FIELD TESTING THE EFFECTIVENESS OF USING HALOPHYTES GROWING IN BIOCHAR-AMENDED SOIL TO CAPTURE AND REMOVE SALT FROM HIGHWAY AND PARKING LOT STORMWATER RUNOFF

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Disclaimer

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
ASTM	American Society for Testing Materials
C	Celsius
Ca^{2+}	Calcium ion
CaCl	Calcium Chloride
CI Bounds	Confidence Interval Bounds
Cl^{-}	Chloride ion
cm	Centimeter
CMA	Calcium Magnesium Acetate
Cu^{2+}	Copper ion
EC	Electrical conductivity
ECe	Electrical Conductivity of a saturated soil extract
EPA	Environmental Protection Agency
ERA	Ecoregional Revegetation Application
ESP	Exchangeable sodium percentage
Fisher's LSD	Fisher's least significant difference
F-value	Ratio of two variances
GHG	Greenhouse gas
HB	House Bill
HNO_3	Nitric Acid (Hydrogen Nitrate)
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
K^{+}	Potassium ion
kg	Kilograms
m2	Square meters
mg/g	Milligrams per gram
mg/kg	Milligrams per kilogram
mg/L	Milligrams per liter
Mg^{2+}	Magnesium ion
MgCl	Magnesium Chloride
mM	Millimolar
Mn^{2+}	Manganese ion
N	Nitrogen
Na^{+}	Sodium ion
NaCl	Sodium Chloride
NOVA	Northern Virginia
p	Probability value
p-adj	Adjusted probability (*p*) value
pH	Potential of hydrogen
PLANTS (database)	Plant List of Accepted Nomenclature, Taxonomy, and Symbols

S	Siemens, e.g., mS/cm, dS/cm, & μS/cm
S^{2-}	Sulfur ion
SaMS	Salt management Strategy
SAR	Sodium absorption ratio
SO_4^{2-}	Sulfate ion
USDA	United States Department of Agriculture
VA DCR	Virginia Department of Conservation and Recreation
VDOT	Virginia Department of Transportation
VT	Virginia Tech
VT CRC	Virginia Tech Corporate Research Center
VTTI	Virginia Tech Transportation Institute
YSI	Yellow Springs Instruments
Zn^{2+}	Zinc ion
α	Significance level

EXECUTIVE SUMMARY

LEGISLATIVE IMPETUS

The Virginia Department of Environmental Quality (DEQ) was tasked with commissioning a study to "field test the effectiveness of using halophytes growing in biochar-amended soil to capture and remove salt from highway and parking lot stormwater runoff" per Virginia General Assembly HB29 Item 377 #2h for the Commonwealth's Natural Resources Budget for fiscal year 2022. In July 2021, DEQ engaged Virginia Tech to conduct this work via a one-year interagency contract with the primary stated objective of demonstrating at a field site in Virginia how halophyte cultivation and harvest may be implemented in real world scenarios to remove salt-based contaminants such as sodium and chloride from the roadside environment to prevent further degradation of critical water resources. It should be noted that this study was originally planned as a two-year project to account for the effects of working within natural systems and allowing perennial plants a sufficient amount of recovery time after transplanting. The research team has revised the originally conceived workplan to provide optimized results given the one-year shortened schedule.

BACKGROUND

More than 22.6 million tons of salt (primarily as sodium chloride [NaCl]) and other de-icing agents are applied annually to American roadways to maintain safety during inclement weather (Mullaney, 2009). These chemicals eventually wash from the pavement onto shoulders and into nearby soil and waterways. In soil, plant growth may be adversely affected by elevated salt concentrations and leaching may lead to contamination of groundwater and compromising of water supplies. Salt in surface water runoff adversely affects aquatic organisms and airborne salt particulates may impact nearby roadside vegetation, including crops. Promising alternative methods for de-icing and anti-icing application are constantly being developed and tested. However, for the foreseeable future there are no economically viable alternatives to NaCl.

OBJECTIVES

The work conducted in this study sought to establish proof-of-concept for recovery of applied de-icing salt through roadside phytoremediation using halophytic (salt loving) plants already, or intentionally, established in stormwater management structures near roadways. Phytoremediation is a widely demonstrated process that employs plants, associated microbes, sometimes in combination with soil amendment, to mitigate environmental contamination through sequestration, extraction, or chemical transformation. Biochar is evaluated herein as a soil amendment that may enable more effective capture of salt by halophytic phytoremediation. Biochar is a charcoal-like material that is a byproduct of low oxygen combustion processes such as those used to produce biofuels. Like charcoal, biochar has an exceptional capacity to absorb and retain certain types of chemicals. Biochar is typically added to soil as an amendment to increase soil porosity, water holding, and fertility.

Numerous types of plants have demonstrated a capacity to assimilate salt components into their tissues that can subsequently be harvested from the environment using standard agricultural practices. Unfortunately, the ability of halophytes to intercept salt that might otherwise enter the waterways from roads and parking areas washed by precipitation is significantly limited during the winter when these plants are dormant. This mismatch in timing between when deicer application and surface washing occur and when halophytes are active critically inhibits potential salt phytoremediation strategies. Biochar has been proposed for use as a soil amendment for halophytes where salt migrating from surface areas to receiving waters during the spring and winter is sequestered temporarily in the root zone until active halophyte growth in the warmer seasons allows uptake into plant tissues. Thus, the primary objectives of this study were to:

- Identify and characterize indigenous Virginia halophytes that may be used to recover salt constituents from pavement drainage conveyances and structures.
- Assess whether the addition of biochar to the soils in which halophytes are grown will enable timely capture of salts and potential subsequent removal through plant top harvest.
- Perform a Virginia-based field demonstration of a combinative system of halophytes and biochar for removal of salt constituents.

PROJECT APPROACH

A comprehensive literature review was conducted to inform the work that would be done in this study. This literature review was compiled from a recently completed student thesis and additional content obtained as part of this study. The full literature review is provided in the appendix and a summary is provided in the following section.

The primary project demonstration objectives were achieved using both field and laboratory study methods. Plant species growing in and around saline wetland and detention ponds at multiple sites in both Blacksburg and Northern Virginia (NOVA) were characterized to identify plants adapted to these unique environments. These surveys were conducted as part of this study for two reasons. The first is to identify candidate species for phytoremediation of road salts in stormwater detention basins. The best candidates are likely to be species that are already present in detention basins in Virginia, as these species are capable of surviving the myriad stressors these basins present, including, but not limited to, transient inundation, erosive flows, sedimentation, and toxicants. Of particular interest was the identification of species that are both native and salt tolerant, as planting such species has additional biodiversity benefits and supports local ecosystems. The second reason is to increase the likelihood that our field experiments in Blacksburg, focused on evaluating salt uptake by plants growing in detention basins (with biochar and without), will be successful. By conducting plant surveys first and using them to guide selection of the plants evaluated in our field experiments, we open the door to using locally adapted species in our experimental plots rather than young nursery specimens that are not guaranteed to establish and overwinter well. In effect, the experiment becomes more a measure of what established local plants can do from a phytoremediation standpoint (a principal project goal) and less about how plants assimilate salt when struggling to adapt to new conditions (not the intended focus of this study).

Field demonstrations were conducted at three natural sites in Blacksburg where commercially acquired biochar was used as a soil amendment in high saline runoff areas to temporarily sequester sodium ions and chloride compounds to increase removal rates and the overall effectiveness of phytoremediation. Native plant species already existing in situ, or introduced, were used in wetland or pond locations along with soil biochar augmentation in experimental configurations to account for varying site conditions, including salt loading, and to reduce any bias that might occur.

Biochar and other natural compounds that bind charged molecules like Na^+ and Cl^- were placed in environmental containment fabric sleeves or ‘socks’, to filter salt runoff in paved areas as a complementary salt removal strategy. This work was performed at the Virginia Tech CRC, Saunders Hall on the VT Campus in Blacksburg, and at Grants Pass, Oregon as part of a graduate student’s thesis work.

Experiments were also conducted in Virginia Tech lab under controlled conditions to determine the maximum amount of salt constituents that subject halophytes would intake and whether the presence of biochar would enhance or inhibit that process.

NOTABLE FINDINGS

1) Salt concentrations in stormwater collected in detention basins along highways in NOVA were higher than those measured in Blacksburg. The salt content (measured as electrical conductivity) in some systems was in excess of seawater.
2) Salt concentrations in detention basin soils only exceeded thresholds for salt-sensitive plants in NOVA systems.

3) Roadside stormwater facilities offer an opportunity for phytoremediation of salt:
   a) Cattail (*Typha latifolia*) accumulates significant Na^+ and Cl^- in their above ground tissues and are good candidates for phytoremediation where deicing salts accumulate.
   b) Yellow dock (*Rumex crispus*) is a prevalent exotic species listed as a low-risk invasive that showed potential to perform a useful salt removal service.
   c) Common rush (*Juncus effusus*), an identified potential salt accumulator, is salt tolerant but excludes salt and is not suited for phytoremediation.
   d) Across all detention basins surveyed, six plant species were identified as both native and salt tolerant that could be candidates for further phytoremediation studies (*Typha latifolia*, *Amelenchier canadensis*, *Andropogon virginicus*, *Elocharis acicularis*, *Calystegia sepium*, and *Parthenocissus quinquefolia*).
4) Over half of the plant species growing in and near ponds and wetlands where saline runoff collects were populated by plants classified as exotic, invasive, or of unknown native status in both NOVA and Blacksburg locations.
5) Biochar, manufactured clay beads, and hemp fibers used as filler in environmental containment fabric sleeves (also called socks) can bind significant amounts of sodium (all materials) and chloride (all save clay) and can filter saline water before entering parking lot drains and other environmentally sensitive areas.

LITERATURE REVIEW

A comprehensive literature review was performed as part of this project, and other previous work completed by the authors on this topic. A summary of literature review findings is included below. The full literature review is included herein as Appendix A.

SUMMARY

Roadway deicing salts are a source of ionic pollutants. Their application has the potential to negatively affect nearby ecosystems, human health, and transportation infrastructure. Since deicing materials are water soluble, movement from road surfaces into the surrounding environment in melt and rainwater is rapid. One possible solution is phytoremediation of salt-affected soils along roads and parking areas. Halophytes are plants that thrive in highly saline environments. Some plants can hyper-accumulate salt in their tissues effectively removing it from the environment. Efficient halophytic plants may accumulate as much of 20% of the dry weight as sodium. Other halophytes exclude salt and are not effective for phytoremediation. Phytoremediation is an inexpensive and sustainable approach to mitigating roadway salt pollution. Intentionally cultivating halophytes in salt contaminated soils along roads could reduce saline contamination, increase soil organic matter to improve soil health, and improve soil structure. There are many species of halophytes that are candidates for phytoremediation. Many halophytes are native to desert areas of the US where saline soils are common. However, these plants are not native to Virginia and not candidates for phytoremediation in the Commonwealth.

Another challenge is that plants are quiescent during the winter when deicing salts are most widely used and concentrations are at their highest. Studies have shown that deicing salts are leached away by spring rains and are lost into the environment before most plants are actively growing. One approach to this problem would be to enhance the ability of typically poor soils along roads to retain salts by adding soil amendments. Biochar is a lightweight, stable, carbon-based, sustainable material made by heating organic biomass to high temperatures in an oxygen-limited environment. Biochar is very stable and sequesters carbon in a form that doesn't contribute to greenhouse gases. Adding biochar to soils in roadway ecosystems should allow sodium and other ions to be held until halophytic plants are actively growing and can remove salts. Halophytes with high salt concentrations can be harvested and processed to recover salts and produce more biochar. Fuels can be recovered as a byproduct of the pyrolysis process that produces biochar (Figure 1).

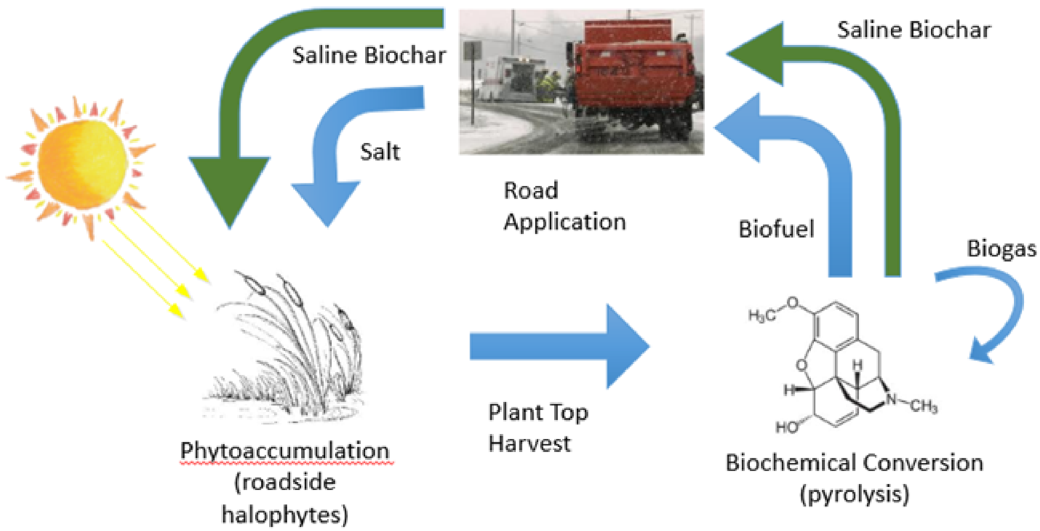

Figure 1. Conceptual Diagram of a Potential Deicing Salt Reclamation and Reuse Cycle.

For a plant-based salt recovery system to be sustainable, it must rely on native plants. Unfortunately, the list of halophytes native to Virginia is short. Many Virginia halophytes are native to coastal areas where saline soils are common. However, many of these species lack the winter hardiness to survive in western Virginia where deicing salts are most used. Two candidate native plants that have been identified for phytoremediation of deicing salts are cattail (*Typha latifolia*) and rush (*Juncus effusus*). Both species prefer wet soils and are well suited for runoff detention ponds and wetlands that collect roadway and parking lot runoff. In urban areas, phytoremediation in heavily paved areas is challenging because space for plants is limited.

In urban areas, environmental containment "socks" filled with natural materials that selectively bind salts could be strategically placed near drains and other areas of high runoff. As water flows through these porous socks, salts would be filtered out before water passes into storm drains. The socks would periodically be collected, and salts recovered to create a continuous recycling system. Significant quantities of deicing salts could be recovered using a combination of salt absorbing halophytes native to Virginia and salt collecting filtration socks in heavily paved areas with concentrated saline runoff. Informational programs could educate the general populations about the dangers of overusing deicing salts to reduce behaviors contributing to salt pollution.

NOVA FIELD ACTIVITIES

SITE DESCRIPTIONS

Site selection in northern Virginia was conducted using randomized stratified sampling approaches, specifically, hierarchical cluster analysis [package FactoMineR, R Core Team version 3.6.3; Le et al., 2008]. Hierarchical clustering was used to group all known detention ponds in Fairfax County (as of 2017) into six categories based on their drainage area classification (i.e., reflecting acres of open space drained, acres of parking lot drained, and acres of roadway drained). Drainage area classifications were sourced from drainage area layers provided by Fairfax County. These layers can be viewed using the County's Drainage Basin Delineation Tool [DBT, 2021].

Three of the six groups of sites identified using cluster analysis represented end members (i.e., clusters of sites that drained primarily open space, parking lots, or roads, respectively). Five sites were selected at random from each of these three end members as possible sampling locations. In the end, one of the road sites wound up being excluded from the sampling pool due to access constraints imposed by ongoing construction, leaving a total of four sampling locations in the road category. We also wound-up reclassifying one of the five sites purportedly draining open space, as a parking lot site due to the observation that drainage from a nearby parking lot backed up into the site during heavy rains. This left us with a final set of 14 sites, four draining open space, four draining roads, and six draining parking lots (Figure 2).

Figure 2. Photos of NOVA Research Sites

<u>Sites Receiving Runoff from Unpaved Areas (Open Space)</u>

Our sites draining open space include 0086DP (site name: Great Falls or GF), which is a 762m^2 detention basin located in Great Falls, VA that receives runoff primarily from 55,588 m^2 of fields and greenspace, including yards and a golf course, with minimal input from impervious surfaces, mainly residential parking lots (322 m^2). Our second open space site was DP0612 (site name: Camelot), which is a 408 m^2 basin located in Annandale, VA that receives runoff from a sports field (9,931 m^2), but not parking lots or roads. We also sampled BMP 0238DP (site name: Twin Knolls), a 349 m^2 basin located in Lincolnia, VA that receives residential runoff, primarily from yards and open space (3,850 m^2), with minor contributions from roads and parking lots (53 m^2 and 160 m^2, respectively), and BMP 1687DP (site name – Clermont), a 1,399 m^2 basin located in Alexandria, VA, which primarily receives runoff from yards and other pervious surfaces (21,338 m^2), with lesser contributions from roads and parking lots (604 m^2 and 669 m^2, respectively). None of these sites have more than 6% of their drainage area classified as impervious.

<u>Sites Receiving Parking Lot Runoff</u>

Our sites draining parking lots include two detention basins (DP0009, site name: Sully 1, and DP0008 – site name: Sully 2) located in a large shopping mall in Chantilly, VA. The first of these basins is 1,623 m^2, and principally drains the southern end of the mall parking lot (26,859 m^2), with some drainage from landscaping around the mall (8,587 m^2) and minor inputs from roads (90 m^2). The other basin is 1,513 m^2 and receives most of its runoff from the north side of the mall parking lot (17,307 m^2) as well as nearby landscaping (6,713 m^2). Our third parking lot site (DP0549 - site name Quarles) is a 359 m^2 detention basin, also located in Chantilly, VA. It receives runoff from 6,102 m^2 of parking lot in a small shopping center and 2,666 m^2 of nearby landscaping. Site four (DP0663 – site name: McLean) is a 529m^2 detention basin located in McLean, VA, that drains the parking lot (9,854 m^2) and landscaping (4,373 m^2) surrounding an office building. Site five (DP0680 – site name Uhaul) is a 305 m^2 basin in Lorton, VA, that drains 5,359 m^2 of parking lot where rental trucks are stored, 204 m^2 of roads, and 2,635 m^2 of pervious landscaping. Finally, site six (DP0061 – site name NOVA.W) is a 357 m^2 detention basin in Chantilly, VA that is intended to drain 5,281 m^2 of open space, but also winds up draining around 1,646 m^2 of parking lot when flows back up into it during large storms (i.e., this site is our re-classified open space site). The site is best thought of as a mixed site (i.e., the primary drainage area is pervious, but there are substantive (24%) parking lot contributions, significantly higher than the imperviousness present in sites belonging to our open space category).

Sites Receiving Roadway Runoff

Our sites draining roads are all managed and maintained by the Virginia Department of Transportation (VDOT) and receive runoff from major highway systems (i.e., I-95 N and S, and I-495 E and W). The first two sites (29I109, pond E – site name: VDOT1, and 29I101, pond F – site name: VDOT2) are large extended detention basins (2,804 m^2 and 1,228 m^2 in size, respectively), located in Alexandria, VA. VDOT1 primarily receives runoff from I-95N and I-495E (i.e., it lies at the intersection of the route 241 onramp and these interstates; highway area drained = 13,881 m^2). It also receives runoff from 6,677 m^2 of open space. VDOT2 primarily receives runoff from the opposite side of this interchange (i.e., it lies on the opposing cloverleaf at the intersection of the route 241 offramp and I-95S/495W; highway area drained = 18,777 m^2). It also receives runoff from 7,041 m^2 of open space. The third road site (29I088, pond AC-4 – site name: VDOT3) is a 1,271 m^2 extended detention basin located in Annandale, VA, that receives runoff from the I-495S (i.e., it lies along the bottom loop of the clover leaf near route 236, Little River Turnpike; highway area drained = 3,503 m^2). The fourth and final highway site (29I119 - site name: VDOT4) is a 2,751 m^2 extended detention basin located near the Occoquan Historic District along I-95S. It receives runoff from the I-95 and the I-1, which pass by on either side (highway area drained = 13,801 m^2). It also receives runoff from nearby open space (15,580 m^2).

Site Sampling Roadmap (how different site types were used):

Two of the 14 sites (Sully 1 and Sully 2, both parking lot sites) were sampled regularly over the course of the year (every two to three months from Fall, 2021 to Spring, 2022) to determine how soil electrical conductivity and ion composition changes over the course of a winter season and into the growing season. Plant surveys were also conducted at these sites in Fall 2021 to get a sense of community composition and halophyte abundance in northern VA detention basins.

Water samples were collected twice (once during winter -*mid January, following a snow event*-, and a second time during the growing season -*late April*-), across any of the 14 sites where stormwater was present. Depending on the sampling date, either electrical conductivity and pH (Winter), or electrical conductivity, pH, cation concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and anion concentrations (Cl^-, SO_4^{2-}) (Spring), were measured.

Plant tissues were collected at most (13 of 14) sites during the growing season and evaluated for cation and anion concentrations (Na^+, Mg^{2+}, Ca^{2+}, and Cl^-) to determine plant uptake of these salts.

PLANT SURVEYS

Field Methods

All plant survey work at Sully 1 and Sully 2 was conducted using a modified version of the FIREMON point intercept sampling method developed by the USDA Forest Service [Caratti, 2006], which can be used to evaluate plant species cover. Briefly, the vegetated region to be sampled is measured to determine its area, which guides point spacing (our goal was to survey a minimum of 200 points per site, allowing species-specific cover to be resolved within +/- 0.5%; Robertson et al., 1999). Once point spacing has been determined, a transect tape (the hub) is placed at the edge of the site. If site species composition appears random, the hub is run along the short axis of the site so that all transects emerging from the hub will be oriented along its longest dimension. If species composition is structured (i.e., different on 1 side of the system than the other), the hub is oriented so that all transects emerging from it will pass through those different regions, sampling the range of plant species at the site as completely as possible.

Once the hub has been established, a second transect tape is used to create the first (and all subsequent) sampling transects. One sampler and one recorder will walk each transect, stopping to make measurements at 0.5-, 1-, or 2-meter intervals, depending on the size of the site (see 200-point rule noted above). Measurements are made by placing a narrow diameter sampling pole (the point pole) on the same side of the transect tape at each measurement location. All plant species that touch the pole are written down by the recorder. If the pole touches only bare ground or infrastructure (i.e., a concrete path or an outlet structure), these points are coded as such. Once all plant species or other point pole hits have been transcribed, the team moves down the tape to the next measurement location.

In the event that a plant species that touches the point pole cannot be identified in the field, a sample of the species is collected, stored in a cooler to preserve it, and returned to the lab where it can be viewed under a microscope and identified to the species level. In the event that species-level identification is not possible, plant genus is recorded.

Statistical and Analytical Methods

Plant richness (the total number of species) and Shannon's diversity index (which takes into account both species number and their relative abundance) were evaluated at all sites using the R-package iNEXT [Chao and Jost, 2012]. iNEXT allows sample coverage to be assessed, for each site, which is a measure of the completeness of sampling, and uses coverage-based extrapolation to estimate metrics of plant richness and Shannon's diversity at different sites at the same level of sample coverage (i.e., if one site wound being sampled more completely than another, this measure facilitates comparison between sites (with error) at the coverage level of the more completely sampled site or higher [Chao and Jost, 2012]). This approach can only be applied if the original sample coverage of each site is at least 50% (any lower and the extrapolation error becomes too great; Chao and Lee, 1992).

Plants identified at each site were classified into 3 categories that represent their origins (i.e., native to Virginia, naturalized to Virginia (species that are prevalent, but not invasive), and invasive (prevalent species that harm their new environment by displacing other, native species). Plants identified at each site were also classified into 3 categories that represent their salt tolerance. Plants were considered salt tolerant if they were 1) recognized in the eHALOPH database [Santos et al., 2016] as true halophytes defined in accordance with Flowers and Colmer [2008] (i.e., plants that can complete their life cycle in a salt concentration of at least 200 mM NaCl); 2) recognized in the eHALOPH database as salt tolerant species (i.e., species tolerant of at least 80 mM NaCl); or 3) have been identified as having at least medium salt tolerance in the Ecoregional Revegetation Application produced by the U.S. Department of Transportation Federal Highway Administration (Armstrong et al., 2017) or the USDA PLANTS database (Mohlenbrock, 1997), where medium reflects a median tolerance of around 6 dS/m or 60 mM NaCl. Plants were considered salt sensitive if their tolerance classification was Low or None in the Ecoregional Revegetation Application, USDA PLANTS database, or local bioretention plant lists (e.g., the bioretention plant list for Fairfax County, FCPW, 2006). Any plant that could not be identified as salt sensitive or salt tolerant in accordance with the above, was classified as unknown.

<u>Results/Discussion</u>

Plant diversity was relatively high in both of the Northern Virginia parking lot detention basins we evaluated (i.e., Sully 1 and Sully 2 represent complex polycultures, not monocultures). When compared at 99% sample cover, plant richness at Sully 1, was less than half of plant richness at Sully 2 (i.e., 31.4 unique species were detected at Sully 1 *-95% confidence bounds: 26.2 to 36.6*, whereas 70 unique species were detected at Sully 2 *-95% confidence bounds: 65.4 to 74.6*). Shannon's diversity index was also higher at Sully 1 than Sully 2 at 99% sample coverage (i.e., 13.4 (11.8-14.5 CI bounds) at Sully 1 vs 29.7 (27.4-31.9 CI bounds) at Sully 2).

The dominant plant species at Sully 1 was a salt-tolerant native rush *Eleocharis acicularis* (needle spikesedge) which was present across 26% of the site. Other common species included a native salt-sensitive forb *Ludwigia palustris* (common water primrose), present across 16% of the site, and a non-native, salt-tolerant grass *Echinochloa crus-galli* (barnyard grass), present across 10% of the site. All other species were present in less than 10% of samples (see Table 1 at the end of this section for a detailed plant list). Thirty-eight percent of the overall plant species identified at this site were native species, and 34% were halophytes (see Figure 3, panel A & Figure 4, panel A). Only 14% of species met both criteria (i.e., were both salt tolerant and native). These species are: *Typha latifolia* (cattail – tolerates 290 mM NaCl [Santos et al., 2016]), *Scirpus validis*, also known as *Schoenoplectus tabernaemontani* (soft stemmed bulrush – tolerates 300 mM NaCl [Santos et al., 2016]), *Panicum dichotomiflorum* (fall panicgrass – tolerates 60 mM NaCl [Mohlenbrock, 1997]), and *Elocharis acicularis* (needle spikesedge – also tolerates 60 mM NaCl [Mohlenbrock, 1997]).

The dominant plant species at Sully 2 was a non-native, salt-tolerant grass, *Festuca arundinacea* (tall fescue), present across 13% of the site. Other common species included a non-native sedge with unknown salt tolerance, *Kyllinga gacillima* (green kyllinga), present across 11% of the site, and native, but not salt tolerant, grass *Leersia oryzoides* (rice cutgrass), present across 10% of the site. All other species were present in less than 10% of point-pole samples (Table 1). The percent of plant species at this site that were native to Virginia was approximately equal to (but slightly higher) than what we observed at Sully 1 (44% at Sully 2 vs. 38% at Sully 1; Figure 3). A smaller fraction of plant species were halophytes (17% at Sully 2, compared with 34% at Sully 1; Figure 4), and only 7% were both salt tolerant and native. This said, because Sully 2 was actually more biodiverse than Sully 1, the same total number of plant species Sully 2 meet both target criteria. Two of these species (*Typha latifolia* and *Elocharis acicularis*) have been described previously for Sully 1 (see above). The other two include *Calystegia sepium* (wild morning glory – tolerates 110 mM NaCl [Santos et al., 2016]) and *Parthenocissus quinquefolia* (Virginia creeper – less salt tolerant: 60 mM NaCl [Mohlenbrock, 1997]).

