M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04i Project Abstract
For the Period Ending June 30, 2022

PROJECT TITLE: Phytoremediation for Extracting Deicing Salt from Roadside Soils
PROJECT MANAGER: Bo Hu
AFFILIATION: University of Minnesota
MAILING ADDRESS: 1390 Eckles Ave
CITY/STATE/ZIP: St Paul, MN, 55108
PHONE: 612-625-4215
E-MAIL: bhu@umn.edu
WEBSITE: https://bbe.umn.edu/directory/faculty/bohu
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04i

APPROPRIATION AMOUNT: $ 360,000
AMOUNT SPENT: $ 360,000
AMOUNT REMAINING: $ 0

Sound bite of Project Outcomes and Results
This project screened and evaluated several halophytic plants that can extract sodium chloride, the deicing salt, from soil, and accumulate it into the leafy biomass. The information can be used to develop phytoremediation methods to address the environmental pollution caused by the application of roadside deicing agents.

Overall Project Outcome and Results

Massive applications of road salts to melt the snow and ice on sidewalks and roads can negatively affect the health of surrounding ecosystem as the salts are leached into lakes, rivers, and groundwater, causing significantly increased salinity and high salt conditions can also negatively affect both plant growth and soil structure. Many agricultural fields have similar concerns over the growing salinity in the soil, especially under the conditions of prolonged drought and improper irrigations. We collaborated with the Minnesota Department of Transportation (MnDOT) to screen and evaluate several halophytic plants that can extract sodium chloride, the deicing salt, from soil, and accumulate it into the leafy biomass. The information can be used to develop phytoremediation methods to address the environmental pollution caused by the application of roadside deicing agents. The research detailed in this project showed that common sunflower and pitseed goosefoot so far are the most promising species for phytoremediation of deicing salt. It is recommended they be mixed in with perennials from MnDOT's seed mixes to improve soil structure and help prevent the salt from reaching the soil surface or the groundwater. Another high salt accumulating plant species, sugar beet and beets in the other cultivar groups, are more suited for agricultural and thus could be used to remediate salt from the growing number of salt-impacted agricultural fields. The harvest and utilization of each of these plants can provide additional value such as animal feed, oil, or reuse of salt in ash if burned for energy. This project and the following phytoremediation method developments can provide a long term sustainable solution to the de-icing salt pollution to our Minnesota environment.

Project Results Use and Dissemination
The detailed research results are in the final report, and we are drafting two manuscripts for possible publications. Leif was accepted for presenting this work at AIChE Annual Meeting in November 2022 in Phoenix, Arizona and he was invited to give a presentation at the MECA (Minnesota Erosion Control Association) Annual Conference in January 2023. We have been working at MnROAD site with support and help from MnDOT. Cindy Dorn, writer/producer with Prairie Sportsman, an outdoor show produced by Pioneer PBS that airs on all Minnesota PBS stations is drafting a story on absorbing deicing salts with plants.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan Final Report (Main Document)

Today's Date: September 30, 2022

Date of Next Status Update Report: Final Report

Date of Work Plan Approval: June 5, 2019

Project Completion Date: June 30, 2022

PROJECT TITLE: Phytoremediation for Extracting Deicing Salt from Roadside Soils

Project Manager: Bo Hu

Organization: University of Minnesota

College/Department/Division: College of Food, Agricultural and Natural Resource Sciences / Department of Bioproducts and Biosystems Engineering

Mailing Address: 1390 Eckles Ave

City/State/Zip Code: Saint Paul, MN, 55108

Telephone Number: 612-625-4215

Email Address: bhu@umn.edu

Web Address: https://bbe.umn.edu/directory/faculty/bohu

Location: The experiment will be primarily done at Biological Agricultural Engineering Building (BAE) 320, 1390 Eckles Ave, St Paul, MN, 55108. The impact of the project will be statewide

---

Total Project Budget: $360,000

Amount Spent: $360,000

Balance: $0

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04i

Appropriation Language: $360,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to collaborate with the Department of Transportation to evaluate potential native plants that can be grown on roadsides to adsorb and remove toxic salts accumulated from deicing roads and assess uses for the harvested material.

I. PROJECT STATEMENT:

The icy conditions of Minnesotan winters require aggressive applications of road salts to melt the snow and ice on sidewalks and roads. It is estimated that 365,000 tons of salt is sprinkled in the Twin Cities Metro Area each year (2017). The continued contamination of salt can negatively affect the health of surrounding ecosystem. It is easy to leach into lakes, rivers, and groundwater, causing significantly increased salinity (Cooper, Mayer et al. 2014). Many lakes (for instance, Loring pond and Diamond lake) around Metro have already been reported the chloride concentrations consistently surpassing the environmental standard(Novotny 2007) of 230 mg/L. High salt conditions can also negatively affect both plant growth and soil structure. Contaminated soil can affect up to 10 m off of a road side increasing soil density and alkalinity causing problems with erosion and vegetation (2017). Similar to road salt, improper irrigation can also cause salt contamination. Irrigation waters tend to have high concentrations of calcium, magnesium, and sodium ions. Use of this brackish water, particularly without adequate drainage management, results in the accumulation of salts in the rooting zone of plants due to evapotranspiration. This typically results in substantial global agricultural and economic losses, sustenance issues for subsistence farmers, and ecosystem imbalances (Qadir, Quillérou et al. 2014, Gerhardt, MacNeill et al. 2017). Planting salt tolerant species can be one way to address this issue. For instance, Dr. Eric Watkins at the University of Minnesota is currently developing salt tolerant turf grasses so that they can grow better for roadsides (Friell, Watkins et al. 2013). Another approach is to develop technologies to remove salt from the soil. This approach will not only address the challenge for the sustainable urban restoration of roadsides and waterways but also provide an opportunity to regain agricultural croplands, revitalize rural economy and increase global food security (Cooper, Mayer et al. 2014).

Phytoremediation is an emerging method to extract salts from the soil by utilizing the growth of certain plants and remove salts by harvesting the plant biomass. These plants are typical halophytes, which excrete salt ions through specialized leaf glands(Hasanuzzaman, Nahar et al. 2014). Phytoremediation has numerous advantages over the conventional techniques for salt remediation, such as removing the contaminated soil to landfill while replacing it with clean soil, leaching, chemical amendments, and organic amendments. Phytoremediation is environmental friendly than the landfill of affected soil since this soil will have the opportunity to be re-used. It can also be more easily applied and less costly than the leaching and amendment methods. The harvested halophytic plant biomass may have some industrial applications, for instance, serving as animal feed or energy source.

Glasswort *Salicornia rubra (S. rubra)* is a succulent halophyte which is found growing in Kittson County, Minnesota. It grows on the saline areas such as salt flats, alkaline depressions, exposed shores of alkaline lakes, and saline swales . Despite not being commonly found in central Minnesota, recently *S. rubra* has been observed growing next to major highways in the Twin Cities . It is predicted that this is a result of the increased salinity of roadside soil, which is the ideal growing condition of *S. rubra* in its natural habitat. Since it is a native grass, *S. rubra* is suitable to MN climate and does not pose any economic threat to the local ecosystem. We believe that *S. rubra* has a great potential to be used for phytoremediation to remove and stabilize salts from the soil surrounding MN roads and lakes. *S. rubra* can uptake salts from the soil, bringing it into the above-ground plant tissues, and then reduce salt contamination through grass mowing and collection. This project will study the potential of *S. rubra* and other native species for the phytoremediation to remove salts from roadside soil and farmland (2017).

II. OVERALL PROJECT STATUS UPDATES:

Amendment Request (06/30/2022):

We are requesting to move $1,231 from Personnel and $1,335 from Travel expenses, transfer $279 to Professional/Technical/Service Contracts, and transfer $2,269 to Equipment/Tools/Supplies for the third year. With the amendment, the budget for Personnel will decrease $327,969 to $326,756. The budget for Professional/Technical/Service Contracts will increase from $4,365 to $4,644. The budget for Equipment/Tools/Supplies will increase from $23,215 to $25,484. The budget for Travel expenses in Minnesota will reduce from $4,451 to $3,116. This is some final budget adjustment.

Amendment Request (03/01/2022):

We are requesting to move $5,000 from Professional/Technical/Service Contracts, $8,000 from Equipment/Tools/Supplies, and $3,122 from printing/publication cost, to personnel for the third year. With the amendment, the budget for Personnel will increase from $311,847 to $327,969. The budget for Professional/Technical/Service Contracts will reduce from $9,365 to $4,365. The budget for Equipment/Tools/Supplies will reduce from $31,325 to $23,215. The budget for Printing will reduce from $3,122 to $0. The reason for this increase was due to the increased labor needs for us to work more greenhouse studies and then we have more soil and plant samples to measure. We will also have a new PhD student Sarman Gultom to help with this project, and Sarman will make this research as his PhD dissertation topics. He is an international student from Indonesia and currently receives scholarship to cover his tuitions. The monthly stipend from his scholarship is $550 lower than the standard UMN graduate student stipend. Due to his visa status, we will pay him travel stipend to cover the difference.

Amendment Approved by LCCMR 3/28/2022

First Update March 1, 2020

We did the inventory study to search Minnesota native plants that can be either classified as halophytic plants or in more general category of “salt tolerant plants”. The inventory also include some potential plants which are not native to Minnesota, but can potentially grow in our Minnesota climate. Then we did some greenhouse growth of some selected plant species, including, two halophytes: Salicornia europaea (glasswort), and Suaeda maritima (herbaceous seepweed); some regular salt tolerant plants: alfalfa, barley, sugar beets, turf grass; and some other possible plant species: wild sunflowers, smooth sumac, and alkali grass. We also involved with two undergraduate student design groups to study the possible designs on how to implement these plants into the roadside remediation. Some preliminary planning was carried out to grow small area of glasswort at the entrance roadside to the MN State Fair ground. The lab experiments are interrupted and delayed due to the campus shutdown, but we have built solid ground to move forward for the full outdoor plantation next year.

Second Update September 1, 2020

We continue working on the inventory study to include more Minnesota native plants that can be either classified as halophytic plants or in more general category of “salt tolerant plants” as well as some potential plants which are not native to Minnesota but can potentially grow in our Minnesota climate. The PhD student Leif van Lierop is taking the project as his PhD research topic and he has been working on his dossier and preparing for his preliminary exam. The lab experiments are interrupted and delayed due to the campus shutdown. We are working finished up the sample analysis for the greenhouse growth of some selected plant species, including *Salicornia europaea* (glasswort), alfalfa, barley, sugar beets, turf grass, wild sunflowers, smooth sumac, and alkali grass. We will postpone the full outdoor plantation to next year.

Third Update March 1, 2021

The greenhouse studies that included the plant species Salicornia europaea (glasswort), alfalfa, barley, sugar beets, and turf grass were fully analyzed. This included measuring the plant tissue and soil sodium chloride content. We have also thoroughly researched which plant species will most likely be the best candidates for the roadside field experiment which is expected to begin around the middle to end of April. This research is presented in a decision matrix. The species ultimately chosen for the field study will be based on how the best rated species from the decision matrix perform in a new greenhouse study which is currently being set up. Also, we have been working on the experimental design of our roadside experiment so that we will be fully prepared for the growing season when we will begin. The PhD student Leif van Lierop has also spent more time writing and editing his dossier so that he will can soon complete his preliminary exam.

Fourth Update September 1, 2021

A third greenhouse experiment was performed using the highest scoring plant species from the decision matrix. The plant biomass and plant tissue chloride content have been analyzed for this experiment. Plant tissue sodium content and soil salinity have yet to be measured. The field study preparations began in late April, plots were seeded and plants transferred to the field site in May, and they will be harvested in the middle of September. Plots have been checked and maintained once per month. Also, we are beginning writing a manuscript of this research.

Fifth Update March 1, 2022

A fourth greenhouse experiment showed that common sunflower is able to accumulate salt into its aboveground biomass at a concentration of roughly 16.5% by dry weight when grown in soil irrigated with 100 mM NaCl. This is comparable to sugar beets which have shown a salt content of up to 20% by dry weight in aboveground leaves.

Initial soil results from the field site showed that the soil salt content of the Mainline roadside was much lower than those found in literature for highly salted roadsides in the TCMA. Therefore, salt solution was added to the Mainline plots in the first week of June and another larger dose in the third week of August. The plants from the field were harvested in the final week of September. The harvested dry biomass results from the seeded plots showed that common sunflower had the highest biomass at both the LVR (control) and Mainline (salted) sites. Most other species (sugar beet, big bluestem, side-oats grama, and slender wheatgrass) struggled to germinate, likely due to the hot weather during the summer and lack of rainfall. Pitseed goosefoot was the only other species with significant germination. The biomass results from the transplant plots were similar to the seeded plots, except that pitseed goosefoot had lower growth at the Mainline compared to the LVR. Overall, the common sunflower showed the highest potential for germination, survival, and high growth on the high and low salt treated roadsides. There was also some potential for pitseed goosefoot. The salt content of the biomass and soil samples are in the process of being analyzed.

Also, two additional greenhouse experiments were performed. One experiment was chosen since the sugar beet shows promise for remediating salt contaminated agricultural soil due to its ability to accumulate salt into aboveground biomass while keeping a low salt content within the beet itself. This led to an experiment using other kinds of beets within the five different cultivar groups of the *Beta vulgaris subsp. vulgaris* subspecies to determine if these other groups show similar salt accumulation abilities. The second additional experiment looked at the potential of promising salt accumulators to compete with invasive roadside plants. Sugar beet, pitseed goosefoot, and common sunflower were grown in pots containing either smooth bromegrass or purslane. Salt was also added to half of the pots to determine if high salt content in soil would impact the competition.

Final Report between project end (June 30) and August 15, 2022

In the final months of this project, another roadside study was set up to run for the 2022 growing season that would be located much closer to the University of Minnesota campus than last year's roadside experiment. The closer location allows for more regular maintenance and watering of the plots to account for large stretches of dry weather that was encountered last year. The plants from this 2022 field study will be harvested in September.

Also, a greenhouse experiment testing several perennial sunflowers for their salt uptake potential was completed. It is advantageous to have perennials because they do not need to be replanted each season and established perennials are more likely to uptake the salt from roadside soil during early spring when soil salt concentration are highest. The perennial sunflower species tested include tall sunflower, Maximillian's sunflower, and western sunflower. The results of the experiment showed there was some salt tolerance of these perennial sunflowers yet they did not exhibit the significant salt uptake ability seen from the annual common sunflower.

Overall Project Outcomes and Results

Massive applications of road salts to melt the snow and ice on sidewalks and roads can negatively affect the health of surrounding ecosystem as the salts are leached into lakes, rivers, and groundwater, causing significantly increased salinity and high salt conditions can also negatively affect both plant growth and soil structure. Many agricultural fields have similar concerns over the growing salinity in the soil, especially under the conditions of prolonged drought and improper irrigations. We collaborated with the Minnesota Department of Transportation (MnDOT) to screen and evaluate several halophytic plants that can extract sodium chloride, the deicing salt, from soil, and accumulate it into the leafy biomass. The information can be used to develop phytoremediation methods to address the environmental pollution caused by the application of roadside deicing agents. The research detailed in this project showed that common sunflower and pitseed goosefoot so far are the most promising species for phytoremediation of deicing salt. It is recommended they be mixed in with perennials from MnDOT's seed mixes to improve soil structure and help prevent the salt from reaching the soil surface or the groundwater. Another high salt accumulating plant species, sugar beet and beets in the other cultivar groups, are more suited for agricultural and thus could be used to remediate salt from the growing number of salt impacted agricultural fields. The harvest and utilization of each of these plants can provide additional value such as animal feed, oil, or reuse of salt in ash if burned for energy. This project and the following phytoremediation method developments can provide a long term sustainable solution to the de-icing salt pollution to our Minnesota environment.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: Screening of native Minnesota halophytic plants

Description:

We first want to study the halophytic plant inventory and screen more native species *via* lab growth tests for phytoremediation purpose. Around 200 distinct halophytic species are reported in the U.S., growing in coastal and inland regions (Glenn, Riley et al. 1994). Several studies have been done on these plant species, covering wide ranges of topics including halophyte ecology and physiology, and their utilization in farming systems. MNDNR publishes on their website about all the plant species living in the state of MN and we will compare with the reference to identify more halophytic species suitable for MN conditions. We will grow some plants in the lab to screen more species that are native in MN and suitable to grow on the roadside. Potential species may include two halophytic plants: *S. rubra* and *Suaeda maritima*; two crop plants: sugar beet and alfalfa; and two grass plants: wild barley and turf grass. Detailed plant species may change as we obtain more detailed information on the literature search. These potential specimen will be planted under varying salt concentrations to study optimal growing conditions. The plants will be kept in the same room with lights placed over the pots to simulate day and night. Plant height and leaf chlorophyll will be measured every 10 days. Salt content of the biomass will be measured after two month of growth at each conditions to determine the best candidate for road salt removal from soil.

ACTIVITY 1 ENRTF BUDGET: $ 116,000

Outcome	Completion Date
1. Select two halophyte, two crop and two grass plants with the ability to grow in MN	Year 1 - 01/2020
2. Screen these plants with lab growth for salt removal capability	Year 1 - 05/2020

First Update March 1, 2020

Step 1: Inventory study of halophytic and salt tolerant plants available in Minnesota.

We have been working on the inventory study to look for suitable halophytic plants that can be grown in the state of Minnesota. Halophytic plants are salt-tolerant plants normally found in coastal areas. They thrive in soils with high salinity. There are many different types of halophytes, including salt excluders and salt accumulators. Salt excluders adapt to salt salinity by avoiding the salt being accumulated in the plant, by dropping leaves with high salt content or having specialized roots. Salt accumulators have adapted salt glands to store the absorbed salt and then releases the salt to the outside of the stem when the plant has reached salt capacity (3). Through compartmentalization, we want to utilize this group of halophytes to absorb the salt dissolved in water and store it in the cell. Some parts of Minnesota have saline soils, which can be from natural salt, fertilizer, stream water, lake water, or irrigation, and therefore has many native species of halophytic plants. Some potential salt accumulating halophytic plants are listed below:

Glasswort *Salicornia rubra (S. rubra)* is a succulent halophyte which is found growing in Kittson County, Minnesota. It grows on the saline areas such as salt flats, alkaline depressions, exposed shores of alkaline lakes, and saline swales . Despite not being commonly found in central Minnesota, recently *S. rubra* has been observed growing next to major highways in the Twin Cities . It is predicted that this is a result of the increased salinity of roadside soil, which is the ideal growing condition of *S. rubra* in its natural habitat. Since it is a native grass, *S. rubra* is suitable to MN climate and does not pose any economic threat to the local ecosystem. Besides regular halophytic plants, there are also a list of saline-tolerant plants that can potentially be screened for salt adsorption.

Suaeda maritima (herbaceous seepweed) is a widely studied halophytic plant. It accumulates, without injury, concentrations of the order of 400 mM NaCl in its leaves (Wang, Zhang et al. 2007). It is a species of flowering plant in the family Amaranthaceae known by the common names herbaceous seepweed and annual seablite. It is a yellow-green shrub with fleshy, succulent leaves and green flowers. It grows to about 35 cm in salt marshes. It is native to the Manitoba province of Canada, so it may be able to grow in the MN climate (https://plants.usda.gov/core/profile?symbol=SUMAM).

The common sunflower is native to Minnesota and commonly grows in the region (common sunflower, n.d.). There have been numerous studies and reports stating the sunflower's ability to uptake salts (Bhatt and Indirakutty 1973). Sunflower seeds have a 25-50% oil yield and are one of the most valuable oil seed in the world. They can be used for both food and biofuel, according to the National Sunflower Association ("Biodiesel", n.d.). The only area where the common sunflower lost points in the matrix was for cost. While it is inexpensive, it does require routine maintenance because it is not a perennial plant and must be harvested and replanted yearly.

The spear saltbush is native to California and is not recommended for temperatures below 33°F ("Spear Orache," n.d.). However, it has been found in several locations across the Midwest, so it did receive some points for possible survivability. It is not known to be invasive. An Australian source from 2019 highlights the use of the saltbush shrubs for salinity management, which strongly indicates the potential for effectiveness, but is not specific to the spear saltbush species (https://www.agric.wa.gov.au/soil-salinity/saltbushes-dryland-salinity-management). No information was found on use for bioproducts. The spear saltbush is relatively easy to maintain but it proved difficult to find a supplier selling seeds or young plants ("Spear Orache," n.d.).

The smooth sumac is native to 48 states including Minnesota, and commonly grows in similar roadside environments. It has been evaluated extensively for its significant ability to survive in saline soils, but the literature on its specific application to phytoremediation is less robust (Sheridan 2010). Sumac has a high lignin content, which makes it non-ideal for some bioproduct uses, there is a potential for some medicinal bioproduct applications (Jesus, Danko et al. 2015). The young plants are more expensive than some of the alternatives, but they are perennials so they do not require yearly planting, reducing maintenance cost.

Narrowleaf cattail is unique in that it is common in Minnesota and able to thrive in the climate, but it is prone to invasive spread. The narrowleaf cattail has been studied for its phytoremediation properties extensively and it was actually the most effective of three plants studied for salt uptake in one particular paper (Morteau, Triffault-Bouchet et al. 2009). There are several ways to utilize cattail biomass, including biofuel for renewable energy. While the narrowleaf cattail is inexpensive, significant maintenance cost would be expected in order to manage and prevent invasive spread.

The Canadian sandspurry is similar to the spear saltbush in that it is native to coastal climates, which may cause survival issues in Minnesota ("Canadian Sandspurry," n.d.). It is not known to be at risk for invasive spread. This was also evaluated in the same study as the narrowleaf cattail, but it was not as successful in salt uptake (Morteau, Triffault-Bouchet et al. 2009). There are no prominent sources stating its utility for bioproduct applications. Similar to the spear saltbush, it is low maintenance but quite difficult to find a supplier for, making costs higher.

We are still evaluating several other halophytic plant species as shown in the table 1. For instance, Atriplex species grown under rangeland conditions have leaf ash concentrations of 130–270 g salt/kg and if grown in salt-affected soils, the species can have leaf ash concentrations as high as 390 g salt/kg (Malcolm, Clarke et al. 1988, Qadir, Oster et al. 2007).

Table 1. Halophytic plant species

NAME	SEEDS? (Y o N)	WEBSITE	QUANTITY/PRICE	ABILITY OF SALT ACCUMULATION
Atriplex hortensis(Orach)	Y	outside pride	1000 seeds for 5 USD	Red - 13% reduction leaf area(15g/L) green - 47% reduction leaf area(15g/L)
Atriplex patula	Y	b and t world seeds	25 grams for 33 Euros	8-12 dS/m
Atriplex prostrata	Y	b and t world seeds	5 grams for 19 Euros	in lab setting, 0.3M NaCl max
Bassia scoparia	WEEDY, do not recommend	b and t world seeds	5 grams for 8 Euros	WEEDY PLANT, do not recommend
Beckmannia syzigachne	Y	b and t world seeds	7 grams for 8 Euros	"medium"
Borago officinalis	Y	b and t world seeds	10 grams for 8 Euros	
Distichlis spicata	Y	great basin seeds/amazon	1 pound for 42 USD/1oz for 22 USD(32,438 seeds)	>12 dS/m
Eleocharis palustris	Y	prairie moon nursery	1 packet for 2.50 USD	can handle 12g/L NaCl
Juncus acutus	Y	the original garden	1 gram for 1.5 Euros	
Leptochloa fusca	N			
Spartina pectinate	Y			good reason to believe is halophyte
Portulaca oleracea	WEEDY, do not recommend	wild garden seed	packet for 3 USD	

Puccinellia nuttalliana	Y	stevenson intermountain	need quote	Very High
Sarcocornia ambigua	N			
Sporobolus anglicus	WEEDY, do not recommend			
Sporobolus foliosus	N			
Sporobolus montevidensis	WEEDY, do not recommend			
Sporobolus pumilus	WEEDY, do not recommend			
Typha angustifolia	Y	b and t world seeds	25 grams for 96 Euros	
Typha latifolia (broadleaf cattail)	Y	seed rack	1 packet for 2.85 USD	
Xanthium strumarium	WEEDY, do not recommend	b and t world seeds	25 grams for 75 Euros	

Besides the halophytic plants listed from above inventory, there are many crop plants well known for their modest soil tolerance and they are widely planted in MN, including *Medicago sativa* (alfalfa), *Beta vulgaris* (sugar beets), and *Hordeum vulgare* (barley). These crops will decrease their yield once the slat concentration reaches to certain level. But they have established market and growing practice, and therefore may have great potential for post treatment if they are proved to absorb salt. Minnesota Stormwater Manual at Minnesota Pollution Control Agency ranked cold climate plant materials of the upper Midwest with known salt tolerance. Several types of grass are marked high, including Karl Foerster reed grass (*Calamogrostis acutifolia* 'Karl Foerster'), Blue grama grass (*Bouteloua hirsuta*), Little bluestem (*Schizachyrium scoparium*), Alkali grass (*Puccinella distans*), Tall wheatgrass (*Agropyron elongatum*), and Western wheat grass (*Elytrigia smithii*). Some of these grass types have already been recommended for landscaping.
(https://stormwater.pca.state.mn.us/index.php?title=Minnesota_plant_lists#Salt_tolerance)

Step 2: Greenhouse study of several selected plant species on the salt adsorption:

We have been working with two rounds of plant growth study for selected plant species to study their salt removal capacity. The plants selected for first round of growth include *Salicornia europaea* (glasswort), *Medicago sativa* (alfalfa), *Beta vulgaris* (sugar beets), Hordeum vulgare (barley), *Schedonorus arundinaceus* (turfgrass), *Puccinellia distans* (alkaligrass), *Elymus trachycaulus* (slender wheatgrass), *Helianthus annuus* (common sunflower), *Rhus glabra* (smooth sumac). *Sueda maritima* (herbaceous seepweed) was another halophyte that was chosen (native to Manitoba, not MN)- however the seeds that were obtained did not germinate.

Greenhouse experiment with non-leaching pots:

Experimental Design: Plants used in Non-leaching experiment include: glasswort, turfgrass, barley, sugar beets, and alfalfa. Suaeda maritima (herbaceous seepweed) seeds did not germinate in germinating soil or on filter paper inside petri plates. Four different salt treatments were applied to water the plants, including: 0, 50, 100, and 200 mM NaCl. With each treatment, we had three replicates. However, there were not enough well-growing Salicornia (glasswort) for all 4 conditions so we only had control and high salt treatment for this specific plant (250 mM NaCl, different from the other high salt concentrations). Altogether, we had 54 pots of plants in the first greenhouse experiment for non-leaching study.

Germination of Seeds: Due to the slower growth and germination of Salicornia europaea seeds, they were first germinated on filter paper inside petri plates for 1-2 months at 5 degrees Celsius**.** Then the germinated seedling were transferred to germinating mix soil. The other plant seeds involved in the experiment were immediately planted in germinating soil in large trays. These seeds grew for a few weeks before being transplanted to their own pots with general mix planting soil.

Greenhouse growth experiment: 300g of fresh soil (general mix) were added into each pot for the greenhouse study*.* After one week of growth in the non-leaching pots, each plant was watered with half strength Hoagland solution and one of four salt concentrations (control, low, medium, and high strength). Composition of the Hoagland solution includes the following macronutrients: KNO_3, $Ca(NO_3)_2*4H_2O$, KH_2PO_4, $MgSO_4*7H_2O$, Fe-EDTA and these micronutrients: KCl, H_3BO_3, $MnSO_4*H_2O$, $ZnSO_4*7H_2O$, $CuSO_4*5H_2O$, H_2MoO_4 (Hoagland and Arnon 1950). The first application of nutrient solution was 500 mL (soil was very dry), no salt added**.** Then the application of nutrient solution with salt treatments (330 mL) were added once per week for a total of 6 irrigations. Then a final nutrient application that did not contain salt treatment. The total growth in individual pots is 8 weeks and then plants were then removed from pots and stored in fridge until analysis

Plant fresh and oven dry weight: We measured the fresh (air dry) weight of both above ground and below ground biomass, and then determined the oven dry (placed in 105 degrees C oven overnight) weight of above ground and below ground biomass

Soil Analysis: Two different methods of NaCl extraction were evaluated to determine the appropriate methods for our sample analysis. The first method was a fixed ratio method or 5 mL deionized water to 1 g of air dry soil (Fixed Ratio Extracts Method- Janzen 1993; Rhoades 1996). One gram of air-dry soil was taken into a flask, then added 5 mL deionized water, and was shaken for 30 minutes. The soil suspension was then filtered with Whatman number 2 filter paper and stored filtrate at 5 degrees C before analysis. The second method was based on (Basta and Tabatabai., 1985) for exchangeable bases: 3 g of air-dried soil was weighed into a flask, then added 40 mL of 1 M ammonium acetate and was shaken for 30 minutes. The supernatant was filtered through a Whatman number 2 filter paper. Filtered samples from both methods were analyzed with ion chromatography for their concentration of NaCl

Table 2. Soil Analysis Extraction Methods:

samples	**Plant**	**NaCl treatment (mM NaCl)**	**replicate**	**Extraction method**	**Na (mg/L) in filtrate**	**Cl (mg/L) in filtrate**	**Na (mg) in 1g air dry soil**	**Cl (mg) in 1g air dry sample**
1	Turf	200	1	NH4OAc	10.5	20.75	17.50	34.58
2	Glasswort	250	3	NH4Oac	9.21	18.44	15.35	30.73
3	Turf	200	1	DI water	5.23	8.54	7.63	12.46
4	Glasswort	250	3	DI water	4.85	7.62	7.07	11.12

As shown in the soil analysis at Table 2: Ammonium acetate extraction was significantly more effective than the DI extraction. This is evidenced by the Na in 1g of soil having values more than 2 times as high for the ammonium acetate extraction and Cl measurements being roughly 3 times higher for the ammonium extraction.

Therefore, we determined that the salt analysis in the soil will be measured by the ammonia acetate extraction method.

Plant Tissue Analysis: Two different methods of plant tissue NaCl extraction were performed and compared. The first method consists of boiling plant tissue in distilled water (based on Marteau et al., 2015 method). Half a gram of oven dry plant material was crushed with a mortar and pestle, mixed with 35 mL of distilled water, and boiled for 2 h in a water bath at 100°C. Extracts were then filtered with Whatman no. 2 filter paper and analyzed. The second method using digestion of plant tissue in HNO_3 (based on Muchate el al., 2016). About 100 mg of oven-dried plant material was digested in 30 mL of 0.5% nitric acid at 100 degrees C for 30 min.

Table 3. Plant Analysis Extraction Methods:

Plant	**Salt conc. (mM)**	**Replicate**	**Extraction**	**Na (mg/L) in filtrate**	**Cl (mg/L) in filtrate**	**mg Na/g plant**	**mg Cl/g plant**
Barley	200	3	DI water	19.16	36.51	38.33	73.02
Barley	200	3	HNO_3	12.27	19.90	49.10	79.59
Alfalfa	200	1	DI water	11.65	18.26	23.31	36.51
Alfalfa	200	1	HNO_3	5.66	10.13	22.65	40.53

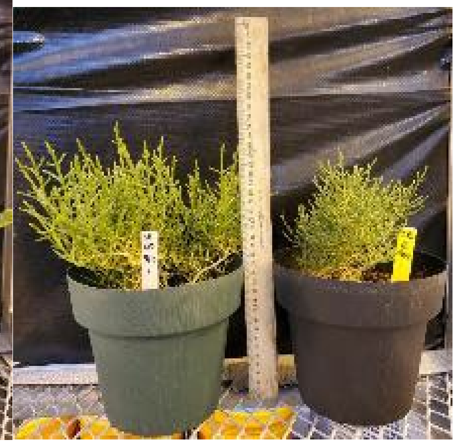
The results obtained from both of these extraction methods (Table 3) show that the nitric acid extraction resulted in higher milligrams of sodium and chloride per gram of plant material compared to the distilled water extraction method. Therefore, nitric acid extraction was more effective (higher extraction of salt) for all samples except for the Alfalfa Na measurement (although the nitric acid extracted sample was close). Also, since the plant samples for the nitric acid extraction were not ground, will perform experiment again with ground samples to reach optimal extraction of NaCl.

Greenhouse Photos: The left pot in each figure are the high salt condition while the right pots are the control.

Figures 1 barley

Figure 2 turfgrass.





Figures 3 alfalfa **Figure 4** shows sugar beets. **Figure 5**: *S. europaea*

Results from Non-leaching Pot Experiment:

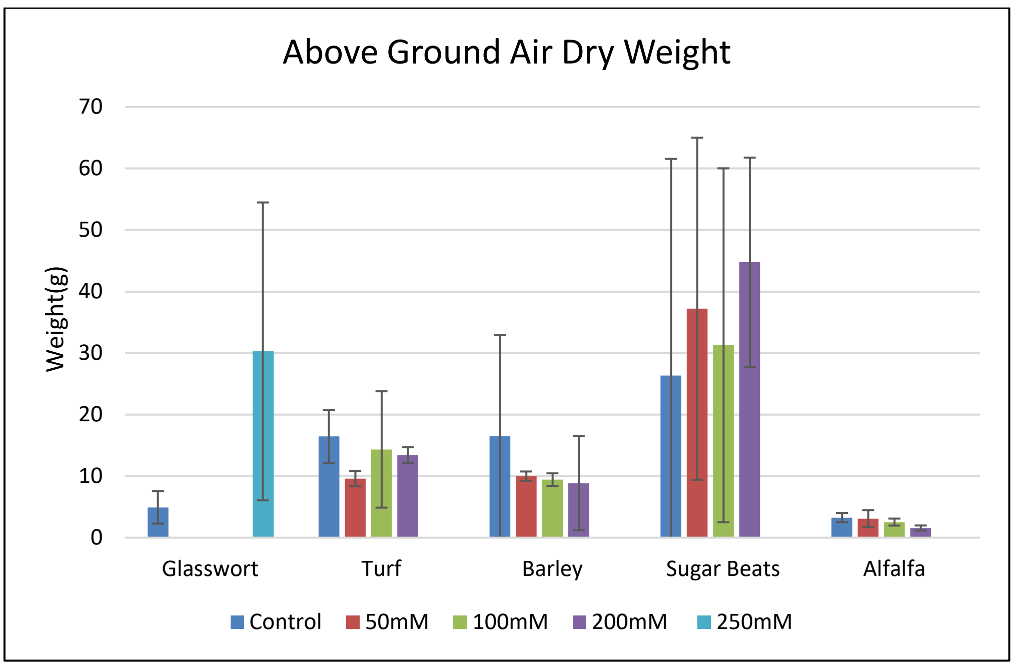


Figure 6: Air-dry weight of above ground biomass in the non-leaching experiment

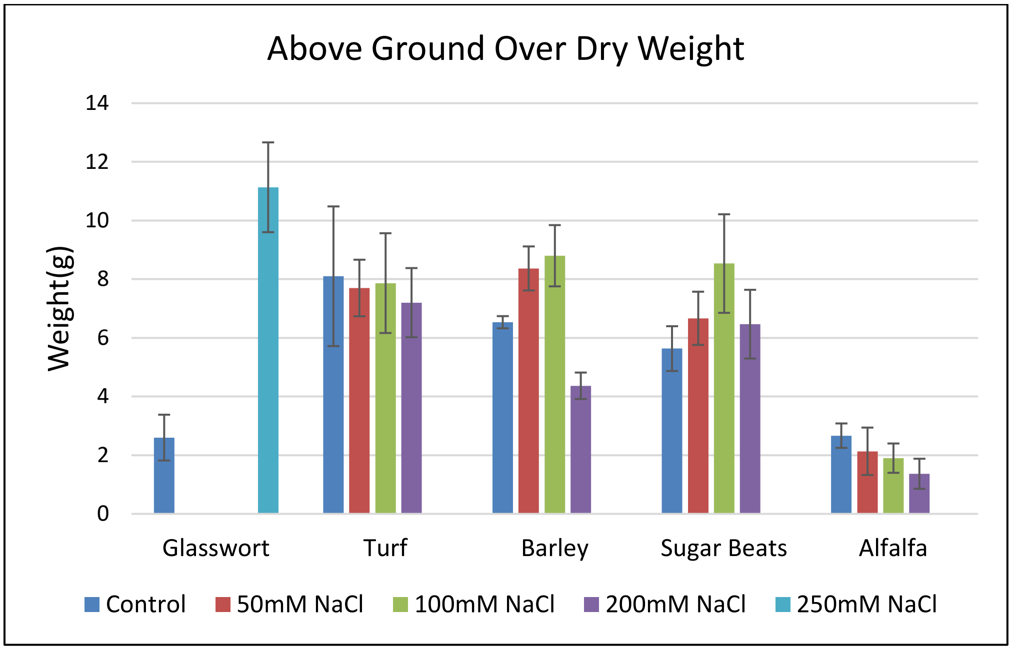


Figure 7: Oven dry weight of above ground biomass in the non-leaching experiment.

