The effects of urine and urine-separated plant nutrient sources on growth and biomass production of perennial ryegrass (*Lolium perenne. L)*

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DEDICATION

This work is dedicated my late family role models (MamaZibuko, Nokuthula, Tshengisile, Staff, George Phakade and Mthandeni Mchunu), and to my children (Nonhle, Nosipho and Mthandeni Junior Mchunu). Most of all this work is dedicated to my God the men Christ Jesus Almighty, Thank you so much father God for all the strength, wisdom, love and care you provided during times of this study. In Jesus name Amen.

SUMMARY

Urine contains plant essential nutrients, which may pose pollution problems if disposed of in the environment. Struvite is a concentrated phosphorus fertilizer produced by precipitation of Magnesium Ammonium Phosphate after addition of magnesium salts into urine. The struvite effluent has been shown to contain high concentrations of mineral elements such as nitrogen, which are important for plant growth. Urine can further be nitrified directly to produce a nutrient source with more of nitrate- N than ammonium- N. There is little information on use of these urine product for agriculture particularly in South Africa. The aim of this study was to determine the effect of urine and urine products, struvite (S), struvite effluent (S.E) and nitrified urine concentrate (NUC), on the growth and biomass production of perennial ryegrass. The specific objectives were (1) to determine nitrogen release pattern of urine and urine products (S, S.E and NUC) in two different soils (Cartref and Inanda soil), (2) to determine the effect of the application of urine and urine products on growth and biomass production of perennial ryegrass. A soil incubation experiment was set up under controlled room temperature at 25^{o}C and 80% atmospheric humidity to determine nitrogen release pattern of urine and the urine products in two different soils. The experiment was designed as a 6 x 2 x 2 factorial treatment structure with five nutrient sources (urine, struvite effluent, struvite effluent + struvite, nitrified urine concentrate) and no fertilizer treatment as a control). The fertilizer materials were applied at two levels (recommended rates and double the recommended rates based on N rate). The two soil types used were the Inanda (acidic clay soil) and Cartref (sandy soil). The treatments were replicated three times giving 72 experimental units (in 2 kg ventilated containers). Data was collected on the ammonium and nitrate- N release on weekly basis for the period of 70 days.

A pot trial was set up in 1 kg pots in the tunnel at 26^{o}C air temperature and 65% atmospheric humidity to determine the effect of the application of urine and urine products on growth and biomass production of perennial ryegrass. The pot experiment was also designed as a 6 x 2 x 2 factorial treatment structure with six nutrient sources consisting of urine, struvite effluent, struvite effluent + struvite, nitrified urine concentrate and two controls; an inorganic fertilizer source (NPK 2:3:2) and no fertilizer treatment. The nutrient sources were either applied once off split applied three times. The fertilizer materials were applied at two levels (recommended rates and double the recommended rates) based on N rate. The treatment combinations were replicated three times. Plants were cut back to 5cm above the ground after attaining a cutting height of 20

cm height, and were allowed to regrow this was repeated four times. Soil moisture was maintained at 70-100% field capacity.

The soil incubation experiment showed that there were significant ($P<0.05$) differences observed among treatments- U, S.E, S.E+S, NUC, NPK and Zero fertilizer, and among application rate. In Inanda soil, ammonium- N declined with incubation time while nitrate-N and mineral- N did not increase significantly. In the Catref soil ammonium- N declined with incubation time while nitrate- N and mineral- N increased significantly. The findings suggested that the Cartref soil released more nitrogen than Inanda soil hence it had more total mineral- N than Inanda soil

Pot experiment result showed that there were significant ($P<0.05$) differences observed in dry matter production and plant height among the treatments, application method (once- off and split rate), application rates (recommended and double rate) and cuts (harvest), likewise in plant height. Dry matter production increased significantly with days after cuts- 1, 2 and 3 and it declined with time after cut 3 at cut 4. Split rate application method had significantly more dry matter than once- off application method. The recommended rate had significantly more dry matter than double rate. Treatment NUC responded significantly different within cuts. NUC treatment at recommended had significantly higher dry matter yield then all treatments at cut 1 and 3. At the same time there were no significant differences in dry matter production between NPK and urine and urine products. All treatments had however significantly higher dry matter than zero fertilizer treatment.

The findings of the study suggested that urine and urine products are equally as effective as mineral fertilizer especially in sandy soil and that splitting the application is a useful strategy to manage urine and urine products for optimum dry matter production.

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CHAPTER 1

GENERAL BACKGROUND OF THE STUDY

1.1. Introduction

The increase in population and urbanization has resulted in increased need for food, water and sanitation (Heinonen-Tanski and Wijk-Sijbesma, 2005). Food and water shortages continue to rise especially in developing countries (Udert and Wachter, 2011). There is a pressure in crop lands to produce more with limited resources, in that crop productivity has to meet food production that equals to the population growth and urbanization rate (Heinonen-Tanski and Wijk-Sijbesma, 2005). The increase in demand for food with increasing population, particularly in cities, has resulted in the greater use of fertilisers. This has resulted in elevated fertilizer prices (Antonini and Clemens, 2010). Mining and processing of phosphate rock for commercial fertiliser production is expensive, and it can be a threat for future fertilizer production as it may run out (Bonvin, 2013). Similarly, production of nitrogen fertilisers from atmospheric N is also expensive. While biological N fixation has great potential, its use also requires other nutrients to grow the legumes (Adler *et al.*, 2005 and Barret *et al.*, 2000). There is need to find ways to a sustainable nutrient recycling to address these challenges.

South Africa is a water scarce country and there is need to conserve water resources (Udert and Wachter, 2011). The quest for sustainable ways of food production has led to the exploration of nutrient cycling through reuse of wastes and waste water (Guzha *et al.*, 2005). Human urine has been shown to contain nutrient equivalent to plant requirement (Schouw *et al.*, 2002). In ancient times human urine has been used in agriculture for food production. For example, for many years famers in China have been using urine to fertilize their crop lands up until when their population increased enormously (Drangert, 1998). This suggest that urine has the potential to rescue and sustain many nations' food security particularly developing countries like South Africa, especially if the nutrient recycling strategy is implemented successfully.

Urine diversion toilets that separate urine from feces at source use low amounts of water (Maurer *et al*, 2006), which is particularly important for water-scarce countries like South Africa, and the strategy is also useful for recovering nutrients from feces and urine (Maurer *et al.*, 2003). In recent years science has been able to treat human urine and extract phosphorous in concentrated form as struvite (Mihelcic *et al.*, 2011). Since then urine has gained new attention and interest as

a source of plant nutrients (Winker *et al.*, 2009). Treating waste streams particularly human urine to derive plant nutrient product is a potential innovative strategy to solve sanitation problems in cities while meeting food production demand (Tanski *et al.*, 2005).

1.2. Problem statement

Human urine has been shown to contain plant nutrients which pose a problem when disposed of in the environment (Drangert, 1997). Urine is reported to cause blockages of municipal pipes through spontaneous formation of struvite crystals, and is also forms part of water stream contaminants (Maurer *et al.*, 2006). The eThekwini Municipality collects and stores about ten thousand litres of urine every month from urine diversion toilet technology. The net NPK average concentration of this urine is: 2800-220-1080 (mg/L) respectively. The NPK concentration equals to the plant nutrient requirement hence it is a potential plant nutrient source (Gardener, 1998). Hence treating urine for fertiliser (urine based fertiliser) is perhaps the innovative idea to mitigate the problem.

Struvite ($NH_4MgPO_4 \cdot 6H2O$) is a phosphorus fertiliser precipitated from the addition of magnesium salts (MgO, $MgSO_4$ or $MgCl_2$) into urine (Barak and Stafford, 2006). The average removal of N and P from this process is 10 and 90%, respectively (Etter *et al.*, 2011 and Jimenez *et al*, 2009). The resulting effect of struvite production further leads to the need for disposal of the effluent (struvite effluent) (El Diwani *et al.*, 2006). The correct nutrient content in the effluent is not known due to different urine composition and different struvite formation reaction (Kirchmann and Pettersson, 1995). Urine can further be nitrified directly to produce a nutrient source with more of nitrate- N than ammonium- N giving a product called Nitrified Urine Concentrate (NUC), which could be important for plant production.

After struvite formation 90% of original nitrogen that was in urine is left in the effluent. When struvite effluent is disposed it has the potential to still contaminate water streams since it contains lots of nitrogen hence can cause environmental problem of eutrophication (Etter *et al.*, 2011 and Jimenez *et al*, 2009). Nitrogen is one of the most limiting macro nutrients in agriculture (Hue and Silva, 2000). The question that arises is: "why dispose of the struvite effluent while its value can be beneficial for agriculture?", hence struvite effluent can be a potential nitrogen fertiliser because of it high mineral nitrogen content. In addition to N and P, urine also contains high salt content including NaCl, and these salts are soluble and most do not

precipitate during production of struvite (Kirchmann and Pettersson, 1995 and Etter *et al.*, 2011 and Jimenez *et al*, 2009). Struvite effluent could, therefore, also contain high salt concentrations, which could be detrimental to soil and crops treated with the effluent. Moreover excess Mg is added during the process, which suggests that high levels of Mg could be in the effluent, which could also affect the Ca:Mg ratio of soils irrigated with the effluent affecting Ca uptake.

The question that arises is that can we minimise negative impact of struvite effluent by innovatively using it for plant production as plant nitrogen source? There is little information on use of urine and urine nutrient products (struvite, NUC and struvite effluent) for agriculture, particularly in South Africa. Hence application method and application rates becomes the important factor in determining environmental impact. Therefore one of the important task of this study is to determine whether applying the nutrient sources at double the recommended N application rate and whether split application could be more appropriate for crop production.

1.3. Justification of the research work

Much research work on waste water treatment has been based on urine and struvite use in agriculture, crop production particularly. There is no data that relates new urine nutrient products such as nitrified urine with agriculture. There is no data or information that proves that struvite effluent and nitrified urine can be used for crop production to provide N supplement. There is no information that relates growth and dry matter production with struvite effluent as it is. The only recent work that touches struvite effluent is work by Bonvin (2013). A Synthetic Urine Nitrified Solids (SUNS) was produced by treating struvite effluent in a nitrifying reactor and distilled to form a concentrated nitrogen rich fertiliser Bonvin (2013). Pot experiment findings showed that dry matter increased significantly and the product was useful as a fertiliser. No one has tested the unmodified struvite effluent and nitrified urine in crop production. As stated by Bonvin (2013) limited studies have been done on the soil mineralisation processes of the urine based fertiliser (UBF). Basically there is limited literature, if any, relating struvite effluent and nitrified urine with agronomy.

1.3. Research Aim

To determine the effect of urine and urine separated plant nutrient sources on growth and dry matter production of perennial ryegrass.

1.4. Objectives

- To determine nitrogen release pattern of urine and urine separated plant nutrient sources on two soil types.
- To determine the effect of application of urine and urine separated plant nutrient sources on growth and dry matter production of perennial ryegrass.

1.5. Hypotheses

- There is no significant increase in nitrogen release between two soil types treated with urine and urine separated nutrient sources
- There is no significant growth nor dry matter production increase in perennial ryegrass when applied with urine and urine separated nutrient sources.

1.5. Research questions

- What is the nitrogen release pattern of urine and urine separated plant nutrient product?
- What is the effect of soil characteristics on ammonium- N mineralization?
- What is the effect of soil pH on the ammonium- N mineralization?
- Why separate phosphorous from urine when its production causes problems?
- What nutrient compositions and forms of nutrients in struvite effluent?
- Can urine or urine nutrient product be a complete replacement of commercial fertilizer?
- What is the effect of application method and application rate on dry matter production?

1.6. Experiments

Two experiments were conducted to accomplish the research aims and objectives of this study:

1. Soil incubation experiment
2. Pot tunnel experiment

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

There is limited literature, data nor agronomic information, if any, relating urine and urine separated plant nutrient sources use in agriculture particularly in South Africa. However urine nutrient composition and struvite production reaction provide valuable information in understanding the nature and potential of urine plant nutrient product as a soil amendment for dry matter production. High rate of population growth and urbanization has resulted into unmanageable waste streams in cities and in urban areas (Antonini and Clemens, 2010). This is attributed to lack of proper sanitation system (Drangert, 1998)

Urine forms part of the waste stream contaminants (Drangert, 1998), urine pose a problem when dispose of into the environment. Through research, science has been able to extract phosphorous from urine in a form of a struvite (Karaka and Bhattacharyyab, 2011). The process remove approximately 90% phosphorous from urine (Doyle and Parsons, 2002). However the process leaves out significant ammonium- N in the supernatant solution which is disposed in grains as the affluent (El Diwani *et al.*, 2006). Disposing the effluent can still pose a problem to the environment. Urine can further be nitrified directly to produce a nutrient source with more of nitrate- N than ammonium- N. As such these urine product can be used as soil amendment for crop production.

This literature review will be exploring urine and urine based plant nutrient sources. The literature will be looking at potential of urine based fertiliser to contribute into agriculture, crop production in particular. The literature will further explore factors and processes that leads to successful mineralization of nutrient materials like urine and urine separated plant nutrient product. Topics that will be covered here will be used to analyse research topic, objectives and hypotheses. The success of this research study will come up with efficient method of disposing urine and urine separated nutrient product in a way that environmental natural processes are not disturbed.

The aim of this study is to find innovate way to dispose waste stream products such as urine and urine separated nutrient sources, to innovatively minimise negative environmental problems

associated with contamination of ecological and natural processes (eutrophication and hydrological cycle).

2.2. Overview of urine and urine separated plant nutrient sources

2.2.1. Urine

Urine has been shown to contain nutrients concentrations equivalent to plant nutrient requirement (Schouw *et al.*, 2002 and Kirchmann and Pettersson, 1995), hence it is the potential to compliment inorganic fertilisers. Evidently many people have studied using urine to fertilise crop lands, Tanski *et al.*, (2005), reported that the cucumber yield after human urine (collected from local households) fertilization was similar and slightly better than the yield obtained from control rows fertilized with commercial mineral fertiliser, and none of the cucumbers contained any enteric microorganisms (coliforms, enterococci, coliphages and clostridia). In agreement with Tanski *et al.,* (2005) findings, Winker *et al.*, (2009) also recommended urine as a complete fertiliser after field trials with vegetables, the study also revealed that urine can outperform inorganic fertiliser if fertiliser management strategies (soil testing, calculation of application rate and method) are practiced well.

Furthermore Guzha *et al.*, (2005), also reported that growing maize with the help of toilet compost and urine on poor sandy soils was beneficial to low income areas. The yield he achieved was not significant different from those of synthetic fertiliser. The findings agree with strategy of using urine as complement of commercial fertilisers. However there could be challenges using urine directly as it is to fertilise crop lands. One of the common problem of all nitrogen fertiliser is volatilisation of ammonium- N as ammonia gas (Heinonen-Tanski and Wijk-Sijbesma, 2005). Hence this may result into significant losses in mineral N that is associated with dry matter production in crop production.

In response to this Karaka and Bhattacharyyab (2011) suggested that, one should assume 15% or 20% nitrogen loss in conjunction with the calculation of the application rate as to achieve maximum yield. However as much as this idea of assumption can be beneficial to achieve yield, what about environmental sustainability? What happens to the extra nitrogen if it not taken up by plant or if not held by the soil? What are the environmental implication of this? Nevertheless the finding from the literature and findings presented by UKZN pollution research group agrees with strategy that urine have the potential to compliment inorganic fertilisers, since it contains nutrients concentration equivalent to plant requirement.

There are limited studies relating urine with soil mineralisation process for crop production (Bonvin, 2013). This pose challenges in understating the environmental impact of urine if disposed of in the environment. This is in agreement with Mkeni *et al.*, 2008, who reported Electric Conductivity increase in soil fertilized with urine and suggested that overtime this may lead to saline soil, evidently sodium uptake was observed in the plant tissue analysis. This suggest that even the urine separated plant nutrient sources may have the same effect. This also suggest that specific application rate and method need to be maintained to minimize negative environmental impact.

2.2.2. Struvite

Struvite is a concentrated phosphorus fertilizer which is produced by addition of magnesium salts into urine (figure 1), this reaction process remove approximately 90% phosphorous from urine and precipitate it as struvite (Tilley *et al.,* 2008) (figure 1). Struvite is the successful product of urine based fertiliser known till today, with the evidence of being a slow release fertiliser (El Diwani *et al.*, 2006 and Doyle and Parsons, 2002).

Struvite has been suggested as a fertilizer but its use has been limited to high value crops because of the additional cost of manufacture (Barak and Stafford, 2006). Struvite is known as an annoyance in sewage treatment plants when it forms blockages in pipes (Jaffer *et al.,* 2002). However with the arrival of new interest in removing phosphorus from waste streams- urine before land application, the recovery of phosphorus as struvite has gained new interest (Barak and Stafford, 2006 and Jimenez *et al.*, 2009).

Evidently Etter *et al.*, 2011 reported a successful implementation of struvite as the fertiliser in Nepal community as the low cost production of struvite. Furthermore Barak and Stafford, 2006 showed that struvite overtakes diammonium phosphate on a unit-for-unit basis in terms of dry matter production and P uptake. This suggest that struvite is a valuable source of P fertiliser. Struvite is the composition of magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$) and has a solubility of 0.2 g/L in water. The low solubility of struvite in water makes it an ideal slow release fertiliser (Rahman *et al.*, 2013).

Struvite contains 5.7% N and 12.6% P by mass (Doyle and Parsons, 2002). However struvite also appears to form in soil upon fertilization with other ammonium phosphate fertilizers,

particularly when neutral or alkaline conditions prevail (Barak and Stafford, 2006 and Jaffer *et al.,* 2002). The finds suggests that urine derived plant nutrient source have the potential to compliment commercial fertilisers while mitigating the environmental hazard of pollution. However there is limited information reported on struvite mineralisation- nutrient release in the soil. This suggest that the environmental implication of struvite is not fully understood, hence affect environmental decision making.

Figure 2: Struvite reactor as Mg added to urine to precipitate struvite at EThekwini municipality waste water centre at Newlands KwaMashu.

2.2.3. Struvite effluent

Struvite effluent is the byproduct of struvite formation, hence urine nutrient composition and struvite precipitation process provide clear insight of the nature and nutrient composition of struvite effluent. The recent work that touches struvite effluent is work by Bonvin 2013. A Synthetic Urine Nitrified Solids (SUNS) was produced by treating struvite effluent in a nitrifying reactor. It was distilled to form a concentrated nitrogen rich fertiliser (SUNS). The experiment trial of SUNS showed that dry matter production increased significantly and nutrient uptake was similar to those of synthetic fertiliser. This proves that urine and urine separated nutrient sources are a valuable source of nutrient for plants and are as good as inorganic fertilisers. However the process to form SUNS is expensive and technical demanding which may add manufactures price (Bonvin, 2013). This may still not be suitable nor affordable for low income areas. The idea may be useful for poor rural communities that rely on agriculture to live as there is no extra manufactures price. However public acceptance and perception is a factor in determining the successful implementation of the project (Udert and Wachter, 2011).

