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Human urine as a crop fertilizer under saline conditions

Dissertation

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The aim (of education) must be
the training of independently
acting and thinking individuals who,
however, can see in the service to the
community their highest life achievement.

[Albert Einstein 1879 – 1955]

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Author's Declaration

I, **Michael Yongha BOH**, hereby declare that this thesis entitled "Human urine as a crop fertilizer under saline conditions" was written independently as my dissertation for a doctoral degree at the Faculty of Agricultural Sciences in the University of Hohenheim, Stuttgart – Germany.

The works of other authors used in this dissertation have been duly credited. This thesis has not in part or wholly been presented to any other examination board for the award of an academic degree.

Stuttgart, 2nd October 2013 Michael Yongha Boh

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Overview of publications

In compliance with the requirements for a cumulative PhD thesis by the Faculty of Agricultural Sciences, University of Hohenheim, this thesis is based on publications and manuscripts resulting from the PhD research project. Each publication has been compiled and edited according to the requirements of the different publishers. Therefore, the methods for citation and bibliographic style vary between chapters.

Chapter 2:

Michael Yongha Boh, Jörn Germer, Torsten Müller and Joachim Sauerborn (2013). Comparative effect of human urine and ammonium nitrate application on maize (*Zea mays* L.) grown under various salt (NaCl) concentrations. *Journal of Plant Nutrition and Soil Science,* 176:703-711.

Chapter 3:

Michael Yongha Boh, Torsten Müller and Joachim Sauerborn (Expected 2013). Interacting effect of urine, ammonium nitrate and NaCl salinity on sorghum growth and shoot nutrients concentrations. *Journal of Soil Science and Plant Nutrition*. Current status: in review.

Chapter 4:

Michael Yongha Boh and Joachim Sauerborn (expected 2014). Effect of NaCl-induced salinity and human urine fertilization on cultivation substrate chemical properties. *Open Journal of Soil Science*. Current status: accepted.

Chapter 5:

Michael Yongha Boh, Torsten Müller and Joachim Sauerborn (2013). Maize (*Zea mays* L.) response to urine and wood ash fertilization under saline (NaCl) soil concentrations. *International Journal of AgriScience, Vol. 3(4): 333-345*.

1 General introduction

1.1 The problem

Worldwide, salinity and low soil fertility limit the potential harvest of crops in agricultural systems. As the world's population continues to grow rapidly, overcoming these constraints is mandatory if the demand for biobased products (food, feed, fibre, fuel) from agriculture should be secured. Salt affected (saline and sodic) soils which constitute more than 6% of the earth's land surface are common in semiarid and arid areas where precipitation is often insufficient to flush salts from the root zone. Salts cause to decrease crop yields through specific ion toxicity, osmotic pressure and nutrient imbalances (Robbins and Gavlak, 1989; Yadav et al., 2011). Though soluble salts and exchangeable cations in the soil are natural products of rocks and mineral weathering (Bui, 2013) the use of saline irrigation water and poor fertilizer management practices are the main causes of increased soil salinity worldwide (Epstein et al., 1980). An estimated 20% of irrigated land and 2% of rain-fed agriculture areas of the earth's land surface are salt affected (Yildirim et al., 2006). This is expected to increase with an increase in the number of mouths to feed (Munns and Tester, 2008).

Whereas much research has already been carried out on irrigation-induced salinity and crop yields (Grattan and Grieve, 1998), only little is known of the effect of fertilizer application on soil salinity (Jacobs and Timmer, 2005; Mortvedt, 2001). The interactive effects of salts and exchangeable sodium with fertilizer nutrients on crop yields is complex and often depends on the degree of salinity/sodicity, soil nutrients concentrations and availability, the type and amount of fertilizer applied and salt tolerant behaviour of the crop (Feigin, 1985; Flores et al., 2001; Grattan and Grieve, 1998). In salinity-fertility interaction studies, it has been shown that up to a certain level of salinity, the application of fertilizers can mitigate the deleterious effect of salinity on crop growth (Bernstein et al., 1974; Villa-Castorena et al., 2003). Ironically, most fertilizers are salts or can easily become salts through decomposition (e.g. urea to ammonium ions) or oxidation (e.g. elemental sulphur to sulphate). Moreover, commercial inorganic fertilizers are either inaccessible or often too expensive for most farmers in the south (Morris et al., 2007). Poor farmers therefore, often resort to alternative fertilizer sources which necessitate research on how using these alternatives can maximize crop yields under existing saline soil conditions.

The use of human excreta is an age-old practice, but in the last two decades, farmers both in the developed and developing countries have shown a heightened interest in human urine as a source of plant fertilizer (Lienert and Larsen, 2010; Richert et al., 2010; Roma et al., 2013). According to Vinnerås et al. (2006) an adult human being excretes 1.5 l of urine per day which is equivalent to 550 litres per annum. It has been estimated that if harvested and recycled in agriculture, urine can offset the current pressure on fertilizers demand by replacing about 20-25% of commercial fertilizers currently in use for food production globally (Jönsson, 1994; Lind et al., 2001).

There is ample evidence from extensive research both at greenhouse and field scale that urine can substitute commercial inorganic fertilizers (Kirchmann and Pettersson, 1995; Mnkeni et al., 2008; Morghan, 2007; Pradhan et al., 2007). Although urine is a multi-component fertilizer, users of urine often target N as the main nutrient (Lind et al., 2001; Münch and Winker, 2009). Unfortunately, the concentrations of Na^+ and Cl^- salts in urine are often too high, placing an additional risk if used on salt affected soils. On this premise, the World Health Organization has recommended only a restricted use of urine under saline conditions (World Health Organization, 2006). However, there is limited knowledge on the interactive effect of urine and salinity on plant nutrition, growth and changes in soil chemical properties. This thesis seeks to answer the following question: Can urine be used as fertilizer under saline (here NaCl) soil conditions and if yes, to what extent?

1.2 State-of-the-art

1.2.1 Use of human excreta in agriculture – Historical background

The use of human excreta as a source of plant nutrients has existed since ancient times. For thousands of years Chinese farmers collected mixed excreta and applied it onto their farms untreated (Krepp, 1867). This practice became popular among Japanese farmers in the 12th century who started trading in urine and faeces from urban dwellers. They also placed buckets at street corners of villages and towns from whence they collected free human excreta while simultaneously providing public toilets to pedestrians (Matsui, 1997). Today, even with the availability of mineral fertilizers, about 50% of the excreta in Japanese municipalities are collected and returned to agricultural land (Drangert, 1998).

By the second half of the 19th century it was already known that the plant nitrogen fraction of urine was six times higher than that of faeces (Krepp, 1867). However, it was not until the last

two decades that human urine was first used as a fertilizer in scientific experiments (Karak and Bhattacharyya, 2011). Since then there has been a growing interest in urine fertilizer research. Sweden, Germany, Switzerland and Austria are among the leading countries in Europe where urine is promoted as an alternative to mineral fertilizer (Lienert and Larsen, 2010). In some African countries like Republic of South Africa, Ghana, Mali, Burkina Faso and Senegal, human excreta is currently gaining more attention as an agricultural input (Müllegger et al., 2010). Large scale systems for collection and recycling human excreta are in use in Chinese eco-villages from where collected urine is used in fields (Kvarnström et al., 2006).

1.2.2 Content of human urine

Urine is a liquid waste product excreted by the kidney and eliminated from the body in the process of urination. Urine is made up of 95% water and 5% organic solutes and inorganic ions. The main organic solutes in urine are urea, uric acid, creatinine, trace amounts of enzymes, carbohydrates and fatty acids meanwhile inorganic ions are sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), ammonium (NH_4^+), sulphate (SO_4^{2-}) and phosphates (PO_4^{3-}). The concentration of nutrients in urine varies with sex, age, diet, drinking water consumption and region which are the reasons for variations in reports from different researchers (Karak and Bhattacharyya, 2011). Though urine accounts only for 1% of the domestic wastewater by volume, it contains 80% nitrogen (N), 55% phosphorus (P) and 60% potassium (K), which are the major plant nutrients (Jönsson et al. 1997; Jönsson et al., 2000). It is therefore, apt to collect urine separately from domestic wastewater sources.

1.2.3 Urine collection and treatment

Three main principles characterize the handling of human excreta today, namely, “flush-and-discharge”, “drop-and-store” and “sanitize-and-use” (Drangert, 1998). The flush-and-discharge is a water-born sewage system, which involves flushing excreta from toilets to a centralized treatment plant where it is treated and discharged into open water bodies. The high piping costs involved and the demand made on water resources especially for water-scarce regions makes this system expensive and unaffordable to inhabitants in most countries in the southern hemisphere (Ganrot, 2005). According to the World Bank (1992, cited in Drangert, (1998)), less than 5% of sewage in developing countries undergo any form of treatment. Open defecation, pit latrines and their variations represent the drop-and-store systems, which are mainly waterless safe for anal cleansing systems. Experts have blamed

today's sanitation crisis on the improper handling of human excreta, which became a priority issue during the United Nations Millennium Development Summit in the year 2000 (United Nations General Assembly, 2000).

The concept of ecological sanitation (ECOSAN) considers human excreta as a resource rather than a waste. It promotes the safe collection, treatment and recycling of human excreta in agriculture (Esrey et al., 1998; Esrey et al., 2001). Because of the relatively high nutrients and low pathogen content of urine compared to faeces, various systems for source separation and collection of human excreta have been developed and are in use around the world (Kvarnström et al., 2006). These "no-mix" systems differ both in design and sophistication and can be adapted to suit local sanitation requirements. For example, using locally available materials Morghan (2007) suggested models for the construction of low-cost "no-mix" systems that are suitable for rural communities. Urine can be collected pure or diluted with little flush water but for a rapid sanitization process, it is recommended to collect urine undiluted (Maurer et al., 2006).

Unlike faeces, urine from a healthy person is generally sterile and can be used as a plant fertilizer without recourse to any further hygienization. However, even sterile urine can get contaminated from faeces during collection due to dysfunctional collection systems or improper use of urine diversion toilets. It is therefore recommended to sanitize urine before use for crop fertilization (Schönning et al., 2002; Vinnerås et al., 2008). According to Vinnerås et al. (2008) storage periods up to 6 months at about 4 °C or 3 month at temperatures above 20 °C are necessary for a safe handling of urine. A review by Maurer et al. (2006) appraising different treatment processes for collected urine observed that apart from storage, acidification, evaporation at high temperatures, membrane separation and biological processes such as nitrification and anaerobic ammonium oxidation can significantly improve the hygienic status of contaminated urine.

1.2.4 User and crop consumer acceptance

The use of urine based fertilizer and consumption of crops fertilized with human excreta in general is influenced by cultural perception, religious believes and hygienic concerns. However, where the concept of recycling nutrients in human excreta for agricultural purposes has been well comprehended, most farmers prefer urine to faeces; the common argument being that the former is less repulsive and much easier to handle (Duncker et al., 2007; World

Health Organization, 2006). A survey conducted for seven European countries by Lienert and Larsen (2010) showed that over 85% of 900 respondents liked the idea of using urine as a fertilizer and about 70% were willing to purchase food grown with it. In the developing countries most excreta recycling projects have recorded success. This can be explained by the fact that recycling of human excreta is not an entirely new concept (Müllegger et al., 2010).

1.2.5 Salinity of human urine

A potential problem associated with the use of urine based fertilizer is salinity and sodicity. The perceived risk of urine-fertilized soils becoming saline and/or sodic is related to the content of soluble salts especially Na^+ and Cl^- inherently in urine. Germer et al., (2011) have reported a slight increase in soil Na^+ and Cl^- concentration resulting from urine application in a field study with sorghum (*Sorghum bicolor*) in Ghana. The application of 200 mg urine-N significantly increased the concentration of Na^+ in maize plant tissues and of soluble salts in the soil-substrate during a pot experiment in South Africa (Mnkeni et al., 2008). However, it is not clear how urine as a fertilizer would interact with salinity and high exchangeable sodium in a growing medium.

1.3 Objectives

The objectives of this thesis were to a) determine whether urine can be used as a fertilizer under NaCl-saline conditions b) compare the effect of urine and ammonium nitrate fertilization on maize (*Zea mays*) and sorghum growth under different levels of NaCl salinity treatments c) assess the effect of urine fertilization on cultivation substrate chemical composition and as influenced by NaCl treatment d) determine whether supplementary wood ash fertilization can enhance salt and Na^+ tolerance of urine fertilized maize seedlings. To realize these objectives four experiments were conducted and are reported in chapters 2 to 5.

1.4 Outline of thesis

Chapter 1 is an introductory chapter than states the problem under investigation followed by a review of the state-of-the-art knowledge in urine fertilizer use. In chapter 2, the vegetative response of maize to urine compared to ammonium nitrate treatments under different NaCl substrate concentrations is reported. In this study it was hypothesized that i) compared to ammonium nitrate, urine fertilizer will increase substrate salinity and ii) both fertilizers will

enhance maize growth until salinity limits growth and development. Using two amounts of N applications (180 and 360 mg N kg^{-1} substrate) and three NaCl imitated salinity treatments (EC_e 1.3, 4.6 and 7.6 dS m^{-1}), the effects of both N fertilizer sources on substrate electrical conductivity, pH, biomass yield, leaf nutrients concentration, plant height and green leaf area were studied.

The goal of the second study reported in chapter 3 was to evaluate the cumulative effect of urine fertilization and its interaction with NaCl treatment on sorghum growth. In this study sorghum was grown on a substrate previously cultivated with maize and fertilized with either urine or ammonium nitrate to mimic a maize-sorghum rotation system. Sorghum was chosen as a second crop as it is generally more tolerant to salinity than maize. Here, it was hypothesized that i) urine-induced increase in substrate salinity reduces growth of urine-treated sorghum plants compared to those treated with ammonium nitrate, ii) the effect of urine and ammonium nitrate on sorghum growth depends on the level of NaCl salinity. Growth indices measured in this study were total yield, root/shoot ratio and shoot nutrients concentration. Nitrogen use efficiency (NUE) for both fertilizers was compared and the relationship between shoot nutrient concentration and biomass accumulation was evaluated.

In chapter 4, the effect of successive urine application on changes in the chemical properties of the cultivation substrate from two cycles of maize cultivation (cycle I and II) was assessed. The soil properties investigated were exchangeable cations, cation exchange capacity (CEC_p), electrical conductivity, water soluble cations and anions and substrate pH_{KCl}. In this study, it was proposed that i) due to their content in urine, exchangeable cations, water extractable cations and anions in the cultivation substrate will increase with higher urine fertilizer application and from cycle I to cycle II, ii) urine imposes a risk on sodicity which might increase under NaCl-imitated salinity.

The objective of chapter 5 was to test whether the fertilizing effect of urine under NaCl salinity can be improved by addition of wood ash. It was hypothesized that i) supplementary wood ash application would enhance the salt and sodium tolerance of urine-fertilized maize plants ii) regardless of salinity level, combined urine and wood ash fertilization optimizes plant nutrition and improves plant growth compared to urine- or wood ash stand-alone treatments. The growth factors investigated in this study were plant height, SPAD values as an indicator of leaf chlorophyll concentration, shoot dry weight as a determinant of plant

vigour and shoot nutrients concentration. General discussions on the main finding, conclusions and limitations of this study are given in chapter 6.

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2 Comparative effect of human urine and ammonium nitrate application on maize (*Zea mays* L.) grown under various salt (NaCl) concentrations

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Abstract

The present study investigates the effect of urine and ammonium nitrate on maize (*Zea mays* L.) vegetative growth, leaf nutrient concentration, soil electrical conductivity and exchangeable cations contents under various concentrations of NaCl in a soil substrate. The experiment was arranged in a completely randomized block design with eight replications under greenhouse conditions. The experimental soil substrate was made from a 1 : 1 : 1 volume ratio mixture of compost, quartz sand and silty-loam soil. Salinity was induced by adding 0, 15 and 30 ml of 1 M NaCl solution per kg of substrate to achieve electrical conductivities (EC) of 1.3 (S0), 4.6 (S1) and 7.6 (S2) dS m^{-1}, respectively. Nitrogen sources were urine and ammonium nitrate applied at 180 and 360 mg N (kg soil substrate)$^{-1}$. Basal P and K were added as mono potassium phosphate in amounts equivalent to 39 mg P and 47 mg K (kg substrate)$^{-1}$, respectively. In the S0 treatment, a 3-fold increase in EC was measured after urine application compared to an insignificant change in ammonium nitrate-fertilized substrates 62 d after sowing. Under saline conditions application of 360 mg N (kg soil)$^{-1}$ as urine significantly decreased soil pH and maize shoot dry weight. At the highest salt and N dose (S2, N360) 50% of urine-fertilized plants died. Regardless of salinity there was no significant difference between the two fertilizers for investigated growth factors when N was supplied at 180 mg (kg soil)$^{-1}$. Leaf N and Ca contents were higher after urine application than in ammonium nitrate-fertilized plants. At an application rate of 180 mg N (kg soil)$^{-1}$, urine was a suitable fertilizer for maize under saline conditions. Higher urine-N dosages and/or soil salinity exceeding 7.6 dS m^{-1} may have a deleterious effect on maize growth.

Keywords: Ammonium nitrate / electrical conductivity / fertilization effect of urine / sodium toxicity / soil salinity

2.1 Introduction

Soil salinity is a worldwide threat to crop production (*Läuchli* and *Lüttge*, 2002), as it affects plants through osmotic stress, ion toxicity and nutritional imbalances. Soil sodicity caused by excessive soil exchangeable sodium and high pH can further compromise plant vigor by destroying soil aggregates and decreasing permeability (*Pascale* et al., 2005). Saline soils originate naturally from weathering of salt-bearing rocks (*Richards*, 1954) though the use of saline irrigation water and fertilizers with high salt indexes also frequently and significantly increase root-zone salinity and/or sodicity (*Bunt*, 1988). *Mortvedt* (2001) has cautioned that the addition of salt should be considered in irrigation and fertilization planning. This is particularly important in semiarid and arid environments where precipitation amounts are usually too low to leach salts from the top soil. In low-income countries, subsistence farmers often tend to use the most affordable fertilizers without considering their salt effect (*Penov* et al., 2011). Even so, under intrinsically saline conditions and where nutrients are limiting, proper use of fertilizer may ameliorate the detrimental effect of salinity (*Villa-Castorena* et al., 2003; *Grattan* and *Grieve*, 1998).

Nitrogen is an important nutrient often needed in large amounts by plants. The form and rate at which N is supplied to plants under saline conditions is important. *Elgharably* et al. (2010) reported a beneficial effect on wheat growth following the addition of 100 mg N (kg soil)$^{-1}$ as NH_4-N or NH_4NO_3-N, whereas NO_3-N caused depression in saline soils. Increasing N rates and soil salinity levels interacted to reduce pod yields of Chile pepper (*Villa-Castorena* et al., 2003), leaf area, dry weight and P concentration of *Ficus benjamina* L. (*Cokuysal* et al., 2006) and shoot and root dry weights of sorghum (*Elgharably* et al., 2010).

Human urine is a rich source of plant-available N with appreciable amounts of P, K, Mg, Ca, Zn, and Fe. Therefore, it can substitute commercial inorganic fertilizers (*Kirchmann* and *Pettersson*, 1995; *Ganrot* et al., 2007) especially in developing countries where fertilizers are often too costly for subsistence farmers. Except from faecal cross-contamination, urine from a healthy person poses no hygienic risks to users (*Schönning* et al., 2002). For a safe and unrestricted use of human urine in agriculture, storage at temperatures above 20°C for 6 months and/or acidification is recommended (*Maurer* et al., 2006; *World Health Organization*, 2006; *Vinnerås* et al., 2008). The efficacy of human urine fertilizer to enhance crop production has been validated by trials with maize in Zimbabwe (*Morghan*, 2007),

sorghum (*Sorghum bicolour* (L.) Moench) in Ghana (*Germer* et al., 2011), maize and vegetables in South Africa (*Mnkeni* et al., 2008), and pumpkins (*Cucurbita maxima* L.) in Finland (*Pradhan* et al., 2009). In the aforementioned studies, urine was primarily a source of N while supplemental P and K were added. So far, negligible attention has been paid to the effect of other plant nutrients in urine on plant performance and soil cation status. Urine is inherently saline and its use as fertilizer may increase soil Na and Cl concentrations *(Germer* et al., 2011). *Haynes* and *Williams* (1992) have reported that the concentration of exchangeable cations in the soil solution can be influenced by urine deposition on pasture land. Therefore, the salinity and sodicity status of soil and irrigation water should be considered to determine if and when urine can be used as a fertilizer.

In spite of the existing studies on crop fertilization with human urine, little is known about its effect on soil salinity. Our study aimed to compare the effect of N supplied as urine or ammonium nitrate on changes in soil substrate EC and exchangeable cations and on the growth of maize under saline and non-saline conditions. We hypothesized that 1) compared to ammonium nitrate, urine fertilization will increase soil salinity and 2) both urine and ammonium nitrate fertilizers will enhance maize growth until salinity limits plant growth and development. This study should serve as an important basis for future considerations when determining the levels of soil salinity and urine application on maize production.

2.2 Material and methods

The experiment was conducted in a greenhouse at the University of Hohenheim in Stuttgart, Germany, and constituted a completely randomized block design with three soil substrate salt concentrations, two fertilizer types and two fertilizer amounts with eight replicates for each treatment (Tab. 2.1). The substrate was composed of quartz sand, silty-loam and bio waste compost mixed in a 1 : 1 : 1 volume ratio to mimic arable soil. The components were air-dried, passed through a 2 mm sieve and homogenized with a concrete mixer. Total N and C in the final substrates were measured by infrared absorption using an elemental analyser (Vario EL, Elementar, Hanau, Germany). Plant-available P was determined using the Bray-II method (*Bray* and *Kurtz*, 1945). P in the extracts was measured with inductively coupled plasma optical emission spectrometry (ICP-OES) using a Varian Vista Pro instrument. Exchangeable K^+, Na^+, Ca^{2+}, and Mg^{2+} in the soil were extracted with 1 M NH_4Cl (1 : 10; soil to solution ratio) for 1 h at room temperature using a mechanical shaker and filter paper (Whatman No. 1). The

concentrations in the extracts were measured with ICP-OES (*VDLUFA*, 2009). Substrate electrical conductivity (EC) was measured from a saturated extract and pH in 1 M KCl, 1 : 2.5 (soil to solution) suspensions. Salinity was induced by adding 0, 15 and 30 mL of 1 M NaCl solution per kg of substrate during homogenization with a concrete mixer to achieve EC values of 1.3 (S0), 4.6 (S1) and 7.6 (S2) dS m^{-1}. Nitrogen fertilization was 180 and 360 mg N (kg soil substrate)$^{-1}$, supplied either as urine or ammonium nitrate (Sigma-Aldrich Chemie GmbH, Germany). Due to their low content in the prepared soil substrate, basal P and K were added as mono potassium phosphate in amounts equivalent to 39 mg P and 47 mg K (kg soil substrate)$^{-1}$ during homogenization. Twelve kg of prepared substrate were filled into each of the 10 L Mitscherlich pots.

Table 2.1: Experimental design (*n* = 8).

Substrate electrical conductivity (EC) /dS m^{-1}	**Nitrogen dosages** /mg (kg soil substrate)$^{-1}$	
	Urine	Ammonium nitrate
1.3 (S0)	180	180
	360	360
4.6 (S1)	180	180
	360	360
7.6 (S2)	180	180
	360	360

Urine was collected from eight students over several weeks and subsequently stored in an air-tight plastic container in the greenhouse for three months, at a daily mean temperature of 25 ± 2°C for sanitization. Chemical analyses of the same elements as in soils were carried out for urine samples taken just before the first dose of application (Tab. 2.2). Additionally, the nitrogen concentration of the added urine was measured prior to each application to detect potential losses that might have resulted from volatilization. Less than 5% of N content was lost from urine between the first and final urine application. At the time of first application, over 95% of N in urine was in the form of ammonium. One third of the targeted N was applied a week before sowing and the remainder in two dosages 21 and 55 d after sowing (DAS), respectively.

Table 2.2: Chemical composition of unsalinzed soil substrate and urine at the start of experiment.

Element	Soil substrate	Element	Urine
Total N / %	0.1±0.0[a]	Total N / g L^{-1}	8.4±0.1
Total C / %	1.6±0.1	Total C / g L^{-1}	7.2±0.1
Ca^{2+} / g kg^{-1}	5.7±0.6	Ca^{2+} / g L^{-1}	0.3±0.0
Mg^{2+} / g kg^{-1}	0.5±0.0	Mg^{2+} / g L^{-1}	0.1±0.0
K^{+} / g kg^{-1}	0.2±0.0	K^{+} / g L^{-1}	1.4±0.1
Na^{+} / mg kg^{-1}	33±4.5	Na^{+} / g L^{-1}	2.7±0.1
Cl^{-} / mg kg^{-1}	4.6±1.0	Cl^{-} / g L^{-1}	3.4±0.1
Bray II-P / mg kg^{-1}	6.8±1.3	Bray II-P / g L^{-1}	0.5±0.0
EC / dS m^{-1}	1.3±0.0	EC / dS m^{-1}	25±1.7
pH	7.2±0.3	pH	8.3±0.8

[a] Values are means of four samples ± one standard deviation.

