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Desalination Concentrate Management for Sustainable Agriculture: A Preliminary Study on Transport behavior and Plant Viability at BGNDRF

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by

Author(s)

Manoj K Shukla, Professor, New Mexico State University
Alison Flores, graduate student, New Mexico State University

U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Water and Environmental Services Division
Water Treatment Engineering Research Team
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Glossary

Brackish Groundwater National Desalination Research Facility – Facility in Alamogordo, NM that was created to develop technologies for the desalination of brackish and impaired groundwater in the inland states. Abbreviated as BGNDRF.

Cl^- – Abbreviation of chloride ion.

Deep percolation – Amount of water leached through soil past the root zone. Abbreviated as DP.

Desalination – Many different processes where salt and other minerals are removed from saline water.

Electrical conductivity – A measure of the ability of water to conduct electrical currents via dissolved ions. Abbreviated as EC.

Evapotranspiration – The sum of the water evaporation from the soil and the plant transpiration to the atmosphere. Abbreviated as ET.

Germinability – The capacity of a seed sample to germinate.

Germination Index – A measure of the time for a seed sample to germinate that uses the number of seeds germinated by the end of the experiment to cancel the effect of the seed sample size. Abbreviated as GI.

Halophyte – Plant species that grows and thrives in high salinity.

Irrigation – Amount of water applied to plants as water source. Abbreviated as IR.

K^+ – Abbreviation of potassium ion.

Leaching fraction – The volumetric ratio of water applied (irrigated) to water leached (via deep percolation). Abbreviated as LF.

Mean germination time – The weighted mean of the time a seed sample takes to germinate. Abbreviated as MGT.

Na^+ – Abbreviation of sodium ion.

Reverse osmosis – Water purification process where water is forced through a semipermeable membrane to remove various molecules and ions, leaving both potable water and a saline concentrate. Abbreviated as RO.

Sodium Adsorption Ratio – A measure of the suitability of water for use in agricultural irrigation determined by concentrations of dissolved solids. Measures sodicity of water and soils. Abbreviated as SAR.

Timson's Index – A measure of the time it takes a seed sample to germinate that combines germination rate with the final germination percentage. Abbreviated as T.

Timson's Modified Index – A measure of time for a seed sample to germinate that minimized the effect of final germination percentage in Timson's index. Abbreviated as T_{mod}.

Ks- saturated hydraulic conductivity of soil

Executive Summary

Water scarcity in arid regions has led to a decline of surface water available for agriculture and put constraints on saline groundwater resources. It has also necessitated the use of nontraditional water sources for augmenting irrigation water portfolio. One way to expand the irrigation water supply is to pump saline groundwater, run it through an inland reverse osmosis (RO) system, and utilize fresh water to grow food crops and saline RO concentrate to grow salt-tolerant plants including forage crops.

The objective of the study was to improve knowledge of six candidate halophyte species (*Atriplex canescens*, *Hordeum vulgure*, *Lepidium alyssoides*, *Distichlis stricta*, *Panicum virgatum*, and ×*Triticosecale*) for cultivation on wastewater land application sites. Study was divided into three parts, a germination study, pore clogging study, and a plant survival study and utilized water from the Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, NM.

The purpose of the germination study was to improve knowledge of halophyte germination by comparing time-integrated measurements of germination among seed populations exposed to saline groundwater and RO concentrate. The germination of six halophyte species was studied in 22-day germination tests conducted in growth chambers set to 25/15°C day/night temperatures with 12-hour photoperiods. Seeds of each species were placed in Petri dishes lined with filter papers moistened with one of the four saline treatments (electrical conductivity [EC] = 0, 0.6, 4.0, and 10.0 dS/m). Germinability, mean germination time, germination index, Timson's index, and Timson's index modified were calculated. Results showed that although final germination percentages remained similar within a species across treatments for all species except *L. alyssoides*, germination time varied to some degree, showing a germination time dependency.

The pore clogging study was conducted to determine how concentrated saline water affects the saturated hydraulic conductivity (Ks) of two soils (sand and clay) over time and to observe the effects of concentrated saline solution on soil. The effect of saline water application to two soils was studied in a 24-day test. Columns measuring 3.8 cm in diameter and 10 cm in height were packed with either a clay or sand and received one pore volume of concentrate each week for 20-weeks, then every two weeks for 4-weeks. Columns were allowed to dry between applications. The Ks was measured every 4-weeks for the duration of the study. Comparisons of the Ks over time showed initial decreases in Ks that were variable in the sand but fairly consistent in the clay. The salt deposition was observed on the soil surface as well as in the pores indicating that problems with Ks could arise with regular application of saline RO concentrate to soil.

The plant survival study was conducted in the greenhouse because NMDA does not allow land application of water above 4 dS/m. Columns were packed with two contrasting soils, clay and sand, to a constant bulk density. Seeds of the six halophyte species were planted and allowed to grow for 30-days prior to the start of the experiment using control water to ensure a consistent growth pattern. Plants were arraigned in a completely randomized design and treatments were applied for 90-days.

The objectives greenhouse study were to components of water balance including amounts of irrigation, drainage (or deep percolation), evapotranspiration (ET), and volumetric leaching fractions (LF) under a salinity gradient. ET for the six species was obtained from water balance equation. Results showed that the ET was higher and DP was lower for the control plants than the saline water-grown plants and values were more evident in the sand than in the clay. Additionally, with increasing irrigation water salinity, ET decreased but LF increased.

Other objectives of the greenhouse study was to quantify plant growth and ion uptake due to irrigation with saline groundwater and RO concentrate. Five physical measurements (height, number of stem nodes, average internodal length, number of leaves, and leaf length), four non-destructive measurements (photosynthetic rates, stomatal conductance rates, leaf temperatures, and transpiration rates), and five destructive measurements (stem water potential, osmotic potential, dry biomass, ion uptake, and chloride content) were made at the different imes and frequency. Plant height was measured along the main live stem from the base of the stem to the highest node. The number of leaves were counted for the entire plant. A LiCor LI-6400XT was used to determine the photosynthetic rates, stomatal conductance, leaf temperatures, and transpiration rates. Dry biomass was determined after cutting the plant at the soil surface and drying at 65°C for 3-days. The Na^+ and K^+ ion concentrations were determined though microwave digestion and Cl^- ion concentration was determined by mixing samples with 2% acetic acid. Results showed that *A. canescens* and *L. alyssoides* saw an increase in biomass while the others saw a decrease. *P. virgatum* was the only species to see a consistent decrease in plant height with increasing salinity and all species (except *A. canescens* which showed an increase) noted a decrease in the number of leaves per plant. There were no clear trends with respect to photosynthetic rates for the species. The concentrations of Na^+ and Cl^- both increased with increasing salinity, whereas K^+ decreased. Based on these results, all of the tested species have the potential for establishment on land application sites.

Introduction

In arid and semi-arid regions around the world, water is a limited resource. These areas are characterized by low rainfall and high evaporation. A significant amount of water is required for agricultural production, and thus, any water, even saline, must be used. (Smedema and Shiati, 2002). In southern New Mexico,

groundwater is often the saline water source utilized for agricultural irrigation. The groundwater salinity is highly variable and about 75% of available groundwater is saline having an electrical conductivity (EC) > 3 dS/m (Lansford *et al.*, 1990; WRRI, 1997). Low quality groundwater, along with persistent drought and diminishing fresh water supplies, has prompted urgent searches for novel water resources in arid and semi-arid regions (Schwabe *et al.*, 2013). Such searches have led to increased efforts to produce water suitable for both human consumption and cropland irrigation through the use of reverse osmosis (RO) desalination. The RO desalination of brackish groundwater produces potable, low saline water, and high saline-sodic RO concentrate (UNEP, 1998). Land application of RO concentrate, either by itself or mixed with wastewater effluent, is one approach to its disposal (Nemmers *et al.*, 2012) but the likelihood of deleterious environmental impacts has made the disposal of wastewater from inland desalination systems challenging and limits the widespread implementation of inland groundwater desalination (Soliz *et al.*, 2011).

The Brackish Groundwater National Desalination Research Facility (BGNDRF) located in Alamogordo, New Mexico utilizes a reverse osmosis (RO) treatment to desalinate groundwater from its four saline wells and produce potable water. The resulting concentrated saline wastewater, also known as concentrate, typically has an EC of 10 dS/m. In arid and semi-arid environments, the waste is often disposed of using evaporation ponds but this method of disposal is expensive and causes the loss of valuable water (Gonzalez et al., 2009; Soliz *et al.*, 2011). Another possibility is to reuse it for irrigating salt-tolerant plants (Noaman and El-Haddad, 2000; Babcock *et al.*, 2009).

Unlike plant species intolerant of salinity (i.e., glycophytes), halophytes can germinate, grow and survive under osmotic tensions potentially limiting to plant water uptake and ionic conditions possibly toxic to plant columns (Ries and Hofmann, 1983; Hussain *et al.,* 1997; Kim *et al.,* 2012). Halophytes have been introduced for revegetation on salt-contaminated soils because they are capable of growing on soil with more than 0.2% salt concentration; however, such revegetation efforts are often characterized by low rates of seedling survival and variability among halophyte species in plant establishment (Barbour *et al.*, 1987; Keiffer and Ungar, 2002). Plants tend to grow more slowly when subjected to salinity and often end up stunted but there is less growth inhibition in salt tolerant plants because they can store the salts within the plant tissue or excrete the salt through salt glands (Miyamoto *et al.*, 1994). Furthermore, application of highly saline-sodic water can produce contiguous patches of soil with high sodium content and low soil hydraulic conductivity (Adhikari *et al.*, 2012).

Germination of an individual seed is the sequence of physiological processes from imbibition to radicle protrusion (Bewley and Black, 1994). Typically, radicle protrusion is treated as a binary variable and is combined across individual seeds to provide insights on the germination percentages across seed populations. Final germination percentages have often been used to determine salinity effects on

seed germination; however, more information on the alleged impacts of salinity on population-level germination dynamics can be discerned by utilizing binary measures of radicle protrusion integrated over time. Studies have shown that despite comparable final germination percentages of seeds treated with water of increasing salinity, there is often a delay in germination (Shalaby, 1993; Almansouri, 2001; Flores *et al.*, 2015). Delayed germination under field conditions may increase the potential for seed mortality caused by pathogen infection (Dalling *et al.*, 2011). Thus, revegetation efforts on salt-contaminated soils should utilize halophyte species capable of rapid germination at the population level.

Another major concern with adding saline solution to a soil system is the affect the aqueous salts could have on soils. A study of factors affecting saturated hydraulic conductivity (Ks) showed that an increase in the sodium absorption ratio (SAR) decreased Ks (McNeal *et al.*, 1968). Soils with higher clay contents, like most agricultural soils, tend to show a larger decrease in Ks (Pupisky and Shainberg, 1979). The high salt content of RO wastewater concentrate could potentially accumulate within the soil and cause pore clogging. This could subsequently lead to further reduction in Ks and effectively change the soil profile. For this reason, concentrate-applied soil would also need to be monitored for pore clogging.

It has been shown that total shoot biomass and cumulative ET are directly related (Allen *et al.,* 1998). A recent study showed that increasing salinity decreased the yield of broccoli plants and the ET also decreased as a consequence of the salt increase (Smith *et al.*, 2013). Diaz *et al.* (2013) found that plants producing higher biomass under lower salinity levels generally had higher ET. However, they also noted that some halophytic species could tolerate high salt levels to produce acceptable biomass yields while maintain ET. A leaching fraction of 0.30 is reported to maintain a salt distribution conducive to plant growth, but some species, like many in the genus *Distichlis*, prefer wetter soils and others, like many *Atriplex* species, prefer drier soils (Miyamoto *et al*., 1994). Studies have shown that the average leaching fraction is not only related to biomass but also ET: as leaching fraction increases, dry biomass increases and ET increase (Khan, 1996; Noaman and El-Haddad, 2000). However, ET still decreased with increasing salinity when leaching fractions were comparable among treatments.

Plants exposed to salinity tend to grow slower and are often stunted in growth. A study on bell peppers showed that as the EC of the irrigation water increased from 0.5 to 7 dS/m, the shoot and fruit weight both decreased (Ben-Gal *et al.*, 2008). Similarly, a study on *Panicum virgatum* and *Spartina pectinata* saw a decrease in plant growth with increasing salinity and increased ion uptake (Kim *et al.*, 2012) and another study on broccoli found that there was a decrease in the plant yield as a consequence of a salt increase (Smith *et al.*, 2013). Sometimes, the plants do not show any signs of growth interference at higher salinity and differences such as

thicker leaves or decreased grain yields are not apparent until they are compared to unaffected plants (Bernstein, 1975; Noaman and El-Haddad, 2000).

It has been noted that for moderate levels of salinity, photosynthetic rates were unaffected by salinity, although an eventual decrease with increasing salinity is sometimes observed (Alvarez *et al.*, 2012; Koyro *et al.*, 2013; Ge *et al.*, 2014). It was often seen that the photosynthetic rates had little effect on plant growth, an indication of salt tolerance (Koyro *et al.*, 2013; Ge *et al.*, 2014). Conversely, it has been seen that growth and osmotic potential are related: the higher the osmotic potential, the taller the plants (Saberi *et al.*, 2011). Osmotic potential is reported to be a better index for ion affect on plants because different concentrations of salts cause similar reactions (Souza *et al.*, 2012). In general, salinity causes a reduction in leaf water potential as well as osmotic potential (Scholberg and Locascio 1999; Souza *et al.*, 2012) and the uptake of ions has been hypothesized to cause this reduction (Hussain *et al.*, 2014).

