Response of *Salix purpurea* growth to fixed schedules of salinity exposure

Sophie Georgel-Walz

Laboratory Section C10

Rebecca Robich

3/21/2018

Abstract

Global pollution has been increasing in across land and sea. Concerns have emerged over the increased salinization of the ocean, and even freshwater-sheds. Plants have experienced negative effects ranging from stunted growth to death due to unnaturally high concentrations of salt in soil and water. Thus, this study was designed to assess various concentrations (low, medium, and high) of salt on *Salix purpurea* growth and survival over two different time intervals. Hydroponic systems were implemented to imitate the riverbank environment common to willows. Data was collected to determine root and shoot growth, overall biomass and phytoremediative abilities. Significant differences were found between salt concentration groups when tested for leaf length, root length, and shoot length. There were also significant interactions between concentration and duration. The low group showed little distress while the high groups perished. The medium groups, which originally seemed to die off as well, showed strong resilience and recovered substantially during the recovery weeks. The conductivity did not show any significant reduction. This suggests that willows are in fact tolerant to high concentrations of salt, however, it cannot be inferred that willows are good filters for NaCl based on this data. Results suggest that willows are a viable choice for designing systems to filter pollution and thrive.

Quick solutions to common issues are often implemented before proper research is completed. The use of road salt as a deicer, for example, seemed like a great option when it was first discovered. The idea that sodium chloride could be detrimental to the entire biosphere was not considered, however, the ecological research that focuses on impacts of road deicing has finally come to light with shocking results.

Highways in the United States have a huge impact on surrounding ecosystems. In winter, this cyclic effect is primarily from the 200 to 500 pounds of deicing salt spread per mile. This accumulates to anywhere from 8 million to 12 million tons per year (Novotony *et al*. 2008; FHWA 1974; TRB 1988). Even the storage of salt during the summer has been known to be detrimental to the surroundings (Wilcox 1986). The constant exposure to salt causes substantial and toxic above and below surface build-up in concentrations. Often, failures in irrigation structure design can create ruts in which salt can accumulate over time (Appleton *et al.* 2015). An average of 55% of the salt is left behind after removal to disperse into the surrounding land (Howard & Haynes 1993; Kelly *et al.* 2008). Salt use has also been connected to an increase in zinc, phosphorus, and lead depending on the location of use (Oberts 1986; Ramakrishna & Viraraghavan 2005). The spread and increase in salinity over the years does massive damage to the surrounding vegetation.

Through many routes, the high salinity water from the roads finds its way into the ocean water. Hills and mountains lead to low lying valleys where the salt accumulates until the next heavy rain washes it further on its path (Appleton *et al.* 2015). In New Zealand, an exceptionally hilly country, erosion has been a serious issue. Sheet erosion, earth flow, soil slips, and gully erosion are just some of the issues that plague the country. Not only can salt exposure increase this, but the land pollutes the waterways with more unnecessary ions (McIvor *et al.* 2011).

Because New Zealand is a small island, it can be inferred that it does not take the polluted water long to stream into the ocean.

Ocean acidification is currently a hot topic in the world of ecological research. Because the oceans are all connected, the harmful effects cannot be contained (Poertner 2008). The long-term use of road deicing salt has added to the damaging effects of over-salinization on many aquatic species (Kaushal *et al*. 2005; Kroeker *et al*. 2013). For example, aquatic species across the planet are suffering from lower levels of reproduction and eventually death to the animal, and in the long run the species, due to increases in ocean salinity (Kroeker 2010). Lower level invertebrates are very sensitive to these environmental changes. These organisms are very sensitive to changes in their ecosystem, such as ion imbalances or heat changes. This limits their geographical niche in addition to their impacted growth and reproductive stages (Poetner 2008). While ocean water does experience an increase in salt concentration, the impact on freshwater ecosystems is substantially worse.

Freshwater salinization syndrome is a phenomenon characterized by a 37% increase in salinity in the United States river water systems (Kaushal *et al*. 2018). In areas like Appalachia, these rivers are the principle source of drinking water, as well as the habitat for many species of fish and amphibians, and have had noticeable increases in salt contamination (Stets *et al*. 2018; Wilcox 1986). The sodium chloride concentrations encourage the release of other toxic chemicals into the drinking water (Novotny *et al.* 2008). The Appalachian region, in general, suffers from greater damages caused by high salinity. The topological aspects of the Appalachian Mountains make them an ideal place for hillside watershed contamination (Jones & Patrick 1998). In these areas, where coal mining is still common, there is an additional contribution of excess salt from mine disturbances (Novotony *et al*. 2008). Some of these disturbances are even

caused by the higher salinity in water, which has the ability of corroding man-made structures and releasing even more pollutants into the stream (Stets *et al*. 2018). While waterways are substantially affected by over-salinization, the surrounding ecosystems also take a hit.

In Western Australia, it has been reported that freshwater ecosystems, important for their agricultural land, have been heavily influenced by localized salinization (Nielsen *et al*. 2003; George *et al* 1997). The high salt concentrations restrict key components to plant growth such as access to water and metal availability. This impact in crop growth has led to upwards of 50% mortality rates (Simon 1978; Pitman & Laeuchli 2000). In the more colonized urban areas, vegetation has also shown negative responses to increases in salt in the environment. This effect has even been noticed in areas of low natural salt levels (Ordonez-Barona *et al*. 2018; Novotny *et al* 2008). The majority of new salt that enters these environments is distributed with a purpose, to deice the roads (Obert 1986).

