THE EFFECT OF OILFIELD BRINE ON SOIL PROPERTIES AND PLANT GROWTH

A Dissertation

by

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ABSTRACT

Uganda is an emerging petroleum-producing country, and protecting vulnerable ecosystems in the impacted areas is paramount. In this study, simulated Ugandan petroleum brine was applied to soils to duplicate the conditions of brine spills. The brine to be used in Ugandan oil fields will be dominated by Na^+, K^+, Cl^-, and HCO_3^- with lower concentrations of potentially toxic metals including Sr and Ba. When brine was applied to the soil at rates high enough to reach electrolytic conductivities of EC 3 and EC 9 dS m^{-1}, respectively, soil properties and plant growth were heavily impacted. Redistribution of exchangeable cations and soil dispersion were observed for both rates of brine application. SAR was elevated to 12 and 18, and ESR was increased to 0.22 and 0.46 for EC3 and for EC9 treatments, respectively. The impacts on plant biomass were dependent upon the target species: cowpea (*Vigna unguiculata* L.) biomass decreased significantly, Bermudagrass (*Cynodon dactylon*) was slightly impacted, and sorghum sudangrass (*Sorghum* × *drummondii* hybrid) increased in biomass with increasing brine additions. Brine enhanced Ca^{2+} and Mg^{2+} content in root but not in shoot, while K^+ and Na^+ increased in shoot and root for all the species. Ba and Sr application did not have significant impact on soil properties or plant growth. From this study, we concluded that a single spill of brine solutions can result in significant damage to soils and vulnerable plants. Careful management will be required to avoid environmental problems associated with petroleum exploration and extraction, but brine spills can be addressed with proper soil and plant management.

CONTRIBUTORS AND FUNDING SOURCES

Collaborators

This work was supervised by a dissertation committee consisting of Dr. Paul Schwab, Dr. Julie Howe, and Dr. Girisha Ganjegunte from the Department of Soil and Crop Sciences and Dr. Bruce Herbert from the University Libraries, Texas A&M University.

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ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AG	Albertine Graben
ANOVA	Analysis of Variance
CAM	Crassulacean Acid Metabolism
CEC	Cation Exchange Capacity
CMC	Criteria Maximum Concentration
CNOOC	China National Offshore Oil Corporation
CUL	CNOOC Uganda Limited
DMRT	Duncan's Multiple Range Test
EC_e	Electrolytic Conductivity (measured)
ESR	Exchangeable Sodium Ratio
FAO	Food and Agriculture Organization of the United Nations
ICP	Inductively Coupled Plasma
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
KFDA	Kingfisher Field Development Area
LSD	Least Significant Difference
MCL	Maximum Contamination level
MPL	Maximum Permissible limit
NH_4OAc	Ammonium Acetate
ns	Non-significant
RO	Reverse Osmosis

ROS	Reactive Oxygen Species
RSLs	Regional Screening Levels.
SAR	Sodium Adsorption Ratio
TDS	Total Dissolved Solids
TSS	Total Soluble Salts
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Overview of petroleum production waste

Petroleum production activities generate varied waste products that have diverse effects on the environment and require proper management to minimize harm. Petroleum pollutants are persistent in the environment, and their clean-up from the contaminated sites is costly, difficult, and time consuming (Reynoso-Cuevas, Gallegos-Martínez, Cruz-Sosa and Gutierréz-Rojas, 2008; Luo, Cai, Qi, Wu and Gu, 2017; Kaur, Erickson, Ball and Ryan, 2017). The wastes include drilling muds, brines, naturally occurring radioactive materials, sludge, sediments, as well as utility, storage, and other associated wastes (Kaur *et al*., 2017). The waste composition varies widely depending on the nature of deposits and production processes (Masakorala *et al*., 2013), which consequently influences the impacts to the impacted ecosystem. This research focuses on oilfield brine, analyzing its effects on vegetation, by investigating the effects of brine and trace metals on soil and the growth of bermudagrass (*Cynodon dactylon*), sorghum sudangrass (*Sorghum* × *drummondii* hybrid), and cowpea (*Vigna unguiculata L.*) plants. This study used simulated oilfield brine to be produced by China National Offshore Oil Corporation (CNOOC) Kingfisher project, Uganda.

1.2 Oilfield brine composition

Oilfield brine is produced water from drilling fluids, geologic formations of salt deposits, and hydrofracturing fluids (Dudášova, Flåten, Sjöblom and Øye, 2008; Kelly, Findlay and Harris, 2018; Taylor, Elliott and Navitsky, 2018). It contains a wide range of contaminants and concentrations associated with the type of oil/gas well geological formation and production fluids (Dudášova *et al*., 2008; 2018; Taylor *et al*., 2018). Generally, oilfield brine is composed of injection water, oil, suspended solids, salts, natural matter, and other organic and other inorganic substances (Chittick and Srebotnjak, 2017; Kelly *et al*., 2018; Taylor *et al*., 2018). Dissolved salts in brines may include calcium, sodium, magnesium, potassium, bromide, fluoride, nitrate, phosphate and sulfate ions (Chittick and Srebotnjak, 2017; Taylor *et al*., 2018). In addition, brines are enriched in trace elements, metals, metalloids and other potentially harmful substances, such as NH_4, Ba, Sr, Ra, free cyanide, hydrocarbons, polycyclic aromatic hydrocarbons (PAH), and alkylphenols (Lauer, Harkness and Vengosh, 2016; Chittick and Srebotnjak, 2017; Kelly *et al*., 2018).

Petroleum-based brines (Table 1.1) are characterized by high salt concentrations (about 10 times the salinity of ocean water), electrolytic conductivities (EC_e >200 dS m^{-1}), sodium adsorption ratios (SAR) (SAR > 300) and total dissolved solids (TDS > 10,000 mg kg^{-1}) (Lauer *et al*., 2016; Whittemore, 2007; Taylor *et al*., 2018). Predominantly, Na and Ca chlorides contribute to the high salinity of the brines (Chittick and Srebotnjak, 2017; Taylor *et al*., 2018). In comparison, irrigation water of $EC_e > 3$ dS m^{-1}, SAR > 9, B > 2.0 mg L^{-1}, and $Cl^- > 350$ mg L^{-1} are injurious to most plant species (Ayers and Roy, 1977, Cera *et al*. 1985; Munn and Stewart, 1989). Thus, oil brines can have severe impact on soils and plant communities.

The compositions of sea water and oilfield brines are generally similar (Table 1.1) with some variation due to differences in geologic formations and production processes. The concentrations in seawater are usually 20% or less than in oilfields. The ion concentrations in oilfield brine expected to be produced by CNOOC Kingfisher project, Uganda (CUL, 2018), are much lower than other oilfield brines, particularly Na concentrations, and these concentrations are not likely to be representative of the brines that will be used during exploration and extraction. Nevertheless, The CNOOC Kingfisher concentrations are still high enough to hamper normal plant growth.

1.3 Effects of petroleum waste to the environment

Contaminants generated by petroleum oil development activities have various ecological effects. The pollutants are rapidly transferred into the environment through multiple pathways such as volatilization, aerosols, contaminated dust, penetration into and percolation through the soil, dissolution in surface and groundwater, and bioaccumulation along the food chain (Luo *et al*., 2017; Tapia *et al*., 2017). The dominant transport mechanism depends upon the medium, and the impact of contamination is dependent on the composition and the sensitivity of the exposed organism or ecosystem. Marine, surface water, groundwater, forest, grassland, and agricultural ecosystems, as well as soil and urban environments are threatened by pollution from petroleum development activities (Tapia *et al*., 2017).

Exposed ecosystems may suffer losses of flora and fauna species, altered function, microclimate change, impaired human health, and extreme impacts involving unrecoverable ecological damage. Persistent bio-accumulative toxic contaminants can also have lasting effects on the environment. Soils are in immediate contact with the pollutants and are a physical platform for petroleum activities. As such, soils are highly vulnerable to the consequences of these activities. Plant communities growing on the soils are equally affected.

Table 1.1. Comparison of brine concentrations (mg L^{-1}) in seawater and brines from conventional wells and oil fields in Uganda, Iraq, and Kuwait

Element/ion	**Seawater**[1]	**Range of oilfield mean concentration**[1]	**Conventional well brine**[2]	**Expected oilfield brine in Uganda**[3]	**Rumaila (Iraq) oil field**[4] **brine**	**Kuwait oilfield brines**[5]
Sodium	10,760	23,000 - 57,300	74,000	1,724	25,940	68,959
Calcium	416	2,530 - 25,800	--	268	4,540	19,014
Chloride	19,353	46,100 - 141,000	229,000	3,969	64,220	150,948
Magnesium	1294	530 - 4,000	--	5.8	574	3,198
Potassium	387	130 - 3,100	--	1,760	410	2,851
Sulfate	2712	210 - 1,170	--	105	122	122
Barium	--	--	4,370	2.3	214	2,000
Strontium	0.008	46 - 1,000	13,000	4.7	604	535,000
Acetate	--	--	--	697	--	--
Bromide	87	46 – 1,200	--	49.8	--	--
Phosphate	--	--	--	<1	--	--
Bicarbonate	142	77 - 560	--	257	638	--
Carbonate	--	30 - 450	--	0	--	--
Ammonium	--	23 - 300	--	--	--	--
Iodide	167	3 - 210	--	--	--	--
Lithium	0.17	3 - 50	--	0.2	--	2,000
Iron	--	--	--	<0.5	--	--
Copper	--	--	--	<0.5	89	--
Zinc	--	--	--	2.2	113.4	--
Manganese	--	--	--	0.6	--	--
Aluminum	--	--	--	<1	--	--
Boron	4.45	8 - 40	--	<3	--	--
Cadmium		--	--	--	26.2	--
Chromium		--	--	--	97	--
Lead					204	204
Nickel					162	162
Formate	--	--	--	5.2	--	--
Propanoate	--	--	--	51	--	--
Toluene	--	--	<0.005 – 1.770	--	--	--

Table 1 Continued

Element/ion	**Seawater[1]**	**Range of oilfield mean concentration[1]**	**Conventional well brine[2]**	**Expected oilfield brine in Uganda[3]**	**Rumaila (Iraq) oil field brine[4]**	**Kuwait oilfield brines[5]**
Benzene	--	--	<0.005 – 1.730	--	--	--
Ra-266	--	--	0 – 5,300	--	--	--
Ra-288	--	--	0 – 24,000	--	--	--
Total Iron	--	--	--	4.2	--	--
Phosphorus	--	--	--	<2	--	--
Silicon	--	--	--	27	--	--
Sulfur	--	--	--	38	--	--
Cation/Anion Balance %	--	--	--	101.67	--	--
Cl:Br	--	--	--	80	--	--
pH	--	--	--	7.32	8.36	8.36
EC_e dS m^{-1}	--	--	--	12.4	--	--
Salinity	35,000	5,000 - 300,000	--	--	7,820	--

[1] Neff, Lee and DeBlois *et al*., 2011; [2] Kelly *et al*., 2018; [3] CUL, 2018; [4] Al-Haleem, Abdulah and Saeed, 2010; [5] Alfarhan and Duane, 2011

Brine contaminants in soil are prone to leaching to surface and groundwater; sediment erosion; salt intrusion, especially to shallow water table; build-up in soils; and consequently, accumulation in plants (Murillo-Amador and Troyo-Diéguez, 2000). As a result of the impacts of high salt, toxic metals, and trace elements on soil and vegetation, impacted sites suffer from a decline in plant growth. Rehabilitation of brine contaminated landscapes, therefore, needs to address the salinity and phytotoxic effects of the contaminants to levels conducive for plant growth.

1.4 Management of petroleum waste

Petroleum waste is produced in large volumes, which poses challenges to its management and environmental risks to plants, animals, human life, and ecosystems. Disposal, storage, and transportation of the wastes can lead to risks for spills, leakage from storage tanks, and storm water discharges that add to the environmental danger. Once exposed to the environment, there are natural mechanisms for dissipation and retention of the pollutants. However, the fate of petroleum contaminants depends upon the characteristics of the environment into which the contaminants are exposed and the composition of the contaminants. Possible dissipation mechanisms include microbial degradation, microbial co-metabolization, irreversible adsorption, formation of solid phases, volatilization, fixation in place, chemical transformation, photodegradation, and phytodegradation (Desjardins *et al*., 2017; Kaur *et al*., 2017). Despite these pathways of loss, some pollutants are persistent and difficult to remove completely. Various physical (i.e., incineration, thermal desorption, excavation, and landfill), chemical, and biological methods (i.e., phytoremediation and mycoaugmentation) have been used to clean up petrochemical contamination (Arévalo-Gardini, Arévalo-Hernández, Baligar and He, 2017;

Petrová, Rezek, Soudek and Vaněk, 2017; García-Sánchez, Košnář, Mercl, Aranda and Tlustoš, 2018). The physical and chemical methods can be expensive and harmful to the environment. Biological methods are generally less expensive, environmentally safer, and widely acceptable, but they are slower in removing pollutants from the environment (Kaur *et al*., 2017). Regardless of the clean-up methods used, vegetation cover is essential for appropriate management of the affected landscape.

Vegetation controls soil degradation, minimizes erosion, facilitates recovery, enhances various soil properties, supports viable microbial populations, and improves the aesthetics of the affected landscape. Despite the necessity to maintain vegetation on landscapes, plant growth on contaminated soils can be hampered by the oil contaminants. Restoration processes, therefore, should aim at ensuring site conditions that can facilitate plant growth. This requires a clear understanding of the contaminant effects on specific plants and their growth conditions.

1.5 Petroleum oil production in Uganda

Petroleum exploration within the Albertine Graben (AG) region of Uganda estimated commercially viable oil reserves at over 6.5 billion barrels in 2014 (Economic Policy Research Center, 2015; Tilenga Project, 2018). The major oil production companies involved included CNOOC Uganda Limited (CUL), operating in the Kingfisher Field Development Area (KFDA); Tullow Uganda Operations Pty Ltd (Tullow), working in the Tilenga License Area; and Total E&P Uganda Ltd (Total), in the Kaiso-Tonya Development Area (Figure 1.1). Commercial petroleum oil production is anticipated to commence in 2022.

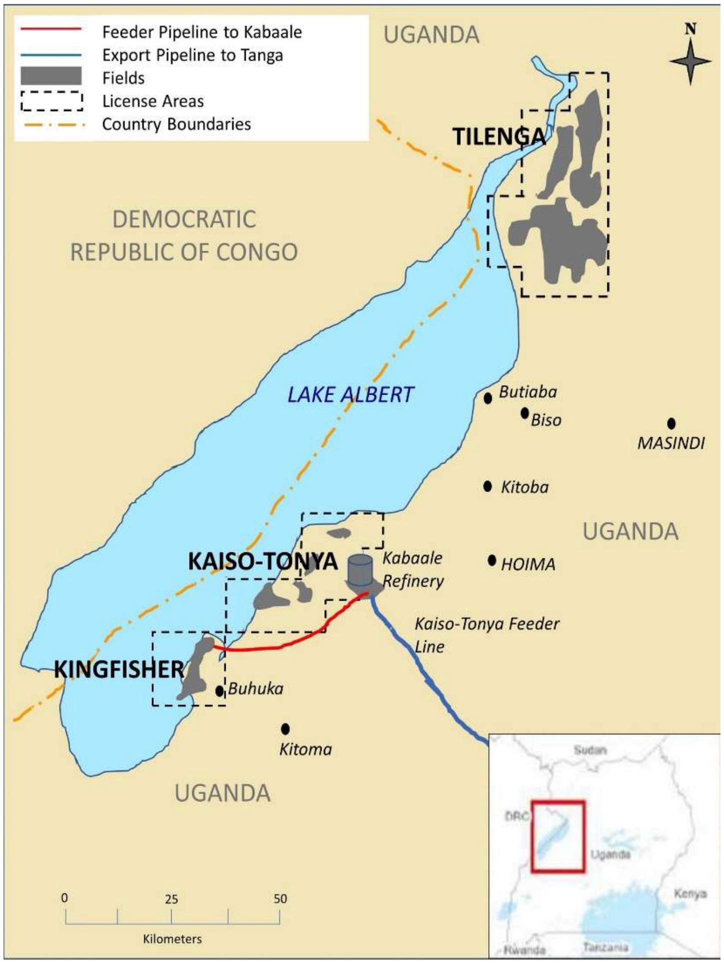

Figure 1.1. Oil project areas in Uganda. (Adapted from: CUL, 2018)

1.5.1. Socio-ecological conditions of the Albertine Graben region of Uganda

The AG region has a tropical climate, generally characterized as hot and humid, with mean monthly temperature range of 16 and 38°C, and mean monthly humidity 60% and 80% (CUL, 2018; National Environment Management Authority, 2009). There are two distinct rainy seasons – March to May and September to November, although rainfall patterns have become more erratic in recent years due to climate change. Annual rainfall range is about 750 to 1500 mm, but there are variations due to landscape, whereby, the rift valley is in a rain shadow, with mean annual rainfall of less than 875 mm y^{-1}; the rift valley escarpment areas receive about 1400 mm y^{-1}; while the Rwenzori mountain slopes receive more than1500 mm y^{-1} (CUL, 2018; National Environment Management Authority, 2009). High air temperatures result in high evaporation rates which, combined with low precipitation, cause a negative hydrological balance, in the rift valley floor and lower escarpment areas (CUL, 2018; National Environment Management Authority, 2009). These parts thus require special attention regarding the management of soil salinity because salts tend to accumulate in upper soil layers due to excessive evaporation.

The soils of the AG area are about 54% Ferralsols (FAO Soil Classification System, or Oxisols - USDA taxonomic system), which is also the dominant soil type in Uganda (about two thirds), then Vertisols (36%), Gleysols (Inceptisol and Mollisol – USDA system) (10%) and some Lithosols (Entisol - USDA system) (CUL, 2018; TP, 2018). Soil pH ranges from 4.4 to 7.6, $EC_e<1.0$ dS m^{-1}, CEC 5 – 15 $cmol_+$ kg^{-1} (1 *M* ammonium acetate- NH_4OAc, pH 7), and mostly sandy and clay loam texture (CUL, 2018; Tilenga Project, 2018). Levels of Ba and Sr are considerably low in soils and waters of the AG region. Measured Ba^{2+} concentration in Tilenga project area ranged between 15 and 200 mg kg^{-1} in soil, 11 to 230 mg kg^{-1} in riverbed sediments, 0.01 to 2 mg L^{-1} in surface water, and < 0.05 to 1.7 mg L^{-1} in groundwater (Tilenga Project, 2018).

The AG is of significant ecological importance, endowed with numerous mountains, valleys, freshwater lakes, protected areas, and vast biodiversity (about 14% reptile, 19% amphibian, 35% butterfly, 52% bird, 39% mammal, 128 fish, and 14% plant species of Africa) (Thomassen and Hindrum, 2011; National Environment Management Authority, 2012; Ericson, 2014; Economic

Policy Research Center, 2018). These provide significant livelihood resources for agriculture, fishing, fuel wood, water, tourism, and ecological services, which are potentially threatened by oil exploitation (Economic Policy Research Center, 2018; Tilenga Project, 2018). Uganda has set some standards and also adapted international standards where national standards are not yet set, to regulate disposal of petroleum waste (Table 1.2).

Produced water for the CNOOC Kingfisher project is expected to be about 415 to 756 m^3 h^{-1} (approximately 3500 m^3 per well annually) (CUL, 2018). The water is to be treated and reinjected back into the reservoir or re-used in oil production processes (CUL, 2018). Sufficient treatment of brine is difficult, moreover discharges of brine are common due to technological failures, inadvertent spills, or irregular discharges, these events are likely to have a negative effect on the ecosystem (Neff *et al*., 2011; Mao, Zhang, Yang and Zhang, 2018; Netherlands Commission for Environmental Assessment, 2018). The biophysical characteristics of the AG combined with the present-day unpredictable climate variability, makes the area prone to more frequent floods, overland flow, steep erosion and leaching, which can facilitate widespread of the petroleum waste in case of exposure. The AG is ecologically sensitive, thus its protection against potential toxicity and salinity of oilfield brine is critical to minimize ecological harm.

1.6 Research problem

Vegetation cover is important for the management and restoration of oilfield brine contaminated lands. However, plant growth on land contaminated with oilfield brine can be hampered by the toxicity of the brines. Understanding the effects of brine on the soil and specific plants and their growth conditions can lead to more effective ways to manage vegetative cover. This research seeks to determine the effect of synthetic oilfield brine salinity and toxicity of barium (Ba) and strontium (Sr) on the growth of bermudagrass (*Cynodon dactylon*), sorghum sudangrass (*Sorghum* × *drummondii* hybrid) and cowpea (*Vigna unguiculata L.*) and on soil properties.

Table 1.2. Regulation standards for selected brine constituents

Parameter	WHO standards	USEPA standards	Uganda standards
EC_e(dS m^{-1})	-	-	1.5 for treated potable water and 2.5 for natural potable water [6]
Ba (mg L^{-1})	- 1.3 for drinking water [7] - 0.7 for surface water [7]	- 2 MCL for drinking water [8] - 0.1 CMC for surface water [8]	- 0.7 for treated potable water, natural potable water, or surface water [6] - 10 MPL for discharge of effluent or waste water in water or land [9] - 1.5 proposed MPL for discharge of effluent in public sewer [10]
	-	15,000 mg kg^{-1} Residential RSL in soil [11]	-
Ca (mg L^{-1})	-	-	- 150 for treated potable water, natural potable water and surface water [6] - 100 proposed MPL for discharge of effluent in public sewer [10]
Mg (mg L^{-1})	-	-	100 for treated potable water, natural potable water and surface water [6]
Na (mg L^{-1})	-	-	200 for treated potable water, natural potable water and surface water [6]
Sr (mg L^{-1})	-	- 1.5 proposed MCL for drinking water [12]	-
	-	- 47,000 mg kg^{-1} Residential RSL in soil [11]	-
Chloride (Cl^-) (mg L^{-1})	-	- 250 secondary MCL in drinking water [8]	- 250 for treated potable water, natural potable water and surface water [6] - 500 MPL of effluent or waste water in water or land [9] - 250 proposed MPL of effluent into public sewer [10] - 1200 MPL in oil and gas produced water effluent [10]
TSS (mg L^{-1})	-	-	- 700 for treated potable water and 1500 for natural potable water [6] - 35 proposed MPL for oil and gas produced water effluent [10]
TDS (mg L^{-1})	600 for drinking water [7]	500 secondary MCL in drinking water [8]	300 proposed MPL in effluent [10]
pH	-	6.5 to 8.5 secondary MCL for drinking water [8]	- 6.5 to 8.5 for treated potable water, and 5.5 to 9.5 for natural potable water [6] - 6.0 to 9.0 proposed MPL of effluent into public sewer [10]

[6] UNBS, 2014; [B] WHO, 2017; [8] USEPA, 2009; [9] National Environment Management Authority, 1999; [10] National Environment Management Authority, 2014; [11] USEPA, 2017; [12] Colvin, 2020. CMC Criteria Maximum Concentration; MCL Maximum Contamination level; MPL Maximum Permissible limit; TDS total dissolved salts; TSS total soluble salts; USEPA RSLs Regional Screening Levels.

1.7 Objectives and hypotheses

Objective 1. Determine the effect of oilfield brines, barium and strontium contamination in soil on the growth of bermudagrass, sorghum sudangrass and cowpea.

Hypotheses:

1a. Addition of brines will reduce plant growth.

1b. Presence of barium and strontium in brine will further reduce plant growth.

Objective 2. Evaluate the effects of oilfield brines, barium and strontium contamination on soil properties and how growing plants can influence these effects.

Hypothesis:

2a. Addition of brine will degenerate soil properties.

2b. Plant growth will moderate the degeneration of soil properties by brine.

1.8 Effects of oilfield brine on soil

High salt and sodium contents in petroleum brines negatively impact soils in many ways. Salinity/sodicity alters the chemical and physical properties of soil, and can lead to more strongly negative osmotic water potential, loss of soil structure, soil compaction, high bulk density, impeded water infiltration and aeration, buildup of salt and toxic ions, mineral displacement, and ion imbalance in soil solution (Strawn, Bohn and O'Connor, 2015). The effects of brine on soil vary depend on soil type, soil conditions, brine composition, and the quantity of brine deposited onto the soil (El-Mageed, Mohammed, El-Samnoudi and Ibrahirm, 2018). Generally, $EC_e > 4$ dS m^{-1} or SAR > 15 adversely affects soils and inhibit plant growth (George, Horst and Neumann, 2012; Strawn *et al*., 2015). Salt-sensitive plants, however, can be affected in soils whose saturation extracts have EC_e 2 to 4 dS m^{-1} (Shahid, Zaman and Heng, 2018).

Soluble salts in the soil solution will result in decreasing (more negative) osmotic pressure, reducing water availability and limiting the absorption of water by seeds or plant roots. Resultantly, plants suffer water stress and root dehydration, causing physiological drought, salt damage, and inhibition of seed germination and plant growth (Murillo-Amador, Lo´pez-Aguilar, Kaya, Larrinaga-Mayoral and Flores-Herna´ndez, 2002; El-Mageed *et al*., 2018).

Exchangeable sodium percentage (ESP) > 15% or SAR >13 hinders aggregate formation and can cause soils to disperse (George *et al*., 2012). This loss of structure leads to soil compaction, clogging of pores and surface crusting, impeded air and water infiltration, and enhanced potential for soil erosion (Muhammad, Anwar and Razzaq, 2002; Islam, Anusontpornperm, Kheoruenromne and Thanachit, 2014; El-Mageed *et al*., 2018). Compacted soils tend to be of high bulk density due to reduction in total porosity (El-Mageed *et al*., 2018).

In saline+sodic soils where $EC_e > 4$ dS m^{-1} and SAR >15, the high salinity will overcome the sodicity and flocculate the colloids. Thus, the typical dispersion of colloids and accompanying soil physical problems will not be present in saline+sodic soils structure (George *et al*., 2012; El-Mageed *et al*., 2018). Thus, testing impacted soils for both salinity and sodicity is critical, particularly when considering remediation options.

Remediation strategies should be carefully staged to avoid dispersion: a) when necessary, Na content is first reduced by amending the soil with a soluble Ca source, such as gypsum, and b) the salt content is reduced by heavy leaching out of the root zone or below a critical level (Munn and Stewart, 1989; Glenn, Waugh and Pepper, 2006; Plaut, Edelstein and Ben-Hur, 2013; Alam, Juraimi, Rafii and Hamid, 2015). Calcium promotes soil aggregation, thereby improving soil structure and the ratio of plant available Ca/Na in soil (Munns, 2002; George *et al*., 2012; Zehra, Gul, Ansari and Khan, 2012).

In addition to salts, heavy metals, metalloids, and trace elements in oil brines influence the biochemical properties of soil. Most heavy metals and trace elements are not required for plant growth or are required in minute levels. When these elements are present in concentrations are greater than optimal values, they can lead to toxicity and poisoning. The retention of large amounts of soluble, toxic soluble species (such as H_3BO_3, Ba^{2+}, Sr^{2+}, CO_3^{2-}, HCO_3^-, Br^-, Na^+, and Cl^-) from the brine can cause toxicity, and/or nutritional imbalance to plants (Murillo-Amador *et al*., 2002). High levels of exchangeable Na^+ and Cl^- tend to displace other mineral nutrients including K^+, Ca^{2+}, PO_4^{3-}, Fe^{3+}/Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, which decreases their bioavailability, leading to deficiencies (Munn and Stewart, 1989; El-Mageed *et al*., 2018).

High soluble Na^+ concentrations lead to increased Na^+ flux that competes with Ca^{2+} at binding sites on plant cells (Zehra *et al*., 2012). However, where Ca^{2+} concentration is also elevated, the result is a rise of cytoplasmic and extracellular Ca^+ which alters Na^+ influx and maintains Na^+ and K^+ homeostasis (Zehra *et al*., 2012). Inhibition of Na^+ uptake by Ca^{2+} provides protection against salt stress by regulating physiological and cellular events, thereby moderating the adverse salt effects and can also stimulate plant growth (Rengel, 1992; Colmer, Fan, Higashi and Läuchli, 1996; Tobe, Zhang, and Omasa, 2003). Alleviation of salt toxicity on germination or seedling growth by Ca^{2+}, through reduced K^+ efflux and influx of Na^+, has been reported in several plants like peas, wheat, sunflower, tomato, barley, shrubs, grass and several other halophytic species (Suhayda, Redmann, Harvey and Cipywnyk, 1992; Mehta, Malik, Khurana and Maheshwari, 1993; Tobe *et al*., 2003; Bonilla, El-Hamdaoui and Bolanos, 2004; Turkmen, Dursun, Turan and Erdinc, 2004; Li-Yun and Ming-You, 2010; Zehra *et al*., 2012).

1.9 Effects of oilfield brine on plants

Major constraints of soil salinity on plant growth result from highly negative osmotic potentials and ionic stress (Glenn and Brown, 1999; Bernstein, Shoresh, Xu and Huang, 2010). As soil salinity increases, plants are forced to overcome an increasing gradient in water potential between the soil and roots, which restricts plants' ability to take up water and nutrients, hence causing the plants to exhibit symptoms of drought and nutrient deficiency (Munns and Tester, 2008; George *et al*., 2012). Osmotic stress inhibits water uptake; reduces cell expansion; diminishes leaf, root and lateral bud development; disrupts protein synthesis and enzyme activity (Munns and Termaat, 1986; Yeo and Flowers, 1986); induces nutritional disorders, ion toxicity, oxidative stress, membrane disorganization; obstructs cellular metabolism, photosynthesis and energy production; inhibits seed germination; retards plant growth; and reduces yield (Munns, 2002; Ortega, Fry and Taleisnik, 2006; Singh and Kalamdhad, 2011; Masakorala *et al*., 2013).