Some researchers have been studying the effects of land application of saline-sodic waters for agriculture but often use wastewaters with ECs between 4 and 6 dS/m which is similar to that of groundwater (Soliz *et al.*, 2011; Picchioni *et al.*, 2012; Ghermandi *et al.*, 2013). There is little guidance, however, concerning species selection and management. Because of the potential problems that could arise due to the land application, a better knowledge of candidate species and their responses to increasing salinity as well as management techniques can be crucial to making wastewater disposal safer and ultimately making inland desalination possible.

Because one of the main problems limiting the implementation of inland groundwater desalination systems is sustainable management of the highly saline RO wastewater concentrate, we believe that finding an acceptable method of disposal is the first step to employing these processes. Most studies on wastewater land application utilize various salt mixtures to determine the effects of salinity. This study improves our knowledge of candidate species for land application sites by making use of both saline groundwater and wastewater from the RO process.

Our general hypothesis is that with proper plant selection and the adoption of appropriate management techniques, saline groundwater and RO concentrate can be used to irrigate halophytes year-round. The objectives of the study were to: 1) improve knowledge of candidate species for cultivation on land application sites by comparing time-integrated measurements of germination among seed populations exposed to saline groundwater and concentrate from RO facilities, 2) determine how concentrated saline water affects the saturated hydraulic conductivity of two soils over time and to observe the effects of concentrated saline solution on soil, 3) evaluate the ET and leaching fractions under a salinity gradient, and 4) determine suitable species for land application sites by comparing growth parameters and ion uptake among greenhouse grown plants exposed to saline groundwater and concentrate from RO facilities.

Methods and Materials

Plant selection

Six plant species were selected for the study due to their high levels of salt tolerance: *Atriplex canescens* (Pursh) Nutt. (also known as fourwing saltbush or atriplex), *Hordeum vulgare* L. (barley), *Lepidium alyssoides* A. Gray (mesa pepperwort or lepidium), *Distichlis stricta* (Torr.) Rydb. (inland saltgrass or nipa grass), *Panicum virgatum* L. (switchgrass), and ×*Triticosecale* Wittm. (triticale). All seeds were purchased from Curtis & Curtis Inc. in Clovis, NM with the exception of the *L. alyssoides* seeds which were collected locally in Las Cruces, NM (32°16′N, 106°54′W).

To improve the germination rates for the *D. stricta* and the *A. canescens*, seeds of both species were pretreated prior to planting (Flores *et al.* 2015). The *D. stricta* seeds were placed into mesh packets which were then placed in a hydrated soil mixture at 4°C for 30 days. The soil mixture consisted of a 1:1, sand to soil volumetric ratio of QUIKRETE all-purpose sand (No. 1152) and soil from Leyendecker Plant Science Center in Las Cruces, NM that had been passed through a 4 mm sieve. The *A. canescens* seeds were treated using a method described by Twitchell (1955) that consisted of taking 30g of seed and soaking in 3L of water for two hours, followed by rinsing with 3L of distilled water, and air drying the seeds for seven days. Seeds of *D. stricta* feature a physiological dormancy that is presumably reduced during cool seasons under natural conditions. For this study, seeds were stratified in a hydrated soil mixture at 4°C for 30 days prior to the start of germination assays (Baskin and Baskin, 1998). For the *A. canescens* seeds, Twitchell's (1955) method of taking 30 g of seeds and soaking in 3 L of water for two hours, rinsing with 3 L of distilled water and air-drying for seven days to reduce seed dormancy was used to prepare the seeds.

Water treatments

This study utilized groundwater taken from the Brackish Groundwater National Desalination Research Facility (BGNDRF) located in the Tularosa Basin in Alamogordo, New Mexico (32°52'N, 105°58'W) which uses RO to desalinate well water. Four water treatments were selected to create a salinity gradient for the germination study: deionized water (electrical conductivity [EC] = 0 dS/m, sodium adsorption ratio [SAR] = non-detectable, pH = 5.6), university greenhouse irrigation tap water (EC = 0.6 dS/m, SAR = 2.1, pH = 8.1), BGNDRF well water (EC = 4 dS/m, SAR = 4.3, pH = 7.9), and BGNDRF RO concentrate (EC = 10 dS/m, SAR = 6.1, pH = 8.2). The germination study utilized the deionized water as the control whereas the greenhouse study considered the tap water as the control. The well from BGNDRF chosen for the study provided both the well

water and, after the RO process, the concentrate. The dominant cation in all treatments was calcium followed by sodium.

Treatment water samples were analysed for pH and EC according to EPA method 150.2 and EPA 120.1, respectively. The concentrations of Mg^{+2}, Na^{+1}, and Ca^{+2} ions were determined by analysing samples in a PerkinElmer Optima 4300 DV ICP-OES according to EPA 200.7 and the sodium adsorption ratios (SAR) were subsequently calculated (Robbins, 1983):

$$SAR = \frac{[Na^+]}{\sqrt{\frac{([Ca^{2+}][Mg^{2+}])}{2}}} \quad (1)$$

where $[Na^+]$ is the concentration of sodium ion (meq/L), $[Ca^{2+}]$ is the concentration of calcium ion (meq/L), and $[Mg^{2+}]$ is the concentration of magnesium ion in the sample (meq/L).

For the greenhouse study, each treatment was mixed with a half strength Hoagland's solution, giving a fertilizer to source water ratio of approximately 1:5. This resulted in ECs of approximately 0.9 dS/m, 4.1 dS/m, and 8.0 dS/m for the control, well water, and concentrate treatments, respectively. The fertilizer was applied to the plants with every irrigation.

Germination Experiment

The effects of the salt treatments on the germination of the species were determined using an experimental set-up consisting of 72 Petri dishes, each lined with two Whatman #2 filter papers (90 mm-diameter) and 3 mL treatment water. The experiment included two runs separated in time. For the first run, three samples of 20 seeds were separated out in each dish while 25 seeds were used for the second experiment. The Petri dishes were sealed with parafilm to reduce evaporation loss, but water was added as needed. The dishes were randomly arranged in a germination chamber set with 25/15°C (day/night) alternating temperatures, with a 12 hour photoperiod. Photosynthetic photon flux density within the chamber was approximately 30 μmol m^{-2} s^{-1}. Seeds were considered germinated and removed once the length of the radicle surpassed the length of the seed. Ungerminated seeds remained in the dish until the conclusion of the study and their viability was determined using the imbibed crush test. The study ran for 22 days.

Parameters used to evaluate the effect of water treatment were germinability, mean germination time, germination index, Timson's index, and Timson's index modified and calculated using the following formulas (Ranal and Santana, 2006):

$$Germinability = \left(\sum_{i=1}^{k} n_i/S\right) \times 100\% \quad (2)$$

$$MGT = \sum_{i=1}^{k} n_i\, t_i / \sum_{i=1}^{k} n_i \quad (3)$$

$$GI = \sum_{i=1}^{k} |(23 - t_i)n_i| / S \quad (4)$$

$$T = \sum_{i=1}^{K} \left(g_i(K - j)\right) \quad (5)$$

$$T_{mod} = T / \sum_{i=1}^{K} g_i \quad (6)$$

Where k is the last day of germination, n_i is the number of newly germinated seeds on day i, S is the number of seeds in the experiment (germinated and viable, non-germinated), t_i is the number of days from the start of the experiment to day i, 23 is the number of days spent in the germination test plus 1, g_i is the number of newly germinated seeds in the time interval i, K is the total number of time intervals (days) and j = 1-i.

These parameters were chosen to: show the total percentage of viable seeds in a sample that complete the germination process (germinability), measure the time it takes seeds to germinate (GI and T), and give a time measurement that accounts for the total number of seeds that germinated within the Petri dish (MGT and T_{mod}). GI is limited because it includes non-germinated seeds in the calculation, making the calculation dependent on seed sample size. T is limited because it is only suitable when germination percentages of seed samples are comparable. MGT and T_{mod} both take into account the final germinability, which can vary from trial to trial, which minimizes the effect of germination percentage.

Soil Sampling

Soil samples were collected from BGNDRF on July 31, 2013 (32:53.081N and 105:58.624W). Soil samples were collected from the top 30 cm of soil, and were air-dried and sieved through a 2-mm sieve. A commercially available silica sand was selected to provide a contrasting soil for the experiment. Texture analysis was performed for both soils using the hydrometer method (Gee and Bauder, 1986), and Ks using the constant head method (Klute and Dirksen, 1986). For the soil moisture characteristics, a pressure plate extractor was used and soil moisture contents were determined for the potentials of 0, -0.03, -0.1, -0.2, -0.3, -0.5, -1.0, and -1.5 MPa (Klute, 1986) and a van Genuchten curve was fitted to the data using the equation (Shukla, 2014):

$$\theta_i = \theta_r + \frac{\theta_s - \theta_r}{[1+(\alpha\phi_i)^n]^m} \quad (7)$$

Where θ_i is the moisture content at pressure i, θ_r is the residual water content at the permanent wilting point (PWP), θ_s is the water content at saturation, α is a fitting parameter, ϕ_i is the ith pressure, n is a fitting parameter, and m = 1 – 1/n.

Saturated paste extracts from soil samples were prepared to determine the pH, EC, and the concentrations of Mg^{+2}, Na^{+1}, and Ca^{+2} ions, also analyzed in a

PerkinElmer Optima 4300 DV ICP-OES. The sodium adsorption ratio (SAR) was then calculated according to equation (1).

Pore Clogging Experiment

Sixteen columns (3.8 cm-diameter, 10 cm-height) were packed for the study: eight with clay, eight with sand. A piece of cheesecloth and small gravel were packed first to prevent the soil loss through the drainage holes at the bottom of the columns. Soil was loosely packed in the columns, giving a height of soil of approximately 8.3 cm.

The bulk density of each column was determined and assuming a particle density of 2.65 g/cm^3, the porosity was calculated for each column. Prior to any treatments, the clay was washed with 3 pore volumes of deionized water to remove salts in the soil. The 16 columns were then wetted from the bottom over the course of 24 hours to ensure that there was no entrapped air in the sample. The initial Ks of the samples was determined using the constant head method (Klute and Dirksen, 1986).

The soil columns were irrigated with one pore volume of the concentrate and given a week to dry. The day of treatment and the following 2 days columns were left to air drying in the laboratory. The following 3 days drying was done in a chamber to simulate the southern New Mexico climate. The chamber was set to maintain a 35°C/25°C (day/night) temperatures, with a 12 hour photoperiod. The columns were removed from the chamber on the day prior to the subsequent irrigation and were allowed to equilibrate with room temperature. This cycle was continued for 20 weeks. For weeks 21-24, the time between irrigation was reduced to one application every two weeks. The same procedure was followed throughout irrigation –drying events.

The change in Ks was assessed by conducting tests every 4 weeks. The second and all subsequent Ks tests were performed using concentrate rather than tap water to avoid clearing any salt-clogged pores.

Greenhouse Plant Survival Cylinder Preparation

Larger size cylindrical columns (6.4 cm-diameter, 25.4 cm-height) were used for the greenhouse study and cheesecloth and small gravel were placed at the bottom of the columns to prevent soil loss. Columns were then packed with two contrasting soils, one collected from the BGNDRF site and a commercially available fine silica sand, to a constant bulk density. Prior to the planting of seeds, it was determined that the columns containing clay contained a large amount of salts and therefore, the columns were leached until the average EC of the leachate was < 5 dS/m. The columns with sand were slowly saturated from the bottom through sub- irrigation.

The experiment was conducted at NMSU Fabian Garcia greenhouse located in Las Cruces, NM (32°16′43"N, 106°46′23"W). The first sand experiment ran from January 2014 to April 2014 and the second experiment ran from June 2014 to September 2014. The first clay experiment was conducted from March 2014 to June 2014 and the second experiment from June 2014 to September 2014.

Seeds were planted in the top 1 cm of soil. Control water was used during the plant establishment to ensure a consistent growth pattern. Fertilizer was not applied to the seedlings until at least one leaf had established to prevent burning. To prevent shock, treatments were gradually introduced to the plants after a seedling establishment period of four weeks. The plants were arranged in a completely randomized design by generating random number using Data Analysis in MS Excel (2013).

Water Balance Experiment

Plant species were irrigated with the same volume of treatment water and with the same frequency within an experiment. This was done to maintain a consistent irrigation schedule rather than a consistent leaching fraction to represent a field containing all species intermixed. The volumetric leaching fractions for the plants were determined using the following equation (Ayers and Wescott, 1985):

$$LF = V_{drainage}/V_{irrigation} \quad (8)$$

Where $V_{drainage}$ is the volume of water applied (cm^3) and $V_{irrigation}$ is the volume of leachate (cm^3).

The evapotranspiration (ET) rates of the plants were calculated for each species and water treatment. The following water balance equation was used for this calculation (Shukla, 2014):

$$ET = IR + R - \Delta S - RO - DP \quad (9)$$

Where IR is the irrigation depth (cm; = 0), R is rainfall (cm), ΔS is the change in soil water content (cm), RO is runoff (cm; = 0), and DP is the deep percolation (cm; leachate collected from the bottom of the column).

