COMPARISON OF EFFECT OF STRUVITE PREPARED FROM SOURCE-SEPARATED HUMAN URINE WITH INORGANIC FERTILIZER ON AMARANTHUS CAUDATUS

BY

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ABSTRACT

In Nigeria, use of urine for fertigation has been limited because of its liquid form, odour and the unsanitary disposal methods. This poses great hazard to man and the environment. Converting urine into dry fertilizer product (struvite) helps extract nutrients contained in urine and also eliminates handling problems. The study was aimed at converting urine to struvite and comparing its effectiveness on plant growth with inorganic fertilizer (NPK).

The study involved the extraction of struvite from human urine and its use as fertilizer for raising *Amaranthus caudatus* in a screen house. Fourteen students selected randomly from the students' hostel at the University College Hospital produced 100 litres of urine which was stored for three months in order to reduce the microbial load. Triplicate samples of fresh and stored (stale) urine collected from the same set were analyzed for physico-chemical [pH, ammonia (NH₃), nitrogen (N), phosphate (PO₄³⁻) and potassium (K)] parameters using standard methods at the point of collection and three months after. Magnesium Chloride (MgCl₂) with concentrations: 1.2M, 1.5M and 1.8M was added to 20 litres of stale urine to produce struvite of varying concentrations. Amaranth vegetable seeds were planted in 15 pots (of three replicates each) containing 2kg of soil treated with struvite and inorganic fertilizer while soil without either struvite or inorganic fertilizer was used as control. Plant height and stem width were measured and number of leaves were counted the second week of planting. Plants were harvested after the fourth week; with the wet and dry weight of the leaves, stem and root taken. Data were analyzed using descriptive statistics, t- test and ANOVA at p=0.05.

Mean pH of fresh and stored urine were 6.50 ± 0.10 and 9.03 ± 0.15 respectively. Mean concentration (mg/l) of NH₃, N, PO₄³⁻ and K were: 0.26 ± 0.20 , 1080.33 ± 145.22 ; 46.00 ± 1.00 , 32.00 ± 3.00 ; and 29.66 ± 4.72 , 1358.66 ± 183.90 , 45.66 ± 3.05 and 35.33 ± 0.57 for fresh and stored urine respectively. Mean concentration (mg/l) of N, PO₄³⁻, K present in struvite were 193.33 ± 56.5 , 471.66 ± 61.71 and 34.56 ± 2.18 , with PO₄³⁻being significantly different when compared with stored urine. Mean plant height (cm) for control, struvite produced from 1.2M, 1.5M, 1.8M of MgCl₂ solution and inorganic fertilizer at the fourth week were 20.00 ± 0.90 , 20.16 ± 4.36 , 29.08 ± 0.87 , 28.00 ± 0.86 and 20.00 ± 7.76 respectively. Mean stem width (cm) of control, struvite 1.2M, 1.5M and 1.8M and inorganic fertilizer were 0.34 ± 0.02 , 0.39 ± 0.07 , 0.48 ± 0.02 , 0.46 ± 0.03 and 0.40 ± 0.21 respectively while the

mean numbers of leaves were 13.00 ± 0.50 , 12.50 ± 1.50 , 15.16 ± 0.76 , 15.16 ± 0.76 and 13.00 ± 2.64 respectively. Plant height and stem width of struvite 1.5M and 1.8M treated vegetable were significantly (p=0.05) higher and thicker respectively when compared with other treatments. Mean wet and dry weights (g) were: 11.58 ± 2.77 and 1.76 ± 0.38 (control), 14.90 ± 11.09 and 2.39 ± 2.19 (struvite 1.2M), 32.11 ± 5.35 and 5.85 ± 0.77 (struvite 1.5M), 28.81 ± 3.84 and 5.45 ± 0.92 (struvite 1.8M), 9.97 ± 8.87 and 1.34 ± 1.24 (inorganic fertilizer) respectively. Weights of struvite-treated vegetable were significantly higher when compared with other treatments.

The highest dry weight (5.85 ± 0.77) was obtained with *Amaranthus caudatus* planted on struvitetreated soil. Struvite production and use in agriculture should be explored on a larger scale and could be employed as organic fertilizer to improve plant yield and indirectly improving environmental sanitation.

Keywords: Struvite, *Amaranthus caudatus*, Inorganic fertilizer (NPK)Word count: 500

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CERTIFICATION

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DEDICATION

This research work is dedicated to GOD ALMIGHTY, the Alpha and Omega for his protection, provision and mercy during the course of this programme.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Ecological sanitation (EcoSan) is a strategic and comprehensive sanitation approach that integrates all aspects of sanitation such as human waste, solid waste, waste water and drainage. This approach links sanitation with agriculture and food production. Hannan and Andersson (2002) defined ecological sanitation as "an ecosystem approach to waste disposal based on three key principles – that sanitation be safe from a health perspective; 'green' or non-polluting; and be based on principles of re-use and recycling of the valuable nutrients in human excreta. Chaggu and John (2002) defined ecological sanitation as "a system that makes use of human 'waste' and turns it into something 'useful and valuable', with minimum pollution of the environment". In essence, it consists of using latrines that are safe and ecologically sound and designed in such a way that the end products can be easily transferred into agriculture or forestry (Lind, *et al.*, 2000; Austin *et al.*, 2005).

The concept of ecological sanitation (referred to as EcoSan) and its worldwide promotion to improve the sanitary situation of people and communities in developing countries has been a success story in many aspects. Ecological sanitation involves the collection, treatment and reuse of human excrement as agricultural fertilizers. The collection and treatment of human waste has helped to improve the environmental situation as well as to decrease the proliferation of vector-borne diseases thus largely helping to establish better living condition for rural and urban areas (Gantenbein and Khadka, 2009). It is a sustainable closed-loop system that treats human excreta as a resource, not as a waste product. Excreta is processed until it is free of disease causing organisms. The nutrients contained in the excreta may be recycled and used for agricultural purposes (Dunker *et al.*, 2007).

Human waste removal is an important part of daily life and it is an important factor in human health (Esrey *et al.*, 2001). The goal of most modern day sanitation systems is to prevent exposure of humans to the harmful pathogens that are found in excrement. Most systems in

the developed world seek to carry away waste, remove pathogens and pollutants in an energy-intensive treatment system and then release the contents back into nature in large volumes thus causing eutrophication. In the developing world, latrines are often used in various ways that concentrate the excrement and still pose a health risk (though this is better than excrement left out in the open). In addition, when sewer systems are used in the developing world, they often focus more on carrying away waste than adequately treating the waste; discharging pathogens that will contaminate the food and water of people downstream (WHO, 2006). Also, sewers may not be an appropriate technology in areas of the world that are water stressed (Fry *et al.*, 2008). Beyond these very real concerns, there is also a growing awareness of the valuable nutrients being lost in human waste streams. A new paradigm shift in the water and wastewater management sector is focusing on the resources that can be recovered from wastewater rather than the constituents that must be removed (Guest et al., 2005). The modern human waste collection systems do much to minimize human contact with the pathogens in excrement but little to ensure that those nutrients will be returned to natural systems in a way that benefits food production soils. According to Vaccari (2009) our modern society separates food production and consumption, which limits our ability to return nutrients to the land. Instead, we use them once and then flush them away.

At the same time, soils are losing nutrients at an alarming rate, especially in Africa (Connor, 2006). Chemical fertilizers of many kinds are widely used but the materials to create them are becoming increasingly more difficult to obtain. The nitrogen, phosphorus and potassium that make up the majority of these fertilizers come from finite resource pools; the majority of nitrogen is made from natural gas and is subject to price changes and availability of methane, while global potassium mines might run out several centuries. Global phosphorus mines are set to run out in less than a century and that of U.S. may run out in a few decades (Vaccari, 2009). The cost of making and buying fertilizer is further exacerbated by fluctuations in oil prices. From 2006 to 2008, increase in petroleum prices made fertilizers and/or importing foods from outside the farming community will become more and more expensive as attached resources become less available.

Human urine is a natural resource which is available in all human societies. Urine from human beings is a valuable resource that could be better utilized than to end up in surface waters as well as groundwater where they can cause significant problems (Kashekya, 2009). In regions where there is a lack of domestic financing of fertilizers for subsistence production of food, treated urine can contribute significantly to food security and health (Johansson *et al.*, 2001).

Urine is a valuable source of nutrients used since ancient times to enhance the growth of plants, notably leafy vegetables. It is best used as fertilizer to nitrogen demanding crops but most garden crops respond well to urine as a fertilizer. Urine has been used with good results on lawns, roses, berry bushes, vegetables as well as annual and perennial flowers. A one month holding period is recommended between last application of urine and harvest of food crops consumed raw for safety reasons (WHO, 2006; Kvarnstrom *el at.*, 2006)

Urine used directly or after storage is a high quality, low cost alternative to the application of N-rich mineral fertilizer in plant production. The nutrients in urine are in ionic form and their availability to plants compare well with chemical fertilizer (Johansson *et al.*, 2001; kirchmann and peterson, 1995; kvarmo, 1998; Richert Stintzing *et al.*, 2001; Simons and Clemens, 2004). Urine also contains large amounts of phosphorus, potassium, sulphur and micronutrients but due to its high content of N, urine is best utilized as a direct fertilizer for N-demanding crops and leafy vegetables. If crops and region-specific recommendations are available for the use of N fertilizers (urea, ammonium or nitrate), a good way of how to use urine, is to translate the recommended to urine. The translation is simplified if the N concentration of the urine is known and if it is not, then as a rule of thumb, a concentration of 3-7 grams of N per litre of urine can be expected (Vinneras, 2002; Jonsson and Vinneras, 2004). Plant trials with urine have been carried out with various vegetables in Zimbabwe (Morgan, 2003 and 2008).

Urine is often considered not to be of much use for a good number of people and even if it is, many are discouraged to use it because of its inconvenient odour and liquid form (Thapa, 2004). Urine has been found to act as a source for Phosphorus recovery as struvite. Udert (2003a) and Tilley (2008) concluded that the storage of urine is an essential step in recovering struvite and that by allowing the pH to attain an ideal working range (9 - 9.3) naturally. However magnesium needs to be added to recover a pure struvite product. Struvite is by mass 44% crystal water, 39% phosphate (PO₄³⁻), 10% magnesium and 7% ammonium (Kealan *et al*, 2010). As far back as 1858, struvite has been proposed as a potential phosphorus source for agriculture. In fact, struvite appears to form in soil upon fertilization with other ammonium phosphate fertilizers, particularly when neutral or alkaline conditions prevail. Struvite formation in soil upon addition of ammonium polyphosphate has also been reported (Ghosh *et al.*, 1996). When struvite is added to soil, phosphorus release appears to be largely the result of microbial nitrification of the ammonium constituent rather than simple dissolution (Bridger *et al.*, 1962). An investigation by Johnston and Richards (2004) revealed that recycled struvite has a potential to substitute for inorganic fertilizer in the market.

1.2 PROBLEM STATEMENT

Literature on human waste and its use had concentrated largely on the dangers of its use, the negative aspects of short-term epidemiology rather than on long-term soil recovery (Tanner, 2001). Little documented research concerning the use of urine as fertilizer makes the development of set guidelines difficult. However, these products have been used in agriculture since ancient times, and there is a lot of undocumented knowledge based upon practice (Jonsson *et al.*, 2004).

In Nigeria, urine is a waste resource. The order of the day is indiscriminate urination into drains, bushes, garbage dumps or streams due to limited organized public urinals. This constitutes a form of pollution to the environment thus making urine a public health nuisance. Human urine is the largest contributor of nutrients to household wastewater containing approximately 80% of the nitrogen, 60% of the phosphorus if no phosphorus detergents are used (Sundberg, 1995; Loosdrecht, 2007) and dissolved potassium, the main macronutrients required by plants. About 75-90% of the nitrogen is excreted as urea while the remainder is either in the form of ammonia or creatinine. (Pradhan *et al.*, 2007). The Nitrogen present in human urine is over 90% as ammonium ion and crops take up this form

of nitrogen very easily. The re-use of urine as a fertilizer in agriculture would reduce the load on wastewater treatment plants and the impact on recipients. Cultivation trials have shown that crops very easily take up the phosphorus which is found in human urine. Human urine also contains a large amount of micronutrients and trace elements which are valuable in plant cultivation (Alfred, 2010).

However, in Nigeria, the handling of human excreta and the use of human excreta for food production are still very foreign ideas and generally not acceptable. There is still a phobia for harvesting and utilizing urine for growing edible crops (Sridhar *et al.*, 2003) as human excreta are seen as waste products, unhealthy, unhygienic and detrimental to humans. The use of urine is not widely acceptable in crop production. Earlier research works showed that urine harvesting is feasible at institutional level and once educated communities accept the collection and use. Several studies have addressed the recovery of phosphate as struvite from industrial and domestic wastewater (CEEP, 2004; Jaffer, *et al.*, 2002; Ronteltap *et al.*, 2007). The precipitation of struvite may present some problems in wastewater treatment plants causing deposits in pipe walls (Loewenthal, 1994). However, struvite has a potential use as a fertilizer. It has been shown to be a highly effective source of nitrogen, magnesium and phosphorus for plants and can be used as a slow release fertilizer at high application rates without damaging plant roots (Gaterell, 2000).

1.3 JUSTIFICATION

The quantities of nutrients present in urine are more when compared to those in mineral fertilizers used in agricultural practice. In Sweden, the yearly production of human urine equals 15-20% of the mineral fertilizer consumption (Jönsson *et al.*, 1996), referring to nitrogen, phosphorus and potassium. Nitrogen is mainly in form of urea, phosphorous as phosphates and potassium as ion. Micronutrients are also present in balanced form in human urine. Moreover, human urine is free from cadmium and other heavy metals (Palmquist and Johnson, 2003) and in a healthy person urine is sterile when leaving the body (Jönsson *et al.*, 2004). From the hygienic point of view, human urine is a "safe" fertilizer with less concern regarding risks for disease transmission when handling (Kvarnstrom *et al.*, 2006) and it is an uncommon transmission route for disease. Fertilizing experiments have indicated that the

fertilizing effect of stored human urine is comparable to that of inorganic fertilizers for wheat and barley (Kirchmann and Pettersson, 1995; Kvarmo, 1998) and can replace inorganic fertilizers (Johansson *et al*, 2000; Johansson *et al.*, 2001; Kirchmann and Pettersson, 1995; Simons and Clemens, 2004; Richert *et al.*, 2010). Also the use of urine as fertilizer leads to a reduction in the use of chemical fertilizers hence reducing pollution resulting from leachates of chemical fertilizer and pesticides (Serpil, 2012).

An advantage of urine in comparison with organic fertilizers is that the phosphorus exists in forms that can easily be taken up by plant. This means that urine is quite efficient as a phosphorus fertilizer which has implications for the future with regard to the concept of Peak Phosphorus and the fact that phosphorus is a finite resource (Richert *et al.*, 2010). Serious environmental hazards are often associated with the use of chemical fertilizers. In industrialized countries for example, indiscriminate use of chemical fertilizer has polluted water supplies (Serpil, 2012). Human urine can be used as an alternative to chemical fertilizers to reduce pollution in air, water and soil and help avoid or control other environmental hazards which surface due to the use of chemical fertilizers. Human urine contains nitrogen, phosphorus and potassium at a much higher ratio than in chemical fertilizers and is environmentally safe to use (Jönsson *et al.*, 2004; Cofie *et al.*, 2010).