Urine contains average plant nutrient requirement as tasted by UKZN pollution research group in mineral form. Evidently Tanski *et al.,* (2005), reported that urine contains high amounts of nitrogen with some phosphorus and potassium also see table 1 & 2. Furthermore Kirchmann and Pettersson, (1995) also proved that urine contains average plant nutrient (NPK) in mineral form. When struvite is formed by addition of magnesium salts (MgCl, $MgSO_4$ or MgO) into urine, the process remove P almost completely from urine and precipitate it as struvite (Jaffer *et al.,* 2002). After the struvite formation, the supernatant solution is left with approximately 90% ammonium-N and 100% K from the process (Doyle and Parsons, 2002). The supernatant solution is regarded as the struvite effluent. Ideally struvite effluent has the potential to be a nitrogen soil amendment or a complete NPK fertiliser if P corrected or supplemented by P sources such as Struvite (S), Single Super Phosphate (SSP) and Diammonium Phosphate (DAP) because of mineral nutrient composition. However nutrient composition of struvite effluent varies with 1) different struvite formation reaction and 2) different urine nutrient composition.

However using struvite effluent directly as it is can also pose risk of ammonia volatilisation as other nitrogen fertilisers if surface applied or when it is not incorporated in the soil (Nielsen, 2006). This suggest that course grained soils like sandy soil with low clay and organic matter are at high risk of ammonia volatilisation. Thus careful nutrient management is vital to minimise losses. Hence application rate, application method and maintaining certain soil moisture content are the primarily options to manage struvite effluent for maximum mineralisation (conversion of ammonium- N into nitrate- N which is plant available). However there is no information or data that relate struvite effluent use as it is in agriculture particularly agronomy. Hence the environmental and economic impact is not understood.

2.2.4. Nitrified Urine Concentrate (NUC)

Nitrified Urine Concentrate (NUC) is made by directly removing water from urine by evaporation method through a reactor (figure 2). This process removed significant quantities of water leaving out only nutrients behind, i.e. taking 1000 litres of urine evaporate water and only get 500 ml in a concentrated nutrient form (see table 1). This process quantifies urine in small volume that can be easily carried anywhere for transport purposes. Further this concentrated urine is nitrified through the nitrifying reactor that converts half of the ammonium- N in urine into nitrates- N. The fraction of ammonium- N and nitrate- N varies with different urine nutrient composition (see table 1). This type of urine nutrient product is not largely dependent on soil mineralisation. Since half of the nitrogen is available as nitrates which are readily available for plant uptake. However this suggest that, if the rate of plant nutrient uptake is slow or if over applied it can thus lead to the nitrate leaching, since nitrate are not held tight into soil colloid because of negative charge. Hence water holding capacity, application rates and application method needs to be calculated carefully to mitigate the risk of nitrate leaching. There is no data or information that relate NUC with agriculture particularly soil mineralisation for crop production. This suggest that the effect of NUC is not known nor understood in the concept of agronomy, there is great need to put such product into test particularly in countries like South Africa which have limited water resources and fertile lands.

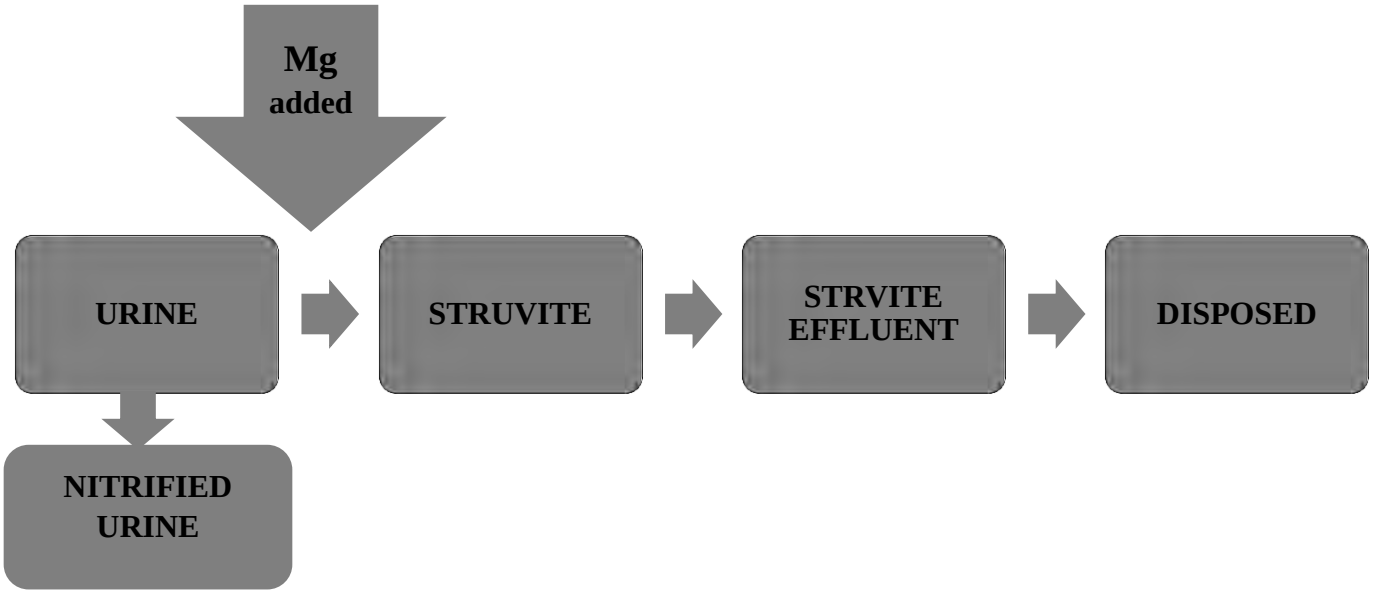

Figure 3: Urine and urine separation processes

Table 1: N and P concentration of urine used for the study

Nutrient	Urine (U) Mg L^{-1}	Struvite Effluent (S.E) Mg L^{-1}	Nitrified Urine Concentrate (NUC) Mg L^{-1}	pH
N	4656	4578	35483	9.76
P	231	7	3847	

Table 2: Average urine nutrient analysis of households in Newlands area in Durban

Nutrients	N Mg L^{-1}	P Mg L^{-1}	K Mg L^{-1}	Na Mg L^{-1}	SO_4 Mg L^{-1}	EC	pH
	2800	220	1080	2472	1259	26.6	9.76

2.3. Mineralization in general

Mineralization is the process by which organic matter breaks down in the soil (Premi and Cornfield, 2003). There are five main mechanisms that are responsible for breakdown of organic matter in the soil. These are nitrification, immobilisation, denitrification, ammonification and volatilisation. Mineralization occurs quickly- less than a week (3- 7 days) when conditions are perfect for bacteria to reproduce. The conditions that favours optimum mineralization are, high aeration, adequate moisture, good pH, and balanced mineral nutrients (wolkowski *et al.*, 1995). Environmental and soil mineralogy factors affect the microflora players and their actions, which in turn determine the rate of mineralization in the soil and thus the amount mineralized over time (Stanford and Smith, 1972). Microbial activity is limited at soil temperature near freezing and at low pH less than 5.5 and increases with rising soil temperature and pH (Leirosa *et al.*, 1999). Maximum N mineralization occurs when the soil temperature reaches 30–35 (Curtin *et al.*, 1998). The decline in N mineralization indicates low microbial activity and a degradation of the biological properties of the soil (Hue *et al.*, 1986).

2.3.1. The potential of urine and urine separated plant nutrients sources as the soil amendment

The appropriateness of mineral nutrient materials such as urine and urine based nutrient source as a fertilizer relies to a great extent on its promptness of mineralization and invigorating the nutrients present in them (Murugan and Swarnam, 2013). Mineralization of nitrogen is the conversion of nitrogen from organic into inorganic form and the immobilization is the setback of the process (Murugan and Swarnam, 2013 and Mamo *et al.*, 1998). These processes are biochemical in nature and are mediated through the activities of microorganisms (Murugan and Swarnam, 2013). The resulting effects of these two processes are expressed as net mineralization or net immobilization which decides the nitrogen supply to the growing crops. However the mineralization and immobilization turnover in the soil is affected by soil properties such as temperature, soil moisture and pH (Medina, 2006 and Murugan and Swarnam, 2013). However nitrogen cycle provide close understanding of how this occurs.

2.4. Nitrogen cycle

2.4.1. Nitrification

Nitrification is the transformation of NH_4^+ into NO_3^- (Gulser *et al.*, 2010). The requirement for optimum nitrification includes, soil moisture (water), aeration, alkaline soil pH range and warm soil temperature. There are two bacteria's or microorganisms that contributes to nitrate formation (nitrification). These are *Nitrosomonas europeana* which oxidises ammonium- N into nitrite- N, nitrite- N is further oxidised by *nitrobacter winogradskyi* into nitrate- N (figure 3). Nitrification is inhibited at high temperature. High temperature result into availability of N as ammonia, which than contributes to increased ammonium- N volatilization losses and reduced nitrification rate (Gastal and Lemaire, 2001) (figure 4)

The optimum temperatures for nitrification in the soil vary between 25-30^{o}C, the pH for both processes is at 8.5 but steps differs with regards to their tolerance ranges (Medina, 2006). However, Tully *et al.*, (2013) reported that in acidic condition at pH less than 5.5 the nitrification is low and weak. Nitrification needs a sufficient oxygen supply, restricted aeration to compaction or drenching delays nitrification, oxygen determines the speed of the process, and metabolisms are increased with increase in oxygen to the bacteria's (Nielsen, 2006 and Medina, 2006)

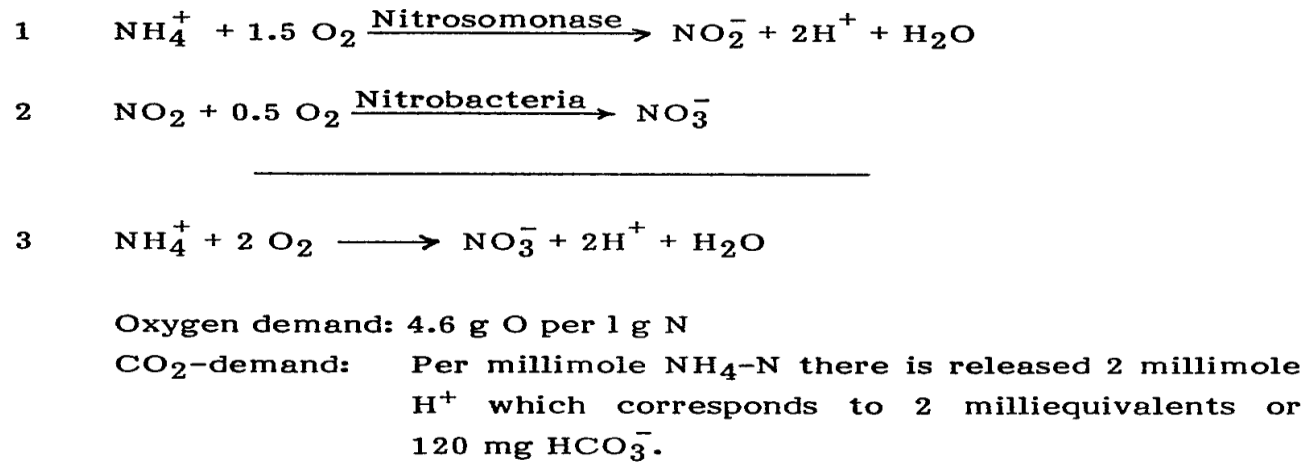

Figure 4: Nitrification process as bacteria transform ammonium- N into nitrate- N (Mamo et al, 1998)

2.4.2. Denitrification

Denitrification is the gaseous loss of nitrogen to the atmosphere via a microbial respiration process (Hue and Silva, 2000) (figure 4). This process occurs under anaerobic conditions where microbes obtain their O_2 from NO_2^- and NO_3^- with the accompanying release of N_2 and N_2O (Leirosa *et al.*, 1999) (figure 4). Environmental concerns about emission of nitrous oxides are mainly related to the effect on global warming and the role of nitrous oxides in ozone destruction. The destruction of O_3 is catalysed by NO, halogens, hydroxyl, and hydrogen. A possible source of NO is from N_2O, the product of denitrification, which can diffuse into the upper atmosphere and lead to atmospheric holes, hence causing problems for plants and animal life from excessive exposure to ultraviolet radiation (medina 2006 and Mamo *et al.*, 1998).

2.4.3. Volatilization

Volatilization is the gaseous loss of ammonia from surface applied with ammonium- N and urea fertilizers (Nielsen, 2006 and Medina, 2006) (figure 4). Volatilization occurs in the first 48 hours after application and conditions during this time are critical to the amount of nitrogen that is lost. Conditions that lead to greater volatilization include; warm and windy weather, moist and drying soils, high residue levels, high soil pH, no rainfall after application to wash the urea into the soil, low soil exchange capacity, and high application rates (Nielsen, 2006 and Medina, 2006) (figure 4). Ammonia volatilization is favoured in sandy soils with low buffering capacity, since the ability of NH_4^+ to form electrostatic bonds with clay minerals and organic colloids to impair losses of soil and fertilizer N is low (Nielsen, 2006 and Medina, 2006). Medina, 2006 reported that in well-drained ridge soils with high pH, volatilization losses can account for 10 to 15% of NH_4^+- N applied to the soil surface (figure 4). Furthermore Medina (2006) reported that ammonia volatilization increased significantly with an increase in NH_4^-N application rate, by 2- and 3- fold, respectively. He also noted that, the environmental conditions such as aeration also favour ammonia volatilization through air circulation (Medina, 2006). Ammonia volatilizing from fertilized fields can accumulate in neighbouring natural ecosystems, possibly causing damage to the vegetation. Some of the ammonia may be converted into nitric acid that can affect plants directly and can acidify lakes, resulting in aluminium toxicity in fish and plants (Medina, 2006). Ammonia volatilization increases with soil temperature and soil pH.

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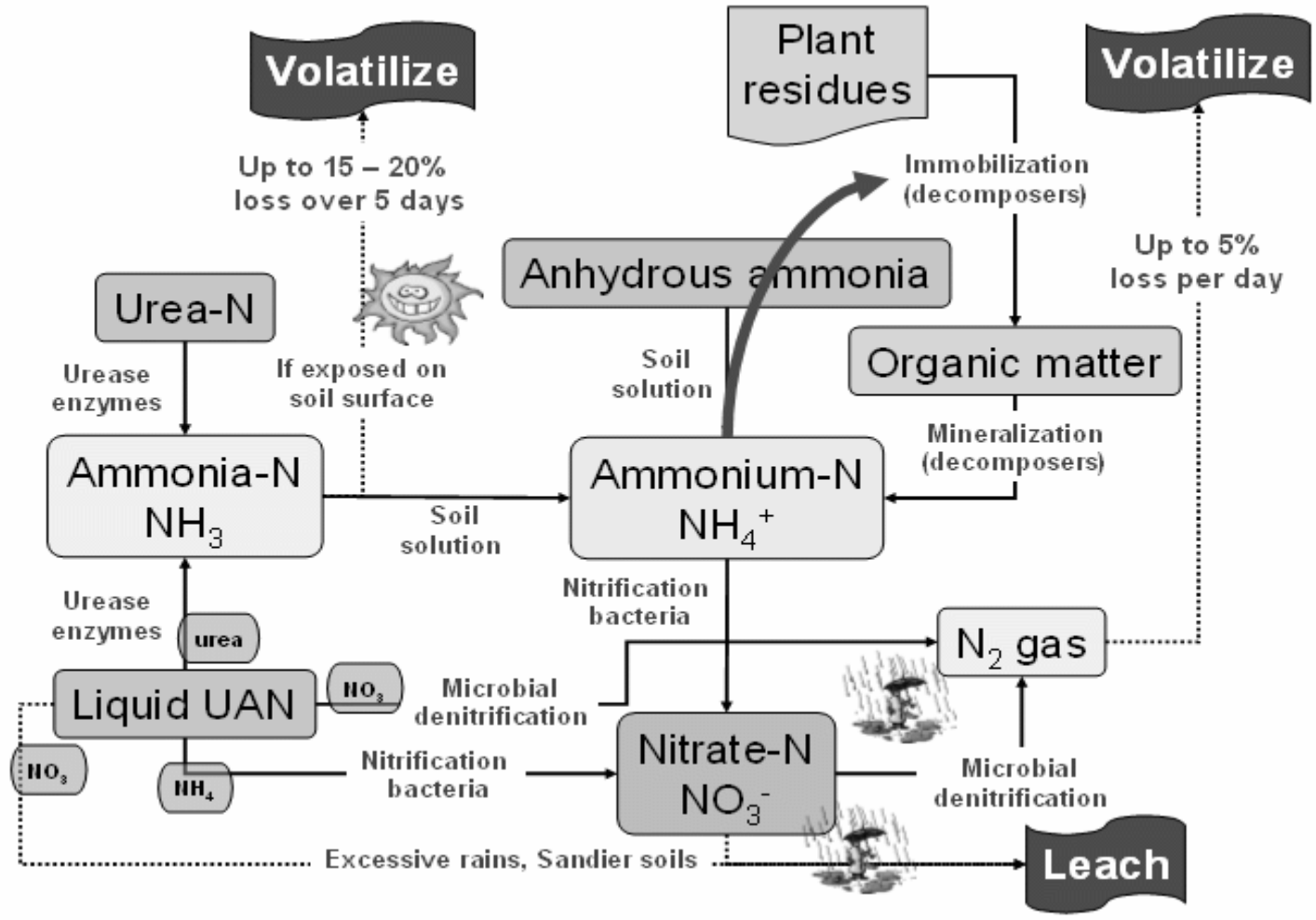

Figure 5: Nitrogen cycle as it relates to nitrogen losses in the soil (Volatilisation and denitrification) (Nielsen, 2006)

2.5. Mineralization in soils

Every soil has billions and billions of microorganisms to act upon anything that enters the soil system (Gulser *et al.*, 2010). However the ability of these microorganisms to work varies with soil types hence mineralization also varies with soil type (Hue and Silva, 2000). The characteristics of the soil determine the amount of nutrients released overtime (Hue *et al.*, 1986). The rate of mineralization in the soil depends on the nature of materials applied (Hue and Silva, 2000), some materials are easily decomposable by microorganisms some are difficult. Those that are easy decomposable by microorganisms tend to be quick available as plant available visor verse (Hue and Silva, 2000). Liquid organic substance are easily decomposable by soil microorganisms than solid substance such as those which carry lignin. (Jimenez *et al.*, 2009). Soils with ideal condition for microorganisms to work will have more nutrient overtime visor verse (Karaka and Bhattacharyyab, 2011). Ideal soil for optimum mineralization must have good fraction of clay and sand particles and neutral to alkaline pH range (Leirosa *et al.*, 1999). The high usage of synthetic fertiliser in soils has resulted into loss of fertility in many crops lands (Bonvin, 2013). There is a need to invest much research work on soils with low fertility status such as sandy and acidic soils.