Sensitive plants may die due to salt stress if exposed to $EC_e > 4$ dS m^{-1}, Na > 70 mg L^{-1} in water, or Na contents of 5% in plant tissue, or 230 mg Na L^{-1} in soil (George *et al*., 2012; Zaman, Shahid and Heng, 2018). However, threshold salinity levels and effects on plant growth vary among species (or cultivars) and growth stages (West and Francois, 1982; Orlovsky, Japakova, Zhang and Volis, 2016). Salt sensitive plants can be harmed severely even by apparently low salinity ($2 < EC_e < 4$ dS m^{-1}). Many glycophytes (plants tolerant to low salt concentrations) are able to tolerate moderate soil salinity ($4 < EC_e < 8$ dS m^{-1}), while some halophytes (salt-tolerant plants) are able to grow and reproduce in soils of $EC_e > 20$ dS m^{-1} (West and Francois, 1982; Reddy *et al*., 2017). Bermudagrass is among the most salt tolerant of plants with threshold for general growth EC_e 6.9 – 18 dS m^{-1}, while sorghum sudangrass is moderate (6.8 – 13 dS m^{-1}) and cowpea is salt sensitive (4.9 – 13 dS m^{-1}) (Wilson, Liu, Lesch and Suarez, 2006; Strawn *et al*., 2015; Shahid *et al*., 2018). A useful parameter in the study of the effects of salinity on crops is the C50, the EC_e above which crop yields are reduced by 50%. The C50 for cowpea is 2.6 dS m^{-1}, 12 dS m^{-1} for sorghum sudangrass, and 18 - 30 dS m^{-1} for bermudagrass (Wilson *et al*., 2006; Strawn *et al*., 2015).

Salt-induced stress in plants is manifest by chlorosis, necrosis in old leaves, premature senescence of leaves, increased leaf mortality, distorted leaf or stem growth (tufted and stunted appearance), delayed flowering, and delayed fruit development (Glenn and Brown, 1999; Hasegawa, Bressan, Zhu and Bohnert, 2000; Wilson *et al*., 2006; Panuccio, Jacobsen, Akhtar and Muscolo, 2014). Increasing salinity has the effects of decreased leaf number and size among cowpea cultivars, increased leaf-firing in bermudagrass, and inhibited leaf expansion in beans (Adavi, Razmjoo and Mobli, 2006; Wilson *et al*., 2006). In sorghum, salinity stress is manifested by reduced germination, impeded emergence and elongation, fewer seedling roots, sudden wilting, marginal burn on leaves, leaf yellowing, leaf injury, increased leaf fall, decreased plant biomass, and potentially death of the plants (Netondo, Onyango and Beck, 2004; Läuchli and Grattan, 2007; Daffalla *et al*., 2014; El Sanousi, Abdelmula, Mohammed, Mishra and Hamza, 2015; Alloudane, Ezzakkioui, El Mourabit and Barrijal, 2018).

1.9.1 Brine effect on germination

Plant sensitivity to salinity varies between species, growth stages, and salt concentration (Nieman and Clark 1976; Munn and Stewart, 1989; Murillo-Amador *et al*., 2002). Germination is a highly sensitive to salinity; thus, salt tolerance at germination or early seedling growth is an important adaptation of species (Khan and Gul, 2006; Zehra *et al*., 2012). Salt can have various physicochemical effects upon the seed that may result in slowed and reduced germination (Waisel, 1972; Zehra and Khan, 2007; Panuccio *et al.,* 2014). High salinity restricts moisture availability for the seed germination due to decreased water potential gradient between the external environment and the seed (Murillo-Amador *et al*., 2002; Plaut *et al*., 2013; Orlovsky *et al*., 2016). Although seeds contain all the essential mineral nutrients required for germination and seedling emergence, nutrient mobilization may be hampered under salinity stress, thus hindering seedling emergence (Knight and Knight, 2001; Zehra *et al*., 2012). Additionally, accumulation of excess salt or specific toxic ions might hinder germination of seeds through accumulation of soluble salts in the cytoplasm or in cell wall that inhibit enzyme and/or hormonal activity of the seed; alter carbon metabolism; or dehydrate the cell (Waisel, 1972; Tobe *et al*., 2004; Zhu, 2003; Panuccio *et al.,* 2014).

Young seedlings also will have trouble absorbing moisture for growth against the more negative osmotic potential associated with salinity (West and Francois, 1982; Munn and Stewart, 1989). Salinity inhibits embryo-axis growth due to delayed reserve mobilization and membrane disturbance as evidenced by increased leakage of materials from the embryo-axis, eventually leading to loss of viability of the developing embryo (Murillo-Amador *et al*., 2002; Patanè, Saita and Sortino, 2012). Overall, osmotic stress and ion toxicity can lead to seed dormancy, embryo damage, delayed or failed seedling emergence, inhibition of seed germination and seedling survival, and later contribute to low plant yield (Munn and Stewart, 1989; Patanè, Saita and Sortino, 2012; Plaut *et al*., 2013; Orlovsky *et al*., 2016).

Munn and Stewart (1989) reported that, relative to a control, 10% sea water (SW) dramatically reduced germination of soybean and tall fescue, 30% SW reduced germination in oat, wheat and peas, while 100% SW completely inhibited germination, and SW $\leq$ 2% did not affect germination percentage. Germination in cowpea was significantly reduced by $EC_e > 12.0$ dS m^{-1}, while $EC_e \leq 12$ dS m^{-1} did not, but simply delayed the germination (West and Francois, 1982; Murillo-Amador *et al*., 2002). In some species, slightly elevated salinity can stimulate germination (West and Francois, 1982; Orlovsky *et al*., 2016).

1.9.2 Brine-induced ion toxicity in plants

Ion toxicity results mainly from excessive uptake of ions or toxicity of specific species such as Cl^-, Na^+, H_3BO_3, and HCO_3^-. Excessive ions are toxic to plants and have potential to affect plant enzymes, displace other ions from binding sites of enzymes, impair enzyme and cellular functioning, and decrease activity of cellular metabolism, chlorophyll production and photosynthesis (Yeo and Flowers 1986; Glenn and Brown, 1999; Munns, 2002; George *et al*., 2012; Panuccio *et al.,* 2014). Cowpea is sensitive to Na, sorghum is semi-tolerant, while bermudagrass is tolerant (Shahid *et al*., 2018). Tolerence to boron is 0.3 – 1.0 mg L^{-1}, 1.0 – 2.0 mg L^{-1} and 2.0 – 4.0 mg L^{-1} for sensitive plants, semi-tolerant and tolerant plants, respectively (Wilcox and Durum, 1967; Food and Agricultural Organization, 1973; Strawn *et al*., 2015). Tolerance to Cl^- is 70 – 140 mg L^{-1}, 141 - 350 mg L^{-1} for sensitive and moderately tolerant plants respectively, while Cl^- > 350 mg L^{-1} can cause severe problems for most plants (Pearson, 1960; Wilcox and Durum, 1967; Food and Agricultural Organization, 1973; Strawn *et al*., 2015; Shahid *et al*., 2018). Excessive salt ion levels interfere with normal nutrient uptake from the soil and transport and internal distribution of nutrients in plant parts, and might induce nutrient deficiency and nutrient imbalance (Silva and Uchida, 2000; Shaul, 2002; Singh and Kalamdhad, 2011).

The ratio of Na^+ to cations and Cl^- to anions can increase significantly under high salt concentrations (Murillo-Amador *et al*., 2002). Chloride toxicity is probably the major limitation in legumes such as clover (*Trifolium*) and *Medicago* (e.g., burclover), and in many fruit trees such as citrus, while Na^+ toxicity limits growth in cowpea, sorghum, bermudagrass and other plants (Alexander and Groot-Obbink, 1971; Ackerson and Youngner, 1975; Dudeck, Singh, Giordano, Nell and McConnell, 1983; Boursier and Läuchli, 1990). Additionally, salinity may also impair the transfer of PO_4^{3-} into the xylem thereby depressing P utilization efficiency and inducing P deficiency (Nieman and Clark, 1976; Shahid *et al*., 2018).

High levels of Na^+ lead to increased Na^+ flux that competes with Ca^{2+} and K^+ at binding sites, or compete with Ca^{2+} for plant uptake via non-selective cation channels thereby inhibiting the movement of Ca^{2+} through the apoplasm of root tissues and may disrupt the integrity of root membranes, alter their selectivity, nutrient acquisition, and increase leakage of intracellular solutes (Rengel, 1992; Guimarães *et al*., 2012; George *et al*., 2012; Zehra *et al*., 2012). Excess Na^+ might impair ribosomal attachment to rRNA and disrupts K^+ ion balancing within the plant (Qian, Wilhelm and Marcum, 2001; Chattopadhyay *et al*., 2002; Tester and Davenport, 2003;

Davenport, James, Plogander, Tester and Munns, 2005). The ability of plants to maintain low Na^+ and high K^+ concentration (high K^+/Na^+ ratio) within the cytoplasm plays a crucial role in K homeostasis under salinity stress, commonly observed in salt-tolerant species as they tend to have higher K^+/Na^+ compared to salt sensitive plants (George *et al*., 2012; Bafeel, 2014).

Increased salinity led to accumulated Na^+ with subsequent reduction in K content of bermudagrass and sorghum shoot (Boursier and Läuchli, 1990; Adavi *et al.*, 2006; Peoples *et al*., 2014). However, in some bermudagrass cultivars, a high accumulation of K and low Na accumulation in shoot was observed under salinity, which resulted in higher salt-tolerance in those cultivars, that also showed superior top growth and low or no leaf-firing (Dudeck *et al*., 1983; Adavi *et al.*, 2006). Additionally, even where Na increased while K decreased with increased salinity, the total Na + K content was not affected, which suggested partial substitution for K^+ by Na^+ in bermudagrass nutrition (Adavi *et al*., 2006). Guimarães *et al*. (2012) found significant reduction of Ca^{2+} content in cowpea plants due to salinity but observed, higher K^+ content in salt-stressed cowpea plants in comparison to control plants; while in sorghum, salinity induced depression in shoot concentrations of K and Mg (Boursier and Läuchli, 1990; Wallace, Mueller, Cha and Romney, 1980; Peoples, Richardson, Simpson and Fillery, 2014).

1.9.3 Brine effect on plant physiology

Salinity stress may result in physiological changes such as chlorophyll degradation, interference with stomatal conductance, transpiration and photosynthesis and cell/tissue damage (Schwarz and Gale, 1981; Kurban *et al*., 1999; Alam *et al*., 2015). Increased salinity may cause significant reduction in total leaf area due to salinity-induced leaf loss or tissue damage, which leads to decline in mean stomatal conductance, transpiration, chlorophyll content, and net CO_2 fixation per unit photosynthetic tissue and intercellular CO_2 concentrations, consequently, photosynthesis is directly or indirectly lowered (Yeo and Flowers, 1986; Kurban *et al*., 1999; George *et al*., 2012; Kumari, Arya, Pahuja, Joshi and Sharma, 2016). Some salt tolerant plants (e.g., salt brush, *Atriplex lentiformis*) are able to shift from C3 to C4 or CAM (crassulacean acid metabolism) metabolism under salinity, triggered by low CO_2 concentration (due to reduced rates of net CO_2 fixation) and osmotic potential in leaf tissue during the light period, while dark respiration may remain unaffected or may even increase (Kurban *et al*., 1999; Kholodova *et al*., 2002; George *et al*., 2012; Alam *et al*., 2015). Plants that can fix CO_2 even at low intercellular CO_2 concentrations via the C4 or CAM pathway often have higher growth rates in saline soil than C3 plants (Katerji, van Hoorn, Hamdy, Karam and Mastrorilli, 1996; George *et al*., 2012; Alam *et al*., 2015).

Similarly, salt tolerant species protect themselves from salt-induced enzymatic degradation of chlorophyll and other pigments, but salt-sensitive species commonly severely deteriorate from such degradations (Alam *et al*., 2015). The root cortex plus the epidermis, being the outermost cell layers, are the first tissues to encounter the excess salts and are potentially either the first site of damage or first line of defense (Kurth, Cramer, Läuchli and Epstein, 1986; Alam *et al*., 2015). Some salt tolerant plants develop thicker cortex to minimize entry of salt into the plant and/or cytoplasm, but in salt-sensitive species, the cortex thickness tends to be reduced with decreased xylem development (Roychoudhury and Chakraborty, 2013; Bafeel, 2014; Alam *et al*., 2015).

1.9.4 Effect on biomass

As a result of physiological deterioration (e.g., through chlorophyll degradation or hampered stomatal conductance), plant growth, biomass, and yield are depressed. Salinity-induced reduction in fresh and dry mass accumulation in plant parts is common for most plants (Bernstein, 2013; Alam *et al*., 2015). Salinity stress potentially leads to reduced root, stem, leaf growth and reduced flowering, grain/pod-filling, and seed set, which contribute to decline in fresh and dry mass in these parts and eventually low yield (West and Francois, 1982; Mass and Poss, 1989; Adavi *et al*., 2006; Panuccio *et al.,* 2014).

Increasing salinity caused significant decrease in growth, grain/seed mass, and protein content of bean and maize plants (Plaut *et al*., 2013; Blanco *et al*., 2008; El-Mageed et al., 2018). Reduction in roots, shoot growth, shoot mass, and shoot length in bermudagrass and sorghum cultivars was reported under increased salinity levels, but stimulation of dry matter accumulation in bermudagrass and other turfgrass species under moderate salinity have also been documented (Youngner and Lunt, 1967; Dudeck *et al*., 1983; Adavi *et al*., 2006; Uddin and Juraimi, 2013; Bafeel, 2014; Alam *et al*., 2015). Elevated salinity caused significant reduction in fresh mass and dry matter content of sugar beet cultivars and fountain grass (*Pennisetum alopecuroides)* (Alam *et al*., 2015).

1.10 Effects of barium on soil and plants

Barium is relatively abundant in the Earth's crust, with mean values ranging between 265 and 835 mg kg^{-}1 dry mass in the upper continental crust, and 100 to 3000 mg kg^{-1} in natural soils depending on soil type; high levels are common with contaminated soils (Environmental

Company of the State of Sao Paulo, 2001; Lide, 2005; Kabata-Pendias and Mukherjee, 2007; Ong, Yap, Mahmood, Tan and Hamzah, 2013). Barium is relatively immobile in soil systems due to formation of sparingly soluble salts and the inability to form soluble complexes with humic or fulvic materials. Barium readily reacts with metal oxides and hydroxides, is strongly adsorbed onto soil solids, and easily displaces other adsorbed alkaline earth metals (Ca and Mg) and K (Ong *et al*., 2013).

Generally, the concentration of Ba in terrestrial plants is lower than its concentration in soils, resulting in a bioconcentration factor <1 (U.S. Environmental Protection Agency, 2003; Abreu, Cantoni, Coscione and Paz-Ferreiro, 2012; Lenntech, 2005; Ong *et al*., 2013). Barium is found in most plants despite being nonessential. Barium is listed among elements that pose risk to human health (U.S. Environmental Protection Agency, 2003; Environmental Company of the State of Sao Paulo 2001; WHO, 2001; Abreu *et al*., 2012; U.S. Environmental Protection Agency, 2009). Concentrations of Ba in plants range from 2 to 13 mg kg^{-1}, but some plants such as nut trees, can bioconcentrate Ba to concentrations as high as 3000 to 4000 mg kg^{-1} (Kabata-Pendias and Mukherjee 2007; Parekha, Khana, Torresa and Kittoa, 2008). At concentrations <200 mg kg^{-1}, Ba is generally not toxic, Ba levels of about 200-500 mg kg^{-1} are moderately toxic, while >500 mg kg^{-1} can be toxic in plants (Pais, Benton and Jones, 1998; Ong *et al*., 2013). Significant concentrations of Ba in edible plants constitute a risk because ingestion of Ba can result in health problems to animals and humans, including muscular paralysis, gastrointestinal disturbances, heart damage, high blood pressure, and, in some cases, even death (Lenntech, 2005; Oskarsson and Reeves, 2007; Ong *et al*., 2013). Thus, monitoring of Ba accumulation in soils and plants attracts attention (Ong *et al*., 2013). Barium in plants is strongly accumulated by legumes, grain stalks, forage plants and trees, and because of its low mobility, the highest levels are found in lower plant parts (roots) rather than in upper parts (leaves and stems) (Yap, Mohd, Mazyhar and Tan, 2010; Ong *et al*., 2013).

1.11 Effects of strontium on soil and plants

Estimated Sr content in the Earth's crust is 340 mg kg^{-1} with about 61% concentrated in the upper 5 cm of the rooting zone (Skupiński and Solecki, 2014; Dubchak, 2018). Usually, Sr forms complex compounds with humic acid, and organic matter fixes Sr on the surface of organic colloids, which influences its distribution and mobility in soil (Dubchak, 2018). Accordingly, Sr is usually firmly fixed in soils of high organic matter, clay or silt content, and the greater part of Sr is retained in the upper soil layers, mainly the humus illuvial layer below the litter (Dubchak, 2018). In saline soils, Sr is retained by the salt crust in the upper soil horizon (Dubchak, 2018). Strontium competes with other cations for sorption by the soil solid phase ($Al^{3+} > Fe^{3+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+} > K^{+} > NH_4^{+} > Na^{+}$), which also influences its availability and migration (Sanzharova, 2005). Strontium is more strongly retained on exchange sites than Ca and Mg, and elevated levels of Sr salts will displace these essential nutrients from the exchangeable surfaces, subjecting them to leaching. The solubility, bioavailability, and migration rate of strontium increase with increased soil moisture and acidity (Dubchak, 2018).

Strontium is more mobile than Ba in soil and predominantly exists in easily accessible forms to plants (Kashparov, Lazarev and Polischuk, 2005; Ong *et al*., 2013; Abreu *et al.,* 2012; Dubchak, 2018). Also, Sr and Ca are natural analogues and similar in their intake into plants and distribution across plant organs. However, a smaller amount of Sr (about 10^{-2} to 10^{-3} % of dry mass) is transferred to plants than Ca due to the stronger Sr fixation in soil (Annenkov and Yudintseva, 2002; Dubchak, 2018). Strontium is not known to be essential for plants, and it is not significantly toxic to plants at low concentrations. High levels of Sr in edible plants can have health consequences to humans, mainly because Sr and Ca have similar chemical behavior, in that Ca can be replaced by Sr in the skeletal system, bone tissue and the bone marrow which can damage (weaken) bones (Ageets, 2001; Annenkov and Yudintseva, 2002; Skupiński and Solecki, 2014; Dubchak, 2018). Because of the mobility of Sr, it easily penetrates through the root system into all plant parts with higher levels accumulating in leaves and stem but relatively lower in spikelets and grains, and lowest in roots (Kashparov *et al*. 2005; Dubchak, 2018). Strontium is accumulated more in legumes, root crops and weakly accumulate in cereals, grains, grasses and vegetable crops (Ageets, 2001; Vasilenko and Vasilenko 2002; Lazarevich and Chernukha, 2007; Tieplyakov, 2010; Skupiński and Solecki, 2014;).

1.12 Plant adaptations to salinity

Plants developed mechanisms either to exclude or minimize entry of salt into their cells/tissues or avoid high concentrations in sensitive organs or to tolerate salts within cells/tissues (Bafeel, 2014; Alam *et al*., 2015;). Plant species/cultivars differ in the evolution of adaptation mechanisms that dictate their degree of tolerance to salinity (George *et al*, 2012; Bafeel, 2014; Alam *et al*., 2015). Salt-sensitive plants have less developed adaptation mechanisms whereas salt tolerant species have advanced adaptations (Alam *et al*., 2015). Within a plant, the effectiveness to salinity adaptation is influenced by the sensitivity of affected organ, and type and concentration of salt (George *et al*., 2012). Under high saline conditions, uptake of high salt amounts is generally inevitable; therefore, plants must tolerate the salts within their tissues to survive the salinity stress (George *et al.*, 2012). Salinity adaptation mechanisms in plants may involve: compartmentation of excess/toxic ions, osmotic regulation by synthesis or accumulation of compatible osmolytes e.g., sugars, Na^+ and Cl^-, accumulation of antioxidants and salt exclusion (Cuin and Shabala, 2005; Kaya, Tuna, Ashraf and Altunlu, 2007; George *et al.*, 2012; Plaut *et al.*, 2013; Hua *et al.*, 2015).

Compartmentation of excess/toxic ions in plants can take various forms. For example, Na^+ and Cl^- can be effectively partitioned between old and young leaves, leaf sheath and leaf blades, cell types within leaf blades, vegetative and reproductive organs (inflorescences and seeds), shoot and root, or vacuole and cytoplasm (Hasegawa *et al.*, 2000; George *et al.*, 2012; Lv *et al.*, 2012; Bafeel, 2014). Halophytes survive high salt levels by regulating their transport or storing salts safely in special compartments of their tissues (e.g., vacuoles) (George *et al.*, 2012). Restricted import of Na^+ and Cl^- into young leaves is characteristic of salt-tolerant species that accumulate excess salt in older leaves so it can be shed with the leaves, as reported in wheat and maize (Gorham *et al.*, 1986; Hajibagheri, Harvey and Flowers, 1987; George *et al.*, 2012). Chloride particularly is accumulated in the leaf sheath and epidermal cells of the leaf blade, with lower concentrations in the mesophyll and bundle sheath cells to protect the photosynthetic tissues from salt stress, as is the case in sorghum and barley (George *et al.*, 2012). Under salt stress, Na^+ ions were retained mainly in root and stem of sorghum plants, but the distribution of excess Na^+ to leaves was prevented, and instead allocated to leaf sheath (Netondo *et al.*, 2004; Ashraf, Athar, Harris and Kwon, 2008; Chaugool, Naito, Kasuga and Ehara, 2013; Panuccio *et al.,* 2014).

Retranslocation of Na^+ from shoot to roots is another mechanism that contributes to low Na^+ concentrations under saline conditions, observed in salt sensitive species such as beans and salt-tolerant species such as the common reed and berseem clover. However, the proportion of Na^+ that is translocated and the rate of transfer from leaves back to roots is higher for salt-sensitive than for salt-tolerant species (Matsushita and Matoh, 1992; Davenport *et al*., 2005; George *et al*., 2012). Accelerated phloem retranslocation of K^+ with restricted xylem import of Na^+ from mature leaves to young leaves aids maintaining high K^+ and lower Na^+ concentrations in young leaves and reproductive organs (Wolf, Munns, Tonnet and Jeschke, 1991; George *et al*., 2012).

Transfer of considerable amounts of salt ions into the vacuole or prevention of leakage of ions through the tonoplast back to the cytoplasm also aids in avoidance of toxic concentrations in the cytoplasm (Gorham *et al*., 1985; George *et al*., 2012). Efficient reduction of leakage of ions through the tonoplast may be enhanced by amino acids or polyols that function to stabilize the membrane (George *et al*., 2012). The ability of plants to maintain low Na^+ and high K^+ concentration (high K^+/Na^+ ratio) within the cytoplasm is crucial in K homeostasis under salinity stress, which is commonly observed in salt-tolerant species, while in some species Na can replace K in its functions (Gibson, Speirs and Brady, 1984; Ligaba and Katsuhara, 2010; Bafeel, 2014).

Enhanced enzymatic and non-enzymatic activation of antioxidants, such as superoxide dismutase, ascorbate peroxidase, glutathione peroxidases and catalase that scavenge and detoxify excessive amounts of reactive oxygen species (ROS) (e.g, hydrogen peroxide, superoxide or hydroxyl radicals), also helps to counteract oxidative stress damage (Khatkar and Kuhad, 2000;

Duan *et al*., 20013; Heikham, Kapoor and Giri, 2009; Plaut *et al*., 2013). Signal transduction due to salt stress stimulates increased production of ROS that triggers antioxidant synthesis to counter the damaging effect of ROS and mitigates salt stress effects (de Azevedo Neto, Prisco, Enéas-Filho, de Abreu and Gomes-Filho, 2005). When exposed to salinity, antioxidant scavenging in C4 plants appears to be more effective than that in C3 plants (Stepien and Klobus, 2005; George *et al*., 2012). In some plants, salt tolerance is associated with accumulation of organic solutes, commonly polyamines, whereby increased biosynthesis and reduced degradation of polyamines in the cytoplasm balance the osmotic pressure of ions in the vacuoles and detoxify ROS (Hasegawa *et al*., 2000; Bhatt, Patel, Bhatti and Pandey, 2008; Heikham *et al*., 2009; Plaut *et al*., 2013). Proline accumulation is a well-known response to water deficit and to salt stress in glycophytes and halophytes suggested to be responsible for alleviation of the harmful salinity effects (Khatkar and Kuhad, 2000). In bermudagrass and other turfgrass species, proline and glycine betaine levels increased as salinity increased (Kaya *et al*., 2007; Cuin and Shabala, 2008; Huang, Bie, Liu, Zhen and Wang, 2009; Shevyakova, Bakulina and Kuznetsov, 2009).

Many halophytes (such as mangrove) develop enhanced barriers particularly in roots against passive influx of salts so as to exclude uptake of excess salts, for example, through enlarged width of the Casparian band (is usually 2 to 3 times in halophytes greater than in glycophytes), and differentiated inner cortex cell layer into a second endodermis (Gorham, 1987; Inan *et al*., 2004; Greenway and Munns, 1980; George *et al*., 2012; Alam *et al*., 2015). Recretohalophytes (e.g., mangroves and zoysia grass) excrete salts through salt glands on leaves and remove excess salt (Waisel, Eshel and Agami, 1986; Ball, 1988; Marcum, Anderson and Engelke, 1998). Other mechanisms initiated by signal-enzymes in response to salinity stress may include leaf drop to reduce water demand, improved tissue water content (succulence) to counter osmotic stress, hydraulic permeability and nutrient acquisition, and molecular changes such as enhanced expression of plasma membrane intrinsic protein (PIP) genes and encoding (Plaut *et al*., 2013; Uddin and Juraimi, 2013).

CHAPTER II

MATERIALS AND METHODS

The effect of brine, Ba, and Sr on bermudagrass (*Cynodon dactylon*), sorghum sudangrass (*Sorghum × drummondii* hybrid), and cowpea (*Vigna unguiculata L.*), and on soil properties was tested in a greenhouse experiment. Plants were grown in soil mixed with synthetic oilfield brine with and without Ba and Sr spiking solutions. The species of plants were selected because of their varied adaptability to salinity: bermudagrass is highly adapted, sorghum sudangrass is moderately adapted, and cowpea is poorly adapted to salinity (Tabosa *et al*., 2007; Huang, 2018).

2.1 Planting material

Plant varieties grown for this study were IT90k – 277-2 cowpea, SP 4105 sorghum sudangrass, and common bermudagrass. Oilfield brine effect was tested on the germination and vegetative growth phase of the plants. The plants were grown from seed to flower initiation stage. Dried and stored seeds were obtained from Texas A&M University (Texas A&M University) cereal seed bank, Department of Soil and Crop Sciences. Bermudagrass seed was hulled and coated with a fungicide, sorghum sudangrass seed was also coated, while cowpea seed was uncoated.

2.2 Synthesis of CNOOC oilfield brine

Brine was synthesized from ultrapure water and selected salts to mimic brine expected to be produced by the CNOOC Kingfisher project in Uganda (Table 2.1). Ion concentrations projected by CNOOC for their brine are lower than the mean concentration range of oilfield brines across the world (Table 1.1); therefore, the synthesized brine concentrations were increased by a factor of 10 to match with the global mean range.

Table 2.1. Composition of synthesized CNOOC oilfield brine and Ba, Sr solution

Brine Solution			
Salt	**mmol$_c$ L^{-1}**	**mmol L^{-1}**	**g L^{-1}**
Na acetate	118.1	118.1	9.69
Na propionate	6.98	7.0	0.67
NaBr	6.2	6.2	0.64
NaCl	615.0	615.0	35.94
K_2SO_4	23.3	11.7	0.14
KCl	426.8	426.8	31.82
$MgCl_2 \cdot 6H_2O$	4.8	2.4	0.24
$CaCl_2 \cdot 2H_2O$	134.0	67.0	4.93
Ba, Sr Spiking Solution			
Salt	**mmol$_c$ L^{-1}**	**mmol L^{-1}**	**g L^{-1}**
$BaCl_2 \cdot 2H_2O$	14.6	7.3	0.89
$SrCl_2 \cdot 6H_2O$	22.8	11.4	1.52

2.3 Soil Characterization

A sample of the soil of the Hearne series: Fine, mixed, semiactive, thermic Typic Haplustults (Soil Survey Staff, 2003**)**, obtained from Robertson County, Texas (Figure 2.1), was chosen for the greenhouse experiment because it has characteristics similar to those of soils of the AG region (Table 2.2). The site was under natural vegetation cover (i.e., trees and grass, Figure 2.1). Soil was collected from the top 6 inches (< 15 cm), air dried, and ground to <2 mm.

