Ecological sanitation: performance evaluation of human urine as a fertilizer under laboratory and field conditions

By

Jenna Senecal-Smith

Degree of Master of Science

Department of Bioresource Engineering,
McGill University, Montreal

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Abstract

Seven billion people are urinating every day, excreting 28 million tonnes of nitrogen (N) a year (Vinneräs, 2002). This mass of N excreted is equal to 26% of the annual global N fertilizer demand, a value of $21.3 trillion USD (based on $350 USD per metric tonne of urea) (Alibaba, 2012, FAO, 2011). The use of human excreta as N fertilizer would allow the recovery of nutrients, resulting in savings for farmers, and counter the threat to people's health by reducing contact with untreated human waste. This practice, termed ecological sanitation (EcoSan), has a long history of application in traditional agricultural contexts, but has a limited scientific knowledge base. This thesis builds upon the scientific knowledge base by: (1) analyzing the chemical changes in soil from long-term use (nine years) of human urine as a fertilizer compared to mineral fertilizer; (2) optimizing the human urine application rate for spinach in Himachal Pradesh, India; and (3) performing a theoretical quantitative microbial risk assessment (QMRA) for the use of human urine on spinach in northern rural India.

A sensitivity experiment, comparing synthetic human urine, mineral fertilizer and in combination and with the increasing application rates, observed that spinach was able to withstand significantly higher EC soil levels than were commonly reported. Brown mustard biomass production rates did decrease with the increased application rates of the three fertilizer treatments. Tissue samples from field trials in India had no significant difference between N concentrations for the three fertilizer treatments (human urine, mineral fertilizer and combination of the two) and were all significantly higher than the control (no fertilizer). The dry biomass production of spinach from the human urine treatments were significantly higher than the control and were not significantly different to mineral fertilizer treatments, confirming that human urine can substitute mineral fertilizer. The optimal human urine application rate in Himachal Pradesh, India, was 59.3 m^3 ha^{-1} of human urine, resulting in 360 kg N, 570 kg P_2O_5 and 720 kg K_2O per ha per season which is higher than the current guidelines. Of the three fertilizer treatment, the optimal fertilizer was the combination treatment (human urine with additional phosphate (P) and potassium (K)) at 360 kg N ha^{-1} as it had significantly higher biomass productions to that of the human urine and the mineral fertilizer treatments.

The novel finding was the use of sodium (Na) by the spinach plants as a supplement for K deficiencies in the human urine fertilizer treatments. The tissue samples from the human urine treatments had significantly higher Na concentrations than the other treatments, with no signs of toxicity. This finding illustrates that other crops with the ability to compensate for limited K will perform well with EcoSan systems by using the Na contained in urine. The results of this thesis illustrated that the 28 million tonnes of excreted human N is an effective substitute to expensive mineral fertilizer. Though, based on the QMRA, areas prone to high occurrence of diarrheal disease should use human urine for crops that will be processed, such as rice, or for cash-crops and not for edible crops grown close to the ground, such as spinach.

Résumé

Sept milliards de personnes urinent chaque jour, excrétant 28 millions de tonnes d'azote (N) par an (Vinnerås 2002). Cette masse d'azote excrétée est égale à 26% de la demande annuelle d'engrais azoté global, d'une valeur de $ 21,3 trillions USD (sur la base de 350 $ USD par tonne métrique d'urée) (Alibaba 2012, FAO, 2011). L'utilisation des excréments humains comme engrais permettrait la récupération des nutriments, ce qui permettrait aux agriculteurs de faire des économies, et contrer la menace pour la santé des personnes en réduisant le contact avec les déchets humains non traités. Cette pratique, appelée assainissement écologique (EcoSan), a une longue histoire d'utilisation dans des contextes agricoles traditionnelles, mais dispose d'une base de connaissances scientifiques limitée. Cette thèse s'appuie sur la base de connaissances scientifiques par: (1) l'analyse des changements chimiques dans le sol à partir de l'utilisation à long terme de l'urine humaine comme engrais par rapport aux engrais minéraux, (2) optimiser le taux d'application de l'urine humaine pour les épinards dans l'Himachal Pradesh, Inde, et (3) d'effectuer une évaluation quantitative des risques microbiologiques théorique (QMRA) pour l'utilisation de l'urine humaine sur les épinards dans le nord de l'Inde rurale.

Une expérience de sensibilité, en comparant l'urine humaine synthétique, d'engrais minéraux et la combinaison avec l'augmentation des taux d'application, ont montré que les épinards étaient capable de résister à des niveaux de CE dans le sol nettement plus élevés que ce qui a fréquemment été rapporté. Les taux de production de biomasse de moutarde brune ont diminué avec l'augmentation du taux d'application des trois traitements de fertilisation. Des échantillons de tissus provenant d'essais sur le terrain en Inde n'avaient pas de différence significative entre les concentrations d'azote pour les trois traitements de fertilisation (urine humaine, engrais minéraux et la combinaison des deux) et étaient significativement plus élevés que le contrôle (sans engrais). La production de biomasse sèche des épinards dans les traitements de l'urine humaine a été significativement plus élevée que le contrôle et n'était pas significativement différente aux traitements d'engrais minéraux, confirmant que l'urine humaine peut remplacer les engrais minéraux. Le taux d'application de l'urine humaine optimale dans l'Himachal Pradesh, en Inde, était de 59,3 m3 par hectare d'urine humaine, résultant en 360 kg d'azote, 570 kg de P2O5 et 720 kg K2O par hectare et par saison, ce qui est plus élevé que les lignes directrices actuelles. Parmi les trois traitement d'engrais, l'engrais optimal était le traitement combiné (urine humaine avec du phosphate supplémentaire (P) et le potassium (K)) à 360 kg N ha-1 puisque les taux de production de la biomasse étaient sensiblement plus élevés pour celle de l'urine humaine et de l'engrais minéraux.

La nouvelle découverte a été l'utilisation de sodium (Na) par les plants d'épinards comme un supplément pour les lacunes en K dans les traitements de fertilisation d'urine humaine. Les échantillons de tissus provenant des traitements d'urine humaine avaient des concentrations de Na significativement plus élevés que les autres traitements, sans aucun signe de toxicité. Ce résultat montre que d'autres cultures qui ont la capacité à compenser la quantité limité en K se développeront bien avec les systèmes ecosan en utilisant le Na contenu dans l'urine. Les résultats de cette thèse ont illustré que les 28 millions de tonnes

de N humain excrété est un substitut efficace à l'engrais minéral cher. Bien que, sur la base du QMRA, les zones sujettes à la fréquence élevée des maladies diarrhéiques devraient utiliser l'urine humaine pour les cultures qui seront transformées, tels que le riz, ou de cultures de rente, et non pour des cultures comestibles poussant au ras du sol, comme les épinards.

"The day that every one of us gets a toilet to use, I shall know that our country has reached the pinnacle of progress."

First Prime Minister of India (date unknown), Jawaharlal Nehru (Government of India, 2006)

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'It's time to stop pissing around and get into the real shit'

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List of abbreviations and acronyms

B	Boron
Ca	Calcium
$CaCO_3$	Calcium carbonate
$Ca_5OH(PO_4)^3$	Struvite
Cd	Cadmium
CEC	Cation exchange capacity
Cl	Chloride
Co	Cobalt
CO_3^-	Carbonate
CRRAQ	Centre de référence en agriculture et agroalimentaire du Québec
Cu	Copper
EC	Electric conductivity
$EC_{1:2}$	Electric conductivity measured by dilution method (one part soil to two parts water)
EC_{SE}	Electric conductivity measured by saturation extraction method
EcoSan	Ecological sanitation
ESP	Exchangeable sodium percentage
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Gg	Mass, equivalent to 1 000 000 kg
ha	Hectare
HCO_3^-	Hydrogen carbonate
Hg	Mercury
HPO_4^{2-}	Monohydrogen phosphate
$H_2PO_4^-$	Dihydrogen phosphate
K	Potassium
K_2CO_3	Potassium carbonate
KCl	Potassium chloride
K_2SO_4	Potassium sulphate
mdb	Maximum disease burden
Mg	Magnesium
$MgNH_4PO_4$	Hydroxyapatite
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
N_2	Dinitrogen or atmospheric nitrogen
Na	Sodium
Ni	Nickel
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate

P	Phosphorus
Pb	Lead
$pH_{1:2}$	pH measured by dilution (one part soil to two part water)
pH_{SE}	pH measured by saturation extraction method
PPE	Personal protective equipment
pr	Risk of diarrheal disease given infection
r	Dose-response
RCBD	randomized complete block design
S	Sulphur
SAR	Sodium absorption ratio
Se	Selenium
sf	Susceptible fraction
SO_4	Sulphate
Trt	Treatment
QMRA	Quantitative microbial risk assessment
v/v	Volume to volume ratio
Zn	Zinc

1 Introduction

1.1 Introduction

The excreta of humans contain nutrients essential for plant growth (Jönsson et al., 2004). Rather than regarding this source of nutrients as a valuable component in food production systems, it is regarded as a waste for which management is costly, in economic and health terms. Of the seven billion global humans, 4.1 billion are discharging sewage into the environment without treatment, resulting in environmental degradation and adverse human health effects (Baum et al., 2013).

The use of human excreta as fertilizer is a cradle-to-cradle system: humans consume food, the unused nutrients are excreted, the excreta is used to grow food, and the cycle repeats (McDonough et al., 2002, Winblad et al., 2004). This system, termed ecological sanitation (EcoSan), has a long history of traditional knowledge of how to use human excreta as a fertilizer as it was once prevalent in China and other parts of the world (King, 1911). However, there is a limited scientific knowledge base for EcoSan (Jönsson et al., 2004). Experiments have demonstrated that human urine is as efficient as mineral fertilizer but little is known about how human urine alters crop production and soil chemistry with repeated use (Chapter 2). This thesis is focused on building the scientific knowledge base of using human urine as fertilizer on a long-term (nine year) basis (Chapter 3 &4).

1.1.1 Plant nutrient requirements

The productivity of a crop is dependent on having adequate nitrogen, phosphorous and potassium (N-P-K) and without the addition of these fertilizers, crop production decreases after each harvest as the naturally available elements in the soil are depleted (Drangert et al., 2012). There are large differences in yields when fertilizer is not used. For example, in Bandundu, Democratic Republic of the Congo, farmers that were not using fertilizer had an average maize yield of 1.0 Mg ha^{-1} while farmers using fertilizer had more than twice the yield at 2.2 Mg ha^{-1} (FAO, 2006). Fertilizers can be used as a global indicator of a country's economic development; developed countries have high levels of phosphate in the soils, a result of intensive fertilization application in agriculture practices, while a low level of phosphate in soils typically occur in developing countries (Ryan et al., 2006). There are several reasons for the differences in fertilizer application rates including high

costs, lack of access to credit or insurance, and rural locations that have increased distribution costs (FAO, 2005, Marenya et al., 2009).

1.1.2 Global food production

In many developing nations, farmers cultivate land without fertilizers due to the high input costs (Pradhan et al., 2010) and this lack of fertilizer application negatively impacts the yields (FAO, 2006). The average quantity of fertilizer used globally varies from 9 kg ha^{-1} in sub-Saharan Africa, 73 kg ha^{-1} in Latin America to 100-135 kg ha^{-1} in Asia (Marenya et al., 2009). Nitrogen is consumed more than phosphate and potassium (FAOSTAT, 2010) due to the better cost-yield ratio in terms of mass of fertilizer applied to increase yields (Camberato et al., 2011). Agricultural soils are typically deficient in nitrogen because nitrogen is volatile and can be released into the atmosphere, or can easily be leached from the soil (Camberato et al., 2011, Prosser, 2011). As the production cost of mineral fertilizer is correlated to the cost of fuel, increased fuel costs will further limit farmers' ability to purchase the required nutrients for optimal crop production (Winblad et al., 2004).

The supply of nitrogen is not limited, but the available sources of phosphate and potassium are finite, and their costs of production are also increasing (Crowson, 1992, Guzha et al., 2005, Power et al., 1997). Phosphate is mined as phosphorite in natural deposits and 90% of the mineral sources are located in five countries (China, South Africa, Morocco, United States and Jordon) (Drangert et al., 2012). The quality of reserves and cost of production are increasing (Drangert et al., 2012) and the global supply is expected to be completely mined within the next 60 to 200 years (Tilley et al., 2008). Potassium is typically mined as potash with Canada producing one third of the global trade, followed by Russia and Belarus (Ober, 2007). The world estimates for the lifespan of mining potassium for is 300 years (Crowson, 1992).

1.1.3 Introduction to the sanitation sector

The goal of sanitation is to reduce the burden of disease and illness-related expenditure, improve water quality, and ultimately result in a higher quality of life (SACOSAN, 2008). Adequate sanitation is defined by the United Nations (2008) as having access to sanitation facilities that isolates the excreta from human contact (includes pit latrine, latrine with slab; and composting toilet). Unimproved sanitation facilities are flush toilets that are not connected to a sewer, latrine without slab (open pit), bucket, hanging toilet

and having no access to facilities (UN 2008). The delivery of safe and effective sanitation services includes infrastructure (e.g. latrines, sewers), associated behaviors (e.g. toilet usage, hand-washing) and an enabling environment (e.g. public health regulations, fiscal incentive schemes for achieving sanitation outcomes) (SACOSAN, 2008). Currently, 2.6 billion people live without adequate sanitation based on the above definition (Baum et al., 2013). But of the 4.3 billion people using toilets connected to a sewer, 1.5 billion people still have their waste not treated (Baum et al., 2013). Globally, it is estimated that 64% of people are dumping raw sewage into the environment without treatment (Figure 1.1) (Baum et al., 2013).

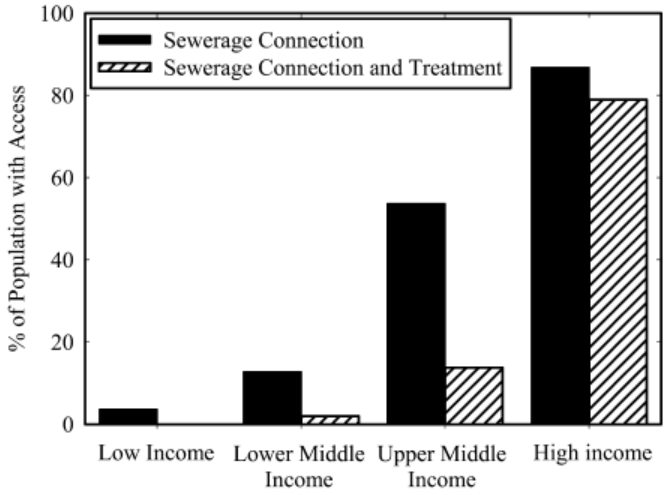

Figure 1.1 – Global access to sewerage connection.
The difference between sewage connections without end treatment and connections with sewage treatment in 2010, by country income group (Baum et al., 2013).

Contaminated water from inadequate waste management (human excreta, organic and inorganic solid and liquid) results in the spread of diseases and environmental degradation (Baum et al., 2013, Gandhi, 1998, Government of India, 2006, SACOSAN, 2008). The meagre sanitation conditions occurring in developing countries have been directly correlated as one of the leading causes of infant mortality, as well as impacting early cognitive and motor development and undermining educational achievement (SACOSAN, 2008).

Poor sanitation has been recognized as both a global issue and a global responsibility, and was included in the Millennium Development Goals (Mutagamba, 2003). The 2015

Millennium Development Goal for access to safe drinking water is currently on target, but the sanitation target will be missed by 1.9 billion people (Baum et al., 2013). The global sanitation situation has been labeled "the silent crisis" since more resources are directed to other, more attractive sectors than sanitation, such as water, health and education (Drangert et al., 2012). Sanitation should be a basic human right, but instead it is used as a means to divide social classes (Mutagamba, 2003). For those with financial means, flush toilets are a luxury that is expected; however, at the other end of the spectrum, finding a safe place to squat is a daily conundrum. Access to adequate sanitation is not only about health but necessary for maintaining personal dignity (UNICEF, 2008).

Over the centuries, the resources and technology available to manage human waste products have changed and so have the objectives, e.g. from avoiding stepping in faeces, to controlling smell, to complying with municipal effluent laws (Drangert et al., 2012). The principle of isolating faeces to avoid human contact and to avoid the contamination of drinking water has remained the same over the centuries but, with increased population density, more sophisticated methods of isolation and treatment have been required (Drangert et al., 2012).

Hygiene habits differ globally, and specific EcoSan toilets have to be designed and accepted within the context of local cultural practices (Winblad et al., 2004). Cultural issues around hygiene practices include: the feeling of intrusion on a private/personal issue; a lack of consistency in separating human urine from faeces; and superstitions, such as believing that toilet use may lead to fertility problems (Mutagamba, 2003). Flush toilet are seen as the gold standard around the world (Drangert, 2003), signifying class and wealth so that even in water-stressed areas, such as South Africa (Republic of South Africa, 2011), people want flush toilets. This inclination needs to be taken into account.

1.1.4 Introduction to sanitation sector of India

Annually, 400 000-500 000 children in India die from diarrhea due to poor sanitation (UNICEF, 2008). The poor sanitation situation in India greatly impacts the ability of people to work, and is a heavy toll on the government health care system (SACOSAN, 2008). In India, only 31% of the population uses adequate sanitation systems (21% in rural areas) and over 50% of the population is practicing open defecation (UNICEF,

2008). These figures use the UN (2008) definition of adequate sanitation and do not include the number of people using toilets connected to sewer that are not treated before disposal into the environment as this is a new definition and data was not available. Open defecation in India occurs due to limited access to toilets and increases the health risks to everyone within the community (WHO et al., 2008). Often fresh water sources become polluted from this human waste and are used as a transport system to remove the excreta (WHO et al., 2008).

1.1.5 Environment

Using rivers and streams to remove waste can lead to an excess of N-P-K (Winblad 2004). These nutrients can cause algae blooms in these waterways, leading to an effect called eutrophication that results in a depletion of oxygen in the water (Tidaker et al., 2005). Ecosystems across the globe are under stress due to eutrophication (Grifo et al., 1997). In India, the lack of wastewater treatment facilities and poor waste water management has lead to 75% of the country's surface water (and drinking water) being polluted (SACOSAN, 2008).

1.1.6 Ecological sanitation

EcoSan is defined as human waste management system that recovers the nutrients present in the waste as fertilizer for plants while reducing the potential of disease and pathogens. The concept of EcoSan has been used in agriculture for centuries and projects are currently being implemented in various parts of the world (Winblad et al., 2004), but the lack of peer-reviewed research limits the current acceptance rate (Jönsson et al., 2004). In an EcoSanRes Publication Series by Jönsson (2004), serious knowledge gaps were identified on the effects of human urine on crops production, soil quality, fertilizer strategies, and application techniques. Human urine contains salts, that can have negative impacts on plant production and soil chemistry, but there is limited understanding of the effects of urine-specific salt content. A better understanding of the long-term impact is required to allow this concept to be fully accepted.

1.2 Objectives

The primary objective of this study was to assess the changes in the soil chemistry and biomass production rates when using human urine as a fertilizer compared to mineral fertilizer on a long-term basis (simulation of nine years of repeated use). The secondary

objective was to find the optimal human urine fertilizer application rate for spinach (*Spinacia oleracea* L.) production in Himachal Pradesh, India.

1.3 Hypothesis

To assess the changes in the soil chemistry and biomass production rates, two experiments were performed. The first was a sensitivity assessment of two plant species, spinach and brown mustard (*Brassica juncea* L.), to increasing concentrations of synthetic human urine, mineral fertilizer and the combination of the two. The hypothesis of the first experiment was that as the fertilizer application rates increased, the EC would increase more in the human urine and combination fertilized treatments than in the mineral fertilizer treatments and would cause a decrease in biomass production rates. The second experiment was designed to observe the changes in the biomass of spinach and the chemical changes in soil from regular uses of human urine as a fertilizer. This was performed by simulating nine years of repeated fertilizer use in the field through increased concentrations of applied fertilizer. The effect of human urine as a fertilizer was compared to mineral fertilizer and the combination of urine with mineral fertilizer by measuring the differences in the spinach emergence rate, survival rate, yield, tissue nutrient concentration and the differences in the soil nutrient concentration and chemistry. For the second experiment, there were three hypotheses. One, the spinach seedlings of the three fertilizer treatments (human urine, mineral fertilizer, and combination) will be equivalent in biomass production at each simulated year and significantly higher than the control for all simulated years. Two, the electric conductivity (EC) of the soil will increase for each simulated year and the human urine and combination treatments will have higher soil EC than the mineral fertilizer treatments. Three, the pH of the soil for the three fertilizer treatments will decrease at equivalent rates at increased simulated years.

1.4 Scope

The research project focuses on the application of urine in India and is specific to the climate in India. It does not address handling or spreading of faeces. The sanitation risks are considered in the literature review with a Quantitative Microbial Risk Assessment (QMRA), but are not the main object of this thesis. This project does not focus on the practical issues of human waste collection, or how to separate faeces from urine. The cultural preferences are specific to India and further sociological and scientific research would be required to design practical and aesthetical acceptable toilets.

1.5 References

ALIBABA. 2012. *Country Search > Canada > urea* [Online]. Hong Kong: Alibaba Group. Available: http://www.alibaba.com/countrysearch/CA/urea.html [Accessed September 21, 2012].

BAUM, R., LUH, J. & BARTRAM, J. 2013. Sanitation: A Global Estimate of Sewerage Connections without Treatment and the Resulting Impact on MDG Progress. *Environmental Science & Technology,* 47**,** 1994-2000.

CAMBERATO, J. J. & PAN, W. L. 2011. Bioavailability of calicum, magnesium, sulphur, and silicon. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

DRANGERT, J.-O. Year. Requirements on sanitation systems – the flush toilet sets the standard for ecosan options. *In:* International Symposium on Ecological Sanitation, 2nd, 2003 Lübeck, Germany.

DRANGERT, J.-O., SCHÖNNING, C. & VINNERÅS, B. 2012. *Sustainable Sanitation for the 21st Century* [Online]. Available: http://www.sustainablesanitation.info/m3.php [Accessed January 10 2013].

FAO 2005. Fertilizer use by crop in India. *Land and Plant Nutrition Management Service.* Rome: Food and Agriculture Organization of the United Nations.

FAO 2006. Fertilizer use by crop. *Fertilizer and Plant Nutrition Bullentin.* Rome: Food and Agriculture Organization of the United Nations,.

FAO. 2011. *Current world fertilizer trends and outlook to 2015* [Online]. Rome: Food and Agricultural Organization of the United Nations. Available: ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf [Accessed September 20, 2012].

FAOSTAT. 2010. *Compare Data* [Online]. Food and Agriculture Organization of the United Nations. Available: http://faostat3.fao.org/home/index.html#COMPARE [Accessed February 12, 2013].

GANDHI, R. 1998. Water and Sanitation - A Baseline Survey. *Indian Institute of mass communication* New Delhi: Rajiv Gandhi National Drinking Water Mission.

GOVERNMENT OF INDIA. Year. A movement towards total sanitation in India. *In:* South Asian Conference on Sanitation, 20-21 September 2006 2006 Islamabad, Pakistan. Government of India.

GRIFO, F. & ROSENTHAL, J. 1997. *Biodiversity and Human Health,* Washignton, D.C., Island press.

JÖNSSON, H., STINTZING, A. R., VINNERAS, B. & SALOMON, E. 2004. Guidelines on the Use of Urine and Faeces in Crop Production. *EcoSanRes Publication Series.* Stockholm: Stockholm Environment Institute.

KING, F. H. 1911. *Farmers of Forty Centuries; Or, Permanent Agriculture in China, Korea, and Japan*, Project Gutenberg eBook.

MARENYA, P. P. & BARRETT, C. B. 2009. Soil quality and fertilizer use rates among smallholder farmers in western Kenya. *Agricultural Economics,* 40**,** 561-572.

MCDONOUGH, W. & BRAUNGART, M. 2002. *Cradle to Cradle: Remaking the Way We Make Things,* New York, Macmillan.

MUTAGAMBA, M. Year. Ecosan – what kind of advocacy is needed. *In:* International Symposium on Ecological Sanitation, 2nd, 2003 Lübeck, Germany.

OBER, J. A. 2007. Potash. *In:* UNITED STATES GEOLOGICAL SURVEY (ed.) *2006 Minerals Yearbook.*

PRADHAN, S. K., HOLOPAINEN, J. K., WEISELL, J. & HEINONEN-TANSKI, H. 2010. Human Urine and Wood Ash as Plant Nutrients for Red Beet (Beta vulgaris) Cultivation: Impacts on Yield Quality. *Journal of agriculture and food chemistry,* 58**,** 2034-2039.

PROSSER, J. I. 2011. Nitrogen Transformations. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Sciences: Properties and Processes.* 2 ed. Boca Raton, FL: CRC Press.

REPUBLIC OF SOUTH AFRICA. 2011. *World Cup Legacy Report: Water* [Online]. Republic of South Africa. Available: http://www.environment.gov.za/sites/default/files/docs/water.pdf [Accessed February 12, 2013].

RYAN, J. & RASHID, A. 2006. Phosphorus. *Encyclopedia of Soil Science.* Taylor & Francis.

SACOSAN 2008. Sustaining the Sanitation Revolution - India Country Paper. *In:* DEPARTMENT OF DRINKING WATER SUPPLY & MINISTRY OF RURAL DEVELOPMENT (eds.) *SACOSAN III.* New Delhi: Rajiv Gandhi National Drinking Water Mission.

TIDAKER, P., MATTSSON, B. & JONSSON, H. 2005. Environmental impact of wheat production using human urine and mineral fertilisers - a scenario study. *Journal of Cleaner Production,* 15**,** 52-62.

UNICEF. 2008. *India: Water, Environment and Sanitation* [Online]. United Nations. Available: http://www.unicef.org/india/wes_209.htm [Accessed January 23, 2013].

WHO & UNICEF. 2008. *Progress on Drinking Water and Sanitation: Special Focus on Sanitation* [Online]. World Health Organization

UNICEF. Available: http://www.who.int/water_sanitation_health/monitoring/jmp2008.pdf [Accessed February 12, 2013].

WINBLAD, U., HEBERT, M., CALVERT, P. & MORGAN, P. 2004. *Ecological sanitation*, Stockholm environment institute.

2 Literature review

2.1 Introduction

Ecological sanitation (EcoSan) is a holistic approach to sanitation in which human excrement is used as fertilizer (Calvert, 2004, Jackson, 2005). Just like cows and chickens, humans have plant available nutrients in our excreta (Jönsson et al., 1997). EcoSan has a long history in traditional agricultural practices as human excrement has been used as a fertilizer for thousands of years (King, 1911). However, there is limited scientific knowledge on the long-term impacts of EcoSan practices on soil chemistry and biomass production. This literature review provides an overview of how plants use fertilizers (Section 2.2), the impact that fertilizers have on soil chemistry (Section 2.3), an overview of the history and design of EcoSan systems (Section 2.6), and chemical composition of urine and the nitrogen (N) cycle will be highlighted (Section 2.7). This review will include an overview of studies performed with human urine (Section 2.8), and considerations that are important to the implementation of EcoSan, such as the scale of operations, climate change, and potential changes in taste are discussed (Section 2.8). And finally, the sanitation risks from using human urine as fertilizer was modelled in a Quantitative Microbial Risk Assessment (QMRA) (Section 2.9).

2.2 Fertilizers

Macronutrients are elements that plants require in large quantities. There are six elements that are identified as macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) (Camberato et al., 2011). N, P, and K are the three most required elements and are often limiting in a soil based growing media thus requiring additional applications for optimal production. Ca, Mg and S are the next three most required elements and generally are not limiting factors for plant growth, but at low concentrations can negatively impact crop yield and quality (Camberato et al., 2011). Micronutrients (boron (B), chloride (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn)) are required in smaller quantity than macronutrients and generally are not limiting factors in soil based crop production (Bolan et al., 2011).

Plants absorb most nutrients in ionic form from the soil into the roots (Jönsson et al., 2004). Plants are dependent on the activity of microorganisms to convert many of the nutrients in the soil into optimal forms for uptake by the plant (Mufwanzala et al., 2010).

Nutrients in the soil can vary in concentration spatially and temporally, and the addition of fertilizers are required to provide adequate levels of nutrients across the field, which allows for more uniform plant growth and higher plant production (Jones et al., 2005).