2.5.1. Acidic soil

Acidic soils in general are known for their adverse effect on plant growth and mineralization processes (Sahrawat, 1980. Nitrification is the crucial processes to be completed successfully in mineral nutrient sources for the plant available (nutrient- N) (Tully *et al.*, 2013). Most of the urine and urine separated nutrient sources are mineral materials in nature, and their nutrient availability is greatly dependent on nitrification. Most nutrients that are essential for mineralization (nitrification) are available at 5.5 and 7.5 pH range, pH below 5.5 is not ideal for both mineralization and plant growth. However some microorganisms are tolerance to acidity (Hue *et al*, 1986 and Sahrawat, 1980).

Microorganism's activity is suppressed at acidic soil condition. Most of the microorganisms that are responsible for nitrification are not active at acidic condition because of aluminium toxicity (Gulser *et al.*, 2010). Aluminium is prevalent at acidic soil condition (Motavalli *et al.*, 1995). Aluminium impair and paralyze the functioning of microorganisms (Hue *et al.*, 1986). Moreover in acidic condition most nutrients are not available and some of these nutrients are needed by microorganisms for their own metabolism (Hue *et al.*, 1986). This is also supported by Tully *et*

al., 2013 they reported that quantities of nitrogen, calcium, phosphorus and magnesium are generally lower at pH of 5.5 or less. This suggest that one can anticipate to see poor mineralization process in acidic soil.

2.5.2. Sandy soil

Sandy soil are generally characterised by poor physical and chemical properties thus poor aggregate stability. Sandy soils have low clay, low organic matter, low CEC, poor water and nutrient retention capacity hence low mineralization potential (Darby 1999). In sandy soil NH_4^+-N has low ability to form electrostatic bonds due to low clay and organic colloids to impair losses of fertilizer N (Medina, 2006). This suggests that significant ammonium- N may not be held to the soil thus is maybe prone to leaching and volatilisation. This suggest that one can anticipate to observe significant N loss in soils or fields fertilised with ammonium- N fertiliser. Thus poor yields are mostly likely to occur if nutrient source is not managed very well.

2.6. Nutrient uptake mechanism by plants

Nutrient uptake is one of the main factors that determine growth and biomass production in most plants. There are three major ingredients of a plant food, these are nitrogen, phosphorus and potassium. Nitrogen is extremely important for leaf growth; phosphorus promotes development of roots, flowers and seeds or fruit; and potassium is necessary for the growth of strong stems and movement of water in plants, in addition to promoting flowering and fruiting (Hue and Silva, 2000). Hence there are three main crop uptake mechanisms; roots interception, mass flow and diffusion. Plants are able to take up nutrients with these mechanisms provided that the nutrients are available in the right form for uptake, and that the growing media is able to hold and slowly release nutrients to plants. Plants are able to take most of the nitrate-N than ammonium- N because most of the ammonium- N is attracted to the soil particles rather than soil solution (Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001).

Mass flow is the first most predominant nutrient uptake mechanism by most plants (table 3). Mass flow is the movement of dissolved nutrients into a plant as the plant absorbs water for transpiration. The process is responsible for most transport of nitrate, sulfate, calcium and magnesium (Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001).

Mass flow is related to environmental conditions, on a clear sky where relative humidity is low and the air is dry, the environment press pressure on stomata plant leaves for water. These conditions make the plants to transpire in excess. As the plant transpires it create suction force to the soil solution to take up water to cool itself, as it takes ups water most of the nitrates, calcium and magnesium is taken as well (Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001).

Diffusion nutrient uptake is categorized as the second mechanism that is typical adopted by plants to take up nutrient (table 3). Diffusion is defined as the movement of nutrients to the root surface in response to a concentration gradient. When nutrients are found in higher concentrations in one area than another, there is a net movement to the low-concentration area so that equilibrium is reached. High concentration in the soil solution and a low concentration at the root cause the nutrients to move to the root surface, where they can be taken up. This is important for the transport of phosphorus and potassium (Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001). Third plant uptake mechanism is root interception (table 3), this mechanism occurs when growth of a root causes contact with soil colloids which contain nutrients. The root then absorbs the nutrients. It is an important mode of transport for calcium and magnesium, but in general is a minor pathway for nutrient transfer (Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001).

Table 3: Nutrient uptake pathways

Nutrient	Mass Flow	Diffusion	Root Interception
Nitrogen	X		
Phosphorus		X	
Potassium	X	X	
Calcium	X		X
Magnesium	X		X
Sulfur	X	X	
Boron	X		
Copper	X		
Iron	X	X	X
Manganese	X		X
Zinc	X	X	X
Molybdenum	X		

2.7. Dry matter production

Dry matter production is one of the main factor that determines crop productivity and profitability (Huisman *et al.,* 2011). Dry matter is the accumulative nutrients weight in plant structure that the plant was able to absorb during the growth season (Huisman *et al.,* 2011 and Barber, 1962). Dry matter production is directly proportional to nutrient uptake (Barber, 1962, Leirosa *et al.*, 1999 and Huisman *et al.,* 2011). This means that dry matter production increases with the increase in nutrient uptake. Typically dry matter production increases with time after fertiliser application and decline overtime (Leirosa *et al.*, 1999 and Huisman *et al.,* 2011). The decline in dry matter production overtime is attributed depletion of nutrients in the soil solution due to uptake or nutrient loss effect, sometime the decline is attributed to the senescence stage of the plant growth (Barber, 1962 and Leirosa *et al.*, 1999).

2.8. Fertiliser management

Fertiliser is the agricultural input tool that is continuously used in agriculture to maintain crop productivity per area. However fertiliser can result into reduced yield if not management well. There are two main fertiliser management strategy, 1) soil testing and 2) calculation of application rate and method. Fertiliser application is associated with global warming, eutrophication, acid rains and yield per hectare. Hence fertiliser management factors are important to mitigate challenges. Thus suggests that fertiliser negative environmental impact must be reduced while obtaining and maintaining optimum yield per giving area.

Population increase and economic opportunities in agricultural enterprise has resulted into pressure to produce more per given area (Drangert, 1997). Some of the options to increase yield per giving area is to increase fertiliser application rate. However increasing fertiliser application over time has resulted into diverse environmental pollution since nutrient holding capacity is limited to the available nutrient holding sites (colloid) and problems associated with toxicity levels brought by increasing the recommended application rate and possible leaching (Barber, 1962 and Leirosa *et al.*, 1999). Hence over application of fertilizer particularly ammonium- N fertiliser will lead to volatilization, elevated nutrient toxicity and leaching. However, lower yields from reduced N fertiliser rates (kg ha-1) are likely to prevail, hence application rate needs to be precisely calculated (Kindred *et al.*, 2008). Reducing N fertiliser rates is beneficial to reduce the greenhouse gas (GHG) emissions associated with volatilization.

Nielsen, 2006, mentioned that volatilization is increased with the increasing application rate of fertilisers. However fertiliser effect is directly proportional to the nature of the fertiliser (Wolkowski *et al.,* 1995). Liquid fertiliser evaporates more quickly to the air and is also subjected to leaching beyond root zone if management factors are not adopted. The impact is more severe in coarse sandy soil than fine textured soil. Hence choosing appropriate application rate and using medium and fine textured soil is the primary management consideration for fertiliser rate application.

"Virtually all fertilizer materials are salts. When they dissolve in the soil they increase the salt concentration of the soil solution. An increase in salt concentration increases the osmotic potential of the soil solution. The higher the osmotic potential of a solution, the more difficult it is for seeds or plants to extract soil water they need for normal growth. Excess fertilizer application can cause seed and seedling injury. Moreover fertilizers that produce free ammonia

(urea, UAN, DAP) will significantly increase seed and seedling stress leading to injury or possible death" (FFS, 2002 and Craighead, 1990).

2.9. Summary

Urine contains nutrient concentration equivalent to plant nutrient requirement. Urine product have the potential to be soil amendment for dry matter production. Mineralization varies with soil types and the appropriateness of nutrient materials such as urine and urine based nutrient source as a fertilizer relies to a great extent on its promptness of mineralization and invigorating the nutrients present in them. Plant take-up most of nitrate- N than ammonium- N, most uptake mechanisms adopted by plants is mass- flow. Over application of ammonium- N fertiliser can lead to leaching and volatilization hence significant losses in total mineral- N are likely to occur. Dry matter production increases with time after fertiliser application and decline overtime.

CHAPTER 3

NITROGEN RELEASE PATTERNS OF URINE AND URINE SEPARATED PLANT NUTRIENTS SOURCES IN TWO CONTRASTING SOILS

ABSTRACT

Determining nutrient release pattern is very important when dealing with unknown or new sources of plant nutrients, particularly organic waste products. Urine has been shown to contain significant concentrations of nitrogen in mineral form, which is important for plant growth. Studying nitrogen release pattern helps to time and optimize nitrogen application. A laboratory incubation experiment was conducted to determine the nitrogen release pattern of urine and urine-derived nutrient sources in two contrasting soils for a period of 70 days. The study was designed as a 6 x 2 x 2 factorial experiment, replicated three times. The two soils used were an acidic Inanda and a sandy Cartref. Five N fertiliser sources used in the study were urine, struvite effluent, struvite effluent + struvite, and concentrated nitrified urine and a no fertiliser treatment (control). The fertilisers were applied at the recommended and double the recommended rates. In Inanda soil ammonium- N declined with incubation time while nitrate-N and mineral- N did not increase significantly. Ammonium- N declined with incubation time while nitrate- N and mineral- N increased significantly, in the Catref soil. The findings suggested that the Cartref soil released more nitrogen than Inanda soil.

Key words: Ammonium- N, Application rate, Mineral- N, Nitrate- N, Urine products

3.1. INTRODUCTION

Fertilizer is one of the most crucial and critical input tool that is continuously used in crop and pasture production (Sahrawat, 1980). To maintain and improve agricultural productivity to enhance food security and economic production (Stanford and Smith, 1972). However, due to the evolution of agriculture, population growth, high food demand and the need for economic production, many agricultural systems have adopted inorganic fertilizers in contrast to the use of organic inputs (Drangert, 1997). Nitrogen, phosphorous and potassium (NPK) are the main essential nutrients that are utilized in high quantities to produce food by plants (Mengel, 1995). However these nutrients are becoming more and more limiting in agriculture as it expected that in future phosphate rock may run out, and production of nitrogen fertiliser become more expensive (Karaka and Bhattacharyyab, 2011). Alternative sources of these nutrients need to be sought if sustainability is to be achieved.

Municipalities in recent years have reported problems with high unmanageable waste water, including urine (Udert and Wachter, 2011), eThekwini municipality has been one of them. The NPK that is in urine 80% - 90% nitrogen, 50% - 70% phosphorous and 60% - 80% potassium is plant available (Maurer *et al.*, 2003 and Schouw *et al.*, 2002). In response to this, technology has introduced urine diversion (UD) toilet system that separates urine from faeces at source. This system helps to collect and store urine in large quantities. The eThekwini Municipality in KwaMashu centre collect about 10 000 litres of urine per month from UD toilets. Science has seen the potential in urine as a source of nutrient for plants. In doing so, science has innovatively and successfully removed phosphorous from urine by adding magnesium salts to precipitate P in the form of struvite (Udert & Wächter 2011 and El Diwani *et al.*, 2006).

While struvite has a potential of being used as a phosphorous fertilizer, the process of its formation leaves large quantities of nitrogen in the effluent, which makes struvite effluent a potential source of environmental contamination. In order to make good use of the N in urine, different approaches could be used to make urine based N fertilizer materials.

Urine based nutrient sources, including urine, struvite, struvite effluent and concentrated nitrified urine, have some of the N in organic form and as such could mineralize and, together with the inorganic N in these materials, improve the potential to support complete life cycle of plants (Murugan and Swarnam, 2013). The suitability of these materials as fertilizer depends on

mineralization of the nutrients present (Murugan and Swarnam, 2013). Mineralisation and immobilization processes are biochemical in nature and are mediated through the activities of microorganisms (Murugan and Swarnam, 2013). The resulting effects of these two processes are expressed as net mineralization or net immobilization which determines the nitrogen supply to the growing crops. The mineralization and immobilization processes are affected by soil properties such as temperature, soil moisture and pH (Murugan& Swarnam, 2013). When temperature, soil moisture and pH is favourable for microorganisms to metabolize will results into Minerilasation, the opposite of the process will lead to immobilisation.

The objective of this study was to determine the nitrogen release patterns of urine and urine based nutrient sources in two different soils.

3.2. MATERIALS AND METHODS

3.2.1. Soil preparation and mixing

Cartref sandy soil was collected from an arable field in KwadinaBakubo area (Hillcrest, South Africa) (29°46′48″S and 30°45′46″E) and Inanda acidic soil was collected from arable land at Worlds View area (29°33′28″S and 30°18′36″E). The soil samples were taken from the 0 to 30 cm depth using 3m soil sampling auger. Table 1 below shows the characteristics of the soils used. Both soils are characterized by low fertility hence a low cation exchange capacity, both soils were collected at cool weather condition.

Table 1: Characteristics of soils used in the study

Soil property	Inanda	Catref
Sample density g/mL	0.75	1.42
P (mg/kg)	12	0.7
K ($cmol_c$/kg)	0.08	0.02
Ca ($cmol_c$/kg)	3.23	0.51
Mg ($cmol_c$/kg)	0.87	0.32
Exch. Acidity ($cmol_c$/kg)	1.75	0.33
Total cations ($cmol_c$/kg)	5.92	1.19
Acid saturation (%)	30	28
pH (KCl)	4.11	4.0
Zn (mg/kg)	2.80	0.14
Mn (mg/kg)	10.7	1.41
Cu (mg/kg)	3.6	0.35
Organic C (%)	6	0.5
N %	0.56	0.08
Clay %	23	11

The soils were sieved using a 5 mm sieve and 20 g each soil was used to determine the water holding capacity of the soil following the method of Haney and Haney (2010). The experimental units (2 kg soil containers) were then maintained at 70-100% field capacity by weight loss balancing method.

3.2.2. Urine based fertilizer materials

Most of the urine based plant nutrient sources used in this study were obtained from experimental site at Newlands Mashu, Durban. These consisted of stored urine (U) collected from households around Durban using UD system, struvite (S) processed from source-separated urine and the resultant struvite effluent (SE) remaining after the precipitation of struvite. The struvite and struvite effluent were processed at the reactor plant at Newlands Mashu. Urine used in this study was two weeks old stored in jojo tank completely siled by a rubber stopper, the tank were exposed to outside environmental condition. The concentrated nitrified urine, also used in this study, was obtained from EAWAG Switzerland. Prior to application to the soil, the nutrient sources (U, SE and NUC) were characterized for N concentration. Samples were analyzed by gallery machine and results were obtained in mg/l. As it was expected samples were very high in total ammonium N. Struvite contained 5.7% N, in treatment where struvite effluent was added with struvite, half of the total N requirement was supplied by struvite and another half was supplied by struvite effluent.

Phosphorus content was also analyzed following Robert 2002 method. For each fertilizer source (U, SE and NUC), 2ml aliquot of the extract was placed in a 50ml beaker in triplicates and diluted 100 times, as P was expected to be high in all samples except for SE, where P was expected to be very low since most of the P was precipitated in struvite production. Colour reagent (10 ml) was added slowly to the solution and mixed by shaking and allowed to stand for 45 minute, after which the absorbance was read at 670 nm on a spectrophotometer. From the standard curve, concentration of phosphorous was calculated in mg/l (Robert, 2002).

3.2.3. Experimental procedure and incubation design

The aerobic incubation experiment was conducted with fertilizer-treated soils over a 70-day period under controlled room temperature conditions maintained at 25 ^{o}C and at 80% relative humidity. This experiment was designed as a 6 x 2 x 2 factorial treatment structure replicated 3 times giving 60 experimental units. Two kg of each of the two soils was filled in ventilated containers. The soils were amended with either Urine, Struvite effluent, struvite effluent + struvite, concentrated nitrified urine and no fertilizer (the control). The treatments were applied either at the recommended N rate or at double the rate. The treatment combinations were replicated three times. Moisture was periodically corrected throughout the study period based on weight loss. Non-destructive sampling was used to collect soil samples from the treatments at days 0, 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days of the incubation.

3.2.4. Extraction and analysis of ammonium-N

The soil samples (5 g) were weighed into conical flasks, 50ml potassium chloride (2M KCl) dispensed, and the flasks were shaken at 180 cycles per minute on the reciprocal shaker for 30 minutes (Dahnke and Johnson, 1990). Samples were then filtered through Bowmen 250 nm filter papers, and soil sample extracts were then analysed using 2011 Thermo Scientific Gallery sample analyser.

3.2.5. Data analysis

Data analysis was carried out using the General Linear Model, Repeated Measures using the Genstat 16th edition Statistical Package to compare treatment means and their interactions. This was done for each soil type, and differences in means were separated by Least Significant Different (LSD). Significant tests were done at the 5% level of significance. Contrasts were used to compare the mean treatment means.

3.3. RESULT

3.3.1. Nitrogen and phosphorus in fertilizer materials

Nitrogen in the different materials differed significantly. Nitrogen was the highest in the nitrified urine treatment, with urine and struvite effluent having similar levels. Phosphorous in materials was also the highest in nitrified urine followed by urine, with struvite effluent having the lowest.

Table 2: Nitrogen and phosphorus contents of the fertilizer materials used

Fertiliser material	NH_4-N content (mg L^{-1})	Phosphorus content (mg L^{-1})
Urine	4656	231
Struvite effluent	4578	7
Nitrified Urine	35483	3741

Struvite contained 5.7% N and 12.6% P.

3.3.2. Soil pH

There were no significant differences observed among fertilizer treatments at the different rates in terms of pH in the sand soil. Soil pH increased with incubation time (Figure 1). The initial pH was 4.0 and it increased to between 5.6 and 6.6 after incubation with the fertilizer N sources. Like in the Cartref soil, there were no significant differences among the treatments in the Inanda soil (acidic). Soil pH only slightly increased with incubation time but remained in the acidic range at the end of the incubation period (Figure 2). The initial soil pH was 4.1 and it increased to between pH 4.4 and 5.2.

