ESTABLISHMENT AND GROWTH OF HALOPHYTIC SHRUBS WITH INCREASING CONCENTRATION OF TOTAL DISSOLVED SALTS

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ABSTRACT

Saltwater spill sites on west Texas rangelands caused by the oil and gas industry can reduce vegetation and weaken the soil structure to the point no vegetation can survive. This study assessed three halophyte shrubs, Four-winged saltbush (*Atriplex canescens* (Pursh) Nutt.), Salt cedar (*Tamarix chinensis* Lour.) and Willow baccharis (*Baccharis salicina* Torr. & A. Gray) response to increasing water salinity. Plants were divided into four groups and watered with either 0, 3,000, 5,000 or 10,000 ppm sodium chloride (NaCl) solution for 34 days. Plant height, drainage volume, drainage electrical conductivity and soil electrical conductivity were measured throughout the study. Nutritional analysis of each plant species was also recorded at the end of the study. All species survived and removed sodium (Na) from the soil. Salt cedar top growth had the highest Na content in its leaves and lowest salinity in the soil making it a preferred shrub specie to plant on saltwater spill sites.

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INTRODUCTION

Rangelands in Texas and the remaining western United States are utilized primarily for livestock grazing and wildlife habitat. Most of the rangelands in western Texas and the southwestern U.S. are semi-arid to arid thereby limiting forage production for livestock or wildlife. Many areas in this region are also characterized by forage loss from oil and gas exploration.

During the production of crude oil and natural gas, salt water, often exceeding 80,000 ppm total dissolved salts, is produced. Normally salt water is pumped back into the same formation after separation from crude oil or natural gas. Unfortunately, saltwater spills do occur both below and above soil surface, resulting in complete vegetation loss and the formation of bare ground.

Wastewater is also produced through fracking (Dornbusch et al. 2020). While total dissolved salts tend to be much lower in frack water (3.000 to 10,000 ppm) little is known regarding its impact of native vegetation growth and recovery. Along with the excess salt, other materials like heavy metals, and other potentially toxic constituents can be found in both salt and frack water (Dornbusch et al. 2020). These compounds may be harmful to forage production or to livestock consuming these forages.

Biological remediation can be applied to reclaim areas where frack water has been stored or areas where salt water was spilled. The process of bio-remediation consists of planting grasses, forbs, or shrubs that have higher salt tolerance in the areas of saltwater

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spills. If the plants establish, soil organic matter and water infiltration rates typically improve, allowing for additional plant establishment. In addition, some plant species tend to uptake salts from the soil thereby reducing soil salinity.

Recent work has shown that two grass species, Alkali Sacaton (*Sporobolus airoides* (Torr.) Torr.) and Giant Sacaton (*Sporobolus wrightii* Munro ex Scribn.) will both establish and persist in areas where salt water has been spilled or frack water was stored (Kennedy 2020). For this study, three shrubs were utilized. Each was tested and evaluated to determine persistence as soil salinity increases from 0 to 10,000 ppm. In addition, the proposed study evaluated salt uptake by three halophytic shrub species.

OBJECTIVES

1. Established three halophytic shrubs (*Atriplex canescens* (Pursh) Nutt., *Tamarix chinensis* Lour., *Baccharis salicina* Torr. & A. Gray) in a simulated environment and watered with an increasing amount of salt from 0 to 10,000 ppm.
2. Monitored saline levels in leachate, soil and vegetation.

LITERATURE REVIEW

When saltwater is released on rangelands, soil quality and structure are altered, thereby reducing the potential for vegetation required for livestock and wildlife. Initially, mortality of herbaceous and woody plants occurs, resulting in bare ground. The increased amount of salt after spills also weakens soil aggregate stability and promotes the dispersion of soil particles leaving the soil highly susceptible to erosion, compaction, and crust formation (Pessarakli 1991; Atalay et al. 1999). This leaves a vast, barren landscape with poor water infiltration and little to no vegetation. Unless salts are removed through infiltration or overland water flow, saline content of these soils remains high, limiting vegetation for decades. To combat these areas, research shows bio-remediation of rangelands can be successful using halophytic plants that tolerate increased amounts of salt.

Halophytes have a high tolerance to an increased amount of salt in contaminated soils. Halophytes can survive in saline soils where 99% of other plant species cannot (Flowers and Colmer 2008). Some halophytes can extract salt from saltwater spills by concentrating salts in above ground growth. Livestock grazing can then be used to remove salts from the location; as livestock consume above ground growth, salts would be removed off site.