A major factor that influences nutrient availability is soil pH as it controls the solubility of the ions (Power et al., 1997). Optimal plant growth occurs at a pH range from 6.0-7.0, as most of the beneficial elements are chemically available and toxic elements are least available (Figure 2.1) (Power et al., 1997). Application of ammoniacal N (ammonium, NH_4^+-N, and ammonia, $NH_{3,aq}$-N) based N fertilizer decreases the soil matrix pH through the release of hydrogen atoms, to increase the soil matrix pH, this can be balanced by the application of limestone (lime) or ash as a buffer to maintain a pH between 6.0-7.0.

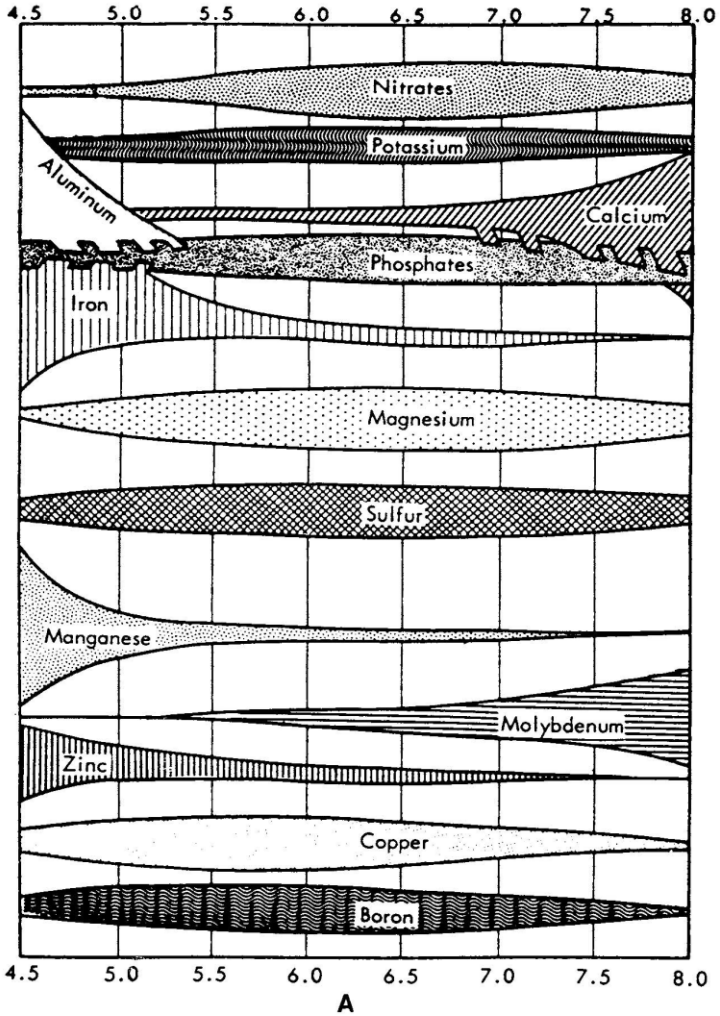

Figure 2.1 – Soil pH and relative plant nutrient availability. The wider the bar, the greater the availability of the nutrient to the plant (Power and Prasad, 1997).

Fertilizer application is required to achieve maximum crop yields. Fertilizer is applied before and during each growing season to replace any nutrients that crops uptake from the soil and that are removed from the field during harvest. These nutrients can also be leached from the soil, washed away with surface runoff, blown away or volatized which result in the need to replace these lost nutrients. Without fertilizers, crop yields tend to be 1/3 to 1/4 of the yields produced with the optimal level of fertilizer (Pradhan et al., 2009). Even with fertilization, seasonal variations, soil type and cultural practices can influence the crop yield each year (Jones et al., 2005).

Fertilizers are a composition of different elements and include salts which can alter the soil chemistry. Parts of these fertilizers can accumulate and alter the chemistry of the soil with repeated use (Whipker et al., 2000), increasing the electric conductivity (EC) of the soil beyond optimal levels and negatively impacting biomass production (Section 2.3). Understanding how the chemistry of the soil is altered by a fertilizer is required to maintain an optimal EC and pH, and maximize plant biomass production.

2.2.1 Nitrogen fertilizer

N is the most abundant element in the atmosphere and 99% is in the inert form dinitrogen (N_2) (Camberato, 2011). The N cycle is the process of N transformation between its different forms (Figure 2.2). Atmospheric N (N_2) is fixed by microbes to NH_3 and used by plants for biochemical reactions (Cleemput et al., 2006). Mineralization is the decomposition of N-compounds in plant residue by microbes to form ammonium (NH_4^+), and nitrification is the further conversion to nitrate (NO_3^-) (Cleemput et al., 2006). Denitrification occurs in anaerobic soils when NO_3^- is converted to N_2 gas (Cleemput et al., 2006). Immobilization is the conversion of inorganic N (NH_4^+ and NO_3^-) to organic N (Prosser, 2011). Mineralization-immobilization turnover follows the microbes' life cycle (Prosser, 2011) and factors that influence this cycle are the organic matter content of soil, temperature, and pH (Camberato, 2011). The high carbon to N content (>20-30:1) in crop residue increases immobilization and decreases the amount of biovailable N (Camberato, 2011). Plants utilise two inorganic forms of N (NH_4^+ and NO_3^-) and while most plants can use both typically NO_3^- is favoured (Jönsson et al., 2004, Lasa et al., 2000).

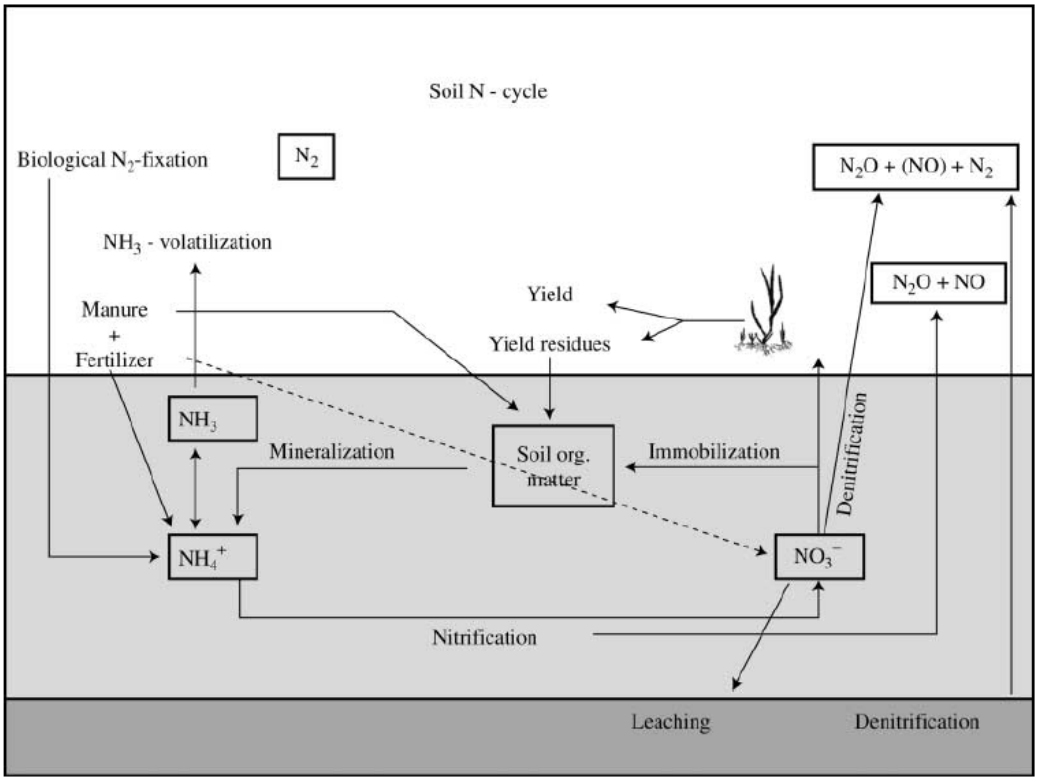

Figure 2.2 – Nitrogen cycle
Transformation between organic and inorganic N (Cleemput et al., 2006).

There are only a few species of bacteria and algae that can utilize N_2 (Prosser, 2011). There are three dominant paths to fix atmospheric N into a form that is more usable in the biosphere: lightning (5×10^9 kg N year^{-1}); Haber-Bosch manufacturing process of urea and ammonium nitrate (1.1×10^{11} kg N year^{-1}); and biological N fixation by an enzyme in prokaryotic microbes called nitrogenase ($1.0\text{-}1.4 \times 10^{11}$ kg N year^{-1}) (Camberato, 2011). N is required by plants more than any other nutrient (based on number of atoms) and is often a limiting nutrient for non-leguminous plants (Camberato, 2011, Jönsson et al., 2004). N is an important nutrient as increasing fertilization levels of bioavailable N results in more plant growth than increased fertilization with any other nutrients (Camberato, 2011). The concentration of organic N in the tissue of plants vary among species and part of the plant; ranging from 20-50 g of N per kg of tissue (Camberato, 2011). Legumes[1] have a symbiosis relationship with specialized N fixing bacteria and have higher concentrations of N in the tissue; > 40 g of N per kg of tissue (Camberato, 2011).

[1] Legumes were not used in the experiment and are not further discussed.

Insufficient N reduces plant growth and causes yellowing, chlorosis, because of reduced chlorophyll in the tissue (Camberato, 2011). Chlorosis is more common in older leaves as N is translocatable from older to newer leaves to maximize the productivity of the plant (Camberato, 2011).

Excessive N application can delay flowering, reduce fruit set, increase shoot lodging (plants too top heavy to support on weight, (Science-dictionary, 2008)), impair crop quality and increase NO_3^- accumulation in the leaves (Camberato, 2011). At high levels in plant tissues NO_3^- is toxic to humans and animals (Camberato, 2011, Lasa et al., 2000). For example, spinach is efficient in taking up NO_3^- from the soil, but, once absorbed spinach is not efficient in relocating or reducing NO_3^-, resulting in high concentrations of NO_3^- in the leaves (Stagnari et al., 2007). NO_3^- can be reduced to NH_4^+ in plant roots and, in some species shoots, to be used in amino acid synthesis (Lasa et al., 2000). NH_4^+ is incorporated into the plant and stored in organic forms: structural N, soluble proteins, and amino acids (Lasa et al., 2000).

The different molecular forms of N sources have varying positive and negative impacts on plant physiological processes (Lasa et al., 2000). Accumulation of NH_4^+ can result in toxic effects that reduce the growth rates, compared to excess NO_3^- that is accumulated in plant tissue with little adverse effects (Lasa et al., 2000). The negativity depends on the species ability to assimilate NH_4^+ (Lasa et al., 2000). One toxic effect of NH_4^+ is the acidification of the rhizosphere, when the plant takes up NH_4^+ ions there is an outflow of hydrogen ions from the roots resulting in increased acidity of the area around the roots (Lasa et al., 2000). The tolerance of a plant to NH_4^+ is correlated to its carbon and N metabolisms, when sufficient carbohydrates (carbon) are available in the root system then more NH_4^+ can be assimilated (Lasa et al., 2000). For example, sunflowers have a higher tolerance to NH_4^+ than spinach due to sunflowers having a higher photosynthetic efficiency that provides a larger source of carbohydrates (Lasa et al., 2000).

Environmental consequences of over application of NO_3^- include leaching, which leads to the potential contamination of surface and subsurface water, and increased release of greenhouse gases, through the release of N_2O by denitrification (Jones et al., 2005, Lasa et al., 2000, Prosser, 2011). NO_3^- leaching occurs more readily in sandy soils than more

complex structured soils (specifically ones with small pores) and is greatly influenced by warm humid climates and rainfall (Camberato, 2011). NH_4^+ ions are less prone to leaching and denitrification losses and from an environmental perspective are a more appropriate source of N (Camberato, 2011, Lasa et al., 2000). NH_3 will volatilize, easily reaching 25% loss of the initial N applied with pH and temperature the primary environmental factors (Camberato, 2011). Increasing pH causes NH_4^+ to react with free hydroxyl groups and is converted to NH_3 (Camberato, 2011). An increase in temperature results in greater NH_3 volatilization (Camberato, 2011).

Urea applied to soils with high crop residue can intensify volatilization due to the increased presence of urease (enzyme in bacteria used for hydrolysis) which raises pH (Camberato, 2011, Orwin et al., 2010). In the N cycle, the hydrolyzed urea (now NH_3 and NH_4^+) undergoes nitrification to NO_3^- which decreases the pH (Haynes et al., 1993). The nitrification rate is reduced at a pH lower than 6.0 (Camberato, 2011). At high pH, > 8, volatilization increases and reduces available N and during hot, dry and windy conditions 100% of the applied N can be volatilized if the fertilizer is not incorporated into the soil (Camberato, 2011). Drying soils amplifies volatilization because the NH_4^+ concentration increases; while rainfall or irrigation after application decreases volatilization by transporting nutrients into the soil (Camberato, 2011). Incorporating fertilizer 5-10 cm into the soil versus broadcasting limits volatilization (Camberato, 2011). NH_3 can volatilize from tissue, potentially 10-20% of the N applied, but is rarely considered during nitrogen balance calculations (Camberato, 2011).

2.2.2 Phosphorus fertilizer

Available P in soil is generally low but the total P is generally high (soluble form: 0.01-0.30 mg L^{-1}) as the availability is dictated by sorption, desorption and precipitation in the P cycle (Figure 2.3) (Power et al., 1997, Ryan et al., 2006, Sharpley, 2011). Inorganic P is the dominant form for plant uptake (Sharpley, 2011). Inorganic forms of P are dependent on pH but the availability does not change greatly (Figure 2.1). At pH 4.0-6.0, the most soluble form is $H_2PO_4^-$ (dihydrogen phosphate) and is easily absorbed by plants; at pH 6.5-7.5 the mix is partly $H_2PO_4^-$ and partly HPO_4^{2-} (monohydrogen phosphate); and at pH 8.0-10.0 HPO_4^{2-} is the dominant form with poor absorption through the roots (Jönsson et al., 2004, Power et al., 1997). Higher soil temperatures increase the microbial activity, for every increase of 15^{o}C there is up to 33% more available inorganic P (Power

et al., 1997, Sharpley, 2011). Mineralization of organic P is typically higher in the tropics with distinct wet and dry seasons (Sharpley, 2011). In tropical areas, additional P is required to maintain its availability to the plants and often a growth limiting factor, potentially only 15-30% of fertilizer applied is available for plant up-take as it quickly precipitates into insoluble compounds or immobilized by clay particles (Power et al., 1997, Ryan et al., 2006, Sharpley, 2011). The correlation between total P and plant available P is small or not significant, as the driving factor is the texture of the soil (changing the absorbance of P) (Ryan et al., 2006, Sharpley, 2011).

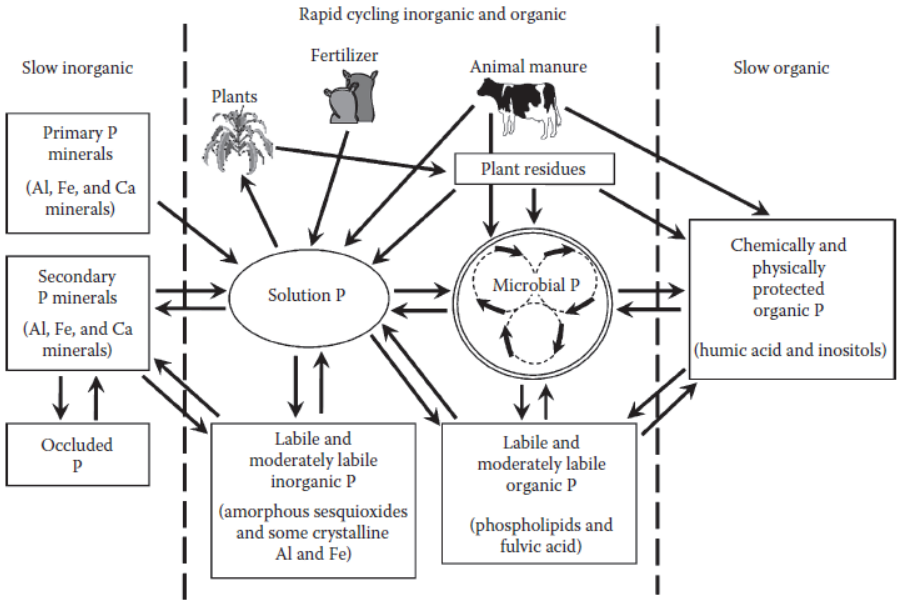

Figure 2.3 – Phosphorus cycle: components, forms, and flows
The labile phase is a group of phosphorus compounds in the soil having various degrees of solubility (Ryan et al., 2006). Source of image: (Sharpley, 2011)

P tends not to leach or volatilize like N as it is predominately immobile due to reactions with calcium, iron and aluminum ion in soil solution (Power et al., 1997). Calcium compounds dominate sorption and precipitation reactions in neutral to basic soil; while iron, manganese and aluminium dominate P sorption in acidic soils (Power et al., 1997, Sharpley, 2011). Sodium will react with P to create monosodium P and disodium P, and in this form P is easily absorbed by the plants (Power et al., 1997). P is mobile in plants and is transferred from old tissue to the active region when deficient occurs (Power et al., 1997). Deficiency symptoms are first noticed in the older leaves and include delayed

maturity, small darker green leaves with reddish purple colouring and dying tips (Power et al., 1997, Ryan et al., 2006).

2.2.3 Potassium fertilizer

K in plants is used for osmotic regulation, cation-anion balance, protein synthesis and activation of enzymes (Hinsinger, 2006). In plant tissue, the concentration of K ranges from 5-50 g K kg^{-1} of tissue (Hinsinger, 2006). K is highly water soluble (Jönsson et al., 2004) and the most plentiful of the other macronutrients in the soil, ranging from 3 000-100 000 kg K ha^{-1} in the top 0.2 meter of soil (Sparks, 2011). High application rates create localized salinity issues which can negatively impact the seeds and plants (Section 2.3).

In soils, K is found in inorganic forms, such as K carbonate (K_2CO_3), K chloride (KCl), and K sulphate (K_2SO_4) (Power et al., 1997). Generally 98% of soil K is in mineral form and 2% is in solution and exchangeable phases (Sparks, 2011). There are four forms of K in soil, listed in order of plant availability: soluble > exchangeable > fixed > mineral. An equilibrium between these four forms occurs in the soil and is based on soil moisture content and concentration of divalent cations (Sparks, 2011). K solution, typically found at low concentrations (2-5 mg K L^{-1}), can leach easily depending on the cation exchange capacity (CEC), pH, application rates and plant adsorption (Hinsinger, 2006, Sparks, 2011). Leaching is less prevalent with higher CEC and in soils with a pH range of 6.0-6.5 (Sparks, 2011). At pH levels above neutral the higher concentrations of oxides and hydroxides of iron and aluminum that bond to K ions result in a decrease of the soluble solution of K (Power et al., 1997).

2.2.4 Calcium and magnesium

Ca and Mg can be in the same section as both are similar in several ways; both have two valence electrons, are absorbed by plants as cations, and are base-forming elements (Power et al., 1997). The bioavailabilities of Ca and Mg are similarly impacted by parent material, ion-exchange reactions, biological transformation, and leaching (Camberato et al., 2011). In soils, Ca and Mg elements are predominantly present as mineral forms (derived from rocks such as sedimentary rock, basic igneous rock and acid igneous rock) and as ions in the exchange complex or in solution (Camberato et al., 2011). Similarly to K, occurrence in organic complexes is rare (Camberato et al., 2011). Ca and Mg ions are transported through the soil to the roots by mass flow and diffusion which can result in

accumulation of ions in the rhizosphere (Camberato et al., 2011). Low uptake rates of Ca and Mg due to low soil solution availability can be aggravated by high levels of K, low P, low soil temperature and wet soils (Camberato et al., 2011, Power et al., 1997). Acidification, such as the nitrification of ammoniacal N (NH_4^+-N and $NH_{3,aq}$-N), can accelerate leaching of calcium and magnesium as the aluminum and hydrogen ions displace them from the soil exchange complex (Camberato et al., 2011). Deficiencies in soils are rare and when observed it is often caused not by a lack of calcium or magnesium but by aluminum interfering with its uptake (Power et al., 1997). Abundant Ca can increase magnesium leaching as Ca is more strongly bonded to soils (Camberato et al., 2011).

In plants, Ca and Mg differ in locations and functions. Ca is required for structural rigidity, organic acids and regulation of several enzymes and concentrations in plant tissue range from 2.0-35.0 g per kg of tissue (Camberato et al., 2011). Transportation of Ca between leaves is low making plants dependent on its constant availability in the soil for uptake to maintain growth and development (Camberato et al., 2011, Power et al., 1997). Limited Ca decreases plants rooting abilities resulting in limited exploration of soil for other nutrients and moisture (Camberato et al., 2011). Negative impacts on storage organs and fruits occur with deficient levels of Ca, for example pod rot in peanuts and blackheart in celery (Camberato et al., 2011, Power et al., 1997). Deficiencies are intensified by temperature and moisture stress during specific crop development stages resulting in yearly variations of deficiency levels (Camberato et al., 2011). Mg is used as part of enzymes, used structurally in ribosome, and as the central cation in chlorophyll molecules (fundamental to photosynthesis) (Camberato et al., 2011, Power et al., 1997). Mg is mobile in plants, so deficiencies are more commonly seen in older, lower leaves (Camberato et al., 2011, Power et al., 1997). A symptom of Mg deficiency is intervinal chlorosis in lower leaves in which leaves lose green pigment except in the veins (Power et al., 1997). Concentrations of Mg in plants range from 1.5-10.0 g per kg of tissue (Camberato et al., 2011).

2.2.5 Micronutrients

Trace elements are divided into two groups for plants: biologically essential (Cu, Cl, Mn, Fe, B and Zn) and nonessential (lead, Pb; cadmium, Cd; and mercury, Hg) (Bolan et al., 2011). Biological essential elements, or micronutrients, are typically found in adequate

concentrations in the soil and by mineralization of organic matter (Jönsson et al., 2004). Micronutrients are required at low concentrations for different plant functions and formation of different enzymes, for example chloride controls the opening/closing of stomata in leaves, balances K, is required as part of photosynthesis, and reduces susceptibility to disease (Bolan et al., 2011). High concentrations of essential elements can be toxic, while nonessential elements can be phytotoxic at low concentrations (Bolan et al., 2011). Trace elements are transformed and released into soils, which act as a sink, through weathering, pedogenic and anthropogenic process and do not generally volatilize (Bolan et al., 2011). The mobility and bioavailability of trace elements in soils are similarly influenced as described in the macronutrients (Sections 2.2.2 and 2.2.3).

2.3 Soil and salinity

Fertilizers and human urine contain salts that can negatively impact plant productivity. Salinity is a general term used to describe the concentration of dissolved inorganic solutes (chiefly Na^{+}, Mg^{++}, Ca^{++}, K^{+}, Cl^{-}, SO_4, HCO_3^{-}, NO_3^{-} and CO_3) in an aqueous solution dispersed in the root medium (Rhoades et al., 1999). Saline soils occur with increased levels of neutral soluble salts and may be visually identifiable during dry periods when salts accumulate on the surface as white crystals (Power et al., 1997). The term sodic refers to soils with high concentrations of sodium and may be identified visually as organic matter (humus) in the soil is diffused on the surface of soil, giving it a black facade (Power et al., 1997). Three criteria are used to determine and classify soil as saline, sodic, or both: salt concentration measured through EC; sodium status measured either by exchangeable sodium percentage (ESP) or by sodium absorption ratio (SAR); and pH (Power et al., 1997).

Soil is saline if EC is > 4 dS m^{-1}, SAR is < 13, and pH of saturation extract is < 8.5. Soil is sodic when EC is < 4 dS m^{-1}, SAR is > 15 and a pH of saturation extract is > 8.5 (Power et al., 1997). Sodic soils with clay content > 20% will crust and cake, decreasing infiltration rates (Power et al., 1997). Sodium ions bond to the clay causing aggregate destruction, decreasing the soil physical structure (Power et al., 1997). Soils can be both saline and sodic with EC > 4 dS m^{-1}, SAR > 15 and a pH of saturation extraction < 8.5 (Power et al., 1997).

The impacts of saline conditions on plant growth are dependent on the salt composition, the salt concentration, the physiological stage of the plant at exposure, and the plant species (Huang et al., 1995). Fertilizers that contain potassium chloride (KCl), NO_3^-, NH_4^+, and P, increase the EC of the soil (Whipker et al., 2000). The EC soil levels are also increased by the decay of organic materials, including urea, as the insoluble salts are chemically changed into soluble form (Whipker et al., 2000). Natural salinity levels vary depending on the landscape, the precipitation levels and the soil formation. Chemical weathering releases salts into a soluble form; this process is accelerated by human activities (DERM, 2011, Power et al., 1997).

Salt tolerance of a plant is defined as the ability of the plant to complete its life cycle without significant negative effects when its root zone is in a medium containing a given concentration of soluble salts (Figure 2.4) (Parida et al., 2005, Shannon et al., 1999). Different species of plants have varying salt tolerance and can be evaluated three ways: plant survival rate; absolute plant growth or yield; or relative growth compared to non-saline soils (Power et al., 1997, Shannon et al., 1999). The classification of tolerance is based on the yield of the plant at the given EC (Ayers et al., 1994). For example, carrots are salt sensitive (Mnkeni et al., 2008).

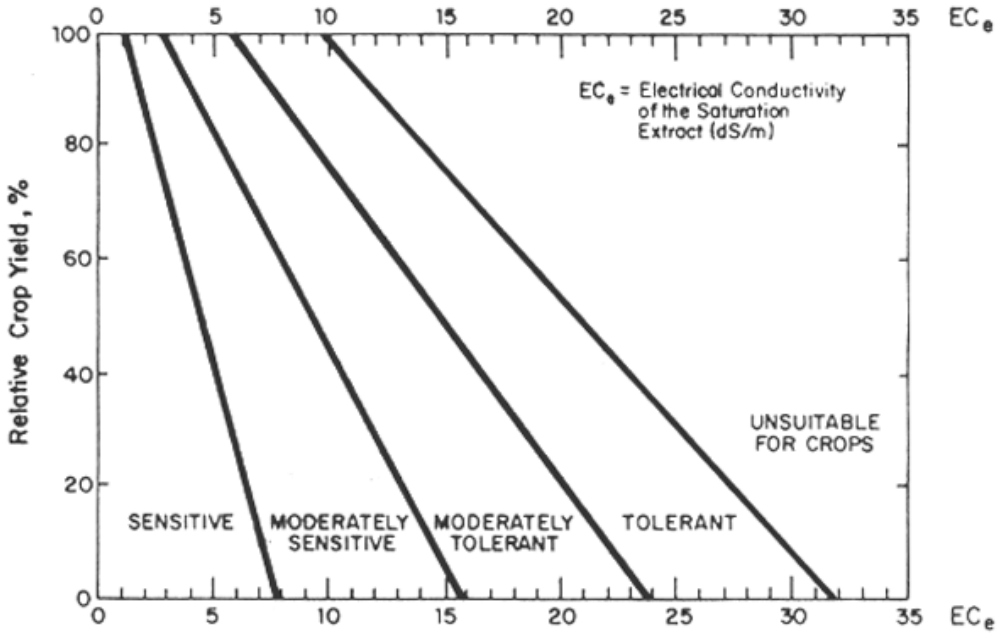

Figure 2.4 – Plant salt tolerance
Classifications for salt tolerance (EC_e) ratings of agricultural crop (Ayers et al., 1994). Note: EC_e in this figure is equivalent to EC_{SE} in the rest of the thesis.

Plants uptake nutrients as soluble salts, and increased salinity can result in a broad array of disturbances during all stages of development, including during germination, at the cellular and the whole plant level (Bajji et al., 1998, Huang et al., 1995, Power et al., 1997). Initial evidence of salinity impacting a crop is impeded height and/or number of leaves (Mnkeni et al., 2008). With higher levels of salinity leaves will wilt, even in moist soils, and exhibit leaf burn due to the inability to extract water (Mnkeni et al., 2008). Root growth is generally not as affected as the leaves, and under severe conditions plants may die (Mnkeni et al., 2008).