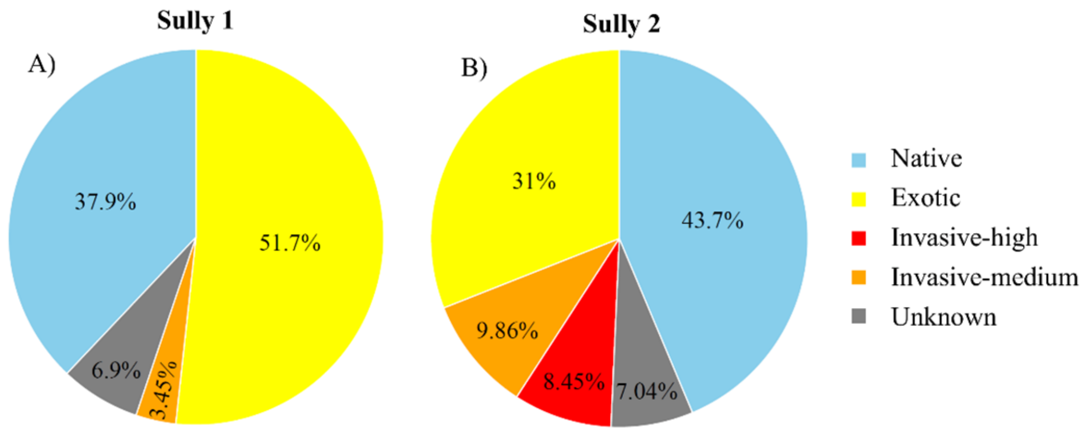

Figure 3. Percent of plant species at A) Sully 1 and B) Sully 2 that are native (blue), exotic (yellow), invasive (high - red or medium - orange) or could not be classified to a species level such that their native status is unknown (gray)

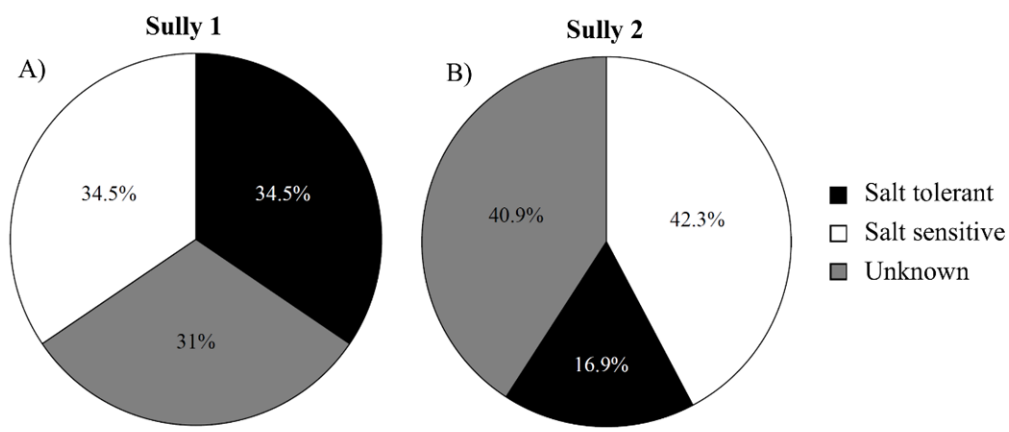

Figure 4. Percent of plant species at A) Sully 1 and B) Sully 2 that are salt tolerant (black), salt sensitive (white) or whose level of salt tolerance is unknown (gray)

At both parking lot sites, several salt tolerant species were observed that can be considered naturalized exotics (i.e., they are not native, but are also not considered moderate or high-risk invasive species in Virginia in accordance with the Virginia Invasive Plant Species List; Heffernan et al., 2014). The most salt tolerant of these species are *Cynodon dactylon* (bermudagrass – tolerates 400 mM NaCl [Santos et al., 2016]) and *Rumex crispus* (curly dock – tolerates 300 mM NaCl [Santos et al., 2016]).

Neither was particularly abundant (0.4 – 6% cover for *Cynodon dactylon* and 1.4-3% cover for *Rumex crispus*; Table 1), but both were often casually observed at many of the 14 detention basins we evaluated Northern Virginia. In both Sully sites (and elsewhere) bermudagrass tended to occur only on the outer edge of sites, which may limit its utility for phytoremediation. Curly dock, however, favored basin bottoms, and might therefore perform a useful salt removal service.

It's also worth noting that several invasives (high or medium risk) were identified at the Sully sites and may be "recruits of concern" for detention basins in the region (Figure 3). One of these, *Persicaria longiseta* (long bristled smartweed – medium-risk invasive), was identified at both sites. The remaining invasives were only detected at our more biodiverse site, Sully 2. These include seven high-risk species (*Ailanthus altissima* – tree of heaven, *Elaeagnus umbellate* – autumn olive, *Lonicera japonica* – Japanese honeysuckle, *Microstegium vimineum* – Japanese stiltgrass, *Sorghum halapense* – johnsongrass, *Rosa multiflora* – mutifloral rose, and *Taraxacum officinale* – common dandelion), as well as six medium-risk species in addition to the afore-mentioned long bristled smartweed (*Arthraxon hispidus* – hairy jointgrass, *Euonymus fortunei* - winter creeper, *Glechoma hederaceae* – ground ivy, *Pyrus calleryana* – Callery pear, *Rumex acetosella* – sheep sorrel, and *Stellaria media* – common chickweed).

Table 1. Detention Basin Plant Species and Characteristics

Species Name	Native/Exotic/Invasive	Salt tolerant	Sully 1 (% cover)	Sully 2 (% cover)	Site-P (% cover)	Site-W (% cover)
Acalypha rhomboidea	Native [1]	No [8]	0.2	0.8	0.4	--
Agrimonia eupatoria	Exotic [1]	UNK	--	--	0.4	1.9
Ailanthus altissima	Invasive-High [2]	Yes [4]	--	0.2	--	--
Allium vineale	Exotic [1]	UNK	--	--	--	0.2
Amelanchier canadensis	Native [3]	Yes [4]	--	--	--	0.3
Ammannia coccinea	Native [3]	No [5]	0.7	--	--	--
Andropogon virginicus	Native [1]	Yes [4]	--	--	0.4	0.3
Arthraxon hispidus	Invasive-Medium [2]	UNK	--	1.3	--	--
Barbarea vulgaris	Exotic [3]	UNK	--	--	0.4	1.4
Bromus sterilis	Exotic [1]	UNK	--	0.1	--	--
Bryophyta	UNK	UNK	--	--	0.4	--
Calystegia sepium	Native [1]	Yes [6]	--	0.6	--	--
Campsis radicans	Native [1]	No [5]	--	0.2	--	--
Carex spp.	UNK	UNK	--	0.2	--	2.4
Centaurea jacea	Exotic [1]	UNK	--	--	1.1	--
Cirsium arvense	Invasive-High [2]	Yes [7]	--	--	13.7	3
Cirsium vulgare	Invasive-Medium [2]	UNK	--	--	0.4	--
Cornus florida	Native [3]	No [5]	--	0.4	--	--
Cynodon dactylon	Exotic [3]	Yes [6]	6	0.4	--	--
Cyperus difformis	Exotic [3]	UNK	3.6	--	--	--
Cyperus esculentus	Native [3]	No [4]	--	5.1	0.4	0.3
Cyperus iria	Exotic [3]	UNK	6	--	--	--
Cyperus spp.	UNK	UNK	0.5	--	--	--
Cyperus strigosus	Native [3]	No [4]	--	--	2.5	1.1
Dactylis glomerata	Exotic [3]	Yes [4]	--	0.1	--	--
Daucus carota	Exotic [1]	Yes [6]	--	--	0.7	--
Digitaria sanguinalis	Exotic [3]	UNK	--	1.3	--	--
Dipsacus fullonum	Invasive-Medium [2]	UNK	--	--	0.4	4.3
Echinochloa crus-galli	Exotic [1]	Yes [6]	10.1	3.4	--	--
Eclipta prostrata	Native [3]	No [5]	2.2	--	--	--
Elaeagnus umbellata	Invasive-High [2]	No [4]	--	0.4	--	--
Eleocharis acicularis	Native [1]	Yes [5]	26.2	1.7	--	--
Eleocharis spp	UNK	UNK	--	--	11.2	1.4
Eleusine indica	Exotic [1]	Yes [6]	4.6	--	--	--
Epilobium coloratum	Native [1]	No [5]	--	--	2.2	4.6
Erigeron annuus	Native [3]	UNK	--	--	0.7	--
Euonymus fortunei	Invasive-Medium [2]	No [4]	--	0.6	--	--
Eupatorium serotinum	Native [1]	UNK	--	0.1	--	--
Euphorbia maculata	Native [1]	UNK	0.7	0.3	--	--
Festuca arundinacea	Exotic [3]	Yes [4]	0.2	12.9	9.4	5.1
Festuca rubra	Native [3]	No [5]	--	7.1	--	--
Galium aparine	Native [3]	No [5]	--	0.1	--	--
Galium mollugo	Exotic [3]	UNK	--	--	4	3.2
Galium verum	Exotic [1]	UNK	--	--	0.4	--
Glechoma hederacea	Invasive-Medium [2]	UNK	--	0.4	0.4	--
Hieracium caespitosum	Exotic [3]	UNK	--	0.6	--	--

Table 1 continued

Species Name	Native/Exotic/Invasive	Salt tolerant	Sully 1 (% cover)	Sully 2 (% cover)	Site-P (% cover)	Site-W (% cover)
Impatiens capensis	Native [3]	No [5]	--	--	0.7	1.4
Ipomoea pandurata	Native [1]	UNK	--	0.2	--	--
Juncus dudleyi	Native [1]	UNK	--	0.1	--	--
Juncus effusus	Native [3]	No [5]	--	--	1.4	6.5
Juniperus spp	UNK	UNK	--	0.8	--	--
Kummerowia striata	Exotic [1]	UNK	2.2	--	--	--
Kyllinga gracillima	Exotic [1]	UNK	1	10.9	--	--
Leersia oryzoides	Native [3]	No [5]	--	9.8	0.4	4.1
Lolium perenne	Exotic [3]	Yes [4]	--	0.3	10.8	2.7
Lonicera japonica	Invasive-High [2]	No [4]	--	0.7	--	4.6
Lonicera morrowii	Invasive-High [2]	UNK	--	--	--	0.3
Ludwigia palustris	Native [1]	No [5]	15.9	0.5	--	0.3
Lycopus americanus	Native [1]	No [4]	--	--	--	0.3
Lysimachia ciliata	Native [1]	UNK	--	--	--	0.3
Medicago lupulina	Exotic [3]	No [4]	1.4	0.4	--	--
Medicago spp.	UNK	UNK	0.2	--	--	--
Mentha spicata	Exotic [3]	UNK	--	--	--	2.4
Microstegium vimineum	Invasive-High [2]	UNK	--	0.9	--	--
Mimulus ringens	Native[1]	UNK	--	--	--	0.3
Morus rubra	Native [3]	No [4]	--	1.8	--	--
Muhlenbergia schreberi	Native [3]	No [5]	--	0.6	--	--
Oxalis stricta	Native [1]	UNK	3.4	0.5	0.7	--
Panicum dichotomiflorum	Native [1]	Yes [5]	1.7	--	--	--
Parthenocissus quinquefolia	Native [3]	Yes [5]	--	0.4	--	--
Paspalum dilatatum	Exotic [3]	Yes [4]	0.2	--	--	--
Paspalum laeve	Native [1]	No [5]	--	4.9	--	--
Paspalum setaceum	Native [1]	UNK	--	--	0.7	2.4
Persicaria longesita	Invasive-Medium [2]	No [4]	1.2	1.9	0.7	1.4
Persicaria punctata	Native [3]	No [4]	1.2	0.4	--	--
Physalis heterophylla	Native [1]	UNK	--	0.1	0.4	0.8
Picea abies	Exotic [3]	UNK	--	--	--	0.5
Pinus spp.	UNK	UNK	--	1.8	--	--
Plantago lanceolata	Exotic [3]	UNK	1.2	2.3	11.5	0.5
Plantago major	Exotic [1]	Yes [6]	--	0.4	--	--
Polygala sanguinea	Native [1]	UNK	--	0.5	--	--
Potentilla indica	Exotic [1]	UNK	--	0.5	--	--
Potentilla simplex	Native [1]	UNK	--	0.3	--	--
Prunus spp.	UNK	UNK	--	--	0.4	--
Pyrus calleryana	Invasive-Medium [2]	No [4]	--	2.8	--	--
Ranunculus parviflorus	Exotic [3]	UNK	--	0.4	--	--
Ranunculus repens	Exotic [3]	UNK	--	--	1.8	--
Rosa multiflora	Invasive-High [2]	UNK	--	0.1	--	--
Rosa palustris	Native [1]	No [5]	--	--	0.7	25.4
Rubus ursinus	Exotic [3]	No [4]	--	--	0.4	0.3
Rumex acetosella	Invasive-Medium [2]	UNK	--	0.6	--	--

Table 1 continued

Species Name	Native/Exotic/Invasive	Salt tolerant	Sully 1 (% cover)	Sully 2 (% cover)	Site-P (% cover)	Site-W (% cover)
Rumex crispus	Invasive-Low [2]	Yes [6]	1.4	3	--	--
Salix alba	Exotic [1]	UNK	--	--	0.7	--
Scirpus validus (tabernaemontani)	Native [1]	Yes [6]	0.7	--	--	--
Securigera varia	Invasive-Low [2]	UNK	--	--	2.9	--
Setaria pumila	Exotic [3]	No [4]	0.2	0.4	9.4	0.3
Solidago gigantea	Native [1]	No [5]	--	--	2.9	1.4
Sonchus arvensis	Exotic [3]	UNK	--	0.2	--	--
Sorghum halepense	Invasive-High [2]	No [4]	--	0.8	--	--
Stellaria media	Invasive-Medium [2]	UNK	--	0.1	--	--
Symphyotrichum lanceolatum	Native [3]	No [5]	--	1	--	--
Symphyotrichum pilosum var pilosum	Native [3]	No [5]	--	0.1	0.4	--
Tanacetum balsamita (aka Balsamita major)	Exotic [3]	UNK	--	0.3	--	--
Taraxacum officinale	Exotic [3]	No [4]	0.5	3.2	--	--
Toxicodendron radicans	Native [1]	UNK	--	0.7	--	--
Tridens flavus	Native [1]	No [5]	--	0.5	--	--
Trifolium pratense	Exotic [1]	No [4]	--	0.6	--	--
Trifolium repens	Exotic [1]	No [4]	0.2	4.6	--	--
Typha latifolia	Native [1]	Yes [6]	5.5	0.1	3.2	13.2
Ulmus americana	Native [3]	No [4]	--	1.3	--	--
Verbascum thapsus	Exotic [1]	UNK	--	--	0.4	--
Verbena hastata	Native [3]	UNK	--	--	--	1.1
Vernonia gigantea	Native [3]	No [5]	--	0.3	--	--
Vernonia noveboracensis	Native [1]	No [4]	--	--	--	0.3
Veronica serpyllifolia	Exotic [1]	UNK	--	--	0.4	--
Veronica spp.	UNK	UNK	--	0.1	--	--
Vicia cracca	Exotic [1]	No [4]	--	0.2	0.4	--
Vitis vulpina	Native [3]	No [5]	--	0.4	--	--

[1] Go Botany Native Plant Trust, https://gobotany.nativeplanttrust.org/
[2] Virginia DCR, https://www.dcr.virginia.gov/natural-heritage/invsppdflist
[3] Bplant.org
[4] USDA PLANTS database, https://plants.usda.gov/home
[5] ERA toolkit, Armstrong et al., 2017
[6] eHALOPH database, eHALOPH, 2022
[7] Wilson, 1979
[8] nativevegetation.org

FIELD OBSERVATIONS

<u>Salt in Water</u>

Field and Analytical Methods

Five separate snow events occurred in Northern Virginia in winter/spring 2022. These events occurred in early January (largest event), mid-January, late-January, February (smallest event), and March (Figure 5). Our winter water samples were collected after the second snow event in mid-January, and our growing-season water samples were collected in late-April, approximately 1 month following the final snow event.

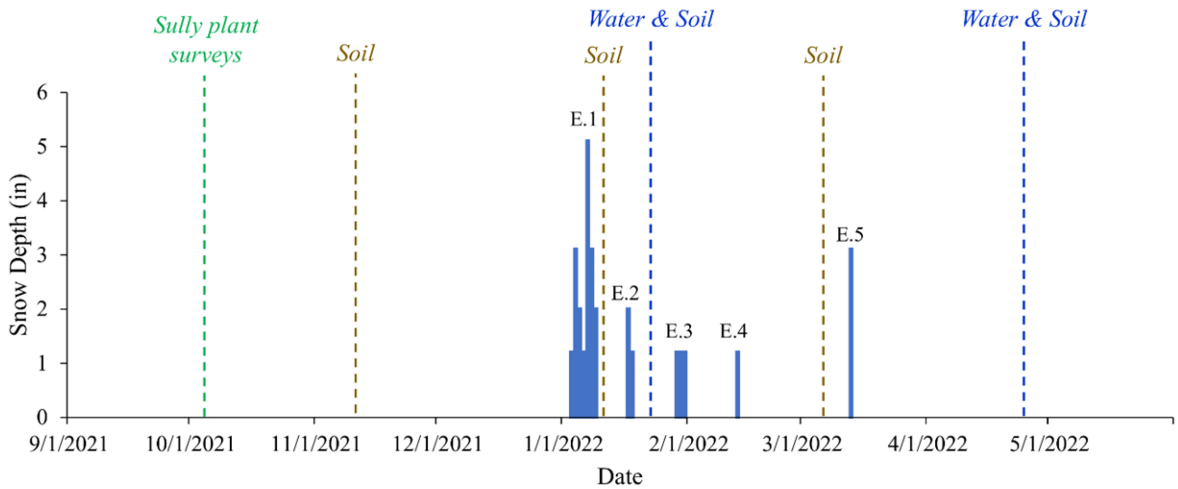

Figure 5. Snow depth in Northern Virginia over the sampling duration (data from USW00093738 station, Dulles Airport). All snow events are labeled (E.1 through E.5). Sampling dates (and the nature of the work conducted) are indicated.

In winter, only conductivity, pH, and temperature were measured, using a YSI 556 Multiparameter probe. The conductivity range for the probe is between 0 and 200 mS/cm (none of our measurements were in excess of the maximum range). The probe was calibrated for conductivity using a 2-point calibration immediately prior to use in the field. Measurements were made at any of the 14 detention basins in Northern Virginia where water was present. Attempts were made to sample at inlets, outlets, and mid-basin, to get a sense of "whole-of-site" conductivity values.

In the growing season, the same multiparameter probe was used to measure conductivity, pH, and temperature at sites where water was present (sampling inlets, outlets and mid-basin, whenever possible). In addition, 50 ml water samples were collected at the same locations where conductivity was measured and transported back to the lab for subsequent analysis of ionic composition (cations: Na^+, K^+, Mg^{2+},

Ca^{2+}, and anions: Cl^-, SO_4^{2-}). Analyses were performed by the Occoquan Watershed Monitoring Lab using an ion chromatograph 5000 (ThermoFisher Scientific) using standard methods (e.g., ASTM 4110 for anions and ASTM D6919-09 for cations; Rice et al., 2011, ASTM, 2004).

Statistical Methods

A nonparametric bootstrap comparison of means (corrected for multiple comparisons; false discovery rate approach, Benjamini and Hoschberg, 1995), was used to determine if conductivity or ion concentrations differed by site class (open space, parking lot, road) or by season (winter season, growing season) [Huang et al., 2018]. This approach was used in lieu of traditional parametric or nonparametric approaches because it is robust to small sample sizes and unbalanced sampling designs; the latter is a consequence of stormwater being transiently present across sites. Nonparametric bootstrapping is employed in this report whenever sampling is unbalanced to a degree that makes use of traditional parametric (e.g., ANOVA) or nonparametric (e.g., Kruskal Wallis) tests inappropriate.

Results and Discussion

During winter, stormwater conductivity was significantly higher at sites draining parking lots and roads than it was at sites draining open space (see Figure 6). Conductivity was slightly lower at parking lot sites than at road sites, but not significantly so. Indeed, most parking lot sites had lower conductivity than road sites, the exception being McLean (third red point from the end in Figure 6), which contained a discarded, partially used, bag of rock salt when the site was sampled, which appears to have dramatically elevated its conductivity. The highest conductivity measured was at VDOT 1 (72,300 µS/cm near the outlet), which is above the average conductivity of seawater (around 50,000 µS/cm [Zheng et al., 2018]). The lowest conductivity measured was at Camelot (102 µS/cm), which is below typical values in many of Northern Virginia's urban streams [Kaushal et al., 2005, Kaushal et al., 2022, Bhide et al., 2021].

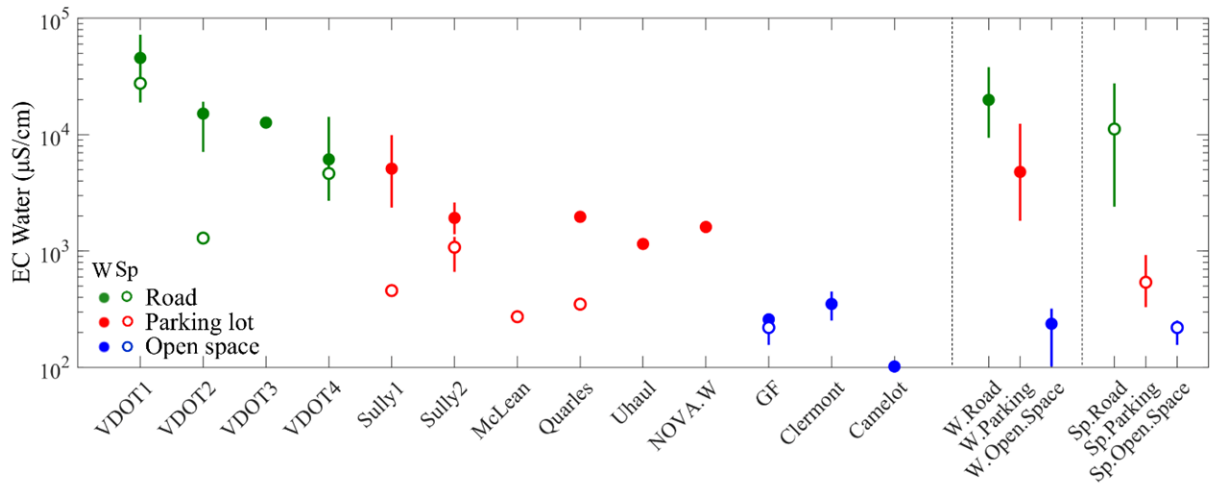

Figure 6. Electrical conductivity in stormwater from basins in Northern Virginia during winter (closed symbols) and spring (open symbols). Site specific conductivities are shown to the left of the dashed lines. Bulk comparisons by site class (open space: blue, parking lot: red, or road: green) are shown to the right, first for winter (W), then for spring (Sp). All error bounds are bootstrapped 95% confidence bounds.

Although fewer sites had stormwater present during the spring growing season, complicating spring to winter comparisons on a site-by-site basis, stormwater conductivity did tend to be significantly lower during the growing season than it was during winter at most sites (Figure 6). There were some exceptions to this general rule, including great falls (the only open space site where stormwater was present in both seasons, and where conductivity was uniformly low), and two VDOT sites (1 and 4), where the elevated conductivity levels observed in winter persisted into the growing season. Indeed, the most dramatic decreases in conductivity during spring occurred at parking lot sites, which in winter were comparable to sites receiving road runoff, but in spring exhibited significantly lower conductivities. Road sites and open space sites were somewhat more stable by season.

Looking beyond conductivity, and considering the concentration of specific ions, we see that there are two ions (sulfate and magnesium) that are largely invariant across sites (i.e., concentrations at roads, parking lots and open space sites were effectively the same; Figure , panels D & E). Two additional ions (calcium and potassium) have significantly higher concentrations at sites that drain roads than sites that drain open space, with parking lots falling somewhere in-between (i.e., not significantly different than either endmember; Figure 7, panels C & F). The last two ions (sodium and chloride), the principal constituents in deicing and anti-icing agents used in the region, have significantly higher concentrations in stormwater at sites draining roads than sites draining parking lots or open space (Figure 7, panels A & B). In the case of sodium, concentrations at parking lot sites also exceed concentrations at open space sites (this difference was not significant for chloride).

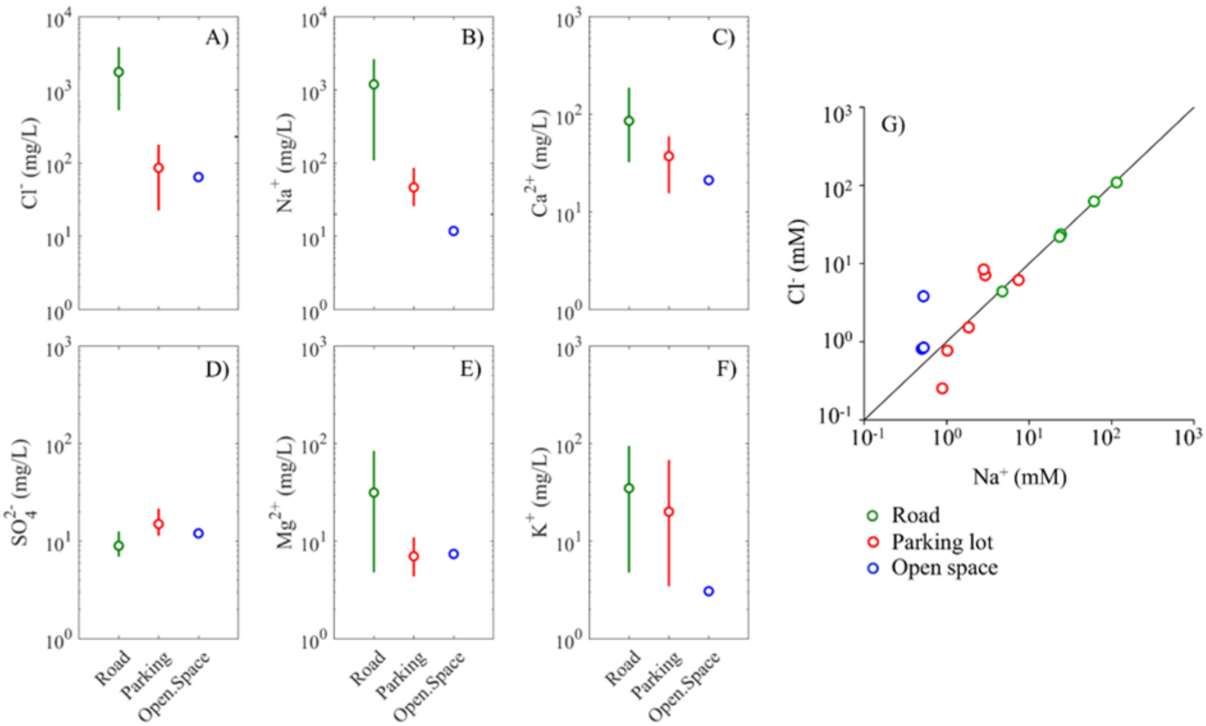

Figure 7. Panels A-F illustrate stormwater ion concentrations during the growing season at sites draining roads (green), parking lots (red), and open space (blue). Panel G) compares molar concentrations of sodium and chloride across sites. The black line shows the molar ratio for the sodium brine used by VDOT. Error bars are bootstrapped 95% confidence bounds.

Sites draining parking lots and open space had comparable chloride concentrations that were consistently below outflows from basins that drain roads. To determine the extent to which the molar ratio of sodium and chloride in detention basin stormwater still reflects winter deicer and anti-icer application during the growing season, the sodium to chloride molar ratio of the sodium brine used by VDOT was compared to the sodium to chloride molar ratio of stormwater across detention basin types (colored points in Figure 7, panel G). Stormwater samples across all VDOT sites had a sodium to chloride molar ratio of 0.98 to 1.08, consistent with sodium brine. Stormwater samples from parking lot sites were more variable (three were largely consistent with sodium brine: 1.2-1.3; two were depleted in sodium relative to chloride: 0.33-0.41, and one was enriched in sodium relative to chloride: 3.49). All stormwater samples from open space sites were depleted in sodium relative to chloride: 0.14-0.62. Given that chloride is more conservative than sodium, low sodium to chloride ratios in stormwater may be indicative of prior ion exchange with soils [Haq et al., 2018, Snodgrass et al., 2017]. This is more likely in sites that drain open space because interflow (subsurface runoff) as well as overland flow contribute to stormwater runoff in pervious catchments [Askarizadeh et al., 2015].

Salt in Soil

Field and Analytical Methods:

Soil collection at Sully1 and Sully2 occurred five times over the course of this study. During each sampling event, a ½ inch diameter T-handle probe was used to collect soil cores at each basin to a depth of 6 inches. Ten to fifteen cores were collected at each site per sampling event and composited to get a single representative sample of each basins soil conditions at each sampling time. All samples were collected from the basin bottom, not its side slopes, as the latter are only exposed to runoff during large storm events. Samples were stored on ice and transported back to the Occoquan Watershed Monitoring Lab for further processing.

Upon receipt at the lab, soils were placed in clean aluminum tins and air dried for 1-2 weeks as recommended by Robertson et al., 1999. Once dry, each composite sample was ground using a Gilson soil grinder (SA-45, Gilson Company Inc.), and shipped to Logan labs, where saturated paste analysis was conducted [Robbins, 1990]. Briefly, water was added to soil, mixed until a saturated paste was obtained, and left standing for 4-12 hours. Subsequently, the paste was filtered under vacuum (Whatman #5 filter paper) and the extract was evaluated for electrical conductivity, cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and soluble chloride. Electrical conductivity of the saturated paste extract (ECe), a measure of soil salinity, was quantified using a specific conductance meter with a dynamic range from 0.01 to 100 dS/m (Method S-1.20, Miller et al., 2013). Soluble chloride was measured using ion chromatography (Method S-1.40, Miller et al., 2013), and cation concentrations were measured using Inductively Coupled Plasma Emission Spectrometry (ICP-AES; Method S-1.60; Miller et al., 2013).

These measurements were used to calculate the exchangeable sodium percentage (ESP), defined as the molar proportion of cation-exchange sites in soil that are occupied by sodium (U.S. Salinity Laboratory, 1954). ESP is an indicator of soil sodicity, which differs from soil salinity in that it is more a function of salt composition than salt concentration [Lauchli and Grattan, 1990]. Sodic soils have poor structure, slow permeability of water and nutrients, and reduced aeration, which can result in anoxic or hypoxic conditions for roots [Qadir et al., 2007]. Saline soils, on the other hand, principally impact plants by making osmoregulation more difficult, although soil organic matter content, structure, and permeability can also be adversely affected [Lauchli and Grattan, 1990, Srivastava et al., 2019].