Some halophytes tolerate salts while others avoid salt in soils. Some adaptations they have to tolerate saline soils include reducing the amount of sodium that enters the plant, compartmentalizing and dispersing Na in different areas of the plant and secreting Na ions through salt glands in the leaves or other parts of the plant (Flowers and Colmer 2008; Flowers et al. 1976). Halophytes with compartmentalized salts in above ground growth would facilitate removal of salts through livestock grazing.

Atriplex canescens (Pursch) Nutt. (Four-winged saltbush) is a native, perennial, semi-evergreen shrub that is highly nutritious to livestock and wildlife. Four-winged saltbush can be found in saline areas and has extensive root systems to promote excretion of Na as well as promoting soil permeability (Powell 1998). These shrubs are extremely valuable since they may be one of the few plants in saline areas that can be consumed by livestock and wildlife. When successfully transplanting these shrubs onto spill sites it is optimal to have around 14% soil moisture by weight to have an 80% survival rate (Aldon 1972). After transplantation, Four-winged saltbush can thrive and reproduce by seeds as long as overgrazing does not occur.

Tamarix chinensis (Lour.) (Salt cedar) is an introduced evergreen shrub found in the southwestern U.S. Introduced in the early 1800's, Salt cedar was used in the control of erosion and windbreaks with little success. Salt cedar also thrive in saline soils and has salt excreting glands in its leaves (Diggs et al. 1999; Di Tomaso 1998). The plant can tolerate salt concentrations ranging from 6,000-36,000 ppm giving it a distinct advantage over other plants and a low selectivity for salts. After excretion, the salts form white residues on the surface of the leaves (Di Tomaso 1998). Salt cedar leaves are readily consumed by livestock, providing a mechanism of salt removal (Knight et al. 2018, Parker and Scott 2021). While very beneficial in the uptake of salts in soils, Salt cedar is an extremely hardy and easily spread plant. Infestations of Salt cedar can happen rapidly if not monitored closely and can turn a barren landscape into an area that is impassable, making it impossible for other native shrubs and grasses to grow.

Baccharis salicina (Torr. & A. Gray) (Willow baccharis) is a native shrub that can be found along waterways and moist environments. Despite preferring mesic sites, Willow baccharis is drought tolerant. Willow baccharis reproduces by seeds and rhizomes and is prolific when established. While livestock typically avoid Willow baccharis, goats will consume the plant when activated charcoal or additional protein supplementation is fed (Smith 2020). Willow baccharis can be found in areas of low salinity, ranging from .02 to .63% of salt on a dry weight basis. Germination also requires the temperature to be between 15℃ and 32℃ and in adequate, ambient light (Ungar 1968).

METHODS

This study was conducted in the greenhouse at the Angelo State University Management, Instruction and Research (MIR) Center (N 31.54330, W -100.51289) in San Angelo, Tx. The shrubs used included Four-winged saltbush, Salt cedar and Willow baccharis.

Salt cedar and Willow baccharis were transplanted and established from established stands at the MIR Center into 8-liter plastic containers and moved into the greenhouse. Four-winged saltbush was established using purchased seeds in the same size containers. The containers have 1 cm diameter holes on the bottom to help excess water drain out. The containers were filled with a mixture of commercially available potting soil (75%) and sand (25%) to help stimulate plant growth and water infiltration. Each container housed an individual shrub for the study. Before transplanting into the new container, the roots were washed off to prevent contamination and provided with a root stimulate to facilitate establishment.

Ten containers of each shrub species were assigned to each of four treatment groups (n = 30 per treatment). After transplanting, each shrub was watered with 2.5 cm (1 inch) of well water every day for 28 days to ensure establishment. The well water that is used is from a well located at the MIR Center and is high in calcium carbonate. After establishment, the shrubs were split up into the treatments, with each treatment watered their respective aqueous solution mixture (0, 3,000, 5,000, 10,000 ppm sodium chloride) to represent common saltwater contamination on rangelands. Shrubs were watered every seven days for the entirety of the study (34 days). The water mixture was produced from the same well water used in the establishment period. The solution that drained completely through the soil profile was collected using a 19 L container placed under each plant.