Urine is almost free from heavy metals – for example, cadmium when ingested, they will tend to bind to the liver and kidneys, making the urine much lower in such contaminants than commercial fertilizers (Jönsson *et al.*, 1997). Phosphorus, for example, is an essential element for plant growth and external phosphorus from mined phosphate is usually supplied in agriculture in order to increase productivity. World supplies of accessible mined phosphate are diminishing. Approximately 25% of the mined phosphorus ends up in aquatic environments. This discharge into aquatic environments is damaging, as it causes eutrophication of water bodies (WHO, 2006; Gumbo *et al.*, 2001). Urine alone contains more than 50% of the phosphorus excreted by humans. Thus, the diversion and use of urine in agriculture can aid crop production and reduce the costs of and need for advanced wastewater treatment processes to remove phosphorus from the treated effluents (WHO, 2006). Since

phosphorus is a non-renewable resource, recovering phosphorus from human waste (urine) is a significant technological breakthrough.

However, the recycling of urine and the nutrients it contains is poor due to a lack of awareness of the people but this is just because urine does have properties which make its use less convenient. These properties (liquid urine handling, difficult storage and transportation as well as bad odour) are essential and inherent barriers for widespread acceptance of liquid urine as fertilizer (Sridhar *et al.*, 2003). Therefore the conversion of urine into struvite (MgNH₄PO₄·6H₂O), a white odourless powder derived from source separated urine by the addition of magnesium, is an option to convert urine into a more desirable form and thereby promoting and optimizing the usage of urine as fertilizer in agriculture. More so, struvite has a potential use as a fertilizer. It has been shown to be a highly effective source of nitrogen, magnesium and phosphorus for plants and can be used as a slow release fertilizer at high application rates without damaging plant roots (Gaterell, 2000). A slow-release fertilizer is more convenient since less frequent application is required. Fertilizer burn is not a problem with slow-release fertilizers even at high rates of application (Nelson *et al.*, 2003).

1.4 Objectives

1.4.1 Broad objective

The broad objective of this study was to assess the effectiveness of urine after conversion into dry fertilizer product (struvite).

1.4.2 Specific objectives

The specific objectives of the study were to:

- i) Determine urine composition before and after storage for five months.
- ii) Change urine to struvite (Magnesium ammonium phosphate hexahydrate).
- iii) Determine the percentage recovery of struvite from urine.
- iv) Assess the effectiveness of struvite and inorganic fertilizer on the cultivation of *Amaranthus caudatus*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Source and historical uses of urine

Urine is the by-product or fluid excreted by the kidneys and transported by the ureters to the urinary bladder where it is stored until it is voided through the urethra. Urine is a dilute aqueous solution of metabolic wastes such as urea, salts and organic compounds. In total the dissolved material amounts to about 5% by weight. Fluid and materials being filtered by the kidneys, destined to become urine, come from the blood or interstitial fluid (Torondel, 2010). Urine is used by the body as a balancing medium for liquids and salts and the amount of urine therefore varies with time, person and circumstances. For example, excessive sweating results in concentrated urine while consumption of large amounts of liquid dilutes the urine (Jönsson *et al*, 2004). The amount of urine produced per person and day depends on the amount of liquid a person drinks but usually lies within the range of 0.8 to 1.5 L per day for an adult and about half as much for children, respectively (WHO, 2006). On the average, an adult produces around 500L (550 kg) of urine per year thus approximately 4 kg of N, 0.5 kg of P and 1 kg of K per person per year (Joensson, 2004).

A big share of the soluble substances in urine consists of essential plant nutrients like Nitrogen (N), Phosphorus (P) and Potassium (K) as well as some trace micro-nutrients that can be easily used in agriculture. Urine can therefore be considered as a nitrogen-rich liquid fertilizer due to its comparably high N and low organic matter content. It is often recommended to complement urine application with other nutrient and organic matter source (Palmquist *el al*, 2003). Urine is a pure nutrient solution which contains very low levels of heavy metals and pathogenic organisms. For additional safety, a storage time of one month in a completely sealed container is generally acceptable to guarantee that it will be safe for agricultural application at the household level. If urine is used for crops that are eaten by those other than the urine producer, it should be stored for at least 6 months (WHO, 2006).

A German alchemist, Henning Brand in 1669, tried distilling fermented urine which led to the discovery of white phosphorus. In 1773 the French chemist Hilaire Rouelle, also discovered the organic compound urea by boiling urine dry (Dahlstrom, 2007). According to Höglund (2001), during the 19th Century urine was stored and used as a detergent for washing clothes in Denmark (Hansen, 1928; Drangert, 1998). In Sweden urine has been used to smear wounds and dry skin and to some extent to drink as therapy (Frode-Kristensen, 1966). Other historic uses of urine include tanning of hides and production of gun powder (Stenström, 1996). Dahlstrom (2007) categorized the use of human urine into four:

I. Immune Booster:

In the past and even now, there are some people who regularly drink their morning urine in order to boost their immune system. Such individuals believe that reintroducing the body to agents it has already seen reminds the immune system to be prepared the next time. This urine therapy is believed to have benefited personalities like Mohanadas Gandhi, Jim Morrison, and Steve McQueen. The medicinal properties of urine have been observed and are also used in China as a part of holistic medicine (Dahlstrom, 2007). In Siberia, to communicate with the spirits, the Koryak people drank the urine of another who has consumed fly agaric (an entheogenic mushroom that is occasionally fatally poisonous) or of one who has in turn drunk urine of like source.

II. Skin Treatment

Using urine for skin treatment is much more common than consuming the liquid. Urine can be used as a topical disinfectant on wounds. It can also be used several times a day on a rash or a blister. Blisters and rashes will heal much faster when using urine than when not using urine. Other people use urine to soften dry skin or to alleviate eczema. There are some that give their tired, dry feet daily urine soak (Dahlstrom, 2007).

III. Bleaching Agent

The ancient Romans used urine as a bleaching agent for cleaning clothes and even isolated reports as a teeth whitener. According to Dahlstrom (2007), some people still use urine and consider it much more natural than chemical bleaching detergents.

IV. Agricultural Fertilizer

For over 4000 years, people in many countries of Eastern Asia and the Western Pacific have been applying human excreta to the land which has helped to maintain soil fertility (Mara and Cairn cross, 1989). Urine is rich in nutrients that can be used in agriculture and horticulture. Urine contains the major plant nutrients (N, P and K) in proportions suitable for plants and the nutrients are readily available since the major proportion is present in mineral form (Tidåker *et al.*, 2007).

2.1.1 Urine Composition

Urine consists of 95% water with the remaining 5% made up of soluble wastes and excess substances of the human body like urea, creatinine, dissolved ions (e.g. chloride, sodium, potassium), inorganic and organic compounds or salts (Richert *et al.*, 2010). An average person produces about 500 litres of urine per year (Jönsson *et al.*, 2004). Urine contains the major part of the daily excretion of nitrogen (N), Phosphorous (P) and potassium (K) (Schönning, 2001). This indicated that it should be considered a valuable fertilizer. The plant availability of N in urine is the same as that of chemical urea or ammonium fertilizer (Jönsson, 2004). The nitrogen efficiency of urine is approximately 90% of that of inorganic fertilizer (Johansson *et al.*, 2001).

Bigger shares of soluble substances in urine are essential plant nutrients like N, P and K often referred to as macro-nutrients as well as smaller fractions of micro-nutrients, in a plant available form. Generally it can be said that the average nutrient concentration in the urine of a population reflects the nutrient ratio contained in the consumed crops and thus the ratio in which nutrients should be returned to the soil to maintain the fertility (Jönsson *et al.*, 2004). Those nutrients are coming from the food consumed everyday and almost all consumed nutrients leave the body again with the excreta (Joensson *et al.*, 2004). As there is nearly a mass balance between nutrient consumption and excretion, the nutrient content in urine strongly depends on the food intake (Gethke and Pinnekamp, 2007).

Since diets can differ from region to region, the actual nutrient content in urine varies among countries, regions as well as individuals and even at the time of the day when excreted. Urine

is the dominating source for the major agricultural nutrients nitrogen, phosphorus and potassium and holds 50% to 90% of these essential elements (Larse and Gujer, 1996). Urine is therefore a prime target for achieving a more sustainable handling of nutrients from urban wastewater. Table 2.1 shows the changes in the composition of fresh and stored urine, which can be seen in the pH and ammonium.

Parameter	Fresh urine	Stored urine
рН	6.2	9.1
Total Nitrogen, TN (mg/l)	8830	9200
Ammonium/ammonia-N,		
NH4 ⁺ and NH3 (mgN/l)	460	8100
Nitrate/nitrite NO ₃ + NO ₂		
(mg/l)	0.06	0
Chemical oxygen demand,		
COD (mg/l)*	6,000	10,000
Total phosphorus, TP (mg/l)	800 - 2000	540
Potassium, K (mg/l)	2740	2200
Sulphate, SO ₄ (SO ₄ mg/l)	1500	1500
Sodium, Na (mg/l)	3450	2600
Magnesium, Mg (mg/l)	120	0
Chloride, Cl (mg/l)	4970	3800
Calcium, Ca (mg/l)	230	0

 Table 2.1 Average chemical composition of fresh urine (literature values) and stored

 urine

Source : Udert *et al.*, (2006).

* COD is a measure of the organic components

2.2 The history of urine separation

Techniques for the separation of urine from the wastewater flow have been applied for many thousands of years in different parts of the world. The reasons for this vary and the system solutions vary too. In China, for example, the objective has been to use the nutrients present in human excreta (Mats *et al.*, 2000).

At the time when latrine contents were collected in buckets from each household in Swedish Cities, urine was often collected separately and poured into the drain to avoid smells and to prevent the latrine from filling too quickly (Sondén 1889). In 1867, it was known that "The proportion of value of the fertilizing ingredients held in solution in urine to that contained in faeces is as six to one" (Krepp 1867, in Drangert 1998) while Müller, a German scientist at that time, saw it as a necessity to separate the urine from the faeces in order to produce a fertilizer that was of manageable proportions (Müller1860, in Mårald, 2002). In many other parts of the world, it is also a tradition to keep the urine and faeces apart. The old Japanese practice of night soil recovery from urban areas separated urine and faeces since urine was regarded as a valuable fertilizer (Matsui, 1997). In Yemen, the urine is drained away and evaporated to obtain the faeces as a dry fraction without smell for later use as fuel, a system that has been in use for hundreds of years (Esrey *et al.*, 1998).

Human urine collected in separating systems can be used directly as a liquid fertilizer (Kirshmann *et al.*, 1995; Kirshmann, 1998; Jönsson *et al.*, 2000; Wilsenach *et al.*, 2007). The fertilizer value of pure urine is similar to that of NPK in which the ratio of nitrogen to phosphorus and potassium is 18:2:5 (Lindén, 1997). Guidelines for agricultural use of urine are available (Simons *et al.*, 2003; Tidåker, 2003; Vinnerås *et al.*, 2003). Loss of nitrogen through ammonia evaporation during storage and spreading can be reduced from well above 20% to below 10 % with better management and agricultural practice (Johansson, 2000; Jönsson *et al.*, 2000; Richert *et al.*, 2001).

2.3 Nutrients in urine

Most of the essential plant nutrients in human excreta can be found in the urine, roughly 80% of Nitrogen, 60% of Potassium, and 55% of Phosphorus (Richert *et al.*, 2010). Based on a

recent study by Gensch et al. (2011), the average nutrient content per person/year in Philippine urine is 2.18 kg of Nitrogen, 0.20 kg of Phosphorus and 0.87 kg of Potassium. Due to the high nutrient content and the low and manageable health risks, urine can be used almost immediately as a liquid, quick-acting complete mineral fertilizer that would allow substituting considerable amounts of synthetic fertilizers. The nutrients in urine are plantavailable with a formulation similar to ammonia- and urea-based fertilizers and comparable results on plant growth. The nutrients excreted in urine are all readily plant-available and thus a quick-acting fertilizer if applied correctly (Jönsson, 2004): N 75-90% of N is excreted as urea and the rest mainly as ammonium and creatinine ($C_7H_4N_3O$). If applied immediately to the field, the urease present in the soil degrades the urea into ammonium. The same process takes place, if urine is stored, given that urease is present nearly anywhere in a natural environment. Ammonium is directly available to plants. Using urine as a source of phosphorus fertilizer will preserve the world's limited geological sources of phosphorus. Additionally urine is sterile unless it gets contaminated through handling during use (Olsson, 1996; Lennartsson and Ridderstolpe, 2001). Urine fertilization used to be rare, however, it has gained attention in some areas as farmers embrace organic production methods and try to reduce use of synthetic fertilizers.

2.3.1 Advantages of the use of human urine as fertilizer

- Human urine is a quick-acting fertilizer that can replace mineral fertilizer.
- Nutrients are directly available to plants.
- Relatively low cost.
- Low risk of pathogen transmission.
- Additional income generation.
- Easy to understand technique.
- Contributes to self-sufficiency and food security.
- Urine contains micro-nutrients which the soil may need for higher soil fertility.

2.3.2 Disadvantages of the use of human urine as fertilizer

Whilst urine is a proven fertilizer, it has some drawbacks compared to artificially manufactured chemical fertilizers:

- Large volume compared to artificial fertilizer.
- Cultural taboos could hinder use of urine.
- Urine is a relatively heavy medium (low value/weight) and difficult to transport.
- Smell may be offensive.
- Application of urine is labour intensive.
- Requires space for storage and agricultural activity.

2.4 Plant availability of nutrients in urine

The urine has been filtered by the kidneys and contains only low molecular weight substances.

At excretion, the pH of urine is normally around 6 but can vary between 4.5 and 8.2 (Lentner *et al.*, 1981). Of the N, 75-90% is excreted as urea and the remainder mainly as ammonium and creatinine (Lentner *et al.*, 1981). In the presence of urease, urea is quickly degraded to ammonium and carbon dioxide (Equation 1) and the hydroxide ions produced normally increase the pH to 9-9.3. Normally urease accumulates within the urine piping system and therefore the above transformation is very swift, usually within hours (Vinnerås *et al.*, 1999; Jönsson *et al.*, 2000).

 $CO (NH_2)_2 + 3H_2O \longrightarrow 2NH_4^+ + OH^- + HCO_3^+ - (Equation 1)$ Urea Water Ammonium hydroxide Carbonate

Ammonium is directly plant-available and an excellent N fertilizer which is verified by the fact that urea (which is degraded to ammonium by urease in the soil) and ammonium are two of the most used N fertilizers in the world. Many crops prefer nitrate to ammonium but this is no problem as ammonium applied to arable soil is transformed within a few days to nitrate (Equation 2-4). In soils with very low microbial activity, these transformations take longer since they are performed by microbes.

$NH_4^+ + 1.5 O_2 \Rightarrow NO_2^- + 2H^+ + H_2O$	Nitrosomonas (Equation 2)
$NO_2^- + 0.5 O_2 \Rightarrow NO_3^-$	Nitrobacter (Equation 3)
$NH_4++2 O_2 \Rightarrow NO_3^-+2H^++H_2O$	(Equation 4)

The plant availability of urine N is the same as that of chemical urea or ammonium fertilizers. This is to be expected, as 90-100% of urine N is found as urea and ammonium and has been verified in fertilizing experiments (Kirchman and Pettersson, 1995; Richert *et al.*,2001). The P in the urine is almost entirely (95-100%) inorganic and is excreted in the form of phosphate ions (Lentner *et al.*,1981). These ions are directly plant-available and thus it is no surprise that their plant availability has been found to be at least as good as that of chemical phosphate (Kirchmann and Pettersson, 1995). K is excreted in the urine as ions which are directly plant-available. This is the same form as supplied by chemical fertilizers and thus their fertilizing effect should be the same. Sulphur (S) is mainly excreted in the form of free sulphate ions (Lentner, 1981; Kirchmann and Pettersson, 1995) which are directly plant-available. This is the S in most chemical fertilizers and thus the fertilizers and thus the same form as the S in most chemical fertilizers and thus the fertilizers and that in chemical S fertilizers should be the same.