While studying extended salt exposure, Kelly *et al*. found that stress on the plant will continue to increase until it ultimately dies (2008). These environments never return to baseline and have noticeably elevated salinity through the entire winter and spring causing increasing damages to the surrounding vegetation as the season progresses (Rife 2016). This is backed by the idea that repeated and extended exposure are both correlated with poor plant growth when compared to shorter or single-time exposures (Appleton *et al*. 2015). By altering the wavelength reflection, stress impairs the plants ability to photosynthesize and create sustainable energy, as well as take up nutrients vital for plant growth and reproduction (Wang, Wilson & Shannon 2010; Aronsson & Perttu 2001; Golldack *et al*. 2014). The more salt that is present, the more energy the plant needs to draw in water. Without a sufficient way of holding water, the plant cannot maintain homeostasis and will likely wither and eventually perish (Letey 2007).

Kodikara et al. tested salt exposure on mangrove trees for 30 weeks and discovered high salinity also correlated with stunts in shoot growth, leaf area, and dry weight. The tolerance levels varied based on the age of the saplings, those exposed to lower levels actually seemed better established by the 20 week mark (2017) Young transplants also have a tendency for being sensitive to increases in salinity. Only plants that are halophytic, or salt lovers, have been tested for their significantly enhanced photorespiration and osmoprotectants (Appleton *et al.* 2015; Parida & Das 2005). Essentially, a plant must be highly resilient to survive in this new high salinity environment.

Salix species have a surprisingly high tolerance to poor growth conditions. When grown in areas of high nitrogen, they have actually worked to reduce the levels of nitrogen in the soil (Aronsson & Perttu 2001). Several species, similar to willows in many aspects, also manage to grow and thrive without fertilizer and in the presence of heavy metals (Scholz & Ellerbrock 2002).

Known for their useful phytoremediation properties, willows are an amazing ecological filter (Elowsona 1999; Kasuga *et al.* 1999; Licht & Isebrnds 2005). Additionally, Salix does not lose large sums of biomass when used as a natural filter, making it a good candidate for harvesting as biofuel (Vyslouzilova *et al.* 2003). These species have also been used for hybridization and cloning purposes. Using genetic manipulation, scientists have been able to find and isolate genes specific to salt and metal tolerance (Mitchell, Sennerby-Forse & Zsuffa 1988; Simon 1978). If these genes can be enhanced, willows would be an excellent short rotation biomass source for biofuels. They have already been used to reduce nitrogen in irrigation water and greenhouse gasses (Scholz & Ellerbrock 2002; Sune 1999; Karp *et al.* 2011). Given their

tolerance to poor conditions, manipulation possibilities, and phytoremediation capabilities, willows are a strong candidate for environmental restoration.

The objective of this study is to determine the tolerance of Salix purpurea in different concentrations and durations of exposure. The results can be used to determine if this strain of willow could be used to fight issues like erosion, pollution, and the on-going depletion of non-renewable fuels. *S. purpurea* is a specifically tolerant species of willow. In order to determine its use as a filter for excess salt, the properties must be tested. Two separate salt durations will be used as well as different levels of salt. We hypothesize that the plants that are exposed long-term to the highest concentration of salt will have the most apparent stunts in growth. The *S. purpurea* at low concentrations will show fewer signs of stress when compared to the higher concentrations. The plants in lower dosages are expected to filter salt from the water on a weekly basis. If the willow successfully endures the salt exposure, it can be explored further as an effective tool for the filtration of soil and water (Baker 2009).

Methods

Preparation and Soaking

Cuttings of *Salix Purpurea* were collected from Fish Creek at West Virginia University's Agronomy farm. These were trimmed into 64 individual 6-inch cuttings and were soaked overnight (less than 24 hours) in a container filled with tap water. Once dried, the cuttings were dipped in 1:100 ZeroTol for approximately 1 minute and allowed to dry again. The top end, which was chosen based on the buds with triangles pointing upwards, was dipped in melted paraffin wax to prevent deterioration of the cut end. The bottom halves of the cuttings were

placed in 0.4 mM calcium nitrate solution in 7 L of deionized water. This soaking solution was used for two weeks.
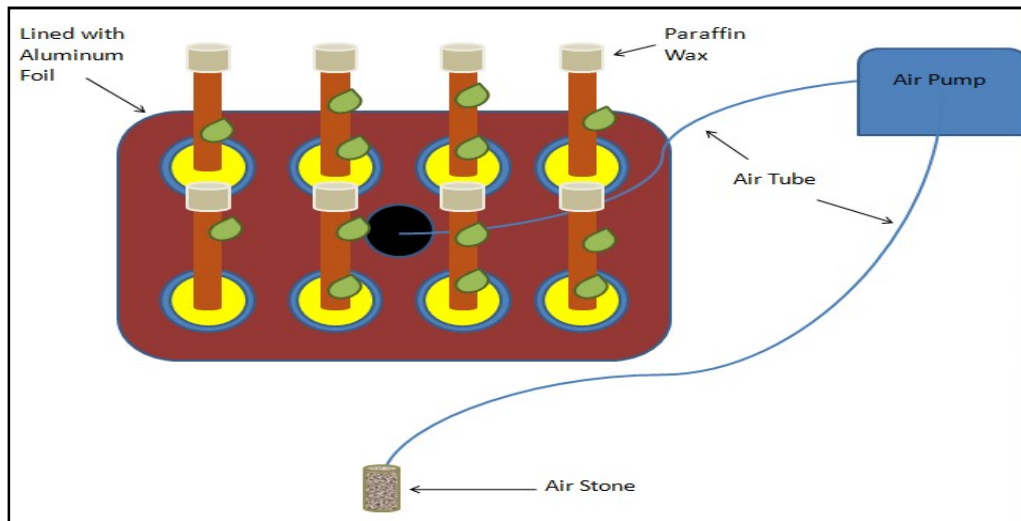
Hydroponic Setup

Figure 1. Hydroponic Set-Up. Each bucket received 8 cuttings and an air pump equipped with an air stone. Each air pump supplied two buckets with air. Buckets were filled with 7L of Hoagland's solution (Hoagland & Arnon 1950) which was diluted in deionized water.