To measure the aqueous solution, soil and leachate, electrical conductivity was used. Electrical conductivity is the ability of a material to conduct or prevent an electrical current passing through it. The electrical conductivity (EC) of the solution and leachate was measured by using a Hach Co. CDC401 Hydraulic Conductivity Probe. Soil EC was measured using a Hanna Instruments Groline H198331 Direct Soil Conductivity and Temperature Meter. EC measurements of the aqueous solution and soil were evaluated prior to watering with the aqueous solution. After a period of seven days of watering with the aqueous solution, EC measurements of the soil was taken again and re-recorded.

After completion of the study, aboveground biomass was assessed. In addition, nutritional quality and salt content was measured and compared among treatments. This was completed by clipping the aboveground growth. Plant materials were dried at 60 degrees Celsius for 48 hours and ground into 2mm granules (50 g sample). The samples were sent to Dairy-One Laboratory to test for the crude protein, total chlorides, Sodium content, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Total Digestible Nutrients (TDN) of each individual shrub.

Data were analyzed using repeated measures analysis of variance. Salt concentrations served as the main effect (treatment) with species as the subplot. Individual plants nested within treatments and species serving as error term. Day of collection was used as the repeated measures. Means were separated using Tukey's Protected LSD when $P \leq 0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

RESULTS

All plant species readily established and persisted throughout the study; no mortality of any plant was recorded. Salt cedar and Willow baccharis seedlings were transplanted into pots while Four-winged saltbush plants were germinated from seeds. Plants were watered at weekly intervals with their respected solution (0, 3,000, 5,000, 10,000 ppm NaCl).

Plant heights (cm) were taken prior to each watering to measure amount of growth between each treatment and each day of collection. Plant height differed by species and by day. Plant heights steadily increased and differed each day of collection (Fig. 1) ($P < 0.05$). Salt cedar plants were taller (96.7 ± 2.4 cm) than Four-winged saltbush (50.6 ± 2.8 cm) or Willow baccharis 48.8 ± 2.8 cm). Four-winged saltbush and Willow baccharis were similar for plant height. All other interactions were similar ($P > 0.05$).

Drainage volume was taken 48 hours after each watering and recorded in milliliters. Drainage volume was not recorded on day 0. After the first collection, leachate volume appears to differ among different plant species. Thereafter, leachate volume was measured 48 hours after each collection. Salt cedar drainage volume was not recorded throughout the study because no leachate was observed. Species by treatment (Table 1), day by treatment (Fig. 2) and species by day (Table 2) interactions all differed ($P < 0.05$). Four-winged saltbush watered with 10,000 ppm had lower drainage than the plants watered 0, 3,000 and 5,000 ppm solution. Willow baccharis in the control showed a higher drainage volume compared to the 3,000, 5,000 and 10,000 ppm group (Table 1). In general, the control typically had the highest drainage volume, especially on day 7 and 21 (Fig. 2). All drainage volumes tended to be lower on day 14. All other volumes were similar. Four-winged saltbush had a lower drainage volume on day 14. Willow baccharis drainage volume differed