2.5 Global use of human urine in Agriculture

Different authors have shown that urine contains more nutrients than faeces. For example, Drangert (1998) indicated that the nutrient content value of urine as one of the three reasons to counteract "urine blindness" (a negative attitude towards urine). According to Esrey and Anderson (2001), urine has been used as a resource in many parts of the world for centuries.

In some societies, however, human excreta (particularly faeces) have for many centuries been considered dirty. However, experience has shown that urine diversion sanitation is acceptable and the handling of urine poses far fewer taboos than that of faeces. According to Winblad (1997), urine diluted with water can be used directly in the garden or it can be stored and used at a later date. Olsson (1996) warned that the risk of disease transmission has to be taken into account when recirculating human urine for agricultural production because small amounts of faecal material may be introduced into the urine fraction.

2.5.1 Use of urine in Latin America

In Mexico, fermented urine is recommended as a fertilizer. Before sealing the container to avoid loss of nitrogen, users often add a handful of soil as a catalyst for the fermentation process. According to Ceballos (1997) in a case study of dry sanitation in Morelos, Mexico,

fermented urine was diluted before watering plants. For fertilization purposes, users have reported varied dilution ratios of urine to water (from 1:5 to 1:4) (Clark, 2003). Unfermented urine can be sprayed as a fungicide. Indigenous people in southeastern Mexico claim that the use of urine as a fungicide was a traditional Mayan practice (Clark, 2003).

Esrey et al., (1998) mentioned the fact that urine used in urban family scale gardening has taken place for a number of years through the ANADEGES network in Mexico City. This project, managed by CEDICAR (Centro de Investigaci`ony Capacitati`on Rural A.C. – Rural Research and Training Centre) which is an ANADEGES affiliate, involved 1200 families who were producing vegetables in reused containers with worm composted kitchen waste, urine and leaves. This was done in response to rapid inflation, high unemployment and inadequate nutrition. In Mexico City studies with the use of fermented urine to grow food showed that leafy vegetables did very well (Esrey and Andersson, 2001). These included lettuce, cilantro (coriander), parsley, celery, fennel, scented herbs, prickly pear and chile piquin (bird peppers). Good results were also obtained with cauliflower, broccoli, cabbage and root produce (turnips, carrots, beets and onions). Sawyer (2003) demonstrated in a pilot project in the municipality of Tepoztlán in Morelos that it is feasible to harvest urine and develop a reuse system in the urban context. Achievement of the pilot project included harvesting urine in public places for use in agriculture. Through the project, various technologies have become available in the local market for the collection and storage of human urine. In Mexico, people now experiment with urine in urban agriculture, as well as in growing traditional Mayan food grains (Strauss, 2000). CEDICAR promoted containers for use with different types of compost and "liquid organic fertilizers" including human urine for household and community production of vegetables and herbs (Esrey and Andersson, 2001).

Esrey and Andersson (2001) reported that other people in Cuernavaca, Mexico, experimented with human urine as a source of nitrogen in organic vegetable production. Esrey et al., (1998, as noted in Austin and Duncker, 2002) mentioned that the urine, which has been stored in separate receptacles for three weeks, was applied to the vegetable containers after dilution with water on a 1:10 ratio. After several years of study it became clear that plants fertilized

with urine grew more rapidly and were larger and healthier than those grown using conventional agricultural techniques. Less water was used in this instance as well.

2.5.2 Use of urine in Europe

Urine was used in Europe in the olden times for household cleaning, softening wool, hardening steel, tanning leather and dyeing clothes. The Greeks and Romans used it to colour their hair and European farmers used it in fermenting plants to produce dyes (Esrey and Andersson, 2001). Sweden is probably the country with the most advanced system of collection and reuse of human urine where it is practiced by farmers on a large, mechanized scale. In a number of settlements (called eco-villages) or apartment blocks in the country, the residents have ecological sanitation systems with urine diversion toilets. The urine from the houses or apartments is collected in large underground tanks, and what the residents do not use themselves is collected by farmers in road tankers and used for fertilizing their crops. The usual practice is to spray it onto the lands while they are being prepared for planting and then harrow it into the soil before sowing the seed (Austin and Duncker, 2002). It has been found to be a valid substitute for mineral fertilizers in growing cereals with no negative impact on the crop or the environment (Esrey and Anderson, 2001). Frode-Kristensen (as quoted by Drangert, 1998) reported that in Sweden, urine was used to smear wounds and to some extent drunk as a therapy. Drangert (1998) also noted Hansen, who reported that in the Danish countryside in the19th century, urine was stored and used as a detergent for washing clothes and dyeing. In a fertilizer experiment on the growth of barley using urine to compare with manure, urine was examined as a fertilizer. The growth experiment showed that the urine had the expected fertilization value (Jansen and Koldby, 2003).

A project conducted in Vaxholm, Sweden, in 2004 with the objective of achieving a system for the use of urine in agriculture concluded that it is possible for municipalities to organize stable systems for agriculture. The study also concluded that it is not a problem to find a use for human urine in agriculture, even in a large city in Sweden (Stintzing, 2005). The farmers' perception of the use of urine in Sweden is that the more concentrated the urine is, the better it is. They also concluded that establishment of a quality control system such as certification of urine and other source diverted waste water fractions is extremely important for the use of fertilizers of organic origin in the Swedish context. Jönsson (2005) indicated in the guideline for the use of urine and faeces on crops that urine can be applied raw or diluted. They stressed that the application rate should always be based on the desired N application rate and the urine or urine mixture should be quickly incorporated into the soil to minimise ammonia loss. For hygienic measures, Schönning (2005) recommended that crops consumed raw should not be fertilized with urine closer than one month to harvest. In addition, there might be pathogens that may be present in the urine, thus requiring inactivation. According to Schönning (2005), the inactivation of pathogens in urine will be dependent on the pH, concentration and temperature. She further clarified that dilution should be avoided for a more efficient treatment influenced by ammonia. It should be noted that the treatment she is referring to was mainly done in Nordic climates thus recommending that adaptation of guidelines to tropical climates should be discussed.

In terms of how to apply urine in agriculture, Jönsson (2004) in his Swedish experiments found that it is better to mix the urine into the soil as quickly as possible. He indicated that the best method of doing this is by applying urine to farrows or holes which have to be covered over immediately after application. The latter has been demonstrated in Mexico, in the Tepoztlán Municipality (Sawyer, 1998). Simons and Clemens (2004) have tested urine as a fertilizer in barley in both field and greenhouse trials in Germany. Their trials found that the fertilizing effect of urine was much higher than that of a mineral fertilizer. The study also showed no difference in yield between plots fertilized with untreated urine and acidified urine.

2.5.3 Use of urine in Asia

Esrey and Andersson (2001) indicated that the Chinese pharmaceutical industry used urine to make blood coagulants. They further highlighted anecdotal evidence from several locations that indicated that people preferred vegetables grown with urine fertilization and in China people were willing to pay more for vegetables grown with urine. Rooftop gardeners used only urine to grow vegetables such as tomatoes, cabbages, beans and pumpkins. Faeces were carted to the fields. Farmers have commonly used urine, often untreated, to grow food (Esrey and Andersson, 2001).

In a pilot project in Kerala, India, urine from toilets was diverted into a growing area attached to the back of the toilet. Bitter gourds were grown, which were sliced, fried and eaten (Esrey and Andersson, 2001). In Manipur State, harvests of potatoes and chillies planted with urine were very good compared to those fertilized with chemical fertilizer such as urea and potash (Singh, 2003).

In Sri Lanka, urine was used for plants such as banana, coconut, vegetables, flowers or fuel wood. In Matale town, the Nandawathi family used urine and wash water to grow chillies but only used the chillies after drying, not fresh (Calvert *et al.*, 2002). Getting people in Thailand to accept the application of human urine as a fertilizer was not easy. The main issue was the sociological difficulty as the common belief is that human excreta are dirty and a pathway for disease transmission (Pinsem and Vinnerås, 2003).

Matsui, (1997, as quoted by Austin and Duncker, 2002) mentioned the fact that farmers in Japan placed buckets at street corners in the towns and villages collecting free urine from pedestrians and providing a simple public toilet at the same time.

In Nepal, the Environment and Public Health Organisation (ENPHO) has conducted experiments in the application of urine on various seasonal crops including potatoes, radishes and rice. The study concluded that there is a possibility of growing potatoes with the application of urine only. The study showed that higher yields of potatoes were reported with the application of chemical fertilizers than urine (Shrestha and Morgan, 1993). The study might be too early to draw conclusions from the study on the impact of urine on crops because urine application on crop yield can only be observed after several years of application and many crop cycles.

2.5.4 Use of urine in United States of America (USA)

Experiments in the USA found that maize, which was grown using substantial quantities of urine grew 50% taller than maize grown using no urine at all (BBC News, 2005).

2.6 Use of human urine in Agriculture in Africa

2.6.1 Use of urine in Tanzania

Urine is now gradually being applied as a fertilizer in Majumbasita, Dar es Salaam, Tanzania. A garden has been established at the school compound and most of the parents and members of communities passing by have learnt the importance of urine as a readily available fertilizer. Some of the people channel urine into the shallow pit near a fruit tree or close to their garden. It could as well be applied in places with woody perennials that are fully grown to provide more than one significant contribution to the production and/or service functions of a land-use system (Chaggu and John, 2002).

Study gardens have been established in Majumbasita, Dar es Salaam at Karakata Primary School and at individual homes to test the recycling of nutrients from EcoSan latrines. The plants in the gardens include eggplants, banana trees, cassava plants etc. For comparison purposes, the gardens were divided into two similar portions. Urine was applied to only one portion to determine its efficiency as a fertilizer and it was observed that the yield from urine were better (Shayo, 2003). In the Kagera area in Tanzania, urine has been used as an antidote when somebody has inhaled and ingested poison by giving that person fresh urine to drink. It has also been used as a pesticide to kill banana weevils (Chaggu and John, 2002).

Chaggu (2004) also mentioned that in Bukoba, Tanzania, the tradition of visitors was for visitors to urinate in the host's home garden which was much appreciated and considered a gesture of respect. This practice has disappeared with the adoption of modern hygiene. He further quoted Missaar (1997) and Chaggu and John (2002) that though excreta is accessible, there is not much written about the subject of humanure (human excreta) and attempts to address this question in Tanzania have been mainly the result of difficulties faced in terms of a high water table.

2.6.2 Use of urine in Zimbabwe

Morgan (2003) reported on trials performed on varieties of vegetables and maize using urine diluted with water at a ratio of three parts water to one of urine as a liquid feed. Seedlings were planted in containers (buckets or cement basins) and irrigated with water first, to

stabilise them in their new environment, and thereafter with a water and urine mix. This was compared with similar vegetables and maize irrigated with water only. After a specified growing period, the crop was harvested and weighed. The yields of the vegetables and the maize irrigated with the urine/water mix were higher. The trial showed the great value of urine when used as a liquid feed for various plants and particularly for leafy vegetables (lettuce, spinach, covo – a type of spinach). There is huge potential for urine application as an enhancer of vegetable and crop growth.

A study conducted in the informal settlement of Hatcliffe extension in Harare, Zimbabwe showed that 61.3% of the households said they would not use urine as a fertilizer because they thought it would burn crops, 17.5% said it had a bad smell, 55% said they did not know that it could be used, 11.3% said urine is a good fertilizer and 12.5% said they would use urine because fertilizer was expensive (Guzha, 2001).

Studies on people's attitudes toward excreta use were carried out in urban and rural areas of Marondera and Zvishane districts with interesting findings covering traditional human excreta reuse, attitudes towards crops grown using human excreta, fears, myths and taboos on excreta use. A few respondents said urine had medicinal properties or could be used as a pesticide. Others indicated that urine has traditionally been used as medicine in the treatment of athlete's foot, sore eyes, impotence, burns, runyoka (illness caused by having sex with someone else's wife) and as a love potion (Guzha, 2004).

2.6.3 Use of urine in Botswana

A study established that 18 of 24 families in Paje used urine for fertilizing purposes; some as trials to learn the new concept while others used fresh overnight urine in growing trees and flowers (Dunker, 2007). However, experience in the village showed that people reacted unfavourably towards the use of urine and treated faecal matter as a fertilizer and soil conditioner. A reason for this rejection might be found in education and tradition which believe that urine is something to keep out of one's territory (Dunker, 2007).

In Botswana, there was strong belief that urine and faecal matter were something very dirty. The consideration that it could be very valuable after treatment was quite erroneous in Botswana understanding. On the other hand, there are also superstitious reasons for the negative attitude for example, a widespread belief in witchcraft which holds that urine as a substance could be harmful. Even the fear of spreading HIV/AIDS through the use of urine in the garden was mentioned (Hanke, 2003).

Hanke (2003) conducted pilot trials for the agricultural use of urine whereby three plots were prepared in 16 locations. One was fertilized with urine, the second one with urine and compost and the third one without any kind of fertilizer for comparison purposes. In all the cases, the plots were planted with spinach (*Swiss chard*). After a certain period, the best results were achieved with the use of compost and urine together. This resulted in participants starting to use urine after the demonstration and even those without toilets started to collect urine for further use. Hanke (2003) reported that with the use of urine and compost, the nutrient supply was more balanced than with the use of urine alone; resulting in a higher yield. Another important aspect reported during the study was the different level of acceptance of using urine directly as opposed to pouring it on the compost and then using it in the garden. The indirect use seems to be easier to accept. A paper presented at the Ecosan Conference in Durban, South Africa by Bolaane and Tiroyamodimo (2005) pointed out that in a few instances urine has been and is still being used in backyard gardening. Experiments for urine fertilized vegetables were conducted in West Hanahai and Paje. However, composted faecal matter was not yet used pending further sampling for pathogenic content.

2.6.4 Use of urine in Ethiopia

Sundin (1999) as noted by Jönsson *et al.* (2004) indicated that urine has been tested as a fertilizer on *Swiss chard* in Ethiopia. The yields of the fertilized plots were up to four times those of the unfertilized.
2.6.5 Use of urine in Mali

Urine has also been tested as a fertilizer on cotton and sorghum in Mali and results are promising (Jönsson *et al.*, 2004). The first Peace Corps urine fertilizer project in the fall of 2007 was done with a women's gardening group made up of 30 women in Diallola, a periurban area on the outskirts of Mahina (a town of 7,000). The women who tried fertilized with urne ended up seeing great improvements. They said their tomatoes and peppers were coming out earlier and growing faster with urine. One of the women had also applied urine on a portion of her corn and was amazed at how the corn fertilized only with urine fertilizer was growing even better than the corn she had only fertilized with cow manure. Urine is best for corn, millet, sorghum, salad greens, and spinach, because nitrogen helps the leaves and stalks grow large and strong (Jonsson, 2004).

2.6.6 Use of urine in Uganda

An EcoSan project carried out in Kampala showed that 22 farmers were identified and agreements signed with them to carry out demonstrations and trials on their farms. These farmers were involved in the plot demarcation and decision making on the types of crops to be grown. The plots were 9 m^2 and the first trials were been conducted with urine since urine is more acceptable than faeces for the farmers to use. The demonstrated areas studied the physical changes in plants grown with urine. In another season more trials were conducted to test the quantity of urine per period, the concentration of urine applied to crops, the application of urine with other organic fertilizers and project structure. One of the challenges experienced in this study was the unacceptability of using faecal products (Project management unit members: Kampala Ecosan Project, 2005). The crops planted with urine grew well as well as the ones with urine and organic fertilizer.