The following thresholds for saline and sodic soils are used in this report (definitions from Levey et al., 1998 and Shainberg and Letey, 1984):

1. soils with ESP > 15 (> 10 for clay soils) and ECe < 4 dS/m are considered sodic;
2. soils with ESP < 15 (< 10 for clay soils) and ECe > 4 dS/m are considered saline;
3. soils with ESP < 15 (< 10 for clay soils) and ECe > 2 dS/m are considered slightly saline;
4. soils with ESP > 15 (> 10 for clay soils) and ECe > 4 dS/m are considered saline sodic;
5. all other soils were classified as neither saline nor sodic

Statistical Methods

Timeseries of electrical conductivity and ESP in soils were prepared to determine if there is evidence that winter salt application at Sully1 or Sully2 increases soil salinity or sodicity (defined in accordance with Levey et al., 1998 and Shainberg and Letey, 1984, see above), and, if so, whether these effects persist into the growing season. Because soil data were heteroscedastic (but the sampling design was balanced), a Kruskal Wallis test, followed by Dunn's test with a Benjamini-Hoschberg correction for multiple comparisons (Benjamini-Hoschberg, 1995), was used to determine if electrical conductivity, ion concentrations (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, K^+), SAR or ESP in soil samples collected immediately following winter storms differed significantly from samples collected during non-winter conditions. The same test was used to determine if soil characteristics at Sully1 and Sully2 were significantly different.

To determine if there is evidence of ion exchange in detention basin soils (i.e., adsorption of excess sodium and release of calcium, magnesium, or potassium), we compared the molar ratio of sodium and other cations (calcium, potassium, magnesium) in detention basin soils collected before winter storms (pre-salting baseline) to those collected during or after winter storms. Biases towards more sodium and away from calcium, magnesium, or potassium during winter and spring were taken as evidence of ion exchange.

<u>Results/Discussion</u>

Ion concentrations (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, K^+) measured in detention basin soils at Sully1 and Sully2 did not significantly differ ($p = 0.08$ to $p = 0.91$, depending on the ion). Concentrations of monovalent ions (Na^+, Cl^-, K^+) were significantly higher in soils during winter than during fall or spring, suggesting a winter source (Figure 8, panels A-C). Concentrations of divalent cations (Ca^{2+}, Mg^{2+}) were also somewhat elevated during winter, but not significantly so (Figure 8, panels D & E). Because each site was only visited once in fall and once in spring, there is insufficient data to statistically test differences in ion composition spring to fall. This said, separation of fall samples (lower concentration) and spring samples (higher concentration) is evident for several ions, particularly sodium and chloride, suggesting that concentrations of these ions do not return to their fall baseline before the start of the spring growing season (i.e., soils appear to retain certain salt ions into the growing season making phytoremediation a possibility).

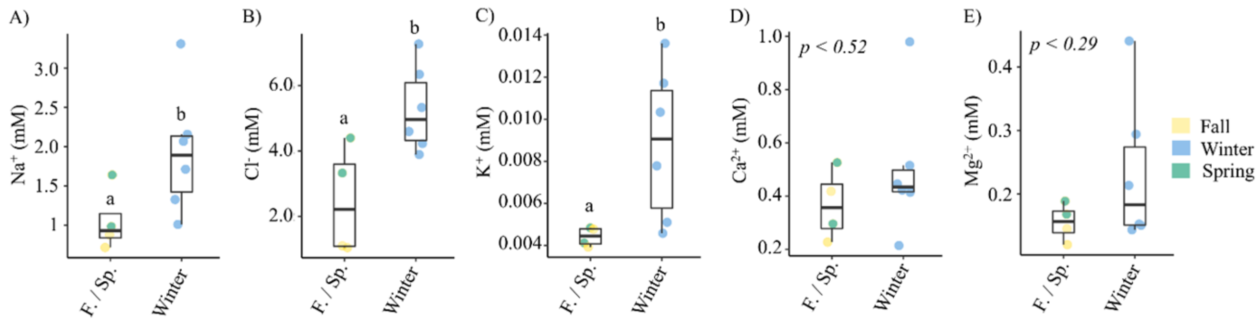

Figure 8. Boxplots comparing soil ion concentrations by season (winter vs spring/fall) across both Sully sites. a,b notation is used to indicate significant differences ($p < 0.05$ level). p-values are provided for non-significant comparisons.

At both Sully1 and 2, we see evidence that the charge balance shifts towards sodium during winter (right of the 1:1 line in Figure 8), and back towards calcium, magnesium and potassium during spring (reversing left towards the 1:1 line Figure 8). This is particularly evident for Sully2, where the soil cation balance is dominated by sodium only during winter months. Importantly, we are not seeing loss of plant-available calcium, magnesium, or potassium in soils during winter, which would be evidenced by a trajectory down and to the right of the black line in Figure 8. Rather it appears that more sodium is bound up in soils during winter, without displacing other cations.

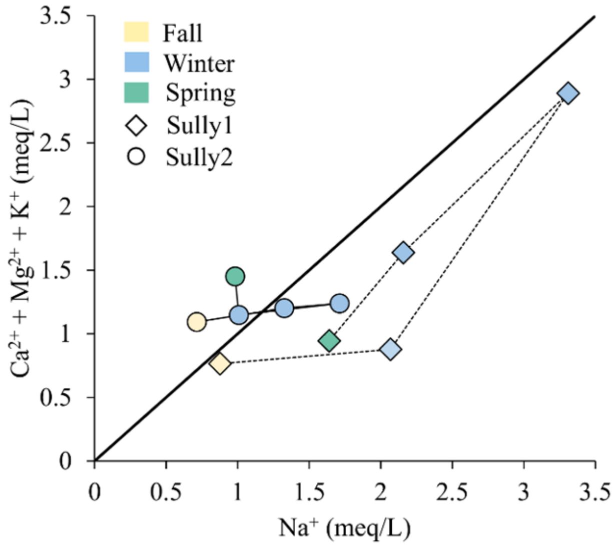

Figure 9. Comparison of sodium charge equivalents (x-axis) to calcium, magnesium, and potassium charge equivalents (y-axis) in detention basin soils at Sully1 (diamonds) and Sully2 (circles). The 1:1 line indicates even contributions of sodium and other cations to overall charge.

Electrical conductivity of soils at Sully1 and Sully2 (max of 0.68 dS/m) was below the reported threshold for slightly saline soils (2 dS/m), where salt sensitive species begin to experience difficulties with osmoregulation [Levey et al., 1998] (Figure 10, panel A). Electrical conductivity did not differ significantly by site ($p < 0.21$) but was significantly elevated in winter (Figure 10, panel B), consistent with our results for monovalent ions and a winter salt source.

Detention basin soils at both Sully sites were predominantly clay (field plasticity test; Robertson et al., 1999), making 10% exchangeable sodium the appropriate sodicity threshold at these sites (dashed line, Figure 10, panel C). ESP at Sully1 was significantly higher than at Sully2 and exceeded the 10% threshold twice during the winter season. ESP remained near this threshold at Sully1 during the growing season (9.1 %) but did not exceed it. ESP at Sully2 returned to baseline levels. Given the elevated richness of salt tolerant species at Sully1 (see Figure 4), and the relatively high ESP, we expect that plants at this site are under more salt stress during the growing season. Although ESP was elevated during winter (relative to spring and fall) at both Sully sites, this effect was not significant, likely reflecting the persistence of high ESP at Sully1 into spring (see Figure 10, panel D).

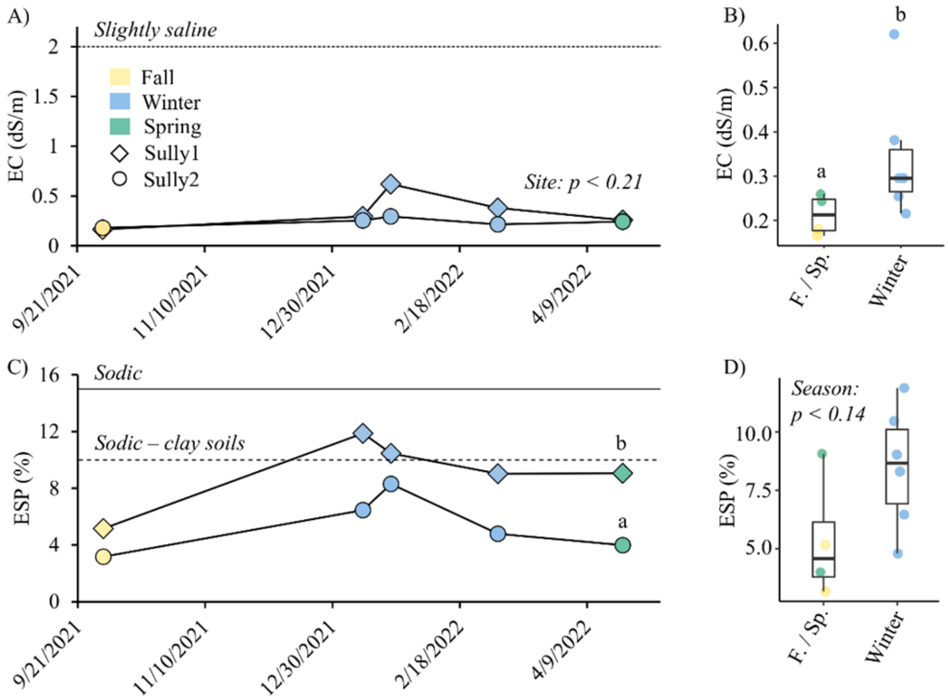

Figure 10. Timeseries of A) electrical conductivity (EC) and C) exchangeable sodium percentage (ESP) at Sully1 (diamonds) and Sully2 (circles). Boxplots illustrate seasonal differences in B) EC and D) ESP. a,b notation is used to indicate significant differences by site or season (p < 0.05 level). p-values are provided for non-significant comparisons.

<u>Salt in Plants</u>

Field and Analytical Methods

Above ground plant tissue samples were collected during the growing season in late April across 13 of the 14 Northern Virginia detention basins evaluated. The 14th site was skipped because it was dominated by an invasive English ivy, not suitable for use for phytoremediation in detention basins. Sample collection targeted native, salt tolerant species like *Typha latifolia*, native species often used for phytoremediation like *Juncus effusus*, salt tolerant exotics like *Rumex crispus*, and a variety of grass species (primarily fescues) because they were observed to be relatively common, and some (like tall fescue) are known to be salt tolerant. To ensure enough tissue was collected for analysis, each sample actually represents a composite of three nearby individual plants. Whenever possible, three or more such composites were collected per site.

Following collection, samples were dried in a drying rack at low heat (60 °C) for two days and shipped to a lab at the North Carolina Department of Agricultural and Consumer Services Division, North

Carolina State University for analysis. Plant samples were ground on a cutting mill (IKA Works, Inc., Wilmington, NC) and then analyzed for chloride and sodium.

Chloride concentration was determined using acetic acid extraction (2%, Miller, 1998), followed by filtration and evaluation of filtered extract on a segmented flow analyzer (San++ Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands) using the thiocyanate displacement method [Skalar, 2018]. Sodium concentration was evaluated using acid digestion (10 ml 15.6N HNO_3 for 30 minutes at 200 °C) followed by filtration (Whatman #2 filter) and Inductively Coupled Plasma-Optical Emission Spectrometry (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical, Ametek; Mahwah, NJ) [Donohue and Aho, 1992, EPA, 2001].

Statistical Methods

Tissue samples for each major plant species collected (*Typha latifolia*, *Juncus effusus*, *Rumex crispus*, and assorted grasses, principally fescue) were pooled across all detention basins, and compared to get a sense of which species accumulate more sodium or more chloride, respectively (i.e., which species have the most phytoremediation potential with respect to common winter maintenance chemicals in Northern Virginia [SaMS, 2020, Burgis et al., 2020]). Because plant tissue data were determined to be heteroscedastic (similar to the soils data detailed above), we employed the same statistical approach to analyze them as we did for soils but focusing on the extent to which ion concentrations in plant tissues differ significantly by plant species (test performed: Kruskal Wallis, followed by a post-hoc Dunn's test, with a Benjamini-Hoschberg correction for multiple comparisons [Benjamini-Hoschberg, 1995].

To compare ion concentrations in aboveground plant tissues by site type (open space, parking lot, and road) we elected to use the same nonparametric bootstrap comparison of means employed to compare stormwater ion concentrations by site type. Our goal was to determine if plant species growing in sites that appear to receive saltier stormwater (see Figure 7) assimilate more salt than plant species growing in sites where stormwater is less salty. This is not necessarily a given, as assimilation by plants occurs via both passive and active transport processes. Plants make use of active transport pathways to preferentially accumulate or exclude ions, whereas passive transport is a function of plant water use, with ions being assimilated in roughly the same concentrations they have in the surrounding environment [Nobel, 2009].

For VDOT road sites, where estimates of typical salt application rates are available, a rough salt budget was computed to determine the fraction of sodium and chloride mass load detention basins receive over the winter season that might subsequently be assimilated by *Typha latifolia* (cattail) over the growing season (see Appendix B for calculation details). These estimates should be interpreted as best-case scenarios, as they assume that all sodium or chloride that enters a detention basin stays there until the growing season and could potentially be taken up by plants. As noted previously, the temporal offset between salt application and the growing season makes this unlikely if additional measures to adsorb salt are not implemented. This is particularly true for chloride which does not readily undergo ion exchange.

Results and Discussion

When all Northern Virginia sites are pooled (i.e., detention basins draining open space, parking lots, and roads are combined), we find that *Typha latifolia* contains significantly more sodium and chloride in its above-ground tissues than the other species we evaluated (Figure 11). The exotic plant *Rumex crispus* is the next best salt accumulator (sodium and chloride), followed by *Juncus effusus* and grass species, predominantly fescue varietals.

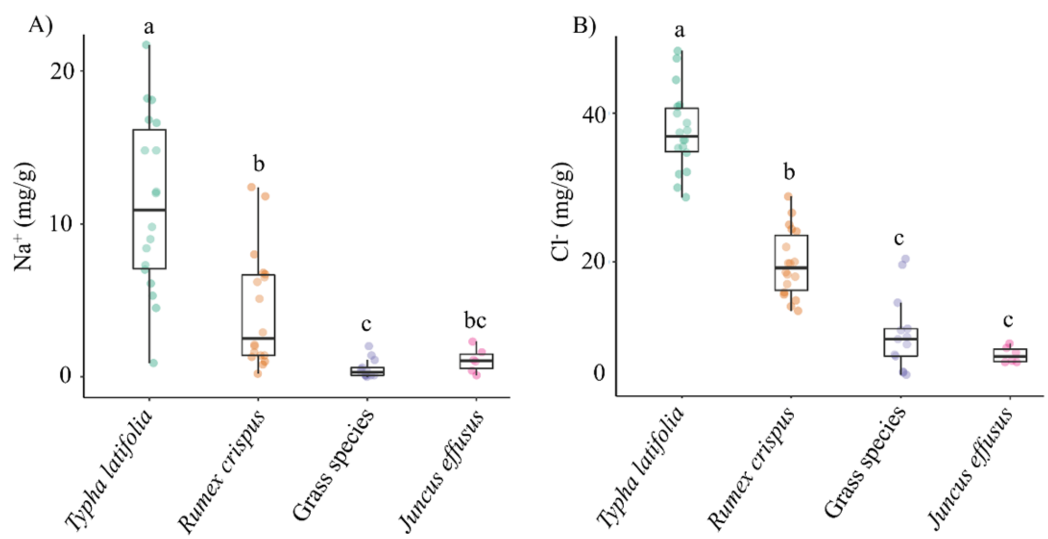

Figure 11. Comparison of the mean ion concentration (mg/g) in above-ground plant tissues from NOVA sites: A) Na+, B) Cl-. Letters above each box and whisker plot indicate whether ion concentrations differ significantly by species (different letters illustrate significant differences).

Some plant species, most notably the native halophyte *Typha latifolia*, have significantly higher sodium concentrations in their tissues in sites that receive more salt (i.e., road sites and parking lot sites) than sites that receive less salt (i.e., open space sites) (Figure 12, panel A). This pattern was also significant (but the concentrations involved were low) for *Juncus effusus*, and grass varietals. No pattern can be resolved for *Rumex crispus*, which was not present at open space sites; all we can say for this species is that sodium concentrations in curly dock tissues are not significantly different in road and parking lot sites.

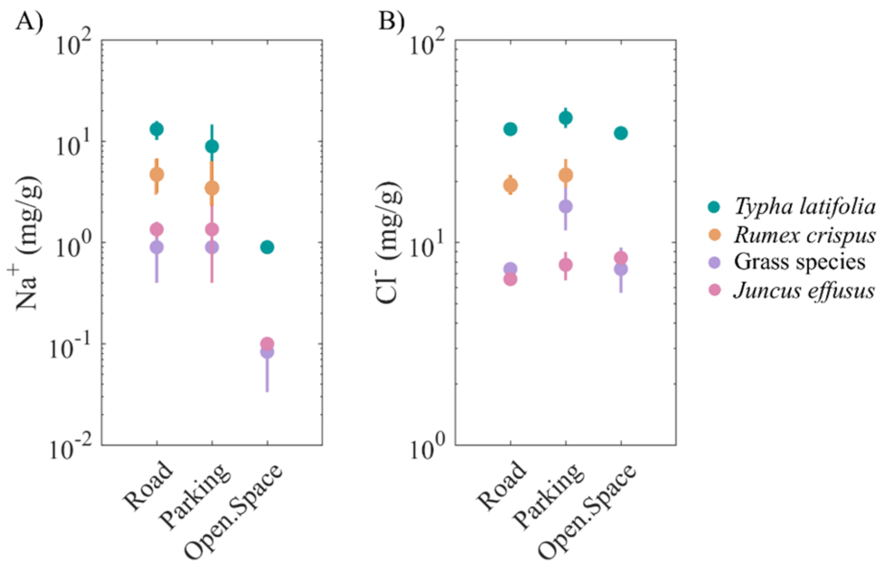

Figure 12. Concentrations of A) Na^+ and B) Cl^- in above ground plant tissues collected at road, parking lot and open space sites in NOVA. Species are denoted by color (teal: cattail, orange: dock, lavender: grass varietals, pink: common rush). All error bars are bootstrapped 95% confidence bounds

Unlike sodium, chloride concentrations appear to be relatively stable across most plant species, regardless of degree of salt application in the site they were sourced from (i.e., open space sites, parking lot sites, and road sites don't tend to significantly differ; Figure 12, panel B). The only exception is for grasses, which had significantly higher chloride concentrations at parking lot sites than road or open space sites. Given that the grass species we collected represent a mixture of varietals, we expect that this difference is due to variability in the composition of grass species collected by site type, rather than more efficient chloride uptake by a single grass species at parking lot sites only.

Rough estimates of sodium uptake by *Typha latifolia* (our species with the highest uptake potential; see Figure 11 & Figure 12) indicate that over the course of the growing season *Typha* has the potential to assimilate between 20 and 45 kg of sodium at VDOT detention basins, corresponding to roughly 0.4-2.4% of the sodium that entered these basins this winter season (Table 2). These estimates will be overly optimistic if sodium is not retained in the detention basin and available for uptake during the growing season (i.e., in the event that plants run out of sodium to assimilate over the course of the growing season). On the other hand, they may prove overly conservative if mature plants exhibit higher sodium concentrations in tissues than juveniles (the estimates made here are based juvenile tissues), as has been observed for chloride [Delattre et al., 2022].

Rough estimates of chloride uptake by *Typha latifolia* are somewhat higher than sodium (Table 2). We estimate that over the growing season (using tissue measurements made on-site) *Typha* has the potential to assimilate between 54 and 123 kg of chloride at VDOT detention basins, corresponding to roughly 0.8-4.3% of the chloride that entered these basins this winter. If in lieu of our on-site measurements we use literature values for chloride concentration in mature cattail plants grown to adulthood in saline conditions, these values increase. Upper estimates for chloride assimilation by *Typha* at VDOT basins are between 178 and 407 kg of chloride per growing season, approximately 2.6-14.3% of estimated chloride mass loading this winter.

Table 2. Estimates of potential salt phytoremediation by Typha latifolia at NOVA sites

Site	[1]M_{db} (kg Na^+ per winter season)	M_{db} (kg Cl^- per winter season)	[2]M_{cat} (kg Na^+ per growing season)	M_{cat} (kg Cl^- per growing season)	**% Na^+ mass assimilated**	**% Cl^- mass assimilated**
VDOT1	3323	5124	45	123 *(max: 407)*	1.4	2.4 *(max: 7.9)*
VDOT2	4496	6932	20	54 *(max: 178)*	0.4	0.8 *(max: 2.6)*
VDOT3	839	1293	20	56 *(max: 185)*	2.4	4.3 *(max: 14.3)*
VDOT4	3304	5095	44	121 *(max: 399)*	1.3	1.3 *(max: 7.8)*

[1]M_{db}: salt mass load to detention basin during winter

[2]M_{cat}: salt mass uptake by cattail during the growing season

max values based on tissue chloride concentrations from Delattre *et al.,* 2022

BLACKSBURG TESTING ACTIVITIES

SITE DESCRIPTIONS

Blacksburg Field Evaluation Sites

Three study sites near Blacksburg were chosen for including in the study based on numerous criteria as shown in Figure 13. Two sites, Pond (P) and Wetland (W) located at the Virginia Tech Corporate Research Center in Blacksburg VA were selected for naturalistic study. These sites were selected, in part, due to the expectation that they would be impacted by deicing salts applied to adjacent roads and parking lots. A third field site, Transportation (T), located at the Virginia Smart Roads facility in Montgomery County, VA was selected for experimental study. This site was chosen to allow salt dosing for testing if winter conditions were such that salt was not applied to pavements near Sites W and P. Since multiple winter storms in the area resulted in the application of deicing salts near Sites W and P, salt dosing was not performed at Site T. Site T was prepared for study with biochar soil amending and planting, but plant and soil samples were not collected from the site for final analysis as was done at Sites W and P.

Figure 13. Map of the Blacksburg area showing the locations of field sites P (pond) and W (wetland) and T (transportation).

Pond (P) and Wetland (W) Sites

Sites P and W are natural field sites where normally occurring winter road maintenance activity is expected to provide field plantings with exposure to applied salts. Sites W and P are located between U.S. Highway 460 and the Virginia Tech Corporate Research Center as shown in Figure 14. Also shown in Figure 14 are the arrows that indicate the observed surface and culvert water flow pathways. Stormwater from the adjacent office building area to the north of the sites flows southward into two Site P ponds separated by a constructed berm. Stormwater from areas west of the sites and from U.S. 460 empties into Site W and then flows southeast across the site in a combined surface/subsurface flow. Water from both sites exits to an intermittent surface stream located to the southwest of the sites. The use of deicing salt on all pavement areas near the site was observed during this project.

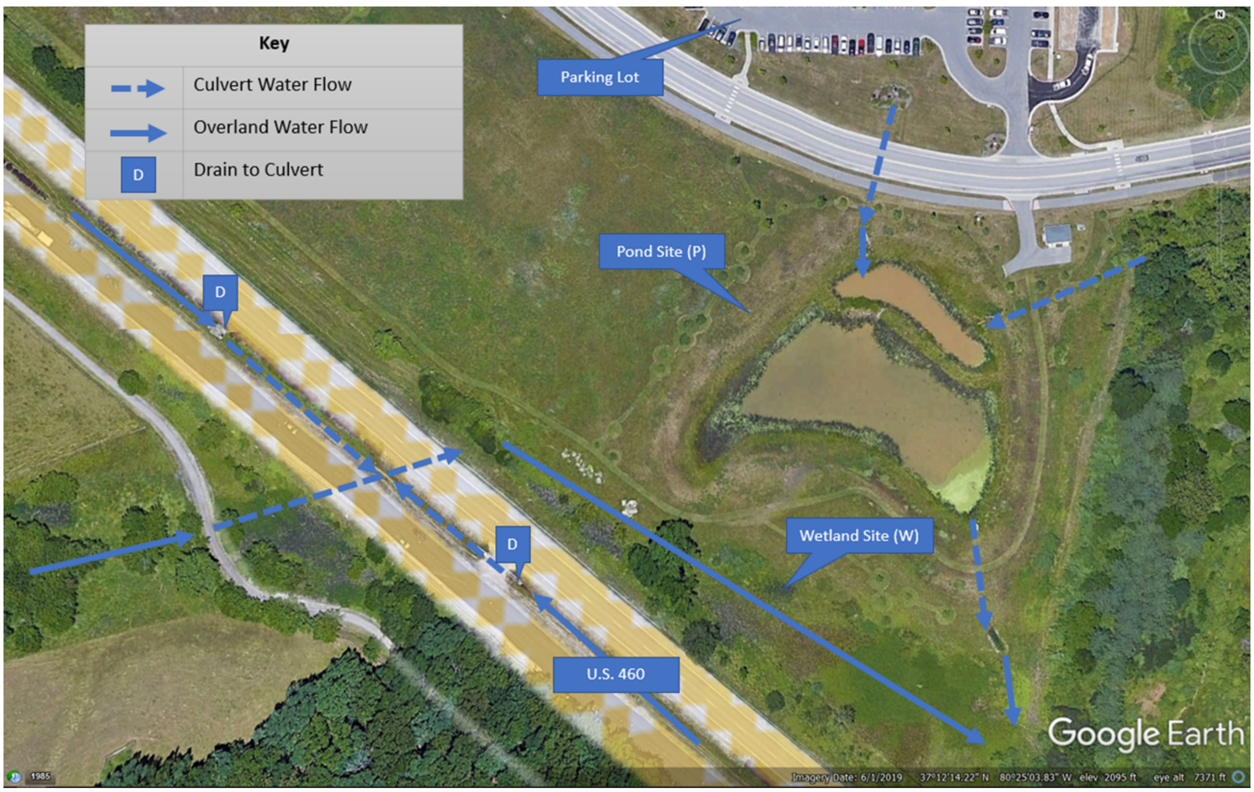

Figure 14. Overhead view of Sites W and P showing potential deicing salt sources and water drainage features.

Views of testing Sites P and W are shown in Figure 15 and Figure 16, respectively.

Figure 15. A view of Site P (Pond) looking northward towards office buildings located at the Virginia Tech Corporate Research Center.

Figure 16. A view of Site W looking west across the wetland area towards the adjacent U.S. Route 460.

Site T (Transportation)

Site T (Figure 13 and Figure 17) was selected as backup testing location that would be used for experimental study if a mild winter occurred and no salt was applied at roads and facilities adjacent to the Sites P and W. This contingency would provide for manual dosing of NaCl at Site T to ensure that soil and plant exposure to salt would occur during the course of the project.

Figure 17. An overhead view of Site T showing the planting area, drainage flow, and the nearby pond.

Soil augmentation with biochar and planting were performed at Site T as they were at the other Blacksburg Sites W and P (Figure 18), as described in the following section. However, since significant winter precipitation occurred in Blacksburg and NOVA and road salts were applied in response, experimental application of NaCl at Site T was unnecessary and collection and analysis of soil, water, and plant tissues were not undertaken.

Figure 18. View of Site T at the Virginia Tech Transportation Institute looking to the northwest. Also shown is the halophyte planting being done at the site.

A summary of Blacksburg test sites characteristics is included as Table 3. This list includes all three field test sites as well as the Virginia Tech lab. Information regarding general site characteristics, the intended purpose of the site utilization, what plants would be used, descriptions of salt sources, and experimental treatments are provided.

Table 3. Summary of site characteristics for the Blacksburg testing locations

Site	General Description	Purpose	Plants	Salt Source	Treatments
Pond (P)	Constructed stormwater detention site located within a corporate research development	Field testing with impact of biochar amendment with existing native halophytes and typical salt application practices	Cattail Common rush	Traditional winter road/walkway maintenance within the CRC	In situ (not removed) Biochar addition (plant removed and replanted) No biochar (plant removed and replanted, control)
Wetland (W)	Semi-natural wetland located adjacent to US Route 460 with direct drainage			Traditional DOT winter road maintenance along US 460	
Transportation (T)	Semi-natural wetland located within a transportation testing site.	Field testing with impact of biochar amendment with existing native and introduced halophytes	Cattail Common rush	Applied by researchers (if required) with the potential for some background sources from nearby private and public roads	In situ (not removed, excl. rush) Biochar addition (plant removed and replanted) No biochar (plant removed and replanted, control)
Lab	Experimental in a controlled indoor laboratory environment	Evaluate plants not suitable for field testing and the effects of biochar and salt dosing	Cattail Common rush Saltmarsh Mallow	Researcher experimental application	Biochar No biochar Salt No salt

PLANT SURVEY

<u>Field methods and Statistical Approach</u>

Plant surveys were conducted at both Site P and Site W using the same methods and statistical/analytical approaches described for the Sully sites sampled in Northern Virginia. No plant survey work was performed at Site T.

Results and Discussion

Consistent with plant survey results from detention basins in Northern Virginia, plant diversity was relatively high in both Blacksburg sites (i.e., Site-W and Site-P are both complex polycultures). Species richness determined at 99% sample cover did not differ significantly between these sites, was statistically comparable (with 95% confidence) to species richness at Sully 1, and significantly lower than species richness at Sully 2 (*values provided above*). In total, 33.6 species (95% confidence bounds: 28.5 to 38.7) were identified at Site-P, and 36.8 species (95% confidence bounds: 30.6-43.1) were identified at Site-W. Shannon's diversity was also comparable across Blacksburg sites (i.e., 16.3 (14.3-18.2) at Site-P and 16.8 (14.9-18.8) at Site-W). Diversity was marginally, but significantly higher at Site-W than at Sully 1, whereas no significant difference was observed between Site-P and Sully 1. As was observed for richness, diversity of plants at both Blacksburg sites was significantly lower than observed at Sully 2.

The dominant plant species at Site-P was an invasive salt tolerant thistle *Cirsium arvense* (field thistle), which was present across 14% of the site. Other common species included a small rush that could not be classified to the species level (*Eleocharis spp.*), present across 11% of the site, a non-native forb with unknown salt tolerance, *Plantago lanceolata* (narrow leaf plantain), which was also present across 11% of the site, and non-native grasses with unknown salt tolerance (*Lolium perenne*: perennial ryegrass), present across 11% of the site. All other species were present across less than 10% of the site (see Table 1 for a detailed plant list). Thirty-nine percent of the overall plant species identified at this site were native species, and 15% were salt tolerant (Figure 19, Figure 20). The fraction of natives observed at Site-P is comparable to both Sully sites in Northern Virginia (36-42%). The fraction of salt tolerant plants (15%) is less than half of what was observed at Sully 1 (35%) but is more or less consistent with Sully 2 (17%). Very few native species at Site-P were also salt tolerant (2 species, 5% of the total). These include: *Typha latifolia* (also observed at Sully 1 and Sully 2), and a grass *Andropogon virginicus* (broomsedge – tolerates 60 mM NaCl [Armstrong et al., 2017] that was not observed at either Sully site.