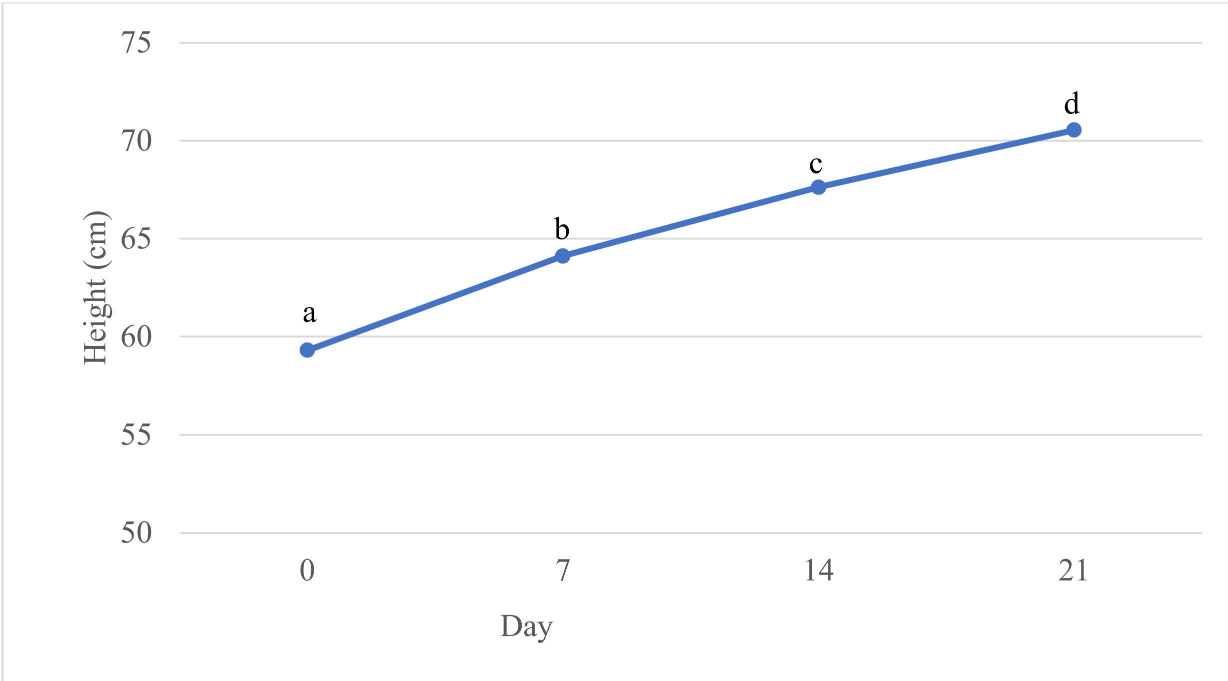

Figure 1. Average height of all plants taken in centimeters during each collection date. Means[a-d] represent letters of significance ($P < 0.05$).

Table 1. Average drainage volume (mL) of brine solution (ppm) that percolated through the soil profile of each individual plant. Collections were made 48 hours after watering.

	Treatment (ppm)				
Species	**0**	**3,000**	**5,000**	**10,000**	**SEM**
Four-winged saltbush	139.0^{ab}	103.1^{ab}	126.3^{ab}	56.0^{cd}	7.2
Willow baccharis	179.8^{a}	81.0^{bc}	89.4^{bc}	91.5^{bc}	7.2
Salt cedar	1.7^{d}	0^{d}	0^{d}	0^{d}	6.2

Means$^{a\text{-}d}$ within rows and columns with different superscripts differ ($P < 0.05$).