2.6.7 Use of urine in Nigeria

Traditions in Nigeria prohibit collection of urine by strangers for fear that the urine may be used against the people through 'black magic' or 'evil spirits' (Sridhar *et al.*, 2005). There is still a phobia of using urine for growing edible crops (Sridhar, *et al.*, 2005 cited Sridhar, 2003). In a study carried out in Ibadan, Southwest Nigeria, urine was collected from a tertiary institution (the Federal Polytechnic, Ede in Osun State) and the contents used for growing the

most popular edible crops. The test crops grown were a fruit-yielding Okro (*Hibiscus esculentus*), Tete (a green amaranth, a leafy vegetable) and maize (*Zea mays*), a cereal demanding high N inputs. In the greenhouse experiments, plant height and number of leaves were recorded as indicators of growth. In the case of okro, urine performed better than the other treatments. For green amaranth, urine was comparable to organo-mineral fertilizer (OMF); the NPK chemical fertilizer performed better. In the case of maize, urine was comparable to OMF and NPK (Sridhar *et al.*, 2005).

Based on the acceptability of urine-grown crops, initially about 73.1% of the community felt that urine was a body waste and may have pathogens and therefore should be disposed of in the conventional way. Only 7.69% accepted that vegetables and other crops could be grown using urine as fertilizer. Once the experiment was completed the community members were taken around the farm to see for themselves the quality of crops obtained. All the respondents were surprised to see the yields obtained from urine which was found to be much better and fresher in appearance and they believed that urine was a good alternative to fertilizer. About 80% of the respondents showed a willingness to build a urine-diversion toilet on their premises (Sridhar *et al.*, 2005).

2.7 Urine Processing Techniques

Treatment options for collected urine should be taken into consideration for basically two reasons:

Firstly, urine contains a large portion of water so that the concentrations of nutrients are relatively low compared to the concentrations of inorganic fertilizers. For example, one kilogram of NPK (15-15-15) fertilizer contains 150 g N, 66 g P and 125 g K. One liter of urine contains only about 9.5 g nitrogen, 0.8 g phosphorus and 1.9 g potassium thus the mass fractions are between 15 to 83 times lower. This results in an unfavorable ratio of fertilizing effect to weight which makes handling and transportation of urine-fertilizer relatively expensive. A volume reduction would reduce the handling costs and improve the market chances for urine-fertilizer (Maurer, 2006).

Secondly, pure urine is not a well marketable product because people consider it as a smelly and worthless waste product and often have hygienic concerns on the reuse. The prices that can be achieved for one m³ of urine are far below the actual nutrient values. A liquid non-odorous substance would be more readily accepted by the customers and thus higher prices could be realized for such a product. Hygienic concerns and the unpleasant smell of urine can also be eliminated by several treatment methods. In recent years different techniques have been developed with the aim either to concentrate the urine or to recover its main nutrients. Basic research is still ongoing in this field and most of the processes are still at the laboratory stage (Maurer, 2006). The energy requirement of each treatment method of urine and it final products are presented in Table 2.2.

2.7.1 Storage

Storage reduces the pathogen concentration in urine. Urine itself has usually a very low germ concentration but in separation toilets it may be contaminated with faecal pathogens. Höglund *et al.*, (2001) stated that probably all pathogens (viruses and protozoa) die when urine is stored for at least six months and that urine then can be regarded as hygienized and ready to be applied to all kinds of crops. As the pathogenic decay strongly depends on the temperature, urine that is stored at a higher temperature needs less storage time (Höglund *et al.*, 2001).

The storing process also affects the chemical quality of the available nitrogen and phosphorus. Fresh urine leaves the human body with an average pH of 6.2 (Larsen and Gujer (1996)) and nitrogen is predominantly present in the form of urea. During storage organic matter is degraded by microbial activity and urea is hydrolyzed into ammonia.

The reaction causes a pH increase of up to 9 and after 24 hours 90% of total nitrogen is present as ammonia (NH₃) or ammonium (NH₄⁺) depending on the temperature and the pH (high temperatures and a high pH shift the equilibrium towards ammonium ion) (Udert, 2002). In order to avoid ammonia evaporation, urine should be stored in airtight-sealed tanks. The pH increase in turn leads to precipitation of phosphorus. If no flush water is added to the urine, phosphorus can only precipitate in form of struvite (MgNH₄PO₄) where the availability of magnesium in urine is the limiting factor. (Udert, 2002) showed that in undiluted urine 30% of the soluble phosphate is precipitated. Hydrolysis and precipitation can be inhibited by keeping the pH value low for example by acidification (Maurer *et al.*, 2006) but high quantities of acid (11.3 cm³/l) is required (Otterpohl and Stegmann, 2008).

2.7.2 Volume Reduction

2.7.2.1 Reverse Osmosis

If two liquids of different concentrations are separated by a partially-permeable membrane, water diffuses from the solution of low solute concentration to the solution with high solute concentration. Urine can be concentrated by inverting this process (reverse osmosis) so that urine is pressed through a membrane and solids like salts and nutrients remain in the retent. For the process, stabilized urine is more suitable than hydrolyzed urine since the retention is better for ammonium than for ammonia and the precipitation of salts in the membrane (scaling) is hindered by the acidification (Maurer *et al.*, 2006). For a fivefold concentration of urine, Dalhammer [cited in Maurer *et al.*, (2003)] calculated an electricity demand of 5 to 10 kilowatt hour (kWh) equals metre cube (m^3) which corresponds to a specific demand for primary energy of 29 Mega Joules equal one kilogram newton (1 kgN).

2.7.2.2 Evaporation

Evaporation of urine can help increase its concentration 60 times but again stabilized urine is needed in order to achieve high nitrogen fractions in the concentrate. Besides acidification, n-depleted urine as a substrate is a suitable alternative (Tettenborn et al., 2007). Based on experimental results Maurer *et al.*, (2003) calculated an energy requirement of 34 Mega Joules equal one kilogram newton (1 kgN) for a tenfold concentration of urine in a vapour condensation evaporation plant. In case urine cannot be stabilized (e.g. for monetary or logistical reasons), evaporation is a feasible method to concentrate phosphorus and potassium-rich urine after recovering nitrogen in a first step.

2.7.3 Nutrient Recovery

2.7.3.1 Struvite Production

Magnesium ammonium phosphate (MAP or struvite) contains two major plant nutrients (nitrogen and phosphorus) plus magnesium and is therefore a very suitable and marketable fertilizer. Struvite precipitation allows recovery of ammonia and phosphorus from urine at the same time. Urine contains much more ammonia than phosphorus hence 98% of phosphorus but only 3% of ammonia can be recovered by precipitation (Maurer *et al*, 2003).

By adding magnesium oxide (MgO) or chloride (MgCl₂) to urine the precipitation is triggered without pH of adjustment since a pH between 8.5 and 9 is optimal for struvite production (Maurer *et al.*, 2006). The process is relatively simple and energy is only required for stirring. (Maurer *et al.*, 2003) converted the necessary amount of magnesium oxide into energy equivalents and calculated a total energy demand of 25 Mega Joules equal one kilogram newton (1 kgN) for quantitative P-fixation.

2.7.3.2 Ammonium Adsorption with Zeolite

Zeolite can serve as a cation exchanger and adsorbs the ammonium ions from urine. Bán and Dave (2004) combined the addition of clinoptilolite, a naturally occurring zeolite, and magnesium oxide. They reported that recovery rates of 80% were achieved with dosages of 0.5 mg/l MgO and 15 g/l zeolite. The process handling for using zeolite adsorption is very simple and thus qualifies it for use in regions where technologically sophisticated treatment plants are difficult to realize due to a lack of technological know-how and infrastructure. The major shortcoming of the process is the relatively high amount of zeolite that is needed to adsorb ammonium ions. In large scale systems, e.g. if the urine from 1,000,000 people is collected, 7,500 kg of zeolite would be needed per year to reach an ammonium recovery rate of 80%. In order to avoid high handling and transportation costs, further research is needed on the optimization of the recovery efficiency or on the applicability of synthetic zeolite. Unfortunately no energy equivalent is available for zeolite, so that an energetic comparison with other processes is not possible (Bán and Dave, 2004).

2.7.3.3 Steam Stripping

Steam stripping is another technique to recover nitrogen from human urine. Heated urine is injected at the top of a stripping tower and travels downwards while steam that is injected at the bottom of the tower rises upwards. The intimate contact between the two phases causes volatile ammonia to transfer into the vapor phase so that a condensate enriched with ammonia can be gained at the top of the stripping tower (Tettenborn *et al.*, 2007). Experiments at the Teaching University Hamburg-Harburg (TUHH) showed that 120 g NH₃/l ammonia solution was produced from human urine condensed to 3% of its original volume. The pH value of the N-depleted substrate decreased to a value below 6 and the nitrogen concentration was reduced by 90% to 98% (Otterpohl and Stegmann, 2008).

The process needs hydrolyzed urine as a feed substance with most of the nitrogen being present in form of volatile ammonia (NH₃). A combination of struvite production and steam stripping seems reasonable as very high removal rates of both nutrients (nitrogen and phosphorus) can be achieved that way. If the energy efficiency of a stripping tower is optimized, a specific demand for primary energy ranges between 30 MJ/kgN and 40 MJ/kgN (Otterpohl and Stegmann, 2008).

Process	Specific Energy	Energy	Product
$(m^J = kgN)$	(mJ/m^3)		
Storage	0	0	hygienized urine
Reverse osmosis	29	27	concentrated nutrient solution
Evaporation	34	180	concentrated nutrient solution
Struvite production	25	50	struvite
Zeolite adsorption	n.a.	n.a.	ammonia laden zeolite
Steam stripping	35	150	(ammonia solution)

 Table 2.2: Energy requirements of different urine treatment methods and their final products

Source: Tettenborn et al., (2007) and Maurer et al., (2006)

2.8 Struvite: Recovered and Recycled Phosphorus

Struvite, magnesium ammonium phosphate hexahydrate (MgNH₄PO₄·6H₂O) —is a biogenic mineral of low solubility. For 150 years, it has been proposed as a fertilizer but its use has been limited to high value crops because of the additional cost of manufacture. Struvite is an effective phosphorus fertilizer (Johnston and Richards, 2004). With the advent of new interest in removing phosphorus from waste streams before land application, recovery of phosphorus as struvite has gained new interest (Stafford and Barak, 2006). Struvite contains 5.7% N and 12.6% P by weight; the phosphate is entirely citrate-soluble (Bridger *et al.*, 1962). As long ago as 1858, struvite has been proposed as potential phosphorus source for agriculture, and repeatedly since then (Stafford and Barak, 2006).

Amongst the possibilities for nutrient recovery from urine, struvite precipitation is technologically most accessible as it does not necessarily involve any sophisticated apparatus (Etter, 2009). Combined with the concept of EcoSan toilets, this technology will hopefully provide some improved sanitation coverage.

Struvite precipitation represents a possibility to produce a solid fertilizer from urine. Struvite $(MgNH_4PO_4.6H_2O)$ is formed by the addition of magnesium, usually in the form of MgO, $Mg(OH)_2$ or $MgCl_2$ (Udert *et al.*, 2003b). There are much more equivalents of ammonium than phosphate present in urine and therefore only 3% of the nitrogen can be eliminated by magnesium addition. Struvite is known to be a slow-release fertilizer (El Diwani *et al.*, 2007) and is a solid product which can easily be transported. Pure struvite occurs as white crystalline powder but also can occur either as large single crystals, very small crystals, curds or a gelatinous mass (Munch and Barr, 2001; Udert *et al.*, 2003c; Le Corre *et al.*, 2007). Yellowish or brownish white, orthorhombic or pyramidal crystals or in platey mica-like structures of MAP are also available (Lee *et al.*, 2009).

It has been shown that micro pollutants present in urine are not included in the struvite but remain completely in the liquid phase which becomes available as a supernatant after precipitation (Ronteltap *et al.*, 2007). Apart from the micro pollutants, the supernatant also contains all of the organic compounds and most of the ammonia (97%) as well as other salts

except P and Mg (Pronk and Koné, 2008). Struvite precipitation holds great potential for phosphate recovery but only a small fraction of the nitrogen can be recovered this way (Jaffer *et al*, 2002).

2.8.1 Why produce struvite

In comparison with directly applied urine, struvite compacts the nutrients contained in urine into a white odourless powder. Therefore weight and volume are reduced to a strict minimum in order to facilitate transportation, storage and handling over longer time periods. Concerns on health risks due to heavy metal content or pharmaceutical residues in struvite fertilizer have been clarified as no such harmful substances were found (Ronteltap, 2007).

2.8.2 Characterization and Solubility of struvite

Struvite is the common name for magnesium ammonium phosphate hexahydrate, or MAP (MgNH₄PO₄.6H₂O). Table 2.3 lists some selected physical and chemical properties of struvite. Struvite crystals are usually stable, white and orthorhombic (Le Corre *et al.*, 2005) precipitating as magnesium, ammonium and phosphorus react in 1:1:1 molar ratios according to the simplified reaction Equation (5):

 $Mg^{2+} + NH_4^+ + HPO_4^{2-} + 6H_2O \longrightarrow MgNH_4PO_4.6H_2O + H^+$ ------- (equation 5) The distinctive orthorhombic structure of struvite crystals allow them to be readily identifiable through X-ray diffraction (XRD) by matching the position and intensity of the peaks produced to a reference library of standard patterns (Doyle and Parsons, 2002). Struvite crystals develop in two chemical stages: nucleation and crystal growth yet various factors control this development including solution pH, supersaturation, temperature, mixing energy, component molar ratios, reaction kinetics and the presence of interfering ions (Booker et al., 1999; Doyle and Parsons, 2002; Le Corre *et al.*, 2005; Forrest *et al.*, 2008).The key factor for determining crystallization is supersaturation which is dependent upon solution pH and reactive solution concentration.

Property	Struvite
Colour	White, yellowish white or brownish white
Specific density	1.73g.cm ⁻³
Solubility at 25°C	Very insoluble in water: 0.018 g . 100 ml^{-1}
Soluble in acid	0.178 g.mol ⁻¹ in 0.01 N HCl
Formular weight	245.41 g.mol ⁻¹
$\Delta H^{o}f$	-880.0 kcal.mol ⁻¹

Table 2.3 Physical and chemical properties of struvite.

Source: Wu and Bishop, 2004 and Le Corre et al., 2009

2.8.3 Effects of struvite production process on Agriculture

In general, the application of Mg fertilizers tends to increase the pH in soils. As a positive side-effect, P becomes more plant-available in alkaline soils. Mg compounds such as dolomite (CaMg)CO₃ have been used for correction of low pH in agricultural soils. Due to the slow nutrient release, these fertilizers can be applied to plants at superior rates without risk of burning the roots or leaves. Bridger *et al.*, (1962) tested the use of different metal ammonium phosphates as fertilizer. They obtained promising yield improvements after application of struvite in forest and agricultural species.

Struvite has been compared to commercial fertilizers in greenhouse and field studies. The effectiveness of the fertilizer powder recovered from urine was comparable to the one of commercial P fertilizers (di-ammonium phosphate DAP) (Ganrot, 2005 and 2007). At certain instances, struvite was even proved to be more stimulating for plant growth than commercially available NPK fertilizers (Ghosh *et al.*, 1996; El Diwani, 2007). Also, the N-rich effluent of the struvite precipitation process may be used as liquid fertilizer (El Diwani, 2007).

2.9 Requirements for plant growth

Apart from the easily available carbon dioxide and light, plants require primarily water, adequate soil structure for the roots to grow in, and nutrients in suitable quantities for their growth. Since nutrients are taken up from the soil by the plants and finally leave the fields with the harvested products, it is essential that these nutrients be replaced in an amount corresponding to the amount removed during the harvest. It is important to note that fertilization increases crop yield only if the respective plant nutrient supplied is one of the limiting growth factors. If factors other than nutrients are limiting e.g. water, light, pH, salinity, light or temperature, adding more nutrients will not increase the yield. As Liebig's law of the minimum goes: "Plant growth is controlled not by the total of resources available but by the most limited resource" (Gensch *et al*, 2011).