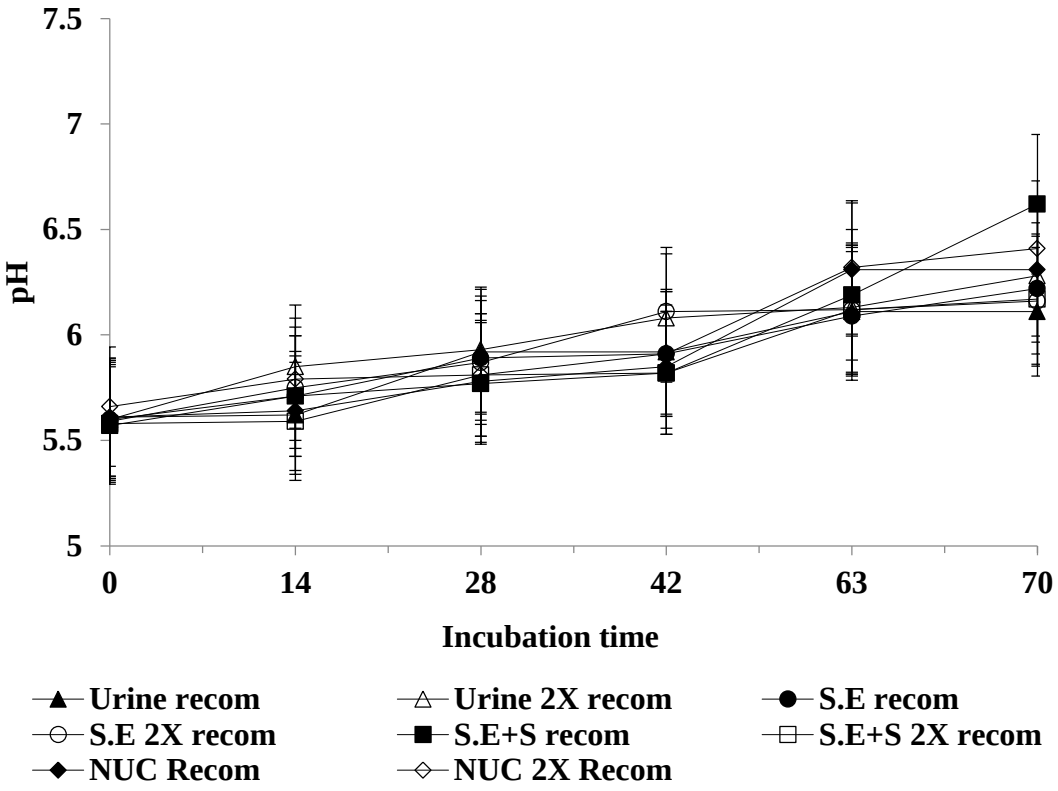

Figure 1: Cartref soil pH level with incubation days

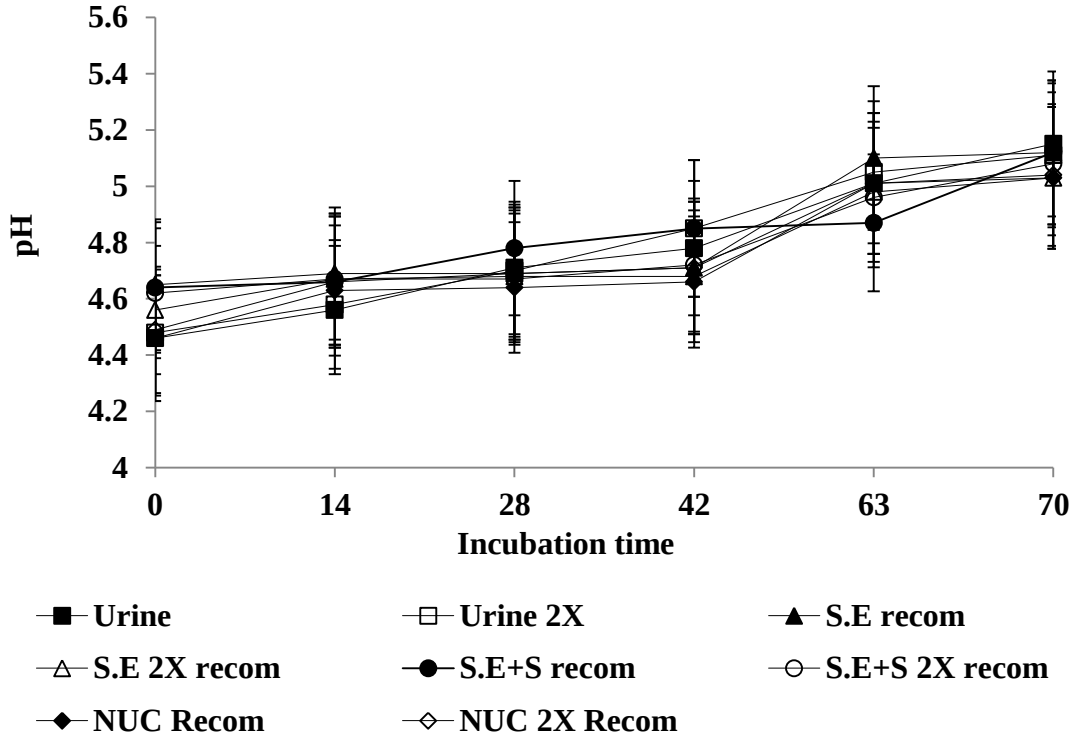

Figure 2: Inanda soil pH level with incubation days

3.3.3. Soil electrical conductivity

There were significant differences among the treatments and between the application rates in terms of in EC. Electrical conductivity was significantly higher where the rates were doubled than at the recommended rate in the Cartref soil (Figure 3). The highest EC was recorded in the NUC at double the N rate, followed by the other treatments at double rate. In the Inanda soil (acidic), there were no significant differences between the treatments. Treatments where the application rate of fertiliser was doubled did not differ significantly from treatments where recommended rate was applied (Figure 4). However, EC also increased with incubation time.

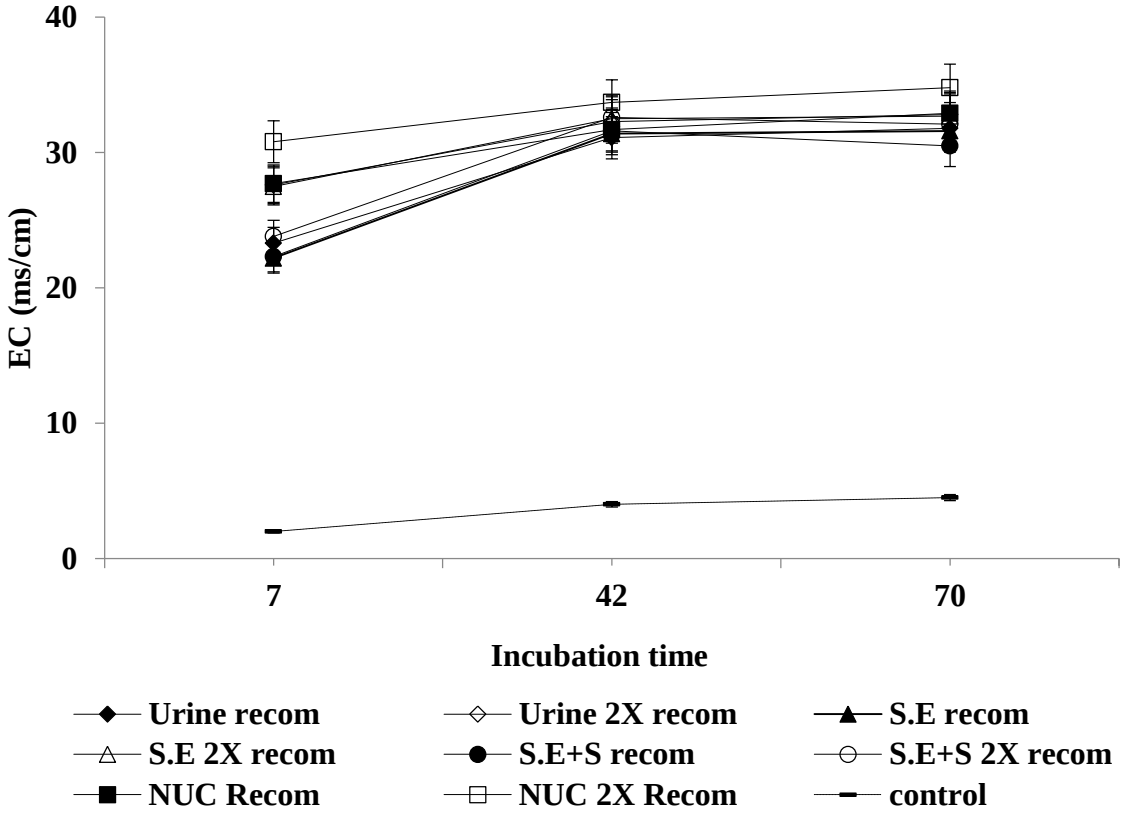

Figure 3: Electrical conductivity of Cartref soil during the incubation study of 70 days

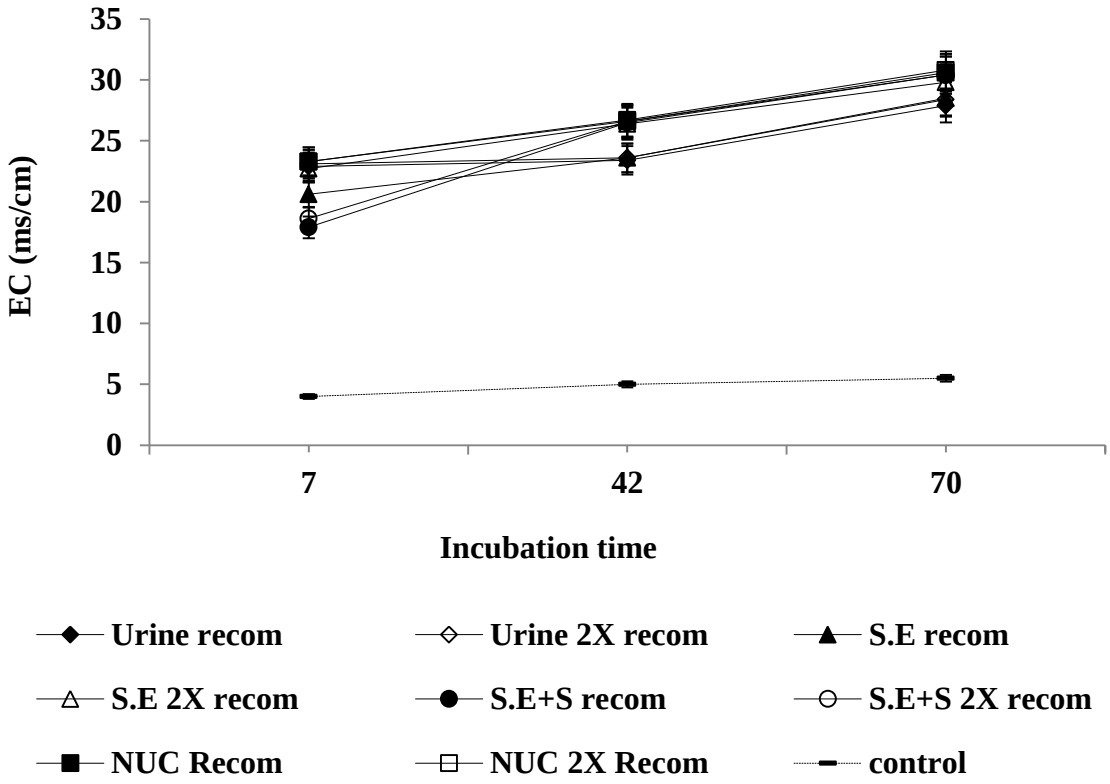

Figure 4: EC for Inanda soil during the incubation study of 70 days

3.3.4. Ammonium-N and nitrate-N concentrations in Cartref soil

The incubation results showed that ammonium- N was disappearing with incubation time. There were significant differences between with the recommended application rate and double the rate in terms of ammonium- N concentration in the soil. In treatments where the rate of application was doubled released significantly more ammonium- N than treatments where the rate of application was at recommended (Figure 5). Treatments where struvite effluent was added with struvite powder had significantly lower ammonium- N release than other treatments. The rest of the treatment did not differ from each other in terms of ammonium- N release, but they had higher ammonium N than the control up to 63 days of incubation.

Nitrate- N production increased significantly with incubation time (Figure 6). In treatments where the rate of application was doubled did not differ significantly from treatments where recommended rate was applied. Nevertheless there were significant differences when comparing treatment alone, with nitrified urine having significantly more nitrate- N than all other treatments. Evidently nitrified urine was the first (after 28 days) to show significant nitrate- N increase among treatments (Figure 6). Other treatments did not differ from each other, but they all had higher nitrate-N than the control after 49 days of incubation. Significant decline of total mineral- N was also observed, but all the treatments had higher levels than the control throughout the study (Figure 7)

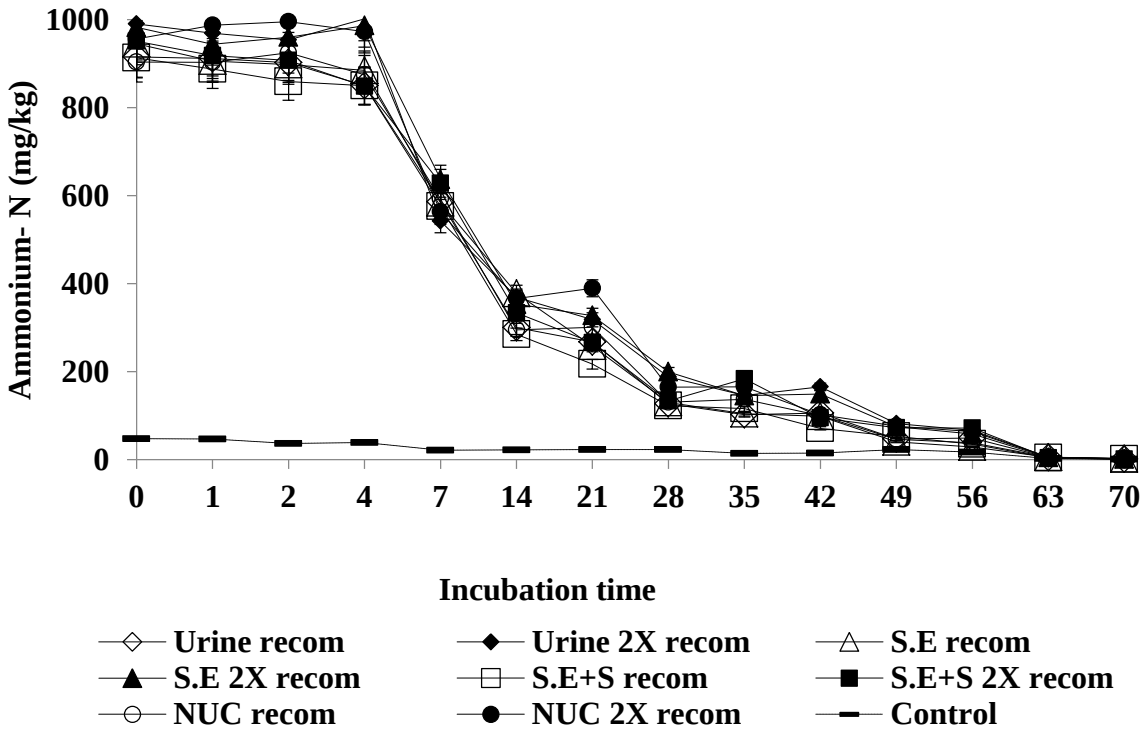

Figure 5:NH_4 depletion during incubation at 70 days for all treatments in Cartref soil.

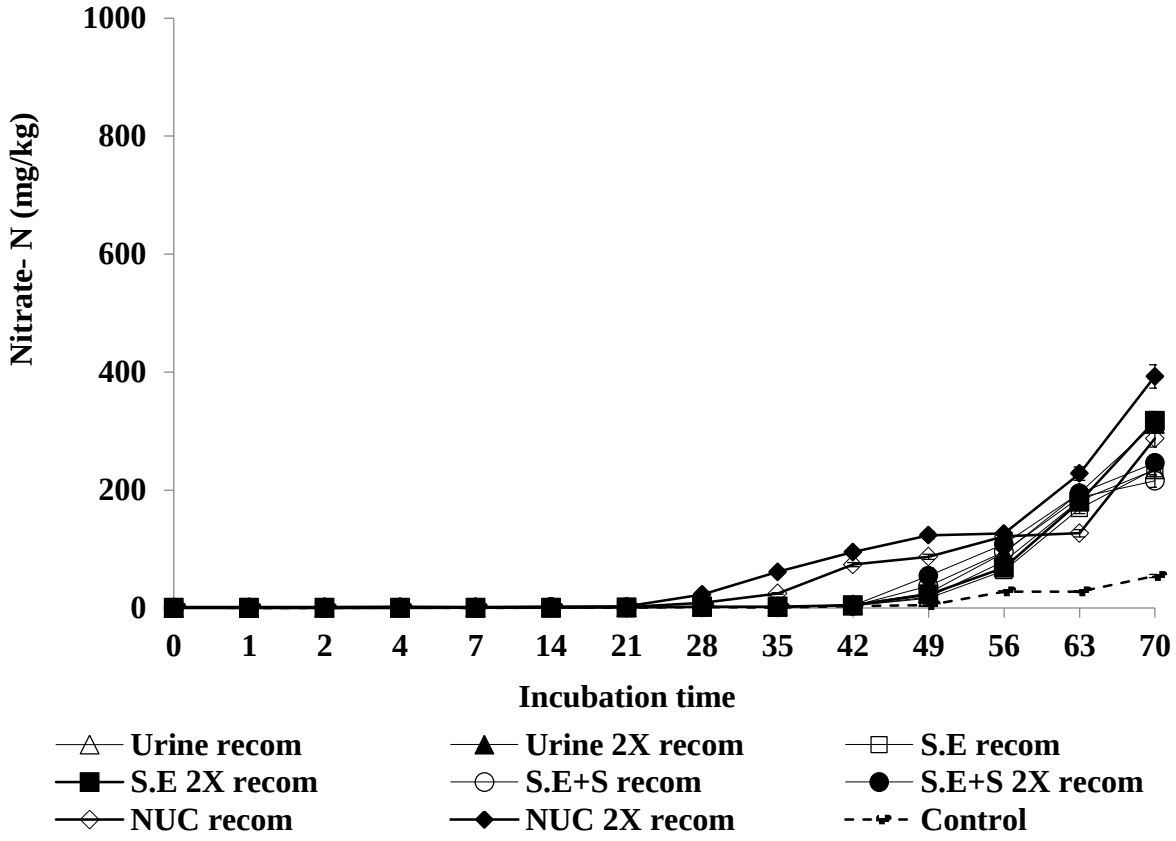

Figure 6: NO_3 release pattern for all treatment during the incubation study of 70 days period in Cartref soil.