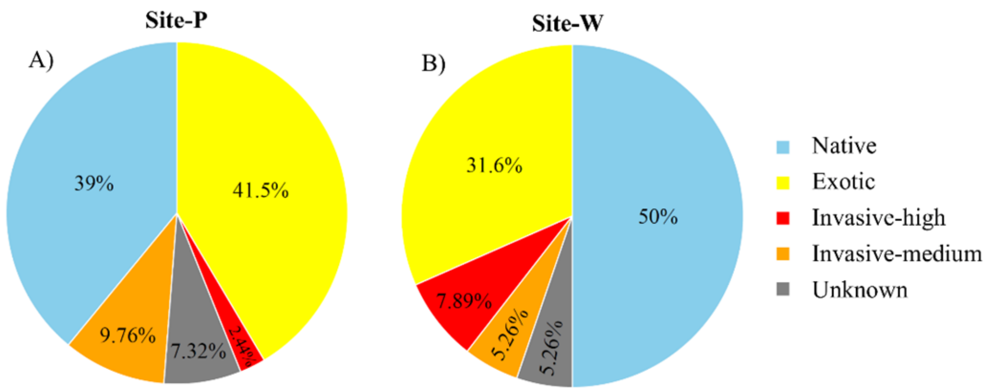

Figure 19. Percent of plant species at A) Site-P and B) Site-W that are native (blue), exotic (yellow), invasive (high - red or medium - orange) or could not be classified to a species level such that their native status is unknown (grey)

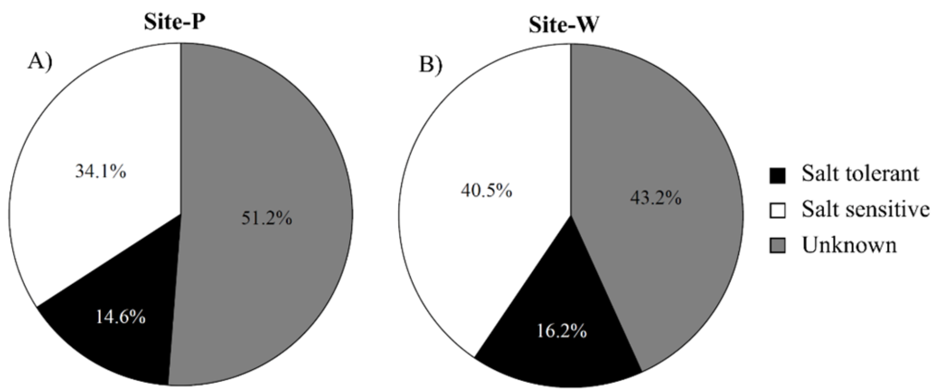

Figure 20. Percent of plant species at A) Site-P and B) Site-W that are salt tolerant (black), salt sensitive (white) or whose level of salt tolerance is unknown (grey)

The dominant plant species at Site-W was a native, non-salt tolerant forb, *Rosa palustris* (swamp rose), which was present across 25% of the site. The other common species was the native halophyte, *Typha latifolia* (cattail), present across 13% of the site. All other species had less than 10% cover at Site-W (see Table 1).). Fifty percent of plant species at this site were native, the largest fraction of natives detected at any site (compare Figure 3 to Figure 19). Fourteen percent were salt tolerant, comparable to Site-P and Sully 2 (compare Figure 4 to Figure 20). Only 8% of species (three plants) were both native and salt tolerant, again comparable to Site-P. Indeed, two of the salt tolerant natives present at Site-P were also present at Site-W. The other salt tolerant native plant was *Amelanchier canadensis* (service berry – tolerates 60 mM NaCl [Armstrong et al., 2017]), which was not detected at any other basin. The only salt tolerant native present across all four sites was *Typha latifolia* (cattail).

At both Blacksburg sites, several salt tolerant species were observed that can be considered naturalized exotics (Heffernan et al., 2014; Figure 19). The most salt tolerant of these exotics is *Daucus carota* (queen Anne's lace – tolerates 200 mM NaCl [Santos et al., 2016]). This species was quite rare (present at only 1 of the two Blacksburg sites, 1% of the time), and was only detected along basin edges, making it an unlikely candidate for phytoremediation.

Several invasive species (high or medium risk) were identified at the Blacksburg sites (Figure 19). Site-P is particularly notable in this regard because the dominant species (field thistle) was actually a high-risk invasive [Heffernan et al., 2014]. This species was also detected at Site-W but was less abundant there (Table 1). In addition to field thistle, two other high-risk invasives were detected, both at Site-W. These species are Lonicera morrowii (Morrow's honeysuckle), and Lonicera japonica (Japanese honeysuckle). Four medium-risk invasives were also detected. Two of these were present at both sites (Dipsacus fullonum – teasel, and Persicaria longesita – long bristled smartweed). The other two were only detected at Site-P (Cirsium vulgare – bull thistle, and Glechoma hedracea – creeping charlie) (Table 1).

FIELD EVALUATION

The Blacksburg sites were south of the town and VT campus (Figure 13). The detention pond and constructed wetland were at the Virginia Tech Corporate Research Center (VT CRC) south of campus and south and west of the Virginia Tech airport. The wetland area was constructed in 1987 when the VTCRC was built. The wetland area is fed by runoff from US Route 460, a stream that runs through conduit underneath the highway, and natural and constructed drainage fed by runoff from the VT CRC and the VT airport (Figure 14). The detention pond is newer and was constructed in 2010 as construction on Phase III of the VTCRC was begun (Figure 15). The pond is constructed in two sections to specifically collect output for drainage from parking lots and buildings across Tech Center Drive, a road, sidewalk and bicycle path that connects VT CRC with the main campus and US Route 460 (Figure 13 & Figure 14) Experiments conducted in Blacksburg are summarized in Table 3.

Experimental Design Concept

Disturbed soils along roadways and other paved surfaces typically contain subsoil and are not fertile. This makes establishing and growing plants for phytoremediation difficult. Disturbed soils often have lower cation exchange capacities and leach minerals used as deicing salts rather than retain them. Deicing salts are applied during winter months when vegetation is dormant or quiescent. Spring rains leach applied salts into the environment, so they are not available for uptake by actively growing plants in April and May (Gonsalves et al., 2014). We intentionally amended soils with biochar, to improve characteristics for nutrient retention during winter months when plants are quiescent. Biochar has many active positive and negative charged sites that bind cations and anions like Ca^{2+}, K^{+}, Na^{+} and Cl^{-} to retain these minerals (Lawrinenko and Laird, 2015). Over time, ions bound to biochar exchange with the soil solution where they can be taken up by plants. As plants resume active growth in spring, they can uptake ions held by biochar through the winter for phytoremediation. Biochar is a solid, very stable carbon-based material obtained from the thermochemical conversion of biomass in an oxygen-limited environment (Lawrinenko and Laird, 2015). Since the carbon in biochar is stable it is removed from the carbon cycle and does not decompose into carbon dioxide and become a greenhouse gas like other soil organic matter. Converting organic matter to biochar is one strategy proposed to combat global warming. The biochar used in this project did not contain heavy metals or other toxic compounds that might be introduced to the environment. A laboratory analysis of the biochar is included in Appendix C. Our hypothesis is that soils amended with biochar immediately absorb deicing salt ions. These ions are then slowly released over time so that plants growing in spring can absorb them for phytoremediation.

Native plants cattail (*Typha latifolia*) and rush (*Juncus effusus*) native were selected for phytoremediation of deicing salts at the Blacksburg sites (Santos et al., 2016). These plants are salt tolerant as described previously in this report and were already growing in the CRC wetlands and detention ponds before this study began. This is an indication that why are well adapted to saline environments. Both species prefer set soils and are well suited for runoff detention ponds and wetlands that collect roadway and parking lot runoff.

Rush and cattails were transplanted into shallow hand-dug holes with permeable coconut coir liners filled with either native soil or a 1-to-1 mixture of native soil amended with biochar around the root ball (Figure 21). Established plants that were not transplanted served as a control. Eight plants each were planted in the wetland area (Site W) and around the detention pond (Site P) at the Virginia Tech CRC, in Blacksburg (Figure 14, Figure 15, & Figure 16). Soil samples were analyzed in Fall after establishment and again in Spring at the resumption of plant growth.

Figure 21. Field planting methods shown where a coconut coir is used with native soil, biochar, or both for in-ground planting at test sites.

<u>Salt in water</u>

At the Blacksburg locations, water samples of 1/2 to 1-liter volumes were collected from a drainage conduit, fed by run-off from parking lots at the Virginia Tech Corporate Research Center that entered the detention pond at the Virginia Tech Corporate Research Center (CRC) or the wetland area adjacent to route U.S. 460 (Figure 14). Some water samples were also taken directly from the detention pond shown in Figure 14. Water samples were collected in Fall before winter weather to determine baseline water quality, after winter weather events when deicing salts were applied to paved surfaces, and periodically throughout the winter season. Samples were analyzed by the Occoquan Watershed Water Quality Lab in Northern Virginia for mineral content.

A timeline of electrical conductivity readings from the wetland and pond retention is shown in Figure 22. Readings from different locations (inlet, middle, outlet) within each site location were averaged. Concentrations of different ions in stormwater at the pond and wetland site are shown in Figure 23. These samples were collected at inlets only, during winter months when road salts were applied (i.e., January through February)

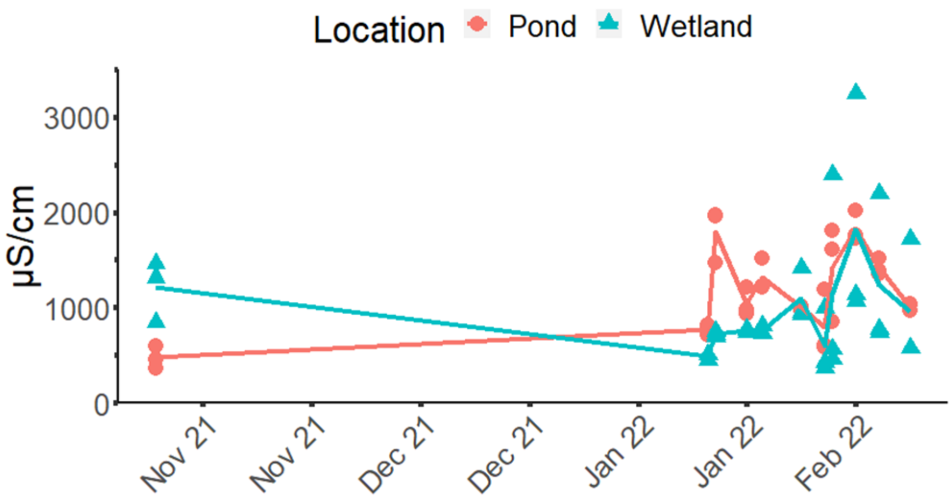

Figure 22. Electrical conductivity of water samples in Blacksburg from the pond and wetland sites. The lines represent average conductivity as measured at different locations within each site (inlet, middle, outlet) and points are discrete measurements.

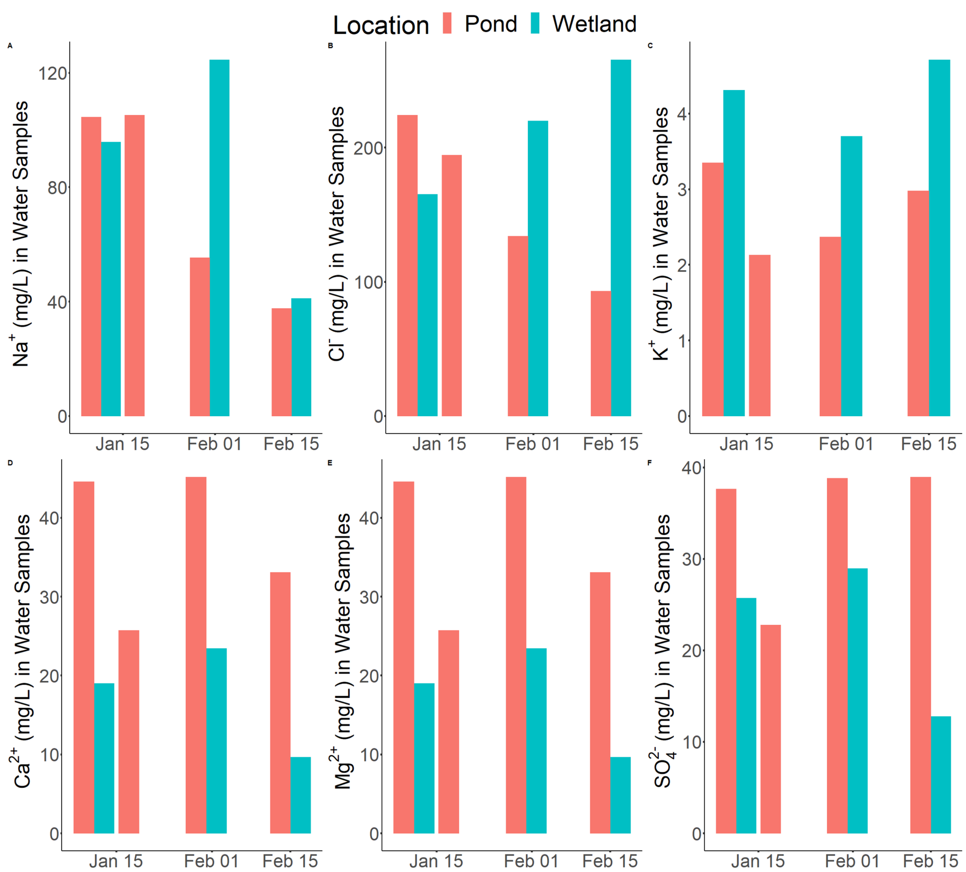

Figure 23. Changes in Na^{+}, Ca^{2+}, Cl^{-}, Mg^{2+}, K^{+}, $S0_4^{2-}$ concentrations in water collected from Blacksburg, Pond and Wetland from January 15 to February 15, 2022.

Results and Discussion

Electrical conductivity fluctuated across seasons with higher values recorded in the VT CRC wetland than the pond (Figure 23). The wetland drains US Route 460, so spikes in conductivity likely resulted from roadway deicing salt applications. The pond collects runoff from nearby parking lots as well as overflow from a stream that drained the west side of the VT CRC, both of which appear to contain less salt (Figure 23). Only four winter weather events in Blacksburg required use of deicing salts in 2022: January 16-17 (1.1 inches), January 20 (0.1 inches), January 28 (0.2 inches) and February 18 to 24 (1.1 inches). While increases in conductivity are associated with these events, conductivity quickly returned to baseline levels (Figure 22). Compared to conductivity results from NOVA (particularly NOVA highway sites), Blacksburg readings were relatively low (see. Figure 6).

Water samples were lower in sodium, calcium, chloride, magnesium, potassium, and sulfur compared to NOVA as well (compare Figure 7 to Figure 23). Sodium, chloride and potassium were generally higher in the wetland than the pond except for the Jan. 15 measurement. The maximum values were 122, 280, and 4.8 mg/L for sodium, chloride, and potassium, respectively. In contrast, calcium, magnesium, and sulfur were higher in the pond than the wetland (all dates, maximum values of 48, 47, and 38 mg/L respectively). The lower salt and conductivity measurements at Blacksburg locations may reflect lower winter salt applications and/or their large pervious drainage areas (both the pond and the wetland are fed by large areas where deicing salts were not applied which would substantially dilute pond and wetland salts).

Salt in Soil

Unamended soil cores were collected from the edge of the CRC pond and wetland in Blacksburg (Figure 14, Figure 15, Figure 16). Soil samples were tested during Fall 2021 (before winter weather) to determine baseline soil nutrient concentrations. Soil samples from the same locations were tested again in May 2022 for comparison. Soils were tested for the following treatments: 1) native unaltered soils (control), 2) biochar-amended soils (rush and cattails were transplanted into native soil amended 1:1 (volume to volume) with biochar and placed in porous, biodegradable, coconut coir liners to ease plant recovery and prevent soil mixing. Coconut coir liners are not significant physical barriers and can be penetrated by roots. Biochar was obtained from Ecotone, Forest Hill, MD made from wood feedstocks) and 3) non-biochar amended amalgamate (unamended native soil in a coconut liner to account for unexpected effects of coir). When we mention soil treatments in later sections of this report, it is these three soil types we are referring to.

Results and Discussion

There were few differences in mineral concentrations between the native soil samples and those amended with biochar in Spring 2022 (Table 4; Figure 24 & Figure 25). This is in part because of statistical differences that occurred among blocks (Table 4). Block variation is due to changes in soil type and moisture conditions at different locations around the pond and wetland.

Table 4. Comparison of mineral composition of amended and native soils growing cattail (Typha latifolia) and common rush (Juncus effusus) plants at the Pond site.

Ion	Factor[1]	F – value	Probability (>F)
Na^+	Block	8.8432	0.0003**
	Species	0.0990	0.7564
	Soil Treatment	0.2205	0.8041
	Soil Treatment x Species	3.375	0.0557
K^+	Block	7.167	0.0010**
	Species	1.835	0.1913
	Soil Treatment	2.673	0.0948
	Soil Treatment x Species	7.802	0.0033**
Ca^+	Block	5.795	0.0031**
	Species	1.972	0.1763
	Soil Treatment	1.819	0.1892
	Soil Treatment x Species	0.756	0.4827
Mg^{2+}	Block	2.110	0.1193
	Species	2.190	0.1552
	Soil Treatment	4.135	0.0322*
	Soil Treatment x Species	2.978	0.0749
Mn^{2+}	Block	2.140	0.1153
	Species	0.855	0.3668
	Soil Treatment	1.209	0.3204
	Soil Treatment x Species	1.788	0.1943
S^{2-}	Block	5.108	0.00575**
	Species	0.390	0.5398
	Soil Treatment	0.192	0.8267
	Soil Treatment x Species	0.004	0.9960
Zn^{2+}	Block	3.830	0.0190*
	Species	2.436	0.1350
	Soil Treatment	0.367	0.6969
	Soil Treatment x Species	2.405	0.1171
Cu^{2+}	Block	0.755	0.5666
	Species	0.226	0.6394
	Soil Treatment	0.803	0.4624
	Soil Treatment x Species	3.446	0.0528

significant at p values of **0.01 or *0.05

[1]Where Blocks are replications;
Species: cattail or common rush plants;
Treatment: soils: 1. biochar-amended, 2. unamended, native soil in coconut coir liners (amalgamated), or 3. control, unamended, native soil.

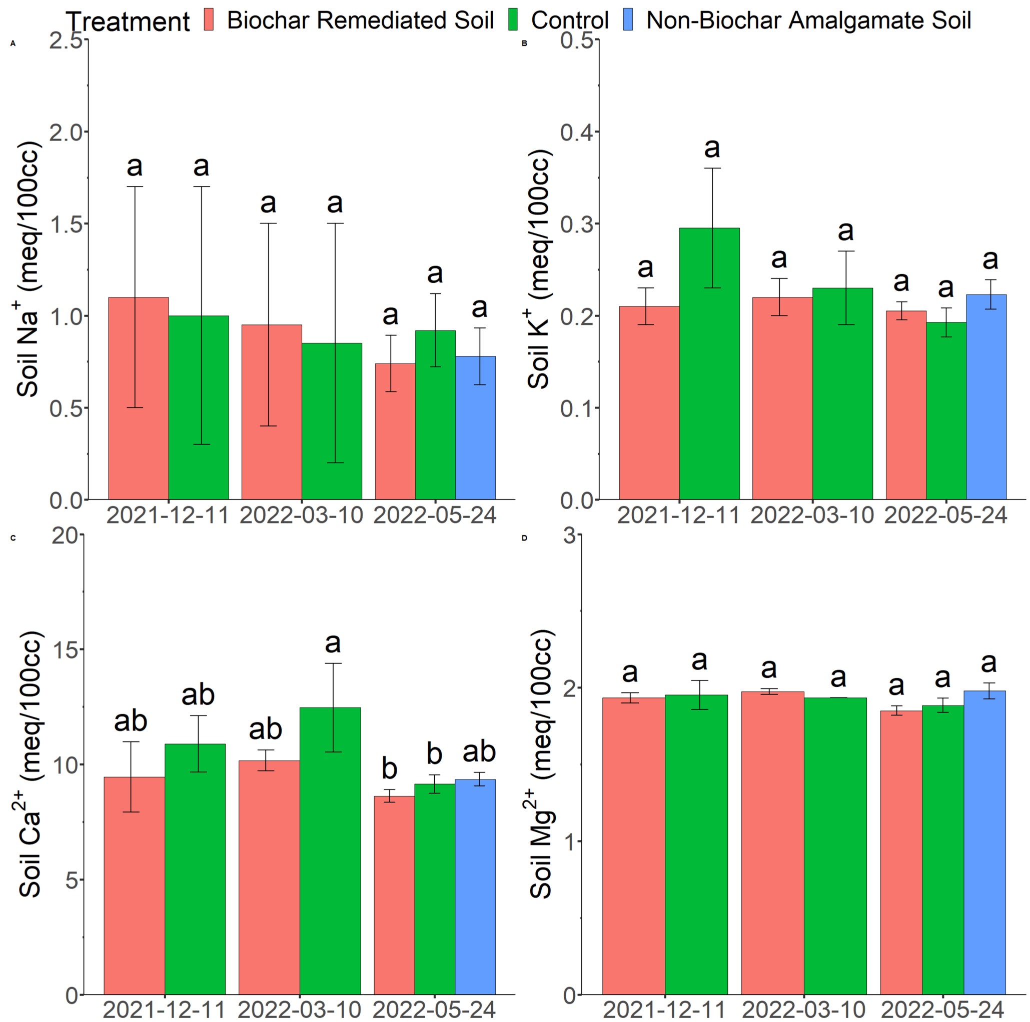

Figure 24. Comparison of the pre and post season soil ions of Na^+ (A), K^+ (B), Ca^+ (C) and Mg^{2+} (D) via Fisher's LSD (α = 0.05, p-adj = Bonferroni) in wetland and pond locations. The values shown are means (+/- standard error). A square root transformation was applied to the Mg^{2+} data prior to analysis.

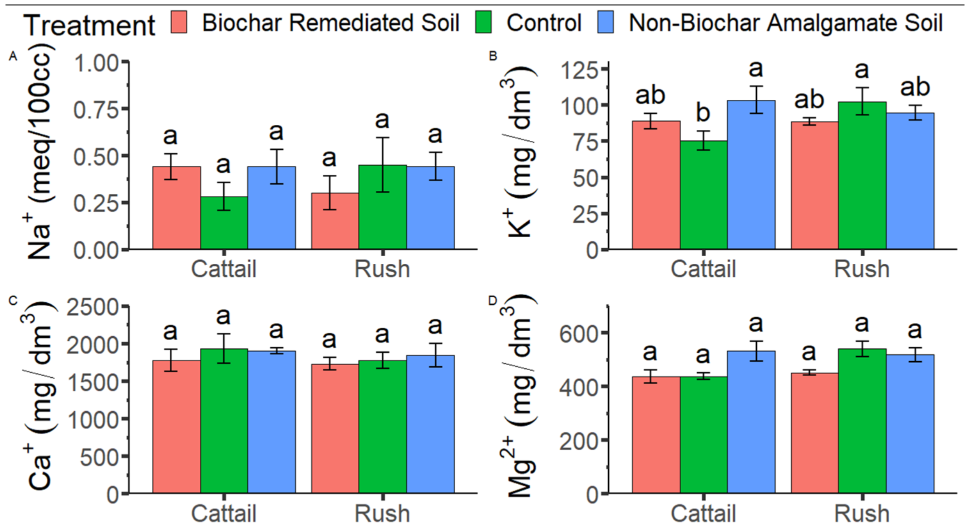

Figure 25. Spring 2022 Na^+ (A), K^+ (B), Ca^+ (C) and Mg^{2+} (D) concentrations in native soils or soils amended with biochar (1:1 ratio) for rush and cattail plants in the pond location. The values shown are means (+/- standard error). Letters show Fisher's LSD (α = 0.05, p-adj = Bonferroni) comparison of means. A log transformation was applied to the Na^+ data prior to analysis.

Focusing on common cations used for anti-icing and deicing applications (Na, Ca, Mg, K), we see that ion retention was statistically comparable in all soil treatments across seasons (pooled pond and wetland soils; Figure 24). The only exception was calcium; marginally less calcium was measured in soils during winter months (see control and biochar treatments). Looking at pond soils only (Figure 25) we see that there were no statistically significant differences amongst treatments for Na^+, Ca^{2+} and Mg^{2+}. There was a marginal effect on the retention of K^+ in plots planted with cattail, but not rushes, with the control holding on to less K^+ than non-biochar amalgamated soil. Overall, where species were planted within the pond (block in Table 4), plant species identity, and interactions between species and soil treatments played a more important role in soil ion retention than soil treatments alone.

The low accumulation of soil ions in soils amended with biochar should not be taken as a sign that the biochar did not work as intended. Rather, we believe salt levels in the environment were insufficient for effects of biochar to be detected. We expect that biochar had benefits even though salt concentrations were low because it improves soil quality, which can enhance survivorship and growth of halophytes used for phytoremediation (plants in biochar amended soils appeared less stressed than plants in control treatments following initial transplantation in early fall; *personal observation*). The ability of biochar to bind Na^+ and Cl^- when used to fill conjunction with environmental containment socks at high concentrations of NaCl is demonstrated in Figure 26 and Figure 27 below.

Salt in plants

Unamended and Biochar-amended

In the retention pond, cattail had significantly higher ion uptake than *Juncus* (Figure 26). For root tissues, ion accumulation was rarely significantly different across treatments, the exception being potassium where uptake was significantly higher in the control than in non-biochar amalgamated soil. Root uptake of potassium in control and biochar treatments did not significantly differ.

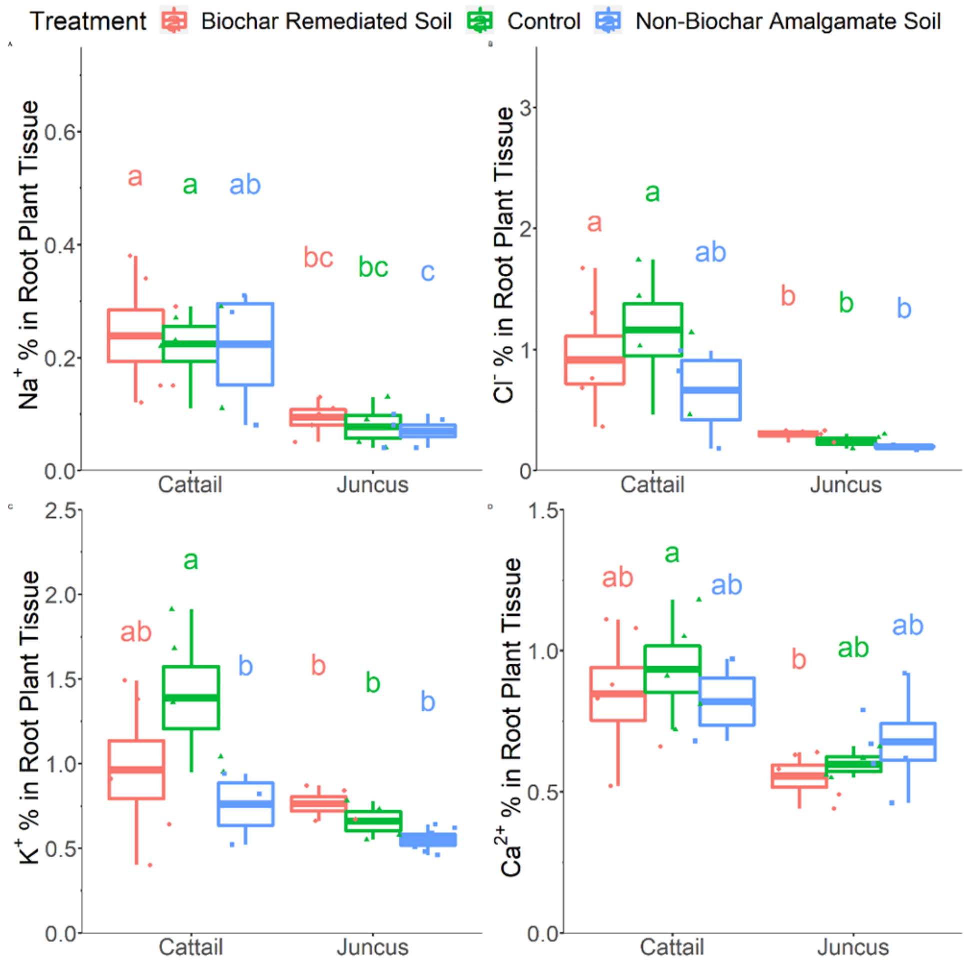

Figure 26. Fisher's LSD (α = 0.05, p-adj = Bonferroni) comparison of the ion content (%) of Na^+ (A), Cl- (B), K^+ (C) and Ca^{2+} (D) in root tissue of plants from the Pond site. The midline of each boxplot is the mean and whiskers show the standard error. Square root transformations were applied to Na^+ and K^+ prior to analysis.