Plant Growth Measurements

Five physical measurements were taken to measure the plant growth over the course of 90 days: height, number of stem nodes, average internodal length, number of leaves, and leaf length. These measurements were taken at day 30, 60, and 90 from the start of each experiment. The height of the main, live stem was measured from the base of the stem to the highest node. The stem nodes were designated as any area where one or more branches away from the stem were noted and were counted and recorded. For the average intermodal length, the

distance between the nodes was measured along the main stem and the mean was calculated and recorded. The number of leaves for each plant were counted and recorded. The leaf length was determined by measuring from the node, up the midrib, to the apex of the third leaf from the top of the main stem. The exception for this measurement was the *P. virgatum* plants grown in the clay soil due to the fact that several of these plants did not grow more than two leaves. In this case, the second leaf from the top of the main stem was measured.

Using a LiCor LI-6400XT Portable Photosynthesis System unit, photosynthetic rates, conductance rates, leaf temperatures, and transpiration rates were measured. These measurements were taken on days 60 and 90.

Other measurements include, stem water potential, osmotic potential, dry biomass, ion uptake, and chloride content. The stem water potential of the plants were measured using a pressure bomb and were measured after 90 days of growth had been achieved. Osmotic potential was determined by taking leaf samples, placing them in a freezer for at least 24 hours, crushing the leaves, and then centrifuging the samples for 5 minutes at 13000 rpm to extract the cell sap. A Wescor Vapro osmometer was used to measure the concentration of the solution and the following equation was used to calculate the osmotic potential (Ψ_s) (Shukla, 2014):

$$\Psi_S = -CiRT \qquad (10)$$

Where C is the concentration of the solution (as determined by the osmometer), i is the ionization constant (taken as 1.8 for saline solutions), R is the gas constant (0.00831 kg MPa mol^{-1} K^{-1}), T is temperature in Kelvin.

At the conclusion of the greenhouse study, the plants were harvested by cutting the plant at the soil surface. The plants were dried at 65°C for 3 days and weighed to determine the dry biomass. The dry biomass was used to determine the ion uptake via microwave digestion by taking 0.5 g of plant matter ground to pass through a 40 mesh and mixing with 5 mL of concentrated nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide (H_2O_2). The mixture was heated in a Microwave Accelerated Reaction System (MARS5 HP-500 Plus) and following a cooling period, the samples were filtered, diluted, and analyzed using the PerkinElmer Optima 4300 DV ICP-OES according to EPA methods 3051A and 200.7. This process determined the concentrations of S, B, Zn, P, Fe, Mn, Mg, Ca, Cu, Al, Na, and K. The dry biomass was also used to determine the chloride content of the plant samples and was determined by taking 0.2 g of plant matter (< 40 mesh) and mixing with 50 mL of 2% acetic acid. The solution was shaken for 30 minutes before being filtered and analyzed on a Technicon Autoanalyzer II.

Saturated soil paste extracts were prepared at the conclusion of the experiment. One sample was analyzed for each soil-species-water treatment combination for the first experiment with clay, however, both soils were analyzed during second

experiment. The extract was prepared according to the method described by Gavlak *et al*. (1994) and samples were analyzed for pH and EC. Using the PerkinElmer Optima 4300 DV ICP-OES, saturated paste extracts were analyzed for Mg^{+2}, Na^{+1}, and Ca^{+2} ions according to EPA 200.7. The sodium adsorption ratio (SAR) was calculated according to equation (1).

Statistical Analysis

Statistical analyses for the germination study were performed using the open source statistical software program R (R Core Team, 2015). For each species, solution effects on germination metrics were determined using analyses of variance. To meet the assumptions of constant variance as indicated by visual inspections of plots of residuals versus predicted values, specific datasets were square-root transformed prior to analysis.

Statistical analyses for the water balance and greenhouse study were performed using SAS software, v 9.2 and v 9.4, respectively. For each species, differences due to water treatments were determined using analyses of variance (ANOVA). Means were separated by least significant difference (LSD) and were considered significant for an alpha (α) value of 0.05. Results were modeled using a general linear model.

Results and Discussion

Water treatments

The ECs for the deionized, greenhouse, well, and concentrate waters, prior to the addition of fertilizer, were 0.6, 4.0, and 10.0 dS/m, respectively (Table 1). The SAR was 2.1, 4.3, and 6.1 for the greenhouse water, well water, and RO concentrate, respectively. However, SAR was <13 for all waters, making the greenhouse tap neither saline nor sodic, and the well and concentrate water treatments saline but not sodic.

Table 1 – Treatment analysis of waters used in study: deionized water; water from the Fabian Garcia (FG) greenhouses in Las Cruces, NM; well water from Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, NM; RO waste concentrate from BGNDRF. *ND indicates non-detection.

Type of Water	Mg (meq/L)	Ca (meq/L)	Na (meq/L)	K (meq/L)	EC (dS/m)	SAR	pH
Deionized	ND*	ND	ND	ND	0	ND	5.6
FG Greenhouse	0.7	2.5	2.6	4.5	0.6	2.1	8.1
BGNDRF Well	13.1	17.4	16.9	6.9	4.0	4.3	7.9
BGNDRF Concentrate	42.7	52.1	42.2	12.8	10.0	6.1	8.2

In the well water, the dominant cation was calcium with 17.4 meq/L, followed by sodium (16.9 meq/L) and magnesium (13.1 meq/L). The dominant cation in the RO concentrate was calcium (52.1 meq/L), while sodium and magnesium were 42.2 and 42.7 meq/L, respectively. These results were consistent with the large amount of calcium in the soil in the form of $CaCO_3$ (Gypsum).

Germination Study

The five response variables MGT, GI, T and T_{mod} were analyzed at $\alpha = 0.05$ for each halophyte species across saline water treatments. There were statistically significant differences noted in all species but for different indices and to different extents (Table 2).

Table 2 – Effect of salinity on germination parameters of seeds of *A. canescens*, *H. vulgare*, *L. alyssoides*, *D. stricta*, *P. virgatum* and ×*Triticosecale*. Abbreviations: MGT, mean germination time; GI, germination index; T, Timson's index; T_{mod}, Timson's modified index. The values followed by different letters were significantly different within a species at $P < 0.05$. Results are means of six replicates and standard errors (SE) were calculated using the formula $SE = S / \sqrt{n}$, where S is the standard deviation of the replicate mean and n is the number of replicates.

		Germination Indices				
		Germinability	MGT	GI	T	T_{mod}
Species	EC	(%)± SE	(day) ± SE	(seeds day^{-1}) ± SE	(% day) ± SE	(day) ± SE
A. canescens	0	36.5 ± 5.6	7.11 ± 0.94	5.7 ± 0.8	70.00 ± 12.44	8.45 ± 0.47
	0.9	28.3 ± 3.4	6.36 ± 0.46	4.7 ± 0.5	56.67 ± 8.09	8.82 ± 0.23
	4.1	26.7 ± 3.4	6.80 ± 0.48	4.3 ± 0.6	51.67 ± 7.00	8.60 ± 0.24
	8	31.8 ± 6.7	6.43 ± 0.20	5.2 ± 1.1	63.83 ± 13.55	8.78 ± 0.10
H. vulgare	0	99.3 ± 0.7	4.14 ± 0.14 a	18.7 ± 0.2 a	221.50 ± 9.28 a	9.93 ± 0.07 a
	0.9	99.2 ± 0.8	4.37 ± 0.15 a	18.5 ± 0.2 ab	218.83 ± 10.63 ab	9.81 ± 0.07 a
	4.1	98.3 ± 1.7	4.74 ± 0.17 b	18.0 ± 0.3 bc	213.00 ± 10.95 bc	9.63 ± 0.08 b
	8	98.7 ± 1.3	5.06 ± 0.14 c	17.7 ± 0.3 c	209.67 ± 8.48 c	9.47 ± 0.07 c
L. alyssoides	0	92.0 ± 2.1 a	5.98 ± 0.23 bc	15.6 ± 0.3 bc	186.33 ± 9.95 bc	9.01 ± 0.12 ab
	0.9	92.0 ± 2.6 a	6.96 ± 0.26 a	14.8 ± 0.5 c	175.50 ± 7.14 c	8.52 ± 0.13 c
	4.1	98.5 ± 1.0 b	5.60 ± 0.22 c	17.1 ± 0.3 a	203.83 ± 10.16 a	9.20 ± 0.11 a
	8	97.2 ± 1.4 b	6.51 ± 0.16 bc	16.0 ± 0.2 b	190.83 ± 8.65 b	8.74 ± 0.08 bc
D. stricta	0	84.8 ± 5.0	6.67 ± 0.34 a	13.9 ± 1.0	164.17 ± 9.31	8.66 ± 0.17 a
	0.9	81.7 ± 4.8	6.59 ± 0.34 a	13.5 ± 1.0	158.17 ± 6.95	8.70 ± 0.17 a
	4.1	85.0 ± 4.8	6.70 ± 0.36 a	13.9 ± 0.9	163.83 ± 8.01	8.65 ± 0.18 a
	8	84.3 ± 3.7	7.26 ± 0.48 b	13.3 ± 0.9	157.17 ± 5.02	8.37 ± 0.24 b
P. virgatum	0	76.0 ± 4.1	7.08 ± 0.15 a	12.1 ± 0.7	145.50 ± 12.50	8.46 ± 0.08
	0.9	74.2 ± 5.9	7.09 ± 0.23 a	11.8 ± 0.9	141.50 ± 13.74	8.46 ± 0.12
	4.1	76.7 ± 5.1	7.20 ± 0.21 a	12.1 ± 0.7	146.17 ± 14.98	8.40 ± 0.11
	8	78.5 ± 4.6	8.43 ± 0.17 b	11.4 ± 0.5	138.17 ± 12.19	7.78 ± 0.09
×Triticosecale	0	99.3 ± 0.7	3.85 ± 0.11 a	19.0 ± 0.2	224.83 ± 9.71	10.08 ± 0.05 a
	0.9	99.3 ± 0.7	4.01 ± 0.09 ab	18.9 ± 0.2	223.00 ± 9.65	9.99 ± 0.04 ab
	4.1	97.5 ± 1.7	4.17 ± 0.08 b	18.4 ± 0.3	218.00 ± 13.19	9.91 ± 0.04 b
	8	96.8 ± 1.6	4.22 ± 0.18 b	18.2 ± 0.2	215.33 ± 11.05	9.89 ± 0.09 b

Because the weighted MGT and T_{mod} take into account the final cumulative germinability of the seed samples which minimizes the effect of the germination percentage, these differences were more pronounced. Analyses of the final

germination percentages determined that the difference among water treatments within a species was not significant for five of the six halophytes studied. The *L. alyssoides* seeds were the only ones to show a difference in final germination with higher germinations observed for the higher salinity treatments.
Although the final germination percentages across the treatments were comparable within the species, there was evidence of variability among treatments between the onset of germination and the final seed germination, indicating a delay for some species (Figure 1). This is supported by the results from the MGT, GI, T, and T_{mod} comparisons for each species (Table 2). Significant differences were noted in the MGT of all species except *A. canescens*. The largest differences were noted for *H. vulgare*, *D. stricta*, and *P. virgatum*. The GI and T, which take into account total final germinability, only showed differences for two species, *H. vulgare* and *L. alyssoides*. Like the weighted MGT, T_{mod} takes into account the cumulative germination of the seed sample and showed a significant difference for four species: *H. vulgare*, *L. alyssoides*, *D. stricta*, and ×*Triticosecale*.

Seeds from *L. alyssoides* were most sensitive to changes in water salinity, as indicated by the significant differences observed in all five calculated indices. *A. canescens* was the least susceptible with no significant differences noted in indices. *H. vulgare* had the second highest number of significant differences with four of the five indices showing significance. *D. stricta* and ×*Triticosecale* each contained significant differences for MGT and T_{mod} while *P. virgatum* noted a significant difference solely in MGT. All the indices, aside from the germinability, are related to the germination time of a species. These results show that increasing salinity delayed germination of all species, except *A. canescens*, and germination was time dependent, indicating a delay. The final germination percentage (germinability) showed no significant differences for the species (except *L. alyssoides*) indicating that the germinability was not affected by the water treatment.