Four maize (*Zea mays* L. cv. Okomasa) seeds were sown per pot and thinned to two seedlings at 7 DAS. Pots were regularly irrigated with tap water (EC: 0.05 dS m^{-1}) to maintain a soil-moisture content of about 80% throughout plant growth. When it occurred, leachate was collected by means of plastic bowls placed below each pot and returned to the respective soils to maintain the same level of soil salinity as at start of experiment and allow for salt build-up resulting from fertilization.

Mean plant height per pot was measured from soil surface to collar of the youngest fully expanded leaf at 20, 40, 80 and 100 DAS. Green leaf area was determined as product of the length and width of all fully expanded leaves per plant at 40 and 80 DAS using the formula: $A = L \times W \times 0.75$, where A = leaf area, L = leaf length measured from collar to tip of the leaf, W = maximum leaf width. Summation of individual leaf area per plant gave the total plant green leaf area (*Pearce* et al., 1975).

At the onset of tasseling, the above-ground biomass of one plant per pot was harvested, separated into leaves and stem, weighed, oven-dried at 60°C to constant weight and weighed again to determine fresh and dry matter weight. The remaining plants were harvested 2 weeks after the onset of tasseling, separated into stem and leaves, oven-dried for fresh and dry matter determination and analyzed for N, P, K, Ca, Mg and Na. Nitrogen was analyzed with the previously explained method. Leaf P, K, Ca, Mg and Na were measured with ICP-OES from samples digested in a microwave-heated high-pressure digestion system (Ultra Clave II, MLS GmbH, Germany; *VDLUFA*, 2009). Soil EC was measured 1 week following each fertilizer dose as described above. At the end of the trial, soil from each pot was hand-crushed and passed through a 2 mm sieve. Homogeneous samples of each pot were analyzed for the same elements as at the start of the experiment.

2.2.1 Data analyses

Data were subjected to two- and three-way ANOVA using SAS statistics (SAS Institute, version 9.2) with substrate salinity (S), nitrogen source (N_S) and nitrogen fertilization amount (N_R) as fixed factors and their interactions (S × N_S, S × N_R, N_S × N_R and S × N_S × N_R) are shown. Dependent factors were above-ground fresh and dry matter yield, leaf nutrient concentration, plant height, green leaf area, substrate EC, pH and exchangeable cation level. A square root transformation of data for leaf P and Na and log transformation of leaf Ca data was carried out to fulfil the conditions for normality. Tukey's tests were carried out to detect significant differences of means at $P \leq 5\%$. As half the urine-fertilized plants in the S2 treatment that received 360 mg N (kg substrate)$^{-1}$ were dead at 60 DAS (that is, after the second urine dose), statistical analyses for this variant were performed on $n = 4$.

2.3 Results

2.3.1 Soil chemical composition

a. Electrical conductivity (EC)

In the S0 treatment, no significant difference in EC between urine and ammonium nitrate-fertilized soils was measured at 7 DAS. However, a significantly higher EC was measured in urine than ammonium nitrate-fertilized soils in the S1 and S2 treatments (Fig. 2.1). Electrical conductivity increased with subsequent applications of both urine and ammonium nitrate at all substrate salinities as measured at 28 and 62 DAS. In the S0 treatment, application of urine resulted in an approximately 3-fold increase in EC at 62 DAS compared to an insignificant change due to ammonium nitrate application.

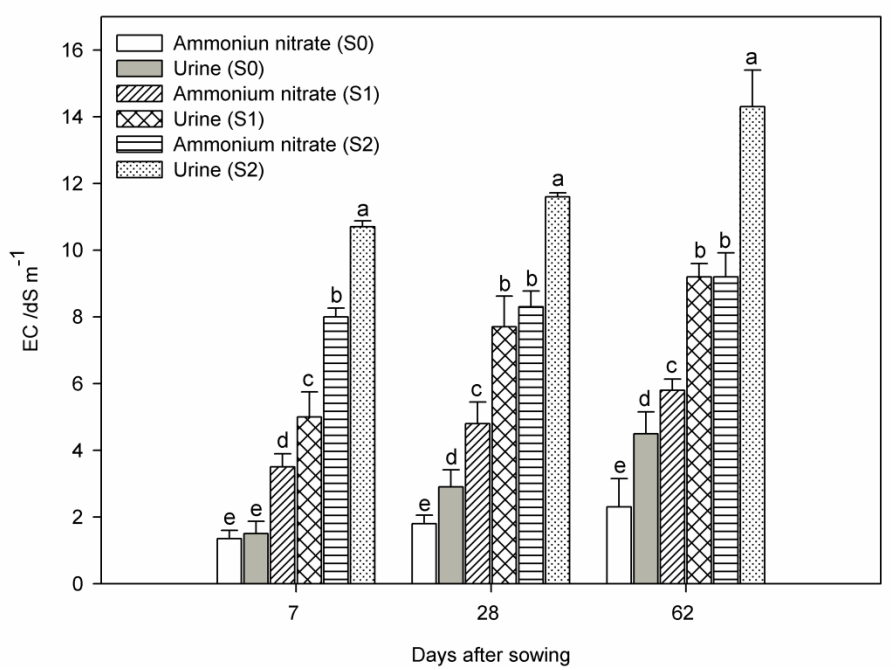

Figure 2.1: Effect of urine and ammonium nitrate application at different substrate salinity levels (S0, S1 and S2) on substrate electrical conductivity (EC) during pot trial. Significant differences between fertilizer types at different substrate EC were determined separately for each sampling day. Values represent means ± SE; *n* = 12. Bars with different letters are significantly different at $P \leq 5\%$.

b. Exchangeable cations in the substrate

Exchangeable Na^+ increased as salinity rose, but there was no significant effect of fertilizer type or amount (Tab. 2.3). Substrate exchangeable K^+ was significantly affected by salinity × N source × N amount interactions. Following an increase in urine application, exchangeable K^+ increased by 18% and 20% in the S1 and S2 treatments, respectively (Tab. 2.3). Furthermore, exchangeable K^+ was 25% and 32% higher in urine than ammonium nitrate-fertilized soil substrates at S1 and S2, respectively. Compared to ammonium nitrate treatments, substrate exchangeable Mg^{2+} and Ca^{2+} were higher in urine treatments at S2 level and with the application of 360 mg N (kg substrate)$^{-1}$. A significant increase in soil exchangeable Ca^{2+} was also observed at S2 level following an increase in urine application (Tab. 2.3).

c. pH

There were significant salinity × N source, salinity × N amount and N source × N amount interaction effects on substrate pH (Tab. 2.3). In the S1 and S2 treatments, significant reductions by 0.2 and 0.3 pH units, respectively, were measured for urine-fertilized substrates that received 360 mg N (kg substrate)$^{-1}$ compared to those that received 180 mg N (kg soil substrate)$^{-1}$. Compared to 180 mg N (kg substrate)$^{-1}$, the application of 360 mg urine-N (kg

substrate)$^{-1}$ significantly reduced substrate pH by 0.2 and 0.3 units in the S1 and S2 salt treatments, respectively. Furthermore, soil substrates fertilized with 360 mg urine-N (kg substrate)$^{-1}$ were by 0.2 and 0.4 pH units significantly lower than those fertilized with the same amount of N in the form of ammonium nitrate in the S1 and S2 treatments, respectively.

Table 2.3: Effect of substrate salinity level (S0, S1, S2), nitrogen source (urine, U; ammonium nitrate, AN) and nitrogen amount on exchangeable cations and pH

Substrate salinity level (EC)/ dS m^{-1}	**N Source/amount** /mg (kg soil substrate) $^{-1}$	**Na^+** /g kg^{-1}	**K^+** /g kg^{-1}	**Mg^{2+}** /g kg^{-1}	**Ca^{2+}** /g kg^{-1}	**pH**
1.3 (S0)	U1 (180)	0.11±0.08f	0.17±0.01ed	0.39±0.01b	5.4±0.16ab	6.8±0.07ab
	U2 (360)	0.25±0.05def	0.18±0.02ed	0.43±0.02ab	5.7±0.16a	6.7±0.07bc
	AN1 (180)	0.15±0.20ef	0.16±0.00e	0.39±0.02b	5.5±0.11ab	6.8±0.04ab
	AN2 (360)	0.08±0.09f	0.16±0.02e	0.39±0.02b	5.5±0.13ab	6.7±0.08ab
4.6 (S1)	U1 (180)	0.49±0.30bcd	0.19±0.02ed	0.40±0.02b	5.4±0.20b	6.8±0.06ab
	U2 (360)	0.48±0.07bcd	0.24±0.02b	0.42±0.02ab	5.6±0.12ab	6.6±0.06c
	AN1 (180)	0.36±0.04cd	0.17±0.02ed	0.40±0.01b	5.4±0.11b	6.8±0.04a
	AN2 (360)	0.40±0.06de	0.18±0.02ed	0.40±0.01b	5.5±0.12ab	6.8±0.08a
7.6 (S2)	U1 (180)	0.83±0.25a	0.23±0.04bc	0.42±0.02ab	5.4±0.14b	6.7±0.11abc
	U2 (360)	0.82±0.10a	0.28±0.04a	0.45±0.04a	5.7±0.16a	6.4±0.10d
	AN1 (180)	0.72±0.15ab	0.19±0.02cd	0.41±0.02b	5.3±0.10b	6.8±0.07a
	AN2 (360)	0.67±0.14abc	0.19±0.02ed	0.40±0.01b	5.4±0.10b	6.8±0.08ab
S		***	***	*	ns	*
N_S		ns	***	***	***	***
S × N_S		ns	***	ns	*	***
N_R		ns	***	*	***	***
S × N_R		ns	*	ns	ns	ns
N_S × N_R		ns	***	***	***	***
S × N_S × N_R		ns	*	ns	ns	ns

Values are means ± SD; *n* = 8. Means within columns followed by different lower-case letters are significantly different at $P \leq 5\%$ according to Tukey test. The results of a two and three-way ANOVA with substrate salinity (S), nitrogen source (N_S) and nitrogen fertilization amount (N_R) as fixed factors and their interaction; S × N_S, S × N_R, N_S × N_R and S × N_S × N_R are shown: $^{***}P < 0.1$; $^{**}P < 1\%$; $^{*}P < 5\%$; ns, not significant. This applies to all subsequent tables.

2.3.2 Biomass yield

Leaf, stem and shoot fresh and dry weights were significantly reduced by increasing substrate salinity (Tab. 2.4). At an application rate of 360 mg N (kg substrate)$^{-1}$ in the S1 treatment, the fresh weights of leaf, stem, shoot and shoot dry weight were 64%, 66%, 67% and 28% lower in urine than in ammonium nitrate-fertilized plants, respectively. Except for stem fresh weight, a similar pattern of biomass reduction was observed in the S2 treatment at the same N application rate. Here, shoot dry weight was 37% lower in the urine than in the ammonium nitrate treatment (Tab. 2.4).

Table 2.4: Effect of substrate salinity (S0, S1 S2), urine (U) and ammonium nitrate (AN) fertilization amounts on fresh and dry matter yield of maize leaf, stem and shoot[a]

Substrate salinity /dS m^{-1}	**N source amount** /mg (kg soil substrate)$^{-1}$	**Leaf**		**Stem**		**Shoot (Leaf + Stem)**	
		Fresh weight /g $plant^{-1}$	Dry weight /g $plant^{-1}$	Fresh weight /g $plant^{-1}$	Dry weight /g $plant^{-1}$	Fresh weight /g $plant^{-1}$	Dry weight /g $plant^{-1}$
1.3 (S0)	U1 (180)	63.6±12.4ab[§]	20.7±3.0abc	83.6±22.6ab	7.9±1.2a	147.2±34.4ab	28.6±3.7a
	U2 (360)	61.5±14.5ab	22.0±3.1a	88.7±10.6ab	7.5±1.5ab	150.2±23.4ab	29.5±3.6a
	AN1 (180)	75.8±13.3a	20.5±4.1abc	96.0±18.1a	9.0±1.0a	171.8±29.5a	28.3±2.8ab
	AN2 (360)	67.5±11.5a	21.1±6.3ab	76.1±12.6abcd	7.5±1.2ab	143.6±22.6ab	28.6±6.2a
4.6 (S1)	U1 (180)	58.7±10.8ab	15.2±3.4bcde	80.2±17.1abc	6.9±1.2abc	140.0±26.3abc	22.2±2.7bcd
	U2 (360)	22.4±14.9de	13.3±5.1de	32.2±18.1e	5.2±1.1c	50.6±33.6ef	19.3±5.3de
	AN1 (180)	63.1±04.8ab	18.3±3.1abcd	93.6±11.9a	7.7±1.2ab	156.7±15.6ab	26.1±2.9abc
	AN2 (360)	61.6±09.1ab	19.5±2.6abcd	93.4±15.6ab	7.2±1.8abc	155.1±29.4ab	26.7±2.4abc
7.6 (S2)	U1 (180)	33.7±16.2cde	11.2±3.6e	51.9±15.4cde	5.1±1.0c	72.7±43.1def	17.0±4.3de
	U2 (360) †	18.9±07.9e	08.9±3.3e	30.3±11.2e	4.0±1.0c	30.1±23.9f	12.6±1.2e
	AN1 (180)	43.6±14.9bc	13.9±2.5cde	50.0±17.3de	5.0±1.0c	93.6±24.8bcd	18.8±2.3de
	AN2 (360)	45.1±16.0bc	14.1±2.8de	63.1±22.5bcde	5.7±1.6bc	108.3±36.1cde	20.1±3.0cd
S		***	***	***	***	***	***
N_S		***	***	***	**	**	***
$S \times N_S$		ns	**	***	ns	**	**
N_R		***	ns	**	ns	ns	ns
$S \times N_R$		ns	ns	ns	ns	**	ns
$N_S \times N_R$		**	ns	**	ns	**	ns
$S \times N_S \times N_R$		*	ns	***	ns	**	ns

[a]Values are means ± SD (n = 8). †Signifies n = 4 due to death plants after second urine dose. [§]Means within columns followed by different lowercase letters differed significantly at $P < 5\%$ according to Tukey test.

2.3.3 Leaf nutrient concentrations

Substrate salinity significantly affected total leaf N, P, K, Ca and Na concentrations (Fig. 2.2A-E). Leaf N was higher in urine than in ammonium nitrate-fertilized plants in the S0 and S2 treatments. The highest N concentration (approximately 3%) was measured in leaves of maize plants fertilized with urine in the S2 treatment. Leaf P concentration rose with an increase in substrate salinity level from S1 to S2 for both fertilizers (Fig. 2.2B). While salinity increased leaf Na, it decreased leaf K concentrations (Fig. 2.2C and D). N source did not affect leaf concentrations of both elements though there was a tendency towards higher Na concentrations with urine fertilization. Higher leaf Ca concentrations were measured in urine compared to ammonium nitrate-treated plants at all substrate salinity levels (Fig. 2.2E). In the S2 treatment, leaf Mg was significantly higher in urine than in the ammonium nitrate-fertilized plants (Fig. 2.2F).

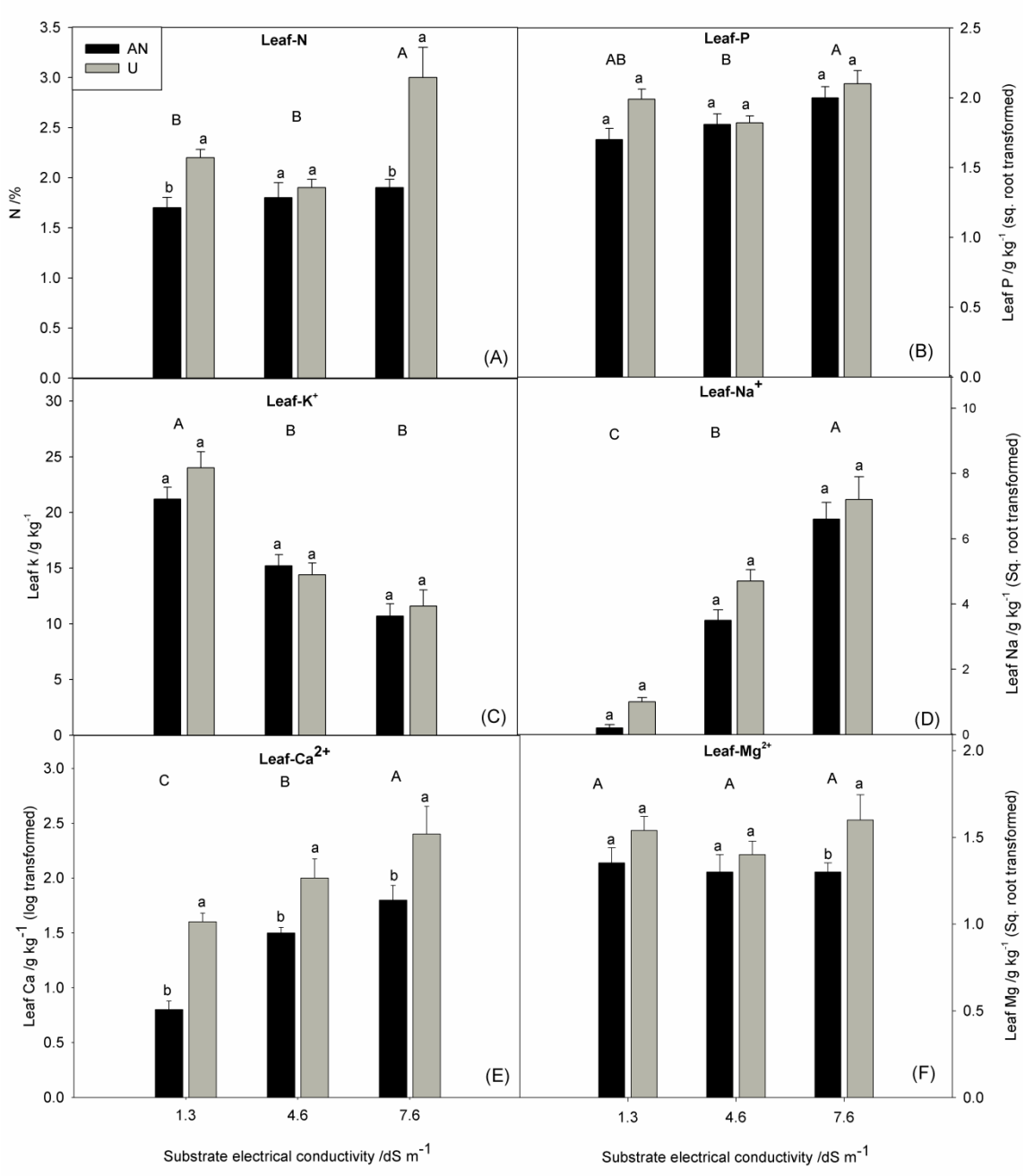

Figure 2.2: Effect of urine (U), ammonium nitrate (AN) and substrate electrical conductivity on maize leaf concentrations of N, P, K, Na, Ca and Mg at the end of pot trial. Values are averages of two N amounts and 8 replications (i.e. *n* = 16; ± SE). Different lower-case letters indicate significant differences ($P \leq 5\%$) between U and AN application within one salt level. Different upper case letters indicate significant differences between salt concentrations at $P \leq 5\%$.

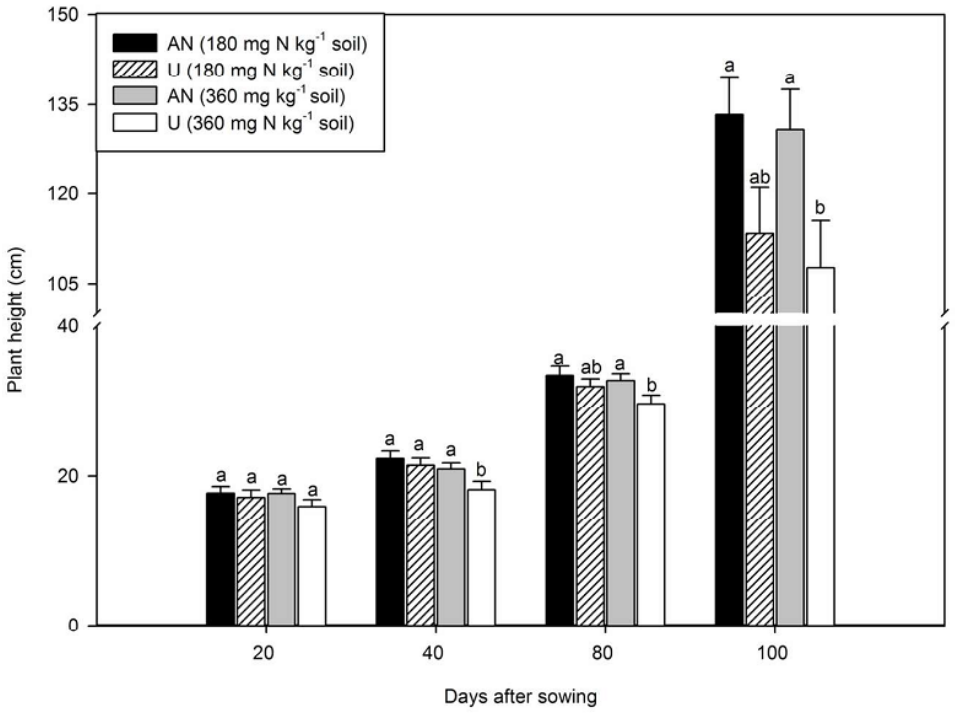

Figure 2.3: Effect of ammonium nitrate (AN) and urine (U) application amount on maize plant height measured during growth. Mean differences between fertilizer type and application amounts were determined separately for each respective number of days after sowing. Histograms represent means ± SE (*n* = 24). Bars with different letters are significantly different at *P* ≤ 5%.

2.3.4 Plant height

Regardless of salinity, an increase in urine application from 180 to 360 mg N (kg substrate)$^{-1}$ significantly reduced maize height ($P < 5\%$) at 40 DAS; ammonium nitrate-fertilized plants at the two application levels were not significantly different (Fig. 2.3). Nitrogen amount had no significant effect on maize height at 20, 80 and 100 DAS. With the application of 360 mg N (kg substrate)$^{-1}$, urine-fertilized maize plants were significantly shorter than those fertilized with ammonium nitrate 40, 80 and 100 DAS. An increase in substrate salinity significantly reduced maize height in all measurements ($F = 72$, $df = 2$, $P < 0.01\%$; data not shown). In the S2 treatment, half of the urine-fertilized plants receiving 360 mg N (kg substrate)$^{-1}$ died one week after the application of the final dose.

2.3.5 Green leaf area

At S2 and 40 DAS, green leaf area of urine-fertilized plants was significantly reduced by 43% compared to S0, while ammonium nitrate-fertilized plants were not significantly different (Tab. 2.5). A similar trend was observed at 80 DAS, when the green leaf area of urine-fertilized plants receiving 360 mg N (kg substrate)$^{-1}$ was lower in S2 than S0 by 28 %.

Table 2.5: Effect of urine (U) and ammonium nitrate (AN) fertilization and substrate salinity level (S0, S1, S2) on mean leaf area of maize 40 and 80 d after sowing (DAS).

Substrate salinity (dS m^{-1})	N source/amount (mg kg^{-1} soil substrate)	Leaf Area (cm^2 $plant^{-1}$) 40 DAS	Leaf Area (cm^2 $plant^{-1}$) 80 DAS
1.3 (S0)	U1 (180)	178±77a	128±26ab
	U2 (360)	159±66abc	95±38ab
	AN1 (180)	182±92a	118±21ab
	AN2 (360)	168±66ab	146±25a
	Mean (*n* = 32)	172A	128A
4.6 (S1)	U1 (180)	121±49abcd	96±28bc
	U2 (360)	111±55bcd	87±18bc
	AN1 (180)	137±52abcd	95±33bc
	AN2 (360)	126±46abcd	93±17bc
	Mean (*n* = 32)	123B	93B
7.6 (S2)	U1 (180)	101±53cd	95±30bc
	U2 (360)	91±57d	68±38c[§]
	AN1 (180)	120±59abcd	81±37bc
	AN2 (360)	135±62abcd	101±26abc
	Mean (*n* = 32)	112B	86B
S		***	***
N_S		*	ns
$S \times N_S$		ns	ns
N_R		ns	ns
$S \times N_R$		ns	ns
$N_S \times N_R$		ns	*
$S \times N_S \times N_R$		ns	ns

Values are means ± SD; *n* = 8. [§]n = 4 due to death plants resulting after second dose of urine. Means with different lower-case letters within the same column are significantly different at $P \leq 5\%$. Different upper-case letters within the same column indicate significant differences between salinity at $P \leq 5\%$ according to Tukey test.

2.4 Discussion

Maize is a field crop and is seldom grown in containers except for the purpose of experimentation. Under field conditions, the effect of fertilization on soil properties is hardly noticeable in the short term due to weather dynamics and the small quantities of fertilizer applied per season (*Ray* and *Sinclair*, 1998; *Haynes* and *Naidu*, 1998). It is anticipated that in the long term salt accumulation from urine fertilization may occur. We therefore conducted an investigation on the potential effect of urine fertilization on soil salinity and maize growth under controlled conditions.