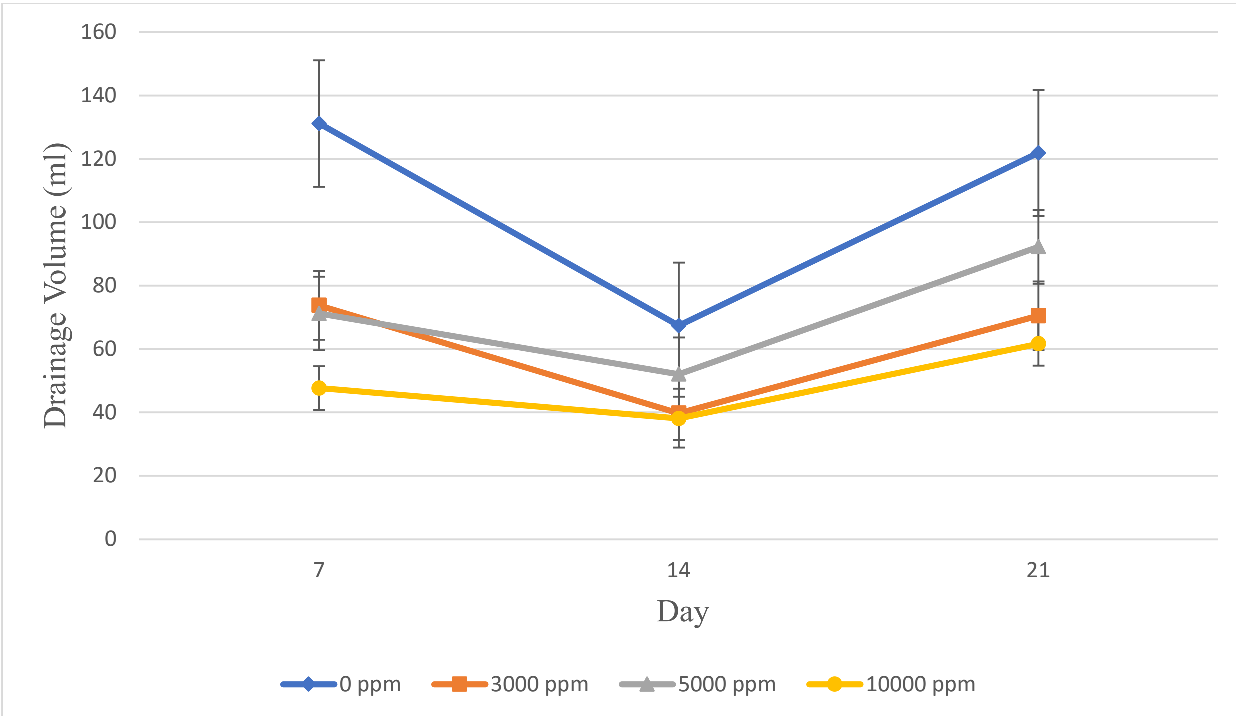

Figure 2. Average drainage volume (mL) represented by date of collection and treatment. The treatment by day interaction differed ($P < 0.05$).

Table 2. Average drainage volume (mL) represented by date of collection and species type.

	Day			
Species	**7**	**14**	**21**	**SEM**
Four-winged saltbush	126.2^{a}	73.3^{b}	118.8^{a}	8.1
Willow baccharis	116.7^{b}	74.8^{c}	139.7^{a}	8.1
Salt cedar	0^{d}	0^{d}	1.3^{d}	7.0

Means$^{a\text{-}d}$ within rows and columns with different superscripts differ (P < 0.05).

each day (Table 2). Collectively, results suggest that Salt cedar resulted in the greatest uptake of water followed by Four-winged saltbush and Willow baccharis. As the amount of salt in water increased, the amount of leachate tended to decline.

Drainage electrical conductivity (EC) (mS/cm) was recorded immediately following leachate volume measurement. Salt cedar drainage EC was not recorded due to lack of drainage. Treatment (data not shown), day by treatment interaction (Fig. 3) and species by day interaction (Table 3) all differed. Drainage EC was higher throughout the study for plants watered with the 10,000 ppm solution (Fig. 3). Drainage EC was similar for plants watered with 0, 3,000, and 5,000 ppm saline solution on day 0 but differed on subsequent days (Fig. 3). Drainage EC typically increased ($P < 0.05$) across days of watering for both Four-winged saltbush and Willow baccharis (Table 3). Drainage EC was not recorded for Salt cedar because no water was collected as drainage.

Saline concentration in the soil differed for species by treatment (Table 4), day by treatment (Fig. 4) and species by day (Table 5). As water salinity increased to 10,000 ppm, soil salinity increased accordingly for all species (Table 4). Soil salinity was typically lower for Salt cedar regardless of water salinity. Soil salinity was similar for all treatments on day 0, but varied thereafter (Fig. 4). For plants watered with 10,000 ppm solution, soil salinity was higher on days 7 through 21 and tended to increase with each collection. For plants watered with 0 ppm solution, soil salinity remained low. For plants watered with 3,000 and 5,000 ppm solution, soil salinity increased after day 0 and then remained fairly constant.

Nutrient analysis was analyzed on a dry matter basis. Percent (%) dry matter, crude protein, and sodium (Na) differed across species (Table 6). Percent dry matter was similar between Four-winged saltbush and Willow baccharis but lower in Salt cedar (Table 6). Crude

