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WASTE NOT, WANT NOT: USING SOURCE-SEPARATED URINE TO CULTIVATE MAIZE IN
THE SOUTHERN HIGHLANDS, TANZANIA

By

Lucy Hatfield

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

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This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Biological Sciences.

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Abstract

Human populations are increasing exponentially and this is having significant repercussions for societies worldwide, although developing countries are the most impacted. Two of the issues that increasing populations generate are food insecurity and ineffective sanitation. When I was serving as a Peace Corps education volunteer in Hagafilo village in the Njombe region of the Southern Highlands of Tanzania (East Africa), I was dismayed by the villagers' struggles with food security (abundance and quality). In this village, maize is the primary food crop. Unfortunately, yields are poor and nutritional content is low, while access to chemical fertilizers that might increase yields and nutritional content are scarce and not an option for most families. Working with my community, we developed a research project to investigate whether use of human urine fertilizer could increase maize crop yield and nutritional content (food security).

In Chapter 1, I explore the concept of ecological sanitation (EcoSan), focusing on human urine as a potential safe and effective fertilizer. Specifically, I evaluate the potential benefits and limitations of its use, explore the economic and ecological value of EcoSan, and discuss its use as a viable low-cost fertilizer for crop improvement. I also present the safety protocols for use of urine fertilizer. I conclude by suggesting that EcoSan and recycling of human urine for fertilizer might be a viable and cost-effective strategy to improve crop yield in smallholder farms. I present the issues pertaining to larger-scale urine fertilizer implementation, infrastructure, and nutrient recovery.

In Chapter 2, I provide an overview of the history, geography, ecology, and people of Tanzania and explore the potential utility and problems of implementing EcoSan. Specifically, I discuss the particular challenges the country faces with sanitation, health, and food security as well as the noteworthy benefits that ecological sanitation can provide. Lastly, I focus on peri-urban areas in Tanzania to explore the utility of small-scale ecological sanitation and note that human acceptance and willingness to implement safe, efficient, and consistent practices may pose significant barriers for implementation.

Lastly, in Chapter 3, I present the research I conducted as a Peace Corps Master's International Student in which I examined whether diluted human urine applied as a fertilizer can increase maize crop yield and nutritional content. I worked with participating farmers, who collected, sterilized, and applied their own urine to their maize plots. In total, 8 plots were maintained with half of each plot receiving urine fertilizer and water, while the other half received water only. I found that urine fertilizer resulted in increased maize yield which suggests that urine application could be an effective, low cost fertilizer for this village. Research from this chapter will be submitted for publication with Drs. Hersch-Green and Cavaleri as co-authors.

CHAPTER 1: FOUNDATION FOR ECOLOGICAL SANITATION AND USE OF HUMAN URINE AS FERTILIZER FOR CROPS

Introduction

The world's population is growing at an ever-increasing rate, with a global estimated population of 9 billion by 2050. The majority of this growth is set to take place in the urban centers of the Southern Hemisphere (Mihelcic, Fry et al. 2011). When urban populations expand rapidly, the challenges of securing food, shelter, and a safe environment become pressing, and the solutions are often intertwined. Providing hygienic living conditions through use of proper sanitation is critically important. One of the solutions proposed seeks to marry wastewater treatment, resource recovery, and food security through a paradigm shift. This shift takes the goals of traditional sanitation (removal of wastes without exposure to pathogens) and enhances them to receive the maximum benefit from the system. Modern sanitation systems are again designed to remove waste and harmful pathogens while also trying to minimize the negative impacts to aquatic ecosystems by limiting the byproducts of waste treatment (hormones, oxygen-consuming pollutants and excess nitrogen and phosphorous). Traditional waste treatment is costly, water intensive and has high energy requirements. It relies upon massive infrastructure to remove excreta and the use of end-of-the-pipe technologies to dispose of them (Simha and Ganesapillai 2016). End-of-the-pipe wastewater treatment plants are typically placed near bodies of water. The nitrogenous waste input to aquatic ecosystems from wastewater treatment plants has resulted in an overload of nitrogen in coastal and estuarine waters and has contributed to the proliferation of harmful algae bloom species (Glibert, Harrison et al. 2006).

Closed-loop sanitation systems, or ecological sanitation (EcoSan), share the same goals as modern sanitation, however, instead of only attempting to reduce the negative impacts on the aquatic environments, closed-loop systems use collection methods to process excrement into beneficial products that can generate income and increase food security (Mihelcic, Fry et al. 2011). Closed-loop sanitation systems require an adjustment from seeing excreta as a waste product to thinking of it as a resource with economic value (Simha, Lalander et al. 2017). Addressing the need for proper sanitation is extremely important, especially in developing countries where infrastructure is lacking and proper sewer systems are uncommon. Globally, 36% of the world's population does not have access to sanitation facilities (Simha and Ganesapillai 2016) and 50% do not have access to systems that meet modern standards (Hu, Fan et al. 2016). Annually, 801,000 children under the age of 5 die from diarrheal disease, and 88% of those deaths are attributed to lack of proper sanitation and safe drinking water (CDC 2016). Surface

water contamination by nitrogen and phosphorous is expected to increase dramatically in the future owing to the increasing world populations, urbanization, and lagging infrastructure. Difficulties in dealing with waste are exacerbated by human's increased use of chemical products in their daily lives and water shortages (Drangert 1998). Effective waste disposal has had a positive impact in developing countries by reducing the instances and severity of diseases (Esrey, Potash et al. 1991). EcoSan systems are able to address many of the challenges facing the world's expanding urban centers.

EcoSan systems allow for the recycling of the resources present in waste through recovery and then reuse in agricultural systems. Ecological sanitation uses the nutrients present in human excreta to make a safe fertilizer that is applied to soils to increase their fertility. Food crops can then be grown in those soils and the food that is produced is consumed by humans. EcoSan creates a circular flow of nutrients from essentially toilet to table. Urine is rich in nitrogen (N), phosphorus (P) and potassium (K). The composition of urine reflects the nutrients required for plant growth (Maurer, Pronk et al. 2006). A study in Sweden found that a person in a year excretes in the form of urine 2.5-4.3 kg nitrogen, 0.7-1.0 kg of phosphorus and 0.9-1.0 kg of potassium (Kirchmann and Pettersson 1994). The urea/ammonium ratio in urine compares to the urea/ammonium found in mineral fertilizers, with 90-100% of N in the form of urea or ammonium (Pradhan, Nerg et al. 2007). The P and K found in urine is almost entirely (95-100%) in inorganic form (Pradhan, Nerg et al. 2007). Considering the nutrient composition of urine, if recycled it has the ability to be a good plant fertilizer. The majority of population growth is happening in countries that do not have access to chemical fertilizers. Urine is a nitrogen rich fertilizer that has been shown to be as effective, if not more so, than chemical fertilizers (Richert, Gensch et al. 2010). Urine fertilizer can help meet the demand for food while conserving water and energy (Pradhan, Nerg et al. 2007).

Principles of waste disposal

There are three main strategies for disposing of waste. The first strategy is flush-and-discharge (most prestigious, developed world with infrastructure present, wastes are transported to a treatment facility for processing and disposal). However, in many urban centers in the Southern Hemisphere "flush-and-forget" is more accurate with raw waste transported through sewers to a body of water. In some developing countries, sanitation and waste removal mainly focuses on the idea of dilution of the waste. In rural Chinese communities, rain water is used to dilute and transport waste from villages, many of which don't have access to sewers and a centralized infrastructure for processing waste products (Gao, Zhou et al. 2015). Even when there is a treatment

plant at the end of the pipe, one study looking at the sustainability of sanitation systems found that water treatment plants built in Africa and Asian largely became dysfunctional after only a few years due to poor management, and lack of capital needed for maintenance (Koné 2010). In 1992, the World Bank estimated that only 5% of waste from cities in the Southern Hemisphere was properly treated before it was discharged into the environment (Drangert 1998). The second type of sanitation system is drop-and-store sanitation and is commonly employed in rural environments through the use of latrines. In Sub-Saharan Africa, 80% of cities and 100% of towns rely on onsite sanitation facilities (Koné 2010). This type of sanitation does not require extensive infrastructure or expertise to operate. The third system is sanitize-and-reuse, and it is from this idea that EcoSan developed (Drangert 1998).

Urine as an alternative fertilizer

Urine composition and quality

In order for urine to be used as an alternative fertilizer it must have nutrients that are essential to plant growth and function (the same nutrients that are supplied in chemical fertilizers) and be safe. The composition of urine is determined by a number of factors including the amount of fluids ingested, exercise level, diet, climate, and an individual's metabolism (Jönsson, Stintzing et al. 2004, Richert, Gensch et al. 2010, Shaw 2010). In Scandinavian countries, an adult produced between 0.8-1.5 L of urine per day (Kirchmann and Pettersson 1995, Richert, Gensch et al. 2010), while in Thailand average urine production was lower at 0.6-1.2 L per day (Schouw, Danteravanich et al. 2002). This observed difference could be impacted by the climate in Thailand where the average temperature far exceeds that of Nordic countries, so loss of fluids due to sweating or physical exercise is increased. Regional differences also affect urine composition by determining elemental proportions within urine. Generally, individuals living in a region share a similar diet. Growth requires nutrients, which for animals must be acquired from the environment. For developing animals, the body removes the nutrients it needs from the food it ingests. For example, N is required for protein synthesis, P is necessary for bone and muscle development, while K is used in nerve and muscle growth (Richert, Gensch et al. 2010). However, once those tissues are fully developed, the body process food differently; greatly reducing the nutrients absorbed. This means that there is an equal balance between nutrients ingested and nutrients excreted (Jönsson, Stintzing et al. 2004, Richert, Gensch et al. 2010). Table 1.1 shows the elemental compositions of urine used in a number of EcoSan studies. In Finland, urine has a composition of 8.36 g of N/L, .7 g of P/L and 2 g of K/L with a pH of 9.2 (Pradhan, Nerg et al. 2007, Pradhan, Pitkänen et al. 2008, Pradhan, Holopainen et al. 2009, Pradhan, Holopainen et al. 2010). However, in Burkina Faso which has a largely

plant based diet, nitrogen concentrations were less at 3-7 g N per L of urine (Richert, Gensch et al. 2010) and in Uganda, nitrogen concentrations were even lower at 2.3 g N per liter of urine (Andersson 2015). Urine contains 50% of all phosphorus and 90% of all nitrogen excreted from the human body (Bonvin, Etter et al. 2015). Fresh urine is 85% nitrogen in the form of urea and 5% ammonia with creatinine, amino acids, and uric acid comprising the remaining nitrogen compounds (Udert, Larsen et al. 2006). However during storage, the pH of urine rapidly changes from 6 to 9 due to the conversion of urea to ammonia/ammonium (Höglund, Ashbolt et al. 2002) by the enzyme urease (Pradhan, Nerg et al. 2007). This conversion happens very quickly, with time estimated between 5-20 days depending on temperature (F. Maggi 2013), however, one study reported that the conversion of urea to ammonia occurred in only 24 hours when the urine was stored at 37 °C (Vinnerås, Nordin et al. 2008).

Many of the changes that occur in urine during storage are a result of microorganisms that are found within the urine collection system. The three main processes urine undergoes during storage are urea hydrolysis, mineral precipitation, and ammonia volatilization. The microorganisms found in the collection systems produce enzymes that function as the catalyst for the hydrolysis of urea to ammonia and bicarbonate (Udert, Larsen et al. 2006). The nitrogen found in fresh urine is mainly in the form of urea, however, after hydrolysis the composition is 90% ammonium (NH_4^+) (Udert, Larsen et al. 2006). Other elements present in urine are found in their ionic form. Phosphorus is commonly found in P_2O_5 or PO_4^{3-} and potassium in K_2O or K^+ (Richert, Gensch et al. 2010), sulphur in SO_4^{2-} , calcium as Ca^{+2} and magnesium as Mg^{+2} (F. Maggi 2013). The P and K found in urine is almost entirely (95-100%) in inorganic form, which is available directly for plant uptake (Pradhan, Nerg et al. 2007, Bonvin, Etter et al. 2015). Urine is also rich in sodium with Na^+ concentrations of 2,170 mg L (Kocatürk and Baykal 2012) and 8.8 g of NaCl per liter of urine (Richert, Gensch et al. 2010). If the urine is stored with flush water, which can add calcium and magnesium to the urine, its composition is altered (Maurer, Pronk et al. 2006). The addition of Ca and Mg to the urine results in spontaneous precipitation of phosphorus into a struvite which begins once the pH increases above 7 (Etter, Tilley et al. 2011, Pradhan, Mikola et al. 2017). The last compositional change that can occur as a result of urine storage is loss of volatilized ammonia (NH_3). Volatilization is low during storage and transport of source-separated urine assuming that the container is airtight, however, the majority of ammonia loss occurs during application of the fertilizer and can be as high as 10 percent (Udert, Larsen et al. 2006).

Table 1.1: Urine composition of P, K, N, Na, Ca, Mg, and pH measured in urine used in ecological sanitation studies

Parameter	Value (mg/L)	Reference
Total-P	208	Udert and Wächter 2012
	689	Ganesapillai, Simha et al. 2015
	415	Kocatürk and Baykal 2012
	150 - 230	Heinonen-Tanski, Sjoblom et al. 2007
	700	Pradhan, Holopainen et al. 2009
	240 - 280	Akpan-Idiok, Udo et al. 2012
	63	Pradhan, Nerg et al. 2007
Total-N	2,390	Udert and Wächter 2012
	8,360	Pradhan, Holopainen et al. 2009
	5,700	Kocatürk and Baykal 2012
	9,734.10	Simha Zabanitou 2017
	940	Pradhan, Nerg et al. 2007
	4,280 - 4,970	Akpan-Idiok, Udo et al. 2012
	2,400 - 3,100	Heinonen-Tanski, Sjoblom et al. 2007
K	1,410	Udert and Wächter 2012
	760 - 940	Akpan-Idiok, Udo et al. 2012
	2,000	Pradhan, Holopainen et al. 2009
	1,200	Kocatürk and Baykal 2012
	1,488	Ganesapillai, Simha et al. 2015
	360	Pradhan, Nerg et al. 2007
	590 - 1,700	Heinonen-Tanski, Sjoblom et al. 2007
	1,498	Simha Zabanitou 2017
Ca	16	Udert and Wächter 2012
	16 - 18	Akpan-Idiok, Udo et al. 2012
Mg	< 5	Udert and Wächter 2012
	1.36 - 1.61	Akpan-Idiok, Udo et al. 2012
Na	1,740	Udert and Wächter 2012
	3,185	Ganesapillai, Simha et al. 2015
	3,186	Simha Zabanitou 2017
	750 - 940	Akpan-Idiok, Udo et al. 2012
pH	8.6	Pradhan, Nerg et al. 2007
	8.69	Udert and Wächter 2012
	9.04	Mnkeni, kutu et al. 2008
	8.9-9.2	Akpan-Idiok, Udo et al. 2012
	6*	Kocatürk and Baykal 2012
	9.2	Pradhan, Holopainen et al. 2009

* Indicates pH was measured from fresh urine, pH not measured in mg/L

Urine fertilizer

Urine is a mixture of over 200 organic and inorganic compounds, and it contains many of the macro- and micro-nutrients necessary for plant growth (F. Maggi 2013). Fresh urine contains 75-90% of nitrogen excreted by the body, with the urea/ammonium ratio in urine comparable to the urea/ammonium found in mineral fertilizers (Pradhan, Nerg et al. 2007). However, the P/N and K/N ratios are lower than that of chemical fertilizer, but due to the P and K solubility (increase availability for plants) it is thought that they perform similarly (Richert, Gensch et al. 2010). Generally, the composition of fresh human urine results in an NPK fertilizer ratio of 18:2:5 (Lindén 1997, Ganrot, Dave et al. 2007). However, when it is mixed with flush water its fertilizer value is 15:1:3 (Palmquist and Jönsson 2004). One study found that plant utilization of N was lower in urine compared to ammonium nitrate fertilizer; 42% and 53% respectively, however, this finding was attributed to a 6-7% gaseous loss of urine during application (Kirchmann and Pettersson 1994). The same study found that urine had 28% more phosphorus available to the plants compared to other P fertilizer sources (Kirchmann and Pettersson 1994). The mass balance between consumption and excretion makes it possible to calculate the nutrients present in urine from food intake. Two equations were developed by Jönsson, Stintzing et al. (2004) to estimate N and P excreted by a human based of food ingested. These equations allow individuals to estimate nutrients present in urine in order to determine application amount. The first equation is used to estimate total nitrogen excreted from the body ($N = 0.13 * \text{total food protein}$) and the second estimates total P ($P = 0.011 * (\text{total food protein} + \text{vegetable food protein})$). The composition of waste products is determined by the food consumed. Thus, in regions that grow and eat few crops, the nutrients present in waste will have the composition of required nutrients to sustain the crops produced in that region (Richert, Gensch et al. 2010).