Generally, fertilizer application increases the content of soluble salts in the soil and the World Health Organization has cautioned against the use of urine fertilizer in saline soils (*Jacobs* and *Timmer*, 2005; *WHO*, 2006). However, it is not clear at what dosage and/or soil salinity level urine fertilizer application becomes problematic. Our results showed that substrate EC was

higher in urine than ammonium nitrate-fertilized soils. An increase EC of urine-fertilized substrates was expected as urine is inherently saline. *Mnkeni* et al. (2008) reported an increase in soil EC of up to 13.35 dS m^{-1} resulting from fertilization of carrot (*Daucus carota* subsp. *Sativus* L.) with human urine. The comparatively high EC measured in that experiment can be explained by the extremely high urine dosages used. When used as the main source of N, the contribution of urine to soil salinity depends mainly on the proportion of N to Na and Cl in the urine. A relatively low N concentration of applied urine entails higher Na and Cl which may result in soil salinity.

At the end of our experiment we observed an average reduction of the initial soil pH by 0.4 and 0.5 units in the S1 and S2 treatments respectively. These reductions were generally higher in the urine than ammonium nitrate-treated substrates. Though soil pH was not measured shortly after each urine dose, it is likely that the relatively high pH of urine (8.3) initially increased that of the soil substrate, which eventually decreased due to nitrification (*Van Miegroet* and *Cole*, 1984; *Ball* et al., 1979). *Ritchey* et al. (2008) reported a decrease in soil pH resulting from goat urine fertilization on grasslands.

In general, the application of fertilizers can increase exchangeable levels of cations in the soil and has been corroborated by *Lombin* (1981), who has shown that the application of K^+ and Mg^{2+} fertilizers increased their exchangeable levels in soil. The comparatively higher exchangeable K^+, Mg^{2+} and Ca^{2+} observed at the highest salt and N level (S2, N360) in urine-fertilized substrates was expected because of their higher concentrations in urine. At the application of 360 mg N kg^{-1} of substrate as urine, 60 mg K, 12 mg Ca and 4 mg Mg (kg substrate)$^{-1}$ were simultaneously added. *Haynes* and *Williams* (1992) also measured increases in soil exchangeable K^+ and Na^+ on urine-treated pasture lands. However, they did not find any significant effect of urine on exchangeable Ca^{2+} and Mg^{2+} as revealed by our findings, which was probably due to leaching in the field, which was not the case in our experiment as leachate was collected and returned to the respective pots.

The observed reduction in leaf K as substrate salinity rose may be explained in terms of antagonistic effects of Na^+ uptake. This is common under saline-sodic and sodic conditions (*Neto* et al., 2006). Besides, elevated tissue Na may cause a disruption in membrane integrity and thus enhance K^+ efflux from the plant tissue into the growth medium (*Cramer* et al., 1985). Studies have shown that NaCl salinity induces tissue Ca^{2+} deficiency (*Rengel*, 1992;

Fortmeier and *Schubert*, 1995). Contrarily, we measured an increase in leaf Ca^{2+} as substrate salinity rose, which could be due to higher solution Ca^{2+} that is being displaced by Na^{+} on the cation exchange sites. We further observed higher Ca^{2+} and a tendency towards elevated Mg^{2+} concentrations in the leaves of urine-fertilized maize plants, probably due to their inherent concentration in urine. However, additional Ca^{2+} and other nutrients supplied through urine did not enhance maize plant growth as was hypothesized.

Our study reveals that increasing NaCl concentrations reduced fresh and dry matter production of leaves and stems. Accordingly, *Turan* et al. (2009) have shown that soil salinity reduced maize dry matter accumulation. Reduced dry matter accumulation in sodic and saline sodic soils can be associated with toxicities in plants caused by high concentrations of Na^{+}. An important toxicity component of Na^{+} is its ability to inhibit K^{+} uptake by plants especially when the former is present in relatively high concentrations in the soil solution (*Luan* et al., 2009). Assuming that symptoms were stronger with increasing salt concentration and independent of the fertilizer high Na^{+} and reduced K^{+} uptake, leaf-tip burning, early leaf senescence and death of plants indicate the occurrence of Na toxicity in our study. Lower shoot fresh and dry matter yields in urine compared to ammonium nitrate-fertilized plants at the highest N application under saline conditions was due to stress or toxicity resulting from increased salt concentration in the substrate solution added with urine application.

Although leaf N concentration was higher in urine- than ammonium nitrate-treated plants, this effect did not translate to shoot biomass yield due to increased salinity induced by urine application. Reduction in leaf area expansion is an early response to salinity stress. Plant leaves may expand up to a certain threshold and cease as salinity intensifies (*Taleisnik* et al., 2009; *Parida* and *Das*, 2005). The smaller leaf area of urine-fertilized maize plants measured at S2 compared to S0 at the highest N application rate (N360) can be explained by nutritional disorders resulting from higher Na uptake over K, the latter being an important nutrient for leaf cell expansion (*Jordan-Meille* and *Pellerin*, 2004). *Fricke* et al. (2006) have also reported a reduction in barley (*Hordeum vulgare* L.) leaf elongation resulting from NaCl-induced stress.

2.5 Conclusion

Urine can substitute ammonium nitrate fertilizer under saline (here NaCl) conditions. The application of 180 mg urine-N (kg soil substrate)$^{-1}$ was suitable for maize fertilization at soil

salinity up to (EC) 7.6 dS m^{-1}. It was shown that application of 360 mg N (kg soil substrate)$^{-1}$ at this salinity exceeds the critical threshold for using urine as a fertilizer for maize. Our results emphasize the need for a customized urine fertilizer management system that takes into account N content, application amount and soil salinity level. This can be achieved by constructing a model that assists in predicting the effects of urine fertilization on a wide range of soil types and crops in semiarid and arid areas where salinity is often a problem.

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3 Interacting effect of urine, ammonium nitrate and NaCl salinity on sorghum growth and shoot nutrient concentrations

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Abstract

In a greenhouse pot experiment, we compared the influence of urine- and ammonium nitrate-N fertilization on sorghum [*Sorghum bicolor* (Moench) L.] shoot nutrient concentrations, biomass yield and root:shoot ratio under different levels of NaCl addition. The two nitrogen application levels were 90 and 180 mg N kg^{-1} soil substrate. Cultivation substrate used was a mixture (1:1:1 v/v/v) of bio-waste compost, quartz sand and silty-loam soil; with addition of NaCl solution to achieve target EC_e of 1.3 (no salt added – Low), 4.6 (Medium) and 7.6 (High) dS m^{-1}, imitating different levels of salinity and sodicity. Sorghum was grown as a second crop after maize (*Zea mays* L.). Shoot and root biomass were harvested 12 weeks after sowing and oven-dried for dry matter determination. Shoot nutrients concentrations were assessed from ashed samples. Salinity significantly decreased biomass yield regardless of N source. At an application rate of 90 mg N kg^{-1} substrate, total biomass (shoot and root) was by 49, 34 and 40 % higher in plants treated with urine compared to ammonium nitrate in the low, medium and high salinity treatments, respectively. In the NaCl-treated substrates, application of 180 mg urine-N kg^{-1} raised soil salinity and caused a significant decrease in shoot biomass yield. Correlation analyses showed a relatively weak relationship between shoot N concentration and shoot biomass yield in urine (r = 0.34; p > 0.05) if compared to ammonium nitrate (r = 0.91, p< 0.0001). In the high salinity treatment nitrogen use efficiency (NUE) was by 33 % significantly lower in the urine compared to ammonium nitrate treatments. We conclude that urine fertilization restricts sorghum growth under NaCl salinity of EC_e 7.6 dS m^{-1}. However, where urine fertilization is under consideration, lower dosaging (90 mg N kg^{-1} soil) and/or conductivity $EC_e \leq 4.6$ dS m^{-1} is recommended.

Keywords: Nitrogen use efficiency, root:shoot ratio, salinity, sodicity, urine nitrogen

3.1 Introduction

Salt accumulation in soils is an important growth-limiting factor in crop production worldwide. Salinity affects plant growth by reducing the osmotic potential of the soil, causing nutritional imbalances and specific ion toxicity in plants (Yadav *et al*. 2011). The relationship between salinity and bioavailability of nutrients is complex and variable, and often depends on plant species, level of salinity and soil nutrient status (Grattan and Grieve 1999). Sorghum [*Sorghum bicolour* (Moench) L.] is a major food and fodder crop in most semiarid and arid regions where salinity is a threat, and is moderately salt tolerant (Reddy *et al*. 2010). Some studies have shown that up to a certain level of salinity, yields increase with the application of N fertilizer to an optimum especially when nitrogen supply is growth-limiting (Esmaili *et al*. 2008; Nathawat *et al*. 2007). However, in poorer countries commercial fertilizers are either too costly or unavailable for most farmers which partly accounts for the low and declining yields of agricultural products in these areas (Morris *et al*. 2007).

Human urine which is available at little cost is increasingly gaining attention as an alternative liquid N fertilizer. Different models of urine diversion toilets aimed at separately collecting, sanitizing and using human urine in agriculture have been developed and are in use around the world today (Kvarnström *et al*. 2006). When excreted, N in urine is in the form of urea but rapidly hydrolyzes to ammonia and ammonium after storage; a process recommended for urine sanitization prior to use as fertilizer (Kirchmann and Pettersson 1995; Vinnerås *et al*. 2008). The potential of urine as a fertilizer has been well proven (Germer *et al*. 2011; Morghan 2007; Pradhan *et al*. 2009). Despite this, salinity and sodicity concerns have always been raised whenever urine is under consideration for use as fertilizer due to its inherent content of Na and Cl salts which is highly related to intake (World Health Organization 2006).

Salinity-mineral nutrition interaction effect on plant nutrients uptake and yields has been extensively studied (Bernstein *et al*. 1974; Grattan and Grieve 1992; Irshad *et al*. 2008). In these studies, it has been well noted that plant response to N fertilizers depends on initial soil fertility, severity of the salt stress and N form. Accordingly, Tshivhandekano and Lewis (1993) reported that maize (*Zea mays*) and wheat (*Triticum aestivum*) plants grown in an ammonium solution were more sensitive to salinity than nitrate-fed plants. Meanwhile, Irshad *et al*. (2002) observed that mixed applications of ammonium and nitrate were more favourable for wheat plants in salt-affected soils. Silberbush and Lips (1988) however, did not find any

relationship between salinity and the sensitivity of peanut (*Arachis hypogaea*) plants treated with different ammonium/nitrate ratios.

Once applied to the soil ammonium is rapidly converted to nitrates but the process of nitrification can be significantly delayed by salinity (McClung and Frankenberger 1985). In an alfafa (*Medicago sativa*) amended sandy loam soil study, McCormick and Wolf (1980) reported a significant reduction in nitrification following the application of 6 mg NaCl g^{-1} and a complete inhibition at 10 mg NaCl g^{-1} soil. It has been argued that chloride salts are more toxic to nitrification than sulphate salts (McClung and Frankenberger 1985; Sindhu and Cornfield 1967) but in a calcareous soil treated with cow urine Monaghan and Barraclough (1992) found that urinary chloride concentration up to 7.4 g l^{-1} did not affect nitrification whereas at an osmotic pressure of -0.2 MPa nitrification was completely inhibited. Delayed nitrification due to salinity and/or osmotic pressure may affect the form in which plants take up and eventually assimilate nitrogen.

Generally, plants require up to 4 times more energy to assimilate nitrate than ammonium. This energy demand on plants has been implicated for the comparatively low dry matter accumulation in nitrate-fed plants (Huffman 1989). According to Irshad *et al*. (2008), this effect could be more severe under saline conditions due to competition with other anions like chloride.

In spite of the advancement in urine fertilizer research, we did not find any published information on the interaction between NaCl salinity and urine fertilization on sorghum growth. Therefore, a maize-sorghum crop sequence was carried out which aimed to evaluate the cumulative effect of urine fertilization and its interaction with salinity on biomass yield and nutrient concentration in sorghum. The specific objectives of our experiment were to 1) compare the effect of urine and ammonium nitrate nitrogen sources on sorghum growth, measured as biomass yield and 2) determine the relationship between shoot biomass yields and shoot nutrient concentration under different levels of NaCl salinities. We hypothesized that i) urine-induced increase in soil salinity reduces sorghum growth of urine-treated plants compared to those treated with ammonium nitrate ii) the effects of urine and ammonium nitrate fertilization on sorghum growth depend on the level of NaCl salinity. Information thus gathered would enhance understanding of NaCl salinity and urine fertilizer interaction which is relevant for urine fertilizer management in salt vulnerable areas.

3.2 Materials and methods

A greenhouse experiment was conducted at the University of Hohenheim, Stuttgart – Germany (48.7114°N 9.2095°E) on a NaCl-treated cultivation substrate previously cropped with maize as a first crop and fertilized with urine or ammonium nitrate as N sources as described in Boh *et al*. (2013). Briefly, the cultivation substrate was made of a mixture of bio-waste compost, quartz sand, and silty loam soil in a 1:1:1 volume ratio. The experimental treatments consisted of 3 levels of NaCl addition, 2 N sources (urine and ammonium nitrate) and 2 N amounts (180 and 360 mg N kg^{-1} soil). Salinity was imitated by adding predetermined amounts of NaCl solutions to a known soil substrate weight following a laboratory calibration study to achieve targeted conductivities of EC_e 1.3 (Low – no salt added), 4.6 (Medium) and 7.6 (High) dS m^{-1}.

Mitscherlich pots were used in these experiments for a cropping sequence. At the end of the first maize cropping cycle, root and shoot biomass were harvested and the soil substrate from each pot was emptied, hand-crushed and homogenized. 11 kg were refilled in the same 10 l Mitscherlich pots at the start of the experiment. As a second crop, six sorghum seeds were sown in each pot, thinned to two healthy seedlings two weeks after emergence and fertilized with urine- (U) or ammonium nitrate-N (AN) 1 week later. The experimental treatments (Table 3.1) were the same as for the preceding maize trial briefly described above except that N supply was 90 and 180 mg kg^{-1} soil substrate. Experimental setup was a completely randomized block design.

Table 3.1 Experimental treatments (*n*=4). Electrical conductivity (EC_e dS m^{-1}) and fertilizer addition to the cultivation substrate (mg kg^{-1}).

Salinity level (EC_e)	**Ammonium nitrate** mg kg^{-1}		**Urine** mg kg^{-1}	
	(AN1)	(AN2)	(U1)	(U2)
Low - no NaCl added (1.3 dS m^{-1})	90	180	90	180
Medium (4.6 dS m^{-1})	90	180	90	180
High (7.6 dS m^{-1})	90	180	90	180

U1 and U2 = 90 and 180 mg urine-N kg^{-1} substrate respectively; AN1 and AN2 = 90 and 180 mg ammonium nitrate-N kg^{-1}substrate respectively.

Urine collected from students in a hostel of the University of Hohenheim was stored at mean daily temperatures of 25±2 °C for 12 months and hence, was considered hygienically safe for unrestricted use as a fertilizer (Vinnerås et al., 2008). Prior to application, the urine container was thoroughly shaken and samples were taken for chemical analyses. Urine contained 6.0 g

total N (mainly NH_4), 1.42 g K (as K_2O), 0.41 g P (as P_2O_5), 0.32 mg Ca, 0.43 mg Mg, 2.3 g Na and 3.2 g Cl l^{-1}. A 25 % reduction in total urine N compared to its content shortly after collection was observed. This can be attributed to loss of ammonia through volatilization. Ammonium nitrate ≥ 98 % (Sigma-Aldrich Chemie GmbH, Germany) was applied as a solution prepared by dissolving the salt in deionised water to contain an equivalent amount of N as 1 l of the urine used.

Day and night temperatures in the greenhouse were set at 30 and 18 °C with 12 hours photoperiods throughout the growing period. To maintain an adequate water supply to the plants throughout the growing period, pots were irrigated regularly with tap water and when leachate accumulated in the plastic bowls placed below each pot it was returned to prevent nutrient losses and salinity changes.

3.2.1 Data collection and analyses

Sorghum plants were harvested 12 weeks after sowing and separated into roots and shoots. Roots were washed with tap water to remove soil and both plant parts were oven-dried at 70 °C to constant weight. Dried shoot tissues were ashed at 500 °C for 5 hours and digested with 1:3 (solution: de-ionized water) concentrated HCl. In the ash solution, P, K, Ca, Mg, Na concentrations were measured. P concentration was measured colorimetrically (Ryan et al., 2001). K and Na were measured by flame photometry while the concentrations of Ca and Mg were measured by atomic absorption spectrometry (AAS). Chloride was extracted from the samples by boiling water and measured by ion chromatography with conductivity detection (VDLUFA 2009). Nitrogen use efficiency (NUE) was calculated as described by (Maranville *et al*. 1980): NUE = SDW/SN, where SDW is shoot dry weight (g pot^{-1}) and SN is total shoot nitrogen (g pot^{-1}). Substrate electrical conductivity and pH were measured from samples taken from each pot at the end of the trial. Electrical conductivity was measured from a saturated paste according to Richards (1954) while pH was measured in a 1:2.5 (soil:solution) suspension of 1 *M* KCl.

A three-factor (salinity, N source and N amount) factorial statistical analysis with four replications was carried out using the SAS statistical software package (SAS Institute version 9.3). ANOVA was conducted using PROC GLM procedure and Fisher's LSD ($\alpha = 0.05$) and Tukey-Kramer adjusted *post hoc* analyses were used to compare statistical significance between means at $p \leq 0.05$. Where ANOVA results did not show significant interactions, data

were pooled and further analyses carried out. Data for shoot P concentration were square root-transformed for statistical calculations. Regression (PROC REG) and correlation (PROC CORR) analyses were carried out to assess the relationship between shoot nutrient concentration and biomass accumulation.

3.3 Results

3.3.1 Substrate pH_{KCl} and electrical conductivity (EC_e)

Substrate pH_{KCl} was significantly affected by salinity x N source x N amount interactions (*df* = 2; *F* = 7.05; *p* = 0.003) (Table 3.2). Compared to the low salinity treatment, the pH_{KCl} of urine-treated cultivation substrate which received 90 mg N kg^{-1} substrate was by 0.2 units significantly higher than those in the high salinity treatment (Table 3.3). Cultivation substrate pH_{KCl} significantly decreased from 7.2 to 6.9 pH_{KCl} units when the amount of urine applied was raised from 90 to 180 mg N kg^{-1} substrate in the high salinity treatment whereas ammonium nitrate-fertilized substrates were not significantly affected. Furthermore, in the high salinity treatment the pH_{KCl} of substrates fertilized with 180 mg ammonium nitrate-N kg^{-1} was 0.3 units significantly higher than those which received the same amount of N in the form of urine.

Table 3.2 Summary of *F*-values from three-way ANOVA of sorghum biomass and nutrient concentration as influenced by salinity (S), nitrogen source (NS) and nitrogen amount (NA).

Variables	S	NS	S x NS	NA	S x NA	NS x NA	S x NS x NA
Root:shoot ratio	1.5 ns	2.0 ns	16.6 ***	0.0 ns	1.2 ns	15.4 ***	10.8 ***
Total biomass	16.1 ***	72.2 ***	2.3 ns	2.5 ns	6.7 **	15.6 ***	0.3 ns
N	2.5 ns	44.3 ***	3.2 ns	9.2 **	1.7 ns	0.1 ns	0.8 ns
P	30.1 ***	12.4 **	14.0 ***	0.6 ns	13.2 ***	20.1 ***	5.9 **
K	24.7 ***	16.6 ***	0.8 ns	3.2 ns	0.1 ns	1.4 ns	3.9 *
Ca	3.9 *	15.8 ***	0.1 ns	6.8 *	0.5 ns	0.0 ns	4.7 *
Mg	1.9 ns	81.7 ***	3.4 *	0.2 ns	2.3 ns	10.7 **	8.3 **
Na	423.4 ***	415.2 ***	218.8 ***	147.4 ***	47.8 ***	144.3 ***	55.2 ***
Cl	47.1 ***	9.2 **	1.5 ns	15.9 ***	4.1 *	4.5 *	7.4 **
K/Na	61.1 ***	40.5 ***	2.5 ns	14.5 **	3.4 *	11.4 **	0.7 ns
Ca/Na	97.5 ***	32.5 ***	2.0 ns	11.6 **	4.1 *	11.8 ***	1.5 ns
Mg/Na	91.1 ***	13.8 ***	2.9 ns	16.1 ***	3.7 *	14.7 ***	0.7 ns

For this and subsequent tables, *, ** and *** indicate level of significance at P < 0.05, 0.01 and 0.001 respectively; ns = not significant.

Table 3.3 Effect of urine and ammonium nitrate application on cultivation substrate pH_{KCl} and electrical conductivity (EC_e) measured at the end of the experiment.

Salinity level (EC_e)	Fertilizer treatment	pH_{KCl}	EC_e (dS m^{-1})
Low (1.3 dS m^{-1})	U1	7.0±0.03bcd	3.1±0.09ef
	U2	7.1±0.03abc	4.9±0.25ed
	AN1	6.9±0.04cd	2.9±0.02ef
	AN2	7.1±0.01abc	2.6±0.04f
Medium (4.6 dS m^{-1})	U1	7.1±0.04ab	5.8±0.23cd
	U2	7.1±0.03abc	8.9±0.20b
	AN1	7.1±0.01ab	5.1±0.19ed
	AN2	7.1±0.04ab	5.3±0.25ed
High (7.6 dS m^{-1})	U1	7.2±0.01a	8.5±0.18b
	U2	6.9±0.07d	11.9±0.88a
	AN1	7.1±0.02ab	8.2±0.48b
	AN2	7.2±0.02a	7.8±0.25bc

Values represent means ± standard error (n = 4). U1 and U2 represent 90 and 180 mg urine-N kg^{-1}substrate respectively; AN1 and AN2 represent 90 and 180 mg ammonium nitrate-N kg^{-1}substrate respectively. Different lowercase letters represent significant difference between treatments ($p \leq 0.05$); Tukey-Kramer adjusted test.

There was a significant interaction effect (p = 0.03) of salinity x N source x N amount on substrate EC_e (Table 3.2). It is important to note that the values of EC_e shown here in Table 3.3 are cumulative. With the exception of low salinity treatment, an increase in urine application from 90 to 180 mg N kg^{-1} substrate significantly raised substrate EC_e. Meanwhile the same increase in N application in the form of ammonium nitrate had no significant effect on EC_e regardless of salinity treatment (Table 3.3). Our data further revealed that while substrate EC_e was not different between the two fertilizers at application of 90 mg N kg^{-1} substrate, the EC_e of urine-fertilized substrates was comparatively higher at 180 mg N kg^{-1} substrate.

3.3.2 Biomass yield and root:shoot ratio

Total biomass (shoot and root) yield was significantly influenced by salinity x N source and N source x N amount interactions (Table 3.2). In the low and medium salinity treatments, total biomass yield was significantly higher in urine than ammonium nitrate-fertilized treatments. With an increase in salinity from EC_e 4.6 to 7.6 dS m^{-1}, total biomass yield significantly reduced for both fertilizer sources. Total biomass yield was by 49, 34 and 40 % higher in treatments fertilized with U1 compared to AN1 in the low, medium and high salinity treatments, respectively (Table 3.4). At higher N applications (U2 and AN2) in the low and medium salinity treatments, total biomass yield was by 22 and 17 % higher in urine than ammonium nitrate-fertilized treatments, respectively. With the exception of low salinity treatment, total biomass yield significantly decreased when the amount of N supplied

through urine rose from 90 to 180 mg kg^{-1} substrate but increased for ammonium nitrate-fertilized treatments regardless of salinity treatment (Table 3.4).

Table 3.4 Effect of N nutrition on total biomass (g $plant^{-1}$) and sorghum shoot nutrient concentrations (mg g^{-1}) under different levels of salinity.