Hydroponics systems were utilized, with cuttings placed into 8 buckets, 8 plants each, as seen in Figure 1. The plants were held in the buckets using foam pieces with about 1/3 of the cutting above the bucket lid. Each bucket was wrapped in aluminum foil to discourage algae growth and air stones were placed to keep oxygen in the water. Four pumps were used to run air through the buckets.

Experimental Design

The cuttings were distributed into 8 per treatment group. The different treatments the groups were exposed to were low salt concentrations 1g/L, medium 6g/L, high 12g/L, or the control

0g/L. The groups were split into Group A which was one week on treatment and one week off, and Group B, which was two weeks on treatment two weeks off. A schedule by week can be seen in Figure 2.

Salt Treatment	On-Treatment Schedule Group A (n=32)	On-Treatment Schedule Group B (n=32)
Control (0g/L)	Weeks 1, 3, 5	Weeks 1, 2, 5, 6
Low (1g/L)	Weeks 1, 3, 5	Weeks 1, 2, 5, 6
Medium (6g/L)	Weeks 1, 3, 5	Weeks 1, 2, 5, 6
High (12g/L)	Weeks 1, 3, 5	Weeks 1, 2, 5, 6

Figure 2. Experimental Setup. Group A and B were given salt treatments on the weeks listed above. The water and salt was replaced three times a week with the same concentrations. Each treatment group had 8 replicates and formed a 4x2 factorial design.

The treatments were administered in a mixture of 1L Hoagland's solution and 6L of deionized water. The solution was replaced every Monday, Wednesday, and Friday for six weeks. During the on-weeks, salt was added back into the solution each time it was replaced.

Measurements and Analysis

Average root length (cm) was measured for each treatment group. This was taken from the stem of the cutting at the root base to the tip of the root. The leaf length, from branch to tip of the leaf, average was also taken and recorded in centimeters. Chlorophyll content percentage was determined using a SPAD meter. These measurements were taken from randomly chosen leaves and roots of each treatment type and then averaged.

Each time the solution was replaced, the conductivity (in μS) of the solution was recorded before and after dumping using a conductivity probe. Photos were taken to determine physical changes within the plants, such as recovery after a salt dose. On the final day of the experimental treatment, turgor pressure was recorded using a pressure probe. The probe calculated the rate of gas exchange of the leaf. Additionally, dry biomass for the roots and the shoots of each bucket and leaf count were recorded to determine growth.

Statistical Analysis

The results of the various measurements were analyzed as a two-way factorial. Using a two-way ANOVA the interaction between salinity and duration of treatment was determined. SAS JMP was implemented to test for significant interactions in the data (a < 0.05, p-value < a). A p-value less than alpha indicated that salinity's effect on *S. purpurea* was significant when compared to the exposure duration. Biomass measurements were analyzed and compared using the Tukey-Kramer Honest Significant Difference test. Scatter plots and linear regressions were designed to interpret the results of the SPAD meter measuring chlorophyll content per μM/m² of leaf and conductivity probe.

Results

The two groups, one week treatments and two week treatments, showed significant differences relative to concentration. A two-way factorial ANOVA was completed for leaf length, chlorophyll content, root length, and shoot length. SPAD meter readings were only comparable between the control and the low groups, seen in Figure 7, due to a lack of leaves. No significant differences were found between the different plant groups. Chlorophyll content between the control and low group had no significant differences. While there were no individual

significant results due to concentration and time, as seen in Figure 6, the effects test revealed a correlational significance of the interaction between the two factors and the leaf length (p=0.0163). Root length (salt: p=0.0009; time: p=0.0011) and shoot length (salt: p=0.0367; time: p=0.0283) each had significant results based on concentration of salt and time individually. No correlations were found between the two factors and root or shoot length (p=0.1351). When the root length was tested, the results (p=0.0041) indicated a correlation between the factors. The changes in root length can be seen in Figure 2.

The biomass of the groups was the combined dry weight of the roots and shoots. The comparison of the weights between the different concentration and time groups can be seen in Figure 3. Biomass was collected at the end of the experiment and composed of the cumulative roots and shoots of each test group; significance was determined using the Tukey-Kramer method. There were no significant differences between the control and low groups, or the medium and high groups. Significant differences were found between the low and control groups and the medium and high groups.

Discussion

Based on these results, it is safe to conclude that the hypothesis that lower concentrations of salt would have fewer symptoms of stress like chlorosis, wilting, and stunted growth of roots and shoots, has been supported. The results of the study do have some important implications. Regarding leaf length, there was a correlation between duration of time that the plants were exposed to salt and the concentration of salt applied. The plants exposed to low salt for longer periods of time had significantly shorter leaves (M=8.1) than the plants exposed to low salt for

short periods of time (M=8.9) and those that were not exposed to salt (M=8.98). The chlorophyll content did not show any note-worthy differences among the control and low groups.