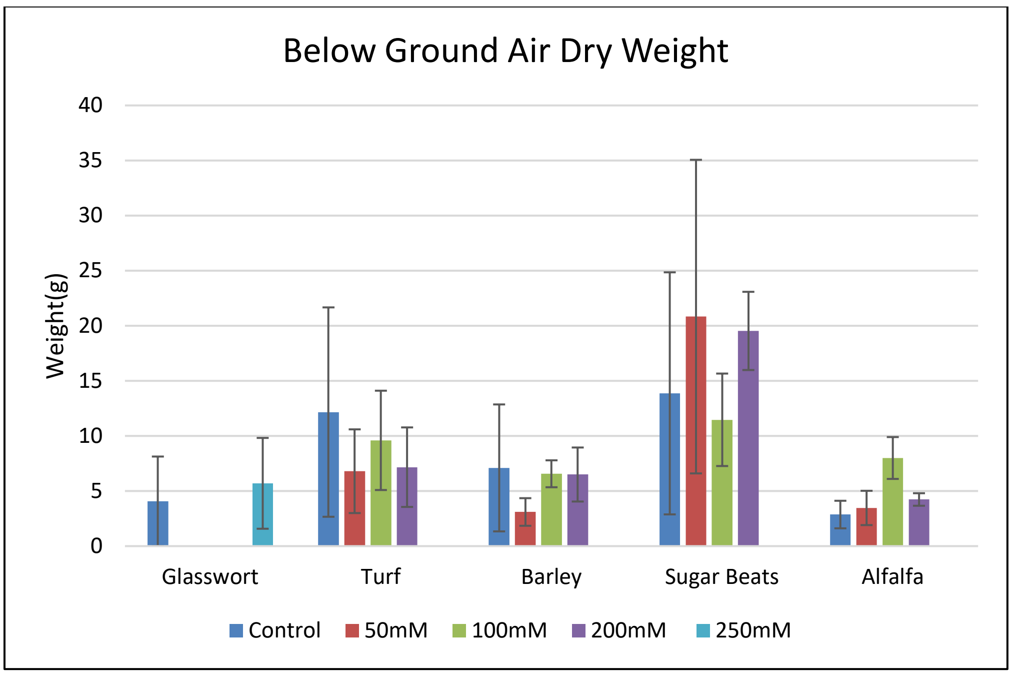


Figure 8: Air-dry weight below ground biomass in the non-leaching experiment.

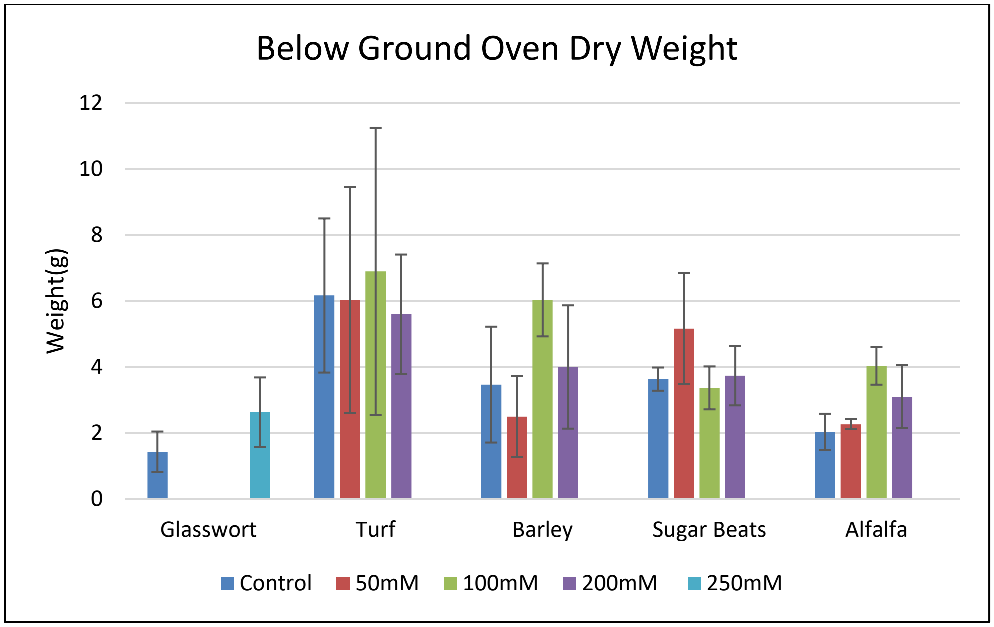


Figure 9: Oven dry weight below ground biomass in the non-leaching experiment

Discussion of Results: In general, the oven dry results from the experiment (Figures 7 and 9) show similar results to the air dry weights (Figures 6 and 8), but the oven dry data has less variance which is seen in the smaller error bars in the figures. This is likely due to some plants being more wet after cleaning their roots and therefore having different drying times before measuring the air dry weight leading to more varied results. The exception to this observation is that some of the turf grass and barley data have some larger error bars for the oven dry results. However, in most of the results, the trends seen between different treatments are similar for both the air dried and oven dried measurements.

In particular, the glasswort plants are shown to have significantly higher above ground oven dry biomass for the high salt (250mM NaCl) treatment compared to the control which is a trend also seen in the air dry data. For the turf grass, the below ground and above ground biomass remains relatively stable (no statistically significant difference) throughout the different salt treatments. The barley showed significantly higher oven dry above ground biomass for the 50 and 100mM NaCl treatments but then significantly decreases for the 200mM condition. A similar trend in growth was seen for the barley roots except for the 50mM treatment. Sugar beet growth also showed nearly significant increase for above ground oven dry biomass for the 100mM salt condition. For the alfalfa plants, there was a decrease in above ground biomass for the high salt condition compared to the control while the below ground biomass increased for the 100mM treatment but then slightly decreased with the highest salt condition.

In conclusion, the glasswort above ground biomass is greatly benefitted by high salt concentration. However, the turf grass is not significantly affected at the various salt levels used. The trends for both barley and sugar beets showed an increases in growth for the low and medium salt condition (50 and 100mM) but then the 200mM would then cause it to decrease growth or remain similar to the control. Alfalfa plants showed decreases in above ground biomass with increasing salt but increasing below ground biomass up unto the 100mM treatment after which it would decrease.

Greenhouse experiment with leaching pots

The leaching pot experiment design was intended to determine whether the Chloride ions of the salt solution pass through the soil more easily than the sodium ions. Also, underneath each leaching pot was a container for catching the irrigation water that passed through the soil and pot so that it could also be analyzed for sodium and chloride content.


Figure 10: Leaching pots with catching container

Experimental Design: Plants used in leaching experiment include: glasswort, turfgrass, barley, sugar beets, and alfalfa. Suaeda maritima (herbaceous seepweed) seeds did not germinate in germinating soil or on filter paper inside petri plates. Four different salt treatments were applied to water the plants, including: 0, 50, 100, and 200 mM NaCl. With each treatment, we had three replicates. However, there were not enough well-growing Salicornia (glasswort) for all 4 conditions so we only had control and high salt treatment for this specific plant (250 mM NaCl, different from the other high salt concentrations). Altogether, we had 54 pots of plants in the first greenhouse experiment for leaching study.

Germination of Seeds: Identical procedure as the non-leaching experiment

Greenhouse Growth Experiment: 150 g of fresh soil (general mix) were added into each leaching pot. After a few weeks of growth, we cut plants to 12cm height (sugar beets and glasswort were not cut) and watered with half strength Hoagland nutrient solution, no salt treatment added. The following week was the first irrigation with 150 mL of nutrient solution with salt treatments and this continued for weekly for a total of 4 saline treatments. The plants were removed from pots 40 days after the start of nutrient additions.

Results from leaching pot experiment:

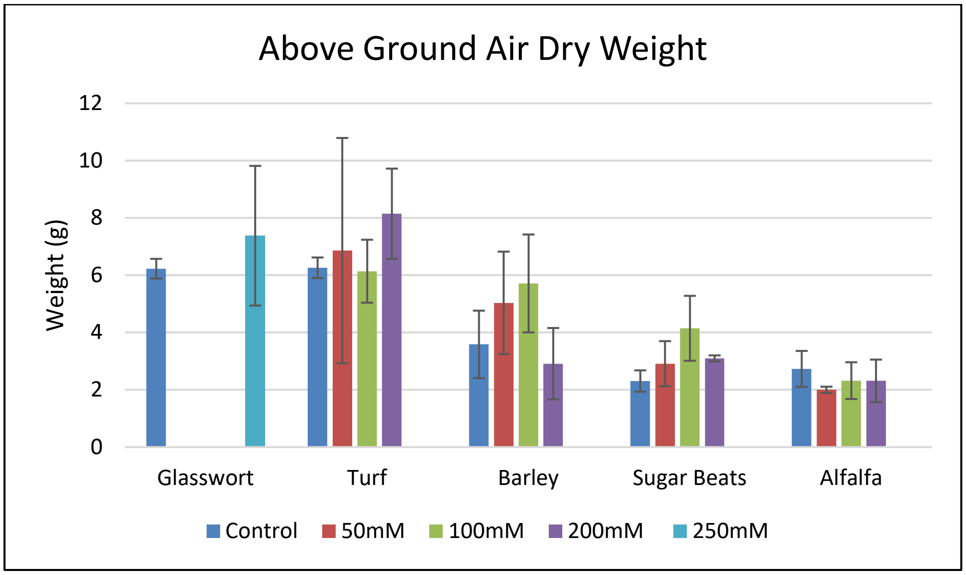


Figure 11: Air dry weight of above ground biomass in the leaching experiment.

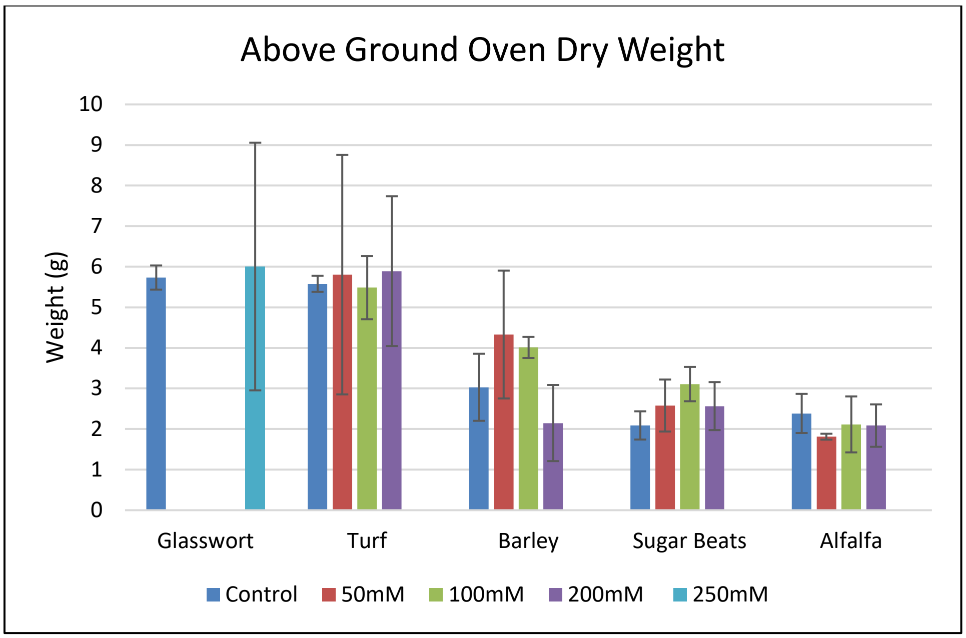


Figure 12: Oven dry weight of above ground biomass in the leaching experiment.

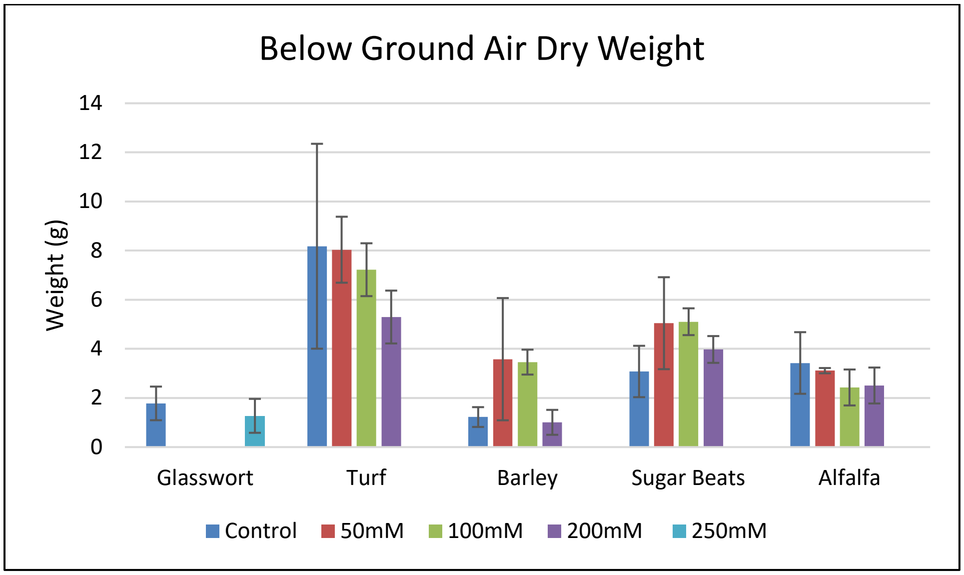


Figure 13: Air dry weight of below ground biomass in the leaching experiment

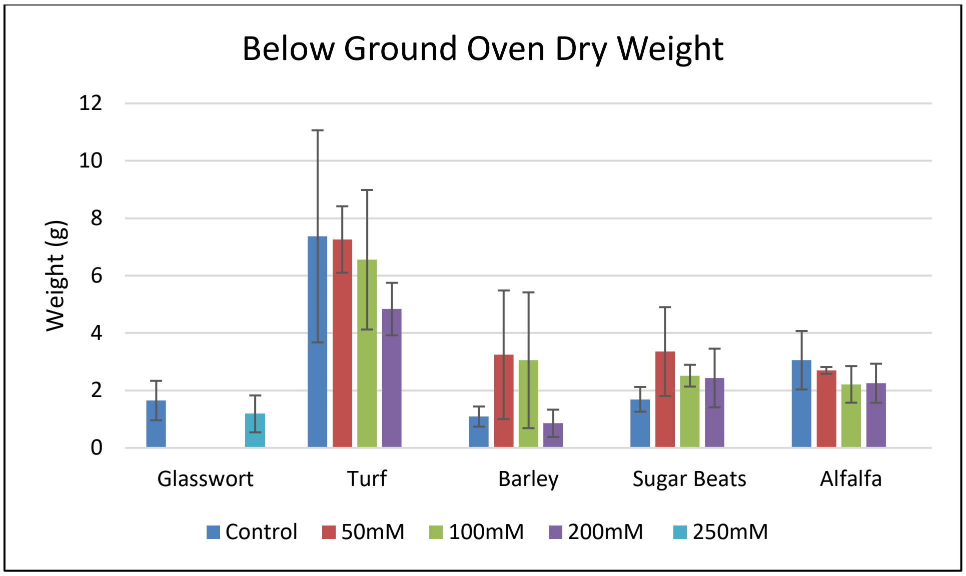


Figure 14: Oven dry weight of below ground biomass in the leaching experiment

Discussion of Results: The glasswort plants did not show statistically significant difference between the control and 250mM salt treatments for either above or below ground biomass (Figure 11-14). The turf grass did not show significant difference between treatments except for a decreasing trend for below ground biomass. For the barley, the above ground plants showed a similar trend seen in the results of the non-leaching pot experiment where the biomass increased with high salt concentrations but decreased for the 200mM treatment. Similarly, the sugar beets showed an increase of above and below ground biomass with increasing salt and then a trend towards decreasing biomass for the 200mM salt condition. With the alfalfa, no statistical difference was shown between the salt treatments (Figure 11-14).

Next greenhouse experiment plan: The next greenhouse experiment will consist of a similar design to the previous experiments but will instead investigate other species for their growth and uptake of salt. These species will include alkaligrass, slender wheatgrass, common sunflower, and smooth sumac and other potential halophytes mentioned in Table 1 such as *Atriplex hortensis.*

In another experiment, we will examine how fungal and bacterial endophytes affect the accumulation of Na^+ in plants. Endophytes will be isolated from halophytic species, like *S. europaea*, and tested for their salt tolerance by growing them on NaCl-rich media. Salt-tolerant isolates will then be tested as rhizosphere inoculants. We have identified a potential collaborator who has already isolated some fungal endophytes with potential to improve Na^+ uptake and plan to test these endophytes in our plants. Selected endophytes will be cultured in liquid broth and inoculated into the rhizospheres of halophytic or salt-tolerant plants growing in a range of NaCl concentrations. Sterile inoculum will be used as controls. After a period of growth, plants will be tested for endophytic colonization using trypan blue staining, and various ecophysiological traits, including photosynthesis rate, transpiration rate, and water use efficiency, as well as plant biomass and Na^+ content, will be measured. In order to determine potential mechanisms of Na^+ accumulation, RNA sequencing will be performed to identify genes that are overexpressed in endophyte-colonized plants that accumulate more Na^+. Quantitative PCR (qPCR) will be performed to confirm differential expression of specific genes identified through RNA sequencing. We expect to find that certain endophytes improve salt tolerance and salt accumulation in several of our test plant species and to correlate these finding with genes whose expression levels are modified by endophytic colonization.

Second Update September 1, 2020

We continue working on the inventory study to include more Minnesota native plants that can be either classified as halophytic plants or in more general category of "salt tolerant plants" as well as some potential plants which are not native to Minnesota but can potentially grow in our Minnesota climate. The updated inventory is shown in Table 4, and several more plant species are selected as following:

Atriplex prostrata (triangle orache) is a native annual herb, growing in both wetland and non-wetland. It has great phytoremediation potential as it can contain 85.1 mg Na/g dry weight, 117.4mg Cl/g dry weight, and 41.4 g Na/100cm2 (all at 2.5% NaCl conditions). It is edible as it could be used as potash/fertilizer.

Chenopodium berlandieri (Pitseed Goosefoot) is an annual forb/herb. It grows in all types of environments, such as part shade, sun; disturbed soil; roadsides, railroads, waste places, cliffs, river banks, and open woods. Its salt removal capability is unknown. Its seeds can be ground into powder with flours, and leaves and young shoots can be eaten.

Pascopyrum smithii (western wheatgrass) is a native perennial grass. Forage quality is high for pasture or range seedings. Western wheatgrass is an excellent erosion control plant because of its spreading rhizomes. It is widely used in seed mixtures for range seeding, revegetation of saline and alkaline areas. It can grow in high levels of salt but no accumulation data could be found.

Phragmites australis (common reed) is a perennial grass. grows in marshes and swamps, on banks of streams and lakes, and around springs. It grows best in firm mineral clays and tolerates moderate salinity. The plant biomass can be used to produce biofuel and animal feeds

Puccinellia nuttalliana (alkaligrass) is a perennial grass native to MN, mostly in the wet saline grassland in western counties. Based on the literature, it has 8.3 g/m2 potential Cl removal and 32mg Cl/g dry weight ion concentration in their biomass. This grass is hardy and can grow in various different types of soil and water environment. It has potential for forage or turf use. This can be a high potential grass for roadside plantation since it is easy to maintain, effective in sodium chloride removal and the biomass can be used as feeds for cattle and horse.

Spergularia marina/salina (salt sandspurry) is a native annual herb. It has a great potential for salt phytoremediation as its biomass contains 78.2 mg Na/g dry weight, 113.03 mg Cl/g dry weight, 28.8 g Na/100cm2 (all at 2.5% NaCl). Its plant biomass use is not known.

Suaeda calceoliformis (Seablite) is a native annual herb. It has a great potential for salt phytoremediation as its biomass contains 94.3 mg Na/g dry weight, 149.3 mg Cl/g dry weight, 46 g Na/100cm2 (all at 2.5% NaCl).). Its plant biomass use is not known.

Thinopyrum ponticum (Tall wheatgrass) is an introduced long-lived perennial grass. It accumulated greater Na^+ in roots than in shoots. Its plant biomass is a high quality forage and this grass is often used on the roadside for erosion control.

Typha latifolia (broadleaf cattail) is a native perennial grass. It has some potential for the salt phytoremediation. Its main application is to help stabilize marshy borders of lakes and ponds; help protect shorelines from wave erosion. The starchy roots, young flowering spikes, and pollen can be eaten.

These plants have great potential and we are planning to test these plants for the next round of greenhouse studies.

Table 4. Parameters for potential halophytic plants for salt removal

NAME	Plant Type	Annual /perenial	USDA CLASSIFICATION	Habitat	Uses/applications
Atriplex hortensis (garden orache)	Shrub	Annual	Introduced	Occur in wetlands and non-wetlands	culinary, and could be fodder too (in a mixed diet for ruminants)(source?), Atriplex hortensis has been recommended as a sub-stitute or supplement to spinach (Spinacea oleracea) due to their similar chemical composition (Carlsson and Clark, 1983)
Atriplex patula (spear saltbush)	shrub/herb	Annual	Introduced	Usually occur in wetlands, but may occur in non-wetlands. prefers moist soil	culinary
Atriplex prostrata (triangle orache)	herb	annual	native	FAC: occurs in wetlands and non-wetlands (https://www.wildflower.org/plants/result.php?id_plant=ATPR)	edible and could be used as potash/fertilizer (http://temperate.theferns.info/viewtropical.php?id=Atriplex+prostrata)
Bassia scoparia (burningbush)	forb/herb	annual	Introduced		Drought and grasshopper resistant
Beckmannia syzigachne (slough grass)	grass	Perennial	Native	MW: OBL; sun; wet soil; marshes, shores, stream banks, wet ditches (https://www.minnesotawildflowers.info/grass-sedge-rush/american-slough-grass)	palatable to all classes of livestock and is frequently hayed (https://plants.usda.gov/plantguide/pdf/pg_besy.pdf)
Borago officinalis (Common borage)	herb	Annual	Introduced	preference is a sunny position, mesic conditions, and a fertile loamy soil (https://www.illinoiswildflowers.info/weeds/plants/borage.htm)	medical herb, leaves reportedly used as diuretic, demulcent emollient, expectorant, etc.

Chenopodium album (lambsquarters, Pigweed)	forb/herb	annual	introduced and native	part shade, shade, sun; disturbed soil; **roadsides**, waste places, gravel pits, fields, open woods, shores	both leaves and seeds can be animal feed
Chenopodium berlandieri (Pitseed Goosefoot)	forb/herb	annual	a native of MN close relative of lambsquarters	part shade, sun; disturbed soil; **roadsides**, railroads, waste places, cliffs, river banks, open woods (https://www.minnesotawildflowers.info/flower/pitseed-goosefoot)	seeds can be ground into powder with flours. leaves and young shoots can be eaten (http://tropical.theferns.info/viewtropical.php?id=Chenopodium+berlandieri)
Distichlis spicata (saltgrass)	grass	Perennial	Native	upper/high marsh (irregularly flooded) areas, in which the water levels vary between 2 inches above the soil surface and 6 inches below the soil surface.	Saltgrass is grazed by both cattle and horses and it has a forage value of fair to good because it remains green when most other grasses are dry during the drought periods and it is resistant to grazing and trampling (https://plants.usda.gov/plantguide/pdf/pg_disp.pdf)
Eleocharis palustris (common spike-rush)	flower	Perennial	Native	wetland obligate plant that grows in permanently or seasonally flooded conditions. It grows on fine texture soils in neutral to alkaline or saline conditions	moderately high protein content in the spring and good digestibility. also used in contructed wetlands and restoration (https://plants.usda.gov/plantguide/pdf/pg_elpa3.pdf)
Elymus trachycaulus (Slender Wheatgrass)	grass	short lived (3-5 yr) perennial	native	prefers loams and sandy loams in areas receiving at least 14 inches of annual precipitation. Grows on moist to dry sites and has moderate to good tolerance of alkaline conditions (pH = 8.8). Salinity tolerance depends on environmental conditions and ecotype. It is surpassed in this trait only by tall wheatgrass.	Slender wheatgrass is both palatable and nutritious to livestock. Also erosion control and reclamation. (https://plants.usda.gov/plantguide/pdf/pg_eltr7.pdf)
Helianthus annuus (annual/common sunflower)	forb	annual	native	is a common and widespread **roadside weed**	Seeds used as food and source of oil. Stalks have been used as fuel, fodder for livestock, poultry, and ensilage. Seed hulls could be used for litter for poultry or fuel. (https://plants.usda.gov/plantguide/pdf/cs_hean3.pdf)

Hordeum jubatum (foxtail barley)	grass	short lived perennial	native	FAC, sun; average to moist soil; **roadsides**, waste places, fields, marsh edges, sloughs (https://www.minnesotawildflowers.info/grass-sedge-rush/foxtail-barley)	Before flowering, is palatable to livestock and big game. Up to the time when seedheads develop, it is fair to good forage for cattle and horses and fair for sheep. Foxtail barley seedheads, when dry, are very harmful to all kinds of grazing animals. Has potential for revegetation of saline mine spoils where forage value is of secondary importance. (https://www.fs.fed.us/database/feis/plants/graminoid/horjub/all.html)
Juncus acutus (spiny rush)	herb	perennial	not native or introduced in MN		
Leptochloa fusca (Malabar sprangletop)	grass	annual	Native	wetlands, marshes	been used to remediate heavy metals (Ullah et al., 2019)
Pascopyrum smithii (western wheatgrass)	grass	perennial	native	FACU, Sun or part shade. Moist, alkaline clay, loam, sand. (https://www.wildflower.org/plants/result.php?id_plant=pasm)	Forage quality is high for pasture or range seedings. Western wheatgrass is an excellent erosion control plant because of its spreading rhizomes. It is widely used in seed mixtures for range seeding, revegetation of saline and alkaline areas. (https://plants.usda.gov/factsheet/pdf/fs_pasm.pdf)
Phragmites australis (common reed)	grass	perennial	there are native (subsp. Americanus) and non-native species (subsp. Australis) which are difficult to tell apart	grows in marshes and swamps, on banks of streams and lakes, and around springs. It grows best in firm mineral clays and tolerates moderate salinity	Can be used as biofuel (Vaičekonytė et al., 2014). readily eaten by cattle and horses. It provides high quality warm-season forage but becomes tough and unpalatable after maturity. Animals grazing this grass during winter should be fed a protein concentrate (https://plants.usda.gov/factsheet/pdf/fs_phau7.pdf)
Portulaca oleracea (common purslane)	succulent	annual	not considered native in MN, introduced	Usually occur in non-wetlands, but may occur in wetlands, sun; disturbed soils; roadsides, fields, waste places, gravel pits, gardens	may be eaten as a leaf veggie

Puccinellia distans (weeping alkaligrass)	grass		non-native, **weedy in MN**	sun; moist to wet disturbed soil; **roadsides**, ditches, fields, gravel pits. highly salt-tolerant as evident from where it has been found across North America: salted roadsides and ditches, saline prairies and cow pastures, and usually in wet soils (https://www.minnesotawildflowers.info/grass-sedge-rush/weeping-alkaligrass)	
Puccinellia nuttalliana (Nutall's alkaligrass)	grass	perennial	Native, found in MN western counties (wet saline grasslands)	(Obligate wetland species in Midwest)sun; wet saline soil; prairies, ditches, swales (https://www.minnesotawildflowers.info/grass-sedge-rush/nuttalls-alkaligrass)	potential for forage or turf use (Morphological and agronomic variation of Puccinellia nuttalliana populations from the Canadian Great Plains)
Rhus glabra (smooth sumac)	woody	perennial	native	part shade, sun; dry to average moisture; woodland edges, savannas, prairies, outcrops, along roadsides, railroads, shores (https://www.minnesotawildflowers.info/shrub/smooth-sumac)	serves primarily as a winter emergency food for wildlife (https://plants.usda.gov/factsheet/pdf/fs_rhgl.pdf)
Spartina pectinata (prairie cordgrass)	grass	perennial	Native	found in wet meadows, sloughs, potholes, and drainage ways. typically found on lower, poorly drained soils along roadsides, ditches, streams, marshes and potholes (https://plants.usda.gov/plantguide/pdf/pg_sppe.pdf). FACW in Midwest, common, fast-growing, warm-season grass that has a preference for moist, sandy soil but tolerates a variety of conditions, including dry and saline soils (https://www.minnesotawildflowers.info/grass-sedge-rush/prairie-cordgrass)	stiff stems, vigorous rhizomes and robust size of this species are useful in stabilizing soil, dissipating wave energy and providing cover. not a forage resource or just in early season (https://plants.usda.gov/plantguide/pdf/pg_sppe.pdf), It's been planted for erosion control and cultivated as a biofuel (https://www.minnesotawildflowers.info/grass-sedge-rush/prairie-cordgrass)
Spergularia canadensis (Canadian sandspurry)	forb/herb	annual	native in Canada	Brackish or salt marshes and flats, coastal beaches (sea beaches), intertidal, subtidal or open ocean (https://gobotany.nativeplanttrust.org/species/spergularia/canadensis/)	No found uses

Spergularia marina/salina (salt sandspurry)	herb	annual or sometimes perennial	found in Midwest and Canada	FACW, Usually occurs in wetlands, but occasionally in non-wetlands. Anthropogenic (man-made or disturbed habitats), brackish or salt marshes and flats, coastal beaches (sea beaches), marshes, intertidal, subtidal or open ocean (https://gobotany.nativeplanttrust.org/species/spergularia/marina/)	no found uses
Suaeda calceoliformis (Seablite)	herb	annual	native	FACW in Midwest, sun; saline or alkaline soil; marshes, shores, prairie swales, salted **roadsides** (https://www.minnesotawildflowers.info/flower/seablite)	cannot find
Thinopyrum ponticum (Tall wheatgrass)	grass	long lived perennial	introduced	introduced to the U.S. from Turkey in 1909 and is now found throughout all western states of the U.S. and most Canadian provinces	produces high yields of good quality forage, however it is typically less palatable than other wheatgrasses. It is best suited for early season rotational grazing. often used for erosion control along **roadsides**. has been used to manage salinity in irrigation water recovery systems. currently being evaluated as a possible source of cellulosic ethanol (https://plants.usda.gov/plantguide/pdf/pg_thpo7.pdf)
Typha angustifolia (narrowleaf cattail)	herb	perennial	both native and introduced, may not be introduced into a free-living state without MN DNR approval and thorough evaluation (https://extension.umn.edu/identify-invasive-species/narrow-leaf-cattail)	OBL: Moist to wet. Marshes, ditches. Full sun. (http://www.minnesotaseasons.com/Plants/narrow-leaved_cattail.html)	All parts of the cattail are edible when gathered at the appropriate stage of growth (https://plants.usda.gov/plantguide/pdf/cs_tyan.pdf)

Typha latifolia (broadleaf cattail)	herb	perennial	Native	OBL, sun; marshes, wetlands, along pond and stream edges (https://www.minnesotawildflowers.info/flower/broad-leaf-cat-tail)	Helps stabilize marshy borders of lakes and ponds; helps protect shorelines from wave erosion. the starchy roots, young flowering spikes, and pollen can be eaten by humans

Third Update March 1, 2021

Data analysis of the soil and plant tissues from the non-leaching experiment:

In figures 15 and 16, which show the mass of sodium and chloride within aboveground plant tissues by gram of dry weight, there is a noticeable similarity in the shapes of the figures. Although, the values for chloride uptake are significantly higher (the y axis of figure 15 goes up to 100mg while in figure 16 it goes to 200mg), in most cases the chloride content is more than twice that of sodium for the same species and salinity treatment indicating that Cl may transport more readily into plant shoots than Na.

As is seen in the figures, tall fescue and barley salt uptake increase with higher salinity irrigations. However, for alfalfa, the only clear exception to the sodium and chloride correlation, the chloride tissue concentrations significantly increases for the higher salt treatments while the sodium content does not.

The sugar beet aboveground salt tissue content increases much more significantly than the three plants previously discussed when comparing the control treatment to any of the saline treatments. When adding the mg of Na and Cl per gram of dry aboveground sugar beet biomass together, the NaCl content can reach 20% when watered with 100mM or 200mM saline solution. Then, not surprisingly, the glasswort aboveground biomass salt content also reaches 20% and higher NaCl concentrations for the 250mM salinity treatment.

The sodium chloride content of the belowground beets were also measured to see how they compared with the aboveground biomass. In figure 17, the beet samples are shown to have significantly lower NaCl content than the aboveground leaves. This results could mean that sugar beets may be used to remediate saline soils while also maintaining a nutritious and low salt content beet for possible use as food or fodder.

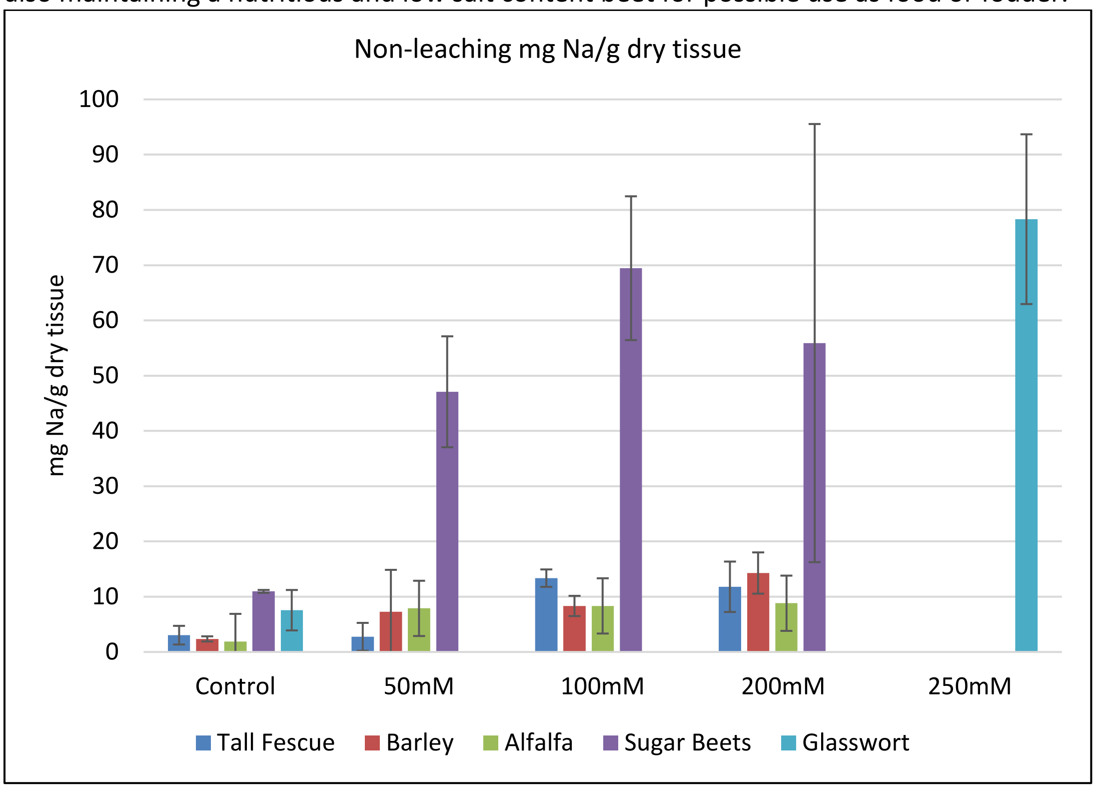


Figure 15: The aboveground sodium content of plant species by dry gram of tissue grown in non-leaching pots and irrigated with various levels of salinity.