Salinity level (EC_e)	Variable	Fertilizer treatment				LSD (0.05)[a]
		U1	U2	AN1	AN2	
Low (1.3 dS m^{-1})	Root:shoot ratio	0.7	0.6	0.3	0.4	0.1
	Total biomass	74.7	80.2	37.9	62.8	4.0
	N	0.7	0.9	0.4	0.7	0.1
	P	0.4	0.3	0.4	0.3	0.0
	K	3.6	3.6	3.2	3.5	0.1
	Ca	2.3	2.6	1.9	2.0	0.1
	Mg	1.1	1.1	0.8	0.9	0.1
	Na	0.04	0.1	0.03	0.04	0.0
	Cl	3.6	3.7	2.1	2.7	0.7
	K/Na	90.0	36.0	107.6	87.5	8.6
	Ca/Na	57.5	26.0	63.3	50.0	4.6
	Mg/Na	27.5	11.0	26.7	22.5	2.4
Medium (4.6 dS m^{-1})	Root:shoot ratio	0.5	0.5	0.5	0.4	0.1
	Total biomass	75.6	70.7	49.6	58.9	4.3
	N	0.8	1.0	0.5	0.8	0.1
	P	0.4	0.4	0.4	0.4	0.0
	K	4.3	4.7	4.1	3.9	0.3
	Ca	2.4	3.3	2.3	2.4	0.1
	Mg	1.0	1.0	0.8	0.8	0.0
	Na	0.1	0.2	0.1	0.1	0.0
	Cl	5.3	6.0	4.9	4.8	0.5
	K/Na	43.0	23.5	41.0	39.0	4.2
	Ca/Na	24.0	16.5	23.0	24.0	2.7
	Mg/Na	10.0	5.0	8.0	8.0	1.2
High (7.6 dS m^{-1})	Root:shoot ratio	0.6	0.3	0.5	0.9	0.1
	Total biomass	65.6	48.2	39.2	44.8	4.3
	N	0.9	0.9	0.4	0.4	0.1
	P	0.4	0.6	0.4	0.4	0.0
	K	4.8	4.6	3.7	4.4	0.1
	Ca	2.7	2.4	1.8	2.5	0.3
	Mg	1.1	0.8	0.7	0.9	0.0
	Na	0.3	0.6	0.1	0.1	0.0
	Cl	5.7	8.7	6.1	6.4	0.3
	K/Na	16.3	7.7	37.0	44.0	7.3
	Ca/Na	9.0	4.0	18.0	25.0	3.4
	Mg/Na	3.7	1.3	7.0	9.0	1.2

Values are means of four replicates. [a]Denotes comparisons within rows. U1 and U2 = 90 and 180 mg urine-N kg^{-1}substrate respectively; AN1 and AN2 = 90 and 180 mg ammonium nitrate-N kg^{-1}substrate respectively.

Root:shoot ratio was significantly affected by salinity x N source x N amount interactions (Table 3.2). Our data showed that with the application of 90 and 180 mg N kg^{-1} substrate in the low salinity treatment, biomass allocation to the roots was by 57.1 and 33.3 % higher in urine- than ammonium nitrate-treatments, respectively (Table 3.4). In the medium salinity

treatment, root and shoot biomass was equitably distributed in the U1, U2 and AN1 fertilized plants but root biomass allocation in the U2 treatment was 20 % higher than in the AN2 treatment. In the high salinity treatment, biomass partitioning to the roots was decreased by 50 % as the amount of urine was raised from U1 to U2 and increased by 44 % following an increase in ammonium nitrate amount from AN1 to AN2. It was further observed that with the application of 180 mg N kg^{-1} substrate in the high salinity treatment, root biomass allocation was by 67 % higher in ammonium nitrate- compared to urine-fertilized treatments.

3.3.3 Shoot nutrient concentrations

Analyses of variance results showed that shoot N concentration in sorghum was significantly influenced by N source and N amount (Table 3.2). Shoot N concentration was significantly higher in urine- than ammonium nitrate-fertilized treatments at all levels of salinity (Table 3.4). With the exception of high salinity treatment, shoot N concentration significantly rose with an increase in N amount for both fertilizer sources. Statistical analyses revealed that shoot N concentration was significantly related to shoot biomass yield regardless of salinity level (Table 3.4). However, a significant positive correlation (r = 0.91; p < 0.0001) between shoot biomass yield and shoot nitrogen concentration was observed only for ammonium nitrate-fertilized treatments (Figure 3.1). Our data further indicated that nitrogen use efficiency (NUE) was significantly influenced by the interaction between salinity and N source (df = 2; F = 3.86; p = 0.03). There was no significant difference (p > 0.05) in NUE between both fertilizers in the low and medium salinity treatments whereas NUE was by 33 % lower in urine than ammonium nitrate treatments under high salinity (Table 3.5).

Table 3.5 Effect of NaCl salinity level (EC_e) and nitrogen source on nitrogen use efficiency (NUE).

Salinity level (EC_e)	Nitrogen source	NUE
Low (1.3 dS m^{-1})	Urine	59.0±2.0ab
	Ammonium nitrate	67.8±4.1a
Medium (4.6 dS m^{-1})	Urine	54.7±4.8ab
	Ammonium nitrate	59.2±2.4ab
High (7.6 dS m^{-1})	Urine	44.8±3.4b
	Ammonium nitrate	67.9±4.1a

Values represent means of four replicates ± S.E. Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$; Tukey-Kramer adjusted test.

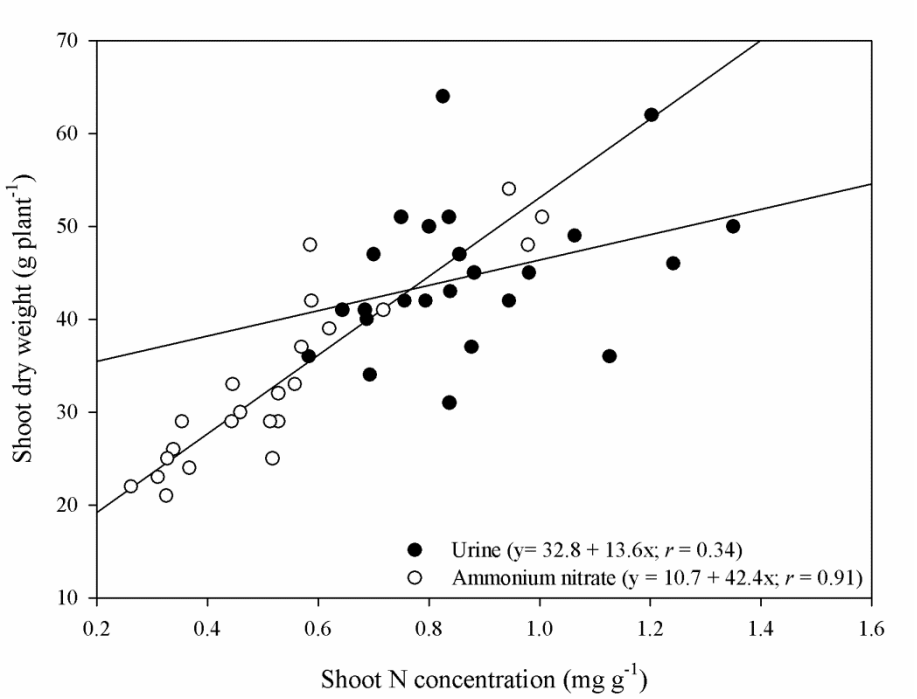

Figure 3.1: Relationship between shoot N concentrations and shoot dry weight of sorghum plants fertilized with urine- or ammonium nitrate-N.

The concentrations of shoot P, K, Ca, Mg, Na and Cl were significantly influenced by salinity x N source x N amount interactions (Table 3.2). Our data in Table 6 showed a significant relationship between shoot biomass yield and shoot concentration of Mg, Na and Cl in the low salinity and P and Na in the high salinity treatments. In the low salinity treatment, shoot P concentration decreased as the amount of N added through both fertilizers rose, but increased significantly under high salinity for urine- while ammonium nitrate-fertilized treatments remained unaffected (Table 3.4). Shoot K concentration was significantly higher in urine than ammonium nitrate-fertilized treatments in the low and high salinity treatments irrespective N amount.

In the low and medium salinity treatments, shoot Ca and Mg concentrations were significantly higher in the urine than ammonium nitrate-fertilized treatments. In urine-fertilized treatments that received 90 mg N kg^{-1} substrate shoot Ca concentration was by 17.4 and 33.3 % significantly higher than in treatments that received an equivalent amount in the form of ammonium nitrate in the low and high salinities, respectively (Table 3.4). At a higher N dose (180 mg N kg^{-1} substrate); shoot Ca concentration was by 23.1 and 27.3 % significantly higher in urine than in the ammonium nitrate treatments at low and medium salinity, meanwhile in the high salinity treatment, there was no significant N source effect. We further observed a significant 27.3 % increase in shoot Ca concentration following an increase in

urine application from U1 to U2 in the medium salinity treatment. A significant 28 % increase in shoot Ca concentration was measured in the high salinity treatment following an increase in ammonium nitrate dosage from 90 to 180 mg N kg^{-1} substrate.

Table 3.6 Regression models, determinants (R^2) and correlation coefficient (*r*) and shoot biomass yields (Y) at different salinity levels.

Salinity level (EC_e)	Variable	Regression equation	R^2	*r*
	N	Y=14.8+39.36N	0.84***	0.92
	P	Y=42.7-2.78P	ns	0.01
Low	K	Y=-10.3+14.91K	ns	0.43
(1.3 dS m^{-1})	Mg	Y=44.5+43.21Mg	62***	0.79
	Ca	Y=11.7+13.60Ca	ns	0.48
	Na	Y=61.7-0.82Na	0.46**	0.68
	Cl	Y=28.9+3.95Cl	0.30*	0.54
	N	Y=21.5+27.56N	0.44**	0.66
	P	Y=54.6-28.79P	ns	0.11
	K	Y=49.3-1.47K	ns	0.09
Medium	Mg	Y=46.4+30.76Mg	ns	0.48
(4.6 dS m^{-1})	Ca	Y=38.9+1.58Ca	ns	0.08
	Na	Y=39.4+0.26Na	ns	0.14
	Cl	Y=24.2+3.61Cl	ns	0.35
	N	Y=16.8+23.81N	0.77***	0.87
	P	Y=10.8+46.25P	0.25*	0.50
	K	Y=-7.00+8.99K	ns	0.50
High	Mg	Y=35.3+26.30Mg	ns	0.48
(7.6 dS m^{-1})	Ca	Y=21.6+4.51Ca	ns	0.27
	Na	Y=39.5-1.06Na	0.42***	0.64
	Cl	Y=29.1+0.46Cl	ns	0.07

With the application of 90 mg N kg^{-1} substrate in the low, medium and high salinity treatments, shoot Mg concentration was by 27.3, 20.0 and 36.4 % significantly higher in urine than ammonium nitrate-fertilized treatments. Our data also showed that with the application of 180 mg N kg^{-1} substrate in the low and medium salinity shoot Mg concentration in urine-fertilized treatments was by 18.2 and 20.0 % significantly higher than those fertilized with ammonium nitrate. There was no significant effect on shoot Mg concentration following an increase in N application from 90 to 180 mg N kg^{-1} substrate in the low and medium salinity treatments for both fertilizers. Whereas in the high salinity treatments, shoot Mg concentration significantly decreased by 18.2 and increased by 22.2 % for urine and ammonium nitrate-fertilized treatments, respectively.

As expected, the concentration of Na in the shoots of sorghum plants significantly increased as salinity rose for all urine treatments but did not change for ammonium nitrate-fertilized treatments as salinity rose from medium to high (Table 3.4). With the exception of U1 in the

medium salinity treatment, shoot Na concentration was higher in urine compared to ammonium nitrate-fertilized treatments and increased as the amount of N applied through urine rose from 90 mg to 180 mg N kg^{-1} substrate (Table 3.4). Like Na, shoot Cl concentration also increased as salinity level rose. Compared to AN1 treatment, shoot Cl concentration was by 44 % higher in U1 in the low salinity treatment. Our data also revealed that with the application of 180 mg N kg^{-1} substrate, shoot Cl concentration was higher by 27, 20 and 26 % in urine-fertilized treatments under low, medium and high salinity treatments, respectively, compared to those fertilized with ammonium nitrate. In the medium and high salinity treatments, shoot Cl concentrations significantly increased by 11.7 and 34.5 %, respectively, when urine-N application was raised from 90 (U1) to 180 (U2) mg kg^{-1} of substrate.

Shoot K/Na, Ca/Na and Mg/Na concentration ratios were significantly affected by salinity x N amount and N source x N amount interactions (Table 3.2). Shoot K/Na, Ca/Na and Mg/Na ratios significantly decreased as salinity level rose and were higher in ammonium nitrate than urine-fertilized treatments in the low and high salinity treatments (Table 4). An increase in urine application from 90 (U1) to 180 (U2) mg N kg^{-1} substrate significantly reduced shoot K/Na ratio by 60, 44.8 and 52.8 % in the low, medium and high salinity treatments, respectively. For ammonium nitrate-fertilized treatments under low salinity, shoot K/Na ratio significantly reduced by 18.7 %. Shoot Ca/Na ratio decreased by 54.8, 31.3 and 55.6 in the low, medium and high salinities following an increase in urine-N application. An increase in the supply of N in the form ammonium nitrate (AN1 to AN2) lowered shoot Ca/Na ratio by 21 % in the low salinity treatments but significantly increased it by 28 % in the high salinity treatment. In the low, medium and high salinity treatments, shoot Mg/Na ratio was lower by 60, 50 and 64.9 %, respectively, following an increase in urine application from U1 to U2. Comparatively, a proportionate increase in N supply as ammonium nitrate significantly reduced shoot Mg/Na ratio by 15.7 % in the low salinity treatments but increased it by 22.2 % on the high salinity treatment.

3.4 Discussion

Fertilizer application often modifies soil chemical and biological properties. How the applied fertilizer affects the soil solution characteristics and the ultimate availability of nutrients to plants depends on the chemical composition of the fertilizer type. The use of ammonium-based N fertilizers has been shown to have a pH-reducing effect on soils mainly due to the

biological conversion of ammonium to nitrate (Zhang and Raun 2006). Though higher nitrification was expected in the urine-fertilized treatment, it is clear from our study that nitrification was not an important determinant of substrate pH_{KCl}. In the urine fertilized treatment (U2), the decrease in substrate pH_{KCl} at salinity treatment EC_e 7.6 dS m^{-1} can be attributed to Na-induced displacement of hydrogen ions from the cation exchange sites considering that Na concentration was higher at this salinity level and urine treatment (Green *et al*. 2008). When plants take up positively charged ions, they excrete positively charged hydrogen ions to maintain a soil electrical balance. Increased concentration of hydrogen ions in the soil has an acidifying effect (Gazey and Davis 2009).

Although application of N fertilizers have been proven to alleviate the deteriorating effect of salinity on crops, over fertilization is known to exacerbate the problem of salinity (Chen *et al*. 2010; Villa-Castorena *et al*. 2003). Apart from nitrogen, urine contains dissolved salts of Na and Cl which explains the increase in substrate salinity at a higher application dosage. This result is consistent with our earlier findings and emphasizes the need to consider the salinizing effect of urine when deciding the amount of urine to be applied to crops in a saline soil (Boh *et al*. 2013).

In this study, we also tested whether compared to ammonium nitrate, urine-N could improve sorghum growth measured as biomass yield at given levels of substrate salt (NaCl) concentrations. It was our expectation that urine salinity would impose a limit on the growth of urine-fertilized treatments. Our results indicated that urine improved the nutrition of sorghum plants shown in higher biomass yield than ammonium nitrate. This can only partly be explained by the addition of K, Mg and Ca alongside urine N though the contents of these nutrients in urine were generally low. Furthermore, apart from Mg in the low salinity treatment, our results (Table 3.3) did not show any significant correlation between the concentration of these nutrients and sorghum shoot biomass yield. However, it is conceivable that synergism between two or more of these nutrients can have a growth-improving effect on sorghum plants. According to Zhong and Lauchli (1994), supplemental external Ca can improve plant tolerance to NaCl salinity by maintaining plasma-membrane selectivity of K over Na. This agrees with the findings of Jafari *et al*. (2009) who observed improved sorghum growth under saline conditions resulting from a combined application of Ca and K.

Increased biomass yield in ammonium compared to nitrate-fed plants has been related to energetic savings (Kant *et al*. 2007). Compared to nitrate, the energy requirement for the assimilation of one molecule of ammonium is 4 times lower. Therefore, reduced biomass yield in ammonium nitrate-treated plants can be related to expended energy involved in nitrate assimilation. Under high salinity the observed decrease in sorghum biomass yield when the amount of applied urine was raised from U1 to U2 can be attributed to the increase in soil salinity induced by urine fertilization. This is in conformity with our previous investigation which showed that under saline conditions higher doses of urine fertilization increased soil salinity and stress in maize plants resulting in lower biomass yield (Boh *et al*. 2013).

According to Bonifas *et al*. (2005), root:shoot ratio which is an important component in crop yields can be affected by N supply. Our results showed that the effect of salinity, N source and N amount on biomass partitioning between roots and shoots was interactive. Reduced root:shoot ratio of urine-fertilized treatments following an increase in application amount under high salinity treatment can be associated with urine-induced increase in soil Na and Cl concentrations which can have synergistic toxic effects on root growth (Martin and Koebner 1995). However, in this study, the critical level at which toxicity sets is not certain. Frechilla *et al*. (2001) found that under NaCl saline conditions root growth inhibition of ammonium-fed pea (*Pisum sativum*) plants was related to increased sodium accumulation in the roots and decreased root organic nitrogen content. Although Kudoyarova *et al*. (1997) have associated higher root:shoot ratios with auxin concentration common in nitrate fed plants, in our study it remains unclear why root:shoot ratio was significantly higher in ammonium nitrate-fed plants in the high salinity treatment. This would require further investigation.

Delayed nitrification due to toxicity and/or osmotic stress caused by urinary salts explains why urine-treated plants are more likely to take up nitrogen in the form of ammonium than nitrate. This however, depends on the time of application (McClung and Frankenberger 1985; Monaghan and Barraclough 1992). The lag phase required by nitrifying bacteria to adjust to salt or osmotic stress is 18 to 20 days after which rapid nitrification will take place following an exponential growth in nitrifying bacteria (Helder and De Vries 1983; Monaghan and Barraclough 1992).

The higher shoot N concentration in urine compared to ammonium nitrate-fed plants can be related to cation:anion balance effect caused by the relatively higher shoot Cl concentration in urine-fed plants. Cation:anion balance effect on plant nutrition has also been reported by Irshad *et al*. (2002) who related high concentrations of P and Cl in the tissues of ammonium-fed wheat plants to a response in ammonium uptake. In the high salinity treatment, reduced shoot N concentration in ammonium nitrate-fed plants could be additionally related to nitrate-chloride antagonism. At high external Cl concentration competition and the toxic effect of Cl salts can cause to reduce NO_3 uptake in plants (Debouba *et al*. 2007).

Even though there was a strong positive correlation between shoot N concentration and shoot biomass yield, when this relationship was compared between the two N forms, urine treated plants showed a weak relationship which suggests that other nutrients in urine could have contributed to increase shoot biomass yield as already mentioned above. Our results further revealed that in spite of the high shoot N concentration, NUE was significantly lower in urine compared to ammonium nitrate-fed plants under high salinity. This can be attributed to growth-inhibitory effects resulting from additional salts applied through urine. In an earlier study, Pessarakli and Tucker (1985) found that NaCl salinity can inhibit ammonium metabolism and protein synthesis in cotton (*Gossypium hirsutum*) plants without affecting its uptake. In a wire-house experiment, Perveen *et al*. (2012) have also shown that salinity can adversely affect nutrient use efficiency in wheat plants.

Leaf K/Na ratio is often used as an indicator of sorghum tolerance to salt stress (Vasilakoglou *et al*. 2011). The observed decrease in shoot K/Na, Ca/Na and Mg/Na ratio as salinity rose was due to an increase in Na uptake which negatively affected plant metabolism and growth (Irshad *et al*. 2008). The lower K/Na, Ca/Na and Mg/Na ratios in urine compared to ammonium nitrate-fertilized treatments was due to the addition of Na through urine. However, the added Na was not enough to cause a growth-reducing effect on sorghum plants treated with urine except when the urine dose was raised to 180 mg N kg^{-1} substrate in the medium and high salinity treatments.

3.5 Conclusion

With biomass production as a growth indicator, our results illustrated that compared to ammonium nitrate, sorghum growth improved under urine-fertilized treatment. Sorghum

growth was inhibited under high salinity treatment. It was shown that in the NaCl-treated cultivation substrate, the application of 180 mg urine-N kg^{-1} substrate increased soil salinity and thus reduced biomass yield. It is apparent that N and other nutrients added through urine interacted to cause an increase in shoot biomass yield of urine-fertilized treatments but this requires further investigation. NUE of urine-fertilized treatments was reduced by 33 % compared to those treated with ammonium nitrate under high NaCl salinity level, suggesting that at NaCl salinity level exceeding 7.6 dS m^{-1} urine fertilization should be restricted for sorghum plants. As sorghum is a field crop and seldom grown in pots, field trials to determine the critical salinity level and urine dosage would be necessary. This study helps to understand the interaction between salinity and urine fertilizer on sorghum growth and stresses the need to keep urine-N application doses low on saline soils.

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4 Effect of NaCl-induced salinity and human urine fertilization on substrate chemical properties

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Abstract

We evaluated the effect of NaCl-induced salinity and successive urine fertilization on changes in cultivation substrate chemical properties in a greenhouse study. The substrate was composed of an equal volume ratio mixture of bio-waste compost, quartz sand and silty loam soil. Salinity was imitated by adding NaCl solutions to a known substrate weight achieving three target salinity treatments of EC_e 1.3 (S0 – no NaCl), 4.6 (S1) and 7.6 (S2) dS m^{-1}. Cultivation substrate had been cropped with two cycles of maize (*Zea mays* L.) (crop cycles I and II) and fertilized with human urine at N amounts of 0 (U0 – no urine), 180 (U1) and 360 (U2) mg kg^{-1} substrate in the first cycle and half of the urine-N dosages in cycle II. Substrate samples collected at the end of each cycle were analyzed for pH_{KCl}, EC_e, exchangeable and water extractable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), cation exchange capacity, water extractable anions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) and exchangeable sodium percentage (ESP).

Exchangeable Na^+, K^+ and Ca^{2+} were significantly ($p < 0.05$) affected by salinity x urine interaction. Following an increase in urine application from U0 to U2, EC_e significantly increased by 7.3, 5.3 and 7.6 dS m^{-1} in the S0, S1 and S2 treatments, respectively, ESP increased in the order U0 < U1 < U2. Extractable NO_3^- and Cl^- were significantly affected by crop cycle, salinity and urine interactions ($p < 0.05$) whereas the effect of urine fertilizer on extractable SO_4^- and PO_4^{3-} depended on crop cycle alone. There was a tendency towards increasing soil sodicity with mounting urine fertilization. The level of NaCl salinity and the amount of urine applied are important determinants of substrate chemical properties. Adoption of appropriate management techniques to avoid salinity/sodicity build up should be included in urine fertilization planning.

Keywords: *Soil sodicity, electrical conductivity, urine nitrogen, nutrient recycling*

4.1 Introduction

Soil salt accumulation constitutes a major problem in agricultural production worldwide (Lobell et al., 2007). Salt affected soils are generally low in available plant nutrients and would require adequate fertilizer application and management to achieve optimal yields (Hague, 2006; Agarwal and Gupta, 1968). However, for farmers in low income countries, commercial inorganic fertilizers are too expensive and unaffordable (Morris et al., 2007). The ecological sanitation (ECOSAN) concept promotes the use of human urine as an alternative plant fertilizer due to its rich content in readily available plant nutrients (Kirchmann and Pettersson, 1995). Human urine is a multi-component fertilizer containing N, P, K^+, S, Ca^{2+}, Mg^{2+}, Na^+, Cl^- and other micronutrients in amounts that vary depending on the diet (Münch and Winker, 2009).

Research has shown that by applying human urine as a fertilizer, plant growth and crop yields were improved (Boh et al., 2013; Morghan, 2007; Pradhan et al., 2010). Concerns regarding hygienic safety, pharmaceutical residues and hormones have been addressed and guidelines for a safe use of urine in agriculture are well documented (Jonsson et al., 2004; World Health Organization, 2006). Na^+, Cl^- and SO_4^{2-} are among the most salt-bearing ions in fresh urine and after storage urine salinity can rise three-fold due to urea degradation (Beler-Baykal et al., 2011). For this reason, restrictive use of human urine as a fertilizer under saline conditions has been recommended (World Health Organization, 2006).

Indeed, some researchers have reported an increase in soil salinity resulting from human urine fertilization and have related a decline in crop growth to urine-induced salinity (Boh et al., 2013; Mnkeni et al., 2008). In these studies, soil EC_e as an index of salinity and pH were among the main investigated soil parameters. So far, very little attention has been accorded the effect of human urine fertilizer application on soil chemical properties and it is not clear how NaCl salinity and urine fertilizer application interaction will affect soil chemical properties. While salinity is commonly thought of as a problem, Na^+ applied with urine can increase the risk of soil sodicity especially under NaCl saline conditions.

The objective of this study was to assess the effect of NaCl-induced salinity and fertilization using urine on cultivation substrate cation exchange capacity (CEC_p), electrical conductivity (EC_e), exchangeable cations, water soluble cations and anions, pH_{KCl} and exchangeable

sodium percentage (ESP). We hypothesized that i) due to their contents in urine, water extractable anions and cations and substrate exchangeable cations will increase with mounting dosages of urine and ii) urine fertilization imposes a risk on soil sodicity which might increase under NaCl-induced saline conditions. Results from this study should enhance urine fertilizer management to improve crop yields while being conscious of the risk of soil degradation.

