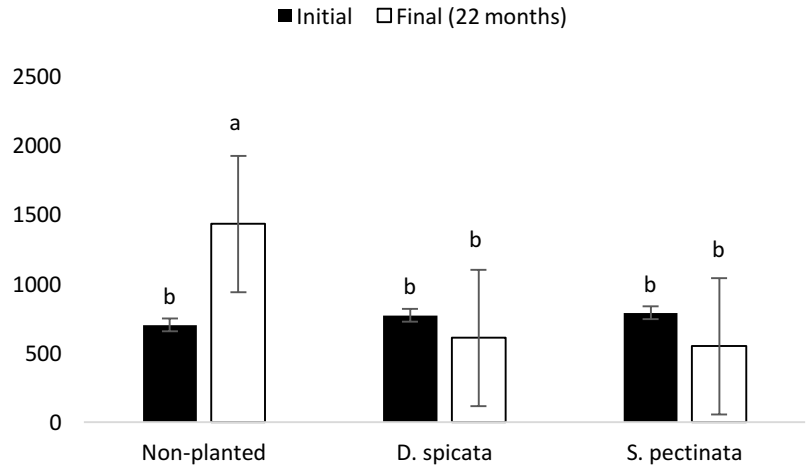

Figure A-3 Chloride levels in control soil spiked with diesel and oil lubricant. Significant differences represented by lower case letters ($p<0.05$).

APPENDIX B

Raw Data for Chapter 4

Characterization of Excreted Salt from the Recretohalophytes Distichlis spicata and Spartina pectinata,