The three underlying causes of salinity disturbances are: a decrease in osmotic potential, salt toxicity and a decrease in bioavailability of nutrients. First, increased salt concentration can change the osmosis gradient and limit the ability of plants to absorb water, even from moist soil (Bajji et al., 1998, Mufwanzala et al., 2010, Orwin et al., 2010). The osmotic stress on plants can cause a decrease in yields in three ways: the stomata close to conserve water, resulting in a decrease of photosynthesis; the plant uses excess energy and carbohydrates for synthesizing organic solutes to regulate internal osmotic potential; and sequestered salts interfere with plant cell function (Shani et al., 2001). Figure 2.5 shows the impact on the yield of several crops irrigated with salt water three and seven weeks after planting (Shannon et al., 2000). Second, higher salt concentrations are correlated to a decrease in yield as some salts are toxic to plants at high concentrations (Mnkeni et al., 2008, Shani et al., 2001). And third, salt ions decrease the biological availability of nutrients, such as K, Mg, P and N (Bajji et al., 1998, Mufwanzala et al., 2010).

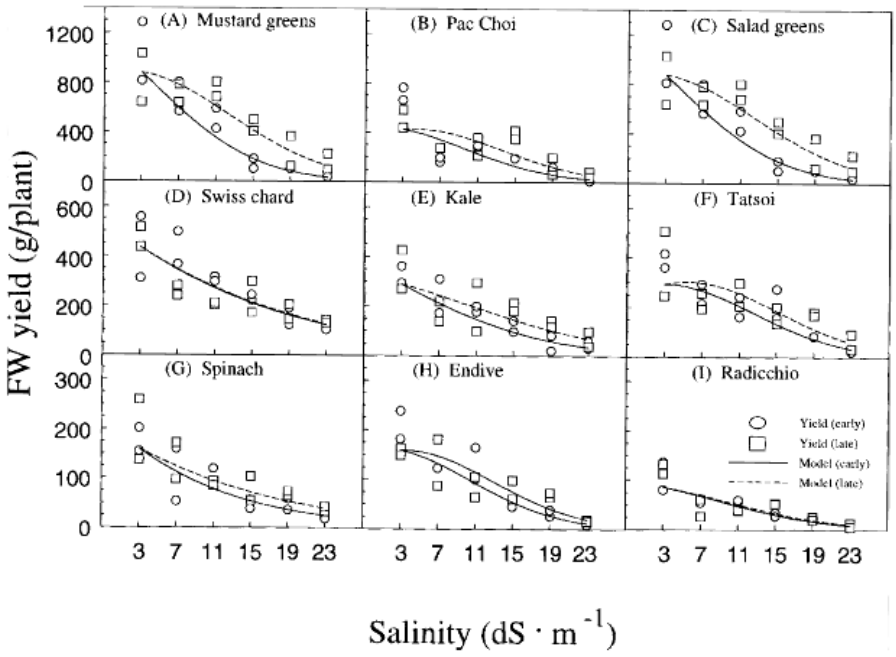

Figure 2.5 – Salinity impact on yield
Salt tolerance of (A) Mustard greens, (B) Pac Choi, (C) Salad greens, (D) Swiss chard, (E) kale, (F) Tatsoi, (G) Spinach, (H) Endive, and (I) Raddicchio planted in sand and irrigated with drainage water from 3 (early, open circle) and 7 (late, open square) weeks after planting (Shannon et al., 2000).

2.4 Measuring electric conductivity and pH

There are two popular methods for measuring the EC and pH of a soil, the dilution (v/v) extraction method (denoted as $EC_{1:2}$ and $pH_{1:2}$, respectively) and the saturated soil extraction paste (denoted as EC_{SE} and pH_{SE}, respectively) (Shannon et al., 1999). The dilution extraction method is fast and simple to process (add water, stir for 30 s and let sit for 1 hr before measuring), versus the saturated soil extraction paste method where water is added until water saturation point and then the water is extracted and filtered by vacuum (Shannon et al., 1999). The saturated soil extraction paste method is time consuming and researchers' perception of saturation end-point can vary, resulting in poor reproducibility (Hogg et al., 1984). The dilution extraction method provides the precise changes in the EC and the pH, while the saturation extraction method provides more accurate concentrations and better representative of soil conditions (Rhoades et al., 1999, Shannon et al., 1999).

The challenge with the dilution extraction method is to correlate the values with literature-reported values that used the saturation paste method for the set thresholds of

spinach and mustard (Power and Prasad 1997; Rhoades, Chanduvi et al. 1999; Shannon and Grieve 1999; Steppuhn, Genuchten et al. 2005). Several different formulas are available to convert the dilution extracted values to the saturation paste values based on the chemistry of solutions and soil types (BC Ministry of Agriculture, 1999, DERM, 2011, Gavlak et al., 2003, Rhoades et al., 1999). Equation 2.1 was selected to convert the $EC_{1:2}$ values measured in suspension to EC_{SE} based on the correction available for soil texture (fine soil texture) and resulted in a strong correlation coefficient (0.97) at a 1% significant level (Hogg et al., 1984):

$$\boldsymbol{EC_{SE} = 3.12 * EC_{1:2} - 0.59} \qquad \textbf{Equation 2.1}$$

$EC_{1:2}$ values less than 0.19 dS m^{-1} were below the range for Equation 1 and the rough estimate of multiplying the $EC_{1:2}$ value by two was used (Gough, 1996). To convert the $pH_{1:2}$ to pH_{SE}, Equation 2.2 was used (Gavlak et al., 2003):

$$\boldsymbol{pH_{SE} = pH_{1:2} - 0.25} \qquad \textbf{Equation 2.2}$$

2.5 Soil requirements for plants selected for experiment

Spinach was selected for its availability, its popularity in the rural area, its short growth period (25-55 days to maturity (Zvalo et al., 2008)), and its performance in the greenhouse experiment in Chapter 3. Spinach prefers well-drained sandy loam soils, with high organic content and a pH range of 6.0-6.8 (optimal range: 6.6-6.8) (Mufwanzala et al., 2010, Zvalo et al., 2008) and is moderately sensitive to saline conditions (Shannon et al., 1999). Spinach is a popular cultivar and is grown predominantly during the post-monsoon seasons in India, with mid-September being the typical sowing time for this crop in Himachal Pradesh (Viswanathan et al., 2011). On a smaller scale, spinach is grown during the monsoon season with rain shelters (personal communication).

2.6 Ecological Sanitation

EcoSan is the management of human excrement to produce a safe and reusable product – the transformation of a waste into a valuable resource (Winblad et al., 2004). Human health is protected by sanitizing the excrement (limiting the spread of disease) and the environment is protected by limiting pollution (Chapter 1). EcoSan is a cradle-to-cradle (cyclical) concept: humans consume food, waste is excreted, the excrement is sanitized and used to grow food, and the cycle repeats (McDonough et al., 2002, Winblad et al.,

2004). EcoSan systems, particularly urine separation at the source, are more efficient at recovering nutrients than conventional waste management systems (Ganrot et al., 2006). Communities with limited toilet access would not only benefit from cheaper sources of nutrients, but from the health benefits that follow decreasing the frequency of open defecation and the reduced risk of contaminating drinking water sources (Höglund, 2001).

2.6.1 Brief history of the use of human excrement as fertilizer

Human excrement was once a valuable resource. For example, in 1908 the city of Shanghai sold one contract, at the time worth $31 000 in gold, for the rights to collect, remove and resell 78 million kg of human excrement (King, 1911). While most other countries were still trying to figure out a way to dispose of excrement and household garbage, the Chinese had had an efficient recycling system for over three thousand years (King, 1911). In Japan, human excrement was also collected and applied to the fields as early as the 12th century (Höglund, 2001, King, 1911). In 1908, it was estimated that over 23 trillion kg of human excrement was applied to agricultural fields (King, 1911).

Attitudes towards the use of human excrement have changed because of three developments during the 19th century. First was the introduction of water closets that limited the access to excrement (in that excrement was mixed and transported away with water) (Höglund, 2001). Second was the increase in knowledge regarding the connection between bacteria and the spread of disease (Höglund, 2001). The third was the increase in animal husbandry and the development of synthetic fertilizers, particularly after the green revolution (Shiming, 2002). These developments transformed the cradle-to-cradle system to the cradle-to-grave system currently used around the world, where the potential of human waste as a resource is being ignored, and the nutrients used to fertilize agricultural crops are being extracted from non-renewable resources (Chapter 1).

2.6.2 Physical design of urine diverting toilets

Urine is automatically diverted away from faeces during excretion just by the design of our bodies. Figure 2.6 shows examples of toilets that collect urine separately from faeces. While the physical designs of the urine diverting toilets, collection processes and distribution systems are beyond the scope of this thesis, the following provides a brief explanation of how urine is diverted away from faeces during excretion and how urine and faeces are collected (Figure 2.7 and Figure 2.8).

Figure 2.6 – Examples of urine-diverting toilets
Urine-diverting toilets function by separates the urine in to the front bowl and the faeces into the back, larger, bowl during excretion. The western style toilet (left) is designed to be used while sitting (for males and females). This toilet uses 80% less water than conventional toilet to flush (BB Innovation, 1991). The center (Vinnerås, 2002) and right (Winblad et al., 2004) are examples of squatting toilets and use no water for flushing.

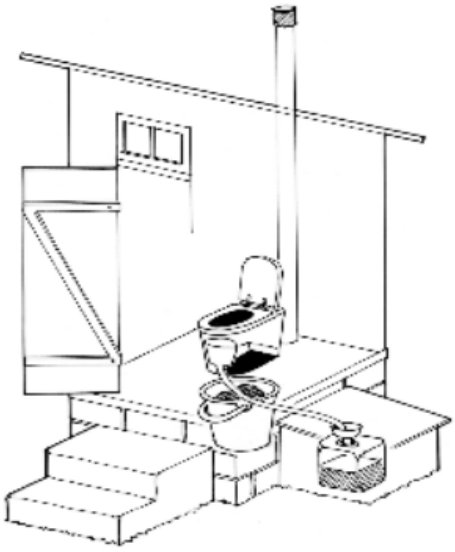

Figure 2.7 – Small-scale urine-diverting collection system
This toilet design is being implemented in Zimbabwe and requires no connection to water or sewer (Winblad et al., 2004). The buckets require regular emptying to other storage facilities until sterilization is achieved.

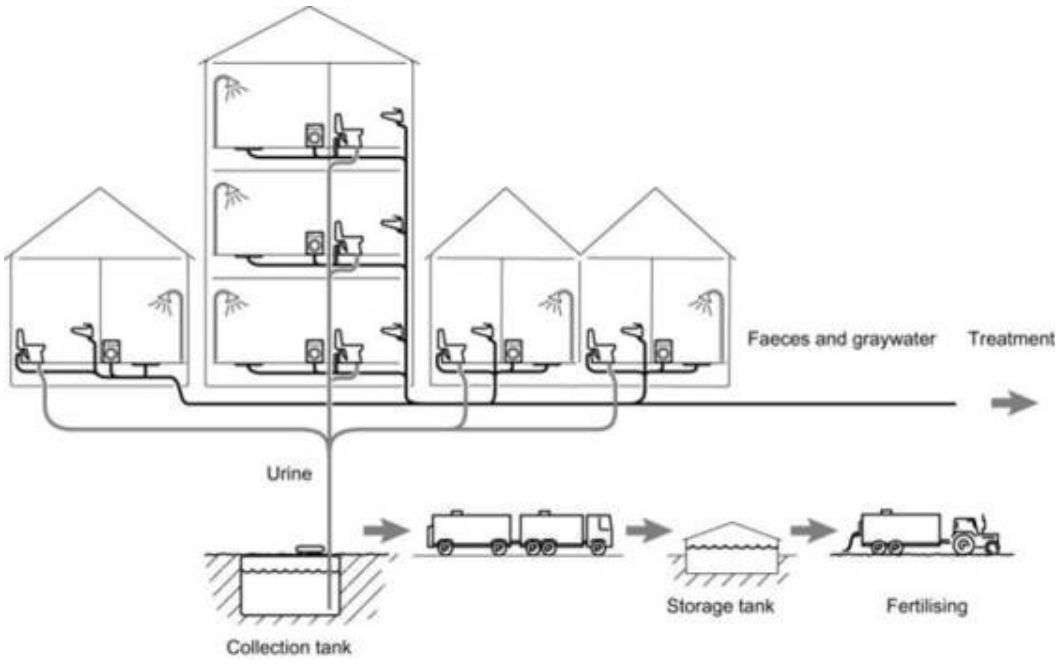

Figure 2.8 – Large-scale urine-diverting collection system
An example of a urine-diverting collection system in Sweden. The collection tank is attached to the building unit. The urine is mechanically transported and applied to agricultural fields (Höglund, 2001).

EcoSan designs for the collection and storage of human excrement range from simple (planting a tree over a disused pit latrine) to complex (sophisticated separation and collection systems in Sweden). The small-scale system, as depicted in the example from Zimbabwe (Figure 2.7), is simple to install and maintain, and requires more frequent handling of the excrement. The Swedish example (Figure 2.8) is a more complex system, with the emptying of the tank and application of the urine being mechanised. Although mechanised systems may be more difficult to maintain they usually decrease human exposure to the excrement. As this

2.6.3 EcoSan in India

There are several EcoSan projects developing in India and, already, there are several companies supplying EcoSan toilets in India (Government of India, 2011). Details on the success and failures of EcoSan projects in India do not seem to be well documented, but there are some lists of completed projects. Examples of projects in Tamil Nadu include: 550 dry composting toilets in Tuticorin District, Tamil Nadu; 30 demonstration dry composting toilet in Kancheepuram, Tamil Nadu; and 420 composting toilets in Cuddalore District, Tamil Nadu (Calvert, 2007). Examples of companies supplying toilet

are Eco-Solutions in Kerala, Prakash Ceramis in Gujarat, and ARIES in Madhya Pradresh (Government of India, 2011).

2.7 Human urine composition

2.7.1 Chemical composition

Urine is a by-product from the filtration of blood in the kidneys and contains macro- and micronutrients after they are used by the body. In human excrement, urine contains 80-90% of the N, 50-80% of the P and 80-90% of the K; the rest is excreted in the faeces (Vinnerås et al., 2002). The concentration of elements (Table 2.1) in human urine has diurnal fluctuations and varies widely between people depending on dietary habits, hydration, perspiration, and level of physical work in the day (Heinonen-Tanski et al., 2007). The range of macro and micronutrient concentrations in the urine highlights the impacts of dietary habits and complications of designing nutrient recycling systems and application rates (Vinnerås, 2002). In Sweden, Vinneräs (2002) observed a 30% increase from the typical values of K in human urine due to the addition of K supplement in table salt. People in cultures with higher meat consumption will excrete higher concentrations of N because of the large amounts of N contained in the amino acids that meat is composed of (Heinonen-Tanski et al., 2007, Pradhan et al., 2007). Mineral supplements contain essential trace elements such as Cu, Ni and Zn, and when supplements are consumed these minerals can be concentrated in human urine (Vinnerås, 2002). In published studies the reported concentrations of nutrients in urine vary due to different methodologies during collection, storage, and measurement. For example, poor storage can result in N loss through NH_3 emissions, and P can precipitate out of solution in the form of struvite ($Ca_5OH(PO_4)^3$) and hydroxyapatite ($MgNH_4PO_4 \cdot 6H_2O$) on the bottom of collection tanks (Vinnerås, 2002).

Table 2.1 – Compounds in human urine
Compounds present at >10 mg L^{-1} of male human urine in decreasing concentration (Putnam, 1971)

	Range (mg L^{-1})	
Total solutes	36 700	46 700
Urea	9 300	23 300
Chloride	1 870	8 400
Sodium	1 170	4 390
Potassium	750	2 610
Creatinine	670	2 150
Sulfur, inorganic	163	1 800
Phosphorus, total	410	1 070
Ammonia	200	730
Uric acid	40	670
Bicarbonate	20	560
Creatine	0	530
Sulfur, organic	77	470
Calcium	30	390
Magnesium	20	205
Glucose	30	200

2.7.2 Nitrogen cycle in human urine

The N in freshly excreted urine is predominately found as urea (~85%), NH_3, and uric acid; these three account for 90-95% of the total N (Kirchmann et al., 1995). Fresh human urine is unstable because of the high concentration of urea (Feng et al., 2008) and is typically acidic at a pH 6 (range 4.5-8.2) (Kirchmann et al., 1995). After the urine is excreted from the body, the urea is hydrolyzed (ureolysis) by the enzyme urease found in bacteria, such as *Proteus mirabilis* (Feng et al., 2008, Ganrot et al., 2006, Jönsson et al., 2004, Kirchmann et al., 1995, Tilley et al., 2008, Vinnerås, 2002) and is transformed into ammoniacal N (ammonia (NH_3) (Tilley et al., 2008) and ammonium (NH_4^+) (Feng et al., 2008, Ganrot et al., 2006, Jönsson et al., 2004, Kirchmann et al., 1995, Vinnerås, 2002)). The concentration of ammoniacal N remains at equilibrium and the equilibrium is dependent on atmospheric pressure and pH (Hellstrom et al., 1999, Tilley et al., 2008). The ureolysis reaction is alkaline and increases the pH to 9.0-9.3 (at 20-25^{o}C), which leads to further precipitation of compounds, such as struvite, urate and uric acid (Feng et al., 2008, Heinonen-Tanski et al., 2007, Jönsson et al., 2004, Kirchmann et al., 1995, Tilley et al., 2008). The ureolysis reaction rate is dependent on temperature and biological activity (Jönsson et al., 2004, Tilley et al., 2008). In locations where urease is able to

accumulate, such as in plumbing systems, the reaction occurs more quickly (Jönsson et al., 2004). Higher cross-contamination from faeces increases the quantity of bacteria with the urease enzyme and increases the rate of the ureolysis reaction (Tilley et al., 2008).

After the urea is hydrolyzed, the total N concentration is 95% ammoniacal N, 5% amino acid-N, and small traces of nitrite (NO_2^-) and NO_3^- (Kirchmann et al., 1995). Hydrolysized urine has a strong odour due to a high concentration of NH_4^+ (Tidaker 2005). Any urea still present in urine at field application is transformed to NH_4^+ by the urease present in the soil (Jönsson et al., 2004). Within three days of application, nitrification transforms NH_4^+ to NO_3^- (nitrosomonas or short nitrification: NH_4-N to NO_2-N followed by nitrobacts: NO_2-N to NO_3-N) (Feng et al., 2008, Jönsson et al., 2004). The nitrification process decreases the pH of soil as both reactions release hydrogen ions (Mnkeni et al., 2008).

2.7.3 Nutrient recovery

The macro- and micronutrients found in urine are present in inorganic and free ionic forms (Berger, 1960; Lentner et al., 1981) and available for plant uptake (Pradhan et al., 2007). The volatilization of NH_3 can decrease the concentration of N in urine due to poor handling practices during storage and application (Ganrot et al., 2006, Jönsson et al., 2004). To avoid NH_3 volatilization, gas exchange needs to be limited during storage; during spreading the urine should be applied close to the soil and incorporated quickly (Hellstrom et al., 1999, Jönsson et al., 2004). Such practices benefit the plants by limiting contact with urine, and benefits humans by reducing the odours (Jönsson et al., 2004). Pradhan (2007) reported that multiple applications of urine to the soil during the growing season decrease volatilization, potentially due to optimal application times where by the plants require the most N and absorb the N before excessive volatilization occurs. NH_3 evaporation can be prevented by reducing the decomposition of urea to ammoniacal N through acidification of the urine, but, during plant trials, there was no significant difference in yields between the acidified urine and untreated urine (Hellstrom et al., 1999).

To increase efficiency in transportation and application of human urine, researchers are developing methods for concentrating the nutrients in human urine. Examples include, the formation of struvite from the precipitation of P (Ganrot et al., 2006, Hellstrom et al.,

1999, Ronteltap et al., 2007); and the recovery N from fresh urine with specific adsorbents (zeolites, activated carbon) (Ganrot et al., 2006). These systems are not yet cost efficient and improvements in costs would make human urine a more competitive nutrient source (Ganrot et al., 2006).

2.8 Human urine as fertilizer

Nutrients present in human urine are typically found in ionic form, providing plants with a quick-acting fertilizer that is comparable to synthetic products (Jönsson et al., 2004, Kirchmann et al., 1995, Mnkeni et al., 2008, Pradhan et al., 2007, Tidaker et al., 2005). Unlike granular fertilizers, urine is not dependent on irrigation or rainfall to dissolve and is readily available to plants even during a dry season (Pradhan et al., 2007). The following is a summary of trials that have compared the production rates of crops fertilized with human urine to crops with no fertilizer, mineral fertilizer, and combinations of human urine with other sources of P and K. The use of human urine as a fertilizer produced significantly higher biomass than crops without fertilizer for beets (Mnkeni et al., 2008), tomatoes (Jönsson et al., 2004), and wheat (Jönsson et al., 2004). The beets and tomato trials were conducted in Zimbabwe, where access to fertilizer can be financially challenging for the average farmer. Increased use of human urine as a fertilizer could dramatically boost crop yields in areas where fertilizer is not currently being used. Crops fertilized with human urine did not produce significantly different biomass to crops fertilized with mineral fertilizer and production was significantly higher than for crops with no fertilizer application. Examples of these trials include cabbage (Pradhan et al., 2007), cucumber (Heinonen-Tanski et al., 2007), maize (Guzha et al., 2005, Mnkeni et al., 2008), tomato (Mnkeni et al., 2008, Pradhan et al., 2009), and wheat (Tidaker et al., 2005). Two studies reported barley (Jönsson et al., 2004) and beets (Pradhan et al., 2010) produce significantly higher yields with human urine than with mineral fertilizer. In the case of cabbage, the plants fertilized with urine matured earlier due to the bioavailability of nutrients, with no reports of disease (Pradhan et al., 2007).

As human urine is lower in P and K than N, some studies have looked at the effectiveness of combining human urine with additional sources of P and K. A trial in Zimbabwe observed no significant difference in the yield of maize with three different fertilizer treatments: mineral fertilizer, human urine, and combinations of human urine combined with faeces; the greatest yield was the combination (Guzha et al., 2005). In Finland, the

tomato yields (Pradhan et al., 2009) and beet yields (Pradhan et al., 2010) were compared to three fertilizer treatments: mineral fertilizer, human urine and combination of human urine and ash, had no significant difference.

2.8.1 Application recommendations

Timing and quantity of application for urine is based on recommended N timings and quantities for the geographic location and crop variety. Predicting the N bioavailability of manure (including animal and human) is more difficult than doing so for synthetic fertilizers (Camberato, 2011). Guidelines from EcoSanRes (Stockholm Environmental Institute) suggest that urine can be undiluted or diluted (1:3 or 1:10, v/v, urine to water) and be applied before sowing or in several smaller dosages up until 30 days before harvesting (Jönsson et al., 2004). Storage and spreading equipment that are currently used for animal manure can be used for human excrement (Tidaker et al., 2005). A study with leeks showed application timings did not have a significant difference on yields (Jönsson et al., 2004). During application, urine should be at a 10-cm distance from the plants for sanitary reasons, and to decrease potential damage to plants from the alkaline urine (Pradhan et al., 2007).

For areas or projects where analysis of the nutrient concentration in urine is not available, EcoSanRes uses a rule of thumb for applying fertilizer that the urine produced by one person in one day is applied to one metre squared per growing season based on the assumption of 3-7 g N L^{-1} (Jönsson et al., 2004).

2.8.2 Agronomic value

Around the world seven billion people are urinating every day, producing 28 million tonnes of N a year (Vinneräs 2002). These unused nutrients could be used to save famers $21.3 trillion USD a year, by supplying 26% of global N demands, based on $350 USD per metric tonne of urea (Alibaba, 2012, FAO, 2011). As human urine is high in N, but low in P, it would be good fertilizer in areas where there are already high concentrations of P in the soil (Heinonen-Tanski et al., 2007), or in combination with animal manure, as animal manure is high and P and K (Tidaker et al., 2005).

Challenges with increasing the scale of urine use, from single household units to a community-wide systems, include efficient storage, transportation and spreading of large volumes of urine (Ganrot et al., 2006, Tidaker et al., 2005). Human urine on average

contains ~0.6% N, compared to anhydrous NH_3 as a liquid fertilizer at 82% N. The low concentration of nutrients increases transportation and spreading costs, and soil compaction in the fields – these large issues need to be considered in the design of the system for successful large-scale use of urine as a fertilizer (Tidaker 2005).

2.8.3 Heavy metals & pharmaceutical products

Using human excrement as fertilizer is a heavily debated subject, as people are cautious of the presence of heavy metals and organic pollutants (Tidaker et al., 2005). For this reason human excrement is not allowed on organic farms in Europe (Tidaker et al., 2005) and Canada (Martin, 2009). However, human urine does not have hazardous concentrations of chemical compounds and heavy metals (Ganrot et al., 2006) and contains lower concentrations than most chemical fertilizers (Jönsson et al., 1997, Jönsson et al., 2004, Kirchmann et al., 1995, Ronteltap et al., 2007). Urine separation at the source eliminates contamination as it is not mixed with the pollutants from industries and runoff from storm pipes (Vinnerås, 2002). By manipulating the pH, metals that are present could be precipitated out of solution before application to the fields. When heavy metals are excreted from human bodies, human urine has a lower concentration than human faeces, and also has a lower concentration than cattle manure (Table 2.2) (Jönsson et al., 2004).

Table 2.2 – Concentration of heavy metals in excrement
Concentration of copper, zinc, chromium, nickel, lead and cadmium in urine, faeces and farmyard manure from organic cattle farms in Sweden in $\mu g\ kg^{-1}$ wet mass (Jönsson et al., 2004)

	Copper	Zinc	Chromium	Nickel	Lead	Cadmium
Urine ($\mu g\ kg^{-1}$)	67	30	7	5	1	0
Faeces ($\mu g\ kg^{-1}$)	6667	65000	122	450	122	62
Manure ($\mu g\ kg^{-1}$)	5220	26640	684	630	184	23

As all mammals produce hormones, vegetation and microbes have evolved abilities to degrade naturally-produced human hormones (Jönsson et al., 2004). The active ingredients in pharmaceutical products not used by the human body are excreted in the urine, averaging 64% from a screening assay of 212 pharmaceuticals, and averaging 35% in the faeces (Jönsson et al., 2004, Lienert et al., 2007). The excretion rates vary greatly; for example, radioactive ingredients do not degrade and 94% of them are excreted in the urine, while for other pharmaceuticals only 6% of their active ingredients are excreted through the urine (Lienert et al., 2007). The degradation of pharmaceuticals vary in soils:

ibuprofen degrades to non-detectible levels in soils three months after application, while 53% of the original Carbamazepine present in urine was detected in soil samples after the same amount of time (Winker et al., 2010).

There is limited knowledge on how heavy metals and pharmaceutical products present in the urine are taken up by plants, diffused into the hydrological system (into the groundwater and/or into the surface water as runoff), or diffused into soils (Lienert et al., 2007, Ronteltap et al., 2007, Winker et al., 2010). The mobility of a compound is influenced by their molecular weight (Winker et al., 2010), the pH, and the presence of organic molecules (Ronteltap et al., 2007). Currently there are no set thresholds as to what amounts of pharmaceuticals should be let into the environment through fertilizer application (Ronteltap et al., 2007). Further research is required to improve processing techniques, such as struvite and steam stripping, for the removal of heavy metals and pharmaceuticals (Winker et al., 2009).

Consumption of pharmaceuticals is concentrated in developed countries: 15% of the world's population in rich countries consumes 90% of total medicines (WHO, 2012). As developing countries consume fewer pharmaceutical products a year on average per capita (WHO, 2012), human urine could be used as fertilizer in these areas without major concerns regarding the concentration of pharmaceutical products, but should be monitored as consumption habits may change.

2.8.4 Environment

EcoSan, especially urine separated at the source, has several environmental benefits. It reduces fresh water usage while flushing, ideal for water stressed areas (BB Innovation, 1991). Human urine can be separated from the waste water stream, which can lead to a decrease in the nutrient load of the waste water at treatment facilities, and allow the nutrients to be applied for other uses (Ronteltap et al., 2007). Efficiency of waste water treatment facilities is improved, particularly in locations with sub-par systems, as 80% of the N and 50% of the P load is removed (Kirchmann et al., 1995). In cities such as Montréal, Québec, Canada, nutrients from human waste are still present in the effluent as the facilities are not able to remove all the nutrients, changing the concentration of nutrients in the waterway (Marcogliese et al., 2003). Municipalities would have reduced costs of operation for drinking water and waste water treatments facilities by removing

human urine from the waste stream (Tidaker et al., 2005). The greatest environmental benefit of removing human urine from the waste water would be the decrease in eutrophication due to the reduction of the nutrient load entering fresh water sources (Tidaker et al., 2005).