4.2 Materials and methods

We conducted our investigations on cultivation substrate from two pot experiments carried out in a greenhouse at the University of Hohenheim, Stuttgart – Germany (48.7114 °N 9.2095 °E). Cultivation substrates had been sown twice with maize (*Zea mays* L.) and fertilized with human urine in 2010 and 2011, hereafter, referred to as crop cycle I and crop cycle II, respectively. The substrate constituted of bio-waste compost, quartz sand and silty loam soil, homogeneously mixed in a 1:1:1 volume ratio to improve organic matter content and to enhance nutrients availability (Boh et al. 2013). Substrate samples from the homogenous mixture were taken and analyzed for chemical properties (Table 4.1). For both crop cycles, the experimental factors were salinity and nitrogen applied as urine. Salinity was imitated by adding NaCl solutions to the cultivation substrate. The amount of salt solution to be added was determined in a calibration study whereby different volumes of NaCl solution were added to known weights of cultivation substrate, incubated for 72 hours and their electrical conductivities measured from a saturated paste extract (EC_e) according to Richards (1954). Targeted NaCl-salinity treatments achieved were EC_e 1.3 (S0 – no NaCl added), 4.6 (S1) and 7.6 (S2) dS m^{-1}.

Table 4.1 Chemical composition of untreated substrate at the beginning of the experiment

Element	Soil substrate
Total N *(%)*	0.1±0.0[§]
Total C_{org} *(%)*	1.6±0.1
Exchangeable Ca^{2+} *($cmol_c\ kg^{-1}$)*	13.7±0.6
Exchangeable Mg^{2+} *($cmol_c\ kg^{-1}$)*	1.9±0.0
Exchangeable K^{+} *($cmol_c\ kg^{-1}$)*	0.9±0.0
Exchangeable Na^{+} *($cmol_c\ kg^{-1}$)*	0.5±0.0
CEC_p *($cmol_c\ kg^{-1}$)*	14.1±1.3
ESP *(%)*	0.1±0.0
Water extractable SO_4^{2-} *(mg kg^{-1})*	112.3±9.5
Cl^{-} *(mg kg^{-1})*	25.2±3.0
Bray II-P *(mg kg^{-1})*	58.6±1.3
EC_e *(dS m^{-1})*	1.3±0.4
pH_{KCl}	7.2±0.3

[§] *Values are means ± standard deviation (n = 4)*

Urine was collected from male and female students in a hostel of the University of Hohenheim, Stuttgart in April 2009 and stored in an air-tight container at mean daily temperatures of 25±2 °C for 12 months before usage. This duration and temperature were according to Vinnerås et al. (2008) sufficient for inactivation of bacteria and viruses. Prior to application, the urine container was thoroughly shaken and samples taken for chemical analyses. At first crop fertilization (crop cycle I), urine contained 8.4 g total N (mainly NH_4^+ and NH_3), 1.42 g K (as K_2O), 0.49 g P (as P_2O_5), 0.35 g Ca^{2+}, 0.53 g Mg^{2+}, 2.70 g Na^+, 3.37 g Cl^- and 2.3 g S (as SO_4) l^{-1}. Total nitrogen content in urine decreased by 29 % before the start of crop cycle II in 2011 assumingly due to volatilization losses.

For crop cycle I, 12 kg of the prepared soil substrate was filled into 10 l Mitscherlich pots, sown with maize (*cv*. Okomasa) seeds and fertilized with urine. Urine treatments were 0 (U0) 180 (U1) and 360 (U2) mg urine-N kg^{-1} substrate. P and K in the substrate were augmented by adding mono potassium phosphate in amounts equivalent to 39 mg PO_4^{3-} and 47 mg K_2O kg^{-1} substrate. Eight replications of the 3 x 3 salinity urine-N factorial combinations were arranged in a completely randomized block design. This experiment was terminated at the onset of tasseling. Plant biomass (root and shoot) was harvested, substrate from each pot was hand-crushed and samples collected for chemical analyses.

Eleven kilograms of the homogenized cultivation substrate was refilled into the Mitscherlich pots for the second crop (crop cycle II). Cultivation substrates were not treated with NaCl any further and salinity remained at levels induced initially or as influenced by urine fertilization at the end of crop cycle I. Maize was sown as a second crop and substrates fertilized with half as much urine-N as used in crop cycle I. Urine dosages were 0 (U0), 90 (U1) and 180 (U2) mg N kg^{-1} substrate. Experimental design was the same as in crop cycle I except that there were 4 replications per treatment. Whole plant (root and shoot) biomass was harvested at tasseling and substrates from each pot was hand-crushed, homogeneously mixed and samples also taken for chemical analyses.

Substrate samples collected at the end of both trials were air-dried and sieved through a 2 mm merge. Water soluble cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) were measured from a 1:5 substrate:water extract after shaking 4 g of air-dried soil substrate end-over-end in 20 ml deionized water for 1 hour and centrifuged for 10 minutes at 2500 rpm. The concentrations of soluble Na^+, K^+ and Ca^{2+} in the extract were measured by flame

photometry while Mg^{2+} was measured by atomic absorption spectrophotometry (AAS). Water soluble anions were measured by ion chromatography [861 Advanced Compact IC with Metrohm Suppressor Module II (MSM II) and 853 Metrohm CO_2 Suppressor (MCS), Metrohm Ltd, Switzerland]. Substrate pH was measured in 1 *M* KCl with a glass electrode using a 1:5 substrate:solution suspension according to standard procedure. Electrical conductivity (EC_e) was measured by same method as mentioned above.

4.2.1 Exchangeable cations and cation exchange capacity (CEC_p)

Exchangeable cations and potential cation exchange capacity (CEC_p) were measured using a modified $BaCl_2$ and $MgCl_2$ method described in Hendershot and Duquette (1986). Five grams of air-dried substrate samples were weighed into 50 ml centrifuge tubes. Three centrifuge-washing steps were carried out as follows: 25 ml of 0.1 *M* $BaCl_2$-triethanolamine solution was added to the substrate in the centrifuge and tubes were shaken on an orbital shaker for 10 min and centrifuged at 2500 rpm for 10 min. The clear supernatant solution was filled into 100 ml volumetric flask after each washing step. After the last washing, the volumetric flask was filled to exactly 100 ml with deionised water, hand-shaken and filtered through ash-free Whatman filter paper N° 42 (Macherey-Nagel GmbH & Co., Germany). The solution was collected in PE-bottles for measurement of exchangeable Na^+, K^+, Ca^{2+} and Mg^{2+}.

Substrates were washed twice with deionised water and decant discarded. Three centrifuge-washing steps with 25 ml of 0.15 *M* $MgCl_2$ solution was carried out following the same procedure as described for $BaCl_2$ above. At the end, the filtrate was collected in PE-bottles for measurement of exchangeable Ba^{2+}. CEC_p was calculated after accounting for the dilution caused by entrained $BaCl_2$ solution using the formula:

$$CEC_p = [C\ (Ba^{2+})*z*V_{Extr.}] / [W*M\ (Ba)];$$

Where, CEC_p is potential cation exchange capacity ($mmol_c\ kg^{-1}$), $C(Ba^{2+})$ is Ba^{2+} concentration in the extract ($mg\ l^{-1}$), z is the valence of Ba^{2+} (2), $V_{Extr.}$ is the volume of the extraction solution (ml), W is the weight of the soil and M(Ba) is the molar mass of Ba^{2+} ($g\ mol^{-1}$).

Exchangeable Na^+, K^+, Ca^{2+} and Ba^{2+} were measured by flame photometry and Mg by AAS. As an index of sodicity, exchangeable sodium percentage (ESP) was calculated according to Seilsepour et al. (2009) using the formula:

$$ESP = (Na^+/CEC_p)*100;$$

Where, Na^+ is the measured exchangeable sodium in $cmol_c\ kg^{-1}$ and CEC_p is cation exchange capacity in $cmol_c\ kg^{-1}$.

4.2.2 Data analyses

Data were analysed using the SAS statistical software (SAS Institute version 9.3). The effect of crop cycle, salinity and urine fertilization on pH_{KCl}, EC_e, water extractable and exchangeable Na^+, K^+, Ca^{2+} and Mg^{2+}, water soluble anions, CEC_p and ESP were assessed using the GLM procedure for analysis of variance (ANOVA). The three main factors crop cycle, salinity, urine fertilization and their interactions were considered statistically significant at $p \leq 0.05$ level of probability. When the three-way interactions were not statistically significant, data were pooled for two- or one-way ANOVA. The data for water extractable cations, anions and CEC_p were log-transformed while the data for exchangeable Na^+ was square root-transformed for statistical calculations. Where ANOVA indicated significant differences, mean differences between treatments were compared using the adjusted Tukey-Kramer *post-hoc* test.

4.3 Results

4.3.1 Effect of crop cycle, salinity and urine treatment on substrate pH_{KCl}

Substrate pH_{KCl} was significantly affected by crop cycle x salinity ($F = 3.54$; $p = 0.034$), crop cycle x urine fertilization ($F = 8.47$; $p = 0.0005$) and salinity x urine fertilization ($F = 12.28$; $p < 0.0001$) interactions. Though substrate pH_{KCl} was generally higher at the end of the crop cycle II, there was no significant salinity or urine application effect. At the end of crop cycle I, substrate pH_{KCl} units significantly decreased from 6.8 (U0) to 6.6 (U2) in the S1 and from 6.8 (U0) to 6.4 (U2) in the S2 treatments (Table 4.2).

4.3.2 Effect of crop cycle, salinity and urine fertilization on exchangeable cations, EC_e, CEC_p and ESP

There was no significant crop cycle, salinity and urine interaction effect on exchangeable cations, CEC_p and ESP ($p > 0.05$). Exchangeable Na^+, K^+ and Ca^{2+} were significantly affected by urine x salinity interactions (Figure 4.1A, B and C). Compared to the unfertilized treatment (U0), average substrate exchangeable Na^+ increased by 33.6 (U1) and 76.6% (U2) in the S0 treatment (Figure 4.1A). Furthermore, a 62% increase in average exchangeable Na^+ was measured as urine fertilization amount was raised from U1 to U2. In the S1 treatment, an 11.3% significant increase in mean exchangeable Na^+ content was measured following the application of U2 compared to U0. Though there was a tendency towards an increase in mean

exchangeable Na^+ in the S2 treatment as the amount of applied urine rose, the effect was not statistically significant.

Table 4.2 Effect of crop cycle (cycle I and II), NaCl salinity level (S0, S1 and S2) and urine fertilization (U0, U1 and U2) on selected water extractable ions

Crop cycle	NaCl Salinity level	Urine treatment	Water extractable ions					
			$logK^+$ (mg kg^{-1})	$logMg^{2+}$ (mg kg^{-1})	$logCa^{2+}$ (mg kg^{-1})	$logCl^-$ (mg kg^{-1})	$logNO_3^-$ (mg kg^{-1})	pH_{KCl}
Cycle I	S0	U0	3.4±0.05de	3.2±0.04fg	4.8±0.14hij	2.3±0.10j	2.7±0.13h	6.7efg
		U1	3.3±0.02de	3.4±0.06ef	5.0±0.14fgh	3.7±0.07h	5.1±0.14f	6.8c-g
		U2	3.4±0.04de	3.8±0.03cd	5.40±12de	4.8±0.06g	6.4±0.08bc	6.7g
	S1	U0	3.3±0.04de	3.3±0.03f	5.0±0.12hi	6.1±0.03ef	4.2±0.09g	6.8b-e
		U1	3.5±0.06de	3.6±0.06de	5.2±0.17efg	6.3±0.05de	5.7±0.18de	6.8c-g
		U2	3.9±0.03b	4.0±0.04ab	5.8±0.14bc	6.5±0.03cd	6.8±0.07ab	6.6g
	S2	U0	3.5±0.06dc	3.6±0.05d	5.3±0.21de	7.0±0.06ab	5.3±0.11ef	6.8b-f
		U1	3.8±0.03bc	3.9±0.06bc	5.6±0.18cd	7.0±0.05ab	6.2±0.12cd	6.7d-g
		U2	4.2±0.06a	4.3±0.07a	6.0±0.15a	7.2±0.06a	7.2±0.10a	6.4h
Cycle II	S0	U0	3.1±0.11ef	3.0±0.01gh	4.7±0.03hij	2.9±0.10i	2.4±0.07h	6.9a-d
		U1	2.7±0.05g	2.9±0.02h	4.7±0.06j	3.1±0.06i	2.3±0.09h	6.9abc
		U2	2.9±0.09fg	2.9±0.08h	4.9±0.07hij	4.4±0.11g	4.8±0.04fg	7.0ab
	S1	U0	2.8±0.09fg	2.9±0.07gh	4.6±0.11j	5.8±0.03f	2.5±0.21h	7.0a
		U1	2.8±0.06fg	3.0±0.03gh	4.7±0.09hij	6.1±0.03ef	2.3±0.00h	7.1a
		U2	2.9±0.09fg	3.1±0.05fgh	5.0±0.10fgh	6.1±0.02def	2.5±0.06h	7.1a
	S2	U0	2.8±0.11fg	3.3±0.01fg	5.0±0.03ghi	6.9±0.01ab	2.3±0.00h	7.1a
		U1	2.7±0.12g	3.1±0.06gh	4.8±0.18hij	6.7±0.10bc	2.1±0.14h	7.1a
		U2	4.0±0.07ab	4.1±0.10abc	6.0±0.23ab	7.0±0.05ab	7.0±0.13ab	6.8b-g

Different lowercase letters within the same column indicate significant differences at $p \leq 0.05$, according to Tukey-Kramer adjusted test.

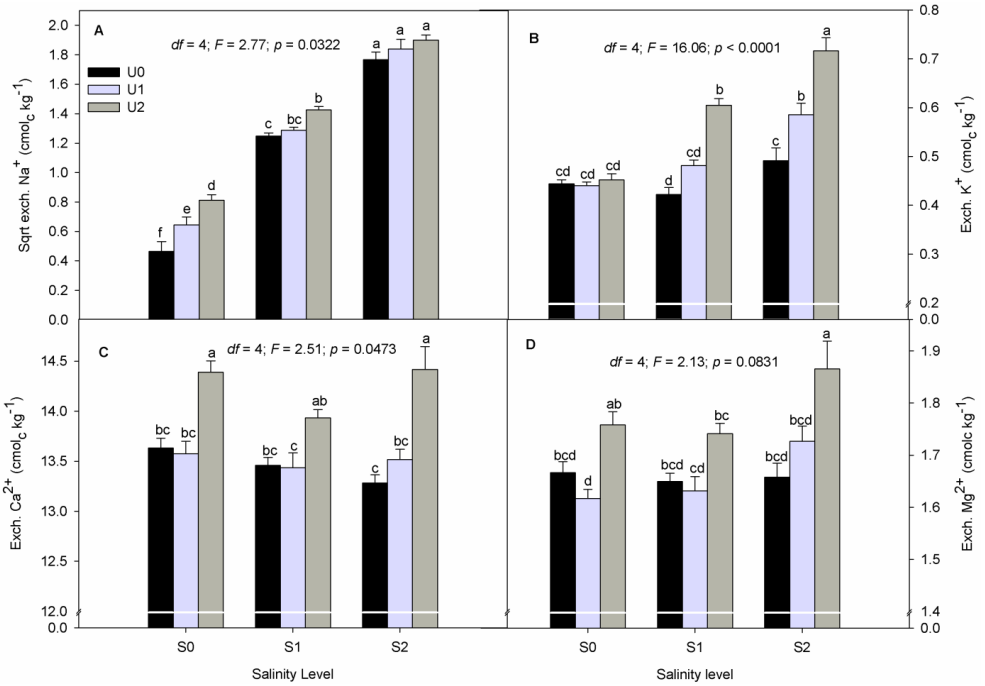

Figure 4.1 Interactive effect of salinity (S0, S1 and S2) and urine fertilization (U0, U1 and U2) on substrate exchangeable Na^+ (A), K^+ (B), Ca^{2+} (C) and Mg^{2+} (D). Data are means of two crop cycles ± S.E. (*n* = 12).

Average exchangeable K^+ content was not affected by urine fertilization in the S0 treatment whereas in the S1 treatment it increased by 0.18 $cmol_c\ kg^{-1}$ (30%) as urine fertilization was raised from U0 to U2 (Figure 4.1B). The effect of urine application was strongest under S2 treatment where mean substrate exchangeable K^+ increased by 16.9% from U0 to U1 and 18.1% from U1 to U2.

Compared to U0, U1-treated substrates had no significant effect on average exchangeable Ca^{2+} concentrations at all salinity levels ($p > 0.05$) (Figure 4.1C). Meanwhile, in the S0 and S2 treatments, the mean concentration of exchangeable Ca^{2+} significantly increased by 0.76 and 1.13 $cmol_c\ kg^{-1}$ following an increase in urine application from U0 to U2, respectively.

Under all salinity treatments, U1 had no significant effect on exchangeable Mg^{2+} concentration compared to the unfertilized treatment (U0) (Figure 4.1D). Exchangeable Mg^{2+} significantly increased by 0.14 $cmol_c\ kg^{-1}$ as urine application rose from U1 to U2 in the S0 treatment meanwhile in the S2 treatment, average exchangeable Mg^{2+} concentration was by 0.21 $cmol_c\ kg^{-1}$ significantly higher in U2 compared to U0 treatment.

Crop cycle did not have a significant effect of substrate EC_e ($p > 0.05$) whereas statistical analyses showed that EC_e was significantly affected by a salinity x urine interaction. Substrate

EC_e significantly increased by 7.3 (83%), 5.3 (52%) and 7.6 (49%) dS m^{-1} as urine fertilization rose from U0 to U2 in the S0, S1 and S2 salinity levels, respectively (Table 4.3).

Table 4.3 Interactive effect of NaCl salinity (S0, S1, S2) and urine fertilizer treatment (U0, U1, U2) on substrate electrical conductivity (EC_e)

NaCl-salinity treatment	Urine treatment	EC_e (dS m^{-1})
	U0	1.5±0.2f
S0	U1	4.9±0.3e
	U2	8.8±0.5bc
	U0	4.8±0.1e
S1	U1	7.9±0.1cd
	U2	10.1±0.2b
	U0	7.8±0.3cd
S2	U1	9.8±0.1b
	U2	15.4±0.7a

Values are means ± S.E. (*n* = 12). Different lowercase letters indicate significant differences at $p \leq 0.05$ (Tukey-Kramer adjusted test)

There was a tendency towards an increase in CEC_p with mounting urine application at all NaCl treatment levels (Figure 4.2A). However, the effect of urine fertilization was only significant with the application of U2 which resulted in an increase in CEC_p from 15.9 (U0) to 17.8 (U2) $cmol_c$ kg^{-1} in the S0 and from 18.6 (U0) to 20.6 (U2) $cmol_c$ kg^{-1} in the S2 treatment. Additionally, CEC_p was by 1.4 $cmol_c$ kg^{-1} significantly higher in the U2 compared to U1-fertilized soils in the S2 treatment.

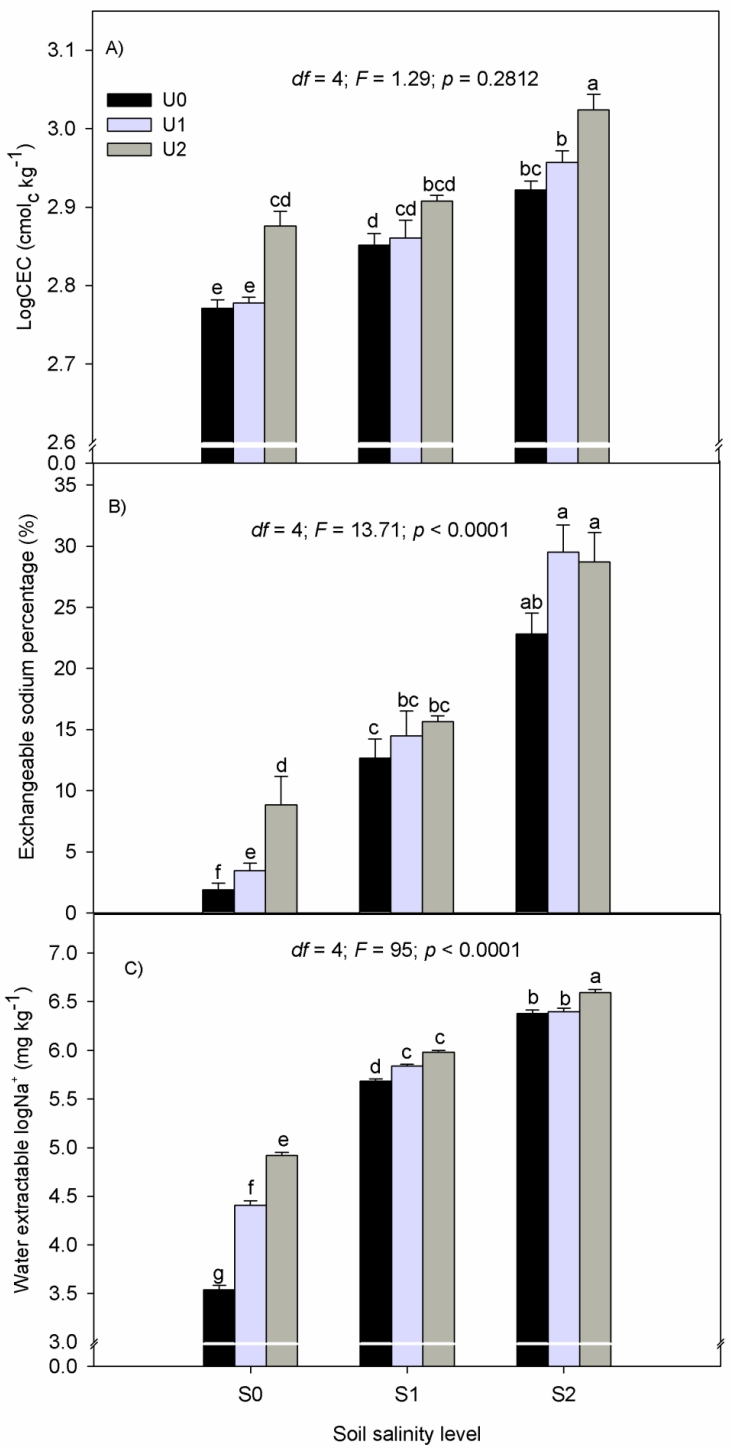

Figure 4.2 Interactive effect of salinity (S0, S1 and S2) and urine fertilization (U0, U1 and U2) on substrate A) cation exchange capacity, B) Exchangeable sodium percentage and C) water extractable Na^+. Data are means of two crop cycle ± S.E. (*n* = 12).

As expected, ESP increased as salinity level rose but the effect of urine fertilization on ESP was significant only in the untreated substrate (Figure 4.2B). Compared to U0 in the S0 treatment, ESP rose by 1.6 (U1) and 6.9 (U2) representing a 46 and 78% increase.

4.3.3 Effect of crop cycle, salinity and urine fertilization on water extractable cations

There was no significant interaction effect of crop cycle x salinity treatment x urine fertilization on water extractable Na^+. Meanwhile, the interaction effect of NaCl treatment and urine application on mean water extractable Na^+ was significant (Figure 4.2C) ($p < 0.05$). Our data showed that the effect of urine fertilization was strongest in the S0 treatment where extractable Na^+ rose by 59.3 (U1) and 66.8% (U2) compared to U0 treatment. In the S1 and S2 treatments, the application of U2 increased mean extractable Na^+ by 25.7 and 19.2% compared to the U0 treatment.

ANOVA results showed that water extractable K^+, Mg^{2+} and Ca^{2+} were significantly affected by crop cycle, salinity treatment and urine fertilization interactions ($p < 0.05$) (Table 4.2). With the exception of S0:U0 and S2:U2 treatment variants extractable K^+ was higher in cycle I than in cycle II (Table 4.2). In cycle I, urine application significantly raised extractable K^+ from 26.2 mg kg^{-1} (U0) to 47.5 mg kg^{-1} substrate (U2) and from 33.8 (U0) to 66.6 mg kg^{-1} substrate (U2) in the S1 and S2 treatments, respectively. Meanwhile, in cycle II average extractable K^+ decreased by 22.9% from 23.1 (U0) to 17.8 mg kg^{-1} substrate (U1) in the S0 treatment. At the end of cycle II, there was no significant urine fertilization effect on extractable K^+ content in S1 whereas in the S2 treatment it was higher by 69.9% in U0 compared to U2 treatment.

Average water extractable Mg^{2+} content was significantly affected by crop cycle x salinity treatment x urine interaction ($F = 3.41$; $p = 0.0123$). Extractable Mg^{2+} was generally higher in crop cycle I compared to cycle II and increased with mounting urine application at all salinity levels (Table 4.2). In cycle I, compared to U0 mean extractable Mg^{2+} content under U2 fertilization rose by 48.3, 50.1 and 46.0% in the S0, S1 and S2 treatments, respectively. In cycle II, whereas urine fertilization did not affect extractable Mg^{2+} concentration in the S0 and S2 treatments, extractable Mg^{2+} concentration more than doubled in the S2 treatment as urine fertilization was raised from U0 to U2 and U1 to U2.

Similarly, water extractable Ca^{2+} increased as urine application amount rose in crop cycle I with significantly higher mean values for U2 compared to U0 under all salinity treatments (Table 4.2). Mean extractable Ca^{2+} was by 107.6, 176.8 and 220.3 mg kg^{-1} substrate higher in U2 than U0 in the S0, S1 and S2 treatments, respectively. Meanwhile, at the end of cycle II the effect of urine application was only significant with U2 application in the S2 treatment.