Application of urine fertilizer has been shown to increase the soil's water holding capacity and elemental composition (Pradhan, Holopainen et al. 2009). Increases in soil organic matter have been found in studies that apply urine and compost. An increase in growth and yield with the addition of compost was attributed to the increased capacity of the soil to retain moisture in the dry climate of southern Ghana (Germer, Addai et al. 2011). Organic matter in soil helps with water and topsoil conservation which can be beneficial during water scarcity (Pimentel, Hepperly et al. 2005). Maize plants in Zimbabwe showed greater water use efficiency if they were cultivated with urine fertilizer compared to chemical fertilizer and those that only received water (Guzha, Nhapi et al. 2005). Soils in Uganda showed three times more phosphorous and increased nitrogen content compared to the control (only irrigated) after urine fertilizer was applied for a growing season (Andersson 2015). In soils treated with urine, increased electrical conductivity, pH, exchangeable K^+ , Mg^{2+} , and Ca^{2+} were reported.

Additionally, the increases became more pronounced as the experiment was conducted. These results indicated that urine fertilizer may have a residual effect on soil properties (Yongha Boh, Germer et al. 2013).

The effect of urine fertilizer on vegetable growth and yield

The effects of urine fertilizer have been studied across various plant species and at a range of application rates. The results have been mixed and are largely dependent on the type of plant, its saline tolerance and its nitrogen requirement (Mnkeni, Kutu et al. 2008, Richert, Gensch et al. 2010). Plants with a high nitrogen requirement and which are tolerant to salinity perform better than plants that are sensitive to salinity or do not require high rates of nitrogen fertilization. For example, beet root continued to accumulate biomass until an application rate of 800 kg N/ha was reached while carrots reached peak biomass at 50 kg N/ha (Mnkeni, Kutu et al. 2008). Beet root has a high nitrogen requirement and is tolerant to salinity, while carrots have a low N requirement and are not tolerant to saline conditions. Every vegetable responds to urine fertilizer differently. Table 1.2 displays the biomass and yield results from past research into urine fertilizer efficacy. It also displays the vegetable under study, its nitrogen requirement and its salinity tolerance. Plants like green amaranthes that have a high nitrogen requirement and a moderate salinity tolerance, perform much better with the addition of urine fertilizer compared to the tomato which has a low nitrogen requirement and is sensitive to salinity.

Many studies compared the effects of urine fertilizer across multiple treatments involving additional amendments. In tomatoes, Pradhan, Holopainen et al. (2009), found that the total dry aboveground biomass was significantly larger in the treatments that received urine + wood ash and mineral/chemical fertilizers compared to the treatment that only received urine. This difference is attributed to the increased concentrations of P, K, Ca^{2+} , and Mg^{2+} . However, the increase in dry biomass did not result in an increase in yield. This study also found a significant increase in the total aboveground biomass of the plants in the urine fertilizer treatment group compared to the plants that did not receive any fertilizer. Researchers in Finland found that beets grown with the addition of urine, urine + wood ash and chemical fertilizer did not differ significantly from each other in growth and biomass measurements. However, all three treatments were different from the control plants that were not given any fertilizer (Pradhan, Holopainen et al. 2010). Similar results were achieved in Zimbabwe where researchers measured the effects of chemical fertilizer, urine and urine + humanure (feces) on growth, yield and water usage in maize. These studies found that all three treatments did not differ significantly in growth measurements or yield. However, they did note that the urine+ humanure produced 3.5 tons/ha, the urine 3.0 tons/ha and the chemical fertilizer produced just 2.5 tons/ha. Maize that was grown with the addition of

fertilizer, either chemical or organic, demonstrated high water use efficiency compared to the plants that were grown without any fertilizer (Guzha, Nhapi et al. 2005). In many Sub-Saharan countries the primary method for irrigation is rainwater (Shekania Bisanda 1998). The ability of a crop to use water efficiently is critical for that crop's success and its ability to withstand drought. Urine fertilizer has not only affected the plants use of water urine fertilizer has been shown to alter the plant's growth rate.

Growth rates between fertilizer sources were also found to be different. In cabbage, the growth rate for the chemical fertilized treatment ceased at the midpoint of the experiment while the cabbages fertilized with urine continued to grow (Pradhan, Nerg et al. 2007). Similar results were found in cucumbers, with both chemical fertilizers and urine showing similar growth rates until the midpoint and then the urine fertilized cucumber growth outpaced the chemical fertilizer treatment (Heinonen-Tanski, Sjoblom et al. 2007). These results maybe attributed to the plant's use of the elemental compounds present in the different fertilizer sources (Richert, Gensch et al. 2010). The urine fertilized cabbage achieve their maximum growth faster than the chemical and non-fertilized cabbages (Pradhan, Nerg et al. 2007). This truncated cultivation time means that multiple crop cycles can be grown on the same season.

Effects of urine fertilizer on vegetable taste

The effect of urine fertilizer on taste and texture of different vegetables has been assessed across a number of studies. Participants were able to tell the difference between vegetables grown with urine fertilizer, chemical fertilizer or no fertilizer in cucumber, cabbage (Heinonen-Tanski, Sjoblom et al. 2007, Pradhan, Nerg et al. 2007) but not in beets (Pradhan, Holopainen et al. 2010). In all studies, participants did not prefer the vegetables cultivated with any particular fertilizer over the other.

Table 1.2: Results of past research on the effects of urine fertilizer on vegetable growth and yield. The table also displays the vegetable variety, its nitrogen requirement and its tolerance to saline.

Source	Vegetable	Nitrogen Requirement	Salinity Tolerance	Fertilizer Application Rate	Biomass	Yield
Pradhan, Neng et al. 2007	Cabbage (<i>Brassica oleracea</i>)	HIGH	MODERATELY SENSITIVE	180 kg N/ha	UF = CF UF > C	UF = CF UF > C
Pradhan, Pitkanen et al. 2010	Pumpkin (<i>Cucurbita maxima</i> L.)	MEDIUM	MODERATELY TOLERANT	113 kg N/ha	UF > CF UF > C	UF < CF UF = C
Pradhan, Holopainen et al. 2010	Red Beet (<i>Beta vulgaris</i>)	HIGH	MODERATELY TOLERANT	133 kg N/ha	UF = CF UF > C	UF = CF UF > C
Pradhan, Holopainen et al. 2009	Tomato (<i>Solanum lycopersicum</i>)	LOW	SENSITIVE	135 kg N/ha	UF < CF UF > C	UF = CF UF > C
Andersson 2015	Maize (<i>Zea mays</i> L.)	HIGH	MODERATELY SENSITIVE	60-75 kg N/ha	-	UF = CF
Heinonen-Tanski, Sjöblom et al. 2007	Cucumber (<i>Cucumis sativus</i> L.)	LOW	MODERATELY SENSITIVE	Recommended	-	UF = CF
Mhkeni, Kutu et al. 2008	Maize (<i>Zea mays</i> L.)	HIGH	MODERATELY SENSITIVE	Recommended	UF = CF UF > C	UF = CF UF > C
Ranasinghe, Karunaratne et al. 2016	Bushita Bean (<i>Phaseolus vulgaris</i> L.)	LOW	SENSITIVE	Recommended	UF > CF	UF < CF
Shrestha, Srivastava et al. 2013	Sweet Pepper (<i>capsicum annuum</i> L.)	MEDIUM	MODERATELY SENSITIVE	100 kg N/ha	UF = CF	UF = CF
Akpan-Idiok, Udo et al. 2012	Okra (<i>Abelmoschus esculentus</i>)	MEDIUM	SENSITIVE	60-68 kg N/ha	UF = CF UF > C	UF < CF UF > C
AdeOluwa and Cofie 2012	Green Amaranthes (<i>Amaranthus caudatus</i>)	HIGH	MODERATELY TOLERANT	100 kg N/ha	UF > CF UF > C	UF > CF UF > C
Guzha, Nhapi et al. 2005	Maize (<i>Zea mays</i> L.)	HIGH	MODERATELY SENSITIVE	Recommended	-	UF = CF

UF = urine fertilizer, CF = chemical fertilizer, C = control

Economic value of urine fertilizer

The value of urine can be measured in a number of ways, including its economic value as a fertilizer (the nutrients it contains), the positive environmental impact from removing it from sanitation systems, and its increase to plant yields. Chemical fertilizer use results in a heavy economic burden for many governments. For example, in Sri Lanka, the government subsidizes chemical fertilizers in order to meet the food requirements at a cost of 2.24% of total government expenditure (Ranasinghe, Karunaratne et al. 2016). In Africa, purchasing chemical fertilizer is simply not possible for many farmers because of its high cost, with chemical fertilizer costing African farmers 2-6 times more than a European farmer because of storage and transport costs (Sanchez 2002, Cordell, Drangert et al. 2009).

The value the nutrients in urine can have on an agricultural system is different across the world. Nutrient inputs from urine can replace 19%, 20% and 29% of N, P, and K, respectively for chemical fertilizers applied in Swedish crop production (Vinnerås, Nordin et al. 2008). But in Sub-Saharan Africa, urine can completely replace chemical fertilizer use at current application rates (Rockström, Axberg et al. 2005). In Zimbabwe, they found that one person is able to produce enough P and N in their urine to fertilize the amount of maize required to feed themselves (Guzha, Nhapi et al. 2005). In Burkina Faso, the nutrients present in a single 20 L jerk can of urine was estimated to be worth 25 cents and assuming yearly urine production of 500 liters, an individual excretes \$6-7 USD worth of nutrients in their urine per year. However, that value increases to \$50 USD when the increase in crop yield is factored in (Richert, Gensch et al. 2010).

Urine fertilizer can result in crop yields that are sufficient in meeting yearly nutritional requirements and urine has positive affect on both soil and plants. One person's average annual urine production is enough to fertilize 6,300 tomato plants resulting in 2.41 tons of fruit (Pradhan, Holopainen et al. 2009). Urine has a nitrogen content of 2.9 g N/L which is enough for a single person to grow 160 cabbages in 90 m² in one year. That is equivalent to 752 kg of cabbage which is 64 kg more cabbage than can be produced from the chemically fertilized plot (Pradhan, Nerg et al. 2007). Comparing urine to chemical fertilizer is more than just comparing NPK ratios; urine adds water, micronutrients, and a wide variety of organic and inorganic compounds, many of which benefit the soil as well as the plant (Germer, Addai et al. 2011).

Salinity

Soil salinity is defined as a measure of the total amount of soluble salt in the soil. The concentrations of salt in the soil can affect the health of a plant in a variety of ways. Within the soil, Na^+ can alter the chemistry, thereby altering the pH, electrical conductivity, osmotic stress and exchangeable cations (Yongha Boh, Germer et al. 2013). A soil's composition naturally differs from place to place and is affected by the parent rock, climatic conditions, types of usage and amendments added. In the developing world, use of chemical fertilizers is beyond the financial reach of many smallholder farmers (Andersson 2015). However, the use of organic methods to maintain soil fertility is gaining traction (Pimentel, Hepperly et al. 2005). The use of human urine fertilizer is growing in popularity owing to its minimal cost, availability, and effectiveness as a fertilizer (Simha, Lalander et al. 2017). However, urine is inherently saline and the effect of its use on crops needs to be considered. Urine fertilizer increases soil salinity and sodicity (Na^+ concentrations within the root zone of the plant) and in excess it has been found to have negative consequences for plant growth (Pradhan, Holopainen et al. 2009). The effect of salinity on a plant is determined by a number of factors and a plant's tolerance is a major factor, along with the age of the plant, and available irrigation (Richert, Gensch et al. 2010). Seedlings, recently transplanted plants or plants that are subjected to other environmental stress (diseases, insects, dry conditions), are more susceptible to high salinity (Kotuby-Amacher, Koenig et al. 2000). All of these factors work together to determine a plant's threshold value, which is the value that if surpassed begins to have yield-reducing effects.

Na^+ also has an impact on the plant itself. With increased salinity above the threshold value, negative growth responses were reported in maize, with leaf, stem (wet and dry weights) and plant height all significantly reduced under high saline conditions (Yongha Boh, Germer et al. 2013). Additionally, elemental concentrations of P, N, K, Mg and Ca in leaf tissues were effected by increasing salinity. Elemental concentrations of N, Mg, P and Ca were all increased while K was decreased. The decrease in K indicates that as salinity increases the Na^+ inhibits the uptake of K by the plant. Plants grown with urine fertilizer treatment were found to have higher N, Mg, Ca concentrations than the plants that were treated with ammonium nitrate fertilizer while P and K showed no effect (Pradhan, Holopainen et al. 2010, Yongha Boh, Germer et al. 2013).

Safety concerns with using human urine fertilizer

Urine is considered sterile when it is eliminated from the body. It contains few enteric pathogens and with the proper precautions it can be used as a safe and effective

fertilizer. The primary source of pathogen introduction is through cross-contamination with fecal material which can introduce bacteria, viruses and parasites into the urine. Urine is produced in the kidneys by filtering metabolic wastes out of the blood. During the filtration process, excess water, salts, minerals, hormones and pharmaceuticals are removed from the body. The urine is then transported through the ureter to the bladder and is eliminated from the body by passing through the urethra (Karak and Bhattacharyya 2011). The pathogenic risks associated with utilizing human urine in agriculture are largely dependent on the collection method (the possibility of cross-contamination), temperature and duration of storage, dilution (if the urine is being stored neat or if it is mixed with flush water), pH and safety precautions taken during application. What constitutes proper storage is determined by the intended use of the urine and the collection method. For example, if urine is being collected by a single family and its intended use is for their personal garden, no storage is necessary (WHO 2006), however, if the urine is being collected from many unrelated individuals and its intended use is to be applied on commercial crops then a 6-month storage period is recommended to ensure that the urine is free of pathogens (Höglund, Ashbolt et al. 2002, Chandran, Pradhan et al. 2009). It is critical to determine the scale of the project (garden/commercial and family/public urine collection) to properly determine the safety precautions necessary for implementing urine fertilizer.

Multi-barrier method

An adaptable multi-barrier approach has been proposed by WHO 2006 and improved by Richert, Gensch et al (2010) to reduce the health risks associated with using human urine in crop production at a small scale. The intended use of the multi-barrier method is not for every individual seeking to implement urine fertilizer to adopt every barrier, but for every individual to examine which barriers and to what extent they should be implemented for their particular project (Fig.1.1). The 9 barriers include: 1) source separation, 2) storage and treatment, 3) application techniques, 4) crop restriction, 5) withholding period, 6) protective equipment, 7) hand washing, 8) food preparation and cooking, and 9) health and hygiene promotion.

Source: (Richert, Gensch et al. 2010)

Figure 1.1: Diagram depicting the 9 steps of the multi-barrier method proposed for the safe use of urine fertilizer in crop production

Source separation

The first barrier is source separation. Source separation is one of the most important steps in producing a safe fertilizer free of pathogens. The purpose of source separation is to store urine and feces separately and decrease the possibility of cross-contamination. Ecological sanitation toilets are commonly used for this purpose and present a user-friendly experience. They can be designed to store urine neat (without dilution) or diluted (mixed with flush water). They also can be designed for recycling feces as well as urine. EcoSan toilets use two-chamber systems to separate liquid and solid wastes, however, due to their cost they are not feasible for many developing nations (Morgan 2003, Jönsson, Stintzing et al. 2004). In developed nations with greater infrastructure, two-chamber toilets divide the urine and solid matter. The urine, with a small amount of flush water, is piped to a holding tank where it is held for some time or emptied and transported to a secondary location for storage (Höglund, Stenström et al. 2002). Fecal contamination is still possible with two-chamber toilets, especially when used by children and individuals with diarrhea (Höglund, Stenström et al. 2002, Richert, Gensch et al. 2010). Although fecal contamination of source-separated urine was found to be uncommon, (Höglund, Stenström et al. 2002, Jönsson, Stintzing et al. 2004, WHO 2006) the risk assessment should always acknowledge the possibility of contamination and take the steps necessary to sterilize the urine. The greater the number of individuals that use a system, the greater chance there is for cross contamination. A study in Sweden found that 17% of household holding tanks showed signs of cross-

contamination, compared to 38% of tanks in an eco-village and 58% of tanks associated with a public space (work or school). The same study collected samples from two areas within the holding tank; the top, which contained the urine mixed with flush water, and the bottom where the sediment collected. They found that 8 out of 36 (22%) samples collected from the urine mixture compared to 11 out of 30 (37%) collected from the sludge were contaminated (Schönning, Leeming et al. 2002). The authors also noted that in the majority of urine samples that tested positive for contamination, that the source of the contamination was visible. Urine with noticeable contamination and the sludge from larger systems can be discarded and thereby drastically reduce the risk of spreading pathogens. The ability to visually confirm contamination is important for smaller systems controlled by one family so they can adjust the storage time accordingly and avoid applying contaminated fertilizer. Rinsing the collection container to remove the sludge and monitoring the container for signs of contamination are effective measures to decrease the risk of fecal contamination.