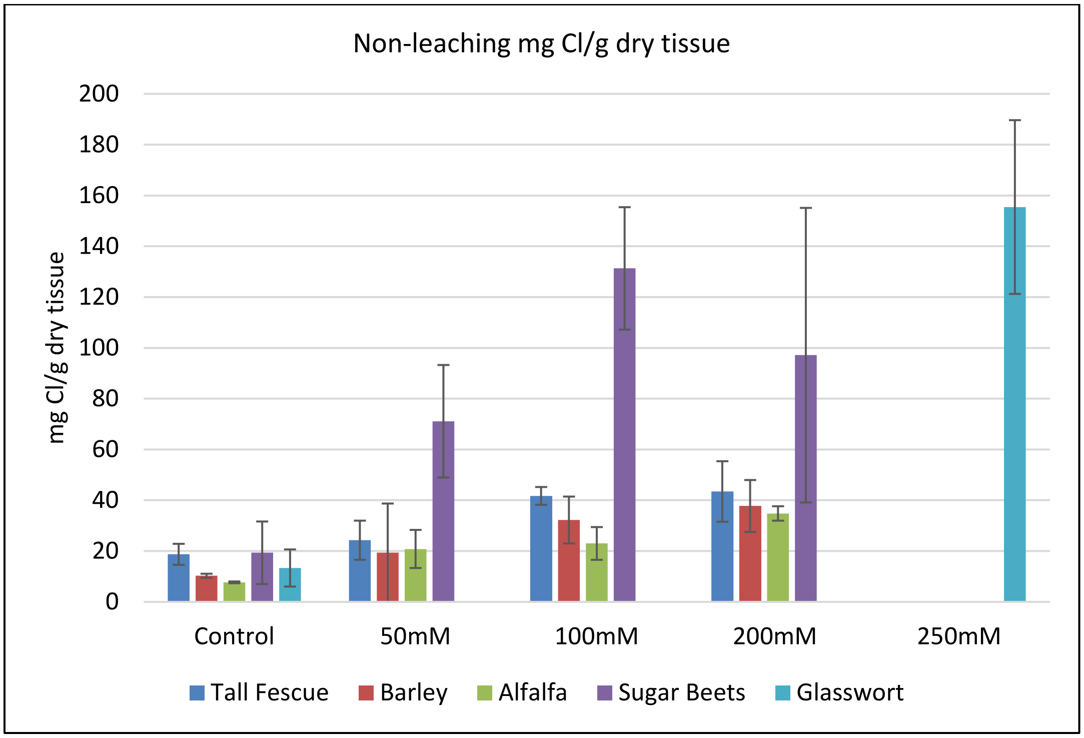


Figure 16: The aboveground chloride content of plant species by dry gram of tissue grown in non-leaching pots and irrigated with various levels of salinity.

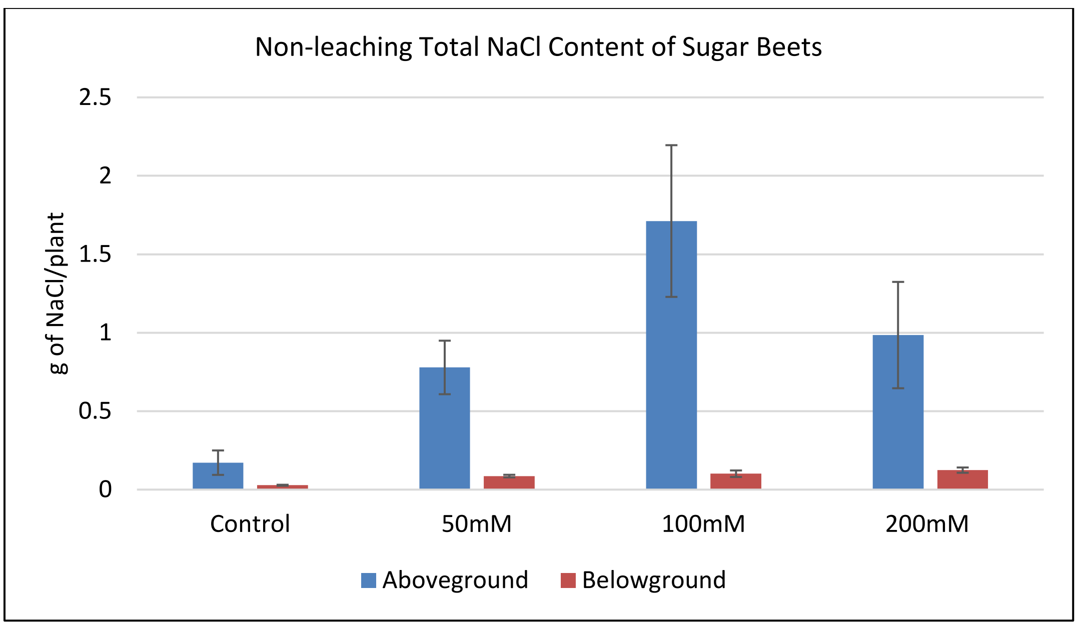


Figure 17: The total NaCl content aboveground and belowground biomass of sugar beets in non-leaching pots.

Figures 18 and 19 show the amount of sodium and chloride remaining in soil at the end of the non-leaching pot experiment. For most of the soil analyzed, the amount of chloride taken up by the plant is directly correlated to the uptake of sodium. The difference being the molar mass between the two ions; chloride has a molar mass 1.54 times that of sodium.

As expected, the soil salinity increased as the salinity concentration of the irrigation water increases. The exception to this being that the glasswort plants were able to remove enough NaCl that the amount in the soil is below or similar to that of the 200mM NaCl pots. When comparing the individual plant species effect on the soil

NaCl content for a given salt treatment, tall fescue and sugar beets appear to decrease the salt content more consistently than barley and alfalfa. These results match up fairly well with figure 5 although the amount of salt remaining in the sugar beet pots would be expected to have significantly lower amounts than tall fescue. However, since the beet salt content (the majority of belowground biomass) is much lower than aboveground (figure 17), it is likely that the turf grass contains more sodium chloride belowground than sugar beets resulting in soil salt content that is similar between the two plant species pots.

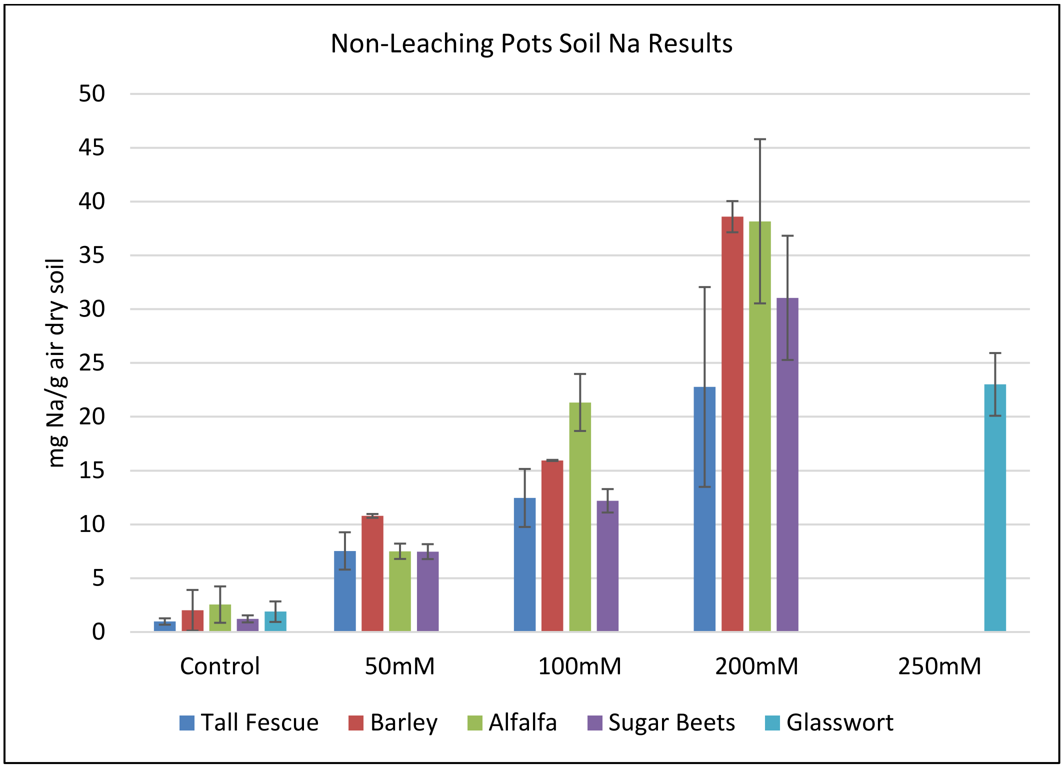


Figure 18: The sodium content of soil in non-leaching pots containing different species of plant and irrigated with varying levels of salinity.

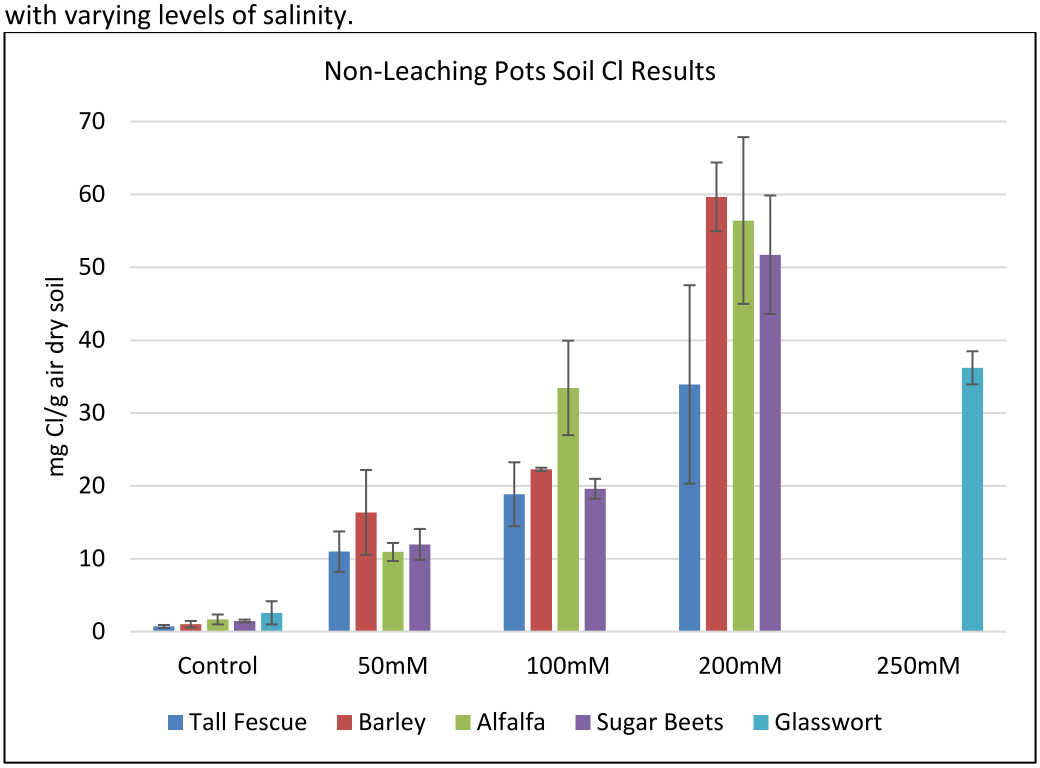


Figure 19: The chloride content of soil in non-leaching pots containing different species of plant and irrigated with varying levels of salinity.

In the leaching experiment, the oven dry aboveground biomass of the glasswort plants surprisingly did not differ from the control and 250mM NaCl treatment (figure 20). It was expected that the sodium chloride would benefit the halophyte's growth. Although the error bars for the 250mM salt treatment are quite high compared to the control and therefore it is difficult to obtain a clear answer. The other plant species in the leaching study showed similar trends in their response to increasing salinity as they did in the non-leaching experiment. Again, turf grass growth is largely unaffected by salinity while the barley and sugar beets have some small increases in aboveground biomass up to the 100mM salt condition. The alfalfa aboveground growth in the leaching pots remained low compared to the other plant species but had no statistical difference between its salt treatments.

Similarly to the belowground biomass results for the non-leaching pot experiment, figure 21 of the leaching experiment also shows large error bars for the oven dry weight of the root systems. There is no significant difference between the treatments for each plant species except between the 50mM and 200mM salt treatments for turf grass signifying a downward trend in root growth. However, the turf grass belowground biomass is still greater than the other species even at the 200mM NaCl condition.

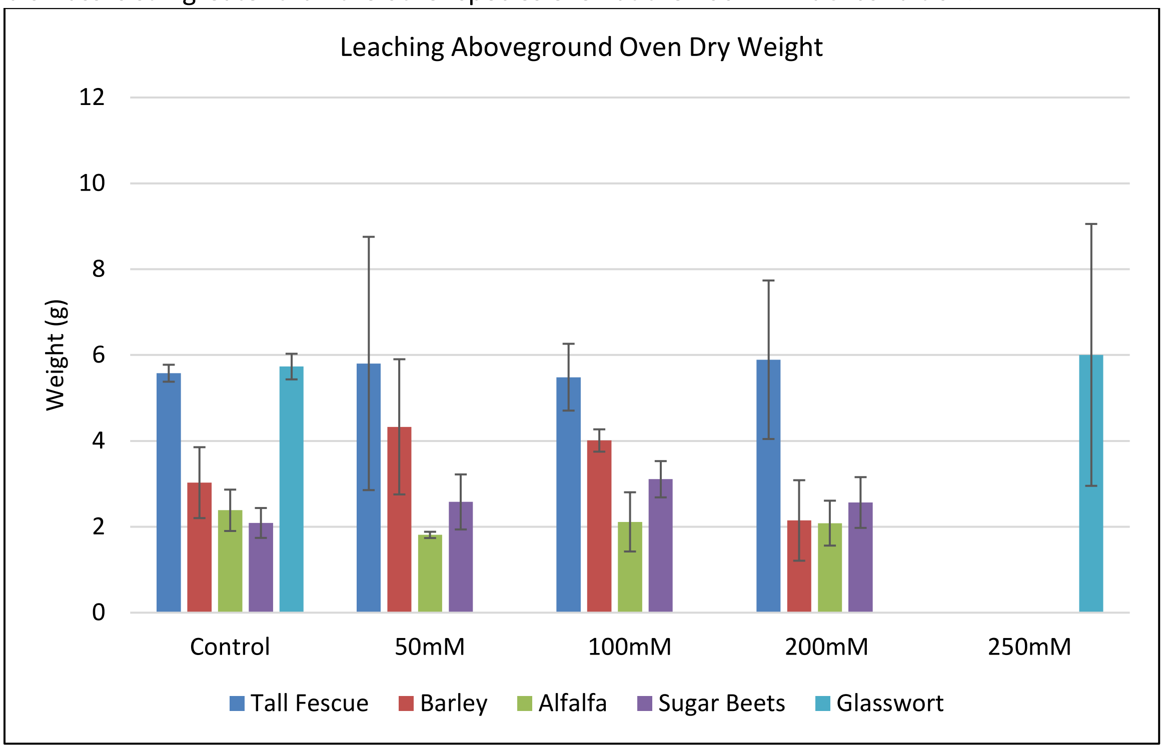


Figure 20: The aboveground oven dry biomass of various plant species that were grown in leaching pots and irrigated with increasing levels of salinity.

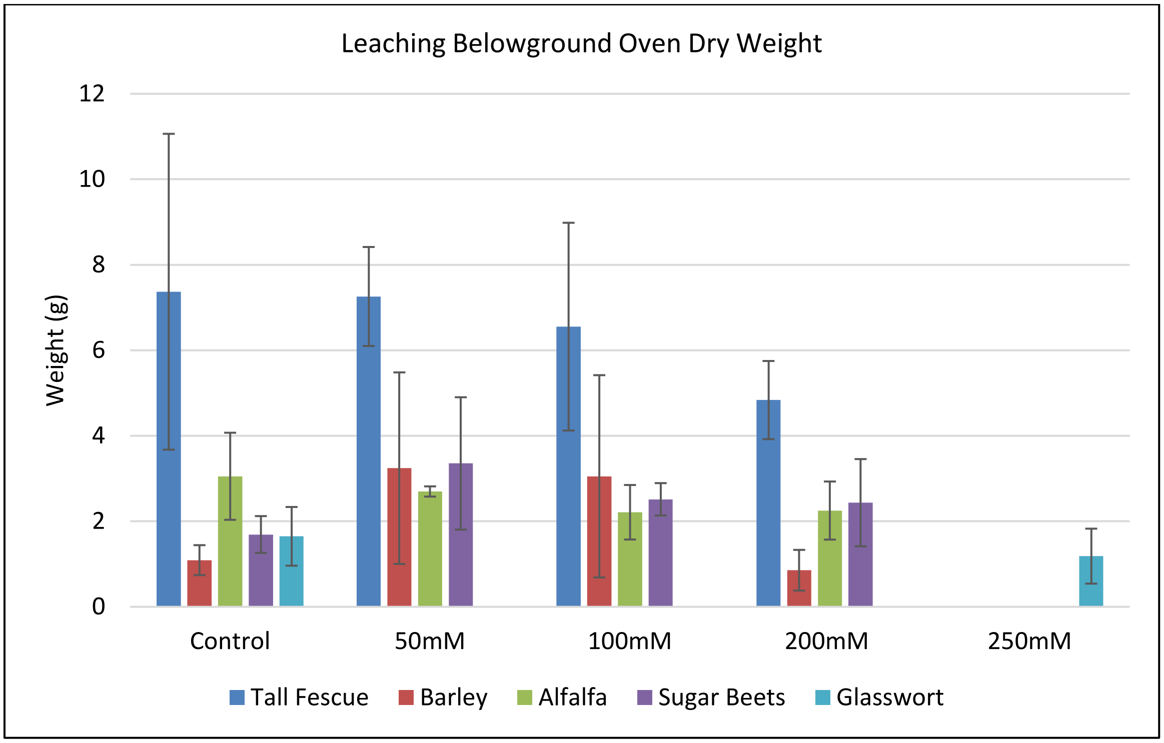


Figure 21: The belowground oven dry biomass of various plant species that were grown in leaching pots and irrigated with increasing levels of salinity.

Like the non-leaching pot study, the chloride content of plant tissues in the leaching pot experiment are nearly all significantly higher than that of sodium when comparing the same plant species and treatment (figures 22 and 23). The exception to this being the sugar beet chloride content. While sugar beet aboveground biomass sodium uptake was significantly greater than the other species the chloride content was much. This result could suggest that in a non-leaching pot, the sugar beet roots which are a relatively small amount of the belowground biomass, are not so effective at keeping the salt ions from leaching out of the pot and therefore less NaCl is taken up by the plant. Also, the total NaCl content of sugar beet aboveground biomass compared to the beet is not as significantly different as it were for the non-leaching pot experiment (figure 24). The leaching pot sugar beets did not taken up as much salt and therefore did not send high concentrations of it to its aboveground leaves.

The salt uptake for tall fescue, barley and alfalfa varied across the different salt treatments but not greatly. Turf grass showed increasing Cl content but not significant difference between treatments for the Na content. The sodium and chloride content of barley increased for the 50 and 100mM salt treatments but not for 200mM NaCl. Alfalfa salt tissue concentration increased for both sodium and chloride at the higher salt treatments compared to the control. Glasswort salt content significantly increased at the higher concentration although the large error bar made it less significantly higher than other species salt levels especially sodium content of sugar beet.

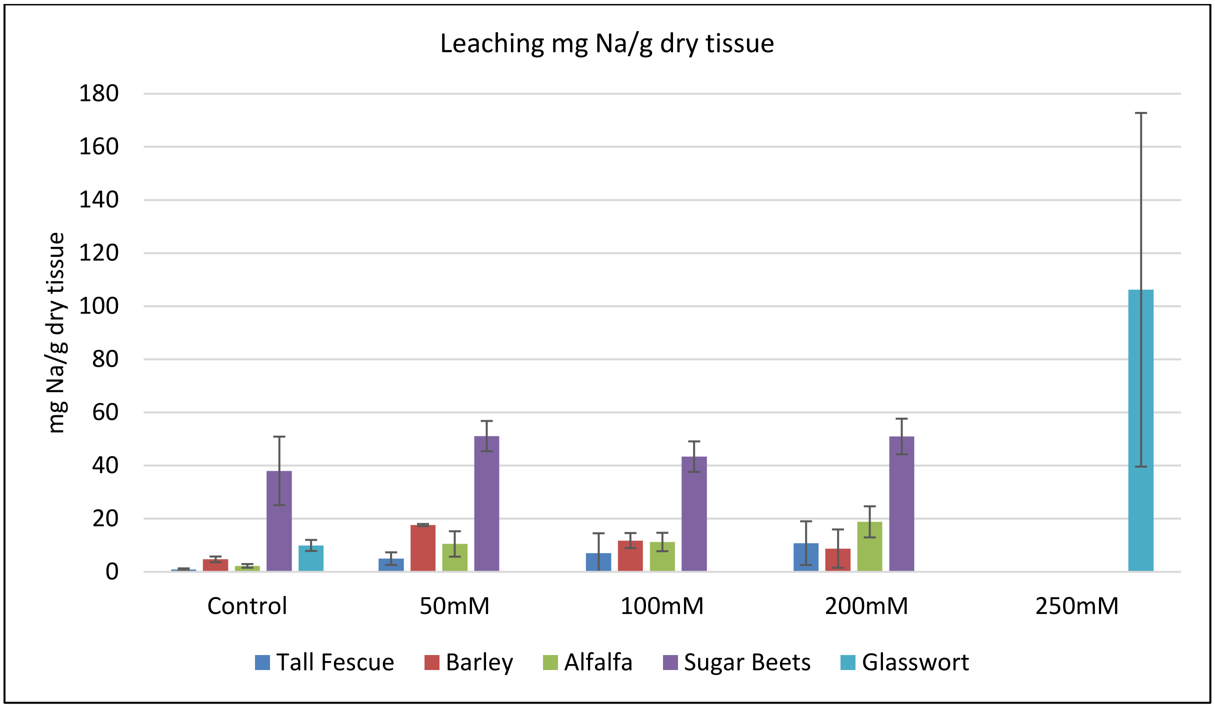


Figure 22: The aboveground sodium content of plant species by dry gram of plant tissue grown in leaching pots and irrigated with increasing levels of salinity.

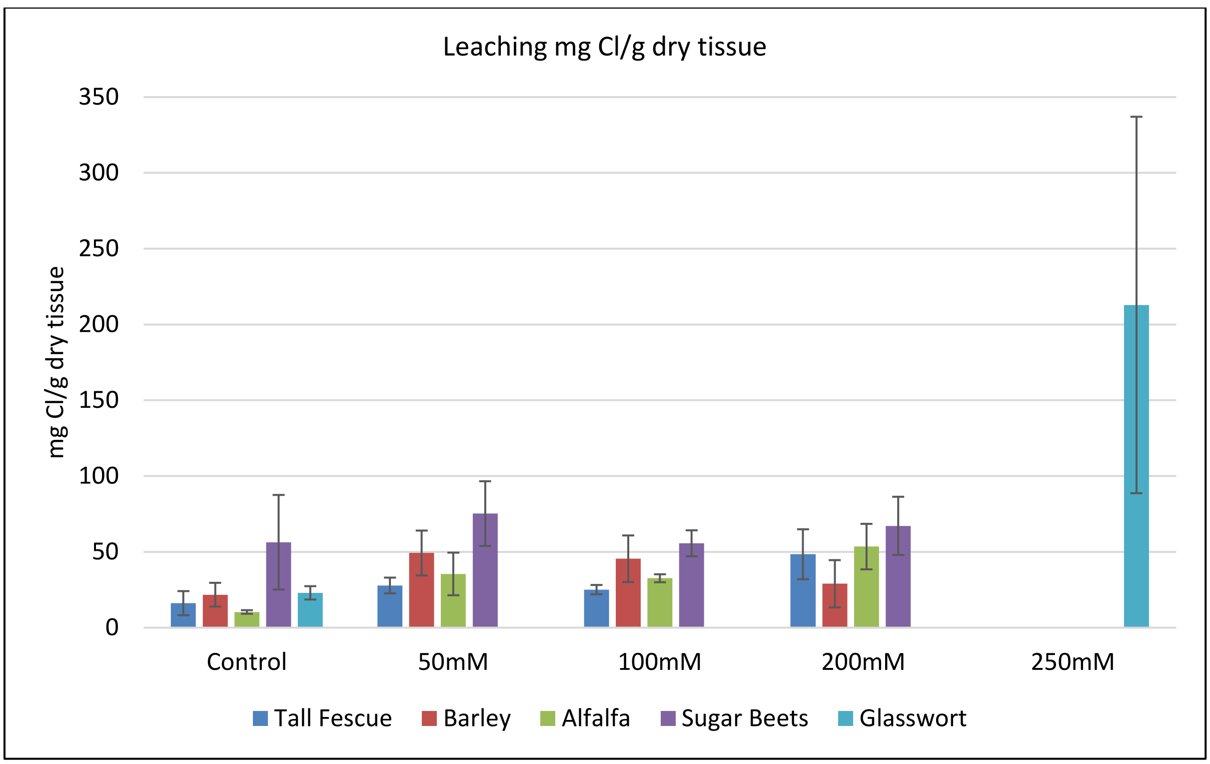


Figure 23: The aboveground chloride content of plant species by dry gram of plant tissue grown in leaching pots and irrigated with increasing levels of salinity.

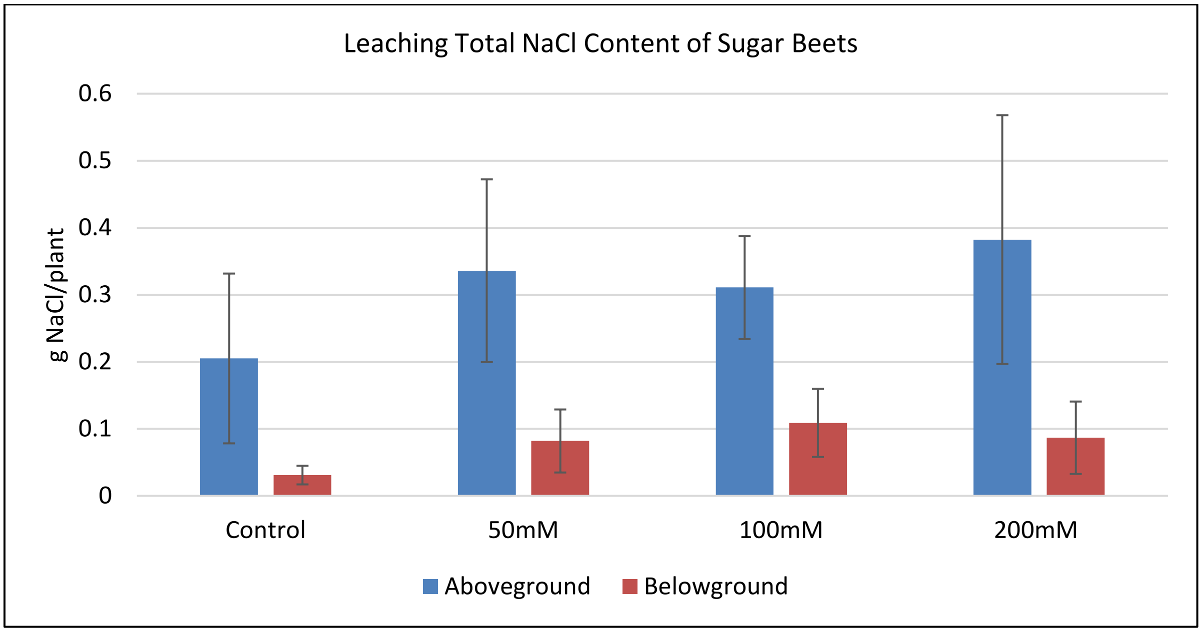


Figure 24: The total NaCl content aboveground and belowground biomass of sugar beets in leaching pots.

The concentration of sodium and chloride left over in the soil is shown in figures 25 and 26. As was the case with the non-leaching pot soil results, these two figures are also directly correlated with the chloride concentration around 1.54 times sodium.

At higher salt treatments the sodium and chloride in the soil increases. Within each treatment, there is some difference between plant species. The most notable difference being the soils with sugar beet compared to the others. Sugar beet pots have significantly lower NaCl content than the other pots for the 50, 100, and 200mM salt treatments with the only deviation from this being the alfalfa pots at the higher salt level. These results demonstrate that the salt uptake of the sugar beets and leaching of the salt through the soil both likely contributed to the lower amounts in the soil.

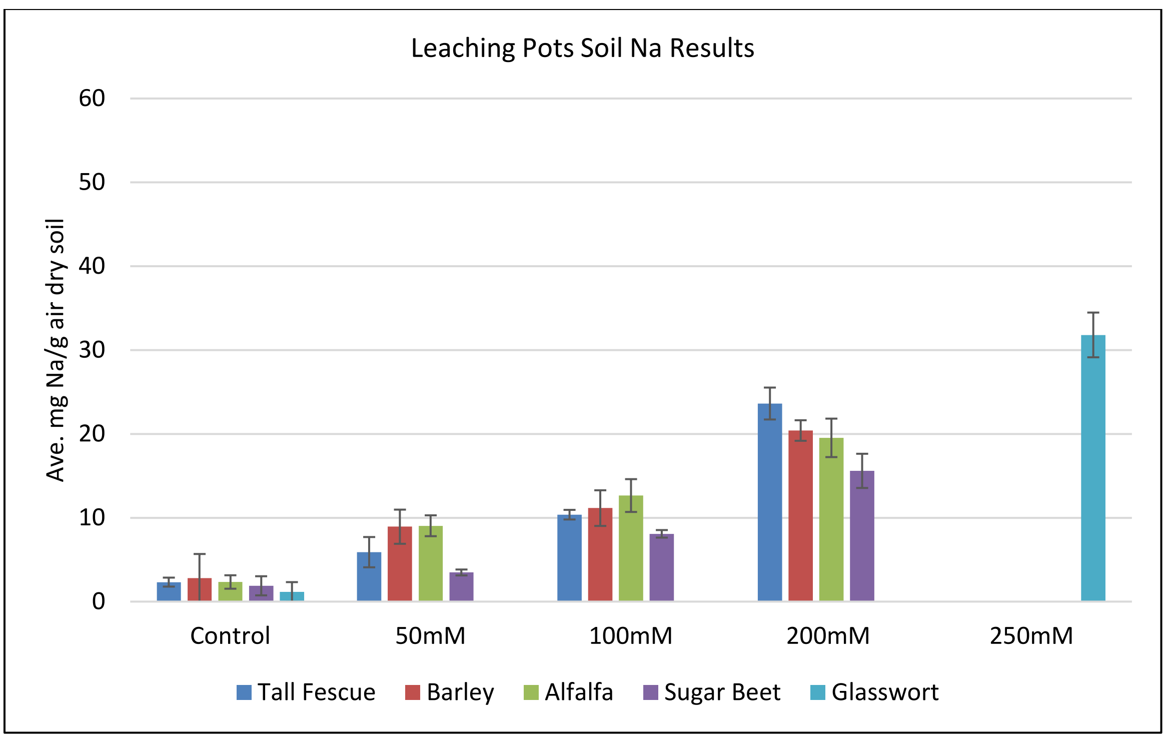


Figure 25: The sodium content of soil by gram of air dry soil left over in the leaching pots at the end of the experiment.

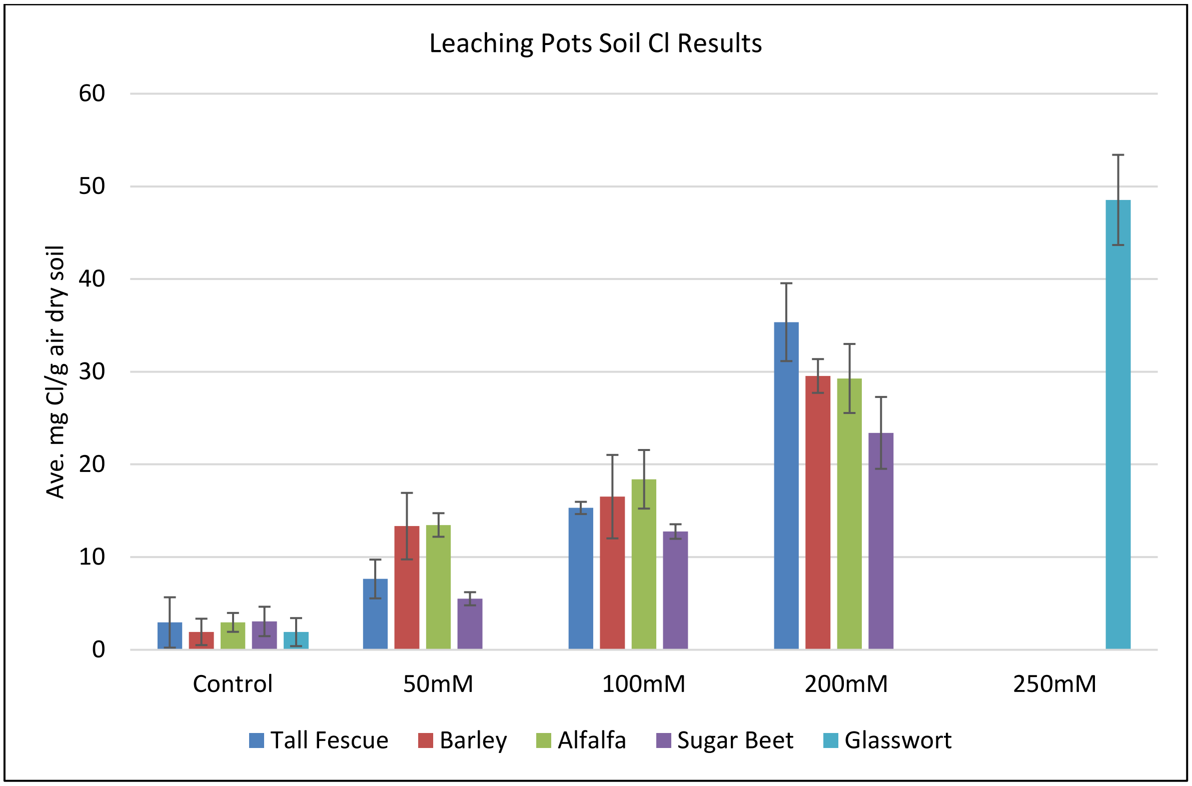


Figure 26: The chloride content of soil by gram of air dry soil left over in the leaching pots at the end of the experiment.

Plant species matrix:

Table 5 below is a decision matrix showing potential plant species for this project and evaluating them based on the four following criteria; the level of safety regarding sowing seeds outside in MN, ease of establishment on roadside soils, phytoremediation potential, and the value of harvested biomass. The matrix values given for each plant species and criteria were estimated based on information in literature and plant guides written by the United States Department of Agriculture's Natural Resources Conservation Service.

Higher values in the matrix mean that the plant species are more desirable with respect to the criteria listed. Total matrix values greater than 150 are highlighted in shades of red with the exception being the values in the lightest shade. These are values that are greater than 140 with no matrix number for the phytoremediation potential and therefore shown as "NA" on the table. There was no available information found in the literature research that would indicate the phytoremediation ability of these species. Due to the promising other attributes of these plant species for this study, it is worthwhile to include these species in the next greenhouse experiment. The species matrix can then be updated according to their salt uptake performance. Plant species with the highest matrix values will ultimately be used in the field experiment.

The plant species that will be tested in the next greenhouse experiment are *Andropogon gerardii* (big bluestem), *Atriplex prostrata* (triangle orache), Bouteloua curtipendula (Side Oats Grama), *Chenopodium berlandieri* (pitseed goosefoot), *Elymus trachycaulus* (slender wheatgrass), *Helianthus annuus* (common sunflower), and *Salicornia europaea* (common glasswort). *A. prostrata, C. berlandi*, and *H. annuus* have been recognized as promising candidates from earlier in the plant species selection process and have been written about in the previous updates.