Root length, seen in Figure 2, was compared with the medium salt concentration groups included. There were significant correlations between longer roots and the concentration of salt added. When concentration effects were paired with the effects of duration, there was an interesting trend. For the low groups, the shorted concentrations had significantly longer roots; however, in the medium group, the longer duration groups had the larger roots. This might implicate the idea that the plants that had longer recovery time managed to adapt easier than the groups that were thrown back into the salt after a week.

Shoot length had significant differences among the concentration groups and the timing at which the concentration was applied. No correlation between the two factors was found. This was likely due to the exclusion of the medium and high results. Biomass had several significant differences among groups. Groups with less salt did much better in terms of growth mass of roots and shoots than those with higher concentrations. Additionally, the biomass was dependent on duration. While low had a higher mass on the one week schedule, medium had a higher biomass for the two week group, shown in Figure 3.

However, these results, excluding root length (which has the medium group), have excluded the medium and high groups. The average leaf length, chlorophyll content, and shoot length for these groups could not be recorded. The leaves died off quickly with the continued addition of salt which did not allow for accurate measurements. This does not mean that the results were not important. On the contrary, it gives an idea of what levels of salt might be lethal for *S. purpurea.* Interestingly, the roots for the medium groups were still attempting to sprout

during the weeks where they did not receive salt treatment. This implies that the plants were attempting to recover and had not completely been wiped out by the salt.

The higher concentrations were hypothesized to show more of these signs of stress. Wilting was certainly apparent among all medium and high concentrations; however, chlorosis could not be measured once the leaves had died. Surprisingly, the plants exposed to salt over longer terms had regeneration of their roots disproving the hypothesis that they would have more stunted growth than the groups that had shorter salt durations of one week. The experiment completed by Mirck and Volk may have some answers. For their five different concentrations of salt exposure, *S. Purpurea* seemed to be suffering until the 4 week point, when it began to adapt (2010). Similar recovery patterns can be seen in Figure 4.

No conclusive significant results could be drawn based on water conductivity. The salt concentrations fluctuated at a seemingly random rate. This may have been due to salt build up in the containers or faulty measurements of the conductivity probe. Additionally, it may have been caused by a condition known as salt burn, where the excess salt in the water actually leaches the rejected salt from the roots (Appleton *et al.* 2015). Regardless of the cause, this disproves our hypothesis that willows could be used as salt filters.

Though the salt concentration changed over time, as seen in Figure 5, it showed an overall increase rather than decrease. Further research has been found suggesting that willows can pull other pollutants from waterways. The same research also found that soil surrounding willow farms has lower concentrations in other potentially harmful sources such as nitrogen (Elowsona 1999). Willows could still have potential uses for removing other harmful chemicals and elements that are released via salt corrosion.

Pollution in urban areas is becoming a very common issue. A natural source of filtration, such as, *S. purpurea,* might be key to reducing the negative impacts without adding more chemicals to the environment (Baker 2009). Some cities have begun to implement rain gardens to reduce pollutants in the water. They already have a solid reputation as natural filtration systems (Good *et al.* 2011). Reducing factors such as over salinization of environments will benefit surrounding vegetation making them more resilient to other negative impacts (Baker 2009). If the genes that code for filtration could be found, they may have the ability to be altered to filter out salt as well. This gene could then be spliced into the willow and similar plants (Kasuga *et al.* 1999).

Willows have the amazing ability to hybridize without human interference. This is essential for Salix species survival in difficult environments. This self-modification can only spread within the same genus, making artificial gene modification an essential tool in current scientific studies (Kuzovkina & Volk 2009). Adding salt tolerant genes from willows to other plants could create more resilient crops (Pitman & Laeuchli 2000). Specific genes have already been located and analyzed for their ability to adapt to colder, drier, and saltier environments. For example, the DERB1A gene (dehydration response element) can be promoted using rd29A to increase survival by over 60% (Kasuga *et al.* 1999). Promoting the genes in willows or splicing them into agricultural plants could essentially save various crops from toxic levels of pollution (Pitman & Laeuchli 2000).

Salix purpurea has been cultivated for its large biomass yield. A genetically mutated plant could be planted in old mining zones to create bioenergy farms or along road sides with run-off pollution (Hangs *et al*. 2011). After the willow cleans the soils of other toxic nutrients, it can later be harvested (Elowsona 1999; Kasuga *et al.* 1999). As the world runs low on non-

renewable resources, solutions must be found. The current methods of creating biomass are slow moving. The efficiency can be increased by planting trees with increased biomass to decrease the energy lost on pretreatment (Serapiglia *et al.* 2013; Zhu *et al.* 2010). The International Energy Agency is utilizing short-rotation plants with efficient growth rates for biofuels (Wright 2006). Willows have the perfect structure to match these criteria (Licht and Isebrands 2005).

In an attempt to find more water in a salinized environment, willows spread their roots. The biomass of these roots is not negatively impacted by the salt ions. Additionally, these roots help them to thrive in nutrient poor soils (Cosio *et al.* 2005). Their strong overlapping systems of roots are used to reduce river bank erosion in addition to their biomass capabilities. The radial distributions of *S. purpurea*'s roots give it a superior and dense structure for preventing landslides (McIvor *et al.* 2011). Additionally, as perennial plants, the roots do not die off during the seasons. Instead the limbs go dormant through the winter (Elowsona 1999).

The innate ability of *S. purpurea* that allows for substantial and quick growth of biomass and roots are vital for new research. Their tolerance to withstand nutrient deficiencies and pollution are worthy of further study to decrease current ecological issues.