No significant differences in ion accumulation in above ground tissues were detected across treatments. Here, however, biochar does appear to reduce the variability in ion uptake, particularly by cattail (note reduced spread of box and whisker plots; Figure 27). In cattail, mean concentrations of chloride and potassium were both higher in biochar treatments than control treatments, but not significantly so. The inverse was true for calcium. Ion concentrations in *Juncus* were substantially less variable than cattail across all treatments.

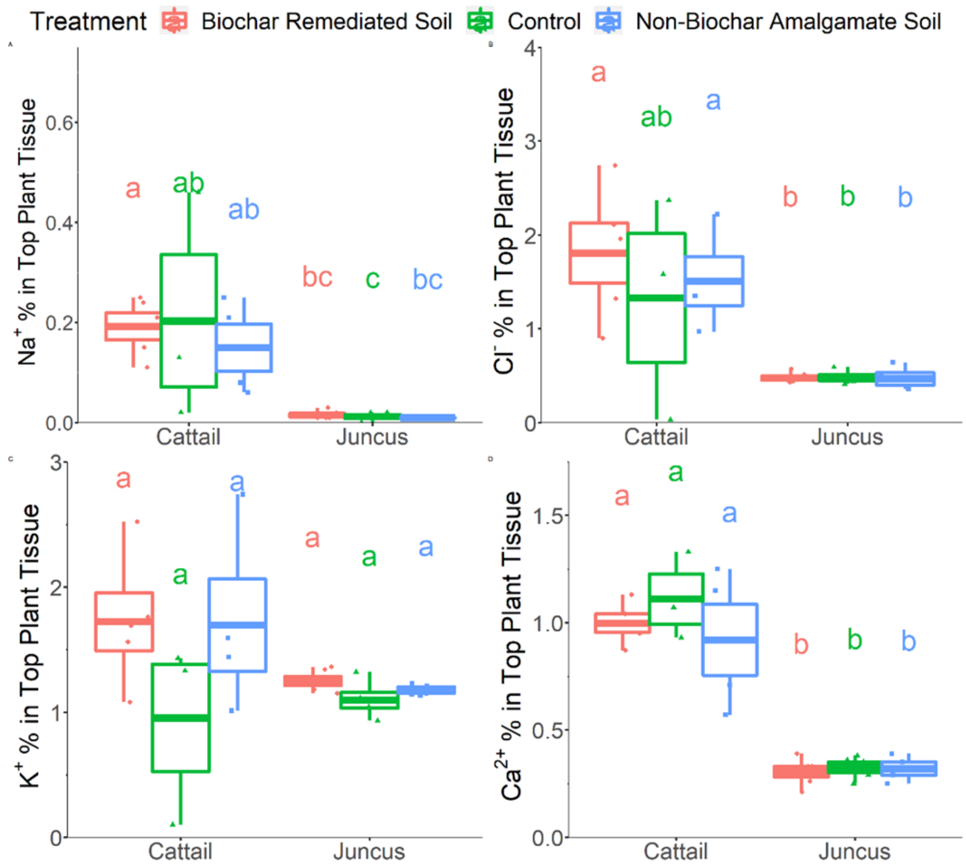

Figure 27. Fisher's LSD (α = 0.05, p-adj = Bonferroni) comparison of the ion content (%) of Na^+ (A), Cl^- (B), K^+ (C) and Ca^{2+} (D) in above-ground plant tissue from the Pond site. The midline of each boxplot is the mean and whiskers show the standard error. A square root transformation was applied to Cl^- and a log transformation was applied to Na^+ and Ca^{2+} prior to analysis.

Ion concentrations in above-ground plant tissues collected prior to winter salt application and in the following growing season did not significantly differ for sodium or chloride (Figure 28), consistent with our observation of relatively low accumulation of these ions in soils at these sites over the winter season (Figure 24). Potassium concentrations in above ground tissues did significantly differ by season; higher in plants grown in biochar (both species) and non-biochar amalgamate soils (cattail only) following winter salt application than plants growing in natural soils (control) prior to winter salt application (Figure 28).

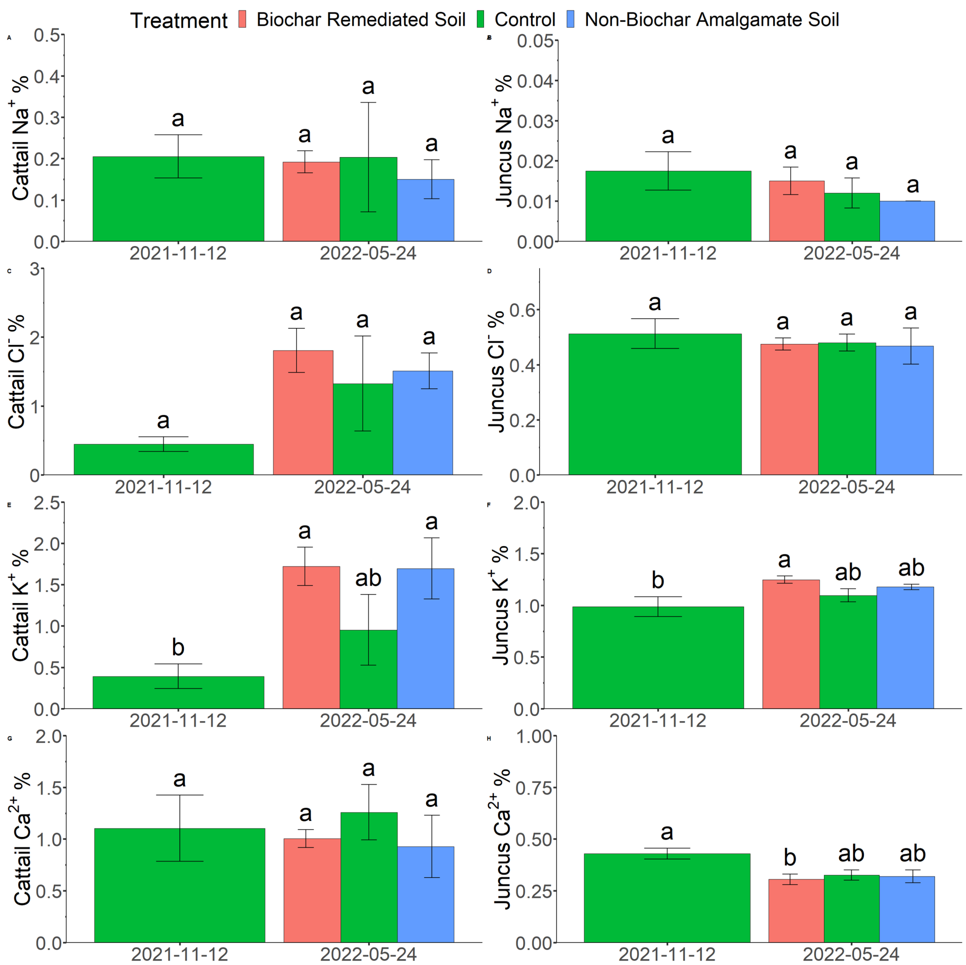

Figure 28. Fisher's LSD (α = 0.05, p-adj = Bonferroni) comparison of the ion content (%) of Na^+ and Cl^- of above-ground plant tissue in the pond site pre (2020-11-12) and post (05-24-2022) road salt exposure. Results are shown for cattail (left) and Juncus (right).

In closing, tissue analysis from field experiments suggests that rush plants may be salt excluders. Although they tolerate high salt concentrations they are not suited for phytoremediation because they cannot remove salt from the environment (Figure 26). Cattails do accumulate salt from the environment and have the potential for phytoremediation of deicing salts in wetland areas (Figures 22 & 26).

LABORATORY EVALUATION – MAXIMUM SALT UPTAKE BY PLANTS

Since environmental salt levels were lower than anticipated in Blacksburg field locations, a series of controlled experiments were conducted to determine the maximum salt accumulation possible through phytoremediation. Small rush and cattail plants were grown in 400 mM sodium chloride solutions at 25 to 28°C in a laboratory on the Virginia Tech campus. This concentration was chosen because it is near the maximum reported to not inhibit growth of cattails and is similar to the highest concentrations measured along some roadways. Five liters of 400 mM salt solution were added to 19-liter buckets containing either biochar-amended or natural wetland soil (soil treatments) and vegetative cattails with three to five leaves (Figure 29). Cattails were transplanted with soil root ball from natural stands at the Blacksburg wetland site (one to three plants per bucket). Rush plants were purchased in 24 count plug trays from Meadows Farms Nurseries in Chantilly, VA. Rush plants, one per 4-liter pot filled with Sunshine Mix potting soil, with or without biochar (soil treatment), were placed on trays flooded with 3 cm of 400 mM sodium chloride solutions in a sub-irrigated system. Pots and trays were incubated under T8 fluorescent lights producing 2500 lumens at plant height for 8 weeks. At the end of the experiment, samples were divided into above and below ground tissues, dried at 60° C, and shipped for tissue analysis to the NC State University plant tissue laboratory. Some of these samples have yet to be processed, but results for cattail are presented below.

Figure 29. Cattail plants growing in 400 mM sodium chloride solution to determine the maximum absorption possible

Results and Discussion

Rush and cattail plants added new leaves and continued to grow in 400 mM sodium chloride solutions, indicating both species are able to grow in high saline environments. Both above and below ground cattail tissues contained a higher percentage of sodium when grown in native soils than biochar amended soil, but these results were not statistically significant (0.7% Na+ in top tissue and 1.1% in root tissue - native soils; 0.4% Na+ in top tissue and 0.6% in root tissue - biochar amended soils; Figure 30A & B). For chloride, the same pattern was observed for root tissue (i.e., chloride uptake was slightly elevated in the control - 3%, relative to biochar amended soils 2.4%) (Figure 30C & D). However, the inverse was observed for above ground tissue, with higher chloride in biochar amended than control soils (4.2% and 3.8%, respectively) (Figure 30C & D). Neither of these differences were statistically significant.

The lower sodium values for cattail grown in biochar were somewhat unexpected but may have been caused by the strong binding of sodium to biochar (Amini et al., 2016, Moradi et al., 2019), release of competing cations from biochar that reduced sodium uptake by cattails, or greater disturbance of cattail root systems during the mixing of biochar and native soil that reduced sodium uptake. Uptake results should be interpreted as early growth stage estimates from a controlled setting (i.e., they may not reflect rapid growth of larger plants in the environment later in the season). However, cattails grown in containers in elevated NaCl did accumulate more Na+ and Cl^- than established plants from the pond and wetland sites in Blacksburg (compare Figure 30 with, 25, 27, and 28). Concentrations were somewhat lower, however, than those observed at highway and parking lot sites in NOVA, particularly for chloride (to compare Blacksburg results in % to NOVA results in mg/g, simply multiply the former by 10).

The container experiment illustrates that greater phytoremediation potential exists when plants are exposed to higher salt levels than those encountered in our Blacksburg test sites. Results of the container experiment also suggest that while biochar can effectively bind sodium, bound molecules may not be immediately accessible to plants (Amini et al., 2016, Moradi et al., 2019). Future work should explore the effect of repeat stormwater rinses on sodium and chloride adsorption to biochar to see whether or not sequestered salts are eventually released to plants, slowly over time.

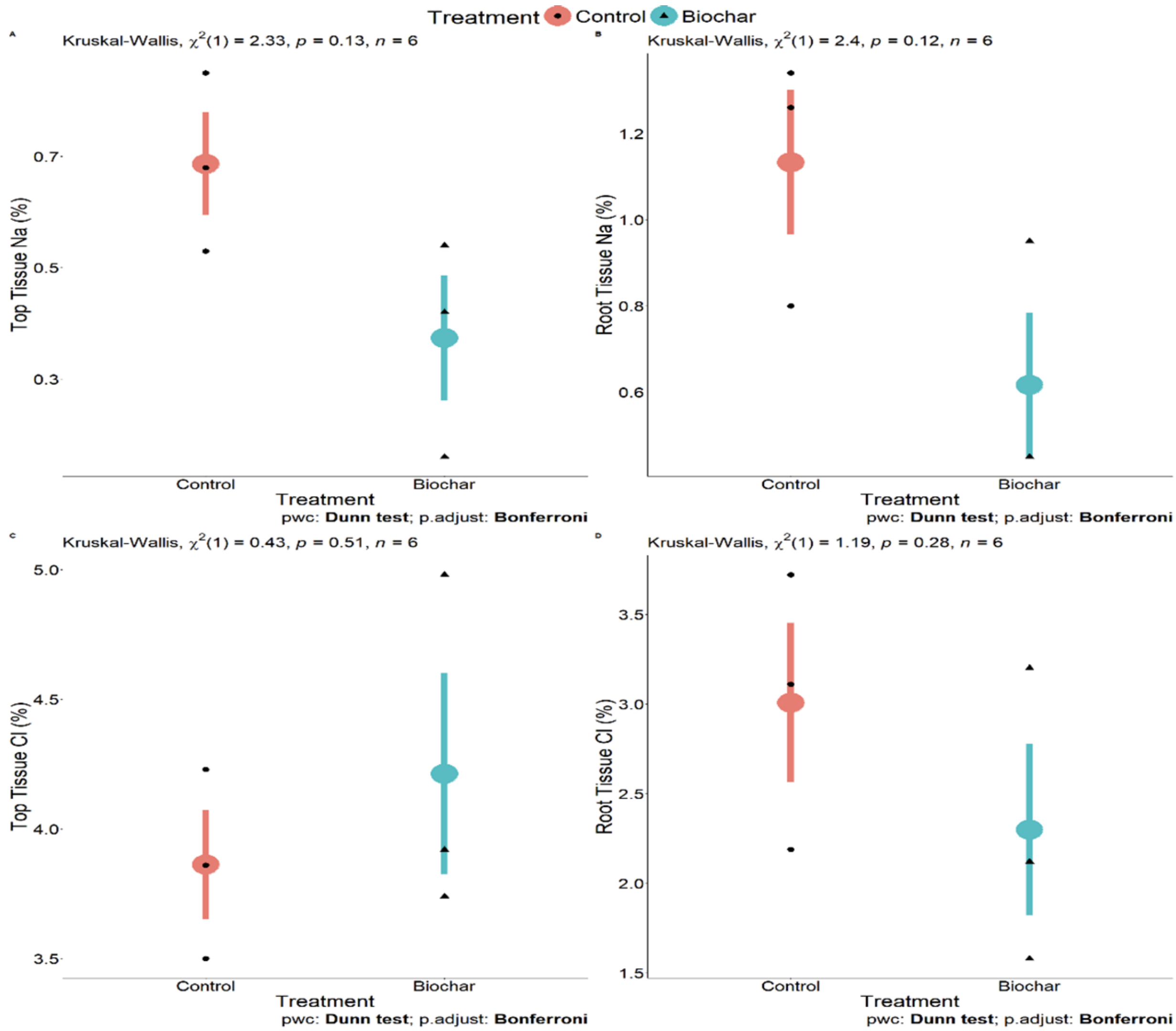

Figure 30. Percentage of Na^{+} and Cl^{-} accumulation by container grown cattail root and above ground tissue. Control plants were grown in native soil saturated with 400 mM NaCl. Colored circles are means with ± standard error bars. Black circles and triangles represent individual data points for control and biochar media treatments, respectively.

Using Environmental Containment Socks to Sequester Salt

Environmental containment socks are porous flexible fiber sleeves filled with highly absorbent materials. The filled socks are placed around drains to absorb chemical runoff in sensitive areas (Figure 31 & Figure 32). They are required by law in some locations at construction sites to protect storm sewers from runoff from harmful pollutants.

Experimental Design and Concept

One focus of this research was to establish salt-loving plants, or halophytes, as part of water management strategy to capture deicing salts near roadways, parking lots, and detention ponds. Some halophytes can naturally recover salts from runoff between the point of salt use (e.g., roads, parking lots) and nearby surface and ground water resources where they accumulate. However, alternative strategies are needed to capture deicing salts particularly in paved urban areas where plants cannot be easily grown.

Another approach is to use environmental containment 'socks' to capture salt from runoff water. Traditional absorbent 'socks' are long polypropylene tubes containing high absorbency materials like cellulose or corn cob filler. They are often used to build containment dikes that stop spills from spreading into the surrounding environment. We envisioned using 'socks' filled with porous substrate with high ionic binding capacity to filter and bind deicing ion species sodium, calcium, potassium, magnesium, and chloride as they flow off pavement before entering nearby watersheds. Environmental socks could be placed in any high flow runoff areas, such as parking lots, to maximize capture. The ability of socks to filter and chemically bind ions from runoff is called ion exchange capacity. Since sodium is positively charged and chloride negatively charged, the best substrate would contain a mixture of both positive and negative binding sites. For example, materials like clay and organic matter particles have a net negative charge although some positive charged sites exist as well. Thus, negatively charged substrate particles will attract and hold positively charged ions like sodium. Positively charged substrate sites hold negatively charged ions like chloride. These attractions are much like how the opposite poles of a magnet attract each other. By the same token, they will repel like charged ions, just like the same poles of a magnet repel each other.

The general concept is that porous containment socks of various shapes filled with ionic binding materials are strategically placed in drainage conduits and other areas of high flow runoff. Saline tainted water would flow through the sock and charged deicing molecules sodium, calcium, potassium, and magnesium would bind to the filler material before it enters the surrounding environmentally sensitive areas such as ponds, streams, and ground water. Filler materials inside socks may include but are not limited to zeolites, biochar, constructed clay beads, hemp fibers, diatomaceous earth, or electrically charged plastic resins. We filled socks with some of these materials, including biochar, and placed them in drainage ways to filter runoff from parking lots at the VT CRC and also in a laboratory in saline solutions to estimate their ion binding capacity. This experiment also allowed us to test the effectiveness of biochar to bind sodium and chloride under controlled conditions to estimate its effectiveness in bioremediation.

The tough fine mesh fiber socks can be positioned and collected repeatedly without breaking. Environmental socks can reversibly chemically bind deicing salt ions. Socks saturated with salt residue could periodically be recharged at a recycling facility. Once socks are rejuvenated, they can be redeployed to capture more salt ions in a sustainable recycling system.

Figure 31. Containment "socks" (Left) were placed in or around parking lot drains (Right) to filter surface runoff and keep sodium, chloride, or other ions out of the environment. Knots in the sock fabric isolate different fillers that have high ion binding capacity in this experimental system.

<u>Results and Discussion</u>

Environmental containment sock filler materials differed in their ability to bind sodium and chloride. Manufactured absorbent clay beads retained about 20,000 mg Na^+/kg sample followed by the 1:1 vermiculite-biochar mix that retained about 1,259 mg Na^+/kg sample (Figure 33, panel A). Biochar contained 200 mg Cl^-/kg sample, the most of any treatment (Figure 33, panel B). The vermiculite mixture (1:1, vermiculite-biochar) absorbed 158 mg Cl^- /kg sample the second most (Figure 33, panel B). Clay beads only retained 13.5 mg Cl^- /kg sample. This value is predictably small because clay is composed overwhelmingly of negatively charged particles which would absorb positively charged ions like Na^+ but repel negatively charged ones like Cl^-.

In a separate experiment designed to test ion binding capacity, socks were soaked in 400 mM NaCl. Biochar made from wood feedstock captured over 11,000 mg Na^+/kg sample (Figure 34, panel A).

Hemp biomass (coarsely ground) and hemp fiber (finely ground), and a mixture of biochar and hemp fibers captured less sodium than biochar alone in a range between 6,310 and 10,000 mg Na^{+}/kg sample (Figure 34, panel A). These materials were also effective at removing Cl^{-} from water. Biochar capturing 12,589 mg Cl^{-} /kg sample (Figure 34, panel B). The mixture of hemp fiber and biochar was second with 11,482 mg Cl^{-}/kg sample. Hemp fibers and hemp biomass captured just less than 10,900 mg Cl^{-}/kg sample (Figure 34, panel B).

The containment sock experiments also show the potential for binding salt with materials with high ionic binding capacities. Biochar amendments to soil at the VT CRC sites failed to improve phytoremediation by cattail and rush plants. This was mainly because Na^{+} and Cl^{-} ion concentrations in the environment were very low. In the presence of high Na^{+} and Cl^{-}, the ability of biochar to retain these ions when concentrations were elevated was clear. High amounts of Na^{+} and Cl^{-} were retained because of ionic interactions with charged sites on the substrate materials. The socks in Figure 34 were harvested when saturated with NaCl solution and dried. A significant volume of salt water was physically captured in the free spaces of each sock. The salts dissolved in this trapped water were concentrated as water evaporated during drying. The sock system has potential to capture salt runoff in parking lots and other paved areas. Salt can be captured and reclaimed in a recycling system, so the socks can be reused repeatedly. Virginia Tech Intellectual Properties is looking for collaborators among companies who design and sell environmental containment socks to develop a commercial product specifically for remediation of deicing salt.

Figure 32. An environmental containment "sock" positioned in drainage ways to filter saline runoff at the VT CRC. In March, socks were collected and analyzed for mineral composition.

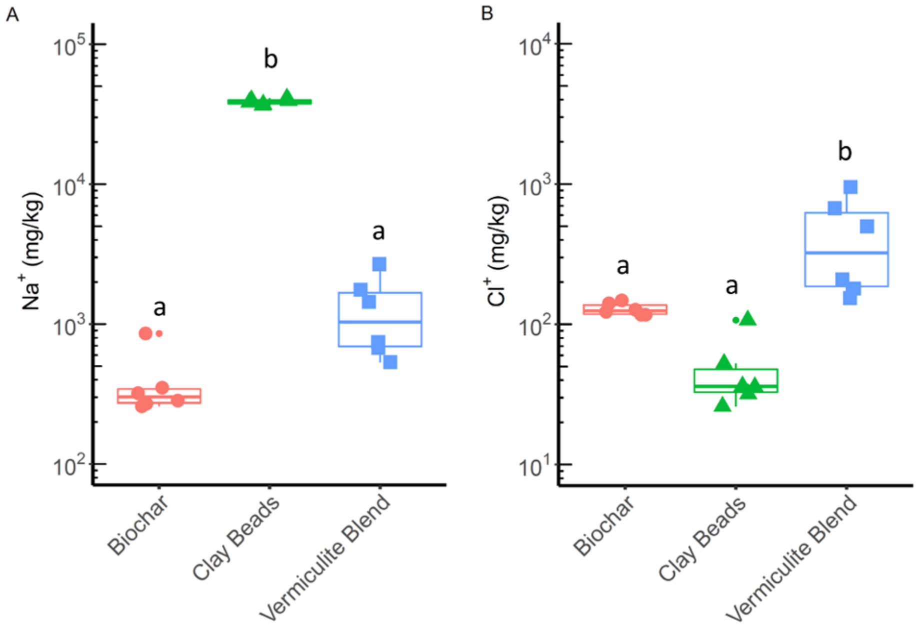

Figure 33. Capacity of environmental containment sock filler materials to bind sodium (A) or chloride (B). Mean Separation by Kruskal Wallis (α = 0.05, p-adj = false discovery rate). Ion concentrations in mg/kg.

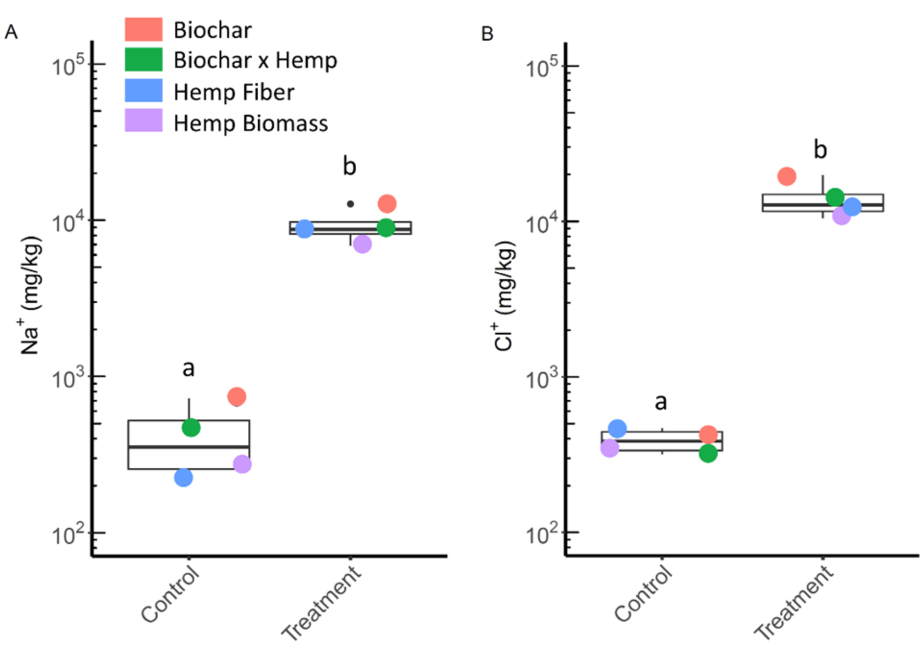

Figure 34. Comparison of sock filler materials ability to bind Na^+ or Cl^-. "Control" indicates the ion content in socks not exposed to NaCl. "Treatment" indicates the ion content of socks exposed to NaCl. The difference between hemp biomass and hemp fiber is the size of the particles. Hemp biomass is coarsely ground and hemp fiber is finely ground.

CONCLUSIONS

Salt presence in the environment

Salt concentrations varied widely depending on location. Highway sites in NOVA typically had higher salt concentrations than parking lot sites, which in turn had higher salt concentrations than sites draining open space. Salt concentrations at Blacksburg sites were in-between parking lot and open space sites in NOVA. Of the Blacksburg sites, higher salt concentrations were observed in the wetland than the pond site, which is consistent with the hierarchy we observed in NOVA (major road > parking lot).

Sodium and chloride concentrations in detention basin soils were rarely above concentrations known to cause harm to salt sensitive species. This was only observed at the NOVA parking lot site Sully 1, which had sodic (high sodium), but not saline, soils during winter months. Notably, soil information for NOVA highway sites was not available at the time this report was completed. Given the elevated salt concentrations observed in stormwater at highway sites relative to parking lot sites, it is entirely possible that soil salinities at highway detention basins will be comparable to Sully 1 or higher, posing some degree of risk to salt sensitive species.

Salt uptake by plants

Juncus effusus does not accumulate Na^+ and Cl^- ions and is not a candidate for phytoremediation of deicing salts. *Typha latifolia* does sequester significant Na^+ and Cl^- and has potential for phytoremediation in bioretention areas where de-icing salts are applied. In NOVA sites, we estimate that a single harvest of *Typha* could sequester between 0.4-2.4% of the sodium and 0.8-14.3% of the chloride added to highway sites this winter. Furthermore, because *Typha* showed substantial growth over a short period of time (i.e., almost six feet over 1.5 months), two cattail harvests per season may be possible, which would double these estimates. Future work should evaluate this possibility and better characterize the ion assimilative capacity of both juvenile (our focus) and adult cattail specimens to better quantify the capacity of *Typha* to phytoremediate deicing salts in the state of Virginia.

Biochar as a soil amendment showed no significant effect on cattail Na^+ or Cl^- ions uptake largely because there was little salt in the runoff water at VT CRC. The potential of biochar and other natural materials to absorb salt was illustrated in the environmental containment sock experiments where significant amounts of Na^+ and Cl^- ions were accumulated in biochar, vermiculite, clay, and hemp fiber filled socks (Figure 29 & Figure 33). Environmental containment socks have the potential to filter saline water runoff from parking lots and other paved surfaces before it enters environmentally sensitive areas.

NOTABLE FINDINGS

1) Salt concentrations of water samples collected in stormwater detention basins along roadways in NOVA were higher than those observed at Blacksburg field sites. The salt content (measured using electrical conductivity as a surrogate) in some systems was in excess of that typically observed in seawater.
2) Roadside stormwater conveyance and storage facilities offer an opportunity for phytoremediation of salt. Additionally:
   a) Cattail (*Typha latifolia*) accumulates significant Na^+ and Cl^- in their above ground tissues and are good candidates for phytoremediation where deicing salts typically accumulate.
   b) Curly dock (*Rumex crispus*) is a prevalent exotic species listed as a low-risk invasive that showed potential to perform a useful salt removal function.
   c) Common rush (*Juncus effusus*), an identified potential salt accumulator and one used in this study, is salt tolerant but excludes salt and is not suited for phytoremediation.
   d) Over half of the plant species growing in and near stormwater conveyance and storage structures where saline runoff accumulates were populated by plants classified as exotic, invasive, or of unknown native status at both NOVA and Blacksburg locations.
   a) Across all stormwater basins surveyed, six plant species were identified as both native and salt tolerant that are potential candidates for future phytoremediation studies:
      i) Cattail (*Typha latifolia)*
      ii) Canadian Serviceberry *(Amelenchier canadensis)*
      iii) Broomsedge *(Andropogon virginicus)*
      iv) Dwarf hair grass *(Eleocharis acicularis)*
      v) Hedge bindweed *(Calystegia sepium)*
      vi) Virginia Creeper *(Parthenocissus quinquefolia*)
3) Biochar, manufactured clay beads, and hemp fibers used as filler in environmental containment fabric sleeves (also called socks) can bind significant amounts of sodium (all materials) and chloride (all save clay) and can filter saline water before entering parking lot drains and other environmentally sensitive areas.

FUTURE WORK

Planned Site Closure

A task for site closure was included in the original scope of work. These activities will be completed before the end of this project. This includes restoration of test sites through the addition of soil to level the ground where plants were removed, and the removal of wooden identification stakes. Any remaining study plant materials will be left in place to avoid further site disturbance. It should be noted however that the sites should readily support future related investigations with respect to the effects of salt on plants and the ability of those plants to sequester salts. This includes plantings augmented with biochar in the soil. Additional sampling and analysis of plants in the summer of 2022 and spring of 2023 would likely provide valuable information that is unavailable under the time frame of this one-year study.