4.3.4 Effect of crop cycle, salinity and urine fertilization on water extractable anions

Our data further showed that water extractable Cl^- and NO_3^- were significantly affected by crop cycle, salinity and urine interactions ($p < 0.05$). At the end of cycle I, water extractable Cl^- increased by 87.9 and 28.9% as urine application rose from U0 to U2 in the S0 and S1 treatments, respectively. Extractable Cl^- also increased from 17.6 (U0) to 86.1 mg kg^{-1} of substrate (U2) in the S0 treatment at the end of crop cycle II. No significant changes due to urine application were observed in the S1 and S2 treatments at the end of cycle II.

There was a significant crop cycle x salinity x urine interaction effect on water extractable NO_3^- ($F = 6.35$; $p = 0.0002$). Soil nitrate was generally lower at the end of cycle II compared to cycle I and the effect of urine application on extractable NO_3^- was stronger at the end of cycle I compared to cycle II (Table 4.2). At the end of cycle I, extractable NO_3^- increased in the order of U0 < U1 < U2 regardless of salinity level. Meanwhile in cycle II, the effect of urine application was significant only at highest urine application (U2) in the S0 and S2 treatments.

As shown by our data in Figure 4.3A, mean water extractable SO_4^{2-} was not affected by any interactions but the main factors (crop cycle and urine application) were significant. Compared to the unfertilized treatments (U0), SO_4^{2-} content increased by 19.5 (U1) and 32.5% (U2) in cycle I. Like in cycle I, SO_4^{2-} content tended to increase with mounting urine application in cycle II though the effect became significant (19.9% increase) only with the application of U2 compared to the unfertilized treatment.

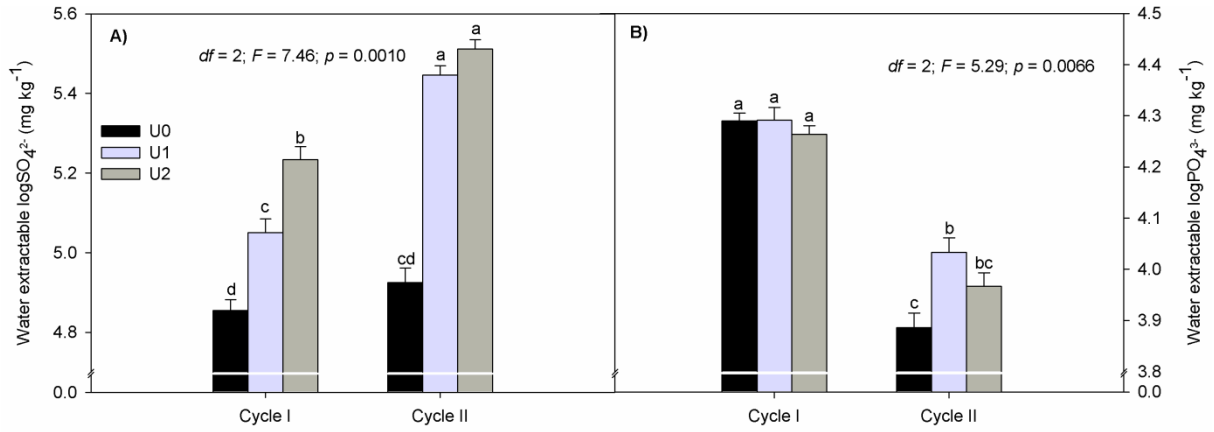

Figure 4.3 Effect of crop cycle (cycle I, II) and urine fertilization (U0, U1 and U2) on substrate extractable A) SO_4^{2-} and B) PO_4^{3-}. Data are means ± S.E. ($n = 12$: cycle I; $n = 24$: cycle II). Significant differences ($p < 0.05$) between treatments are indicated by small case letters.

A significant crop cycle x urine fertilizer interaction affected average water extractable PO_4^{3-} content (Figure 4.3B). In cycle II, water extractable PO_4^{3-} significantly increased from 48.9

(U0) to 53.0 mg kg^{-1} substrate (U1) but a higher dose of urine did not have a significant effect on PO_4^{3-}. In contrast, PO_4^{3-} was not affected by urine rates in the cycle I.

4.4 Discussion

This paper reports the interactive effect of urine fertilization and NaCl-induced salinity on changes in substrate chemical properties under controlled greenhouse conditions. Of the changes that affect soil chemical properties, pH is considered very important as it influences the availability of plant nutrients (Bagayoko et al., 2000). At the end of cycle I, a slight decrease in substrate pH_{KCl} at higher urine application in S1 and S2 treatments can be associated with nitrification and the release of H^+. This effect was only temporal and by the end of cycle II substrate pH_{KCl} increased and the effect of urine fertilization was no longer significant. Fluctuating pH resulting from urine deposition in pasture lands has also been reported by Haynes and Williams (1992) while Hoglund (2001) has argued that the net effect of urine fertilizer on soil pH is small due to the release of hydroxides when plants take up the supplied N in the form of nitrate ions.

Our results showed that substrate EC_e rose as urine fertilizer amount increased in agreement with earlier research findings (Beler-Baykal et al., 2011; Boh et al., 2013; Mnkeni et al., 2008). As urine is inherently saline, high dosages of urine entailed higher amount of salts added to the cultivation substrate. Although under field conditions leaching of salts from the soil may occur due to precipitation, the high electrical conductivities resulting from the application of urine in our experiments suggests that in the long term salt build up may occur even in fields. To prevent soils degradation due to urine-induced salinity, salinity management measures should be included in urine fertilization programs. Mnkeni et al. (2008) have suggested that a well-adapted crop rotation system with salt-tolerant varieties and halophytic vegetables might reduce the potential risk of an eventual salt build resulting from urine fertilization.

The application of ammoniacal N fertilizer has been shown to cause a decrease in soil exchangeable Ca^{2+}, Mg^{2+} and cation exchange capacity due to leaching losses (Barak et al., 1997). Increased exchangeable Ca^{2+}, Mg^{2+} and K^+ observed in our study resulted from their addition through urine and were expected as leachate was returned to the substrate throughout the experiments. An increase in soil cations due to urine fertilization has also been reported in field trials (AdeOluwa and Cofie, 2012; Germer et al., 2011). The high

content of water extractable Ca^{2+} and Mg^{2+} in the substrate at the end of crop cycle I due to urine fertilization represent the portion of these cations that can potentially be leached from the soil profile. However, if they are not leached as was the case in our experiments these nutrient cations can become available to the second crop or next season. Reduction in water soluble Ca^{2+} and Mg^{2+} at the end of crop cycle II can partly be explained by their precipitation in the soil solution in agreement with Miyazawa et al. (2001).

Generally, the concentration of Na^{+} in urine is manifold higher than that of Ca^{2+} and Mg^{2+} and therefore, requires attention in urine fertilizer planning especially under saline conditions. Although Na^{+} is noted for its functional role as a plant nutrient, high concentrations in the growing medium promotes competition with K^{+}, Ca^{2+} and Mg^{2+} that can result in toxic concentrations in plant tissue (Brown et al., 2006; Grattan and Grieve, 1999; Subarao et al., 2003). The N to Na^{+} mass-ratio of the urine used in this study was 3.1:1 but decreased to 2.2:1 following N losses from urine prior to the start of crop cycle II. The risk of raising Na^{+} supply through urine application increases with N losses and should be prevented. This is because more urine will have to be applied if the N content is lowered. Lower N to NaCl mass ratio for urine collected in Ghana has been reported by Germer et al. (2011). Therefore, the increase in exchangeable and water extractable Na^{+} with mounting urine application was no surprise. Haynes and Williams (1992) reported an increase in the concentrations of exchangeable K^{+} and Na^{+} in pasture land due to sheep urine application. It was notable that in the S2 treatment, the effect of urine fertilization on exchangeable Na^{+} concentration was masked by the high content of Na^{+} in the soil substrate solution which is related to the use of NaCl to induce salinity. This suggests that there is a critical limit to the effect of Na^{+} supplied through urine on the concentration of exchangeable Na^{+} in a NaCl-dominated soil substrate. However, this critical limit cannot be determined from our study and would require further investigation.

In this study, it was also expected that due to the high concentration of Na^{+} compared to Ca^{2+} and Mg^{2+}, urine fertilization will increase sodicity with a more severe effect in the NaCl-treated substrates. According to Abrol et al. (1988) an ESP of 15% and a pH greater than 8.5 are critical for soils to be classified as sodic or saline-sodic if in addition EC_e is greater than 4 dS m^{-1}. Our results showed that though in the S0 treatment ESP increased as the amount of urine fertilizer rose, the effect of urine fertilization became less important in the S1 (EC_e 4.6

dS m^{-1}) and S2 (EC_e 7.6 dS m^{-1}) treatments due to the dominating effect of Na^+ added through NaCl. Besides, as noted above, overall substrate pH_{KCl} remained below 8.5. In spite of the amount of Na^+ added through urine it is clear from our results that salinity is of greater concern than sodicity.

Results of this study suggest that Cl^- content of urine fertilizer is an important factor in determining the concentration of Cl^- in the soil. Chloride is generally considered as an essential micronutrient; however, excessive supply of Cl^- can have a toxic effect on plant growth (White and Broadley, 2001). Therefore regulations for the application of urine fertilizers should consider the risk of elevated chloride levels that may cause toxicity in plants (Tavakkoli et al., 2010).

The increase in substrate SO_4^{2-} with mounting urine application was expected due to the high concentration of SO_4^{2-} in urine. Of the secondary nutrients contained in urine, SO_4^{2-} had the highest concentration and can therefore be used to correct S deficiency. However, in areas where precipitation is high, the leaching of SO_4^{2-} supplied through urine can become an important concern and should be considered in urine fertilization planning.

Phosphate concentration was not affected by urine application in crop cycle I probably due to the basal application. The significant decrease in substrate PO_4^{3-} at the end of crop cycle II is due to plant uptake and precipitation (Wandruzska, 2006). Meanwhile, higher concentration of PO_4^{3-} in urine treated substrate is related to addition through urine.

Our results showed an increase in substrate NO_3^- as urine application amount rose which is in agreement with the findings of Decau et al. (2004) and Williams et al. (1998) who measured high concentrations of soil NO_3^- in grasslands following urine deposition from cattle. Once applied to the soil urine-N undergoes different changes. Ammonium which is the major form of N in stored urine is either taken up directly by plants or nitrified to NO_3^- which is also an available form for plant uptake. Some of the NO_3^- can be taken up by microorganisms (immobilization) or microbially reduced (de-nitrification) to produce gaseous nitrogen that is eventually lost from the soil through volatilization. In a lysimeter study, Di and Cameron (2007) measured significant NO_3^--N leaching losses as the amount of urine-N application increased. No N losses in our experiment can be attributed to leaching as leachate was returned to the respective pots. Therefore, lower substrate NO_3^- concentration measured in

our substrates at the end of the cycle II can be explained by immobilization or volatilization losses. The significantly high substrate NO_3^- concentration in the S2:U2 treatment variant in both cropping cycles can be attributed to the plant's inability to take up supplied nitrogen due to high salinity (Bowman et al., 2006; Ward et al., 1986).

As pot sizes and the artificial environment under which these investigations were carried out are limiting, our results are meant to present a snapshot of the changes in soil chemical properties that may result from urine fertilization at different level of NaCl salinity. An understanding of the changes imposed by urine fertilization on soil chemical properties under saline conditions is important for urine fertilization planning and management.

4.5 Conclusion

The potential of urine to cause an increase in exchangeable Ca^{2+} and Mg^{2+} depends on the amount of urine applied regardless of NaCl concentration level. An average urine dosage of 270 mg kg^{-1} substrate over two crop cycles can increase soil EC_e by up to 3.5-folds representing a severe salinity risk if urine is used to fertilize potted plants. In confirmation with our hypothesis, urine fertilization induced an increase in substrate exchangeable Na^+ content and ESP under non-saline conditions (EC_e 1.3 dS m^{-1}) but the perceived effect under NaCl treatments was minimal. However, regularly monitoring salinity and/or sodicity build up and adopting appropriate management strategies must also be thought of during urine fertilizer planning. To develop a guideline for urine use under saline conditions, a long term investigation on urine x salinity interaction under field conditions would be necessary.

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5 Maize (*Zea mays* L.) response to urine and wood ash fertilization under saline (NaCl) soil conditions

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Abstract

An experiment was conducted under controlled environmental conditions to test whether supplementary wood ash application could improve the fertilizing effect of urine on maize (*Zea mays* L.) under saline conditions. The study consisted of three salinity levels (EC_e) 0.6 (S0), 4.2 (S1), 9.6 (S2) dS m^{-1}, three nitrogen (given as urine) treatments 0 (no urine), 75 (U1) and 150 mg urine-N kg^{-1} soil (U2) and two wood ash treatments; 0 (no wood ash) and 150 mg (WA) kg^{-1} soil. In total, six fertilizer treatments F0 (no urine, no wood ash), U1, U2, WA, U1+WA and U2+WA were tested at each salinity level. Maize height and SPAD-values were measured 35 days after sowing. Dry weights and shoot concentrations of K, P, Na, Mg, Ca, Mn, Cu and Zn of treated plants were measured 45 days after sowing. Results showed that salinity stress significantly decreased plant height and shoot dry weight. At salinity level (S1), the combined application of wood ash and 75 mg urine-N kg^{-1} soil (U1) increased shoot dry weight 1.8-fold compared to F0. No beneficial effect of wood ash was observed under 150 mg urine-N kg^{-1} soil and/or at salinity level S2 (9.6 dS m^{-1}). The results also revealed that under salinity level S1 (4.2 dS m^{-1}), application of WA or U1+WA increased shoot concentrations of K, Mg and Ca. In conclusion, application of wood ash alone or of 75 mg urine-N kg^{-1} soil (U1) plus wood ash proved suitable to foster Na and salt tolerance of maize plants when salinity does not exceed 4.2 dS m^{-1}.

Keywords: *urine fertilizer; NaCl salinity; urine salinity interaction; K and Na antagonism; maize*

5.1 Introduction

Soil salinity is a severe abiotic stress factor limiting agricultural production worldwide (Yadav et al., 2011). Salinity offsets nutritional balance in plants, reducing nutrient uptake in less tolerant crops with a consequent decrease in plant growth (Nandy et al., 2007). Saline soils are inherently poor in nutrients (Hague, 2006); hence, most salinity-fertility interaction studies concluded that fertilizer application can alleviate the deleterious effect on plants caused by salinity (Villa-Castorena et al., 2003; Esmaili et al., 2008). Paradoxically, most mineral fertilizer materials are salts and their use often contributes to salinity in the growing medium (Jacobs and Timmer, 2005) and this should be considered in fertilizer planning under saline soil conditions (Bunt, 1988; Mortvedt, 2001).

In coastal, semiarid and arid areas of developing countries where soil salinity is common and commercial fertilizers are unaffordable, increasing crop yields remains a great challenge (Irshad et al., 2002). In many regions, human urine and wood ash, which are available to households as waste, present a valuable alternative fertilizer for crop production (Pradhan et al., 2009). Urine fertilizer has gained an increased attention during the last two decades and its agronomic importance has been validated both by field trials (Germer et al., 2011; Morghan, 2007; Pradhan et al., 2010) and under controlled conditions (Mnkeni et al., 2008). Urine collected from healthy persons is a hygienically safe fertilizer (Schönning et al., 2002). However, in case of contamination, urine can be sanitized by acidification and/or storage prior to use as fertilizer for food crops (WHO, 2006; Maurer et al., 2006; Vinnerås et al., 2008). The simultaneous presence of macronutrients (NPK) and Na and Cl salts in human urine could be a limitation to its use in crop production under saline conditions. Therefore, where crop fertilization with human urine under saline conditions is envisaged, strategies to improve salinity and Na tolerance should be considered. It has been suggested that by improving the soil cationic balance especially K/Na and/or Ca/Na, crop Na and salinity tolerance may be enhanced (Hague, 2006; Bar-Tal et al., 1991).

Wood ash, produced by combustion of woody vegetation is low in N but comprise cations such as K, Ca and Mg (Awodun et al., 2007; Nieminen et al., 2005) and has a pH-increasing effect (Adekayode and Olojugba, 2010; Ojeniyi et al., 2010; Saarsalmi et al., 2012). Wood ash application can significantly affect N mineralisation process by altering the activity and changing the composition and population of soil microbes through changes in soil chemical

properties (Saarsalmi et al., 2010). In South-West Nigeria, Owolabi et al. (2003) found that the application of wood ash from sawdust alone significantly increased yield of tomato (*Solanum lycopersicum* L.) and okra (*Abelmoschus esculentus* (L.) Moench) and their leaf nutrient content. Wood ash has also been used in combination with urine as fertilizers in Finland with significantly beneficial results. For example, Pradhan et al. (2009) have reported a significantly higher root biomass production for red beet (*Beta vulgaris* L.) fertilized with urine and ash compared to stand-alone treatments with urine or mineral fertilizer.

To date, there is no published information on the effect of urine in conjunction with wood ash under saline soil conditions. Hence, the objective of this study was to test whether the fertilizing effect of urine on shoot nutrient concentration and maize growth under saline conditions could be improved by addition of wood ash. We hypothesized that 1) the salt and Na tolerance of urine fertilized maize can be enhanced by supplementary wood ash application, and 2) regardless of salinity; combined urine and wood ash fertilization optimizes plant nutrition and improves plant growth compared to urine- or wood ash stand-alone treatments.

The results from this study should serve as important bases for future research on urine-wood ash fertilization under saline soil conditions and should be a useful guide to potential users of urine and wood ash in saline soils.

5.2 Materials and methods

A pot experiment was conducted in growth chambers (EA 75HIL Emersacker, Germany) using a silty loam soil derived from Loess collected from the uppermost 30 cm at Stuttgart-Echterdingen, Germany (48°40” N, 9°13” W). The soil was air-dried and passed through a 2 mm sieve. Chemical analyses from a representative sample of the bulk soil were carried out before the beginning of the experiment (Tab. 5.1). Soil total N was measured by infrared absorption using an elemental analyzer (vario EL, Elementar, Hanau, Germany). Plant available P was extracted as described by Bray and Kurtz (1945) and P in the extract was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Varian Vista Pro instrument. Exchangeable cations in the soil (K^+, Na^+, Ca^{2+}, Mg^{2+}) were extracted with 1 *M* NH_4Cl and measured by ICP-OES (VDLUFA, 2009). Soil electrical

conductivity was measured from a saturated paste extract (EC_e) according to Richards (1954) while soil pH was measured in a 1:2.5 soil: water suspension (pH_w).

Table 5.1 Chemical and physical properties of soil, urine and wood ash used in this study

Element	Soil (mg kg^{-1})	Wood ash (mg kg^{-1})	Urine (mg l^{-1})
Total N	1.1	0.06	6325
Total P	7.2	<1	492
K^+	28	59156	1418
Ca^{2+}	ND	19317	53
Mg^{2+}	ND	6781	347
Na^+	ND	1333	2702
Cl^-	ND	544	3370
Zn	0.64	437	70[a]
Cu	0.46	63	98[a]
Mn	5.0	1204	BDL
pH_w	6.2	12.8	11.8
EC_e (dS m^{-1})	0.6	1.7	47.6

[a] values are in µg kg^{-1}; BDL = below detection limit; ND = not determined

The experiment was arranged in a completely randomized block design with three factors and four replications. As a first experimental factor, three soil salinity levels: S0 (no salt added), S1 and S2 were established by adding pre-determined amounts of NaCl solution to known soil weights, achieving target salinities of (EC_e) 0.6 (S0), 4.2 (S1) and 9.6 (S2) dS m^{-1}, respectively. Briefly, to determine the amount of salt to be added to the soil, NaCl was dissolved in distilled water and different amounts applied to known soil weights. Treated soils were incubated for 3 days and the electrical conductivities of saturated paste extracts were measured. Two kg each of the salinized soil were filled in 2 l non free-draining plastic pots.

The second and third experimental factors were urine (U) and wood ash fertilization (WA). Urine for fertilizer application was collected from male and female students of the University of Hohenheim, Stuttgart, Germany in April 2009, stored in an air-tight plastic container at a mean daily temperature of 25±2 °C as recommended by Vinnerås et al. (2008). Prior to application, the urine container was thoroughly agitated and samples were taken for chemical analyses (Tab. 5.1). Wood ash derived from burning twigs and branches of apple (*Malus domestica*, Borkh.) and spruces (*Picea* spp.) was collected from the furnace of a household in Stuttgart-Möhringen during the winter of 2010. The ash was passed through a 2 mm sieve and samples from the bulk were analyzed for chemical content by aforementioned methods (Tab. 5.1). Urine was supplied as an N fertilizer assuming that the effect of other macronutrients was negligible (Mnkeni et al., 2008). Treatments corresponded to 0 (no urine added), 75 mg N (U1) and 150 mg N kg^{-1} soil (U2). Wood ash treatments were 0 (no wood ash

added) and 150 mg wood ash (WA) kg^{-1} soil. The entire experimental setup constituted 18 treatments (3 salinities x 3 urine-N x 2 wood ash) with 6 fertilizer treatments per salinity level, viz, F0 (no fertilizer added), U1, U2, WA, U1+WA and U2+WA. 1/3 of the targeted N dosage was applied seven days before sowing and the remainder 14 days after sowing (DAS). Wood ash was incorporated into the upper 5 cm layer of the soil in each pot a day before sowing.

Maize seeds (*Zea mays* L. *cv.* Ronaldinio) were surface sterilized by soaking for 10 minutes in 6 % sodium hypo-chloride solution, washed with de-ionized water to remove detergent and soaked overnight in distilled water to stimulate germination prior to sowing (Janmohammadi et al., 2008). Six seeds were sown per pot to a depth of 2 cm and later thinned to three healthy seedlings 8 days after onset of emergence. Soil moisture was maintained at 80 % max. water holding capacity throughout the experiment by adjusting the weight of the pot daily with de-ionized water. Day (12 hours) and night temperatures in the growth chambers were set to 30°C and 22°C, respectively, to resemble growing conditions in tropical climate.

5.2.1 Data collection

Investigated growth factors were maize plant height, SPAD values as indicators of leaf chlorophyll content and shoot dry weight, as determinants of plant vigour. Seedling height and SPAD values were measured 35 days after sowing with a ruler and SPAD-502 chlorophyll meter (Konica Minolta, Japan) respectively. The SPAD-502 meter was used according to Schlemmer et al. (2005). Briefly, measurements were taken from the youngest fully developed leaf, halfway from the leaf base to tip and halfway from the midrib to the leaf margin. Three measurements were taken per pot, that is, one measurement each on all three plants growing in each pot. The results were averaged to achieve a single value that represents the pot.

Plant shoots were harvested from 0.5 cm above the ground 45 days after sowing and then oven-dried at 70°C to constant weight. Dried shoots were milled and ashed at 500°C, digested with 1:3 concentrated HCl and analyzed for K, Ca, Na, Mg, Cu, Zn, Mn and Zn content. K, Ca, Na in the ash solution were measured with a flame photometer while Mg, Cu, Zn, Mn and Zn were measured by atomic absorption spectrometry (AAS). P was measured colorimetrically according to the method of Ryan et al. (2001). Soil EC_e and pH_w were measured from samples taken from each pot at the end of the experiment that had been processed according to the methods already described above.

5.2.2 Data analysis

The data were analyzed with the SAS statistical software (SAS Institute, version 9.2) using the GLM procedure for ANOVA. The three main factors salinity (S), urine (U), wood ash (WA) and their interactions were considered statistically significant at $p \leq 0.05$ level of probability. Data for shoot dry weight was log transformed prior to statistical analyses to fulfil the conditions of normality. Where significant differences were found, the differences of means across treatments were further compared using the post-hoc Tukey test.

5.3 Results

5.3.1 pH_w and EC_e

Analysis of variance showed that soil pH_w measured at the end of the trial was significantly affected by urine x wood ash interaction while soil EC_e was affected by urine fertilization alone (Tab. 5.2). In the salinity level S0, wood ash stand-alone fertilizer raised soil pH_w by 20 % compared to U1 treatment. Meanwhile an increase of 21 % in soil pH_w was observed under salinity level S1 in the WA and U1+WA treatments compared to the unfertilized soil (Tab. 5.3). Compared to the unfertilized soils, application of 150 mg urine-N kg^{-1} soil significantly increased soil EC across salinity levels (Tab. 5.3).

Table 5.2 *P*-Values for the ANOVA conducted to determine the interactive effects of salinity (S), urine (U) and wood ash (WA) application on ion content in shoots of maize plants grown in a growth chamber. *P*-values for soil pH_w and EC_e at the end of the experiment are also shown.