Acknowledgments

References

Appleton, B., V. Greene, A. Smith, S. French, B. Kane, L. Fox, A. Downing, and T. Gilland. 2015. Trees and Shrubs that Tolerate Saline Soils and Salt Spray Drift. Virginia Cooperative Extension.

Aronsson, P., and K. Perttu. 2001. Willow vegetation filters for wastewater treatment and soil remediation combined with biomass production. The Forestry Chronicle 77(2): 293-299.

Baker L.A. 2009. New concepts for managing urban pollution. The Water Environment of Cities: 69-91.

Cosio, C., P. Vollenweider., and C. Keller. 2005. Localization and effects of cadmium in leaves of a cadmium-tolerant willow (Salix viminalis L.)" Macrolocalization and phytotoxic effects of cadmium. Environmental and Experimental Botany 58(3): 25-40.

Elowsona, S. 1999. Willow as a vegetation filter for cleaning of polluted drainage water from agricultural land. Biomass and Bioenergy 16(4): 281-290.

FHWA. 1989. Highway statistics 1988. U.S. Department of Transportation.

George, R., D. McFarlane, and B. Nulsen. 1997. Salinity threatens the viability of agriculture and ecosystems in western Australia. Hydrogeology Journal 5: 6-21.

Golldack, D., C. Li, H. Mohan, and N. Probst. 2014. Tolerance to drought and salt stress in plants: unraveling the signaling networks. Frontiers in Plant Science 5(151): 1-10.

Good, J., A.D. O'Sullivan, D. Wicke, and T.A. Cochrane. 2011. 'Appreciating' drainage assets in New Zealand cities: rain garden treatment and hydraulic performances. UC Research Repository.

Hangs, R.D., J.J. Schoenau, K.C.J. Van Rees, and H. Steppuhn. 2011. Examining the salt tolerance of willow (*Salix spp.*) bioenergy species for use on salt-affected agricultural lands. Canadian Journal of Plant Science 91: 509-517.

Hoagland D.R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station: 4-32.

Howard, K.W., and J. Haynes. 1993. Groundwater contamination due to road de-icing chemicals – salt balance implications. Journal of the Geological Association of Canada 20: 1-8.

Jones, J.B., and M.J. Patrick. 1998. Influence of drainage basin topography and elevation on carbon dioxide and methane supersaturation of stream water. Biogeochemistry 40(1): 55-72.

Karp, A., S.J. Hanley, S.O. Trybush, W. Macalpine, M. Pei, and I. Shield. 2011. Genetic improvement of willow for bioenergy and biofuels. Journal of Integrative Plant Biology 53(2): 151-165

Kasuga, M., L. Qiang, S. Miura, Yamaguchi-Shinozaki, and K.K. Shinozaki. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnology 17: 287-291.

Kaushal, S.S., G.E. Likens, M.L. Pace, R.M. Utz, S. Haq, J. Gorman, and M. Grese. 2018. Freshwater salinization syndrome on a continental scale. National Academy of Science: 1-10.

Kelly, V.R., G.M. Lovett, K.C. Weathers, S.E. Findlay, D.L. Stayre, D.J. Burns, and G.E. Likens. 2008. Long-term sodium chloride retention in a rural watershed: legacy effects of road salt on streamwater concentration. Environmental Science and Technology: 410-415.

Kodikara, K.A., L.P. Jayatissa, M. Huxman, F. Dahdouh-Guebas, and N. Koedam. 2017. The effects of salinity on growth and survival of mangrove seedlings changes with age. Acta Botanica Brasilica 32.

Kroeker, K.J., R.L. Kordas, R. Crim, I.E. Hendriks, L. Ramajo, G.S. Singh, C.M. Duarte, and J.P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology 19: 1884-1896.

Kroeker, K.J., R.L. Kordas, R.N. Crim, and G.G. Singh. 2010. Meta-analysis reveal negative yet variable effects of ocean acidification on marine organisms. Ecology Letters 13: 1419-1434.

Kuzovkina, Y.A., and T.A. Volk. 2009. The characterization of willow (*Salix L.*) varieties for use in ecological engineering applications: Co-ordination of structure, function and autecology. Ecological Engineering 35(8): 1178-1189.

Letey, J. 2000. Soil salinity poses challenges for sustainable agriculture and wildlife. California Agriculture 52(2):43-48.

Licht, L.A., J.G. Isebrands. 2005. Linking phytoremediation pollutant removal to biomass economic opportunities. Biomass and Bioenergy 28: 203-218.

Mirck, J., and T.A.Volk. 2010. Response of three shrub willow varieties (*Salix spp.*) to storm

    water treatments with different concentrations of salts. Bioresource Technology 101(10):

    3484-3492.

Mitchell, C.P, L. Sennerby-Forse, and L. Zsuffa. 1988. Biomass qualities and potential for

    genetic improvement in poplars and willows. Biomass 17: 21-37.

McIvor, I., G. Douglas, J. Dymond, G. Eyles, and M. Marden. 2011. Pastoral Hill Slope Erosion

    in New Zealand and the Role of Poplar and Willow Tree in Its Reduction. Soil Erosion

    Issues in Agriculture 11: 257-279.

Nielsen, D.L.,M.A. Brock, G.N. Rees, and D.S. Baldwin. 2003. Effects of increasing salinity on

    freshwater ecosystems in Australia. Australian Journal of Botany 51: 655-665.

Novotny, E.V., D. Murphy, and H.G. Stefan. 2008. Increase of urban lake salinity by road

    deicing salt. Science of the Total Environment 406: 131-144.