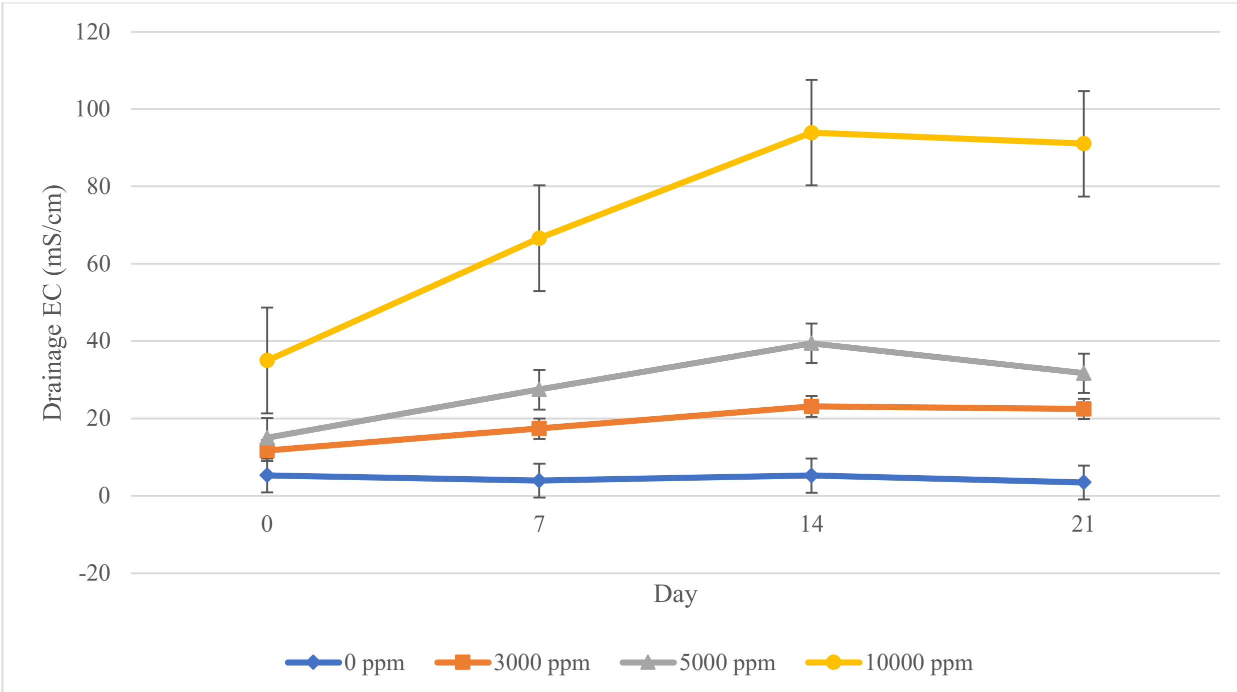

Figure 3. Average drainage electrical conductivity (mS/cm) represented by date of collection and treatment. The treatment by day interaction differed (*P* < 0.05)

Table 3. Average drainage electrical conductivity (mS/cm) represented by day and species. Salt cedar was not included in this table because no drainage was seen.

	Day				
Species	**0**	**7**	**14**	**21**	**SEM**
Four-winged saltbush	17.8^{def}	27.6^{cde}	37.5^{ab}	41.3^{ab}	2.5
Willow baccharis	15.7^{ef}	30.1^{bcd}	43.5^{a}	33.1^{bc}	2.6

$^{a-f}$ Means within rows and columns with different superscripts differ ($P < 0.05$).

Table 4. Soil electrical conductivity (mS/cm) taken after watering each treatment (0, 3,000, 5,000 or 10,000 ppm saline solution).

	Treatment (ppm)				
Species	**0**	**3,000**	**5,000**	**10,000**	**SEM**
Four-winged saltbush	$.115^{cd}$	$.267^{cd}$	$.318^{c}$	$.537^{b}$	0.42
Willow baccharis	$.107^{de}$	$.174^{cd}$	$.262^{cd}$	$.753^{a}$	0.37
Salt cedar	$.031^{e}$	$.071^{e}$	$.092^{e}$	$.131^{de}$	0.42

$^{a\text{-}e}$ Means within rows and columns with different superscripts differ ($P < 0.05$).