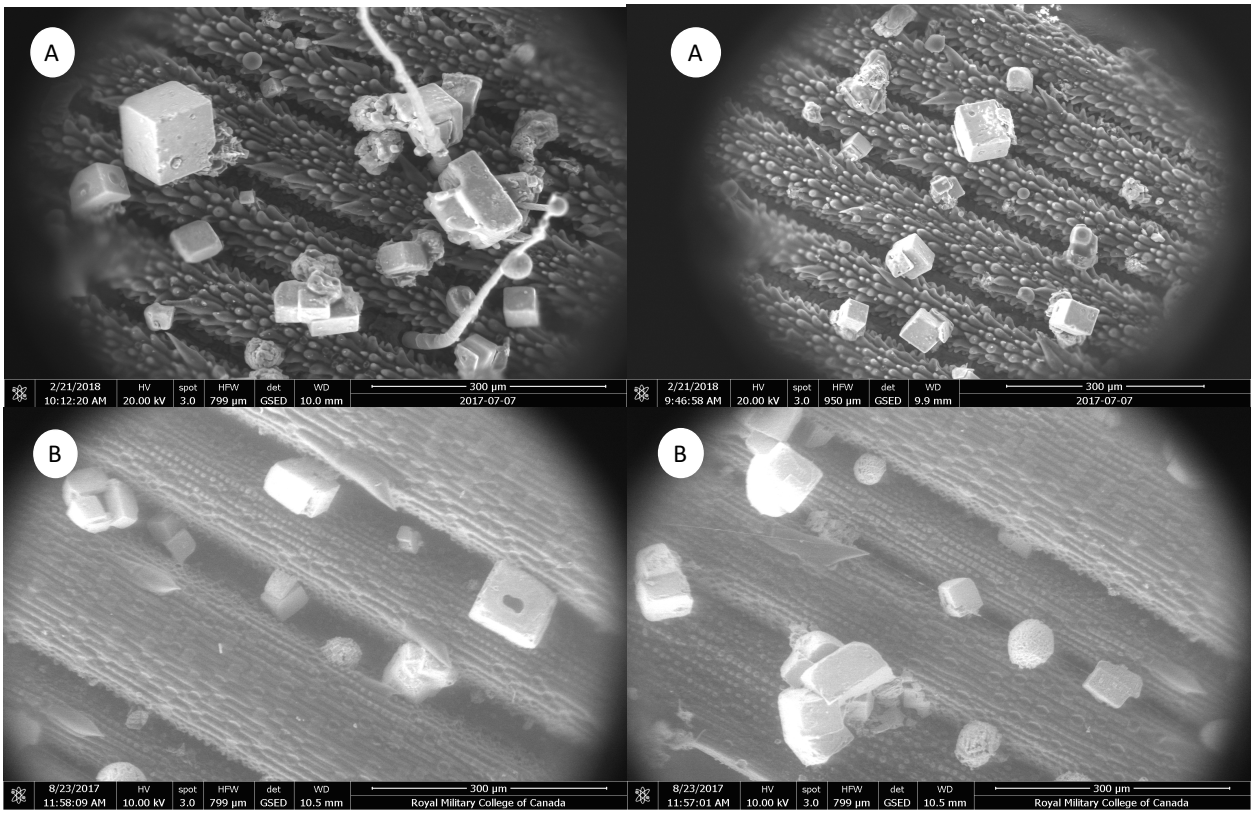

Figure B-1 Examples of photos with crystals that were measured under ideal conditions (A) *D. spicata* (B) *S. pectinata*.

Table B-1 Summary of salt crystal diameter measurements

	Diameter (µm)				
n, N	**Plant Type**	**Min**	**Median**	**Max**	**Mean**
109, 2048	*D. spicata*	5	45	188	49
41, 2557	*S. pectinata*	1	25	254	31

Conversion Factor Calculation of # crystals/ mm^2 = $\left(\frac{1}{(Mean\ Photo\ Area\ (\mu m^2) * \left(\frac{1\ mm^2}{10000000\ \mu m^2}\right))}\right)$

Spartina pectinata Conversion Factor = $\left(\frac{1}{((433472 \mu m^2) * \left(\frac{1\ mm^2}{10000000\ \mu m^2}\right))}\right)$

= 2.31

Distichlis spicata Conversion Factor = $\left(\frac{1}{((348881\ \mu m^2) * \left(\frac{1\ mm^2}{10000000\ \mu m^2}\right))}\right)$

= 2.87

Figure B-2 Sample calculation for the conversion factor used to extrapolate and calculate number of crystals per mm^2

Table B-2 Summary of salt crystal density per 1 mm^2.

	Diameter (µm)				
n	**Plant Type**	**Min**	**Median**	**Max**	**Mean**
109	*D. spicata*	6	23	132	27
41	*S. pectinata*	12	44	129	60

Table B-3 Summary of photo area, number of crystals per photo and the mean diameter of the crystals for *S. pectinata.*

Micrograph Name	Area (μm^2)	Number Crystals in the photo	Mean Diameter (µm)
Spectinata(1)	353794	40	19
Spectinata(2)	359404	46	16
Spectinata(3)	354404	8	43
Spectinata(4)	342609	52	19
Spectinata(5)	355440	11	48
Spectinata(6)	345988	45	19
Spectinata(7)	358490	11	41
Spectinata(8)	340082	56	19
Spectinata(9)	332327	7	65
Spectinata(10)	349245	55	17
Spectinata(11)	356946	49	16
Spectinata(12)	257137	48	19
Spectinata(13)	250841	53	14
Spectinata(14)	358453	44	19
Spectinata(15)	356222	25	36
Spectinata(16)	351898	10	34
Spectinata(17)	352363	10	40
Spectinata(18)	348446	10	41
Spectinata(19)	353969	10	35
Spectinata(20)	353968	8	42

Spectinata(21)	375067	49	22
Spectinata(22)	354567	12	35
Spectinata(23)	711875	31	63
Spectinata(24)	2836184	48	55
Spectinata(25)	102634	10	27
Spectinata(26)	371837	10	47
Spectinata(27)	385185	31	45
Spectinata(28)	387907	45	42
Spectinata(29)	406846	7	58
Spectinata(30)	414484	13	49
Spectinata(31)	415454	11	44
Spectinata(32)	414171	16	53
Spectinata(33)	424127	5	81
Spectinata(34)	481209	11	86
Spectinata(35)	349299	51	26
Spectinata(36)	344108	18	42
Spectinata(37)	323108	14	45
Spectinata(38)	379972	23	49
Spectinata(39)	377487	29	41
Spectinata(40)	380934	19	38
Spectinata(41)	703874	20	68

Table B-4 Summary of photo area, number of crystals per photo and the mean diameter of the crystals for *S. pectinata*