The use of fertilizer on fields can have negative impacts on the environment. Human urine and mineral fertilizers can leach NO_3^- from the field into waterways causing eutrophication (Orwin et al., 2010, Tidaker et al., 2005); good fertilizing management would mitigate this issue. The production of N fertilizer requires large quantities of fossil fuels for production; using human urine as N fertilizer therefore decreases greenhouse gas emissions (Tidaker et al., 2005). A potentially negative environmental impact of human urine is the doubling of atmospheric acidification compared to conventional fertilizer regimens, due to releases of nitric oxide, N dioxide and nitrous oxide gases during storage and transportation (Orwin et al., 2010, Tidaker et al., 2005). As previously mentioned, improvements in handling techniques would decrease N losses and acidification.

2.8.5 Taste

Taste of vegetables grown with human urine as a fertilizer was beyond the scope of this project, but experiments on taste have been performed and are briefly summarized here. In a cabbage taste test, the tasters did not detect a difference in flavour from plants fertilized with minerals or urine or plants with no fertilizer (Pradhan et al., 2007). No differences were detected in a similar taste test with beets (treatments with no fertilizer produced too small of a mass to be used in the taste test) (Pradhan et al., 2010). In a taste test with cucumbers fertilized with human urine and minerals, the tasters did detect a difference in taste but did not prefer an individual cucumber sample and appraised all the samples as tasty, with good form and texture (Heinonen-Tanski et al., 2007). In a taste test for tomatoes fertilized with three different treatments (mineral, urine and ash, and urine), tasters remarked upon a difference between urine and ash and urine treatments, and no difference between mineral and urine treatments (Pradhan et al., 2009). The tasters concluded that all the samples were equally tasty and did not have a preference (Pradhan et al., 2009). The conclusion of no significant difference in taste supports the previous statement of human urine being equivalent to mineral fertilizer.

2.9 Sanitation: a Quantitative Microbial Risk Assessment (QMRA) case study of the use of human urine as a fertilizer for spinach in northern rural India

2.9.1 Introduction

Many disease-causing pathogens originate from faeces, and can survive outside the human body long enough to infect others through transmission by food or water (Höglund, 2001). Human urine typically does not contain pathogens transmittable through the environment (Höglund, 2001). In urine-diverting toilets, there is risk of cross-contamination from faeces (Höglund, 2001). The risk of cross-contamination is greater with instances of diarrhea and with young children using the toilet (Höglund et al., 2002). The possibility of cross-contamination puts people's health at risk during the six stages in EcoSan (Figure 2.9). During the first four stages (collection, storage, transportation, and application) there is risk of accidental ingestion of urine (urine-oral transmission). In the final two stages (harvest and consumption), there is a risk of harmful pathogens being present on crops (food-oral and hand-oral transmission).

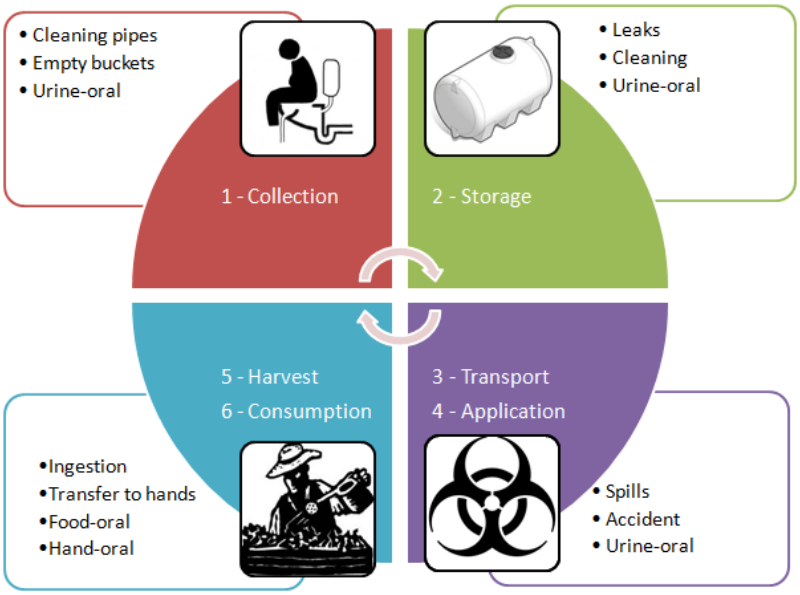

Figure 2.9 – The mode of accidental transmission of pathogens
At each stage of the process, there are different risks of exposure.

Höglund (2001) reported that the use of human urine as a fertilizer in temperate climates has a low health risk. Calculations were based on statistics in Sweden, where the procedure of collection, transportation, and application of urine is mechanized (Höglund, 2001), and few cases of mortality from diarrheal diseases are reported, compared to

developing countries, such as India (Howard et al., 2006). However, EcoSan projects are being promoted in developing countries (Winblad et al., 2004) with more diarrheal outbreaks, where risk assessments are not performed (Howard et al., 2006). A criticism of EcoSan as a system is how the sanitization of the urine and faeces are neglected (no confirmation that the end product is safe for field application) (Höglund et al., 2002).

To understand the risks of using human urine as a fertilizer for spinach in developing countries, a QMRA was used to calculate the potential disease burden from exposure to pathogens at each stage. The QMRA was performed for the urine fertilization of spinach, a higher risk crop that grows closer to the ground. This QMRA was based on several wide-range factors, such as the distribution and the occurrence of indicator pathogens. This method is not yet widely used in developing countries, due to the limit on available data and its complexity (Howard et al., 2006). MatLab (R2009b, Natick, MA) was used to model the QMRA with a recommended acceptable disease burden of $< 10^{-3}$ (1 person infected per 1000 persons a year) from using human urine as a fertilizer (Höglund et al., 2002).

2.9.1.1 Pathogens excreted in urine

Excreted urine of a healthy individual is sterile (Höglund, 2001), but from an unhealthy person microorganisms can be transmitted to the environment and potentially cause infectious disease; however, the chances of urine-oral transmission is low (Feachem et al., 1983). There are four commonly known pathogens excreted in urine: *Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi* and *Schistosoma haematobium* (Feachem et al., 1983). *Leptospira* is a more serious risk for sewage and farm workers in developing countries and is deemed to be an occupational hazard for individuals in these fields (Höglund, 2001). The occurrence of infection is low and urine-oral transmission is not a key route (Feachem et al., 1983). *Salmonella* is a common cause of gastroenteritis (food poisoning), with the non-typhoidal form of *Salmonella* causing 1.3 billion cases and three million deaths annually (PHAC, 2010). Mortality rates in developed countries are as low as 1%, while in developing countries the mortality rates are as high as 24% (PHAC, 2010). The highest probability of transmission of *Salmonella* is faecal-oral (Feachem et al., 1983). For *Schistosoma*, the eggs are excreted in the urine and are then dependent on snails to continue their life cycle, making urine-oral transmission a low risk (Feachem et al., 1983).

There are other examples of pathogens being excreted in urine, such as *Escherichia Coli* (the cause of > 80% of urinary tract infections), *Mycobacterium tuberculosis*, *Mycobacterium bovis* and Microsporidia (protozoa found in HIV-positive individuals), but there is little evidence of urine-oral transmission (Höglund, 2001). Venereal diseases have in some cases been reported to be caused by pathogens excreted in urine, but their potential survival outside of the body is low (Feachem et al., 1983).

2.9.1.2 Pathogens excreted in faeces

Pathogens from faeces are the principal concern of microbial activity when setting safety standards (WHO, 2011). Examples of parasites with the ability to transmit diseases to humans by food-oral and soil-oral methods are: *Campylobacter, Salmonella, Giardia, Yersinia, Shigella, Balantidum coli* and helminths (*Fasciola, Fasiolopsis, Echinococcus*) (Höglund, 2001, WHO, 2011). Helminths have higher rates of infection in developing countries, causing morbidity and mortality (Höglund, 2001). The viruses excreted by faeces are estimated to cause 80% of the gastrointestinal infections in humans in the United States (Höglund, 2001). One of the most commonly identified viral pathogens is the rotavirus which can be transmitted in waterborne outbreaks (Höglund, 2001). The reported cases are typically underestimated as not everyone goes to the hospital each time they are sick, and the aetiological agent is also typically not known (Höglund, 2001). Some diseases are zoonoses, making their transmission difficult to track and control (Höglund, 2001).

2.9.1.3 Survival of microorganisms

After excretion, the number of enteric pathogens declines due to death or loss of reproductive ability (viability), but the complete life cycle is complex and difficult to approximate (Höglund, 2001). By collecting and storing urine and faeces separately, the number of pathogens in human urine is limited and the sterilization storage period is decreased (Höglund, 2001). The main factors affecting the pathogens' survival in urine are temperature, pH and NH_3 concentration (Höglund, 2001). After a few days, the stored human urine pH rises to 9.0, creating a noxious environment for pathogens, increasing decay rate, and sterilizing of the urine over time (Höglund, 2001). Figure 2.10 is an example of the decay rates of three pathogens at a pH of 9, at 4°C and 20°C (Höglund et al., 2002).

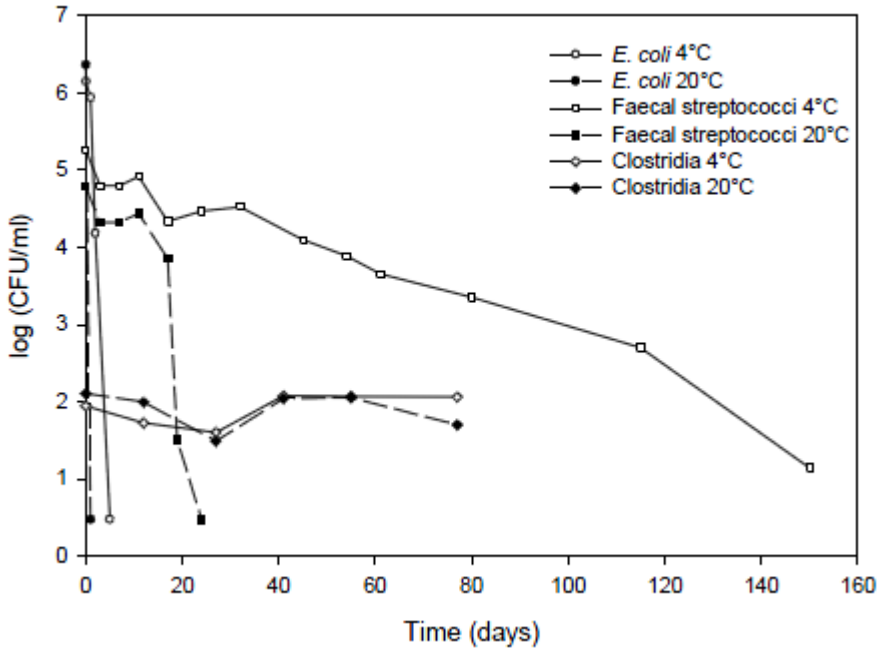

Figure 2.10 – The decay rates for three pathogens
The decay rates for *E. coli*, faecal streptococci and *C. perfringens* spores (clostridia) at 4°C and 20°C in source separated human urine at pH 9 (Höglund et al., 2002).

As an example of the required storage period to inactivate pathogens, guidelines specific to Sweden are summarized in Table 2.3. The survival of the pathogens once the urine is applied to soil as a fertilizer is assumed to be marginal (Höglund, 2001). The risk of pathogens contaminating a water source downstream was also assumed to be low risk due to the high dilution (Höglund, 2001).

Table 2.3 – Guidelines for the storage period of human urine[a] to be used for larger crop systems[b]
Developed for Sweden with the assumptions of a pH of ≥ 8.8 and a nitrogen concentration of ≥ 1 g L^{-1} (Höglund, 2001)

Storage temperature	Storage time	Possible pathogens in the urine mixture	Recommended crops
4°C	≥1 month	viruses, protozoa	food and fodder crops that are to be processed
4°C	≥6 months	viruses	food crops that are to be processed, fodder crops[c]
20°C	≥1 month	viruses	food crops that are to be processed, fodder crops[c]
20°C	≥6 months	probably none	all crops[d]

[a] Gram-positive bacteria and spore-forming bacteria are not included.
[b] A larger system in this case is a system where the urine mixture is used to fertilise crops that will be consumed by individuals other than members of the household from which the urine was collected.
[c] Not grasslands for production of fodder. Use of straw is also discouraged, further discussed below.
[d] For food crops that are consumed raw it is recommended that the urine be applied at least one month before harvesting and that it be incorporated into the ground if the edible parts grow above the soil surface.

2.9.1.4 Microorganisms indicators

As cross-contamination from faeces to urine is challenging to estimate (Höglund, 2001), indicators of faecal contamination are used, rather than testing for a large range of enteric pathogens that may or may not be present, which is difficult and costly (Haas et al., 1999, Höglund et al., 2002, Howard et al., 2006). The indicator organisms need to meet the following criteria: only be present when faecal contamination is present; have the same or greater survival rate as the enteric pathogens while not multiplying in the environment; and can be detected with a simple, fast, and inexpensive method (Copper et al., 1998).

Campylobacter jejuni, *Crytospordium parvum* and rotavirus fit the three criteria of an ideal indicator and have been well characterized, making them ideal for estimating the potential health effects from ingestion of a set number of the organisms (Höglund et al., 2002, WHO, 2011). *Campylobacter jejuni* is one of the most common bacterial aetiological agents of acute gastroenteritis worldwide (Acheson et al., 2001). From infected agents (humans and animal), large numbers of organisms are excreted in the faeces and only a relatively low number are required to infect another agent (WHO, 2011). *Cryptosporidium* is an aetiological organism causing diarrheal diseases in children, but is also a leading cause of infection and disease for people living with HIV/AIDS (Howard et al., 2006, WHO, 2011). *Cryptosporidium parvum* is relatively resistant to chemical treatments and causes infections at low doses (WHO, 2011). Cysts

and oocysts of protozoa have a prolonged survival period after being excreted from a human host (Copper et al., 1998). The human host sheds rotavirus in high concentrations; it has a low median infectious dose (N_{50} = 5.6) (Höglund, 2001). Rotavirus is the most common cause of gastroenteritis in infants in the world, leading to 527 000 deaths annually (29% of all deaths due to diarrhea) (WHO, 2011). There are many types of human rotaviruses which are transmitted from person to person, by aerosols and by faecal contaminated water, leading to repeated infections (WHO, 2011). The rotaviruses are also resistant to chemical treatments (WHO, 2011).

2.9.2 Methodology for the QMRA

The output of the QMRA is a number called the disease burden and is based on the characterization of the exposure and hazards from using human urine as a fertilizer (Höglund, 2001). The acceptable disease burden was set at $\leq 10^{-3}$ (Höglund, 2001) and calculated by the method described in Howard et al. (2006) and the WHO 3rd edition of the Guidelines to Drinking Water Quality (2004). The variables used to calculate the disease burden are summarized in Table 2.4 and then explained in more detail below.

Table 2.4 – The variables used in the QMRA
The variables were derived from Howard et al. (2006) and WHO (2004)

Variable	Label	Formula
Raw urine (organisms L^{-1})	q	Table 2.5
Decay rates	k	Table 2.6
Urine quality after treatment	u	q * (1-k)
Consumption of urine	v	from literature
Exposure by urine (organisms L^{-1})	e	u * v
Dose-response	r	Table 2.7
Risk of infection per day	pd	E * r
Risk of infection per year	py	1 - (1-pd)^365
Risk of diarrheal disease given infection	pr	Table 2.7
Risk of diarrheal disease	p	py * pr
Maximum disease burden	mdb	Table 2.7
Susceptible fraction	sf	from literature
Disease burden	DB	p * mdb * fs

2.9.2.1 Indicator microorganisms used

The three indicator pathogens used were: *Campylobacter jejuni*, *Crytospordium parvum* and rotavirus. Epidemiological data was used to estimate the likelihood of pathogens

being present in the faeces for a given population in northern rural India. Data was difficult to obtain and estimations were used to assume the number of organisms per L in urine (Table 2.5). Two scenarios were calculated: the first was for the worst case, where there was a high rate of cross-contamination; the second was for the best case, where there a minimum amount of cross-contamination.

Table 2.5 – Number of organisms per L in urine for worst and best case scenarios

Variable	*C. jejuni*	*C. Parvum*	Rotavirus
q (worst-case)[z]	500000	90700	10000
q (best-case)[y]	32	30	900

[Z] (WHO, 2011)

[Y] (Howard et al., 2006)

2.9.2.2 Inactivation of pathogens

The inactivation of the pathogens were calculated based the decay rates of the three indicator pathogens during the six stages of EcoSan and are summarized in Table 2.6. Decay rates are calculated based on the initial population, the final population and the time required. The actual values were difficult to estimate, as no region-specific data was collected on initial indicator pathogen concentration. These were conservative estimates based on the report of Höglund (2001) with an assumed urine pH of 9. The decay rates provide an estimate of the remaining pathogens which were used to estimate the risk of using the urine.

Table 2.6 – The assumed decay rates for two temperatures in human urine at pH 9
The rotavirus was the only indicator impacted by the change in temperature (4°C to 20°C) and only at Stage 2. Stage 6 had two decay rates for fresh and processed consumption (Höglund, 2001).

Stage & Season	*C. jejuni*	*C. Parvum*	Rotavirus
1 – Collection	0.00	0.00	0.00
2 – Storage (4°C/20°C)	0.99	0.90	0.00/0.70
3 – Transportation	0.00	0.00	0.00
4 – Application	0.00	0.00	0.00
5 – Harvesting	0.90	0.90	0.90
6[z] – Consumption (fresh)	0.00	0.00	0.00
6[y] – Consumption (Processed)	0.99	0.99	0.99

[z] For crops consumed fresh: no cooking and no washing

[y] For crops processed (i.e. cooking) before consumption

2.9.2.3 Consumption of urine

The accidental consumption of human urine was assumed to be 1 ml per exposure for Stages 1 through 4 and 10 ml per 100 g crop consumed for Stages 5 and 6 (Asano et al., 1992). In this QMRA, the method of applying the urine to the field was chosen to be non-mechanised, i.e. the urine was applied by hand. By not spraying, there are no aerosols produced at Stage 6 and no risk of inhalation of pathogens (Höglund, 2001).

2.9.2.4 Variables

Without being able to collect specific data for northern rural India, assumptions for the dose-response (r), the risk of diarrheal disease given infection (pr) and the maximum disease burden (mdb) variables were made based on available literature (Table 2.7). The 'r' is the correlation of the number of pathogens consumed to the number of incidences of adverse health effects in a given exposed population and provides the risk of infection per day (Höglund, 2001). The maximum disease burden considers the severity (from having diarrhea to mortality) and duration of each pathogen and by knowing the risk of contracting the disease, the actual disease burden can be calculated (Howard et al., 2006). For *C. jejuni* and rotavirus, the proportions of watery diarrhea and bloody diarrhea were used to calculate the maximum disease burden. For *C. parvum*, bloody diarrhea is rare, so the proportion was assumed to be zero (Howard et al., 2006).

Table 2.7 – The dose-response, risk of diarrheal disease and maximum disease burden

The values were assumed based on available literature and are unitless

Variable	*C. jejuni*	*C. Parvum*	Rotavirus
Dose-response (r)	0.001[Z]	0.004[Z]	0.27[Z]
Risk of diarrheal disease given infection (pr)	0.3[Y]	0.3[Z]	0.5[Z]
Maximum disease burden (mdb)	0.32[Z]	0.15[Z]	0.32[Z]

[Z] (Howard et al., 2006)

[Y] (WHO, 2011)

2.9.2.5 Susceptible fraction

Human urine used as a fertilizer in northern rural India, was assumed to be at household scale. The people living in a house were identified as the susceptible fraction (sf). The same susceptible fraction was used for all three indicators; the number does not take into account the greater impact that *Cryptosporidium* has on children and people suffering from HIV/AIDS (Howard et al., 2006). A MatLab program was developed for this case

study and designed to be flexible: it prompts the user to type in the number of inhabitants of the household and the number of external people helping on the farm. This was to include the number of workers handling the urine, farmers applying the urine, and people consuming fertilized crops. The average rural household size was rounded up to 6 people (Census, 2001). The number of external helpers varies widely with location and seasons and, for this example, was assumed to be 1.

2.9.3 Results and discussion of the QMRA

Table 2.8 – The disease burden[z] at each stage of exposure
The decay rates at 20°C for the worst-case and best-case scenarios. Numbers in red indicate that the disease burdan was above the accepted limite of 10^{-3}

Stage	*C. jejuni*		*C. Parvum*		Rotavirus	
	Worse	Better	Worse	Better	Worse	Better
1	0.0240	2.79E-04	0.0113	2.30E-03	5.20E+82	0.0400
2	0.0101	1.40E-06	0.0056	1.30E-04	0.0200	0.0200
3	0.0604	8.41E-06	0.0338	7.79E-04	0.1200	0.1200
4	0.0604	8.41E-06	0.0338	7.79E-04	0.1200	0.1200
5	0.0806	1.12E-05	0.0450	2.10E-03	0.1600	0.1600
6	0.0017	1.12E-07	0.0331	2.10E-05	0.1518	0.0374

[z] The recommended acceptable disease burden was set to < 10^{-3} (Höglund et al., 2002)

Table 2.8 summarizes the disease burden at each stage of EcoSan at 20°C for the worst-case and best-case scenarios. For the worst-case scenario, the disease burdens for all indicators were above the set target during the six stages, except at Stage 2 for *C. parvum* which was at an acceptable disease burden. For the best-case scenario, the disease burden of *C. jejuni* and *C. parvum* was < 10^{-3} at each stage. The disease burden for the rotavirus was above target at each stage. For the decay rates at 4°C, the disease burden of the rotavirus increased in severity. As the data shows that the rotavirus is a risk, the results from 4°C were not displayed.

The disease burden will change depending on the local conditions (temperature, mortality rates, disease outbreaks, etc.) and it is impossible to have exact data for every variable. The study by Heinonen-Tanski, Sjöblom et al. (2007) concluded that human urine collected in tropical countries may not need to be stored the recommended minimum of one month because the atmospheric heat would be sufficient at reducing the survival of enteric microorganisms. This calculated QMRA demonstrates the significance of the risks

when using human urine as a fertilizer in countries with greater frequencies of diarrheal diseases.

Past studies, which detected no coliforms on crops grown close to the soil and fertilized with human urine, were conducted predominantly in developed countries. Growth trials with human urine in Finland had adequate sanitation quality for cucumber; on the cucumber there were no detectable coliforms (>10 microorganisms per fresh cucumber) such as somatic coliphage, enterococcus, and faecal coliforms (Heinonen-Tanski et al., 2007). Similar trials using human urine on tomato found no significant difference in the microbial and chemical compounds, such as antioxidants, when compared to tomato fertilized with mineral fertilizer (Pradhan et al., 2009).

The number of exposures a person experiences in a year would vary depending on the EcoSan system being used. The values calculated at each stage of the QMRA were for one-time exposure only. Based on this QMRA, it was clear that standards need to be put in place to limit handling time with human waste. At each stage simple personal protective equipment (PPE), such as a mask and gloves, should be used to limit exposure. Models using centralized systems with bigger reservoirs would decrease the number of times a person has to empty and transport the urine. A two-tank system would allow one tank to sit for more than one month, leaving more time for the decay of pathogens, while the other tank was in use. In high risk areas, the urine could be used for cereal crops to avoid the consumption of food riddled with surviving pathogens. Repeated and up-to-date QMRA data is a part of proper management and good communication between implementing organizations and communities involved. Specific guidelines for individual countries are recommended to address the local conditions and cultural practices, in terms of associated risk, farming practices, collection and application systems, and hygiene customs (Höglund, 2001).

2.10 Conclusion

By transforming human urine into a tradable good, nutrients can be better managed and can decrease the negative impacts on the environment. Globally, societies are not adequately managing the disposal of human excrement, while large amounts of money and resources are used to extract those same nutrients out of the air and ground for fertilizer. The cradle-to-cradle system of EcoSan has demonstrated that equivalent

biomass is produced compared with mineral fertilizer. Proper handling of human urine in areas with low rates of water-borne disease can lead to an extremely effective and safe fertilizer for vegetable crops. In developing countries, the risks of disease are higher, and greater care is required by the personnel handling human urine. Until further developments in processing, the urine can be used effectively for cereal crop production. Human urine has the potential to be equivalent to mineral fertilizers, but issues with the long-term impacts on the soil, particularly increased EC and decreased pH, need to be better understood.

2.11 References

ACHESON, D. & ALLOS, D. 2001. Campylobacter jejuni infections: update on emerging issues and trends. *Clinical Infectious Diseases,* 32**,** 1201-1206.

ALIBABA. 2012. *Country Search > Canada > urea* [Online]. Hong Kong: Alibaba Group. Available: http://www.alibaba.com/countrysearch/CA/urea.html [Accessed September 21, 2012].

ASANO, T., LEONG, L., RIGBY, M. & SAKAJI, R. 1992. Evaluation of the California wastewater reclamation criteria using enteric virus monitoring data. *Water science and technology,* 26**,** 1513-1524.

AYERS, R. S. & WESTCOT, D. W. 1994. *Water quality for agriculture: Salinity Problems* [Online]. Rome: Food and Agricultural Organization of the United Nations. Available: http://www.fao.org/docrep/003/t0234e/T0234E03.htm [Accessed February 13, 2013].

BAJJI, M., KINET, J.-M. & LUTTS, S. 1998. Salt stress effects on roots and leaves of Atriplex halimus L. and their corresponding callus cultures. *Plant Science,* 137**,** 131-142.

BB INNOVATION. 1991. *WC-Dubletten* [Online]. Föder Idéer Innovation & Co AB. Available: http://www.dubbletten.nu/wc-dubbletten-en.html [Accessed February 15, 2013].

BC MINISTRY OF AGRICULTURE. 1999. *On-site Testing of Growing Media and Irrigation Water* [Online]. Abbotsford: Ministry of Agriculture and Food of Bristish Columbia,. Available: http://www.agf.gov.bc.ca/ornamentals/floriculture/testing.pdf [Accessed November 22, 2012].

BOLAN, N., BRENNAN, R., BUDIANTA, D., NAIDU, R. & SUMMER, M. E. 2011. Bioavailability of micronutrients. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

CALVERT, P. 2004. *Ecological Solutions to Flush Toilet Failures,* Pickering, UK, EcoSolutions.

CALVERT, P. 2007. *Press* [Online]. Trivandrum, India: Eco-solutions. Available: http://www.eco-solutions.org/press.html [Accessed June 21, 2013].

CAMBERATO, J. J. 2011. Bioavailability of nitrogen. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

CAMBERATO, J. J. & PAN, W. L. 2011. Bioavailability of calicum, magnesium, sulphur, and silicon. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

CENSUS. 2001. *Data highlights* [Online]. Census of India. Available: http://censusindia.gov.in/Data_Products/Data_Highlights/Data_Highlights_link/data_highlights_hh1_2_3.pdf [Accessed April 8th 2012].

CLEEMPUT, O. V. & BOECKX, P. 2006. Nitrogen and Its Transformations. *Encyclopedia of Soil Science.* Taylor & Francis.

COPPER, R. & OLIVIERI, A. 1998. *Infectious disease concerns in wastewater reuse,* Lancaster, PA, Technomic Publishing Company, Inc.

DERM 2011. Salinity management handbook, 2nd edition. *In:* DEPARTMENT OF ENVIRONMENT AND RESOURCE MANAGEMENT (ed.) 2nd ed. Brisbane: State of Queensland.

FAO. 2011. *Current world fertilizer trends and outlook to 2015* [Online]. Rome: Food and Agricultural Organization of the United Nations. Available: ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf [Accessed September 20, 2012].

FEACHEM, R., BRADLEY, D., GARELICK, H. & MARA, D. 1983. *Sanitation and disease - health aspects of excreta and wastewater management,* Chichester, UK, John Wiley and Sons.

FENG, D., WUB, Z. & XU, S. 2008. Nitrification of human urine for its stabilization and nutrient recycling. *Bioresource Technology,* 99**,** 6299-6304.