Storage

The second proposed protective barrier in the multi-barrier approach is storage. Storage of source-separated urine has been a major area of study in recent years since the benefits of a closed-loop system have been widely recognized. The primary purpose of urine storage is to create a harsh environment to eliminate organisms (e.g. bacteria, virus) from the urine. Urine should be stored undiluted in a sealed container for the duration of the storage period. If diluted urine is left in an unsealed container, it can provide a habitat for insects like mosquitos to develop (Richert, Gensch et al. 2010). During storage, urea is converted to ammonium and the pH of the urine increases and these changes result in the die-off of many pathogens. The underlying effectiveness of storage as a sterilization tool is controlled by the pH, temperature and time. The dilution ratio also contributes to the effectiveness of storage. One study found that bacteria and coliphage MS2 experience a higher die-off rate in undiluted urine (1 week) compared to diluted urine (6 weeks) when stored at 30°C (Chandran, Pradhan et al. 2009). While increased pH has been found to be an effective measure for inactivation of bacteria in urine across storage temperatures, it has been less effective with virus and phage. A storage temperature of at least 20°C has been found to be an effective promoter of viral and phage die-off (Höglund, Ashbolt et al. 2002, Vinnerås, Nordin et al. 2008). In Burkina Faso, urine collected from 120 different households was stored in the sun for 1 month before being applied as a fertilizer for local farmers. No evidence of fecal contamination or pathogens were identified in the urine fertilizer or in the foods produced (Richert, Gensch et al. 2010).

Several viruses and pathogens are commonly associated with urine when contamination by fecal material has occurred. The three most prevalent pathogens

present in feces are (1) *Campylobacter jejuni*, which is the most common cause of bacterial gastroenteritis in the world, (2) *Cryptosporidium parvum*, which is a parasite, and (3) rotavirus. All of these pathogens cause diarrhea and intestinal problems, especially in children. Inactivation rates for rotavirus were 4-10 times greater in urine stored at 20°C than for the urine stored at 5°C (Höglund, Ashbolt et al. 2002). However, die-offs reported for *C. jejuni* and *C. parvum* when stored at 4°C and 20°C, with the *C. jejuni* bacteria inactivation nearly equal between the temperatures and the *C. parvum* being almost completely eliminated when stored at 20° (Höglund, Stenström et al. 2002). These results make it clear that determining safety protocol is dependent on the exact pathogenic concerns for the urine. An increase in pH has been found to be sufficient for eliminating bacterial risk at a range of storage temperatures, however, viruses may pose a greater health risk in source-separated urine compared to bacterial and protozoan pathogens which require a longer storage time (Höglund, Ashbolt et al. 2002).

In Africa and in other developing countries, a major health concern is the risk of the parasites, *Schistosoma heamatobium* and *Schistosoma mansoni*, which are excreted through urine (Esrey, Potash et al. 1991). *Schistosoma* parasites use can be found in species of freshwater snails in 53 different counties including Tanzania. The parasite eggs are excreted through urine and in order to complete its parasitic life cycle, the contaminated urine must return to a fresh water source. The schistosoma parasite uses fresh water snails as a host. When the parasitic cercaria emerge from their snail host, the free swimming larva can then penetrate the skin of the humans who contact the fresh body of water. Storage for two days has been shown to kill the schistosoma parasite in urine (CDC 2012). Increasing the temperature of urine by storing it in the hot tropical sun or applying it to hot soil, has also shown to eliminate the parasite (Heinonen-Tanski and van Wijk-Sijbesma 2005). The liquidity of urine makes its transport to fresh water a greater risk compared to solid fertilizers (Maurer, Pronk et al. 2006). If the urine is being directly applied, it is recommended to not be used near a fresh water source (Stenström 2004).

E. coli is found in the lower urinary tract of humans, thus urine frequently contains the bacteria, however, *E. coli* has a very rapid die off within urine. Similarly to *E. coli*, *Salmonella typhi* and *Salmonella paratyphi* are transmitted through an infected individual to their urine but also have a very rapid die off. Additionally, transmission of *Salmonella spp.* is frequently associated with fecal-oral, not urine-oral pathways (Stenström 2004). The greatest risk associated with enteric pathogens comes from viruses because they show greater resistance to die-off from increased pH, temperature and time, are found in high numbers in urine and can cause infection in a low dose (Höglund, Stenström et al. 2002). The “infective dose” of a pathogen, which is the

amount of a particular pathogen required to infect a human, varies by pathogen. Bacteria's infective dose is medium to high requiring $\pm 10^4$ and $>10^6$, respectively, compared to helminthes, protozoa and viruses, which have an infective low dose ($<10^2$) (Austin 2001). This is important to consider when evaluating the health risks of a system. The majority of enteric viruses do not display inactivation at low (3.5) or high (10) pH's, however, ammonia created during storage has been shown to have the ability to inactivate enteric viruses (Höglund, Ashbolt et al. 2002).

Application techniques

Proper application of the urine fertilizer is an important barrier to reduce the risk of pathogen transfer to the crop. The application technique adopted should take into consideration the crop, the organization of the field and the most efficient methods of delivery. When crops are planted in rows, the farmer can simply travel plant to plant incorporating the fertilizer in a circle around the base of the plant (i.e. maize) taking care to avoid direct contact with the plant. When the crop is a leafy variety, the fertilizer must be applied at a great enough distance to avoid contact with the portions of the plant that are eaten yet close enough to allow the roots' access to the fertilizer. When urine fertilizer is applied close to the ground and incorporated into the soil quickly, using either water or tilling, the formation of aerosols and loss of ammonia from evaporation are decreased (Höglund, Ashbolt et al. 2002). However, when the fertilizer is sprayed, aerosols form and they become a vector for pathogen transport. For example, rotavirus was identified in aerosols from unstored urine and urine stored at 4°C for 6 months (Höglund, Stenström et al. 2002). Of the ammonia that is lost as gas, 50% occurs during application of the fertilizer, but if the fertilizer is applied directly to the ground, that loss can be reduced by 30-60% and if the fertilizer is administered directly into the soil, 90% of the ammonia can be retained (Udert, Larsen et al. 2003, Udert, Larsen et al. 2006). During storage, phosphorus changes forms and settles to the bottom the storage container in a sludge. Simply mixing the storage container is not sufficient to homogenize the stored urine. In many cases, the storage container is too large to be manually shaken, and opening and mixing the urine by hand is not an option due to exposure to hazardous gasses (from volatilizing ammonia) and pathogens. Additionally, the majority of the pathogens found in the stored urine are found within the sludge. For large scale farming, stored urine can be applied using the same equipment used when applying farmyard slurry. Care must be taken to ensure that the individual applying the slurry wears personal protection equipment and that the slurry spreader be flushed after every use to avoid clogging of the pipes. At larger scales, drip irrigation has also been tested, however, blockages in the system were common which led to malfunction in the system. Diluting the urine introduces Ca and Mg into the mix, which precipitates spontaneous granular formation that can clog the equipment. In both of

the above examples, malfunction of the system could have been prevented if the urine had been applied undiluted, preferably before a rainfall (Richert, Gensch et al. 2010).

Crop restriction

The risks associated with using urine as a fertilizer are impacted by many factors. For instance, the larger (greater number of people contributing) the collection system, the greater the risk of contamination by pathogens. Another consideration is the type of crop the urine is being applied to and how that product is used (whether it is a fiber, feed or food crop). If there are concerns about the safety of the fertilizer, one option is to only apply it to crops not intended for human consumption. Human urine can be safely applied to fodder crops because there is no risk of pathogen transmission from humans to agricultural animals (Höglund, Ashbolt et al. 2002). Another option is to only apply it to crops that are processed by removing the plants' outer skin/leaves and then cooking the remaining edible portions. For example, avoid applying urine fertilizer on crops that are eaten without cooking or which are harvested while still growing (spinach) (Höglund, Ashbolt et al. 2002). Despite the minimal pathogenic risks associated with implementing urine as a fertilizer, there are fewer risks associated with human urine than with other fertilizers like livestock manure (Richert, Gensch et al. 2010). Additionally, adopting the first two steps in the multi-barrier approach greatly diminishes any negative health risk associated with using urine as an alternative fertilizer.

Withholding period

The withholding period is the amount of time between final fertilizer application and harvest. Researchers found that a 1-week withholding period was sufficient to reduce the risk of transmission of pathogens from ingesting food grown with urine fertilizer of the *C. jejuni* and *C. parvum* regardless of storage time or temperature. However, the withholding period needs to be increased to 3 weeks to reduce the prevalence of rotavirus to negligible levels (Höglund, Stenström et al. 2002). The withholding period is affected by UV-light and temperature which affect the die-off of pathogens on the surface of leaves and at the surface of the soil. For plants that grow underground, like carrots and potatoes, the withholding period should be increased because the pathogen die-off attributed to UV and temperature is decreased. Also, with crops like carrots and potatoes, post-harvest food processing plays an important role in reducing health risks (Richert, Gensch et al. 2010). Use of a withholding period is also important because of further inactivation of pathogens through soil microbe activity and dilution through irrigation (Höglund, Ashbolt et al. 2002).

Protective equipment

The greatest risk of exposure to pathogens is associated with handling and transporting fresh urine prior to storage (Höglund, Stenström et al. 2002). There are minimal risks associated with applying properly stored urine, however, if the urine was cross-contaminated by feces then there is a risk of exposure to the applicator through direct skin contact (Richert, Gensch et al. 2010). It is recommended that workers wear shoes and gloves when handling pretreated urine.

Hand washing

All people along the urine pathway from toilet to table are encouraged to wash their hands after coming into contact with any of the products or equipment used in the process. Hand washing after using the toilet and before meals is already common in many cultures. Effective implementation of this barrier entails hand washing after transporting, applying, or harvesting which can greatly reduce an individual's exposure risk (Richert, Gensch et al. 2010).

Post-harvest food processing

Properly washing/peeling and/or cooking fruits and vegetables before ingestion can reduce the risk of exposure by 2-6 logs (or 100-1,000,000 times) (Richert, Gensch et al. 2010). In food like cabbage, post-harvest processing is extremely important. One study found that all fertilizer treatments (water, chemical and urine) had similar contamination of *E. coli* which would indicate that contamination was introduced from a different environmental form (ie. bird guano) but once the consumer properly cleaned and prepared the food, no *E. coli* was found (Pradhan, Nerg et al. 2007). However, a study found that as long as the correct precautions were taken during the source separation and no fecal contamination occurred, cucumbers grown in a family garden could be eaten without cooking. Microbial analysis indicated that there were no microbes present in or on the food (Heinonen-Tanski, Sjöblom et al. 2007).

Pharmaceuticals and hormones

The majority of pharmaceuticals and hormones are eliminated from the body through urination (Jönsson, Stintzing et al. 2004). Christiansen et al. via Maurer, Pronk et al. (2006) found that 80% of all natural estrogen and 67% of artificial hormones were excreted in urine. In traditional waste management systems, urine and fecal material are stored together and water filtration is used to separate the material waste from liquid waste but in the process, estrogen, estrogen-like and pharmaceutical compounds are commonly introduced into aquatic environments (Richert, Gensch et al. 2010) through sewage effluent. These compounds can have a profound detrimental effect on

many fish species causing a feminization of their reproductive tract (Christiansen, Winther-Nielsen et al. 2002). Whereas, when hormones are deposited in terrestrial systems which have been breaking down hormones from excreta since the start of terrestrial life, the microbes present in the soil have the ability to do so. The soil microbes are also able to promote quicker biodegradation of compounds because terrestrial microbes have a 50,000 times higher oxygen level than water microbes (Richert, Gensch et al. 2010). However, research has found that if urine contains antibiotics, these compounds can have a harmful effect on soil microorganisms (Drangert 1998).

The amount of pharmaceuticals an individual excretes is largely dependent on the amount they ingest, so more pharm chemicals are excreted in developed countries compared to the undeveloped. Pharmaceuticals are excreted through two pathways; 2/3 in urine and 1/3 in feces (Richert, Gensch et al. 2010). One study looked at 212 different pharmaceuticals and found that 64% of each was excreted through urine as their parent compound (Ronteltap, Maurer et al. 2007) or as metabolites (Landry and Boyer 2016). To date, there has been no research conducted on what effect pharmaceuticals have on crops. But, when considering the amount of hormones and antibiotics used in industrial agriculture which are included in the manure that is used in modern farming, the concentrations in human urine are much less. Winker, Vinnerås et al. 2009, found that animal manure contained antibiotics in concentrations nearly 100 times that found in human urine. Additionally, in developing countries where use and access to pharmaceuticals is low, the concentration found in urine would also be low.

Economics of EcoSan

EcoSan toilets have been successfully implemented in countries all around the globe, providing low energy sanitation resource recycling solutions (Simha and Ganesapillai 2016). Source-separating toilets (toilets that separate and store solid and liquid waste separately) have saved institutions the size of the University of Florida \$231,000 USD annually from reduced water consumption. This reduction in water usage was then transmitted to the wastewater treatment plant, where there was a 17% reduction in processing volume. This reduction in processing also created savings in electricity usage at the plant (Landry and Boyer 2016). There are many designs for separating toilets with a wide range of costs; for example dryflush (\$3,000-5,000 USD), flushing (\$8,000-12,000 USD), low-flow non-separating waterless toilets (\$600-1,000 USD), cistern low-flush toilet (\$12,000-18,000 USD), and composting toilet (\$1,000-4,000 USD) (Hu, Fan et al. 2016). In Tanzania, urine diversion toilets were constructed using locally available resources in Dar es Salaam for \$570 USD (John 2003). Each toilet

design processes waste products differently (Austin 2001), but they are all designed around resource recycling and reuse. In China, they found that although composting and source-separating toilets had higher initial costs than traditional modern toilets, they had lower operational costs and consumed less energy (Gao, Zhou et al. 2015).

Large scale EcoSan implementation

Urine is an effective fertilizer, however, there are several issues that need to be overcome in order to realize the potential of closed-loop sanitation at a large scale. Currently, only localized small scale closed-loop sanitation systems are being used in developing countries. The major hurdle facing closed-loop sanitation is geographical; how to deliver the nutrients present in urine to the agricultural fields that need them. To collect the most nutrients, urine needs to be harvested from high-density areas, but large scale cultivation of crops does not take place in cities. Studies suggest that large scale EcoSan systems can have a greater impact on climate change than some new high efficiency wastewater treatment systems because of the increased greenhouse emissions associated with transportation of excreta from cities to processing locations and then to the crop lands (Benetto, Nguyen et al. 2009). However, scientists are developing methods for recovering the nutrients present in urine, which can be more easily transported to crops fields for use as a fertilizer.

Nutrient recovery

Several methods have been developed to overcome the issue of transporting urine, and in particular, the nutrients urine contains from populated areas to agricultural areas. Many of these methods also aim to overcome hygienic concerns, as well as the contamination of urine by micropollutents (synthetic hormones, pharmaceuticals and their metabolites) that are commonly associated with using human urine fertilizer (Bonvin, Etter et al. 2015). These solutions involve processing the urine using various methods, and then the product of each method can be used as an effective fertilizer. Each method has its own drawbacks in terms of time, cost, required knowledge and infrastructure, but each is useful in particular situations.

Struvite precipitation

Struvite is a white odorless powder that is an effective phosphorus fertilizer (Etter, Tilley et al. 2011). Struvite or magnesium ammonium phosphate hexahydrate can be stored, transported and used without any of the safety concerns associated with direct use of urine. Forming a struvite or a slow-release fertilizer relies on Mg^{+2} from

different sources (e.g. wood ash, bittern, magnesium sulfate, magnesium oxide, magnesium chloride) to create a chemical equilibrium of constant ions in a solution (Sakthivel, Tilley et al. 2012). Typically, struvite results in a 90% phosphorus recovery and a 5-10% nitrogen recovery (Ganrot, Dave et al. 2007, Pradhan, Mikola et al. 2017), with 1kg of struvite being produced from 640L of urine (Etter, Tilley et al. 2011). In a greenhouse study comparing struvite to conventional fertilizers, 26-28% of phosphorus was recovered by Italian ryegrass (*Lolium multiflorum*) from both P struvite derived from human urine as well as traditional phosphorus fertilizers (Bonvin, Etter et al. 2015). Struvite precipitation is the most widely accepted form of nutrient recovery from urine. Additionally, struvite precipitation can reduce nutrient emission losses and cadmium emissions by offsetting chemical fertilizer use as well as reducing chemical fertilizer production (Landry and Boyer 2016). The primary limiting factor to affecting the implementation of phosphorus precipitation is the cost of the precipitate; magnesium salts are very expensive (Sakthivel, Tilley et al. 2012). One way to reduce costs is to eliminate waste, so knowing the initial concentrations of phosphorus in the urine is key to developing efficient struvite precipitations by knowing the exact amount of Mg necessary to recover the phosphorus from the urine. Ranges of 1.5 mol of Mg/ mol P^{-1} to 1.8 mol of Mg/ mol P^{-1} was enough to collect 95 to 98% of the phosphorus in urine (Etter, Tilley et al. 2011). Another way of making struvite production more affordable is to find alternative precipitates. In India, researchers experimented with wood ash as a source of Mg to facilitate P precipitation, however, the Mg content was too low to generate a P precipitate of high enough quality to warrant use as a fertilizer (Sakthivel, Tilley et al. 2012). The wood used to generate the ash had high concentrations of heavy metals and was not a safe fertilizer for food production. Bittern is also identified as a possible alternative to magnesium salts. Bittern is a waste product of salt production and can be found in abundance near salt production facilities (Etter, Tilley et al. 2011). However, despite bittern's effectiveness in P precipitation, bittern and $MgSO_4$ were also found to have heavy metals present (Etter, Tilley et al. 2011). Heavy metals can be absorbed into the precipitate with roughly 20–63% of the heavy metals present in bittern found in the final precipitate (Ronteltap, Maurer et al. 2007). This makes the fertilizer they produce not suitable for application to crops. However, when struvite is precipitated with magnesium salts, hormones and pharmaceuticals (>98%) were not incorporated into the struvite (Ronteltap, Maurer et al. 2007). It is also important to consider that the phosphorus in urine only accounts for 30% of the total value of urine. The remaining liquid is still rich in nitrogen and potassium which can be recovered and used in agriculture (Etter, Tilley et al. 2011).