2.9.1 Macro-nutrients

Elements essential for the growth of plants are called nutrients. The nutrients used in the largest amounts are the non-mineral elements, i.e. carbon, hydrogen and oxygen. These elements are mainly taken up as carbon dioxide (CO_2) from the air, and water (H_2O) by the roots. Increasing the supply of light, carbon dioxide, water and mineral nutrients from the deficiency range increases the growth rate and crop yield.

Nutrients can be divided into the two categories; macro-nutrients and micro-nutrients.

The six elements normally classified as macro-nutrients which are nutrients needed in large amounts by plants: nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg). These nutrients are mainly taken up from the soil by the roots in ionic form. The uptake of macro-nutrients is about 100 times that of micro-nutrients (Marschner, 1997).

Nitrogen is frequently the most limiting nutrient for plant growth and the use of N is usually higher than the total use of the other macro-nutrients and micro-nutrients together. N is taken up by the plant as ions of nitrate (NO₃⁻) and ammonium (NH₄⁺). The natural sources of plant available N are degradation of organic matter in the soil and N fixation by microorganisms living in the roots of legumes (Jönsson *et al.*, 2004). Phosphorus is taken up by the plants as phosphate ions (at pH 5-7 mainly as HPO₄²⁻ and H₂PO₄⁻). The natural supply of plant-available P comes from dissolution of soluble phosphates in the soil and from mineralization of organic matter.

The high water solubility of potassium often results in a good supply of plant-available K. However, many crops, such as vegetables, need large amounts of K and therefore additional K fertilization may improve plant growth. S is also highly water-soluble and most crops need it in somewhat smaller amounts than P (Jönsson *et al.*, 2004).

2.9.1.1 Phosphorus and its role

Phosphorus is a major limiting plant nutrient which is available in large concentrations in urine. Because phosphorus is highly reactive, it does not naturally occur as a free element, but is instead bound in phosphates. Phosphates typically occur in inorganic rocks (Ganrot *et al.*, 2007). Most phosphorus is obtained from mining phosphate rock. Crude phosphate is now used in organic farming whereas chemically treated forms such as superphosphate triple superphosphate or ammonium phosphate are used in non-organic farming (Barak and Stafford, 2006).

Dissolved phosphorus is present in urine as orthophosphate (PO_4^{3-}) and precipitates out of solution in urine mainly in the form of struvite ($MgNH_4PO_4 \cdot 6H_2O$) and hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Udert *et al.*, 2003a). Although these solid forms of phosphorus can break down over time and become bio-available, the form most directly available to plants is the phosphate ion in solution. Phosphorus is one of the three major nutrients required for plant growth: nitrogen (N), phosphorous (P) and potassium (K). Fertilizers are mostly labeled for the amount of N-P-K they contain. In the literature, estimates before depletion of available phosphorus resources is expected to be 100 years (UNEP, 2001). This date seems conveniently far enough in the future but immediate action is required to combat the situation. Since phosphorus is a non-renewable resource, recovering phosphorus from human waste (urine) is a significant breakthrough technology.

2.9.1.2 Magnesium

Struvite is a naturally occurring precipitate in aged urine. It is formed out of some of its compounds when the pH level of the urine exceeds a distinctive level due to chemical changes happening during storage. The struvite molecules are formed through a combination of dissolved magnesium, ammonium and phosphate ions. Since the concentration level of magnesium in urine is considerably lower than the concentration of the other compounds, this natural process stops when all the magnesium ions in solution are fixed. However, this process can be stimulated again by artificially adding magnesium to the stored urine. By this, the precipitation of struvite continues until one of the other in the process involved components (ammonium and phosphate) are depleted. In normal urine, the concentration of

phosphate is subsequently the limiting factor. The artificial addition of magnesium to urine is therefore a very convenient way of fixing the phosphorus and a part of the nitrogen that is contained in urine (Tilley *et al.* 2008b; Udert 2002; Udert *et al.*, 2006; Gantenben *et al.*, 2009).

2.9.1.2.1 Sources of magnesium

In line with the above mentioned criteria, three main sources of possible magnesium have been identified: magnesium salts, magnesium carbonates and magnesium oxides. Magnesium salts are produced in abundance and are the sources for many other magnesium products. The two most important magnesium salts are magnesium chloride (MgCl₂) and magnesium sulphate (MgSO₄). They are produced and traded in the market in the form of magnesium chloride hexahydrate (MgCl₂·6H₂0) and magnesium sulphate heptahydrate (MgSO₄·7H₂O). Since these products are commonly of high grade and differ only by their hydrate form from the original salts, the distinction between the magnesium source and the actual products is sometimes omitted (Etter *et al.*, 2011).

Magnesium carbonate (MgCO₃) on the other hand is a naturally occurring compound which is present in sedimental deposits. It is closely related to calcium carbonate and can be found in vast parts of the world. In higher concentration, the magnesium carbonate is present in the minerals magnesite (MgCO₃) and dolomite ((CaMg)CO₃). Since both minerals naturally occur in pure form, one can also speak of magnesite and dolomite stone. Magnesite is the mineral/stone name for magnesium carbonate and is therefore by definition almost completely made up of magnesium carbonate. Normally it only contains smaller amounts of side products especially iron, manganese, calcium, cobalt and nickel (Mindat, access 22.02.2009). Dolomite on the other hand is a compound of magnesium carbonate and calcium carbonate. The ration of magnesium to calcium ions is normally around 1:1. Minor occurring impurities are Fe, Mn, Co, Pb and Zn (Mindat, access 22.02.2009). Besides magnesium salts and magnesium carbonates, the magnesium oxides can be regarded as a third group of magnesium sources. Magnesium oxide (MgO) and magnesium hydroxide (Mg(OH)₂) are the most common compounds of this group. Magnesium hydroxide is normally produced from watery solutions containing magnesium chloride (e.g. seawater) whereas magnesia oxide is produced by heating up magnesium hydroxide or magnesium carbonate (a process called calcination) (Webelements, 2009). If magnesium carbonate is used as a source three grades have to be distinguished depending on the temperature of burning: caustic, hard burned and dead burned magnesium oxide. Since hard and dead burned magnesium oxides show practically no reactivity anymore, only caustic magnesium oxide is of interest as a magnesium source as both substances are gained through processing the traded grade is normally high, lower grade products mainly contain silicium dioxide, sulphate, calcium oxide and ferric oxides.

2.9.2 Micro-nutrients

Micro-nutrients are as essential for plant growth as macro-nutrients but are taken up in small (micro) amounts. The elements normally considered as micro-nutrients are boron, copper, iron, chloride, manganese, molybdenum and zinc (Frausto da Silva and Williams, 1997; Marschner, 1997). Most of the micronutrients are needed for formation of different enzymes. These nutrients are normally available in sufficient quantities through initial soil content and mineralization of organic material. Only in special circumstances do scarcity of micronutrients limit plant growth. When human excreta is used as a fertilizer, the risk for such deficiency is minimal as excreta contains all micronutrients necessary for plant growth. Table 2.4 shows the macro and micro-nutrients required by plants while Table 2.5 shows the functions and deficiency of various plant nutrients.

Macro-Nutrients		Micro-Nutrients
Primary Nutrients	Secondary Nutrients	
Nitrogen (N)	Calcium (Ca)	Zinc (Zn)
Phosphorus (P)	Sulphur (S)	Calcium (Ca)
Potassium (K)	Magnesium (Mg)	Copper (Cu)
		Molybdenum (Mo)
		Manganese (Mn)

Table 2.4: Macro and micro plant nutrients

Source: MoAC (2003)

Nutrient	Major functions	Deficiency symptoms of crop
Nitrogen	 i) Necessary in large quantity for all growing crops to promote growth of leaf and stem. ii) Increase plant vigour, improve succulence in leafy crops and improve their quality. iii) Increase protein content of food and fodder crops. 	Yellowing of old leaves, reduced tillering of cereals. If deficiency is severe, the whole crop appears yellowish and growth is stunted.
Phosphorus	i) Needed for flowering, fruiting, seed formation, root development and early plant development.ii) Strengthens straw and decreases loading.	Stunted growth, purple colour of older leaves, new leaves dark green, cereals' tillering is drastically reduced.
Potassium	i) Imparts increased vigour.ii) Makes plant stalks healthy and strong.iii) It is very important in reducing the effect of drought.	Deficiency symptoms are seen in older leave first. Overall slow growth, increased susceptibility to decreased loading.
Calcium	 i) More important in alkaline and acidic soils. ii) Necessary for normal cell division. iii) Helps in the development of the terminal buds. 	New leaves become white, growing points curl and die.
Sulphur	i) Required for the formation of chlorophyll; especially important for soy bean.	Pronounced retarding effect on plant growth, results in uniformly chlorotic plants, first chlorosis of younger leaves, under severe deficiency, the whole plant shows symptoms.
Magnesium	 i) More important in acidic soils ii) key element in chlorophyll molecule without which photosynthesis cannot occur. 	Marginal of interveinal chlorosis with pinkish colour of older leaves, sometimes leaf rolling like drought effect, plant susceptible to winter injury.

 Table 2.5: Functions and deficiency symptoms of macro plant nutrients

Source: MoAC (2003)

CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study design was experimental with field and laboratory components. The experiment was carried out in the laboratory of the Department of Environment Health Sciences at the College of Medicine, University of Ibadan.

3.2 Experimental Method

Experimental method included urine collection and storage, analysis of fresh and stored urine, the design of a reactor, preparation of magnesium chloride solution (MgCl₂), struvite production and green house experiment with *Amaranthus caudathus*.

3.2.1 Urine collection and storage

Urine was collected from a students' hostel at the University College Hospital for two months. To determine the average concentration of nutrients, the urine was thoroughly mixed together in an 100 litres plastic drum and analyzed for both physical and chemical parameters before storage. The urine was then stored in an airtight 100 litres plastic drum for three months. At the end of the third month, sample of the urine was analyzed. The urine was then stored for another two months. For each of the two months, the composition of the urine was analyzed in order to study the effect of storage on it.

Calculation of volume of urine required for the experiment;

From laboratory analysis of the urine, amount of phosphate (PO₄³⁻) in urine

= 45 mg/l = 0.045 g/l

Concentration in $mol/dm^3 = no$ of moles of solute/volume of solution (litre)

No of moles = mass/molar mass

Molar mass of $PO_4^{3-} = 31 + (4 \times 16)$

= 31 + 64 = 95

No of moles $= \frac{0.045}{95} = 0.0004737 \text{ mol/l}$

i. e, 0.0004737 M = 1000 ml of urine

to know the volume of urine with 1M concentration

1M = Vol

To get the volume of urine of 1M Concentration required for the experiment

 $= 1 \times 1000 \text{ ml} = 2111040.7 \text{ ml}$ approximately = 2000000 ml of urine

0.0004737

1000 ml = 1 litre

V = 2111 L approximately = 2000 L of urine

3.2.2 Physico–Chemical and Microbiological Analysis of fresh and stored urine **3.2.2.1** Total Solids

A clean dish of suitable size was dried in an oven at a temperature of $103 - 105^{\circ}$ C until a constant weight was obtained and cooled to room temperature in a desiccator. The weight was noted (w₁). Urine sample (250 ml) was then pipetted into the dish, after mixing thoroughly and then evaporated to dryness on a steam bath. The residue was then dried in an oven for an hour at 103-105°C. The dish was quickly transferred to a desiccator, cooled and weighed. The dish was returned to the oven and was further dried for another 20 minutes and reweighed after cooling to room temperature. The process was repeated until the weight of the dish plus residue was constant to within 0.05 mg (w₂). Total solids were obtained by subtracting the weight of dish from the weight of the dish and residue.

Total solids $(mg/l) = (w_1 - w_2) \times 1000$ ml sample ------Equation 6

3.2.2.2 Electrical Conductivity

Standard potassium chloride (0.01 M) – reference solution was prepared by dissolving 745.6 mg of dry anhydrous potassium chloride (A.R.) in freshly boiled CO_2 free double – distilled water, made up to one litre and stored in glass-stoppered Pyrex bottles. This standard reference solution has conductivity of 0.001413 μ Scm⁻¹ or 1,413 μ Scm⁻¹.

The above solution was used to calibrate the conductivity meter (Jenway 470, England) at a temperature of 25°C. Urine sample (100 ml) was measured into a beaker and the conductivity meter probe was inserted making sure it did not touch the beaker. The reading was then recorded from the liquid crystal display after it had stabilized

3.2.2.3 рН

The pH of the urine sample was determined using a Jenway pH meter (370 Model) manufactured in England. Urine sample (200 ml) was measured into a beaker. The pH electrode and meter were calibrated using buffer solutions of pH 7 and 4. After calibration the pH electrode was immersed in the urine sample making sure it did not touch the beaker. The pH reading was then taken from the liquid crystal display after it had stabilized.

3.2.2.4 Determination of copper, iron and zinc.

Copper, iron and zinc were determined using Atomic Absorption Spectrophotometric (AAS) method. The AAS has the (model number 210/211 vgp 2005 by Buck Scientific).

The reagents used were deionized water for preparation of all solutions; concentrated nitric acid and stock metal solution. Standard metal solutions were prepared by appropriate dilution of the stock metal solutions, a series of standard metals solutions in the range $5 - 1,000 \mu g/l$ i.e.

- 1. Zinc (Zn) standards 0,0.2, 0.4, 0.6, 0.8, 1.0 mg/I
- 2. Iron (Fe) standards 0,1,2,3,4,5 mg/I
- 3. Copper (Cu) standards 0,1,2,3,4,5 mg/I

Working standard of each of the elements was prepared from stock standard solution (1000 mg/I). At each time the hollow cathode lamp was fixed and the required wavelength was set. The instrument was put on for about 15 minutes in order to stabilize while the compressor to supply air at a regulated pressure was set on. The fuel acetylene was on and regulated, then the ignition control knob was pressed for flame to alight. Blank control was introduced flame which aspirated into flame. The blank control was adjusted to set zero absorbance. Working standards were introduced differently and adjusted until agreeable readings were obtained. The absorbance of standards was recorded against concentration in milligram per litre (mg/I). A graph of absorbance against concentration of standard was plotted. The elements in urine were determined by acidifying 100 ml of urine with 10 ml of concentrated nitric acid and the mixture was autoclaved at 121° C for one hour to solubilize the matter content and cooled. The digest was filtered then introduced into the Atomic Absorption Spectrophotometer (AAS) and the absorbance were recorded.

3.2.2.4.1 Copper

Stock Copper solution was prepared by dissolving 3.9296 g of copper sulphate $5 - hydrate CuSO_4.5H_2O$, in some water and made up to 1 litre (1 ml = 1 mg Cu). The standard copper solution was then prepared by withdrawing 5ml of the stock solution into a 100 ml flask and made up to mark.

3.2.2.4.2 Iron

Stock iron solution was prepared by dissolving 5.0503 g of iron (II) ammonium sulphate, Fe $(NH_4)_2$ (SO₄) in distilled water and made up to 1litre (1 ml = 1 mg Fe). The standard iron solution was then prepared by withdrawing 20ml of the stock solution and made up to 1 litre.

3.2.2.4.3 Zinc

Stock zinc solution was prepared by dissolving100mg 30 – mesh zinc metal in slight excess of (1+1) HCI – about 1ml is required and diluted to 1 litre with distilled water [1 ml = 100 μ g Zn]. The standard zinc solution was then prepared by withdrawing 10 ml of the stock solution was pipetted and diluted to1 litre with distilled water.

3.2.2.5 Determination of Ammonia

Ammonia was determined by direct nesslerization method, nessler reagent was prepared by adding cold saturated solution of mercury (II) iodide was prepared in 100 ml of distilled water and 25 g of potassium iodide in about 100 ml distilled water together. This solution was thoroughly mixed and added slowly while stirring to a cold solution of 100 g potassium hydroxide in 250 ml distilled water. The total mixture was diluted to 1 litre water and stored in a rubber – stoppered pyrex glass – ware and kept in a refrigerator.