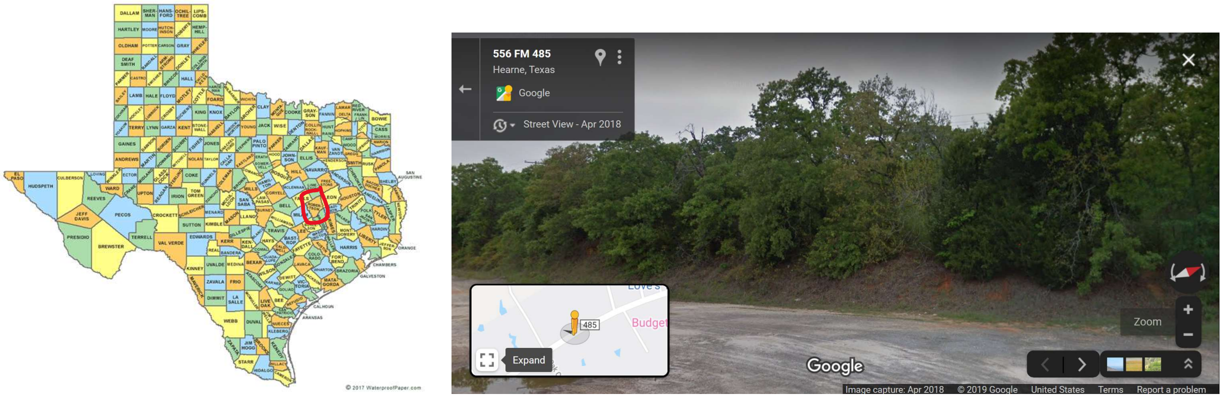

Figure 2.1 Robertson County on Texas map (left) and soil collection site (right).

2.3.1 Basic properties of Hearne soil

Prior to planting the plants in the greenhouse, the soil was characterized at the Texas A&M Soil, Water, and Forage Testing Laboratory (https://soiltesting.tamu.edu/) for soil pH, texture, EC_e, organic matter content, nutrients and salinity. Soil texture was determined using the hydrometer method (Day, 1965), and organic matter content was determined by the combustion method at 400 °C overnight (Storer, 1984; McGeehan and Naylor, 1988; Schulte and Hopkins, 1996). Soil water pH and EC_e were determined on a saturated soil paste with DI water, and measured the pH using a hydrogen ion selective electrode and the EC_e by a conductivity probe (Schofield and Taylor, 1955; Rhoades, 1982). Detailed soil salinity was evaluated on the saturated soil paste extract for soluble cations (Ca^{2+}, K^+, Mg^{2+} and Na^+) by induction coupled plasma (ICP) (Rhoades and Clark. 1978), and sodium adsorption ratio (SAR) was calculated. Boron was extracted using hot-water extraction method and determined by ICP (Bingham, 1982; de Abreu, de Abreu, van Raij, Bataglia and de Andrade, 1994).

Plant available P, K, Ca, Mg, Na and S were extracted using Mehlich III multi-nutrient extractant method and determined by ICP (Mehlich, 1978). Extractable Cu, Fe, Mn and Zn were extracted by DTPA extractant method and determined by ICP (Lindsay and Norvell, 1978). Nitrate-nitrogen (NO_3-N) was extracted using a 10:1 ratio of 1 M KCl solution to soil and determined by nitrate reduction method followed by spectrophotometric measurement (Keeney and Nelson, 1982; Markus, McKinnon and Buccafuri, 1985; Kachurina, Zhang, Raun and Krenzer, 2000).

2.3.2 Mineralogical analysis of Hearne soil

The mineralogy of the study soil was evaluated by the Soil Minerology Laboratory and the Soil Characterization Laboratory, Soil and Crop Sciences Department, Texas A&M University. The soil was pretreated to remove cementing materials and disperse soil particles (Whittig and Allardice, 1986). Particle size fractionation was done by sieving and centrifugation (based on Stokes' Law) (Deng and Arvide, 2011). Cation exchange capacity (CEC) of the clay fraction was determined by $CaCl_2$/$MgCl_2$ saturation/replacement method, and CEC of bulk soil determined by NH_4OAc (pH 7) method (Chapman, 1965; Holmgren, Juve and Geschwender, 1977; Soil Survey Staff, 1996). Soil minerals in the bulk, silt, and clay fractions were characterized using X-ray diffraction; Fourier transformation infrared Spectroscopy; scanning electron microscopy; sodium dithionite, sodium citrate and sodium bicarbonate (DCB) for Fe oxides; and total potassium determination for quantification of mica (Mehra and Jackson, 1960; Bernas, 1968). Mineral identification was aided by mineral identification database manuals/software such as the International Center for Diffraction Data cards, manuals, and web-based mineral searches (http://webmineral.com/ websites).

Table 2.2. Characterization of soils of the Albertine Graben (AG) and Hearne Series

SOIL PROPERTY	AG SOIL [13, 14]	HEARNE SOIL
Dominant group (order)	Ferralsol (Oxisol)	Ultisol [15]
Texture (hydrometer method)	sandy to clay loams	sandy clay loam
CEC (cmol$_+$ kg^{-1}) (1 *M* NH$_4$OAc, pH 7)	5 to 15	3.9
Mineralogy	kaolinite, Fe & Al oxides	quartz, kaolinite, feldspars, mica, anatase smectite
EC$_e$ (dS m^{-1})	< 1.0	< 1.0
pH	4.4 to 7.6	5.7 to 6.0

[13] CUL, 2018, [14] Tilenga Project, 2018, [15] Soil Survey Staff, 2003

2.4 Greenhouse experimental design

The brine solution (section 2.2) was added at rates aimed to bring the Hearne soil to EC 3 or 9 dS m^{-1}. The brine solution had an EC$_e$ of 134 dS m^{-1}, and the soil Hearne soil contained 37.5% water at saturation. Therefore, the amount of brine solution to be added to 1 kg of soil to achieve these EC$_e$ values was calculated to be approximately 8.4 and 25.2 mL. The BaSr spiking solution also referred to earlier was added at target concentration of Ba and Sr in the soil for "Low" level = 1.08 mg kg^{-1} and "High" level = 4.3 mg kg^{-1} of Ba and Sr. So, 0.63 and 2.52 mL kg^{-1} of the Ba and Sr spiking solution was required for the low and high target Ba, Sr levels respectively.

The greenhouse experiment involved 7 treatments including an untreated control (neither brine nor Ba, Sr solution added); addition of brine to reach EC$_e$ 3 dS m^{-1} ("EC3"); addition of brine to reach EC$_e$ 9 dS m^{-1} ("EC9"); addition of the Ba, Sr spiking solution only at a low level ("BaSr_low"); addition of the Ba, Sr spiking solution only at a high level and ("BaSr_high"); spiking with a combination of brine to reach EC$_e$ 9 dS m^{-1} plus a low level of Ba, Sr

(“EC9+BaSr_low”); and spiking with a combination of brine to reach EC_e 9 dS m^{-1} plus a high level of Ba, Sr (“EC9+BaSr_high”) (Table 2.3).

The brine levels for the greenhouse experiment were selected based on a prescreening study in the laboratory to determine the brine levels that would impact germination but would neither completely eliminate germination nor seedling growth. The preliminary study involved 6 treatments including an untreated control (neither brine nor Ba, Sr solution added), 3 levels of brine (EC_e 3, 9 and 18 dS m^{-1}), 2 levels of BaSr spiking solution (low and high, at target concentration 25 and 100 mg L^{-1} of Ba and Sr spiking solution in brine, respectively). The brine level of EC_e 18 dS m^{-1} prohibited germination of most of the seeds and thus was not included for the greenhouse experiment, while the BaSr did not negatively affect germination.

Table 2.3. Treatments of brine and a Ba, Sr solution used in the research experiment

Treatment	Target EC_e (dS m^{-1})	BaSr addition
Control	-	-
EC3	3	-
EC9	9	-
BaSr_low	-	Low
BaSr_high	-	High
EC9+BaSr_low	9	Low
EC9+BaSr_high	9	High

Low = 1.08 mg BaSr kg^{-1} soil; High = 4.3 mg Ba,Sr kg^{-1} soil

Cowpea and sorghum sudangrass were grown in 2 L pots with 2.8 kg of soil, while bermudagrass was grown in 1 L pots with 1.25 kg of soil. Required volumes of brine and BaSr solution for the respective treatments were mixed with ultrapure water to ensure 37.5% water at saturation (Table 2.4). However, the BaSr spiking solution was initially mixed with 25 mL of ultrapure water; therefore, 25 mL water were added to all non-BaSr treatments to ensure uniform initial water contents in all pots. The treatments were surface applied to soil in the pots to simulate a spill. The solutions were allowed to infuse prior to sowing seeds. Each treatment was replicated four times for each plant species.

Table 2.4. Volume (mL) of solutions added to soil for each treatment in cowpea and sorghum sudangrass (2800 g soil per 2 L pot) and bermudagrass (1250 g soil per 1 L pot)

Solution	**Brine**	**BaSr solution**	**Water added**	**Total solution added**
Treatment	*Volume (mL)*			
	Cowpea and Sorghum sudangrass			
Control	0.00	0.00	595.0	595.0
EC3	23.51	0.00	571.5	595.0
EC9	70.52	0.00	524.5	595.0
EC9+BaSr_low	70.52	1.763	524.5	596.8
EC9+BaSr_high	70.52	7.052	524.5	602.1
BaSr_low	0.00	1.763	595.0	596.8
BaSr_high	0.00	7.052	595.0	602.1
	Bermudagrass			
Control	0.00	0.00	265.6	265.6
EC3	10.49	0.00	255.1	265.6
EC9	31.48	0.00	234.1	265.6
EC9+BaSr_low	31.48	0.787	234.1	266.4
EC9+BaSr_high	31.48	3.148	234.1	268.7
BaSr_low	0.00	0.787	265.6	266.4
BaSr_high	0.00	3.148	265.6	268.7

Plants were grown in a greenhouse for approximately three months in the summer. The treatments (amended pots) were arranged in a completely randomized design. The summer growing period was preferred because temperature and light conditions are similar to those in the AG region and are conducive for the growth of study plants. Bermudagrass and sorghum sudangrass were sown on 24 June, 2019; cowpea was sown on 25 June 2019. The greenhouse humidity was not modified and temperature was vented to prevent it from going too high. Mean daily daytime temperature during June to October 2019 ranged between 28 and 36 °C, and nighttime temperatures ranged between 15 and 23 °C (https://www.weather.gov/hgx/climate). The mean monthly relative humidity during June to October 2019 was between 77 and 79% (https://www.weather.gov/hgx/climate). Supplemental lighting of approximately 1000 μmol m^{-2} s^{-1} was provided when evening sunlight intensity was <300 W m^{-2} (~ 606 μmol m^{-2} s^{-1}) until 8 pm and, in the morning after 6 am, the lights were switched off when sunlight intensity exceeded 600 W m^{-2} (~ 1213 μmol m^{-2} s^{-1}).

Ten seeds of sorghum sudangrass and cowpea were sown in each pot and later thinned to two plants per pot. Approximately 0.27 g pot^{-1} (3 lb sq ft^{-1}) of bermudagrass seed (about 452 seeds per pot) were sown in each pot and not thinned. The sowing depth of seeds was approximately 5 cm for cowpea, 3 cm sorghum sudangrass, and 0.5 cm for bermudagrass. The plants were watered with reverse osmosis (RO) water to avoid addition of salts from tap water. The quantity of water was carefully controlled to avoid loss of salts by leaching, which was ensured by placing all pots in small trays to capture any water that emerged from the bottom of the pot. Fertilizers (monoammonium phosphate -MAP, urea and KCl) were added based on agronomic recommendations made by the Soil, Water and Forage Testing Laboratory, Texas A&M

University (Table 2.5). However, only N and P fertilizers were added to pots treated with brine to limit addition of salts beyond the treatment levels.

Table 2.5. Fertilizer application rates for cowpea, sorghum sudangrass and bermudagrass in the greenhouse

	Cowpea	**Sorghum sudangrass**	**Bermudagrass**
	Nutrient application rate		
Nutrient	Lbs./acre	Lbs./acre	Lbs./1000 ft^2
Nitrogen	15	75	0.9
Phosphorus (P_2O_5)	60	40	2.2
Potassium (K_2O)	40	80	2.3

2.5 Measuring plant growth parameters in the greenhouse

The number of seedlings per pot were counted every two days until the thinning date. The number of leaves per plant per pot for sorghum sudangrass and cowpea were counted. Sorghum sudangrass was thinned after 26 days and cowpea after 28 days from sowing. Seedling/plant height (i.e., vertical height to growth point and tallest plant point) of each plant per pot for sorghum sudangrass and cowpea was measured every two weeks. For bermudagrass, plant height was measured by taking vertical height of shoots per pot (Figure 2.2). Observations of qualitative changes in plant morphology (e.g., leaf burn, leaf discoloration and plant deformation) were noted.

Figure 2.2. Measurement of plant height in bermudagrass

2.6 Harvesting and preparation of plant and soil samples

Plant shoots and roots were harvested just before flowering stage. Cowpea was harvested after 44 days from sowing, sorghum sudangrass after 65 days at the initiation of heading, and bermudagrass was allowed to grow for more 16 days (harvested after 81 days) to accumulate sufficient biomass. The harvested plant samples were oven dried at 65 °C for 48 hrs. Dry mass of the plant samples was measured after which, the samples were ground and homogenized for chemical analysis. Soil samples also were collected at harvest from two depths, as follows: a) from 0-2 cm ("upper") and >2 cm ("lower") for bermudagrass or b) 0-4 cm (upper) or >4 cm (lower) for cowpea and sorghum sudangrass. The harvested soil samples were air dried for 2 days, homogenized, and ground to pass 2-mm sieve.

2.7 Analysis of plant and soil samples

2.7.1 Digestion and chemical analysis of plant samples

Plant samples (approximately 0.5 g) were digested in 10 ml of nitric acid and analyzed by ICP following EPA method 3051A- microwave assisted acid digestion (Kingston and Jassie, 1988; U.S. Environmental Protection Agency, 2007). The digests were poured over a funnel and #2 Whatman filter into 100 mL volumetric flasks, diluted with ultrapure water to volume, and transferred into 50 mL centrifuge tubes. The resulting extracts were diluted 1:10 to minimize possible interferences at high ion concentrations during ICP analysis. Cation (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Ba^{2+}, and Sr^{2+}) concentrations in plant samples were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES), model Thermo Scientific™ iCAP™ 7400 ICP-OES, with an auto sampler ASX-560 Autosampler - Teledyne CETAC Technologies.

The internal standard used for ICP-OES was yttrium. Sample blanks, standard blanks, quality control standards and calibration standards were analyzed to check for matrix effects and possible contamination sources. The analytical standard solutions for Ca, Mg, Na and K were obtained from BDH- VWR analytical (VWR international, LLC), USA; for Ba and Sr, the standard solutions were purchased from Alfa Aesar -Specpure (Thermo Fisher Scientific), USA.

2.7.2 Extraction and analysis of soil samples

Soil samples from each of the two pot depths (upper and bottom) were analyzed for pH, EC_e, soluble and exchangeable cation (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Ba^{2+}, and Sr^{2+}) concentrations, and SAR. Soil pH and EC_e were measured on saturated soil paste (Rhoades, 1996; Thomas, 1996).

Soluble salts were extracted by saturated paste extract method, using RO water provided by the central RO unit in the building (Rhoades, 1996). For each soil sample, about 55 mL of water was added to 200 g soil to approach saturation of soil and let stand overnight. The condition of saturation was met by adding water or soil until the soil was of the proper consistency. The moisture content at saturation was determined for each sample. Soil pH was measured in the saturated paste using a combination of pH probe (VWR 89231-614 pH Benchtop Probe (Electrode), VWR International) and a pH meter (sympHony™ Benchtop Meter B30PCI model, VWR International, Inc. 2012) (Thomas, 1996). The soil was vacuum extracted, and EC_e was measured on extracted solute using a combination of an EC probe (YSI 3252 Conductivity Probe, YSI Incorporated) and an EC meter (YSI 3100 Conductivity Instrument, YSI incorporated, 1997) (Rhoades 1996). Soluble cation (Ca, Mg, K, Na, Ba and Sr) concentrations in the extracted soil samples were determined by ICP analyses (ICP-OES) as described earlier (Helmke and Sparks, 1996; Rhoades, 1996; Suarez, 1996).

SAR (concentrations were in $mmol_c\ L^{-1}$) was calculated according to the following equation:

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

Exchangeable salts were extracted by 1 *M* ammonium acetate extraction at pH 7 method (Rhoades, 1996). Briefly, 2.5 g soil were extracted three times in succession with 10 mL NH_4OAc, centrifuged, and the supernatant decanted into a 50 mL plastic tube. After the third extraction, nitric acid was added to achieve 2% HNO_3 in the final volume (50 mL). Concentrations of soluble and exchangeable cations in the extracted soil samples were determined by atomic absorption spectroscopy (AAS) and ICP-OES analyses, as described previously (Helmke and Sparks, 1996; Rhoades, 1996; Suarez, 1996).

2.8 Statistical analysis

One-way analysis of variance (ANOVA) and two-way ANOVA were conducted on data for single factor or two factor analyses. Duncan's Multiple Range Test (DMRT) and least significant difference (LSD) at $\alpha = 0.05$ were used to separate means. The statistical analyses were done using CoHort Software, Monterey, California (v. 6.4), and Excel version 2007 (Microsoft Excel 2019 MSO).

CHAPTER III

RESULTS

3.1 Effect of brine, barium and strontium in soil on the growth of cowpea, sorghum sudangrass and bermudagrass

3.1.1 Germination

3.1.1.1 Prescreening laboratory study on germination

During the prescreening germination study in the laboratory, germination was highest under Ba, Sr without brine than under control and brine treatments. The control, EC3 and BaSr treatments had higher germination than EC_e 9 and 18 dS m^{-1} (results not shown). Germination in the EC_e 18 dS m^{-1} treatments was very low in sorghum sudangrass and failed completely in cowpea and bermudagrass; therefore, the 18 dS m^{-1} treatment was not used in further experiments.

3.1.1.2 Germination and emergence of cowpea

Seed emergence in cowpea commenced on day 3 after sowing for the control, EC3 and both BaSr without brine treatments (Figure 3.1). For EC9 and EC9+BaSr_high, seed emergence began on day 4, while at EC9+BaSr_low, seed emergence commenced on day 6 after sowing. The emergence rate was slower for all EC9 treatments, although final emergence in all treatments was realized on about the same day (day 8 after sowing). More than 60% emergence was attained by day 4 in the non-brine and EC3 brine treatments, while under EC9 brine treatments, < 40% emergence had been attained by day 4. Mean final emergence percentage in cowpea ranged from 53% (in EC9+BaSr_low) to 85% (in BaSr_high).

Table 3.1. Effect of oilfield brine on mean emergence of cowpea, sorghum sudangrass and bermudagrass

Treatment	**Emergence (%)**		
	Cowpea	Sorghum sudangrass	Bermudagrass
Control	82.5	90.0 ^{ab}	17.8 ^{a}
EC3	75.0	85.0 ^{ab}	17.3 ^{a}
EC9	65.0	62.5 ^{c}	5.9 ^{b}
EC9+BaSr_low	52.5	75.0 ^{abc}	4.7 ^{b}
EC9+BaSr_high	72.5	67.5 ^{c}	2.5 ^{b}
BaSr_low	75.0	90.0 ^{ab}	18.2 ^{a}
BaSr_high	85.0	95.0 ^{a}	20.6 ^{a}
One-way ANOVA	0.074 ^{ns}	0.002	< 0.001

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$.

The mean emergence percentage in cowpea was significantly lower in EC9+BaSr_low than in the control and BaSr_high treatments, but emergence in all other treatments were statistically equivalent to the control ($P = 0.074$) (Table 3.1). Seedling mortality in cowpea was noted in all treatments, but it was highest within brine treatments at EC9 (Figure 3.1). About 20% (EC9 and EC9+BaSr_low) and 30% (EC9+BaSr_high) of the germinated seedlings died by day 14. A lower mortality (about 10%) was observed within the non-brine and brine in EC3 treatments.

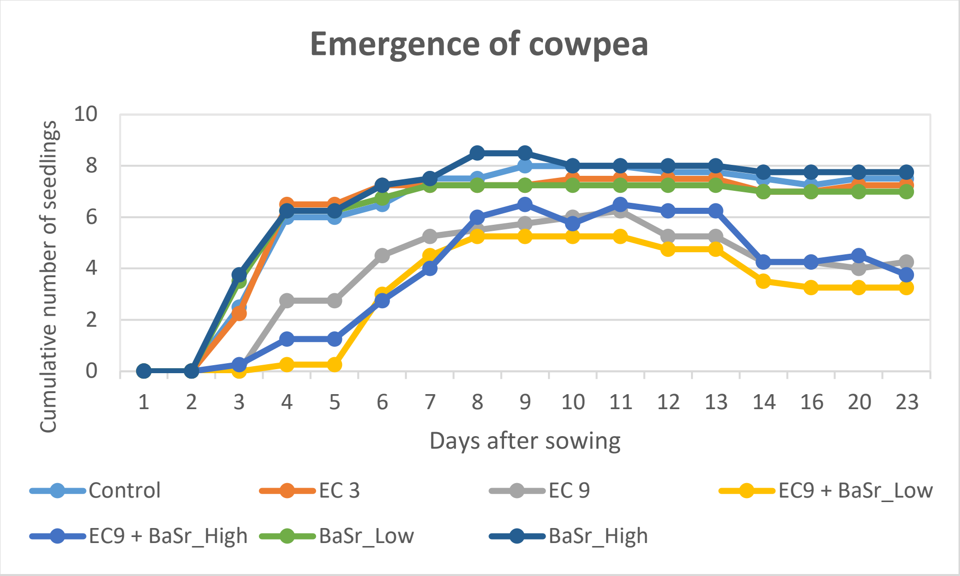

Figure 3.1. Emergence of cowpea as affected by oilfield brine

3.1.1.3 Germination and emergence of sorghum sudangrass

Seed emergence in sorghum sudangrass commenced on the same day in all sorghum sudangrass treatments (i.e., day 3 after sowing) and progressed at a similar rate in all treatments (Figure 3.2). Final emergence was realized by day 5 for most of the treatments, although it was attained earlier (on day 4) in Ba,Sr treatments without brine. More than 70% emergence was observed upon initiation of seed emergence on day 3 within non-brine and EC3 treatments, while < 30% emerged under brine in all treatments with EC9 (without and without BaSr). Mean final emergence percentage was significantly lower than the control in all brine treatments at EC9 (with or without BaSr striking solution), but not at EC3 (*P* = 0.002) (Table 3.1). The emergence percentage in sorghum sudangrass ranged from 62.5% (in EC9) to 95.0% (in BaSr_high). Seedling mortality was noted within in treatments containing brine at EC9 (about 10% EC9, and 20% EC9 with BaSr at -high and -low levels and within control but was not distinct under brine at EC3 and Ba, Sr without brine treatments (Figure 3.2). Salt-affected seedlings of sorghum sudangrass were thinner than the unaffected seedlings.

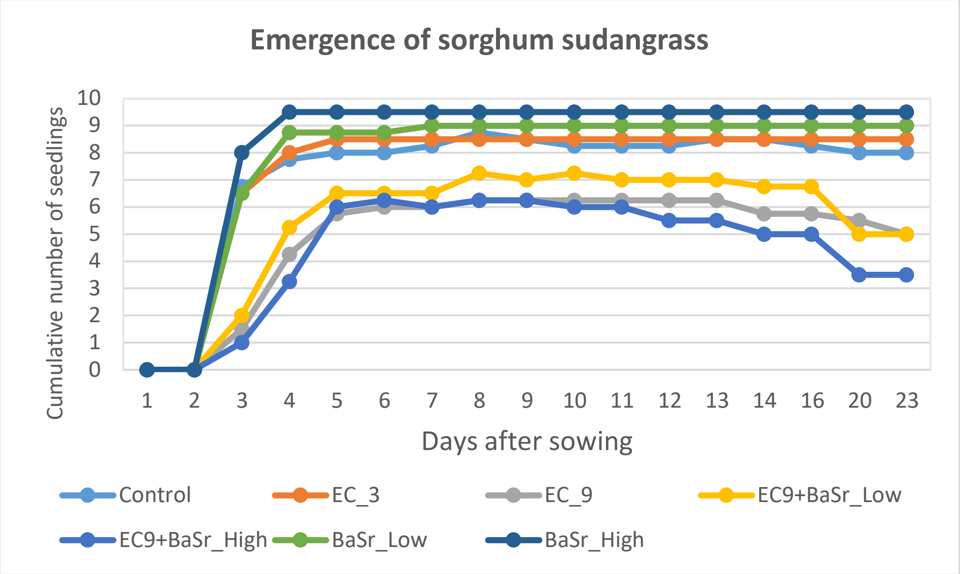

Figure 3.2. Brine effect on emergence of sorghum sudangrass

3.1.1.4 Germination and emergence of bermudagrass

Seed emergence in bermudagrass started on day 6 after sowing for non-brine treatments, and around day 10 in brine treatments (Figure 3.3). Emergence in bermudagrass steadily increased with time in the non-brine treatments and EC3 but stagnated in brine treatments at EC9 until day 20 when it revived slowly. However, it is not clear whether the emerged seedlings after day 20 sprouted from rhizomes (of the few seedlings that germinated earlier on day 10) or from sown seeds. Although hulled bermudagrass seed is expected to germinate within 5 to 10 days, the seeds can as well remain viable (in soil) ungerminated for about 28 days (approximately a month) (SFGATE, 2019). Therefore, final emergence considered for the case of bermudagrass was that on day 31 after sowing. Seed viability is generally maintained in the presence of salinity, although some seeds may lose viability while many of them enter into salt induced dormancy (Zehra and Khan, 2007; Zehra *et al*., 2012).

Similar to cowpea and sorghum sudangrass, seedling mortality was noted in bermudagrass. Contrary to cowpea and sorghum sudangrass, bermudagrass seedling death was more pronounced among non-brine treatments which were overcrowded, but it was difficult to quantify (Figure 3.3). Tiny, dead seedlings would be spotted on the soil surface, but more new seedlings emerged every day; the mortality could not be quantified by mere subtraction of numbers. Bermudagrass seedlings in brine treatments had the most vigorous growth (seem to have explored the available growth resources under uncrowded environment); with no visible signs of salt injury compared to non-brine treated seedlings.

The emergence percentage in bermudagrass was significantly lower in brine treatments at EC9 (with and without BaSr solution) (Table 3.1). Percentage emergence under brine at EC9 (with and without BaSr solution) was more than 3 times lower than in control, while in BaSr without brine, emergence percentage was higher by about 13%. Mean emergence percentage in bermudagrass ranged from 2.5% in EC9+BaSr_high to 20.6% in BaSr_high (Table 3.1). According to one-way ANOVA, emergence percentage of bermudagrass was highly significant among treatments ($P < 0.001$). Between the species, emergence percentage was highest in sorghum sudangrass, then cowpea and lowest in bermudagrass (Figure 3.4).

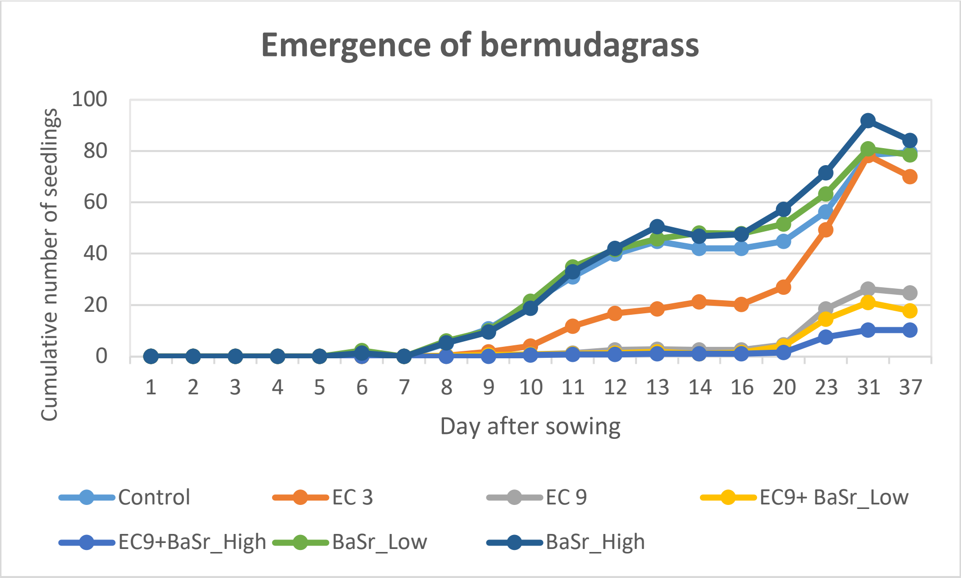

Figure 3.3. Brine effect on emergence of bermudagrass

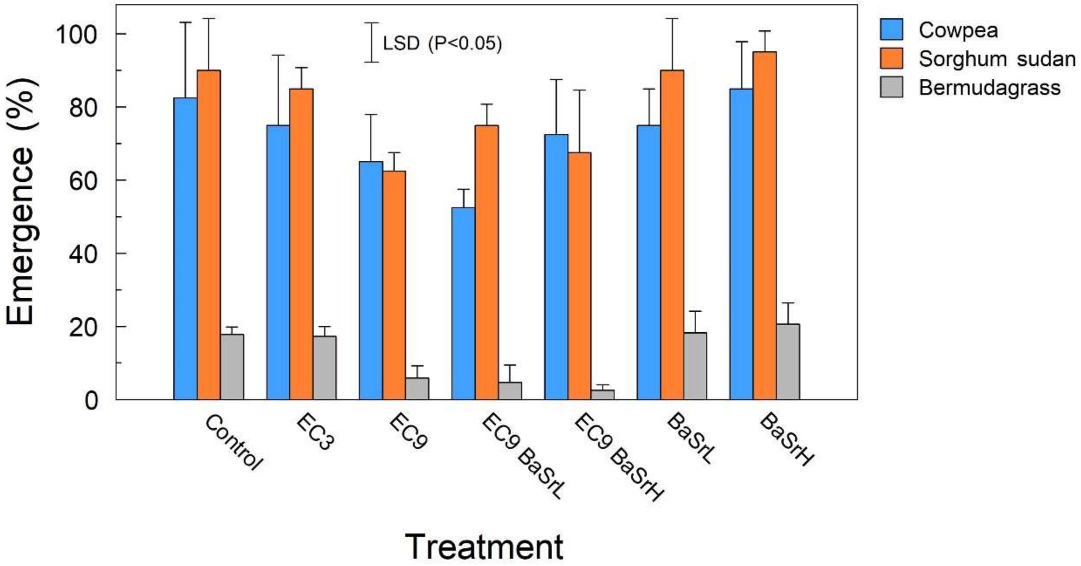

Figure 3.4. Brine effect on emergence of cowpea, sorghum sudangrass and bermudagrass. Error bars represent one standard deviation

3.1.2 Effect on vegetative growth

3.1.2.1 Effect on plant morphology, shoot and root growth of cowpea

Cowpea plants treated by brine at EC9 had smaller, deformed and discolored (yellow spotted-chlorotic) leaves with multiple foliates, while plants under all other treatments had the typical trifoliate morphology (results not shown). Most of the brine (EC9) treated plants were stunted with deformed (bent) stems. Harvested roots were observed to be smaller, shorter, deformed (bent) with fewer and smaller root nodules compared to plants under other treatments. The morphology of cowpea plants treated with Ba,Sr (without brine) was not different from control plants but exhibited slightly more vigorous growth than the control. For the EC3 treatment, the plants had less growth vigor than the control but did not show distinct salt-induced morphological symptoms.