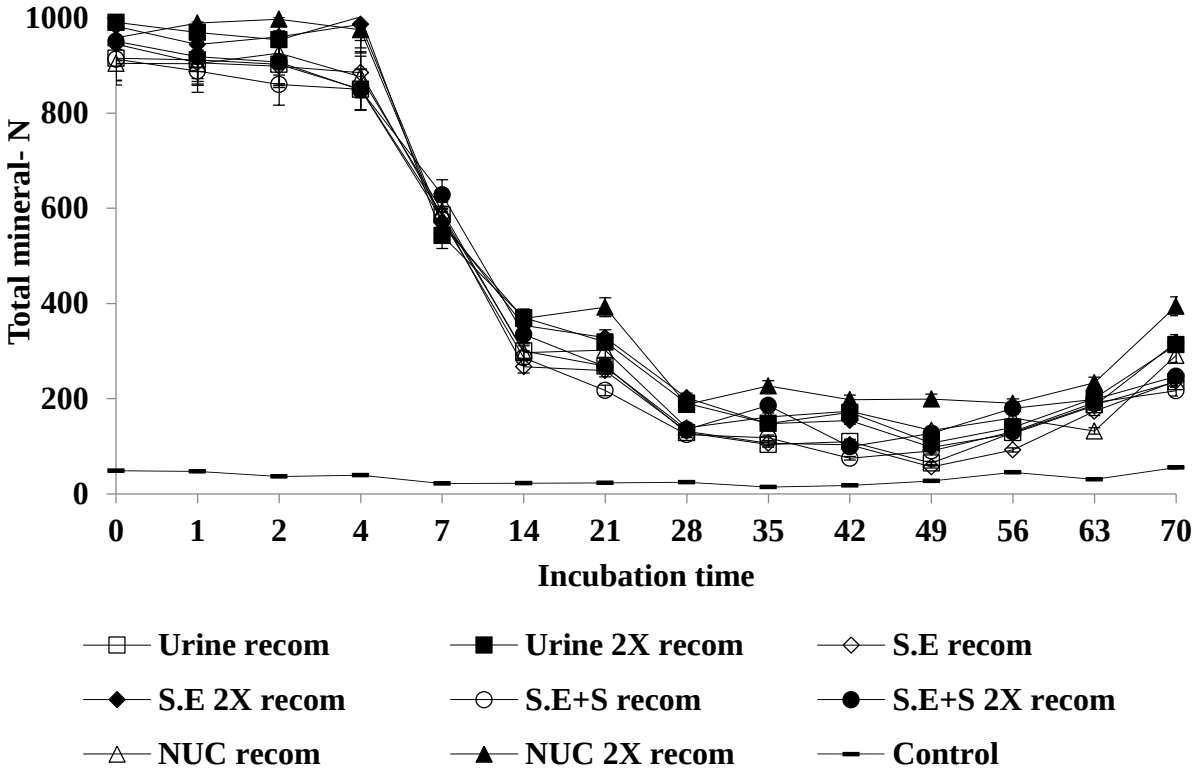

Figure 7: Total mineral nitrogen pattern for all treatment in Cartref soil for incubation study of 70 days.

3.3.5. Ammonium-N and nitrate-N concentrations in Inanda soil

The incubation results showed that ammonium- N was disappearing with incubation time. There were significant differences between treatments at recommended rate and double the rate in terms of ammonium- N release in the soil. Treatments where the rate of application was doubled released significantly more ammonium- N than treatments where the rate of application was at recommended (Figure 8). NUC released significantly higher nitrates than other treatments, other treatments did not differ significantly, there were no significant differences in nitrate- N among other treatments nor between recommended and double the rate (Figure 9). The NUC treatment had higher nitrate N than the control throughout the study. Total mineral- N declined with incubation time and was higher for double the rates than the recommended rate. All the treatments had great mineral N than the control up to 42 days of incubation (Figure 10).

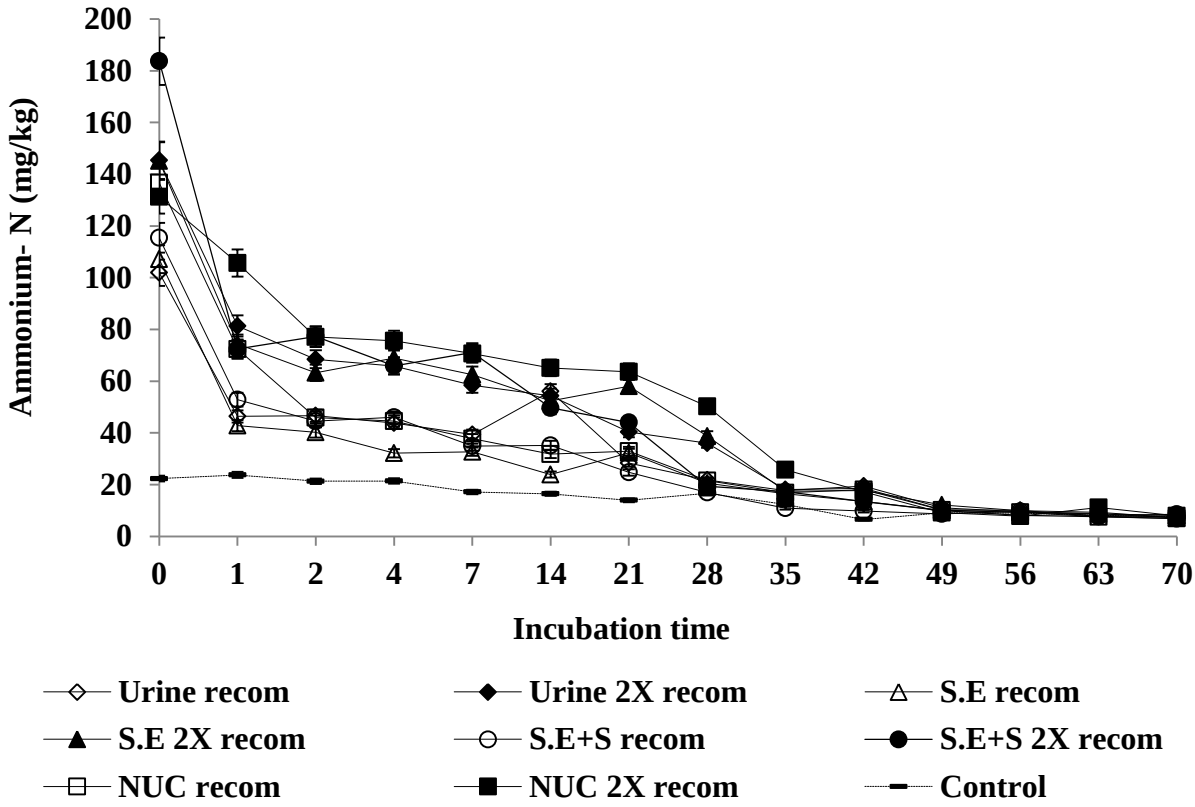

Figure 8: NH_4 depletion results during incubation study of 70 days for all treatment in Inanda acidic soil

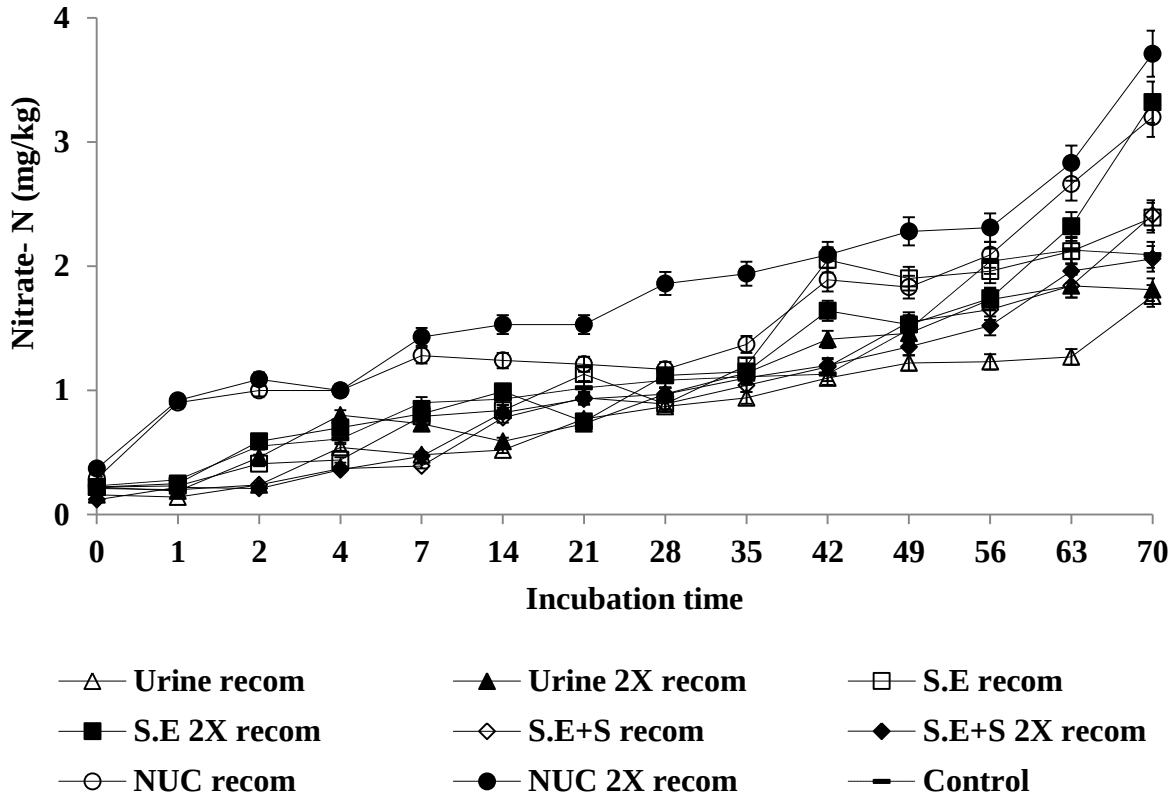

Figure 9: NO_3 release during incubation in Inanda acidic soil

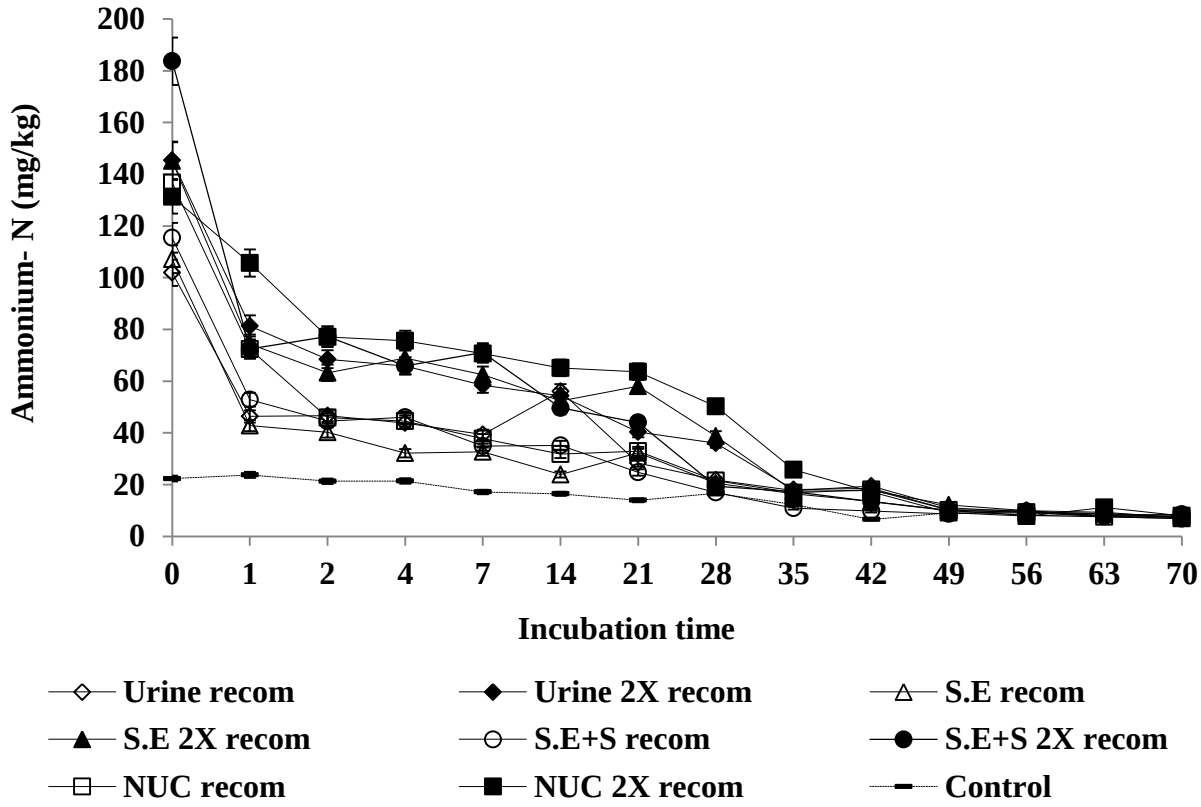

Figure 10: Total mineral- N for Inanda soil during incubation

3.4. DISCUSSION

Urine contains significant concentrations of nitrogen in mineral form (ammonium- N) about 80% (Schouw *et al.*, 2002). The similarity in ammonium-N between struvite effluent and urine could be explained by the high N in the urine from which they were all derived. The significant high concentration of nitrogen in nitrified compared to urine and struvite effluent could be explained by the nature in which nitrified urine was made, nitrified urine is a concentrated nutrient source made by removing water from urine i.e. taking 1000 litres of urine remove water and get 500 ml concentrated nutrient solution from urine. Struvite formation uses low quantities of N and high amounts of P from the urine (Udert and Wachter, 2011), this explains the lower P content in the struvite effluent than urine.

The high ammonium- N observed at the initial stages during incubation in both soil types could be explained by the high urea in urine and its conversion to ammonium-N (Kirchmann and Pettersson, 1995). The decline in ammonium-N and the associated increase in nitrate-N could be explained by nitrification. During the mineralization process ammonium- N is first released into the soil in which the nitrifying bacteria act and oxidize ammonium into nitrate-N, resulting in decreases in ammonium content with the increase in the nitrate- N (Murugan and Swarnam, 2013). However more ammonium- N tends to accumulate if the nitrification process is inhibited. The decline in total mineral N was as a result of lower nitrate-N produced relative to the ammonium-N decline, particularly in the Cartref soil. Such decline in mineral N suggested that N was being lost from the soil possibly due to volatilization and ammonification by converting ammonium- N into ammonia- N gas (Mamo *et al.*, 1998). Furthermore in sandy soil the ability of NH_4^+ to form electrostatic bonds with clay minerals and organic colloids to impair losses of soil and fertilizer N is low, this favors ammonium volatilization (Medina, 2006 and Nielsen, 2006). The liquid nature of the N sources used also could also have impacted on significant N- loss, in agreement with Argo and Biernbaum, (1995), who reported that liquid ammonium- N fertilisers evaporate quickly to the air.

Nitrogen volatilization is catalysed by urease enzymes in the soil converting the urea component to free ammonia gas, and is favored in temperature above 22^{o}C (Wolkowski *et al.,* 1995). Medina (2006) noticed that aeration which is necessary for microorganism's metabolism to nitrify during the incubation also promotes the losses of ammonium nitrogen via air circulation

in the incubation system. This suggest that even the temperature that was maintained at 25^{o}C also contributed to the significant ammonium- N losses. In Cartref soil the pH increased significantly, Curtin *et al*., (1998) reported that the increase in pH favors the formation of ammonia gas which is the ammonification process. Same findings were observed in this study thus suggest that ammonification also contributed to significant ammonium- N and mineral- N losses. These findings further justify and agrees with the finding of this study that significant ammonium- N and mineral- N losses in Cartref soil was attributed to volatilisation and ammonification.

The lack of increase in nitrate production and total mineral- N as a result of doubling rate of fertiliser application suggest that over application or doubling the rate of nitrogen fertilizer will lead to N volatilization as ammonia- N gas. This is supported by Leirosa *et al*., (1999), who reported that high or over application of nitrogen fertiliser promotes further volatilization. The significantly higher nitrate in the NUC treatment than other treatments was attributed to more availability of nitrogen as nitrate-N because NUC is already nitrified before application in the soil. Similarities between treatments in terms of ammonium- N and nitrate- N release could also be explained by mineral- N in the urine from which they were all derived. Treatment S.E+S released significantly low ammonium- N compared to other treatments- U, S.E and NUC. This could be explained by organic N that is in struvite which could have nitrified or volatilised which suggest that most of the ammonium- N was released by struvite effluent rather than struvite.

In Inanda the lack of increases in nitrate-N and mineral-N were attributed to lack of microorganism's activity to initiate and facilitate mineralization process, due to acidity as shown by the low pH which did not increase with incubation time. Most of the microorganisms that are responsible for nitrification are not active at acidic condition because of aluminium toxicity (Gulser *et al.*, 2010). Aluminium is prevalent at acidic soil condition (Motavalli *et al.*, 1995). Aluminium impair and paralyze the functioning of microorganisms (Hue *et al.*, 1986). Moreover in acidic condition most nutrients are not available and some of these nutrients are needed by microorganisms for their own metabolism (Hue *et al.*, 1986). This is also supported by Tully *et al*., (2013) they reported that quantities of nitrogen, calcium, phosphorus and magnesium are generally lower at pH of 5.5 or less. The higher ammonium N and nitrate N at the early and later stages, respectively, than the control suggested that all the urine based nutrient sources could contribute significantly in supplying N for crop growth, irrespective of soil type.

3.5. CONCLUSION

Amending soils with urine-based nutrient sources increases ammonium N, nitrate N and total mineral N, particularly where nitrified urine treatment is used. Ammonium N and total mineral N declined while nitrate N increased with incubation time particularly in the Cartref soil. There are high N losses from the soil during incubation of urine-based nutrient sources. Doubling the rate of urine and urine separated plant nutrient sources does not increase total mineral- N during the incubation.

CHAPTER 4

GROWTH AND BIOMASS PRODUCTION OF PERENNIAL RYEGRASS (*LOLIUM PERENNE. L)* IN RESPONSE TO THE APPLICATION OF URINE BASED FERTILISER

ABSTRACT

Dry matter production is one of the main factor that determines crop productivity and profitability. Biomass is the accumulative nutrients weight in plant structure that the plant was able to absorb during the growth season. Urine has been shown to contain nutrients equivalent to plant requirement. Ryegrass tunnel planting pot experiment was conducted to study the effect of application of urine and urine separated plant nutrient sources on growth and biomass production of perennial ryegrass. The study was designed as a 6 x 2 x 2 factorial treatment structure with the following factors: fertilizer sources (6 levels- Urine, struvite effluent, struvite effluent + struvite, nitrified urine concentrate and two controls, an inorganic fertilizer source (NPK 2:3:2) and no fertilizer treatment). The second factor was the application method of fertiliser sources (2 levels–once off and split application). The split application was done 3 times. The third factor was the application rates (2 levels – recommended rates and double the recommended rates) and treatments were replicated 3 times. Dry matter production increased significantly with time after harvest and it declined with time after harvest 3 at cut 4. Where the split application method was used had significantly high dry matter production than where all nutrient sources was applied all at once. The recommended application rate had significantly high dry matter production than the double application rate. The findings suggested that urine and urine nutrient sources are equally as effective as a mineral fertilizer for dry matter production, especially when split applied, on a sandy soil.

Key words: Dry matter production, urine, application method, application rate

4.1. INTRODUCTION

Elevated fertiliser prices due to costs of production and limited sources of plant nutrients (phosphate rock and N sources) (Heinonen-Tanski and Wijk-Sijbesma, 2005), have resulted in the need for alternative sources of nutrients. Urine has been shown to contain nutrient equivalent to plant nutrient requirements (Karaka and Bhattacharyyab, 2011 and Maurer *et al.*, 2006). Moreover urine can further be nitrified directly to produce a nutrient source enriched in nitrate-N than ammonium- N called nitrified urine. Phosphorous, in urine, has been precipitated and concentrated into struvite (Jaffer *et al.,* 2002), a potential P fertilizer material. However the resulting effect of struvite production leads to challenges on the disposal of the effluent (Etter *et al.*, 2011). Struvite effluent has been shown to contain significant N and K in mineral forms, which are important for plant growth (Doyle and Parsons, 2002). The question that comes up is, "Why dispose struvite effluent while it value can be beneficial to plants of economic importance perennial ryegrass?"