Future Research

Some key questions remain unanswered due to pending laboratory analyses and other time constraints. The project was designed to last two years to accommodate perennial plant establishment/growth and extensive analysis of field samples. Due to administrative constraints, the acquisition of materials and supplies, and time needed for site establishment, however, the experimental period was foreshortened, occurring in a period when perennial plants are typically slow to establish and grow (September 2021 until May of 2022). Most biomass growth (and presumably phytoremediation) occurs over summer, a time period we were not able to observe. This limitation of our study is important to consider when interpreting our results.

Other limitations and key questions to guide future research

1) Juvenile vs adult: The results presented above are largely based on seedlings or small plants. Analyzing salt accumulation in rapidly growing adult specimens may produce different results. Furthermore, the rapid growth of cattail observed in the final month of the study raises questions about how often this species might be harvested to remove salts from roadside basins. Species capable of multiple harvests have the potential to be very high value from a phytoremediation standpoint.
2) Native halophytes: We were somewhat constrained by the requirement to use only native salt tolerant plants, which are not always available from commercial nurseries and may not always survive well in stormwater detention basins and ponds. Plant samples from our NOVA sites indicate that curly dock (*Rumex crispus*), a non-native, may be both a useful salt accumulator and well-adapted to saline soils near roadways. More information about the salt accumulation capacity of *R. crispus* and other non-natives may lead to better management of weedy species, including, perhaps their utilization for phytoremediation. We have also identified six candidate salt-tolerant native species for future evaluation in the phytoremediation of salt.

The research team appreciates the support of the Virginia Department of Environmental Quality and the Virginia Legislature in conducting this study and hope the results are useful and help inform future work.

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APPENDIX A - LITERATURE REVIEW

INTRODUCTION

Salinization of land is a major environmental issue that is growing rapidly. Salt contaminated soils are increasing with climate change (Hasanuzzaman *et al*., 2014, Karakas *et al.,* 2020). It is estimated that half the world's arable land will be impacted by salinization by 2050 if efforts are not taken to mitigate salt pollution (Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020; Litalien & Zeeb, 2020; Singh *et al.*, 2021). Salt pollution is a complex, interdisciplinary, global issue. Desalination of soils and water is often prohibitively expensive, difficult, and labor intensive (Hasanuzzaman *et al.,* 2014; Litalien & Zeeb, 2020). Salinity comes from both natural sources such as weathering parent material and sea salt deposition as well as anthropogenic sources such as changing water tables from human activity, irrigation with salt water, agricultural practices, soil drainage issues from construction, and the use of de-icing agents (Arora *et al.,* 2014; Hasanuzzaman *et al.,* 2014; Karakas *et al.*, 2020; Litalien & Zeeb, 2020; Singh *et al.*, 2021). This review will focus on ways of mitigating effects of roadway deicing salts.

Roadway deicing salt runoff is one of the major pollutants of transportation ecosystems in the United States. Magnesium chloride (MgCl) and sodium chloride (NaCl) are used commonly, though NaCl comprises the majority of road salt applied (Gonsalves *et al.*, 2014). After deicing salts are applied (often in excess), they are moved through precipitation events and wind and deposited into soils and water surrounding roadways. Salt pollutant negatively impacts crops, native plants, soil biota, and manmade and natural water systems, as well as human health (Hasanuzzaman *et al*., 2014; Litalien & Zeeb, 2020; Singh *et al.,* 2021) and is especially dangerous for people who suffer from high blood pressure (Gonsalves *et al.*, 2014). Salt also damages urban structures such as road surfaces, bridges, buildings, vehicles, and more (Baeckstroem *et al.,* 2004). There is great need to reduce salt pollution from deicing salt runoff (Ashraf *et al*., 2010; Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020; Litalien & Zeeb, 2020; Singh *et al.*, 2021).

Salinity inhibits seed germination, plant growth, and reproduction. High concentrations can kill mature plants, often leaving roadsides barren. Saline soils are more likely to leach into groundwater (Hasanuzzaman *et al*., 2014). One cost-effective and environmentally responsible solution is phytoremediation, or the use of plants and/or microbes to remove or neutralize pollutants (Ashraf *et al*., 2010; Hasanuzzaman *et al*., 2014; Karakas *et al.,* 2020; Rabhi *et al*., 2008, Suaire *et al*., 2016; Young *et al.*, 2011). Phytoremediation is defined as the use of plants and/or microbes to reduce the concentrations and toxicity of environmental pollutants (Ashraf *et al*., 2010; Hasanuzzaman *et al*., 2014; Rabhi *et al*., 2008; Suaire *et al.,* 2016). Phytoremediation as a term refers to many processes and approaches including phytostabilization, phytodegradation, phytovolatilization, and phytoextraction (Suaire *et al.,* 2016). "Phytoextraction" is the ability of some plants to extract pollutants such as salt ions, or "phytodesalination" which is the phytoextraction of salt, from the environment and accumulate them within their biomass and some halophytes, or salt-loving plants, are capable of phytoextraction (Litalien & Zeeb, 2020; Suaire *et al.,* 2016). Phytoremediation of salt-affected roadside soils is possible and an attractive possibility. Chemicals used in traditional salt Na^+ remediation approaches, such as gypsum, increase soil calcium (Ca^+) content replacing Na^+ ions on exchange sites. However, this approach is expensive and increasing in price. (Hasanuzzaman *et al.,* 2014; Litalien & Zeeb, 2020; Rabhi *et al.,* 2008). Na^+ displaced by Ca^+ can still become a pollutant in some cases. Phytoremediation can also include other inputs such as microbes and soil amendments, which support plant growth or remediation goals.

This overview is made with the intention of facilitating the future research needed to create standardized phytoremediation treatments for roadway salt pollution in Virginia. I will discuss candidate halophytes that may be suitable as one approach to remediating deicing salts from the environment. The other focus is how soil amendments can help hold salts at peak application times during winter until they can be sequestered by plants.

LITERATURE REVIEW

Roadway Salt Runoff Pollution in Virginia

NaCl salt is one of the most common salts applied to roadways because it is easily available and inexpensive (Snodgrass *et al.,* 2017). The Virginia Department of Transportation (VDOT) maintains more than 57,000 miles of roadway, the majority of which is subject to the anti-icing program which includes snow removal and the use of deicing agents such as NaCl salt to deice roads for driver's safety (Fitch *et al.,* 2005). Most public works departments follow a "bare pavement" policy, as advocated by the Salt Institute (Fitch *et al.,* 2005). For deicing purposes, salt application rates are in the tens of Mg/year/km of roadways (Fitch *et al*., 2005; Litalien & Zeeb, 2020), and it is estimated that more than 10 million tons of salt are applied to roadways nationally each year (Fitch *et al.,* 2005; Snodgrass *et al.,* 2017). Most of this salt dissociates into Na^+ and Cl^- ions polluting both soil and water. One study by Jahan & Pradhanang (2020) showed that runoff Cl^- concentrations were highest in the winter and early spring. The highest concentrations were found year-round close to salt input areas such as parking lot sites, showing that approximately 70% of applied road salts stay within local watersheds. Salt pollution contributes to the trend of increasing salt concentrations in urban areas due to de-icing agents. Once dissolved, salt ions cannot be removed from the water using natural processes (Jahan & Pradhanang, 2020).

While deicing salts are effective at melting frozen precipitation, they are also a concerning source of environmental pollution. In addition to NaCl, which is damaging on its own, deicing agents often contain other chemicals such as anti-caking agents like sodium ferrocyanide or ferric ferrocyanide, as well as sodium hexametaphosphate and chromate salts to prevent automobile corrosion (Fitch *et al.,* 2005; Karakas *et al*., 2020; Litalien & Zeeb, 2020; Robinson & Thomson, 2015). As precipitation washes salt from roadways, the saline runoff contaminates groundwater, surface water, and has adverse effects on roadside vegetation (Fitch *et al.,* 2005, Karakas *et al.,* 2020; Litalien & Zeeb, 2020; Robinson & Thomson, 2015). Runoff polluted with salt has wide ranging effects, negatively impacting urban areas (Fitch *et al.,* 2005; Baeckstroem *et al.,* 2004), agricultural lands (Hasanuzzaman *et al.,* 2014; Litalien & Zeeb, 2020), streams and other water systems (Baeckstroem *et al.,* 2004; Litalien & Zeeb, 2020; Suaire *et al.,* 2016), human health and public water systems (Baeckstroem *et al.,* 2004; Litalien & Zeeb, 2020; Singh *et al.,* 2021; Snodgrass *et al.,* 2017). Only an estimated 35% of deicing salts used on roadways make it to detention sites for treatment. The majority are released to the environment, either by soaking into soil before it reaches the detention pond or overflowing once it's been contained within detention ponds (Suaire *et al.,* 2016).

The term for soils impacted by salt pollution is "salt-affected soils." This describes saline, sodic, and saline-sodic soils and refers to the accumulation of salts in soil to the extent that plant growth and other soil processes are limited (Karakas *et al.,* 2020; Litalien & Zeeb, 2020). Excessive salt negatively affects the physicochemical properties of soil, causing soil particle dispersion, reduced hydraulic capabilities, salt crusting, increased erosion, increased pH, mobilization of trace metals, and electrical conductivity (EC) (Baeckstroem *et al.,* 2004; Karakas *et al.,* 2020; Litalien & Zeeb, 2020). These negative effects contribute to groundwater contamination (Baeckstroem *et al.,* 2004; Robinson & Thomson, 2015). Salt pollution and decreased plant matter from salt-killed vegetation negatively impact biodiversity in soil and aquatic systems to the point of loss and reduces richness of detritivore, macroinvertebrate, and fish populations. Salt pollution also impacts nutrient cycling and water quality in both soil and aquatic systems (Gonsalves *et al.,* 2014; Litalien & Zeeb, 2020; Snodgrass *et al.,* 2017). Salt pollution remobilizes trace metals in soil, increases the bioavailability of trace metals in water (Baeckstroem *et al.,* 2004; Suaire *et al.,* 2016), and depletes soil carbon stocks with an average of 3.47 tons lost per hectare (Litalien & Zeeb, 2020). Salt pollution decreases the soil's capability to act as a carbon sink contributing to global climate change by releasing CO_2 into the atmosphere. Thus, desalination of roadway runoff is imperative to global environmental health (Litalien & Zeeb, 2020; Suaire *et al.,* 2016).

Phytoremediation of Salt-Affected Soils

Phytoremediation of salt-affected soils using halophytes is advantageous for many reasons. Using this strategy, the need to purchase expensive chemical amendments is eliminated. There is potential for financial return by utilizing halophyte crops or products of secondary value harvested during the amelioration process. Vegetation, soil, and water impacts are lessened through phytoremediation. Vegetation-stabilized soil encourages beneficial soil aggregation and macropore formation which improves hydraulic properties of the soil which subsequently improves soil structure, drainage, and biota supporting capabilities of soil (Ashraf *et al.,* 2010; Karakas *et al.,* 2020; Young *et al*., 2011). Carbon is also sequestered in soil post-remediation. Another advantage of this strategy is that the burden of cost and maintenance for phytoremediation projects are eased over time as the vegetation systems become more established (Hasanuzzaman *et al.,* 2014).

Phytoremediation of salt-affected soils has been found to be comparable to remediation with chemical amendments (Hasanuzzaman *et al.,* 2014). However, there are some challenges to overcome for phytoremediation of salt-affected soils. Phytoremediation is time consuming, taking one or more growing seasons to be effective. Remediation ability is limited to the rhizosphere, where plants can actively glean pollutants from cation exchange sites. Phytoremediation is proportional to the biomass that can be produced as well as the accumulation capacity of that aboveground biomass (Hasanuzzaman *et al.,* 2014). Also, there is a seasonal aspect to phytoremediation of salt-affected soils (Snodgrass *et al.,* 2017). Seasonal salt concentrations vary, as most salt applications occur in the winter when plants are quiescent (Alden, 2021; Baeckstroem *et al.,* 2004; Snodgrass *et al.,* 2017). Hence, there is a need to find a method to sequester salt in the rhizosphere until salt uptake can occur during active growth periods. Increasing the cation exchange capacity of soil using amendments that can adsorb salt ions is one approach which is covered later in this review.

Lastly, amelioration of saline soils using halophytes increases over time. As halophytes age, the salt-storing capabilities and size of salt glands of their leaves increase and plateau (Litalien & Zeeb, 2020). Dicotyledonous plants are more capable of storing large amounts of salt in older leaves than monocots because of higher respiration rates, more Na^+ transporter activity in some plant tissues, and higher vacuole densities (Albert, 1975; Dashtebani *et al.*, 2014; Sleimi & Abdelly, 2003). Additionally, halophytes with previous salt exposure have higher capacities for phytodesalination (Barcia-Piedras *et al.,* 2019) which increases phytoremediation potential over time.

<u>Halophytes Overview</u>

Halophytes are useful in reclaiming salt-affected soils (Hasanuzzaman *et al.,* 2014). Some halophytes are able to decrease the soil EC of the soil solution by absorbing and accumulating or conducting soluble salts (Rabhi *et al.,* 2008). Phytoextraction of salts in this manner can bring damaged or unusable land back into cultivation and increase sustainability of the land in question (Hasanuzzaman *et al.,* 2014). This is a strategy long used by the Dutch to reclaim sea land along the coast of the Netherlands for cultivation (Welbaum, 2021). Studies have shown that cultivating halophytes on saline soils will reduce salt contamination, increase soil organic matter (OM), and increase stability through improved structure (Ashraf *et al*., 2010; Hasanuzzaman *et al.*, 2014; Karakas *et al.,* 2020).

Some halophytes can complete their entire life cycles under saline conditions close to sea water (Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020). There is a spectrum of halophytic plants, including a range of obligate, facultative, and habitat-indifferent halophytes. Obligate halophytes require saline environments for optimal performance (Hasanuzzaman *et al.,* 2014). There are many obligate halophytes within the Chenopodiaceae family (Hasanuzzaman *et al.,* 2014; Shekhawat *et al*., 2006). Facultative halophytes are able to grow and establish in saline environments, but it is not their optimal environment. There are many facultative halophytes within the Poaceae, Cyperaceae, and Brassiceae families. Habitat indifferent halophytes are able to tolerate saline soils, but prefer non-saline soils (Hasanuzzaman *et al.,* 2014). For phytoremediation of roadside runoff, halophytes which are obligate or facultative which can tolerate fluctuating salinity throughout the season.

To be most effective for phytoremediation, halophyte species that actively uptake large amounts of Na^+ and/or Cl^- are needed. Some halophytes can compartmentalize salt ions within cell vacuoles to avoid salt stress or to use for osmotic adjustment and water uptake. There are three classifications of halophytes based on how they deal with salt stress: 1. salt excluding (those whose roots possess ultra-filtering mechanisms), 2. salt excreting halophytes or "recretohalophytes" (those which regulate salt levels by excretion through foliar salt glands), and 3. salt accumulating halophytes (those which build up salts within cells, tissues, or organs such as salt bladders to minimize salt toxicity through succulence) (Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020; Litalien & Zeeb, 2020). Salt exclusion is a very efficient means of preventing excessive buildup of salts in plant tissues. Exclusion is much more common strategy used by halophyte species compared to accumulating or excreting plants

(Hasanuzzaman *et al.,* 2014; Litalien & Zeeb, 2020). Salt excretors and accumulators are the classifications of highest value for phytodesalination because of their ability to remove salt from the soil system. Some species are capable of both accumulating and excreting salts (Shabala & Mackay, 2011).

<u>Salt excreting halophytes</u>

Salt excreting halophytes, or recretohalophytes, exist within the families Asterid, Caryophalles, Rosid, and Poaceae. There are four types of salt glands within these families. The most basic is a type of salt bladder found exclusively within Aizoaceae and Amaranthaceae members. Next are plants with multicellular salt glands that exist in the families Plumbaginaceae, Acanthaceae, and Tamariceae. The last two types are bicellular and unicellular salt glands such as those found in monocot grass species *Chloridoid* and *Porteresia* (Litalien & Zeeb, 2020). Salt glands (bicellular and unicellular) are present in trichomes, known as microhairs, that contain many small vacuoles within cap and basal cells that store salts (Hasanuzzaman *et al.*, 2014). Salt excretors generally are capable of taking up more salt from soils than accumulators. By excreting salts from their leaves, wind can disperse crystals over large areas effectively reducing soil concentrations to less damaging levels. This method is called "haloconduction" and can be incorporated into long-term, natural attenuation approaches to phytoremediation of salt-affected soils (Litalien & Zeeb, 2020). Salt excretors are useful for some types of phytoremediation such as agricultural soils, but are not solely ideal for roadside remediation. Excretors may have some value in a mixed or established, stratified system. They can reduce the salt load of soils surrounding roadways where the salt stress will be the highest. Examples of species that excrete salt that may be useful for roadside phytoremediation in Virginia include *Spartina* and *Distichlis* (Table 1) (Litalien & Zeeb, 2020).

<u>Salt accumulating halophytes</u>

Salt accumulating halophytes are able to uptake and hold salt within plant tissues. Salt bladders in leaves, which are comprised of a swollen epidermal cells with large vacuoles, or within cell vacuoles sequester salts that are used for osmotic adjustment (Hasanuzzaman *et al*., 2014; Karakas *et al.*, 2020; Litalien & Zeeb, 2020; Shabala & Mackay, 2011; Rabhi *et al.,* 2008; Shekhawat *et al.,* 2006; Zhao *et al*., 2005). Epidermal salt bladder cells, or EBC, are characteristic of the families Chenopodiaceae, Oxalidaceae, and Mesembryanthemaceae. These salt bladders accumulate excess Na^+, Cl^-, and K^+ ions, metabolic compounds like malate, flavonoids, cysteine, pinitol inositol, and calcium oxalate crystals. At least 50% of halophytes do not use EBC to tolerate salt stress (Shabala & Mackay, 2011). Cell vacuoles likely take the role of salt storage in bladderless species, and even in species which possess bladders, salt may still be stored largely in cell vacuoles of mature leaves (Shabala & Mackay, 2011). EBC storage and vacuole storage mechanisms often result in succulence (Karakas *et al.,* 2020; Litalien & Zeeb, 2020). Salt bladders may rupture once maximum salt content has been reached. Physical disturbance on leaves, such as touch, wind, precipitation, or other forces, will leave salt deposits on the leaf surface. Salt residues from ruptured bladders do not expel salt in the same volume as recretohalophytes do (Litalien & Zeeb, 2020). Once salt has been accumulated by the plant, it can be mechanically harvested to effectively remove salt from the soil (Karakas *et al.,* 2020; Rabhi *et al.,* 2008; Suaire *et al.,* 2016). Harvested plant tissues can then be disposed of in a different location, which relocates the salt to less sensitive areas. Alternatively, harvested tissues can be recycled into other materials such as biochar, which will be covered in a later section of this review (Shabala & Mackay, 2011).

Hyperaccumulators

Hyperaccumulators are halophytes which accumulate or conduct amounts of salts large enough to be used reliably for phytoremediation of salt-affected soils (Robinson *et al.*, 2003). Some can accumulate more than 20% of their dry weight biomass as salt ions (Litalien & Zeeb, 2020; Shabala & Mackay, 2011; Suaire *et al.,* 2016; Zhao *et al.*, 2005). Some can haloconduct over 90% of salt taken up (Litalien & Zeeb, 2020; Sleimi & Abdelly, 2003). Examples of well-studied hyperaccumulating halophytes, not all native to Virginia, include *Atriplex* spp*., Suaeda* spp., *Salsola* spp., *Chenopodium* spp., *Mesembryanthemum crystallinum*, and *Portulaca* spp. (Karakas *et al.,* 2020; Litalien & Zeeb, 2020). One study (Suaire *et al.,* 2016) recorded *Atriplex* species (*A. halimus* and *A. hortensis*) that accumulated more than 50 mg of Na^+ ions in their aerial tissues over 60 days when exposed to a 2g/L NaCl solution. This demonstrates the promise for using these species for phytodesalinization of saline road runoff. *Salicornia europaea* accumulated up to 426-475 kg salt/ha in biomass in one study (Yucel *et al.,* 2017) and 139g Na^+/kg dry weight and 180g Cl^-/kg dry weight in another (Litalien & Zeeb, 2020). Ravindran *et al.* (2007) studied six halophytes for their capacity to desalinate the upper 40 cm of soil. *Suaeda maritima* and *Sesuvium portulacastrum* decreased the EC of the soil solution from 4.9 to 1.4-2.5 dSm^{-1}. Shekhawat *et al.,* (2006) observed effects of salinity on biomass production, water content, and ion accumulation of six members of the Chenopodiaceae family (*Atriplex amnicola, Atriplex calotheca, Atriplex hortensis, Chenopodium album, Salsola kali,* and *Suaeda nudiflora*). They found that all species accumulated salt and survived a 6000 mg/L NaCl treatment. Of the six species, the most suitable for phytodesalination was *Suaeda nudiflora* as it accumulated the most Na^+ ions with increasing salinity treatments and produced the greatest biomass overall (Shekhawat *et al.* 2006). Rabhi *et al.* (2008) observed halophytes *Anthrocnemum indicum, Suaeda fruticosa,* and *Sesuvium portulacastrum* can desalinate soils under non-leaching conditions, such as arid and semiarid soils where precipitation is too low to leach salts from the rhizosphere. All halophytes used in the Rabhi *et al*. (2008) study had the ability to accumulate significant amounts of sodium from their substrates, though their absorption and accumulation effectiveness varied.

There is plenty of evidence showing the potential of halophytes in phytodesalinization projects. However, more studies are needed to determine extraction and accumulation rates for halophyte species, as well as their bioregional suitability. There is also a need for more studies on the efficacy of pure accumulator, pure recretohalophyte, and mixed halophyte stands for different phytoremediation needs. It is essential to avoid invasive species during phytoremediation efforts. More research is needed on native halophytes that can be used in phytoremediation in Virginia and the mid-Atlantic region.

Halophyte Candidates for Phytoremediation of Roadway Salt Runoff in Virginia

In order to fulfill the need to identify bioregionally suitable candidate halophytes for Virginia and the mid-Atlantic region, the parameters for "ideal" had to be identified. The ideal halophytes for roadway salt runoff phytoremediation in Virginia would be native or non-invasive, perennial or reliably self-seeding annuals, obligate or facultative halophytes that are low maintenance, low growing, winter hardy, easily established by seed, drought resistant, tolerant of roadside stress conditions such as high traffic and low nutrition, and have a substantial and explorative root system to maximize salt uptake potential (Alden, 2021; Hasanuzzaman *et al.,* 2014; Welbaum, 2021). Candidate halophytes must also be prolific biomass producers that are easily mechanically harvested. Hyperaccumulators are particularly desirable, as they are well suited for this type of remediation (Hasanuzzaman *et al.,* 2014; Litalien & Zeeb, 2020).

A mixed stand of hyperaccumulators and recretohalophytes could be useful for dispersing salt to a density which can be more readily remediated within plant uptake limitations. A possible approach is to stratify vegetation according to salt levels. Hyperaccumulators closer to the road may desalinate higher salt loads before leaching. Salt excretors planted farther away from the road may dilute salt concentrations in soil (Litalien & Zeeb, 2020). Another stratified system to consider is using plants which prefer dry soils near roadsides and plants which prefer wetter soils along ditches. For the most effective phytoremediation effort, candidate halophytes should be active in the early spring. One challenge to the phytoremediation of deicing salts is that saline runoff is highest in the winter and early spring months when most plants are dormant (Hasanuzzaman *et al.*, 2014; Jahan & Pradhanang, 2020; Wahls *et al*., 2016).

Additionally, halophytes which have higher economic importance and offer secondary value would be most desirable for phytoremediation projects (Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020; Litalien & Zeeb, 2020; Snyder, 1991). Some halophytes have additional value as products, such as a substitute for conventional crops, fuel, forage, fodder, timber, or fiber. Additional potential uses could be essential oil extraction, or medicinal uses (Hasanuzzaman *et al.,* 2014; Karakas *et al.,* 2020; Litalien & Zeeb, 2020; Snyder, 1991).

Candidate Halophytes List

The following is a list of candidate halophytes. Most are native to Virginia, those which are not are introduced or naturalized. The halophytes chosen were suitable to be used in an immediate roadside vegetation band or in seeps and wet ditches close to the road in Virginia and the mid-Atlantic region. Candidate halophytes and their salt accumulation were found in a search containing or combining keywords such as: halophyte, salt, NaCl, ion, compartmentalization, content, accumulat***, hyperaccumulating, mechanism, removal, sequestering, stress, tolerance, transport, uptake; sodium, chloride; roadway, roadside, road salt. A more concise layout of this information can be found in Table 1.

Table 1. An alphabetical list of candidate halophytes by species, common name, growth cycle, status, salt tolerance classification, halophyte type, and accumulation rates found from literature review.

Table 1. Alphabetical list of candidate halophytes by species, common name, growth cycle, status, salt tolerance classification, halophyte type, and accumulation rates

Species	Common name	Growth Cycle	Status	Salt Tolerance Classification	Halophyte Type	Approximate Salt Accumulation Rates
Agropyron smithii/Pascopyrum smithii	Barton' Western wheatgrass	Perennial	Native	Facultative	Recretohalophyte	Unknown
Alopecurus arundinaceus	Garrison' creeping meadow foxtail	Perennial	Introduced	Facultative	Likely Recretohalophyte	17 mg Na/g DW (Riedell, 2016)
Atriplex glabriuscula	Smooth orach	Annual	Native, threatened	Facultative	Accumulator	>35 g Na/kg FW (Ievinsh, 2020)
Atriplex patula	Spear orach	Annual	Native	Facultative	Accumulator	4826 µmol Na/g DW (Glenn & O'leary, 1984); 490 kg Cl/ha/season (Litalien & Zeeb, 2020); Mean concentration of 40 mg Cl/g DW and mean mass of 50 mg Cl/plant (Morteau *et al.*, 2009); 5-20% Na DW biomass (Young et al., 2011)
Baccharis halimifolia	Groundsel-tree	Perennial	Native	Obligate	Accumulator	Up to 50 mg Na/g DW (Caño *et al.*, 2016)
Bolboschoenus robustus	Sturdy bulrush	Perennial	Native	Facultative	Unknown	Similar species *B. maritima* showed ~400 mmol Na/L and 500 mmol Cl/L (Albert, 1975); 200-400 mmol L Na (Ievinsh *et al.*, 2021)
Chenopodium album	Lambsquarters	Self-seeding annual	Introduced	Facultative or obligate	Accumulator	570 kg NaCl/ha/season (Litalien & Zeeb, 2020); 7.63 mg Na ions/g DW (Shekhawat *et al.*, 2006)
Distichlis spicata	Saltgrass	Perennial	Native	Obligate	Recretohalophyte	64.10±3.14 mg Na/g DW plant in shoot and 30.35± 1.66 mg Na/g DW root (Sabzalian *et al.*, 2018)
Eleocharis parvula	Drawf spikerush	Perennial	Native	Likely facultative	Unknown	1.0 mol Na (Ievinsh *et al.*, 2021)
Festuca rubra	Red fescue	Perennial	Native	Facultative or indifferent	Likely Recretohalophyte	9.6 mg Na/g DW (Cooper, 1982); Crowns accumulated >30 mg Na/g DW, young leaves accumuated >30 mg Na/g DW, old leaves accumulated up to 35 mg Na/g DW (Krishnan & Brown, 2009)
Hibiscus moscheutos	Swamp rose-mallow	Perennial	Native	Unknown	Unknown	Unknown
Hordeum jubatum	Foxtail barley	Perennial	Native in Western US, naturalized in Eastern US	Facultative	Likely Accumulator	166.9 mEq Na/100g DW and 216.0 mEq Cl/100 g DW stem tissue, 152.9 mEq Na/100g DW and 246.2 mEq Cl/100g DW leaf tissue (Badger & Ungar, 1990)
Impatiens capensis	Jewelweed	Self-seeding annual	Native	Unknown	Unknown	Unknown
Juncus gerardii	Saltmarsh bulrush	Perennial	Native	Obligate	Likely Accumulator	66.3 mg Na/g DW (Cooper, 1982); ~300 mmol Na/L and >400 mmol Cl/L (Albert, 1975); 40-150 mmol Na in leaf sap (Shabala & Mackay, 2011)
Medicago sativa	Alfalfa	Perennial	Introduced, naturalized	N/A	Accumulator	>450 mmol Na/kg DW and 450 mmol Cl/kg DW (Grieve et al., 2004); up to 43.1% Na concentration in salt intolerant cultivars and 40.8% Na concentration in salt tolerant cultivars (Winicov, 1991)
Panicum virgatum	Shawnee' switchgrass	Perennial	Native	Facultative	Accumulator or Recretohalophyte	Between 9.92-38.86g Na/kg DW depending on cultivar (Cordero *et al.*, 2019); 25 mg Na/g DW (Riedell, 2016)

Table 1. Continued

Species	Common name	Growth Cycle	Status	Salt Tolerance Classification	Halophyte Type	Approximate Salt Accumulation Rates
Puccinellia distans	Weeping alkali grass	Perennial	Introduced	Obligate	Accumulator	125-250 mg Na/g DW (Dashtebani *et al.,* 2014); 400 mmol Na/L and 500 mmol Cl/L (Albert, 1975); 50-130 mmols Na in leaf sap (Shabala & Mackay, 2011)
Salicornia virginica	Jointed glasswort	Perennial	Native	Obligate	Accumulator	19.17-37.94 mmol/L Na (Ownbey & Mahall, 1983; Shabala & Mackay, 2011); Accumulated Cl concentration up to 30% of DW tissue (Ralph & Manley, 2006)
Schoenoplectus pungens var pungens	Common threesquare	Perennial	Native	Unknown	Accumulator	Unknown
Solidago mexicana	Seaside goldenrod	Perennial	Native	Facultative	Accumulator	Unknown
Spartina alterniflora	Smooth cordgrass	Perennial	Native	Obligate	Recretohalophyte	~1050 µg Na/g DW (Chai et al., 2013); 2.0 mmol Na/kg 0.4 M NaCl treatment (Vasquez et al., 2006); 1.5 mM NaCl/g DW and 1.5 mM Cl/g DW (Sleimi & Abdelly, 2003)
Spartina patens	Saltmeadow cordgrass	Perennial	Native	Obligate	Recretohalophyte	~2.7% of leaf tissue in Na (Tobias *et al.,* 2014)
Sporobolus airoides	Alkali sacaton	Perennial	Native	Facultative	Recretohalophyte	62.12-200.87 mmol Na/g leaf FW weekly, 16.22-75.31 mmol Cl/g leaf FW weekly (Weragodavidana, 2016); ~550 mmol Na/kg DW and ~525 mmol Cl/kg DW per season (Grieve *et al.,* 2004); 0.318-0.637% Na of aboveground biomass (Burris, 2017).
Suaeda maritima	Herbaceous sea-blite	Annual/perennial	Native	Obligate	Accumulator	504 mg NaCl/plant/season (Hasanuzzaman et al., 2014); 147 mmol Na in 200 mmol NaCl treatment (Clipson & Flowers, 1986); 700 mmol cations in biomass after 7 weeks (Flowers *et al.*, 1986); 380-660 mmols Na in leaf sap (Shabala & Mackay, 2011)
Typha latifolia	Common cattail	Perennial	Native	Facultative	Accumulator	Mean concentration of 65 mg Cl/g DW, mean mass of 70 mg Cl/plant (Morteau *et al.*, 2009)

Agropyron smithii (also referred to as *Pascopyrum smithii*), or Western wheatgrass, is a cool-season, rhizomatous grass native to the western U.S. which grows to 1-3' tall (Aschenbach, 2006). It comes out of dormancy in March for Virginia (Alden, 2021; Wahls *et al*., 2006). It is generally a prairie plant and is found in its natural habitats alongside plants such as *Sporobolus airoides, Buchloe dactyloides, Koeleria macrantha, Hesperostipa comata, Nassella viridula,* and *Schizachyrium scoparium*. Western wheatgrass handles drought, flooding, and cold, and shade stress (Alden, 2021; Tirmenstein, 1999) such as what is found on many Virginia roadsides. It has secondary value as a forage and fodder plant and is used in grazing pastures. It is an aggressive sod-forming grass, and rhizomes can penetrate soil depths to 7' or more, making it a very suitable plant for erosion prevention. It has been used in reclamation of disturbed sites such as surface coal mines and other sites with weak soil structure. It is also used for revegetation of saline-alkali areas. It rapidly establishes on abandoned or disturbed land. Western wheatgrass is not highly dependent upon vesicular arbuscular mycorrhiza, or "VAM," for survival. It establishes well through broadcast seeding in either the fall or spring. It has strong, rapid vegetative growth in the early spring, flowers in June, and fruits from August to September (Tirmenstein, 1999). There are many cultivars available for use, as it is commonly used in seeding mixtures for revegetation projects. The best candidate varieties for salt-polluted roadside soil phytoremediation in Virginia are expected to be: "Barton," a native variety from clay bottomlands in Kansas, and "Recovery," a variety which has excellent seedling vigor and establishment and is intended for use in revegetating disturbed rangelands, high traffic areas, and areas with high soil erosion and disturbance events (Ogle *et al.*, 2009). This plant tolerates high salinity and salt fluctuations well and is deemed to be a good candidate plant for salt remediation projects (Aschenbach, 2006; Deeter, 2002). Salt accumulation has not yet been determined for this species.