GANROT, Z., DAVE, G. & NILSSON, E. 2006. Recovery of N and P from human urine by freezing, struvite precipitation and adsorption to zeolite and active carbon. *Bioresource Technology,* 98**,** 3112-3121.

GAVLAK, R., HORNECK, D., MILLER, R. O. & KOTUBY-AMACHER, J. 2003. Soil pH and Electric Conductivity *Soil and Water Analysis Manual, 2nd Edition.* Western States Laboratory Plant.

GOUGH, N. 1996. *Soil and plant tissue testing methods and interpretations of their results for British Columbia agricultural soils* [Online]. Ministry of Agriculture and Food of Bristish Columbia,. Available: http://www.agf.gov.bc.ca/resmgmt/publist/600Series/634200-3_Soil_and_Plant_Tissue_Testing_Methods.pdf [Accessed November 12, 2012].

GOVERNMENT OF INDIA 2011. Ecological Sanitation - Practitioner's Handbook. *In:* SANITATION, M. O. D. W. A. (ed.). New Delhi: Government of India,

UNICEF.

GUZHA, E., NHAPI, I. & ROCKSTROM, J. 2005. An assessment of the effect of human faeces and urine on maize production and water productivity. *Physics and Chemistry of the Earth,* 30**,** 840-845.

HAAS, C., ROSE, J. & GERBA, C. 1999. *Quantitative Microbial Risk Assessment,* New York, USA, John Wiley and Sons.

HAYNES, R. J. & WILLIAMS, P. H. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy,* 49**,** 119-199.

HEINONEN-TANSKI, H., SJÖBLOM, A., FABRITIUS, H. & KARINEN, P. 2007. Pure human urine is a good fertilizer for cucumbers. *Bioresource Technology,* 98**,** 214-217.

HELLSTROM, D., JOHANSSON, E. & GRENNBERG, K. 1999. Storage of human urine: acidification as a method to inhibit decomposition of urea. *Ecological Engineering,* 12**,** 253–269.

HINSINGER, P. 2006. Potassium. *Encyclopedia of Soil Science.* Taylor & Francis.

HOGG, T. J. & HENRY, J. L. 1984. Comparison of 1:1 and 1:2 suspensions and extracts with the saturation extract in estimating salinity in Saskatchewan soils. *Canadian Journal of Soil Sciences,* 64**,** 699-704.

HÖGLUND, C. 2001. *Evaluation of microbial health risks associated with the reuse of source-separated human urine.* Doctoral, Royal Institute of Technology.

HÖGLUND, C., STENSTROM, T. A. & ASHBOLT, N. 2002. Mibrobial risk assessment of source-separated urine used in agriculture. *Waste management & research,* 20**,** 150-161.

HOWARD, G., PEDLEY, S. & TIBATEMWA, S. 2006. Quantitative microbial risk assessment to estimate health risks attributable to water supply: can the technique be applied in developing countries with limited data? *Journal of water and health,* 04.

HUANG, J. & REDMAN, R. E. 1995. Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. *Canadian Journal of Plant Sciences,* 75**,** 815-819.

JACKSON, B. 2005. *A Review of EcoSan Experience in Eastern and Southern Africa* [Online]. Nairobi, Kenya: Water and Sanitation Programme (WSP), World Bank. Available: http://www.wsp.org/sites/wsp.org/files/publications/af_ecosan_esa.pdf [Accessed Feburary 18, 2013].

JONES, C. L., MANESS, N. O., STONE, M. L., SOLIE, J. B. & ZAVODNY, D. 2005. Variable rate nitrogen application on row crop spinach. *American Society for Agriculture and Engineering Annual International Meeting.* Tampa, Florida.

JÖNSSON, H., STENSTRÖM, T.-A., SVENSSON, J. & SUNDIN, A. 1997. Source separated urine-nutrient and heavy metal content, water saving and faecal contamination. *Water science and technology,* 35**,** 145-152.

JÖNSSON, H., STINTZING, A. R., VINNERAS, B. & SALOMON, E. 2004. Guidelines on the Use of Urine and Faeces in Crop Production. *EcoSanRes Publication Series.* Stockholm: Stockholm Environment Institute.

KING, F. H. 1911. *Farmers of Forty Centuries; Or, Permanent Agriculture in China, Korea, and Japan*, Project Gutenberg eBook.

KIRCHMANN, H. & PETTERSSON, S. 1995. Human urine - Chemical composition and fertilizer use efficiency. *Fertilizer Research,* 40**,** 149-154.

LASA, B., FRECHILLA, S., LAMSFUS, C. & APARICIO-TEJO, P. M. 2000. The sensitivity to ammonium nutrition is related to nitrogen accumulation. *Scientia Horticulturae,* 91**,** 143-152.

LIENERT, J., BÜRKI, T. & ESCHER, B. 2007. Reducing micropollutants with source control: substance flow analysis of 212 pharmaceuticals in faeces and urine. *Water science and technology,* 56**,** 87-96.

MARCOGLIESE, D., RASMUSSEN, J. & DEBRUYN, A. 2003. The role of sewage in a large river food web. *Canadian Journal of Fisheries and Aquatic Sciences,* 60**,** 1332-1344.

MARTIN, H. 2009. *Introduction to Organic Farming* [Online]. Guelf: OMAFRA. Available: http://www.omafra.gov.on.ca/english/crops/facts/09-077.htm [Accessed September 19 2012].

MCDONOUGH, W. & BRAUNGART, M. 2002. *Cradle to Cradle: Remaking the Way We Make Things,* New York, Macmillan.

MNKENI, P. N. S., KUTU, F. R., MUCHAONYERWA, P. & AUSTIN, L. M. 2008. Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in Eastern Cape, South Africa. *Waste management & research,* 26.

MUFWANZALA, N. & DIKINYA, O. 2010. Impact of Poultry Manure and its Associated Salinity on the Growth and Yield of Spinach (Spinacea oleracea) and Carrot (Daucus carota). *International journal of agriculture and biology,* 12**,** 489-494.

ORWIN, K. H., BERTRAM, J. E., CLOUGH, T. J., CONDRON, L. M., SHERLOCK, R. R., O'CALLAGHAN, M., RAY, J. & BAIRD, D. B. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. *Applied Soil Ecology,* 44**,** 89-100.

PARIDA, A. K. & DAS, A. B. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety,* 60**,** 324-349.

PHAC. 2010. *Salmonella Enterica SPP* [Online]. Ottawa: Public Health Agency of Canada. Available: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/salmonella-ent-eng.php [Accessed February 11, 2013].

POWER, J. F. & PRASAD, R. 1997. *Soil Fertility Management for Sustainable Agriculture,* Boca Raton, FL, CRC Press.

PRADHAN, S. K., HOLOPAINEN, J. K. & HEINONEN-TANSKI, H. 2009. Stored Human Urine Supplemented with Wood Ash as Fertilizer in Tomato (Solanum lycopersicum) Cultivation and Its Impacts on Fruit Yield and Quality. *Journal of Agricultural and Food Chemistry,* 57**,** 7612-7617.

PRADHAN, S. K., HOLOPAINEN, J. K., WEISELL, J. & HEINONEN-TANSKI, H. 2010. Human Urine and Wood Ash as Plant Nutrients for Red Beet (Beta vulgaris) Cultivation: Impacts on Yield Quality. *Journal of agriculture and food chemistry,* 58**,** 2034-2039.

PRADHAN, S. K., NERG, A.-M., SJÖBLOM, A., HOLOPAINEN, J. K. & HEINONEN-TANSKI, H. 2007. Use of Human Urine Fertilizer in Cultivation of cabbage (Brassica oleracea)—Impacts on Chemical, Microbial, and Flavor Quality. *Journal of agriculture and food chemistry,* 55**,** 8657-8663.

PROSSER, J. I. 2011. Nitrogen Transformations. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Sciences: Properties and Processes.* 2 ed. Boca Raton, FL: CRC Press.

PUTNAM, D. F. 1971. Composition and concentrative properties of human urine. *National Aeronautics and Space Administration Contractor Report.* Huntington Beach, California: McDonnell Douglas Astronautics Company - Western Division.

RHOADES, J. D., CHANDUVI, F. & LESCH, S. 1999. Soil salinity assessment: methods and interpretation of electrical conductivity measurements. *Irrigation and Drainage.* Rome: Food and Agriculture Organization of the United Nations.

RONTELTAP, M., MAURER, M. & GUJER, W. 2007. The behaviour of pharmaceuticals and heavy metals during struvite precipitation in urine. *Water Research,* 41**,** 1859-1868.

RYAN, J. & RASHID, A. 2006. Phosphorus. *Encyclopedia of Soil Science.* Taylor & Francis.

SCIENCE-DICTIONARY. 2008. *Lodging* [Online]. Science-dictionary. Available: http://agriculture.science-dictionary.org/Agricultural-Dictionary/Lodging [Accessed June 20, 2013].

SHANI, U. & DUDLEY, L. M. 2001. Field studies of crop response to water and salt stress. *Soil Sci. Soc. Am. J.,* 65**,** 1522-1528.

SHANNON, M. C. & GRIEVE, C. M. 1999. Tolerance of vegetable crops to salinity. *Scientia Horticulturae,* 78**,** 5-38.

SHANNON, M. C., GRIEVE, C. M., LESCH, S. M. & DRAPER, J. H. 2000. Analysis of Salt Tolerance in Nine Leafy Vegetables Irrigated with Saline Drainage Water. *Journal of the American Society for Horticultural Science,* 125**,** 658-664.

SHARPLEY, A. 2011. Phosphorus availability. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

SHIMING, L. 2002. The utilization of human excreta in Chinese agriculture and the challenge faced. South China Agricultural University, EcoSanRes.

SPARKS, D. L. 2011. Bioavailability of soil potassium. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

STAGNARI, F., BITETTO, V. D. & PISANTE, M. 2007. Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. *Scientia Horticulturae,* 114**,** 225-233.

TIDAKER, P., MATTSSON, B. & JONSSON, H. 2005. Environmental impact of wheat production using human urine and mineral fertilisers - a scenario study. *Journal of Cleaner Production,* 15**,** 52-62.

TILLEY, E., ATWATER, J. & MAVINIC, D. 2008. Effects of storage on phosphorus recovery from urine. *Environmental Technology,* 29**,** 807-816.
VINNERÅS, B. 2002. *Possibilites for sustainable nutrient recycling by faecal separation combined with urine diversion.* Doctoral Doctoral, Swedish University of Agricultural Sciences.
VINNERÅS, B. & JÖNSSON, H. 2002. Faecal separation for nutrient management—evaluation of different separation techniques. *Urban Water,* 4**,** 321–329.
VISWANATHAN, S. R., BHANDARI, B. S., KUMAR, P., SHARMA, V. P., SINGH, S. & SINGH, N. 2011. Agricultural Situation in India. *In:* DEPARTMENT OF AGRICULTURE AND CO-OPERATION (ed.). New Delhi: Director of Economics and Statistics.
WHIPKER, B. E. & CAVINS, T. J. 2000. *Electrical Conductivity (EC): Units and Conversions* [Online]. North Carolina State University. Available: http://www.ces.ncsu.edu/depts/hort/floriculture/Florex/EC%20Conversion.pdf [Accessed November 21, 2012].
WHO 2011. Evaluating household water treatment options: Health-based targets and microbiological performance specifications. Geneva, Switzerland: World Health Organization.
WHO 2012. Chapter 4. World pharmaceutical sales and consumption. *The World Medicines Situation.* World Health Organization.
WINBLAD, U., HEBERT, M., CALVERT, P. & MORGAN, P. 2004. *Ecological sanitation*, Stockholm environment institute.
WINKER, M., CLEMENS, J., REICH, M., GULYAS, H. & OTTERPOHL, R. 2010. Ryegrass uptake of carbamazepine and ibuprofen applied by urine fertilization. *Science of The Total Environment,* 408**,** 1902-1908.
WINKER, M., VINNERÅS, B., MUSKOLUS, A., ARNOLD, U. & CLEMENS, J. 2009. Fertiliser products from new sanitation systems: Their potential values and risks. *Bioresource Technology,* 100**,** 4090-4096.
ZVALO, D. V. & RESPONDEK, A. 2008. Spinach. *In:* AGRAPOINT (ed.) *Vegetable Crops Production Guide for Nova Scotia.* Atlantic Provinces Agricultural Services Coordinating Committee.

3 Sensitivity analysis of spinach and mustard seedlings to increasing application level of synthetic human urine and mineral fertilizer

3.1 Introduction

Several studies have confirmed that human urine successfully increases biomass production rates when used either as an independent or as a supplemental fertilizer (Chapter 2). However, there are only a few studies that have assessed the changes in the soil chemistry from the use of human urine as a fertilizer (Germer et al., 2011, Mnkeni et al., 2008). Human urine has a naturally high salt content, an average of 18.28 g L^{-1} (Putnam, 1971), which is high enough to cause severe damage to plants (Power et al., 1997). Mnkeni, Kutu et al. (2008) is the only published results found by this author, comparing the salinity from human urine with nitrogen fertilizer application levels (0-800 kg per ha) using beets (salt tolerant) and carrots (salt sensitive). The lack of published results highlights the need for baseline data on the changes in electrical conductivity (EC) and pH in soil fertilized with a range of concentrations of human urine. A data-set summarising the changes in soil chemistry, would be valuable information to understand how the human urine will impact soils and plant production rates from long-term use.

The objective of this experiment was to measure the changes in the EC and pH of the soil and assess the sensitivity of two plant species, spinach (*Spinacia oleracea* L.) and brown mustard (*Brassica juncea* L.), to a range of human urine concentrations. Four fertilizer treatments at eight levels of concentrations (15.0 to 683.4 kg nitrogen ha^{-1}) were tested. The treatments included: no fertilizer (control), human urine, mineral fertilizer, and a combination of human urine and additional phosphate and potassium fertilizers. The combination treatment was used to optimize the fertilizing ratio as human urine is higher in nitrogen than phosphate and potassium (Putnam, 1971), and additional sources of phosphate and potassium would be required in normal production to achieve maximum yield (Pradhan et al., 2009). The sensitivity of the plant was measured based on: emergence rate, survival rate, final biomass production, and changes in soil chemistry (EC and pH). The hypothesis was that as the fertilizer application rates increased, the EC would increase more in the human urine and combination fertilized treatments than in the mineral fertilizer treatments and would cause a decrease in biomass production rates.

Spinach and brown mustard seedlings were grown in a greenhouse for a 14-day period to test the null hypothesis. The emergence of the seedlings was recorded every three days. The survival rates were based on the number of emerged seedlings to live to the termination of the experiment. The biomass production (mass and length of the roots and shoots) of the seedlings, and the EC and pH were measured at the termination of the experiment.

3.2 Background

Salinity is a general term used to describe the concentration of dissolved inorganic solutes (chiefly Na^{+}, Mg^{++}, Ca^{++}, K^{+}, Cl^{-}, SO_4, HCO_3^{-}, NO_3^{-} and CO_3) in an aqueous solution (Rhoades et al., 1999). Human urine is saline because of the inorganic salts (average 14.2 g L^{-1}) and organic ammonium salts (average 4.1 g L^{-1}) excreted by the body (Putnam, 1971). Even though plants uptake nutrients in the form of soluble salts, increased salinity in the soils can result in a broad array of soil and plant disturbances (Shannon et al., 1999).

Initial evidence of salinity impacting a crop is impeded height and/or number of leaves (Mnkeni et al., 2008). Leaves will wilt even in moist soils and exhibit leaf burn (Mnkeni et al., 2008). Root growth is generally not as affected, but in acute conditions plants can die (Mnkeni et al., 2008). The three underlying causes of these disturbances are: decrease in osmotic potential (Bajji et al., 1998, Mufwanzala et al., 2010, Orwin et al., 2010), salt toxicity (Mnkeni et al., 2008, Shani et al., 2001) and decrease in bioavailability of nutrients by competing with other similarly charged ions (Bajji et al., 1998, Mufwanzala et al., 2010). The impact of saline environments on plants varies with species, the plant physiological stage, and the composition and concentration of salts (Huang et al., 1995, Shannon et al., 1999).

The tolerance of a plant is dependent on the biomass production rate at a given EC (Figure 3.1). For this thesis, the salt tolerance of a plant species was described by three parameters: threshold, slope and EC_{50}. The threshold refers to an EC point beyond which there is potential for reduction in yield due to stress (Power et al., 1997). The slope refers to the yield reduction in percentage per unit increase of the EC above the threshold (Power et al., 1997). The EC_{50} refers to the salinity level at which point the yield would produce 50% of the non-saline crop yield (Steppuhn et al., 2005). The EC values

measured by the saturated soil paste extract method were denoted as EC_{SE} (Gavlak et al., 2003, Shannon et al., 1999).

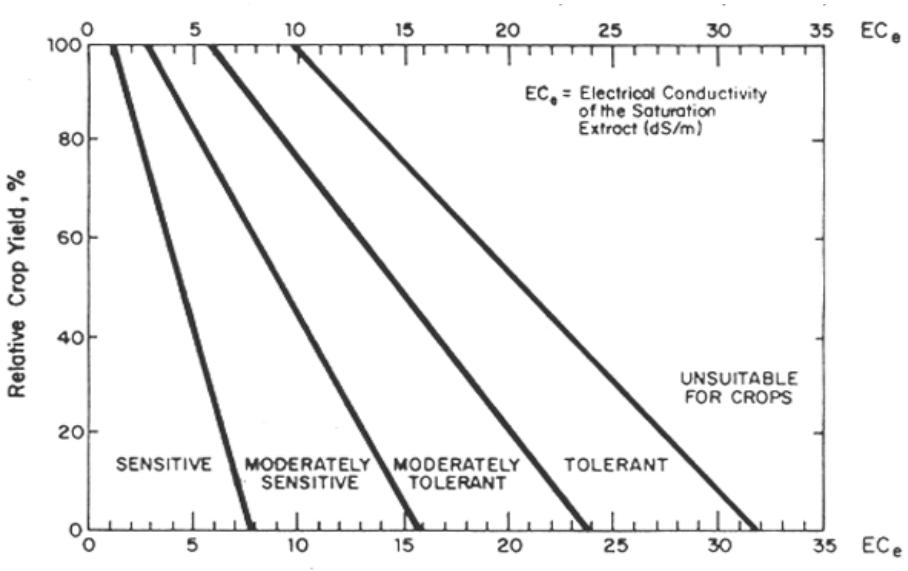

Figure 3.1 – Plant salt tolerance
Classifications for salt tolerance (EC_e) ratings of agricultural crop (Ayers et al., 1994). Note: EC_e in this figure is equivalent to EC_{SE} in the rest of the thesis.

The threshold, slope and EC_{50} values were used in the discussion as a general comparison to assess the overall sensitivity of the plants. Spinach and mustard have been observed to be moderately sensitive to saline conditions (Figure 3.1) (Shannon et al., 1999). The specific values for the salt threshold, slope and EC_{50} for spinach were 2.0 dS m^{-1} (Shannon et al., 1999, Steppuhn et al., 2005), 7.6% (Shannon et al., 1999), and 8.2 dS m^{-1} (Steppuhn et al., 2005), respectively. The salt tolerance of brown mustard varieties can vary from salt sensitive to salt tolerant (Figure 3.1) (Ashraf, et al., 1994), and specific values for the threshold, slope and EC_{50} were not found. Still, brown mustard was used in this study as it is an important oil seed crop (Jain et al., 1993), and for the assessment of the mustard sensitivity an alternative crop within the same family was selected based on the following information. Shannon et al. (1999) reported that canola or rapeseed (*Brassica napus* L.) was salt tolerant; while cabbage (*Brassica oleracea capitata*) and brown mustard were moderately salt sensitive. As cabbage had similar tolerance to brown mustard in the Shannon et al. (1999) study, salt tolerance values of cabbage were used for assessing the sensitivity in the discussion. The salt threshold, slope and EC_{50} for cabbage were 1.8 dS m^{-1}, 9.7% and 7.0 dS m^{-1}, respectively (Power et al., 1997).

Fertilizers typically are not composed of a single element but are a composition of different elements and include salts. Parts of these fertilizers can accumulate and alter the chemistry of the soil with repeated use (Whipker et al., 2000), increasing the EC of the soil beyond optimal levels. As human urine has a high average EC of 19.5 dS m^{-1}, understanding how the chemistry of the soil is altered by a fertilizer is required to maintain an optimal EC and pH, maximizing plant biomass production.

3.3 Methodology

Artificial soil was mixed and was based on a fine sandy loam recipe (initial EC_{SE} = 0.3 dS m^{-1}) provided by Environment Canada's Biological Test Method (Environment Canada, 2005). The soil mixture, by mass, consisted of: 70% grade 70 sand (half coarse/half fine); 20% kaolin clay (particles > 40 μm); and 10% sphagnum peat moss (Environment Canada, 2005). Calcium carbonate ($CaCO_3$) was added, at the rate of 10.0 g kg^{-1} of sphagnum peat to increase the $pH_{1:2}$ to 6.5 (tested after three days). The $pH_{1:2}$ was measured by adding one part soil to two parts distilled water, stirred for 30 s and left to settled for 1 hr (Rhoades et al., 1999, Shannon et al., 1999). A pH of 6.5 was ideal for both spinach and brown mustard (Zvalo et al., 2007, Zvalo et al., 2008). The soil was moistened with water and wrapped in a black plastic bag for a three-day equilibrium period. The artificial soil was placed in four-inch pots and the surface was smoothed but not compressed. The surface of each pot was spiked with 3.0 mg of vermi-compost to establish natural microbial soil activity.

The mass of fertilizer applied to each four-inch pot was based on the following soil characteristics. The mass of soil per pot was approximately 0.34 kg; the volume of a pot was 350 ml; and the bulk density of the artificial soil was approximately 0.98 g cm^{-3} (Environment Canada, 2005). Recommended field fertilizer applications were based on a soil depth of 0.15 m and a bulk density of 1.49 t m^{-3} (CRRAQ, 2010), resulting in 2.24 Gg of soil fertilized per ha. The mass of potted soil (0.34 kg) was divided by the mass of soil fertilized per ha (2.24 Gg) to calculate the mass of fertilizer to be added to each pot of soil.

The synthetic human urine was produced based on a recipe by Chutipongtanate and Thongboonkerd (2010) named ''AU-Siriraj" and contained 6.00 g N, 0.125 g P, 1.18 g K

and 2.164 g Na L^{-1} of liquid solution. A beaker with 1 L of distilled water was placed on a magnetic stir table and chemicals listed in Table 3.1 (purchased from Fisher Scientific, Hampton, NH) were added in no specific order.

Table 3.1 – Synthetic human urine
The recipe ‘‘AU-Siriraj” was used (Chutipongtanate et al., 2010).

Compound	Mass (g L^{-1})
CH_4N_2O	12.14
$C_5H_4N_4O_3$	0.17
$C_4H_9N_3O_2$	0.45
$Na_3C_6H_5O_7$ *$2H_2O$	1.49
NaCl	3.17
KCl	2.25
NH_4Cl	0.81
$CaCl_2$ *$2H_2O$	0.44
$MgSO_4$ *$7H_2O$	0.50
$NaHCO_3$	0.17
$NaC2O_4$	0.02
Na_2SO_4	1.29
NaH_2PO_4 *H_2O	0.50
Na_2HPO_4	0.06

The mineral fertilizer treatment was a premixed 5-20-5 liquid plant fertilizer (Liquid Growth, BIO TLC, Burlington, Ontario, Canada). For the combination treatment, human urine was supplemented with 0-64-0 and 0-0-62, both in pellet form which were provided by the Research Greenhouse at McGill University. The pellets were mixed into the surface of the soil (5 cm). The liquid fertilizers were applied by pipette after the pellet fertilizers, followed by 50 ml of distilled water. The four treatments – synthetic human urine, mineral fertilizer, combination (synthetic human urine and mineral fertilizer) and the control – were applied at eight levels of nitrogen (Table 3.2), with three replicates. In Quebec, the recommended nitrogen application rate for spinach was 120.0 kg of nitrogen per ha (CRRAQ, 2010) and the range of nitrogen tested was from 15.0 to 607.5 kg ha^{-1} (50% intervals above and below the recommended 120.0 kg of nitrogen ha^{-1}). The recommended nitrogen application rate for mustard in Quebec was 135.0 kg ha^{-1} (CRRAQ, 2010) and the range of 16.9 to 683.4 kg N ha^{-1} was tested (50% intervals above and below the recommended 135.0 kg N ha^{-1}).

Table 3.2 – Fertilizer application rates for spinach and brown mustard for all treatments.
The synthetic human urine was the ''AU-Siriraj" recipe in Chutipongtanate and Thongboonkerd (2010). The mineral fertilizer as a liquid mixture, 5-20-5. And the combination treatment was the synthetic human urine with the addition of 0-46-0 and 0-0-62 in pellet form.

		Synthetic human urine (kg ha^{-1})			**Mineral fertilizer** (kg ha^{-1})			**Combination** (kg ha^{-1})		
	Application levels	N	P	K	N	P	K	N	P	K
Spinach	1	15.0	0.3	2.9	15.0	26.2	12.5	15.0	10.1	22.0
	2	30.0	0.6	5.8	30.0	52.4	24.9	30.0	20.1	44.0
	3	60.0	1.2	11.7	60.0	104.7	49.8	60.0	40.2	88.0
	4[z]	120.0	2.5	23.3	120.0	209.5	99.6	120.0	80.5	175.9
	5	180.0	3.7	35.0	180.0	314.2	149.4	180.0	120.7	263.9
	6	270.0	5.6	52.5	270.0	471.3	224.1	270.0	181.0	395.8
	7	405.0	8.3	78.7	405.0	707.0	336.2	405.0	271.5	593.7
	8	607.5	12.5	118.1	607.5	1060.5	504.3	607.5	407.3	890.6
Brown mustard	1	16.9	0.3	3.3	16.9	67.5	0.4	16.9	12.7	20.6
	2	33.8	0.7	6.6	33.8	135.0	0.8	33.8	25.5	41.2
	3	67.5	1.4	13.1	67.5	270.0	1.6	67.5	51.0	82.4
	4[y]	135.0	2.8	26.2	135.0	540.0	3.2	135.0	102.0	164.7
	5	202.5	4.2	39.4	202.5	810.0	4.8	202.5	152.9	247.1
	6	303.8	6.3	59.0	303.8	1215.0	7.2	303.8	229.4	370.6
	7	455.6	9.4	88.5	455.6	1822.5	10.8	455.6	344.1	555.9
	8	683.4	14.1	132.8	683.4	2733.8	16.1	683.4	516.2	833.8
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[z] Recommended fertilizer application level for spinach is 120.0 kg N ha^{-1}, 82.9 kg P ha^{-1} and 199.2 kg K ha^{-1} (CRRAQ, 2010)

[y] Recommended fertilizer application level for brassica family is 135.0 kg N ha^{-1}, 104.7 kg P ha^{-1} and 190.9 kg K ha^{-1} (CRRAQ, 2010)

Spinach seeds were sourced from Veseys (Spinach, cv. Tyee, 2010; York, PE). The brown mustard seeds were sourced from Export Packers, (Canadian N1 Brown Mustard seeds, 2007; Brampton, ON). The seeds were washed separately in distilled water with 0.2% bleach, rinsed three times, and soaked in distilled water for seven hours in darkness at room temperature (Environment Canada, 2005). The soaked seeds were placed on moist paper towel in container, with a loose lid for air circulation, and kept in darkness for 36 hours (Environment Canada, 2005). Seeds were deemed germinated when the radical had emerged by 2.0 mm (Huang et al., 1995). After 36 hours, germinated seeds were transplanted into pots at a depth of 1.5 cm (Environment Canada, 2005).

The pots were placed on benches in nine rows by 16 columns in the glass greenhouse (latitude 45.5^{o}N, Ste-Anne-de-Bellevue, QC). The environment was maintained at 22.2 +/- 3^{o}C during the day and 20.0 +/- 3^{o}C during the night (Boese et al., 1990, Zvalo et al., 2007, Zvalo et al., 2008), with an average relative humidity of 58%. High pressure sodium (HPS) lights were operated for 16 hours a day. Seedlings were watered every second day, or as needed, with distilled water, by spraying the surface of the soil until 0.5 cm of water was temporarily visible (<1 hr), excess water pooling in the bottom of the collection tray. The emergence rate was recorded every three days, and the survival rate was recorded on day 7 and day 14.