Oberts, G.L. 1986. Pollutants associated with sand and salt applied to roads in Minnesota.

    Journal of the American Water Resources Association 22: 479-483.

Ordonez-Barona, C., V. Sabetski, A.A. Millward, and J. Steenberg. 2018. De-icing salt

    contamination reduces urban tree performance in structural soil cells. Environmental

    Pollution 234: 562-571.

Parida, A.K., and A.B. Das. 2005. Salt tolerance and salinity effects on plants: a review.

    Ecotoxicology and Environmental Safety 60: 324-349.

Pitman, M.G., and A. Laeuchli. 2000. Global impact of salinity and agricultural ecosystems.

    Salinity: Environment – Plants – Molecules: 3-20.

Poertner, H.O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series 373: 203-218.

Ramakrishna, D.M., and T. Viraraghavan. 2005. Environmental Impact of Chemical Deicer – A Review. Water, Air, and Soil Pollution 166: 49-63.

Rife S.R. 2016.An evaluation of chloride movement at a rain garden. Proquest: 1-68.

Scholz, V., and R. Ellerbrock. 2002. The growth productivity, and environmental impact of the cultivation of energy crops on sandy soil in Germany. Biomass and Bioenergy 23(2): 81-92.

Serapiglia, M.J., M.C. Humiston, H. Xu, D.A. Hogsett, R.M. deOrduna, A.J. Stipanovic, and L.B. Smart. 2013. Enzymatic saccharification of shrub willow genotypes with differing biomass composition for biofuel production. Plant Sciences.

Simon, E. 1978. Heavy metals in soils, vegetation development and heavy metal tolerance in plant populations from metalliferous areas. New Phytologist Trust 81(1): 175-188.

Stets, E.G.,C.J. Lee, D.A. Lytle, and M.R. Schock. 2018. Increasing chloride in rivers of the conterminous U.S. and linkages to potential corrosivity and lead action level exceedances in drinking water. Science of the Total Environment 614: 1498-1509.

Sune, E. 1999. Willows as a vegetation filter for cleaning of polluted drainage water from agricultural land. Biomass and Bioenergy 16(4): 281-290.

TRB. 1974. NCHRP Synthesis of highway practice 24: Minimizing deicing chemicals use. National Research Council.

Wang, D., C. Wilson, and M.C. Shannon. 2010. Interpretation of salinity and irrigation effects on soybean canopy reflectance in visible and near-infrared spectrum domain. International Journal of Remote Sensing 23(5): 811-824.

Wilcox, D. A. 1986.The Effects of Deicing Salts on Water Chemistry in Pinhook Bog, Indiana. Water Resources Bulletin 22(1): 57-65.

Wright, L. 2006. Worldwide commercial development of bioenergy with a focus on energy crop-based projects. Biomass and Bioenergy 30(8): 706-714.

Zhu, J.Y., X. Pan, and R.S. Zalesny. 2010. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. Applied Microbiology and Biotechnology 87(3): 847-857.

Figure Legends

Figure 2. The average root length of each group by week. The graph above plots the differences in root length over the duration of the experiment. There was a significant correlation between concentration of salt and the amount of time salt was applied (p=0.0041). The results were compared using a Tukey-Kramer test. Group A control was significantly longer by week 6 than the other groups. Group B control and the low groups were significantly longer than the medium and high groups.

Figure 3. The total dry biomass for each group. The above graph displays the combined root and shoots biomass for each individual group. The A and B groups were compared using a Tukey-Kramer test. The control groups and low groups were significantly different from the medium and high groups, but similar to each other.

Figure 4. Groups after recovery period. The Group A high is pictured after one week of recover (left) and Group B is pictured after the two week recovery (right).

Figure 5. Percent change in water conductivity. The graph above depicts the percent change in water conductivity over the 6 weeks. This measure was determined by comparing the conductivity before and after replacing the water every three days.

Figure 6. Shoot length growth over time. The above graph is the average shoot length per week over the final 3 weeks of testing. Both high groups and medium groups had shoot lengths of 0cm and therefore cannot all be seen. There were significant differences between Control A and Low B, as well as between both low and control groups versus the medium and high groups.

Figure 6. Average leaf length over time. The average leaf length for each group was recorded weekly. Both high and medium had lost all leaves by week 5. The remaining plants had no significant differences between the groups.

Figure 7. Average SPAD meter readings over time. The SPAD meter was used to measure chlorophyll content per $\mu M/m^2$ per leaf. Each plant was measured weekly. No significant differences were found between the low and control groups. The medium and high groups did not have leaves after week 5 and therefore had no readings.