Additional nutrient recovery methods

Past research has employed precipitation of struvite to recover phosphorus and then subsequent processing was employed to recover nitrogen. Several methods have

been experimented with to enhance nutrient recovery. These methods can occur one after the other (activated carbon and zeolite) or simultaneously (ammonia stripping and phosphorus precipitating).

Ammonia stripping

Ammonia stripping can be conducted after the precipitation of the struvite to recover ammonia from the urine using H_2SO_4 . However, nitrogen can be stripped from fresh and diluted urine using NaOH (Pradhan, Mikola et al. 2017). Urine can also be stripped under vacuum pressure and the ammonia gas stream can pass through sulfuric acid which results in a 10% ammonia solution (Maurer, Pronk et al. 2006). Currently, research is focusing on combining P and N recovery. Traditional struvite precipitation has relied on Mg, however, $\text{Ca}(\text{OH})_2$ has been used which increases the urine's pH to above 12 and this converts ammonium to ammonia gas and precipitates P as Ca-P compound. The gas is then stripped, and ammonium sulfate is formed. Using $\text{Ca}(\text{OH})_2$ allows for simultaneously recovering N (85-99%) and P (99%) by "stripping and absorption" for N and "precipitation and sedimentation" for P (Pradhan, Mikola et al. 2017). However, this method requires expertise, electricity, and access to chemicals and a laboratory, which makes the process unattainable for the majority of individuals.

Zeolite

Zeolite nutrient recovery requires a stable pH of 7, and if the pH exceeds 7, then precipitation of struvite can occur and the urine will become granulated which makes it unable to pass through the column filled with zeolite. In this method, unprecipitated urine is injected into a column containing zeolite which can recover up to 90% of nitrogen, phosphorus and potassium. The nutrient laden zeolite can then be dried and applied to agricultural fields as a slow release fertilizer (Ramesh and Reddy 2011). Zeolite recovery is sensitive to the initial concentrations of the urine. If the urine dilution is greater than 50% this method is not effective (Hu, Fan et al. 2016). Combining zeolite with struvite precipitation is effective at increasing nutrient recovery. One study found that 0.5 mg/L of MgO and a zeolite concentration of 15 g/L resulted in P recovery of 10 g m^{-3} and N recovery of $1,000 \text{ g m}^{-3}$ (Maurer, Pronk et al. 2006).

Activated carbon

In India, researchers used activated carbon found in crushed coconut shells to facilitate the capture of urea from urine. Crushed coconuts are rich in carbon and are a locally available resource. The research found that a capture of nearly 80% of the urea was achieved by running urine through 30cm of activated crushed coconut (Simha, Lalander et al. 2017) and in increasing the column depth to 45.45 cm they were able to increase the nitrogen capture from urine to 87.53% (Simha, Mathew et al. 2016). Simha, Lalander et al. (2017) were also able to recover 90% of the phosphate through

introducing MgO to the urine after it filtered through the column which facilitates precipitation of phosphate struvite. Using urea-absorbed activated carbon has been shown to have positive effects on soil properties with increases in soil nitrogen and electrical conductivity, pH (6.23 to 8) and cation exchange capacity improved in the soils that were given urea-adsorbed activated carbon as an amendment. In addition to the altered soil characteristics, plant growth was effected. The lentil plants with the treatment had twice the biomass of the control and increased seed germination (Ganesapillai, Simha et al. 2015). Using activated carbon, urea recovery based on an individual's average yearly urine production of 500 L would be 4.5 kg N (urea). In developed countries, the yearly demand for N fertilizer for food production is 22.3 kg N, which means activated carbon can recover 1/5 of the N demand. However, in the developing world, the yearly demand for N fertilizer is much less. For example, in Kenya the yearly N fertilizer demand is 6.5 kg N, so this method of urea recovery can produce nearly 70% of the required N for food production (Maurer, Pronk et al. 2006).

Volume reduction

The following methods are designed to reduce the volume of urine by eliminating the H₂O component of urine, which accounts for 93-96% of volume (Drangert 1998). The removal of water is the only processing method that maintains all the nutrients found in urine (Udert and Wächter 2012). Also, for many of these methods, acidification of the urine must be done first to avoid hydrolysis of urea into ammonium (Maurer, Pronk et al. 2006). Keeping the urine pH below 4 inhibited hydrolysis. Maurer, Pronk et al. (2006) found that 2.9 g of concentrated sulphuric acid was able to keep 1 liter of fresh urine pH below 4 for 250 days. However, once urine has been stored the process of acidification requires significantly more acid, 13 g H₂SO₄ to 1 L of stored urine resulted in a pH of 4.5 (Udert and Wächter 2012). In addition to preventing hydrolysis, a low pH has been shown to have a positive impact on sterilizing the urine. An additional benefit of a reduced pH is that achieving a pH below 2 results in the inactivation of pharmaceuticals (antibiotics and anti-inflammatory drugs) of between 50-95% (Maurer, Pronk et al. 2006).

Reverse osmosis

In this method, the acidified urine is repeatedly passed through ionically charged membranes resulting in the urine volume being reduced by a factor of 5 and recovery of 70-97% of N, 73-98% of P and 71-98% of K. However, the membranes used in the process may retain micropollutents from the urine and this process is energy intensive and requires use of chemicals to avoid the scaling on the membranes (Maurer, Pronk et al. 2006). One study reported an 80% water reduction using acidified urine at an high energy cost of 30 W h per liter of urine (Udert and Wächter 2012).

Freeze/thaw volume reduction

When water molecules freeze they undergo a transformation from an irregular structure to a stable tetrahedral shape (Lind, Ban et al. 2001). When the freezing process of urine occurs slowly enough, the water molecules reorganize into their tetrahedral shape and in doing so will displace the other compounds into the liquid portion that remains. Through controlled freezing to -14° C and thawing researchers have discovered that they are able to concentrate 80% of the nutrients found in urine into only 25% of its original volume (Lind, Ban et al. 2001). However, this method is the most energy intensive of all of the volume reduction methods requiring 790 W h to process 1 liter of urine (Udert and Wächter 2012), but the energy demand can be reduced in cold climates by harnessing natural cold temperatures.

Environmental impacts

Plants require nutrients to grow, with macronutrients like nitrogen, potassium and phosphorus needed in larger quantities than micronutrients. Plants accrue the majority of the nutrients they need from the soil in which they are grown, however, the increased demand for food has led to unsustainable farming practices. Where farmers were once able to manage their crops through natural methods (rotation, letting the land go fallow) they now increasingly rely on fertilizers to boost the nutrients in the soils that are available to plants. Modern farmers use chemical fertilizers often derived from fossil fuels to meet the plant demand for macronutrients; NPK (Nitrogen Phosphorus and Potassium) fertilizers are the most prevalent. In the developed world, agriculture is heavily dependent on chemical fertilizers to a degree that is not sustainable, with use increasing 100-fold in the past four decades (Glibert, Harrison et al. 2006). Improper and/or excessive fertilizer use has resulted in eutrophication of water bodies, algae blooms, decreased soil fertility, loss of topsoil and loss of biodiversity (Glibert, Harrison et al. 2006, Cordell, Drangert et al. 2009).

Current P demand is 15 million metric tons, however, by 2050 demand may be as high as 67 million metric tons (Mihelcic, Fry et al. 2011), with phosphate rock reserves only projected to last for 50-100 years (Cordell, Drangert et al. 2009). In the past half century, nearly one-half billion tons of phosphorus element has been removed from phosphate rock to meet the rising global food demand. To guarantee food security, more sustainable phosphorus sources need to be utilized. Peak phosphorus is estimated to occur by 2035 and then slowly decline (Cordell, Rosemarin et al. 2011). Although the exact reserves of phosphate rock remain unknown, the fertilizer industry acknowledges that the reserves are declining and the costs of extraction, transport, and processing are increasing (Cordell, Drangert et al. 2009). Like other nonrenewable

resources, eventually the demand will out-strip the supply, however, unlike the use of fossil fuels for production of N fertilizer (N fertilizer can be produced without fossil fuels), global food security is depended on phosphate rock. Avoiding this crisis hinges on a proactive anticipation of future phosphorus supplies. Current approaches to reduce P use or recover P from waste include, removing phosphorus from waste, reusing agricultural debris, implementing alternatives wherever possible, and making dietary choices that use less P as well as optimizing P efficiency. The P available in urine in 2009 was estimated to be 1.68 million metric tons which accounts for 11% of yearly demand, however, by 2050 with the increase in population the amount of P in urine is conservatively estimated at 2.16 million metric tons (Mihelcic, Fry et al. 2011). Use of human urine fertilizer has the ability to not only reduce agriculture's dependence on fossil fuels but also to alleviate some of the strain on the world's limited phosphorus resources (Germer, Addai et al. 2011).

Conclusion

In summary, urine has been used as an effective fertilizer in small-scale farming around the world and across a variety of crops. Its effectiveness as a fertilizer is determined by the particular crop's nitrogen requirement and salinity tolerance. Urine is generally safe to use except when it is contaminated with fecal material or excreted from an unhealthy individual. The risks associated with urine fertilizer can be minimized by following the 9 step adaptable multi-barrier safety protocol recommended by the WHO for using human urine in agriculture on small farms. Its composition is similar to NPK fertilizer and its value can be determined in the worth of the nutrients it contains and by the increased crop yields that can result by its use. Urine can completely replace chemical fertilizer use in Africa and between 19-29% in western countries. The phosphorus in urine can help reduce our agricultural systems' reliance on phosphate rock. Ecological sanitation can reduce the nutrients that enter traditional wastewater treatment systems. EcoSan can have a positive effect on health and hygiene in developing countries by reducing or removing human waste from normal sanitation flows and instead redirecting it to agricultural systems. Currently, only small scale EcoSan systems are being used because of the greater difficulties associated with large scale urine collection and the processing for recovering nutrients and eliminating pathogens from urine. When urine is collected from many unrelated individuals, additional safety procedures must be followed to ensure the urine's safety. These additional procedures result in the need to recover the nutrients from the stored urine. Another major hurdle to large scale EcoSan is overcoming the geographic distance between urban centers where urine is produced and the agricultural fields that need it. The recovery of nutrients from human urine and their reuse in agriculture is the goal of

EcoSan systems, and it is the systems that address multiple needs like food security and sanitation in an eloquent solution that hold the greatest potential for developing countries.

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CHAPTER 2: TANZANIA

History and colonial influence

The United Republic of Tanzania was established in 1964 when two territories, Tanganyika on the continent of Africa and the offshore archipelago of Zanzibar, joined to form Tanzania. Tanzania has a long history of colonial influence dating back to 1498 when the Portuguese explorer, Vasco da Gama first surveyed the coast and islands. In 1505 the Portuguese captured the critical trading post of Zanzibar, also known as the Spice Islands, and held the territory until the island was overtaken by the Oman Empire in 1699; a strong Middle Eastern connection is still present today. Under Arab rule, the Island of Zanzibar flourished as it became the center of East African slave trade (Lodhi 1994). Mainland Tanzania was the stage for many of the great tales of exploration of the African continent. Notable excursions into the Tanzanian wilderness were led by famous Scottish explorer and missionary, David Livingston in 1866, closely followed by journalist Henry Morton Stanley, who was sent by the New York Herald to locate the elusive Livingston. In 1871, while working at his mission on the shores of Lake Tanganyika, Stanley found Livingston with the iconic introduction, “Dr. Livingston, I presume” (Mckenna 2011). A little over a century later in Northern Tanzania, archaeologist Mary Leakey uncovered footprints in volcanic ash of an hominid ancestor (*Australopithecus afarensis*) (Leakey and Hay 1979).

Exploration for the purpose of colonization of the territories occurred simultaneously in the late 1800's by both Great Britain and Germany. Germany eventually established influence primarily along the coast and at key trading settlements. One such settlement was Iringa, which was a key access point to the fertile lands of the Southern Highlands, and the trading ports on Lake Nyasa (Mckenna 2011). German colonialism was not accepted by the inhabitants in the interior of the territory, who mounted two major resistances to the German occupation. Both rebellions took place in the southern regions of the territory (Lliffe 1979). The first resistance occurred from 1891-1894 and was organized by Uhehe Chief Mkwawa. The Wahehe people of Iringa were able to mount a substantial force against the better equipped German army which gave the tribe the reputation of being fearsome warriors. By 1894, the official resistance had ended, but Chief Mkwawa was able to evade capture and he mounted an annoyance campaign employing guerilla-like tactics. However, in 1898 the Germans had closed in on the Chief. To avoid capture, Chief Mkwawa shot himself. In a brutal, symbolic gesture intended to crush future rebellion, the Germans removed Chief Mkwawa's head and sent it to Berlin (Redmayne 1968, Lliffe 1979). Despite repeated attempts by the Germans to crush their spirit, the southern tribes again united from 1905-1907 in the Maji Maji Resistance. The Maji Maji rebellion is noted as one of the most important anti-colonial movements in African history (Monson 1998). The Maji Maji rebellion grew to include tribes that ranged over 10,000 square miles. It is

estimated that 200,000 to 300,000 people; nearly one-third of the population, perished in the fighting and famine that resulted (Lliffe 1979). Germany's 40-year occupation of Tanganyika ended with the signing of the Treaty of Versailles, in 1919 at the end of the First World War. In 1920, the League of Nations gave control of Tanganyika to the British under a mandate. Tanganyika gained its independence from the British in 1961 as a gesture of gratitude for its involvement with the allied forces in WWII (Gewald 2008).

The politician and anti-colonial activist Mwalimu (Swahili for teacher) Julius Nyerere took charge of Tanganyika, first as its prime minister from 1961-1963 and then as the President of Tanzania for 21 years from 1964-1985 (Mckenna 2011). At the unification of Tanganyika and Zanzibar, Mwalimu Nyerere adopted both Swahili and English as the country's official languages, but English was almost exclusively used in foreign affairs. By choosing Swahili and the uniquely African culture it represents, Nyerere returned pride to the people as being an African nation and continued their defiance of colonial influence (Petzell 2012). This decision, to unify through an African language, made Tanzania unique from the other neighboring East African countries such as Kenya and Zambia, both of whom adopted English as their primary language after gaining independence. Nyerere is referred to as "Baba wa Taifa" or father of the nation, although his popularity among the people is divided. On one hand, he did return pride and peace to the country, but on the other, his socialist policies established reliance on foreign aid that is still being felt today. When Nyerere finally stepped down in 1985, Tanzania was one of the poorest countries in the world and almost completely dependent on foreign aid (Mckenna 2011).

Physical geography

Tanzania is the largest country in East Africa covering 885,800 square km, which is more than twice the size of California. Tanzania has shoreline on Lake Victoria, Lake Tanganyika and Lake Nyasa (Lake Malawi). In addition to its fresh water shoreline Tanzania boasts nearly 1,424 km of coastline on the Indian Ocean (CIA 2016). It shares land boundaries with Kenya, Mozambique, Burundi, Democratic Republic of the Congo, Malawi, Rwanda, Uganda, and Zambia (Fig. 2.1).

Fig. 2.1: Map of Tanzania displaying its location in East Africa, as well as Njombe town and region; where the study was conducted

The people

Tanzania is the 26th most populous country in the world with a current population of 53,950,935 and is ranked 219 out of 229 countries for fastest growing populations (CIA 2016). Tanzania's population is not only increasing, it is becoming more urbanized. Approximately 33% of the population resides in cities but this percentage is increasing due to migration from rural areas to urban centers at a rate of 5% annually (Partner 2015). Many factors likely contribute to this dramatic increase in population, such as medical advancements, increased life expectancy, high fecundity rates, and early age of reproduction (CIA 2016).

Tanzania is home to 130 different tribes, many of which have their own dialect. Swahili, which is a member of the Bantu family of languages, is the most commonly spoken language across the mainland, and in Zanzibar. Swahili developed on the coast and spread across East Africa along the trade routes (Petzell 2012). English is used in business and among the upper classes of society (personal communication). Arabic is spoken along the coast and in Zanzibar, particularly within the Muslim communities. Roughly 35% of the population is Muslim, mostly concentrated along the coast and on the islands, where Islam is the major religion (>99%). Christianity is the predominant religion in the interior of the country, making up 61% of the population (CIA 2016).

Tanzania is currently undergoing a nationalist movement. For example, the country has two official languages; Swahili and English, however, last year the government made the transition from using English to Swahili in meetings and governmental documents (personal observation). There is a movement in Tanzania to reject western culture and promote national pride. The current administration has banned the importation of used clothes from western countries, implemented strict dress codes, and increased the tax by 3-fold on all imported goods and services directed at the tourism industry. Additionally, the government has made it increasingly challenging for foreign companies to invest in the country (personal communication).