A. gerardii, known by the common name big bluestem, is a perennial warm season grass native to MN. It grows on average to dry soil on plains, prairies, railroads, and roadsides. It has been mixed with other native prairie species for prairie and highway revegetation. Big bluestem is also one of the most palatable warm season grasses with crude protein content of 16-18% maintained from May through August (Wennerberg, 2004). The grass has rhizomes that are typically 1 to 2 inches below the ground and its main roots can extend 10 feet downward which is why it has been used for erosion control. Since the seedling vigor is weak compared to weeds and cool season grasses, control of competition is likely necessary for it to establish successfully. It is rated as being moderately tolerant of saline soil.

Another native to MN perennial warm season grass species *B. curtipendula*, called Side oats grama, produces high quality, nutritious forage that remains somewhat palatable into the winter months making it one of the most importance range grasses (Wynia, 2017). Similar to big bluestem it is also used as erosion control due to its deep roots. Side oats grama can establish quickly and be utilized for forage the second year after planting. The grass is moderately salt tolerant but has also been found to accumulate high levels of sodium and chloride in its shoots (Marcum, 2008).

E. trachycaulus or slender wheatgrass, is a native short-lived perennial grass. Due to its fast growth and quick establishment, it is commonly used as a filler in seed mixtures that container slower growing long-lived perennials. Slender wheatgrass has also been used for erosion control and revegetation of roadsides. This grass is both palatable and nutritious as forage with crude protein percentages ranging from 22 to 25 in the spring and drops below 10% in late summer to fall (Tilley et al., 2006). It is tolerant of saline soils ranging from 10 to 20 mmhos/cm.

The succulent annual herb *S. europaea*, commonly known as common glasswort or glasswort, is similar to the halophyte *S. rubra* which has been found on salted roadsides in MN. However, being that there is no source for *S rubra* seeds other than finding them in the wild, common glasswort was seen as another potential option. Common glasswort has been widely studied for its phytoremediation potential and its use as biodiesel once harvested because of its high oil content that can reach 30% (Abideen et al., 2015). It also has potential for use as forage (Gunning, 2016). The salt tolerance of glasswort is incredibly high and when irrigated with 2.5% saltwater, the NaCl content of its dry biomass can reach 25% (Kieffer and Ungar, 1997).

Table 5: Plant species decision matrix

	Level of safety regarding sowing seeds outside in MN	**Ease of establishmen t on roadside**	**Phytoreme-diation potential**	**Value of harvested biomass**	
Criteria Weight	**5**	**5**	**10**	**8**	**Total Matrix Value**
Andropogon gerardii (big bluestem)	10	7	NA	7.5	145
Atriplex prostrata (triangle orache)	10	5.5	8	4	189.5
Bouteloua curtipendula (Side Oats Grama)	10	6.5	NA	8	146.5

Chenopodium album (lambsquarters)	3	7	6.5	3	139
Chenopodium berlandieri (pitseed goosefoot)	10	7	5	3	159
Distichlis spicata (saltgrass)	9	7	1	5.5	134
Elymus trachycaulus (slender wheatgrass)	10	7.5	NA	7	143.5
Helianthus annuus (common sunflower)	10	8	3	6.5	172
Hordeum jubatum (foxtail barley)	7	7.5	4	3	136.5
Panicum virgatum (switchgrass)	10	7	0.5	7.5	150
Phragmites australis (common reed)	-5	3	9	7	136
Portulaca oleracea (common purslane)	-2	9	7	4	137
Puccinellia nuttalliana (Nutall's alkaligrass)	10	4	3	4	132
Rhus glabra (smooth sumac)	8.5	8	2	3	126.5
Salicornia europaea (common glasswort)	10	1	5.5	5.5	154
Spartina pectinata (prairie cordgrass)	10	4	1	6.5	132
Typha latifolia (broadleaf cattail)	-5	2	10	6	133

Design of next (3rd) greenhouse experiment:

In the next greenhouse experiment before the field study, the 8 plant species with the highest decision matrix values will be evaluated. The experimental design will be similar to that of the non-leaching experiment with a few changes. The salinity treatments will be based on the salt content of actual roadside soils in the Twin Cities metro area. Therefore, three different salt levels will be used. There will be the control condition with no salt added, a 15mM NaCl treatment similar to that of literature values, and a higher salt treatment of 50mM NaCl. Four replicates for each species and treatment will mean that the total number of pots will equal 96. After a few weeks of growth with weekly half strength solution, the plant will be irrigated with the saline treatment. In order to obtain some results before the start of the field study, samples of aboveground biomass will be removed the plants after around 4 to 6 weeks from the start of the salt irrigations and tested for salt content to determine which species are most efficiently taking up salt. This will provide more information along with the species matrix into which species will ultimately be used for the field experiment. The greenhouse experiment will continue for two months of total growth under the varying saline conditions after which they will be harvested and analyzed.

Fourth Update September 1, 2021

A third greenhouse experiment was conducted during the months of March through May earlier this year. The experiment was indented to provide results that could determine which species to use in the field trial. The experiment was planned to include all 8 of the highest performing plant species from the decision matrix (shaded in red in the matrix). Although, because the start of the field trial was planned for May, the species that were growing too slowly or did not germinate were not included in this initial greenhouse study. The following 6 species were included: *Andropogon gerardii* (big bluestem), *Bouteloua curtipendula* (Side Oats Grama), *Chenopodium berlandieri* (pitseed goosefoot), *Elymus trachycaulus* (slender wheatgrass), and *Beta vulgaris* (sugar beet). Sugar beet was included because of its strong performance in the previous greenhouse experiments and it was also added to the updated matrix shown below. The high scoring matrix species that were not included in this greenhouse study were: *Atriplex prost*rata (triangle orache), *Helianthus annuus* (common sunflower), and *Salicornia europaea* (common glasswort). The only known available source of triangle orache seeds that we purchased did not germinate. The common glasswort seedlings grew very slowly and so it had a much lower amount of biomass compared to the other species when the experiment was ready to start. The first set of sunflower seeds that were purchased did not germinate (a second set of seeds that has high germination rates will be used for the following experiments).

Experimental Design: The plant seeds of the 6 species mentioned above were germinated in seedling trays with germinating mix soil. When the seedlings were large enough to be transferred, they were carefully moved to their own individual 4-inch diameter pots filled with 75 g of fresh general mix soil. These pots contain drainage holes on the bottom, and each sat on a dish to catch the liquid that drains out of the holes. Pictures of the plants growing in the greenhouse are shown in Figures 27-31. Plants grew for a total of 4 to 8 weeks, depending on their growth rate, before the start of the saltwater irrigations.

Figure 27: Big bluestem   Figure 28: Side-oats grama

Figure 29: Slender wheatgrass   Figure 30: Pitseed goosefoot   Figure 31: Sugar beet

Three different salt levels were used: control (no added salt), 8 mM NaCl, and 24 mM NaCl. The pots were irrigated weekly with 100 mL of water containing one quarter strength Hoagland solution and their respective salt concentration. After 4 saline irrigations of 100 mL per pot, the total amount of salt added for the 8 and 24 mM NaCl salt treatments will roughly equal 3.6 and 10.7 mg NaCl/g air dry soil. The 3.6 mg NaCl/g is a rough average of the salt concentrations one meter from the road measured by Biesboer & Jacobson, (1994) in the month of May (5.1 mg NaCl/g) and during the growing season from May to September (2.0 mg NaCl/g). Salt addition in the high salt treatment is nearly equal to the highest measured value during the month of May (11.8 mg NaCl/g). The plants were harvested two weeks after the final salt irrigation. Soil and plant tissue analysis was performed following the same methods describes in the first update for this section.

Results and Discussion: Data analysis of the plant tissues from this new greenhouse experiment with 4-inch pots is shown in Figures 32-35. The salt content of the soil in each pot has not yet been measured but will be completed in the near future. Also, the salt content of the plant biomass shown in Figures 34 and 35 do not include sodium. This was due to the ion chromatography instrument not functioning properly for the cation measurement. The graphs for plant tissue sodium content and the salt content of the soil will be added when it becomes available.

The measured oven-dry biomass for each plant species is shown in Figures 32 and 33 below. When looking at each individual plant species, there was no significant difference found between treatments. This is an indication that each of these species are tolerant of the salt levels that were used. Also, it shows that the difference in salt levels used did not have nearly as high an impact on plant growth as the levels used in the previous leaching and non-leaching pot greenhouse experiments (0, 50, 100, and 200 mM NaCl). Although, the results do show a significant difference between the plant biomass of pitseed goosefoot and the other species. The pitseed goosefoot had significantly higher aboveground biomass than the other plants, other than side-oats grama, which had high error bars showing a lack of consistent growth observed. However, for the belowground root growth, the pitseed goosefoot had significantly lower biomass than both sugar beet and slender wheatgrass. Big bluestem and side-oats grama also seemed to have more root biomass than pitseed goosefoot, although statistical significance was not observed.

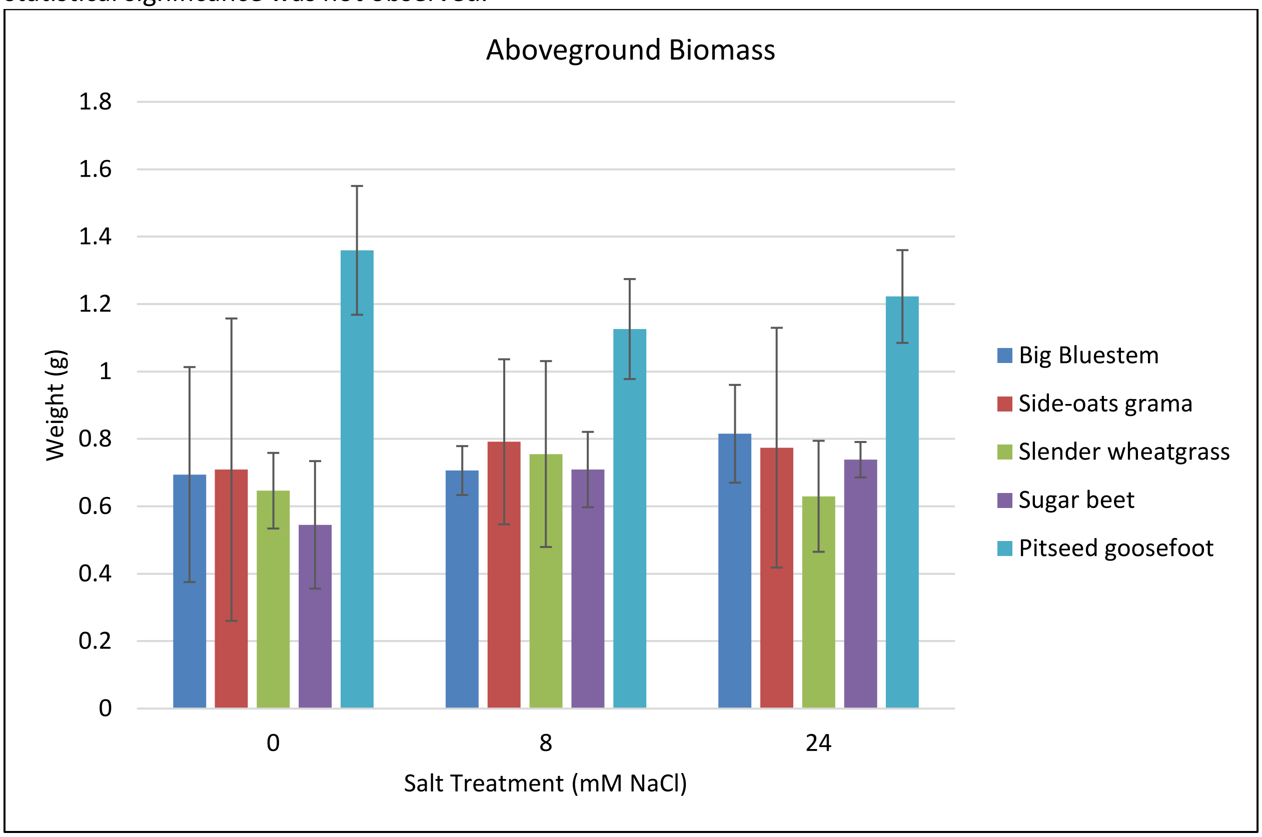


Figure 32: The amount of oven-dry aboveground plant biomass of each species grown in the 4-inch diameter pots and irrigated with increasing levels of salinity.

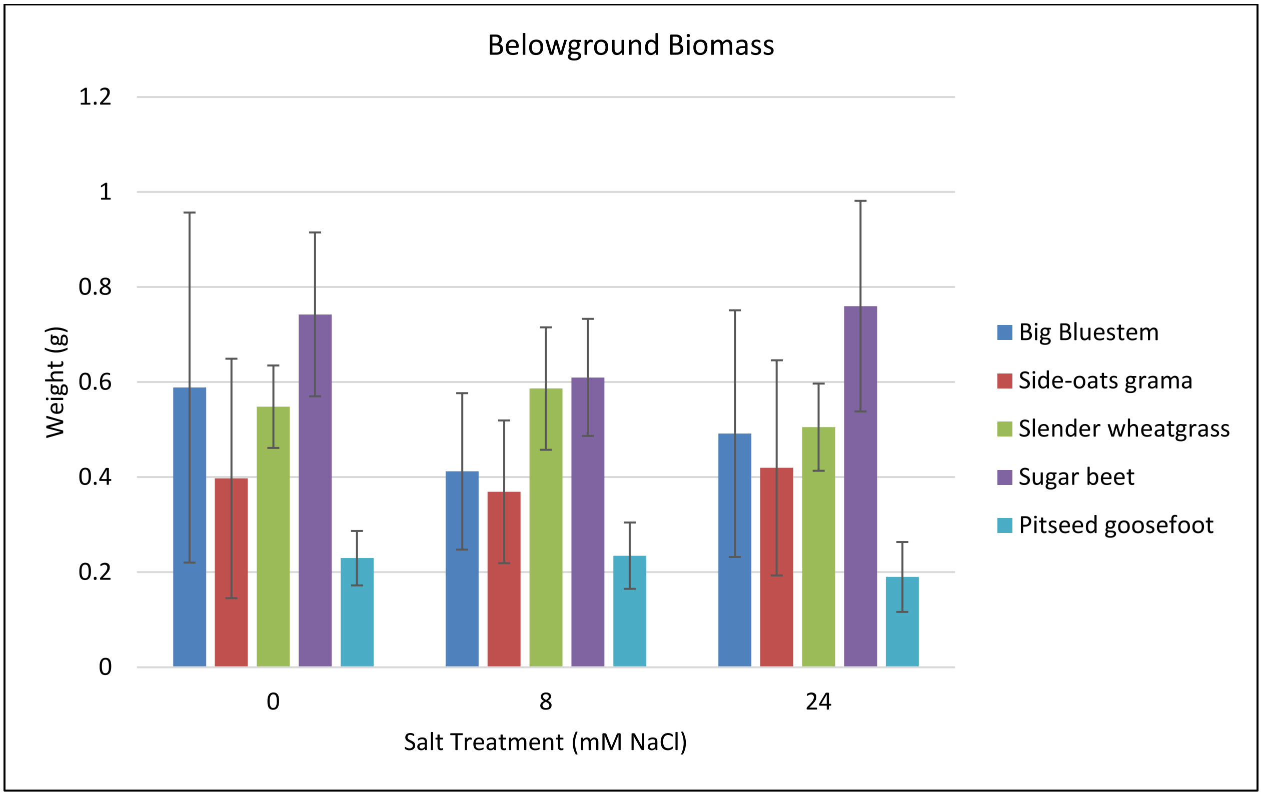


Figure 33: The oven-dry belowground plant biomass of each species grown in the 4-inch pots and irrigated with increasing levels of salinity.

In Figures 34 and 35, the chloride content is shown both in regards to the mg Cl/g DW (Figure 34) and the total mg of chloride taken up into the aboveground biomass (Figure 35). No statistical significance was shown between salt treatments for the grass species (big bluestem, side-oats grama, and slender wheatgrass). Also, the chloride content for the grass species was mostly in a range lower than that of sugar beet and pitseed goosefoot for both the 8 and 24 mM salt treatments. The sugar beet Cl content per g of dry biomass was significantly higher than all of the other species, including pitseed goosefoot. However, since pitseed goosefoot had a higher amount of aboveground biomass, the total chloride content per plant was about equal to the sugar beets (Figure 35).

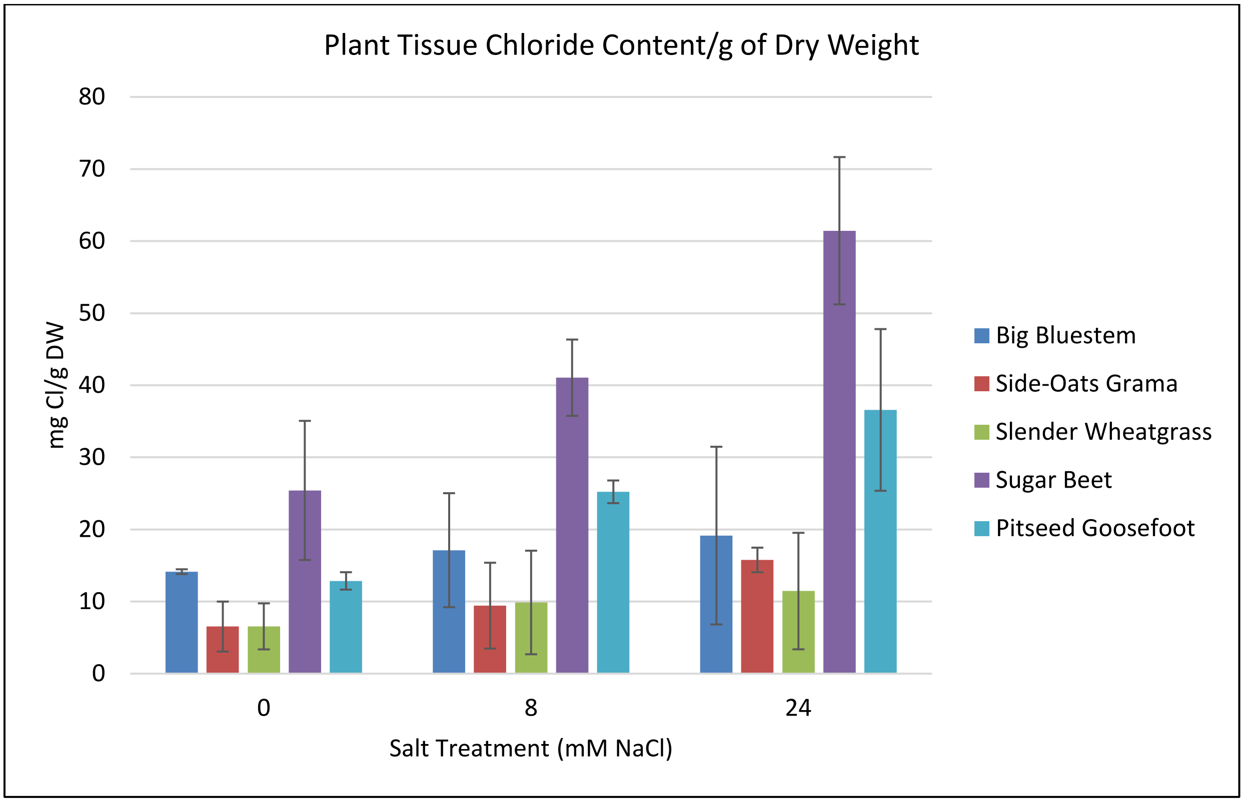


Figure 34: The Cl content per g of dry plant tissue in the aboveground biomass of the 4-inch pot experiment.

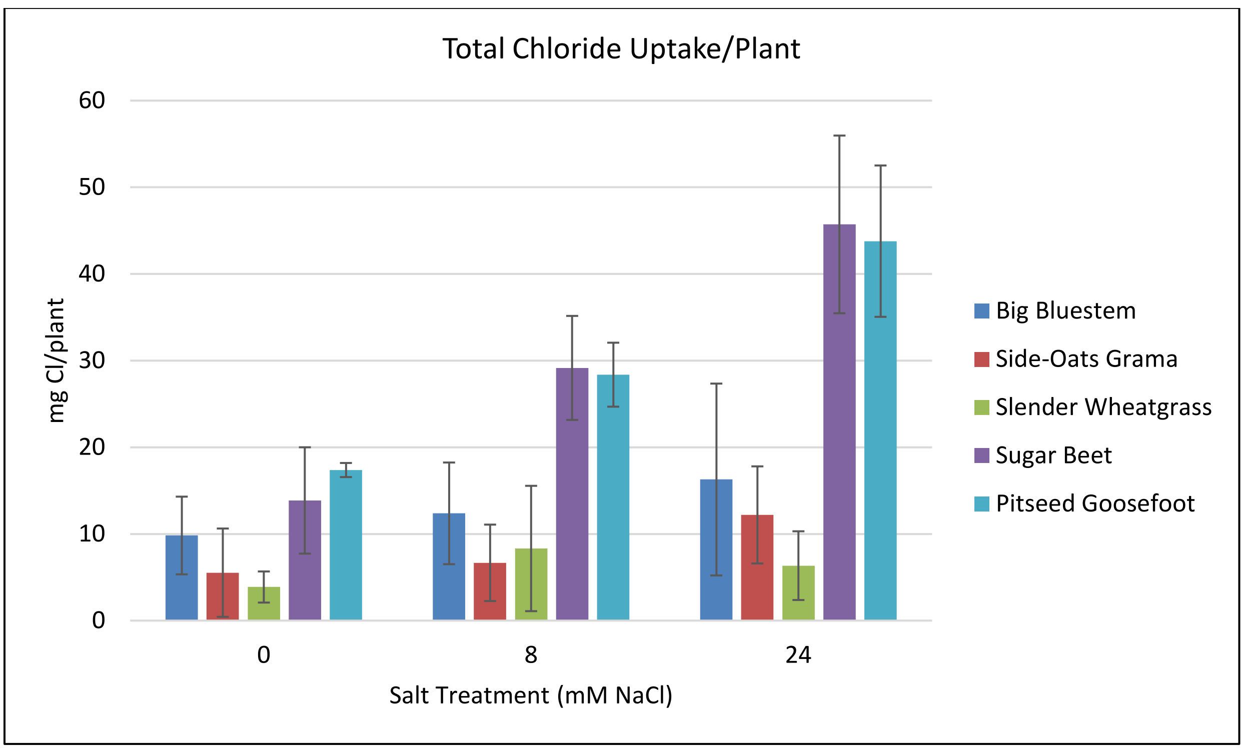


Figure 35: The total Cl content in the aboveground biomass of each plant species grown in the 4-inch pots.

The experimental data above was used to update some of the scores of the plant species decision matrix. The updated decision matrix is below (Table 6). The phytoremediation ability matrix scores were updated for the grass species. Also, *A. prostrata* (triangle orache) was removed from the matrix because the seeds from the only found source did not germinate.

Table 6: Updated Plant Species Decision Matrix:

	Level of safety regarding sowing seeds outside in MN	**Ease of establishment on roadside**	**Phytoremediation ability**	**Value of harvested biomass**	
Criteria Weight	**5**	**5**	**10**	**8**	**Total Matrix Value**
Andropogon gerardii (big bluestem)	10	6	1.5	7	151
Beta vulgaris (sugar beet)	10	1	8.5	7	196
Bouteloua curtipendula (Side Oats Grama)	10	5.5	1	7	143.5
Buchloe dactyloides (Buffalo grass)	9	7	1	6	138
Chenopodium berlandieri (pitseed goosefoot)	9	5	5	4	152
Distichlis spicata (saltgrass)	9	5	1	5.5	124
Elymus trachycaulus (slender wheatgrass)	10	8	1	7	159
Festuca rubra ssp. Littoralis (slender creeping red fescue)	9	7.5	1	4	124.5
Helianthus annuus (common sunflower)	10	6	3	6.5	162
Hordeum jubatum (foxtail barley)	7	7.5	4	3	136.5
Panicum virgatum (switchgrass)	8.5	7	0.5	7.5	142.5
Pascopyrum smithii (western wheatgrass)	10	6.5	NA	6.5	134.5

Puccinellia nuttalliana (Nutall's alkaligrass)	10	3	3	4	127
Rhus glabra (smooth sumac)	8.5	8	2	3	126.5
Salicornia europaea (common glasswort)	10	1	5.5	5.5	154
Spartina pectinata (prairie cordgrass)	10	4	1	6.5	132
Thinopyrum ponticum (Tall wheatgrass)	9	6.5	2	6	145.5

Next (4^th^) Greenhouse Experiment Design: Another experiment will soon be performed in the greenhouse. This next experiment will include the best performing species from the 4-inch pot experiment: *Chenopodium berlandieri* (pitseed goosefoot), and *Beta vulgaris* (sugar beet) and also common glasswort and sunflower. Without the rush of trying to get results before a field experiment, there will be plenty of time to germinate and grow the glasswort plants to be around the same size as the others. Also, new sunflower seeds with a high germination rate were purchased for the new experiment.

The 4th greenhouse experiment will also use higher salt treatments than the 8 and 24 mM NaCl used in the 4-inch diameter pot experiment. It was found that the MnROAD salted roadside had salt contents much lower than was expected (Table 7). Also, a significant amount of weedy growth was intruding on the plots at our field site even after an addition of salt to levels around Biesboer & Jacobson, (1994). This has led us to believe that these saline conditions are not high enough. It makes the most sense for us to have our plant species grow in areas with high enough salinity where most other species will not survive so that maintenance of the sites would be minimal.

Therefore we will switch our focus from replicating the salt content results found in Biesboer & Jacobson, (1994) and will instead use higher salt treatments such as 50 and 100 mM NaCl for the next greenhouse experiment. These higher levels may be closer to extremely salt contaminated roadsides where growth of most species, especially non-salt tolerant species, is greatly reduced.

Fifth Update March 1, 2022

The 4th greenhouse experiment was performed with the following plant species: big bluestem (*Andropogon gerardii*), sugar beet (*Beta vulgaris*), pitseed goosefoot (*Chenopodium berlandieri*), and common sunflower (*Helianthus annuus*). Each pot was watered weekly with 100 mL of nutrient water containing one of three salt treatments (0, 50, and 100 mM NaCl) for a total of four weeks. The results from this experiment are shown below.

In this greenhouse experiment, the aboveground biomass growth was highest for both pitseed goosefoot and common sunflower, followed by sugar beet and then big bluestem had the lowest aboveground dry biomass (Figure 36). Sugar beet and pitseed goosefoot were not significantly impacted by the increasing salt concentration, while big bluestem showed lower aboveground biomass for the highest salt treatment and common sunflower showed a decreasing trend with the 50 and 100 mM salt treatments. The belowground biomass results similarly showed that sunflower and big bluestem were more significantly impacted by the increase in salt than the other two species (Figure 37).

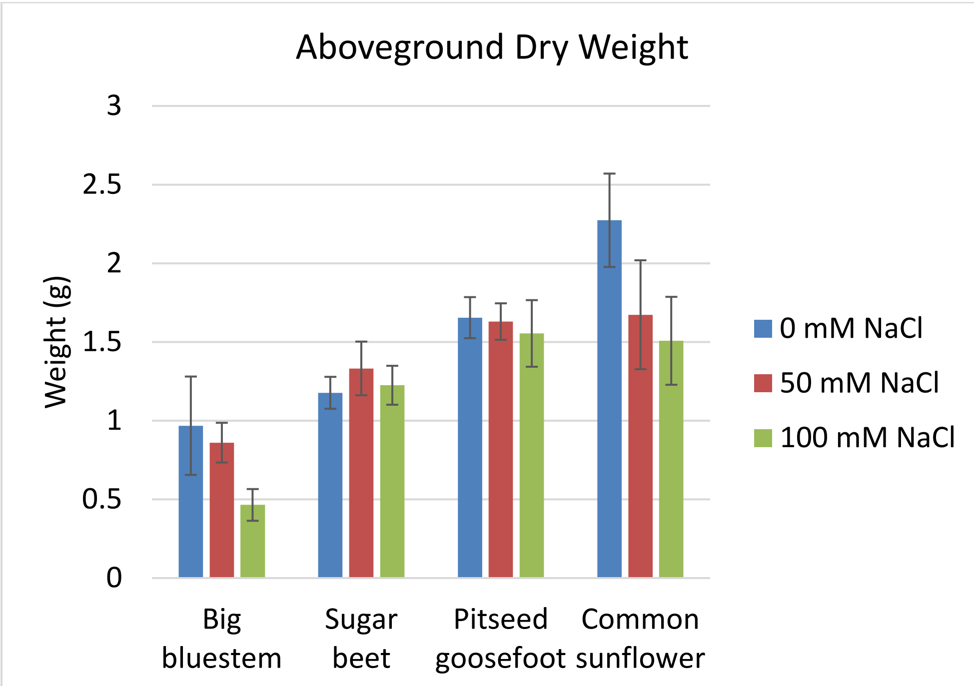


Figure 36: The aboveground dry biomass (g) of each plant species at 3 different salt concentration (0, 50, and 100 mM NaCl).

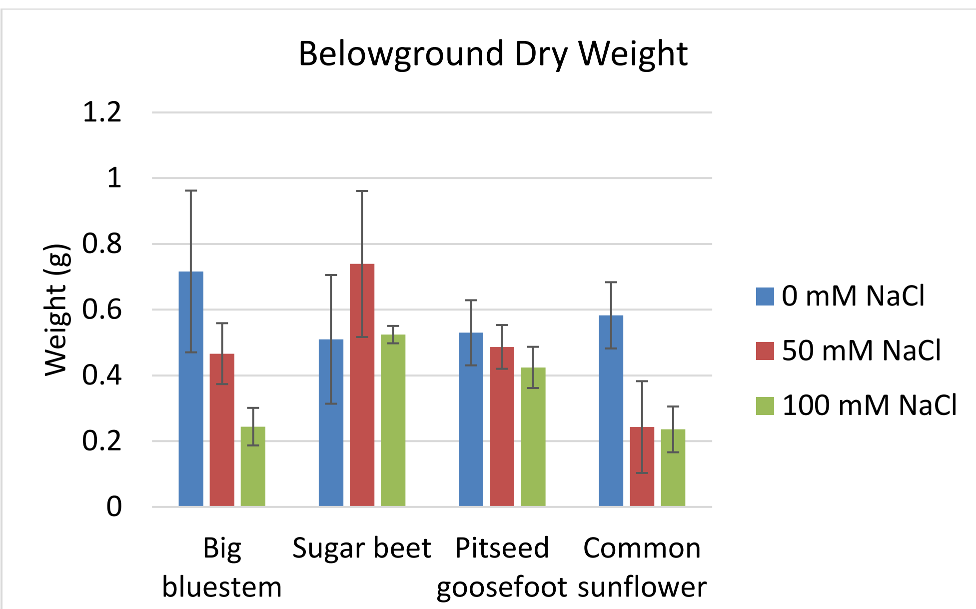


Figure 37: The belowground dry biomass (g) of each plant species at 3 different salt concentration (0, 50, and 100 mM NaCl)

The sodium uptake per gram of dry biomass was highest for sugar beet followed by common sunflower, then pitseed goosefoot, and finally big bluestem with the lowest (Figure 38). Chloride concentrations in aboveground biomass followed a similar trend, except that sugar beet chloride content was not significantly higher than common sunflower (Figure 39). The salt concentrations of sugar beet are consistent with those obtained in greenhouse experiments 1 and 2.

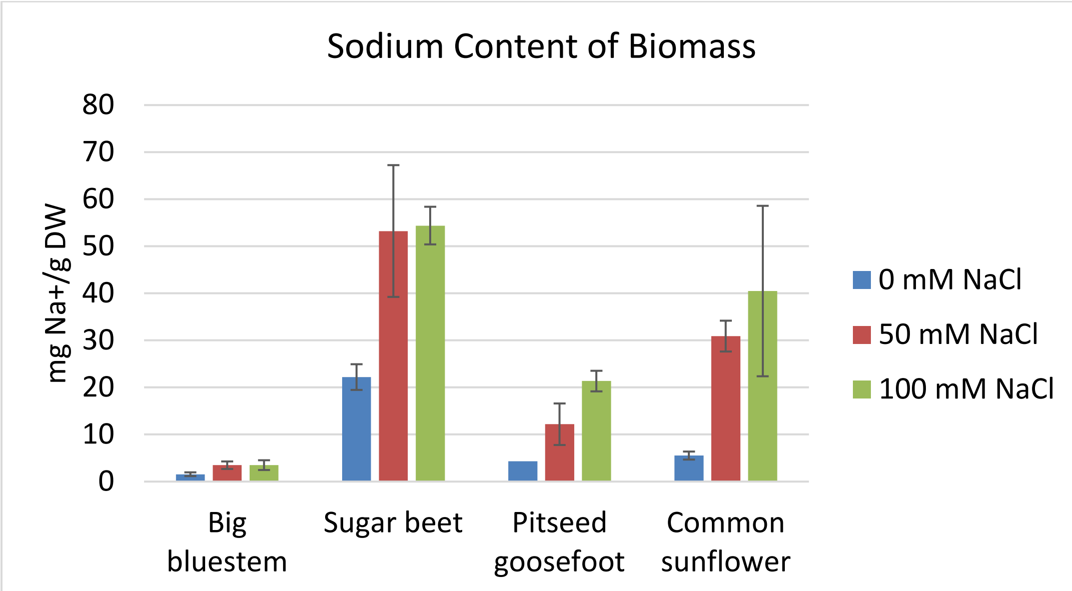


Figure 38: Sodium content of the aboveground biomass per gram of dry weight of each species grown at the three different salt treatments.

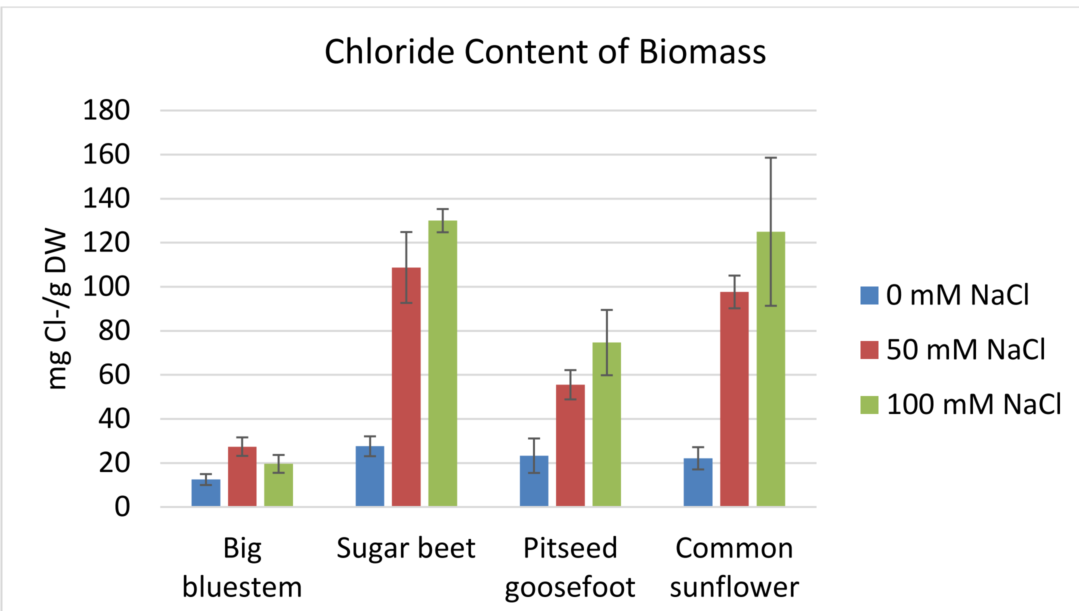


Figure 39: Chloride content of the aboveground biomass per gram of dry weight of each species grown at the three different salt treatments.

The total salt uptake per plant was calculated by multiplying the total dry weight of each plant by the salt concentration per dry gram. Sugar beet and common sunflower showed the highest total sodium uptake (Figure 40). However, common sunflower had significantly higher chloride uptake per plant (Figure 41). Pitseed goosefoot took up salt at quantities in between big bluestem and sugar beet.