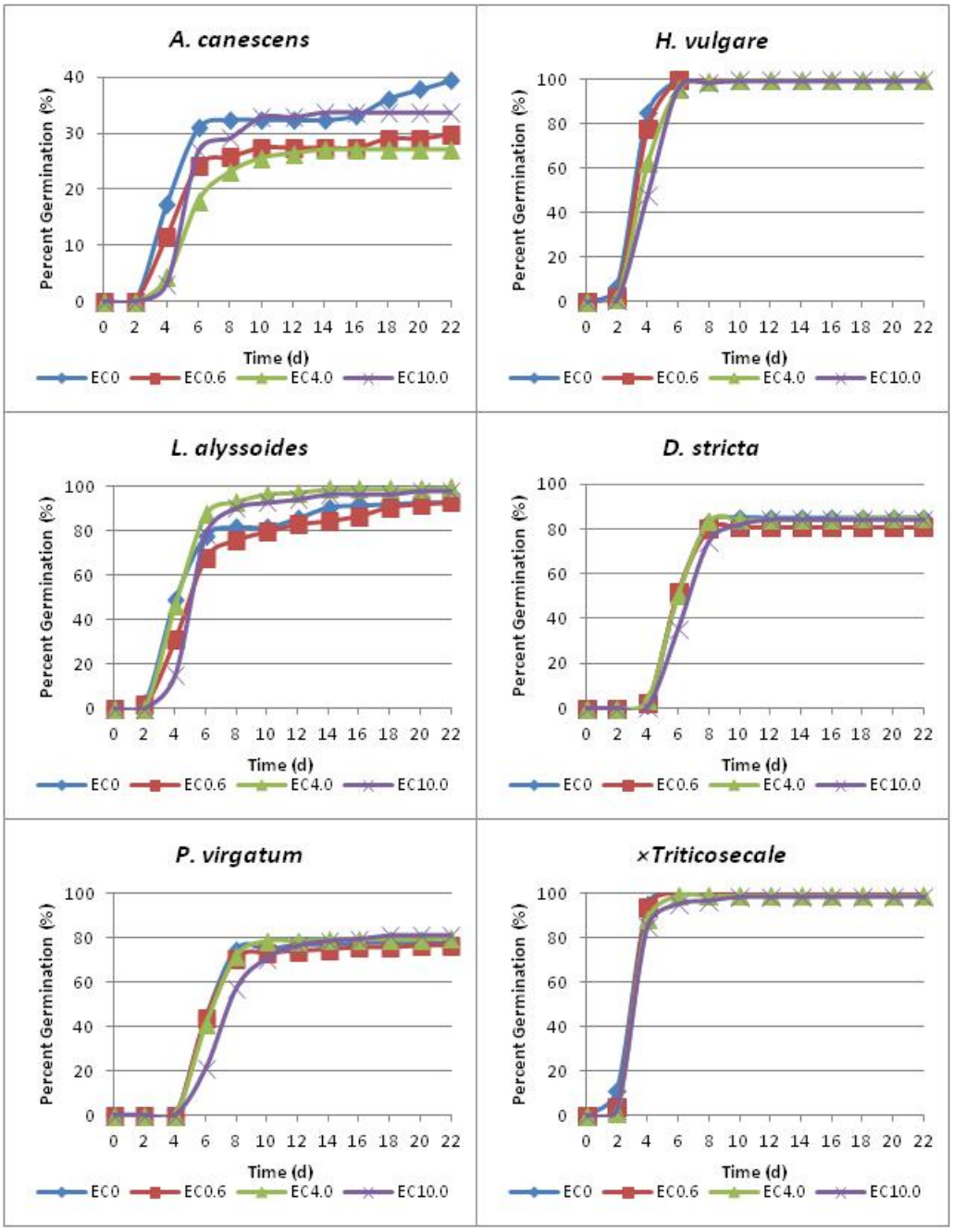

Figure 1 – Cumulative germination of halophyte seeds of *A. canescens*, *H. vulgare*, *L. alyssoides*, *D. stricta*, *P. virgatum* and ×*Triticosecale* over 22 days, under different salt concentration treatments: EC 0, 0.6, 4.0, and 10.0 dS/m. Results are means of six replicates across two runs.

Soil Sampling

According to USDA textural classification, the soil collected from BGNDRF was classified as clay and commercial soil was sand (Table 3). The Ks was 5.9 ± 2.5 cm/h from clay and 150.4 ± 13.5 cm/h from sand. The clay soil from BGNDRF contained large amounts of salts. The concentrations of magnesium, calcium, and

sodium were found to be 70.1, 155, and 127 meq/L, respectively (Table 4). With an EC of 28.9 dS/m, the soil was classified as highly saline and with an SAR of 11.95 and it was classified as borderline sodic. The dominant cation found in the clay soil was calcium, which was consistent with the groundwater at the site. The commercial sand had a significantly lower amount of salt than the clay with magnesium, calcium, and sodium concentrations of 0.8, 4.7, and 5.2 meq/L, respectively. Although dominant cation was sodium, sand had an EC of 1.2 dS/m and an SAR of 3.15 making it neither saline nor sodic.

Table 3 – Hydrometer method, texture analysis results for two soils in the study. Bulk density results are the packing readings for the study's plant column preparations.

Property	% Sand	% Silt	% Clay	Soil Texture	Bulk Density (g/cm^3)
Soil 1	19.57	28.96	51.47	Clay	1.07
Soil 2	99.60	0.07	0.32	Fine Sand	1.00

Table 4 – Mg^{+2}, Ca^{+2}, Na^{+}, and K^{+} ion concentrations for the clay soil collected at BGNDRF, along with the EC, SAR, and pH readings for the soil.

Property	Mg (meq/L)	Ca (meq/L)	Na (meq/L)	EC (dS/m)	SAR	pH
Clay Soil	70.1	155.0	127.0	28.9	11.95	7.5
Sand Soil	0.9	4.7	5.2	1.2	3.15	7.5

The van Genuchten parameters from soil moisture retention curves (Figure 2) were found to be very different for the clay and the sand. The α value for the clay was 0.002582 ± 0.000073 cm^{-1} whereas the α for sand was 0.009198 ± 0.002340 cm^{-1}. The n values were 1.7425 ± 0.0471 and 3.1466 ± 0.1481 for clay and sand, respectively.

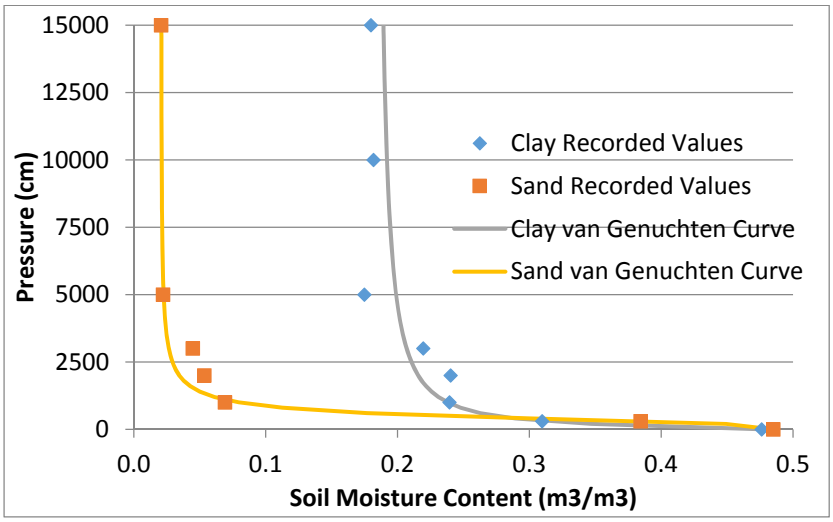

Figure 2 – van Genuchten drying curves for the clay and sand. 1 cm water = 0.001 MPa.

Pore Clogging

Overall, the soils maintained Ks with the application of the saline RO concentrate, although both showed a decrease in Ks. Both the sand and clay samples experienced a decrease in Ks from week 0 to week 4 (Figure 3). Some of this decrease can be attributed to the accumulation of salts within the pore which was also visually evident around the drainage holes (Figure 4). Additionally, the settling of soil particles within the samples, particularly in the clay could be the cause of the decrease in Ks as the movement of finer particles into pores can cause blockage (Pupisky and Shainberg, 1979). A high amount of sodium in water is another cause of hydraulic conductivity reduction shown in previous studies (McNeal *et al.*, 1968; Pupisky and Shainberg, 1979; Adhikari *et al.*, 2014). The pore clogging mechanism study by deVries (1972) suggested that allowing soils to drain daily may have helped maintain Ks. It has also been observed that when calcium and magnesium are present in similar amounts as sodium in the saline solution (like the BGNDRF RO concentrate), the deterioration of soil structure is reduced, helping permeability (Singh *et al.*, 2011).

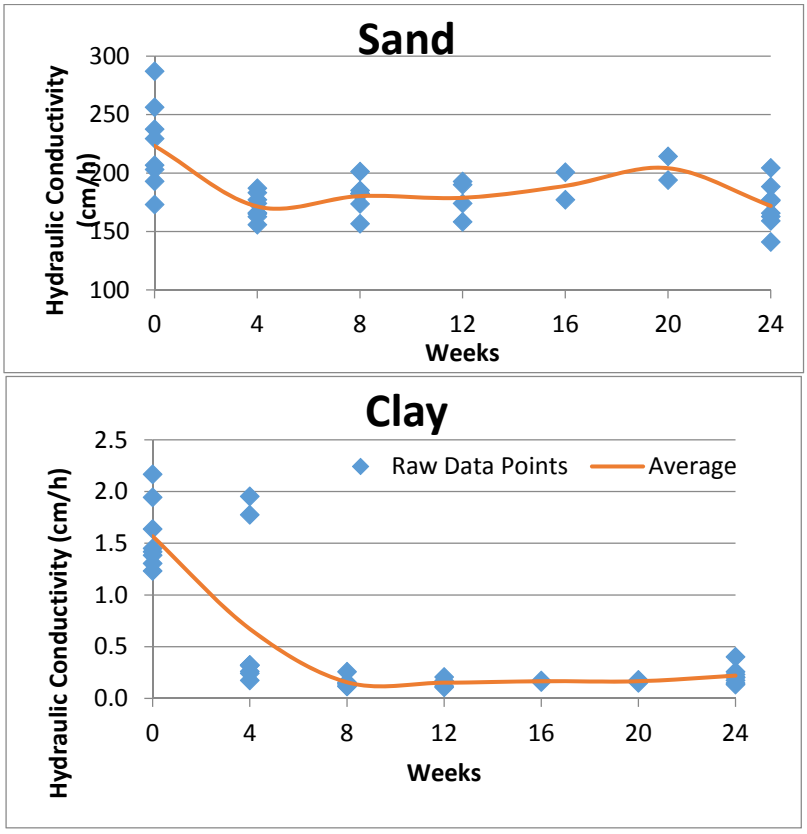

Figure 3 – Graphs showing the change in hydraulic conductivity over time for sand and clay samples.

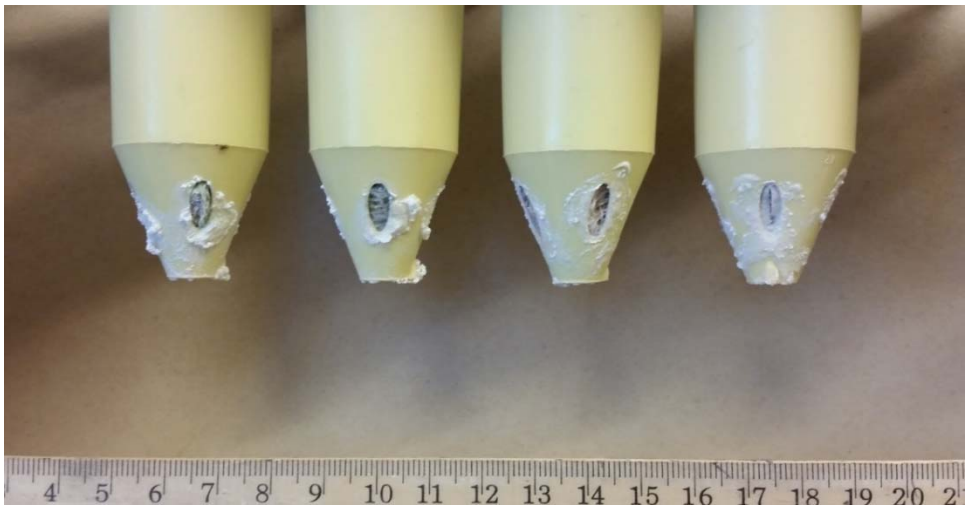

Figure 4 – Salt accumulation at drainage holes of columns. Salt buildup was evident in all clay and sand samples.

The clay soil showed little variability in Ks after the initial drop as opposed to the sand soil. The clay soil also had a much lower Ks than the sand which can be expected as other studies have noted that an increase in clay content can cause a decrease in Ks (McNeal *et al.*, 1968). Although the clay showed some cracks with drying, this likely did not increase Ks as the clay swelled with the addition of concentrate, closing the cracks (Figure 5). However, it has been observed that clay swelling can lead to a sealing of pores which could, in addition to salt accumulation, lower permeability (Pupisky and Shainberg, 1979).

Figure 5 – Cracks formed in clay samples following a drying cycle. All clay samples showed evidence of cracking with drying. On the left, dry clay just prior to concentrate application. On the right, wet clay just after application.

The sand soil was much more variable than the clay and showed increasing as well as decreasing Ks over time. This could be due to the surface crusting that was observed in the sand columns (Figure 6). The buildup of salt and the clogging of pores at the soil surface can lead to decreased Ks. The surface clogging is typically responsible for most of the decrease in Ks (Rice, 1974). Because the

majority of the Ks issues are at the surface of the soil, any disruption in the layer could temporarily increase water flow (deVries, 1972; Lal and Shukla, 2004). However, despite the varying Ks over time, the sand soil maintained a high level of permeability, something that has been previously observed (deVries, 1972).

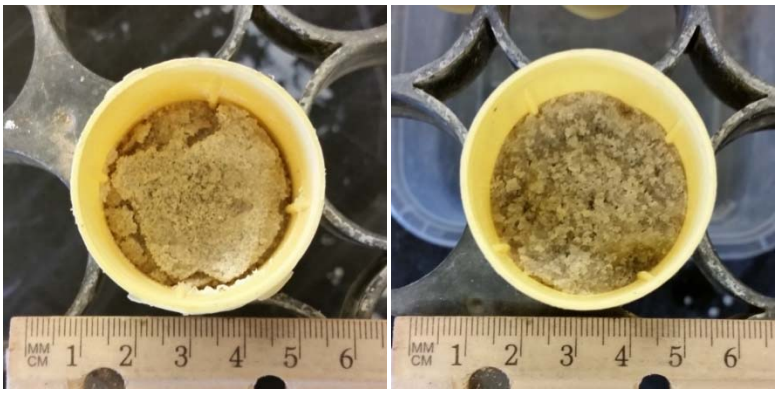

Figure 6 – Surface crusting in the sand samples. Crusting was evident in all sand samples. On the left, dry sand just prior to concentrate application. On the right, wet sand just after application.

This section of the study has shown that a problem with water retention and water movement can arise with land application of RO concentrate and thus a management plan, such as leaching, is needed.