EDTA Reagent was prepared by dissolving 50 g of disodium ethylene-diamine tetraethanoate (acetate) dehydrate in 60 ml distilled water containing 10 g sodium hydroxide. Heat was applied gently to complete the dissolution, cooled at room temperature and diluted to 100 ml.

One drop of EDTA reagent was added to 50 ml of urine sample to remove the precipitate of cations. Nessler reagent (2 ml) was added to the sample, a colour developed. Some of the solution was put into Lovibond tube and insert into casing. Distilled water was used as blank and placed in another tube and inserted into the casing. Urine sample was placed at the right hand side of the Lovibond comparator set. The permanent glass standards were turned until a match was obtained and the readings taken.

3.2.2.6 Determination of Chloride

Chloride was determined by Mohr's method, the principle behind this experiment is the titration of a known volume of $AgNO_3$ against a known volume of water sample. After which 1 ml or 2-3 drops of indicator was added to obtain a pinkish-yellow colour end-point.

Silver Nitrate (AgNO₃) solution was prepared by dissolving 1.1975 g of AgNO₃ in a beaker with a little distilled water. The mixture was thoroughly mixed and made to 500 ml in a flask.

Potassium chromate indicator was prepared by dissolving 5 g of Potassium chromate in a beaker containing distilled water, and then $AgNO_3$ solution was added drop wise until a definite reddish colour appeared. The mixture was then filtered and diluted to 100 ml with distilled water

Urine sample (50 ml) was measured and introduced into a conical flask and 3 drops of indicator was introduced using a 1ml pipette. AgNO₃ of 0.014 M was introduced into a burette mounted on a retort stand and the volume noted and recorded. The AgNO₃ was then titrated against urine sample until a pinkish-yellow end-point was obtained, the procedure was repeated for the blank sample and the volume of AgNO₃ used noted and recorded.

Calculations

 $Cl (mg/l) = (A - B) \times M \times 70,900$ Sample

Where $A = ml AgNO_3$ used for titrating sample

 $B = 0.2 \text{ ml AgNO}_3$ used for titrating blank

 $M = molarity of AgNO_3$.

3.2.2.7 Determination of Sulphate

Sulphate was determined by gravimetric method using Barium chloride solution and Hydrochloric acid as reagents. Urine sample (100 ml) was filtered using sintered-glass crucible having fine porosity with a maximum pore size of 5 microns. 1:1 HCl was added in drops until acid to litmus, three drops was added in excess and evaporated to 50 ml. The solution was boiled and boiling barium chloride solution was added until all the sulphate was

precipitated. The solution was digested on a water bath until the precipitate was settled. A sintered-glass crucible was dried to constant weight. The filtering equipment was connected to the vacuum pump and the precipitate was filtered through the sintered-glass crucible. The filtrate was washed many times with hot water until the filtrate was chloride-free (AgNO₃ test). The precipitate with crucible was dried in an oven at $103 - 105^{\circ}$ C to constant weight.

Calculation

 $SO_4 (mg/l) = mg BaSO_4 \times 411.5 / ml.$ sample

3.2.2.8 Determination of Calcium and Magnesium

Calcium and magnesium were determined by ETDA titration method. EDTA Solution of 0.01 M was prepared by dissolving 3.712 g of disodium dehydrate in water and made up to 1 litre mark with distilled water in 1000 ml flask. Standardization of EDTA was done by pipetting 25 ml zinc solution into a conical flask. Ammonium chloride (1 ml) buffer solution and a few drops of the indicator were added. The mixture was shaken and wine red color was produced, this was titrated with EDTA until the color changed from wine red to bluet. The volume of EDTA solution used was noted. The correct molarity of EDTA was calculated. Eriochrome (or Solochrome) Black T indicator Solution was prepared by dissolving 0.1 g of eriochrome black T indicator in 25 ml AR CH₃OH.

Distilled water (50 ml) was placed into 250 ml Erlenmeyer flask. 25 ml of conc. ammonia solution'10 drops of Ca and Mg solution, and 3 drops of eriochrome black T was added. The mixture was shaken to obtain wine red color. Urine sample (50 ml) was added to the Erlenmeyer flask and shaken (the initial wine red color was revived). Potassium cyanide (1.5 M KCN) of 2 ml was added to masked Zn, Cu, Co etc.; 2 ml of 2% hydroxyl amine hychloride to masked Mn and 1ml, 30% triethanol amine to masked Fe, Al etc. the mixture was titrated against standard EDTA with gentle shook to avoid splashing until blue end point was obtained. The volume of standard EDTA used was recorded. This volume was for both Ca and Mg This was labeled A.

Distilled water (50 ml) was placed into another 250 ml Erlenmeyer flask. 25 ml, 20% KOH was added, 10 drops of Ca and Mg solution was added, a few grains of 1% calcein indicator. The mixture was shaken to obtain light standard EDTA to obtain fluorescent yellow end point.

Urine sample (50 ml) was added to the flask and shaken to revive the initial light or leaf green color. Potassium cynide (1.5 M KCN) of 2 ml was added to masked Zn, Cu, Co etc.; 2 ml of 2% hydroxyl amine hydrochloride was added to masked Fe, Al etc. the solution was titrated against the standard EDTA, while shaken gently to avoid splashing, to obtain fluorescent yellow end point. The volume of standard EDTA used was recorded. This was for Ca alone. This was labeled B. Volume for Mg alone = A-B

Calculation

Ca (mg/l) = $40080 \times M \times B/$ ml. sample Mg (mg/l) = $24320 \times M \times (A-B)/$ ml. sample Where A = volume (ml) of EDTA for Ca and Mg titration B = volume (ml) of EDTA for Ca titration M = molarity of EDTA

3.2.2.9 Determination of Sodium

Sodium was determined by flame photometry method. Stock sodium solution was prepared by dissolving 2.542 g of dried NaCI crystals in a beaker with little water and diluted to 1 litre with distilled water in 1000 ml flask.

Intermediate sodium solution was prepared by pipetting 10 ml of stock solution into a flask and diluted to 100 ml with distilled water. Thus, 1 ml gave 0.1 mg also gave 100 ugNa. This was use to prepare calibration curve in the sodium range of 1.0 to 10 mg/l.

Standard sodium solution was prepared by dilution of 10 ml of the intermediate sodium solution to 100 ml with distilled water. This gave 1ml = 10 ugNa. This was used to prepare calibration curve in the sodium range of 0.1 to 1.0 mg/l.

The flame photometer was placed in a place where there is no direct ray of sunlight or constant light emitted by an overhead fixture and free from dust and tobacco smoke.

Urine sample (100 ml) was acidified 10 ml of concentrated nitric acid in a erlenmeyer flask, swirled to mix properly, heated for one hour and cooled. The mixture was then filtered, the digest was placed in the flame photometer and the emission was measured at 589 nm. A blank was ran in the same manner and the calibration curve was constructed from the sodium standards.

Calculation

Calculate Na (mg/l) = concentration reading on curve × D/volume (ml) sample

- D = dilution factor
- = vol. (ml) sample + vol. (ml) distilled water added / vol. (ml) sample
- D is taken as 1 if there is no dilution.

3.2.2.10 Determination of Total Nitrogen

Total nitrogen was determined using distillation followed by titration method, the preparation of the reagents are as follows;

Digestion solution was prepared by dissolving 134 g of potassium sulphate, K_2SO_4 , in 650 ml hot distilled water, cooled, and 200 ml conc. H_2SO_4 was added, stirred gently, diluted to 1 litre and mixed. Ammonia free water was obtained by adding 0.1 ml conc. H_2SO_4 to 1litre of distilled water and redistilled. Phosphate buffer solution, pH 7.4; 14.3 g of KH₂PO₄ and 68.8 of K_2HPO_4 were dissolved in ammonia free distilled water then made up to 1 litre. The NH₃-N on the buffer solution was determined. Dechlorinating agent, 0.007 M Na₂S₂O₃ was prepared by dissolving 0.09 g Na₂SO₃ in ammonia free water and diluted to 100 ml. It was prepared fresh before use.

Neutralization reagent was made by adding 11 tre of 1 M NaOH and 0.5 M H_2SO_4 to ammonia- free distilled water.

Urine sample (5 ml) was pipetted into kjeldahl flask, 10ml digested potassium sulphate solution was added and mixed. A small funnel was placed on the flask to help the digestion

and prevent loss. It was heated gently with under a hood and when the solution was clear, it was boiled briskly for thirty minutes and cooled. A blank was ran in the same manner.

The content of the flask was transferred into a beaker and washed with 36 ml 1 M NaOH to neutralize the acid medium. It was adjusted to pH 7. The sample was transferred into a 100 ml volumetric flask and made up to mark. 25 ml of the digested urine sample was diluted to 25 ml and put into 50 ml volumetric flask.

Micro-Kjeidahl steam distillation apparatus was set up and the distillation of the sample was carried out. The distillate was collected in conical flask. The ammonia in the distillate was titrated against standard acid to the end point.

Calculation

 $Mg/NH_3N = 28$ (A-B) 1,000 M/ml sample

Where

 $A = ml H_2SO_4$ titration for the sample

 $B = ml H_2 SO_4$ titration for the blank

 $M = Molarity of H_2SO_4$

3.2.2.11 Determination of Phosphate

The determination of phosphate was done by Vanado-Molybdo-Phosphoric acid Colorimetric method, using Phenolphthalein indicator solution, Concentrated Hydrochloric acid, Activated carbon, Vanadate-molybdate reagents and spectrophotometer at wavelength of 65-690 nm.

The Vanadate-molybdate reagents was prepared by weighing into a flask 25 g ammonium molybdate, $(NH_4)_6 Mo_7O_{24}.4H_2O$ was weighed and dissolved in 200 ml distilled water (solution A). Solution B was prepared by weighing 1.25 g ammonium trioxo-vanadate (v), NH_4VO_3 into a flask and dissolved by heating to boiling in 300 ml distilled water, cooled and 330 ml conc. HCl was added at room temperature. Solution A was poured into solution B and diluted to one litre.

Standard phosphate solution was prepared by preparing weighing 0.2195 g anhydrous potassium dihydrogen phosphate, KH_2PO_4 into a flask and dissolved in distilled water then diluted to one litre. In this solution 1 ml = 0.05 mg PO_4 – P (or 50 µgPO_4⁻³ – P). From this solution, various concentrations with 100 ml portions were prepared. The color was developed. Absorbance against concentrated was measured and the curve was plotted.

Excess color was removed from urine sample by shaking 50 ml with about 0.2 g activated carbon of type no. 330332 in an Erlenmeyer flask for 5 minutes. The solution was filtered through Whatman filter paper No. 42. 25 ml of the filtered solution was measured into 50 ml volumetric flask. Vanadate-molybdate (10 ml) reagent was added and diluted to the mark with distilled water. A blank was prepared using 25 ml distilled water was substituted for the sample solution. After 10 minutes of the vanadate-molybdate reagent, the absorbance of the sample against the blank was measured at 470 nm.

Calculation

Mg/l $PO_4^{3^-}$ -P = Reading from curve × 1000 × D/ ml sample Where D= dilution factor

3.2.2.12 Determination of Carbonate

Carbon (iv) oxide free distilled water : all stock and standard solutions, and dilution water was prepared for standardization procedure with distilled water freshly boiled for 15 minutes to expel CO_2 and then cooled to room temperature. The pH should not be less than 6.

Standard 0.02M HCI: 8.3 ml conc. HCl was pipetted into 1 litre conical flask and diluted to mark. Stock solution of 0.01 M (200 ml) was diluted to 1 litre with CO_2 free distilled water. The acid 0.02M was standardized against 0.01M sodium carbonate using phenolphthalein and total alkalinity indicator, and the same time interval as for the sample.

Methyl orange indicator, 0.05%: 0.05 of methyl orange was dissolved in about 100 ml CO_2 – free distilled water.

Urine sample (50 ml) was put into a clean conical flask. one drop of 0.05 M sodium thiosulphate solution was added to remove free residual chlorine, 2 drops phenolphthalein

indicator was added to urine sample. The titration was carried out with 0.02 M of standard acid (HCI)

Calculation Total alkalinity as mg/L CaCO₃ = Vp \times M \times 100,000/ml sample Where M is the molarity of the acid used. Vp = volume of acid used

3.2.2.13 Isolation of organisms from fresh and stored urine samples

3.2.2.13.1 Preparation of media

Nutrient Agar, Potatoes Dextrose Agar, De man Rogosa sharpe Agar was used for total fungal count.

Nutrient agar – Two (2 g) of the nutrient Agar was homogenized in 11itre of distilled water using a water bath at 100° c. This was then autoclaved at 121° C for 15 minutes. Medium was cooled to 45° C after autoclaving before pouring into plates and used for subsequent bacteria plating.

MacConkey Agar – Fifty two (52 g) was measured into 1 litre of distilled water and mixed thoroughly. The solution was heated and for one minute to completely dissolve the medium, Autoclaved at 121^oC for 15 minutes. MacConkey agar is a selective and differential medium designed to selectively isolate gram-negative and enteric bacilli bacteria.

De man Rogosa sharpe Agar – Seventy (70 g) was measured in 11itre of water, heated with frequent agitation and boiled for one minute to completely dissolve the medium. The mixture was autoclaved at 121^{0} C for 15 minutes. This type of bacterial growth medium is to favour the growth of lactobacilli and suppress the growth of many competing bacteria.

Potato Dextrose Agar -39 g in 1 litre + streptomycin (added to impede bacteria growth). This is a common microbiological growth medium and made from potato infusion, and dextrose, it is the most widely used for growing fungi.

3.2.2.13.2 Isolation of microorganisms from fresh and stored urine sample

Serial dilution -1 ml of the urine sample was measured and subjected to serial dilutions with the range of 10^{-1} and 10^{-4} . Each sample was thoroughly mixed with 9 ml of distilled water to give 10^{-1} dilution. 1 ml of 10^{-1} dilution was also pipette into another 9 ml of sterile distilled water in screw capped bottles to give 10^{-2} . This was repeated for 2 other screw capped bottles that have been filled with 9 ml of distilled water to give 10^{-3} and 10^{-4} dilution respectively. Isolation organisms on a medium (Nutrient Agar) - This was done using pour plate method (Hamyan and Mucous, 1976). A sterile pipette was used to measure 1ml out of 2 dilutions and it was pipette into sterile petri dishes and molten agar at 45° C was poured on it. It was swirled gently for even distribution of inocula in agar. After solidification, the plates were inverted and incubated in an incubator at 30° C for 48 hours.

The plates containing bacteria using MacConkey Agar were allowed to stay overnight (24 hrs) at 45°C while that of PDA was incubated for 3-5 days at 30°C. It is expected that bacteria will grow on nutrient Agar while fungi will grow on PDA.

Total Bacterial and total fungi count were done by counting the different colonies on the agar plates after incubation and multiplying with dilution factor.

Preparation of pure cultures of isolates.

Brilliant green lactose bile broth produced by OXOID Limited, England was used as culture media for the confirmation test.

Preparation of culture media

The culture media (MacConkey Broth, MacConkey Agar, Nutrient Agar and Brilliant Green) were prepared according to manufacturers' instructions and autoclaved at 121°C/15 PSI for 30 minutes. The brilliant green lactose bile broth used was prepared carefully by weighing 28 grammes of the powdered broth and dissolved in 1 litre distilled water.