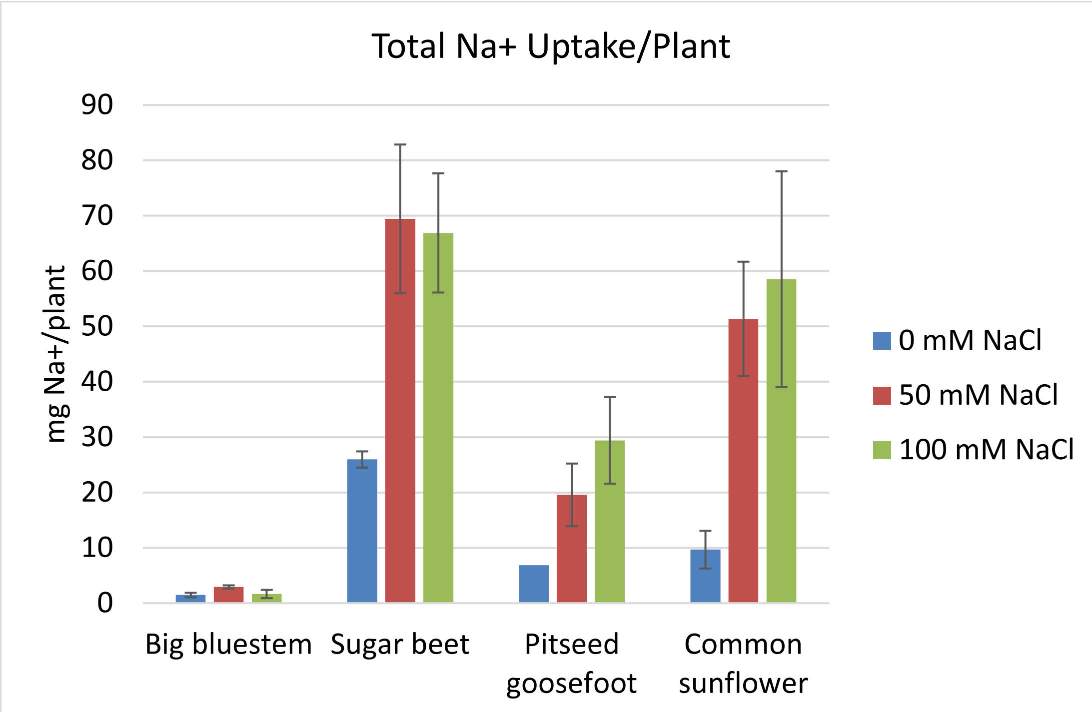


Figure 40: Total sodium uptake per plant, calculated based on the salt concentration of the biomass and the aboveground dry weight.

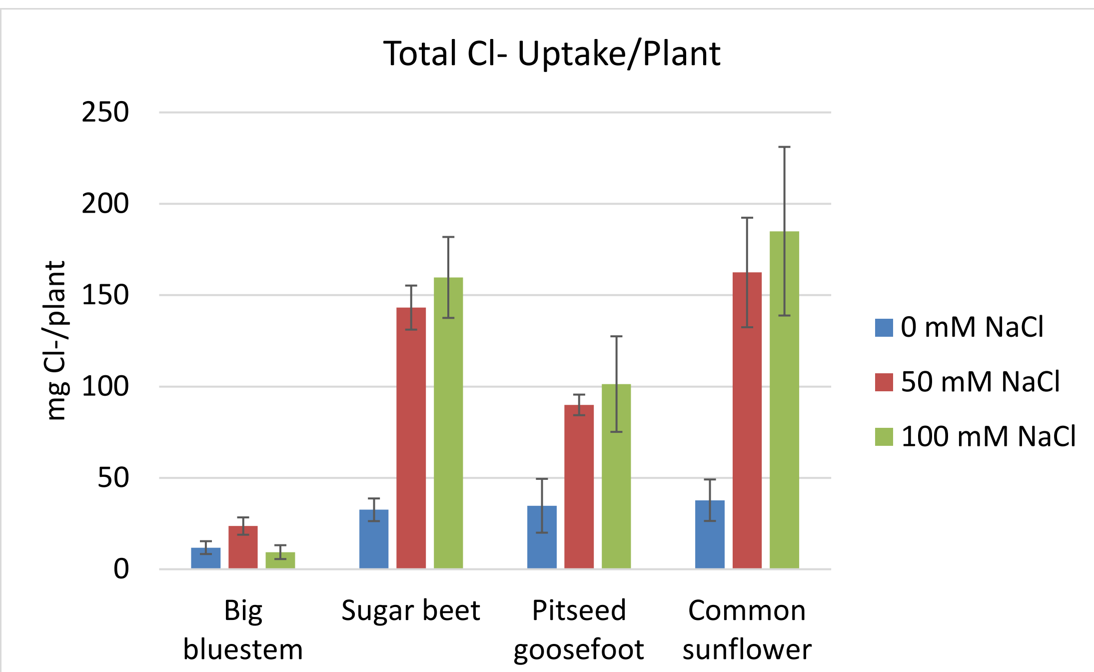


Figure 41: Total chloride uptake per plant, calculated based on the salt concentration of the biomass and the aboveground dry weight.

Figures 42 and 43 show the sodium and chloride content per gram measured in the soil. While sugar beet and common sunflower took up significantly more salt into the aboveground biomass, the soil salt content results show a trend of higher salt content in the pots with common sunflower and sugar beet. Although, due to the high error bars, there was not much significance between plant species in the same salt treatment.

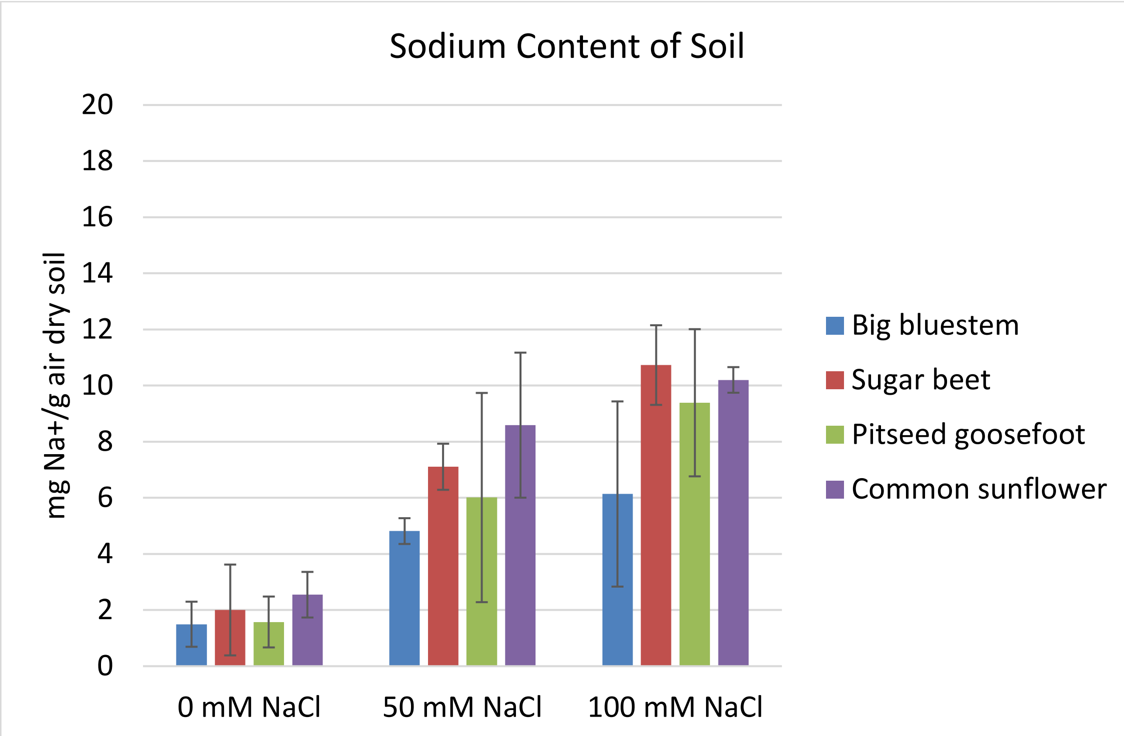


Figure 42: Sodium content of the soil per gram of dry weight.

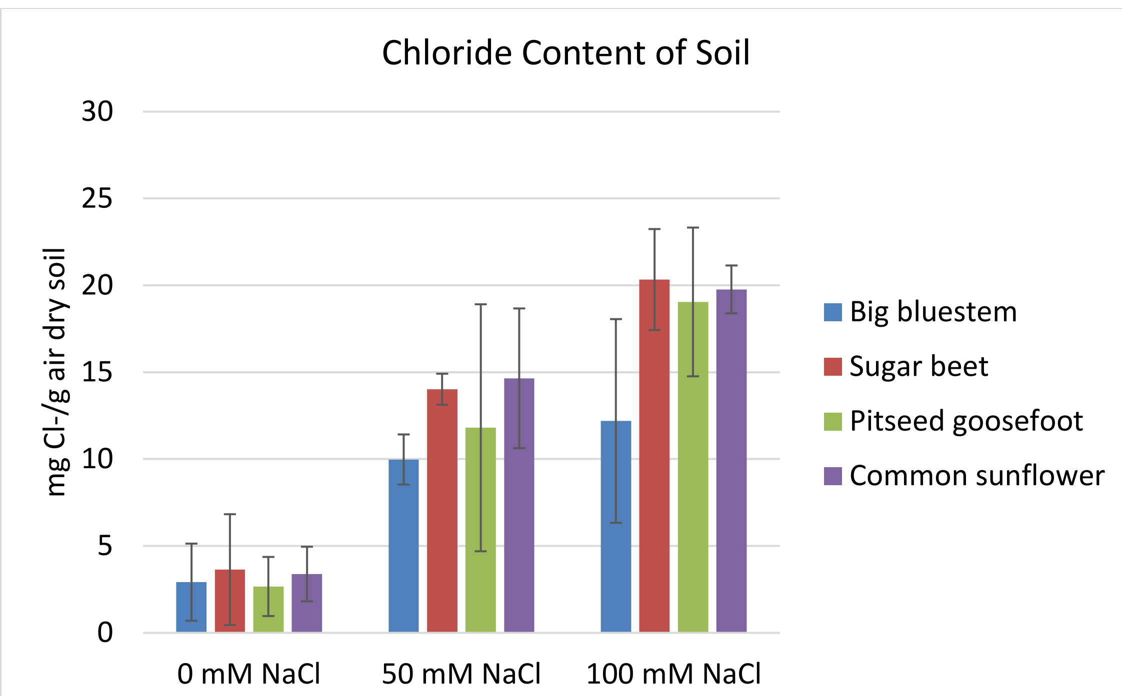


Figure 43: Chloride content of the soil per gram of dry weight.

Table 7 shows the theoretical salt reduction through aboveground harvest based on the amount of salt added to the pots and the total salt uptake per plant. Harvesting the aboveground biomass of sugar beet and common sunflower results in the highest salt reduction for both the 50 mM and 100 mM NaCl treatments. Sugar beet and sunflower can reduce the salt content of the 50 mM NaCl treatment soil by 17.77 and 17.87% respectively. Therefore, it would theoretically take roughly 6 harvests to fully remediate the salt from this soil.

Table 7: Values based on the amount of salt added to the pots and the total salt uptake per plant.

% Salt reduction from harvesting aboveground biomass				
Salt Treatment	**Big bluestem**	**Sugar beet**	**Pitseed goosefoot**	**Common sunflower**
0 mM NaCl	23.13	101.73	72.37	96.02
50 mM NaCl	2.22	17.77	9.15	17.87
100 mM NaCl	0.45	9.26	5.35	9.96

Final Report between project end (June 30) and August 15, 2022

Nothing to update

Final Report Summary

In this project, an inventory of native MN salt tolerant plant species was created to provide a list of potential plant species for remediating deicing salt from roadside soils. This process began with a screening of salt tolerant plants that selected for species with multiple beneficial characteristics in line with the research objective. Then, a decision matrix was created to determine the most favorable remaining species in a somewhat quantitative manner. The plant species with the highest matrix scores were used in the subsequent greenhouse and field studies. Multiple greenhouse experiments were performed to obtain further insight into the phytoremediation capabilities of some common native MN plants and highest ranking species from the matrix. Ultimately, these greenhouse experiments confirmed that common sunflower, pitseed goosefoot, and sugar beet have high potential for phytoremediation of road salt and were used in the field studies in objective two.

ACTIVITY 2 Title: Plant growth test on salt-affected soil

Description:

We will consult with MNDOT to identify a slot of area for some pilot plant growing tests. The selected specimen from the lab tests will be planted in the spring on this pilot testing lot and monitored for the entire growing season. Based on the literature, it will take *S. rubra* 2-3 weeks before they grow to the market height and it is expected the plants can keep removing salts when the grass is mowed and collected. We will measure the plant biomass, nitrogen (TN), phosphorus (TP and PO_4-P), and the salt concentration in the shoots, roots, and soil. We will use this information to develop an implementation plan for how this species will be added into current regional seed mixtures for plantation diversity and how to maintain their growth. The plan will also consider effects of this species on the roadside stabilization and safety, a better outcome for NPDES permit compliance for obtaining a uniform, perennial cover, changes to standard specification for construction activities, structural root system enhancement that increase the shear resistance for reducing soil slides, flood overtopping stability, etc.

ACTIVITY 2 ENRTF BUDGET: $ 120,000

Outcome	**Completion Date**
1. Pilot plant growth in a field	*Year 2 – 12/2020*
2. Evaluation and analysis of samples for pilot growth study	*Year 2 – 05/2021*

First Update March 1, 2020

Some preliminary engineering design study was carried out to determine a location that we will grow our plants outside. One feasibility of employing phytoremediation along a strip of the University of Minnesota - Twin Cities Transitway. This site is the ideal location for looking at the phytoremediation effect on soils due to its proximity to the Sarita Wetland, the well-established ground maintenance infrastructure due to the University's Facilities Management, the topographic geometry along the road, the urban geography creating increased initial soil concentration, and the proximity to the University of Minnesota's storage-lot for most of its road deicers. The theoretical design is based on the plantation of the common sunflower and smooth sumac, two common roadside salt absorbing plants. It was determined that the common sunflower plants should be spaced every two feet in the planting zone and will be planted from seeds in May-June. The smooth sumac will be transplanted from young plants in the fall, with 1 plant every 10 feet. Removal of native plants and tillage will not be necessary due to the minimal amount of growth close to the road. Mulch will be used after planting to hold in moisture for the young sumac plants. Fertilizer is optional, so non-fertilizer growth will be attempted for one year and further evaluation will be required. During sunflower germination daily watering is necessary, but afterward the plants only require moderate weekly monitoring because both are fairly drought resistant. Additional maintenance includes mowing around the growth area to prevent unwanted spread. Smooth sumac will be harvested every 3 years and sunflowers will be harvested every year. We are in communication with UMN Landcare to finalize a testing roadside lot and plantation can be started soon.

Second Update September 1, 2020

The lab experiments are interrupted and delayed due to the campus shutdown, and we could not have enough pot plants grown during the spring season for the plantation outside the lab. We will focus more greenhouse studies this year and postpone the full outdoor plantation to next year.

Third Update March 1, 2021
Field study experimental design:

We will begin sowing seeds for the field study in the middle to end of march so that the plants will be big enough to transfer them outdoors around a month later when the ground is no longer frozen for the beginning of the experiment.

There will be three possible different sites for this study. One will be located in Albertville on interstate 94W where it splits into multiple roadways for experimental purposes such as this. A large amount of salt is used on these roads yet there are times when certain roads are blocked off so that roadside activities can be done safely. Another location for a possible site will be along the Campus Connector route on the University of Minnesota campus. This will serve as a lower salt treated road since the University is known to not use a lot of salt on the roadways. Our research team is already in contact with UMN Landcare and we have been told that we are able to use this site for our research. The third site will be on the roadside of Larpenteur Avenue in St. Paul between Cleveland Avenue North and Fairview Avenue North roads. This roadway is salted by the city and is likely to have a significant amount applied to it throughout the winter. We will obtain the approval of MnDOT for use of this site and will work with them to ensure the study performed there is done safely.

Our experimental setup at each site will consist of plots that are 1.5 ft by 3 ft with the shorter length being parallel to the road. The plots will be 1 meter from the road. The experimental plots containing our desired plant species will have the currently growing vegetation removed either by plowing the soil and removal by hand or mowing and using Roundup to kill the weeds. Each experimental plot will be spaced 1.5 feet apart. Three replicate plots will be made for each desired species and one extra plot will be a positive control that will not be touched. All of the plots at each site will be randomized and 5 plants will end up being placed in each experiment plot.

After preparation of the plots, our plant species will be transferred to the plots and watered with fertilizer during the initial establishment phase. At this starting phase of the experiment we will obtain soil samples from each experimental field plot and also the positive and negative control plots. These soil samples will be analyzed for their sodium chloride content using the procedures described in the earlier soil testing section. This will be one of three sampling times throughout the course of the experiment. The second time to obtain soil samples will be in August and the third will be after harvesting the plants in May of next year.

The plots will be checked on about every other week for the first two months to ensure the plants are able to make it through the establishment phase. Monitoring of the sites in the following months will be done about once every 6-8 weeks. After plant harvest, samples of plant tissue aboveground biomass for each plant species will be analyzed for their salt content.

Fourth Update September 1, 2021

The chosen site for the field experiment is the Minnesota Road Research Facility (MnROAD), located along Interstate 94 (I-94) near Albertville, Minnesota. MnROAD is an outdoor research site operated by MnDOT and contains several roadway segments including a 2.5 mile stretch of Low Volume Road (LVR) and a 3.5 mile I-94 Mainline section where traffic can be redirected from the original westbound lane. The LVR loop is only lightly salted during the winter (around 1 or 2 applications) and so will be the site of the control roadside plots. The Mainline stretch is used to simulate real highway traffic and receives the same amount of salt as the original westbound lane, therefore providing a site for the salt treatment plots. There are specific times when the Mainline of I-94 closes, and traffic is switched to the alternate lane. This allows for us to perform roadside research activities safely.

Experimental Design: The chosen plant species were both directly sown onto the roadside soil as seeds and transplanted to the site as 4 to 6-week-old plugs that were germinated in the greenhouse. A design of plots is shown below (Figure 44).

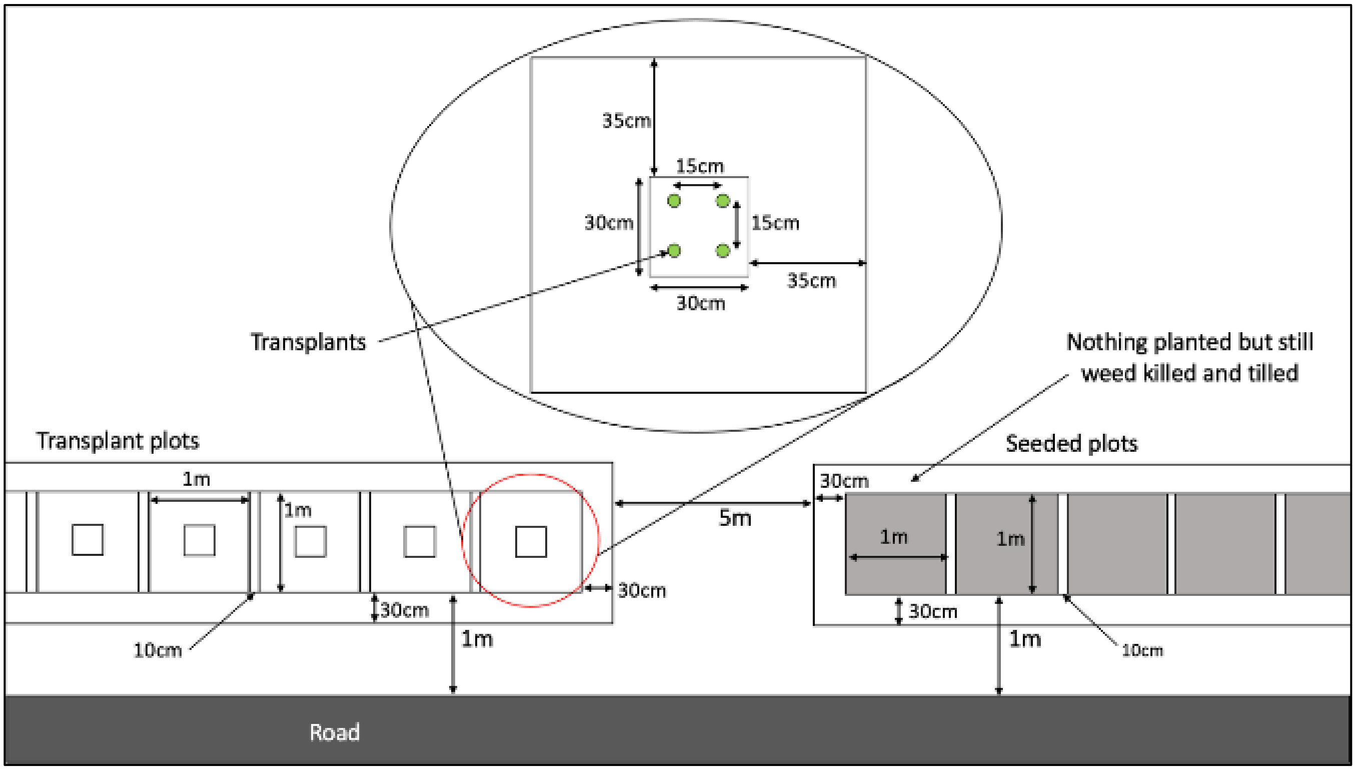


Figure 44: Design of both the seeded and transplant plots for both the LVR and Mainline sites at MnROAD.
For this field experiment, the following species were sown onto the seeded plots: *Andropogon gerardii* (big bluestem), *Atriplex prost*rata (triangle orache), *Beta vulgaris* (sugar beet), *Bouteloua curtipendula* (Side Oats Grama), *Chenopodium berlandieri* (pitseed goosefoot), *Elymus trachycaulus* (slender wheatgrass), *Helianthus annuus* (common sunflower), and *Salicornia europaea* (common glasswort). The same species were grown in the greenhouse and transplanted except for *Atriplex prost*rata (triangle orache), which did not germinate in the greenhouse.

The plants have been growing there since May and we are planning to harvest them in the month of September. The experimental design is as described above in the previous update, except the size of the plots was changed to one square meter and seeded plots were added. Thus, each site has two sections, one that was seeded with our species and the other being the transplants that were initially germinated and grown for a few weeks in the greenhouse.

Figures 45 and 46: Plot preparation included tilling, marking the plots, and adding mulch on top.

Initial Results and Discussion: Soil samples were taken at the beginning of the experiment (May 5th) and initial salt analysis showed that the salt content of both the Mainline plots was much lower than expected (Table 8). Therefore, we added salt to each Mainline plot, excluding three plots that will be non-salted controls. The amount of salt applied was aimed at bringing the soil salinity to around 3.5 mg NaCl/g of soil.

Table 8: Initial salt content of analysis of a few samples from the LVR and Mainline plots.

Initial Soil Salt Analysis	LVR	Mainline
mg Na and Cl/g air dry soil	0.0835	0.295

Some photos of the seeded plots from the field site are show below in Figures 47-54. Currently, the best performing species is sunflower (Figure 47). There was some germination from the pitseed goosefoot and sugar beets but their growth was not as significant as the sunflower plants (Figures 83 and 84). None of the grass species seemed to have germinated in the seeded plots, although the invading grass species made it difficult to concretely determine if our grass species did germinate (Figures 50-54). Similarly, the glasswort and triangle orache plots do not show any sign of germination from the seeds that were sown (Figures 88 and 89). All of the existing vegetation in these plots are most likely not our species.

Figure 47: A photo of a sunflower seeded plot located at the Mainline site. Photo taken on 8/17/21.

Figure 48: A photo of a sugar beet seeded plot located at the LVR site. Small sugar beet plants are circled in black. Photo taken on 6/24/21.

Figure 49: Photo of a pitseed goosefoot seeded plot located at the LVR site. A couple small pitseed goosefoot plants are circled in black. Photo taken on 6/24/21



Figures 50-52. Photos of big bluestem (Figure 50), side-oats grama (Figure 51), and slender wheatgrass (Figure 52) seeded plots located at the Mainline site. None of our sown grasses can be distinctly seen. The plants seen growing in the plots are likely not our species. Photos taken on 8/17/21.


Figures 53 and 54: Photos of glasswort (Figure 53) and triangle orache (Figure 54) seeded plots located at the Mainline site. Neither triangle orache or side-oats grama can be distinctly seen in the photos. The plants seen growing in the plots are most likely not our species. Photos taken on 8/17/21.

So far for the transplant plots, sunflower appears to be the best performing due to its tall height (Figure 55). Pitseed goosefoot, sugar beet, slender wheatgrass, and big bluestem also seem to be growing well (Figures 56-59). Some of the side-oats grama plants have died and all of the glasswort plants are dried out (Figures 60 and 61).

Figures 55-57: Photos of sunflower (Figure 55), pitseed goosefoot (Figure 56), and sugar beets (Figure 57) from the LVR plots. Photos taken on 6/24/21.

Figures 58 and 59: Photos of big bluestem (Figure 58) and slender wheatgrass (Figure 59) from the LVR plots. Photos taken on 6/24/21.

Figures 60 and 61: Photos of side-oats grama (Figure 60) and glasswort (Figure 61) from the LVR plots. Photos taken on 6/24/21.

As was discussion in the fourth update in the previous section, there has been a lot of growth of other plants, especially at the mainline plots. This significant intrusive growth was still observed weeks after the salt addition mentioned above. Thus, salt was again applied, this time roughly four times the amount that was applied before. The intention of this significant salt application is to create conditions that only our salt-tolerant plant species will be able to endure. If the growth of other species remains strong, we can take samples of their biomass and test it for salt uptake as well.

The plants will be harvested in September and the aboveground biomass will be measured along with its salt content. Three soil samples will also be taken from each plot and analyzed for sodium chloride. These results will be compared to the soil samples obtained at the beginning of the experiment.

Fifth Update March 1, 2022

The quantitative results from the 2021 MnROAD field experiment are shown below. On the seeded plots, the plant species with the highest biomass was common sunflower (Figure 62). The sunflower showed significantly higher biomass on the LVR (control) roadside compared to the Mainline (salted) roadside. Pitseed goosefoot was the only other species with significant germination and growth. Sugar beet had very low germination and survival while there was none seen from the grass species.

The biomass results from the transplant plots shows that there is good survival and growth from the sunflower and pitseed goosefoot (Figure 63). Also, the grass species and sugar beet had higher growth and survival than on the seeded plots (Figure 64). Most species demonstrated higher or similar growth and survival on the LVR versus the Mainline. The exception to this being side-oats grama, which shows a possible trend towards higher growth on the Mainline. Big bluestem had the highest biomass of the grass species on the LVR.

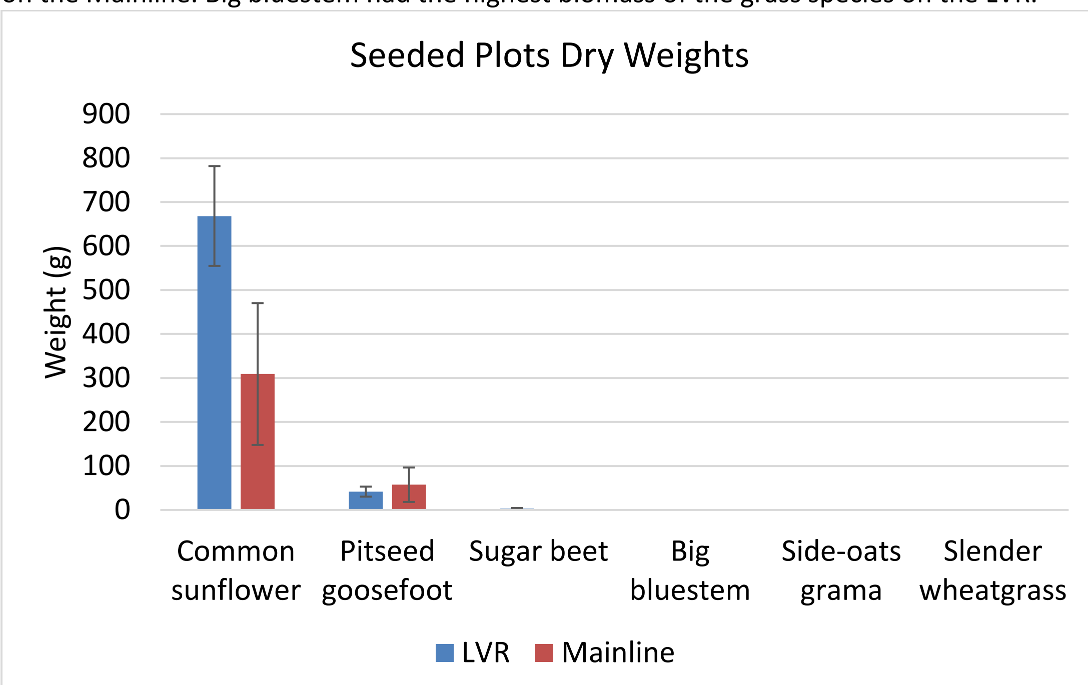


Figure 62: Dry weight of aboveground biomass harvested from the seeded plots.

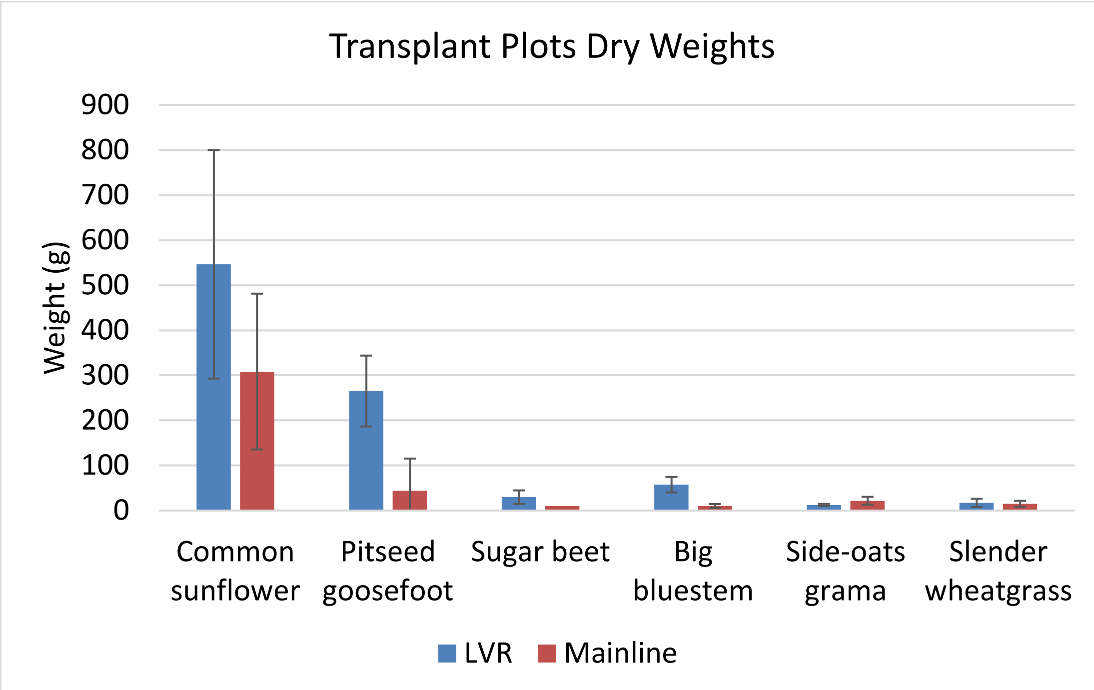


Figure 63: Dry weight of aboveground biomass harvested from the transplant plots (all plant species).

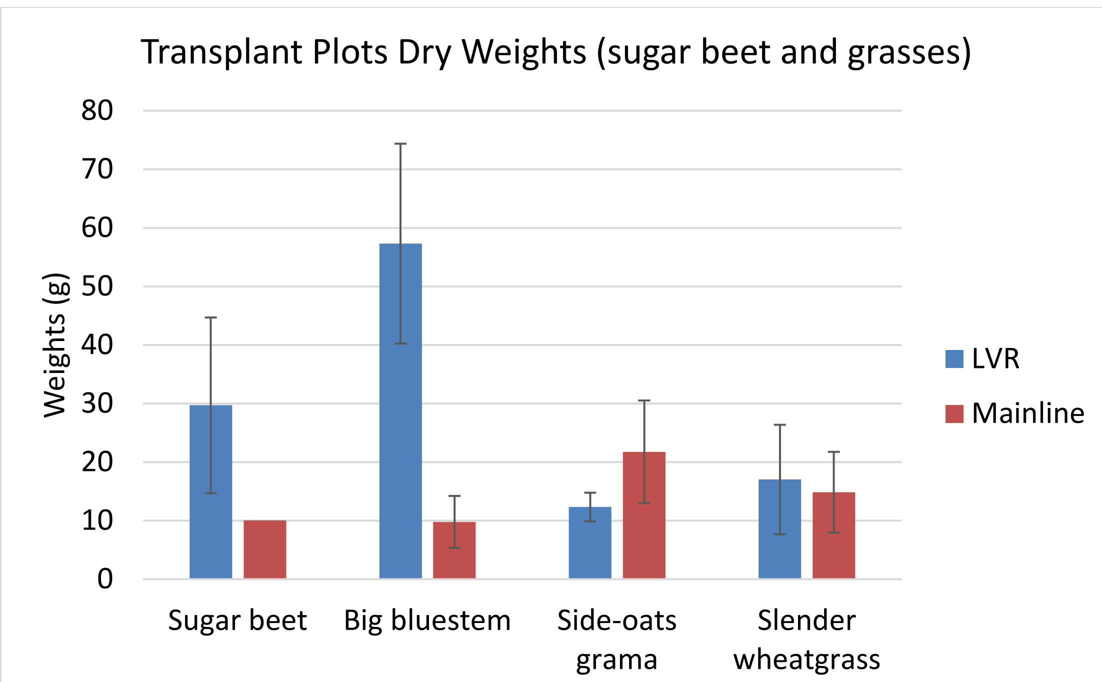


Figure 64: Dry weight of aboveground biomass harvested from the transplant plots (just sugar beet, big bluestem, side-oats grama, and slender wheatgrass).

Figures 65 and 66 show the sodium and chloride concentrations in the aboveground biomass of the seeded plots. There are no results for the grass species because no biomass of these species were distinguished. The sodium concentration in the plant biomass was highest for sunflower grown at the Mainline site (Figure 65). Sodium concentration in aboveground biomass was significantly lower for sunflower and pitseed goosefoot on the LVR compared to the Mainline. Sugar beet sodium content seemed to not have any significant difference between the two sites. Sunflower, pitseed goosefoot, and sugar beet all appear to have a higher chloride concentration at the Mainline site compared to the control (LVR). Also, pitseed goosefoot and sugar beet show chloride concentrations much closer to that of sunflower on the Mainline.

Figures 67 and 68 show the sodium and chloride concentration in the aboveground biomass of the transplant plots. It can be clearly seen that sugar beet took up the highest concentration of sodium ions into its leafy biomass, especially at the Mainline site (Figure 67). The other plant species showed similar concentrations of sodium at both the Mainline and LVR. The chloride concentration of sugar beet was also significantly higher than the other species at both sites, except for sunflower which showed a trend of salt uptake nearing sugar beet levels (Figure 68). Also, pitseed goosefoot had a higher uptake of chloride per gram than the grass species on the salted Mainline roadside.