Micrograph Name	**Area (μm^2)**	**Number Crystals in the photo**	**Mean Diameter (µm)**
Dspicata-1	317731	4	58
Dspicata-2	422290	5	96
Dspicata-3	320397	4	65
Dspicata-4	305378	3	70
Dspicata-5	313361	7	86
Dspicata-6	464439	16	59
Dspicata-7	483617	18	48
Dspicata-8	448155	13	48
Dspicata-9	365247	8	57
Dspicata-10	371832	10	44
Dspicata-11	442378	11	47
Dspicata-12	445165	14	39
Dspicata-13	388715	8	57
Dspicata-14	397692	17	42
Dspicata-15	441171	16	47

Dspicata-16	370145	8	41
Dspicata-17	371368	7	42
Dspicata-18	362993	5	47
Dspicata-19	367103	8	55
Dspicata-20	380021	17	35
Dspicata-21	374982	10	58
Dspicata-22	372526	15	49
Dspicata-23	365130	15	44
Dspicata-24	357249	6	40
Dspicata-25	372917	5	50
Dspicata-26	370290	12	38
Dspicata-27	366290	14	43
Dspicata-28	376697	13	45
Dspicata-29	393200	13	40
Dspicata-30	376682	6	41
Dspicata-31	371439	6	46
Dspicata-32	373840	8	36
Dspicata-33	370163	8	49
Dspicata-34	377944	15	43
Dspicata-35	349578	7	43
Dspicata-36	357539	4	66
Dspicata-37	293795	3	50
Dspicata-38	176920	5	56
Dspicata-39	353344	6	40
Dspicata-40	261651	5	34
Dspicata-41	294834	4	30
Dspicata-42	347635	5	33
Dspicata-43	327543	4	46
Dspicata-44	43859	4	19
Dspicata-45	30690	9	17
Dspicata-46	122521	8	32
Dspicata-47	348353	8	55
Dspicata-48	353862	5	35
Dspicata-49	349874	8	40
Dspicata-50	154314	5	22
Dspicata-51	349900	4	28
Dspicata-52	499966	15	51
Dspicata-53	341320	3	46
Dspicata-54	495685	4	55
Dspicata-55	334104	7	57
Dspicata-56	326689	4	60

Dspicata-57	340107	2	73
Dspicata-58	340016	7	66
Dspicata-59	394327	7	52
Dspicata-60	387340	7	52
Dspicata-61	1499204	46	60
Dspicata-62	111362	8	45
Dspicata-63	347906	7	50
Dspicata-64	341679	4	40
Dspicata-65	348716	4	40
Dspicata-66	377239	17	48
Dspicata-67	323615	17	49
Dspicata-68	335151	12	51
Dspicata-69	240590	8	47
Dspicata-70	328560	10	55
Dspicata-71	467218	15	60
Dspicata-72	310598	10	53
Dspicata-73	321072	7	54
Dspicata-74	265089	9	48
Dspicata-75	285238	7	33
Dspicata-76	323988	9	42
Dspicata-77	327209	2	54
Dspicata-78	309326	11	53
Dspicata-79	304901	7	57
Dspicata-80	318513	11	50
Dspicata-81	323755	11	50
Dspicata-82	328496	8	53
Dspicata-83	316620	7	42
Dspicata-84	275923	8	44
Dspicata-85	299703	7	52
Dspicata-86	331303	12	49
Dspicata-87	331485	9	54
Dspicata-88	356057	11	56
Dspicata-89	350611	8	66
Dspicata-90	334064	5	45
Dspicata-91	292212	10	53
Dspicata-92	348974	7	40
Dspicata-93	339467	14	52
Dspicata-94	344781	17	65
Dspicata-95	351789	10	54
Dspicata-96	331421	16	41
Dspicata-97	340869	12	52

Dspicata-98	317392	11	47
Dspicata-99	263237	11	38
Dspicata-100	332312	10	62
Dspicata-101	357852	18	51
Dspicata-102	350310	11	56
Dspicata-103	350431	15	54
Dspicata-104	349137	10	51
Dspicata-105	294220	11	51
Dspicata-106	308576	11	42
Dspicata-107	344681	15	37
Dspicata-108	351949	18	49
Dspicata-109	348916	11	48

APPENDIX C

Raw Data for Chapter 5

The Collection of the Excreted Salt Particles of Two Recretohalophytes Distichlis spicata and Spartina pectinata.