Figures
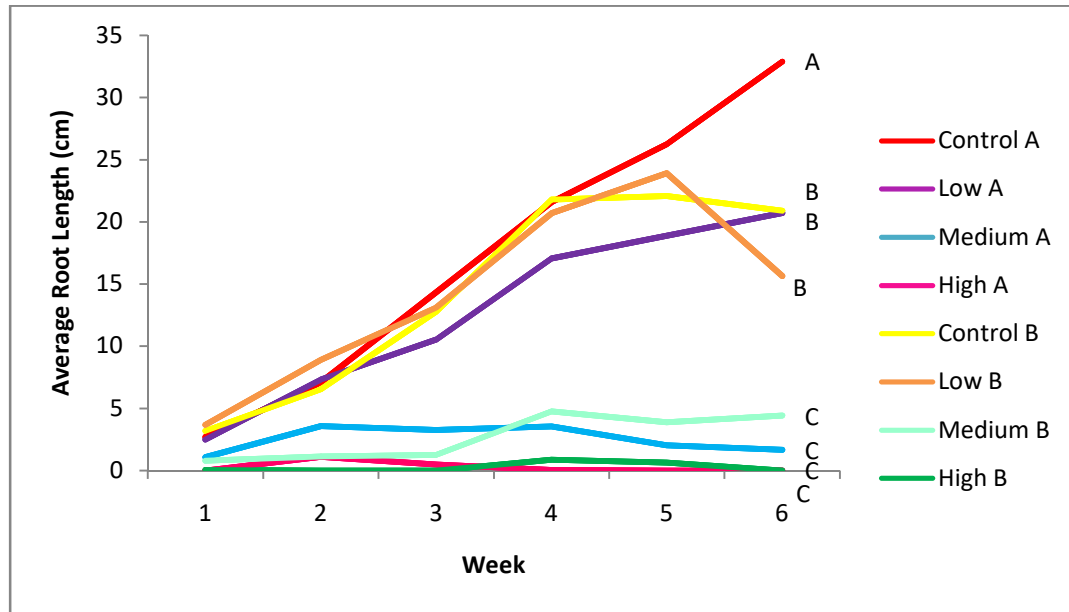
Figure 2. The average root length of each group by week.
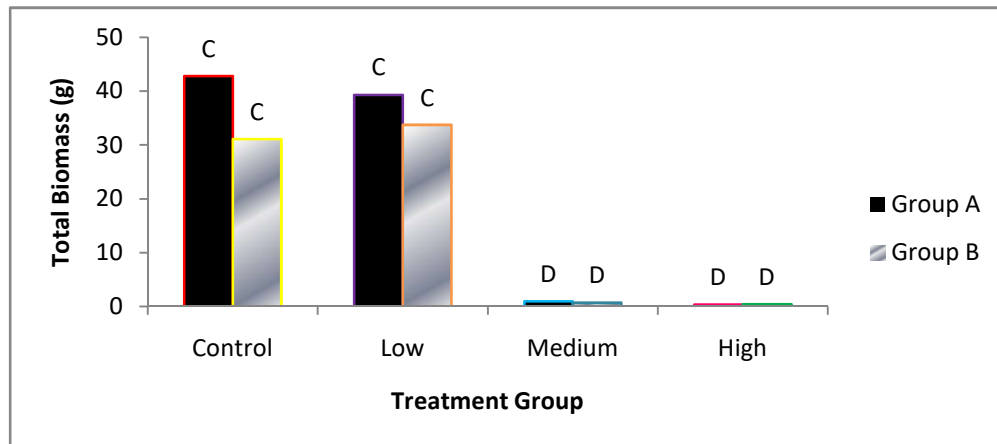
Figure 3. The total dry biomass for each group.
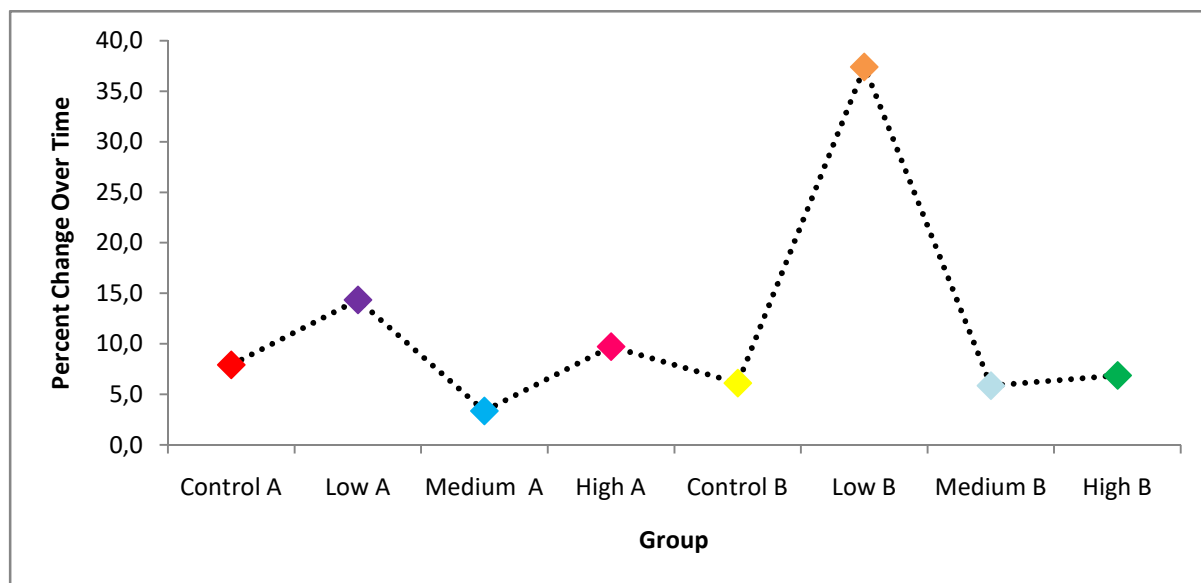
Figure 4. Groups after recovery period.