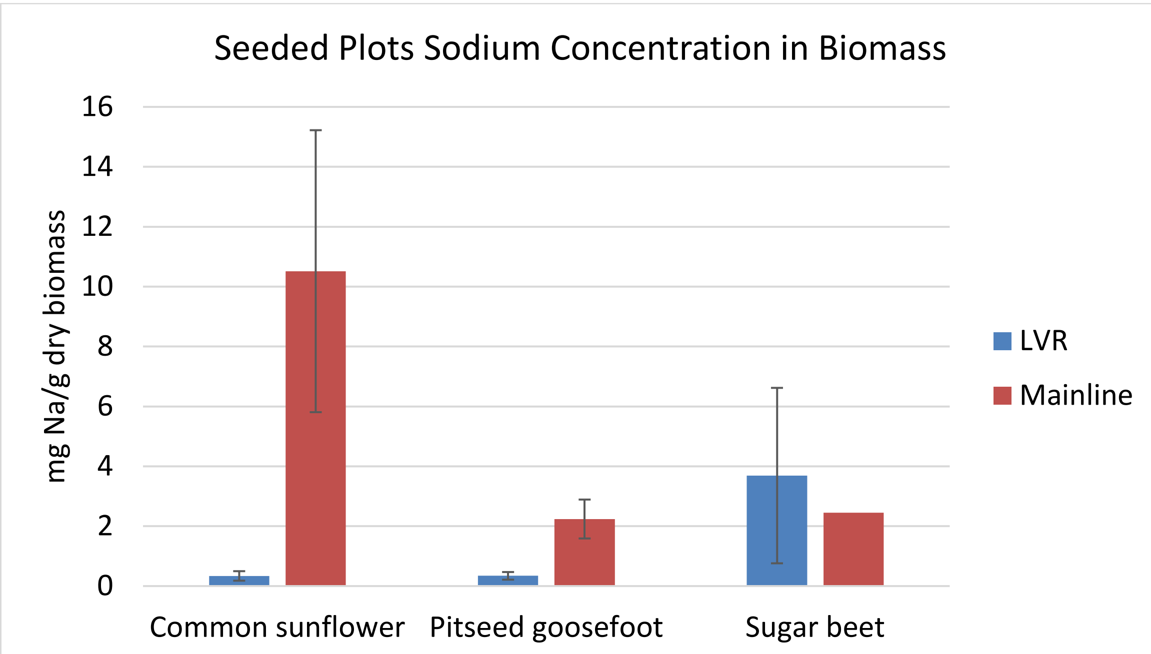


Figure 65: Concentration of sodium ions (mg Na/g dry biomass) measured in the aboveground biomass of the seeded plots.

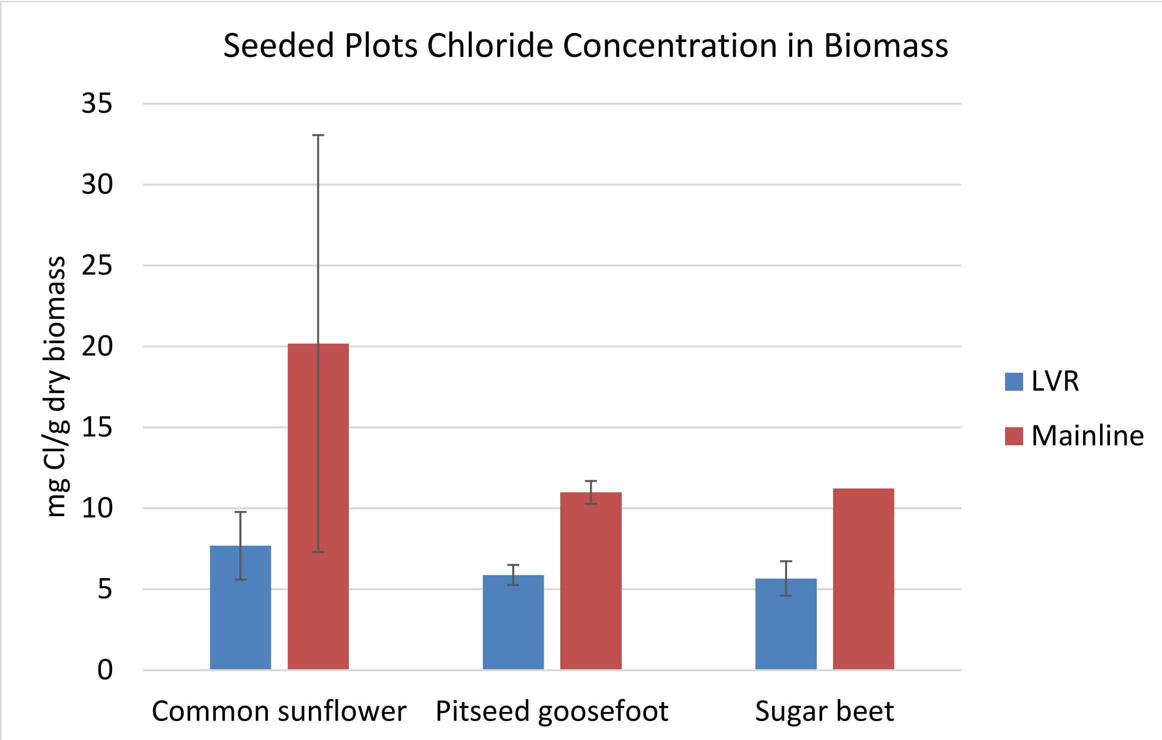


Figure 66: Concentration of chloride ions (mg Cl/g dry biomass) measured in the aboveground biomass of the seeded plots.

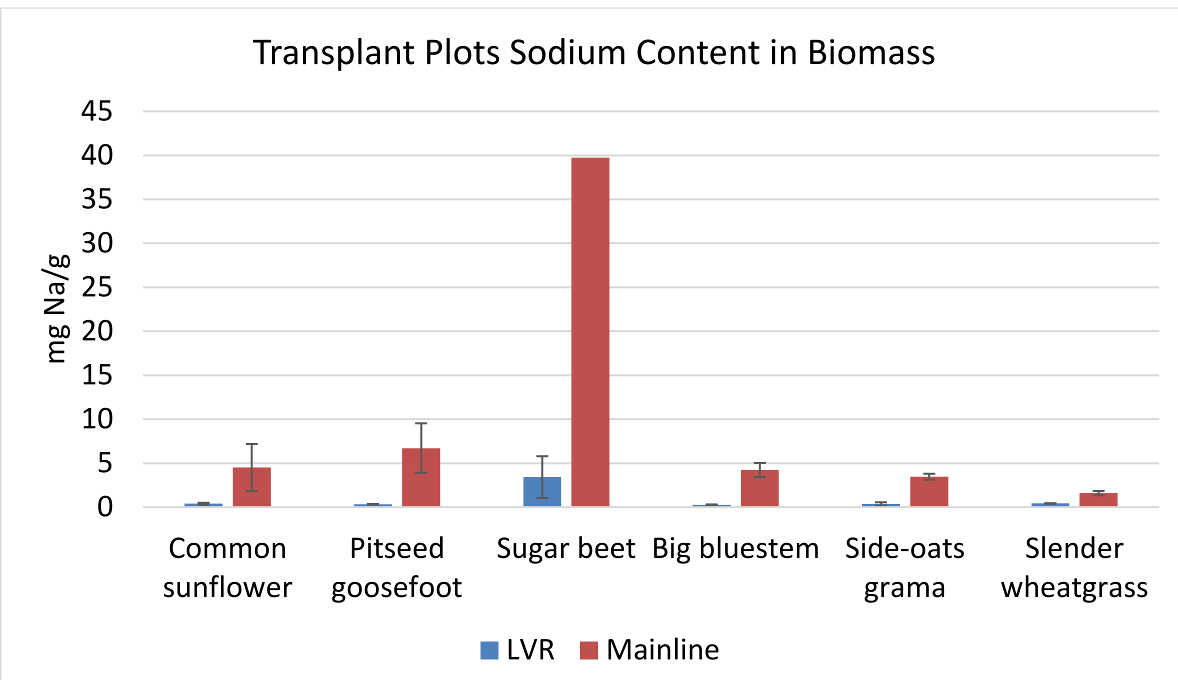


Figure 67: Concentration of sodium ions (mg Na/g dry biomass) measured in the aboveground biomass at the transplant plots.

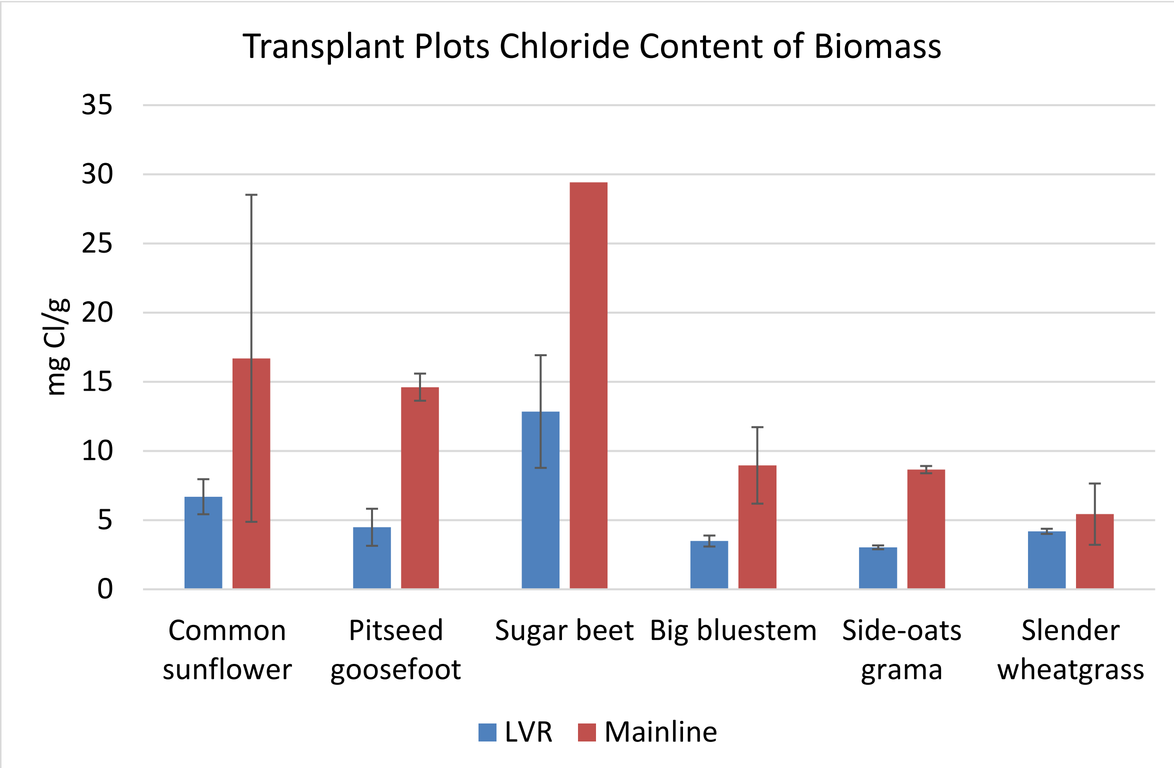


Figure 68: Concentration of chloride ions (mg Cl/g dry biomass) measured in the aboveground biomass at the transplant plots.

Figures 69-72 show the total amount of sodium and chloride uptake per plot by harvesting the aboveground biomass of each species. The values were calculated by multiplying the salt concentration in the biomass together with the total aboveground biomass harvested in each plot. The total sodium and chloride uptake is significantly higher for common sunflower than pitseed goosefoot or sugar beet in the seeded plots (Figures 69 and 70). For the transplant plots, sunflower again shows the highest salt uptake (Figures 71 and 72). However, sugar beet total sodium uptake is far closer to that of sunflower than it was in the seeded plots.

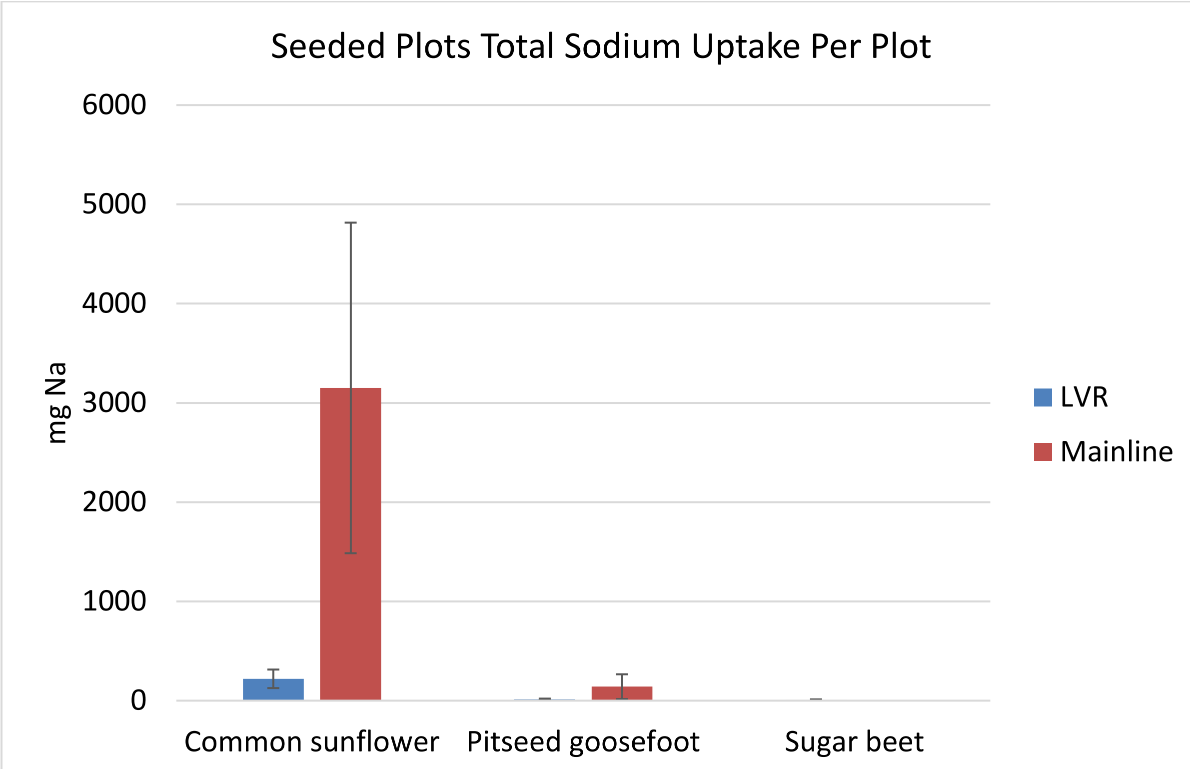


Figure 69: Total sodium uptake (mg Na) per plot based on the concentration of sodium in aboveground biomass and the amount of dry biomass harvested from each plot.

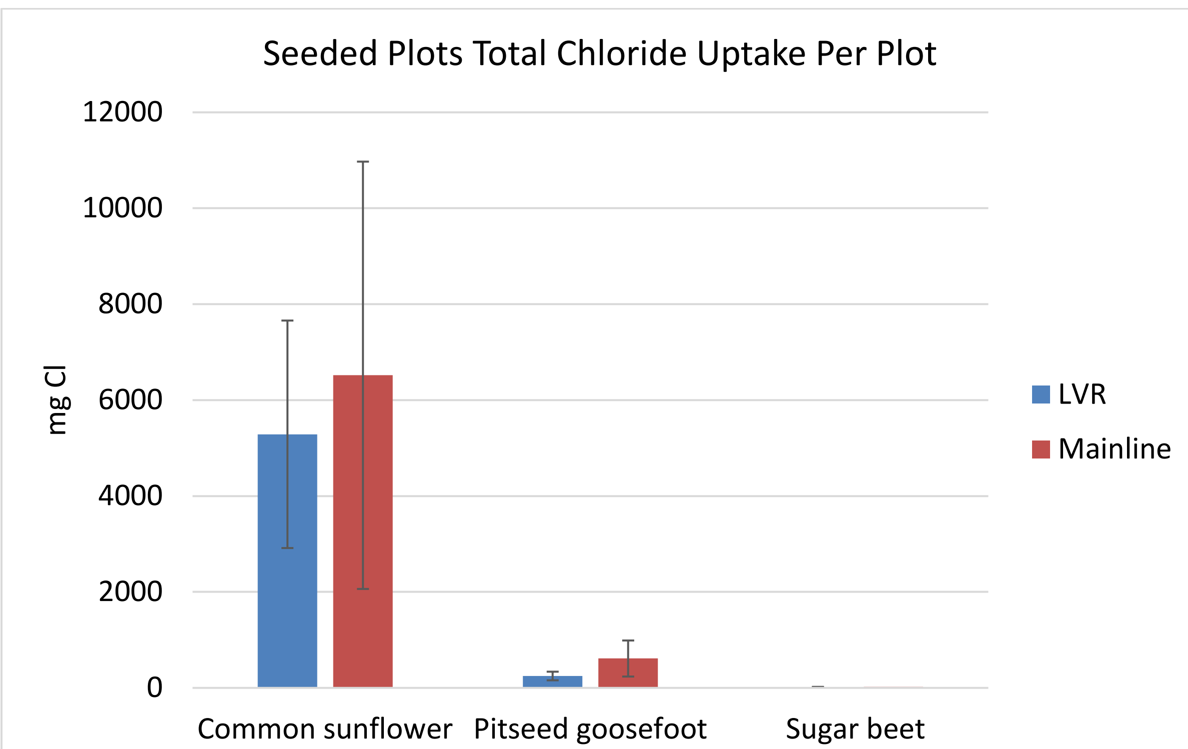


Figure 70: Total chloride uptake (mg Cl) per seeded plot based on the concentration of sodium in aboveground biomass and the amount of dry biomass harvested from each plot.

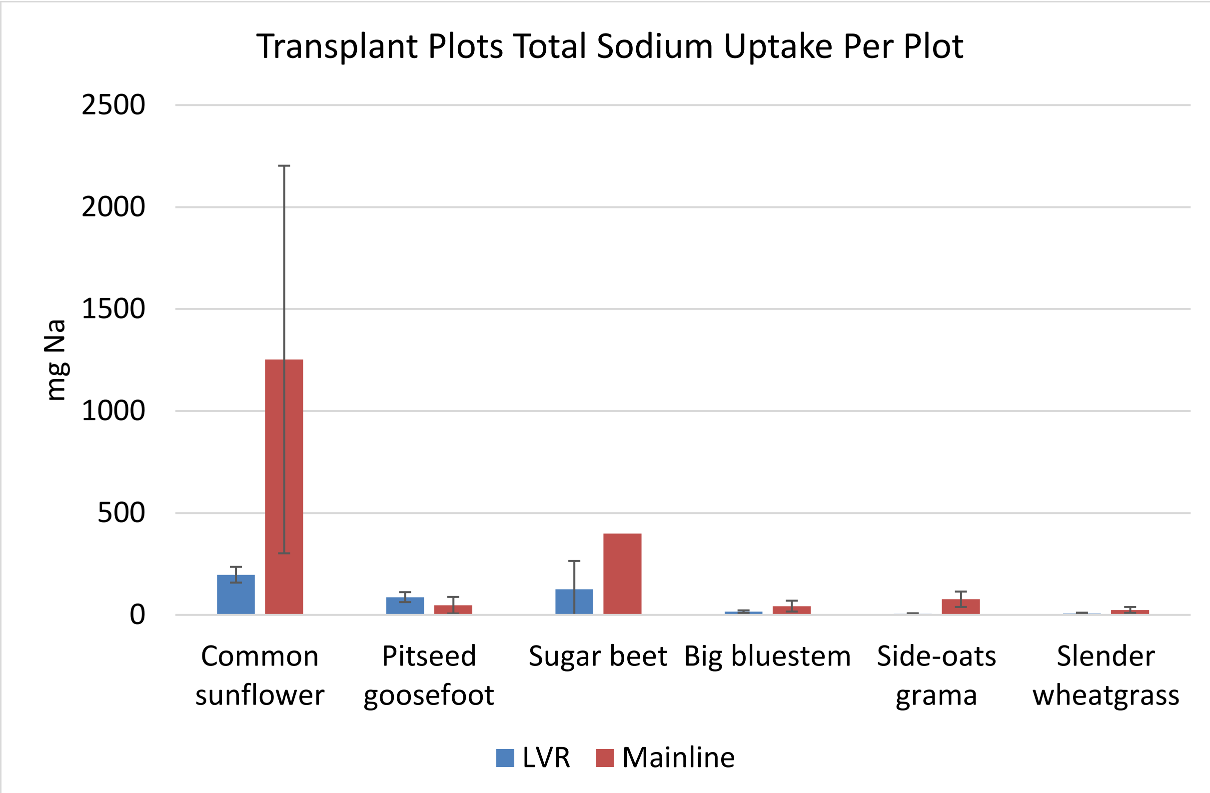


Figure 71: Total sodium uptake (mg Na) per transplant plot based on the concentration of sodium in aboveground biomass and the amount of dry biomass harvested from each plot.

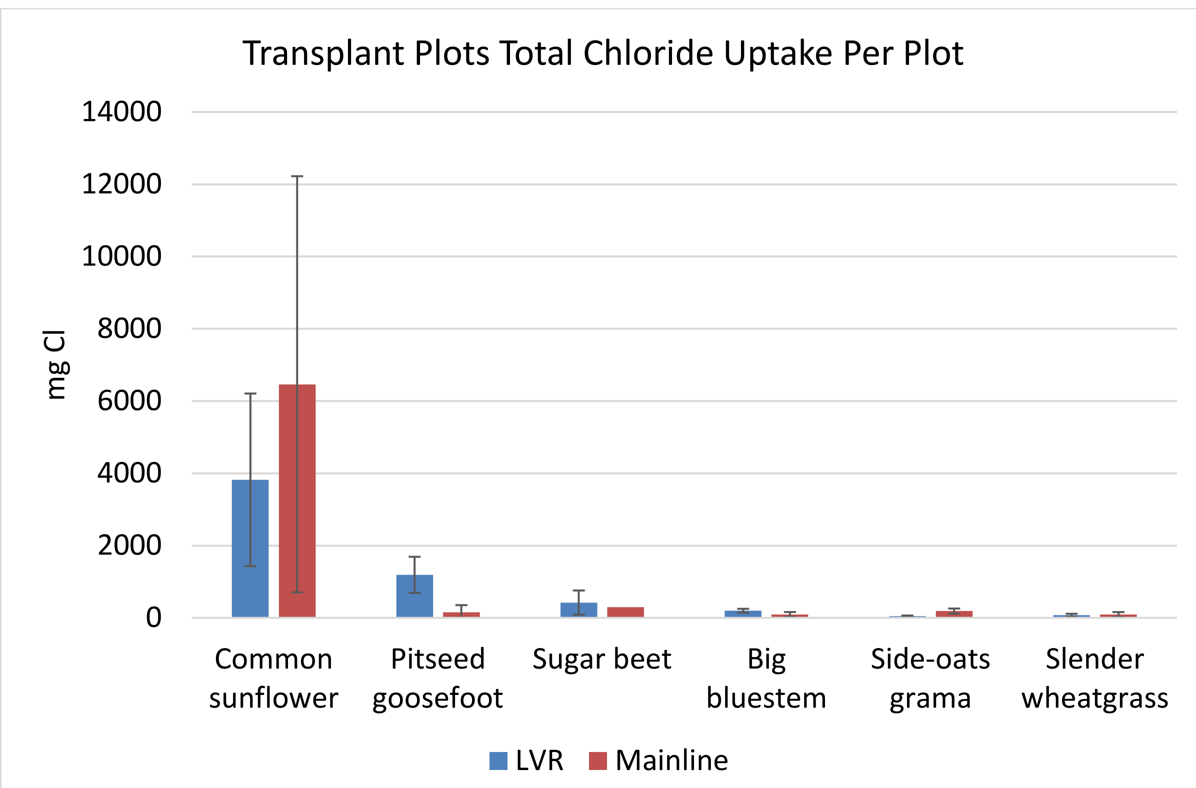


Figure 72: Total chloride uptake (mg Cl) per transplant plot based on the concentration of sodium in aboveground biomass and the amount of dry biomass harvested from each plot.

It can be concluded that most plant species showed higher growth on the LVR roadside. Potential reasons are that there was no heavy addition of salt on the LVR, while there was a significant amount added at one time to the Mainline. This extreme amount of salt may have negatively impacted the species growth. Also, the soil was sandier at the LVR with less clay than the Mainline so there could have been better root penetration through the sandy soil. Lastly, the LVR plots received more plot maintenance because it could be accessed more often than the Mainline due to traffic on the Mainline when it was switched. Plot maintenance included weed removal which may have given some of the plants at the LVR a better chance of survival and higher growth.

Nevertheless, common sunflower and pitseed goosefoot had the highest biomass measured at both sites. Common sunflower showed the highest total salt uptake due to its relatively high salt concentration in its biomass and the large amount of dry biomass that was harvested. Therefore, sunflower appears to have the highest potential for roadside salt remediation of the plant species tested at the MnROAD sites.

Final Report between project end (June 30) and August 15, 2022

The roadside field study located at MnROAD during the 2021 growing season tested the following plant species for their survival and salt uptake ability on actual roadsides. It was found that common sunflower and pitseed goosefoot had the highest survival and growth rate of all the plant species tested. In particular, common sunflower showed the highest overall salt uptake mostly due to the higher amount of biomass that was accumulated over the duration of the study. While sugar beet showed high salt concentrations in its leaves, the overall salt uptake per plot was much lower than common sunflower because of the relatively small amount of biomass. One significant challenge of this study was the lack of rainfall during the 2021 summer and limited access to the plots. The Mainline plots were only accessible for roughly once a week each month and therefore it was difficult to maintain the plots and made watering them unrealistic.

Another roadside experiment was planned for the 2022 growing season that would be located much closer to the University of Minnesota campus. The experimental design is shown below.

2022 Roadside Experimental Design:
Another roadside field experiment is currently running and the results from it will be obtained later this year. The site chosen for this experiment was on the edge of the University of Minnesota, St. Paul campus so that it could be much easier accessed during the study for watering the plots when needed. There are two locations of plots, one location being on Larpenteur Avenue, a road that is managed by the Ramsey County. It receives a significant amount of salt from the county during the winter and is a highly disturbed roadside. The other location, Gortner Avenue is a roadside managed by the University of Minnesota and receives little to no salt during the year and is not nearly as disturbed. The Larpenteur Avenue location contains only seeded plots and was chosen to determine how well the plant species can grow in a disturbed, salted roadside. At the Gortner Avenue location, there are two groups of transplant plots and no seeded plots. One group of transplant plots was a control with no salt added and the other was salted once at a rate of around 290 g NaCl per plot, spread evenly over the entire 1 meter squared plot.

Plant species chosen are the following: common sunflower, pitseed goosefoot, sugar beet, tall sunflower, and stiff sunflower. The transplant plots each contain 4 plants in the same formation as the previous field experiment (Figure 44). There are 3 replicate plots for each.

Photos from the Larpenteur and Gortner Avenue plots are shown below (Figures 73-74). As was the case with the 2021 field experiment, most of the common sunflowers showed great germination and growth in the seeded plots (Figure 73). There is also good germination and growth of sugar beets (Figure 74). At the Gortner Ave transplant plots, common sunflower, pitseed goosefoot, and sugar beet all have great growth, while the two perennial sunflower species showed significantly less (Figures 75 and 76).


Figures 73 and 74: Photos of the Larpenteur Avenue plots which are all seeded plots. Photos taken 6/29/22.


Figures 75 and 76: Photos of the Gortner Avenue plots which are all transplant plots. Photos taken 7/27/22.

Plants will be harvested in September after which they will be analyzed for their dry biomass and salt content. Soil samples taken at the beginning, end, and during the experiment will also be analyzed for their sodium and chloride content.

Final Report Summary

Overall, the field studies have shown that common sunflower and pitseed goosefoot have relatively high survival and growth under disturbed roadside conditions. Also, sugar beet showed some germination in the roadside plots and growth was increased under conditions with more plot maintenance including regular watering of the plots and weed removal. It is expected that sugar beet will exhibit higher overall salt uptake into aboveground biomass with higher growth due to more plot maintenance.

ACTIVITY 3 Title: Develop possible utilization of harvested plant biomass
Description:
It is important to find a utilization of the biomass in order to cover the cost of harvest, and provide an economically sustainable solution. We will study the utilization of the biomass for animal feed supplement, energy source, and for recycled road salts after ashing. *S. rubra* has been reported as the ingredient supplement to improve the flavor and nutrition of the animal feed. The plant biomass will be analyzed for its feed value, including the following parameters: gross energy, fiber, total protein and amino acid profile, phosphate, lipids, and possible accumulation of heavy metals. The plant biomass can also be combusted for heat and power, meanwhile the ash can be recycled as the road salts.

ACTIVITY 3 ENRTF BUDGET: $ 124,000

Outcome	Completion Date
1. Biomass characterization for possible applications	*Year 3 - 01/2022*
2. Business strategies for how to adopt this plant for road side applications	*Year 3 - 06/2022*

First Update March 1, 2020

Nothing to update for this activity

Second Update September 1, 2020

Nothing to update for this activity

Third Update March 1, 2021

Nothing to update for this activity

Fourth Update September 1, 2021

Nothing to update for this activity

Fifth Update March 1, 2022

Some plant species showed consistent and great potentials from both greenhouse studies and MnROAD field experiments. These species include sunflower, sugar beet, and pitseed goosefoot. Each plant shows unique and distinctly different applications and possible business models. We are testing several perennial sunflower species and the results will significant shift possible operational models for the commercial implementation. If we need to use annual sunflower, it needs to be planted in the spring and will be harvested at once in the Fall. The sunflower seeds will have food/feed applications and the biomass can potentially be combusted to harvest heat and ash to reuse as de-icing agent. The sugar beet is also under further study in the greenhouse since the main part to accumulate the salt is the shoots instead of beets. This will have potentially revolutionary applications in the agriculture, especially considering that large portion of agricultural land around the US and the entire world is affected by high salinity in the soil. The pitseed goosefoot has values as animal feed and can be harvested periodically. Further analysis and recommendations will be provided.

As mentioned above, sugar beets show great promise for remediating salt contaminated agricultural soil due to its ability to accumulate salt into aboveground biomass while keeping a low salt content within the beet itself. This led us to investigate whether other kinds of beets such as those within the five different cultivar groups of the *Beta vulgaris subsp. vulgaris* subspecies show similar salt accumulation abilities. The five different cultivar groups are the altissima group (sugar beet), cicla group (leaf beet, spinach, or chard), conditiva group (garden beet or beetroot), crassa group (fodder or mangelwurzel beet) flavescens group (swiss chard). The six beets obtained for the experiment were sugar beet (altissima group), Detroit dark red beet (conditiva group), red mammoth fodder beet (crassa group), Fordhook swiss chard heirloom (flavescens group), ruby red swiss chard (cicla group), and barese swiss chard (cicla group). The two cicla group swiss chards (ruby red and barese) were both chosen for the experiment because they seemed to have significantly different phenotypes and therefore may give varying results.

Beets experimental design:

The experimental setup of this beets experiment was identical to Greenhouse Experiment 4. The three salt treatments were kept at 0, 50, and 100 mM NaCl. Each pot was watered with 100 mL of salt water each week for a total of four irrigations. The results of the experiment are shown below.

Photos of beets in the greenhouse:

Figures 77-79: Individual pots growing in the greenhouse of sugar beet (77), Detroit dark red beet (78), and red mammoth fodder beet (79).

Figures 80-82: Individual pots growing in the greenhouse of Fordhook swiss chard (80), ruby red swiss chard (81), and barese swiss chard (82).

The aboveground and belowground dry weights of each beet show little significant difference (Figures 83 and 84). The greatest differences were a slightly lower aboveground weight for the dark red beet (conditiva) and slightly lower belowground weight for ruby red (cicla). Also, the salt treatments did not appear to have any significant affect on the growth of any of the beets.

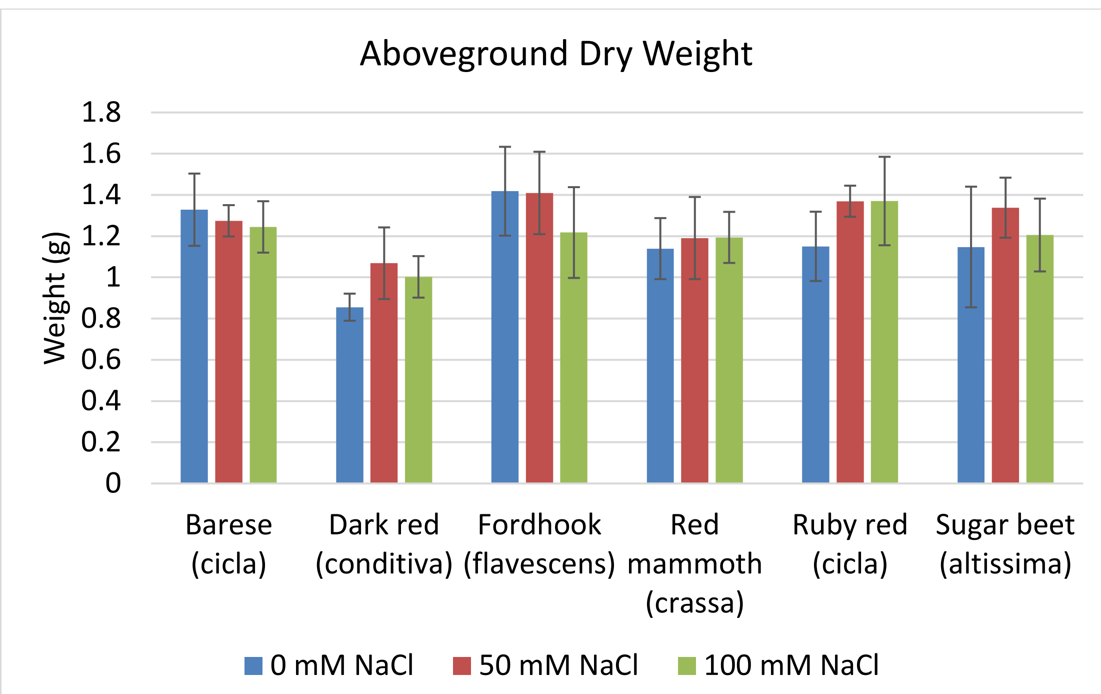


Figure 83: Aboveground dry weight of beets grown in 4” pots at 3 different salt treatment levels.

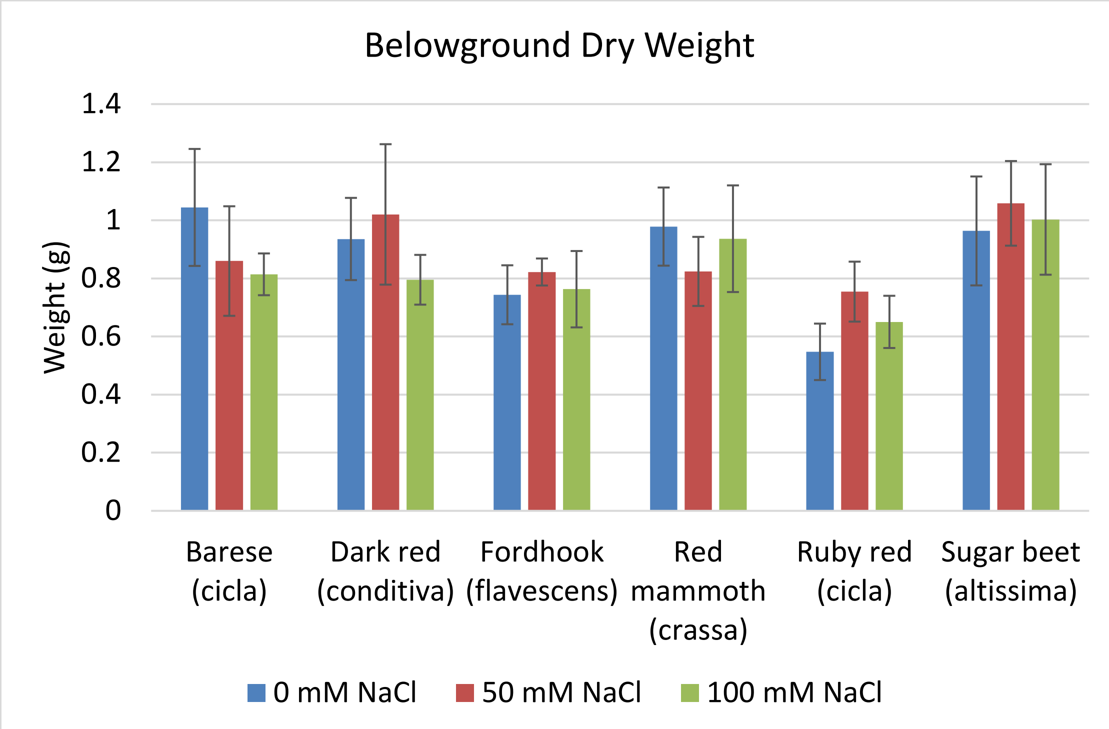


Figure 84: Belowground dry weight of beets grown in 4” pots at 3 different salt treatment levels.