Shoot biomass of cowpea

Dry shoot mass in cowpea declined with increasing brine, while BaSr_low and BaSr_high increased shoot mass (Figure 3.5). Dry shoot mass was highly significant ($P < 0.001$) among treatments in cowpea. The dry shoot mass in cowpea was significantly lower in all treatments with brine, but BaSr_low and BaSr_high were not significantly different from control plants. Shoot mass was highest in the control and BaSr_low but lowest in EC9+BaSr_low (Table 3.2).

Root biomass of cowpea

Dry root mass in cowpea decreased with increasing brine (Figure 3.5). Dry root mass in cowpea was significantly lower in all EC9 treatments, with and without BaSr. Root mass was highest in control and BaSr_low but lowest in all treatments with EC9 (Table 3.2).

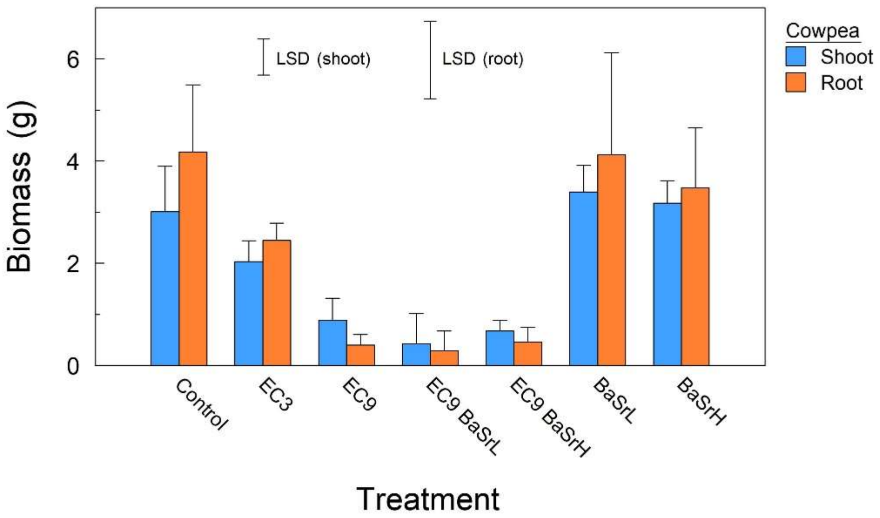

Figure 3.5. Dry shoot and root mass of cowpea as influenced by oilfield brine. Error bars represent one standard deviation

Table 3.2. Mean dry shoot mass, root mass and root to shoot ratio of cowpea as affected by oilfield brine

Treatment	**Shoot (g)**	**Root (g)**	**Root to shoot ratio**
Control	3.1^{a}	4.2^{a}	1.46
EC3	2.0^{b}	2.4^{b}	1.26
EC9	0.9^{c}	0.4^{c}	0.55
EC9+BaSr_low	0.4^{c}	0.3^{c}	0.85
EC9+BaSr_high	0.7^{c}	0.5^{c}	0.64
BaSr_low	3.4^{a}	4.2^{a}	1.20
BaSr_high	3.2^{a}	3.5^{ab}	1.13
One-way ANOVA	$P < 0.001$	$P < 0.001$	$P = 0.067^{ns}$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

Root to shoot ratio of cowpea

Root to shoot mass ratio in cowpea reduced with increasing brine and BaSr levels (Figure 3.8) but it did not differ among treatments (P < 0.067). However, the root to shoot mass ratio at EC9 and EC9+BaSr_high was significantly different from control (Table 3.2). Root to shoot ratio was highest in control and lowest in EC9 (Table 3.2).

3.1.2.2 Effect on plant morphology, shoot and root growth of sorghum sudangrass

Sorghum sudangrass plants under all treatments exhibited relatively similar growth morphology, although plants under brine EC9 generally appeared to be at a leaf stage behind in comparison to other treatments, with fewer number of leaves necrotic older leaves.

Shoot biomass of sorghum sudangrass

Dry shoot mass in sorghum sudangrass increased with increasing brine (Figure 3.6). Dry shoot mass BaSr_low and BaSr_high were statistically equivalent to the control ($P < 0.001$). Dry shoot mass in sorghum sudangrass was highest in EC9+BaSr_high (Table 3.3). Dry shoot mass in sorghum sudangrass for all EC9 treatments (with and without BaSr) was significantly higher than control and BaSr_low, while the rest of the treatments were not significantly different from one another.

Root mass of sorghum sudangrass

Treatments did not have a significant impact on dry root mass in sorghum sudangrass ($P < 0.42$) (Figure 3.6). Root mass in sorghum sudangrass was highest in BaSr_high and lowest in BaSr_low (Table 3.3), but none of the differences were significant.

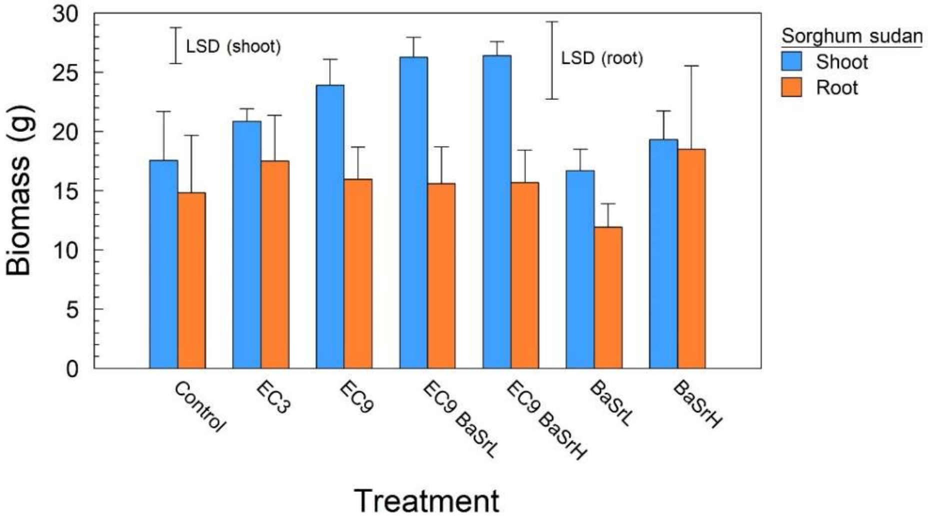

Figure 3.6. Dry shoot and root mass of sorghum sudangrass as influenced by oilfield brine. Error bars represent one standard deviation

Table 3.3. Mean dry shoot mass, root mass and root to shoot ratio in sorghum sudangrass as affected by oilfield brine

Treatment	**Shoot (g)**	**Root (g)**	**Root:shoot ratio**
Control	17.6^{cd}	14.9	0.85^{ab}
EC3	20.8^{bc}	17.5	0.84^{abc}
EC9	23.9 ^{ab}	15.9	0.67^{bcd}
EC9+BaSr_low	26.3 ^{a}	15.6	0.609^{cd}
EC9+BaSr_high	26.4 ^{a}	15.7	0.59^{d}
BaSr_low	16.7^{c}	11.9	0.71^{abcd}
BaSr_high	19.3 ^{cd}	18.5	0.94 ^{a}
One-way ANOVA	$P < 0.001$	$P = 0.419^{ns}$	$P = 0.038$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$.

Root to shoot mass ratio in sorghum sudangrass

Dry root:shoot mass ratio in sorghum sudangrass decreased with increasing brine and BaSr levels (Figure 3.8) and varied significantly ($P = 0.0382$) among and between treatments.

Root:shoot ratio in sorghum sudangrass was highest in BaSr_high and lowest in EC9+BaSr_high (Table 3.3).

3.1.2.3 Effect on plant morphology, shoot and root growth of bermudagrass

Bermudagrass plants had comparable morphology under all treatments with no noticeable salt-induced changes, except that the plants under all EC9 treatments had tougher stems compared to other treatments.

Shoot biomass of bermudagrass

Dry shoot mass in bermudagrass generally increased with increasing brine and BaSr levels, but was not significant (*P* = 0.4754) among and between treatments (Figure 3.7).

Root biomass of bermudagrass

Dry root mass in bermudagrass was lower under brine at EC9 than control, but slightly increased for BaSr_low, BaSr_high, and EC3 (Figure 3.7). Dry root mass in bermudagrass was highest under BaSr_high and lowest in EC9+BaSr_high (Table 3.4).

Root to shoot mass ratio in bermudagrass

Dry root to shoot mass ratio in bermudagrass was lower than control under brine at EC9 (with and without BaSr) and under BaSr (without brine), but was slightly higher at brine EC3 (Figure 3.8). The dry root mass in bermudagrass was different (*P* = 0.002) among and between some treatments. Dry root mass in bermudagrass was highest under EC3 and lowest in EC9+BaSr_high (Table 3.4).

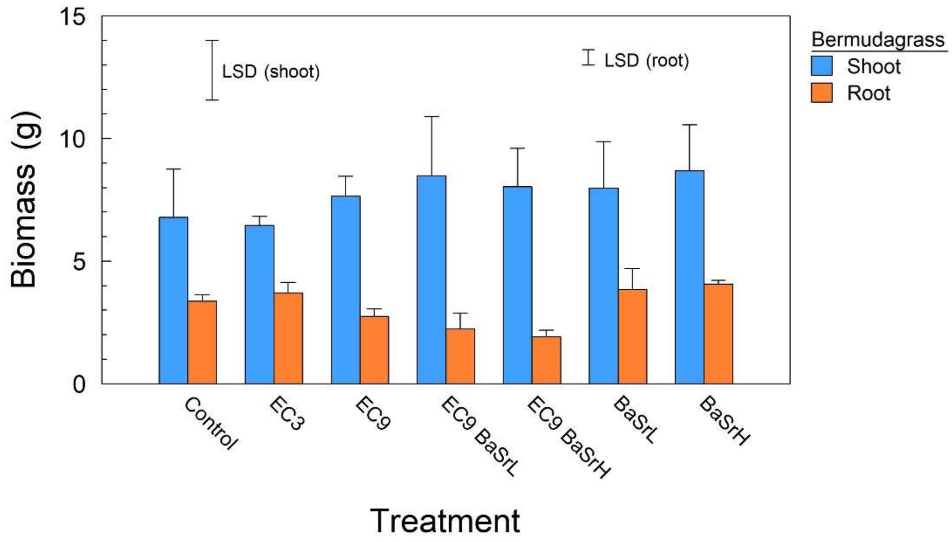

Figure 3.7. Dry shoot and root mass of bermudagrass as influenced by oilfield brine. Error bars represent one standard deviation

Table 3.4. Mean dry shoot mass, root mass and root to shoot ratio in bermudagrass as affected by oilfield brine

Treatment	*Shoot (g)*	*Root (g)*	*Root:shoot ratio*
Control	6.8	3.4 ab	0.52 ab
EC3	6.5	3.7 a	0.58 a
EC9	7.7	2.8 bc	0.36 bc
EC9+BaSr_low	8.5	2.2 cd	0.28 c
EC9+BaSr_high	8.0	1.9 d	0.25 c
BaSr_low	8.0	3.8 a	0.51 ab
BaSr_high	8.7	4.1 a	0.49 ab
One-way ANOVA	$P = 0.475^{ns}$	$P < 0.001$	$P = 0.002$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

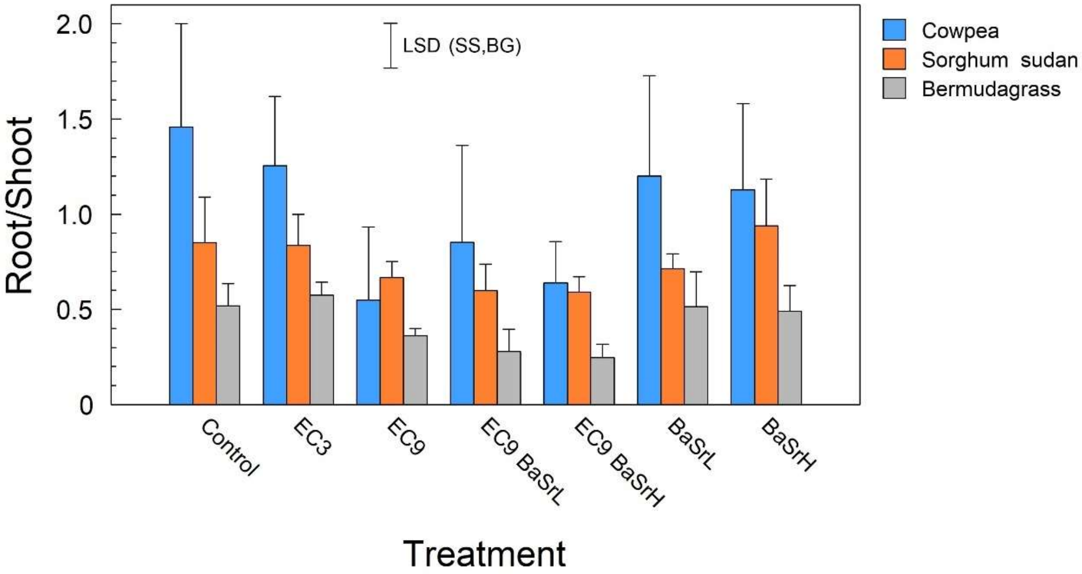

Figure 3.8. Dry root to shoot mass ratio of cowpea, sorghum sudangrass and bermudagrass as influenced by oilfield brine. Error bars represent one standard deviation

3.1.3 Effect of brine, Ba and Sr on cation accumulation in plants

3.1.3.1 Cations in cowpea shoot

The concentrations of Ca^{2+} in shoot of cowpea did not statistically vary in response to brine and BaSr treatments ($P = 0.594$). The concentration of Ca^{2+} in cowpea shoot was highest under BaSr_high (15,360 mg kg^{-1}) and lowest in EC9+BaSr_low (13,580 mg kg^{-1}), but were not statistically different from the untreated control (Table 3.5).

The concentration of Mg^{2+} in shoot of cowpea decreased with increasing brine ($P < 0.001$), but increased slightly under BaSr_low. The concentration of Mg^{2+} in cowpea was highest under BaSr_low (3406 mg kg^{-1}) and lowest in EC9+BaSr_high (2335 mg kg^{-1}) (Table 3.5).

Table 3.5. Accumulation of cations in shoot of cowpea as affected by oilfield brine

Treatment	**Cation concentration in shoot (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	14650	3386^{a}	9867^{d}	396.7^{c}	46.16^{c}	45.01^{c}	0.040^{c}
EC 3	13790	2382^{c}	28210^{c}	995.1^{c}	54.04^{c}	43.36^{c}	0.035^{c}
EC9	14330	2345^{c}	34870^{b}	8468^{b}	98.10^{b}	46.65^{c}	0.24^{b}
EC9+BaSr_low	13590	2839^{bc}	43850^{a}	30330^{a}	153.6^{a}	58.95^{b}	0.57^{a}
EC9+BaSr_high	15310	2335^{c}	33830^{b}	7336^{b}	104.9^{b}	80.09^{a}	0.22^{b}
BaSr_low	15060	3406^{a}	1051^{d}	605.0^{c}	59.97^{c}	51.78^{bc}	0.060^{c}
BaSr_high	15360	3280^{ab}	10030^{d}	436.9^{c}	51.41^{c}	59.11^{b}	0.043^{c}
One-way ANOVA	$P = 0.594^{ns}$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

Potassium concentrations in the cowpea shoots varied significantly with treatment ($P < 0.001$). The concentration of K^{+} in shoot of cowpea increased with increasing brine. The concentration of K^{+} in cowpea shoot was highest under EC9+BaSr_low (43,850 mg kg^{-1}) and lowest in control (9866 mg kg^{-1}), and brine treatments were significantly higher than in control (Table 3.5).

Sodium concentration in cowpea shoot varied significantly ($P < 0.001$) among treatments. The concentration of Na^{+} in shoot of cowpea increased with increasing brine, while slightly decreased under BaSr without brine. The concentration of Na^{+} in cowpea shoot was highest under EC9+BaSr_low (30,330 mg kg^{-1}) and lowest in control (397 mg kg^{-1}) (Table 3.5).

The Na:K ratio in cowpea shoot differed significantly among treatments ($P < 0.001$). The Na^{+} to K^{+} ratio in shoot of cowpea increased with increasing brine, and under BaSr without brine, but slightly decreased at EC3. The Na:K ratio in cowpea shoot was significantly lower under brine treatments at EC9 than in control. The concentration of Na^+ in cowpea shoot was highest under EC9+BaSr_low (0.5712) and lowest under EC3 (0.03487) (Table 3.5).

The concentration of Ba^{2+} in shoot of cowpea increased with increasing brine, and BaSr ($P <$ 0.001), but was not significantly different form control in EC3 and BaSr (without brine) treatments. Ba^{2+} concentration in cowpea shoot was highest under EC9+BaSr_low (153.6 mg kg^{-1}) and lowest in (46.16 mg kg^{-1}) (Table 3.5).

The concentration of Sr^{2+} in shoot of cowpea varied significantly among treatments ($P < 0.001$) but was only EC9+BaSr_low, EC9+BaSr_high, and BaSr_high were significantly different from control (Table 3.5). The concentration of Sr^{2+} in cowpea shoot was highest under EC9+BaSr_high (80.09 mg kg^{-1}), and lowest in EC3 (43.36 mg kg^{-1}) (Table 3.5).

3.1.3.2 Cations in cowpea root

The Ca^{2+} concentration in cowpea root was significantly affected by treatments ($P = 0.022$), but the control was significantly different only from brine EC9+ BaSr. The concentration of Ca^{2+} in cowpea root was highest under control (7226 mg kg^{-1}) and lowest in EC9+BaSr_high (4481 mg kg^{-1}) (Table 3.6).

One-way ANOVA found nonsignificant differences in the Mg^{2+} concentration of cowpea root among treatments ($P = 0.721$). The Mg^{2+} concentration in cowpea root was highest under EC9+BaSr_low (1049 mg kg^{-1}) and lowest in BaSr_high (638.3 mg kg^{-1}), but none of the treatments were significantly different from the control (Table 3.6).

Table 3.6. Accumulation of cations in root of cowpea as affected by oilfield brine

Treatment	**Cation concentration in shoot (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	7226 ^{a}	1574	4046 ^{cd}	3876 ^{b}	130.4	31.79	0.96
EC 3	6243 ^{ab}	1558	7446 ^{c}	6237 ^{b}	139.2	30.76	0.83
EC9	5962 ^{abc}	1643	14060^{ab}	14540 ^{a}	334.9	29.82	1.06
EC9+BaSr_low	4721 ^{bc}	1678	12670 ^{b}	16850 ^{a}	247.4	27.24	1.40
EC9+BaSr_high	4481 ^{c}	1461	16590 ^{a}	14530 ^{a}	200.8	38.07	0.85
BaSr_low	6001 ^{abc}	1443	2376 ^{d}	2772 ^{b}	119.3	29.54	1.14
BaSr_high	6439 ^{ab}	1414	2302 ^{d}	2313 ^{b}	111.7	31.90	1.02
One-way ANOVA	$P = 0.022$	$P = 0.721^{ns}$	$P < 0.001$	$P < 0.001$	$P = 0.18^{ns}$	$P = 0.083^{ns}$	$P = 0.129^{ns}$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

The K^{+} concentration in cowpea root varied significantly ($P < 0.001$) among treatments. Treatments under brine EC9 (with and without BaSr) were significantly higher than control in cowpea root. The concentration of K^{+} in cowpea root was highest under EC9+BaSr_high (16,590 mg kg^{-1}) and lowest in BaSr_high (2302 mg kg^{-1}) (Table 3.6).

The Na^{+} concentration in cowpea root differed significantly among treatments ($P < 0.001$). The Na^{+} concentration in cowpea root was significantly higher for all brine treatments EC9 (with and without BaSr) than in control. The concentration of Na^{+} in cowpea root was highest under EC9+BaSr_low (16,850 mg kg^{-1}) and lowest in BaSr_high (2313 mg kg^{-1}) (Table 3.6).

The ANOVA of Na:K ratio in cowpea root showed nonsignificant differences among treatments ($P = 0.129$). The concentration of Na^{+} in cowpea root was highest under EC9+BaSr_low (1.401) and lowest under EC3 (0.826) and all the treatments were not significantly different from control (Table 3.6).

The concentration of Ba^{2+} in root of cowpea was not significantly different among treatments ($P = 0.175$) and all treatments were not significantly different from control. The concentration of Ba^{2+} in cowpea root was highest under EC9 (334.9 mg kg^{-1}) and lowest in BaSr_high (111.7 mg kg^{-1}) (Table 3.6).

Strontium concentration in cowpea root was not significantly different ($P = 0.083$) among treatments, and all treatments were not significantly different from control. The concentration of Sr^{2+} in cowpea root ranged from 38.07 mg kg^{-1} in EC9+BaSr_high to 27.25 mg kg^{-1} in EC9+BaSr_low (Table 3.6).

3.1.3.3 Cations in sorghum sudangrass shoot

The Ca^{2+} concentration in sorghum sudangrass shoot varied significantly ($P = 0.005$) among treatments, but only brine treatments of EC9 (with and without BaSr) were significantly lower than in control. The concentration of Ca^{2+} in sorghum sudangrass shoot was highest under BaSr_high (7919 mg kg^{-1}) and lowest in EC9+BaSr_low (5237 mg kg^{-1}) (Table 3.7).

The concentration of Mg^{2+} in shoot of sorghum sudangrass varied significantly ($P < 0.001$) among treatments, and the Mg^{2+} concentration in all brine treatments was significantly lower than control. The concentration of Mg^{2+} in sorghum sudangrass shoot was highest under BaSr_high (5978 mg kg^{-1}) and lowest in EC9+BaSr_low (1760 mg kg^{-1}) (Table 3.7).

The concentration of K^{+} in sorghum sudangrass shoot varied significantly ($P < 0.001$) among treatments. The K^{+} concentration in brine treatments was significantly higher than in control.

The concentration of K^+ in sorghum sudangrass shoot was highest under EC9 (18,250 mg kg^{-1}) and lowest in BaSr_high (5635 mg kg^{-1}) (Table 3.7).

The concentration of Na^+ in sorghum sudangrass shoot varied significantly ($P = 0.002$) among treatments, but only brine EC9 (with and without BaSr) treatments were significantly higher than control. The concentration of Na^+ in sorghum sudangrass shoot was highest under EC9 (2025 mg kg^{-1}), and lowest in BaSr_low (440.0 mg kg^{-1}) (Table 3.7).

Table 3.7. Accumulation of cations in shoot of sorghum sudangrass as affected by oilfield brine

Treatment	**Cation concentration in shoot (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^+**	**Na^+**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	7727^{ab}	5637^{a}	6409^{c}	526.0^{c}	67.50^{ab}	24.75^{b}	0.080
EC 3	6433^{b}	3236^{b}	10070^{b}	990.3^{bc}	56.50^{abc}	19.50^{c}	0.10
EC9	5770^{c}	1905^{c}	18250^{a}	2025^{a}	60.50^{abc}	17.00^{c}	0.11
EC9+BaSr_low	5237^{c}	1856^{c}	17580^{a}	1865^{ab}	47.00^{c}	17.00^{c}	0.11
EC9+BaSr_high	5828^{c}	1760^{c}	17620^{a}	1548^{ab}	55.00^{bc}	28.75^{b}	0.088
BaSr_low	66720^{abc}	5020^{a}	6669^{c}	440.0^{c}	70.25^{a}	25.75^{b}	0.066
BaSr_high	7919^{a}	5978^{a}	5635^{c}	478.8^{c}	64.75^{ab}	40.00^{a}	0.085
One-way ANOVA	$P = 0.005$	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.045$	$P < 0.001$	$P = 0.64^{ns}$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

The Na^+ to K^+ ratio in shoot of sorghum sudangrass was not significantly different ($P = 0.638$) among treatments (Table 3.7).

The concentration of Ba^{2+} in shoot of sorghum sudangrass varied significantly ($P = 0.045$) among treatments, but only brine EC9+BaSr_low was significantly different from control. The concentration of Ba^{2+} in sorghum sudangrass shoot was highest under BaSr_low (70.25 mg kg^{-1}) and lowest in EC9+BaSr_low (47.00 mg kg^{-1}) (Table 3.7).

The concentration of Sr^{2+} in shoot of sorghum sudangrass varied significantly ($P < 0.001$) among treatments. BaSr_high and brine treatments, except EC9+BaSr_high, were significantly different from control. The Sr^{2+} concentration in sorghum sudangrass shoot was highest under BaSr_high (40.00 mg kg^{-1}), and lowest under EC9+BaSr_low and EC9 (17.00 mg kg^{-1}) (Table 3.7).

3.1.3.4 Cations in sorghum sudangrass root

The concentration of Ca^{2+} in root of sorghum sudangrass was not significantly different ($P = 0.287$) among and between treatments. The concentration of Ca^{2+} in sorghum sudangrass root was highest under EC9+BaSr_high (5508 mg kg^{-1}) and lowest in BaSr_low (3449 mg kg^{-1}) (Table 3.8).

The concentration of Mg^{2+} in root of sorghum sudangrass varied significantly ($P < 0.001$) among treatments, which were all significantly different from control. The concentration of Mg^{2+} in sorghum sudangrass root was highest under EC3 (1033 mg kg^{-1}) and lowest in BaSr_high (622 mg kg^{-1}) (Table 3.8).

The concentration of K^{+} in the root of sorghum sudangrass varied significantly ($P < 0.001$) among treatments. All treatments with EC9 (with and without BaSr) were significantly higher than control. The concentration of K^{+} in sorghum sudangrass root was highest under EC9+BaSr_high (6639 mg kg^{-1}) and lowest in BaSr_high (1408 mg kg^{-1}) (Table 3.8).

Table 3.8. Accumulation of cations in root of sorghum sudangrass as affected by oilfield brine

Treatment	**Cation concentration in root (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	4455	737.2^{c}	1777^{bc}	2593^{b}	90.00	19.00^{b}	1.46^{a}
EC 3	5386	1033^{a}	2577^{b}	3038^{b}	104.0	21.75^{b}	1.21^{ab}
EC9	4167	930.2^{ab}	5911^{a}	5810^{a}	82.25	16.00^{b}	0.96^{bc}
EC9+BaSr_low	5413	954.6^{ab}	6081^{a}	5525^{a}	96.25	23.00^{b}	0.91^{bc}
EC9+BaSr_high	5508	995.2^{a}	6639^{a}	5727^{a}	106.5	34.25^{a}	0.87^{c}
BaSr_low	3449	850.7^{b}	1856^{bc}	2656^{b}	101.0	20.25^{b}	1.44^{a}
BaSr_high	5338	621.6^{d}	1408^{c}	1913^{b}	105.8	35.50^{a}	1.37^{a}
One-way ANOVA	$P = 0.287^{ns}$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.41^{ns}$	$P < 0.001$	$P < 0.001$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

The concentration of Na^{+} in root of sorghum sudangrass varied significantly ($P < 0.001$) among treatments. Only treatments with EC9 (with and without BaSr) were significantly different from control. The concentration of Na^{+} in sorghum sudangrass root was highest under EC9 (5810 mg kg^{-1}), and lowest under BaSr_high (1913 mg kg^{-1}) (Table 3.8).

The Na^{+} to K^{+} ratio in root of sorghum sudangrass varied significantly across treatments ($P < 0.001$), but only EC9 treatments (with and without brine) were significantly different from the control. The Na^{+} to K^{+} ratio in sorghum sudangrass shoot was highest under control (1.463) and lowest under EC9+BaSr_high (0.866) (Table 3.8).

The concentration of Ba^{2+} in root of sorghum sudangrass was not significantly different among treatments ($P = 0.407$). The concentration of Ba^{2+} in sorghum sudangrass root ranged from 82.2 mg kg^{-1} (in EC9) to 106.5 mg kg^{-1} (in EC9+BaSr_high) (Table 3.8).