On day 14, the experiment was terminated and the roots were separated from the soil by hand with the assistance of a spray water bottle. The shoots were separated from the roots at the transition zone between the hypocotyl and the root, using a scalpel and placed on a moistened paper towel (Environment Canada, 2005). The lengths of the longest roots and longest shoots were recorded. The fresh and dry masses were recorded for the roots and the shoots. The fresh plants were oven-dried at 65^{o}C for $\geq$ 48 hr until constant mass (Ahmadil et al., 2010). The soils were air dried for 48 hours (Brady et al., 2002, OMAFRA, 2011).

The EC (Probe 314, CDM 83 Conductivity meter, Radiometer, Copenhagen, DK) and pH (Accumet probe, cat # 13-620-221, AR10 pH Meter, Fisher Scientific, Hampton, NH) instruments were calibrated with standard solutions of known potentials and measured the soil samples in a 1:2 dilution (soil to distilled water) (Hogg and Henry 1984; Rhoades, Chanduvi et al. 1999; Gavlak, Horneck et al. 2003) . The EC results were converted to

values correlating to the saturated extraction method by Equation 2.1 based on fine soil texture (Hogg et al., 1984). Measure EC values that were less than 0.19 dS m^{-1} were below the range for Equation 2.1 and the rough estimate of multiplying the values by two was used to convert the values to correlate with the saturation extraction values(Gough, 1996). Converted EC values were denoted as EC_{SE}. The measured pH values were converted to the saturation extraction values by Equation 2.2 and denoted as pH_{SE} (Gavlak et al., 2003).

The converted EC_{SE} values, salinity threshold and the slope for the given plant species (Chapter 2) were used to calculate the potential yield lost due to the increased EC levels in the soil by the following formula:

$$\boldsymbol{Potential\ yield\ lost = (EC_{SE} - Threshold) * \frac{slope}{100}} \qquad \textbf{Equation 3.1}$$

The experiment was evaluated as a complete randomized design (CRD), though the pots were not in random order; further explained in Section 3.6. Data were analysed by the standard analysis of variance (ANOVA) using SAS 9.2 software (SAS Institute Inc., Cary, NC). The power of the test was computed by calculating the standard deviation from the coefficient of variance and the mean (SAS 9.2, 2009); a power ≥ 0.8 was accepted. The effects of the treatments were computed by pairwise comparisons with the Duncan multiple range tests at $\alpha = 0.05$. The correlation between measured components was computed using the CORR procedure in SAS. A significant correlation was described by type: positive/negative; and by strength: weak (<0.3), moderate (0.3-0.7) and strong (>0.7).

3.4 Spinach – results & discussion

3.4.1 Spinach results

The power of the experiment for each component was ≥ 0.80 except for the survival rate (power of 0.590) and the root mass (power of 0.613) (Table 3.3).

Table 3.3 – Power of the experiment for the measured spinach components (N = 75)
The power of the test was computed by calculating the standard deviation from the coefficient of variance and the mean (SAS 9.2, 2009); a power ≥ 0.8 was accepted.

Component	Standard Deviation	Power
Emergence rate	0.365	0.925
Survival rate	0.183	0.590
Root (mass)	5.032	0.613
Root (length)	4.728	0.881
Shoot (mass)	7.572	0.867
Shoot (length)	1.661	0.951
EC	564.3	>0.999
pH	0.141	>0.999

The spinach emergence rates were not significantly different. The lowest overall emergence rate was with the mineral fertilizer treatment at 58%; the highest was the combination treatment at 92% (Figure 3.2). Fertilizer application Levels 3 (60.0 kg N ha^{-1}) and 8 (607.5 kg N ha^{-1}) had the lowest emergence rate at 56%; the highest was Level 7 (405.0 kg N ha^{-1}) at 100%. The control had 100% emergence.

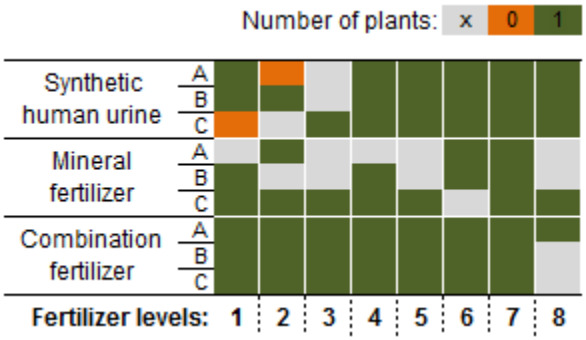

Figure 3.2 – The emergence and survival rate of the spinach seedlings
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control (not shown). Each treatment had three replicates (A, B, C) at each fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5). The seedlings that did not emerge are shaded grey (x). The emerged seedlings that did not survive to the termination of the 14-day experiment are shaded orange (0). Those which survived to the termination are shaded green (1). The control had 100% emergence and survival rate.

The spinach survival rates were not significant. Of the emerged spinach seedlings, 97% survived (Figure 3.2). The control had 100% survival rate. The results show that there

was phytotoxicity in the spinach seedlings. Leaves of the spinach seedlings for the following treatments had curled by day six of the experiment: all three replicates for Levels 7 and 8 of synthetic human urine treatment; one replicate for Levels 6 of combination treatment.

Of the measured spinach biomass components, root length, root mass and shoot mass were not significant. The shoot length was significant ($P = 0.021$). The combination treatment, Level 6 (270.0 kg N ha^{-1}), had the longest shoot at 15.1 cm and was the only treatment significantly different to the control at 9.4 cm (Table 3.4). The longest shoot length for both synthetic human urine and combination treatments was at Level 6, and the longest length for mineral fertilizer was at Level 3. The shortest shoot was from the combination treatment Level 8 at 6.0 cm, which was not significantly different to the control and the mineral fertilizer treatment but was significantly shorter to the synthetic human urine treatment. The synthetic human urine treatment was not significantly different to the control nor the mineral fertilizer treatment at any fertilizer application level. The mineral fertilizer treatment shoot lengths were consistently the shortest in the experiment but not significant (Figure 3.3).

Table 3.4 – Summary of dry biomass components for spinach

The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5) with the standard deviation. The mineral fertilizer treatment Level 6 root mass was zero because the roots were below the minimum threshold for the balance (< 1 mg). Mean separation by Duncan's multiple range test at $P \leq 0.05$. The shoot length had significant model and the different letter indicate statistical significant difference within the fertilizer application level.

	Fertilizer application level							
Root (mass, mg)	1	2	3	4	5	6	7	8
Control	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1	10.3 +/- 8.1
Synthetic human urine	3.0 +/- 1.4	12.0 +/- 0.0	14.0 +/- 0.0	2.3 +/- 4.0	8.3 +/- 12.7	5.3 +/- 0.6	5.0 +/- 5.3	2.3 +/- 2.3
Mineral fertilizer	6.5 +/- 2.1	2.5 +/- 0.7	4.0 +/- 0.0	3.5 +/- 3.0	1.0 +/- 0.0	0.0 +/- 0.0	3.3 +/- 4.9	2.0 +/- 0.0
Combination	7.7 +/- 2.9	6.7 +/- 5.5	3.3 +/- 4.2	1.0 +/- 1.0	10.7 +/- 4.9	8.0 +/- 4.6	7.0 +/- 2.0	1.0 +/- 0.0
Root (length, cm)								
Control	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1	9.2 +/- 4.1
Synthetic human urine	6.6 +/- 1.4	15.3 +/- 0.0	22.4 +/- 0.0	6.4 +/- 4.3	8.0 +/- 2.5	5.5 +/- 1.5	5.2 +/- 1.1	3.5 +/- 1.1
Mineral fertilizer	12.4 +/- 1.1	10.2 +/- 4.1	5.3 +/- 0.0	8.1 +/- 2.8	7.9 +/- 0.0	2.4 +/- 0.5	3.4 +/- 1.8	5.7 +/- 0.0
Combination	12.2 +/- 5.5	17.4 +/- 13.6	9.1 +/- 9.1	5.5 +/- 0.3	8.6 +/- 1.9	7.8 +/- 2.2	6.8 +/- 1.0	3.5 +/- 0.0
Shoot (mass, mg)								
Control	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5	20.3 +/- 11.5
Synthetic human urine	21.5 +/- 0.7	33.0 +/- 0.0	27.0 +/- 0.0	20.3 +/- 5.7	24.7 +/- 13.4	30.0 +/- 6.6	21.7 +/- 4.6	18.3 +/- 3.1
Mineral fertilizer	29.0 +/- 8.5	17.5 +/- 2.1	37.0 +/- 0.0	8.0 +/- 0.0	26.0 +/- 0.0	21.0 +/- 2.8	23.3 +/- 4.0	16.0 +/- 0.0
Combination	22.3 +/- 3.2	33.3 +/- 7.4	24.7 +/- 10.3	19.7 +/- 3.8	33.3 +/- 13.6	27.3 +/- 6.0	18.7 +/- 8.1	6.0 +/- 0.0
Shoot (length, cm)								
Control	9.4 +/- 1.2	9.4 +/- 1.2	9.4 +/- 1.2	9.4 +/- 1.2	9.4 +/- 1.2	**9.4** [b] +/- 1.2	9.4 +/- 1.2	**9.4** [ab] +/- 1.2
Synthetic human urine	10.0 +/- 1.1	12.2 +/- 0.0	10.4 +/- 0.0	10.2 +/- 0.2	11.8 +/- 2.2	**12.3** [ab] +/- 1.3	11.4 +/- 0.6	**10.4** [a] +/- 1.0
Mineral fertilizer	11.1 +/- 0.3	10.3 +/- 0.8	11.2 +/- 0.0	7.8 +/- 0.3	10.7 +/- 0.0	**10.5** [b] +/- 0.1	9.8 +/- 0.1	**9.7** [ab] +/- 0.0
Combination	11.1 +/- 1.0	12.3 +/- 1.4	11.2 +/- 0.9	10.9 +/- 1.3	12.9 +/- 1.5	**15.1** [a] +/- 5.2	11.3 +/- 0.7	**6.0** [b] +/- 0.0

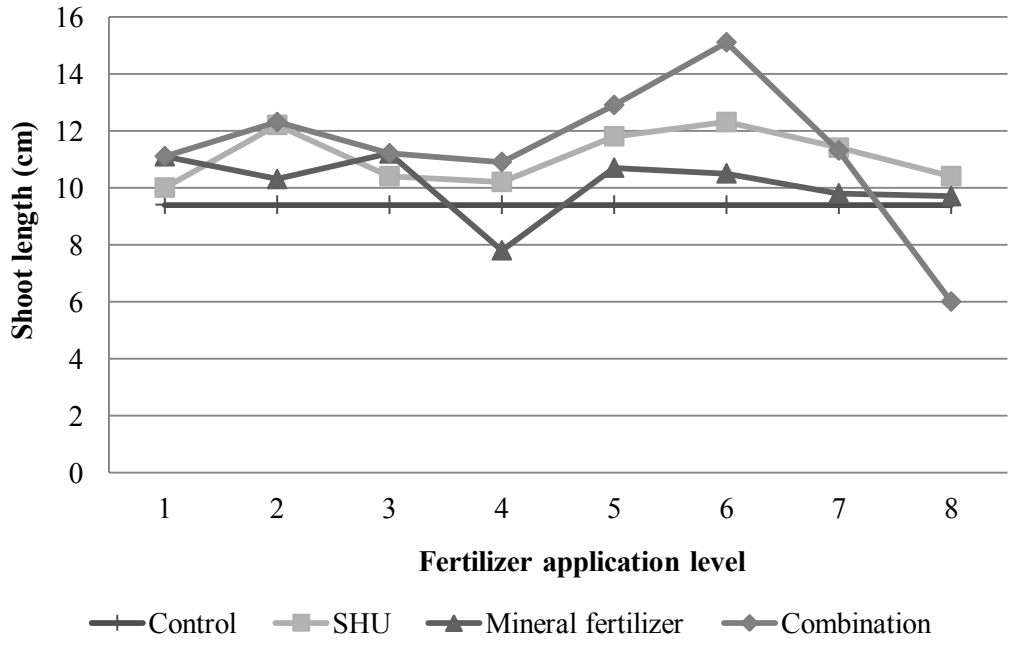

Figure 3.3 – Mean shoot length of spinach under four fertilizer treatments
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5).

The shoot lengths differ significantly at two fertilizer application levels. At Level 6, the combination treatment shoot was significantly longer to the control and the mineral fertilizer treatment. At Level 8, the combination treatment was significantly shorter than all of the synthetic human urine fertilizer treatments. The EC_{SE} model of the spinach soil samples was significant ($P \leq 0.0001$) (Table 3.5). The EC_{SE} readings of synthetic human urine ($R^2 = 0.899$) and combination ($R^2 = 0.919$) treatments had an increasing trend as the fertilizer application levels increased (Figure 3.4). The combination treatment, Level 8, had the highest EC_{SE} reading at 10.9 dS m^{-1}. The mineral fertilizer treatment, Level 6, had the lowest EC_{SE} at 0.7 dS m^{-1}. The EC_{SE} readings of the synthetic human urine and combination treatments were significantly higher compared to the control at 1.1 dS m^{-1} and above the threshold, 2 dS m^{-1} (Steppuhn et al., 2005) for fertilizer treatment application Levels 5-8. At each fertilizer application level, the synthetic human urine and combination treatments EC_{SE} levels were not significantly different to each other. At Levels 6-8, the mineral fertilizer treatments were significantly lower compared to the synthetic human urine and combination treatments.

Table 3.5 – Summary of soil components, EC_{SE} and pH_{SE}, for spinach

The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5) with the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$. The different letter indicates a statistical significant difference within the fertilizer application level.

	Fertilizer application level															
EC_{SE} (dS m^{-1})	1		2		3		4		5		6		7		8	
Control	1.06	+/- 0.87	1.06	+/- 0.87	1.06	+/- 0.87	1.06	+/- 0.87	**1.06^{b}**	+/- 0.87	**1.06^{b}**	+/- 0.87	**1.06^{b}**	+/- 0.87	**1.06^{b}**	+/- 0.87
Synthetic human urine	1.34	+/- 0.22	1.55	+/- 0.28	3.43	+/- 0.43	4.13	+/- 0.78	**6.86^{a}**	+/- 1.58	**6.38^{a}**	+/- 0.97	**6.07^{a}**	+/- 0.75	**8.24^{a}**	+/- 1.18
Mineral fertilizer	3.35	+/- 0.85	3.44	+/- 1.32	3.10	+/- 0.71	3.95	+/- 0.42	**3.91^{ab}**	+/- 1.59	**0.74^{b}**	+/- 0.26	**1.53^{b}**	+/- 0.39	**1.82^{b}**	+/- 0.39
Combination	1.19	+/- 0.90	2.01	+/- 1.28	1.97	+/- 1.25	3.14	+/- 0.57	**5.25^{a}**	+/- 1.75	**7.53^{a}**	+/- 3.21	**7.18^{a}**	+/- 2.45	**10.92^{a}**	+/- 6.39
pH_{SE}																
Control	6.13	+/- 0.29	6.13	+/- 0.29	6.13	+/- 0.29	6.13	+/- 0.29	6.13	+/- 0.29	**6.13^{b}**	+/- 0.29	**6.13^{b}**	+/- 0.29	**6.13^{b}**	+/- 0.29
Synthetic human urine	6.22	+/- 0.15	6.21	+/- 0.12	6.06	+/- 0.04	6.06	+/- 0.09	6.01	+/- 0.19	**6.14^{b}**	+/- 0.13	**6.24^{b}**	+/- 0.04	**6.28^{b}**	+/- 0.09
Mineral fertilizer	5.97	+/- 0.04	6.10	+/- 0.13	6.13	+/- 0.04	6.16	+/- 0.04	6.19	+/- 0.18	**6.60^{a}**	+/- 0.08	**6.64^{a}**	+/- 0.04	**6.71^{a}**	+/- 0.03
Combination	6.16	+/- 0.03	6.16	+/- 0.22	6.18	+/- 0.11	6.24	+/- 0.08	6.04	+/- 0.09	**6.06^{b}**	+/- 0.19	**6.17^{b}**	+/- 0.18	**6.22^{b}**	+/- 0.30

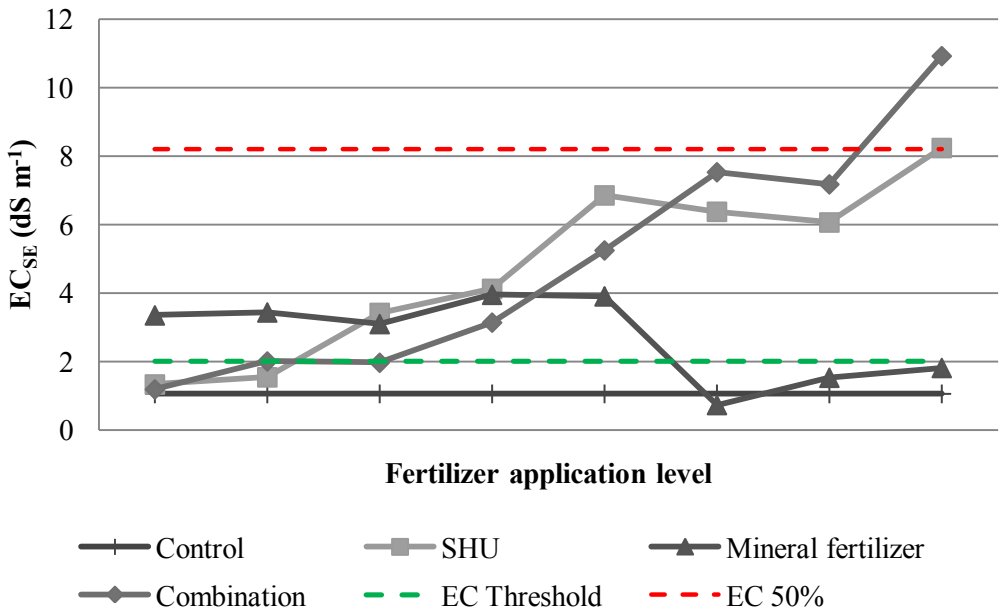

Figure 3.4 – The EC_{SE} of the spinach soil samples
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5). The green line, at 2 dS m^{-1}, is the EC_{SE} threshold and the red line, at 8.2 dS m^{-1}, is the EC_{50} (Steppuhn et al., 2005).

The EC_{SE} measurements of Figure 3.4 were used with the salinity threshold (2.0 dS m^{-1}) and the slope (7.6 %) for spinach to calculate the theoretical potential yield loss due to the increase in salinity (Steppuhn et al., 2005). In Figure 3.5, the negative bars were the potential yield loss (%) compared to the control.

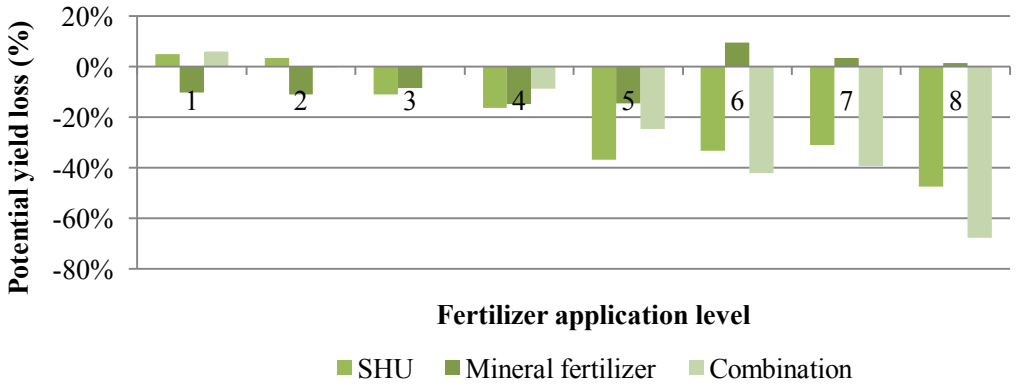

Figure 3.5 – Theoretical potential spinach yield loss (%) due to increased EC_{SE}
Each of the three fertilizer treatments (synthetic human urine, mineral fertilizer and combination) are shown at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5). All negative bars indicate that the EC_{SE} was above the maximum allowable threshold before harm to plant yield (2.0 dS m^{-1}) (Steppuhn et al., 2005).

The pH_{SE} model significant ($P \leq 0.0001$) (Table 3.5). Overall, the soil pH_{SE} had a narrow range (lowest was from the mineral fertilizer treatment, Level 1, at 5.97 and the highest was from the mineral fertilizer treatment, Level 8, at 6.71). The soil pH_{SE} from the synthetic human urine and combination treatments did not change significantly as the fertilizer application levels increased; at no point were the treatments significantly different from each other (Figure 3.6). From Level 6 through 8, the pH_{SE} of the mineral fertilizer treatment was significantly higher compared to the synthetic human urine, the combination and the control.

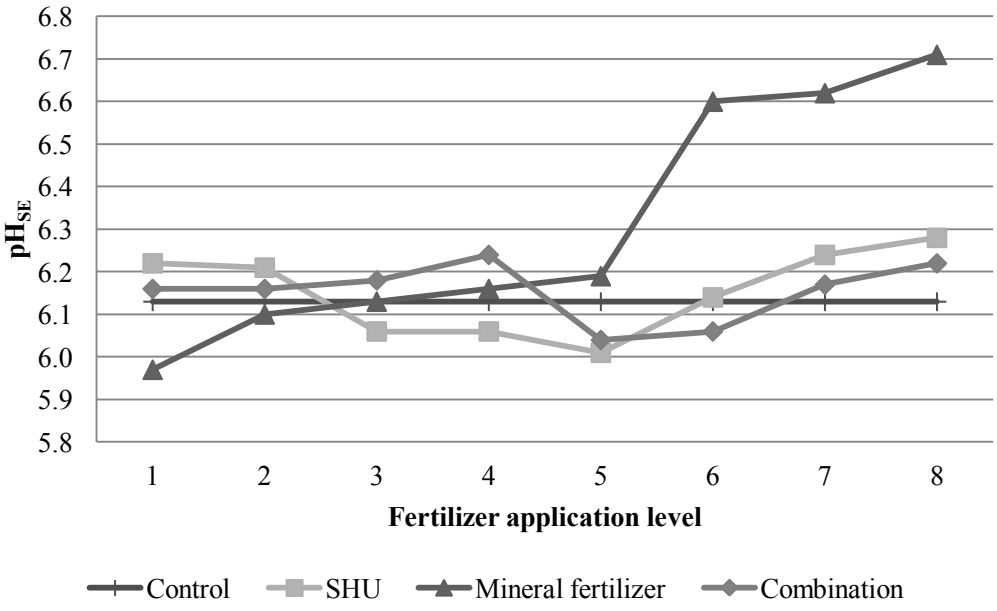

Figure 3.6 – The pH_{SE} of the spinach soil samples
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application levels (kg N ha^{-1}): 1 (15.0), 2 (30.0), 3 (60.0), 4 (120.0), 5 (180.0), 6 (270.0), 7 (405.0), and 8 (607.5).

3.4.2 Spinach discussion

Spinach was not sensitive to the different fertilizer treatments (control, synthetic human urine, mineral fertilizer and combination treatment) at increased application rates. The first part of the hypothesis, that as the EC would increase more in the human urine and combination fertilized treatments than in the mineral fertilizer treatments, was correct; the second part of the hypothesis, that the higher EC levels would cause a decrease in biomass production rates, was not correct as there was no significant decrease in biomass production. At Level 6, the combination treatment had shoots significantly longer compared to the control with an EC three folds above the threslhold. The EC had a moderate positive correlation with the shoot lengths. The correlation was observed with

the spinach seedlings fertilized with the combination treatment: the avearge shoot lengths increased as the EC increased from low to moderate, until the EC_{SE} reached >7.0 dS m^{-1}, at which point the shoot length decreased significantly. Even with the high EC_{SE} values, there was no decrease in the survival rate and only two treatments (mineral fertilizer, Level 4, and combination treatments, Level 8) experienced a non-statistically significant a shoot length decrease compared to the control, at 17% and 36%, respectively. The potential yield losses, calculated based on the measured EC of the soils were not observed. During the 14-day trial, it was clear that spinach was able to withstand significantly higher EC soil levels than were commonly reported.

Spinach has been reported to increase in yield with an increase of EC_{SE} from low to moderate (Nieman, 1962, Shannon et al., 1999), but the values from the current experiment were well above moderate salinity levels. This increase in yield for spinach can be explained by the formulation of the salt present in human urine. Spinach has been found to be less sensitive to NaCl than other single salt formations and showed no significant growth reduction with EC_{SE} up to 8.0 dS m^{-1} (Nieman, 1962). Similarly for this experiment, reduction in growth started at 7.5 dS m^{-1}.

The power of the emergence rate test for spinach was satisfactory (> 0.80) and the ANOVA model was not significant. The spinach seedlings treated by mineral fertilizer had the lowest emergence rates, the lowest EC_{SE}, and the highest pH_{SE} over all fertilizer application levels, but the emergence rate had no significant correlation to the EC_{SE} or to the pH_{SE}. Of the emerged spinach seedlings, only two seedlings did not survive (one from synthetic human urine Level 1 and Level 2). The power of the survival rate test was not satisfactory (< 0.80) and the ANOVA model was not significant, leading to no correlation between EC_{SE} and pH_{SE}. The pH remained predominately in the lower end of the ideal range for spinach (6.0-6.8 (Mufwanzala et al., 2010, Zvalo et al., 2008)) and applied nutrients would have remained available for plant up-take (Power et al., 1997). The lack of significance of the measured spinach components indicated that fertilizer application rates were not large enough to cause a reduction in the emergence and survival rates (Section 3.6).

The soil of the synthetic human urine and combination treatments followed patterns similar to one another in regards to EC_{SE} and pH_{SE}. The similarities suggested that the

synthetic human urine was the driving factor of the two parameters. The similarities demonstrated that the differences between the potassium application rates of the four fertilizer treatments had no significant impact on the EC_{SE} at fertilizer application Level 8, synthetic human urine received 118 kg K ha^{-1} and the combination treatment received 7.5 times more at 890.6 kg K ha^{-1}, yet the pH_{SE} and EC_{SE} were not significantly different. The pH_{SE} remained in the ideal spinach range of 6.0-6.8 for all fertilizer treatments, leading to the assumption that the negative moderate correlation with the shoot length was not directly dependent. The shoot lengths from the synthetic human urine were not significantly different for all eight levels, indicating the range of fertilizer application levels had little effect on initial biomass production of spinach.

The mineral fertilizer treatments received more P than the combination treatments, but there was no significant difference between biomass production. At mineral fertilizer application Level 4 (209.5 kg P ha^{-1}) the spinach seedlings received more than double the recommended concentration of P (82.9 kg P ha^{-1}), while the combination treatment was at the recommended level (80.5 kg P ha^{-1}). This change in P would not have affected the seedlings negatively as P remains predominantly in its immobile form or is adsorbed to clay particles (Power et al., 1997). The P may have remained immobile in the mineral fertilizer, whereas the P in the combination soils had more Na to bind with, transforming into the inorganic form, which is more readily available for plant uptake (Power et al., 1997). The makeup of the fertilizers in the combination treatment was potentially more efficient; further tissue and soil analysis would confirm how the phosphate usage differed between treatments.

The mineral fertilizer treatments received lower concentrations of potassium than the combination treatment. The lower concentrations of potassium explain the lower EC_{SE} readings of the mineral fertilizer treatments. Another reason for the lower EC_{SE} values of the mineral fertilizer was that synthetic human urine and the combination treatments received sodium ions that would increase the EC_{SE}.

Based on the significant shoot lengths for spinach, the optimal fertilizer application level for synthetic human urine was Level 2, for mineral fertilizer treatment it was Level 3, and for the combination treatment it was Level 6. At Level 6, the EC_{SE} of the combination treatment was 7.5 dS m^{-1} and received 607.5 kg N ha^{-1}, 407.3 kg P ha^{-1} and 890.6 kg K ha^{-1} or five times the recommended fertilizer application rate for growing spinach in

Quebec; there was no yield loss compared to the control. In this experiment most of the EC_{SE} values surpassed the threshold and one soil sample surpassed the EC_{50} at fertilizer application Level 8; but no treatments had a significant yield loss compared to the control. A field experiment where the plants could reach maturity would enable a more complete analysis of the sensitivity to the human urine as a fertilizer.

3.5 Mustard – results & discussion

3.5.1 Mustard results

The power of the experiment for each component was ≥ 0.80 except for the emergence rate (power of 0.784) (Table 3.6).