Figures 85 and 86 show the sodium and chloride concentrations of the aboveground beet biomass. All the beets show increasing salt concentrations when watered with the higher salt treatments, indicating that they all have the potential to take up significant amounts of salt similar to sugar beet.

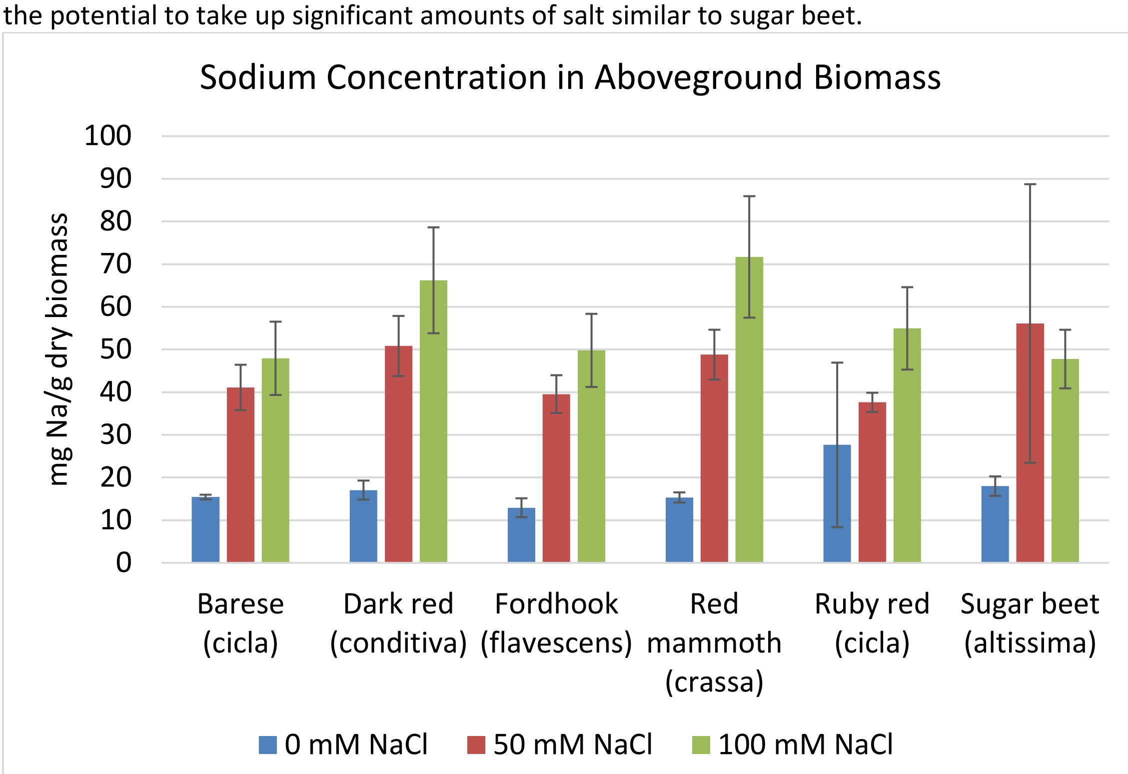


Figure 85: Sodium concentration in biomass per gram of dry biomass in the aboveground beet leaves.

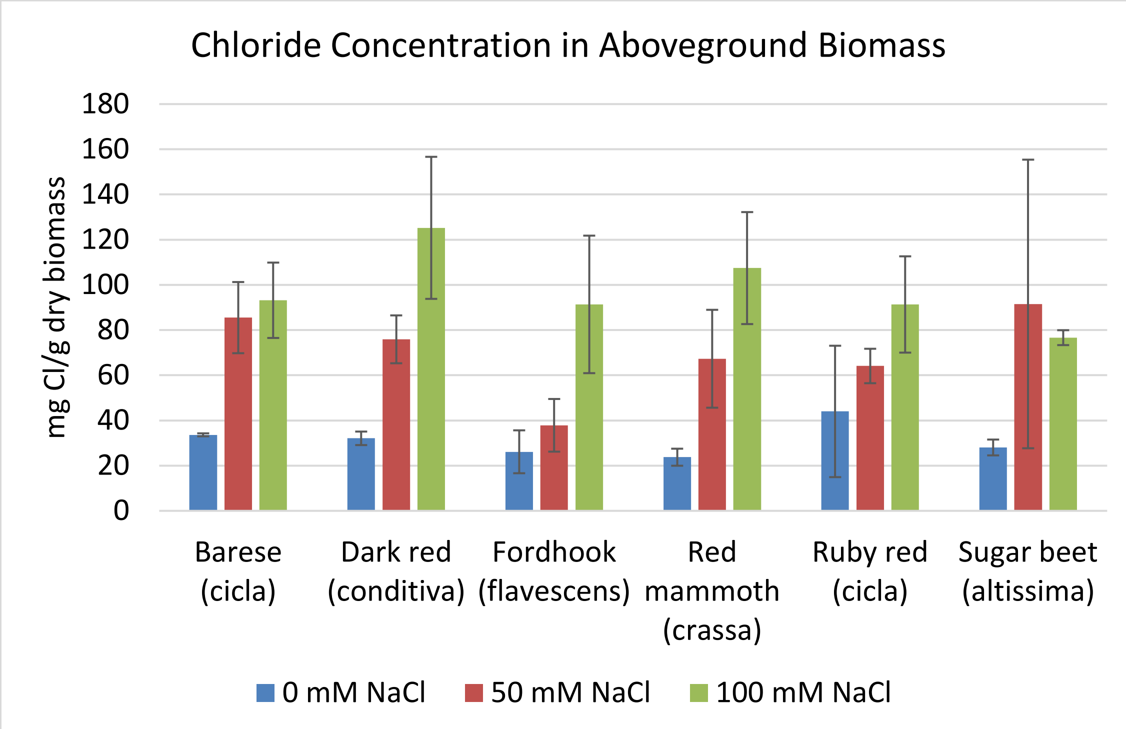


Figure 86: Chloride concentration in biomass per gram of dry biomass in the aboveground beet leaves.

The salt content in the belowground beets was also measured for each beet type to determine if the salt in the beet itself would remain low (Figure 87 and 88). Each kind of beet showed salt content trends similar to sugar beet where the salt concentrations in the beet (9.33 mg Na/g and 15.42 mg Cl/g) were roughly 5 times lower than that of the aboveground leafy biomass (47.76 mg Na/g and 76.64 mg Cl/g). Even red mammoth (crassa), which shows higher sodium and chloride beet concentrations than sugar beet for the 100 mM NaCl treatment, still had salt concentrations between 4.2 and 4.6 times lower than the amount in the aboveground biomass.

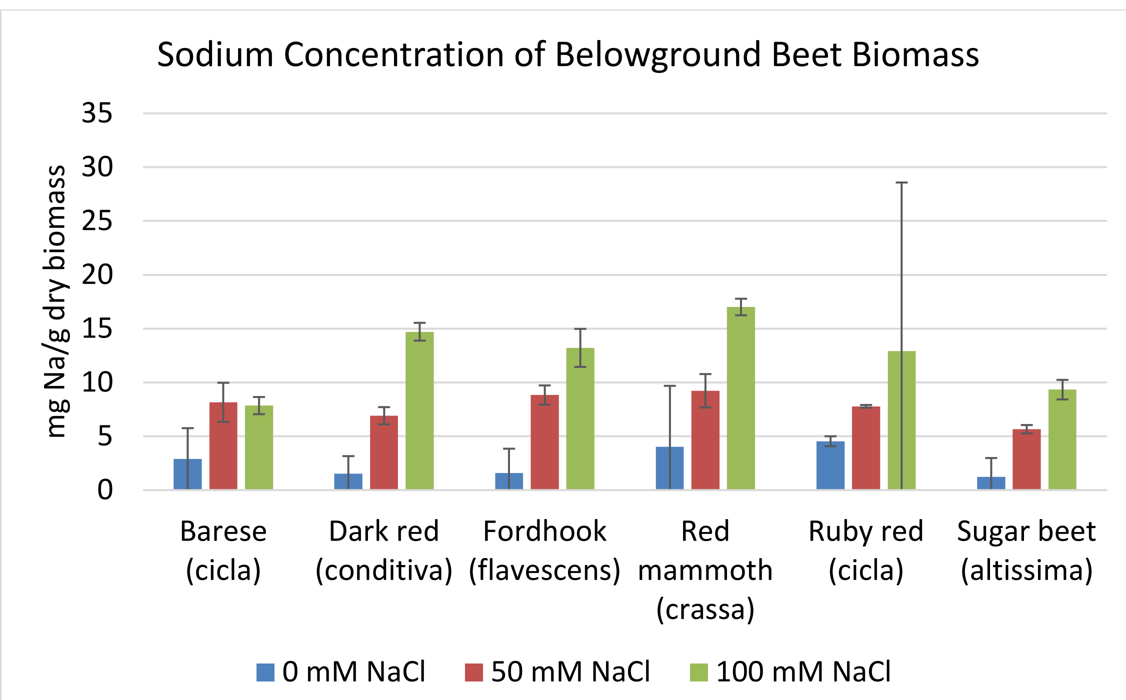


Figure 87: Sodium concentration per gram of dry biomass in the belowground beets.

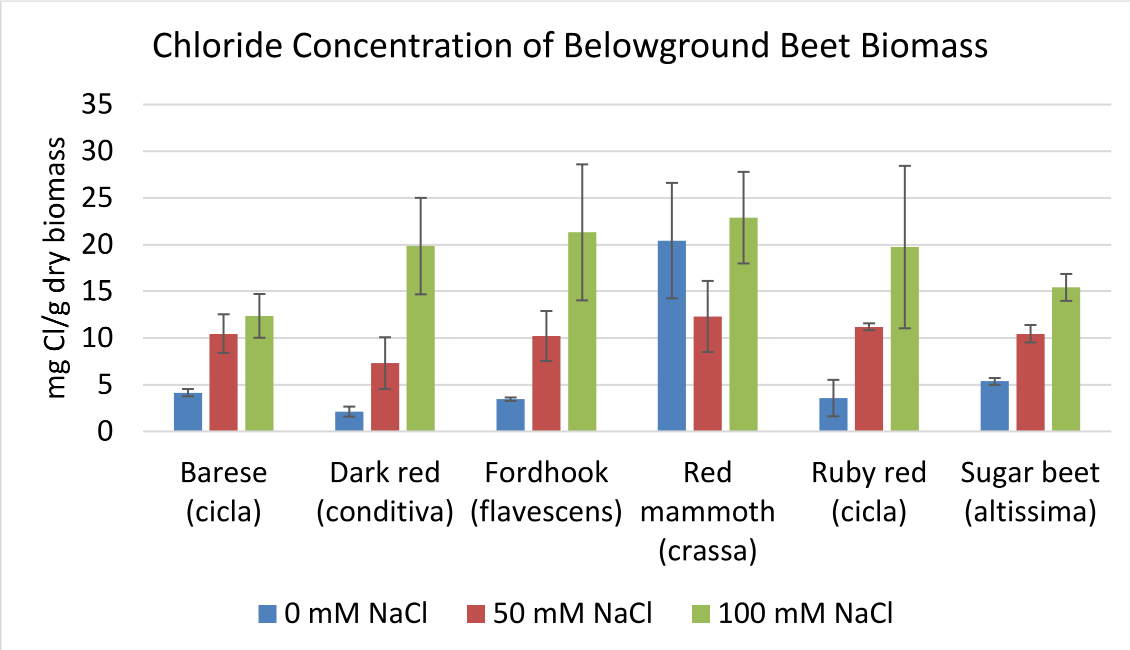


Figure 88: Chloride concentration per gram of dry biomass in the belowground beets.

The sodium and chloride results from the soil testing also showed similar trends between the different beet groups (Figures 89 and 90). Due to the high error bars, there is hardly any significant difference between the salt content in the soil when comparing the pots of different beet types within the same salt treatment.

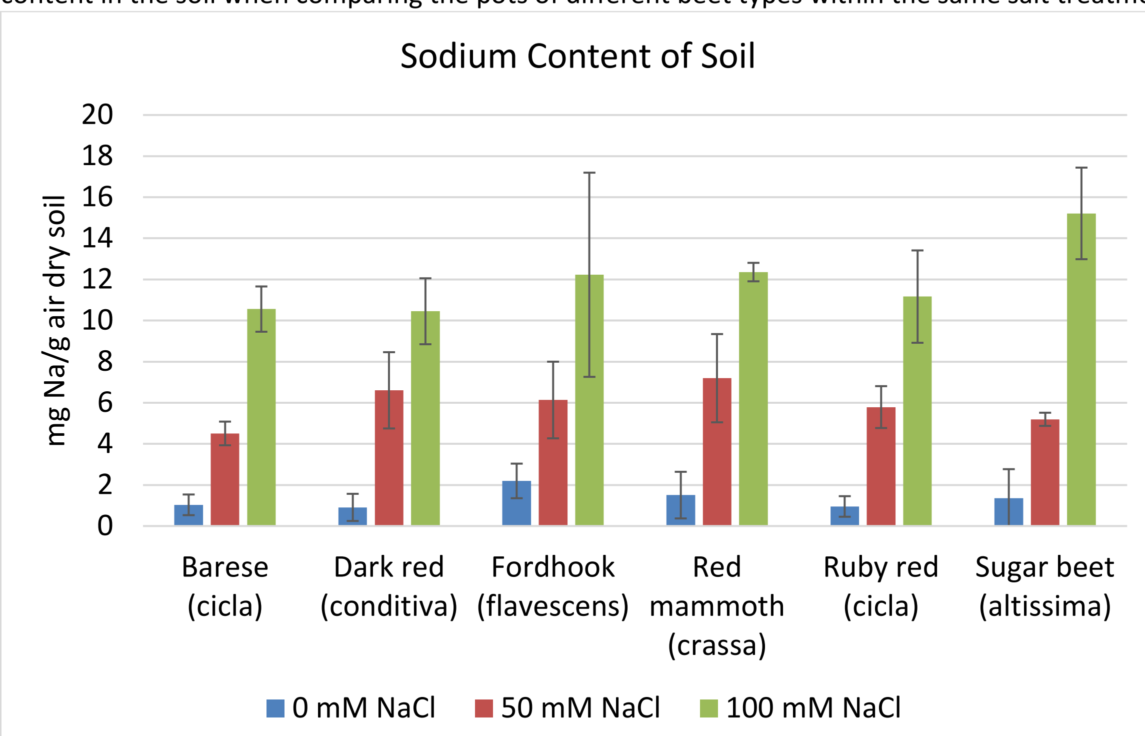


Figure 89: Sodium concentration in the soil (mg Na/g) for each beet at each of the three salt treatments.

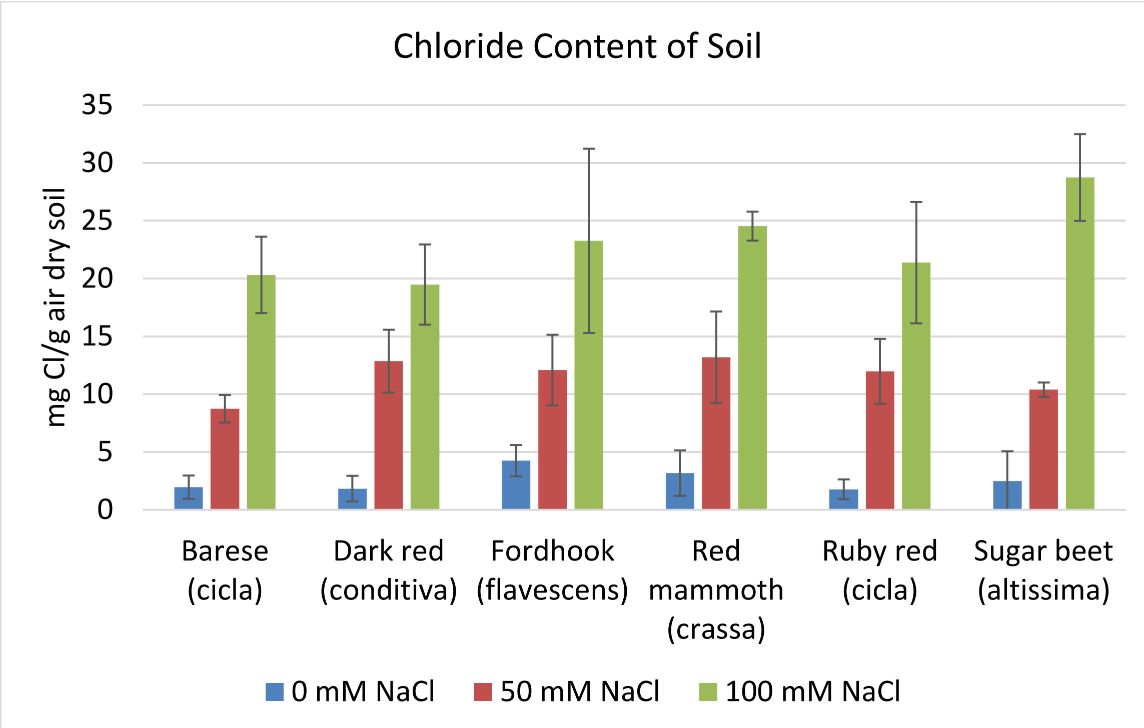


Figure 90: Chloride concentration in the soil (mg Cl/g) for each beet at each of the three salt treatments.

Table 9 shows the percentage of salt reduction through the harvest of aboveground leafy biomass. The sugar beet salt reduction for the 50 mM NaCl treatment in this experiment (15.74%) was slightly lower than the salt reduction in Greenhouse Experiment 4 (17.77%), but still within a standard deviation margin of error (Tables 8 and 9). All the beets from the other groups also showed significant potential for phytoremediation of salt, especially Barese (13.73% at 50 mM NaCl) and red mammoth (9.79% at 100 mM NaCl). These different beets should be tested on agriculture soil that is contaminated with salt.

Table 9: Values based on the amount of salt added to the pots and the total salt uptake per plant

% Salt reduction from harvesting aboveground biomass						
Salt Treatment	**Barese (cicla)**	**Dark red (conditiva)**	**Fordhook (flavescens)**	**Red mammoth (crassa)**	**Ruby red (cicla)**	**Sugar beet (altissima)**
0 mM NaCl	108.90	73.04	89.47	78.26	137.78	91.08
50 mM NaCl	13.73	11.19	9.03	11.26	11.66	15.74
100 mM NaCl	7.18	7.93	6.83	8.79	8.23	6.18

The results here show that these other beets also have significant salt uptake into aboveground leaves while keeping the salt content in the belowground beets relatively low. Therefore, these different cultivar groups could be grown in agriculture areas that they are best suited for in terms of environmental conditions and desired utilization of the aboveground and belowground biomass.

Another important aspect of biomass utilization is how well these plant species can outcompete invasives or other fast growing plants. If there is significant invasive growth then harvest and utilization of the biomass will become more difficult.

Greenhouse competition experiment:
This greenhouse experiment looked at the potential of native salt accumulator species to compete with invasive roadside plants. Sugar beet (*Beta vulgaris*), pitseed goosefoot (*Chenopodium berlandieri*), common sunflower (*Helianthus annuus*) were chosen as native species due to their high potential for phytoremediation based on earlier studies. Smooth bromegrass (*Bromus inermis*) and purslane (*Portulaca oleracea*) were chosen as the invasive species since they are common roadside weeds.

Like the previous greenhouse experiments, the plants were grown in 4" pots with 75 g of fresh general purpose soil but only two salt treatments were used (0 and 100 mM NaCl). The pots were watered with 100 mL of salt solution once a week for a period of four weeks. Two weeks after the last salt irrigation, the plants were harvested, and the aboveground dry biomass of each pot was measured. Every pot consisted of only one species or a mix of two. The number of plants in each pot were based on the recommended seeding rate of each species. The plant densities in each pot were the following: purslane and pitseed goosefoot with 8 seedling per pot, smooth bromegrass and sugar beet with 6 seedlings per pot, and finally common sunflower with only 4 seedlings per pot. Pots containing a mix of two species included half the number of seedlings for both plant species. The pots with two species are shown below in Figures 58-63.

Figures 91-93: The invasive species purslane, growing together with pitseed goosefoot (91), common sunflower (92), and sugar beet (93).


Figures 94-96: The invasive species smooth bromegrass growing together with pitseed goosefoot (94), common sunflower (95), and sugar beet (96).

The results of the pots with only a single plant species are shown in Figure 97. The biomass of common sunflower was the highest for both the 0 and 100 mM NaCl treatments. The other plant species showed similar growth to one another, and the higher salt concentration did not significantly impact their biomass.

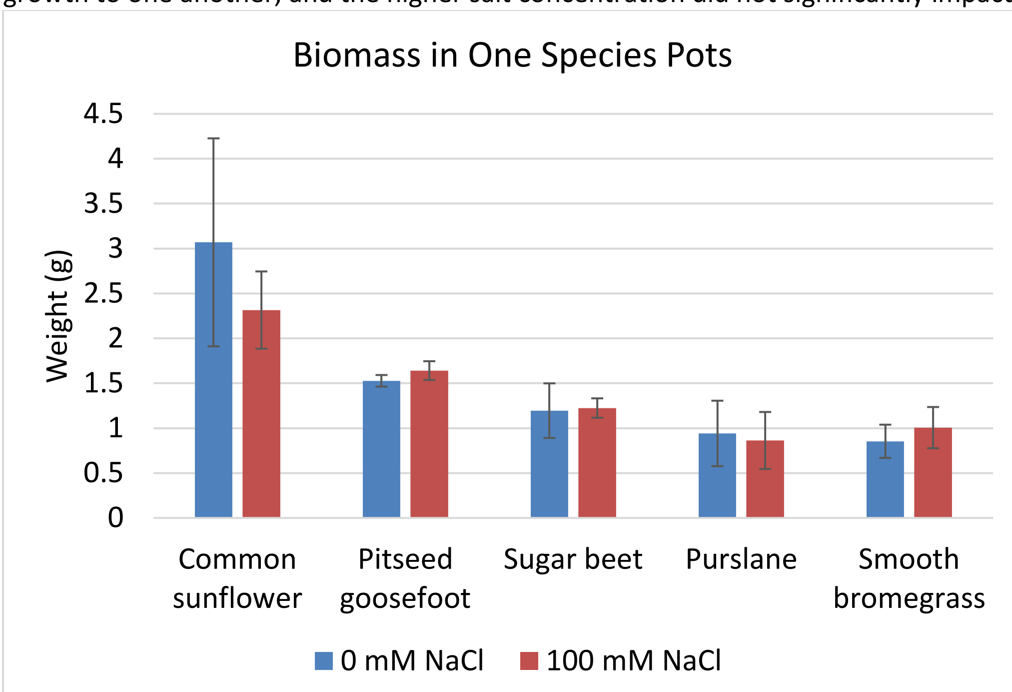


Figure 97: The total aboveground dry biomass harvested from each pot containing only a single species, irrigated with two different salt treatments.

Common sunflower showed higher growth when competing against purslane (Figure 98). Since sunflower grows tall quickly, it may have easily restricted light from hitting the small purslane plants. This was likely not the case against smooth bromegrass because this grass also grows quickly and tall. The higher treatment did not have any significant impact on the results. The pitseed goosefoot results showed inconsistent growth between replicates of the same treatment especially when competing against smooth bromegrass (Figure 99). When transferring seedlings to the 4” pots, a few of the pitseed goosefoot seedlings were not growing as well as the others which may have caused this inconsistency between the replicates. Also, pitseed goosefoot shows slightly higher biomass when competing against purslane. Sugar beet shows a trend of higher growth when competing with invasives at higher salt levels compared to itself (Figure 100).

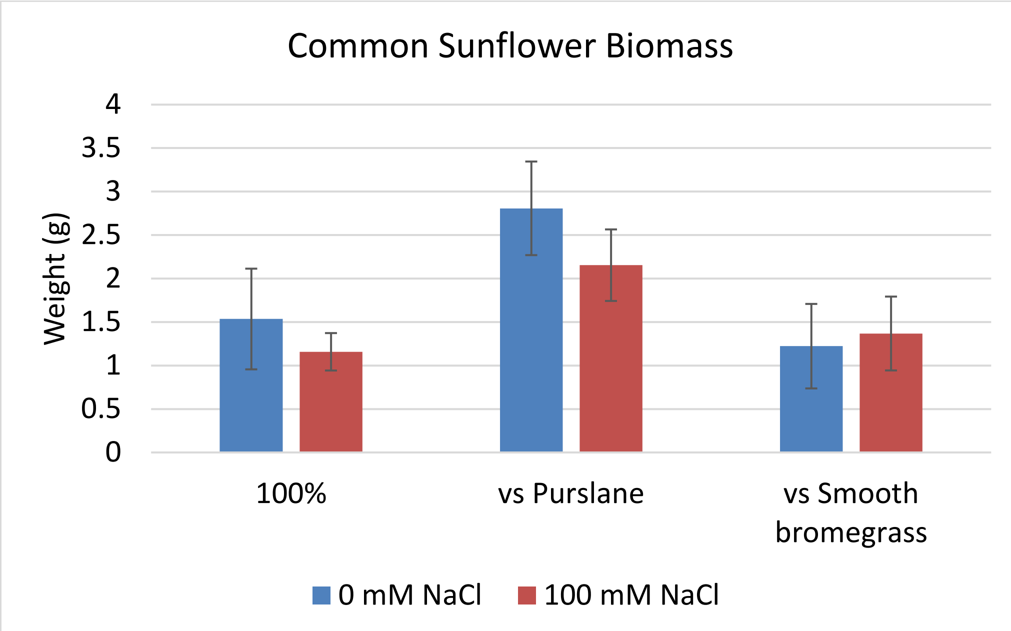


Figure 98: Aboveground biomass of common sunflower that has grown in a single pot with either itself (4 sunflower plants total, total biomass divided by two), purslane (2 sunflower plants) or smooth bromegrass (2 sunflower plants).

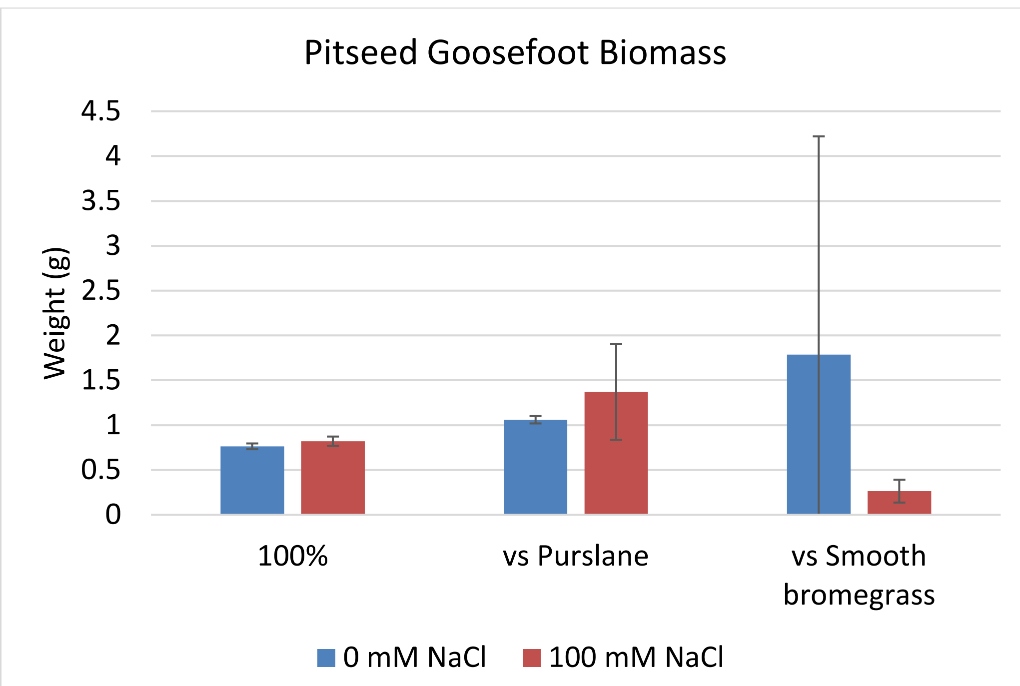


Figure 99: Aboveground biomass of pitseed goosefoot that has grown in a single pot with either itself (8 pitseed goosefoot plants total, total biomass divided by 2), purslane (4 pitseed goosefoot plants) or smooth bromegrass (4 pitseed goosefoot plants).

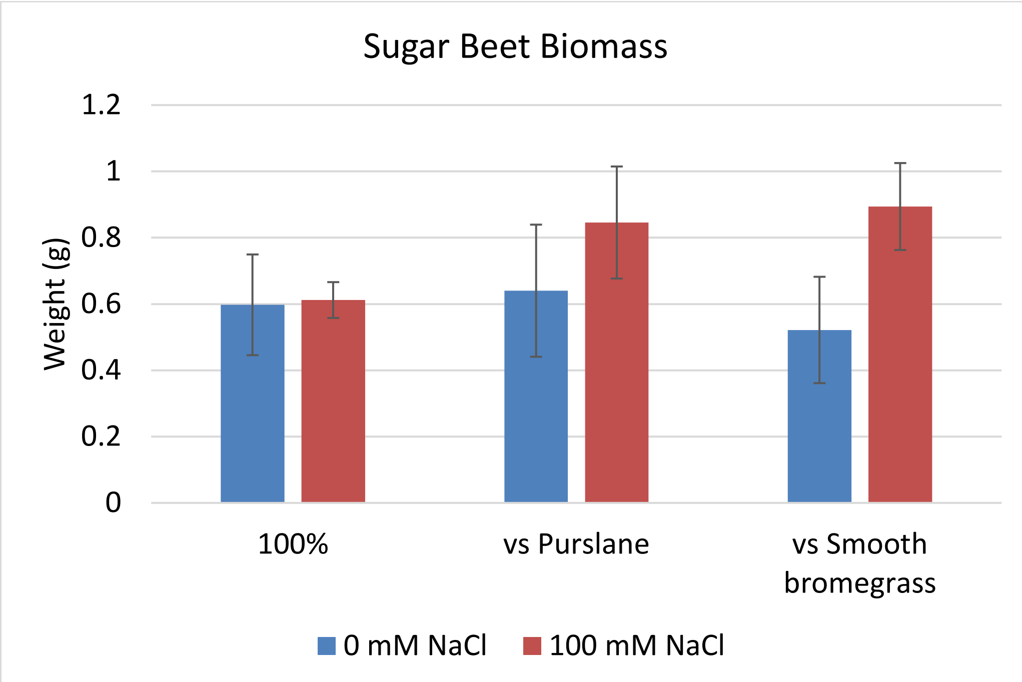


Figure 100: Aboveground biomass of sugar beet that has grown in a single pot with either itself (6 sugar beet plants total, total biomass divided by 2), purslane (3 sugar beet plants) or smooth bromegrass (3 sugar beet plants).

In general, the invasive purslane appeared to have better growth when competing against itself rather than common sunflower and sugar beet at both salt concentrations (Figure 101). However, when competing against pitseed goosefoot, the purslane growth appears quite similar to the growth shown in the 100% purslane pots. Smooth bromegrass growth was similar when competing with itself as with common sunflower and sugar beet (Figure 102).

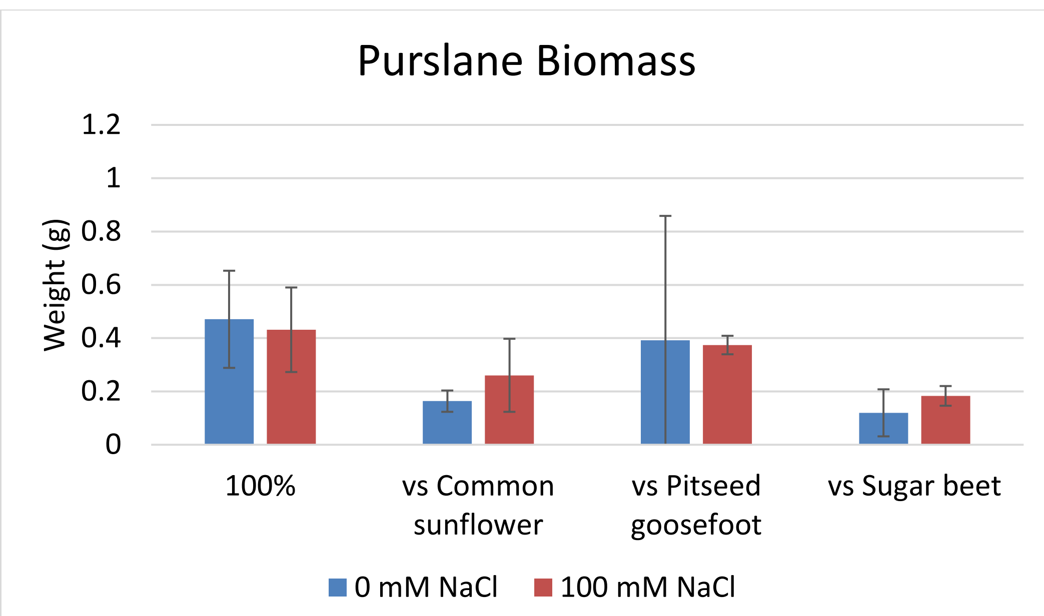


Figure 101: Aboveground biomass of purslane that has grown in a single pot with either itself (8 purslane plants total, total biomass divided by 2), common sunflower (4 purslane plants), pitseed goosefoot (4 purslane plants), and sugar beet (4 purslane plants).

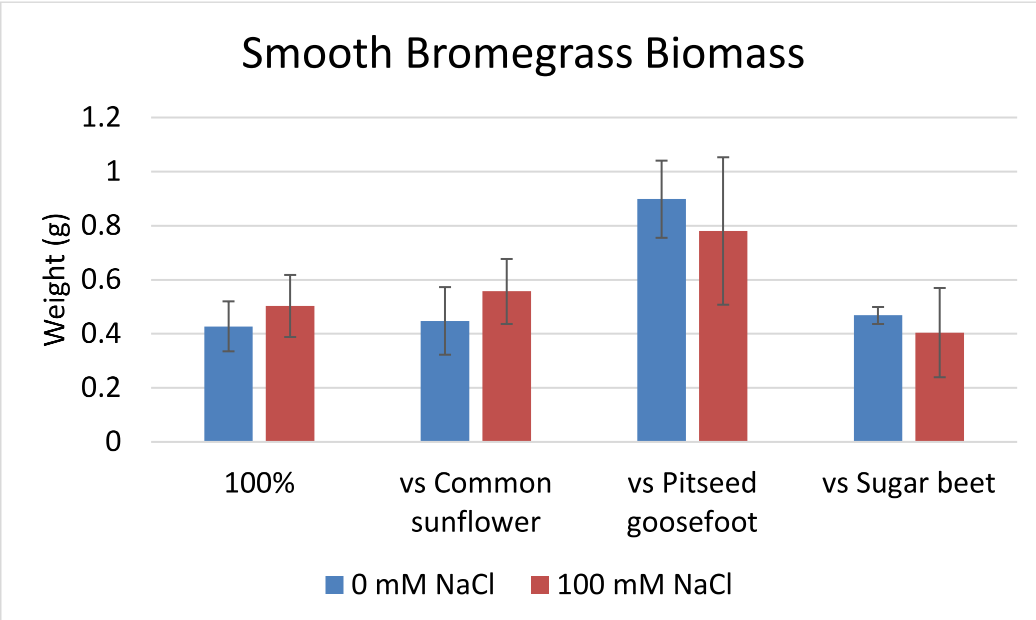


Figure 102: Aboveground biomass of smooth bromegrass that has grown in a single pot with either itself (6 smooth bromegrass plants total, total biomass divided by 2), common sunflower (3 smooth bromegrass plants), pitseed goosefoot (3 smooth bromegrass plants), and sugar beet (3 smooth bromegrass plants).

Invasive species generally did not significantly reduce the growth of the native species, which may have been the results of high levels of nutrients in the general mix soil. The invasives may have competed much better on roadside soil with less nutrients and more stresses. However, the fast growth of common sunflower, pitseed goosefoot, and sugar beet that often outcompeted the invasives suggests that these plant species may be more easily harvested and their biomass utilized due to less maintenance required to remove invasives.