The concentration of Sr^{2+} in root of sorghum sudangrass varied significantly ($P < 0.001$) among treatments, but only BaSr_high and EC9+BaSr_high were significantly different from control. The concentration of Sr^{2+} in sorghum sudangrass root was highest under BaSr_high (35.50 mg kg^{-1}) and lowest in EC9 (16.00 mg kg^{-1}) (Table 3.8).

3.1.3.5 Cations in bermudagrass shoot

The concentration of Ca^{2+} in shoot of bermudagrass was not significantly different ($P = 0.139$) among and between treatments. The concentration of Ca^{2+} in bermudagrass shoot was highest under EC3 (42590 mg kg^{-1}) and lowest in EC9+BaSr_high (2961 mg kg^{-1}) (Table 3.9).

Table 3.9. Accumulation of cations in shoot of bermudagrass as affected by oilfield brine

Treatment	**Cation concentration in shoot (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^+**	**Na^+**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	4152	1853^{a}	7757^{b}	1498	126.9^{a}	10.23^{b}	0.19
EC 3	4251	1183^{b}	9334^{b}	1222	103.6^{ab}	8.076^{b}	0.13
EC9	3476	910.1^{b}	12590^{a}	2016	66.05^{bc}	6.637^{b}	0.16
EC9+BaSr_low	3104	878.0^{b}	14340^{a}	2003	61.11^{c}	6.962^{b}	0.14
EC9+BaSr_high	2961	869.3^{b}	14050^{a}	1552	60.22^{c}	10.77^{b}	0.12
BaSr_low	4140	1857^{a}	7679^{b}	1470	139.8^{a}	11.38^{b}	0.19
BaSr_high	4238	1903^{a}	7793^{b}	1618	132.2^{a}	16.18^{a}	0.21
One-way ANOVA	$P = 0.139^{ns}$	$P < 0.001$	$P < 0.001$	$P = 0.47^{ns}$	$P < 0.001$	$P = 0.002$	$P = 0.37^{ns}$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

The concentration of Mg^{2+} in shoot of bermudagrass varied significantly ($P < 0.001$) among treatments, which was significantly lower under brine treatments than in control. The concentration of Mg^{2+} in bermudagrass shoot was highest under BaSr_high (1903 mg kg^{-1}) and lowest in EC9+BaSr_high (869.3 mg kg^{-1}) (Table 3.9).

The concentration of K^+ in shoot of bermudagrass varied significantly ($P < 0.001$) among treatments, which were significantly higher under brine treatments than in control. The concentration of K^+ in bermudagrass shoot was highest under EC9+BaSr_low (14340 mg kg^{-1}) and lowest in BaSr_low (7679 mg kg^{-1}) (Table 3.9).

The concentration of Na^+ in shoot of bermudagrass increased was not significantly different (P = 0.477) among and between treatments. The concentration of Na^+ in bermudagrass shoot ranged from 2016 to 1222 mg kg^{-1} (Table 3.9).

Similarly, Na^+ to K^+ ratio in bermudagrass shoot was not significantly different ($P = 0.369$) among and between treatments. The concentration of Na^+ in bermudagrass shoot was highest under BaSr_high (0.2099) and lowest under EC9+BaSr_high (0.1169) (Table 3.9).

The concentration of Ba^{2+} in shoot of bermudagrass was significantly different ($P < 0.001$) among treatments, but only brine EC9 (with and without BaSr) treatments were significantly different (lower than) from control. The concentration of Ba^{2+} in bermudagrass shoot was highest under BaSr_low (139.8 mg kg^{-1}) and lowest in EC9+BaSr_high (60.22 mg kg^{-1}) (Table 3.9).

The concentration of Sr^{2+} in shoot of bermudagrass varied significantly ($P = 0.002$) among treatments, but only BaSr_high (without brine) treatments were significantly different from control. The concentration of Sr^{2+} in bermudagrass shoot was highest under BaSr_high (16.18 mg kg^{-1}) and lowest in EC9 (6.637 mg kg^{-1}) (Table 3.9).

3.1.3.6 Cations in bermudagrass root

The concentration of Ca^{2+} in root of bermudagrass was not significantly different ($P = 0.16$) among treatments. The concentration of Ca^{2+} in bermudagrass root ranged from 6186 to 2468 mg kg^{-1}, and all treatments were not significantly different from the control (Table 3.10).

The concentration of Mg^{2+} in root of bermudagrass did not vary significantly ($P = 0.077$) among treatments. The concentration of Mg^{2+} in bermudagrass root was highest under BaSr_low (1012 mg kg^{-1}) and lowest in EC3 (806.1 mg kg^{-1}) (Table 3.10).

Table 3.10. Accumulation of cations in root of bermudagrass as affected by oilfield brine

Treatment	**Cation concentration in root (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**Na:K ratio**
Control	4047	918.1	7799^{b}	1305^{c}	88.83^{bc}	15.44^{cd}	0.17
EC 3	2468	806.1	9247^{b}	1443^{c}	67.58^{c}	9.17^{d}	0.16
EC9	4658	936.1	12750^{a}	3303^{a}	105.75^{ab}	15.75^{cd}	0.26
EC9+BaSr_low	6186	902.8	13820^{a}	2601^{b}	126.0^{a}	23.03^{abc}	0.191
EC9+BaSr_high	4545	991.2	13910^{a}	2996^{ab}	122.2^{a}	29.07^{a}	0.22
Ba, Sr_low	4374	1012	7637^{b}	1374^{c}	101.7^{ab}	18.97^{bcd}	0.18
Ba, Sr_high	5034	899.2	7726^{b}	1403^{c}	98.53^{ab}	28.05^{ab}	0.185
One-way ANOVA	$P = 0.16^{ns}$	$P = 0.08^{ns}$	$P < 0.001$	$P < 0.001$	$P = 0027$	$P = 0.002$	$P = 0.06^{ns}$

Means followed by the same letter do not differ significantly. ns: not significant, $P > 0.050$

The concentration of Mg^{2+} in root of bermudagrass did not vary significantly ($P = 0.08$) among treatments. The concentration of Mg^{2+} in bermudagrass root was highest under BaSr_low (1012 mg kg^{-1}) and lowest in EC3 (806.1 mg kg^{-1}) (Table 3.10).

The concentration of K^+ in root of bermudagrass varied significantly ($P < 0.001$) among treatments; however, only brine EC9 (with and without BaSr) treatments were significantly different (higher than) from control. The concentration of K^+ in bermudagrass root was highest under EC9+BaSr_high (13910 mg kg^{-1}) and lowest in BaSr_low (7637 mg kg^{-1}) (Table 3.10).

The concentration of Na^+ in root of bermudagrass was significantly different ($P < 0.001$) among treatments, which was significantly different from control only under brine EC9 (with and without BaSr) treatments. The Na^+ concentration in bermudagrass root was highest under EC9 (3302 mg kg^{-1}) and lowest in control (1305 mg kg^{-1}) (Table 3.10).

The Na^+ to K^+ ratio in root of bermudagrass was not significantly different ($P = 0.06$) among treatments; however, treatment EC9 was significantly different from control. The concentration of Na^+ in bermudagrass root was highest under EC9 (0.26) and lowest under EC3 (0.16) (Table 3.10).

The concentration of Ba^{2+} in root of bermudagrass was significantly different ($P = 0.027$) among treatments, but only EC9+BaSr_low and EC9+BaSr_high were significantly different from control. The concentration of Ba^{2+} in bermudagrass root was highest under EC9+BaSr_low (126.0 mg kg^{-1}) and lowest under EC3 (67.58 mg kg^{-1}) (Table 3.10).

The concentration of Sr^{2+} in root of bermudagrass was significantly different ($P = 0.002$) differences among treatments, but only EC9+BaSr_high and BaSr_high were significantly different from control. The concentration of Sr^{2+} in bermudagrass root was highest under EC9+BaSr_high (29.07 mg kg^{-1}) and lowest in EC3 (9.17 mg kg^{-1}) (Table 3.10).

3.2 The effect of oilfield brine, barium and strontium on soil properties

3.2.1 Effect on soil pH and electrolytic conductivity (EC_e)

3.2.1.1 pH and EC_e of soil grown with cowpea

Soil pH of cowpea soil varied significantly with treatment ($P < 0.001$), soil depth ($P = 0.002$), and the interaction between treatment and soil depth ($P = 0.048$). Soil pH for cowpea decreased for all EC9 treatments (with and without BaSr) and BaSr_low, but increased under EC3 and BaSr_high in the upper soil depth. In the upper soil depth (0-4 cm), pH was highest under EC3 (6.0) and lowest under EC9 (5.5) and did not differ significantly from the control (Figure 3.9). In the lower soil depth (> 4 cm), soil pH decreased for all brine and BaSr treatments. Soil pH in the lower soil depth was highest under control (5.8) and lowest under EC9+BaSr_low (5.5). Although significant differences exist between some treatments, none of the treatments at either depth resulted in significant differences relative to the pH of the untreated control.

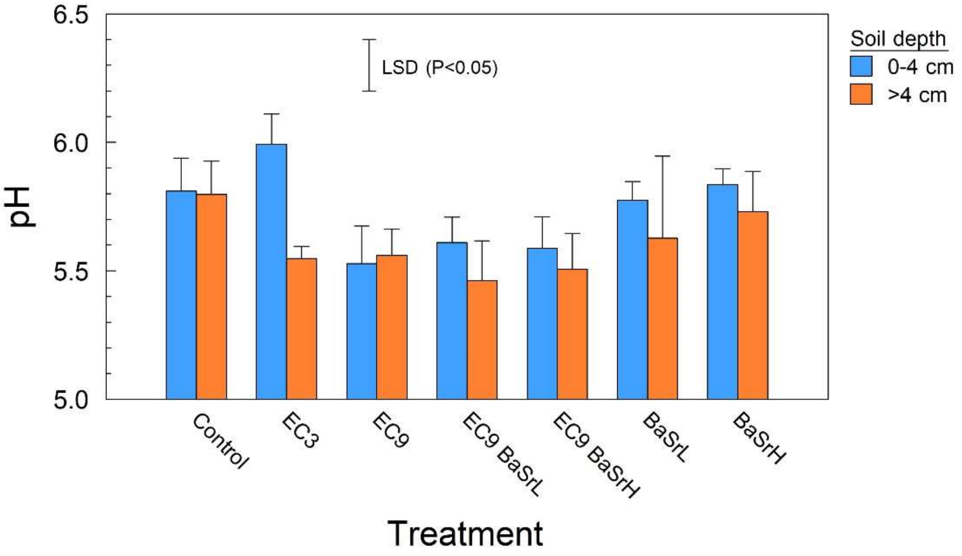

Figure 3.9. Variation of soil pH in response to brine, barium and strontium in cowpea soil. Error bars represent one standard deviation

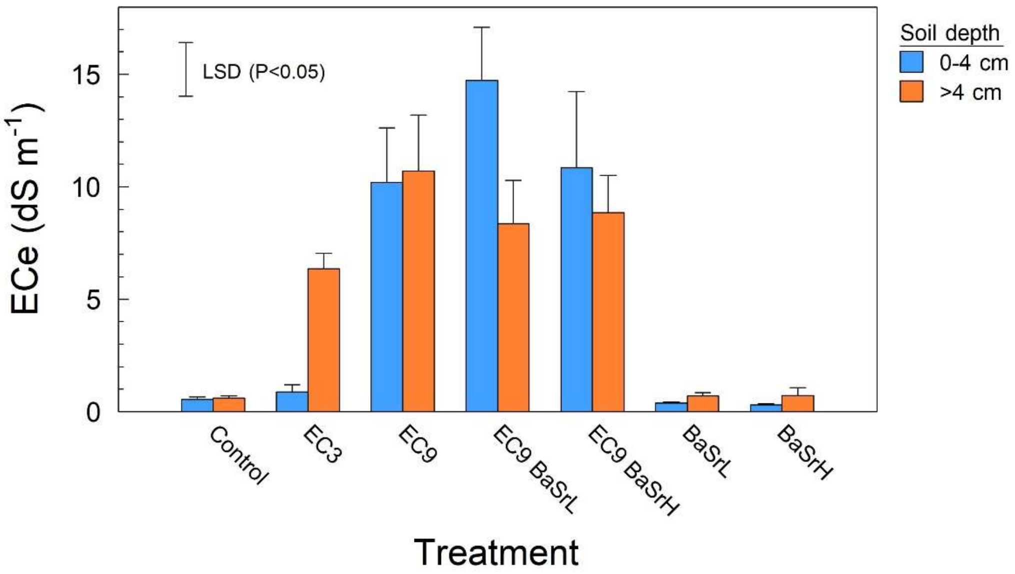

Figure 3.10. Variation of ECe in response to brine, barium and strontium in cowpea soil. Error bars represent one standard deviation

The electrolytic conductivity (EC_e) of the saturated paste extracts for cowpea soils differed by treatment ($P < 0.001$) and significant interactions between treatment and soil depth ($P < 0.001$). The effect of depth was not significant ($P = 0.602$). EC_e for cowpea soil increased with increasing brine and for BaSr without brine. In the upper soil depth, EC_e was highest under EC9+BaSr_low (14.7 dS m^{-1}) and lowest and lowest for all treatments no receiving brine (Figure 3.10). Interestingly, the salinity in the upper depth of the EC3 treatment was not significantly different from the control.

3.2.1.2 pH and EC_e of soil grown with sorghum sudangrass

Soil pH of sorghum sudangrass soil varied significantly with treatment ($P < 0.001$) and soil depth ($P < 0.001$). The interaction between treatment and soil depth was not significant ($P = 0.204$). The pH for sorghum sudangrass in the upper soil depth was not significantly impacted by treatment, though all brine treatments decreased the pH somewhat. In the upper soil depth, pH was highest under control (6.2) and lowest under EC3 (5.9) (Figure 3.11) Similarly, none of the treatments in the lower depth resulted in pH values that were significantly different from the control. Soil pH in the lower soil depth for sorghum sudangrass was highest under BaSr_high (6.2) and lowest under EC9 (5.7). Soil pH for sorghum sudangrass in the upper depth was generally higher than in the lower soil depth.

The EC_e of the soil saturated paste extract for sorghum sudangrass differed significantly due to treatments ($P < 0.001$), and the depth x treatment interaction was significant ($P < 0.013$). The main effect of depth was not significant ($P = 0.646$). Relative to the control, salinity in the non-brine treated soils did not change but was significantly higher in all brine treatments with the lone exception of the EC3 for the lower depth (Figure 3.12).

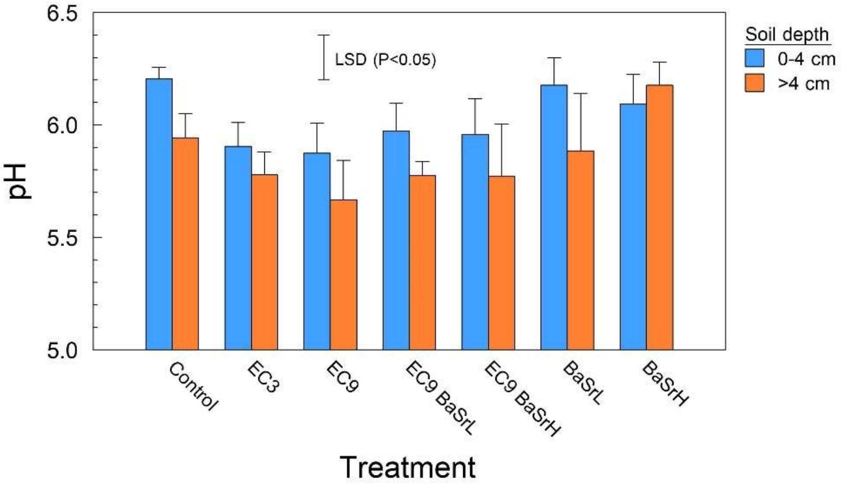

Figure 2.11. Variation of soil pH in response to brine, barium and strontium in sorghum sudangrass soil. Error bars represent one standard deviation

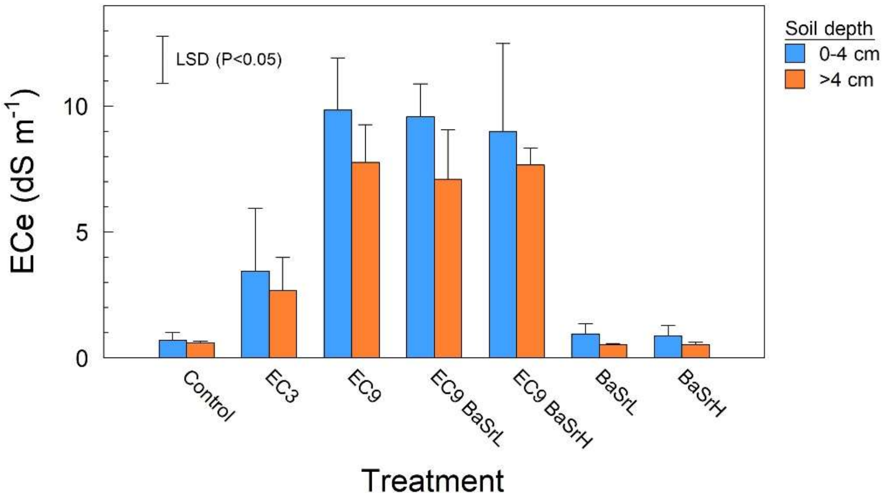

Figure 3.12. Variation of ECe in response to brine, barium and strontium in sorghum sudangrass soil. Error bars represent one standard deviation

3.2.1.3 pH and EC_e of soil grown with bermudagrass

The pH of soil from bermudagrass varied significantly with treatments ($P = 0.003$), and soil depth ($P < 0.001$), but was nonsignificant due to the interaction between treatment and soil depth ($P = 0.851$). In the upper depth, soil pH for all treatments were statistically equal, although some small differences exist (Figure 3.13). Likewise for the lower depth, the treatments did not have a significant impact on soil pH. Soil pH for bermudagrass in the upper depth was generally higher than in the lower soil depth, but within any given treatment, the differences were not significant.

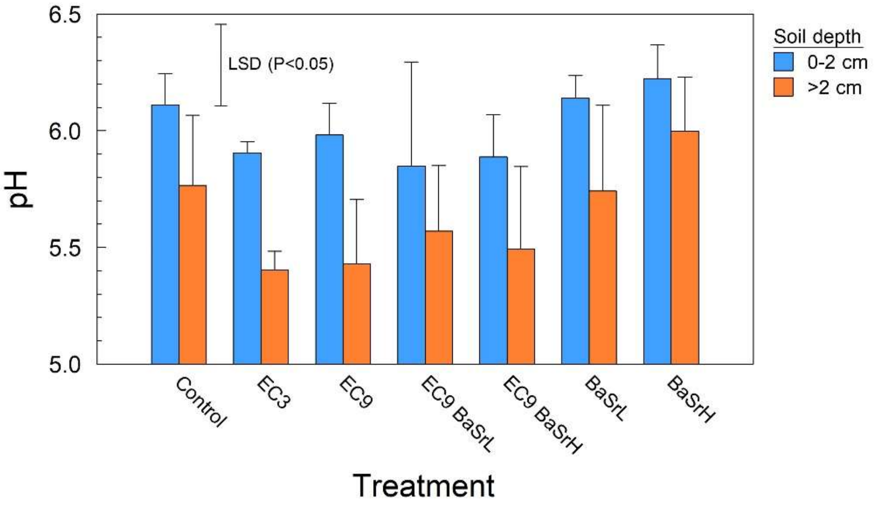

Figure 3.13. Variation of pH in response to brine, barium and strontium in bermudagrass soil. Error bars represent one standard deviation

The EC_e of soil grown with bermudagrass varied significantly with treatment ($P < 0.001$) and soil depth ($P < 0.001$), and the interaction between treatment and soil depth was significant ($P < 0.003$). The EC_e for bermudagrass soils increased significantly under all brine treatments (with and without BaSr) in both soil depths except EC3 in the lower depth. Also, the EC_e for bermudagrass was significantly higher in the upper depth than in the lower depth except under EC9+BaSr_low, where it was not significantly different between the two depths. In the upper soil depth, EC_e was highest under EC9+BaSr_high (15.3 dS m^{-1}) and lowest under BaSr_low (1.1 dS m^{-1}) (Figure 3.15). In the lower depth, EC_e was highest under EC9+BaSr_low (9.2 dS m^{-1}) and lowest under BaSr_high (0.7 dS m^{-1}).

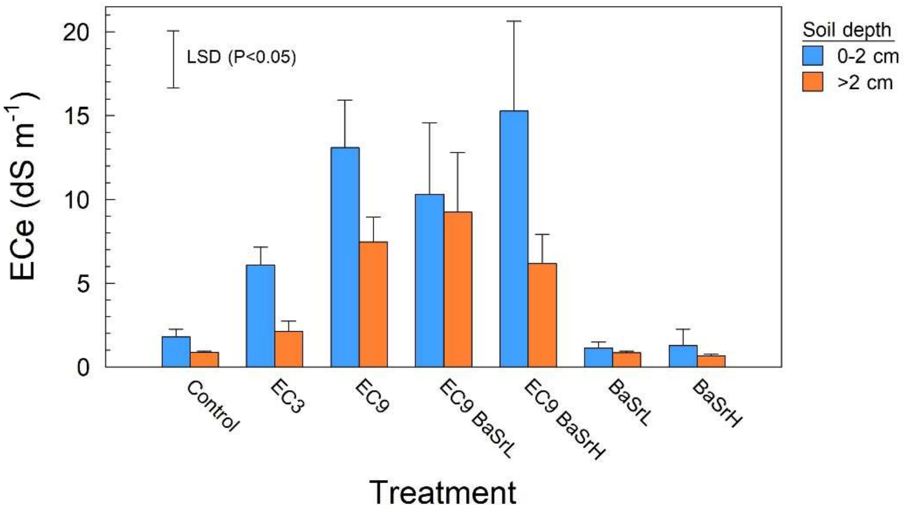

Figure 3.14. Variation of ECe in response to brine, barium and strontium in bermudagrass soil. Any bars across both depths associated with the same letter are not significantly different

3.2.2 Effect of brine, barium and strontium on soluble cations in soil grown with cowpea, sorghum sudangrass and bermudagrass

The saturated paste extract is a procedure developed to yield water-soluble cations that have equilibrated with the soil cation exchange sites. Soils are brought to saturation with clean water and equilibrated for at least 18 hours, then saturated extracts are removed from the soil using vacuum filtration, and the solutions analyzed for Ca, Mg, K, and Na. The saturation step is lengthy, but necessary, because the amount of water added to each soil is directly related to the texture. With procedures using a fixed soil mass-to-water ratio, the amount of dilution is texture dependent: sandy soils will absorb less water than heavier textured soils and will yield a more diluted extract. The saturated approach was developed and tested in the 1950s (U.S. Salinity Laboratory Staff, 1954) and is accepted as the standard means of assessing the salinity of soils.

3.2.2.1 Soluble Ca^{2+}, Mg^{2+}, K^+, and Na^+

The data for the concentrations of the major cations were analyzed by two-way analysis of variance, with depth and treatment as the main effects. Data for each species was analyzed separately. The effects of soil sampling depth and treatment all were significant ($P<0.05$) as were the depth x treatment interactions (with the lone exceptions of Ba^{2+} and Sr^{2+} in sorghum sudangrass). The implications of these results are given perspective by examining trends in SAR. The brine additions consistently and significantly increased all cation concentrations in all EC9 treatments for both soil sampling depths and across all three plant species (Tables 3.11-3.13). Typical increases in Ca^{2+} concentrations were from 1.0-2.5 $mmol_c\ L^{-1}$ in the control to 13 to 29 $mmol_c\ L^{-1}$ treatments. Similarly, concentrations of Mg^{2+}, K^+, and Na^+ increased by 10-fold or more for EC9 treatments versus the controls. The response to the EC3 treatment was much different. The cation concentrations tended to not respond significantly to the EC3 treatment in the 0-4 cm samples, but there was a response for K^+ for cowpea and sorghum sudangrass.

Table 3.11. Soluble cations in cowpea soil as affected by oilfield brine

Treatment	**Mean soluble cation concentration in soil (mmol$_c$ L^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**SAR**
	0 - 4 cm						
Control	2.155 ^{d}	0.4789 ^{c}	0.2405 ^{d}	2.771 ^{e}	0.007618 ^{c}	0.003195 ^{d}	2.78 ^{f}
EC3	1.336 ^{d}	0.1943 ^{c}	0.8892 ^{d}	6.314 ^{e}	0.009810 ^{c}	0.001835 ^{d}	7.25 ^{e}
EC9	20.21 ^{bc}	6.152 ^{ab}	15.63 ^{b}	57.41 ^{bc}	0.03981 ^{b}	0.02813 ^{c}	16.0 ^{bc}
EC9+BaSr_low	25.40 ^{ab}	8.787 ^{ab}	26.90 ^{a}	87.72 ^{a}	0.05656 ^{a}	0.04637 ^{b}	21.2 ^{a}
EC9+BaSr_high	19.54 ^{bc}	6.227 ^{ab}	16.82 ^{b}	59.63 ^{b}	0.04780 ^{ab}	0.06238 ^{a}	16.6 ^{b}
BaSr_low	1.040 ^{d}	2.219 ^{c}	0.2985 ^{d}	2.903 ^{e}	0.004724 ^{c}	0.002085 ^{d}	3.15 ^{f}
BaSr_high	0.6984 ^{d}	0.1194 ^{c}	0.2992 ^{d}	2.242 ^{e}	0.003478 ^{c}	0.003813 ^{d}	3.52 ^{f}
	> 4 cm						
Control	3.139 ^{d}	0.6990 ^{c}	0.09110 ^{d}	3.193 ^{e}	0.01029 ^{c}	0.004529 ^{d}	2.35 ^{f}
EC3	25.49 ^{ab}	8.087 ^{ab}	0.7585 ^{d}	27.29 ^{d}	0.04826 ^{ab}	0.03880 ^{bc}	6.69 ^{e}
EC9	28.76 ^{a}	9.230 ^{a}	13.45 ^{bc}	56.69 ^{bc}	0.04937 ^{ab}	0.04059 ^{bc}	13.4 ^{cd}
EC9+BaSr_low	16.39 ^{c}	5.840 ^{b}	11.72 ^{bc}	45.49 ^{bc}	0.04712 ^{ab}	0.02811 ^{c}	13.6 ^{cd}
EC9+BaSr_high	23.67 ^{abc}	7.364 ^{ab}	9.049 ^{c}	42.46 ^{c}	0.04711 ^{ab}	0.04326 ^{bc}	11.2 ^{d}
BaSr_low	3.018 ^{d}	0.7938 ^{c}	0.1027 ^{d}	3.248 ^{e}	0.006903 ^{c}	0.004702 ^{d}	2.36 ^{f}
BaSr_high	3.634 ^{d}	0.8950 ^{c}	0.1542 ^{d}	2.893 ^{e}	0.007681 ^{c}	0.005474 ^{d}	1.90 ^{f}
Two-way ANOVA							
Treatment	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Soil depth	$P = 0.001$	$P = 0.029$	$P < 0.001$	$P = 0.06$ ^{ns}	$P = 0.001$	$P = 0.369$ ^{ns}	$P < 0.001$
Treatment x Depth	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.002$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

Table 3.12. Soluble cations in soil grown with sorghum sudangrass as affected by oilfield brine

Treatment	**Mean soluble cation concentration in soil (mmol$_c$ L^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**SAR**
Soil depth	**0 - 4 cm**						
Control	1.206 ^{c}	0.2263 ^{b}	0.2023 ^{d}	6.500 ^{d}	0.01410 ^{cd}	0.002555 ^{c}	7.60 ^{e}
EC3	4.740 ^{bc}	1.453 ^{b}	0.5594 ^{d}	16.34 ^{cd}	0.01847 ^{bcd}	0.008660 ^{c}	9.94 ^{cd}
EC9	17.83 ^{a}	4.786 ^{a}	5.336 ^{a}	56.85 ^{a}	0.03950 ^{ab}	0.02801 ^{b}	17.1 ^{ab}
EC9+BaSr_low	17.25 ^{a}	4.445 ^{a}	4.437 ^{b}	56.56 ^{a}	0.04186 ^{a}	0.03304 ^{b}	17.4 ^{ab}
EC9+BaSr_high	16.12 ^{a}	4.101 ^{a}	4.093 ^{bc}	60.86 ^{a}	0.03733 ^{ab}	0.04928 ^{a}	19.6 ^{a}
BaSr_low	1.346 ^{c}	0.3245 ^{b}	0.1931 ^{d}	8.962 ^{d}	0.008469 ^{d}	0.0007715 ^{c}	9.63 ^{cd}
BaSr_high	1.904 ^{c}	0.3662 ^{b}	0.1931 ^{d}	8.147 ^{d}	0.02658 ^{abcd}	0.005694 ^{c}	7.742 ^{cde}
Soil depth	**> 4 cm**						
Control	2.532 ^{c}	0.2704 ^{b}	0.1099 ^{d}	5.908 ^{d}	0.01206 ^{cd}	0.004594 ^{c}	6.16 ^{e}
EC3	8.588 ^{b}	1.776 ^{b}	0.1754 ^{d}	24.52 ^{c}	0.02597 ^{abcd}	0.01152 ^{c}	10.8 ^{c}
EC9	16.95 ^{a}	4.333 ^{a}	3.717 ^{bc}	53.65 ^{ab}	0.03803 ^{ab}	0.02823 ^{b}	17.1 ^{ab}
EC9+BaSr_low	15.47 ^{a}	3.953 ^{a}	3.356 ^{c}	42.77 ^{b}	0.03104 ^{abc}	0.02573 ^{b}	14.1 ^{b}
EC9+BaSr_high	17.45 ^{a}	4.307 ^{a}	3.420 ^{c}	51.64 ^{ab}	0.03219 ^{abc}	0.03409 ^{b}	15.6 ^{b}
BaSr_low	1.059 ^{c}	0.1735 ^{b}	0.05622 ^{d}	5.146 ^{d}	0.007767 ^{d}	0.002709 ^{c}	6.92 ^{de}
BaSr_high	1.533 ^{c}	1.992 ^{b}	0.07995 ^{d}	4.549 ^{d}	0.009825 ^{d}	0.0007800 ^{c}	5.09 ^{e}
Two-way ANOVA							
Treatment	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Soil depth	$P = 0.658^{ns}$	$P = 0.716^{ns}$	$P = 0.407^{ns}$	$P = 0.040$	$P = 0.23^{ns}$	$P = 0.233^{ns}$	$P = 0.002$
Treatment x Depth	$P = 0.82^{ns}$	$P = 0.98^{ns}$	$P = 0.025$	$P = 0.36^{ns}$	$P = 0.64^{ns}$	$P = 0.38^{ns}$	$P = 0.25^{ns}$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