Table 3.6 – Power of the experiment for the measured mustard components (N = 75).

Component	Standard deviation	Power
Emergence rate	0.37	0.784
Survival rate	0.21	>0.999
Root (mass)	7.9	0.807
Root (length)	4.65	0.899
Shoot (mass)	18.48	0.991
Shoot (length)	2.15	0.860
EC	438.5	>0.999
pH	0.14	0.959

The mustard emergence rates were not significant. The overall emergence rate for all four fertilizer treatments was 83% (Figure 3.7). The highest emergence rates were Levels 1 (16.7 kg N ha^{-1}) and 7 (455.6 kg N ha^{-1}) at 100%, and the lowest was Level 8 (683.4 kg N ha^{-1}) at 55%. The overall emergence rate for synthetic human urine was 83%, with low emergence (≥2 replicates did not emerge) at Level 8. The mineral fertilizer treatment had the highest overall emergence rate at 88%. The combination treatment had an overall emergence rate at 79%, with low emergence at Levels 5 (202.5 kg N ha^{-1}) and Level 8. The control had the lowest emergence rate at 67% as one of the three replicates did not emerge.

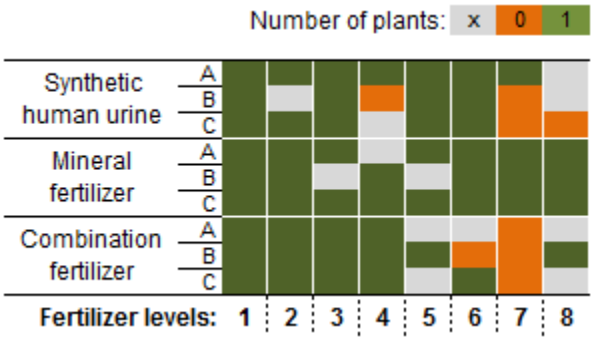

Figure 3.7 – The emergence and survival rate of the mustard seedlings
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination, and the control (not shown). Each had three replicates (A, B, C) at each fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4). The seedlings that did not emerge are shaded grey (x). The emerged seedlings that did not survive to the termination of the 14-day experiment are shaded orange (0). Those which survived to the termination are shaded green (1). The control had 100% emergence and survival rate.

The survival rate of the emerged mustard seedlings were significant ($P < 0.0001$) (Figure 3.7). The highest survival rates were Levels 1, 2 (33.2 kg N ha^{-1}), 3 (67.5 kg N ha^{-1}) and 5 at 100%, and the lowest was Level 7 at 44%. Synthetic human urine had the lowest overall survival rate at 73%. At Level 7, the synthetic human urine survival rate, 33%, and the combination treatment survival rate, 0%, were significantly lower to the control, 100%, and mineral fertilizer, 100%. At Level 8, the synthetic human urine had a 0% survival rate and was significantly lower to the control, the mineral fertilizer treatment, 100%, and the combination treatment, 100%. The mineral fertilizer treatment had the highest survival rate at 100%. The combination treatment overall survival rate was 81%, with low survival at fertilizer application Level 6 (303.8 kg N ha^{-1}) with 50% and Level 7 with 0%.

There was one treatment level with phytotoxicity in the mustard seedlings; mineral fertilizer treatment, Level 7, which had curled and burned leaves. Emerged seedlings varied in size but were predominantly uniform in appearance. Combination treatment, Level 8, contained one plant that emerged and remained yellow during the 14 days.

Of the measured mustard biomass components, root mass and shoot length were not significant. The biomass components were significant for root length ($P = 0.0024$) and shoot mass ($P = 0.0463$) (Table 3.7). The root length from the synthetic human urine treatment Level 1 (27.9 cm) was significantly higher than all other treatments. The rest of

the treatments were not significantly different compared to the control or to each other at all other fertilizer application levels. The root length had a decreasing trend for all treatments after fertilizer application Level 4 (135.0 kg N ha^{-1}) (Figure 3.8).

Table 3.7 – Summary of the dry biomass components for mustard

The four treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4) and the standard deviation. The mineral fertilizer treatment Level 6 root mass was zero because the roots were too small in mass for the balance (> 1 mg). Mean separation by Duncan's multiple range test at $P \leq 0.05$. Different letter indicate statistical significant difference.

	Fertilizer application level							
Root (mass, mg)	1	2	3	4	5	6	7	8
Control	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0
Synthetic human urine	28.3 +/- 8.96	15.5 +/- 7.78	3.3 +/- 2.08	11.0 +/- 0.0	15.0 +/- 5.29	3.7 +/- 2.31	7.0 +/- 0.0	0.0 +/- 0.0
Mineral fertilizer	15.3 +/- 14.01	7.0 +/- 7.81	11.5 +/- 3.54	7.5 +/- 3.54	8.0 +/- 7.07	3.0 +/- 2.65	9.3 +/- 13.65	9.7 +/- 10.97
Combination	4.3 +/- 1.53	7.7 +/- 8.14	11.0 +/- 9.85	11.7 +/- 6.66	3.0 +/- 0.0	7.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0
Root (length, cm)								
Control	**6.2**[b] +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69	6.2 +/- 2.69
Synthetic human urine	**27.9**[a] +/- 7.81	14.1 +/- 1.34	6.9 +/- 6.74	9.9 +/- 0.0	7.7 +/- 3.16	6.1 +/- 2.68	5.4 +/- 0.0	0.0 +/- 0.0
Mineral fertilizer	**7.9**[b] +/- 1.36	6.7 +/- 3.03	11.9 +/- 4.38	13.1 +/- 5.87	8.8 +/- 0.21	7.1 +/- 2.42	6.2 +/- 3.01	9.4 +/- 1.23
Combination	**5.9**[b] +/- 5.0	9.3 +/- 6.81	14.8 +/- 8.64	8.6 +/- 2.75	4.2 +/- 0.0	9.1 +/- 0.0	0.0 +/- 0.0	5.9 +/- 0.0
Shoot (mass, mg)								
Control	**4.0**[b] +/- 0.0	**4.0**[b] +/- 0.0	**4.0**[b] +/- 0.0	**4.0**[b] +/- 0.0	**4.0**[b] +/- 0.0	**4.0**[b] +/- 0.0	4.0 +/- 0.0	4.0 +/- 0.0
Synthetic human urine	**49.0**[ab] +/- 2.64	**44.5**[ab] +/- 3.53	**40.7**[a] +/- 35.7	**37.0**[ab] +/- 0.0	**38.3**[ab] +/- 13.32	**24.7**[ab] +/- 16.26	15.0 +/- 0.0	0.0 +/- 0.0
Mineral fertilizer	**50.0**[a] +/- 25.06	**22.0**[b] +/- 13.23	**72.0**[a] +/- 19.8	**48.0**[ab] +/- 4.24	**57.0**[a] +/- 1.41	**35.3**[ab] +/- 26.27	33.3 +/- 7.09	28.7 +/- 4.93
Combination	**17.0**[ab] +/- 14.11	**56.7**[a] +/- 15.5	**53.3**[a] +/- 35.13	**59.3**[a] +/- 10.79	**10.0**[b] +/- 0.0	**51.0**[a] +/- 0.0	0.0 +/- 0.0	16.0 +/- 0.0
Shoot (length, cm)								
Control	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85	3.8 +/- 0.85
Synthetic human urine	8.6 +/- 0.78	9.3 +/- 0.49	6.5 +/- 4.2	7.8 +/- 0.0	7.8 +/- 0.93	5.9 +/- 3.5	4.9 +/- 0.0	0.0 +/- 0.0
Mineral fertilizer	8.3 +/- 1.21	6.3 +/- 0.8	9.5 +/- 0.71	7.2 +/- 2.4	9.3 +/- 0.28	6.2 +/- 3.86	6.4 +/- 0.67	5.8 +/- 1.07
Combination	5.6 +/- 3.04	10.4 +/- 1.1	9.7 +/- 2.75	9.9 +/- 0.57	4.7 +/- 0.0	9.2 +/- 0.0	0.0 +/- 0.0	5.1 +/- 0.0

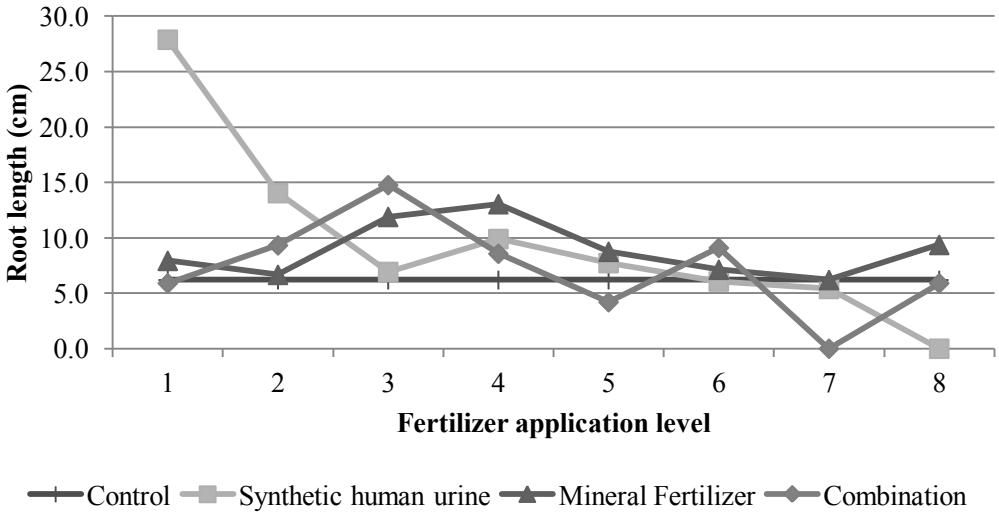

Figure 3.8 – Mean root length of mustard under four fertilizer treatments
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4).

Seven treatments were significantly higher in shoot mass to the control. The mineral fertilizer treatment, Level 3, had the largest shoot mass, 72.0 mg (Table 3.7). There was a downward trend in the shoot mass after fertilizer application Level 4 (Figure 3.9). The eight shoot masses from the synthetic human urine treatments were not significantly different to each other. At Level 5, the combination treatment was significantly lower than the mineral fertilizer treatment. At Level 7, the combination treatment had no surviving seedlings; the synthetic human urine and mineral fertilizer treatments were not significantly different. At Level 8, the synthetic human urine had no surviving seedlings; the mineral fertilizer and combination treatments were not significantly different.

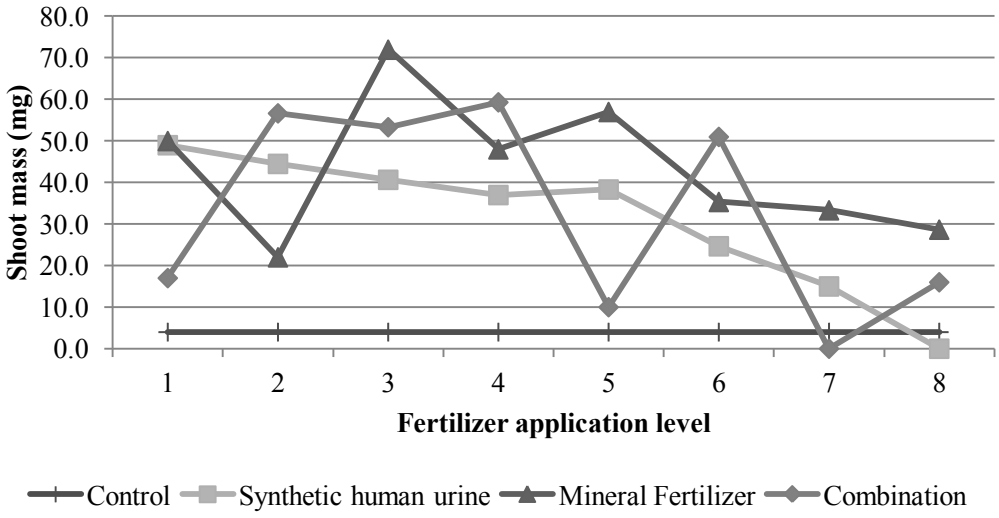

Figure 3.9 – Mean shoot mass of mustard under four fertilizer treatments
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4).

The EC_{SE} model of the mustard soil samples was significant ($P < 0.0001$) (Table 3.8). The three treatments (synthetic human urine, mineral fertilizer and combination) at Levels 5, 6, 7, 8, and the combination treatment, Level 4, were significantly higher to the control. The combination treatment, Level 8, had the highest EC_{SE} at 12.7 dS m^{-1}, and was significantly different to all other treatments. The lowest EC_{SE} reading was the control at 0.19 dS m^{-1}. The EC_{SE} increased linearly as the fertilizer application levels increased (Figure 3.10). At each fertilizer application level, the four fertilizer treatments (synthetic human urine, mineral fertilizer, combination and the control) were not significantly different to each other until Level 7, where the mineral fertilizer treatment was significantly lower compared to the synthetic human urine and combination treatment.

Table 3.8 – Summary of the soil components, EC_{SE} and pH_{SE}, for mustard

The four treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4) and the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$. Different letter indicate statistical significant difference.

	Fertilizer application level															
EC_{SE} (dS m^{-1})	1		2		3		4		5		6		7		8	
Control	1.19	+/- 0.06	1.19	+/- 0.06	1.19	+/- 0.06	**1.19^{b}**	+/- 0.06	**1.19^{b}**	+/- 0.06	**1.19^{b}**	+/- 0.06	**1.19^{c}**	+/- 0.06	**1.19^{c}**	+/- 0.06
Synthetic human urine	1.49	+/- 1.09	2.27	+/- 1.05	1.59	+/- 1.09	**1.82^{ab}**	+/- 1.04	**3.09^{a}**	+/- 0.73	**3.74^{a}**	+/- 1.87	**6.57^{a}**	+/- 3.10	**5.75^{b}**	+/- 2.51
Mineral fertilizer	0.94	+/- 0.73	1.81	+/- 0.44	1.45	+/- 0.85	**2.06^{ab}**	+/- 0.22	**2.48^{a}**	+/- 1.07	**3.61^{a}**	+/- 0.06	**2.93^{b}**	+/- 0.05	**6.13^{b}**	+/- 1.02
Combination	0.69	+/- 0.49	1.37	+/- 1.08	1.88	+/- 1.40	**3.93^{a}**	+/- 1.11	**3.20^{a}**	+/- 0.12	**4.38^{a}**	+/- 0.64	**5.93^{a}**	+/- 1.17	**12.68^{a}**	+/- 3.21
pH_{SE}																
Control	6.25	+/- 0.06	6.25	+/- 0.06	6.25	+/- 0.06	**6.25^{a}**	+/- 0.06	6.25	+/- 0.06	6.25	+/- 0.06	6.25	+/- 0.06	6.25	+/- 0.06
Synthetic human urine	6.25	+/- 0.10	6.16	+/- 0.20	6.24	+/- 0.14	**6.21^{ab}**	+/- 0.20	6.15	+/- 0.09	6.38	+/- 0.13	6.47	+/- 0.12	6.38	+/- 0.19
Mineral fertilizer	6.24	+/- 0.09	6.13	+/- 0.16	6.25	+/- 0.14	**6.21^{ab}**	+/- 0.19	6.28	+/- 0.07	6.31	+/- 0.05	6.49	+/- 0.02	6.29	+/- 0.07
Combination	6.25	+/- 0.04	6.18	+/- 0.23	6.27	+/- 0.21	**5.97^{b}**	+/- 0.06	6.17	+/- 0.11	6.22	+/- 0.13	6.36	+/- 0.12	6.15	+/- 0.16

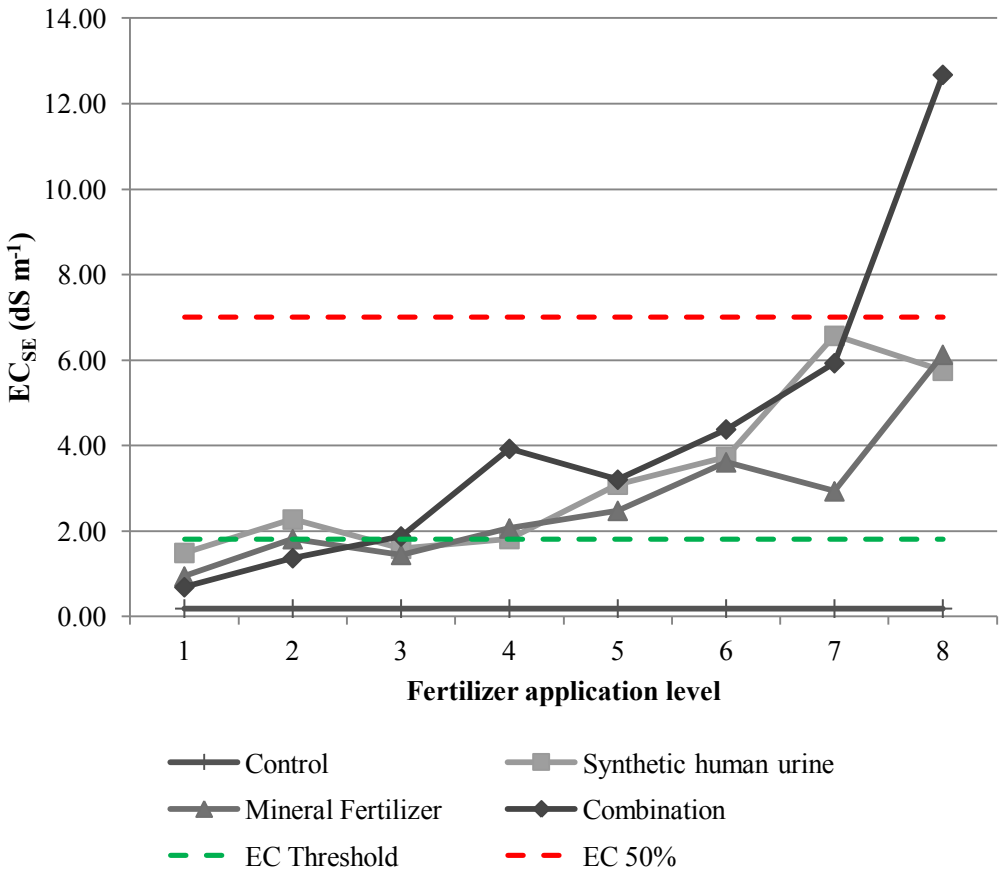

Figure 3.10 – The EC_{SE} of the mustard soil samples
The four fertilizer treatments were synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4). The green line, at 1.8 dS m^{-1}, is the EC_{SE} threshold and the red line, at 7.0 dS m^{-1}, is the EC_{50} (Power et al., 1997).

The majority of the EC_{SE} values were above the threshold 1.8 dS m^{-1} (green line) and the combination treatment, Level 8, surpassed the EC_{50} 7.0 dS m^{-1}. The EC_{SE} measurements of Figure 3.10 were used with the salinity threshold (1.8 dS m^{-1}) and the slope (9.7 %) for mustard to calculate the theoretical potential yield loss due to the increase in salinity (Power et al., 1997). In Figure 3.11, the negative bars were the potential yield loss (%) compared to the control.

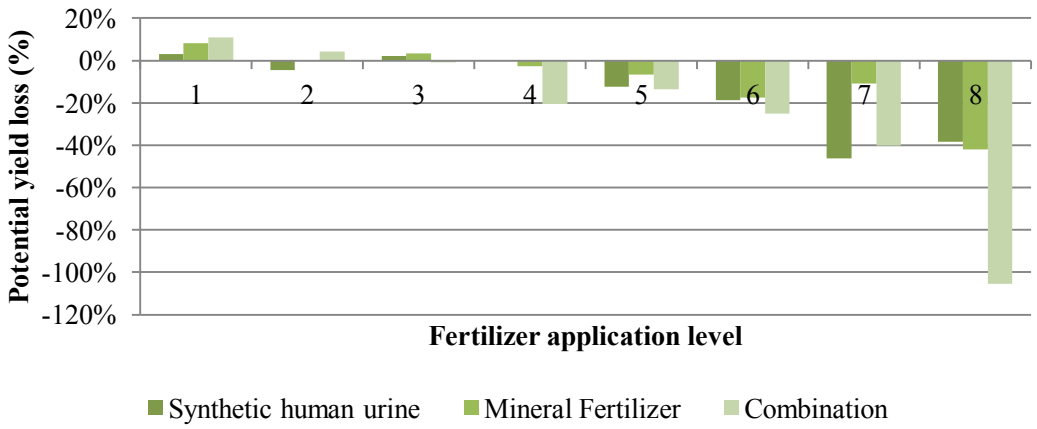

Figure 3.11 – Potential mustard yield loss (%) due to increased EC_{SE}
Each of the three fertilizer treatments (synthetic human urine, mineral fertilizer and combination) are shown at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4). All negative bars indicate that the EC_{SE} was above the maximum allowable threshold before harm to plant yield (1.8 dS m^{-1}) (Power et al., 1997).

The pH_{SE} model was significant (P = 0.0137) within a narrow range: the highest was mineral fertilizer, Level 7, at 6.49, and the lowest was the combination treatment, Level 4, at 5.97 (Figure 3.12). The combination treatment, Level 4, was the only treatment significantly lower to the control and was significantly lower to synthetic human urine treatment, Levels 7, 6, and 8, and to mineral fertilizer treatment, Level 7. At each fertilizer application level, the fertilizer treatments were not significantly different to each other.

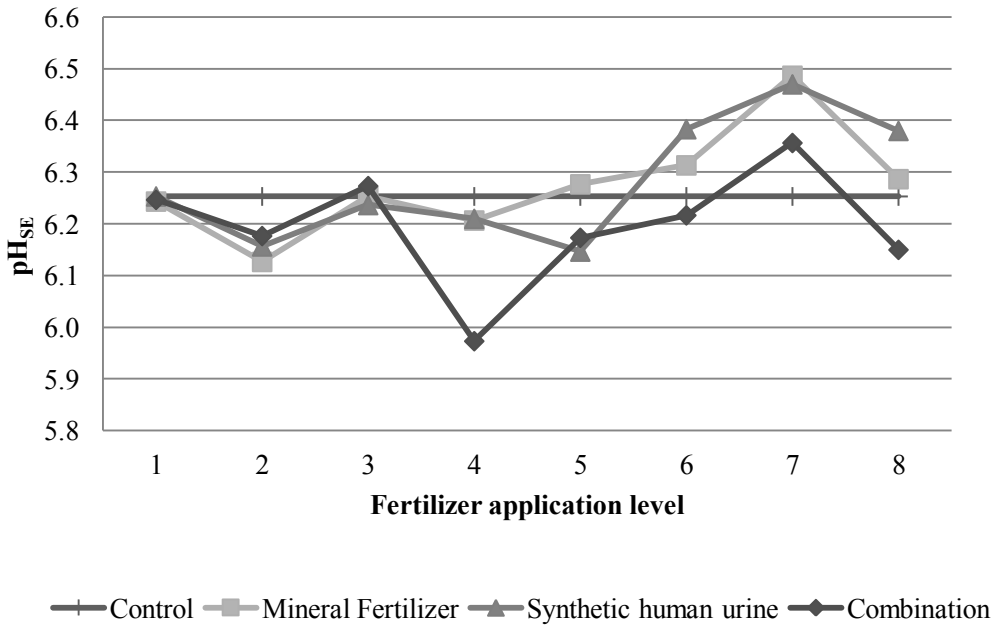

Figure 3.12 – The pH_{SE} of the mustard soil samples
For the four fertilizer treatments: synthetic human urine, mineral fertilizer, combination and the control at increasing fertilizer application level (kg N ha^{-1}): 1 (16.9), 2 (33.8), 3 (67.5), 4 (135.0), 5 (202.5), 6 (303.8), 7 (455.6), and 8 (683.4).

3.5.2 Mustard discussion

Mustard was sensitive to the different fertilizer treatments (control, synthetic human urine, mineral fertilizer and combination treatment) at increased application rates. The power of the emergence test was not satisfied (< 0.8) and the ANOVA model of the emergence rates was not significant, indicating that the ranges of the fertilizer application rates could have been greater. The decrease in the root mass indicated that the germination did not have to be inhibited to have effects on the production rate. The first part of the hypothesis, that as the EC would increase more in the human urine and combination fertilized treatments than in the mineral fertilizer treatments, was correct for Levels 7 and 8; the second part of the hypothesis, that the higher EC levels would cause a decrease in biomass production rates, was correct and there was a moderate negative correlation with the EC and the survival rate. The combination treatment at Level 7 and the synthetic human urine treatment at Level 8 had potential yield losses of 40% based on the measured EC of the soils, but the replicates all died. Even so, the EC was not correlated to the two significant biomass ANOVA models (root length and shoot mass); no treatments with surviving plants had yields significantly lower than that of the control. The increase of pH had no correlation to the biomass production or emergence and had a

weak negative correlation to the survival rate. The highest pH was at fertilizer application Level 7, which had the lowest survival rate for the synthetic human urine and combination treatments. At Levels 7 and 8, signs of stress in the leaves were apparent, signifying adverse effects of fertilizer. Below these levels there were no visual signs of stress. In the 14-day trial, it was clear that mustard was sensitive to the increase in EC and was not able to withstand significant changes of the chemistry in the soils.

In summary, sensitivity of the mustard seedlings was greater than that of the spinach. The mustard biomass production was negatively impacted by the increase of the fertilizer application levels, but was not correlated to the EC_{SE}. Based on the significant root length and shoot mass ANOVA mustard models and the non-significant shoot length ANOVA model, the optimal fertilizer application level for synthetic human urine was Level 1; for mineral fertilizer treatment it was Level 3; and for the combination treatment it was Level 4.

3.6 General discussion

The recipe used for the synthetic human urine was labelled as 'multi-purpose' and was selected for this experiment based on the content of urea and sodium (Chutipongtanate et al., 2010). Other synthetic human urine recipes were designed for specific purposes, such as for dermatology or calcium oxalate crystallization, and do not have the required levels of urea and salts (Chutipongtanate et al., 2010).

Human error may have negatively impacted the final outcome of the biomass production. The researcher did not transplant the seedlings randomly, but in increasing order of fertilizer application levels and started by transplanting spinach into the mineral fertilizer treatment pots. Spinach seedlings of the mineral fertilizer treatments had the lowest emergence rate and were the first to be transplanted. The combination treatments had the highest emergence rate and were the last to be transplanted. Seedlings may not have emerged due to being damaged while the researcher perfected the technique.

The sources of phosphate and potassium were different for the mineral fertilizer and combination treatments. The mineral fertilizer was applied in liquid form, and in the combination treatment phosphate and potassium were applied in pellet form. The pellets should have been liquefied before being incorporated into the soil to eliminate discrepancy between applications. This was not the case as the pH of the soil samples

from the four treatments at each fertilizer application level were not significantly different and the EC was not significantly different from fertilizer application rates 1 through 6. This indicates two things: first that the different forms of fertilizer did not significantly change the pH and EC, but the increase in application rates did; second that the pH and EC did not influence the emergence rate of the mustard seedlings.

The survival rates may have been influenced by the variability of temperature and relative humidity across the greenhouse as pots were not laid out in random order. This error could have had devastating effects on the data collected, but was not found to be an issue. The seedling pots were closely packed on one table (4' by 15') making it easy for frequent observation and regular watering. With the small surface area, dry or stressed zones would have been identifiable, but none were observed during the experiment or within the dataset. There was no correlation between the EC and the pH to the biomass production and emergence. There was a negative correlation between the EC and the pH to the survival rate.

The EC_{SE} readings of the spinach soil samples from the mineral fertilizer treatments dropped at fertilizer application Level 6 and an explanation is presented in the discussion of Section 3.4.2. From the mustard trial, the EC_{SE} continued to increase with the synthetic human urine and combination fertilizer treatments and was only significantly lower compared to those treatments at Level 7. As the pH_{SE} remained in the optimal range for both seedling species, there would have been no significant difference in the availability of nutrients between treatments (Power et al., 1997). The soils were not sodic as the $pH_{SE} \leq 8.5$ (Power et al., 1997).

The root biomass and length were compromised during the time-consuming task of separating them from the soil as the roots were delicate and long, and the artificial soil was difficult to break apart. While separating the roots from the artificial soil, maintaining the longest roots in one section was difficult, and it is likely several roots were broken without the researcher noticing, which might explain the erratic brown mustard root lengths of the combination treatment. However, this error seems not to have negatively influenced the data set as the root masses were not significantly different and the root lengths were not significantly different.