Water Balance

Evapotranspiration and Deep Percolation

The sand-grown plants showed varying results for different species and showed more significant differences for the mean total ET and DP (Table 5). *H. vulgare* and×*Triticosecale* plants responded similarly and showed statistically significant differences with increasingly salinity in both experiments. Both saw decreases in ET with increasing salinity and consequently significant increases in DP as well. In the first experiment, 3% of the control irrigation water was lost to DP for both species, but this number increased during the second experiment to 25% and 14% for the *H. vulgare* and ×*Triticosecale*, respectively. In contrast, the concentrate plants saw DP of 17% and 33% for *H. vulgare* and 20% and 32% for ×*Triticosecale* for the first and second experiments, respectively. These two species saw the greatest ET losses and lowest DP amounts of all species studied. Only the *D. stricta* plants grown in the sand showed any difference in ET or DP. For both experiments, the highest values for ET and the lowest values of DP were found in the control plants. The *L. alyssoides* plants saw a difference in ET and DP for the first experiment only, whereas the *P. virgatum* plants only showed a difference in the second experiment. However, like the other species, the highest ET and lowest DP values were seen in the control. There were no significant differences in ET or DP for either sand experiment for the *A. canescens* plants: the plants treated with well water and RO concentrate showed ET and DP values

close to the control. This species was the only one of the six to show no differences for ET or DP with increasing salinity.

Table 5 – Total water balance readings (cm) for the six halophyte species at the conclusion of each of the two study periods for the sand soil. Different letters across a row correspond to a statistically significant difference in total irrigation (IR), deep percolation (DP), and evapotranspiration (ET) means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		Water Balance Calculations (cm)											
		Sand 1						Sand 2					
Species	Water	EC 0.9 ± SE		EC 4.1 ± SE		EC 8.0 ± SE		EC 0.9 ± SE		EC 4.1 ± SE		EC 8.0 ± SE	
A. canescens	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	22.67 ± 4.49	a	23.31 ± 3.24	a	22.66 ± 1.25	a	40.40 ± 3.30	a	40.57 ± 3.93	a	34.38 ± 2.35	a
	ET (cm)	57.91 ± 4.49	a	57.27 ± 3.24	a	57.92 ± 1.25	a	57.49 ± 3.30	a	57.32 ± 3.93	a	63.51 ± 2.35	a
H. vulgare	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	2.51 ± 0.78	c	6.37 ± 0.27	b	13.85 ± 1.59	a	24.48 ± 1.39	b	28.02 ± 1.30	ab	32.52 ± 1.65	a
	ET (cm)	78.07 ± 0.78	a	74.21 ± 0.27	b	66.73 ± 1.59	c	73.41 ± 1.39	a	69.87 ± 1.30	ab	65.37 ± 1.65	b
L. alyssoides	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	12.00 ± 1.53	b	22.52 ± 1.26	a	25.59 ± 2.01	a	45.40 ± 2.43	a	49.48 ± 2.59	a	50.20 ± 2.22	a
	ET (cm)	68.58 ± 1.53	a	58.06 ± 1.26	b	54.99 ± 2.01	b	52.49 ± 2.43	a	48.41 ± 2.59	a	47.69 ± 2.22	a
D. stricta	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	30.02 ± 0.83	b	30.31 ± 3.14	ab	36.61 ± 1.37	a	52.35 ± 1.39	b	56.62 ± 2.75	ab	60.18 ± 1.71	a
	ET (cm)	50.56 ± 0.83	a	50.27 ± 3.14	ab	43.97 ± 1.37	b	45.54 ± 1.39	a	41.27 ± 2.75	ab	37.71 ± 1.71	b
P. virgatum	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	34.05 ± 1.87	a	36.76 ± 1.81	a	39.56 ± 1.64	a	50.39 ± 1.17	c	53.70 ± 1.12	b	58.42 ± 0.58	a
	ET (cm)	46.53 ± 1.87	a	43.82 ± 1.81	a	41.02 ± 1.64	a	47.50 ± 1.17	a	44.19 ± 1.12	b	39.47 ± 0.58	c
×Triticosecale	IR (cm)	80.58 ± 0.00	a	80.58 ± 0.00	a	80.58 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a	97.89 ± 0.00	a
	DP (cm)	2.80 ± 0.56	c	8.11 ± 1.74	b	16.16 ± 1.91	a	14.06 ± 0.83	c	20.04 ± 1.75	b	30.92 ± 0.50	a
	ET (cm)	77.78 ± 0.56	a	72.47 ± 1.74	b	64.42 ± 1.91	c	83.83 ± 0.83	a	77.85 ± 1.75	b	66.97 ± 0.50	c

The species did not necessarily perform better in the sand than in the clay (Table 6). The ET tended to be lower and the DP tended to be higher for all species grown in clay than in the sand. Both experiments showed a difference in ET and DP with increasing salinity for ×*Triticosecale* and it was the only one of the six species to show a difference for the first clay experiment. Unlike the sand, however, the ×*Triticosecale* saw higher values for the ET and lower values for the DP in the concentrate grown plants than in the control grown plants. For the first experiment, the control plants saw 39% of the irrigation water lost to DP whereas the RO concentrate irrigated plants only lost 33% of the irrigation water to DP. Despite this deviation from the norm for the ET and DP, the values for the species were still the highest ones noted for any of the plants grown in the clay. For the ×*Triticosecale*, it was observed that as salinity increased, ET decreased and DP increased. Like the sand plants, the control plants saw higher ET and lower DP values than the RO concentrate grown plants with 22% and 35% of the irrigation water lost to DP, respectively. The *H. vulgare* and *P. virgatum* plants only saw a difference in ET and DP for the second experiment and like the second experiment for ×*Triticosecale*, an increase in salinity saw a decrease in ET and an increase in DP. The *A. canescens*, *L. alyssoides*, and *D. stricta* plants did not show any difference in ET or DP with increasing salinity in the clay soil. Within these species, the values for the plants treated with saline irrigation water were consistent with the control values.

Table 6 – Total water balance readings (cm) for the six halophyte species at the conclusion of each of the two study periods for the clay soil. Different letters across a row correspond to a statistically significant difference in total irrigation (IR), deep percolation (DP), and evapotranspiration (ET) means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		Water Balance Calculations (cm)											
		Clay 1						Clay 2					
Species	Water	EC 0.9 ± SE		EC 4.1 ± SE		EC 8.0 ± SE		EC 0.9 ± SE		EC 4.1 ± SE		EC 8.0 ± SE	
A. canescens	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	36.04 ± 1.85	a	39.31 ± 1.03	a	36.67 ± 1.35	a	46.32 ± 1.21	a	46.23 ± 0.62	a	43.12 ± 1.59	a
	ET (cm)	44.86 ± 1.85	a	41.59 ± 1.03	a	44.23 ± 1.35	a	38.62 ± 1.21	a	38.71 ± 0.62	a	41.82 ± 1.59	a
H. vulgare	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	29.15 ± 0.46	a	26.06 ± 1.71	a	29.78 ± 1.47	a	29.09 ± 3.71	b	37.25 ± 2.71	ab	38.19 ± 0.83	a
	ET (cm)	51.75 ± 0.46	a	54.84 ± 1.71	a	51.12 ± 1.47	a	55.85 ± 3.71	a	47.69 ± 2.71	ab	46.75 ± 0.83	b
L. alyssoides	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	35.41 ± 2.13	a	35.96 ± 1.98	a	37.04 ± 0.68	a	41.09 ± 0.88	a	41.53 ± 1.14	a	38.22 ± 2.79	a
	ET (cm)	45.49 ± 2.13	a	44.94 ± 1.98	a	43.86 ± 0.68	a	43.85 ± 0.88	a	43.41 ± 1.14	a	46.72 ± 2.79	a
D. stricta	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	38.64 ± 1.24	a	38.71 ± 2.77	a	38.49 ± 0.99	a	46.73 ± 2.56	a	51.45 ± 1.63	a	51.17 ± 1.84	a
	ET (cm)	42.26 ± 1.24	a	42.19 ± 2.77	a	42.41 ± 0.99	a	38.21 ± 2.56	a	33.49 ± 1.63	a	33.77 ± 1.84	a
P. virgatum	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	42.11 ± 1.46	a	41.27 ± 2.05	a	43.77 ± 0.77	a	50.93 ± 0.52	b	54.48 ± 0.37	a	54.84 ± 1.32	a
	ET (cm)	38.79 ± 1.46	a	39.63 ± 2.05	a	37.13 ± 0.77	a	34.01 ± 0.52	a	30.46 ± 0.37	b	30.10 ± 1.32	b
×Triticosecale	IR (cm)	80.90 ± 0.00	a	80.90 ± 0.00	a	80.90 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a	84.94 ± 0.00	a
	DP (cm)	31.81 ± 0.66	a	25.45 ± 1.16	b	26.70 ± 1.13	b	19.34 ± 2.04	b	21.93 ± 1.84	b	29.97 ± 0.70	a
	ET (cm)	49.09 ± 0.66	b	55.45 ± 1.16	a	54.20 ± 1.13	a	65.60 ± 2.04	a	63.01 ± 1.84	a	54.97 ± 0.70	b

The total amount of irrigation water varied depending on the soil type and the experiment, but was consistent within each experiment and soil for all treatments. As was noted, the DP was inversely related to the ET for a given treatment-plant combination. Despite the values of ET and DP varying for different halophytes, the trends remained similar: i) in both soils (with the exception of the first clay experiment), when a significant difference was detected, the ET was higher and the DP was lower for the control plants than that for either the well water or the concentrate irrigated plants, ii) the ET values were higher and the DP values were lower in the sand than in the clay, and iii) the spread of values was much smaller in the clay across treatments within a species than sand.

Leaching Fractions

During the growth period, leaching fractions remained fairly steady for most species in both soils. *D. stricta* and *×Triticosecale* showed results typical of these observations (Figure 7). *H. vulgare* was an exception to this because the plants began to flower around day 60 and subsequently started dying. This decreased the water uptake for the plants and increased the leaching.

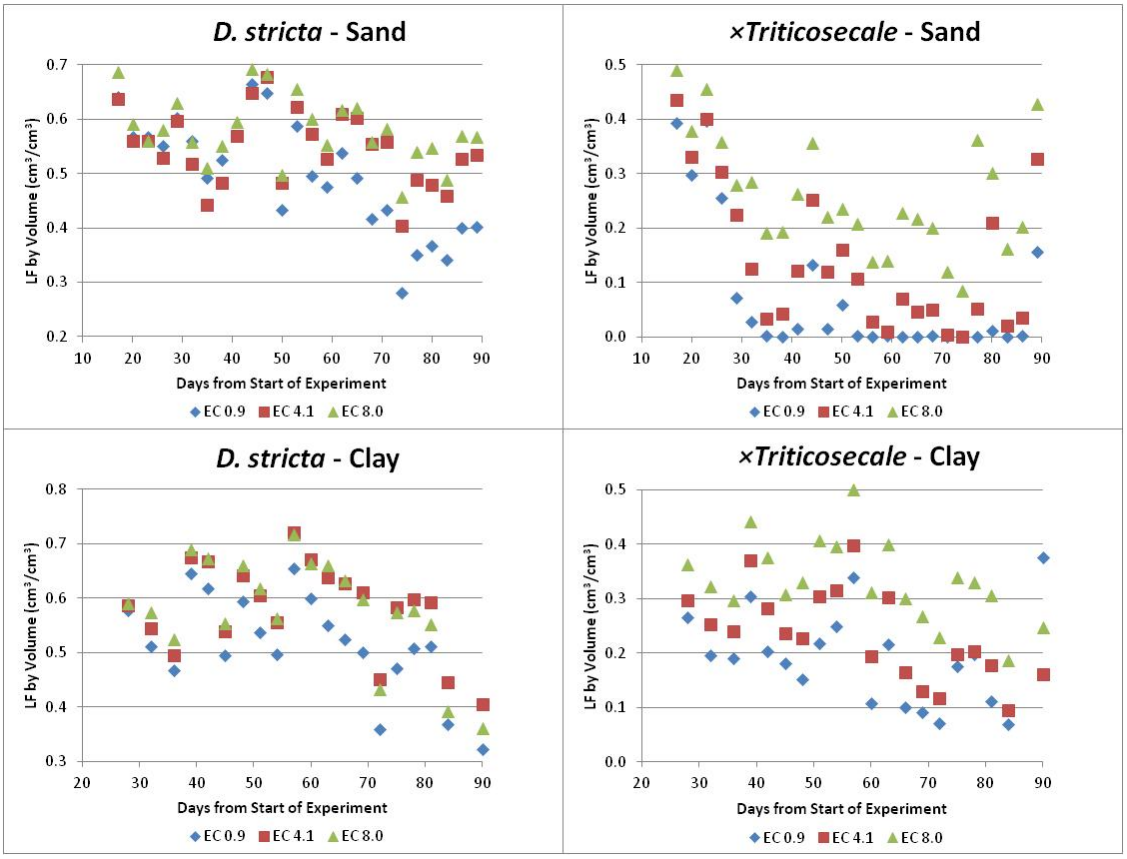

Figure 7 – Graphs of the leaching fractions by volume (volume deep percolation / volume irrigation) for *D. stricta* and ×*Triticosecale* for the second experiment in both sand and clay soil.