Table 3.13. Soluble cations in soil grown with bermudagrass as affected by oilfield brine

Treatment	**Mean soluble cation concentration in soil (mmol$_c$ L^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**SAR**
Soil depth	**0 - 4 cm**						
Control	2.311 ^{ef}	0.3649 ^{f}	0.9204 ^{de}	14.67 ^{d}	0.008080 ^{c}	0.005813 ^{d}	12.6 ^{e}
EC3	8.186 ^{de}	1.523 ^{e}	1.775 ^{de}	49.86 ^{d}	0.01897 ^{c}	0.01459 ^{cd}	22.7 ^{abc}
EC9	21.84 ^{ab}	3.268 ^{b}	14.36 ^{ab}	87.24 ^{ab}	0.05344 ^{b}	0.03480 ^{b}	24.7 ^{ab}
EC9+BaSr_low	18.72 ^{bc}	2.730 ^{bc}	9.933 ^{bc}	67.17 ^{bc}	0.04044 ^{b}	0.03770 ^{b}	20.4 ^{bcd}
EC9+BaSr_high	27.25 ^{a}	4.229 ^{a}	14.96 ^{a}	102.2 ^{a}	0.07217 ^{a}	0.07942 ^{a}	25.5 ^{a}
BaSr_low	1.709 ^{f}	0.2108 ^{f}	0.3036 ^{e}	8.934 ^{d}	0.005681 ^{c}	0.005656 ^{d}	9.37 ^{ef}
BaSr_high	2.078 ^{ef}	0.2454 ^{f}	0.09287 ^{e}	10.31 ^{d}	0.005833 ^{c}	0.008321 ^{d}	9.02 ^{ef}
Soil depth	**> 4 cm**						
Control	1.510 ^{f}	0.2056 ^{f}	0.1168 ^{e}	5.579 ^{d}	0.009285 ^{c}	0.004860 ^{d}	8.31 ^{ef}
EC3	3.085 ^{ef}	0.5731 ^{f}	0.4556 ^{e}	17.08 ^{d}	0.01189 ^{c}	0.007609 ^{d}	12.5 ^{e}
EC9	13.39 ^{cd}	2.034 ^{cde}	6.800 ^{c}	50.55 ^{c}	0.04101 ^{b}	0.02393 ^{bc}	18.2 ^{cd}
EC9+BaSr_low	17.11 ^{bc}	2.505 ^{bcd}	8.053 ^{c}	61.01 ^{c}	0.04356 ^{b}	0.03332 ^{b}	19.3 ^{cd}
EC9+BaSr_high	10.69 ^{d}	1.681 ^{de}	5.340 ^{cd}	43.96 ^{c}	0.03712 ^{b}	0.03238 ^{b}	17.6 ^{d}
BaSr_low	1.396 ^{f}	0.1898 ^{f}	0.06678 ^{e}	7.130 ^{d}	0.007098 ^{c}	0.004946 ^{d}	8.12 ^{ef}
BaSr_high	1.257 ^{f}	0.1786 ^{f}	0.07715 ^{e}	5.348 ^{d}	0.005071 ^{c}	0.005367 ^{d}	6.30 ^{f}
Two-way ANOVA							
Treatment	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Soil depth	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.027$	$P < 0.001$	$P < 0.001$
Treatment x Depth	$P = 0.002$	$P = 0.002$	$P = 0.013$	$P = 0.003$	$P = 0.024$	$P < 0.001$	$P = 0.035$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

3.2.2.2 Ba^{2+} and Sr^{2+}

Concentrations of Ba^{2+} and Sr^{2+} in the soil from the brine and BaSr additions were consistent but somewhat counterintuitive. At both sampling depths, the only significant increases in Ba^{2+} and Sr^{2+} concentration were observed in the EC9 additions for both BaSr_low and BaSr_high (Tables 3.14-3.16). The concentrations were sometimes, but inconsistently, greater in the BaSr_low than BaSr_low.

3.2.2.3 SAR

The SAR of the control samples ranged from 2.3 to 2.8 for the cowpea pots, 6.2 to 7.6 for the sorghum sudangrass, and 8.3 to 13 for bermudagrass (Tables 3.14-3.16). An important observation is that the SAR of the original soil was analyzed prior to planting, and the SAR was quite low (SAR = 0.4). This marked increase in SAR of the untreated control soils is a reflection of the elevated sodium content of the reverse osmosis (RO) water used for irrigation in the greenhouse. The drinking water from the College Station drinking water treatment system is highly elevated in Na^+ (181-210 mg L^{-1}). Although RO systems are capable of removing approximately 90% of the Na, the RO water from the irrigation system in the greenhouse was analyzed and found to contain 20 mg L^{-1} Na. This is a substantial level of Na^+ contamination and could account for the observed increases in the SAR of the control samples.

3.2.3 Effect of brine, barium and strontium on exchangeable cations in soil

The greenhouse experiment in this study was designed to allow testing of the several brine treatments as a function of two depths after the growth of three plant species. As discussed previously, the species were chosen to represent a range in salinity tolerance, and the two sampling depths are used examine whether leaching from the surface of the soil in a predominant mechanism affecting cation concentrations. An initial attempt was made to combine the plant species and break down the 3-way ANOVA, but large differences between species (e.g., time before harvest and biomass) created untenable complications in the interpretation. Although the concentrations of the cations vary significantly from one species to the next, many of the important trends in concentration are similar. The major cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) and observations for Ba and Sr will be separated because of the differences in application patterns. Observations concerning the calculated exchangeable sodium ratios (ESR) has the potential to provide insight into the effect of Na on soil structure.

3.2.3.1 Exchangeable Ca, Mg, K and Na

Treatment and depth main effects were statistically significant ($P < 0.05$ or less) for nearly all cations (Tables 3.12-4.14). Although the 2-way interactions (treatment x depth) were not always significant, the interactions were of the most interest and will be the center of this discussion. The concentrations of exchangeable cations in response to brine treatments follow similar trends for cowpea and sorghum sudangrass soils. In the soils from the 0-4 cm depth, exchangeable concentrations of Ca, Mg and K generally do not differ significantly from the concentrations in the untreated control soils. The only exceptions are the K^{+} concentrations in various EC9 treatments (with and without Ba, Sr). For example, the mean exchangeable Ca^{2+} concentration in the 0-4 cm control is 37 $mmol_c\ kg^{-1}$, and the concentrations in the EC3 and EC9 treatments range from 28-35 $mmol_c\ kg^{-1}$ despite the additions Ca^{2+} in the brine. In contrast, K^{+} concentration in the control is 1.5 $mmol_c\ kg^{-1}$, compared to 9-12 $mmol_c\ kg^{-1}$ in the EC9 treatments.

Concentrations of exchangeable Na^+ in these soils were statistically equivalent for the control and EC3 treatments (3-4 $mmol_c$ kg^{-1}) but increased in the EC9 treatments (14-44 $mmol_c$ kg^{-1}).

Responses to treatments were more pronounced in the >4 cm soil for cowpea and sorghum sudangrass. Significant increases relative to the controls were observed for all EC3 and EC9 treatments for Na^+ and the EC9 treatments for Ca^{2+}, Mg^{2+}, and K^+. Treatment effects were less pronounced in the bermudagrass soils for both depths (0-2 cm and >2 cm). Very few significant differences were observed between any treatments and controls for Ca^{2+}, Mg^{2+}, and K^+ at either sampling depth. For Na^+, all EC3 and EC9 treatments in bermudagrass soils resulted in significantly higher exchangeable Na^+ concentrations. In the 0-2 cm samples, the control Na^+ was 5 $mmol_c$ kg^{-1}; Na^+ in the treated samples are 13 $mmol_c$ kg^{-1} for EC3 and 18-24 $mmol_c$ kg^{-1} for the EC9 treatments. In the >2 cm samples, the control exchangeable Na^+ is 4 $mmol_c$ kg^{-1} and 8-11 $mmol_c$ kg^{-1} in the EC9 treatments. These observations suggest that leaching of the major cations was actively occurring in these soils.

3.2.3.2 Exchangeable Ba, Sr

The response of Ba^{2+} and Sr^{2+} to treatments was fairly consistent across all plant species. The BaSr_low treatment had no impact these exchangeable ions at either sampling depth. The BaSr_high treatments (with or without EC9) increased both Ba^{2+} and Sr^{2+} at least one depth for the three plant species. However, the exchangeable concentrations of these ions are quite small for all treatments with Ba^{2+} never exceeding 0.3 $mmol_c$ kg^{-1} and Sr^{2+} always less than 0.18 $mmol_c$ kg^{-1}.

Table 3.14. Exchangeable cations in cowpea soil as affected by oilfield brine

Treatment	**Mean exchangeable cation concentration in soil (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**ESR**
Soil depth	**0 - 4 cm**						
Control	37.35	4.790	1.538 ^{e}	2.988 ^{g}	0.2642 ^{bc}	0.06713 ^{def}	0.0735 ^{d}
EC3	35.06	4.369	3.332 ^{d}	4.471 ^{fg}	0.2494 ^{bc}	0.05943 ^{ef}	0.116 ^{d}
EC9	31.02	4.343	9.507 ^{b}	13.87 ^{bc}	0.2331 ^{c}	0.05165 ^{f}	0.387 ^{b}
EC9+BaSr_low	28.37	4.607	12.58 ^{a}	22.62 ^{a}	0.2338 ^{c}	0.05963 ^{ef}	0.687 ^{a}
EC9+BaSr_high	33.06	4.572	9.529 ^{b}	15.69 ^{b}	0.2581 ^{bc}	0.1148 ^{b}	0.431 ^{b}
BaSr_low	32.01	4.607	1.524 ^{e}	2.221 ^{g}	0.2529 ^{bc}	0.06834 ^{def}	0.0633 ^{d}
BaSr_high	26.32	4.190	1.404 ^{e}	1.698 ^{g}	0.3015 ^{a}	0.1821 ^{a}	0.0558 ^{d}
Soil depth	**> 4 cm**						
Control	45.27	5.292	1.078 ^{e}	2.587 ^{g}	0.2776 ^{ab}	0.07827 ^{cdef}	0.0554 ^{d}
EC3	54.33	7.907	1.531 ^{e}	7.146 ^{ef}	0.3015 ^{a}	0.09906 ^{bc}	0.114 ^{d}
EC9	46.27	6.923	8.413 ^{b}	13.67 ^{bc}	0.2758 ^{ab}	0.08332 ^{cde}	0.307 ^{bc}
EC9+BaSr_low	29.27	4.410	6.738 ^{c}	10.06 ^{de}	0.2545 ^{bc}	0.05740 ^{ef}	0.323 ^{bc}
EC9+BaSr_high	42.29	6.203	5.749 ^{c}	11.35 ^{cd}	0.2671 ^{b}	0.09178 ^{bcd}	0.242 ^{c}
BaSr_low	41.44	5.316	0.6688 ^{e}	2.260 ^{g}	0.2738 ^{ab}	0.07624 ^{cdef}	0.0540 ^{d}
BaSr_high	50.49	6.256	0.8082 ^{e}	1.400 ^{g}	0.2810 ^{ab}	0.09216 ^{bcd}	0.0246 ^{d}
Two-way ANOVA							
Treatment	$P = 0.092$ ^{ns}	$P = 0.202$ ^{ns}	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Soil depth	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.473$ ^{ns}	$P < 0.001$
Treatment x Depth	$P = 0.295$ ^{ns}	$P = 0.049$	$P < 0.001$	$P < 0.001$	$P = 0.016$	$P < 0.001$	$P < 0.001$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

Table 3.15. Exchangeable cations in soil grown with sorghum sudangrass as affected by oilfield brine

Treatment	**Mean exchangeable cation concentration in soil (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**ESR**
Soil depth	**0 - 4 cm**						
Control	20.64^{cd}	2.473^{efg}	6.284^{bc}	4.219^{e}	0.2790	0.04977^{cde}	0.191^{d}
EC3	23.35^{cd}	3.077^{def}	6.944^{b}	5.061^{de}	0.2323	0.03015^{e}	0.193^{d}
EC9	24.91^{bcd}	3.339^{de}	9.841^{a}	21.64^{a}	0.2430	0.03797^{e}	0.773^{b}
EC9+BaSr_low	26.21^{bcd}	3.407^{de}	9.373^{a}	20.77^{ab}	0.2739	0.05378^{cde}	0.699^{a}
EC9+BaSr_high	29.73^{bc}	3.662^{cd}	4.242^{d}	18.39^{abc}	0.2552	0.06992^{bc}	0.556^{b}
BaSr_low	16.47^{d}	2.819^{def}	1.802^{ef}	5.494^{de}	0.2588	0.02957^{e}	0.283^{d}
BaSr_high	16.99^{d}	2.852^{def}	2.480^{e}	4.597^{e}	0.2881	0.08057^{ab}	0.232^{d}
Soil depth	**> 4 cm**						
Control	30.71^{bc}	2.029^{fg}	6.721^{bc}	3.953^{e}	0.2562	0.02838^{e}	0.154^{d}
EC3	37.00^{ab}	3.649^{cd}	6.025^{c}	8.374^{d}	0.2881	0.04970^{cde}	0.246^{d}
EC9	37.51^{ab}	4.526^{bc}	9.575^{a}	16.47^{c}	0.2621	0.04427^{de}	0.456^{bc}
EC9+BaSr_low	46.47^{a}	5.387^{ab}	9.666^{a}	17.15^{c}	0.2359	0.04599^{cde}	0.329^{bc}
EC9+BaSr_high	48.62^{a}	5.530^{a}	4.408^{d}	17.49^{bc}	0.2979	0.09636^{a}	0.322^{c}
BaSr_low	16.73^{d}	1.705^{g}	2.300^{e}	3.916^{e}	0.2570	0.03612^{e}	0.212^{d}
BaSr_high	32.68^{bc}	2.378^{efg}	1.400^{f}	3.876^{e}	0.2868	0.06713^{bcd}	0.115^{d}
Two-way ANOVA							
Treatment	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.059$ ^{ns}	$P < 0.001$	$P < 0.001$
Soil depth	$P < 0.001$	$P = 0.006$	$P = 0.407$ ^{ns}	$P = 0.053$ ^{ns}	$P = 0.242$ ^{ns}	$P = 0.602$ ^{ns}	$P < 0.001$
Treatment x Depth	$P = 0.228$ ^{ns}	$P < 0.001$	$P = 0.025$	$P = 0.036$	$P = 0.003$	$P = 0.036$	$P = 0.002$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

Table 3.16. Exchangeable cations in soil grown with bermudagrass as affected by oilfield brine

Treatment	**Mean exchangeable cation concentration in soil (mg kg^{-1})**						
	Ca^{2+}	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**Ba^{2+}**	**Sr^{2+}**	**ESR**
Soil depth	**0 - 2 cm**						
Control	26.34	3.646	8.527 ^{bc}	5.320 ^{ef}	0.2278 ^{bcd}	0.04513 ^{c}	0.182 ^{fg}
EC3	24.55	3.912	8.175 ^{bc}	12.96 ^{c}	0.2002 ^{d}	0.04010 ^{c}	0.453 ^{bcd}
EC9	29.03	4.022	12.25 ^{a}	23.50 ^{a}	0.2255 ^{bcd}	0.04445 ^{c}	0.705 ^{a}
EC9+BaSr_low	34.22	4.360	8.251 ^{bc}	17.97 ^{b}	0.2255 ^{bcd}	0.06723 ^{bc}	0.504 ^{bc}
EC9+BaSr_high	34.60	4.916	9.325 ^{b}	20.94 ^{ab}	0.2525 ^{ab}	0.1008 ^{ab}	0.581 ^{ab}
BaSr_low	31.54	3.683	3.200 ^{fg}	3.239 ^{f}	0.2259 ^{bcd}	0.07350 ^{bc}	0.101 ^{fg}
BaSr_high	32.93	3.758	3.136 ^{fg}	2.711 ^{f}	0.2647 ^{a}	0.1444 ^{a}	0.0749 ^{g}
Soil depth	**> 2 cm**						
Control	28.17	3.606	6.139 ^{de}	3.736 ^{f}	0.2546 ^{ab}	0.05069 ^{bc}	0.130 ^{fg}
EC3	22.09	3.347	7.054 ^{cd}	5.250 ^{ef}	0.2594 ^{ab}	0.09451 ^{bc}	0.206 ^{efg}
EC9	27.21	3.419	8.271 ^{bc}	11.20 ^{cd}	0.2336 ^{abcd}	0.04577 ^{c}	0.380 ^{cde}
EC9+BaSr_low	26.30	3.104	5.004 ^{ef}	10.27 ^{cde}	0.2107 ^{cd}	0.05184 ^{bc}	0.361 ^{cde}
EC9+BaSr_high	25.69	3.249	5.490 ^{de}	7.713 ^{def}	0.2116 ^{cd}	0.07518 ^{bc}	0.285 ^{def}
BaSr_low	27.65	3.159	1.869 ^{g}	2.856 ^{f}	0.2373 ^{abc}	0.05288 ^{bc}	0.105 ^{fg}
BaSr_high	37.15	3.926	1.966 ^{g}	3.193 ^{f}	0.2505 ^{ab}	0.08156 ^{bc}	0.0788 ^{g}
Two-way ANOVA							
Treatment	$P = 0.33^{ns}$	$P = 0.65^{ns}$	$P < 0.001$	$P < 0.001$	$P = 0.022$	$P = 0.002$	$P < 0.001$
Soil depth	$P = 0.27^{ns}$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.36^{ns}$	$P = 0.31^{ns}$	$P < 0.001$
Treatment x Depth	$P = 0.77^{ns}$	$P = 0.14^{ns}$	$P = 0.12^{ns}$	$P < 0.001$	$P < 0.001$	$P = 0.045$	$P = 0.016$

Means followed by the same letter in a given column do not differ significantly. ns: not significant, $P > 0.050$

3.2.3.3 Exchangeable Sodium Ratio (ESR)

The ESR is defined as:

$$ESR = \frac{[Na_X^+]}{[Ca_X^{2+}] + [Mg_X^{2+}]}$$

where [Na$_X^+$], [Ca$_X^{2+}$], and [Mg$_X^{2+}$] are exchangeable ion concentrations in mmol$_c$ kg^{-1}. The ESR is a particularly useful parameter because it has been found to be highly correlated to the sodium adsorption ratio (SAR) and gives a measure of the "sodium hazard" for the dispersion of clays in sodium impacted soils. The ESR for the experimental soils is given in the final column of Tables 3.14-3.16.

The response of ESR across all treatments was consistent across plant species. No response to the EC3 brine addition is observed for any depth or any plant species except the 0-2 cm depth for the bermudagrass. The EC9 treatments resulted in significant increases in ESR for all plant species at both depths. For the controls, mean ESR values across species and depths ranged from 0.06 to 0.18. The ESR values associated with EC9 treatments ranged from 0.24 to 0.77. The tendency for ESR to increase with brine additions is expected considering that the brine solution was SAR 75.

CHAPTER IV

DISCUSSION OF RESULTS

4.1 Effect of brine, barium and strontium in soil on the growth of cowpea, sorghum sudangrass and bermudagrass

4.1.1 Germination

The analysis of variance (ANOVA) of percent germination found nonsignificant treatments (main effects) for cowpea ($P = 0.074$) but significant treatment effects for sorghum sudangrass ($P = 0.002$) and bermudagrass ($P < 0.001$). The application of brine generally reduced germination in all the 3 species, and the reduction intensified with increasing brine EC level, which was significant in most treatments at EC9 but not significant at EC3. The same trend was observed during the screening germination study, where germination reduced with increased brine EC level. At the highest EC (18 dS m^{-1}), < 30% sorghum sudangrass germinated, while cowpea and bermudagrass completely failed to germinate, thus EC 18 dS m^{-1} brine level was excluded for the greenhouse experiment.

Brine adversely affected seed germination in this study, which is in agreement with findings of earlier studies in literature (Munn and Stewart, 1989; Panuccio *et al.,* 2014; Orlovsky *et al*., 2016). Munn and Stewart (1989) reported that 100% seawater (SW) completely inhibited germination of soybean, tall fescue, wheat and garden pea, while 10% SW dramatically reduced germination of soybean and tall fescue but not wheat or peas, and ≤ 2% SW did not affect germination in all these species. Results from this study showed varied germination responses to brine concentration among the 3 study species and EC levels. For instance, at the high EC level

(EC9), seed germination was delayed in cowpea and bermudagrass (delayed for 1 to 3 days in cowpea, and for 14 days in bermudagrass) compared to control, but it was not delayed in sorghum sudangrass (Figures 3.1, 3.2 and 3.3). On the other hand, at a lower brine level (EC3), seed emergence was not affected in cowpea and sorghum sudangrass, but was delayed in bermudagrass by 3 days relative to control. Also, brine EC3 did not have significant impact on emergence percentage of the 3 species, but EC9 significantly reduced emergence percentage in bermudagrass, and some treatments in sorghum sudangrass and cowpea.

Several studies have indicated that high salt concentrations affect most of the germination indices, whereas lower salt concentrations may affect only some aspects of emergence (Orlovsky *et al*., 2016). West and Francois (1982) reported that EC exceeding 12 dS m^{-1} significantly reduced the emergence percentage of cowpea, whereas EC ≤ 12 dS m^{-1} simply delayed the emergence. Manohar (1966) also observed a similar response for *Pisum sativum* L. where low EC levels merely delayed emergence but did not affect emergence percentage.

In addition to delayed seed germination, emergence rate was also slowed by the brine especially in cowpea and bermudagrass at EC9. More than 60% emergence of cowpea seed was attained by day 4 in the non-brine and brine EC3 treatments, while < 40% emergence had been attained in EC9 brine treatments, although final emergence in all treatments was attained on about the same day (i.e., day 8 after sowing) (Figure 3.1). Similarly, in bermudagrass, seed emergence stagnated in brine treatments at EC9 until day 20 when it revived slowly (Figure 3.3). On the contrary, in sorghum sudangrass, seed emergence progressed almost at a similar rate in all treatments, although with lower emergence percentages under brine EC9 treatments (Figure 3.2). On day 3, more than 70% seed emergence was attained in non-brine and EC3 treatments, while < 30% in EC9 brine treatments had been reached in sorghum sudangrass.

Although monocots (such as halophytic grasses) can grow under very high salt concentrations, many of them are not as salt tolerant at seed emergence as resistant dicotyledonous plants (Khan and Gul, 2006; Zehra *et al*., 2012). This perhaps explains the lower tolerance of bermudagrass at emergence compared to sorghum sudangrass and cowpea. Future research is necessary to ascertain the salinity tolerance limits of bermudagrass, sorghum sudangrass and cowpea at emergence stage.

Although many seedlings in the experiment were able to emerge, some of the seedlings failed to establish. Seedling mortality was observed among all the 3 species especially under EC9 treatments and was most severe in cowpea. Seedling mortality in bermudagrass was observed mainly among non-brine treatments but not in EC9 treatments, although mortality is difficult to quantify in the highly rhizominous bermudagrass. Seedling death in bermudagrass seems to have been associated with overcrowding rather than salinity, since salt treatments, specifically EC9, had very low seedling density compared to non-brine treatments (Table 3.1). The tolerance of bermudagrass was demonstrated from the vigorous growth of the germinated seedlings.

Seedling emergence in bermudagrass seemed to be more sensitive to salinity than seedling establishment when compared to cowpea and sorghum sudangrass; while sorghum sudangrass displayed good tolerance levels during both seedling emergence and establishment. Murillo-Amador *et al*., (2002) reported that cowpea was as tolerant during emergence stage as establishment stage, which is partially similar to this study results for cowpea that strongly tolerated brine salinity during seed emergence (Murillo-Amador *et al*., 2002).

Salinity may inhibit the growth of embryo axis during seedling establishment as a result of delayed reserve mobilization and/or membrane disturbance, leading to seedling death (Prisco and Vieira, 1976; Gomes-Filho *et al*., 1983; Murillo-Amador *et al*., 2002). Also, under high salt levels, the young seedling may have trouble to absorb moisture for seedling growth against the low moisture gradient due to osmotic stress (Waisel, 1972; West and Francois, 1982; Panuccio *et al.,* 2014). Also, ions such as Na^+ in excessive levels, which was the dominant cation in the brine for this study cause injury to seeds and seedling and fails the developing embryo (Panuccio *et al.,* 2014).

4.1.2 Effect brine additions on vegetative growth

4.1.2.1 Effect on morphology of cowpea, sorghum sudangrass and bermudagrass

Salt-induced stress in plants manifests by symptoms such as chlorosis, necrosis, and premature senescence of leaves (Yeo and Flowers, 1986; Glenn and Brown, 1999; Hasegawa *et al*. 2000; Munns 2002; Panuccio *et al.,* 2014). In this study, cowpea plants grown in soil treated with brine, compared to non-brine treated plants, distinctly exhibited salinity-stress symptoms such as smaller, deformed and discolored leaves. The findings of this study are similar to those of Wilson *et al*. (2006) who observed decreasing leaf number with increasing salinity levels and much smaller trifoliate at higher salt stress levels among cowpea cultivars. Salt stress has resulted in reduced leaf growth and decreased biomass (Murillo-Amador *et al*., 2002; Wilson *et al*., 2006). In beans, high NaCl levels inhibited leaf expansion largely due to inhibition of cell division (Murillo-Amador *et al*., 2002). Decline in leaf growth is the earliest response of glycophytes exposed to salt or drought stress (Murillo-Amador *et al*., 2002). This is a probable a mechanism for minimizing water and nutrient demands for such plants, hence avoiding excessive uptake of salts.

Increased leaf senescence, as was observed in cowpea for this study, also aids to rid excess salt through shedding of leaves where excess ions are partitioned mostly in older leaves to avoid damage of young leaves and other sensitive organs (George *et al*., 2012; Chaugool *et al*., 2013; Plaut *et al*., 2013; Uddin and Juraimi, 2013). Relative to cowpea, brine caused limited morphological effects on sorghum sudangrass and bermudagrass plants. Brine-treated plants of sorghum sudangrass had slightly delayed leaf growth stages and fewer number of leaves, and with dried leaf tips especially on old leaves. Brine-treated bermudagrass plants revealed no salt-induced effects on the plants' morphology, except that the plants had tougher (hard to cut) shoots, compared to non-brine treated plants, which had soft and thinner stems. Adavi *et al.* (2006) reported increased leaf-firing in bermudagrass by 4% when salinity increased to 17.8 dS m^{-1} (Adavi *et al.*, 2006). Reduction of chlorophyll content due to salinity stress is common among salt-sensitive plant species, which causes burning of leaves or other succulent parts and degradation of other pigments, but saline tolerant species can protect themselves from such deterioration (Alam *et al*., 2015).

In this study, brine treated bermudagrass plants (at EC9) displayed more horizontal and branched growth compared to non-brine treated plants that displayed vertical and less branched growth. Horizontal growth among brine treated plants was attributed to fewer number of seedlings/plants per pot (at EC9) rather than a physiological effect of the salt, compared to non-brine treated plants that were overcrowded that probably limited growth space and instead promoted vertical growth in competition for light.

4.1.2.2 Effect on shoot growth

Both reduction and increase of fresh and dry matter contents in response to salinity are common phenomena in cultivated crop plants and trees (Alam *et al*., 2015). Some authors have observed decreases in biomass due to salinity, while others have observed increases (Mass and Poss, 1989; Alam *et al*., 2015). These findings are similar to our results, where compared to control, brine significantly reduced dry shoot mass in cowpea, whereas in bermudagrass and sorghum sudangrass, the brine at EC9 increased dry shoot mass. Brine level EC3 did not have significant impact on dry shoot mass in bermudagrass and sorghum sudangrass relative to control. In similar studies, vegetative growth (dry shoot mass) of cowpea was reduced by 9.0% for each unit increase in EC_e beyond a threshold value of 1.6 dS m^{-1}, and shoot dry-matter accumulation reduced by 52% at salinity –0.35 MPa (about 8.5 dS m^{-1}) (West and Francois, 1982; Mass and Poss, 1989). A 50% decrease in vegetative growth of cowpea was at EC_e 7 dS m^{-1} (West and Francois, 1982), and C50 for leaf dry mass recorded at EC_e 8.4 dS m^{-1} (Wilson *et al*., 2006). Their values compare with this study results on cowpea where the C50 for dry shoot mass would estimate between 3 dS m^{-1} and 9 dS m^{-1}.