Table C-1 Quality assurance data (QC and CRM) of chloride (Cl^-) analysis by Ion-Chromatography for Chapter 5.

Quality Assurance/Quality Control			
Batch 1			
Control (mg/L)	5.31	Cranberry-05 (ppm)	34.71
Control Target (mg/L)	5	Cranberry-05 Target (ppm)	35
% of Target	106	% of Target	99
Batch 2			
Control (mg/L)	4.8	Cranberry-05 (ppm)	33.3
Control Target (mg/L)	5	Cranberry-05 Target (ppm)	35
% of Target	96	% of Target	95
Batch 3			
Control (mg/L)	5.60	Cranberry-05 (ppm)	36.00
Control Target (mg/L)	5	Cranberry-05 Target (ppm)	35
% of Target	112	% of Target	103
Batch 4			
Control (mg/L)	5.4	Cranberry-05 (ppm)	36
Control Target (mg/L)	5	Cranberry-05 Target (ppm)	35
% of Target	108	% of Target	103
Batch 5			
Control (mg/L)	5.0	Cranberry-05 (ppm)	38
Control Target (mg/L)	4.7	Cranberry-05 Target (ppm)	35
% of Target	106	% of Target	109

Table C-2 Quality assurance data (duplicates and blanks) of chloride (Cl^-) analysis by Ion-Chromatography for Chapter 5.

Quality Assurance/Quality Control			
Batch 1			
M-1.5-3-2	11.06	M-1.5-CB-3	7.64
M-1.5-3-2-D	11.17	M-1.5-CB-3 D	7.38
%RSD	0.480	%RSD	1.71
Batch 2			
F-1.5-3-2	4.55	F-1.5-CB-3	5.02
F-1.5-3-2-D	4.67	F-1.5-CB-3-D	5.66
%RSD	1.25	%RSD	6.02
Batch 3			
2-C-6	50		
2-C-6-D	48		
%RSD	2.0		
Batch 4			
S2-7	5.90	S4-7	5.70
S2-7-D	6.10	S4-7-D	5.90
%RSD	1.67	%RSD	1.72
Batch 5			
S7-4	50	S9-7	5.50
S7-4-D	50	S9-7-D	5.60
%RSD	0	%RSD	0.90
All Blanks (mg/L)	<0.05		

Table C-3 Raw data of salt collected (mg/L) in the wind tunnel apparatus during the 48-h *Distichlis spicata* trial collected through swabbing, cheesecloth washing, and plant rinses. Most of the salt that was collected in these trials came from the plant rinse (70%) and only a small percentage of the salt was collected in the cheesecloth (8%).

	Wind Tunnel Trial Number								
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0-30 cm	130	230	140	180	200	120	110	97	170
30-60 cm	140	53	76	87	180	50	53	65	46
60-90 cm	38	36	36	35	43	20	55	24	76
90-120 cm	35	52	36	18	48	42	50	27	19
120-150 cm	44	28	34	36	32	23	49	35	27
Plant Rinse	370	320	730	320	660	580	370	400	540
Cheesecloth	5.4	6.0	5.4	5.8	5.1	5.8	5.5	5.4	5.6
TOTAL	762	725	1057	682	1168	840	691	654	884

Table C-4 Raw data of salt collected (mg/L) in the wind tunnel apparatus during the 48-h *Spartina pectinata* trial collected through swabbing, cheesecloth washing, and plant rinses. Most of the salt that was collected in these trials came from the plant rinse (90%) and only a small percentage of the salt was collected in the cheesecloth (1%).

	Wind Tunnel Trial Number								
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0-30 cm	18	18	63	95	94	67	26	89	19
30-60 cm	20	19	41	12	41	89	47	66	20
60-90 cm	17	15	16	14	14	18	13	15	18
90-120 cm	36	20	16	10	13	11	15	14	36
120-150 cm	17	15.5	20	14	32	42	12	17	18
Plant Rinse	16	16.1	230	75	62	49	150	190	17
Cheesecloth	20	20.4	3.5	5.1	3.4	4.9	3.5	0.74	21
TOTAL	148	124	390	225	259	281	270	390	150