Inoculation of the media

One (1ml) of urine (fresh and stored) and dilutions thereof were inoculated into sterile MacConkey Broth and incubated at 37°C for 24 hours. The presumptive fermentation tube showing positive result were gently shaken and with a sterile (the loop was sterilized between

successive transfers by heating in a flame until it is red hot and was allowed to cool before use) wire loop, one loopful of culture was transferred into a fermentation tube containing brilliant green lactose bile broth. Five (5) mls of the prepared media were measured into each of fermentation tubes in the number of tubes (with Durham tubes) equal to the number of the tubes that were detected positive in the presumptive test. The inoculated brilliant green lactose bile broth tubes were incubated for 24 to 48 hours at 44° C.

Detection and Enumeration

For the presumptive, formation of gas in the Durham tubes and change in odour from purple to yellow were regarded as positive. For the confirmed test; at any time within the period of 24 to 48 hours of incubation, formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube were regarded as positive confirmed test. The number of positive for sample dilution were recorded and the patterns of positive results were compared with a most probable number table.

3.2.3 Design of reactor

A 50 litre plastic bucket and a plastic funnel were melted together to make the reactor (Plate 3.1). At the end of the fabrication, the result was a plastic reactor with a cone shape. A metal stirrer was inserted into the bucket through the cover to allow for thorough mixing and filtration of the mixture.

3.2.4 Preparation of magnesium chloride

Magnesium chloride (1.2 M) was prepared by dissolving 243.6 g of MgCl₂ .6H₂O in distilled water and made up to 1000 ml with distilled water in a standard flask. Thereafter 1.5 M and 1.8 M MgCl₂ .6H₂O were prepared by dissolving 304.5 g and 365.4 g in distilled water respectively. These were transferred into 1000 ml flasks each and made up to mark.

3.2.5 Struvite Production

Struvite production was carried out using different concentrations of magnesium chloride (1.2 M, 1.5 M and 1.8 M) with 20 L of urine. Magnesium chloride solution of 1.2 M was added to 20 L of urine, stirred for 30 minutes and then left to settle for 3hrs. The mixture was

filtered and the precipitate (Plate 3.2) was collected in filter bag attached to the outflow of the reactor. The procedure was repeated for other concentrations; 1.5 M and 1.8 M. The method was adapted from Etter *et al.*, (2011).



Plate 3.1: The plastic reactor used in the production of struvite



Plate 3.2: Struvite prepared from urine

3.2.6 Screenhouse Experiment

Amaranthus caudatus vegetable was planted in the screenhouse of the Department of Agronomy, University of Ibadan (7°24'N, 3°54'E, 234 m above sea level).

3.2.6.1 Experimental design and Treatments

In the screen house experiment, the design was Completely Randomized Block Design with three replicates. There were seven treatments and the control; each treatment was replicated three times making a total of twenty four experimental pots. The experimental layout was randomized in triplicates with seven treatments and control as shown in Table 3.1. The viability of the amaranth seed was tested before planting by springling some of the seed on water-soaked cotton wool, there was growth of the seed on the third day.

The treatments applied were; inorganic fertilizer (NPK 15-15-15 which was collected from Agronomy Department); struvite prepared with 1.2 M, 1.5 M and 1.8 M MgCl₂, and a control with neither inorganic fertilizer nor struvite was put in place. The quantity of struvite required for 2 kg soil for the greenhouse experiment was derived from the following:

100 kg of Nitrogen is required for one hectare of land (Richert *et al.*, 2010; Cofie *et al.*, 2010).

 $100 \text{ kgN} = 2 \times 10^6 \text{ kg of soil} = 1 \text{ hectare of land}$

100 kgN/ha to 2 kg soil

Nitrogen content of struvite (1.2 M MgCl₂) was 1.28%. Therefore, 7812.5 kg of struvite (1.2 M MgCl₂) was required for 2×10^6 kg soil; 7.81 g of struvite (1.2 M MgCl₂) was required for a 2 kg soil. Nitrogen content of struvite (1.5 M MgCl₂) was 2.29% of nitrogen, therefore 2×10^6 kg soil required 4366.8kg of struvite (1.5 M MgCl₂); 4.37 g of struvite was (1.5 M MgCl₂) required for 2 kg soil. Nitrogen content of struvite (1.8 M MgCl₂) was 2.23%. For 2×10^6 kg soil, 4484.3049 kg of struvite (1.8 M MgCl₂) was required for planting, therefore for 2 kg soil; 4.48 g of struvite (1.8 M MgCl₂) was required for planting.

For effluent:

 2×10^6 kg soil = 100 kgN 2×10^4 kg soil = 1 kgN 1kg soil = $\frac{1}{2 \times 10^4}$ = 0.00005 kg 2 kg soil = 0.00005 kg×2 = 0.0001 kgN = 0.1 gN = 1 mlN

Nitrogen content of effluent 1.2 M MgCl₂ was 0.89 ml present; 112 ml of effluent 1.2 M MgCl₂ was required to obtained 1mlN required for 2 kg soil. Nitrogen content of effluent 1.5 M MgCl₂ was 0.97 ml, 103 ml effluent 1.5 M MgCl₂ gave 1 mlN required for 2kg soil. Nitrogen content of effluent 1.8 M MgCl₂ was 0.47 ml, 213 ml effluent 1.8 M MgCl₂ gave 1 mlN required for 2 kg soil.

Quantity of inorganic fertilizer (NPK 15-15-15) require for 2 kg soil:

For every 100 kg of NPK, 15 kg nitrogen was present

100 kgN/ha = 2 kg soil

1ha land $(2 \times 10^6 \text{ kg soil})$ required 666.66667 kg of NPK $2 \times 10^6 \text{ kg soil}$, therefore 0.67 g of NPK is required for 2 kg soil.
Table 3.1: Experimental layout

Struvite 1.2M (6)	NPK (22)	Struvite 1.5M (9)	Effluent 1.5M (16)
NPK (24)	Effluent 1.8M (20)	Struvite 1.2M (4)	Struvite 1.5M (7)
Effluent 1.2M (15)	Struvite 1.2M (5)	Struvite 1.8M (11)	Effluent 1.2M (13)
Struvite 1.5M (8)	Control (1)	Effluent 1.2M (14)	Struvite 1.8M (10)
Control (2)	Control (3)	NPK (23)	Effluent 1.5M (17)
Effluent 1.5M (18)	Effluent 1.8M (21)	Struvite 1.2M (12)	Effluent 1.5M (16)

3.2.6.2 Soil Preparation and application of Treatments

The soil used was sieved using a 2 mm sieve before weighing into the polyethylene pot. Each treatment was thoroughly mixed with 2 kg soil and poured into the polyethylene pot to a height of 15 cm. The treatments were replicated three times yielding a total of 24 pots for planting. After the application of the treatments, the soils in the pots were left for two days before planting but wetting was done for those two days.

3.2.6.3 Determination of Growth Parameters

Plant growth parameters (plant height, stem girth, number of leaves) were taken at second, third and fourth weeks after sowing. At the end of the fourth week, the plants were harvested and the wet weight of the plants were also taken after which the plants were oven dry at temperature of $60 - 70^{\circ}$ C until a constant dry weight was obtained. The Fresh and dry weight of leaves, root and stem were also taken.

3.2.6.4 Statistical Analysis

All parameters measured were subjected to analysis of variance (ANOVA) and means were separated using Least significance difference (LSD) at p <0.05.

CHAPTER FOUR

RESULTS

This chapter presents the findings of the study. Physicochemical analyses of urine sample both fresh and stored were presented first, followed by the morphological characteristics of the plant in the control (soil without either struvite or NPK fertilizers), struvite treated soil and NPK treated soil.

4.1 Characteristics and effect of storage on the physicochemical quality of urine samples

Table 4.1 shows the mean results of the physico-chemical analysis of fresh and stored urine samples, there was a wide range of increase in Ammonia and Total Nitrogen of the stored urine which were also far from the range of a normal urine. It showed there was increase in almost all the parameters of stored urine from the fresh except for copper, zinc, iron, organism isolated, and coliform count. Almost all the parameters increased after storage except for sulphate, carbonate, copper and zinc.

The Table also compares the parameters of fresh and stored urine. It shows that the variation of the parameters in the stored urine samples were not significant (p>0.05) compared to fresh urine sample except for pH which was 6.50 ± 0.10 for fresh and 9.03 ± 0.15 for stored, Electrical conductivity 540.00 ± 0.00 for fresh and 1079.00 ± 41.01 for stored, potassium (K) 32.00 ± 0.00 for fresh, 35.33 ± 0.57 for stored and ammonia (NH₃) 0.26 ± 0.00 for fresh, 29.66 ± 4.72 for stored urine. The pH and calcium of stored urine were within the range of stored normal human urine.

4.2 The microbial quality of urine

The microbiological characteristics also show that neither coliform organism nor fungi was found in the stored urine as shown in Figure 4.1.

4.3 Comparison of the composition of struvite with fresh and stored urine

Struvite production increased with increase in molarity of magnesium chloride (MgCl₂.6H₂O) used. Thus, struvite production from 1.2 M, 1.5 M, and 1.8 M of magnesium chloride yielded 23.303 g, 34.470 g and 41.240 g respectively as shown in Figure 4.2.

The results of laboratory analysis of the quality of fertilizer (struvite) used was presented in Table 4.3 which compared the mineral content present in struvite, fresh and stored urine. It shows that the phosphate, carbonate, magnesium and sulphate present in struvite were higher and significantly different when compared to that of fresh and stored urine with the following values; 471.66 ± 61.71 (PO₄²⁻), 2511.00 ± 228.37 (Mg) and 76.66 ± 10.40 (SO₄²⁻) respectively.

PARAMETERS	FRESH URINE (M±SD)	STORED URINE (M±SD)	*STORED NORMAL HUMAN URINE	T-TEST	P- VALUE
pH	6.5±0.10	9.03±0.15	4.6-8	24.03	0
Total Solids	3.50±0.10	3.03±0.15	36,700-46,700	4.427	0.11
Electrical Conductivity (µs)	534.00±12.16	1079.00 ±41.01		23.25	0
Ammonia (mg/l)	0.26±0.20	29.66±4.72	200-730	10.765	0
Total nitrogen (mg/l)	1080.33±145.22	1358.66±183.90		2.057	0.109
K (mg/l)	32.00±3.00	35.33±0.57	730-2,610	1.89	0.003
PO ₄ ³⁻ (mg/l)	42.66±3.05	45.66±3.05	470-1,070	1.203	0.295
Na (mg/l)	847.00±2.64	690.33± 350.17	1,170-4390	0.775	0.482
Cl (mg/l)	237.66±7.50	253.66±9.29	1,870-8,400	2.32	0.081
SO ₄ (mg/l)	4.83±1.04	4.66±0.28	77-470	0.267	0.802
CO ₃ (mg/l)	13.33±0.76	11.33±0.76	60-100	3.207	0.033
Mg (mg/l)	2.63±0.15	2.68 ± 0.16	20-205	0.391	0.716
Ca (mg/l)	36.16±1.04	41.66±2.88	30-390	3.01	0.04
Cu (mg/l)	0.06±0.01	0.05±0.00	1.732	1.732	0.158
Zn (mg/l)	0.03±0.01	0.02 ± 0.00	1.941	1.941	0.192
Fe (mg/l)	0.39±0.27	0.60±0.00	1.297	1.297	0.264

Table 4.1: The effect of storage on the physicochemical quality of urine

*Characteristics of normal human urine

Source: David, 1971



4.1: Microbial quality of urine



Figure 4.2: Quantity of struvite produced per concentration of magnesium chloride

PARAMETERS	FERTILIZER(STRUVITE (M±SD)	FRESH URINE (M±SD)	STORED URINE (M±SD)	P VALUE
Total Nitrogen (mg/l)	193.33±56.66	1246.00±1.00	1188.66±331.87	*0.001
K (mg/l)	34.56±2.18	32.00±3.00	35.33±0.57	*0.000
P O ₄ ³⁻ (mg/l)	471.66±61.71	46.00±1.00	45.66±3.05	*0.000
SO4 ²⁻ (mg/l)	76.66±10.40	4.60±0.10	4.66±0.28	*0.000
CO ₃ ²⁻ (mg/l)	14.40±2.00	13.33±0.76	11.33±0.76	*0.001
Mg (mg/l)	2511.00±228.37	2.63±0.15	2.68±0.16	*0.000

 Table 4.2: Comparison of the composition of Struvite with fresh and stored urine

*Significant at p<0.05

4.4: Effect of fertilizer treatments on of leaves

Table 4.3 showed that for week two vegetables treated with NPK had the highest number of leaves with the value 11.00 follow by effluent of (1.2 M MgCl₂ and 1.5 M MgCl₂) with values 10.67 and 10.33. Struvite of (1.5 M MgCl₂ and 1.8 M MgCl₂) had the highest number of leaves for week three and four, the values were the same for week four, these were 14.67and 15.33 while struvite of (1.2 M MgCl₂) comes up next with 12.33 for week three and effluent of (1.2 M MgCl₂) for week four.

4.5: Effect of fertilizer treatments on plant height (cm)

Figure 4.2 shows that for week two vegetables treated with NPK had the highest plant height 12.33 cm followed by effluent of (1.2 M MgCl₂) then struvite of (1.8 M MgCl₂) with values 10.00 cm and 9.75 cm respectively, control had the least value of 6.83 cm. In week three, the table also showed an increase in plant height (cm 19.08 >1 8.42 > 14.83 > 12.33 for Struvite (1.5 M, 1.8 M and 1.2 M of MgCl₂) and NPK respectively. Likewise for week four, there was variation in the plant height 29.10 > 28.00 for struvite (1.5 M of MgCl₂ and 1.8 M of MgCl₂). The least value was 12.90 cm for effluent of (1.2 M MgCl₂).

4.6: Effect of fertilizer treatments on stem girth (cm)

Table 4.4 showed that for week two, 0.153 cm is the highest stem girth from vegetable treated with NPK followed by struvite of (1.2 M MgCl₂), the least value of stem girth was 0.087 cm which was from effluent of (1.5 M MgCl₂) and struvite of (1.5 M MgCl₂). For week three, 0.433 cm the highest value was from struvite of (1.8 M MgCl₂) while 0.210 cm from effluent of (1.5 M MgCl₂). The highest value of stem girth for week four which was 0.480 cm was from vegetables treated with struvite of (1.5 M MgCl₂) while 0.257 cm the lowest value was from vegetables treated with effluent of (1.5 M MgCl₂).

4.7: Effect of fertilizer treatments on fresh weight of Amaranth plant (g)

Table 4.6 shows the wet weight of the *Amaranthus caudatus* (vegetable) after harvest with vegetables treated with struvite produced using 1.5 molar concentration of magnesium chloride solution had the highest weight of number of leaves 15.80 g while the vegetable treated with effluent of (1.5 M MgCl₂) had the lowest weight of 2.60 g.

Fresh weight of vegetable root treated with struvite of (1.5 M MgCl₂) had the highest weight of 3.82 g while 0.44 g from vegetable root treated with effluent of (1.5 M MgCl₂) treated vegetables.

The highest fresh weight of the stem was 12.46 g from struvite of (1.5 M MgCl₂) treated vegetables while 1.73 g from effluent of (1.5 M MgCl₂) treated vegetables.

4.8: Effect of fertilizer treatments on dry weight of Amaranth plant (g)

Figure 4.3 below shows the wet weight of the *Amaranthus caudatus* (vegetable) after harvest with vegetables treated with struvite produced using 1.5 molar concentration of magnesium chloride solution had the highest of number of leaves weight of 2.88 g while the leaves of the vegetable treated with effluent of (1.5 M MgCl₂) had the lowest weight of 0.34 g.

Dry weight of root struvite of (1.5 M MgCl₂) treated vegetables had the highest weight of 0.79 g while the least weight was 0.06 g from effluent of (1.5 M MgCl₂) treated vegetables.