Significant effect on shoot-mass of sorghum cultivars was not observed at lower salt concentrations < 12.5% sea water, but 24.4% reduction of shoot length was observed at 50% seawater compared to control (Bafeel, 2014). Non-significant reduction in dry shoot mass of sorghum sudangrass at lower brine level (EC3) in this study, but significant increase at the higher level (EC9), which agrees with some earlier studies. Top growth mass of bermudagrass decreased significantly with each increment of salt in Na and Ca chloride solutions (Youngner and Lunt, 1967). Adavi *et al.* (2006) also recorded 75% reduction in dry mass of top growth of bermudagrass as the salinity level increased to 17.8 dS m^{-1} (Adavi *et al.*, 2006). Other authors also reported reductions in top growth in bermudagrass and other turfgrass species due to increased salinity (Ackerson and Youngner, 1975; Dudeck *et al.*, 1983; Adavi *et al.*, 2006; Youngner and Lunt, 2006).

4.1.2.3 Effect on root biomass

Dry root mass in cowpea in this study significantly decreased with increasing brine relative to control. Sorghum sudangrass root biomass increased EC3 (though not statistically significant), and EC9 root biomass was greater than the control and EC3. Wilson *et al.*, (2006) observed a 50% reduction in root dry-matter accumulation in cowpea cultivars with increasing salinity at 15.2 dS m^{-1}. Mean root length of sorghum cultivars decreased treated when treated with sea water (Bafeel, 2014). The findings of these two studies differ from this study results as dry root mass of sorghum sudangrass was increased by brine treatment. For bermudagrass, our results agree with Youngner and Lunt (1967) who observed increased root growth under intermediate salt treatments. Adavi *et al.* (2006) also reported increased root dry mass in bermudagrass with increasing salinity ≤10.2 dS m^{-1}, but a declined at salinity >10.2 dS m^{-1} to 17.8 dS m^{-1}. The stimulatory effect of moderate salinity, and the inhibitory effect of high salinity levels on root growth in bermudagrass and other turfgrass species have been documented by various authors (Ackerson and Youngner, 1975; Youngner and Lunt, 1967; Dudeck *et al.*, 1983; Adavi *et al.*, 2006).

4.1.2.4 Effect on root to shoot ratio

An increase in root:shoot ratio of many plants due to salinity stress upon exposure to salinity is a common observation (Munns and Termaat, 1986; Wilson *et al*., 2006). A similar observation was registered in this study; brine (EC3 and EC9) increased dry mass root to shoot ratio of sorghum sudangrass, though not significantly. Also, in bermudagrass, at EC9 (especially with BaSr) root to shoot ratio significantly increased, but was reduced by EC3. In cowpea, root to shoot mass ratio was significantly reduced by brine EC9 (without BaSr). Root to shoot mass ratio of bermudagrass varieties increased with increased salinity (Youngner and Lunt, 1967; Youngner and Lunt, 2006). Shoot growth of sorghum and cowpea was reported to be more adversely affected by salt stress compared to root growth (Bafeel, 2014; Wilson *et al*., 2006).

In our study, dry shoot and root mass of cowpea were adversely affected by brine, but the root mass seemed slightly more affected than the shoot. On the contrary, sorghum sudangrass shoot responded positively to salinity at a higher rate than root growth. Enhanced root growth with corresponding decline in shoot growth is an adaptation mechanism in some species/varieties upon exposure to salinity that allows the roots to probe deeper into the soil for search of more water, under water deficit that accompanies ionic toxicity (Bafeel, 2014). Reduction in top growth and simultaneous increase in root growth up to 10.2 dS m^{-1} salinity was suggested that allowed bermudagrass to overcome the osmotic and nutritional stresses caused by salinity (Adavi *et al.*, 2006). The same was observed in our study based on the increased root to shoot ratio at EC9 dS m^{-1} that probably contributed to tolerance to brine that was exhibited in bermudagrass.

Salinity stress on plant vegetative growth can induce physiological changes such as interference with chlorophyll, photosynthesis, stomatal conductance, and transpiration as well as cell/tissue, all of which damage that reduce plant growth (Alam *et al.*, 2015). The vegetative growth of salt sensitive cowpea was affected most by brine compared to sorghum sudangrass and bermudagrass, species that tolerate salinity levels exceeding 9 dS m^{-1} (Strawn *et al.*, 2015; Shahid *et al.*, 2018).

4.1.2.5 Effect of Ba and Sr on the vegetative growth of cowpea, sorghum sudangrass and bermudagrass

Little Ba and Sr is bioconcentrated by plants relative to the amount found in soils, and the plant will try to eliminate excess Ba or Sr from its system to prevent phytotoxicity caused by high Ba/Sr levels (Pais *et al.*, 1998; U.S. Environmental Protection Agency 2003; Environmental Company of the State of Sao Paulo 2001; Kabata-Pendias & Mukherjee 2007; Ong *et al.*, 2013). In our study, no significant effects due to Ba and Sr on the growth of cowpea, sorghum sudangrass and bermudagrass were observed.

4.1.3 Analysis of cation accumulation in plant tissue

4.1.3.1 Calcium in root and shoot

The mean Ca^{2+} concentration in shoot and root generally decreased relative to control in response to brine application, but significant differences were observed only at EC9 in sorghum sudangrass shoot and some treatments for cowpea root. Application of brine (dominated by Na^+ and K^+) considerably increased K^+ and Na^+ concentrations in brine-treated soil, and the high concentrations of these cations may have depressed Ca^{2+} uptake and distribution in plant tissue (Wallace *et al.*, 1980; Rengel, 1992; Silva and Uchida, 2000; George *et al.*, 2012; Bafeel, 2014).

Calcium occurs chiefly in leaves with lower concentrations in roots, (Wallace *et al*., 1980; Rengel, 1992). This is consistent with our results whereby, despite the low mobility of Ca within the plant, Ca^{2+} concentration was higher in shoot of cowpea (about two times) and sorghum sudangrass (about 1.5x) than in root, but slightly lower (almost equal) in bermudagrass shoot than in root. Guimarães *et al*. (2012) found lower Ca^{2+} content in cowpea roots than in shoot even under Ca supplementation.

Additions of Ba and Sr did not have consistent effects on plant tissue Ca^{2+}, and the concentrations of Ba^{2+} and Sr^{2+} in the treatments were probably too small to impact the concentrations of major ions in the plant tissues. Trace metals, such as Ba and Sr, present in excess (especially Sr which is a natural analogue of Ca) can inhibit calcium uptake and transport in plants (Wallace *et al*., 1980; Dubchak, 2018). However, this study suggests minimal influence of Ba and Sr on Ca^{2+} accumulation in these plant species probably because the concentrations of Ba and Sr applied were very low.

4.1.3.2 Magnesium in root and shoot

All brine applications (with or Ba and Sr) suppressed Mg^{2+} concentration in the shoots of all three species relative to Mg in the control plants. The effects of treatments on Mg in the roots was small and inconsistent across the plant species. High levels of Ca^{2+}, K^{+} and Na^{+} in the brine treatments might have interfered with the plant uptake and transport of Mg^{2+}. Similar observations have been reported in other studies in which salinity significantly reduced Mg^{2+} content in leaves and roots of cowpea and sorghum; supplemental Ca^{2+} caused decreases in

Mg^{2+} contents in salt stressed cowpea plants (Boursier and Läuchli, 1990; Marschner and Rengel, 1995; George *et al*., 2012).

Excess levels of K^+, Na^+ and Ca^{2+} compete with Mg^{2+} and reduce its uptake and translocation from roots to upper plant parts (Silva and Uchida, 2000; Shaul, 2002). Also, Ca^{2+} competitively inhibits Mg^{2+} for binding sites on the root plasma membrane which appear to have a lower affinity for the highly hydrated Mg^{2+} than for Ca^{2+} (Wallace and Mueller, 1980; Marschner and Rengel, 1995; Guimarães *et al*., 2012). The Mg^{2+} and Ca^{2+} were added in the simulated brine at a ratio of about 1:30, enough for Ca^{2+} to inhibit Mg^{2+} uptake at the root membrane binding sites.

The concentration of Mg^{2+} was higher in shoot than in root for all three species. Magnesium is very mobile in plants, thus tends to easily move from roots to shoot (Guimarães *et al*., 2012; Silva and Uchida, 2000). Guimarães *et al*. (2012) reported that higher supplemental Ca^{2+} concentrations reduced Mg^{2+} content of cowpea roots, while lower supplemental Ca^{2+} concentration led to higher Mg^{2+} content in stems and petioles of salt-stressed plants. Fast growing plants tend to have increased resource (nutrient) uptake especially under stress conditions compared to slow growing plants, to sustain the faster growth but also to withstand the stresses conditions (George *et al*., 2012).

Trace metals, such as Ba and Sr, influence Mg uptake by plants (Shaul, 2002), and the plant Mg concentrations were impacted in treatments with Ba and Sr. However, the concentrations of Ba and Sr in the brine were too small to impact Mg content. The changes in Mg concentrations were more likely due to the impact of the major cations in the brine rather than the Ba and Sr.

4.1.3.3 Potassium in root and shoot

Additions of brine significantly increased the concentration of K^+ in shoot and root in the three plant species, and the impact was greater in the EC9 treatments. The K^+ concentrations were significantly different from control at both EC3 and EC9 in cowpea and sorghum sudangrass shoot, but not significant for bermudagrass at EC3. In the root, differences in K concentration were significant only for EC9 but not EC3 across all species. Guimarães *et al*. (2012) observed higher K^+ content in salt-stressed cowpea plants in comparison to control plants. In contrast, increased salinity led to reduction in K content of bermudagrass and sorghum shoot (Boursier and Läuchli, 1990; Adavi *et al.*, 2006; George *et al*., 2012).

Potassium can be stored easily without cell damage in the vacuole for distribution to other plant parts (Silva and Uchida, 2000; Morgan and Connolly, 2013). Potassium was the most accumulated cation in shoot and root for all plant species under all treatments, except in sorghum sudangrass and cowpea root where Na^+ content was higher than K^+, especially at brine level EC9. The Na:K ratio decreased in sorghum sudangrass. Potassium uptake and retention in plants is affected by H^+, Ca^{2+}, Mg^{2+}, Na^+ and NH_4^+, and excess of these cations can reduce the net uptake and retention of K^+ (Wallace *et al*., 1980; Peoples *et al*., 2014). Potassium is highly mobile and soluble within plant tissues and aids in ion balancing within the plant (Wallace *et al*, 1980). This partly explains the observed reduction in Ca^{2+} and Mg^{2+} contents in plant tissue for the three species in response to brine application as the K^+ concentration increased in brine treated plants.

The K^+ concentration was higher in shoot of cowpea (about two times) and sorghum sudangrass (more than three times) than in root, while for bermudagrass shoot K^+ was slightly lower than (almost equal) in root. The higher K^+ concentrations in shoot than in root may be attributed to the high mobility of K^+ in plant tissues that probably facilitated an easier transfer of the K^+ from roots to shoot.

Potassium has high root selectivity in uptake compared to Na, but under saline conditions, high levels of Na^+ may disrupt the integrity of root membranes and alter their selectivity (Grattan and Grieve, 1999; George *et al*., 2012; Guimarães *et al*., 2012). Bermudagrass tends to accumulate Na^+ with subsequent reduction in K^+; thus, Na may partially substitute for K in bermudagrass (Adavi *et al.*, 2006). Our support the notion of substitution of K by Na in bermudagrass as brine increased Na:K ratio in bermudagrass root, but the salinity had no adverse effects on vegetative growth. In sorghum, Na-based salinity led to a decrease in growth and in shoot concentrations of K^+ and Mg^{2+} (Boursier and Läuchli, 1990; George *et al*., 2012).

Barium and Sr additions mostly followed the trends of the accompanying treatments. For BaSr_low and BaSr_high (no brine), K concentrations in roots and shoots were similar to the control. For EC9+BaSr_low and EC9+BaSr_high, the K^+ content was similar to plants grown in soils amended with EC9.

4.1.3.4 Sodium in root and shoot

Brine increased the concentration of Na^+ in shoot and root in all three species, which intensified with EC9. In the shoots, Na^+ concentrations were significantly different from the control at both

EC3 and EC9 for cowpea and sorghum sudangrass, but the differences were not significant for bermudagrass. For the roots, all EC9 treatments significantly increased Na concentrations for all plant species, but treatment and control Na concentrations were not significantly different for EC3.

Similar results were observed by other studies, where salinity increased Na^+ content in cowpea, sorghum, bermudagrass and other plants (Alexander and Groot-Obbink, 1971; Ackerson and Youngner, 1975; Dudeck *et al*., 1983; Boursier and Läuchli, 1990; Sibole, Cabot, Poschenrieder and Barcelo, 2003; Adavi *et al.*, 2006; George *et al*., 2012). Unlike Ca, K and Mg, Na is an essential element only for particular plant species (mostly C4 plants but not Cthree species), or under specific conditions, largely depending on the extent to which it can replace K functions in plants (Chattopadhyay *et al*., 2002; George *et al*, 2012). High Na concentrations in the soil solution under saline conditions may be toxic to the plant or result in Na-induced nutrient deficiencies (Alexander and Groot Obbink, 1971; Sibole *et al*., 2003; George *et al*., 2012). Among the major cations, sodium was the least accumulated by the control plants. Na-induced salinity increased Na concentrations but decreased shoot concentrations of K^+ and Mg^{2+} in sorghum and bermudagrass (Ackerson and Youngner, 1975; Dudeck *et al*., 1983; Boursier and Läuchli, 1990; Adavi *et al.*, 2006; George *et al*., 2012).

Bermudagrass and sorghum sudangrass were better able to exclude or avoid uptake/accumulation of Na^+ in their tissue than cowpea. Excessive Na^+ content in cowpea adversely affected vegetative growth whereas bermudagrass and sorghum sudangrass experienced enhanced growth, although root biomass of bermudagrass was reduced at EC9. The results also suggest that bermudagrass and sorghum sudangrass were able to tolerate high Na^+ levels in plant tissue and enhanced growth while cowpea was sensitive to elevated Na.

Under salt stress conditions, there may be preferential accumulation of Na^+ in the shoot, enabling plants to avoid excessive Na^+ accumulation in root tissue (Koyro, 2000; Ashraf *et al*., 2008; Panuccio *et al.,* 2014). The rate of Na^+ transfer from root to shoot (xylem loading) is much lower in salt-tolerant species than in salt-sensitive species; and leaves and roots of salt-tolerant species usually have lower Na^+ concentration compared with salt-sensitive plants (Davenport *et al*., 2005; Roychoudhury and Chakraborty, 2013; Bafeel, 2014).

Sorghum sudangrass and bermudagrass, which had higher Na^+ content in root than in shoot, were more tolerant to Na^+ than cowpea which instead had higher Na^+ content in shoot than in root. Under salt stress, sorghum plants exhibited salt tolerance and Na ions were retained mainly in root and stem, but the distribution of excess Na^+ to leaves was prevented. Instead, the Na was allocated to the leaf sheath, and lower shoot growth was observed at proportionately higher concentrations of Na^+ and Cl^- in leaves (Munns, 2002; Krishnamurthy, Serraj, Hash, Dakheel and Reddy, 2007; Netondo *et al*., 2004; Chaugool *et al*., 2013; Bafeel, 2014).

The high Na^+ levels in cowpea shoot could serve as a mechanism for eliminating excess Na^+ through leaf fall. The increased necrosis and eventually early leaf senescence observed in cowpea leaves under EC9 treatments could be evidence of this mechanism for exclusion of excess Na^+ by shedding the leaves. Retranslocation of Na^+ from shoot to roots is also a possible mechanism that can contribute to low Na^+ concentrations in the shoot under saline conditions, but the proportion of Na^+ that is translocated from leaves back to roots seems to be higher for salt-sensitive than for salt-tolerant plant species (Matsushita and Matoh, 1992; George *et al*., 2012). In this study, retranslocation of Na^+ from shoot to roots seems not be exhibited in cowpea.

Because excess Na^+ competes with K^+ for binding sites in the cytoplasm and impairs ribosomal attachment to rRNA, the ability of plants to maintain low Na^+ and high K^+ concentration (high K:Na ratio) plays a crucial role in K homeostasis under salinity stress (Qian *et al*., 2001; Chattopadhyay *et al*., 2002; Tester and Davenport, 2003; Ligaba and Katsuhara, 2010; George *et al*., 2012; Bafeel, 2014). This is an important aspect of salinity tolerance, commonly observed in salt-tolerant species as they tend to have higher K:Na compared to salt sensitive plants (Ligaba and Katsuhara, 2010; George *et al*., 2012; Bafeel, 2014). Although brine additions led to increased K^+ and Na^+ in all three species, K:Na increased in cowpea (shoot and root), and bermudagrass (root) but decreased in sorghum sudangrass (shoot and root) and bermudagrass shoot. The reduction in K:Na ratio probably aided sorghum sudangrass and bermudagrass to evade Na^+.

Contrary to our findings, Adavi *et al.* (2006) reported an increase in Na content and reduction in K content of bermudagrass shoot, which reduced the K:Na ratio as salinity level increased to 18 dS m^{-1}. However, in some bermudagrass cultivars, a high K and low Na accumulation (high K:Na) in shoot were reported, which resulted in higher salt-tolerance in those cultivars. These cultivars also showed superior top growth and low or no leaf-firing (Adavi *et al.*, 2006). Sodium increased while K decreased with increased salinity levels, but the total Na + K content was not affected (Dudeck *et al*. 1983).

Calcium can control the entry of Na^+ into shoot through apoplastic transpirational bypass flow. Rising cytoplasmic and extracellular Ca^{2+} concentrations can decrease the influx of Na^+ and efflux of K^+ and eventually helps to maintain Na^+ and K^+ homeostasis for most plant species (Rengel, 1992; Kaya *et al*., 2002; Zehra *et al*., 2012). In this study, brine somewhat reduced Ca^{2+} levels and increased Na^+ in plant tissue of the three species, but growth declined in cowpea while in sorghum sudangrass growth was enhanced or unaffected, which imply that either a) brine Ca probably had insignificant influence on Na or b) the Na content of the brine overwhelmed the Ca content. Supplemental Ca^{2+} reduced Na^+ translocation to shoot and retained the Na^+ in roots, partially restored Na^+/Ca^{2+} balance and reduced Na^+ toxicity, but this was insufficient to alleviate the negative effects of Na^+ on cowpea growth, as the benefits were surpassed by the osmotic effects associated with increased total salt concentration (Guimarães *et al*., 2012).

4.1.3.5 Barium in root and shoot

The analysis of variance of mean Ba^{2+} concentrations found significant treatment effects for shoots of all species and in bermudagrass root but nonsignificant effects for cowpea and sorghum sudangrass root. There was mixed response to Ba and Sr additions (with and without brine) treatment whereby Ba^{2+} concentration increased in some treatments and decreased in other cases.

Barium is not toxic at low concentrations; Ba levels of about 200 mg/kg Ba were found to be moderately toxic, while 500 mg/kg Ba could be toxic in plants (Pais *et al*. 1998; Abreu *et al.*, 2012; Ong *et al*., 2013). Barium is relatively immobile, and little Ba is bioconcentrated by terrestrial plants relative to the amount found in soils (Ong *et al*., 2013). Mean Ba^{2+} concentrations range for cowpea was 46 – 154 mg kg^{-1} in shoot and 112 – 335 mg kg^{-1} in root; in sorghum sudangrass shoot 47 – 70 mg kg^{-1} and 82 – 106 mg kg^{-1} in root; and in bermudagrass shoot 60 – 140 mg kg^{-1} and 68 – 126 mg kg^{-1} in root. These Ba^{2+} concentration levels in the plant shoot and root of all the species are not likely to be toxic to plants.

Barium is strongly accumulated by legumes, grain stalks, forage plants, and trees with the highest levels found in roots than in leaves and stems (*Ong et al*., 2013). We observed higher Ba^{2+} concentration in root than in shoot of cowpea (almost 3x) and sorghum sudangrass Ba^{2+} (almost 1.5x); while for bermudagrass, shoot Ba^{2+} was slightly higher than in root. Also, the legume cowpea accumulated the highest Ba^{2+} concentration, then bermudagrass and lowest in sorghum sudangrass.

4.1.3.6 Strontium in root and shoot

The analysis of variance of the mean Sr^{2+} concentration in shoot found significant treatment effects for cowpea, sorghum sudangrass and bermudagrass, bermudagrass and sorghum sudangrass root but nonsignificant effects for cowpea root. Accumulated Sr^{2+} content in shoot and root increased with increasing BaSr spiking solution concentration for all the species. For most times, the Sr^{2+} concentration in shoot was significantly different from control under BaSr_high (with and without brine).

Strontium is a trace metal that is not essential for plants and taken up by plants in very small quantities (about 10^{-2} to 10^{-3} % of dry mass). High concentrations of Sr in edible plants can have health consequences for humans (Ageets 2001; Annenkov and Yudintseva, 2002; Skupiński and Solecki, 2014; Dubchak, 2018). Accumulated Sr^{2+} concentration in cowpea, sorghum sudangrass and bermudagrass was relatively low (< 100 mg kg^{-1}), which is within the expected range in plants and should not cause toxicity to plants. The migration and bioavailability of Sr^{2+} decreases with increasing exchangeable Ca^{2+} and Mg^{2+} due to stronger sorption of Sr^{2+}compared to Ca^{2+} (Skupiński and Solecki, 2014; Dubchak, 2018). The plant intake of Sr increases with increasing Sr concentration in the growth medium (Dubchak, 2018), which is similar to that observed in all three plant species where the highest Sr^{2+} concentrations were recorded in treatments with the high level of BaSr spiking solution applied.

Strontium is analogous to Ca in nature and are both absorbed by plants via the same transport systems; however, a smaller amount of Sr^{2+} is transferred to plants than Ca^{2+} due to stronger Sr^{2+} fixation in soil (Dubchak, 2018). Compared to Ba^{2+}, Sr^{2+} is more mobile and accumulated by plants (Dubchak, 2018). Strontium relatively easily penetrates through the root system into all parts of the plant. It accumulates largely in aboveground plant organs, with higher levels in leaves and stem than in spikelets and relatively little in grains, relative to Ba and other trace metals that bioaccumulate mainly in roots (Kashparov *et al*. 2005; Dubchak, 2018). We also observed higher Sr^{2+} concentration in shoot than root of cowpea and sorghum sudangrass, although for bermudagrass, root Sr^{2+} was higher than in shoot. Strontium accumulates in larger quantities in legumes and root crops but weakly accumulate in cereals > grains, grasses and vegetable crops (Vasilenko and Vasilenko 2002; Lazarevich and Chernukha, 2007; Tieplyakov, 2010; Skupiński and Solecki, 2014; Dubchak, 2018). This is consistent with our results that revealed cowpea (a legume) the highest bioaccumulator of Sr.

4.2 The effect of oilfield brine, barium and strontium on soil properties

4.2.1 Soil pH and electrolytic conductivity (EC_e)

Brine additions (with and without BaSr) slightly reduced soil pH and markedly increased EC_e while the response due to BaSr (without brine) varied marginally from untreated control. The original field soil (unamended) had low EC_e (<1 dS m^{-1}) as did the untreated control soil, whose EC_e remained low (< 2 dS m^{-1}) after harvesting. The EC_e of the BaSr_low treatment did not differ from the control because, at the concentrations added, Ba and Sr have negligible contribution to soil EC_e (Glenn *et al*., 2006; George *et al*., 2012). On the other hand, as expected, addition of brine led to soil EC_e close to the target EC_e of the treatments (EC_e 3 and EC_e 9), which is potentially damaging to soils (Strawn *et al*., 2015; Shahid *et al*., 2018). The threshold soil salinity for initial yield decline is estimated at 1.3 dS m^{-1} for cowpea, 2.8 dS m^{-1} for sorghum sudangrass, and 6.9 dS m^{-1} for bermudagrass (Maas and Hoffman, 1977; Strawn *et al*., 2015; Shahid *et al*., 2018). The observed salt levels accumulated in brine-treated soil are above the threshold levels for all species.

The EC_e values decreased with soil depth for all three plant species, reaffirming that while salts migrated downward during irrigation events, salts accumulated in the surface during evaporation. Salts tend to accumulate in upper soil layers if ET exceeds irrigation plus rainfall (Hariuandi, 1984; George *et al*., 2012; Marschner and Rengel, 2012). Water was not allowed to leach from the greenhouse pots, ensuring that ET was at least equal to irrigation rates.

The RO water used for irrigation contained considerable Na concentration (about 20 mg L^{-1}), but the total salinity was low (<1 dS m^{-1}). Continuous irrigation with this water marginally elevated soil salinity (even in untreated control). Bermudagrass, grown for the longest period in the greenhouse, accumulated the highest concentration of salts in the upper depth. Cowpea was grown for the shortest period of time, had the least water demand, and accumulated the least amount of salt the upper depth.

4.2.2 Soluble cations

The soils in the greenhouse experiment were amended with two quantities of brine with the specific intent of increasing the electrolytic conductivity (EC) of the saturated extracts to 3 dS m^{-1} (EC3 treatment) or 9 dS m^{-1} (EC9 treatments). As discussed in a previous section, this objective was reached in that the mean measured EC_e of the saturated extracts were very close to the intended targets. The discussion of the results first will address the major cations: Ca^{2+}, Mg^{2+}, K^+, and Na^+. Responses will be discussed as a function of treatment and sampling, with differences between plant species noted. The toxic cations, Ba^{2+} and Sr^{2+}, are discussed separately because the approach to adding them to the soil was different than for the major cations. The implications of these results are given perspective by examining trends in SAR.

4.2.2.1 Ca^{2+}, Mg^{2+}, K^+, and Na^+

The two-way analysis of variance for the concentrations of the major cations as impacted by depth, treatment, and plant species showed significant main effects and significant depth x treatment interactions. Our interest was in the interaction soil depth x treatment for each species, and this is the center of discussion for this section. The key aspects of soil chemical properties result from the saturated extract analysis relate to salinity, SAR and BaSr.

Additions of brine (particularly EC9) significantly increased the soluble concentrations of the major cations (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}) as well as the sodium adsorption ratio (SAR) for all plant species. The concentration of soluble cations in the saturated extracts followed the trend: $Na^{+} > Ca^{2+} > K^{+} > Mg^{2+} > Ba^{2+} > Sr^{2+}$, though there was some variation across treatments and plant species.

The observed trend of soluble cation concentrations roughly followed the patterns dictated by cation solubility in soil solution, sorption strength to colloids, and hydrated radius of the cations in soil. Soil colloids have a strong preference for divalent cations over monovalent cations, and within a given valence, preference for cations with a smaller hydrated radius. Using these criteria alone, the expected relative binding strength to the soil solid phase would be $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$; the reverse order would represent anticipated solubility in soil solution, assuming all else being equal (e.g., same initial concentrations) (Marschner, 1995; Johnston and Tombácz, 2002; Guimarães *et al.*, 2012; Dubchak, 2018). The results generally reflect that monovalent cations dominated the soluble pool with the exception of Ca^{2+}, which was the second most soluble species (because Ca^{2+} was the overwhelmingly dominant soluble cation for the unamended soils). In the EC3 and EC9 treatments, Na^{+} was added at much higher concentrations than all other major cations, as is reflected in the analysis of the saturated extracts for these soils. In the soils receiving no brine additions (control, BaSr_low, and BaSr_high), the Ca^{2+} and Na^{+} concentrations were still dominant but not to the extent as the brine amended soils.

At the end of the experiment, the control soil had much higher soluble Na^{+} than the original field soil. This was the result of months of irrigation with RO water containing 20 mg L^{-1}, and this impact was further magnified in the SAR and ESR values discussed below. Although the elevated Na^+ in the RO water used for irrigation impacted soluble Na^+ concentrations in the saturated extracts of all treatments, the total salts in the RO water were low (0.1 dS m^{-1}) and had little impact on the EC_e of the control soils at the end of the experiment.

4.2.2.2 SAR

The saturated extracts of the brine amended soils frequently had an SAR in excess 15, the point beyond which the soils are likely to be sodium impaired. Although the SAR values for the EC3 treatments were statistically equivalent to the control in the cowpea and sorghum sudangrass soils, the SAR values in the EC9 amended soils always were much greater than the control, and the mean values exceeded SAR 15 in every case. This outcome is somewhat alarming considering that it resulted from a single exposure to the simulated petroleum brine. Thus, careful management of the brine during petroleum exploration and extraction will be crucial to ensure the continued productivity of the AG soils.

As discussed previously, elevated SAR of the control samples (2.3 to 2.8 for the cowpea pots, 6.2 to 7.6 for the sorghum sudangrass, and 8.3 to 13 for bermudagrass), was due to the RO water used for irrigation in the greenhouse containing elevated Na^{+-}.

4.2.2.3 Ba^{2+} and Sr^{2+}

Concentrations of Ba^{2+} and Sr^{2+} to brine and BaSr additions at both sampling depths, led to significant increases in Ba^{2+} and Sr^{2+} concentration only in the EC9 additions for both BaSr_low and BaSr_high, and were sometimes, but inconsistently, greater in the BaSr_high than BaSr_low.

However, in all cases, the Ba and Sr concentrations in the Ba,Sr amended soils never became high enough to be of environmental significance.

4.2.3 Exchangeable cations

4.2.3.1 Exchangeable Ca, Mg, K, Na, Ba and Sr

At the initiation of the experiment, Ca^{2+} was the dominant exchangeable cation, and this continued to be the case even after the additions of high levels of brine with significant quantities of Na^+ (for the brine solution, SAR = 75). Had the brine addition been repeated several times, the exchangeable Na^+ eventually would have greatly exceeded Ca^{2+}, particularly in a leaching environment when the displaced Ca^{2+} could have been removed from the brine-impacted soil.