The economy

In 2017, Tanzania reported a GDP of \$51.61 billion and has maintained an average of 7% growth in GDP per year for several years (CIA 2016). The majority of GDP (47%) is generated through the service sector, and 28% from the industrial sector (mining and agricultural processing). Ninety-six percent of exports come from agriculture and include coffee, cotton, sisal, cashew, fruits and vegetables, which represents only a quarter of the GDP (CIA 2016). Despite the growth in GDP, little has

changed for the majority of Tanzanians with the exception of an expanding wealth gap (Alfred Agwanda 2014).

According to the CIA World Fact Book, per capita income is \$3,100 based on GDP, however, that amount is misleading. The nation's increasing wealth is not reaching the majority of the population. Tanzania has an extremely high Gini coefficient, which is a measure of inequality, $(0_{\text{(none)}} - 1_{\text{(total inequality)}})$ at .773 overall. As of 2016, one study found that in two Tanzanian cities, 89% the population lived on less than \$1 per day (Lusambo 2016). It is important to consider that what defines poverty is determined by location. The same study found that when the definition of poverty was scaled to local living requirements, only 55% of the population reported living on less than \$1 per day (the World Bank's definition of absolute poverty). The percentage of people living in poverty in Tanzania differs between urban and rural populations. In 2007, the percentage of the population living in rural settings was 74.5% with 37.6% living in poverty. In urban settings like Dar es Salaam which accounts for 7.5% of the total population, the percentage living in poverty was only 17.7%. In other urban areas the percentage of the population living in poverty is higher at 24.1 %. This study compared 2001 data to 2007 data to identify if there was a decline in poverty headcount, but found no significant reduction in poverty despite an average annual increase of 7% in GDP in the same period. In fact, when the researchers factored in the increase in population from 2001 to 2007, they estimated an increase of 1.3 million people living in poverty (FAO 2008). In 2016, urban centers across the country reported an average of 46.4% of residences lived below the poverty line while 55% of rural residences were impoverished (Lusambo 2016).

Like many nations, the Tanzanian government struggles to decide where to allocate governmental support to achieve the most benefit. Governmental programs that target farmers resulted in an income increase four times greater than programs targeted at industry or service sectors (Salami, Kamara et al. 2010). Agriculture only accounts for 25% of GDP but it employs 65-80% of the country's population (Amare, Asfaw et al. 2012, Kassie, Jaleta et al. 2013, CIA 2016). The majority of the families living in extreme poverty are agricultural workers living in rural settings. In 2010, a study looking into household income found that in rural villages which almost solely rely on agriculture, the average household income was 480,000 TZS (Tanzanian shillings), which based on the 2007 average exchange rate of \$1 USD = 1,214 TZS, equates to roughly \$395 USD (Aikaeli 2010). In Tanzania, the fate of the agricultural sector is directly responsible for the economic development, food security, and poverty reduction (Kassie, Jaleta et al. 2013).

Government assistance programs are the most beneficial when they are used for agricultural subsidies and supporting improved agricultural practices through

agricultural extension officers (Kassie, Jaleta et al. 2013, Kassie, Jaleta et al. 2014). This research indicates the importance of education and outreach in agricultural communities. Tanzania's agricultural sector is dominated by smallholder farms of less than 2.5 hectares, which produce over three-quarters of the country's agricultural products (Salami, Kamara et al. 2010). When evaluating the causes of malnutrition, family income matters, with the lowest income quartile ingesting only 1690 kcals per day compared to the highest income quartile ingesting 3480 kcal/day (Statistics 2010). Decreasing the amount of people in poverty can increase their access to food which can make them less exposed to food insecurity and decrease the incidents of malnutrition (Rockström, Axberg et al. 2005, Affairs 2009, Lusambo 2016).

Climate and biodiversity

The geography and climate of Tanzania is extremely varied ranging from tropical coast to savannah plains, to coral reefs on the islands of Zanzibar and Pemba, to glaciers on the slopes of Kilimanjaro and temperate highlands in the north and south (CIA 2016). Three of Africa's seven wonders can be found within its borders, including the Serengeti plains, Ngorongoro Crater and Mount Kilimanjaro which is the highest point in Africa at 5,895 m. The East Africa Rift Valley runs north to south through the western regions of the country and the Eastern Arc Mountain Range runs through the eastern regions. Tanzania is home to numerous other lesser mountain ranges and volcanoes (CIA 2016). Owing to the heterogeneity of climates and biomes, and relatively low anthropogenic development, Tanzania is a center of biodiversity. It houses numerous endemic species including: 34 bird species, 85 reptile species, 86 amphibian species, 159 freshwater fish species (mostly located in Lake Nyasa), 5 marine fish species, and 24 mammal species (including 5 primate species). As of 2015, Tanzania was home to 1,077 critically endangered, endangered, and vulnerable species (Society 2016). Twenty-seven species of reptiles in addition to 43 species of amphibians have been discovered since the year 2000. In 2005, a new primate species, the Highland Mangabey, was discovered (Jones, Ehardt et al. 2005). The number of species identified in recent years indicates that there are likely more species yet to be discovered. Conservation and preservation supported through policy and education remain a central focus of many nongovernmental organizations and government agencies in Tanzania. Scientists are currently struggling to identify Tanzania's biodiversity before its unique species are lost to deforestation, climate change or poaching.

Health and water sanitation

Tanzania's population is rapidly expanding, with an annual growth rate of nearly 3% (Partner 2015) and an expected population of 70.1 million by 2025 (Alfred Agwanda

2014). Providing food security, sanitation and hygienic living conditions for the growing population are beginning to stress the current systems in place in Tanzania. Sanitation and hygiene are interconnected in Tanzania and both are dependent on water. The most common type of latrine in the country is the pit or drop “choo” (Swahili for toilet). In the peri-urban area surrounding Dar es Salaam (Dar), pit latrines are the predominate sanitary solution with 80% of the residents relying on them. Additionally, the geographic area around Dar has a high water table which makes the chance of water source contamination from sewage pits high. Eighty-five percent of the population within the peri-urban area rely wholly on groundwater, which is accessed through 850 boreholes (John 2003). The risk of water source contamination by waste products is common throughout the country. When the water used for sanitation is the same water used for daily cooking, cleaning, and drinking, hygienic conditions cannot be maintained. Not only is the lack of discrete water sources a problem, many regions of the country face water scarcity issues.

For choos to be hygienic, water must be available for use. Many regions of the country do not have access to a constant source of water and with variable temperature and precipitation patterns brought on by climate change, historically dependable water sources are becoming unreliable (Rowhani, Lobell et al. 2011). Currently, only 54% of Tanzanians have access to improved drinking water. Eighty-seven percent of Tanzanians have no access to modern sanitation, which is defined as a reliable sanitation system that removes wastes and prevents exposure to pathogens. Whether due to lack of sanitation systems or lack of water, 16% of Tanzanians practice open defecation (UNICEF 2009). This practice can contribute to contamination of food and water supplies and has negative implications for the environment, and personal health and hygiene.

Lack of proper and reliable sanitation has negative impacts on health by increasing the transmission of many diseases. The CDC has assigned Tanzania’s risk associated with infectious diseases as very high (CDC 2017). Common diseases in Tanzania are bacterial diarrhea, Hepatitis A, Typhoid fever, Malaria, Dengue fever, and Rift Valley fever (CIA 2016). Parasitic infections by schistosomiasis and leishmaniasis are common in areas where water sources are vulnerable to contamination from human fecal material (CDC 2012). Since 2015, there has been an active cholera outbreak which the government has been unable to eradicate because of poor sanitation and hygiene (CDC 2017). Nearly 1/3 of deaths of children under age 5 are related to poor hygiene with nearly 20% of these deaths stemming from preventable diarrhea. This equates to nearly 5 deaths every hour (UNICEF 2009). Improving sanitation, hygiene and food security is not only important for improving the quality of life of Tanzanians, it is extremely important for the portion of the country’s population who have increased

vulnerability to disease and infections. Nationally, the HIV prevalence is 4.7% in individuals ages 15-49. In women that percentage increases to 5.8%, and in Njombe (which is where this study was conducted) the prevalence is 15.4% (USAID 2017). Individuals with HIV/AIDS have greater susceptibility to diseases, so by improving sanitation and hygiene these individuals are able to avoid exposures that could result in life threatening conditions.

Heath and food security

Increasing food security to support its growing population is also a vital concern for Tanzania. Maize is now the primary food source in the country, replacing traditional grains such as sorghum and finger millet. Maize consumption accounts for 60% of the daily caloric intake and 50% of the daily utilizable protein of an average Tanzanian (Shekania Bisanda 1998). Tanzanian diets depend heavily on vegetables, with 80% of protein, 74% of lipids and 94% of caloric intake coming from vegetable origin (FAO 2008). Protein from plant sources is deficient in the amino acids: methionine, cysteine and lysine, as well as being less digestible than proteins from animal sources (Smil 2002, Feil, Moser et al. 2005). Because of the characteristics of vegetable proteins, children need to consume twice the amount of vegetable protein to equal the nutritional content derived from animal protein. The typical western diet averages 100 grams per day of protein; 55 g from animal sources (dairy, meat, eggs) (Smil 2002). The typical Tanzanian diet averages 55 g/day with only 10 g from animal origin (FAO 2008). Not only has maize replaced traditional food sources, the ingestion of animal products, fruit and vegetables has also decreased which negatively impacts the nutritional content of the diet. In rural areas, vegetables are consumed almost daily but in quantities too insignificant to constitute any real nutritional input. Fruit is not routinely eaten and many Tanzanians view fruit as a special treat without any nutritional importance. FAO data collected between 1986 until 2003 displays several trends in the country's food intake. Ingestion of cereals (maize) increased from 181 g/day to 307 g/day. Fruit and vegetable intake dropped from 283 g/day to 158 g/day and vegetable oils consumption increased from 7 g/day to 14 g/day. Consumption of milk and eggs also decreased from 87 g/day to 72 g/day (FAO 2008). There has been a detrimental effect on nutrition with a maize-based diet.

Cultivating maize is challenging in the poor soils of Sub Saharan Africa (Sanchez 2002) because of the high nutrient requirement of the crop (Shekania Bisanda 1998). Chemical fertilizers are unattainable for the majority of farmers (Salami, Kamara et al. 2010). This results in many crops being cultivated without chemical fertilizers, which subsequently results in very poor yields (Kassie, Jaleta et al. 2014). The majority of

smallholder farms cultivate food crops to feed their families. In Tanzania it is common for the female members of the family to maintain the garden that feeds the family and for men to cultivate land away from the home for income generation (personal observation and communication).

The growing season for maize in Tanzania is different in different areas of the country. In the Southern Highlands, which was where I lived, planting coincides with the onset of the rainy season around the end of November or into December (Shekania Bisanda 1998, Mbululo and Nyihirani 2012). Farming in Tanzania relies on rain-fed agriculture, so droughts have a huge impact on food security. In years with normal rainfall, the country can produce enough food to feed its population but in years of drought, food scarcity is an issue (FAO 2008). Recently, Tanzania has been experiencing variable rainfall patterns. The central regions of the country are projected to experience a reduction of 20% in total precipitation by 2050, while the northern and eastern parts of the country may experience an increase of 50%. This can result in an increase in droughts for the former and in severe flooding for the latter (Rowhani, Lobell et al. 2011). Understanding and adapting to the projected volatility in precipitation for the country is important for farmers to maintain productivity. The Southern Highlands receive a unimodal rainfall pattern from November to April. Recent research has identified a trend of delayed onset rains as well as variability of precipitation events (Mbululo and Nyihirani 2012). These trends have retarded maize growth and result in a reduction of yield (Rowhani, Lobell et al. 2011). The late onset of rains in 2016 affected the start of this current study.

Because of the country's reliance on maize during the wet season, when the last year's harvests are dwindling and the current harvest is drying in the field, hunger is wide spread, especially for children. It is a common practice for families to eat together out of one large pot. Traditionally, men eat first followed by women and children last (Kimanya, De Meulenaer et al. 2010). This practice can result in children not having access to enough food (FAO 2008). Nationwide, nearly 1/3 of children under 5 are affected by stunting and in some regions it is as high as 50% (FAO 2008). Maize is usually ingested in the form of ugali; a stiff porridge which is the staple dish of the country. It is eaten daily and many Tanzanians view it as an excellence food rich in nutrients. Ugali was introduced into Tanzania concurrently with the cultivation of maize. Cultivation of maize in the Southern Highlands of Tanzania began at the beginning of the 20th century in Iringa and by the 1950's it had spread to Mbeya and Ruvuma and by 1970 to Rukwa (Shekania Bisanda 1998). One study investigated the causes of the high prevalence of stunting and malnutrition within the country and found that although lack of food does affect malnutrition in children, it is not the sole cause. The study looked into infant feeding habits and growth rates. The researchers found

that growth retardation is most commonly observed following the introduction of complementary foods during the transition from exclusively breastfeeding to incorporation some soft foods. In that study which surveyed 215 Tanzanian mothers, they found that 191 mothers feed their infants maize on a daily basis once complementary foods were introduced (Kimanya, De Meulenaer et al. 2010). This result would indicate that maize is not a well-balanced source of food and that children who rely on it almost exclusively can experience malnutrition.

Fertilizer use in the country

Fertilizer use in Tanzania is very low, with average fertilizer application at just 5-7 kg/ha/year compared to the industrial worlds' average of 100 kg/ha/year (Salami, Kamara et al. 2010, Partner 2015). A major drawback to minimal fertilizer use has resulted in reduced yields. One hectare of land in Tanzania produced on average 1 ton of cereal crops which is one-half as much as India, a quarter as much as China and only one-fifth as much as an American farmer (Salami, Kamara et al. 2010, Kassie, Jaleta et al. 2013). Farmers attributed the decrease in yield to poor soils and lack of access to fertilizer as well as no money to buy them (Salami, Kamara et al. 2010, Kassie, Jaleta et al. 2013). For example, a survey in 2010 of 1,500 smallholder farms in the northern and eastern regions of Tanzania found that only 4% of farmers used chemical fertilizer, however, 67% reported using improved seeds (Kassie, Jaleta et al. 2013). Another study in 2010 found lower use of improved seeds (23%) but higher reported fertilizer use (13%). Again, the farmers in the study reported poor access, lack of credit, lack of awareness, land scarcity to test new seeds, and lack of knowledge about how they work as barriers to implementing improved farming practices (Salami, Kamara et al. 2010, Amare, Asfaw et al. 2012). Maize cultivation on arable land has increased by 54% from 2000-2010 (Kassie, Jaleta et al. 2014). Maize is cultivated in nearly every region of the country. One survey of smallholder farms found that 96% of farmers grow maize (Amare, Asfaw et al. 2012). Improving education and access to improved farming methods is essential for increasing food security and poverty reduction in the country. One study conducted in Tanzania found that using improved maize seed varieties produced on average 1.2 tone/ha compared to .5 t/ha that was harvested from local varieties (Kassie, Jaleta et al. 2014). Yet another study found that households who used improved seeds had an income increase of 150% (Amare, Asfaw et al. 2012). Despite the improvement in yield seen with improved farming practices, the effects are diminished because of the poor soil quality. Using improved seeds in Africa does not have as large an impact on yield when compared to other countries. In the 38 years since improved seeds became available, Asian, Latin American and Middle Eastern countries have seen an improvement in yield of between 66-88%, however, African countries have only seen a 28% improvement (Sanchez 2002).

Poor soil fertility is the primary limiting factor inhibiting food production in smallholder farms in Tanzania (Kassie, Jaleta et al. 2013). Many of the cultivated lands in developing countries are subject to continuous unplanned farming. This practice, along with others, contributes to depleting soil nutrients (Sanchez 2002). When fertilizers are not used, the land requires longer fallow periods between crops in order to replenish soil nutrients, however, because of rising food demands the fallow periods are being shortened if not eliminated altogether (Kassie, Jaleta et al. 2013). At a time when the African soils need to be producing ever more food, the current farming practices are having the opposite effect. A survey of the Tanzanian farming practices from 1983-2000 found that according to land uses and amendment history, per hectare average annual nutrient balance was negative 22-26 kg nitrogen, negative 2.5-7 kg phosphorus, and negative 15-23 kg potassium (Sanchez 2002, Kassie, Jaleta et al. 2013). A yield of 6 t/ha of maize removed 100-150 kg of nitrogen and 40-60 kg of P_2O_5 from the soil in the Southern Highlands of Tanzania (Shekani Bisanda 1998).

Soil fertility is not only an issue for Tanzania. African soils have the lowest P fertilizer application rate in the world at 9-23 kg/ha with 75% of African soils are P deficient (Cordell, Drangert et al. 2009). In eastern Kenya, 80% of the lands that were primarily planted with maize were found to be extremely P deficient (Sanchez 2002). In the Southern Highlands where the current study was conducted, P and N are the main limiting factor for maize production (Shekani Bisanda 1998). Alternative farming practices hold promise for increasing soil fertility. Many of these practices enable the soil to function as a carbon sink, which increases the soil's water retention and reduces erosion without having a negative impact on yield (Kassie, Jaleta et al. 2013). In Tanzania, over 75% of agricultural products produced were by smallholder farms ≈ 2.5 ha (Salami, Kamara et al. 2010) and over 96% of the food consumed is produced within the country (2008). Considering Sub Saharan Africa's minimal use of chemical fertilizers, recycled nutrients obtained through ecological sanitation will be able to meet the fertilizer requirements without forcing farmers to rely on chemical fertilizer (Rockström, Axberg et al. 2005, Mihelcic, Fry et al. 2011).