Perennial ryegrass (*Lolium perenne*) is the member of Poaceae family (Wyk, 2005 and FFS, 2004). Ryegrass, as it is commonly known in man English speaking countries, is the C3 monocotyledon cool season flowering plant originating from Europe typically used for lawns across the world (Wyk, 2005 and FFS, 2004). Perennial ryegrass grows best on fertile, well-drained soils thus is not productive on low fertility dry soils (Wyk, 2005). Further, perennial ryegrass grows well and rapid in fertile irrigated soils and is high yielding under good environmental conditions and proper fertilization (Craighead, 1987and FFS, 2004).

When perennial ryegrass is fertilized well, it contains high quality nutrients, recovers well after grazing, and is high yielding when is grazed or cut at three leaf stage hence is valuable as hay, silage, and pasture (Craighead, 1987and FFS, 2004). The tissue of ryegrass is highly digestible for all classes of ruminant animals and performs best under a high N status. This suggests that input fertiliser must be rich in nitrogen. Thus the nitrogen in the fertiliser must be easily accessible as plant available (nitrate- N) to effect maximum growth and dry matter production at the time of grazing or harvest (Adler *et al.*, 2005 and Barret *et al.*, 2000).

The aim of this study was to determine the effect of application of urine and urine products on growth and biomass production of perennial ryegrass. Perennial ryegrass was selected because of its potential to grow fast and reestablish quick after cutting which was ideal for this study.

4.2. MATERIALS AND METHODS

4.2.1. Experimental site

The experiment was conducted in Control Environment Facility (CEF) at the Agriculture Campus of the University of KwaZulu Natal, Pietermaritzburg, South Africa. . Tunnel conditions were maintained at 26^{o}C air temperature and 65% atmospheric humidity.

4.2.2. Experimental material

4.2.2.1. Planting material

Seeds of perennial ryegrass ***(Lolium perenne)*** cultivar that were used for this study were purchased from Agric-Solution Company.

4.2.2.2. Soil preparation and potting

The soil used in the pot experiment was collected from an arable field in KwadinaBakubo area, Hillcrest, South Africa (29°46′48″S and 30°45′46″E). The soil was classified as Cartref soil with a sandy loam texture and a low cation exchange capacity. Soil samples were taken from the topsoil horizon (0 to 20 cm) and sieved using a 5 mm sieve. The l characteristics of the soil are in Table 1. Twenty g of soil was used to estimate water holding capacity of the soil following Haney and Haney (2010) method. The experimental units (tubs) were maintained at 70-100% field capacity.

4.2.2.2.1. Water holding capacity description

Twenty grams field-moist soils were placed in a funnels and filter papers fitted into pre-weight collecting flasks, 100.0 g distilled water was added in small portions and allowed to drain. The samples were covered with aluminum foil to prevent and avoid evaporation, and then allowed to stand overnight for complete draining. The following day, adhering water was removed by gently tapping the funnels against the neck of the collecting flasks. The collecting flasks + percolated water were weighed to the nearest 0.001g. The experiment was conducted for soils Cartref soil, allowing three replicates per variable. Three blanks were also run, including funnels and filter papers without soils. For the determination of the soils initial water contents, 20g field dry soils were weighed into pre weighed glass beaker and oven-dried over night at 105^{O}C. Percentage water holding capacity (WHC) of soils were computed using equation (1) below, where W_P is the weight of water percolated in grams, W_i is the initial amount of water contained in soil samples in grams, and *dwt* being the oven-dry soil weights in grams.

(1) WHC = $[(100- W_{P)} \div W_i]/dwt \times 100$

Table 1: Characteristics of soil used to study the effect of urine based fertiliser on the growth and biomass production of perennial ryegrass.

Sample density g/mL	1.42
P (mg/kg)	0.7
K (mg/kg)	0.02
Ca (mg/kg)	0.51
Mg (mg/kg)	0.32
Exch. Acidity (cmol/kg)	0.33
Total cations (cmol/kg)	1.19
Acid saturation %	28
pH (KCl)	4.0
Zn (mg/kg)	0.14
Mn (mg/kg)	1.41
Cu (mg/kg)	0.35
Organic C (%)	0.5
N (%)	0.08
Clay %	11

4.2.3. Fertilizer sources (types)

The plant nutrient sources used in this study were obtained from an experimental site at Newlands Mashu, Durban. These consisted of (i) stored urine (U) collected from households around Newlands area, Durban, using UD system (ii) struvite (S) processed from source – separated urine and (iii) the resultant struvite effluent (SE) remaining after the precipitation of struvite and (iv) nitrified urine concentrate (NUC) was obtained from EAWAG Switzerland produced directly from urine through nitrification reactor. The struvite and struvite effluent concentrate were processed at the reactor plant at Newlands Mashu. Urine used in this study was two weeks old stored in green jojo tank exposed to outside environmental condition. The compound fertilizer 2:3:2 was used as an inorganic plant nutrient source for comparison.

Prior to application to the soil, the plant nutrient sources (U, SE and NUC) were characterized for N and P concentration. Struvite is known to contain 5.7% N (Barak and Stafford, 2006). For N analysis in U, SE and NUC, extracts were prepared in triplicates and diluted 100 times as N was expected to be very high in all samples. Samples were then analyzed for ammonium-N and nitrate-N using a 2011 Thermo Scientific Gallery sample analyser, and results were obtained in mg/L. As it was expected samples were very high in N; Urine, struvite effluent and nitrified urine concentrate contained 4656, 4578 and 35483 mg N/L, respectively.

Phosphorus content was analyzed in U, SE and NUC only. The struvite used in the study contained 12.6% P (Barak and Stafford, 2006). For each fertilizer source (U, SE and NUC), a precise quantity (10 ml) of colour reagent was added slowly to the solution and mixed by shaking and allowed to stand for 45 minute, after which the absorbance was read at 670 nm on a spectrophotometer. From the standard curve, concentration of phosphorous was calculated in mg/l and was found to be; 231, 7 and 3847 mg/L respectively.

4.2.4. Experimental procedure and trial design

The experiment was designed as a 6 x 2 x 2 factorial treatment structure replicated 3 times giving 54 experimental units (1 kg pots). The factors included six nutrient sources (urine, struvite effluent, struvite effluent + struvite, nitrified urine, 2:3:2 NPK and no fertiliser t). The second factor had two application method (once off and split application). The split application was done 3 times, once after each cut. The third factor was the application rates (2 levels – recommended rates and double the recommended rates). However the control treatments (NPK and no fertilizer) did not have a comparison between application rates and methods since the inorganic fertiliser was applied once at recommended rates to meet crop nutrient requirements and there were no rates for the zero fertiliser application.

4.2.5. Application of fertiliser

Phosphorous was found to be highly deficient in these soils and application rates were based on N requirements with the assumption that K won`t be limiting. However P was corrected by adding additional P using SSP (10.5% P) (see Table 2). The correction was done by calculating the amount of P contained in the fertiliser source and then determining the quantity needed to meet crop nutrient requirements if SSP were to be used. However in (S+SE) treatment P was not limiting as extra P was supplied by struvite (S). All additional P supplement (SSP) was all applied at sowing when 24.2 mg was to be achieved. All fertilizers were then added and mixed homogeneously to the soil.

Table 2: Correcting P deficit for ryegrass growth and biomass production

Sources	[N] mg/l	[P] mg/l	N rate ml	[P] mg in N rate	P needed by soil	P short	SSP added (g)	N (ml) and SSP (g) rates
U	4656	231	15.25	3.5	24.2	20.7	0.2	15.25+ 0.2
S.E	4578	7	15.3	0.11	24.2	24.09	0.23	15.3 + 0.23
NUC	35483	3847	2	8	24.2	16.2	0.15	2 + 0.15

Note: [N] stands for nitrogen concentration, [P] stands for phosphorous concentration, SSP Stands for Single Super Phosphate

4.2.6. Planting

Ryegrass seeds were broadcasted at the rate of 25 kg seed per hectare into1kg soil containing pots. This translated into about a gram (g) of perennial ryegrass (*Lolium perenne*) seeds to be sown per pot. Different plant nutrient sources (fertilizers) calculated on the basis of N crop requirements for perennial ryegrass was added to the 1 kg pots containing soil. Deionized water was applied as an irrigation source to maintain 70% moisture content throughout the experiment, by experimental unit weight loss method. Pots were randomized three times a week.

4.2.7. Data collection

Harvesting was done at pre-determined intervals based on crop growth rates (20 cm plant height), at 35, 45, 63 and 79 days after sowing. Before the harvesting, data collection was done on the following crop growth variables; seedling emergence after 14 days and seedling emergence rate (SET). To determine plant height, ten healthy growing leaves from each treatment were marked and measured for height using a 30 cm ruler. At harvest fresh mass and dry mass was determined as follows: Plants were cut 5 cm above soil surface at all cuts, harvested plants were weighed for the determination of fresh biomass and dried at 60 ^{o}C for 72 hours for the determination of dry mass. Plants samples were than subsequently taken to Cedara Department of Agriculture and Environmental Affairs at division of plant fertility analysis for plant tissue analysis.

4.2.8. Data analysis

Data analysis was carried out using the General Linear Model, Repeated Measures using the Genstat 14 Statistical Package to compare treatment means and the interactions. Significant tests were done at the 5% level of significance. Contrasts were used to compare the mean treatment means.

The data were analyzed as follows (Table 3) to compare the urine based N sources:

Table 3: ANOVA table for urine based N sources treatment analysis

Sources of variance	Degree of freedom (n-1)
Factor A (urine based N sources)	3
Factor B (Method of application)	1
Factor C (Application rate)	1
Interaction	
Urine based N sources × method of application	3
Urine based N × application rates	3
Method of application × application rate	1
Urine based N × Method of application × application rate	3
Residual	32
Total	47

The second analysis was comparing the fertiliser N sources with controls (Table 4). The analysis was done as a single factors analysis having the total of 18 treatments and treatment combinations all applied at once- off. There was no interaction in this analysis since controls did not have application rate and application method but contrasts were done to compare (i) urine based N sources and control; (ii) application rates between N fertilizer's sources (iii) differences among the organic sources (iv) whether the controls differed significantly from N sources. The data was analyzed as follows to compare fertiliser with controls:

Table 4: ANOVA table including control treatments (NPK 2:3:2 and control 0)

Source of variance	Degree of freedom (n-1)
Treatment	17
Residuals	36
Total	53

4.3. RESULTS

4.3.1. The effect of urine and urine nutrient sources on dry matter yield

4.3.1.1. Comparison of urine based N sources

There were significant ($P<0.05$) differences, in dry matter production, when comparing treatments- U, S.E, S.E+S and NUC, application method- split and once- off, application rates- recommended and double and cuts. Dry matter production increased significantly with time after each cut and it declined with time after cut 3 at cut 4 (figure 1). Cuts- 1, 2, 3&4 differed significantly among each other, the means were 150, 208, 481 and 321 respectively (figure 2). However it was noted that even though dry matter declined significantly after cut 3 at cut 4 from all treatments- U, S.E, S.E+S and NUC, but all split rate application method gradually declined whereas all once- off application method declined drastically. In application method where the application rate was split had significantly high dry matter production than application method where all nutrient sources was applied all at once (Figure 3). The mean for split rate application was 315 whereas the mean for once- off application method was 265. The recommended application rate had significantly high dry matter production than the double application rate (figure 4). The mean for recommended application rate was 303 whereas mean for double rate was 227. Nevertheless NUC had the highest dry matter production and was followed by S.E, U and S.E+S treatments, the means were: 301, 289, 286 and 284 respectively.

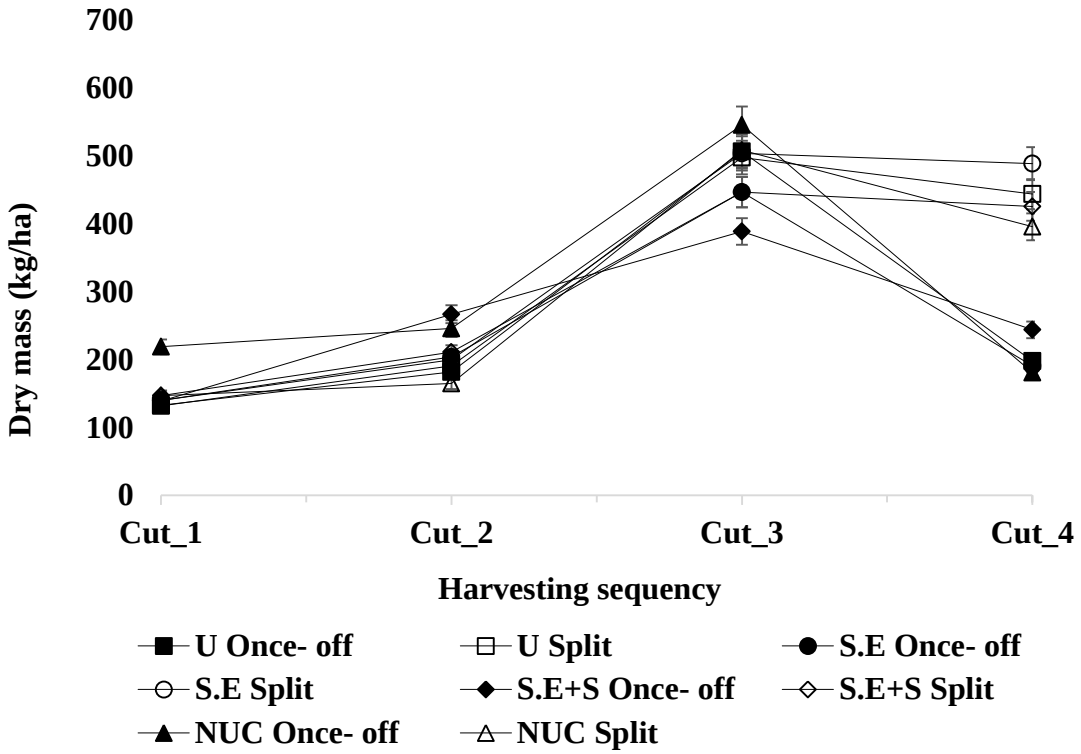

Figure 1: Dry matter production of perennial ryegrass comparing urine plant nutrient sources only.

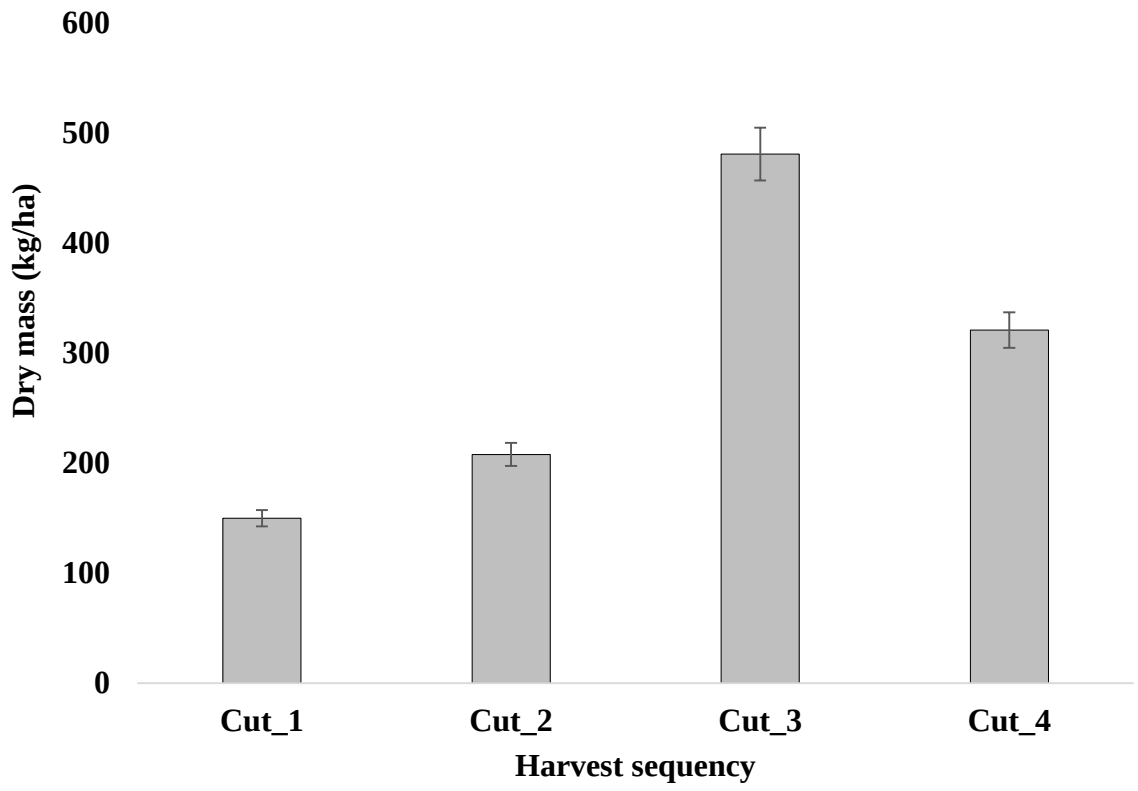

Figure 2: Dry matter production of perennial ryegrass at different cutting stage.

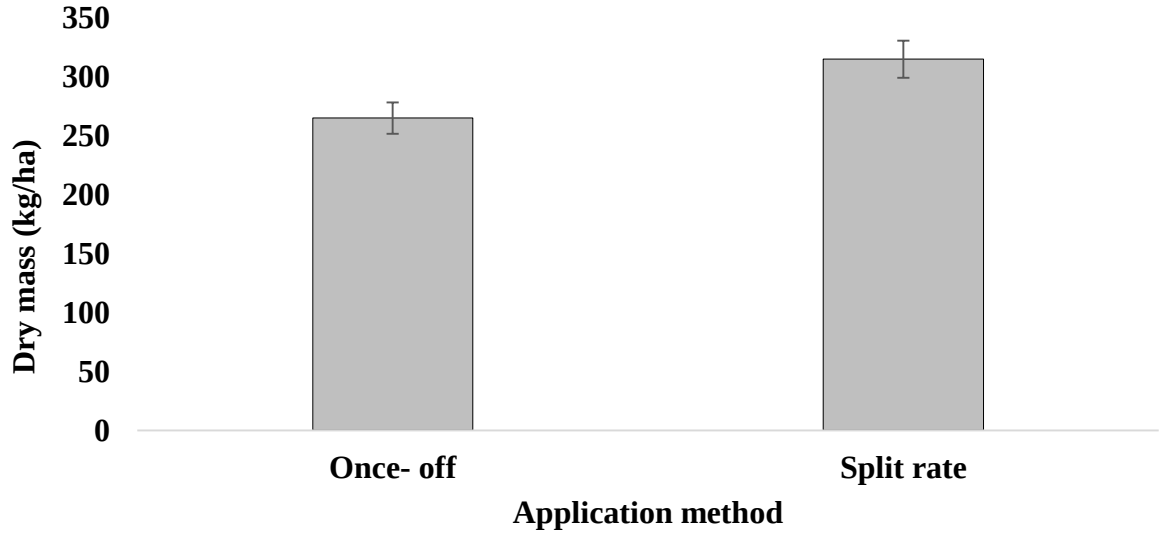

Figure 3: The effect of once- off and split rate fertiliser application method on dry matter production of perennial ryegrass.