The highest dry weight of the stem was 1.82 g from struvite (1.8 M MgCl₂) treated vegetables while 0.12 g from effluent 1.5 treated vegetables. The pictures of *Amaranthus caudatus* planted with struvite of (1.2 M MgCl₂), struvite of (1.5 M MgCl₂) struvite (1.8 M MgCl₂) NPK and control are presented in Plate 4.1 to 4.7 where (a) represent control, (b) represent struvite of (1.2M, 1.5M &1.8M) and effluent of (1.2M, 1.5M &1.8M) and (c) represent NPK. Plate 4.7 shows the overall experiment.

Treatment	Week 2	Week 3	Week 4
Control	9.67	13.00	13.33
NPK	11.00	12.33	13.00
Struvite 1.8	9.33	14.67	15.33
Struvite 1.5	9.67	14.67	15.33
Struvite 1.2	9.33	12.33	12.67
Effluent 1.8	9.33	11.33	13.33
Effluent 1.5	10.33	9.00	11.67
Effluent 1.2	10.67	11.33	14.67
NG Not Significant	NS	NS	NS

 Table 4.3: Effect of fertilizer treatments on leaves of Amaranthus caudatus

NS – Not Significant



Fig. 4.3: Effects of fertilizer treatment on plant height (cm) of Amaranthus caudatus

Treatment	Week 2	Week 3	Week 4
Control	0.097	0.317	0 343
Control	0.077	0.517	0.010
NPK	0.153	0.357	0.403
	0.100	0.400	0.470
Struvite 1.8	0.120	0.433	0.460
Struvite 1.5	0.093	0.413	0.480
Struvite 1.2	0.107	0.317	0.400
Effluent 1.8	0.093	0.330	0.357
Effluent 1.5	0.087	0.210	0.257
Effluent 1.2	0 120	0 347	0.403
Lindont 1.2	0.120	0.5 11	0.105
	NS	NS	NS
NS – Not Significant; Means ($n = 3$) followed by different lower case letter are significant at			

Table 4.4: Effect of fertilizer treatments on stem girth of Amaranthus caudatus

p = 0.05

Not Significant; Means (n = 3) followed by different lower case letter are significant at 05

Treatment	Number of leaves	Root	Stem
Control	5.70	1.62	4.3bc
NPK	4.70	1.25	4.01bc
Struvite 1.8	14.60	3.99	10.86ab
Struvite 1.5	15.80	3.82	12.46a
Struvite 1.2	7.70	1.98	5.21abc
Effluent 1.8	10.40	2.15	7.15abc
Effluent 1.5	2.60	0.44	1.73c
Effluent 1.2	12.90	2.74	10.19ab
	NS	NS	

NS – Not Significant; Means (n = 3) followed by different lower case letter are significant at p = 0.05



Figure 4.4: Effect of fertilizer treatments on dry weight of growth parameters (g) of Amaranthus caudatus



Plate 4.1a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.1b: Amaranthus caudatus grown with struvite 1.2M in week 4



Plate 4.1c: Amaranthus caudatus grown with NPK in week 4



Plate 4.2a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.2b: Amaranthus caudatus grown with struvite 1.5M in week 4





Plate 4.2c: Amaranthus caudatus grown with NPK in week 4

Plate 4.3a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.3b: Amaranthus caudatus grown with struvite 1.8M in week 4





Plate 4.3c: Amaranthus caudatus grown with NPK in week 4

Plate 4.4a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.4b: Amaranthus caudatus grown with Effluent 1.2M in week 4



Plate 4.4c: Amaranthus caudatus grown with NPK in week 4



Plate 4.5a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.5b: Amaranthus *Caudatus* grown with Effluent 1.5M in week 4



Plate 4.5c: Amaranthus caudatus grown with NPK in week 4



Plate 4.6a: Amaranthus caudatus grown without treatment (control) in week 4



Plate 4.6b: Amaranthus caudatus grown with Effluent 1.8M in week 4



Plate 4.6c: Amaranthus caudatus grown with NPK in week 4



Plate 4.7: Overall experiment

CHAPTER FIVE

DISCUSSION

5.1 The effect of storage on the physico-chemical quality of urine

The mean pH value for fresh urine was 6.40 (± 0.00) which was slightly acidic while the mean pH for stored urine was 9.03 (± 0.15) which are similar to the figures started by (Hoglund, 2001; Zaixing Li et al., 2012; Tunay et al., 1997). The authors reported that the pH of fresh urine is normally between 4.8 and 7.5 but after storage the pH increased to 9.0. The main nitrogen source in stored urine is ammoniacal nitrogen, with bicarbonate as the main anion (Kirchmann and Pettersson, 1995). Urea and urate decompose during storage and may account for the high pH value that was measured for stored urine (Kirchmann and Pettersson, 1995). The nitrogen content of the urine used for planting was 1.25 g/l, this was in harmony with the findings of Richert et al. (2010) reported that urine or urine and water, when diluted is assumed that the urine mixture has at least pH 8.8 and a nitrogen concentration of at least 1 g/l. Total solids present in the fresh and stale urine were 3.5% and 3.03% respectively. The values are similar to those of Chaggu, (2004) and Nwaneri et al., (2008) (1.3-4%). Fresh urine showed the concentration of ammonia and phosphates were 0.26 mg/l and 42.66 mg/l respectively. However, during storage, these two parameters were altered due to decomposition process. The concentration of ammonia increased while the concentration of phosphate reduced. The changes agreed with the work of Gethke et al., (2007). The concentrations of calcium in both fresh and stored urine were within the range of normal human urine (David, 1971). The mean concentrations (mg/l) of potassium, sodium, choride, sulphate, carbonate, magnesium, copper, zinc, iron, value for fresh and stored urine were not within the range of normal human urine. The variation might be due to the difference in food consumption. Lennartsson and Ridderstolpe (2001) observed that the exact nutrient content of urine depends on the food consumed by individuals. Alfred, (2010), also confirmed that the actual amounts of these minerals will vary from one person to another and also from country to country depending on the national diet. Ammonia and pH shows significant changes during storage, this agreed with the work of Udert *et al.*, (2006).

5.2 Microbial quality of urine

In a healthy individual, the urine is sterile in the bladder. When transported out of the body different types of dermal bacteria are picked up and freshly excreted urine normally contains <10 000 bacteria per ml (Tortora *et al.*, 1992). The total viable bacterial and fungal counts of fresh and stored urine were 1150 cfu/ml, 165 cfu/ml and 0.00 cfu/ml, 0.00 cfus/ml which are below that found in normal urine. It was also reported by Feachem *et al.* (1983) that pathogens causing venereal diseases may occasionally be excreted in urine but there is no evidence that their potential survival outside the body would be of health significance. According to Höglund (2001), pathogens that may be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are thus not considered to constitute a health risk related to the reuse of human urine in temperate climates. In general, pH values greater than about 9.0 are detrimental to all microbial growth (Prescott *et al.*, 1990).

5.3 Comparison of the composition of Struvite with fresh and stored urine

Comparing the composition of struvite to urine, it was discovered that struvite has the highest value of phosphate being 471.66 mg/l (\pm 61.7) while the value of fresh and stale urine are 46.00 mg/ml (\pm 1.00) and 45.66 mg/ml (\pm 3.05) respectively. This is in agreement with the work of (Etter *et al.*, 2010). The high magnesium content struvite 2511.00 mg/l (\pm 228.37) agree with the work of De-Bashan and Bashan (2004). Magnesium is the vital element of chlorophyll, which is responsible for the green coloration of the plant (Gaterell, 2000).

5.4 Effect of fertilizer treatments on plant growth parameters

The plants in all four treatments grew well. For assessment of the growth rate, the differences among treatments in plant height stem width and numbers of leaves were compared:

5.4.1 Plant height

Amaranthus caudatus planted with struvite produced from 1.2 M of $MgCl_2$ was not significant in height when compared with control and inorganic fertilizer (NPK) from the second week of planting to harvest (week four). The effect of struvite produced using magnesium chloride of 1.5 molar concentrations on *Amaranthus caudatus* was not significant

in the second` week when compared with control but the plant height of *Amaranthus caudatus* treated with NPK was significant in the second week of planting with the value 13.17 cm compared to control with the 6.83 cm and struvite 1.5 M MgCl₂ of 8.58 cm. In the third week, plant height of struvite (1.5 M MgCl₂ treated vegetable was significant with 19.08 cm for struvite 1.5 M MgCl₂, 13.17 cm for NPK and 12.83 cm for control. For week four there was a significant difference (p<0.05) in the plant height when compared with control and NPK 29.08 cm for struvite 1.5M MgCl₂ while 20.00 cm for control and NPK.

Likewise, the effect of struvite produced using magnesium chloride of 1.8 molar concentrations on plant height was significantly different (p<0.05) in the second, third and fourth week when compared with control and NPK with these; 9.75 cm, 18.42 cm and 28.00 cm respectively. For control 6.83 cm, 12.83 cm and 20.00cm; for NPK 12.33 cm, 13.17 cm and 20.00 cm. These agree with the report of Yetilmezsoy *et al.* (2013); Alysa Stafford and Phillip Barak (2006) which says that plants fertilized with struvite grew faster than those planted with other treatments.

Amaranthus caudatus planted with the effluent of the struvite prepared from 1.2 M of MgCl₂ was significantly different (p<0.05) for the second, third and fourth week with these results; 10.00 cm, 12.00 cm, 24.50 cm when compared with control. However vegetable grown with effluent of the struvite prepared from 1.2 M of MgCl₂ was only significantly different (p<0.05) when compared with the value for *Amaranthus caudatus* treated with NPK for week three with 12.00cm and 12.33cm for NPK. The effluent obtained from struvite using 1.8 M of MgCl₂ was only significant in the fourth week (25.20 cm) while the effluent obtained from the production of the struvite using 1.5 M concentration of magnesium chloride was not significant from the second to third week when compared with control and NPK.

5.4.2 Stem girth

Amaranthus caudatus planted with struvite (1.2 M, 1.5 M and 1.8 M) were not significantly different (p<0.05) in stem width when compared with control and NPK from the second to third week of planting. However struvite (1.5 M MgCl₂ and 1.8 M MgCl₂) gave the highest stem girth in the third and fourth week with the value; 0.413 cm and 0.480 cm; 0.433 cm,

0.460 cm respectively. Also the effluent of struvite (1.2 M, 1.5 M and 1.8 M) on *Amaranthus caudatus* were not significantly different (p<0.05) from the second to third week when compared with both control and NPK. The values for the third and fourth week were for effluent (1.2 M MgCl₂); 0.347 cm, 0.403 cm, for effluent (1.5 M MgCl₂); 0.210 cm, 0.257 cm, for effluent (1.8 M MgCl₂); 0.330 cm, 0.357 cm, for NPK; 0.357 cm, 0.403 cm. This finding agree with YingHoa *et al* (2011)'s report that stem circumference for struvite treated plant had the highest value.

5.4.3 Number of leaves

There was no significant difference (p<0.05) in the mean number of leaves produced by *Amaranthus caudatus* using different fertilizers. However, struvite of 1.5 M MgCl₂ and 1.8 M MgCl₂ (15.33) followed by effluent of 1.2 M MgCl₂ (14.67), effluent (1.5 M MgCl₂) (11.67), effluent of 1.8 M MgCl₂ (13.33), NPK (13.33), struvite of 1.2 M MgCl₂ (12.67), control (13.00) gave the highest mean number of leaves. This agree with the work of YingHoa *et al* (2011) which states that Leaf number was significantly different between control and treated groups (p<0.05), but struvite produced the highest number of leaves. The leaf number is dependent on several environmental factors including nutrient levels in the soil. The low number of leaves in control might be due to senescence, which is also caused by the low nutrient status of the soil (Gungula *et al.*, 2005)

5.4.4 Fresh Weights of plant

The wet weights of leaves and root of *Amaranthus caudatus* grown using struvite, effluent, NPK and control were not significantly different when compared with each other. The treatment with the highest weight of leaves and root was struvite of 1.5 M MgCl₂ (15.80 g, 3.82 g) followed by struvite of 1.8 M MgCl₂ (14.60 g, 3.39), effluent of 1.2 M MgCl₂ (12.90 g, 2.74 g), effluent of 1.8 M MgCl₂ (10.40 g, 2.15 g), struvite 1.2 M MgCl₂ (7.70 g, 1.98 g), control (5.70 g, 1.62 g), NPK (4.70 g, 1.25 g) and effluent of 1.5M MgCl₂ (2.60 g, 0.44 g). The wet weights of stem of *Amaranthus caudatus* planted with struvite were significantly different (p<0.05) when compared with NPK and control with the value; struvite of 1.5 M MgCl₂ (12.46 g), struvite of 1.8 M MgCl₂ (10.86g), struvite of 1.2 M MgCl₂ (5.21 g). The stem of effluent treated vegetables were also significant (p<0.05) when compared with NPK

and control with the values; effluent of 1.2 M MgCl₂ (10.19 g), effluent of 1.8 M MgCl₂ (7.1 g). The yield of struvite treated vegetables increased with concentration up to 1.5molar concentration of magnesium chloride then remain constant at 1.8 molar concentration of magnesium chloride. This report is consistent with Jonsson *et al* (2004).

5.4.5 Dry weight of plant

Struvite treated vegetables had the highest dry weight of leaves with these figures; struvite of 1.5 M MgCl₂ (2.88 g), struvite of 1.8 M MgCl₂ (2.85 g). These results were significantly different (p<0.05) when compared with the dry weight of vegetable treated with NPK (0.73 g) and control (1.00 g). These agree with the work of Johnston and Richards (2004) on rye grass using struvite. Vegetables treated with Effluent 1.2 M of MgCl₂ was next to the ones treated with struvite of 1.8 M MgCl₂ in value with the result 1.96 g, followed by *Amaranthus caudatus* treated with struvite of 1.2 M MgCl₂ with the value 1.21 g, these were also significantly different (p<0.05) when compared to NPK and control. Dry weight of *Amaranthus caudatus* treated with effluent of 1.5 M MgCl₂ (0.34 g), NPK (0.73 g) and control (1.00 g) were not significantly different when compared with one another.

Dry weight of root *Amaranthus caudatus* treated with struvite of 1.5 M MgCl₂ had the highest value of 1.25 g followed by that of struvite of 1.8 M MgCl₂ with 0.79 g; these two were significantly different when compared with the dry weight of root of other treatments.

The highest value for dry weight of stem was 1.82 g from vegetable treated with struvite of 1.8 M MgCl₂ followed by 1.73 g of struvite of 1.5 M MgCl₂ treated vegetables then 0.98 g of effluent of 1.2 M MgCl₂ treated vegetables; these results were significantly different (p<0.05) when compared with the stem weight of other treated vegetables.

CHAPTER SIX

CONCLUSION

The study compared the effect of struvite prepared from source separated human urine with inorganic fertilizer on *Amaranthus caudatus*. It was found that struvite gave a better yield when compared with other treatments. The findings from this study may contribute to the development of positive attitudes about the use of Struvite produced from urine as fertilizer - a way to increase crop yield. Struvite crystallization process is effective eco-friendly process which can result reduce environmental pollution caused through the use of inorganic fertilizer. In addition, findings may optimize the use of struvite produced fron urine to fertilize crops, which will help to maintain *Amaranthus caudatus* quality, improve soil characteristics, and reduce the demand for inorganic fertilizers. Struvite is also a highly effective source of nitrogen, magnesium and phosphorus for plants and can be used as a slow release fertilizer at high application rates without damaging plant roots. It was also discovered that the quantity of struvite produced increased with the concentration of magnesium chloride used.

6.1 Recommendation

Struvite production and use in agriculture should be explored on a larger scale and could be employed as organic fertilizer to improve plant yield and indirectly improve environmental sanitation. Struvite is a slow-release valuable fertilizer that can be used in agriculture. So, it is necessary to develop the optimum conditions for small-scale and commercial production of struvite. Struvite can be a good substitute for inorganic fertilizer especially now that urine has been certified as fertilizer.

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