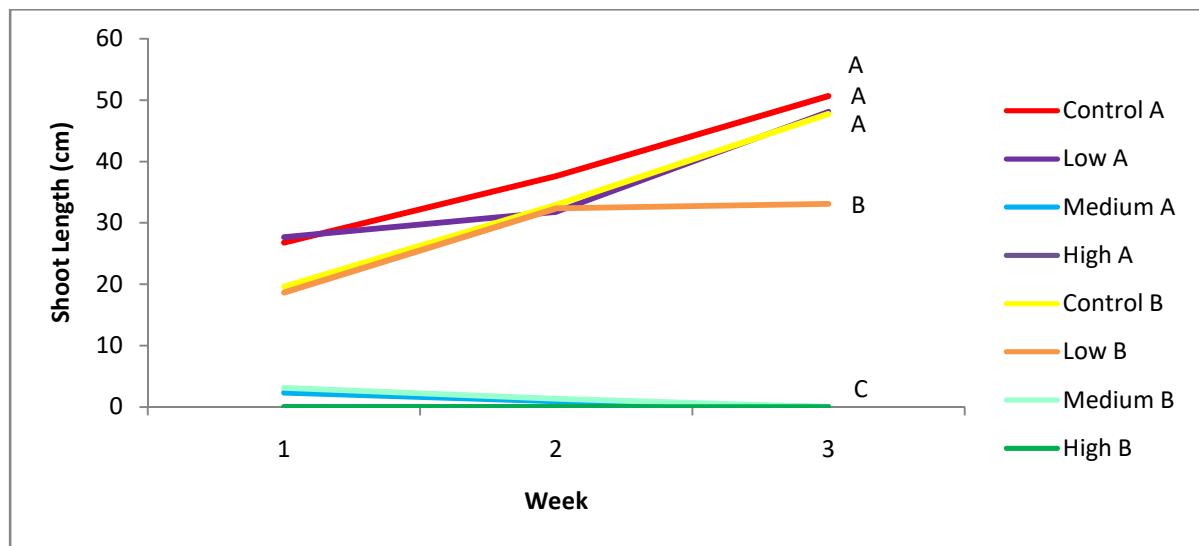**Figure 5. Percent change in water conductivity.**

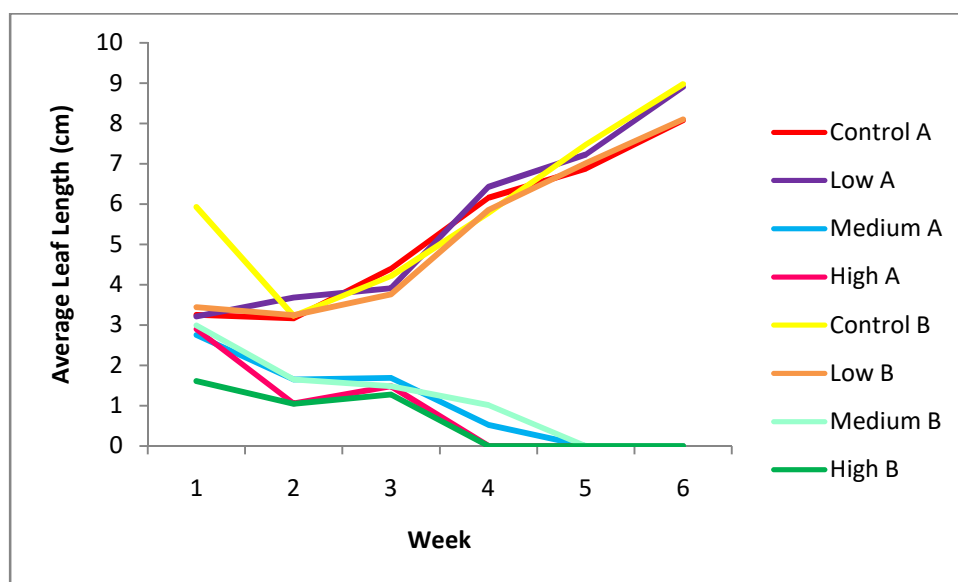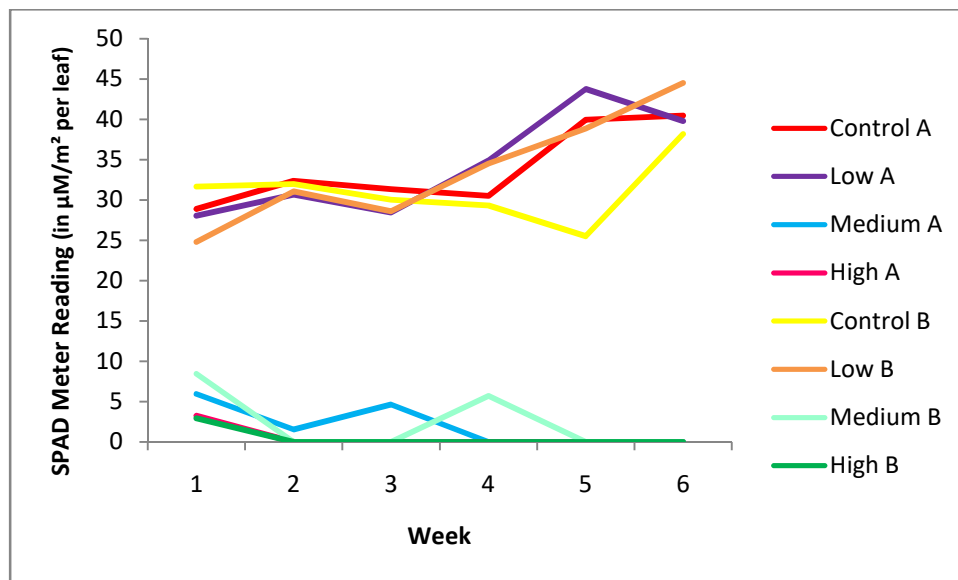**Figure 6. Shoot length growth over time.**

Figure 6. Average leaf length over time.

Figure 7. Average SPAD meter readings over time.