Final Report between project end (June 30) and August 15, 2022

The following greenhouse experiment includes the testing of several perennial sunflowers for their salt uptake potential. Using perennial sunflowers would exclude the need to replant the seeds each season which would likely lead to easier commercial implementation. Also, perennial plant species that have already been established the previous season are more likely to uptake the salt from roadside soil when it is at higher concentrations in the soil during early spring.

Perennial sunflower greenhouse experiment:
The following perennial sunflowers were selected because they are *Helianthus* species and thus assumed to have some similar salt uptake ability as annual sunflowers and the species are already included in MnDOT's roadside seed mixes; tall sunflower (*H. giganteus*), Maximilian's sunflower (*H. maximilianii*), and western sunflower (*H. occidentalis*).

Similar to the previous greenhouse experiments, the plants were grown in 4" pots with 75 g of fresh general purpose soil. This experiment included three different salt concentrations (0, 25, and 50 mM NaCl). The pots were irrigated with 100 mL of salt solution weekly for a total of four weeks. Two weeks after the last salt irrigation, the plants were harvested, and the aboveground dry biomass of each plant was measured. Photos and results of the experiment are shown below.

Figures 103-105: Individual 4” pots growing in the greenhouse of Maximillian sunflower (103) tall sunflower (104), and western sunflower (105).

The aboveground biomass of both Maximillian and tall sunflower species were not significantly impacted by the increasing salt concentrations of the irrigation water (Figure 106). However, western sunflower is shown to have decreased aboveground biomass at the 50 mM salt treatment. Similarly, for the belowground roots systems, the western sunflower roots were smaller at the 50 mM NaCl condition compared to the other salt conditions (Figure 107). Also, while not as significant, the tall sunflower roots show a decreasing trend at the highest salt concentration.

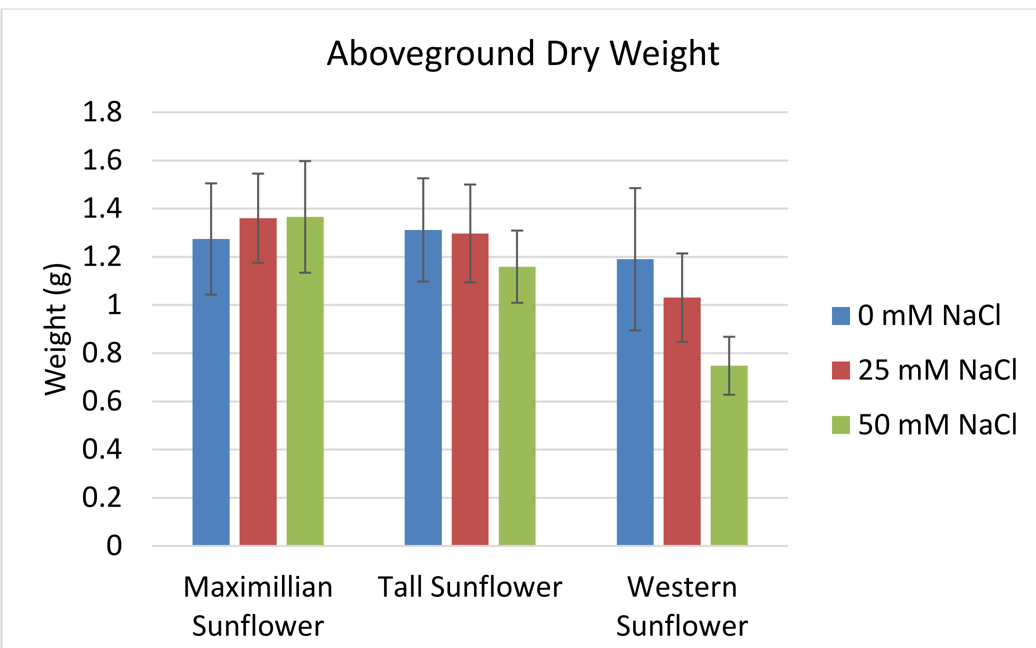


Figure 106: Aboveground dry weight of perennial sunflower species grown in 4” pots at 3 different salt treatment levels.

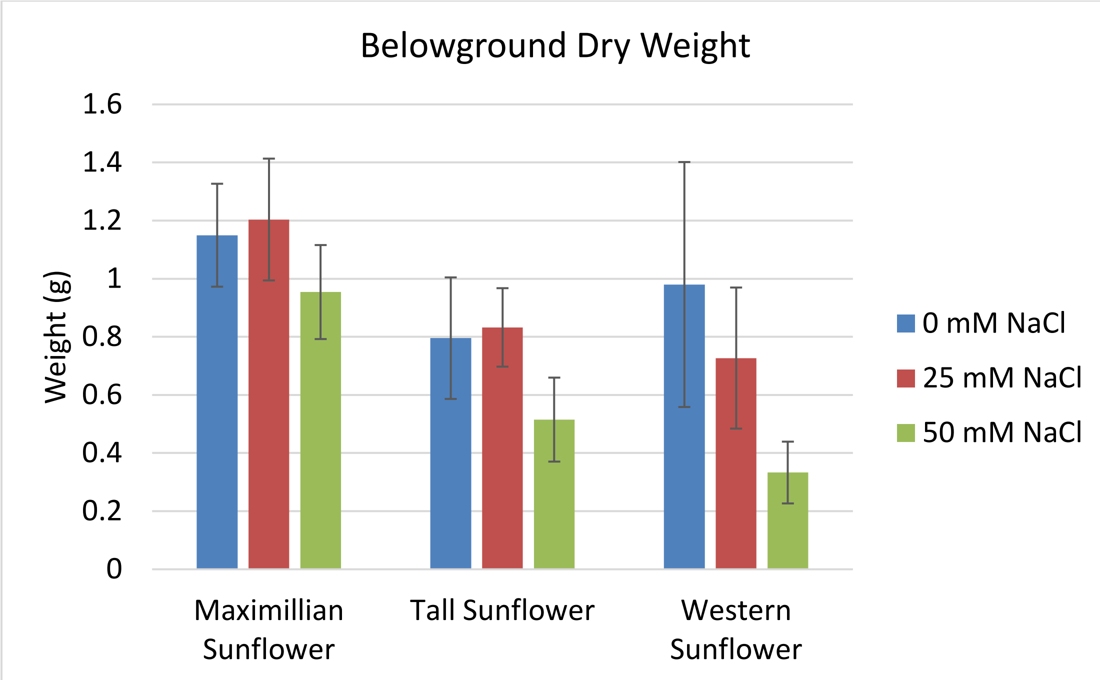


Figure 107: Belowground dry weight of three perennial sunflower species grown in 4” pots at 3 different salt treatment levels.

The sodium and chloride content of the aboveground biomass is shown in figures 108 and 109. Western sunflower had the highest uptake of sodium at the highest salt treatment. Maximillian sunflower consistently showed lower sodium and chloride uptake for each salt treatment compared to the other two species, whereas tall sunflower was closer to the salt content of western sunflower. Also, there is significant difference in the sodium uptake compared to chloride. For example, each of the sunflower species has chloride concentrations in their biomass much higher than sodium, in most cases at least 4 to 5 times higher. Yet, when comparing these biomass salt concentrations to that of common sunflower in Greenhouse Experiment 4, the perennial sunflowers had much lower concentrations. For the 50 mM NaCl salt treatment, these salt concentrations were much lower than the roughly 30 mg Na/g and 97 mg Cl/g that common sunflower exhibited (Figures 38 and 39).

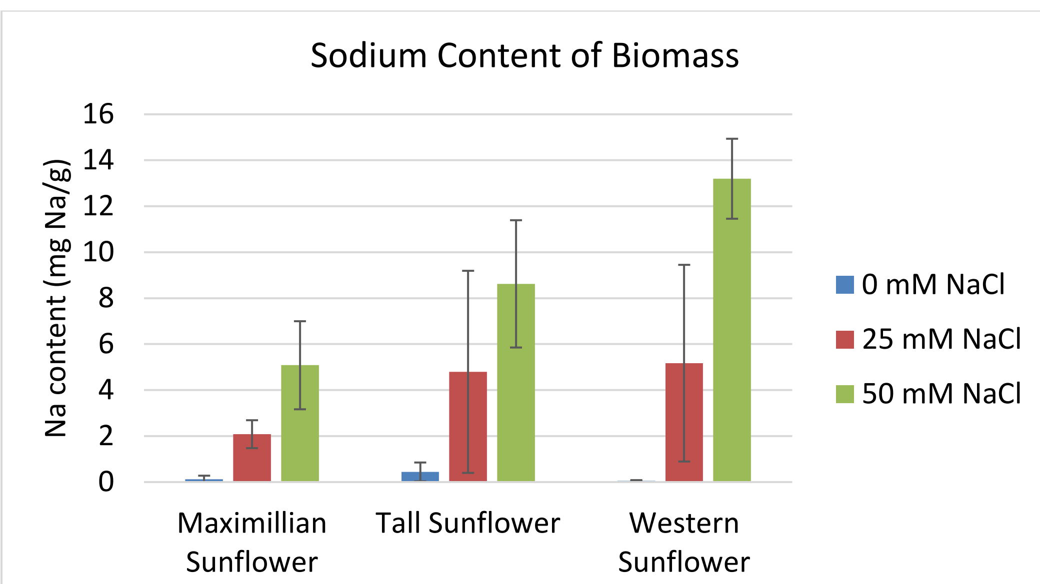


Figure 108: Sodium concentration in the aboveground biomass per gram of dry biomass for the three sunflower species at the three different salt concentrations.

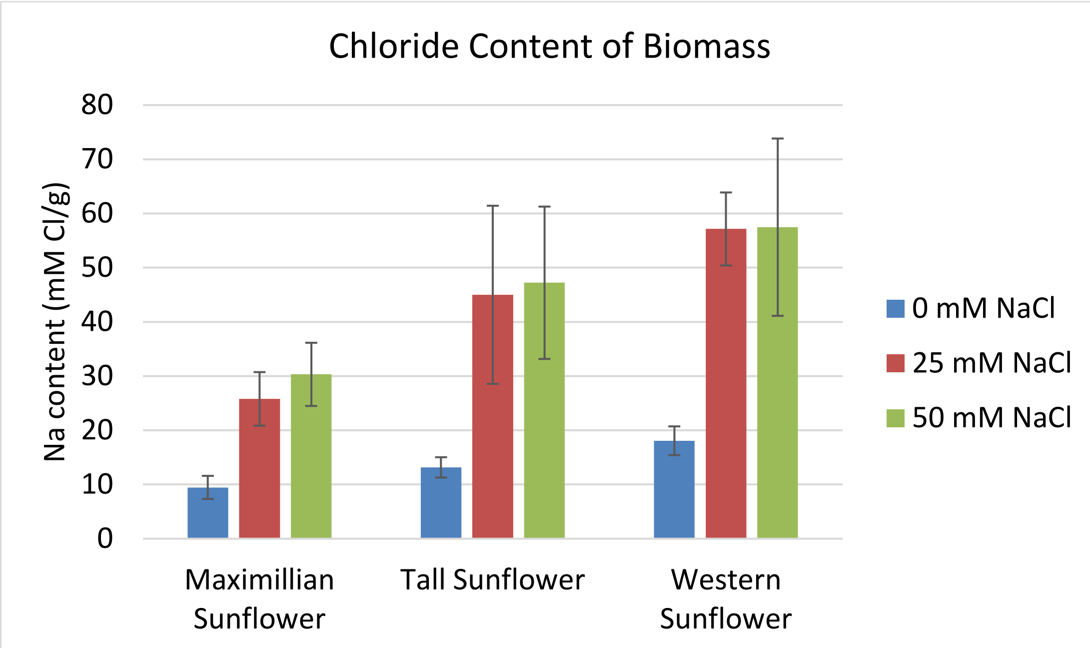


Figure 109: Chloride concentration in the aboveground biomass per dry gram for the three sunflower species at the three different salt concentrations.

In figures 110 and 111, the total sodium and chloride uptake per plant are shown for each species. While there is large variability with much of the data, shown by the large error bars, tall sunflower and wester sunflower seem to be trending a little higher than Maximillian sunflower. The perennial sunflowers total salt uptake results shown here are much lower than for common sunflower which took up around 51 mg Na and 162 mg Cl per plant (Figures 40 and 41).

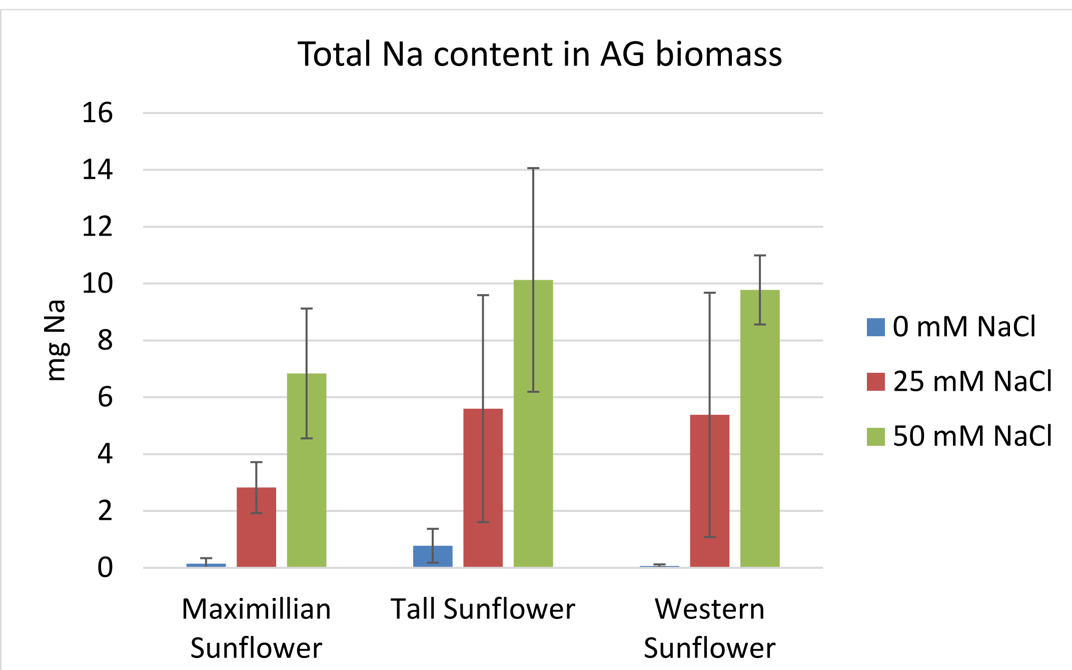


Figure 110: Total sodium uptake in aboveground biomass per plant. Calculated by multiplying the aboveground dry biomass by the sodium content of the biomass.

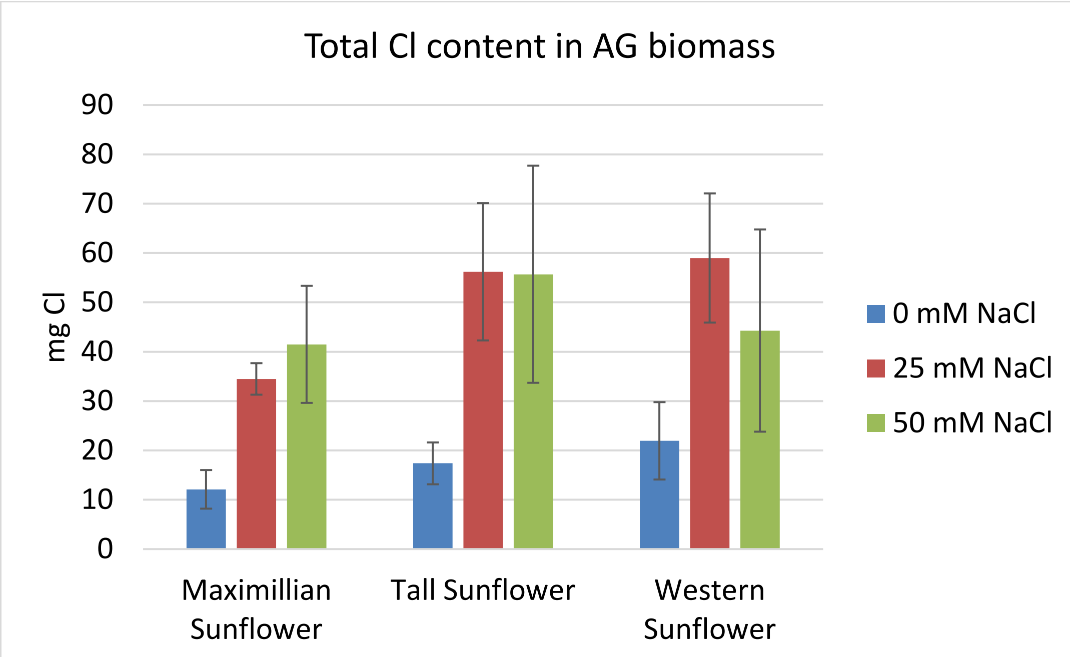


Figure 111: Total chloride uptake in aboveground biomass per plant. Calculated by multiplying the aboveground dry biomass by the chloride content of the biomass.

In summary, the perennial sunflower species in this experiment showed some salt tolerance but did not exhibit significant salt uptake ability seen in the previously performed experiments. Therefore, it is unlikely that these plant species will be strong candidates for phytoremediation. However, perennial sunflowers may have more access to salt during the spring thaw than annuals planted later in the season and are currently being compared with annuals in second field experiment to determine the difference in salt uptake.

Overall, based on the results detailed in this report, the most promising plant species for phytoremediation of deicing salt are common sunflower, pitseed goosefoot, and sugar beet. Common sunflower and sugar beets are commonly grown agricultural plants that have well established processes for utilization of their harvested biomass. Sunflowers are ranked the fourth most important oilseed crop after soybeans, rapeseed, and safflower and the sunflower meal obtained from the oil extraction contains high nutritional value for poultry. Sugar beet is a root crop grown in MN more than any other state in the U.S. The beets are primarily grown for their sucrose content but the byproducts of the sugar making process are also widely used as animal feed supplements. Also, the aboveground leaves of sugar beets can also be consumed by livestock. While pitseed goosefoot is not an established agricultural crop, the leaves and young shoots of pitseed goosefoot can be eaten. Its leaves are ranked high in protein and like alfalfa, It can be mechanically harvested for its dried greens. Pitseed goosefoot could be periodically harvested during the season and then allowed to reach seeding stage at the end of the season so that it can grow back the next year.

Another potential use for the harvested biomass of these plants is to burn them for energy and use the resulting ash with high NaCl content for reuse as deicing salt. Table 10 below shows the salt content of the aboveground biomass of these three species at the different concentrations from the fourth greenhouse experiment. The table also gives an estimate of the potential NaCl obtained from ashing the biomass assuming an accumulation of 500 dry g per meter squared, a value which has been seen in the range for each of these plant species. However, due to the lower than expected salt content of soil measured at our salted roadside sites, it is unlikely that the amount of salt in the roadside plant biomass will reach near these levels. Different remediation designs may however provide the potential for higher salt accumulation in aboveground biomass. Examples of these include rain gardens or constructed wetlands that treat the stormwater runoff. In these designs, the salt levels in the water may reach levels seen in the greenhouse experiments. Harvesting a square meter of common sunflower, sugar beet, and pitseed goosefoot in these designs and then ashing the biomass could result in a significant amount of NaCl available for reuse as deicing salt depending on plant species and salt content (Table 10). Also, different plant species that thrive in wetter conditions such as common glasswort, prairie cordgrass, and Canadian sandspurry could be used and investigated for their salt uptake potential.

Table 10: Final aboveground biomass sodium and chloride content of high potential plant species for phytoremediation in greenhouse experiments watered with 50 and 100 mM NaCl saline solutions. The last column on the right calculates the amount of potential NaCl obtained from ashing 1 m^2 of harvested biomass assuming 500 g dry biomass per m^2 that could be reused as deicing salt.

	Treatment (mM NaCl)	**mg Na/g dry biomass**	**mg Cl/g dry biomass**	**Total potential g NaCl obtained from burning 1 m^2 of biomass (assuming 500 g dry biomass per m^2)**
Common sunflower	50 mM	30.9	74.6	52.8
	100 mM	40.5	125.0	82.8
Sugar beet	50 mM	53.2	108.8	81
	100 mM	54.4	130.0	92.2
Pitseed goosefoot	50 mM	12.2	55.5	33.8
	100 mM	21.4	74.6	48.0

There are likely only a few roadside areas with soil salt levels at concentrations similar to those found by Biesboer & Jacobson, (1994) mostly at the intersections of major highways in the Twin Cities. In these highly contaminated salt areas is where the most promising plant species from this research such as common sunflower and pitseed goosefoot could be utilized to remediate the soil over the course of a few seasons. Also, it is recommended that these plants be used in a mix with perennial salt tolerant roadside species like tall fescue turfgrass, slender wheatgrass, buffalo grass, and big bluestem. Perennials help to stabilize the soil and many salt tolerant grass species have been shown to exclude salt from their roots, potentially providing more availability of the salt to the annual salt accumulators. Deep rooted perennials also continue to grow and use water during the seasons that do not support annual crop plants which helps prevent rising water tables and the movement of salt to the surface. If roadsides are vegetated with deep rooted perennials and annual salt accumulators, it is more probably that the salt will not enter the groundwater or reach the soil surface as easily and therefore there may be more salt uptake from the rooting zone of the plants.

Final Report Summary

In conclusion, the research detailed in this project report illustrate the high potential of some native MN plant species for phytoremediation of deicing salt. Specifically, common sunflower and pitseed goosefoot have so far shown the highest salt uptake and growth during the roadside field studies. These plants could effectively remove significant amounts of salt from highly contaminated roadsides over the course of multiple seasons and harvests. It is recommended that these plant species be mixed in with perennials from MnDOT's seed mixes to aid in soil structure and keeping the salt from ending up on the soil surface or in the groundwater. Harvesting of the plants should be performed towards the end of the summer in August or September to ensure that sunflower and pitseed goosefoot have produced seeds. It should be investigated how well the annual plants re-seed the area as it still may need to be re-seeded each year. Sugar beet, which had less overall growth in the first field study, did have the highest aboveground biomass salt concentration at around 20% in the greenhouse experiments when watered with 100 mM NaCl solution. Also, since sugar and other beets are significant global crops, the results from this research suggest they have great potential for phytoremediation of saline agricultural fields. Each of the promising salt phytoremediation species has the potential for the harvested biomass to be utilized as animal feed, burned for energy and salt reuse from ash, or in the case of sunflower, its seeds can be harvested for oil. The harvest and utilization of these plants will provide additional value and incentive for using these plants to remediate salt contaminated soil.

IV. DISSEMINATION:

Description:
We will publish two to three peer-reviewed manuscripts in the related journals to disseminate our results to the general public. We will also use the university extension website www.extension.umn.edu as well as PI's academic website http://bohu.cfans.umn.edu/ for dissemination of the research. A teaching module will be developed to teach students the concept of phytoremediation, lab plant cultivation, as well as basic analytical techniques on how to measure sodium and chloride concentration via the ion chromatography. The teaching module will be primarily targeting to the new coming freshmen students as well as potential undergraduate candidates to stimulate their interests in the STEM related field. We will also explore the possibilities to add this module to the UMN Summer Camp program or CFANS booth at MN State Fair to showcase the general public about our mission toward the overall environmental protection.

The primary target to disseminate our research results will be the scientific community, MNDOT as well as local community concerned with the road salt pollution. Information obtained from the plant cultivation experiments will be directly applied to establish possible implementations and business models in order to develop a sustainable solution for the road salt remediation. The research results will be fully disseminated to the public and we are not anticipating any patents or revenues from the project. However, any possible royalty, copyright, patent, and sales of products and assets resulting from this project will be subject to revenue sharing requirements with ENRTF according to Minnesota Statutes, section 116P.10.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

First Update March 1, 2020

A poster presentation was made at the Biorpoducts and Biosystems Engineering Annual Department Showcase. PhD student Leif van Leirop will use this project as his PhD dissertation and he is in the process to write his dossier for preliminary defense. Dr. Noah Strom is also currently taken this project for his Postdoc training.

Second Update September 1, 2020

The PhD student Leif van Lierop is taking the project as his PhD research topic and he has been working on his dossier and preparing for his preliminary exam. Dr. Noah Strom is also currently taken this project for his Postdoc training.

Third Update March 1, 2021

The PhD student Leif van Lierop is continuing to write his dossier for preliminary defense.

Fourth Update September 1, 2021

PhD student Leif van Lierop completed his dossier and has begun writing his first manuscript on this research.

Fifth Update March 1, 2022

PhD student Leif van Lierop has continued writing his first manuscript on this research.

Final Report between project end (June 30) and August 15, 2022

We worked with Cindy Dorn for a story at Prairie Sportsman about phytoremediation of road salt

Final Report Summary

The detailed research results are in the final report; and we are drafting two manuscripts for possible publications. Leif was accepted for presenting this work at AIChE Annual Meeting in November 2022 in Phoenix, Arizona and he was invited to give a presentation at the MECA (Minnesota Erosion Control Association) Annual Conference in January 2023. We have been working at MnROAD site with support and help from MnDOT. Cindy Dorn, writer/producer with Prairie Sportsman, an outdoor show produced by Pioneer PBS that airs on all Minnesota PBS stations is drafting a story on absorbing deicing salts with plants.

V. ADDITIONAL BUDGET INFORMATION:
A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours for entire duration of project: 7960	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 1.3

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Contract Personnel Hours for entire duration of project: 0	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: 0

VI. PROJECT PARTNERS:

A. Partners outside of project manager's organization receiving ENRTF funding: N/A

B. Partners outside of project manager's organization NOT receiving ENRTF funding
We are partnering with the MN Department of Transportation for our field study.

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The project will have a broad impact on both academia and industry. The results will provide fundamental knowledge on how these native plants mobilize and excrete salt in the soil. The possible applications will lead to sustainable developments in road salt management and agricultural practices, and alleviate the deteriorating conditions related to road salt application and improper irrigation.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2022

IX. SEE ADDITIONAL WORK PLAN COMPONENTS: N/A

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet
D. Acquisition, Easements, and Restoration Requirements
E. Research Addendum

Reference:

"Minnesota plant lists " Minnesota Stormwater Manual, from https://stormwater.pca.state.mn.us/index.php/Minnesota_plant_lists.

"Red Saltwort, Salicornia europaea ssp. rubra, Rare Species Guide, MN DNR." Rare Species Guide Retrieved Feb 4th, 2018, 2018, from http://www.dnr.state.mn.us/rsg/profile.html?action=elementDetail&selectedElement=PDCHE0J020.

(2017). "Environmental Impacts of Road Salt and Other De-Icing Chemicals, Minnesota Stormwater Manual , Minnesota Department of Natural Resources, ." Minnesota Stormwater Manual Retrieved 15 May 2017, from stormwater.pca.state.mn.us/index.php/Environmental_impacts_of_road_salt_and_other_de-icing_chemicals.

Bhatt, J. G. and K. N. Indirakutty (1973). "Salt uptake and salt tolerance by sunflower." Plant and Soil **39**(2): 457-460.

Cooper, C. A., P. M. Mayer and B. R. Faulkner (2014). "Effects of road salts on groundwater and surface water dynamics of sodium and chloride in an urban restored stream." Biogeochemistry **121**(1): 149-166.

Friell, J., E. Watkins and B. Horgan (2013). Salt Tolerance of 74 Turfgrass Cultivars in Nutrient Solution Culture.

Gerhardt, K. E., G. J. MacNeill, P. D. Gerwing and B. M. Greenberg (2017). Phytoremediation of Salt-Impacted Soils and Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Enhance Phytoremediation. Phytoremediation: Management of Environmental Contaminants, Volume 5. A. A. Ansari, S. S. Gill, R. Gill, G. R. Lanza and L. Newman. Cham, Springer International Publishing**:** 19-51.

Glenn, E. P., J. J. Riley, R. S. Swingle, C. U. Mota, M. C. Watson and V. R. Squires (1994). North American halophytes: Potential use in animal husbandry. Halophytes as a resource for livestock and for rehabilitation of degraded lands. V. R. Squires and A. T. Ayoub. Dordrecht, Springer Netherlands**:** 165-174.

Hasanuzzaman, M., K. Nahar, M. Alam, P. Bhowmik, A. Hossain, M. M. Rahman, P. Majeti, M. Ozturk and M. Fujita (2014). Potential Use of Halophytes to Remediate Saline Soils.

Hoagland, D. R. and D. I. Arnon (1950). "The water-culture method for growing plants without soil." Circular. California Agricultural Experiment Station **347**(2nd edit): 32 pp.

Jesus, J. M., A. S. Danko, A. Fiúza and M.-T. Borges (2015). "Phytoremediation of salt-affected soils: a review of processes, applicability, and the impact of climate change." Environmental Science and Pollution Research **22**(9): 6511-6525.

Malcolm, C. V., A. J. Clarke, M. F. D'Antuono and T. C. Swaan (1988). "Effects of plant spacing and soil conditions on the growth of five Atriplex species." Agriculture, Ecosystems & Environment **21**(3): 265-279.

Morteau, B., G. Triffault-Bouchet, R. Galvez, L. Martel, S. Leroueil, M. Dyer and S. Dean (2009). "Treatment of Salted Road Runoffs Using Typha latifolia, Spergularia canadensis, and Atriplex patula: A Comparison of Their Salt Removal Potential." Journal of Astm International **6**.
Novotny, E. M., Dan; Stefan, Heinz. (2007). "Road Salt Effects on the Water Quality of Lakes in the Twin Cities Metropolitan Area. St. Anthony Falls Laboratory, University of Minnesota Digital Conservancy ", from http://hdl.handle.net/11299/115334.
Qadir, M., J. D. Oster, S. Schubert, A. D. Noble and K. L. Sahrawat (2007). Phytoremediation of Sodic and Saline - Sodic Soils. Advances in Agronomy, Academic Press. **96:** 197-247.
Qadir, M., E. Quillérou, V. Nangia, G. Murtaza, M. Singh, R. J. Thomas, P. Drechsel and A. D. Noble (2014). "Economics of salt-induced land degradation and restoration." Natural Resources Forum **38**(4): 282-295.
Sheridan, R. M., Brian; Harrison, Bryan D; Manteuffel, Chris (2010). RIDOT Salt Tolerant Tree and Shrub Guide. R. I. D. o. Transportation.
Wang, S.-M., J.-L. Zhang and T. J. Flowers (2007). "Low-Affinity Na⁺ Uptake in the Halophyte Suaeda maritima." Plant Physiology **145**(2): 559-571.

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet
Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04i
Project Manager: Bo Hu
Project Title: Phytoremediation for Extracting Deicing Salt from Roadside Soils
Organization: University of Minnesota
Project Budget: $360,000
Project Length and Completion Date: 3 years, June 30th, 2022
Today's Date: August 15th, 2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Budget 3/28/2022	Expenditure 6/30/2022	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 327,969	$ 326,756	$ 1,213
Bo Hu, Project Manager (74.5% Salary, 25.5% Benefits), 10% FTE per year for three years			
Research Associate (74.5% Salary, 25.5% Benefits), 50% FTE for 3 years			
Graduate student, Research assistant, 50% FTE			
Undergraduate student researcher, 400 hours			
Research Associate (74.5% Salary, 25.5% Benefits), 50% FTE for the third year on the field study			
Professional/Technical/Service Contracts			
Professional analysis service for water and solid samples at other UMN analytical labs	$ 4,365	$ 4,644	$ (279)
Equipment/Tools/Supplies			
Supplies for the lab experiments to purchase necessary chemicals, test kits, culture medium, and	$ 23,215	$ 25,484	$ (2,269)
Capital Expenditures Over $5,000			
	$ -	$ -	$ -
Fee Title Acquisition			
	$ -	$ -	$ -
Easement Acquisition			
	$ -	$ -	$ -
Professional Services for Acquisition			
	$ -	$ -	$ -
Printing			
Publication costs for two/three papers, page charges	$ -		$ -
Travel expenses in Minnesota			
In-state travel (Mileage, lodging, and meals) to the site for collecting water samples	$ 4,451	$ 3,116	$ 1,335
Other			
	$ -	$ -	$ -
COLUMN TOTAL	$ 360,000	$ 360,000	0

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		$ -	$ -	$ -
State:		$ -	$ -	$ -
In kind: UM F&A		$ 169,254	$ 169,254	0

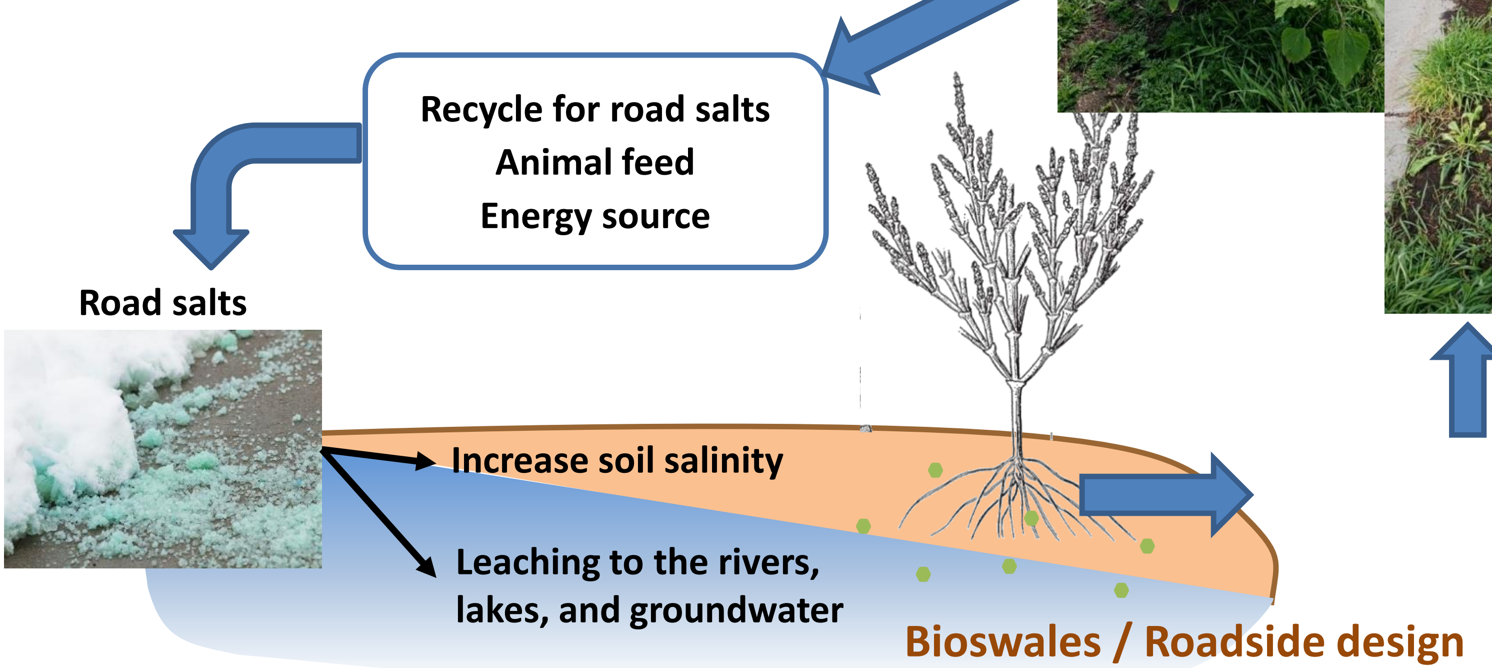
PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:		$ -	$ -	$ -
Past appropriations:		$ -	$ -	$ -

Phytoremediation for Extracting Deicing Salt from Roadside Soils

Bo Hu, Bioproducts and Biosystems Engineering, University of Minnesota

Environment and Natural Resources Trust Fund-2019

Pitseed goosefeet, sugar beet, and sun flowers are proved native species that can effectively extract salt from affected soil



Activity 1: Screening of native Minnesota halophytic plants

Activity 2: Plant growth test on salt-affected soil

Activity 3: Develop possible utilization of harvested plant biomass