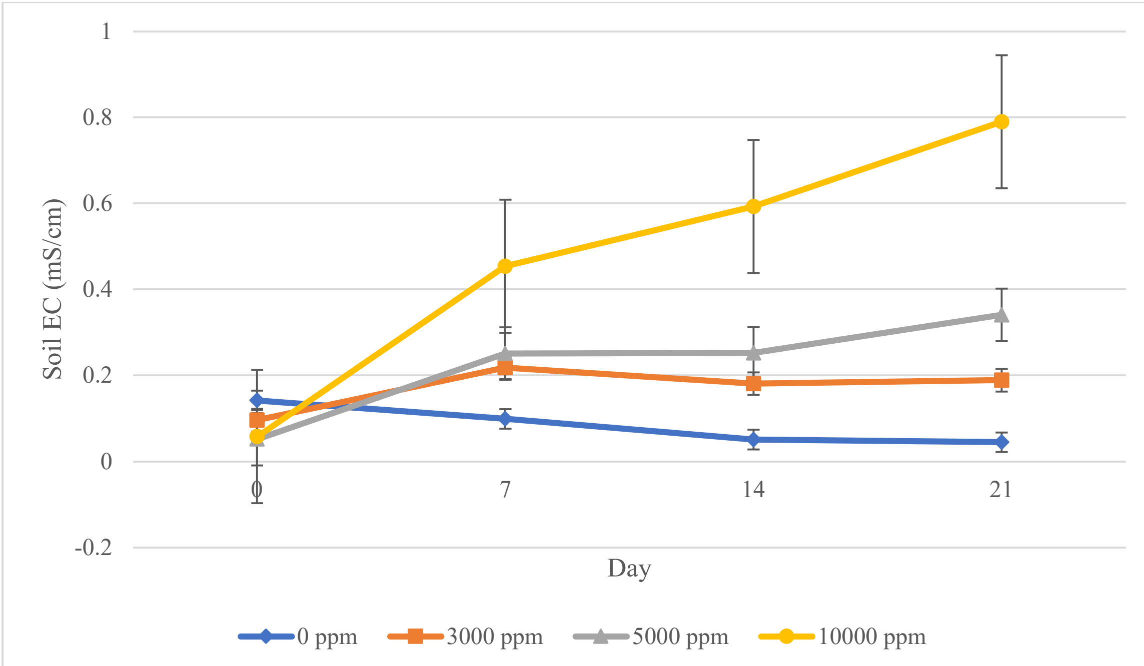

Figure 4. Soil electrical (mS/cm) conductivity represented by day and treatment after watering with their respected solution (0, 3,000, 5,000 or 10,000 ppm saline solution).

Table 5. Soil electrical conductivity (mS/cm) for day by species interaction. Samples were watered with either 0, 3,000, 5,000 or 10,000 ppm saline solution.

	Day				
Species	**0**	**7**	**14**	**21**	**SEM**
Four-winged saltbush	.104^{c}	.357^{ab}	.361^{ab}	.415^{ab}	.029
Willow baccharis	.101^{c}	.331^{b}	.371^{b}	.492^{a}	.025
Salt cedar	.055^{c}	.078^{c}	.076^{c}	.116^{c}	.029

$^{a\text{-}c}$ Means within rows with different superscripts differ ($P < 0.05$).

Table 6. Percent (%) nutrient content for Four-winged saltbush, Willow baccharis and Salt cedar on a dry matter basis.

	Species			
Nutrient Component	**Four-winged saltbush**	**Willow baccharis**	**Salt cedar**	**SEM**
DM (%)	94.0^{a}	93.6^{a}	91.7^{b}	.24
CP (%)	10.5^{a}	6.4^{b}	11.5^{a}	.45
NDF (%)	44.0	43.7	44.0	.92
ADF (%)	33.2	35.0	34.4	.75
TDN (%)	60.5	60.8	60.8	.35
Na (%)	$.64^{b}$	$.53^{b}$	1.3^{a}	.20
Chlorides (%)	3.0	1.4	4.3	1.2

$^{a-b}$ Means within rows with different superscripts differ ($P < 0.05$).

protein of Salt cedar and Four-winged saltbush was higher ($P < 0.05$) than in Willow baccharis. Salt cedar had higher sodium levels compared to Four-winged saltbush or Willow baccharis. Neutral Detergent fiber (NDF), Acid Detergent Fiber (ADF), Total Digestible Nutrients (TDN) and chloride levels were similar among species (Table 6).

DISCUSSION

No mortality was seen in any treatment. Based on the results, these plants can be readily established on brine water sites from 0 ppm to 10,000 ppm soil salinity. Of the plants utilized in this study, Salt cedar produced greater biomass than the other species. Salt cedar typically displays rapid growth rates, even in saline soils (Tomaso 1998). Livestock will readily consume Salt cedar (Parker and Scott 2021; Munoz et al. 2017; Knight et al. 2018). Given its rapid growth rate and livestock's willingness to consume the plant, Salt cedar may contribute more to reducing soil salinity than Willow baccharis or Four-winged saltbush. Once livestock consume Salt cedar foliage, salts collected in the plant would potentially be removed off of the site, diluted, and deposited in urine.

Four-winged saltbush and Willow baccharis both allowed drainage throughout the study. Salt cedar showed no drainage but is known for consuming large amounts of water daily. Some estimate that a mature Salt cedar can absorb up to 760 L of water per day (Tomaso 1998). While this estimate may be high, Salt cedar extends a tap root to available water and adjusts water usage rate based on water availability. Four-winged saltbush and Willow baccharis watered with 0 ppm solution may have seen a higher drainage volume because of the lack of Na in the solution whereas the 10,000 ppm saw a low drainage volume potentially because the additional Na may have affected water uptake rate.