Tanzania is reliant on imported fertilizer sourced mostly from the Middle East, India, and China. The cost of fertilizer increases annually and is subject to taxes, handling fees and transportation costs (2008). Tanzania has a natural supply of phosphate rock (Semu and Singh 1995), but the only fertilizer factory in the country was closed in the 1980's (John 2003). Compared to other continents, fertilizer is 2-6 times more expensive in Africa. For example, in the US and Europe, urea costs \$90 per metric ton, \$400 in western Kenya, \$500 in eastern Uganda, and \$770 in Malawi (Sanchez 2002). Within Tanzania, fertilizer is generally sold in 50-kilo bags. In Iringa town (a major city in the Southern Highlands) a 50kg bag of DAP costs 51,700 TZS, CAN costs 47,000

TZS and Urea is 39,200 TZS. Most retailers do not sell in smaller quantities, however, an estimated price of 1,500 TZS per kilo was reported for DAP. These prices would most likely increase in smaller villages and with further transport (personal communication).

Farmers report many reasons for not adopting improved farming practices. They report lack of understanding, lack of education, inaccessibility to the technology, and most commonly, financial constraints. For many smallholder farms, purchasing fertilizer is not an option (Salami, Kamara et al. 2010, Amare, Asfaw et al. 2012). Using local spacing recommendations for maize cultivation, every hectare has approximately 44,440 plants (Marandu 2015). Recommended fertilizer application is 5 g per plant of each of the three fertilizers (DAP at planting, CAN when the maize plant is knee high and Urea when the plant tassels) (Fig. 2.2). In order to meet the fertilizer requirements, 222 kg of each fertilizer (which is 4.44 bags) would be required (Table 2.1).

Table 2.1: Local cost and cost in USD (exchange rate of \$1 USD = 2,250 TZS) of three chemical fertilizers, and the cost of application using recommended application amounts.

Fertilizer	Cost (TZS) per 50 KG	Cost (TZS) per Hectare
CAN	47,000 (\$20.89 USD)	208,680 (\$92.75 USD)
Urea	39,200 (\$17.42 USD)	174,048 (\$77.35 USD)
DAP	51,700 (\$22.98 USD)	229,548 (\$102.02 USD)
Total per Hectare		612,276 (\$272.12 USD)
Total for 2.5 Hectare		1,530,690 (\$680.31 USD)

The average yearly income for a rural Tanzanian family is 480,000 TZS (Aikaeli 2010), and the average farm size is 2.5 hectares. The cost of fertilizer using local recommendations is 1,530,690 TZS which is out of reach for the majority of farmers. It is clear why the rate of chemical fertilizer application is so low. In order to increase food security and reduce malnutrition, an alternative to chemical fertilizer needs to be found.

Fig. 2.2: Photos of the three fertilizers recommended for maize cultivation. Fertilizers are imported in bulk and once in the port in Dar es Salaam, they are packaged for transport across the country.

Ecological sanitation: a sustainable solution

Ecological Sanitation, which was discussed in detail in Chapter One, presents a sustainable solution which can be implemented on a small scale, thereby enhancing food security, and increasing hygiene and sanitation, while helping the environment. One factor that could constrain the use of EcoSan is the cost of implementation. Source-separating toilets in Dar es Salaam cost \$570 USD/toilet (John 2003). However, in the current study, a low-cost urine collection system was built for each participant at a cost of 15,000 TZS or \$6.66 USD using locally available materials (Table 2.2 and Appendix E). In addition to cost concerns, attitudes about using human urine as a fertilizer for food crops could restrict its use (John 2003), although negative biases can be reduced through awareness campaigns (Andersson 2015, Simha, Lalander et al. 2017). In the current study, all participants expressed curiosity and positive attitudes about urine fertilizer, however, they were educated and employed at a school that taught improved farming practices. Other studies in Tanzania involving the implementation of urine fertilizer found that in many Muslim communities the practice was not widely accepted because it was viewed as unclean to handle human waste (Shayo 2002). In Dar es Salaam only 46% of the 110 households that used EcoSan toilets reported using the urine as a fertilizer in their gardens (John 2003). However, in Uganda, one research group found that shoppers were comfortable buying and eating food fertilized with human urine once the minimal risks were explained (Andersson 2015). The aforementioned study in Dar es Salaam reported similar results; that within the 46% of households that used the source-separated urine to fertilize their garden, the fears of ingesting the food fertilized with urine decreased when they understood the risks and the steps taken to mitigate the hazards (John 2003).

Table 2.2: Cost of materials for the urine collection system given to participants in the study.

Materials	Cost in Tanzanian Schillings (TZS)	Cost in USD (\$)
20-liter Jerry can	3,000	1.33
Sealing plug (plate, twine, bag of sand, handle bamboo or old pipe)	1,000	0.44
10-liter bucket	3,500	1.56
Application cups (2)	1,000 * 2 = 2,000	0.89
1 liter cup (female collection)	1,500	0.67
Rubber ribbon (used for sealing the funnel to the can)	1,000	0.44
funnel	3,000	1.33
Total cost	15,000 TZS	\$ 6.66
Exchange rate \$1 USD = 2,250 TZS		

Health and safety concerns associated with implementing closed-loop sanitation in Tanzania are focused around decreasing the chance of fecal contamination and utilizing a storage period for urine to facilitate pathogen die off. According the WHO guidelines, direct application of source-separated urine to a household garden is safe, but in the current study we added a two-day storage period because of the possible presence of schistosomiasis in the urine. Shaw (2010), successfully used a two-day storage period to eliminate schistosomiasis from source-separated urine in Mali West Africa. In Tanzania, fecal contamination is a risk in many systems due to the likelihood of the water used for cleaning the anus entering the tank for storing urine and thus introducing fecal pathogens into the urine. Culturally, water must be used in cleansing after bathroom use. In the Kilimanjaro region, one study found that 4 of 28 urine storage tanks were contaminated with fecal material from anal cleansing water (Shayo

2002). Proper design of the source-separating toilet can reduce the risk of contamination from anal cleansing water.

Conclusion

Tanzania is a diverse country made of many different tribes. Each tribe has its unique history and culture, and these tribes together weave the fabric of the country. Tanzania has a history of defiance of colonial rule and pride in its African heritage. Within Tanzania's borders many different landscapes can be found as well as an amazing variety of animal diversity. Tanzania has a growing economy built off this plethora of natural resources and its tourism industry, however, there is high economic inequality. Despite Tanzania gaining independence in 1964, many of the country's early socialist policies resulted in restricted economic growth for the majority of the population. This has created an extremely poor populous and inadequate infrastructure, which have held back advancements in agriculture. Agriculture is the primary employer within the country, however, it accounts for only a quarter of the GDP. The vast majority of food eaten in country is grown on family cultivated subsistence farms. Tanzania's population is increasing and the farmers are struggling to meet the increased food demands. The poor crop yield on many farms is caused by low soil fertility and lack of access and capital to purchase chemical fertilizers. These poor yields contribute to malnutrition of children. Also, many of the population are migrating to cities; placing stress on overworked or nonexistent sanitation systems. Low-cost, small-scale ecological sanitation systems can provide sanitation while recovering the nutrients from human urine for use as a fertilizer in agricultural fields. Closed-loop sanitation systems that addresses health, sanitation and food security is emerging as a viable solution in developing countries.

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CHAPTER 3: THE EFFECTS OF A 30% URINE DILUTE FETILIZER ON MAIZE BIOMASS, YIELD AND NUTRIENT GRAIN CONTENT

Abstract

In Sub-Saharan African countries, soil fertility is low due to unsustainable agricultural practices and little to no access to chemical fertilizers. Poor yields have resulted in many of these countries struggling to produce enough food to feed their growing populations. Human urine has been used as an effective, low-cost alternative to chemical fertilizers in greenhouse and plot trials on a variety of vegetables, but its effects on elemental grain composition in cereal crops are largely unknown. Here we tested whether diluted human urine applied as a fertilizer can increase maize crop growth, yield, and nutritional content on small family-owned plots. In Hagafilo village, in the Southern Highlands of Tanzania, participating farmers maintained family plots (n=8) within which half of each plot received urine fertilizer and water, while the other half received water only. The experiment ran for one growing season. We found that urine fertilizer significantly increased growth (above and below ground dry biomass, stem length) and yield (cob length, number of total and developed cobs) metrics. We also found that shoot/root ratio was greater in the control treatment, suggesting that the fertilized plants invested a greater proportion of resources into roots relative to shoots; this could have implications for soil health as extended root systems can minimize soil erosion and have longer term benefits on soil fertility. In contrast, urine fertilizer did not significantly alter maize kernel nutrients. An increase in grain nitrogen is usually observed with increases in fertilizer supplied. This study supports the use of human urine fertilizer as a way to increase food security.

Introduction

Modern agriculture relies on chemical fertilizers to provide nutrients to plants, however, fertilizers are costly and their use is cost-prohibitive in developing countries (Ganesapillai, Simha et al. 2015). For at least one-third of the world's population, having access to N fertilizers makes the difference between food security and malnutrition (Smil 2002). Applying human urine as a substitute to chemical fertilizers may provide a renewable and sustainable option for smallholder farms (Jönsson, Stintzing et al. 2004, Richert, Gensch et al. 2010). Human urine is rich in the elements nitrogen, phosphorus and potassium and these elements are present in forms that are available for direct uptake by the plant (Richert, Gensch et al. 2010). The average adult produces enough urine to fertilize their yearly requirement of cereal crops (Guzha, Nhapi et al. 2005). However, using human urine as a fertilizer is not without risk. Human urine may contain *Campylobacter jejuni*, *Cryptosporidium parvum*, rotavirus, *Salmonella typhimurium*, *E. coli*, heminths and schistosomes eggs, however, it contains very few enteric microorganisms when it is excreted from the body of a healthy individual (Höglund, Ashbolt et al. 2002, Höglund, Stenström et al. 2002, Heinonen-Tanski and van Wijk-Sijbesma 2005, Chandran, Pradhan et al. 2009). The majority of hormones and pharmaceuticals are excreted through urine while heavy metals are eliminated through feces (Jönsson, Stintzing et al. 2004). The impact of pharmaceuticals on agriculture from using human urine fertilizer is largely unknown but the levels of antibiotics found in human urine are 100 times less than that in the manure fertilizer collected from farm animals (Winker, Vinnerås et al. 2009). Avoiding contamination or transfer of any of these substances is as simple as avoiding collecting the urine from unhealthy individuals. Apart from the transmission of pathogens which can be naturally present in urine, the level of contamination is directly proportional to the amount of fecal material introduced during elimination (Schönning, Leeming et al. 2002). Implementing a source separation, a storage period, and/or a withholding period before harvest can greatly reduce any risk associated with human urine fertilizer. During storage, the three main processes urine undergoes are urea hydrolysis, mineral precipitation, and ammonia volatilization. The microorganisms found in the collection systems produce enzymes that function as the catalyst for the hydrolysis of urea to ammonia (NH_3) (Udert, Larsen et al. 2006). The nitrogen found in fresh urine is mainly in the form of urea, however, after hydrolysis the composition is 90% ammonium (NH_4^+) and bicarbonate. Additionally, during this process the urine pH increases to 9 (Ganrot 2005). The increase in pH, and the ammonium concentrations along with the increase in temperature (which can be achieved by setting the stored urine in the sun) results in a harsh environment and a high die-off of many pathogens (Jönsson, Stintzing et al. 2004, Richert, Gensch et al. 2010).

In agriculture, there is general consensus that increasing the supply of nitrogen is positively correlated with increases in plant growth. The vast majority of studies that have investigated the relationship between nitrogen supply and plant growth have relied on chemical fertilizer to supply the nitrogen. Very few studies have used source-separated human urine as fertilizer and even fewer have relied on local farmers as participants. The studies conducted have produced mixed growth results. This may be because of the nutrient requirements for different plant species and how those species allocate nutrients. For example, peak biomass was achieved with application of 50 kg N ha in carrots, while beetroot continued to accumulate biomass until 800 kg N ha (Mnkeni, Kutu et al. 2008). Some studies have found that excessive N fertilizer results in a decrease in root growth (Feil, Moser et al. 2005). Plants grown with urine fertilizer produce plants equal in biomass to the plants that were fertilized with chemical fertilizer in cabbage, beet, and maize (Guzha, Nhapi et al. 2005, Pradhan, Nerg et al. 2007, Pradhan, Holopainen et al. 2010) and larger plants in pumpkin and amaranthes (Guadarrama and Pichardo 2001, Pradhan, Pitkänen et al. 2008). Because of the different plant species' allocation of nutrients, the plant's growth response is not always the same as the yield response.

Increases in yield are ubiquitous with increases in N fertilizer application in maize (Feil, Thiraporn et al. 1992, Thiraporn, Feil et al. 1992, Alfoldi, Pinter et al. 1994, Feil, Moser et al. 2005, Seebauer, Singletary et al. 2009). Nitrogen supply impacts maize yield by determining the number of kernels that can develop per cob. Larger N supplements result in a higher number of kernels developing and a greater accumulation of carbohydrates/starches (biomass) during the filling stage of kernel development (Feil, Moser et al. 2005, Seebauer, Singletary et al. 2009). Urine fertilizer has been used to cultivate a variety of food crops. Urine fertilizer's effectiveness is controlled by the nitrogen requirement for the plant, the application rate, and the plant's salinity tolerance. Urine was found to produce yields comparable to yields achieved through chemical fertilizers in maize (Andersson 2015), cabbage (Pradhan, Nerg et al. 2007), tomato (Pradhan, Holopainen et al. 2009), red beet (Pradhan, Holopainen et al. 2010), sweet pepper (Shrestha, Srivastava et al. 2013), and cucumbers (Heinonen-Tanski, Sjoblom et al. 2007). A number of studies have been conducted on maize in Zimbabwe. One study found that plants fertilized with varying application amounts of urine had a 6 to 35-fold increase in yield compared to the plants that were just irrigated (Morgan 2003). Another study found maize grown with chemical fertilizer produced 2.5 t/ha, compared to 3 t/ha with urine fertilization. Both fertilizers produced larger yields than the plants that were only irrigated (1.5 t/ ha) (Guzha, Nhapi et al. 2005).

Researchers to date have been focused on increasing growth and yield in maize without considering the effect on maize grain nutrient values (Feil, Moser et al. 2005). Plant accumulation and allocation of nutrients in response to different fertilizer sources have been measured in leaves and stems, as well as the fruits of tomatoes and red beets, but never in maize grain. Increases in chemical nitrogen fertilizer have a positive relationship with nitrogen concentration in the maize grain (Thiraporn, Feil et al. 1992, Alfoldi, Pinter et al. 1994, Feil and Fossati 1995, Feil, Moser et al. 2005, Seebauer, Singletary et al. 2009). Despite the yield increase, scientists have proposed that there may be a dilution effect on grain elemental concentrations with increased yield (Bennett, Stanford et al. 1953). In small cereal crop cultivation, decreases in grain protein are often associated with increases in yield (Feil, Moser et al. 2005). Grain protein concentration can be estimated based on grain nitrogen concentration (Seebauer, Singletary et al. 2009). Increased N fertilizer resulted in a decrease of grain phosphorus (Bennett, Stanford et al. 1953) as well as grain calcium and zinc (Feil, Moser et al. 2005). However, Kirchmann and Petterson (1995) found that plant uptake of phosphorus was increased by 28% when sourced from urine compared to chemical fertilizer. As yield increases, the plant's demand for elements increases (Feil, Moser et al. 2005) and if the plant is unable to meet those demands a dilution effect can be observed as a result of the grain filling stage (Seebauer, Singletary et al. 2009). It has been suggested the effect of nitrogen fertilizer on grain composition may be dependent on the form of the N fertilizer (Feil, Moser et al. 2005). To date, there has been limited research on the effects of urine fertilizer on grain elemental concentrations.

Hagafilo village is located in the peri-urban area south of Njombe town. Its population is a mix of people who commute into Njombe town for work, farmers who commute to undeveloped lands for cultivation of potatoes and other food crops and workers at the three schools in the village (Mother Theresa's Pre-Primary and Primary school, Hagafilo Secondary School and Hagafilo College). It is unique in that many of the villages' inhabitants have above average education levels however, the village suffers from lack of arable land to cultivate crops because of its proximity to town. The college in the town offers training in agriculture, animal husbandry and teachers education. The Schools pride themselves on being some of the top schools in the country and they accomplish this by being open to alternative teaching methods and experimentation. Working with individuals who understand the importance of alternative farming practices and also personally struggle with getting the most out of the limited arable land is vital in implementation of urine fertilizer. Participants were able to see a direct benefit to using human urine fertilizer.