Source	K	P	Na	Mg	Ca	Mn	Cu	Zn	pH_w	EC_e
P-values										
model	<0.0001	<0.0001	<0.0001	0.0012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0003	0.0003
Single factors										
S	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0922	<0.0001
U	0.0588	0.0133	0.0030	0.0167	0.1201	0.0952	0.8042	0.0986	0.9280	<0.0001
S x U	0.0004	0.0087	<0.0001	0.0394	0.0058	<0.0001	0.0743	<0.0001	0.3109	0.7299
WA	<0.0001	<0.0001	0.0519	0.0111	0.0002	0.2560	0.0027	0.0005	<0.0001	0.9152
S x WA	<0.0001	0.0001	<0.0001	0.0425	0.0003	0.0031	0.0428	0.0358	0.2684	0.9391
U x WA	0.0383	0.0007	0.1551	0.2326	0.0121	0.0836	0.3765	0.1784	0.0002	0.6380
S x U x WA	0.9883	0.3041	0.5329	0.1017	0.2701	0.0393	0.4550	0.5489	0.4483	0.6263

Table 5.3 Salinity and fertilizer effects on SPAD values, plant height, shoot dry weight, soil pH_w and soil EC_e

Salinity	Fertilizer treatment	SPAD values	Plant height (cm plant^{-1})	Shoot dw (g plant^{-1})	pH_w	EC_e dS m^{-1}
S0	F0	15.5±0.6d	113±10ab	1.5±0.0bc	6.2±0.1bc	0.7h
	U1	30.4±0.9ab	124±16a	1.6±0.1bc	6.0±0.6c	1.0gh
	U2	35.7±1.4a	114±06ab	1.7±0.1ab	6.9±0.2abc	1.8g
	WA	24.7±0.6bc	119±10a	1.9±0.1a	7.5±0.2ab	0.8h
	U1+WA	30.5±1.3ab	100±03abc	1.9±0.0a	7.2±0.1abc	1.1gh
	U2+WA	29.8±1.6ab	116±01ab	1.7±0.0ab	6.8±0.1abc	1.5gh
	Mean (n = 24)	*27.7C*	*114A*	*1.7A*	*6.8A*	*1.1C*
S1	F0	21.1±1.0cd	100±10abc	0.8±0.0fgh	5.9±0.5c	4.9f
	U1	32.7±1.2a	92±03abc	1.0±0.1fgh	6.8±0.3abc	5.3def
	U2	35.5±2.5a	84±05bcd	0.9±0.0fgh	7.1±0.0abc	6.0de
	WA	23.9±2.2bc	94±09abc	1.2±0.1de	7.4±0.1ab	5.0f
	U1+WA	35.5±1.4a	80±01cde	1.4±0.3dc	7.4±0.3ab	5.2ef
	U2+WA	30.3±2.9ab	68±03cdef	1.0±0.1ef	7.0±0.1abc	6.1de
	Mean (n = 24)	*29.8B*	*86B*	*1.0B*	*6.9A*	*5.4B*
S2	F0	31.0±1.5ab	74±02cdef	0.7±0.0gh	6.7±0.1abc	9.8bc
	U1	33.0±1.1a	67±02cdef	0.7±0.1hi	7.2±0.2abc	10.1abc
	U2	32.5±1.0a	49±03ef	0.5±0.0ij	7.0±0.2abc	10.7a
	WA	34.3±1.4a	74±04cdef	0.8±0.1fgh	7.7±0.3a	9.7c
	U1+WA	35.3±0.8a	57±03def	0.7±0.1hi	7.1±0.1abc	10.2abc
	U2+WA	33.0±0.7a	44±04f	0.3±0.1j	6.9±0.2abc	10.6ab
	Mean (n = 24)	*33.2A*	*60C*	*0.6C*	*7.1A*	*10.2A*

Different letters within one row indicate significant difference at $P \leq 0.05$ according to Tukey test. S0, S1 and S2 are soil salt levels. F0 – no fertilizer treatment; WA – wood ash treatment, U1 and U2 – urine-N level 1 and 2, and their combined application with wood ash – U1+WA and U2+WA are fertilizer treatments.

5.3.2 Maize seedling growth

Plant height and shoot dry weight decreased as salinity rose whereas SPAD values increased (Tab. 5.3). Compared to the S0 treatment (no salt added), plant height decreased by 25 and 47 % in the S1 and S2 treatments, respectively. Similarly, a significant decrease of 41 and 65 % in shoot dry matter accumulation was observed in the S1 and S2 treatments (Tab. 5.3). However, mean shoot dry weight was 1.5 and 1.8 times higher in WA and U1+WA treatments compared to the unfertilized control (F0) in the S1 treatments. But, this effect diminished at salinity level S2.

While mean SPAD values generally increased as salinity rose, application of urine and wood ash either as stand-alone fertilizers or in combination significantly further increased leaf SPAD values in the S0 and S1 treatments. The only exception was with WA stand-alone fertilization in the S1 treatment where leaf SPAD values were more or less the same compared with the

unfertilized treatment. Furthermore, SPAD values were twice as high in F0 under S2 compared to S0 (Tab. 5.3).

5.3.3 Shoot K, P, Na, Ca and Mg concentrations

a) Potassium

Maize shoot K concentration was significantly affected by interactions between salinity x urine, salinity x wood ash and urine x wood ash fertilizations (Tab. 5.2). Salinity reduced shoot K concentration in the order S2 < S1 < S0. Comparative analyses showed that shoot K concentration in the U1+WA treatment was 57 and 55 % higher than in the unfertilized control (F0) under S0 and S1 salinity levels, respectively (Tab. 5.4). Additionally, shoot K concentration was significantly higher in U1+WA treatment compared to urine stand-alone (U1) fertilization under S0 and S1 treatments. A higher urine fertilizer application (U2) supplemented with wood ash led to a more than 2.4-fold decrease in shoot K concentration compared to the WA and U1+WA treatments under S1 salinity.

Table 5.4 Effect of salinity and fertilizer treatment on the concentration of nutrients in maize shoots

Salinity	Fertilizer treatment	Shoot nutrient concentration (mg g^{-1} dw)				
		K	P	Na	Mg	Ca
S0	F0	14.6±3.4cdef	0.7±0.2bc	0.1±0.0e	2.2±0.3abc	2.9±0.5bcdef
	U1	15.8±3.5cde	0.6±0.1cd	0.2±0.0e	3.0±0.4ab	3.7±0.6abcde
	U2	22.5±3.1abc	0.7±0.1bc	0.3±0.0e	3.0±0.4ab	4.8±0.7abc
	WA	25.3±2.5abc	0.9±0.1abc	0.3±0.0e	3.3±0.4a	4.5±0.6abcd
	U1+WA	33.8±3.9a	1.1±0.1a	0.4±0.1e	3.4±0.4a	5.7±0.7a
	U2+WA	30.6±1.1ab	1.0±0.0ab	0.2±0.0e	2.9±0.2ab	5.2±0.3ab
	Mean (n = 24)	*23.8A*	*0.9A*	*0.3B*	*3.0A*	*4.5A*
S1	F0	9.8±1.4defg	0.3±0.0def	1.4±0.3cde	1.5±0.3cdef	2.6±0.5cdef
	U1	9.3±0.4defg	0.2±0.0def	1.5±0.1bcde	0.7±0.0defg	2.0±0.1def
	U2	6.6±0.3efg	0.2±0.0ef	1.8±0.2bcde	0.5±0.0efg	2.0±0.4def
	WA	18.4±3.8dc	0.5±0.1cde	2.7±0.5abcd	1.7±0.3bcde	3.7±0.7abcde
	U1+WA	21.8±2.3bc	0.8±0.1abc	4.7±0.7a	2.1±0.2abcd	5.9±0.8a
	U2+WA	9.0±0.8defg	0.4±0.0def	3.4±0.4abc	1.0±0.1cdefg	2.9±0.6bcdef
	Mean (n = 24)	*12.5B*	*0.4B*	*2.6A*	*1.3B*	*3.2B*
S2	F0	6.9±0.9efg	0.3±0.1def	4.3±0.5a	0.8±0.1defg	2.0±0.2def
	U1	4.5±0.4efg	0.2±0.0f	3.6±0.9ab	0.3±0.1fg	1.3±0.3ef
	U2	1.3±0.2g	0.1±0.0f	1.8±0.4bcde	0.2±0.0g	0.9±0.3f
	WA	7.0 ±0.8defg	0.3±0.0def	3.1±0.6abc	0.5±0.1efg	1.3±0.3ef
	U1+WA	3.9±0.7fg	0.2±0.0ef	2.9±0.6abc	0.4±0.1efg	1.2±0.3ef
	U2+WA	0.7±0.3g	0.1±0.0f	0.8±0.2de	0.1±0.0g	0.4±0.1f
	Mean (n = 24)	*4.1C*	*0.2C*	*2.7A*	*0.4C*	*1.2C*

Different letters within one row indicate significant difference at $P \leq 0.05$ according to Tukey test. S0, S1 and S2 are soil salt levels. F0 – no fertilizer treatment; WA – wood ash treatment, U1 and U2 – urine-N level 1 and 2, and their combined application with wood ash – U1+WA and U2+WA are fertilizer treatments. dw = dry weight.

b) Phosphorus

A significant salinity, urine, wood ash and urine x wood ash interaction affected shoot P concentration (Tab. 5.2). Under the S2 treatment, shoot P concentration was 55 and 78 % lower than in the S1 and S0 treatments, respectively (Tab. 5.4). Application of U1+WA significantly increased shoot P concentration compared to the unfertilized control (F0), U1 and U2 as stand-alone fertilizer in the S0 and S1 treatments. In the S1 treatment shoot P concentration was reduced by 50 % in the treatment U2+WA compared to U1+WA treatment.

c) Sodium

Shoot Na concentration was significantly affected by salinity and urine applications. Additionally, there were significant salinity x urine and salinity x wood ash interactions (Tab. 5.2). Average shoot Na concentration was 9 times higher under salinity levels S1 and S2 compared to S0 (Tab. 5.4). Compared with the control (F0) and urine as a stand-alone fertilizer, shoot Na concentration in the S1 treatment was significantly lower than in U1+WA treatment. This effect diminished with the application of U2+WA. In the salinity level S2, application U2 and U2+WA decreased shoot Na by 17 and 71 % respectively, compared to the control, F0. Furthermore, shoot Na concentration was 3.6 times lower in the U2+WA than U1+WA.

d) Magnesium

Mg content in the shoot was significantly affected by salinity, salinity x urine and salinity x wood ash interactions (Tab. 5.2). Salinity decreased shoot Mg concentration in the order S2 < S1 < S0 (Tab. 5.4). Significant effects of fertilizer treatment on shoot Mg concentration were only observed in the S1 treatment.

e) Calcium

Statistical analyses indicated that shoot Ca concentration was significantly affected by salinity, salinity x urine, salinity x wood ash and urine x wood ash interactions (Tab. 5.2). Data presented in Tab. 5.4 showed that compared to the S0 treatment (no salt added), mean shoot Ca concentration decreased by 29 and 73 % in the S1 and S2 treatments, respectively. In comparison with the unfertilized treatments (F0), shoot Ca concentrations were 2.8 and 3.3 mg g^{-1} higher in U1+WA fertilized plants in the S0 and S1 treatments, respectively. Additionally, in the S1 treatment, shoot Ca content was significantly higher in U1+WA

treatment than in urine stand-alone and U2+WA treatments. There was no significant fertilization effect on shoot Ca content under S2 treatment (Tab. 5.4).

5.3.4 Shoot micronutrient content

Mean shoot content of Cu, Zn, and Mn significantly decreased as salinity rose (Tab. 5.5). With regard to shoot Cu content, no significant differences were observed between fertilizer treatments within the respective salinity levels. The content of Zn in maize shoots increased by 9.4 µg g^{-1} plant dw in plants fertilized with 150 mg urine-N kg^{-1} soil (U2) compared with unfertilized plants (F0), in the S0 treatment. Whereas in the S1 treatment, highest shoot Zn concentration was measured in the U1+WA fertilized plants and was 3.2-, 2.1- and 2.5 times higher than in the F0, U1 and U2 fertilized treatments, respectively. Analyses of variance indicated that shoot Mn concentration was significantly affected by salinity, urine and wood ash interactions (p = 0.0393; Tab. 5.2). Compared to the U2 treatment at salinity level S0, shoot Mn concentrations were by 62, 43 and 46 % higher than in the F0, U1 and WA treatments, respectively. Meanwhile in the S1 treatment, Mn concentration was highest in the U1+WA fertilized plants but significantly different only from U2 treatment. There was no significant fertilizer effect on shoot Mn concentration in the S2 treatment.

Table 5.5 Effect of salinity and fertilizer treatments on maize shoots contents of Cu, Zn and Mn.

Salinity	Fertilizer treatment	Shoot nutrient concentration (µg g^{-1} dw)		
		Cu	Zn	Mn
S0	F0	1.4±0.5abcd	8.5±2.1bcd	19.5±3.4bcdefg
	U1	2.5±0.6ab	11.6±1.8abc	29.3±3.9bcde
	U2	3.0±0.4a	17.9±2.5a	51.2±6.3a
	WA	2.9±0.4a	12.1±1.7abc	27.9±4.0bcde
	U1+WA	2.9±0.7a	16.3±1.9ab	35.7±5.1abc
	U2+WA	2.9±0.3a	18.7±1.0a	37.4±2.3ab
	Mean (n = 24)	*2.6A*	*14.2A*	*33.5A*
S1	F0	1.3±0.2abcd	4.9±0.7cd	18.3±3.0cdefg
	U1	1.0±0.1abcd	7.4±0.5cd	17.5±1.1defg
	U2	1.0±0.1abcd	6.3±0.7cd	14.3±1.1efg
	WA	2.2±0.4abc	8.9±1.8bcd	21.2±4.1bcdef
	U1+WA	2.4±0.3ab	15.8±2.3ab	32.9±4.3bcd
	U2+WA	1.9±0.3abcd	9.1±1.2bcd	25.7±5.5bcdef
	Mean (n = 24)	*1.7B*	*8.7B*	*21.7B*
S2	F0	1.1±0.2abcd	5.5±1.0cd	16.2±2.2defg
	U1	0.7±0.1bcd	4.8±0.8cd	11.4±2.1efg
	U2	0.4±0.1cd	2.1±0.2d	7.6±1.4fg
	WA	1.0±0.1abcd	8.0±3.9bcd	9.9±0.7efg
	U1+WA	0.8±0.1bcd	4.1±0.7cd	9.2±1.7fg
	U2+WA	0.3±0.1d	1.0±0.3d	2.1±0.7g
	Mean (n = 24)	*0.7C*	*4.3C*	*9.5C*

Different letters within one row indicate significant difference at $P \leq 0.05$ according to Tukey test. S0, S1 and S2 are soil salt levels. F0 – no fertilizer treatment; WA – wood ash treatment, U1 and U2 – urine-N level 1 and 2, and their combined application with wood ash – U1+WA and U2+WA are fertilizer treatments. dw = dry weight.

5.4 Discussion

This study emphasized the need to adopt appropriate management practices when urine is considered for use as fertilizer under saline (NaCl) soil conditions. Soil salinity may cause diminished plant growth by reducing the plant's ability to take up water and nutrients (Yadav et al., 2011). Results of our study showed that salinity-induced stress reduced plant height and maize shoot dry weight. In addition, an increase in soil salinity induced by urine fertilization was expected due to its high concentration in Na and Cl. The contribution of human urine fertilizer to soil salinity has also been reported by Mnkeni et al. (2008).

Our results further indicated that wood ash application increased soil pH. Soil pH is an important property that affects crop performance and may affect nutrient availability to plants by controlling microbial immobilization rates, de-nitrification and P solubility (Chapin et al., 2004). The increase in soil pH after wood ash application is due to its alkaline nature. Wood ash is rich in oxides, hydroxides and carbonates of Ca and K which and when used as a soil additive may contribute to raise pH. In incubation experiments to determine the effect of wood ash application on soil solution chemistry, Nkana et al. (2002) found that soil solution pH rose with increasing amounts of wood ash. Ojeniyi et al. (2010) have also reported an increase in soil pH from maize fertilization with wood ash.

At salinity level S1, increased shoot dry weight of maize seedlings in the U1+WA treatment compared to the control is an indication that the deleterious effect of salinity can be overcome by a combined application of urine and wood ash. It also suggests an improved salt tolerance of maize plants and better utilization of nutrients in confirmation of our hypothesis. By combining urine and wood ash application, soil content of N, K and Ca and their availability increased due to increased pH (Nottidge et al., 2007; Saarsalmi et al., 2010). However, reduced dry matter yield in the U2+WA treatment indicate that, in this treatment, urine amount exceeded the critical limit beneficial for combined urine and wood ash fertilization of young maize seedlings at S1 salinity. It is apparent that at a higher urine-N application the supplementary effect of wood ash fertilization becomes dominated by increased urine-induced salinity, suggesting that higher dosages of wood ash may be required to improve maize seedling growth. This requires further investigation.

As expected, the application of urine and wood ash in the S0 and S1 treatments increased leaf chlorophyll content (higher SPAD values) due to enhanced N uptake and utilization. However, in the S2 treatment (EC_e 9.6 dS m^{-1}), the fertilizer effect was no longer visible even though the measured SPAD values were high across fertilizer treatments. Here, the high SPAD values can be explained by an increase in the number of chloroplasts which is typical in salt stressed leaves according to findings of Misra et al. (1997) for rice (*Oryza sativa* L.).

5.4.1 Salinity-fertilizer interaction effect on nutrient concentration

Our results further showed that the effect of salinity on shoot K concentration depends on urine application. This can be explained by the fact that increasing urine application in soils with high salt (NaCl) content meant an unavoidable increase in soil Na and Cl salts. Na competes with K uptake when the former is present in large amount in the growing medium (Fricke et al., 2006). Our results also showed a significant salinity x wood ash interaction on shoot K concentration which is an indication that the effect of wood ash application on shoot K concentration depends on the level of soil salinity. Wood ash application is known to increase shoot K concentration (Etiegni et al., 1991). Potassium supplied through wood ash increased the soluble fraction of K in the soil nutrient pool and decreased the potential antagonistic effect of Na on K uptake in S1, which explains the increase in its concentration in the maize shoots (Luan et al., 2009). This effect diminished when the amount of urine added to wood ash was raised to U2 level which meant an increase in soil Na and hence, an enhanced Na-K competition.

The increase in maize shoot Na concentration with mounting soil salinity observed in our study was due to the fact that NaCl was used for salinization which affirms an earlier report by Irshad et al. (2008). A high concentration of Na in the soil solution has an antagonistic effect on K, Mg and Ca uptake (Bernstein, 1975). Antagonism causes a displacement of K, Mg and Ca in the root cells of the maize plant in favour of Na. In our study, NaCl salinity decreased maize shoot K, Mg and Ca concentration in agreement with the findings of Turan et al. (2009). K constitutes an important cation in the plant tissue and is responsible for regulating the cell osmotic potential, translocating sugars and forming starch. Although Na may help maintain cell tugor, it does not substitute the specific enzymatic functions of K and Ca in enhancing plant growth (Subbarao et al., 2003; Pettigrew, 2008). Increased shoot K and Ca concentrations and hence shoot dry matter accumulation in U1+WA-fertilized plants at S0

and S1 can be attributed to their addition through both fertilizers; K, Mg and Ca being higher in the wood ash than in urine. This confirms the findings of Adekayode and Olojugba (2010) who also observed an increase in maize shoot Mg and Ca from wood ash fertilization.

Furthermore, in the S1 treatment the substantial increase in shoot Na concentration in the U1+WA compared to the unfertilized treatment can be attributed to its content in the fertilizer sources combined. Under NaCl salinity, an increase in shoot Na concentration could lead to toxicity and reduce plant growth (Fortmeier and Schubert, 1995). In our study, there was an increase in shoot biomass accumulation in spite of the significant increase in shoot Na concentration in the U1+WA treatment. This can be explained by the dilution effect of K and Ca in the plant tissues considering that the shoot content of these cations also increased in this variant. Supplemental Ca and K added to the soil through wood ash enhanced shoot concentrations. Rengel (1992) has attributed the salt tolerance of plants to their ability to maintain high concentrations of K and Ca and avoid Na toxicity.

In our study shoot P concentration increased in the U1+WA treatment under S0 and S1 salinities in spite of the increase in soil pH. According to Erich and Ohno (1992), wood ash-amended soils can cause an increase or a decrease in plant available P depending on the pH of the soil prior to wood ash application. P availability also tends to be higher in slightly acidic soils; therefore, where soil pH is low, micro doses of wood ash can enhance P availability. For example, Etiegni et al. (1991) reported that wood ash application at rates below 20 g kg^{-1} soil increased P uptake in wheat (*Triticum aestivum* L.). The reason for an increase in shoot P concentration in spite of raised pH following wood ash application remains unclear and would require further investigation.

Proper application of micronutrients can enhance maize growth as they are responsible for vital plant processes such as photosynthesis, respiration and protein synthesis (Salem and El-Gizawy, 2012). However, the solubility of micronutrients under saline conditions is generally low which explains the low concentrations of Cu, Zn and Mn in maize shoots observed in our study. This is in agreement with the findings of Abou El-Nour (2002) who has reported a decrease in concentrations of Cu, Zn and Mn in maize shoots when subjected to saline irrigation water.

Furthermore, micronutrient availability tends to decrease in wood ash-fertilized soils due to increased pH as has been proven under forest soil conditions (Naylor and Schmidt, 1986; Demeyer et al., 2001). Krejsl and Scanlon (1996) also found that Mn and Cu concentrations in bean (*Phaseolus vulgaris* L.) plants decreased following the application of wood ash. In our study, wood ash application had no significant effect on shoot Cu concentration due to the very low content of Cu in the wood ash. However, it increased shoot Mn concentration in the U1+WA compared to the S1:U2 treatment variant. Though it was expected that increased pH_w would reduce Mn availability and hence, its concentration in the shoots of maize seedlings, it is apparent that due to the high content of Mn in the wood ash used, the increase in pH_w had a negligible effect.

5.5 Conclusion

The results of this study revealed that the application of wood ash alone or a combined application of urine and wood ash is beneficial to young maize plants when soil salinity is 4 dS m^{-1} or lower. An improvement in salinity and Na tolerance shown by the ability of maize seedlings to accumulate more biomass can be related to increased K and Ca availability due to wood ash application. No benefit of fertilization was observed under salinity level S2 (9.6 dS m^{-1}). Field trials are recommended to determine the optimum urine and wood ash combination for maize growth under saline soil conditions. Even so, soils should be regularly monitored to avoid any potential salt build up.

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6 General discussion

The decision to apply fertilizer to saline soils depends on whether or not this would lead to an improvement in crop yields. According to Bernstein et al. (1974) three types of salinity-fertility relationships can be distinguished; an independent effect of salinity and fertility on crop yields under sub-optimal and optimal fertility conditions, a decrease in salinity tolerance under sub-optimal fertility conditions and an increase in salt tolerance under low fertility (Fig 6.1). With regards to soil fertility management, nitrogen is one of the most important fertilizers used in crop production and is often the first nutrient to be deficient in poor soils (Irshad et al., 2002). The application of nitrogen fertilizer does not only improve plant growth but may also mitigate the inhibitory effect of salinity (Feigin, 1985; Flores et al., 2001; Villa-Castorena et al., 2003). Plants can take up nitrogen either in the form of NH_4^+ or NO_3^-.

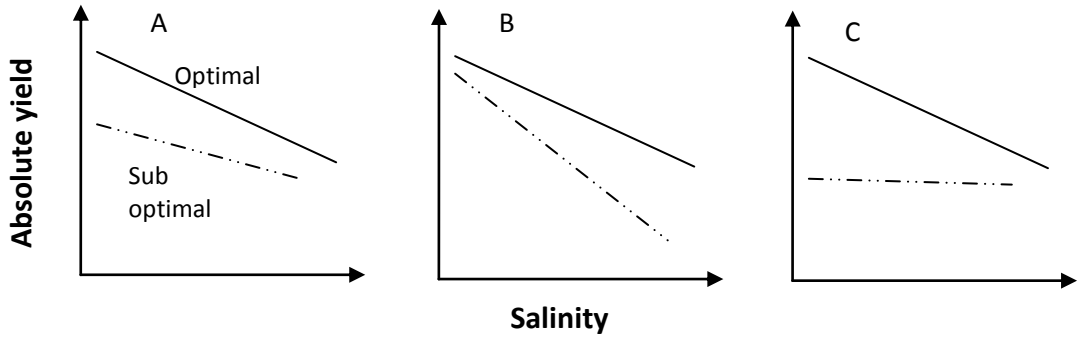

Figure 6.1: Types of salinity-fertility relationships (*Adapted from Bernstein et al., 1974*). (A) Independent effect of salinity and fertility. (B) Decreased salt tolerance and (C) increased salt tolerance, respectively, at a given level of fertility represented by the broken lines of each graph.

It has been argued that growth response of plant to nitrogen application under saline soil conditions depends on whether nitrogen is supplied as NH_4^+ or NO_3^- (Bybordi et al., 2009; Esmaili et al., 2008; Nathawat et al., 2007). Growth inhibition can occur if plants are fed only with NH_4^+ while NO_3^- uptake can be reduced by high concentrations of Cl^- in the growing medium due to antagonism which may result in toxic accumulation of Cl^- in plant tissues (Bar et al., 1997; Iglesias et al., 2004). One of the objectives of this study was to compare the effect of urine and $NH_4^+NO_3^-$ on maize and sorghum growth (see chapters 2 and 3). It was expected that both fertilizers would have the same effect on plant growth until salinity becomes limiting. No difference in dry matter accumulation between urine and ammonium nitrate treated maize plants was measured following the application 180 mg N kg^{-1} substrate whereas, the application of 380 mg urine-N kg^{-1} substrate proved to be comparably unsuitable for maize (see chapter 2). On the other hand, sorghum plants treated with urine

produced comparatively more biomass regardless of salinity level (see chapter 3); even though the two-crop sequence and fertilization with urine increased salinity of urine-treated substrate. The difference in results can be attributed to the fact that sorghum plants are more tolerant to soil salinity than maize (Reddy et al., 2010). Biomass yield of urine-treated plants was not related to additional P, K, Ca and Mg in urine as was expected. However, synergism between two or more of these nutrients could have played a beneficial role (see chapter 3).