Drainage electrical conductivity increased as the amount of Na increased in water solutions (0, 3,000, 5,000 and 10,000 ppm solutions). Drainage electrical conductivity peaked for both Four-winged saltbush and Willow baccharis on day 14 of the study and then declined on day 21. Why drainage electrical conductivity peaked on day 14 for these two plants remains unclear but may be related to ambient conditions on that particular day in the greenhouse. Daily drainage volume declined on day 14 as well for both species. While temperature data was not recorded in the greenhouse, daily temperature fluctuations may have affected the volume and electrical conductivity of drainage. Given that no drainage volume was collected from Salt cedar plants, apparently the plant was utilizing all of the available soil moisture irrespective of the amount of Na in the solution. Thus, Salt cedar may aid in reducing soil salinity by removing more salts from the soil because of its higher uptake of available soil moisture.

Salt cedar had the lowest soil electrical conductivity reading based on treatment and treatment day. Forage analysis taken from Salt cedar also shows that it had the highest Na content present in its top growth. This suggests that Salt cedar is better adapted to saline soil types when compared to Four-winged saltbush and Willow baccharis, and can translocate Na into the plant mass and out of the affected soil. Four-winged saltbush and Willow baccharis reacted similarly between days increasing after each watering, with soil electrical conductivity increasing over days, suggesting that both species may have a limited impact on reducing soil salinity.

Others have noted that Four-winged saltbush established in soils with 80,000 ppm total dissolved salts (Burris 2017). Similar observations have been made with Salt cedar. University Lands was able to establish Salt cedar from cuttings on a spill site with a high content of total dissolved salts (Steve Hartmann, personal communication). Given both species ability to establish in high saline soils, both may be beneficial in revegetation efforts on saline soils.

Crude protein was higher in Four-winged saltbush and Salt cedar. A high crude protein content in these plants make them valuable to livestock and wildlife and are readily consumed by both as well. Willow baccharis established and persisted throughout the entire study but had limited impact on soil salinity. While Salt cedar had a greater impact on reducing soil salinity when compared to Four-winged saltbush, both species could benefit revegetation efforts. Four-winged saltbush is considered a preferred browse species for both livestock and wildlife. Given its crude protein content, palatability, and ability to establish in saline soils, it should be included in revegetation efforts on saline soils. Conversely, Willow baccharis is not normally consumed by livestock or wildlife because of the chemical toxins that protect it, (Munoz et al. 2017) and based on the results of this study, should not be included in revegetation efforts on saline soils.

Previous studies determined Alkali sacaton and Giant sacaton can be established as well at 0, 3,000, 5,000 and 10,000 ppm which are readily consumed by livestock (Kennedy 2020). Burris (2017) established Four-winged saltbush, Alkali sacaton and Giant sacaton on a spill site that exceeded 80,000 ppm total dissolved salts. Adding organic matter to the soil helped to improve seedling establishment rate (Belew 2018). Ripping and furrowing also done by Belew (2018) and Burris (2017) improved the soil structure and infiltration rates ultimately reducing the soil salinity.

MANAGEMENT IMPLICATIONS

All three shrubs survived watering with solutions ranging from 0 ppm to 10,000 ppm sodium chloride solutions. Salt cedar had the highest percentage of Na in its top growth as well as the lowest soil electrical conductivity reading. Re-seeding Salt cedar on brine water spill sites can reduce the amount of Na in the soil profile and provide forage for livestock. Other re-seeding options can include adding in Four-winged saltbush or grasses like Alkali sacaton or Giant sacaton. A combination of these plants will promote foraging from different species of livestock as well as wildlife. Future research should assess these species ability to compartmentalize salts into its top growth on different soil types and how they will react to foraging by livestock/wildlife.

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VITA

Lance Adam Culak was born to Alvin and Malisa Culak. He began his education at Angelo State University in the fall of 2016 and graduated with his Bachelor's degree in Natural Resource Management in 2020. During this time, he was an avid member of the Angelo State University Bass Club, competing in collegiate bass fishing tournaments. He served as president for two years and tournament director for one year. Lance chose to attend graduate school at Angelo State University in the spring of 2020. During this time, he worked for the Management, Instruction and Research center as well as the Rocker b Ranch. Upon completion of his graduate studies, Lance plans to continue working at the Rocker b Ranch as the Range and Wildlife Manager.