The addition of the EC3 treatment generally had little impact on exchangeable Ca^{2+}, Mg^{2+}, Na^+, and K^+. Only in 5 of the possible 24 treatment x species combinations was a significant increase observed, three of which were Na^+. Of the 72 total treatment x species combinations for EC9, 38 resulted in significant increases in exchangeable cations relative to the control, 17 of which were Na^+. These results reflect the fact that Na^+ was the dominant contaminant cation in the brine. For Ba^{2+} and Sr^{2+}, only the BaSr_high treatments (with or without EC9) resulted in significant increases in exchangeable Ba^{2+} and Sr^{2+} relative to the untreated control.

4.2.3.2 Exchangeable Sodium Ratio (ESR)

The impact of the brine treatments on the soil ESR was similar to that of the major cation ions but far more distinct. Of the six EC3 treatment x plant combinations, only one resulted in a higher ESR value compared to the control, whereas 17 of the 18 EC9 instances resulted in significant ESR increases. This result points out the value of the ESR as a sensitive index of Na impact. The high ESR levels observed in brine-treated soils are above threshold levels (ESR 0.13-0.15) for normal soil health that can allow optimum plant growth (Bernstein, 1964; Pearson, 2003). The elevated ESR values also indicate high potential of Na-hazard and the resultant consequences (such as Na-induced nutrient deficiency, soil dispersion, loss of soil structure, restricted permeability and infiltration, swelling of soil clays, and surface crusting) in brine-exposed soil (Pearson, 2003).

4.2.4 Relationships of exchangeable sodium ratio (ESR), sodium adsorption ratio (SAR), soluble salts, and electrolytic conductivity

4.2.4.1 Relationship of exchangeable sodium ratio and sodium adsorption ratio

Cation exchange selectivity equations are used to predict the distribution of exchangeable cations in soils and can be used to predict the sodium hazard. The Gapon exchange equation is the most commonly used relationship to evaluate soil sodicity (United States Salinity Laboratory Staff, 1954; Oster and Sposito, 1980; Strawn *et al*., 2015):

$$Na^{+} + Ca_{0.5}X \leftrightarrow NaX + 0.5Ca^{2+}$$

$$K_G = \frac{[NaX][Ca^{2+}]^{0.5}}{[Ca_{0.5}X][Na^{+}]}$$

In the context of saturated extracts and SAR:

$$K_G = \frac{[NaX][Ca^{2+}+Mg^{2+}]^{0.5}}{[Ca_{0.5}X + Mg_{0.5}][Na^{+}]}$$

rearranging:

$$\frac{[NaX]}{\{[Ca_{0.5}X] + [Mg_{0.5}X]\}} = K_G \frac{[Na^+]}{([Ca^{2+}] + [Mg^{2+}])^{0.5}}$$

Knowing that:

$$ESR = \frac{[NaX]}{([Ca_{0.5}X] + [Mg_{0.5}X])}$$

and

$$SAR = \frac{[Na^+]}{([Ca^{2+}] + [Mg^{2+}])^{0.5}}$$

then

$$ESR = K_G \cdot SAR$$

K_G is the Gapon exchange constant, commonly ranging from 0.010 to 0.015 (liter/mmole)$^{1/2}$ for most alkaline, saline soils.

ESR-SAR data were plotted for the individual greenhouse soils planted with the three species (Fig. 4.1-4.3) and for all soils combined (Fig. 4.4). A strong linear relationship is exhibited between ESR and SAR, exhibited by high correlation coefficients: r = 0.96 for cowpea soils, 0.79 for sorghum sudangrass, 0.91 bermudagrass, and 0.85 for the three species combined. Gapon's selectivity coefficients of Na^+ (the slopes of the regression lines) were 0.0299 for cowpea soil, 0.0342 for sorghum sudangrass, 0.0289 bermudagrass, and 0.0275 for the combined data. These K_G values are higher than the range 0.010 to 0.015 (liter/mmole)$^{-1/2}$ reported by the U.S. Salinity Laboratory (United States Salinity Laboratory Staff, 1954; Sreenivas and Reddy, 2008). This departure is due, in some part, to the fact that this is a slightly acidic soil rather than the alkaline soils used to develop the model. A larger contributing factor to the departure from $K_G = 0.015$ is the contribution of the high concentrations of soluble (non-exchangeable) Na^+ to the apparent exchangeable Na. Under typical soil conditions, NH_4OAc extractions yield only exchangeable cations, but when salinity is very high, interferences can occur. Amending the soils with the EC3 treatment would create less than a 10% bias in the exchangeable Na data, but some soils with EC9 amendments had high enough residual soluble Na^+ to double the apparent exchangeable Na. When the regressions in Figures 4.1-4.3 were re-run excluding all treatments with EC9, the slopes of the regressions became closer to the typical values of K_G, ranged from 0.013 to 0.021 for the individual species. The slope was 0.013 for cowpea ($r = 0.77$), 0.018 for sorghum sudangrass ($r = 0.73$), and 0.021 for bermudagrass ($r = 0.91$). When soils for all species were analyzed as a single data set, the slope was 0.017 ($r = 0.81$), very close to the upper end of the typical K_G range, and the slope was not significantly different from 0.015 ($P < 0.05$) (data not shown).

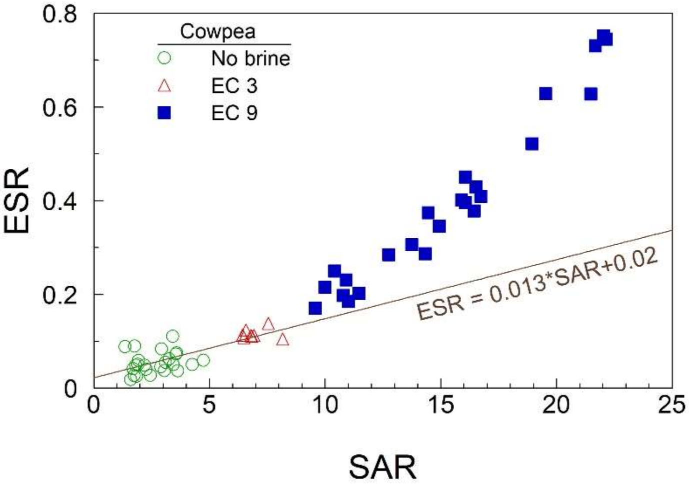

Figure 3.1. Relationship of exchangeable sodium ratio (ESR) with sodium adsorption ratio (SAR) of cowpea soil

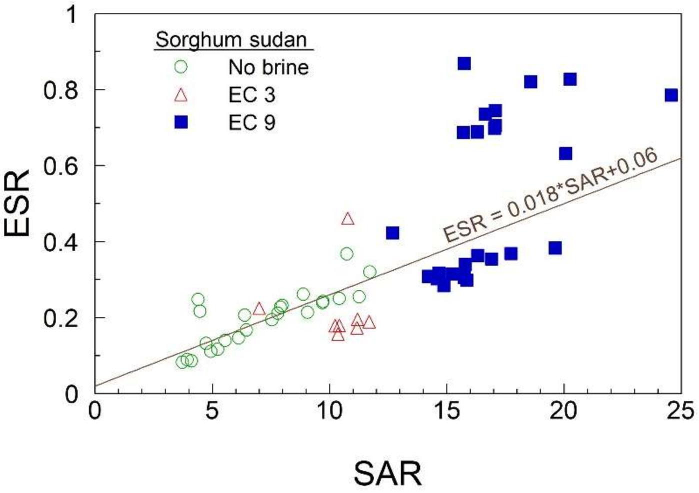

Figure 4.2. Relationship of exchangeable sodium ratio (ESR) with sodium adsorption ratio (SAR) of sorghum sudangrass soil

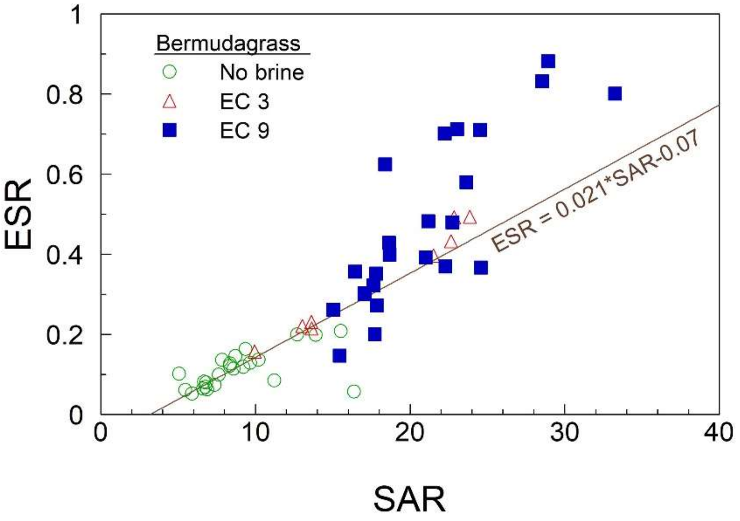

Figure 4.3. Relationship of exchangeable sodium ratio (ESR) with sodium adsorption ratio (SAR) of bermudagrass soil

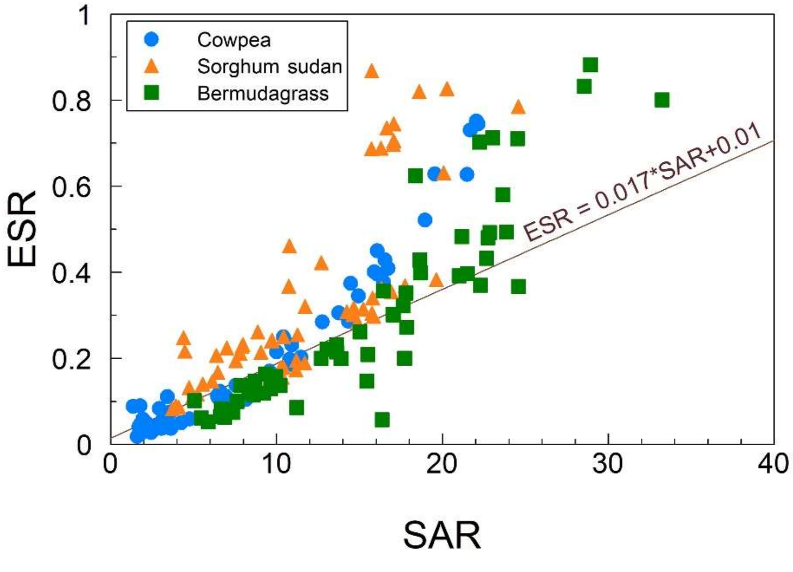

Figure 4.4. Relationship of exchangeable sodium ratio (ESR) with sodium adsorption ratio (SAR) of soil grown with cowpea, sorghum sudangrass and bermudagrass

The ESR-SAR models from the greenhouse data demonstrate that ESR and SAR are directly related and, despite some limitations in the data, the ESR-SAR linear relationship can predict ESR > 0.2 (Evangelou and Coale, 1988). This is of practical significance for the reclamation of sodic soils of ESR > 0.2 to near Na^+ saturation, such as the brine treated soil in our experiment that exceeded threshold SAR = 15 that corresponded to ESR about 0.26 from our models. The ESR-SAR relationship can guide management approaches. For saline sodic soils, one must first leach sodium from the soil while retaining salts in solution and flush them below the root zone, the first step in remediating Na-hazard potential (Sreenivas and Reddy, 2008).

4.2.4.2 Relationship of soluble salts and electrolytic conductivity

The electrolytical conductivity (EC) of saturated extracts is related to the total soluble salts, and several empirical relationships exist. One of the more useful relationships is that between EC_e (dS m^{-1}) and the total cationic charge (Strawn *et al*., 2015; Shahid *et al*., 2018):

$$\Sigma \text{ cations } (\text{mmol}_+ \text{ L}^{-1}) = \Sigma \text{ anions } (\text{mmol}_- \text{ L}^{-1}) = EC_e \text{ (dS m}^{-1}) \times 10$$

From this equation, a solution with $EC_e = 1$ dS m^{-1} would be expected to have a total soluble cationic charge of 10 mmol_c L^{-1} and 10 mmol_c L^{-1} total soluble anionic charge. This relationship was plotted for the saturated extracts of the greenhouse soils (Fig. 4.5-4.8). The regression equations had slopes very close to the theoretical 10 (9.1 to 9.9) with high r^2 values (0.98 to 0.99).

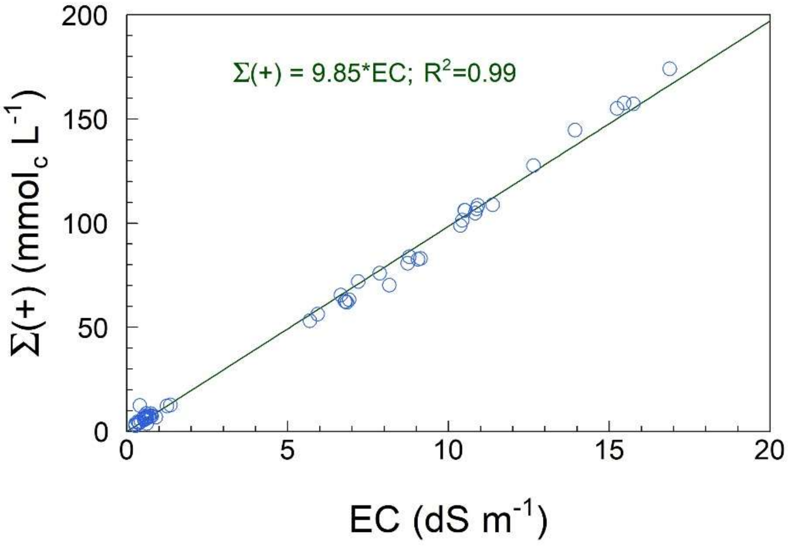

Figure 4.5. Relationship of total soluble salts (TSS) with electrolytic conductivity (ECe) of soil grown with cowpea

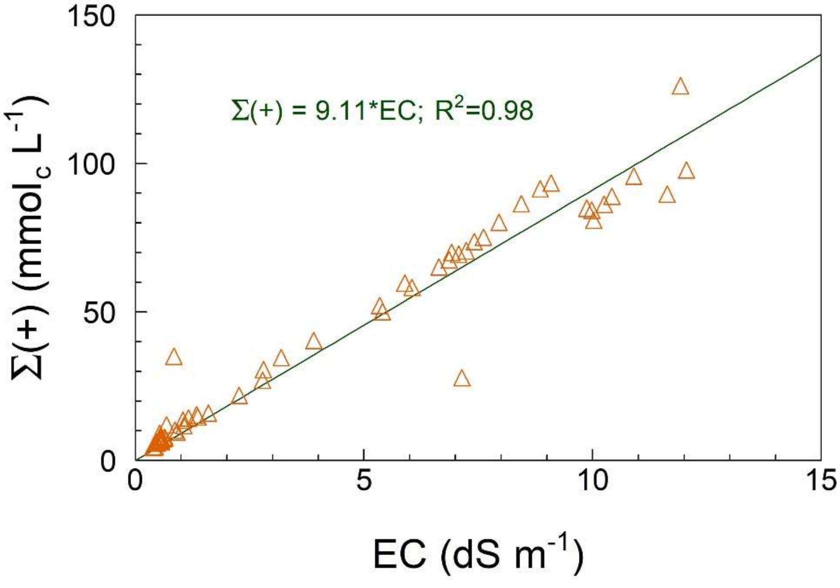

Figure 4.6. Relationship of total soluble salts (TSS) with electrolytic conductivity (ECe) of soil grown with sorghum sudangrass

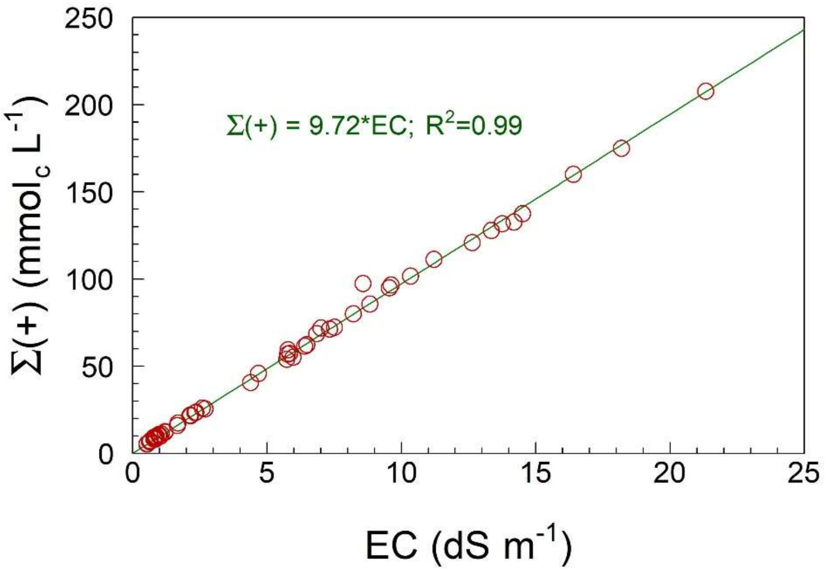

Figure 4.7. Relationship of total soluble salts (TSS) with electrolytic conductivity (ECe) of soil grown with bermudagrass

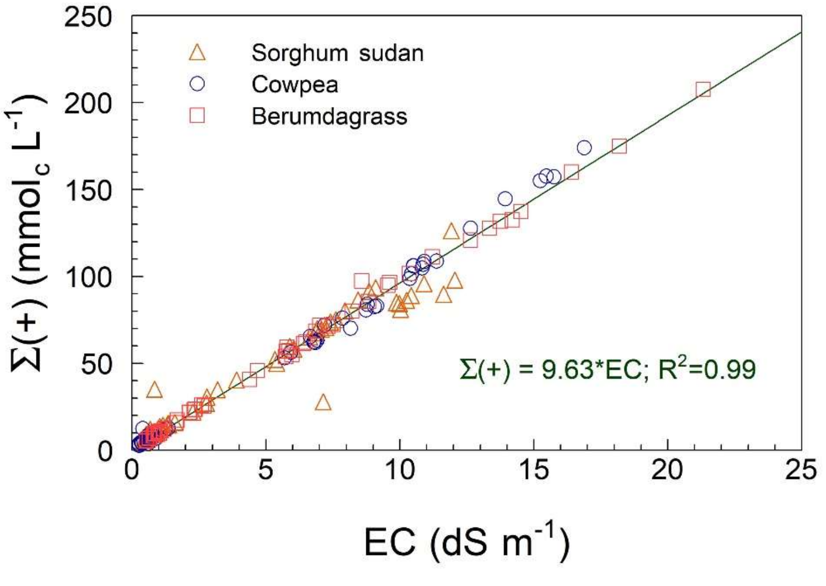

Figure 4.8. Relationship of total soluble salts (TSS) with electrolytic conductivity (ECe) of soil grown with cowpea, sorghum sudangrass and bermudagrass

The great benefit of this relationship is that it is an independent measure of the relative accuracy of the analytical data in the saturation extractions. The close adherence to the theoretical slope and the general lack of outliers (only 4 potential outliers in 165 total data points) suggests an acceptable level of accuracy.

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1. Summary

This study aimed to evaluate the effect of oilfield brine, Ba, and Sr on soil properties and plant growth. Cowpea, sorghum sudangrass, and bermudagrass plants were grown in a greenhouse in soil treated with synthetic oilfield brine at target EC_e levels 3 and 9 dS m^{-1}, and BaSr spiking solution at two concentration levels (high and low). Germination and morphological growth response of plants were recorded. Response to treatments was analyzed and quantified on plants and soil after harvesting: plant biomass and plant tissue cations; soil saturated paste extract pH, EC_e, soluble cations, and SAR; and exchangeable cations and ESR.

The impact of brine application on seed germination varied by species and level of salts. Delayed germination, slow emergence, reductions in percentage emergence, and increased seedling mortality were significant at EC9 but not at EC3. Cowpea was the most severely affected by brine. Bermudagrass particularly suffered stagnated seed germination/seedling emergence (for about 14 days relative to control), while seedling emergence and germination rate were not affected in sorghum sudangrass. Seedling emergence in bermudagrass was more sensitive to salinity than seedling establishment when compared to cowpea and sorghum sudangrass, but bermudagrass exhibited vigorous growth after seedling establishment, while sorghum sudangrass displayed better tolerance during seed germination, seedling emergence, and establishment. Additions of Ba and Sr showed no negative effects on germination of these species

Morphologically, symptoms of salinity stress were highly pronounced in cowpea plants under brine EC9 but less pronounced in sorghum sudangrass and at EC3, while salt-induced morphological effects were unnoticeable in brine-treated bermudagrass plants. In cowpea, brine-affected plants had smaller, deformed and discolored leaves, with multiple foliates instead of the characteristic trifoliate. These plants were commonly stunted, deformed, and possess fewer and/or smaller root nodules compared to non-brine treated plants. Brine-affected sorghum sudangrass plants had slightly delayed leaf growth, fewer number of leaves, and dried leaf tips especially on old leaves. Bermudagrass plants growing in brine EC9 dS m^{-1} displayed the most vigorous growth.

Brine treatments significantly reduced dry shoot and root mass in cowpea; decreased root mass in bermudagrass; but increased shoot and root mass of sorghum sudangrass and shoot mass of bermudagrass. Cowpea biomass decreased for both EC3 and EC9 relative to the unamended control, and sorghum sudangrass biomass decreased for the EC9, but bermudagrass root and shoot biomass were not impacted by treatments. The root to shoot ratio was reduced due to brine in cowpea (at EC3 and EC9) and bermudagrass (at EC3), but increased with both treatments (EC and EC9) for sorghum sudangrass.

Relative to the untreated control, soils treated with brine displayed varied effects on concentrations of major cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) in plant shoot and root, while Ba and Sr had only slight effects in treatments under BaSr_high. Brine enhanced Ca^{2+} and Mg^{2+} content in root but not in shoot, while K^{+} and Na^{+} increased in shoot and root for all the species. The K^{+} and Na^{+} shoot concentrations for brine treatments (at EC9) in all three species and root concentrations (at EC3) in cowpea and bermudagrass were significantly different from control. Under brine, cowpea accumulated the highest Na^+ and K^+ content and bermudagrass accumulated the lowest levels of all cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+). Excessive Na^+ content in cowpea adversely affected growth whereas increased K^+ concentrations appeared to stimulate growth in bermudagrass and sorghum sudangrass.

The Ba+Sr amendment did not result in pronounced effects on the major cations in these plant species probably because of the low concentrations of BaSr applied. Higher Ba^{2+} levels were observed in root than in shoot and higher levels of Sr^{2+} in shoot than in root for cowpea and sorghum sudangrass, while the reverse was observed in bermudagrass. Cowpea (a legume) was the highest bioaccumulator of Ba^{2+} and Sr^{2+}, whereas sorghum sudangrass the weakest for Ba^{2+} and bermudagrass lowest for Sr^{2+}.

Brine additions markedly increased the EC_e, of saturated extracts of the soil and were close to the intended targets 3 dS m^{-1} and 9 dS m^{-1}. All brine treatments tended to significantly increase EC_e with the exception of the EC3 for the lower depth in sorghum sudangrass and bermudagrass. Measured EC_e in brine treated soil (especially for EC9) is above salinity threshold levels most crop species, and cowpea growth was adversely affected. However, sorghum sudangrass and bermudagrass were tolerant to these EC_e levels because their threshold levels are higher than for cowpea.

The brine additions significantly increased concentrations of all the soluble cations in all EC9 treatments for both soil sampling depths and across all three plant species. Typical increases in

Ca^{2+} concentrations were from 1.0-2.5 $mmol_c\ L^{-1}$ in the control to 13 to 29 $mmol_c\ L^{-1}$ in treatments. Concentrations of Mg^{2+}, K^+ and Na^+ increased by 10-fold or more for EC9 treatments versus the controls. The cation concentrations tended to not respond significantly to the EC3 treatment in the 0-4 cm samples. The EC9 treatments consistently increased the salinity to levels high enough for the soils to be considered salt damaged. Soluble Ba^{2+} and Sr^{2+} responded to Ba and Sr additions, but the only significant increases in soluble Ba^{2+} and Sr^{2+} concentration were observed in the EC9 additions for both BaSr_low and BaSr_high. The Ba and Sr concentrations in the BaSr amended soils never became high enough to be of environmental significance.

A complicating factor in the soil analyses was the discovery of high Na concentrations in the irrigation water. The source of the water was a reverse osmosis system implemented to mitigate the very high concentrations of Na in the College Station tap water (approximately 800 mg L^{-1} with an SAR of 28). Although the RO system removed the majority of the salts, the RO water still contained nearly 20 mg/L Na with an SAR of 14. Months of irrigation with this water clearly impacted the control samples, raising the SAR from 0.2 prior to the greenhouse experiment to a mean SAR 7 by the experiment's end. The ESR values of the control soils increased similarly from an initial value of ESR 0.08 to a final mean value of ESR 0.13. This unfortunate problem tended to mask the impact of the EC3 treatments but had less effect on the EC9. The EC3 additions provided solutions of SAR 8.5 (less than the SAR 14 of the irrigation water), whereas the EC9 solutions were SAR 21. As a result, the prolonged source of high SAR irrigation water was enough to lessen the relative impact of EC3 but was still overwhelmed by the EC9 amendment.

The addition of the EC3 treatment slightly (but not significantly) increased exchangeable Ca^{2+}, Mg^{2+}, Na^+, and K^+, while EC9 resulted in significant increases in exchangeable cations relative to the control. Significant increases in exchangeable Na^+ were observed in most treatments at both EC3 and EC9 because Na^+ was the dominant contaminant cation in the brine. Consequently, almost all EC9 treatments resulted in significant ESR increases while only one EC3 treatment x plant combination resulted in a statistically higher ESR value compared to the control. The high ESR levels observed in EC9 treated soil indicate high potential of Na-hazard, above threshold levels for normal soil health.

The Gapon selectivity exchange equation exhibited a strong linear relationship between ESR and SAR for all soils, and the calculated Gapon' selectivity coefficients of Na^+ ranged from 0.027-0.034. These K_G values are higher than the range 0.010 to 0.015 $(\text{liter/mmole})^{-1/2}$ for most alkaline, saline soils. Plotting EC_e versus total cations of saturated extracts of the greenhouse soils also showed strong relationship between EC_e ($dS\ m^{-1}$) and the total cationic charge: Σ cations ($mmol_+\ L^{-1}$) = EC_e ($dS\ m^{-1}$) × 10. The regression equations revealed close adherence to the theoretical slope 10 (9.1 to 9.9).

5.2. Conclusions

The objective of this study was to determine the potential impacts of an accidental spill of petroleum brine on the soils and plants in the Albertine rift basins. Brine is a typical and necessary part of oil well development and extraction, and spills are commonplace. This greenhouse study attempted to replicate such an event using a simulated brine solution based on published compositions and a soil with properties similar to those found in the impacted areas of

Uganda. Two levels of brine were added to the soil to simulate a single minor spill (EC3) and a single severe event (EC9). Concentrations of the major components of the brine were based on those projected for use by the CNOOC oil company. Most of the components are non-toxic salts, so Ba and Sr were added at typical levels to investigate their environmental impacts.

The impacts of the brine additions on soil properties were evident. Changes in pH were slight, but EC_e of the soils at the end of the experiment were similar to the target values of 3 and 9 dS m^{-1} in the EC3 and EC9 treatments, respectively. The EC_e of the controls remained low, <1 dS m^{-1}. The SAR of the treated soils were in concert with the treatments: control SAR values increased to a mean of SAR 7 (due to Na contaminated irrigation water), SAR increased to a mean of 12 in the EC3 treatments; and SAR increased to 18 in EC9 treatments. The trends in the ESR were similar: ESR 0.13 for the controls, 0.22 for EC3, and 0.46 for EC9. The ESR values in EC9 treatments often were excessively elevated due to biases introduced by residual soluble salts from the brine additions.

Three plant species were chosen to represent plants that could be grown in the Albertine Graben, including a salt sensitive edible crop (cowpea), a salt tolerant forage crop (sorghum sudangrass), and a salt tolerant pasture grass (bermudagrass). Seeds of the plants were sown in the treated soils, and after several months of growth in the greenhouse, the samples were chemically analyzed to assess the impacts of the salinity, sodicity, and Ba/Sr on the soils and plants. The germination results seemed to mostly reflect the size of the seeds: cowpea (very large seeds) was unimpacted by the brine additions whereas sorghum (small seeds) and bermudagrass (very small seeds) were negative affected. Final biomass of the plants brought out clear distinctions in the salt tolerance. Cowpea shoot and root biomass were greatly affected by the brine to the point that the EC9 plants were struggling to survive. The roots and shoot biomass of bermudagrass were not affected by either level of brine addition. For bermudagrass, although the roots were unaffected by the brine, shoot biomass actually increased in response to both levels of brine addition.

The inevitable conclusion of this study is that a single spill of brine solutions can result in significant damage to soils and vulnerable plants. The equivalent of an EC3 contamination event significantly elevated the soil salt content and sodicity, but the levels were below those considered to be most damaging to the soil and crops. A spill event similar to the EC9 treatment, however, would be highly damaging to soils both in terms of salinity and resulting exchangeable Na ratios. The greenhouse experiment also demonstrated that some of the damage from a brine spill can be avoided by proper selection of plant species. A salt-sensitive species, such as cowpea, would suffer significant loss in yield. The salt-resistant sorghum sudangrass and bermudagrass would be more likely to maintain productivity.

In regions with significant petroleum production, brine spills are difficult to avoid completely. However, proper soil analyses and soil/crop management can give industry and agricultural producers the necessary tools to minimize the impact.

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