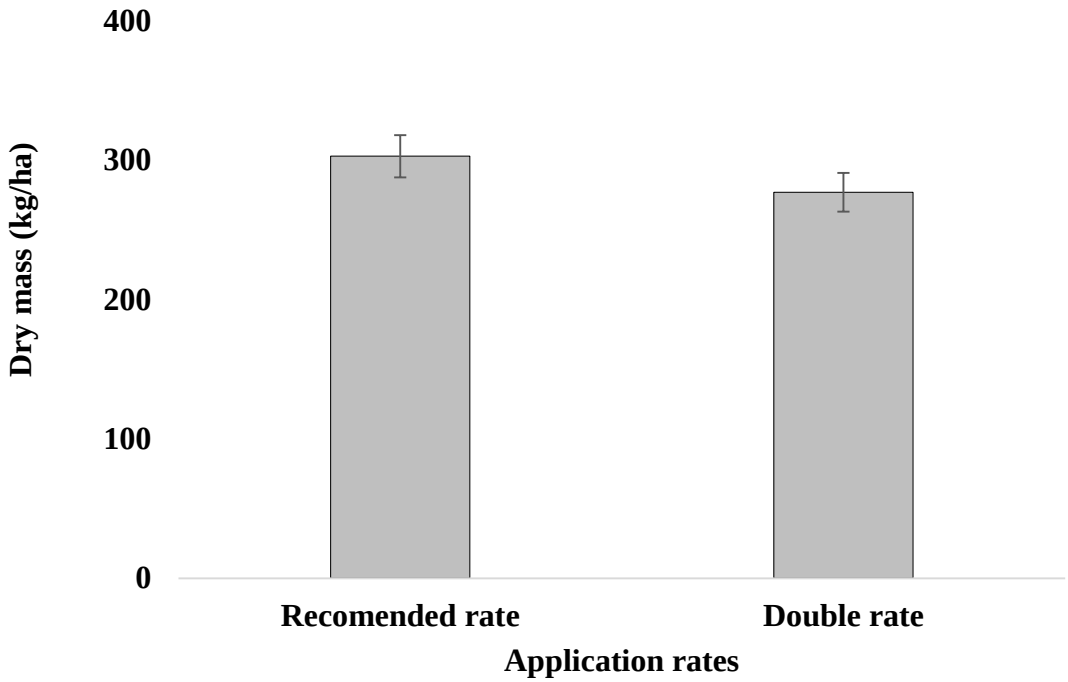

Figure 4: The effect of application rate- recommended and double rate on dry matter production of perennial ryegrass.

4.3.1.2. Comparison of N sources and the controls

In this analysis once- off application method was used to compare N sources with controls, since controls was applied at once and did not had application method and rate. There were significant ($P<0.05$) differences observed among treatments. All treatments- U, S.E, S.E+S, NUC and NPK had significantly higher dry matter than zero fertilizer treatment. However treatment NUC responded significantly different within cuts. Treatment NUC at recommended rate had significantly higher dry matter yield then all treatments at cut 1 and 3. At the same time there were no significant differences in dry matter production between NPK, urine and urine products. At cut 4 dry matter production decline significantly from all treatments- U, S.E, S.E+S, NUC and NPK (figure 5).

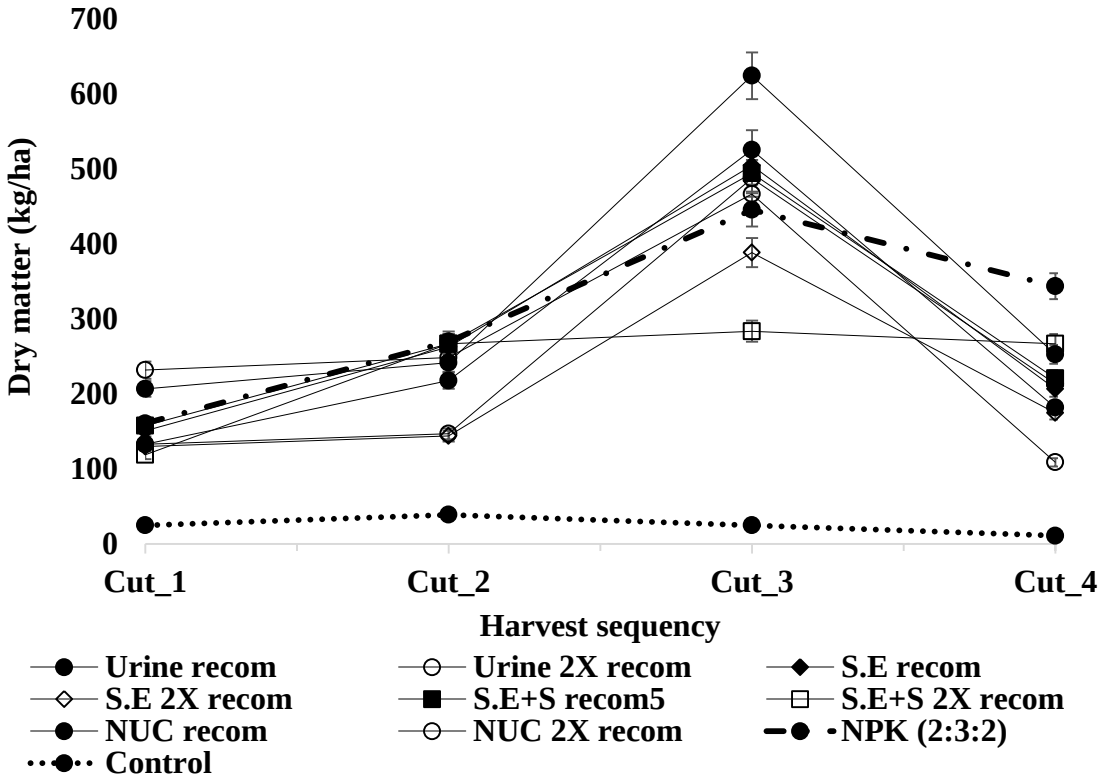

Figure 5: Dry matter production of perennial ryegrass comparing urine plant nutrient sources with controls (NPK and Zero fertiliser treatment).

4.3.1.3. Cumulative dry matter production

Dry matter production increased significantly ($P<0.05$) with time after sowing. In analysis where N sources was compared among each other showed that overtime split application method had significantly high cumulative dry matter than once- off application method (figure 6). However there were no significant differences among treatments within split rate application method likewise in once- off application method. In analysis where N sources was compared with controls showed that all treatment- U, S.E, S.E+S, NUC and NPK had significantly high accumulative dry matter then treatment where no fertiliser was added- the control (figure 7). Moreover the analysis also revealed that recommended rate had significantly high accumulative dry matter production then double rate. Nevertheless dry matter increased significantly after sowing.

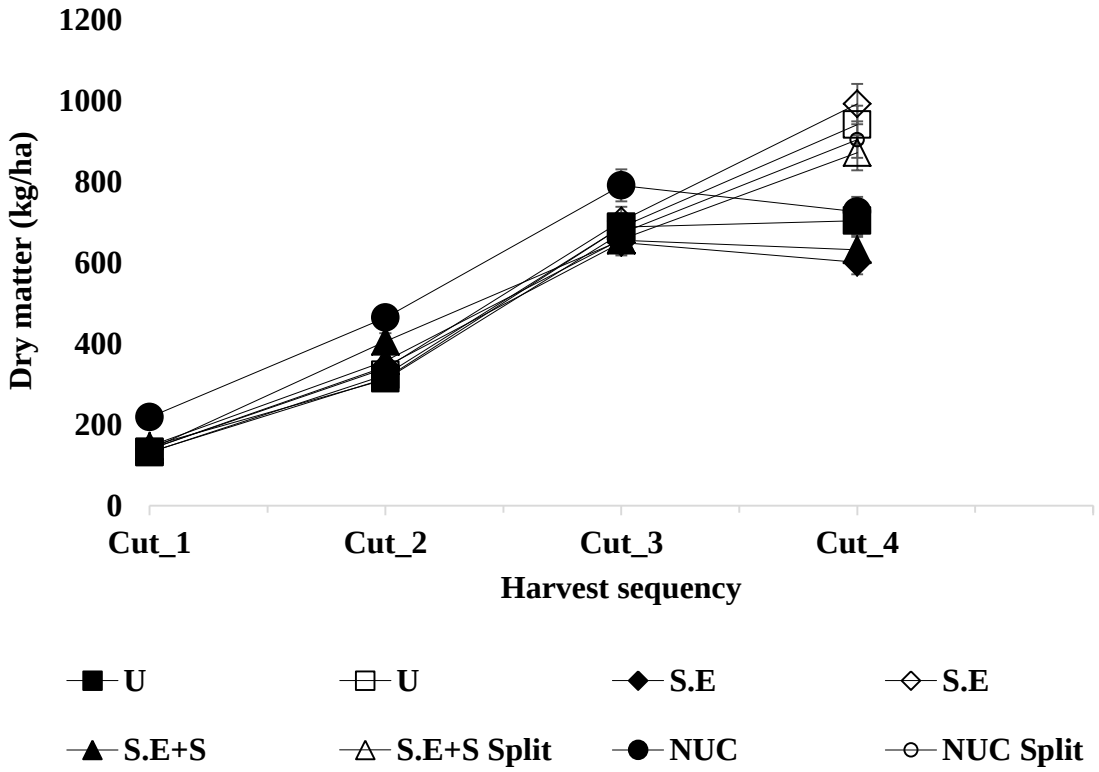

Figure 6: Accumulative dry matter production of perennial ryegrass when comparing N sources among each other.

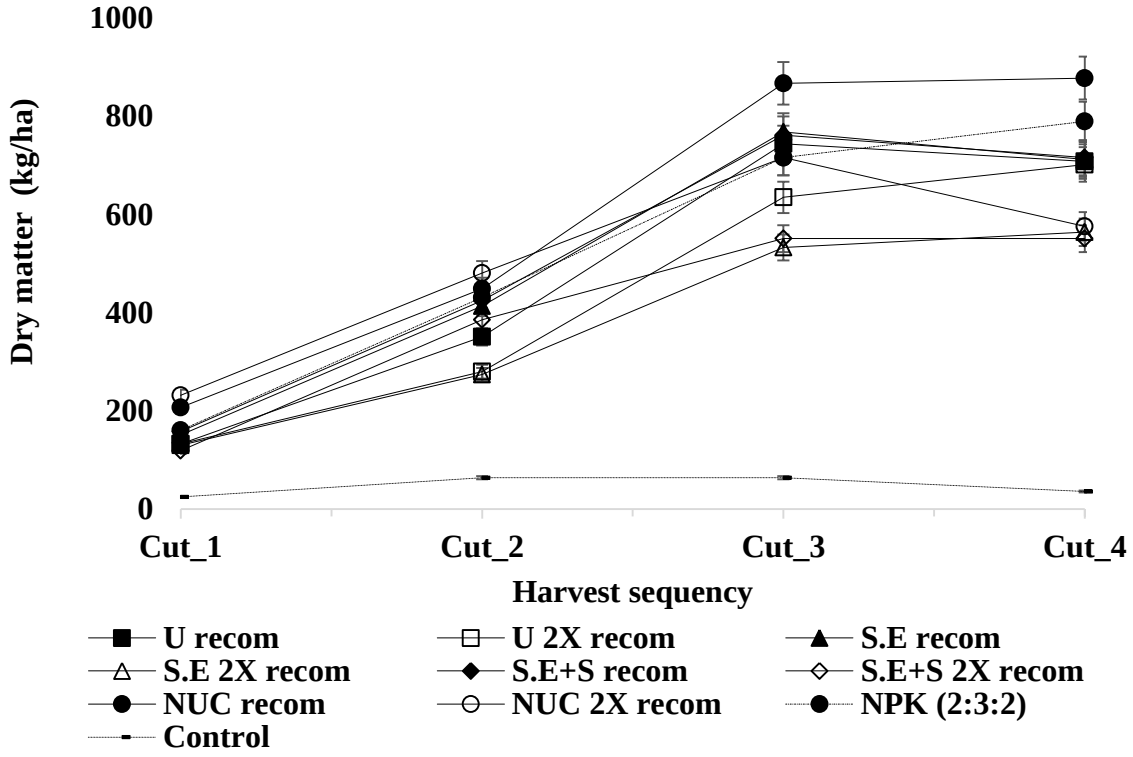

Figure 7: Cumulative dry matter production of perennial ryegrass when comparing N sources with controls (NPK and zero fertiliser treatment)

4.3.1.4. Tissue concentration in perennial ryegrass

Dry matter yield of the different cuts in treatments (U, S.E, S.E+S, NUC, NPK and Zero fertiliser) did not accumulate enough weight for plant tissue analysis. Hence, dry matter yields were combined (cuts 1, 2, 3 and 4) while maintaining replicates, to give one accumulative dry matter yield per treatment for tissue analysis. The zero fertiliser treatment still did not accumulate minimum weight to be analyzed as a results it was not analyzed. The concentrations of P, Mg, K, Na, Zn and Cu showed significance differences among treatments- U, S.E, S.E+S, NUC and NPK. The concentration of N, Al, Ca, Fe and Mn in perennial ryegrass tissue did not differ significantly among all treatments. All urine based fertiliser nutrients sources had similar tissue P and Mg concentrations, the urine treatment had significantly lower P than NPK, with NUC and S.E+S having significantly higher levels of Mg than the NPK, others did not differ significantly from the NPK. All urine based nutrient sources had significantly higher tissue Na

and less Cu and K than NPK. The treatment U had significantly higher Zn than other treatments, which all had similar levels.

Table 5: Tissue concentration of macro nutrients in perennial ryegrass

TRT	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
U	5.29	0.51	3.31	0.41	0.51
S.E	5.36	0.56	3.24	0.41	0.51
S.E+S	4.95	0.59	3.27	0.39	0.58
NUC	5.28	0.58	3.05	0.40	0.59
NPK	4.76	0.64	4.44	0.53	0.46
LSD (0.05)	0.73	0.08	0.62	0.29	0.06

Table 6: Tissue concentration of micro nutrients in perennial ryegrass

TRT	Na (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Al (mg/kg)
U	11188.03	58.83	11.03	110.32	728.83	946.65
S.E	11470.16	55.03	11.96	102.77	671.41	836.48
S.E+S	12510.39	45.31	11.44	124.06	630.18	772.21
NUC	12897.11	50.80	12.30	121.48	677.84	885.16
NPK	6074.40	49.76	14.35	130.07	873.31	944.11
LSD (0.05)	2581.96	7.66	1.09	26.15	470.56	575.09

4.4. DISCUSSION

The increase in dry matter with harvesting period was attributed to nutrient uptake (Craighead, 1987 and Nathan, 2005). The decline in dry matter production at cut 4 was attributed to depletion of nutrient in the soil solution due to nutrient uptake by ryegrass (Stanford and Smith, 1972). The gradual decline in split application method rather than drastic decline in once- off application could be explained by more availability ammonium- N in the soil solution which was timely applied to meet regrowth needs after harvest by split application method.

The effectiveness of fertilizers widely depends on soil properties and plant soil interactions (Bonvin, 2013). The higher dry matter yield in the treatment NUC than all treatments (U, S.E, S.E+S and NPK) up to cut 3 was attributed to more nitrogen available as nitrate- N in the nitrified treatment. As the plant was taking up the readily available nitrate- N, at the same time ammonium- N was being converted into nitrates- N resulting in maximum plant available N (Leirosa *et al.*, 1999, Gastal and Lemaire, 2000; Stanford and Smith, 1972 and Clarkon *et al.*, 1986). The high dry matter among U, S.E, S.E+S, NUC and NPK treatments compared to zero fertiliser treatment was attributed to nutrient characterization of the sources, there are more nutrients in fertilisers sources than in pure water. The similarity in dry matter yield between urine based nutrient sources other than NUC could be explained by the similarity in their N content, as indicated in the results of the tissue composition. The urine based nutrient sources when applied at the recommended rate could be equally as effective as mineral fertilizer.

The greater dry matter production in the split application method than the once- off application method was attributed to continuous supply of ammonium- N for conversion into nitrate- N (Adler *et al.*, 2005). In sandy soil NH_4^+- N has low ability to form electrostatic bonds due to low clay and organic colloids to impair losses of fertilizer N (Medina, 2006). Moreover ammonium- N takes a minimum of 3- 7 days to be converted into plant available nitrate- N (Kizildag *et al.*, 2013 and Barber, 1962). During this time it is susceptible to leaching and quick conversion of ammonium- N into ammonia- N which then evaporates into the air as ammonia gas. Leaching could be explained by low ability of ammonium- N to form electrostatic bonds with the soil due to low clay and organic colloids which suggest ammonium- N was push down by water during irrigation which subsequently lead to leaching. Volatilisation could be explained by Medina (2006) findings that air circulation in a tunnel, which is necessary to maintain tunnel environmental conditions for plant and microorganisms metabolism, promotes volatilisation.

Nitrogen volatilization happens by urease enzymes in the soil converting the urea component to free ammonia gas, and is favored in temperature above 22^{o}C (Wolkowski *et al.*, 1995). This also suggests that even tunnel air temperature that was kept constant above 25 ^{o}C also contributed to N volatilisation. The findings suggest that in once- off application method low dry matter production was attributed to a significant ammonium- N loss by either leaching or volatilization as ammonia- N gas. Therefore, if the different urine-based nutrient sources are to be used as N fertiliser materials, they need to be split applied to avoid N losses. This is because they behave like mineral N fertilisers, which need to be managed that way to avoid volatilization and leaching losses (Argo and Biernbaum, 1995).

The lower dry matter production in treatments where rate of fertiliser was doubled could be explained by toxicity which is brought by too much concentration of nutrients in the soil solution brought by doubling the rate of application (Britto and Kronzucker, 2001 and Barber, 1962 and Leirosa *et al*, 1999). Urine contains high salt concentration including sodium chloride (NaCl) in particular (Maurer *et al.*, 2006), so do the urine nutrient products (Mamo *et al.*, 1998). High salts concentration in the soil inhibit water and nutrient uptake thus leads to low dry matter per given area (Mengel, 1995, Tully *et al.*, 2013, Kizildag *et al*, 2013 and Adler *et al.*, 2005), this is in agreement with the findings of this study that reveled significant sodium uptake by perennial ryegrass, the findings are in agreement with Mkeni *et al.,* 2008 who reported similar findings on most on maize and selected vegetables (tomato, beetroot and carrot). The findings were also supported by Argo and Biernbaum (1995), who reported that the low yield are most likely to prevail in high concentration of salts in the soil solution. There is no yield advantage for doubling the application rate of the urine-based nutrient sources and as such they need to be added at the recommended rate. This is because they behave like mineral N fertilisers, and the bulk of the nutrients in urine are in mineral form (Karaka and Bhattacharyyab, 2011 and Schouw *et al.*, 2002).