Here we tested whether application of urine fertilizer to plants in small family plots can impact maize productivity, yield, and kernel nutritional content. We

hypothesized that maize plants grown with the urine fertilizer would be larger and produce more cobs than plants grown without urine fertilization. Because urine contains vital elements that are incorporated into plants, we also predicted that maize grain from plants grown with urine fertilizer would show an increase in elemental grain concentrations. To test our hypotheses, we had volunteers maintain eight family maize plots for one growing season, and in each plot, maize plants either received urine fertilizer over the growing season or were only irrigated. At the end of the growing season we harvested plants and measured traits related to plant growth, yield, and kernel nutrient content (from a subset of cobs we measured the content of 6 of elements: calcium, carbon, magnesium, nitrogen, phosphorus, and potassium). In accordance with our hypothesis, we did find that urine fertilizer resulted in increased plant growth and cob yield, but surprisingly plants receiving fertilizer invested relatively more into root development per unit shoot than plants not receiving fertilizer. Furthermore, we found the proportion of developed to total cobs was greater in the control treatment. Also, we did not find an increase in grain nitrogen concentrations with application of human urine fertilizer.

Materials and methods

Study site

This study was conducted in Hagafilo village (09.201037 °S, 34.460202 °E) in the Njombe region of the Southern Highlands of Tanzania (elevation of 1,921 m), which has a population of approximately 2,000 (Fig. 3.1). The region's wet season is from November to April, with an average total precipitation of 1,170 mm/year (N.S.S. 1987, Mbululo and Nyihirani 2012). The mean temperature is 16 °C with extremes ranging from 1°C to 30 °C and during the months of June, July and August it is not uncommon for night frost to develop (N.S.S. 1987). The soils in Hagafilo are developed from granite bedrock, composed of loam to clay material, are highly acidic (average PH values 4.1 to 5.5), nutrient poor, and have limited water holding capacity; all of which combine to make the soils undesirable for crop production (Stockley 1948, N.S.S. 1987).

Participants

The participants were from the Hagafilo community and were debriefed on the project purpose, potential risks, and requirements. Participation in the study was completely voluntary and no personal information was gathered (Appendix A). Each participant cultivated maize on land that is traditionally cultivated for maize, provided the urine from their family unit to fertilize their own plots, employed a storage before application to insure sterilization and pathogen die-off, as well as a withholding period before harvest after the final treatment in order to reduce risk of maize contamination

by urine; due to these steps our research was exempt from IRB review (Appendix B). In total, we had six participating families that maintained a total of eight experimental plots.

Experimental design

The experiment was designed using a randomized complete block design: there were eight cultivation plots (“blocks”) and within each plot we administered the treatment in subplots (two levels of treatment: urine fertilizer plus water or water only). Treatment subplots were separated by a buffer zone to prevent leaching between treatments (for experimental design see Fig. 3.2). All participants were supplied with the same hybrid maize seed source for experimentation (single cross C.P. 201, Charoen Pokphand Produce Co., LTD). Due to site differences, plot dimensions and number of maize planted differed between participants, although for each plot, the number of plants in each treatment factor were equal, ranging from 4 plants to 20 plants. Planting took place in December 2016 and January 2017, which is in accordance with the onset of the rains and plant spacing following accepted farming practices (90 cm between rows and 1 hoe length between holes) (Marandu 2015). Maize was grown in rows for ease of treatment application and to allow for easy visual comparison between treatments (Andersson 2015). Treatments (30 percent dilute urine fertilizer solution plus water or water only) were applied every 7 days for 12 weeks, at which time maize ear development had begun and plant nutrient uptake decreases (Richert, Gensch et al. 2010). The control treatment received 0.5 liter of water only while, in the urine fertilizer treatment plots, 0.515 liters of urine fertilizer was applied close to the ground inside a trench at the base of each plant. Once the urine fertilizer was absorbed, 0.5 liters of water was applied. The water served to decrease the smell and loss of ammonia, and also helped facilitate movement of the fertilizer through the soil into the reach of the maize roots. Insecticide (Fentrathion 50EC) was applied 4 weeks after planting to combat African Maize Stalk Borer (*Bosseola fusca*), which is extremely pervasive in the region (Shekania Bisanda 1998), and plots were hand weeded when necessary and uprooted manually at 20 weeks at which time we collected data on maize productivity, and cob yield and elemental nutrient concentrations.

Urine fertilizer application

To determine the amount of urine to apply in order to be consistent with the local chemical fertilizer recommended dosage, we combined data on chemical fertilizer use and estimates of nutrients in urine based on average protein consumption. Using Jösön et al.’s (2004) equation ($\text{Total N} = \text{Protein ingested} * 0.13$) for estimating the concentration of N excreted from the body from total protein ingestion and FAO nutritional data on the average consumption of protein, we calculated the average concentration of N in a liter of urine produced by a Tanzanian. Using the estimated N

per liter of urine and the crops N requirements, we determined the amount of urine required to replace chemical fertilizer. By using that amount and factoring in a 30% dilution rate and application schedule, we determined that applying 0.515 L of dilute fertilizer for 12 weeks would meet maize nutrient requirements. Detailed calculations for determining fertilizer application rates are presented in Appendix D.

To make the urine fertilizer, participants collected their own urine weekly throughout the experiment for five days in provided containers (Appendix D) and then containers were sealed and left to sit for 2 days; this yielded approximately 6 liters. Sealing the container minimized odor and loss of nitrogen as aerosols and likely increased pH to approximately 9, which facilitates the death of potentially harmful bacteria, viruses and pathogens (Kirchmann and Pettersson 1994). To achieve a 30% dilute concentration used for fertilizer treatments, 14L of water was subsequently added to the concentrated urine. When urine is applied directly without dilution, increases in soil salinity are common, as are follicular burns. A 30% urine dilution was found to be the most effective dilution ratio (Morgan 2003, Pinsem, Sathreanranon et al. 2004). All participants used water from naturally occurring.

Maize growth and yield measurements

To examine whether the plants grown in the two treatments differed in productivity and yield, we measured plant weight (above and below ground dry biomass), plant stem length, and plant yield (number of cobs, cob length) attributes. At harvest, the stem was separated from the root, we measured length of the stem (cm) and separated cobs from stems. Aboveground tissues excluding the cobs (husk, silk and cobs) and below ground tissue (roots) were transported separately to a secondary location where roots were rinsed to remove any soil debris. We also counted the total number of developed and undeveloped cobs on each plant and measured individual cob length (cm) within 24 hours of harvest. Plants (above and below ground biomass) were then air-dried and weighed to obtain dry biomass (g); plants were considered dry when they held a constant weight for three consecutive days (drying times ranged from 2-4 weeks). Aboveground biomass did not include the weight of the cobs. All weights were recorded in the afternoon and to the nearest gram using a Salter by Williams-Sonoma 30lb. scale. To determine whether plants in different treatments allocated more or less to aboveground verses below ground biomass we determined the shoot/root ratio using dry biomass

Maize nutrient analysis

To assess the effect of urine fertilizer on nutrient composition of maize, we removed and air-dried six kernels from each cob. After drying, the kernels were ground, sifted, homogenized, and transported to Michigan Technological University for further

processing and analysis. Maize grain percent C and N were determined for 64 (a subset of 144 plants) randomly selected samples by elemental combustion analysis using a Costech elemental analyzer (ECS 4010; Costech Analytical Technologies, Inc., Valencia, CA). The C/N ratio was determined to be used as an estimation of nutrimental value of the grain. Elemental analysis for ion concentrations of P, K, Mg, and Ca was conducted by first combining and homogenizing the samples by plot and treatment and then two sub-samples were taken from each plot for analysis except plot 7, where only one was taken due to insufficient sample quantity. The samples were acid digested using 6mL of concentrated trace metal grade nitric acid and 3 mL of 30% hydrogen peroxide and then microwave digested (using an Anton Paar Multiwave 300) resulting in a clear solution, in accordance with EPA 3052 methods. The digested samples were tested for their concentrations of P, K, Mg, and Ca with ICP-OES analysis by a Perkin-Elmer ICP-OES (Optima 7000 DV; Perkin- Elmer, Waltham, Ma) (Bettinelli, Beone et al. 2000).

Statistical analysis

We first used analysis of variance (ANOVA) models as a randomized complete block design to test the effects of treatment (treated as a fixed effect), plot (“block” – treated as a random effect), and their interaction on four growth variables (dry aboveground biomass, dry below ground biomass, stem length, and ratio shoot/root biomass), and three yield factors (average cob length, developed cobs and proportion developed/total cobs). Analyses were conducted in SAS version 9.4 statistical software (SAS Institute Inc., Cary, NC, USA) using the proc mixed method with Type 3 option and Type 3 Test of Fixed Effects.

Next, to assess whether maize grain elemental compositions differed between treatments (fixed effect) and plots (“block” – random effect), we performed a MANOVA on the elemental concentrations (mg/g) of P, N, Ca, K, and Mg. Prior to analyses we averaged the nitrogen content from 4 samples from each treatment to calculate mg/g per plot and to match the data that we had for the other elements. These analysis were conducted in JMP Pro (version 13.0.0, SAS Institute, Inc.).

Lastly, to determine whether treatment influenced kernel C/N ratio, we used an ANOVA model as a randomized complete block design to test the effects of treatment (treated as a fixed effect) and plot (“block” – treated as a random effect in JMP Pro (version 13.0.0, SAS Institute, Inc.). Prior to all analyses we tested for violations of the assumptions of normality of residuals and homogeneity of variances, and none were detected.

Results

Maize growth and biomass

Application of dilute urine fertilizer and plot had significant effects on maize plant productivity. In general, plants that received urine fertilizer had significant higher below and above ground biomass (Table 3.1) with treatment plants having roughly a 51% increase in dry aboveground biomass and a 70% increase in dry below ground biomass from the control plants (Fig. 3.3 a and b). Also, maize plants grown with dilute urine fertilizer had significantly longer stems with a 16% increase from the control plants (Fig. 3.3 c). Surprisingly, although treatment plants had both higher above and belowground weights, shoot/root ratios significantly differed between treatments (Table 3.1). Shoot/root ratios in the urine fertilizer treatment were significantly less than the control plants ($LSmeans \pm 1 SE$ for treatment and control plants = 6.1685 ± 0.8786 and 8.1889 ± 0.8701 , respectively, Fig. 3.3 d). This indicates that the plants that did not receive fertilizer developed greater shoot biomass to root biomass compared to plants in the urine fertilizer treatment. All of the growth factors had a significant effect of plot (Table 3.1). These results would indicate that despite the intra-plot differences present, the effects of the fertilizer treatment were pervasive enough to alter the growth factors measured.

Maize yield

Urine fertilizer treatment resulted in an increase in all of the yield variables (Table 3.2). Plants in the urine fertilizer treatments produced cobs that were 51% longer than plants receiving only water and urine fertilization resulted in a 41% greater cob production (Fig 3.4a and Fig. 3.4b). Despite the increase in total number of cobs and total developed cobs, the proportion of developed/total cobs were greater in the control treatment (Table 3.2 and Fig. 3.4c). Maize yield was also impacted significantly by the effect of plot in all yield factors (Table 3.2). This result is important to consider because despite the inter-plot differences, the effects of urine on the yield were still present.

Grain nutrient composition

Neither urine fertilizer treatment nor plot significantly affect nutrient concentrations of P, Mg, K, N, and Ca ($F_{5,15} = 2.0246$, $p = 0.1603$) nor the C/N (Fig 3.5).

Discussion

Two of the most challenging problems associated with rapid increases in population are providing sanitation and food to inhabitants of unplanned communities that form around urban areas in developing nations. One solution proposed is ecological sanitation or closed-loop sanitation, where residents have the opportunity to turn their waste into a usable product that has value. Using human urine fertilizer collected from small ecological sanitation systems provides low cost safe sanitation, effective fertilizer, and food security.

The effect of urine fertilizer on maize biomass and yield

As a whole, our findings are similar to past research on urine fertilizer efficacy. We found urine fertilizer produced larger plants and a greater yield compared to control plants. The maize plants that had urine fertilizer applied had an increase in stem length, which was also found by Guzha, Nhapi and Rockstrom in 2005. We found an increase in dry aboveground and below ground biomass in the treatment group, which is in line with research from South Africa (Mnkeni, Kutu et al. 2008). In Zimbabwe, researchers found that the growth and yield of maize increased with urine application (Morgan 2003). The higher biomass allocated to shoots relative to roots in the control treatment was unexpected and indicates that plants within the control treatment allocated more development into their stems compared to the urine fertilizer treatment. It is common for plants to invest greater energy into root development in nutrient-poor soils and it is thought plants do this to increase the plant's ability to remove the available nutrients, so the opposite observation was expected. To date, there has been no other research which has measured shoot/root in plants fertilized with human urine.

In other grain crops like sorghum, an increase in yield of 1,137 kg/ha was identified between the control and urine fertilizer treatments (Germer, Addai et al. 2011). We found a 40.58% increase in number of developed cobs associated with urine fertilizer. This increase in yield between urine and water treatments was also identified in maize cultivated in Uganda, where a 50% increase was identified between control and urine fertilizer treatments (Andersson 2015). However, despite the increase in amount of developed cobs associated with urine fertilizer application, the proportion of developed cobs to total was higher in the control. This would indicate that the treatment plants either had access to more nutrients to begin cob development or possibly cobs developed over a longer time period compared to the control treatment. Beets fertilized with urine showed had a faster growth rate than beets that was only irrigated (Pradhan, Holopainen et al. 2010). Similarly to our study, they found an increase in root biomass within the urine fertilizer treatment, which could help with nutrient absorption. The control treatment may also be displaying better resource use efficiency. Seebauer, Singletary et al. (2009) suggests that deficiencies in N supply have

been shown to decrease the yield by decreasing the number of kernels per cob, however, we did not record number of kernels per cob nor their individual weight. But from personal observation, the urine treatment produced a greater number of cobs, many of which did not reach maturity. This most likely caused the greater proportion of developed/total cobs that was seen in the control.

Effect of urine fertilizer of elemental grain composition

Unlike previous research into the effect of N fertilizer supply on elemental grain composition we did not find an increase in the grain N with increasing N supply (Thiraporn, Feil et al. 1992, Alfoldi, Pinter et al. 1994, Feil, Moser et al. 2005). This could be because of the variety of maize seed used in the study. Researchers have suggested that seed varieties designed for high yield, produce grains which do not demonstrate the increase in grain N commonly associated with increasing N supply (Feil and Fossati 1995). Hybrid seeds require more N supply and it is possible that the increased demand for N which is common in hybrid seeds was allocated into developing more cobs instead of enhancing the nutrient value of the maize grain (Seebauer, Singletary et al. 2009). Grain N can be used to calculate the protein concentrations of the kernel using the following equation: "Grain Protein = Grain N * 6.25" (Seebauer, Singletary et al. 2009). In our study, we believe that due to our seed genotype, grain protein was independent of N supply. Research has shown that elemental grain concentrations are largely determined by the genotype of the parent seed (Feil, Thiraporn et al. 1992). Elemental concentrations of N are highest immediately after silking and then decrease with the increase in endosperm/starch (mass) accumulation within the kernel (Alfoldi, Pinter et al. 1994, Feil, Moser et al. 2005), which has a diluting effect on grain elemental concentrations. The C/N ratio, which can be used to estimate nutrition was slightly higher in the control. Higher C/N ratios are indicative of a less nutritious food source (Luo, Li et al. 2017).

Studies that have compared different seed varieties across treatments of varying N fertilizer supply, have led scientists to believe that in addition to N grain elemental concentration there are correlations between N fertilizer supply and the concentration of other elements (Feil, Thiraporn et al. 1992, Alfoldi, Pinter et al. 1994, Seebauer, Singletary et al. 2009). Feil, Moser et al. (2005) identified a negative correlation between N fertilizer supply and Ca and Mn elemental concentration. Numerous other studies have determined that N-supply is independent of grain concentrations of P, K, (Thiraporn, Feil et al. 1992, Alfoldi, Pinter et al. 1994, Feil, Moser et al. 2005) and Mg (Feil, Moser et al. 2005) despite the increase in yield associated with the increase in N fertilizer supply, indicating that there is not dilution of elemental concentrations in these elements. These findings suggest two possible mechanisms for maintaining grain elemental concentrations. One, increased uptake by the plant from the soil and two,

the plant is demonstrating improved allocation efficiency (Thiraporn, Feil et al. 1992). Our finding agree with past research, we did not find identified a relationship between N fertilizer supply and grain concentrations (mg/g). Preceding research has been conducted using chemical fertilizer which the plant has to convert to a usable form. The N found in urine fertilizer is in a form that the plant can directly remove from the soil and use. Despite the different form the nutrients are found in urine fertilizer, the plants response to the nutrients seems the same.