The sand showed a lot of variability in average leaching fractions within a species for both experiments (Table 7). In the first sand experiment, only one trend appeared: as the salinity of the irrigation water increased, the leaching fraction also increased. This was evident in the *H. vulgare*, *L. alyssoides*, *P. virgatum*, and ×*Triticosecale* plants. Two species, *A. canescens* and *D. stricta*, did not see any statistical differences in the leaching fractions with changing salinity. In the second experiment, three species showed increasing leaching fractions with increasing salinity, *D. stricta*, *P. virgatum*, and ×*Triticosecale*, but none showed any difference in ET. Although it did not show any difference in the first experiment, *A. canescens* saw a decrease in leaching fraction with increasing salinity, which was coupled with an increase in dry biomass. There were no differenced noted in either *H. vulgare* or *L. alyssoides* for the second sand experiment. Despite a modification that increased the amount of total irrigation water in the second experiment, *D. stricta*, *P. virgatum*, and ×*Triticosecale* all showed similar average leaching fractions across the two experiments. The largest decrease was seen in the *H. vulgare* plants which decreased from 0.17 to 0.15 and to 0.09 for the control, well water, and concentrate treatments, respectively. Regardless of how the change affected the average leaching fraction, the trends seen in the first experiment were the same as those in the second experiment, even when no significant difference was detected.

Table 7 – Average leaching fractions by volume (volume deep percolation / volume irrigation) for the six halophyte species over the course of the two study periods for both the sand and clay soils. Different letters down a column correspond to a statistically significant difference in leaching fraction means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		Leaching Fraction by Volume (cm^3/cm^3)							
		Sand 1		Sand 2		Clay 1		Clay 2	
Species	EC	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
A. canescens	0.9	0.28 ± 0.02	a	0.38 ± 0.01	a	0.45 ± 0.01	b	0.53 ± 0.01	a
	4.1	0.28 ± 0.02	a	0.37 ± 0.01	a	0.49 ± 0.01	a	0.52 ± 0.01	a
	8	0.27 ± 0.01	a	0.29 ± 0.01	b	0.45 ± 0.01	ab	0.48 ± 0.01	b
H. vulgare	0.9	0.05 ± 0.02	b	0.22 ± 0.02	a	0.35 ± 0.02	a	0.33 ± 0.01	b
	4.1	0.10 ± 0.02	b	0.25 ± 0.02	a	0.31 ± 0.02	a	0.43 ± 0.01	a
	8	0.21 ± 0.02	a	0.30 ± 0.01	a	0.36 ± 0.01	a	0.46 ± 0.01	a
L. alyssoides	0.9	0.17 ± 0.02	b	0.41 ± 0.01	a	0.46 ± 0.02	a	0.46 ± 0.01	a
	4.1	0.30 ± 0.01	a	0.46 ± 0.01	a	0.45 ± 0.01	a	0.46 ± 0.01	a
	8	0.35 ± 0.01	a	0.46 ± 0.01	a	0.47 ± 0.01	a	0.42 ± 0.01	a
D. stricta	0.9	0.41 ± 0.01	a	0.49 ± 0.01	b	0.49 ± 0.01	a	0.52 ± 0.01	b
	4.1	0.40 ± 0.02	a	0.55 ± 0.01	a	0.49 ± 0.01	a	0.58 ± 0.01	a
	8	0.47 ± 0.01	a	0.58 ± 0.01	a	0.49 ± 0.01	a	0.58 ± 0.01	a
P. virgatum	0.9	0.44 ± 0.01	b	0.48 ± 0.01	b	0.53 ± 0.01	a	0.58 ± 0.01	a
	4.1	0.48 ± 0.01	ab	0.51 ± 0.01	b	0.51 ± 0.01	a	0.63 ± 0.01	a
	8	0.53 ± 0.01	a	0.56 ± 0.01	a	0.54 ± 0.01	a	0.63 ± 0.01	a
×Triticosecale	0.9	0.05 ± 0.02	c	0.09 ± 0.01	b	0.39 ± 0.02	a	0.19 ± 0.01	b
	4.1	0.11 ± 0.01	b	0.15 ± 0.01	b	0.31 ± 0.02	a	0.23 ± 0.01	b
	8	0.21 ± 0.01	a	0.27 ± 0.01	a	0.34 ± 0.01	a	0.33 ± 0.01	a

The clay soil showed much less variability in the leaching fractions within a species than the sand soil (Table 7). In the first experiment, only the *A. canescens* showed a statistical difference; however, there was no corresponding difference in either ET or dry biomass for the species even with this difference in leaching fraction across the treatments. There was more variability seen in the second experiment. The *H. vulgare*, *D. stricta*, and *×Triticosecale* all showed an increase in average leaching fraction with increasing salinity. For *D. stricta* and *×Triticosecale*, there were no differences noted in ET or dry biomass, but the *H. vulgare* plants did see a trend with respect to biomass: as the leaching fraction increased, the dry biomass decreased. *A. canescens* showed a decrease in leaching fraction with an increase in salinity, but this was accompanied by an increase in biomass. Neither *L. alyssoides* nor *P. virgatum* showed any difference with respect to leaching fraction for the second experiment. The change in total amount of irrigation water changed much less for the clay than for the sand from the first to the second experiment; however, average leaching fractions did not change drastically from the first experiment to the second experiment, with the exception of *×Triticosecale*. This exception can be explained by the cracking that occurred when the clay soil dried: if the cracks became too large because the plants were taking up a lot of water and the clay was contracting (which happened most often in the control), then less water penetrated the soil and quickly leached through the cracks, increasing the leaching fraction.

For all species, the leaching fractions were higher in the clay than in the soil. This can be seen in both experiments. Even when similar fractions were seen in both soils as in *D. stricta* for experiment 2 and *P. virgatum* in experiment 1, it was still slightly higher in the clay than in the sand for those cases. Unless otherwise noted, an increase in average leaching fraction was accompanied by a decrease in total final ET and vice versa. Although differences with respect to soils were noted, trends that were seen in the sand could also be seen in the clay for the species.

Plant Growth Measurements

Dry Biomass

The plant species that responded similarly for the biomass results responded similarly for ET and DP. The *A. canescens* plants showed an increase in dry biomass with increasing salinity for both sand experiments, indicating that the plants grew slightly better with the higher salinity treatments (Table 8). Similarly, the *L. alyssoides* plants showed a greater dry biomass for the concentrate treatments for the second sand experiment and although there was not a significant difference for the first experiment, the means for the concentrate treatment were still found to be higher than the control. The *D. stricta* plants showed the least amount of variability in dry biomass yields with no significant differences found for any combination of soils and experiments except for the second sand experiment, which showed lower yields for the saline treatments than the control. The *P. virgatum* plants had the most variability in growth with notably different dry biomass yields for the sand plants. The first sand experiment showed control plants with a mean biomass of over one and a half times the mean biomass for the well treatment plants and three times the mean for the concentrate treatment plants. The *P. virgatum* plants irrigated with the saline treatments grew considerably better in the second sand experiment but no significant difference was detected; however, the biomass yields were still lower in the higher saline treatments than they were for the control. Neither *H. vulgare* nor ×*Triticosecale* showed any significant difference in dry biomass yields for the sand.

The *A. canescens* plants also showed an increase in dry biomass with increasing salinity in the clay (Table 8). There was a statistically significant difference in the second clay experiment for the species and despite not having a significant difference in the means for the first experiment, it was still evident that the average dry biomass yield for the concentrate treatment was higher than that for the control treatment. The *L. alyssoides* plants also showed a greater dry biomass for the concentrate treatments in the second experiment and like the *A. canescens*, although there was not a significant difference detected in the first experiment, the means for the concentrate treatment were higher than the control. In the first clay experiment, both *H. vulgare* and ×*Triticosecale* showed the highest biomass yield for the well water treatment, followed by the concentrate treatment and then the control. For the second clay experiment, both showed the highest biomass yield in the control but only *H. vulgare* showed a statistically significant difference. The

P. virgatum plants had the most difficult time growing in the clay soil of all six halophyte species and the plants barely survived with final mean biomass readings measuring no more than 0.22 g for either experiment in any treatment. However, despite the low dry biomass yields for the species, the control treatment plants still showed a statistically higher mean than the saline treatments. There was no significant difference detected for the *D. stricta* plants in the clay soil.

Table 8 – Dry biomass readings (g) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column correspond to a statistically significant difference in dry biomass means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		Dry Biomass (g)							
		Sand 1		Sand 2		Clay 1		Clay 2	
Species	EC	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
A. canescens	0.9	2.12 ± 0.24	b	2.53 ± 0.18	b	1.73 ± 0.22	a	1.27 ± 0.09	b
	4.1	2.37 ± 0.19	ab	3.02 ± 0.18	b	1.51 ± 0.28	a	1.29 ± 0.13	b
	8	3.00 ± 0.24	a	4.18 ± 0.10	a	1.98 ± 0.17	a	1.82 ± 0.14	a
H. vulgare	0.9	4.97 ± 0.27	a	2.93 ± 0.24	a	2.48 ± 0.23	b	2.59 ± 0.14	a
	4.1	5.29 ± 0.09	a	2.85 ± 0.56	a	3.36 ± 0.16	a	1.83 ± 0.27	b
	8	5.11 ± 0.29	a	3.61 ± 0.20	a	2.88 ± 0.20	ab	1.98 ± 0.09	b
L. alyssoides	0.9	0.86 ± 0.07	a	0.86 ± 0.03	c	0.54 ± 0.09	a	0.56 ± 0.01	b
	4.1	0.91 ± 0.08	a	1.01 ± 0.06	b	0.72 ± 0.14	a	0.63 ± 0.06	ab
	8	1.05 ± 0.11	a	1.17 ± 0.05	a	0.60 ± 0.10	a	0.86 ± 0.13	a
D. stricta	0.9	1.26 ± 0.09	a	1.54 ± 0.10	a	0.87 ± 0.12	a	0.83 ± 0.24	a
	4.1	1.58 ± 0.12	a	0.99 ± 0.18	b	0.78 ± 0.11	a	0.73 ± 0.13	a
	8	1.55 ± 0.11	a	1.17 ± 0.08	ab	0.94 ± 0.04	a	0.91 ± 0.18	a
P. virgatum	0.9	2.99 ± 0.10	a	2.56 ± 0.21	a	0.09 ± 0.02	a	0.22 ± 0.05	a
	4.1	1.72 ± 0.07	b	2.54 ± 0.21	a	0.02 ± 0.01	b	0.04 ± 0.01	b
	8	0.89 ± 0.10	c	1.93 ± 0.40	a	0.02 ± 0.00	b	0.04 ± 0.01	b
×Triticosecale	0.9	4.58 ± 0.34	a	3.09 ± 0.13	a	1.61 ± 0.14	b	1.93 ± 0.09	a
	4.1	4.36 ± 0.14	a	3.38 ± 0.14	a	2.14 ± 0.15	a	1.89 ± 0.09	a
	8	4.20 ± 0.22	a	3.55 ± 0.16	a	1.94 ± 0.09	ab	1.68 ± 0.07	a

Although there was some variability seen in the different soils, the trends tended to be similar across soils. Two plant species, *A. canescens* and *L. alyssoides*, had runs that resulted in higher amounts of dry biomass from the plants irrigated with the higher salinity water. *H. vulgare*, *×Triticosecale*, and *D. stricta* showed little difference in overall final biomass amounts and *P. virgatum* had the most variation, with the control plants containing the highest amounts of biomass. There was not a clear overall trend as some combinations showed the highest yields in the control treatment and some saw the highest in the RO concentrate. Similar to the results for *A. canescens* and *L. alyssoides*, one study showed no statistically significant difference among the dry weights of plants exposed to varying levels of salt but did note a slight increase in means with increasing salinity (Muhammad and Hussain, 2010). However, this is in contrast to the majority of salinity studies which typically show a decrease in dry biomass with increasing salinity (Hussain *et al.*, 1997; Glenn and Brown, 1998; Kim *et al.*,

2012). Other studies have shown that although dry biomass tends to decrease with increasing salinity, many differences are not noticeable until the treatment is higher (waters with ECs higher than 10 dS/m) than what was tested in this study, which could explain the lack of noted differences here (Shalaby *et al.* 1993; Marcum, 1999).

Height and Number of Leaves

At the conclusion of the study, four species (*A. canescens*, *H. vulgare*, *L. alyssoides*, and *D. stricta*) showed no significant difference in height with respect to increasing salinity in either soil or in either experiment (Table 9). The ×*Triticosecale* plants showed a much larger difference in height for the first sand experiment than for the second sand experiment or either clay experiment. This was due to the fact that in the first sand experiment, the plants reached maturity near the end of the plant cycle, whereas they did not in any of the subsequent experiments. The second sand experiment was the only one that showed a difference in heights for the ×*Triticosecale* plants with the control and well water plants being nearly twice as tall as the concentrate plants. The *P. virgatum* plants were the only ones to show a statistical difference in heights for both soils and both experiments. Even when severely stunted as in the clay soil, the plants were still tallest in the low saline treatments and shortest in the saline treatments.

The number of leaves at the conclusion of the study was much more varied than the plant height for the species in the study (Table 10). The *P. virgatum* plants showed a difference in the total number of leaves for the first sand experiment and both clay experiments. The concentrate grown plants had a significantly lower number of leaves than the control or well water grown plants in the first sand experiment, but both saline treatments showed less leaves than the control for the clay experiments. *A. canescens* showed differences in the second experiment for both soils with the concentrate grown plants having the most leaves of the treatments. Although not statistically different, the trend was the same for the first experiments. Three species, *L. alyssoides*, *D. stricta*, and ×*Triticosecale* all showed only one statistical difference in number of leaves but the results varied. *L. alyssoides* showed that the salinity treatments tended to have the higher number of leaves. *D. stricta* showed the most leaves in the well water, control, and concentrate treatments for the first sand, second sand, and both clay experiments, respectively. The ×*Triticosecale* plants had no clear trend either with the first sand experiment showing the most leaves in the control, the second sand with the most in the saline treatments, the first clay with the most in the well, and the second clay with the number of leaves equal for all treatments. The *H. vulgare* plants had no significant differences noted for either soil or either experiment but like the other species, showed varied results with the lowest number of leaves in the control for the second sand and the first clay experiments but the highest number in the control for the second clay experiment.