Alopecurus arundinaceus, or 'Garrison' creeping meadow foxtail, is a perennial, rhizomatous, cool-season grass growing to 3-6' tall (Alden, 2021; USDA-NRCS, 2013). This variety is not native to the U.S. but is introduced and present around the U.S. (USDA-NRCS, 2013). It is vegetative in early spring and is tolerant of drought, flooding, poor drainage, frost, alkalinity, acidity, and salt conditions (Alden, 2021; Markovskaya *et al*., 2020). It is aggressively rhizomatous, able to quickly recover from aboveground damage like harvest, and is valuable for erosion control. It tolerates a wide range of habitats and soil conditions, including a broad range of pH (5.6-8.4) and salinities, usually performing well at moderate salinity. These features made it valuable on critically disturbed areas and difficult terrain such as roadsides and saline seeps. It competes well with species such as *Juncus* spp. and *Carex* spp. It has secondary value as grazing forage. Seeding is most successful with a cover of hay, firm packing of seeds into soil, on moistened soil, and with early precipitation to promote germination. Coated seeds help germination rates. This species can become weedy and requires management in the form of mechanical harvest or control through competition (USDA-NRCS, 2013). Riedell (2016) found that creeping meadow foxtail accumulated 17 mg Na^+/g of dry weight plant material (DW).

Atriplex glabriuscula, or smooth orach, is a native, annual, obligate halophyte growing 20-60 cm in height with a creeping stem (Markovskaya *et al.,* 2020). Smooth orach is a species of concern in some New England states. It is presumed to be extirpated from its previous range in Virginia, thus using this plant for phytoremediation may also have additional conservation value (Flora of North America, 2003b; Kartesz, 1999). Smooth orach prefers sandy and stony soils much like what is found on many roadsides (Markovskaya *et al.*, 2020). *Atriplex* spp. have secretory trichomes or vesicular microhairs as a characteristic feature of the genus (Markovskaya *et al.,* 2020; Weragodavidana, 2016). They also have salt bladders on their epidermal tissue (Markovskaya *et al*., 2020). Smooth orach preferentially accumulates Na^+ in its aboveground biomass for osmotic regulation. It was shown to accumulate approximately >35 g Na^+/kg fresh mass (Ievinsh, 2020). Not all *Atriplex* spp. accumulate Na^+ in this manner, some species in the genus clearly exclude Na^+ from shoots and accumulate the ions in roots instead, such as *Atriplex halimus* (Ievinsh, 2020).

Atriplex patula, or spear orach, is an annual found in most northern and coastal US states (Flora of North America, 2003b; USDA NRCS, 2021) and Canada (Young *et al.,* 2011). It grows to a height of 2-3' tall and prefers full light and moist soils but is drought tolerant. This plant, like many *Atriplex* sp., has secondary value as an edible crop (Flora of North America, 2003b). Spear orach germinates best and produces highest yield when shallow seeded in the spring versus broadcast seeding (Young *et al.*, 2011). Spear orach has been shown to accumulate around 4826 μmol Na^+/g DW (Glenn & O'Leary, 1984), a mean concentration of 40 mg Cl^-/g DW, a mean mass of 50 mg Cl^-/plant (Morteau *et al.*, 2009), and 490 kg Cl^-/ha in a growing season (Litalien & Zeeb, 2020).

Baccharis halimifolia, or groundsel-tree, is a perennial shrub native to the Eastern coastal states that grows up to a height of 5m. It commonly inhabits moist soils with high organic matter such as pond or bay margins, swamps, wet prairies, marshes, salt marshes, and everglade hammocks. It rapidly colonizes disturbed sites and readily regrows if aboveground parts are trimmed (Van Deelen, 1991). *B. halimifolia* associates with both AMF and ectomycorrhizal species, with root colonization rates between 20-45% for AMF and 10-20% for ectomycorrhiza (Younginger *et al.*, 2009). *B. halimifolia* was reported to accumulate up to 60 mg Na^+/g DW and was shown to accumulate more Na^+ as salinity treatments increased (Caño *et al*., 2016).

Bolboschoenus robustus, also known as sturdy bulrush, is a native, rhizomatous, perennial sedge growing 2.5-5' tall. It is an obligate wetland species and a fast spreading halophyte with high germination rates (95% in lower saline environments, inhibited to 50% around 9000 ppm NaCl, and halted around 2,000 ppm NaCl but retains dormancy until conditions are favorable again) and high survival rate (88%) in wetland conditions. It establishes quickly when conditions are suitable, doubling its vegetative cover within a month. It has value as food and habitat for wildlife. Muskrats and waterfowl eat the seeds, and the vegetation is cover for fiddler crabs and nesting ducks. It has recorded use in remediation work to improve habitat for largemouth bass. Sturdy bulrush would be best utilized in detention ponds or wet ditches along roadsides. It thrives in salinities between 3,000-22,000 ppm NaCl with an optimal growth range between 3,000-7,000 ppm NaCl and pH between 4.3-6.4. It tolerates fluctuating water levels and performs best in disturbed environments such as post-fire systems. It sprouts in early spring, flowers in April-August and fruits from July-October. It resprouts in the fall if inundated. Excellent companion plants for sturdy bulrush include: common reed (*Phragmites communis*), switchgrass (*Panicum virgatum),* cordgrass (*Spartina* spp.), American bulrush (*Schoenoplectus americanus*), widgeon grass (*Ruppia maritima*), coastal saltgrass (*Distichlis spicata* var. *spicata*), sedge (*Carex* spp.), buckbrush (*Baccharis halimifolia*), marsh button (*Achyranthes philoxeroides*), seaside goldenrod (*Solidago mexicana*), cattail (*Typha* spp.), bulltongue (*Sagittaria* spp.), and cutgrass (*Zizaniopsis miliacea*) (Snyder *et al.,* 1991). One study by Albert (1975) showed that a similar species, *B. maritimus,* accumulated concentrations close to 400 mmol Na^+/L and 500 mmol Cl^-/L in its aboveground biomass. Another study (Ievinsh *et al*., 2020) showed that *B. maritimus* accumulated 200-400 mmol Na^+/L.

Chenopodium album, or lambsquarters, is an annual, herbaceous, obligate halophyte growing 0.2-2m tall (CABI, 2019; Deeter, 2002). It is an introduced species which has been present in the U.S. since colonization and has a wide distribution with frequent, ubiquitous occurrence throughout the U.S. including Virginia. It establishes better when seeded early in spring. It has secondary value as an edible crop, fodder, and as a traditional medicinal plant (CABI, 2019). *C. album* is very salt tolerant (Deeter, 2002) and is a common volunteer in saline soils (Young *et al.*, 2011). In one study (Shekhawat *et al.,* 2006), increased salinity reduced *C. album* biomass production, but stunted growth from salt stress did not stop the plant from accumulating NaCl at the maximum salinity tested. Relative succulence increased with salinity (Shekhawat *et al.*, 2006). Lambsquarters is an excellent biomass producer capable of producing 3.23 tons of biomass per hectare and 20% of that biomass is accounted for by salt content, which is roughly 570 kg $NaCl^-$/ha (Litalien & Zeeb, 2020).

Distichlis spicata, or saltgrass, is a perennial, strongly rhizomatous, warm-season, low growing, obligate halophyte graminoid. It is native to coastal regions of the U.S. as well as some inland salt marshes and similar saline wet soils. It grows up to 1' tall and forms dense colonies, and rhizomes can grow to depths of 10" into soil, forming a dense sod which makes this species excellent for erosion control (Hauser, 2006; Sabzalian *et al.,* 2018). It can grow in a wide range of habitats including tidal salt marshes, deserts, and grasslands (Hauser, 2006). Saltgrass volunteers on disturbed sites (Perry & Atkinson, 1997) and prefers full light environments (Hauser, 2006). It is most competitive in disturbed areas and is eventually outcompeted as succession advances. It has secondary value as forage as it stays green into cold season and is a source of food for wildlife, namely migrating birds (Hauser, 2006). Rhizomes are its primary means of reproduction. Root systems are notably colonized by VAM fungi which aid its survival in hypersaline soils (Hauser, 2006). Saltgrass deals with salt stress by excretion and is able to significantly reduce the salinity of the top 10 cm of soil over time (Litalien & Zeeb, 2020; Sabzalian *et al.*, 2018). One study (Aschenbach, 2006) showed that saltgrass' growth is stunted at salinity levels above 9.85 dS/m, though it can survive in higher salinities and is deemed a good candidate for salt remediation projects. Another study (Sabzalian *et al.,* 2018) suggested that optimal salinity for this plant was around 12 dS/m. It was shown to survive in substrates of 20 dS/m salt concentrations without showing significant stress (Pessarakli *et al.*, 2012). Saltgrass grows well in nature with many of the halophytes listed here, including but not limited to *Agropyron smithii*, *Salicornia* spp., *Juncus gerardii, Atriplex* spp., *Sporobolus airoides, Puccinellia* spp., and *Spartina* spp. (Bertness, 1988; Hauser, 2006). A study by Sabzalian *et al*. (2018), showed that *D. spicata* accumulated 64.10±3.14 mg Na^+/g DW plant in shoot and 30.35±1.66 mg Na^+/g DW roots.

Eleocharis parvula, or dwarf spikerush, is a perennial, grass-like, herbaceous halophyte native to North America which grows to roughly 10 cm tall (Calflora). Dwarf spikerush is a facultative halophyte usually found in wetlands and coastal soils (USDA). Ievinsh *et al.* (2020) showed that *Eleocharis parvula* accumulated 1.0 mol Na^+ ions.

Festuca rubra, or red fescue, is a perennial, cool-season, rhizomatous grass native to the northern U.S. states. It has value in remediation and often volunteers on severely disturbed sites such as abandoned coal mines and roadsides (Krishnan & Brown, 2009; Walsh, 1995). Red fescue germinates quickly and at high rates. This fescue holds up well against foot traffic and roadside stresses and has high pest and disease resistance (Krishnan & Brown, 2009). It has value as a wildlife food source. It is used to prevent erosion on irrigation ditches, waterways, channels, highways, and hillsides. It can spread through seed or vegetative propagation. It is drought and flood tolerant, and grows on a broad range of soil types. It tolerates soil pH between 4.5-6.0 well and doesn't require much soil fertility but does require high light. It becomes vegetative in early spring and grows slowly until midsummer then grows vigorously until frost. It regenerates readily when aboveground parts have been harvested or removed (Walsh, 1995). A study by Khan & Marshall (1981) showed that red fescue accumulated 371.4 mEq Na^+/100 grams DW and 297.4 mEq Cl^-/100g DW. Another study by Cooper (1982) showed that *F. rubra* accumulated 9.6 mg Na^+/g DW in dry saline substrate. Krishnan & Brown (2009) showed that red fescue crowns accumulated up to 30 mg Na^+/g DW, young leaves accumulated up to 30 mg Na^+/g DW, and old leaves accumulated up to 35 mg Na^+/g DW.

Hibiscus moscheutos, or swamp rose-mallow, is a native shrubby, herbaceous, perennial forb growing 1-2.5' tall and forming a large woody rootstock. It is often found in moderately saline tidal marsh communities in nature. It is able to sprout vegetatively from the caudex. It handles drought and inundation stress well. This plant is aesthetically pleasing with beautiful, prominent blossoms (Reeves, 2008).

Hordeum jubatum ssp. *jubatum*, or foxtail barley, is a short-lived, perennial, fibrous-rooting, cool-season, facultative halophyte grass native to western North America and naturalized in eastern North America due to increasing soil salinity in urban areas as a result of human activity (Badger & Ungar, 1990; Tesky, 1992). It grows 1-2' tall and is capable of producing two cohorts a year, one in spring and the other in the fall. It is native to the Western US and is naturalized in the Eastern states. It grows vegetatively from April onward, with flower and seedset occurring from late May to late July (Tesky, 1991) and grows densely, providing up to 90-100% vegetative cover at moderate salinities (Badger & Ungar, 1990). It commonly volunteers in disturbed, saline soils. It has extensive, aggressive rooting habits which make it valuable for erosion control. It is a prolific seeder and also propagates vegetatively, especially when salinity is high enough to inhibit seed germination (1% salinity and above can impact germination). It has value as food for wildlife, especially the seed, though the dry seed heads can be dangerous to grazing animals due to their spiky nature and ability to penetrate flesh. Mechanical harvest would be a good management strategy to prevent seed issues as well as removing salt-laden biomass from the site. It has potential where forage value is of secondary importance to remediation importance. Additionally, it has value as an ornamental flower when dried (Tesky, 1992). At 1.0% NaCl substrate, *H.*

jubatum was found to accumulate concentrations of 166.9 mEq Na^+/100g DW and 216.0 mEq Cl^-/100 g DW in stem tissue, and 152.9 mEq Na^+/100g DW and 246.2 mEq Cl^-/100g dry weight in leaf tissue (Badger & Ungar, 1990).

Juncus gerardii Loisel, also known as saltmarsh bulrush, is a frost tolerant, rhizomatous, perennial graminoid growing over 1' tall. It is a brackish species native to coastal US regions. It has been naturalized in the Great Lakes region (Cao *et al.,* 2021). It forms extensive colonies in salt marshes and coastal meadows. It is a facultative halophyte with a preference for non-saline soils. It doesn't tolerate inundation well. It is most competitive in high light conditions, with shoots emerging in March and lasting until June, then fruits from May to August (Cao *et al.,* 2021). *J. gerardii* is restricted to coastal distributions in nature. Its vegetative biomass is reduced in saline conditions, but overall number of shoots produced was not affected, showing that *J. gerardii* shows potential to carry out vegetative propagation under saline conditions (Rozema & Blom, 1977). A study by Albert (1975) showed that *J. gerardii* accumulated around 300 mmol/L concentration of Na^+ and >400 mmol/L concentration of Cl^- in its aboveground biomass. Shabala & Mackay (2011) showed that *J. gerardii* contained 40-150 mmols Na^+ in leaf sap within 200-500 mM NaCl range treatments. Another study (Cooper, 1982) showed that *J. gerardii* accumulated 66.3 mg Na^+/g DW in waterlogged saline soils and 41.3 mg Na^+/g DW in dry saline soils.

Medicago sativa, or alfalfa, is a long-lived, perennial legume which is naturalized in much of the U.S. It grows 2-3' tall. Alfalfa commonly volunteers on disturbed sites and is suited to roadside stress conditions. It fixes atmospheric nitrogen and does not need nitrogen fertilizer to perform optimally. It has great value as a food plant for wildlife and livestock. It also supports honey production and pollinator migration. It is regarded as the most valued legume. Seed mixes intended for revegetating disturbed lands often include alfalfa varieties. Alfalfa replenishes soil nutrients, supports growth of other plants, reduces erosion and compaction while stabilizing soil with its deep roots, increases forage value, acts as a soil conditioner for future growth, and handles difficult soils and high traffic well. It seeds easily through broadcasting and does well with a firm seedbed (Sullivan, 1992). There are many varieties and subspecies, some of which have been bred for salt tolerance (Alden, 2021). While alfalfa itself is very salt tolerant (Deeter, 2002), it excludes salt (especially Na^+ ions, making it natrophobic) (Grieve *et al*., 2004; Scasta *et al.,* 2012). However, alfalfa enriches soil and supports the growth of plants around it. For this reason, more salt-tolerant varieties such as 'Ameristand 90' (Alden, 2021), 'Barstow,' 'Salado,' 'Malone,' and 'Mesa Sirsa' cultivars are valuable in salt-affected soils (Greub *et al.,* 1985; Scasta *et al*., 2012)..

‘Ameristand 90’ alfalfa is a recommended variety for Virginia roadside phytoremediation (Alden, 2021). A study by Winicov (1991) showed that alfalfa was able to grow well in 1.0% saline substrate and that salt tolerant varieties were able to accumulate between 2.2-40.8% Na^+ concentration.

Panicum virgatum, or ‘Shawnee’ switchgrass, is a very salt tolerant, native, warm-season, perennial grass growing to 3-5’ tall (Alden, 2021; Deeter, 2002). It is sod and bunch forming. Most growth occurs in the early summer but it grows fast and matures early. It is tolerant to shallow soil, drought, flooding, poor drainage. *P. virgatum* has higher salt tolerance than other switchgrass cultivars (Alden, 2021; Wahls *et al.*, 2006), tolerating up to moderate soil salinities (Uchytil, 1993). It can also tolerate a soil pH range between 4.5-6.5. It is an excellent biomass producer that can produce 2-4 tons of aboveground biomass per acre. It produces both through seed and vegetative propagation. Shawnee switchgrass has secondary value as a valuable grazing pasture and forage plant, especially in early summer. It also serves as a valuable shelter source for many wildlife species. Shawnee switchgrass is used for revegetation on disturbed sites such as abandoned mine lands. It is also used for erosion control on soils with weak structures, along waterways, in areas of high disturbance, and for prairie restoration. There are many cultivars available as this plant is very popular for revegetation efforts. Aboveground harvesting may damage the plant over time. Replanting after a few years may be necessary (Uchytil, 1993). Shawnee switchgrass accumulates salt in its aerial parts but also excretes salt onto leaf surfaces. A study by Riedell (2016) showed that *P. virgatum* accumulated 25 mg Na^+/g DW. Another study found that accumulation rates of Na^+ for *P. virgatum* were found to vary according to cultivar, with ‘Alamo’ accumulating a concentration of 4.53 g/kg in leaves and 5.39 in stems, ‘Kanlow’ accumulating 16.23 g/kg in leaves and 13.55 in stems, and ‘Trailblazer’ accumulating 15.47 g/kg in leaves and 23.39 g/kg in stems (Cordero et al., 2019). Variation in accumulation rates by cultivar are expected to occur in most halophytes, though more studies need done.

Puccinellia distans, or weeping alkali grass, is a non-native introduced species, commonly occurring in Virginia. It is a perennial, cool-season, sod-forming bunchgrass that is well adapted to alkaline and saline soils (Burris, 2017; USDA-NRCS, 2021). It is naturalized in the Great Lakes area (USDA-NRCS, 2021). Weeping alkaligrass has volunteered and migrated along the corridor of interstate I-77 where high volumes of deicing salts are applied (Alden, 2021). Weeping alkaligrass is also excellent for soil stabilization due to its prominent roots (Dashtebani *et al.*, 2014). It’s natural occurrence in roadside environments in addition to its salt, drought, and flood tolerance makes it a candidate for this type of phytoremediation (Alden, 2021; Deeter, 2002). A study by Dashtebani *et al.* (2014) showed that

P. distans produced higher biomass when inoculated with *Claroideoglomus etunicatum*, an arbuscular mycorrhizal fungus species which resides naturally in saline soils alongside *P. distans*. In addition to higher biomass production, AMF inoculated plants showed reduced salt stress compared to plants which were not inoculated. The biomass of inoculated plants was not significantly affected by the higher salinity treatments of the study. In addition to AMF, *P. distans* can avoid salt toxicity with its thick endodermis, vacuolar compartmentalization and sequestration of Na^+ which is used as an osmolyte (Dashtebani *et al.,* 2014; Shabala & Mackay, 2011). A study by Albert (1975) showed that *P. distans* accumulated concentrations around 400 mmol Na^+/L and 400 mmol Cl^-/L in its aboveground biomass. Another study by Shabala & Mackay (2011) showed that *P. distans* contained 50-130 mmols Na^+ in leaf sap within 200-500 mM NaCl range treatments.

Salicornia virginica, or jointed glasswort, is a rhizomatous, perennial, obligate halophyte native to Western US states, growing 1' tall (Ball, 2012; Ownbey & Mahall, 1983). It prefers full sun and moist soils. It flowers from July to November in Western states (Ball, 2012). *S. virginica* was found to take up 4.52-7.56 mOsmol NaCl per unit in dry weight and 19.17-37.94 mOsmol/kg through evapotranspiration (Ownbey & Mahall, 1983). Shabala & Mackay (2011) showed that *S. virginica* had 19-38 mM Na^+ in xylem within 200-500 mM NaCl range treatments. Ralph & Manley (2006) showed accumulated Cl^- concentrations up to 30% of *S. virginica*'s DW tissue.

Schoenoplectus pungens var. *pungens*, or common threesquare, is a rhizomatous, perennial grass-like herb native to most of the US growing 4-6' tall. It is commonly found in floodplains, ditches, streams, marshy areas, and pond or lake margins. It is moderately halotolerant and can tolerate seasonal drought. Seeds are produced from July through August and help in the seed heads for many months if undisturbed. Germination can be difficult, with seeds requiring cold stratification and scarification to germinate. Germination rates can be rather unpredictable (Stevens *et al.*, 2012). A study by Ievinsh *et al.* (2021) that a related species, *Schoenoplectus tabernamontani,* preferentially accumulated Na^+ ions in its aboveground biomass.

Solidago mexicana, or seaside goldenrod, is a native perennial forb growing up to 6'. It produces sprouts early in the season, from February to March, and blooms from August to October and produces a large, clustered spike of yellow flowers. In nature it is often found with some of the species mentioned in this review, such as *Panicum virgatum* and *Spartina patens*. It is a facultative wetland species. Seaside goldenrod can tolerate infertile soils, drought, and pH ranges between 5.5-7.5. It is quite halotolerant and is succulent, so it may have a high rate of salt accumulation. Seaside goldenrod is an important resource for pollinators and other wildlife, serving as food and shelter. It supports the migration of the monarch butterfly as a primary food source in the fall. Seaside goldenrod has stocky, short rhizomes and a root-length of at least 14” making it excellent for erosion control. In sandy areas it contributes to dune formation. It propagates through seed and clonally. Once a stand is established it requires minimal irrigation and little to no maintenance. This species can withstand hot and dry conditions such as what is found on roadside soils. It produces better when broadcast seeded with American beachgrass (*Ammophila breviligulata*). One issue with this plant is that it is suspected to produce root exudates which negatively impact the growth of surrounding vegetation, especially native grasses such as *Triplasis purpurea* and *Cenchrus tribuloides* (Sheahan, 2014).

Spartina alterniflora, or smooth cordgrass, is a perennial, warm-season grass native to North America along eastern coasts and marshes. It grows to 1.5- 8’ tall depending on conditions. Its natural habitat retains surface water year-round and includes plants such as *Distichlis spicata* and *Juncus roemerianis*. Smooth cordgrass has been successfully direct seeded on damaged marsh soils in Virginia to mitigate erosion and remediate the marsh soil by filtering heavy metals from the water column. Smooth cordgrass is quite halotolerant and is used as an indicator of salinity. It is a source of shelter for wildlife species. It germinates from April to June. Smooth cordgrass can germinate in salinities up to 6-8 percent. It establishes well through rhizomatous growth and forms a sod-like layer within the upper 5.9 inches of soil from April to October, with the upper 2 inches having the densest rhizome formations. It dominates where salinities range between 3-5% and prefers wet soil or wetland conditions. It is known for invading wet roadside ditches. While often dominant in saline environments, it is outcompeted by other *Spartina* spp., *Juncus* spp., and *Distichlis* spp. (Walkup, 1991). *Spartina alterniflora* excretes salts onto its leaf surfaces (Smart, 1982). In a study by Chai *et al.* (2013), *Spartina alterniflora* seedlings accumulated about 1050 µg $NaCl^-$/g DW. This study showed that smooth cordgrass can grow well in 600 mM NaCl substrate and likely higher salinities (Chai *et al.,* 2013). Another study by Vasquez *et al.* (2006) showed that *S. alterniflora* has a shoot Na^+ content of 2.0 mmol under 0.4 M NaCl treatment and that the plant seemed to preferentially accumulate Na^+ over K^+ for osmotic regulation. This efficient salt tolerance mechanism made *S. alterniflora* competitive over some invasive species such as *Phragmites australis* (Vasquez *et al.*, 2006). Sleimi & Abdelly (2003) showed that *S. alterniflora* accumulated 1.5 mM NaCl/g DW and 1.5 mM Cl^-/g DW at 800 mM NaCl substrate. Sleimi & Abdelly (2003) also acknowledged that *S. alterniflora*’s capacity for phytodesalination is likely underestimated because an approximate 90% of salt taken up through the plant was excreted through leaves and not sequestered in plant tissues.

Spartina patens, or saltmeadow cordgrass, is a perennial, warm-season grass native to the Atlantic coast, growing 1-5' tall in rhizomatous clumps. It flowers from June to September around Virginia and the Carolinas. It is valuable as a food source for wildlife and serves as natural pasture. It is flood tolerant and has roots that readily develop aerenchyma tissue. It regularly grows in brackish marshes, low dunes, sand flats, beaches, overwash areas, and high salt marshes (Deeter, 2002; Walkup, 1991). Salt content of soils where it occurs in nature ranges between 0.12-3.91 percent. *Juncus gerardii* is competitive against saltmeadow cordgrass and can exclude it from certain habitats (Walkup, 1991). Tobias *et al.* (2004) showed that *S. patens* accumulated up to 2.7% of its leaf tissue in Na^+.

Sporobolus airoides, or alkali sacaton, is a facultative halophyte that is a perennial, warm-season, chloridoid bunchgrass native to the western U.S.. It grows from tillers and seeds to a height of 0.5-3' tall. It is tolerant of salt, drought, and flooding conditions but not shade (Alden 2021; Deeter, 2002; Johnson, 2000; Weragodavindana, 2016). Alkali sacaton has salt glands which excrete salt on leaf surfaces (Weragodavindana, 2016). It readily forms associations with VAM fungi and produces more biomass when inoculated. It can grow in saline and non-saline soils and tolerates salinities between 0.003-3% well with optimal performance between 0.3-0.5%. It is known to invade saline flats and tolerates fluctuating saline inputs well. It flowers between July to October depending on where it's grown and produces seeds in the fall. It grows in a wide range of soils and does not require high fertility or organic matter content. It has secondary value as forage and shelter for wildlife and has grazing value for livestock. It establishes well when seeded on saline sites in mixtures with *Panicum virgatum*. It performs well in riparian zones and has been used in reclamation efforts in saline areas, oil well reserve pits, saline waste areas, sewage sludge sites with bauxite residue, and selenium contaminated sites. Best management practices for establishing alkali sacaton from seed on highly disturbed sites includes having soil moisture above 14%, soil temperature near 30 °C, using seeds at least 1 year old, prewetting site before seed application, and early irrigation to promote germination (Johnson, 2000). Weekly salt gland excretion rates of Cl^- for *Sporobolus* sp. were found to be between 16.22-75.31 mmol NaCl/g dry weight of plant and excretion rates of Na^+ ranged between 62.12-200.87 mmol Na^+/g dry weight of plant (Weragodavindana, 2016).