Involving local farmers

We collected data from 8 plots maintained by 6 participants. Initially, we had 16 participants in the study, however, 4 plots were lost to chickens (scratching the seedling), 4 plots were lost to attrition and another 2 plots were not managed properly (independent treatments were not maintained) so their data could not be included. We attribute the initial starting enthusiasm to curiosity and an eagerness to be a part of a research project. Due to their education level, the participants were engaged in the science, however, many of them were not directly responsible for maintaining their experimental plot; tasking the responsibility of weekly application to their domestic servants. Also, for some of the participants their direct survival was not tied to their level of engagement, being that they had the financial means to purchase food. I attribute 4 of our attritions to this cause. Conducting research using educated participants is a valuable step towards acceptance of the research. Past studies have found that the more education a person has, the greater their willingness to experiment with alternative farming practices. This could be because they are more willing to experiment with new information, have a greater understanding of risk assessment as well as most likely have an alternative income. This financial flexibility allows the farmers to experiment with new methods (Kassie, Jaleta et al. 2013). Education is valued very highly in many cultures, including Njombe region, and those who poses it carry influence. Educated participants can act as information ambassadors and spread the results of the research around the community. Farming groups in Uganda that cultivated maize with urine fertilizer, reported that they were able to spread knowledge and acceptance of the practice to 15 additional individuals through observation and conversation (Andersson 2015). We also found that as the results of the experiment became clear many more community members began to express interest in using urine fertilizer.

In this study we did not compare urine fertilizer to chemical because the participants in the study did not all have experience using chemical fertilizer. Instead, we compared urine fertilizer to rain-fed irrigation plots, which are the most common cultivation situation in the area. We involved local farmers to quantify the effectiveness of urine fertilizer and to investigate the effect of urine fertilizer on elemental grain

composition. Additionally, the urine treatment and control plots received slightly different amounts of water because the urine treatment receives water in the urine dilute in addition to the 0.5 liters applied to the plants after urine application. While, the control plot only received 0.5 liters of water, we viewed this discrepancy as having little implication for the study because of the near daily rainfall all the plots received. For the months of fertilizer treatment application a total 831.7 mm of rain was measured at the airfield 6.7 km away. The rainfall broken down by month was: December 2016- 174.9 mm, January 2017- 260.6 mm, February 2017- 209.3 mm, March 2017- 186.9 mm (Online 2018). Also, no difference was identified between treatments in percent stem moisture, however, a significant difference was found in percent root moisture between treatments (data not shown). Conducting field trials using urine fertilizer transitions recycling ecological sanitation products from theoretical to applied as well as functioning as a showcase for the results of urine fertilizer on maize crops for community members. The direct applicability of the research was evident throughout the study with all participants expressed their surprise with the visual differences of the maize plants and cobs between the two treatments. The urine treatment plants were more lush and had a deeper green colour. The cobs from the urine treatment were much fuller looking and the plump kernels were a rich yellow. One person commented saying that, “they just look like a better food”.

Recommendations

More research is needed to fully understand how plants use urine fertilizer. Investigation needs to be performed to comprehend how different plant species and different genotypes accumulate and allocate the nutrients they receive from urine fertilizer. Preliminary results into the effect of urine fertilizer on the environment are mixed. Ugandan soils that were fertilized with human urine showed an increase in soil N and a 3-fold increase in P post-harvest compared to soils that did not receive urine fertilizer, which might suggest a residual buildup of soil nutrients (Andersson 2015). On the other hand, urine fertilizer was found to have a negative impact on earthworm populations, with their populations decreasing for several months after application (Richert, Gensch et al. 2010). There has not been a study conducted across numerous crop cycles, so the long-term consequences of urine fertilizer are largely unknown. Within the urine fertilizer field, there seems to be a divide in research focus; either studying nutrient recovery from stored urine using numerous recovery methods for implementation at a large scale, or small-scale controlled experiments using variable nutrients supplied from a range of fertilizer sources. Scientists need to remain aware of the direct practical application of this research. For example, in this experiment we used a high yield seed variety which was recommended by the agriculture education officer for the region. However, it may be possible that by using that particular variety of seed we decreased the potential for adding nutrition by increasing elemental grain

concentration. The types of data we were able to collect were limited because not only was the maize grain a focus of the study it was also the food for the participants. We sacrificed collecting a more robust data set for the food security of the participants.

Despite the limited research that has been conducted in the field of human urine fertilizer, the results are compelling. From our research we concluded urine increases yield and is an effective alternative fertilizer that is available at almost no cost. It can be implemented safely at a small scale and produces far greater yields than can be achieved without any fertilizer application. It was accepted by the community and the grain produced was highly coveted. Urine fertilization does not affect grain elemental concentration and there is no evidence of a grain elemental dilution effected due to increasing yield.

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Tables

Table 3.1: ANOVA results for effects of treatment (fixed effect), plot (block, random effect) and their interaction (T*P) on dry aboveground biomass, dry below ground biomass, stem length, and dry shoot/root biomass. Analyses were run in SAS version 9.4 statistical software (SAS Institute Inc., Cary, NC, USA citation) using the proc mixed method with Type 3 option for plot, T*P and error. Type 3 test of fixed effects was used for treatment. Significant *p*-values are indicated in bold.

<i>Factor</i>	Source	df	<i>F</i>	<i>p-value</i>
Dry aboveground biomass (g)	Treatment	1	21.42	0.0024
	Plot	7	7.64	0.0078
	T * P	7	1.77	0.0992
	Error	129		
Dry below ground biomass (g)	Treatment	1	18.08	0.0038
	Plot	7	5.03	0.0246
	T * P	7	1.30	0.2575
	Error	129		
Stem length (cm)	Treatment	1	7.78	0.0270
	Plot	7	3.94	0.0454
	T * P	7	3.89	0.0007
	Error	129		
Dry shoot/root biomass (g)	Treatment	1	10.86	0.0132
	Plot	7	7.22	0.0091
	T * P	7	1.45	0.1896
	Error	129		

Table 3.3: ANOVA results for effects of treatment (fixed effect), plot (block, random effect) and their interaction (T*P) on average cob length, total developed cobs and proportion developed/total cobs. Analyses were run in SAS version 9.4 statistical software (SAS Institute Inc., Cary, NC, USA citation) using the proc mixed method with Type 3 option for plot, T*P and error. Type 3 test of fixed effects was used for treatment. Significant p-values are indicated in bold.

<i>Factor</i>	Source	df	<i>F</i>	<i>p-value</i>
Average cob length (cm)	Treatment	1	22.97	0.0020
	Plot	7	6.14	0.0144
	T * P	7	2.23	0.0356
	Error	129		
Total developed cobs	Treatment	1	16.02	0.0052
	Plot	7	3.74	0.0515
	T * P	7	2.44	0.0220
	Error	129		
Developed/total cobs	Treatment	1	31.19	0.0008
	Plot	7	6.40	0.0128
	T * P	7	0.53	0.8089
	Error	129		

Figures

Figure 3.1: Map of the Southern Highlands of Tanzania indication the location of Hagafilo village. Map created using ArcMap (Esri 2017. ArcGIS. Desktop: Release 10.5.1. Redlands, CA: Environmental Systems Research Institute).

Figure 3.2: Experimental design for plots. Plot is picture is at week 20 of the experiment. Each participant maintained two plots with a buffer between (1) control-water only and (2) urine fertilizer- urine fertilizer and water.

Figure 2.3: Least Square Means ± 1 Standard Error (S.E.) for (a) dry aboveground biomass, (b) below ground biomass, (c) stem length, and (d) shoot/root biomass. * = significance at $p < 0.05$ and ** = significance at $p < 0.005$

Figure 3.4: Least Square Means $\pm$ 1 Standard Error (S.E.) for (a) average cob length, (b) developed cobs and (c) proportion developed/ total cobs. * = significance at $p < 0.05$ and ** = significance at $p < 0.005$

Figure 3.5: Least Square Means $\pm$ 1 Standard Error (S.E.) for carbon/nitrogen ratio (C/N), differences among means were not significant ($F_{1,15} = 0.3898$, $p = 0.5522$)

Appendices

Appendix A: Participant Handout

Investigation into the effects of Urine Fertilizer on Maize plants.

Utafiti kuhusu matokeo ya mbolea ya mkojo katika mimea ya mahindi

Purpose: To determine the effects (growth, output, and nutrient content) of maize plants that have been given a 30% human urine dilute fertilizer.

Lengo: kutambua matokeo (ukuaji, mavuno, na kiasi cha virutubisho) katika mimea ya mahindi yaliyo wekewa asilimia thelasini ya mbolea ya mkojo wa binadamu.

Duration of the study: Three months of urine dilute fertilizer application, once a week.

Muda wa mafunzo: miezi mitatu ya utumiaji wa mbolea ya mkojo, maramoja kwa wiki.

Requirements of participants: Two plots of maize growth approximately 1 meter x 2 meters. One plot for control and the other for treatment With a 1 meter space in between (buffer) the plots with corn planted. See diagram below. The urine dilute fertilizer needs to be applied once a week for 12 weeks. The participants will start collecting urine 1 week prior to planting.

Fertilizer application begins at the time of planting.

Mahitaji ya washiriki: ploti mbili za ardhi zenye ukubwa wa mita 1 kwa mita 2 kwaajili ya kukuza mahindi. Ploti moja kwaajili ya matazamia na nyingine kwaajili ya jaribio, zenye kutenganishwa na ploti yenye nafasi ya mita moja, ploti zote zikiwa zimepandwa mahindi. Angalia mchoro hapa chini. Mbole ya mkojo iwekwe mara moja kwa wiki katika mda wa wiki 12. Washiriki wataanza kukusanya mkojo wiki moja kabla ya upandaji. Matumizi ya mbolea huanza mda wa upandaji.

	1 Meter	1 Meter	1 Meter
2 Meter	Treatment (0.515 L urine fertilizer + 0.5L water)	Buffer	Control (0.5L water)

Making the urine dilute fertilizer: Each participant's household will be given a urine collection system. They will urinate into the system for 5 days or until urine has reached the 6-liter mark on the jerry can. After the 5 days mark the can is closed and the urine left to sit for two days. This kills all potential bacteria, parasites and pathogens. After two days the jerry can is filled with water, thus making a 30% urine dilute fertilizer. The fertilizer is ready to be applied. The participant will then apply 0.515 liters of the fertilizer to the base of each plant in the treatment plot, avoiding contact with the leaves. To help reduce the smell 0.5 liters of water are then applied to the base of the plant. The water will move the urine fertilizer into the soil and reduce smell. The 0.5 liters of water needs to be applied to both the treatment and the control plots.

Utengenezwaji wa mbolea ya mkojo: Kila nyumba ya mshiriki itapewa mfumo wa ukusanyaji mkojo. Watakojoa kwenye mfumo kwa mda wa siku tano au mpaka mkojo ufikie lita 6 kwenye galoni la kuhifadha. Baada ya siku tano galoni lifungwe na mkojo utunzwe kwa siku mbili. Hii inasaidia kuua bacteria hatari, parasaiti na wadudu waenezao magonjwa. Baada ya siku mbili galoni lijazwe maji, ili kufanya mbolea ya mkojo yenye 30%. Mbolea iko tayari kwa matumizi. Mshiriki atatumia lita 0.515 ya mbolea kwenye shina la kila mmea kwenye ploti ya jaribio, epuka mbolea kugusana na majani ya mimea. Ilikupunguza harufu lita 0.5 ya maji iwekwe kwa kila shina la mmea. Maji yatapeleka mbolea ya mkojo kwenye

udongo na kupunguza harufu. Lita 0.5 ya maji iwekwe kwe ploti zote mbili ya majaribio na matazamio.

Participants will be given/ washiriki watapewa:

- urine collection system/ mfumo wa ukusanyaji mkojo
- maize seed/ mbegu za mahindi
- application cup/ kikombe
- rope to mark off plots / kamba ya kupimia shamba

The experiment is the basis for my master's degree thesis in biology. I greatly appreciate your help. Without you none of this could be possible

Utafiti huu ni kwaajili ya udhamili katika fani yangu ya bayolojia. Nathamini sana msaada wako. Bila ushiriki wako hili lisinge wezekana.

Appendix B: IRB Exemption

**Michigan
Technological
University**

Compliance, Integrity, and Safety

302 Lakeshore Center | 1400 Townsend Drive Houghton, MI 49931-1295

906-487-2902 | T. 906-487-2245

Michigan Tech is an EOE which includes protected veterans and individuals with disabilities.

DATE: September 27, 2016

TO: Kari Henquinet, Director Peace Corp and Dept. of Social Science
Lucy Hatfield, Student with Peace Corp

FROM: Cheryl A Gherna, CIP, Coordinator Regulatory Review Boards [REDACTED]

RE: Peace Corp Work in Tanzania conducted by Lucy Hatfield

SUBMISSION TYPE: Project Awareness

STATUS: **APPROVED, Exempt Status**

Thank you for your submission of information for this research study. Your information indicates that you will not use human subjects themselves in the research but the Peace Corp work itself and/or data collected may be used for graduate work for presentations or publication; the information has been reviewed by the Institutional Review Board and the Institutional Biosafety Officer and is determined to have an Exempt status.

When a research project is determined by the Office of Compliance, Integrity, and Safety (CIS) to meet the criteria for an Exempt status, it means that it is exempt from annual continuing review by the Institutional Review Board (IRB). The investigator, however is required to report to the IRB ANY changes, revisions, or modifications in the research activity including personnel changes. ANY changes made may cause the research status to change from Exempt to Expedited or Full Review and must be determined and documented by the CIS Office.

All Unanticipated Problems / Serious Adverse Events to participants or other parties affected by the research must be reported to this office within two days of the event occurrence. All instances of non-compliance or complaints regarding this study must be reported to this office in a timely manner. Please use the INSTRUCTIONS and FORM: Unanticipated Problem / Serious Adverse Event Form found both on our web site and the IRBNet Library.

This approval applies only for this project, and only under the conditions and procedures described in the application. If/when changes become necessary but are not limited to: changes in protocol, personnel, study location, participant recruitment, etc., as set forth in this approval, you must follow the INSTRUCTIONS and submit the FORM for Change Request during approval found in the IRBNet Library. **You must receive notification of approval PRIOR to implementing the change(s).**

If you have any questions, please contact the Compliance, Integrity, and Safety Office at 906.487.2902 or send your message via email through IRBNet using the Send Project Mail feature.

Appendix C: Urine Collection System

Photo of the urine collection system given to every participant. (A) Materials include: 1) a 20-liter jerry can, a funnel with lid connected to a handle for easy removal and suspended plug (from the lip to the base of the funnel) to seal the opening to the can. 2) a cup for female collection 3) a 10-liter bucket for transport of the urine fertilizer from the home to the garden and two measured pour cups (one for fertilizer and one for water). (B) Handled lid with sealing plug to reduce odor and minimize ammonia loss. (C) 6L line on the jerry can. (D) Application cups (0.515 for urine and 0.5 for water).

Appendix D: Calculations for determining urine fertilizer application

In the Southern Highlands, chemical fertilizer is applied 3 times throughout the growing season by incorporating a soda cap worth of each fertilizer into the topsoil surrounding each plant: DAP (Diammonium Phosphate) at planting, CAN (Calcium Ammonium Nitrate) when the maize is knee high and Urea at tasseling. The table below shows the calculations for determining the total nitrogen input from chemical fertilizers.

Table 1. Local amounts of chemical fertilizer added and their respective nitrogen concentrations. Used to calculate total nitrogen added through chemical fertilizers in grams per plant per year

Fertilizer	% Nitrogen	Weight of one cap of fertilizer (g)	g of Nitrogen
DAP	18%	7.03	1.2654 g N
CAN	27%	7.98	2.1546 g N
UREA	46%	6	2.76 g N
Total g N added per year per plant			6.1796 g N

The nutrient composition of urine is dependent upon several factors including, climatic conditions, physical exercise, ingestion of water, and diet. Tanzanians have a predominantly plant-based diet. Total nitrogen excreted (urine and feces) is equal to $N = 0.13 * \text{total food protein}$ from Jönsson et al (2004:5) equation 3.1. The average Tanzanian ingests 55 g /capita/day of total food protein (FAO 2008).

$$N = 0.13 * 55 \text{ g protein/day} = 7.15 \text{ g N/day in excreta}$$

To determine what percent of the total nitrogen is excreted in urine, data from China, Gao et al via Jönsson et al (2004:7) was used, which estimated that 70% of the nitrogen eliminated from the body is in the form of urine.

$$7.15 \text{ g N/day} * 70\% (\text{N found in urine}) = 5.005 \text{ g N/day are excreted in urine}$$

To calculate per capita nitrogen concentration within urine, 1.5 L of urine production was assumed (Jönsson, Stintzing et al. 2004).

$$5.005 \text{ g N/day} \div 1.5 \text{ liters of urine/day} = 3.34 \text{ g N/liter of urine}$$

To determine the nitrogen concentration present in the 30% urine dilute fertilizer

$$20\text{-liter jerry can} * .30 = 6 \text{ liters of pure urine}$$

$$6 \text{ liters} * 3.34 \text{ g N/liter} = 20.04 \text{ g N in 6 liters of urine}$$

$$20.04 \text{ g N} \div 20 \text{ liters} = 1.002 \text{ g N/liter of urine dilute fertilizer}$$

A weekly fertilizer application schedule was used. The total nitrogen input from chemical fertilizers (Table 1) was used to set the required weekly application amount.

$$6.1796 \text{ total g N/plant} \div 12 \text{ weeks for application} = 0.515 \text{ g N/week}$$

$$0.515 \text{ g N/plant/week} \div 1.002 \text{ g N/ liter of dilute fertilizer} = \mathbf{0.515L \text{ fertilizer/plant/week}}$$

Appendix E: Correlations between above and belowground wet and dry biomasses