Table 9 – Average plant height (cm) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column correspond to a statistically significant difference in dry biomass means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		90 Day Plant Height (cm)															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	29.05	±	1.97	a	40.23	±	2.49	a	35.68	±	2.25	a	31.90	±	2.50	a
A. canescens	4.1	34.53	±	5.84	a	45.13	±	7.85	a	33.43	±	4.06	a	32.13	±	3.80	a
	8.0	39.90	±	4.53	a	42.58	±	9.61	a	37.10	±	1.72	a	30.18	±	3.27	a
LSD			NS				NS				NS				NS		
	0.9	44.85	±	1.33	a	42.38	±	1.09	a	42.28	±	1.84	a	33.15	±	1.28	a
H. vulgare	4.1	46.95	±	0.90	a	39.63	±	3.78	a	47.03	±	1.19	a	34.83	±	2.15	a
	8.0	45.88	±	1.10	a	44.25	±	1.67	a	43.93	±	1.79	a	40.28	±	3.90	a
LSD			NS				NS				NS				NS		
	0.9	4.73	±	0.98	a	9.78	±	0.52	a	5.23	±	0.79	a	6.10	±	1.21	a
L. alyssoides	4.1	3.98	±	0.52	a	11.40	±	1.52	a	6.80	±	0.71	a	9.10	±	1.55	a
	8.0	4.95	±	1.54	a	9.60	±	1.48	a	5.13	±	1.24	a	6.58	±	0.55	a
LSD			NS				NS				NS				NS		
	0.9	34.25	±	2.45	a	36.75	±	1.00	a	34.10	±	0.91	a	32.75	±	2.71	a
D. stricta	4.1	34.10	±	4.44	a	34.93	±	2.03	a	29.93	±	3.53	a	27.45	±	7.75	a
	8.0	30.78	±	2.25	a	34.65	±	2.80	a	31.93	±	3.15	a	31.73	±	3.41	a
LSD			NS				NS				NS				NS		
	0.9	35.55	±	5.90	a	43.23	±	2.60	a	5.78	±	1.08	a	11.50	±	2.06	a
P. virgatum	4.1	29.08	±	3.83	a	44.35	±	1.87	a	2.43	±	0.49	b	4.00	±	0.42	b
	8.0	11.50	±	2.10	b	32.70	±	4.04	b	2.30	±	0.37	b	4.20	±	0.95	b
LSD			13.55				9.53				2.50				4.26		
	0.9	97.23	±	0.80	a	36.23	±	4.34	a	9.50	±	0.76	a	16.45	±	2.69	a
×Triticosecale	4.1	90.75	±	3.79	a	33.13	±	5.01	a	11.63	±	0.78	a	12.20	±	0.47	a
	8.0	93.40	±	7.24	a	18.80	±	4.02	b	11.48	±	0.39	a	11.75	±	0.43	a
LSD			NS				14.31				NS				NS		

Typically there is a decrease in growth when plants are exposed to salinity. Many salinity studies have supported this observation. Scholberg and Locascio (1999) saw a decrease in dry plant matter with increasing salinity and Kim *et al.* (2012) found that plants were stunted as the salinity increased. But despite usually showing an obvious difference in growth due to salinity, occasionally plants do not appear stunted until they are compared to control plants (Bernstein, 1975; Noaman and El-Haddad, 2000). For example, it was noted in one study that at salinity levels up to approximately 13.5 dS/m, there was no difference in dry weight between saline affected and unaffected plants (Koyro *et al.*, 2013). Similarly, many studies have shown that salt affected plants generally have fewer leaves than their unaffected counterparts (Saberi *et al.*, 2011; Alvarez *et al.*, 2012); however, some species did show a slight increase in the number of leaves with irrigation salinity up to approximately 21 dS/m (Redondo-Gomez *et al.*, 2007).

Table 10 – Average number of leaves per plant for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column correspond to a statistically significant difference in dry biomass means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		90 Day Number of Leaves															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	151.00	±	14.28	a	180.75	±	11.64	b	118.75	±	11.09	a	109.00	±	9.58	b
A. canescens	4.1	125.50	±	14.48	a	335.00	±	41.65	a	106.25	±	12.85	a	99.50	±	5.50	b
	8.0	164.00	±	20.38	a	306.25	±	28.74	a	137.75	±	12.96	a	168.75	±	15.63	a
LSD			NS				95.90				NS				35.36		
	0.9	3.25	±	1.18	a	3.75	±	2.84	a	6.75	±	1.11	a	7.25	±	0.75	a
H. vulgare	4.1	3.50	±	0.65	a	6.50	±	1.50	a	9.00	±	0.41	a	5.00	±	1.41	a
	8.0	3.00	±	1.29	a	11.25	±	3.42	a	7.00	±	1.29	a	5.25	±	1.89	a
LSD			NS				NS				NS				NS		
	0.9	25.75	±	1.25	a	29.00	±	0.71	b	19.25	±	1.31	a	21.50	±	0.87	a
L. alyssoides	4.1	24.00	±	1.96	a	61.00	±	12.83	a	17.67	±	1.20	a	21.25	±	1.44	a
	8.0	28.50	±	1.26	a	47.25	±	7.95	ab	21.67	±	1.76	a	26.50	±	2.90	a
LSD			NS				27.90				NS				NS		
	0.9	92.25	±	5.36	a	150.50	±	11.03	a	73.75	±	8.05	a	70.50	±	16.98	a
D. stricta	4.1	105.25	±	11.99	a	87.75	±	17.71	b	66.00	±	10.98	a	62.75	±	23.22	a
	8.0	95.75	±	8.08	a	90.00	±	3.94	b	82.00	±	5.21	a	82.00	±	17.15	a
LSD			NS				39.22				NS				NS		
	0.9	18.75	±	1.25	a	18.75	±	1.25	a	4.00	±	0.41	a	7.50	±	0.65	a
P. virgatum	4.1	19.00	±	1.96	a	17.00	±	1.68	a	2.00	±	0.00	b	2.25	±	0.25	b
	8.0	10.50	±	2.63	b	16.50	±	2.66	a	2.50	±	0.29	b	2.25	±	0.25	b
LSD			6.48				NS				1.05				1.36		
	0.9	7.25	±	2.81	a	22.25	±	2.72	a	14.00	±	1.47	b	13.25	±	1.93	a
×*Triticosecale*	4.1	3.00	±	1.08	a	33.00	±	4.78	a	21.25	±	1.31	a	13.75	±	1.84	a
	8.0	2.50	±	0.87	a	31.25	±	2.78	a	17.00	±	0.91	b	12.75	±	1.55	a
LSD			NS				NS				4.02				NS		

Photosynthetic Rates

In the first sand experiment, only *H. vulgare* showed a significant difference in photosynthetic rates (Table 11). It saw the highest rates in the well water treatment, followed by the control then the concentrate. *A. canescens*, *D. stricta*, and *P. virgatum* all showed the highest values in the control, whereas *L. alyssoides* had higher rates in the saline treatments than in the control. The ×*Triticosecale* plants had treatment values much closer to each other than the other species and saw the highest rates in the control, then the concentrate and well water. In the second sand experiment, four species showed significant difference. *A. canescens*, *L. alyssoides*, and *P. virgatum* all showed the highest photosynthetic rates in the concentrate treatment plants whereas *H. vulgare* had the highest values in the control. The *D. stricta* plants saw higher values in the saline treatments than the control and ×*Triticosecale* showed the highest photosynthetic values in the control, despite neither species showing a significant difference.

Table 11 – Average photosynthetic rates (μmol CO_2 $m^{-2}s^{-1}$) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column correspond to a statistically significant difference in dry biomass means within a species at $\alpha = 0.05$. Measurements were not compared across species.

		Photosynthetic Rates (μmol $CO_2 m^{-2}s^{-1}$)															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	19.94	±	3.19	a	7.94	±	0.47	b	7.39	±	0.47	b	5.69	±	1.22	a
A. canescens	4.1	13.70	±	1.69	a	7.91	±	0.61	b	10.37	±	0.42	a	7.18	±	1.29	a
	8.0	13.66	±	1.09	a	10.60	±	1.33	a	11.51	±	1.24	a	7.17	±	1.02	a
LSD			NS				2.62				2.37				NS		
	0.9	16.56	±	0.99	ab	8.47	±	0.92	a	11.43	±	0.15	a	7.83	±	2.26	a
H. vulgare	4.1	18.36	±	0.76	a	6.37	±	0.24	b	10.51	±	0.76	a	3.50	±	1.64	a
	8.0	15.43	±	0.56	b	7.35	±	0.61	ab	7.68	±	0.59	b	9.49	±	1.78	a
LSD			2.37				1.64				2.12				NS		
	0.9	16.79	±	0.69	a	9.33	±	0.88	b	15.92	±	2.00	a	5.44	±	1.21	a
L. alyssoides	4.1	18.17	±	1.87	a	13.48	±	1.41	a	12.69	±	1.43	a	7.60	±	2.26	a
	8.0	18.04	±	3.50	a	14.01	±	1.22	a	13.19	±	3.00	a	8.07	±	1.53	a
LSD			NS				3.49				NS				NS		
	0.9	13.30	±	0.98	a	5.66	±	1.10	a	10.33	±	1.83	b	6.42	±	1.82	a
D. stricta	4.1	12.20	±	1.29	a	9.21	±	1.69	a	13.10	±	1.03	ab	7.57	±	2.43	a
	8.0	11.49	±	0.76	a	6.26	±	1.07	a	14.12	±	0.69	a	9.10	±	1.01	a
LSD			NS				NS				3.75				NS		
	0.9	20.66	±	1.02	a	8.02	±	0.42	b	15.97	±	1.43	a	5.32	±	1.63	a
P. virgatum	4.1	17.82	±	1.39	a	15.59	±	0.88	a	13.30	±	0.25	a	-1.59	±	1.82	b
	8.0	17.36	±	1.17	a	13.48	±	1.06	a	8.43	±	1.78	b	-1.25	±	0.99	b
LSD			NS				2.44				4.80				4.47		
	0.9	13.88	±	0.57	a	13.48	±	0.75	a	7.35	±	0.64	b	10.55	±	0.29	a
×Triticosecale	4.1	12.03	±	0.92	a	11.89	±	0.85	a	13.17	±	2.07	a	9.76	±	0.60	a
	8.0	13.19	±	0.36	a	11.29	±	0.67	a	11.59	±	0.34	a	10.20	±	0.80	a
LSD			NS				NS				3.73				NS		

In the first clay experiment, the photosynthetic rate varied a lot with respect to species and treatment (Table 11). *A. canescens*, *D. stricta*, and *×Triticosecale* showed their highest readings for photosynthetic rate in the concentrate while the highest rates for *H. vulgare* and *P. virgatum* were found in the control. *L. alyssoides* saw no significant difference, but the photosynthetic rates in the control were shown to be higher than the saline treatments. Far fewer differences were evident for the second clay experiment and only *P. virgatum* saw a statistically significant difference. Four plant species (*A. canescens*, *H. vulgare*, *L. alyssoides*, and *D. stricta*) saw the highest values of photosynthetic rate in the saline treatments. *P. virgatum* and *×Triticosecale* had their highest values in the control.

For all combinations of soil and experiment, any significant differences for conductance or transpiration followed the trends that the photosynthetic rate measurements saw. The average leaf temperature did not vary by more than 4°C for any combination of species, soil, and experiment.

There are many contrasting reports concerning salinity and photosynthetic rates in previous studies. Some studies showed that at moderate levels of salinity, especially in halophytic species, photosynthetic rates remained unaffected as compared to the control treatment (Koyro *et al.*, 2013; Ge *et al.*, 2014). Other studies showed little variability in photosynthetic rates for the first several weeks of saline treatments but eventually showed a decrease in rates with increasing salinity (Alvarez *et al.*, 2012). Many halophytes showed an eventual decrease in photosynthetic rates with increasing salinity (Redondo-Gomez *et al.*, 2006; Redondo-Gomez *et al.*, 2007; Ge *et al.*, 2014) but this was not always the case. Some halophytes saw an increase in photosynthetic rate until the EC of the irrigation water reached approximately 14 dS/m (Koyro *et al.*, 2013) and one extreme halophyte showed an increase in rates with increasing salinity up to an EC of approximately 52 dS/m (Redondo-Gomez *et al.*, 2010). For each of these studies, the stomatal conductance and transpiration rates responded in the same way that photosynthetic rates did.

Ion Uptake

Generally, an increase in irrigation water salinity caused an increase in sodium (Na^+) concentration for all species, soils, and experiments, even when no significant difference was detected, but there were two deviations from this trend (Table 12). The first sand experiment showed a decrease in sodium ion concentration with increasing salinity for *H. vulgare* and the first clay experiment for *A. canescens* had a much lower concentration for the well water treatment than either the control or the concentrate treatments. *D. stricta* and ×*Triticosecale* both saw significant differences in both soils and both experiments. *H. vulgare* had a statistical difference for all combinations of soil and experiment except for the one mentioned above. *L. alyssoides* and *P. virgatum* both only had differences for the second sand experiment and *A. canescens* showed no differences for either soil or experiment.