Suaeda maritima, or herbaceous sea-blite, is native, annual, succulent, herbaceous, obligate halophyte growing to 2' in height (Raju & Kumar, 2016). It grows in salt marshes and coastal beaches and is often found near other species such as *Salicornia virginica, Spartina alterniflora*, and *Salsola kali*. It is not a common species in the U.S. and is generally confined to Northeastern coasts (Massachusetts Division of Fisheries & Wildlife, 2015). Herbaceous sea-blite has been shown to accumulate 504 mg

NaCl in a four-month season, and some *Suaeda* spp. can contain up to 10% salt by weight (Litalien & Zeeb, 2020). Flowers *et al*. (1986) showed that *S. maritima* accumulates 700 mmol concentration of cations in aboveground plant parts after 7 weeks. Clipson & Flowers (1986) showed that Na^+ concentrations in *S. maritima* xylem approximated 147 mmol grown in 200 mmol substrate. Shabala & Mackay (2011) showed that S. maritima accumulated 380-660 mmol Na^+ in leaf sap and 46-60 mM Na^+ in xylem within 200-500 mM NaCl range treatments.

Typha latifolia, or common cattail, is a rhizomatous aquatic or semiaquatic perennial growing 3-10' tall native to the US. It pioneers in disturbed soils and establishes quickly, often forming dominant stands. Clonal propagation is their primary means of propagation though it is capable of seeding if flowers present. Salt tolerance varies with growing stage, seeds being most vulnerable to salt. Vegetative cattails are regularly spotted in saline soils and waters and have been known to invade brackish marshes. It is fairly drought tolerant (Gucker, 2008). In one study, common cattail accumulated a mean concentration of 65 mg Cl^-/g DW and a mean mass of 70 mg Cl^-/plant (Morteau *et al.,* 2009)

This list of candidate halophytes is by no means exhaustive but is merely a speculative list of plants which show promise for phytoremediation of Virginia roadside soils with runoff salt pollution.

Halophilic Microbes

Halophiles, or salt-loving microbes, are "halotolerant" which means they have adapted to high saline conditions. By definition, halophiles require at least 0.2 molar (M) salt for growth (Arora *et al.,* 2014). Halophilic microbes exist within a spectrum, much like halophytes, ranging from slightly halophilic which are salt tolerant (around 0.2-0.5M or ~1.3% salt) up to extremely halophilic (2.5-5.2M or 15-32% salt) (Arora *et al.*, 2014). Halophilic bacteria are often moderately halophilic (Arora *et al.,* 2014).

The rhizosphere is a very microbially active place, even when highly saline. Plants contribute primarily to the root exudates and nutritive compounds available in the rhizosphere, such as carbohydrates, amino acids, and sugars (Arora *et al.,* 2014). The microbes most commonly found in the rhizosphere include bacteria, archaea, fungi (VAM being notably prominent), viruses, and actinomycetes (Arora *et al.,* 2014). Because of this relatively high level of activity and diversity even in saline conditions, halophilic microbes should be considered an important aspect of phytoremediation.

Halophiles are conducive to phytoremediation because they can survive in high salinity, accumulate salt ions from soil, and support vegetation against salt stress (Arora *et al.,* 2014; Litalien &

Zeeb, 2020). They are also used industrially for their ability to decontaminate saline or sodic wastewater and degrade toxic compounds in soil and water. They are low maintenance and have simple nutritional requirements. Their hypothesized main value in salt-affected soils comes from supporting plant growth in this harsh environment (Arora *et al.,* 2014; Singh *et al.*, 2021). The rhizosphere distribution of microbes in salt-affected soils is determined by more than the salinity levels (Quesada *et al.,* 1983). Plant-host specificity and soil characteristics such as aeration, texture, moisture, and more play important roles (Arora *et al.,* 2014).

Soil salinity is shown to decrease proportionally to the density of halophiles. Some species can consume salt from substrate (Shukla *et al.*, 2011), such as *Oceanobacillus kapialis*, which is reported to increase phytoextraction capacity and assist salt uptake and accumulation in halophytes under saline conditions (Litalien & Zeeb, 2020). Other species assist plant growth, which subsequently reduces salinity through uptake and improved soil conditions. Rhizobacteria, such as *Azospirillum* spp., are one of the best adapted microbes for living in salt-affected soils and are well-known for their role in supporting plant growth (Tripathi *et al.,* 1998). Halophilic microbes also support optimal plant growth amidst salt stress and are easy to include as inoculum during vegetation efforts (Litalien & Zeeb, 2020). Bacteria are most commonly used for this type of remediation technique, but archaea, actinomycetes, and fungi can be used as well (Arora *et al.,* 2014).

Halophiles are coated in a special protein which allows selective salinity levels into the cell (Shukla, *et al.*, 2011). Salt accumulation is another mechanism utilized by true halophiles and is seen primarily in halophilic archaea and extremely halophilic bacteria. Many microbes respond to high salinity by accumulating osmotica in their cytosol to protect against dehydration. One halophilic bacterium, *Halobacillus,* is Cl^-dependent for activities such as activation of solute accumulation because it can switch osmolyte strategies with environmental salinity by producing compatible solutes (Arora *et al.,* 2014). *Azotobacter chroococum*, are dependent upon Na^+ (Page *et al.*, 1988).

Fungi can also be halophiles. Gunde-Cinerman (2009) defined halophilic fungi as those that are regularly isolated with high frequency on selective saline media from environments with 10% or higher salinity and can grow in environments above 3 M NaCl. Fungi also exist within a halophile spectrum. True or obligate halophilic fungi are those regularly isolated from 1.7 M NaCl salinity in nature, and/or can grow *in vitro* with 17% or higher salinity (Gunde-Cinerman, 2009).

Halophilic fungi were often neglected in hypersaline ecosystem studies until recently (Gunde-Cinerman, 2009). Some of these fungi were identified in Gunde-Cinerman's (2009) study. *Saccharomyces cerevisiae* grows up for 1.2 M NaCl making it halotolerant. *Debaryomyces hansenii* is commonly found in air, soil, and salt-preserved foods and tolerates salt fluctuations and accumulation well without symptoms of toxicity. Black yeast (*Hortaea werneckii*) can grow up to 5M NaCl and persists in even higher salinities. *Aureobasidium pullulans* grows up to 3M NaCl. *Wallemia ichthyophaga* tolerates salinities up to and perhaps above 5.2 M NaCl and requires 1.5M NaCl to grow making it a true halophile. Halophilic fungi commonly found in hypersaline environments include *Cladosporium* spp., *Wallemia* spp., *Scopulariopsis* spp., *Alternaria,* spp. *Aspergillus* spp., and *Penicillium* spp. (Gunde-Cinerman, 2009).

Mycorrhizal fungi, especially VAM, can support plant growth in saline soils by increasing access and uptake of water and nutrient, accumulation of compatible solutes, preventing salt ion toxicity, enhancing photosynthesis, and activating antioxidant enzymes (Dashtebani *et al.,* 2014). One study (Porrias-Soriano *et al.,* 2009) showed that mycorrhiza helped olive plants perform in saline conditions. The most efficient fungus for olive plant growth assistance in saline conditions was *Glomus mosseae* (Porrias-Soriano *et al.,* 2009). The Dashtebani *et al.* (2014) showed that *P. distan*s inoculated with *Claroideoglomus etunicatum* produced higher biomass and showed less salt stress than uninoculated plants. The interaction between hyphae associations and host plants changes with salinity levels. Often, the number and type of fungal spores or fungal infectivity change with different saline concentrations (Arora *et al.,* 2014). In a study by Dashtebani *et al.* (2014), AMF colonization was shown to be lower in salt-treat *P. distans* plants but still efficient for colonization.

Halophilic fungi orders of interest identified in Gunde-Cinerman's (2009) paper include Capnodiales, Dothideales, and Eurotiales within the phylum Ascomycota and Wallemiales, in the phylum Basidiomycota.

Within Ascomycota, Capnodiales and Dothideales often have halophilic expression. The dominant halophilic Ascomycete fungi species are generally regarded to be *Hortanea werneckii, Phaetotheca triangularis, Trimmatostroma salinum,* and *Aureobasidium pullulans*. Eurotiales is another Ascomycete group which has many halotolerant species, such as *Aspergillus niger, Aspergillus sydowii, Eurotium amstelodami,* and *Penicillium chrysogenum* which have been isolated from brines in nature. Other species commonly detected in brines include *Aspergillus versicolor, Aspergillus flavus, Eurotium herbariorum, Penicillium citrinum,* and *Penicillium steckii*. Those with confirmed cosmopolitan distribution include *Cladosporium* spp., *Penicillium chrysogenum* and *Penicillium brevicompactum*. Those with suspected cosmopolitan distribution include *Aspergillus niger* and *Eurotium amstelodami*. Halotolerant yeasts isolated from saline environments include *Candida, Debaryomyces, Metschnikowia*, and *Pichia* spp. The order Saccharomycetales are often associated with plant saps and exudates and have notable osmotolerance, such as *Debaryomyces hansenii. D. hansenii* accumulates more Na^+ than *Saccharomyces cerevisiae* and uses Na^+ to protect itself from other stress factors (Gunde-Cinerman, 2009).

Basidiomycota have three notably halotolerant orders: Trichnonosporales containing *Trichosporon mucoides* which has been isolated in hypersaline waters, Sporiadiales containing *Rhodotorula* spp., and Wallemiales which contains the entirely halophilic/xerophilic genus *Wallemia* (Gunde-Cinerman, 2009).

Some fungi, such as *Wallemia ichthyophaga* and *Debaryomyces hansenii*, accumulate more Na^+ ions than *Hortaea werneckii*, which employs salt exclusion as its primary mechanism for salt tolerance (Gunde-Cinerman, 2009).

Halophilic bacteria generally tolerate a wider range of salinities and osmotic stress than fungi, which are more sensitive and prefer more stable concentrations of saline. However, fungi likely do more for soil structure and plant support than bacteria (Arora *et al.,* 2014).

Few hypersaline environments have been carefully surveyed using molecular methods for microbial diversity. More work is needed to discover which microbes can be most beneficial for supportive use in phytoremediation of salt-affected soils (Arora *et al.,* 2014), particularly for microbes which are capable of salt-accumulation. More research is needed to investigate salt accumulation rates, halotolerance mechanisms, bioregional suitability, and plant-microbe interactions for halophilic microbes to determine suitability for use in remediation projects. Halophilic yeasts and fungi grow best under aerobic conditions with moderate temperatures and acidic to neutral pH (Arora *et al.,* 2014), which is what many Virginia roadside conditions provide.

Microbe assisted remediation may be quite successful on Virginia roadside salt-affected soils. However, more research is needed to identify species which can be most helpful as inoculant amendments. There is also a need for future research regarding genetic manipulation techniques which can imbue plants and other microbes with enzymes from halophiles to help them tolerate and grow in saline environments (Arora *et al.,* 2014).

Soil Amendments

A major challenge of phytoextraction of salt in roadside soils is that many plants are dormant when salt runoff is likely to be the highest in the winter and spring months (Alden, 2021; Welbaum, 2021). In order to best remove salt from the soil, salt must be suspended in the rhizosphere so that plants can reactivate and effectively desalinate the soil before leachates become pollution. The properties of soil determine whether salt is held in the rhizosphere or leached out. Soil aggregates, OM density, and composition all affect the adsorption properties of the soil by increasing cation and anion exchange capacity (CEC and AEC) sites of soil and the subsequent adsorption of Na^+ and Cl^- ions (Ashraf *et al*., 2010; Camberato, 2001).

The VDOT is most likely to apply deicing salts from the months of November to March (Fitch *et al.,* 2005). These 5 months generally have high precipitation, with melting rates increasing into the spring. Most halophytes used in this type of remediation are not active during these colder, wetter months. One possible strategy to overcome this is adding soil amendments with high cation exchange capacity which can temporarily bind Na^+ cations and prevent them leaching until plant dormancy ends. Plants can then glean them from CEC sites before salts leach into the water systems (Alden, 2021). Green, efficient, and low-cost adsorbent amendments are shown to be effective for separating pollutants from water and soil. While more studies need to be conducted on how absorbent materials perform in salt-affected soils, there is much promise for their efficacy in remediation efforts where the aim is holding cations in topsoil for more efficient phytoextraction (Amer & Hashem, 2018; Bée *et al.*, 2017).

Potential soil amendments to hold salt within the rhizosphere covered in this review include biochar, natural fibers and plant materials like cellulose, hemp, and sawdust or other compostable plant material products, chitosan, clay beads and clay composites, and organic cation exchange resins like those used in water decalcification systems.

Biochar is produced via pyrolysis of organic materials, such as plants and manure. Hyperaccumulator biomass can be processed as biochar and recycled back into the roadside environment to further improve soil quality (Litalien & Zeeb, 2020). As a soil amendment, biochar is a green adsorbent with a porous structure, corrosion resistance, and abundant functional groups (Han *et al.*, 2019). It can improve texture, drainage and water holding capacity, soil nutrient retention and binds toxic ions

(Camberato, 2001; Han *et al.*, 2019; Lawrinenko *et al*., 2015; Litalien & Zeeb, 2020). The biochar system is carbon negative, because it sequesters carbon from biomass carbon into stable carbon structures in the soil (“Guidelines for a Sustainable Biochar Industry,” 2012). It is easily compostable and sustainable. Loose biochar added to soil will contribute to the organic matter density of soil, which increases the CEC of soil further contributing to its salt remediation capability (Camberato, 2001; Lawrinenko *et al.*, 2015; Litalien & Zeeb, 2020). While the overall effectiveness of biochar depends upon the feedstock of materials used to produce it, biochar that is produced at low temperatures (below 250°C) was generally found to have the highest number of CEC sites. Low temperature produced biochars retain sufficient functional groups to produce a high negative charge while retaining its structure and an optimal amount of surface area (Weber & Quicker, 2018). Biochars produced from plant biomass and other materials with high cellulose, hemicellulose, and lignin content are more likely to retain their structure during the production process and result in optimal stability, porosity, CEC sites, and water holding capacity in the final product (Weber & Quicker, 2018). There are challenges with using biochar, as it is dusty and can pose a risk to human health (Alden, 2021; Welbaum, 2021). For these reasons, it might be best to use biochar in a contained manner, such as using it in porous bags to function as a roadside salt catching barrier or integrating it into soil to best utilize its remediation potential while avoiding negative side effects.

Compostable plant materials such as sawdust, woodchips, bark, straw, and hemp fibers contain cellulose, hemicellulose, pectin, lignin, and extractives. Hemp fibers have many micropores, microcracks, and “sticky” functional groups which work to bind ions (Na^+ and other metal cations, particularly). These properties also help the fibers to stick to themselves, forming a physical net which aids in filtration and adsorption (Vukcevic *et al.*, 2014). Compost of plant materials supports phytoextraction, improves soil structure, water holding capacity, increased organic matter, pH buffer capacity, and aggregate retention in addition to immobilizing other metals with fulvic and humic acid groups and adsorption of contaminants onto mineral surfaces (Grobelak, 2016).

Chitosan, the deacetylated form of the abundant biopolymer chitin, is a long-chain polysaccharide polymer obtained from insects, fungal cell walls, and marine shellfish (Bée *et al.*, 2017; Chawla *et al.*, 2015). It is inexpensive, non-toxic, and biodegradable (Bée *et al*., 2017; Hamed *et al*., 2016). It has many uses including food processing, medicine, and phytoremediation (Hamed, *et al*., 2016; Pirbalouti *et al*., 2017). For phytoremediation strategies, chitosan is valuable because it has been shown to enhance plant defense against bacteria, fungi, and micropredators, promote plant growth and production, and alleviate certain nutrient deficiencies (Vasconcelos, 2014). Chitosan is of interest for this type of phytoremediation for its capacity to chelate metal ions (Vasconcelos, 2014).

Clay is known for its negative charge, high CEC, and high buffer capacity, giving it great potential for adsorption of loose Na+ ions from runoff (Camberato, 2001). The addition of clay is expected to be a great aid in binding salt to the rhizosphere. However, excessive natural clay can be detrimental to soil structure. This is especially true for high clay soils often found in Virginia, where adding more clay will contribute to drainage issues. For this reason, clay beads are a possible alternative because clay beads will retain their integrity through natural processes while offering many of the benefits of loose clay (Bée *et al.,* 2017; Han *et al.*, 2019).

Clay composites are also of interest for phytoremediation strategies because often they possess characteristics superior to their individual components (Bée *et al*., 2017; Han *et al.*, 2019). There are many types of clay composites, some potentially more suitable than others for soil remediation though more studies need to be performed to evaluate their efficacy in soil (Han *et al.*, 2019). Combining chitosan and clay in the form of magnetic clay-chitosan composite beads show promise for remediation of salt affected soils. Magnetic clay-chitosan composite beads were shown to significantly adsorb positively charged pollutants in studies involving wastewater. These composites are capable of adsorbing cationic and anionic pollutants either separately or together (Bée *et al.*, 2017) and are likely to work similarly in soil environments. Another clay composite of interest for this work is clay-biochar composites. Clay biochar composites are valuable for their high carbon content, multipore structure, compatibility, suitability as a reusable medium, resistance to corrosion, abundant functional groups, non-toxic nature, and inexpensive cost (Han *et al.*, 2019).

Water softener resin is used commercially to remove excessive mineral cations (namely Ca^+ and Mg^+) from water which interfere with tasks such as cleaning (Scherer, 2017). For commercial purposes, the resin is usually charged with NaCl brine, and the Na^+ exchanges with Ca^+ and Mg^+ (Scherer, 2017). Once the resin is coated in water hardening cations, a NaCl brine is once again used to charge the resin (Scherer, 2017). Na^+ and Ca^+ are capable of ion exchange within the soil, with high Ca^+ leachates in soil solutions appearing after applications of deicing salts (Baeckstroem *et al.,* 2004). For remediation of salt-affected soils, resin charged with calcium chloride (CaCl) could potentially be held in porous bags to avoid being released to the environment and placed as a barrier along roadsides to adsorb Na^+ ions from saline road runoff before it infiltrates the soil, reducing the salt load to roadside soils and vegetation bands.

CONCLUSION

This review is intended to be an overview of technologies for future research in the hopes of building a standardized treatment for salt-affected roadside soils in Virginia. Research is still needed to evaluate field performance using an integrated halophyte, halophile, and soil amendment approach to phytoremediation of salt runoff in roadside soils.

For halophytes, determining ideal candidates by their regional suitability and salt accumulating capabilities is essential. Studying how vegetation treatment stands of hyperaccumulators, recretohalophytes and mixed stands of both halophyte types for their biomass production, most effective cover densities, and phytoextraction capabilities could be valuable in determining vegetation choices for roadsides. The impact of salt on soil EC decreases as distance from the pollution sources (roadways, in this case) increases. There is a zone of immediate impact within 10m of the road (Baeckstroem *et al.,* 2004) so having a band of effective vegetation close to the road and within treatment ponds is essential (Gonsalves *et al.,* 2014). Planting a mixture of halophytes may be a beneficial approach because halophytes support other plants, halophytes and glycophytes (plants which are not halotolerant) alike, against saline stress. Also, a stratified vegetation system which utilizes terrestrial and aquatic plants to provide treatment along roadsides as well as ditch seeps and detention ponds could maximize desalination efforts.

Similar research is needed for halophilic microbes to determine their regional suitability and ability to accumulate salt or support growth and performance of hyperaccumulating halophytes. While many halophiles can support growth halophytes and glycophytes in salt-affected soils, the nature of this support is often salt exclusion for the plant which could negatively impact the desired effect of ultimate salt removal using halophytes. However, vegetative support against salt stress may aid plants in establishing and producing sufficient biomass to perform phytoextraction as intended. This may overall neutralize the effect of salt exclusion.

Soil amendments have potential for increasing CEC in soil, but field studies are needed to observe their performance for holding salt within the rhizosphere. Ideal materials have yet to be identified, though biochar, natural fibers, compostable plant material products, clay beads, clay composites, chitosan and organic cation exchange resin seem to be promising amendments for increasing CEC in soil. Estimates for salt retention on ion exchange sites need to be calculated in the field. Additionally, more studies are needed to quantify and qualify halophyte ability to glean salts from soil amendment exchange sites *in situ*.

Salt pollution is a growing global issue and the need for sustainable, reasonable, and safe alternatives grows with it. Road salts affect human health and damage roadside vegetation, contaminate water, damage hydraulic properties of roadside soils, as well as corrode vehicles, bridges, concrete, and road surfaces (Baeckstroem *et al.,* 2004; Gonsalves *et al.*, 2014). Researching effective and safe alternatives to road salt is essential for sustainability, and the only way to truly reduce salt pollution (Snodgrass *et al.,* 2017). Potentially suitable alternatives include, but are not limited to, mixtures from refined corn, MgCl, sand, CaCl, calcium magnesium acetate (CMA), and environmentally sound additives such as carbohydrate byproducts (Robinson & Thomson, 2015). CaCl is a much safer alternative to NaCl salt, doesn't contain as many chemical additives as NaCl salt, and is effective at lower temperatures than NaCl. However, it is more expensive and still produces Cl^- pollution and subsequent damage to water resources. CaCl is often used on bridges and areas which freeze faster than grounded roadways. CMA is a mixture of limestone and acetic acid which works within the temperature range as NaCl and is more sustainable, less corrosive, and less damaging to aquatic systems than NaCl (Gonsalves *et al.*, 2014). Carbohydrate-based products such as beet, corn, molasses, and alcohol byproducts can be used as a prewetting agent and are biodegradable, non-corrosive, and while they don't actively de-ice they do prevent the formation of ice crystals and would make a suitable addition to deicing mixes (Gonsalves *et al.*, 2014; Robinson & Thomson, 2015).

Salt pollution is a complex issue with many factors to consider and requires an interdisciplinary focus for remediation. A combination of effective phytoremediation and salt alternatives could significantly reduce salt pollution and improve quality of water and soils in urban and agricultural areas for the future.

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APPENDIX B - ESTIMATES OF SODIUM AND CHLORIDE UPTAKE BY CATTAIL (NOVA)

To estimate ion-specific plant uptake, the average ion concentration (sodium or chloride; mg/kg) in cattail tissues from sites draining roads was multiplied by literature estimates of dry mass per square meter for adult cattail plants (average of 1.22 kg/m^2; Maddison, 2009, Gagnon et al., 2012, Grosshans 2014), generating an estimate of the total ion mass stored in adult cattail tissues per square meter of planted ground (mg ion per m^2). When multiplied by the square meters of cattail cover at each site that develops over the growing season, this gives an estimate of the total ion mass that could accumulate in plant tissues per growing season (accumulation rate: mg ion per season). The following equation was used:

$$M_{cat:Na,Cl} = C_{cat:Na,Cl} \times 1.22 \times A_{db} \times c \qquad \text{EQ 1}$$

where $M_{cat:Na,Cl}$ is the mass accumulation rate (mg Na^+ or Cl^- per season), $C_{cat:Na,Cl}$ is the concentration of sodium or chloride in above-ground cattail tissue (mg/kg), A_{db} is the detention basin area (m^2), and c is the fraction of the basin covered by cattail (presumed to be 1 for these calculations). This calculation assumes that cattail shoots emerge from rhizomes, grow to adulthood during the growing season, and die back in winter (i.e., the full cycle occurs during a single growing season, consistent with cattail life history [Baldwin and Cannon, 2007]). It also assumes that salt accumulation does not change as juveniles (our measurements) mature to adults; this may not be strictly true for cattail, particularly for chloride, where higher concentrations have been observed in mature specimens, making our estimates somewhat conservative [Delattre et al., 2022]. To account for this, upper estimates of chloride accumulation based on reported values for tissue chloride concentration in mature cattail exposed to road salt [Delattre et al., 2022] are also reported.

Rough estimates of the total mass of chloride and sodium that each of our VDOT road sites received this winter season, were made by assuming road salt was applied during each winter storm detailed in Figure 5, in accordance with recommended guidelines in the Salt Management Strategy for Virginia (SaMS) toolkit (i.e., pounds of salt per road mile given snowfall depth and temperature) [Appendix B**,** SaMS, 2020]. Assuming a standard highway width of 12 ft (0.002m), road miles were translated into impervious area of road (1 road mile ~ 5180 m^2 road), to express salt application guidelines in terms of road area. These were then multiplied by the total area of roadway draining to each detention to generate estimates of basin-specific mass loading. The following equation was used:

$$M_{db:Na,Cl} = \sum_{i=1}^{n} m_{i:Na,Cl} \times 5180 \times DA_{road} \qquad \text{EQ 2}$$

where $M_{db:Na,Cl}$ is the cumulative mass of sodium or chloride delivered to a detention basin during winter storms in a single season (kg/season), n is the total number of winter storms, $m_{i:Na,Cl}$ is the mass of sodium or chloride put down each storm per road mile (kg/mile), 5180 is a conversion factor (road miles to square meters of road), and DA_{road} is the area of road draining to a detention basin (m^2).

Estimates of cumulative salt mass in each detention basin ($M_{db:Na,Cl}$) were compared to estimates of salt mass uptake by cattail ($M_{cat:Na,Cl}$) to determine the fraction of sodium and chloride delivered to each basin that could potentially be phytoremediated by cattail.

APPENDIX C - LABORATORY ANALYSIS OF BIOCHAR

Control Laboratories

42 Hangar Way
Watsonville, CA 95076
www.biocharlab.com
Tel: 831 724-5422
Fax: 831 724-3188

Account No:
10981
Batch:
JUN 20 B
CODE:
BioChar IBI

Tony Marrero
Wakefield Biochar
2101 W Broadway, Suite 103-274
Columbia, MO 65203

Date Received:	6/8/2020
Sample ID:	060520WF-C
Lab ID. Number:	0060310-01

International BioChar Initiative (IBI) Laboratory Tests for Certification Program

	Dry Basis Unless Stated:	Range	Units	Method
Moisture (time of analysis)	37.6		% wet wt.	ASTM D1762-84 (105c)
Bulk Density	7.9		lb/cu ft	
Organic Carbon	65.8		% of total dry mass	Dry Combust-ASTM D 4373
Hydrogen/Carbon (H:C)	0.33	0.7 Max	Molar Ratio	H dry combustion/C(above)
Total Ash	20.0		% of total dry mass	ASTM D-1762-84
Total Nitrogen	0.59		% of total dry mass	Dry Combustion
pH value	8.94		units	4.11USCC:dil. Rajkovich
Electrical Conductivity (EC20 w/w)	0.451		dS/m	4.10USCC:dil. Rajkovich
Liming (neut. Value as-CaCO3)	9.5		%CaCO3	AOAC 955.01
Carbonates (as-CaCO3)	6.6		%CaCO3	ASTM D 4373
Butane Act.	7.0		g/100g dry	ASTM D 5742-95
Surface Area Correlation	357		m2/g dry	G

All units mg/kg dry unless stated:		Results	Range of Max. Levels	Reporting Limit (ppm)	Method
Arsenic	(As)	1.2	13 to 100	0.45	J
Cadmium	(Cd)	0.2	1.4 to 39	0.18	J
Chromium	(Cr)	12.3	93 to 1200	0.45	J
Cobalt	(Co)	**ND**	34 to 100	0.45	J
Copper	(Cu)	8.0	143 to 6000	0.45	J
Lead	(Pb)	1.9	121 to 300	0.18	J
Molybdenum	(Mo)	0.5	5 to 75	0.45	J
Mercury	(Hg)	**ND**	1 to 17	0.001	EPA 7471
Nickel	(Ni)	2.1	47 to 420	0.45	J
Selenium	(Se)	**ND**	2 to 200	0.90	J
Zinc	(Zn)	22.9	416 to 7400	0.90	J
Boron	(B)	20.8	Declaration	4.49	TMECC
Chlorine	(Cl)	87.6	Declaration	20.0	TMECC
Sodium	(Na)	759	Declaration	448.6	E
Iron	(Fe)	5769	Declaration	22.4	E
Manganese	(Mn)	194	Declaration	0.45	J

Particle Size Distribution	Results	Units	Method
< 0.5mm	12.9	percent	F
0.5-1mm	1.3	percent	F
1-2mm	58.9	percent	F
2-4mm	25.8	percent	F
4-8mm	1.1	percent	F
8-16mm	0.0	percent	F
16-25mm	0.0	percent	F
25-50mm	0.0	percent	F
>50mm	0.0	percent	F

Basic Soil Enhancement Properties			
Total (K)	4408	mg/kg	E
Total (P)	958	mg/kg	E
Ammonia (NH4-N)	35.9	mg/kg	A
Nitrate (NO3-N)	1.8	mg/kg	A
Organic (Org-N)	5852	mg/kg	Calc.
Volatile Matter	16.8	percent dw	D

* "ND" stands for "not detected" which means the result is below the reporting limit.

Method A Rayment & Higginson
D ASTM D1762-84
E EPA3050B/EPA 6010
F ASTM D 2862 Granular
G Butane Activity Surface Area Correlation Based on McLaughlin, Shields, Jagiello, & Thiele's 2012 paper: Analytical Options for Biochar Adsorption and Surface Area
J EPA3050B/EPA 6020

Analyst: Nik Zumberge

NZ