Concerns about soil salinization and sodicification issuing from crop fertilization with urine have been raised whenever urine is under consideration. Human urine has a water:nutrient ratio of aprroximately 19:1 with a salinity range between EC 18 – 45 dS m^{-1} depending on whether it is fresh or stored urine (Beler-Baykal et al., 2011). This salinity range, according to Pescod (1992) is 6 to 15 times higher than the maximum EC level for a severe restriction of irrigation water use. Therefore, even with targeted fertilizer nutrient dosages applied, salinity of the growing medium is bound to occur.

In this study it was hypothesized that urine fertilization would increase the risk of soil salinity and sodicity and that the effect on the latter would be severe under NaCl-dominated conditions. Although urine application in moderate amounts was shown to be as good as ammonium nitrate, high dosages of urine increased substrate electrical conductivity and set up extremely adverse conditions for plant growth. Leaf tips burning, stunted growth and dead of urine-fed plants was observed (see chapter 2). The 2 to 3-fold increase electrical conductivity of the saturation extract, measured in urine fertilized substrate can be related to the high contents of soluble salt especially Na^+, Cl^- and SO_4^{3-} added through urine.

The urine used at the beginning of this study had an N to NaCl mass ratio of 1:0.7 but increased to 1:0.9 due to a decrease in N probably from volatilization losses. Germer et al. (2011) reported a 1:1.4 N to NaCl mass ratio in Ghana while in Nepal it was estimated at 1:1.6 (Etter et al., 2011). This implies that with the application of 50 kg of urine-N ha^{-1} of the urine used in this study will result in the addition of 45 kg of NaCl which is lower than the 70 kg and 80 kg in Ghana or Nepal respectively if the same amount of urine N were to be applied as fertilizer. Therefore, the risk of soils turning saline and/or sodic due to urine fertilization depends largely on the N to NaCl ratio and should be considered in urine fertilization planning.

The idea of urine fertilized soils becoming saline or sodic is inseparable due to the fact that while salinity is the concentration of soluble salts, sodicity is a measure of the concentration of Na^+ relative to Ca^{2+} and Mg^{2+} (van de Graaf and Petterson, 2001). Sodic soils generally have lower soluble salt concentration (EC_e < 4 dS m^{-1}) than saline soils (Table 6.1) and have a characteristic particles dispersion which often results in a poor structure of sodic soils making it difficult for water to infiltrate and plant roots to penetrate the soil (Robbins and Gavlak, 1989). Unlike most commercial fertilizer formulations, urine has a high concentration of Na^+. In spite of the high amount of Na^+ added with urine to the cultivation substrate in this study, exchangeable Na^+ concentration was not affected by urine fertilization when compared with $NH_4^+NO_3^-$ (chapter 2). However, it increased during two cycles of urine application (chapter 4) indicating that the effect is additive. Most of the applied N remained in the soil substrate in a water soluble form and represents the fraction that is susceptible to leaching during high rainfall under field conditions. This represents an environmental threat especially during a long dry period in semiarid and arid areas when Na^+ salts could be drawn back to the surface through capillary rise (Kielen et al., 1996).

Table 6.1: Chemical parameters defining salt- and sodium-affected soils *(Adapted from van de Graaf and Petterson 2001)*

Salinity class	EC_e dS m^{-1}	ESP	SAR	pH
Normal soil	< 4.0	< 15	< 13	< 8.3
Saline soil	> 4.0	< 15	< 13	< 8.3
Saline-sodic soil	> 4.0	> 15	> 13	< 8.3
Sodic soil	< 4.0	> 15	> 13	> 8.3

EC_e = electrical conductivity, ESP = exchangeable sodium percentage, SAR = sodium adsorption ratio

The concentration of Na^+ in urine is many times higher than that of Ca^{2+} and Mg^{2+}. However, though in small amounts the content of Ca^{2+} and Mg^{2+} can contribute to offset the potential risk of soils becoming sodic as was assumingly the case in this study. Furthermore, the high concentration of Ca^{2+} in the cultivation substrate used diluted the effect of sodicity issuing from urine fertilization. The addition of SO_4^{3-} through urine fertilization induced the release of tied up Ca^{2+} in the substrate, increasing its solubility and reducing any potential sodicity build up due to urine application (McCauley and Jones, 2005). The pH values remained within the neutral range which is below the benchmark of 8.3 units necessary for soils to be classified as sodic (Abrol et al., 1988; Robbins and Gavlak, 1989). Therefore, under the conditions of the current study, salinity proved to be a greater threat than sodicity.

There was a strong positive correlation between N fertilization and biomass yield regardless of NaCl salinity treatment. However, compared to ammonium nitrate, there was a weak relationship between tissue nitrogen concentration and biomass accumulation of urine-treated sorghum plants (chapter 3). No evidence of additional nutrients through urine was found that should justify higher biomass accumulation in sorghum plants suggesting a synergy between two or more nutrients (e. g. Ca and K) other than nitrogen as reported by Jafari *et al*. (2009). It was further shown in this study that the effect of N source on use efficiency depends on the level of salinity (chapter 3). The critical limit for an efficient use of urine-supplied nitrogen was exceeded at NaCl salinity of 7.6 dS m^{-1} indicating that it will be unwise to fertilize with urine at this level of salinity.

For urine to become a more desirable fertilizer option in saline conditions, measures must be introduced to reduce salinity resulting from urine application. Beler-Baykal et al. (2011) have suggested that by using clinoptilolite, ammonium can be removed from stored urine and the product used as a fertilizer. The weakness with this approach is the loss of other nutrients contents of urine. In this study, it was opined that the salinity and Na tolerance of crops under urine fertilization can be enhanced by improving soil K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ balance in the soil through wood ash application. Results showed that wood ash (150 mg kg^{-1} soil) with or without urine improved Na^+ and salinity tolerance of young maize plants which was related to the increase in K^+ and Ca^{2+} availability (chapter 5). This effect was not evident at NaCl treatment of EC_e 9.6 dS m^{-1} and when the amount of urine-N added to wood ash rose from 75 to 150 mg kg^{-1} soil which could be related to an increase in salinity induced by urine application. This suggests that the tolerance enhancing effect of wood ash which was proposed as a temporal measure can also be limited by urine salinity.

All in all, urine is a highly salty fertilizer and it is not wise to apply high amounts under saline (NaCl) conditions. Under such conditions the local recommendation for N fertilizer especially $NH_4^+NO_3^-$ cannot be adopted for urine. Rather, the decision to use urine and in what amounts should be determined from an evaluation of the risk and benefits that will accrue and urine should be applied only when it reasonably increases yields. The nutrient requirement of the crop to be fertilized, its salt tolerance behaviour and the salt supply power of urine should be taken into consideration. This research stresses the need to develop a urine management system apt for crop, climate and soil salinity and exchangeable sodium conditions. This can be

achieved constructing a model that assists in the prediction of urine fertilizer effect under different salinity levels, climate and crop type.

6.1 Limitations of this study and implication for future research

The conclusions drawn from this study should be interpreted within the following limitations. Firstly, NaCl was used as the only salt for salinization meanwhile in the natural environment though Na^+ is usually the dominant cation and Cl^- the dominant anion, soils contain a mixture of salts and vary depending on the parent rock from which they are formed. Secondly, due to limited time and other resources maize and sorghum which are field crops were grown in pots. Though pot sizes were considerably large, root-bounding was observed in pots where plant growth was more vigorous. Thirdly, regular water supply was assumed in this study which can be obtained only under irrigated systems. This notwithstanding, the water used was portable water which is very costly in semiarid and arid countries of the world where salt affected soil abound.

6.2 References

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Summary

Soil salinity and nutrients deficiency are jointly responsible for low agricultural production in many parts of the world. With a still growing world population and a continuing pressure on arable land, there is need to increase the productivity of salt affected soils. It is known that the application of N fertilizer can mitigate the deleterious effects of salinity on crop growth but this beneficial effect depends on the source of nitrogen and the extent of soil salinity.

Whereas commercial inorganic fertilizers are expensive and often inaccessible to poor farmers especially in developing countries, human urine is an alternative low-cost fertilizer which, if collected and used, can replace up to 20% of current world fertilizer consumption. Its efficacy to increase crop productivity has been validated in scientific experiments. However, the high concentration of soluble salts (especially Na and Cl) in urine may impose a restriction on its use under saline and/or sodic conditions.

The research presented in this thesis investigates the extent to which urine can be used as a fertilizer under saline/sodic conditions. It compares the effect of urine and ammonium nitrate-N sources on nutrients accumulation and growth of maize and sorghum plants in NaCl-saline substrate under controlled environmental conditions. Additionally, the effect of urine fertilization on changes in substrate chemical composition was investigated. Due to the high content of K, Ca and Mg in wood ash which can improve the soil cationic balance in salt affected soils, it was further investigated whether or not supplemental wood ash application can enhance salt- and Na-tolerance of urine-fertilized maize plants.

Regardless of NaCl salinity treatment, no significant difference in investigated growth factors (height, leaf area and shoot biomass accumulation) of maize plants was measured when N was supplied at 180 mg kg^{-1} cultivation substrate as urine or ammonium nitrate. Meanwhile, urine treated maize plants produced comparatively less shoot biomass following the application of 380 mg N kg^{-1} of cultivation substrate and at NaCl salinities of 4.6 and 7.6 dS m^{-1}. As expected, sorghum plants were more tolerant to salinity than maize and produced more biomass under urine than ammonium nitrate fertilization. Though there was a positive relationship between biomass yield and tissue nitrogen concentration regardless of salinity treatment level, this relationship was stronger for ammonium nitrate than urine treatments. Ca and Mg concentration in the tissue of urine-fed maize and sorghum plants was higher than

those treated with ammonium nitrate which can be explained by the inherent content of these nutrients in urine. However, there was no direct relationship with biomass yield of maize plants of either nutrient.

Additionally, urine fertilization significantly increased substrate salinity by 2 to 3-fold. Substrate sodium concentration and a tendency towards mounting sodicity with an increase in urine fertilization were also observed. At a NaCl-salinity of EC_e 4.2 dS m^{-1}, the application of wood ash (150 mg kg^{-1} soil) alone or in combination with 75 mg urine-N kg^{-1} soil fostered Na and salinity tolerance of maize which can be explained by the dilution effect of K and Ca supplied through wood ash. However, at a higher urine-N dosage (150 mg kg^{-1} soil) with or without wood ash enrichment growth inhibition occurred indicating that the tolerance threshold had been exceeded assumingly due to urine-induced increase in Na and salinity.

It was demonstrated that urine can substitute ammonium nitrate as a source of nitrogen for maize and sorghum and can be considered for fertilization if salinity does not exceed EC_e 4.0 dS m^{-1}. Due to urine-induced increase in salinity and Na concentration in the growing medium, regular monitoring for salt build up and the use of salt-tolerant crop varieties should be included in urine fertilizer planning. It was further demonstrated that supplemental wood ash enhances Na and salinity tolerance and where necessary should be incorporated in urine-fertilizer programs.

Zusammenfassung

In weiten Teilen der Erde sind sowohl der hohe Salzgehalt des Bodens als auch die mangelnde Versorgung mit Nährstoffen mitverantwortlich für niedrige Erträge aus der landwirtschaftlichen Produktion. Eine weiter zunehmende Weltbevölkerung und der damit verbundenen steigenden Nachfrage nach biobasierten Produkten aus der Landwirtschaft verlangt nach einer Erhöhung der Produktivität auch von salzhaltigen Böden. Stickstoffdüngung kann bekanntermaßen die negativen Effekte einer Bodenversalzung auf das Wachstum von Nutzpflanzen verringern. Dies wiederum ist stark abhängig von der Stickstoffform und dem Ausmaß der Bodenversalzung.

Eine kostengünstige Alternative zu den mineralischen Düngern, die für Landwirte in vielen Entwicklungsländern oft schwer zugänglich und teuer sind bietet Urin menschlicher Herkunft. Dieser könnte bis zu 20% des weltweiten Düngemittelbedarfs decken. Die Wirksamkeit von Urin zur Steigerung der Produktivität von Nutzpflanzen wurde in verschiedenen wissenschaftlichen Experimenten belegt. Die hohe Konzentration von löslichen Salzen im Urin, insbesondere von Natrium und Chlor, kann eine Einschränkung bei der Nutzung menschlichen Urins unter salzhaltigen und/oder natriumhaltigen Bedingungen darstellen.

Die hier vorgestellte Forschungsarbeit beschäftigt sich mit der Fragestellung in welchem Ausmaß Urin als Düngemittel auf versalzten und/oder natriumhaltigen Böden genutzt werden kann. Die Auswirkungen von Urin im Vergleich zur Ammonium-Nitrat-Düngung werden im Hinblick auf die Nährstoffakkumulation und das Wachstum von Mais und Sorghum auf salzhaltigem (NaCl) Substrat unter kontrollierten Umweltbedingungen untersucht. Zusätzlich dazu wird der Einfluss von Urindüngung auf die chemische Zusammensetzung des Bodensubstrats erfasst. Ein weiterer Schwerpunkt dieser Arbeit liegt in der Untersuchung des Einsatzes von Holzasche zur Verbesserung der Salz- und Natriumtoleranz von Maispflanzen unter Bedingungen einer Urindüngung. Durch den hohen Gehalt an Kalium, Calcium und Magnesium kann diese das Kationengleichgewicht in salzhaltigen Böden verbessern.

Bei einer Stickstoffgabe von 180 mg kg^{-1} Kultursubstrat konnte weder bei Urin noch bei Ammonium-Nitrat als Stickstoffquelle ein signifikanter Unterschied der untersuchten Parameter (Wuchshöhe, Blattfläche und Sprossbiomasse) bei unterschiedlichen Salzkonzentrationen im Boden festgestellt werden. Bei Stickstoffgaben von 380 mg kg^{-1}

Substrat und einer NaCl-Salinität von 4,6-7,6 dS m^{-1} wurde vergleichsweise weniger Sprossbiomasse bei urinbehandelten Maispflanzen erzielt. Wie erwartet zeigten Sorghumpflanzen eine höhere Salztoleranz als Mais und produzierten unter Urinbehandlung mehr Biomasse als unter Ammonium-Nitrat-Düngung. Es konnte eine positive Beziehung von Biomasseertrag und Stickstoffkonzentration im Gewebe festgestellt werden und dies unabhängig von der Salinitätsbehandlung. Dieser Effekt war jedoch deutlicher ausgeprägt unter Ammonium-Nitrat-Düngung. Die Gehalte an Calcium und Magnesium waren im Gewebe von urinbehandeltem Mais und Sorghum höher als bei den Behandlungen mit Ammonium-Nitrat. Dies lässt sich über den naturgegebenen Gehalt dieser Nährstoffe im Urin erklären. Allerdings ließ sich keine direkte Beziehung zwischen dem Biomasseertrag von Maispflanzen und einem der beiden Nährstoffe zeigen.

Die Düngung mit Urin erhöhte die Salinität des Kultursubstrats um das zwei- bis dreifache. Zusätzlich konnte eine Anreicherung des Substrats mit Natrium und eine Tendenz zur Natrium-Übersättigung bei steigender Urinkonzentration beobachtet werden. Bei einer durch NaCl bedingten Salinität von EC_e 4,2 dS m^{-1} führte die Beimengung von Holzasche (150 mg pro kg Boden), allein oder in Kombination mit 75mg Urin-Stickstoff pro kg Boden zu einer verstärkten Toleranz von Mais gegenüber Natrium und Salinität. Dies kann durch den Verdünnungseffekt erklärt werden, der durch die in der Holzasche enthaltenen Kalium- und Calcium-Ionen eintritt. Nichtsdestotrotz konnte ab einer Stickstoffgabe aus Urin (150 mg kg^{-1} Boden) eine Wachstumshemmung festgestellt werden, unabhängig von erfolgter oder nicht erfolgter Holzaschebeimengung. Dies deutet auf die Überschreitung eines Grenzwertes bei der Toleranz gegenüber Natriumkonzentration und Salinität im Boden hin.

Mit dieser Arbeit konnte gezeigt werden, dass Urin Ammonium-Nitrat als Stickstoffquelle bei der Düngung von Mais und Sorghum substituieren kann, insbesondere solange die Salinitätsbedingungen EC_e 4,0 dS m^{-1} nicht überschreiten. Aufgrund der urininduzierten Zunahme an Na-Konzentration und Salinität des Bodens sollten diese Standorte regelmäßig auf ihren Salzgehalt hin untersucht werden. Auch sollten salztolerante Kulturpflanzen in einen Bewirtschaftungsplan mit Urindüngung eingebunden werden. Es wurde außerdem gezeigt dass die Beimengung von Holzasche die Toleranz von Nutzpflanzen gegenüber Natrium und Salinität verbessern kann. Bei Bedarf sollte die Ausbringung von Holzasche in urinbasierte Düngungsprogramme integriert werden.

Résumé

La salinité du sol et la carence d'éléments nutritifs sont conjointement responsables de la faible production agricole dans de nombreuses régions du monde. Avec une population mondiale toujours croissante et une pression continue sur les terres arables, il est nécessaire d'augmenter la productivité des sols salins. L'application d'engrais azoté peut atténuer les effets néfastes de la salinité sur la croissance des cultures. Toutefois, cet effet bénéfique dépend de la source d'azote et de l'étendue de la salinité des sols.

Alors que les engrais inorganiques commerciaux sont coûteux et souvent inaccessibles aux agriculteurs pauvres en particulier dans les pays en développement, l'urine humaine est un engrais alternatif à faible coût. En effet, recueillie et utilisée, l'urine humaine peut remplacer jusqu'à 20% de la consommation actuelle mondiale d'engrais. Son efficacité à augmenter la productivité des cultures a été validée par des expériences scientifiques. Cependant, la forte concentration de sels solubles (surtout Na et Cl) dans les urines peut imposer une restriction sur son utilisation dans des conditions salines et/ou sodiques.

La recherche présentée dans cette thèse examine l'étude de l'utilisation de l'urine comme engrais dans des conditions salines et/ou sodiques. Il compare l'effet d'urine et du nitrate d'ammonium (sources d'azote) sur l'accumulation des nutriments et sur la croissance des plants de maïs et de sorgho dans un substrat saline NaCl. Les conditions environnementales de cette étude ont été contrôlées. En outre, l'effet de la fertilisation de l'urine sur les changements dans la composition chimique du substrat a été étudié. En raison de la forte teneur en K, Ca et Mg dans la cendre de bois qui peut améliorer l'équilibre cationique dans les sols salins, il a été aussi examiné si l'application supplémentaire de la cendre de bois peut améliorer la tolérance en sel et Na des plants de maïs traités à l'urine.

Indépendamment du traitement de la salinité NaCl, aucune différence significative en facteurs de croissance étudiés (hauteur, surface foliaire, accumulation de la biomasse des pousses) des plants de maïs a été mesurée lorsque N a été fournie à 180 mg kg^{-1} de substrat de culture sous forme d'urine ou du nitrate d'ammonium. Pendant ce temps, les plants de maïs traités à l'urine produisent relativement moins de biomasse des pousses après l'application de 380 mg N kg^{-1} de substrat de culture et à des salinités de NaCl 4,6 et 7,6 dS m^{-1}. Comme prévu, les plants de sorgho étaient plus tolérants à la salinité que les plants de

maïs. En outre, les plants de sorgho produisaient plus de biomasse lorsqu'elles étaient traitées à l'urine qu'au nitrate d'ammonium. Bien qu'il y ait une relation positive entre le rendement de la biomasse et de la concentration en azote des tissus indépendamment du niveau de traitement de la salinité, cette relation a été plus forte pour le nitrate d'ammonium que pour les traitements à l'urine. La concentration en Ca et Mg dans le tissus de plants de sorgho et de maïs traité avec l'urine était plus élevée que celle des tissus traités avec du nitrate d'ammonium. Ce qui peut s'expliquer par le contenu intrinsèque de ces nutriments dans l'urine. Cependant, il n'y avait pas de relation directe entre le rendement en biomasse des plants de maïs avec un autre nutriment.

En outre, la fertilisation à l'urine a significativement augmenté la salinité du substrat de deux à trois fois. On a également observé une concentration de sodium de substrat et une tendance croissante de la teneur en sodium avec une augmentation de la fertilisation à l'urine. Lors d'une salinité NaCl de EC_e 4.2 dS m^{-1}, l'application de la cendre de bois (150 mg kg^{-1}), seul ou en combinaison avec 75 mg d'urine-N kg^{-1} de sol favorise la tolérance en Na et à la salinité du maïs qui peut être expliquée par l'effet de dilution de K et Ca fourni par la cendre de bois. Cependant, avec un dosage d'urine N supérieur (150 mg kg^{-1} de sol) avec ou sans cendre de bois, l'inhibition de la croissance a eu lieu indiquant que le seuil de tolérance de l'enrichissement a été dépassé probablement dû à l'augmentation de la salinité et Na induites par l'urine.

Il a été démontré que l'urine peut se substituer au nitrate d'ammonium comme source d'azote pour le maïs et le sorgho et peut être considérée pour la fertilisation des sols si la salinité ne dépasse pas ECe 4.0 dS m^{-1}. En raison de l'augmentation de la salinité et la concentration de Na dans le milieu de culture grâce à l'urine, un suivi régulier de l'accumulation en sel et de l'utilisation de variétés tolérantes au sel devrait être inclus dans le projet d'utilisation d'urine comme engrais. Il a également été démontré que l'emploi supplémentaire de la cendre de bois améliore la tolérance en Na et en salinité et le cas échéant, devrait être intégré dans les programmes d'urine comme engrais.

CURRICULUM VITAE

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PERSONAL DATA

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RESEARCH INTERESTS

Nutrients recycling, composting, sanitizing human excreta, soil salinity, urine fertilizer management, ecological sanitation, human excreta and wastewater use in agriculture.

EDUCATION

04/2009 –	Doctoral fellow. Institute of Plant Production and Agroecology in the Tropics and Subtropics. Faculty of Agricultural Sciences, University of Hohenheim, Germany.
10/2005 – 01/ 2008	Graduate student. University of Hohenheim, Germany. MSc. thesis title: *Optimising faecal sludge co-composting in semiarid Tropics*. Agroecology in the Tropics and Subtropics Funded by Eiselen Foundation, Ulm and BMZ, Germany.
10/1998 – 07/2001	Undergraduate student. Department of Geography, University of Buea, Cameroon. BSc. dissertation title: *Upland farming in Njinikom Subdivision*, Department of Geography.
10/1991 – 06/1993	High school Student. Government High School (GHS), Mbengwi, Cameroon. Certificate obtained: GCE Advanced Level.
09/1986 – 06/1991	Secondary school studies. Kom Secondary Grammar School (KSGS), Njinikom, Cameroon. Certificate Obtained: GCE Ordinary Level.

TRAINING AND EMPLOYMENT

08/2012 – present	Scientific assistant/graduate instructor. Institute of Plant Production and Agroecology in the Tropics and Subtropics. University of Hohenheim, Germany.

08/2008 – 03/2009 Research associate. Project: *Efficiency of different sanitization methods on nutrients preservation and pathogen destruction in faecal matter*. Agroecology in the Tropics and Subtropics, University of Hohenheim, Germany. Funded by BMZ.

01/2007 – 05/2008 Trainee. Advanced International Training course on *Ecological Alternatives to Sanitation*, Phase I, II & III: Sweden, Hohenheim (Germany), Durban & Kimberley, South Africa. Sponsor: Swedish International Development Agency (SIDA) and Stockholm Environmental Institute (SEI).

09/2001 – 09/2005 Teaching associate. Geography Department, Saint Joseph's College, Sasse. Diocese of Buea, Cameroon.

09/1996 – 09/1998 Teacher of primary 4 and 5 at Saint Anthony's Catholic School Buea Town. Diocese of Buea, Cameroon.

07/1993 – 07/1994 Marketing agent for Growth Product Enterprise (GPE). Dealer in Soya bean products. Bamenda Commercial Avenue, Cameroon.

PUBLICATIONS

Boh, M. Y., Germer, J., Torsten, M. & Sauerborn, J. (2013). Comparative effect of human urine and ammonium nitrate application on maize (*Zea mays* L.) grown under various salt (NaCl) concentrations. *Journal of Plant Nutrition and Soil Science*. DOI: 10.1002/jpln.201200486.

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Germer, J., **Boh, M. Y**. & Sauerborn, J. (2007). Co-composting as a disposal solution for faecal sludge from innovative pit latrines. Proceedings of the Conference of International Research on Food Security, Natural resource Management and Rural Development. Witzenhausen, Germany Oct. 9 – 11, 2007.

ADDITIONAL SKILLS

Languages : Fluent in spoken and written English
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Computer : Good knowledge of MS word, excel and PowerPoint

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