Chloride (Cl^-) concentration was much more varied than the Na^+ concentration (Table 13). In general, Cl^- concentration increased with increasing salinity, however, a few instances of decreasing concentration were noted for *A. canescens* in the first clay experiment and *A. canescens* and *P. virgatum* in the second sand experiment, but not all were significantly different. In the second clay experiment, *D. stricta* saw a significant difference with the highest concentration in the well water treatment, and although not statistically different, the same was noted for *H. vulgare* in the second experiment for both soils.

Far fewer differences were noted for potassium (K^+) concentration than for Na^+ or Cl^- (Table 14). The general trend for K^+ was that as irrigation salinity increased, the concentration of the ion decreased. Although a couple species showed slightly different values for the well water treatment than either the control or the concentrate, they still followed the trend in that the treatments exposed to less salinity had higher K^+ concentrations than those exposed to higher salinity. The

one exception to this was the *P. virgatum* plants in the second sand experiment which showed a significantly different increase in ion concentration with increasing salinity.

Table 12 – Sodium (Na^+) ion concentrations (%) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column within a species correspond to a statistically significant difference in concentration at $\alpha = 0.05$. Measurements were not compared across species. Final *P. virgatum* dry biomass samples were combined due to low individual plant biomass for analysis and therefore, only one data point (and thus no standard error data) could be obtained for the species.

		Sodium Concentration (%)															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	1.31	±	0.74	a	1.93	±	0.60	a	1.10	±	0.41	a	0.46	±	0.40	a
A. canescens	4.1	0.83	±	0.54	a	1.54	±	0.78	a	0.50	±	0.34	a	1.12	±	0.67	a
	8.0	0.85	±	0.44	a	2.31	±	0.72	a	1.46	±	0.70	a	1.15	±	0.85	a
LSD			NS				NS				NS				NS		
	0.9	0.32	±	0.12	a	0.19	±	0.01	c	0.19	±	0.01	b	0.18	±	0.03	b
H. vulgare	4.1	0.30	±	0.03	a	0.40	±	0.04	b	0.29	±	0.04	b	0.39	±	0.03	ab
	8.0	0.24	±	0.09	a	0.60	±	0.04	a	0.55	±	0.12	a	0.56	±	0.14	a
LSD			NS				0.10				0.23				0.27		
	0.9	0.07	±	0.03	b	0.05	±	0.01	b	0.03	±	0.02	a	0.12	±	0.07	a
L. alyssoides	4.1	0.09	±	0.02	b	0.13	±	0.03	b	0.31	±	0.21	a	0.11	±	0.04	a
	8.0	0.21	±	0.05	a	0.34	±	0.06	a	0.13	±	0.04	a	0.19	±	0.02	a
LSD			0.12				0.12				NS				NS		
	0.9	0.32	±	0.06	b	0.60	±	0.01	b	0.50	±	0.07	b	0.64	±	0.10	c
D. stricta	4.1	0.57	±	0.07	ab	1.39	±	0.23	a	0.79	±	0.07	ab	0.98	±	0.06	b
	8.0	0.78	±	0.17	a	1.56	±	0.09	a	1.01	±	0.12	a	1.30	±	0.07	a
LSD			0.36				0.46				0.30				0.28		
	0.9	0.03	±	0.01	b	0.06	±	0.03	b	0.06	±	.	.	0.06	±	.	.
P. virgatum	4.1	0.13	±	0.06	b	0.05	±	0.00	b	0.22	±	.	.	0.40	±	.	.
	8.0	0.32	±	0.07	a	0.14	±	0.03	a	1.22	±	.	.	1.68	±	.	.
LSD			0.17				0.08				N/A				N/A		
	0.9	0.13	±	0.03	c	0.07	±	0.01	c	0.07	±	0.01	b	0.11	±	0.01	c
×Triticosecale	4.1	0.29	±	0.04	b	0.42	±	0.06	b	0.48	±	0.12	a	0.31	±	0.07	b
	8.0	0.70	±	0.06	a	0.73	±	0.05	a	0.52	±	0.03	a	0.66	±	0.03	a
LSD			0.15				0.15				0.23				0.14		

With respect to Na^+, Cl^-, and K^+ ion concentrations, *×Triticosecale* and *D. stricta* were the species that were most susceptible to ion uptake due to differing amounts of salt in its irrigation water. These species each noted 9 statistically significant differences for various combinations of soil and experiment for these ions. The least susceptible was *A. canescens* with only 1 difference and the remaining three species, *H. vulgare*, *L. alyssoides*, and *P. virgatum*, were similar in the amount of noted differences with 5, 5, and 4, respectively.

Table 13 – Chloride (Cl^-) ion concentrations (%) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column within a species correspond to a statistically significant difference in concentration at $\alpha = 0.05$. Measurements were not compared across species. No data could be recorded for some species

(noted with a '.') because of low total final dry biomass. Following microwave digestion, there was not enough remaining plant matter for chloride analysis.

		Chloride Concentration (%)															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	2.02	±	0.37	a	4.12	±	0.32	a	2.03	±	0.77	a	2.41	±	0.29	a
A. canescens	4.1	1.76	±	0.44	a	2.66	±	0.54	a	1.62	±	0.25	a	2.48	±	0.88	a
	8.0	2.43	±	0.29	a	3.63	±	0.53	a	1.94	±	0.44	a	2.91	±	0.17	a
LSD			NS				NS				NS				NS		
	0.9	1.08	±	0.09	b	1.64	±	0.13	a	1.88	±	0.08	ab	2.14	±	0.56	a
H. vulgare	4.1	1.13	±	0.06	b	1.93	±	0.09	a	1.62	±	0.16	b	2.29	±	0.18	a
	8.0	1.76	±	0.25	a	1.66	±	0.18	a	2.12	±	0.18	a	2.03	±	0.20	a
LSD			0.51				NS				0.46				NS		
	0.9	0.77	±	0.05	b	0.97	±	0.12	b	.	±	.	.	.	±	.	.
L. alyssoides	4.1	0.84	±	0.07	ab	1.40	±	0.08	a	0.88	±	.	.	.	±	.	.
	8.0	1.37	±	0.24	a	1.47	±	0.11	a	0.71	±	.	.	1.63	±	0.12	.
LSD			0.54				0.32				N/A				N/A		
	0.9	0.91	±	0.12	a	1.38	±	0.15	b	1.21	±	0.29	a	1.44	±	0.08	b
D. stricta	4.1	1.34	±	0.49	a	1.95	±	0.14	ab	1.39	±	0.23	a	2.21	±	0.10	a
	8.0	1.43	±	0.19	a	2.24	±	0.27	a	1.85	±	0.27	a	1.87	±	0.22	ab
LSD			NS				0.62				NS				0.60		
	0.9	0.58	±	0.16	b	1.37	±	0.95	a	.	±	.	.	.	±	.	.
P. virgatum	4.1	0.72	±	0.13	b	0.53	±	0.04	a	.	±	.	.	.	±	.	.
	8.0	1.38	±	0.11	a	0.80	±	0.04	a	.	±	.	.	.	±	.	.
LSD			0.53				NS				N/A				N/A		
	0.9	0.79	±	0.09	b	0.88	±	0.07	b	1.83	±	0.30	a	1.63	±	0.24	b
×Triticosecale	4.1	0.92	±	0.07	b	1.82	±	0.05	a	2.00	±	0.16	a	1.93	±	0.22	b
	8.0	1.64	±	0.16	a	2.03	±	0.07	a	2.39	±	0.12	a	2.69	±	0.14	a
LSD			0.36				0.21				NS				0.65		

In order to facilitate water uptake when the soil solution is saline, many plants take up some of the ions in order to adjust the internal osmotic potential and it is important to look at all the ions and interactions because similar reactions occur within plants even when the individual ion concentrations vary (Alvarez *et al.*, 2012; Souza *et al.*, 2012). Many studies showed that as the salinity of the irrigation water increased, the concentration of Na^+ also increased (Scholberg and Locascio, 1999; Redondo-Gomez *et al.*, 2007; Redondo-Gomez *et al.*, 2010; Alvarez *et al.*, 2012). Chloride was suggested to be more toxic than Na^+ and tended to appear in larger concentrations when salinity was higher (Alvarez *et al.* 2012). Potassium ions are generally excluded from plant uptake in the presence of Na^+ and various salinity studies have shown that an increase in salinity stress causes a decrease in K^+ (Scholberg and Locascio, 1999; Redondo-Gomez *et al.*, 2010; Hussain *et al.*, 2014). However, this was more variable as one study showed little difference after 3 dS/m in K^+ concentration (Redondo-Gomez *et al.*, 2007) and another even showed a slight increase with increasing salinity (Scholberg and Locascio, 1999).

Table 14 – Potassium (K^+) ion concentrations (%) for the six halophyte species at the conclusion of each of the two study periods for both the sand and clay soils. Different letters down a column within a species correspond to a statistically significant difference in concentration at $\alpha = 0.05$. Measurements were not compared across species. Final *P. virgatum* dry biomass samples were combined due to low individual plant biomass for analysis and therefore, only one data point (and thus no standard error data) could be obtained for the species.

		Potassium Concentration (%)															
		Sand 1				Sand 2				Clay 1				Clay 2			
Species	EC	Mean	±	SE		Mean	±	SE		Mean	±	SE		Mean	±	SE	
	0.9	3.37	±	0.54	a	2.83	±	0.18	a	4.63	±	0.35	a	4.74	±	0.51	a
A. canescens	4.1	2.75	±	0.27	a	2.32	±	0.10	b	4.11	±	0.53	a	5.00	±	1.03	a
	8.0	2.23	±	0.20	a	1.65	±	0.11	c	4.33	±	0.29	a	4.40	±	0.39	a
LSD			NS				0.43				NS				NS		
	0.9	1.25	±	0.03	a	1.99	±	0.20	a	2.10	±	0.12	a	2.28	±	0.13	a
H. vulgare	4.1	1.14	±	0.04	a	1.75	±	0.15	a	1.84	±	0.09	a	2.60	±	0.22	a
	8.0	1.35	±	0.13	a	1.67	±	0.09	a	1.95	±	0.07	a	2.36	±	0.22	a
LSD			NS				NS				NS				NS		
	0.9	2.14	±	0.15	a	3.36	±	0.21	a	2.25	±	0.12	a	2.72	±	0.28	a
L. alyssoides	4.1	2.27	±	0.29	a	3.59	±	0.26	a	1.93	±	0.40	a	2.13	±	0.09	b
	8.0	1.89	±	0.21	a	3.04	±	0.39	a	2.37	±	0.12	a	2.49	±	0.07	ab
LSD			NS				NS				NS				0.55		
	0.9	1.17	±	0.01	a	1.38	±	0.04	ab	1.32	±	0.07	a	1.48	±	0.07	a
D. stricta	4.1	1.03	±	0.06	a	1.50	±	0.07	a	1.21	±	0.07	a	1.43	±	0.06	ab
	8.0	1.05	±	0.09	a	1.18	±	0.11	b	0.88	±	0.04	b	1.12	±	0.13	b
LSD			NS				NS				0.20				0.33		
	0.9	0.90	±	0.07	b	1.15	±	0.09	a	1.47	±	.	.	1.60	±	.	.
P. virgatum	4.1	1.13	±	0.12	b	1.01	±	0.09	a	0.23	±	.	.	0.76	±	.	.
	8.0	1.68	±	0.04	a	1.12	±	0.13	a	0.36	±	.	.	0.47	±	.	.
LSD			0.27				NS				N/A				N/A		
	0.9	1.34	±	0.06	a	1.97	±	0.12	a	2.78	±	0.11	a	2.63	±	0.08	a
×Triticosecale	4.1	1.13	±	0.06	b	1.75	±	0.03	a	2.11	±	0.14	b	2.46	±	0.07	a
	8.0	1.02	±	0.04	b	1.72	±	0.10	a	2.15	±	0.08	b	2.51	±	0.18	a
LSD			0.18				NS				0.36				NS		

Conclusions

1. The germination study study showed that for these seed lots, levels of germination obtained with deionized water can also be achieved with saline water treatments up to an EC of 10.0 dS/m. Five of the six species showed some delay in germination with increasing salinity, which is consistent with the findings of previous studies (Ries and Hofmann, 1983; Almansouri *et al.*, 2001). These six species, *A. canescens*, *H. vulgare*, *L. alyssoides*, *D. stricta*, *P. virgatum* and *×Triticosecale*, can potentially survive the germination and re-vegetation process with saline water and are adequate candidate species for land application sites.
2. Pore clogging study showed that continuous application of RO concentrate will cause salt deposition in the pores leading to pore clogging with attendant decreases in hydraulic conductivity of soil.

3. The greenhouse plant survival experiments showed that for these six species, salinity can affect their growth but at the levels of salinity tested, all species were able to tolerate the salt and survive. Two species, *A. canescens* and *L. alyssoides* saw increases in dry biomass with increasing salinity whereas the others saw a decrease. This increase is in contrast to most studies which see a reduction in biomass with increasing salinity (Glenn and Brown, 1998; Kim *et al.*, 2012), but indicates that they are indeed halophytic species and grow slightly better with salts, rather than just tolerating them. K^+ is often replaced by Na^+ in plants when sodium ions are present which is evident in this study as well. Based on the results from this study, these six species (*A. canescens*, *H.vulgare*, *L. alyssoides*, *D. stricta*, *P. virgatum*, and ×*Triticosecale*) could be adequate candidate species for land application sites.

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