The similarities in nutrient concentrations among urine based nutrient sources could be also be explained by the source in which they were all derived. High Na concentrations by perennial ryegrass as compared to NPK could be explained by high sodium concentration in urine and urine based nutrient sources, this was in agreement with Mnkeni *et al.,* (2008) who reported similar findings on maize and selected vegetables (tomato, beetroot and carrot). The similarities in tissue N, P and Ca concentrations between urine based nutrient sources and NPK suggest that

these nutrient sources are as effective as inorganic fertilisers, this is in agreement with Adler *et al.*, 2005, Barret *et al.*, 2000, Barber, 1962 and Gastal &Lemaire, 2001, who reported N, P, and K as major plant ingredient that are utilized by plant in large quantities to produce. As such, this suggests that urine and urine based nutrient sources are useful as N fertiliser in perennial ryegrass for growth and biomass production. The significant higher levels of tissue Zn concentration than all other treatment could be explained by different urine nutrient composition which could have influenced the uptake. The lower concentrations of tissue Mg in NPK compared to S.E+S and NUC treatments could be explained by the nature of make of NUC and struvite. When struvite is produced, additional Mg is added into urine which make struvite a source of Mg which could have influence the uptake of Mg. In NUC treatment, NUC is a concentrated nutrient source by make, this suggest that the level of Mg is also concentrated in NUC which could have also influenced significance the uptake of tissue Mg. The significant dry matter increase among treatment and nutrient concentrations in plant tissue suggest that urine and urine based are useful as a fertiliser in perennial ryegrass.

4.5. CONCLUSION

Urine and urine products, particularly the nitrified urine concentrate, are equally as effective as mineral fertiliser for dry matter production of rye grass. Double the recommended rate using urine based fertiliser does not improve dry matter production. Split application of urine based nutrient sources is more effective strategy to optimize dry matter production, than once off application.

CHAPTER 5

GENERAL DISCUSSION

The greater need to increase food production in response to increases in population has resulted in greater use of fertilizer materials in agriculture, with resulting increase in prices of fertilisers. The need to find alternative sources of nutrients has resulted in research in source separation, and in some cases, processing of human urine. The main objective of this study was to determine availability of N in soils treated with urine and urine separated plant nutrient sources and their effects on growth and dry matter production of perennial ryegrass.

The higher dry matter in the soils treated with urine based nutrient sources, than the control, suggested that these materials can be used as fertilizer materials to supply nitrogen. This was supported by the results of the incubation studies which showed that urine based nutrient sources resulted in higher ammonium N, nitrate N and total mineral N than the control. The greater available N, than the control resulted in greater uptake and yield of ryegrass. This is in agreement with Wyk, (2005) and FFS, (2004) who reported perennial ryegrass grows well and rapid in fertile irrigated soils and is high yielding under good environmental conditions and proper fertilization. The higher dry matter yield in the NUC treatment was in response to greater available nitrogen as supported by the mineral N results from the incubation study. The higher ammonium N and nitrate N during the early and late stages of incubation, respectively, suggested that this treatment would make more N available for plant growth. This was supported by the dry matter yield in this treatment. Plants prefer taking up nitrate N than ammonium N, although both species are plant available. The higher nitrate-N in the NUC treatment therefore supported the ryegrass growth better. While the tissue N concentrations for all the urine based nutrient sources were similar, the dry matter of NUC was higher which suggest a greater N uptake than the other sources.

The decline in ryegrass yield at the fourth harvest suggested that the nutrients were getting depleted. This could be explained by nutrient uptake during earlier harvests and possible losses due to leaching and ammonia volatilization. The decline in total mineral N during the incubation study indicated that N was being lost from the system resulting in decline in its availability to plants. Both volatilization and leaching could explain these changes. In a soil where there are

growing plants, the mineral N could be volatilized, leached or taken up by the plants this is in agreement with Nielsen, (2006) and Medina, (2006) who reported that mineral N is susceptible to leaching beyond root zone and most of can volatilise with two days if not managed well, otherwise plants can take it up for yield production. Over time, the soil mineral N gets depleted resulting in lower uptake. The decline in dry matter after the third harvest suggested that, if this pasture grass is to be fertilized with urine-based nutrient sources, more fertilizer material may need to be added after three harvests. Alternatively, split application may need to be practiced to avoid losses and maximize yields. The need for split application is supported by the results of the pot trial showing better dry matter for split application than for once off, and those of the incubation study where mineral N declined with incubation in soil. This is in agreement with FFS, (2004) who reported that rate of application must be split evenly as possible to meet ryegrass growth needs after harvest.

Although the findings of the incubation trial appeared to show elevated EC levels due to urine based nutrient sources, the salts did not show any negative effects on the ryegrass, as it was explained by Wyk, (2005) and FFS, (2004) that perennial ryegrass is tolerant to wide salinity levels. Even though there were no salinity effects on the ryegrass, such effects need to be monitored if these fertilizer materials are to be repeatedly used. This is in agreement with Mnkeni *et al.*, (2008) who reported E.C increase is soil fertilised with urine which suggest that urine increased soil E.C with the implication that overtime urine if used repeatedly can lead to saline soils.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

Urine and urine nutrient sources, particularly nitrified urine concentrate, can be used as a fertilizer, with split application, in perennial ryegrass for dry matter production, particularly in sandy soils. Doubling the rate of urine based fertiliser application does not increase total mineral-N nor dry matter production in perennial ryegrass. Splitting the rate of urine based fertiliser application is more useful for optimum growth and dry matter production in perennial ryegrass. The current study revealed significant sodium uptake by perennial ryegrass in plant tissue analysis. Hence further studies needs to be done on the effect of sodium and possibly electrical conductivity on soil aggregate stability and plant water use efficiency when applied with urine and urine nutrient product. More studies need to be done to measure forms in which nitrogen losses occur from soils or fields fertilized with urine plant nutrient products.

CHAPTER 7

REFERENCES

Adler PR, Cumming JR, Arora R. 2005. Nature of mineral nutrient uptake by plants. *Journal of Agricultural Sciences* 1: 49-65

Antonini S, Clemens J. 2010. Recycling and fertilizer efficiency of nutrients from human urine**.** Institute of Crop Science and Resource Conservation - Plant Nutrition, German.

Argo WR, Biernbaum JA. 1995. The effect of irrigation method, water-soluble fertilization, replant nutrient charge, and surface evaporation on early vegetative and root growth of poinsettia. *Journal of American Society Horticultural Science* 120 (2): 163-169.

Barak P, Stafford A. 2006. Struvite: a recovered and recycled phosphorus fertilizer. Wisconsin fertilizer, aglime and pest management conference.

Barber S.A. 1962. A diffusion and mass-flow concept of soil nutrient availability. *Journal of Soil Science* 93: 39-49

Barret FD, Justes T, Machets M, Mary, B. 2000. Integrated control of nitrate uptake by crop growth rate and soil nitrate availability under field conditions. *Annals of Botany* 86: 995-1005.

Bonvin C. 2013. Recycling of phosphorus and nitrogen from human urine: evaluation of two urine based fertilizers. Published MSc thesis. Swiss federal institute of technology Zurich. Sweden.

Britto DT, Kronzucker HJ. 2001. NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology* 159(2001): 567-584.

Clarkson DT, Hopper MJ, Jones LH. 1986. The effect of root temperature on the uptake of nitrogen and relative size of the root system in Lolium perenne. I. Solutions containing both NH_4^+ and NO_3^-. *Plant, Cell and Environment* 9: 535-545.

Craighead MD. 1987. Effects of Fertilisers on Seed Germination in New Zealand. *New Zealand Journal of Experimental Agriculture* 5(1).

Curtin D, Campbell CA, Jalil A. 1998. Effects of acidity on mineralization: pH dependence of organic matter mineralization in weakly acidic soils. *Soil Biology and. Biochemistry* 30(1998): 57-64.

Darby S. (1999). Properties of sand soil. Available at http://www.ehow.com/list_7318998_properties-sandy-soils.html. [Accessed on 26 October 2014]

Doyle JD, Parsons SA. 2002. Struvite formation, control and recovery. *Water research* 36(16): 3925-3940.

Drangert JO. 1998. Fighting the urine blindness to provide more sanitation options. Institute of water and environmental studies. Institute of Water and Environmental Studies, Linkoping University, Sweden.

El Diwani G, El RafieSh, El Ibiari NN, El-Aila HI. 2006. Recovery of ammonia nitrogen from industrial wastewater treatment as struvite slow releasing fertilizer. *Desalination* 1: 200-214.

Etter B, Tilley E, Khadka R, Udert KM. 2011. Low-cost struvite production using source-separated urine in Nepal. *Water Research* 45 (2011): 852-862.

Fertiliser Fat Sheet. 2002. Fact Sheet No. 15: fertilizer salt index. A & L Great Lakes Laboratories. Fort Wayne.

Ganrot Z. 2005. Urine processing for efficient nutrient recovery and reuse in agriculture**.** PhD thesis, Göteborg University. Sweden.

Gardner G. 1998. Recycling human waste: fertile ground or toxic legacy? *World Watch* 11(1998): 28-39.

Gastal F, Lemaire G. 2001. N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of Experimental Botany* 370(2001): 789-99.

Gulser C, Demir Z, Serkan I .2010. Changes in some soil properties at different incubation periods after tobacco waste application. *Journal of Environmental Biology* 31(2010): 671-674.

Guzha E, Nhapi I, Rockstrom J. 2005. An assessment of the effect of human faeces and urine on maize production and water productivity. *Physics and Chemistry of the Earth* 30 (2005): 840-84.

Haney RL, Haney EB. 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. *Communications in Soil Science and Plant Analysis* 41(2010): 1493-1501.

Heinonen-Tanski H, Wijk-Sijbesma C. 2005. Human excreta for plant production. *Bioresource Technology* 96: 403-411.

Hue NV, Craddock GR, Adams F. 1986. Effect of organic acid on aluminium toxicity in subsoils**.** *American Journal of Soil Science Society* 50: 28-34.

Hue NV, Silva JA. 2000. Organic soil amendments for sustainable agriculture: organic sources of nitrogen, phosphorus, and potassium. College of tropical agriculture and human resources, University of Hawaii at Manoa.

Huisman GH, Van Rens GLMA, De Lathouder H, Cornelissen RL. 2011. Cost estimation of biomass-to-fuel plants producing methanol, dimethylether or hydrogen. *Biomass and bioenergy* 35(2011): 155-166.

Jaffer Y, Clark TA, Pearce P, Parsons SA. 2002. Potential phosphorus recovery by struvite formation. *Water research* 36(2002): 1834-1842.

Jensena PKM, Phucb PD, Knudsenc LG, Dalsgaardd A, Konradsenc F. 2008. Hygiene versus fertiliser: The use of human excreta in agriculture – A Vietnamese example. *International Journal of Hygiene Environment and Health* 211(2008): 432-439.

Jimenez B, Drechsel P, Kone D, Babri A, Raschid-Sally L, Qadir M. 2009. Wastewater, sludge and excreta uses in developing countries: an overview**.** International water management institute and international development research centre. UK and USA.

Karaka T, Bhattacharyyab P. 2011. Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality. *Resources, Conservation and Recycling* 55(2011): 400-408.

Kirchmann H, Pettersson S. 1995. Human urine - Chemical composition and fertilizer use efficiency. *Fertilizer Research* 40(1995): 149-154.

Kizildag N, Sagliker H, Darici1 C. 2013. Nitrogen mineralization in some saline soils at Eastern Mediterranean coasts, Turkey**.** *Eurasia Journal of Biosciences* 7(2013): 95-100.

Leirósa MC, Cepeda CT, Seoanea S, Sotresa FG. 1999. Dependence of mineralization of soil organic matter on temperature and moisture. *Soil Biology and Biochemistry* 31(1999): 327-335.

Mamo M, Molina JAE, Rosen C.J, Halbach TR. 1998. Nitrogen and carbon mineralization in soil amended with municipal solid waste compost. *Canadian Journal of Soil Science* 9: 535-542.

Maurer M, Schwegler P, Larsen TA. 2003. Nutrients in urine: energetic aspects of removal and Recovery. *Water Science and Technology* 48(2003): 37-46.

Maurer M, Pronk W, Larsen TA. 2006. Treatment processes for source-separated urine. *Water research* 40(2006): 3151-3166.

Medina C. 2006. Nutrient release patterns of coated fertilizers used for citrus production and their effect on fruit yield and foliar nutrition. MSc Thesis. University of Florida. America.

Mengel D. 1995. Roots, growth and nutrient uptake. Pardue University. Australia

Mihelcic JR, Fry LM, Shaw R. 2011. Global potential of phosphorus recovery from human urine and faeces. *Chemosphere* 84(2011): 832-839.

Mnkeni PNS, Kutu FR, Muchaonyerwa P. 2008. Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. *Waste Management & Research* 26(2008): 132-139.

Motavalli PP, Palm CA, Parton WJ, Elliott ET, Frey SD. 1995. Soil pH and organic C dynamics in tropical forest soils: Evidence from laboratory and simulation studies. *Soil Biology and Biochemistry* 27(12):1589-1599.

Murugan AV, Swarnam TP. 2013. Nitrogen Release Pattern From Organic Manures Applied to an Acid Soil. *Journal of Agricultural Science* 5(6):174-188.

Nathan MV. 2005. Soils, plant nutrition and nutrient management. *MU Extension* 573(2005): 882-7216.

Nielsen RL. 2006. N- loss mechanisms and nitrogen use efficiency. Purdue Nitrogen Management Workshops. Purdue.

Nordmeyer H, Richter J. 1985. Incubation experiments on nitrogen mineralisation in loess and sandy soils. *Plant and Soil* 83: 433-445.

Persson T, Wiren A. 1995. Nitrogen and potential nitrification at differe depths in acid forest soils. *Plant and Soil* 168(1995): 55-65.

Premi PR, Cornfield AH. 2003. Incubation study of nitrogen mineralisation in soil treated with dried sewage sludge. *Environmental Pollution* 2(2003): 1.

Rahman MM, Mohammad AM, Salleh UR, Amimul A, Mohammad MH, Chang SR. 2014. Production of slow release crystal fertilizer from wastewaters through struvite crystallization. *Arabian Journal of Chemistry* 7: 139-155.

Sahrawat KL. 1980. Nitrogen Minerilasation in Acidic sulfate soils. *Plant and Soil* 57: 143-146.
Schouw NL, Danteravanich S, Mosbaek H, Tjell JC. 2002. Composition of human excreta _ a case study from Southern Thailand. *The Science of the Total Environment* 286: 155-166.

Stanford G, Smith SJ. 1972. Nitrogen mineralization potentials of soils. *Soil Science Society American Journal* 36(1972): 465-472.

Tanski HH, Sjöblom A, Fabritius H, Karinen P. 2005. Pure human urine is a good fertiliser for cucumbers. *Bioresource Technology* 98: 214-217.

Tully KL, Wood TE, Schwantes AM, Lawrence D. 2013. Soil nutrient availability and reproductive effort drive patterns in nutrient resorption in Pentaclethra macroloba. *Ecology 94*: 930-940.

Udert KM, Wächter M. 2011. Complete nutrient recovery from source-separated urine by nitrification and distillation. *Water Research* 46(2011):.453-464.

Winker M, Vinnerås B, Muskolus A, Arnold U, Clemens J. 2009. Fertiliser products from new sanitation systems: Their potential values and risks. *Bioresource Technology* 100: 4090-4096.

Wolkowski, R.P., Kelling, K.A and Bundy, L.G. (1995). Nitrogen management in sandy soils. Cooperative Extension Publication. Madison, United State.
Wyk BE. 2005. Food plants of the world: Identification, culinary uses and nutritional value. Briza. South Africa.

APPENDICES

CHARACTERISTICS OF THE SOILS USED FOR THE STUDY

Soil property	Inanda	Catref
Sample density g/mL	0.75	1.42
P (mg/kg)	12	0.7
K ($cmol_c$/kg)	0.08	0.02
Ca ($cmol_c$/kg)	3.23	0.51
Mg ($cmol_c$/kg)	0.87	0.32
Exch. Acidity ($cmol_c$/kg)	1.75	0.33
Total cations ($cmol_c$/kg)	5.92	1.19
Acid saturation (%)	30	28
pH (KCl)	4.11	4.0
Zn (mg/kg)	2.80	0.14
Mn (mg/kg)	10.7	1.41
Cu (mg/kg)	3.6	0.35
Organic C (%)	6	0.5
N % =	0.56	0.08
Clay % =	23	11

PLANT TISSUE ANALYSIS FOR PERENNIAL RYEGRASS

Trt code	N %	P (%)	Ca (%)	Mg (%)	K (%)	Na (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Al (mg/kg)
U1	5.43	0.52	0.41	0.50	3.30	10455.04	61.76	11.47	110.29	789.64	952.86
U2	4.99	0.48	0.38	0.50	3.25	12011.97	57.84	10.68	111.22	707.37	978.75
U3	5.45	0.51	0.42	0.53	3.37	11097.09	56.91	10.94	109.44	689.46	908.34
S.E1	5.34	0.52	0.39	0.49	3.09	11434.97	54.77	12.49	96.39	615.56	727.28
S.E2	5.23	0.56	0.44	0.53	3.24	11702.97	59.39	11.66	103.39	802.93	1077.90
S.E3	5.52	0.60	0.40	0.52	3.39	11272.54	50.94	11.74	108.52	595.74	704.26
S.E+S1	4.35	0.62	0.44	0.55	3.80	9623.47	44.25	12.17	117.25	741.12	838.46
S.E+S2	5.42	0.57	0.37	0.61	3.12	14098.74	44.69	10.95	127.36	560.82	721.69
S.E+S3	5.08	0.58	0.36	0.58	2.89	13808.95	47.00	11.19	127.57	588.61	756.47
NUC1	5.27	0.56	0.44	0.63	3.07	12251.56	52.51	13.78	133.45	851.05	1327.98
NUC2	5.34	0.63	0.38	0.55	2.73	14078.59	52.96	10.59	114.75	516.36	538.43
NUC3	5.23	0.57	0.40	0.58	3.36	12361.17	46.94	12.52	116.24	666.12	789.06
NPK1	4.08	0.58	0.36	0.48	4.17	7297.30	43.06	15.18	122.38	659.48	756.93
NPK2	4.75	0.63	0.91	0.43	4.20	5058.48	57.68	14.20	159.74	1437.67	1482.04
NPK3	5.44	0.71	0.32	0.45	4.95	5867.41	48.53	13.68	108.08	522.77	593.36

URINE AND URINE NUTRIENT PRODUCT ANALYSIS

Nutrient product	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	PO_3^- (mg L^{-1})
Urine (U)	LOW	4656	231
Struvite effluent (S.E)	LOW	4578	7
Nitrified Urine Concentrate (NUC)	18483	16462	3847

STRUVITE CONTAINED 5.7% N AND 12.6% P