There were significant differences in the biomass production, and field trial of a similar experimental set-up with a longer maturation period and a higher range of fertilizer application levels would be the next phase in understanding the impacts of the increased fertilizer application levels on the nitrogen accumulation in the biomass. An experiment with a longer duration would produce enough biomass to test the tissue's concentration of elements to provide a better representation of how the plants are affected by human urine as a fertilizer and as a comparison of nutritional content. The combination treatment produced significantly higher biomass at some treatment levels but was not conclusive that combination treatment was the best fertilizer treatment and should be included in the field experiment.

3.7 Conclusion

The soil chemistry was altered by the application of the fertilizers. The spinach plants were not sensitive to the changes but the mustard plants were sensitive to the changes. The changes in pH and EC between the fertilizer treatments were significant at the higher fertilizer application levels (5 through 8) for both plant species. The soil from the spinach plants treatments, the synthetic human urine and combination, Levels 6 through 8, had significantly higher EC values compared to the spinach control and to the soil from the spinach mineral fertilizer treatments, but the biomass production rates were not negatively impacted. During the 14-day trial, it was clear that spinach was able to withstand significantly higher EC soil levels than were commonly reported. The spinach ability to withstand the increased EC values above the threshold supported that the salt formations in urine were impacting the plants differently than the salt formations in the mineral fertilizers. For spinach, the combination treatment at Level 6 produced the highest biomass. For brown mustard, the mineral fertilizer treatment at Level 3 produced the highest biomass.

3.8 References

AYERS, R. S. & WESTCOT, D. W. 1994. *Water quality for agriculture: Salinity Problems* [Online]. Rome: Food and Agricultural Organization of the United Nations. Available: http://www.fao.org/docrep/003/t0234e/T0234E03.htm [Accessed February 13, 2013].

BAJJI, M., KINET, J.-M. & LUTTS, S. 1998. Salt stress effects on roots and leaves of Atriplex halimus L. and their corresponding callus cultures. *Plant Science,* 137**,** 131-142.

CHUTIPONGTANATE, S. & THONGBOONKERD, V. 2010. Systematic comparisons of artificial urine formulas for in vitro cellular study. *Analytical Biochemistry,* 402**,** 110-112.

CRRAQ 2010. *Guide de référence en fertilisation*, Centre de référene en agriculture et agroalimentaire du Québec,.

ENVIRONMENT CANADA 2005. Biological test method: Test for measuring emergence and growth of terrestrial plants exposed to contaminants in soil. *In:* ENVIRONMENTAL TECHNOLOGY CENTER (ed.) *Method Development and Application Section.* Ottawa.

GAVLAK, R., HORNECK, D., MILLER, R. O. & KOTUBY-AMACHER, J. 2003. Soil pH and Electric Conductivity *Soil and Water Analysis Manual, 2nd Edition.* Western States Laboratory Plant.

GERMER, J., ADDAI, S. & SAUERBORN, J. 2011. Response of grain sorghum to fertilisation with human urine. *Field Crops Research,* 122**,** 234-241.

HUANG, J. & REDMAN, R. E. 1995. Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. *Canadian Journal of Plant Sciences,* 75**,** 815-819.

JAIN, S., NAINAWATEE, H. S., JAIN, R. K. & CHOWDHURY, J. B. 1993. SALT-TOLERANCE IN BRASSICA-JUNCEA L .2. SALT-STRESS INDUCED CHANGES IN POLYPEPTIDE PATTERN OF INVITRO SELECTED NACL-TOLERANT PLANTS. *Euphytica,* 65**,** 107-112.

MNKENI, P. N. S., KUTU, F. R., MUCHAONYERWA, P. & AUSTIN, L. M. 2008. Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in Eastern Cape, South Africa. *Waste management & research,* 26.

MUFWANZALA, N. & DIKINYA, O. 2010. Impact of Poultry Manure and its Associated Salinity on the Growth and Yield of Spinach (Spinacea oleracea) and Carrot (Daucus carota). *International journal of agriculture and biology,* 12**,** 489-494.

ORWIN, K. H., BERTRAM, J. E., CLOUGH, T. J., CONDRON, L. M., SHERLOCK, R. R., O'CALLAGHAN, M., RAY, J. & BAIRD, D. B. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. *Applied Soil Ecology,* 44**,** 89-100.

POWER, J. F. & PRASAD, R. 1997. *Soil Fertility Management for Sustainable Agriculture,* Boca Raton, FL, CRC Press.

PRADHAN, S. K., HOLOPAINEN, J. K. & HEINONEN-TANSKI, H. 2009. Stored Human Urine Supplemented with Wood Ash as Fertilizer in Tomato (Solanum lycopersicum) Cultivation and Its Impacts on Fruit Yield and Quality. *Journal of Agricultural and Food Chemistry,* 57**,** 7612-7617.

PUTNAM, D. F. 1971. Composition and concentrative properties of human urine. *National Aeronautics and Space Administration Contractor Report.* Huntington Beach, California: McDonnell Douglas Astronautics Company - Western Division.

RHOADES, J. D., CHANDUVI, F. & LESCH, S. 1999. Soil salinity assessment: methods and interpretation of electrical conductivity measurements. *Irrigation and Drainage.* Rome: Food and Agriculture Organization of the United Nations.

SHANI, U. & DUDLEY, L. M. 2001. Field studies of crop response to water and salt stress. *Soil Sci. Soc. Am. J.,* 65**,** 1522-1528.

SHANNON, M. C. & GRIEVE, C. M. 1999. Tolerance of vegetable crops to salinity. *Scientia Horticulturae,* 78**,** 5-38.

STEPPUHN, H., GENUCHTEN, M. T. V. & GRIEVE, C. M. 2005. Root-Zone Salinity: II. Indices for Tolerance in Agricultural Crops. *Crop Science Society of America,* 45**,** 221-232.

WHIPKER, B. E. & CAVINS, T. J. 2000. *Electrical Conductivity (EC): Units and Conversions* [Online]. North Carolina State University. Available: http://www.ces.ncsu.edu/depts/hort/floriculture/Florex/EC%20Conversion.pdf [Accessed November 21, 2012].

ZVALO, D. V. & RESPONDEK, A. 2007. Broccoli. *In:* AGRAPOINT (ed.) *Vegetable Crops Production Guide for Nova Scotia.* Atlantic Provinces Agricultural Services Coordinating Committee.

ZVALO, D. V. & RESPONDEK, A. 2008. Spinach. *In:* AGRAPOINT (ed.) *Vegetable Crops Production Guide for Nova Scotia.* Atlantic Provinces Agricultural Services Coordinating Committee.

Connecting Statement

The sensitivity analysis of Chapter 3 demonstrated that the high concentration fertilizer increase the soil EC but did not negatively change the emergence, survival and biomass production rates of spinach but did negatively change the biomass production rates for brown mustard. Areas with regular to intense rainfall, such as monsoons, would be ideal in order to leach the salts out of the soils on a regular basis. Chapter 4 develops from the observations of Chapter 3 into a simulated nine year field experiment implemented in a monsoon area of India: Himachal Pradesh. Chapter 4 compares the results of the experiment to current literature guidelines and provides recommendations for use of human urine as a fertilizer in Himachal Pradesh.

4 Human urine increases spinach biomass production rates in simulated long-term use as fertilizer

4.1 Introduction

Seven billion people are urinating every day, producing 28 million tonnes of nitrogen a year (Vinneräs 2002). This mass of nitrogen excreted is equal to 26% of the annual global nitrogen fertilizer demand, a value of $21.3 trillion USD (based on $350 USD per metric tonne of urea) (Alibaba, 2012, FAO, 2011). Yet, human urine is typically considered a waste, not a fertilizer. The use of human urine as a fertilizer is not farfetched – on the contrary – human excrement has been used as a fertilizer for over three thousand years and not that long ago, was considered a highly valued resource (King, 1911). In 1908, the city of Shanghai sold one contract worth $31 000 in gold, for the rights to collect, remove and resell 78 million kg of human excrement (King, 1911). While most other countries were still trying to figure out a way to dispose of excrement and household garbage, the Chinese had an efficient recycling system (King, 1911).

The use of human excrement as fertilizer has now been termed Ecological Sanitation (EcoSan). EcoSan is a broad term encompassing the technologies that use human excreta as fertilizer (Jackson, 2005). The technologies range from simplistic (planting a tree over a disused pit latrine) to sophisticated separation and collection systems (Chapter 2).The concept of EcoSan is to use human excrement in a cradle-to-cradle system: humans consume food, the unused nutrients are excreted, the excrement is used to grow food, and the cycle repeats (McDonough et al., 2002, Winblad et al., 2004).

EcoSan has a long history of traditional knowledge of how to use human excrement as a fertilizer; however, there is limited scientific knowledge (Jönsson et al., 2004). Experiments have demonstrated that human urine is as efficient as mineral fertilizer (Chapter 2) but little is known about how human urine alters crop production and soil chemistry with repeated use. This chapter is focused on building the scientific knowledge base of using human urine as fertilizer on a long-term (nine year) base. Human urine, and not faeces, was the focus for two reasons. First, urine contains the majority of the excreted nutrients (by mass): 80-90% of the nitrogen (N), 50-80% of the phosphate (P) and 80-90% of the potassium (K); the rest is excreted in the faeces (Vinnerås et al., 2002). And second, urine requires less sanitation treatment for safe application on edible crops compared to faeces, as faeces contain potentially harmful pathogens (Heinonen-

Tanski et al., 2007, Höglund et al., 2002, Kirchmann et al., 1995). Human urine is high in N but lower in P and K, so this chapter also observes the use of human urine in combination with mineral P and K fertilizers to achieve the optimal fertilizer application rates.

The primary objective of the experiment was to observe the changes in the biomass of spinach (*Spinacia oleracea* L.) and the chemical changes in soil from regular uses of human urine as a fertilizer. This was performed by simulating nine years of repeated fertilizer use in the field through increased concentrations of applied fertilizer. The effect of human urine as a fertilizer was compared to mineral fertilizer and the combination of urine with mineral fertilizer by measuring the differences in the spinach emergence rate, survival rate, yield, tissue nutrient concentration and the differences in the soil nutrient concentration and chemistry (pH and EC). Three hypotheses were designed to meet the objective. One, the spinach seedlings of the three fertilizer treatments (human urine, mineral fertilizer, and combination) will be equivalent in biomass production at each simulated year and significantly higher than the control for all simulated years. Two, the electric conductivity (EC) of the soil will increase for each simulated year and the human urine and combination treatments will have higher soil EC than the mineral fertilizer treatments. Three, the pH of the soil for the three fertilizer treatments will decrease at equivalent rates at increased simulated years. From this experiment, the secondary objective of finding the optimal human urine fertilizer application rate for crop production in Himachal Pradesh, India, will be determined.

A focused outcome of this thesis was to identify the optimal human urine fertilizer level for spinach in Himachal Pradesh, India. The optimal fertilizer level had the highest biomass production with minimal impacts on the soil, in comparison to the effects of mineral fertilizer. India was selected as the field location as there are already several EcoSan projects occurring (Chapter 2) and Himachal Pradesh was selected based on climate (average temperatures 25-30^{o}C from July to October,(IMD, 2012a)) and benefits to personal health (no malaria). Undiluted human urine was used to fertilize spinach in three replicated blocks in space (Block 1, 2 and 3) and over two growth periods (Run 1 and 2), in Himachal Pradesh, India.

4.2 Background

4.2.1 Importance of soil chemistry

Fertilizer application is required to achieve maximum crop yields. Before and during each growing season, fertilizer is ideally applied to replace any nutrients that the plants have accumulated from the soil, as the plants are removed from the field during harvest, depleting the soil of nutrients. These same nutrients can be leached from the soil, washed away, blown away or volatized, which result in the need to replace these lost nutrients. Without fertilizers, crop yields tend to be 1/3 to 1/4 of a crop grown with the optimal level of fertilizer (Pradhan et al., 2009). Even with fertilization, seasonal variations, soil type and cultural practices can influence the crop yield each year (Jones et al., 2005).

Plants absorb most nutrients in ionic form, from the soil into the roots (Jönsson et al., 2004). Plants are dependent on the activity of microorganisms to convert many of the nutrients in the soil for uptake by the plant (Mufwanzala et al., 2010). Nutrients in the soil can vary in concentration spatially and the addition of fertilizers are required to provide uniform levels of the elements across the field which allow for more uniform plant growth and higher plant production (Jones et al., 2005).

The fertilizers applied are typically not composed of a single element but are a composition of different elements. Components of these fertilizers include salts that can accumulate and alter the chemistry of the soil with repeated use (Whipker et al., 2000), increasing the electric conductivity (EC) of the soil beyond optimal levels which can negatively impact the biomass production (Chapter 2). Understanding how the chemistry of the soil is altered by a fertilizer is required to maintain an optimal EC and pH, maximizing plant biomass production.

The pH of the soil is a major influencing factor of the nutrient availability as it controls the solubility of the ions (Power et al., 1997). Optimal plant growth occurs at a pH range from 6.0-7.0, as most of the beneficial elements are chemically available and detrimental elements are not available (Power et al., 1997). Application of ammonical based N fertilizer decreases the soil matrix pH through the release of hydrogen atoms, to increase the soil matrix pH, limestone (lime) or ash is applied and can be used as a buffer to maintain a pH between 6.0-7.0 (Power et al., 1997).

The optimal EC level varies with plant species. The tolerance of a plant is dependent on the biomass production rate at a given EC (Chapter 2). For this thesis, the salt tolerance of a plant species is described by three parameters: threshold, slope and EC_{50}. The threshold refers to an EC point beyond which there is potential for reduction in yield due to stress (Power et al., 1997). The slope refers to the yield reduction in percentage per unit increase of the EC above the threshold (Power et al., 1997). The EC_{50} refers to the salinity level at which point the yield would produce 50% of the non-saline crop yield (Steppuhn et al., 2005). The EC values measured by the saturated soil paste extract method are denoted as EC_{SE} (Gavlak et al., 2003, Shannon et al., 1999).

The threshold, slope and EC_{50} values are used in the discussion as a general comparison to assess the overall sensitivity of the plants. Spinach has been observed to be moderately sensitive to saline conditions (Shannon et al., 1999). The specific values for the salt threshold, slope and EC_{50} for spinach are 2.0 dS m^{-1} (Shannon et al., 1999, Steppuhn et al., 2005), 7.6% (Shannon et al., 1999), and 8.2 dS m^{-1} (Steppuhn et al., 2005), respectively.

4.2.2 Human urine nutrient concentrations

In domestic waste water, human urine accounts for 80%, 50%, and 60% of the respective masses of N-P-K (Ganrot et al., 2006). The concentration of elements (Table 2.1) in human urine has diurnal fluctuations and varies widely between people depending on dietary habits, hydration, perspiration, and level of physical work in the day (Heinonen-Tanski et al., 2007). The range of elemental concentrations in the urine highlights the impacts of dietary habits and complications of designing nutrient recycling systems and application rates (Vinnerås, 2002). Reported concentrations in published studies vary due to different methodology, poor storage resulting in nitrogen lost through ammonia emissions, and phosphorus precipitation out of solution (Vinnerås, 2002). Nutrients present in human urine are typically found in ionic form and results in urine having a high average EC of 19.5 dS m^{-1} (Putnam, 1971).

Table 4.1 – Elements in human urine
Element present at >10 mg L^{-1} of male human urine in decreasing concentration (Putnam, 1971)

	Range (mg L^{-1})	
Total solutes	36 700	46 700
Urea	9 300	23 300
Chloride	1 870	8 400
Sodium	1 170	4 390
Potassium	750	2 610
Creatinine	670	2 150
Sulfur, inorganic	163	1 800
Phosphorus, total	410	1 070
Ammonia	200	730
Uric acid	40	670
Bicarbonate	20	560
Creatine	0	530
Sulfur, organic	77	470
Calcium	30	390
Magnesium	20	205
Glucose	30	200

4.2.3 Nitrogen cycle in human urine

The nitrogen in freshly excreted urine is predominately urea (~85%), ammonia, and uric acid, as these three account for 90-95% of the total nitrogen (Kirchmann et al., 1995). Fresh human urine is unstable because of the high concentration of urea (Feng et al., 2008) and is typically acidic at a pH 6 (range 4.5-8.2) (Kirchmann et al., 1995). After the urine is excreted from the body, the urea is hydrolyzed (ureolysis) by the enzyme urease found in bacteria (Feng et al., 2008, Ganrot et al., 2006, Jönsson et al., 2004, Kirchmann et al., 1995, Tilley et al., 2008, Vinnerås, 2002) and is transformed into ammoniacal nitrogen (ammonia (NH_3) (Tilley et al., 2008) and ammonium (NH_4^+) (Feng et al., 2008, Ganrot et al., 2006, Jönsson et al., 2004, Kirchmann et al., 1995, Vinnerås, 2002)). The ammoniacal nitrogen remains in an equilibrium balance dependent on atmospheric pressure and pH (Hellstrom et al., 1999, Tilley et al., 2008). The ureolysis reaction is alkaline and increases the pH to 9.0-9.3 (at 20-25^{o}C), which leads to further precipitation of compounds, such as struvite, urate and uric acid (Feng et al., 2008, Heinonen-Tanski et al., 2007, Jönsson et al., 2004, Kirchmann et al., 1995, Tilley et al., 2008). The ureolysis reaction rate is dependent on temperature and biological activity (Jönsson et al., 2004, Tilley et al., 2008). In locations where urease is able to accumulate, such as in plumbing

systems, the reaction occurs more quickly (Jönsson et al., 2004). Higher cross-contamination from faeces increases the quantity of bacteria with the urease enzyme, thus increasing the rate of ureolysis reaction (Tilley et al., 2008).

After the urea is hydrolyzed, the total nitrogen concentration is 95% ammoniacal nitrogen, 5% amino acid-N, and small traces of nitrite and nitrate (Kirchmann et al., 1995). Any urea still present in urine at field application is transformed to $NH_4^+$by the urease present in the soil (Jönsson et al., 2004). Within three days of application, $NH_4^+$is transformed to nitrate by nitrification (nitrosomonas or short nitrification: NH_4-N to NO_2-N followed by nitrobacts: NO_2-N to NO_3-N) (Feng et al., 2008, Jönsson et al., 2004). The nitrification process is important to understand as it decreases the pH of soil as the reactions release hydrogen ions (Mnkeni et al., 2008).

4.2.4 Introduction to agricultural sector of India

The agriculture sector is the foundation of India's economy: two-thirds of the population dependent on it as their source of revenue; agriculture is 22% of the country's gross domestic product (FAO, 2005). In the past 60 years, grain production in India has more than quadrupled (Singh et al., 2002) yet production rates are still lower than other countries. For example, in 2011 the average yields for chickpeas and maize in Canada were 1 827 kg ha-1 and 8 895 kg ha-1 respectively, compared to India's yield of 893 kg ha-1 and 2 967 kg ha-1, respectively (FAOSTAT, 2011). This is partly due to the nutrient deficiency of soils in India (FAO, 2005). Current fertilizer application rates are below the rates of nutrient removal from the crops harvested – an estimated imbalance of ten million tonnes of N-P-K nutrients (FAO, 2005). There are considerable differences of fertilizer application rates from state to state. For example, Himachal Pradesh uses almost half (32.6 kg ha^{-1}) of the national average (59.2 kg ha^{-1}) of nitrogen and proportionately less fertilizer than other states at equivalent geographic and economic rankings, such as Haryana (125.6 kg ha^{-1} of nitrogen) (FAO, 2005). The low fertilizer application rates decreases yields (Pradhan et al., 2009). Small-hold farmers (< 2.0 ha of farmland) are an essential part of the agricultural sector, producing 41% of India's grains on 33% of the gross cultivated land, and constituting 78% of the farmer population (Singh et al., 2002). With little opportunity to increase farm sizes (Wackernagel et al., 1999), increasing yields and efficiencies need to be prioritized (FAO, 2005).

In India, the soils are typically low in organic carbon, deficient in nitrogen, low to medium in phosphates, and increasingly the soils are becoming deficient in potassium, sulfates, and micronutrients (FAO, 2005). Nutrient depletion in the soil is a major factor for low yields in India (FAO, 2005). In the late 1970s, subsidies and price controls on fertilizers were introduced by the government to maintain low input production costs (FAO, 2005). On August 25, 1992, phosphate and potassium were decontrolled and the prices increased significantly, but urea has remained price controlled (FAO, 2005). In 1997/1998, in reaction to the soaring fertilizer prices, the government put into place a fixed maximum retail price on the decontrolled fertilizers to have homogenous pricing across the country (FAO, 2005). Even with the subsidies and maximum retail prices, most farmers cannot afford to buy fertilizer with cash (FAO, 2005).

The government of India has created the Integrates Nutrient Supply System (INSS) program to promote the use of mineral fertilizer in combination with organic manures and biofertilizers (nitrogen fixing plants, phosphate solubilising bacteria and fungi) to increase fertilizer application rates. The program has a number of constraints, such as: cow manure is already used as a source of fuel; residuals from crops are used as animal feed; and there is poor and inconsistent crop response to biofertilizers to the amount of labour put into the system (FAO, 2005). As part of this INSS program farmers in India are using 90% of all cattle manure for either a fuel source or for field application as a fertilizer, but application rates are well below the ideal level at 2 tonnes ha^{-1} instead of the recommended level of 10 tonnes ha^{-1} (FAO, 2005).

There are two growing seasons in India: kharif (includes rice, millet and cotton; grown April-September) and rabi (includes wheat, barley and mustard; grown October-March); cereals and pulse account for approximately 60% of the cropped area (FAO, 2005). The climate varies widely across the diverse landscape of India, and is classified as tropical monsoon with four distinct seasons: summer from March to May; monsoon from June to September; post-monsoon from October to December; and winter including January and February (FAO, 2005). The climate is affected by two seasonal winds (the southwest monsoon and the northeast monsoon) that distribute variable annual rainfall levels (FAO, 2005). Some areas receive as low as 0 mm a year, while other areas receive more than 2 000 mm a year (FAO, 2005). The state of Himachal Pradesh (Table 4.2) on average

receives 825.3 mm of rainfall during the monsoon from June to September, and receives 108.2 mm during the post-monsoon season from October to December (IMD, 2012).

Table 4.2 – The average rainfall of Himachal Pradesh, India.
The long term rainfall (1951-2000) of Himachal Pradesh (IMD, 2012b) and the monthly average rain fall of Kangra district of Himachal Pradesh in 2011(IMD, 2011)

	Long-term	2011
August	283.0	756.6
September	140.0	218.6
October	42.5	28.8

The juxtaposition of the need for increased agricultural output and the sanitation crises identified in Chapter 1 indicates that EcoSan systems can be used to improved crop yields, decreasing household debt and potentially increasing sanitation standards. The purpose of this study was to observe the changes in the biomass of spinach and the chemical changes in soil from regular uses of human urine as a fertilizer to then identify the optimal human urine fertilizer level for spinach in Himachal Pradesh, India.

4.3 Methodology

4.3.1 Collection of human urine

Undiluted human urine was collected (Aug 17th – Sept 7th, 80 L) from 16 volunteers in Arla, Himachal Pradesh, India; following ethics approval from Dawson Student Union (received July 8th, 2011) and McGill University (received August 17th, 2011). The volunteers urinated in buckets in the morning and evening, and the lids to the buckets remained sealed between uses. Each morning, the urine from the buckets was emptied into 10 L containers. The buckets were rinsed with tap water to reduce the smell (no soap was used). Ideally, the urine would have been stored for three months to limit the risk of disease, but as the students were healthy and there were time constraint, the urine was stored in a dark room for 10 days at 25^{o}C before use. The pH was measured using litmus paper every three days.

4.3.2 Field preparations

The field experiment was conducted in the Kangra district of Himachal Pradesh, India, located in the foothills of the Dhauladhar mountain range (N 32°04 E 76°29). A rain shelter (Figure 4.1) constructed from a blue tarpaulin and bamboo sticks and was set up 50 cm above the plants to limit water from the heavy rains (removed when not raining and replaced each night). The plants received an average of 0.75 mm of rain a day during Run 1 and 0.67 mm during Run 2 using the tarpaulin system (Appendix 4.1 and Appendix 4.2). The min/max temperatures, the relative humidity and the rainfall were recorded daily (Appendix 4.1 and Appendix 4.2). During Run 1, the insect infestation from grasshoppers, crickets and caterpillars was observed to limit plant growth and bed nets (Figure 4.2) were used to limit their access.

Figure 4.1 – Example of the rain shelter

Figure 4.2 – Example of the bed nets used to limit pest damage

The experimental set-up was a randomized complete block design (RCBD) consisting of four fertilizer treatments: human urine; mineral fertilizer; combination of the two; and the control (no fertilizer). To model the repeated use of human urine, the worst-case scenario was assumed: none of the salts or nutrients from the previous years of application would leach away, and the fertilizer treatment application rates were increased linearly to simulate 9 years of use (Table 4.3). The recommended Quebec rates were used (as in Chapter 3) as the recommended rates for spinach in Himachal Pradesh could not be sourced at the time of the study. Quebec and Arla, Himachal Pradesh, have temperate climates. The Quebec fertilizer application rate of N (120 kg N ha^{-1} (CRRAQ, 2010)) was 3.7 times higher than the average application rate in Himachal Pradesh in 2005 (32.6 kg ha^{-1} of N(FAO, 2005)). For the field experiment, the application rate started at the recommended fertilizer application rate for Quebec, Canada (CRRAQ, 2010) and the consecutive simulated years (3, 5, 7 and 9) were multiples of the recommended fertilizer application rate. Both Quebec and Arla, Himachal Pradesh a

Table 4.3 – The fertilizer application rate for the nine simulated years (kg ha^{-1})

Simulated yr	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
1[z]	120.0	82.9	199.2
3	360.0	248.8	597.7
5	600.0	414.6	996.2
7	840.0	580.5	1394.7
9	1080.0	746.3	1793.1
Control	0	0	0

[Z] Recommended fertilizer application rate for spinach in Quebec (CRRAQ, 2010)

A total of 16 experimental units per block consisting of five simulated years of fertilizer application rates for each of the three types of fertilizer treatments and the control (Figure 4.3 and Figure 4.4).

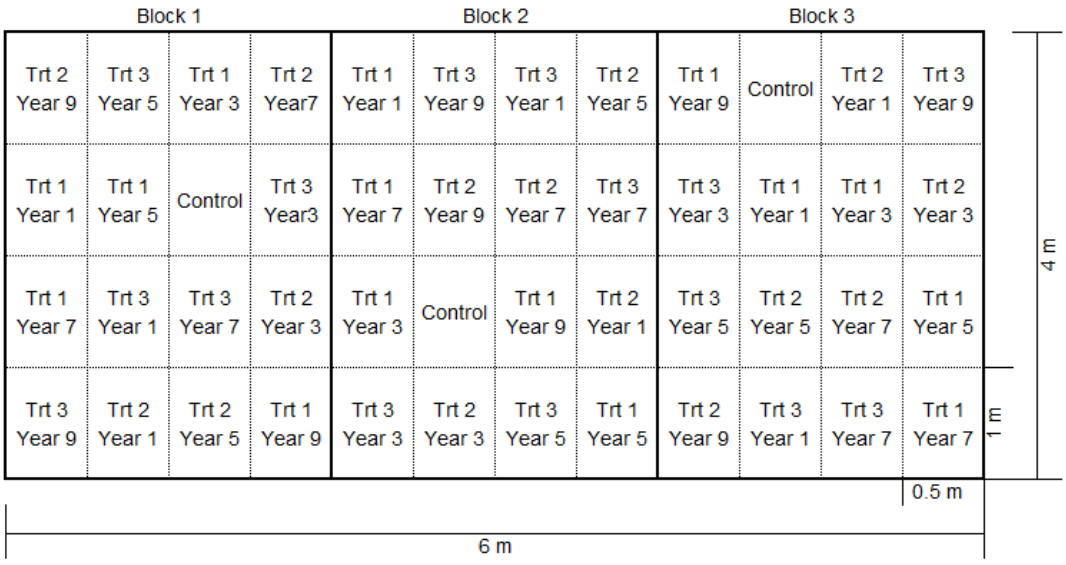

Figure 4.3 – The randomized completed block (RCB) design of the four fertilizer treatments.
Trt 1- human urine; Trt 2- mineral fertilizer; Trt 3- combination of human urine and mineral fertilizer that were applied at five fertilizer levels and the control (as described in Table 4.4).

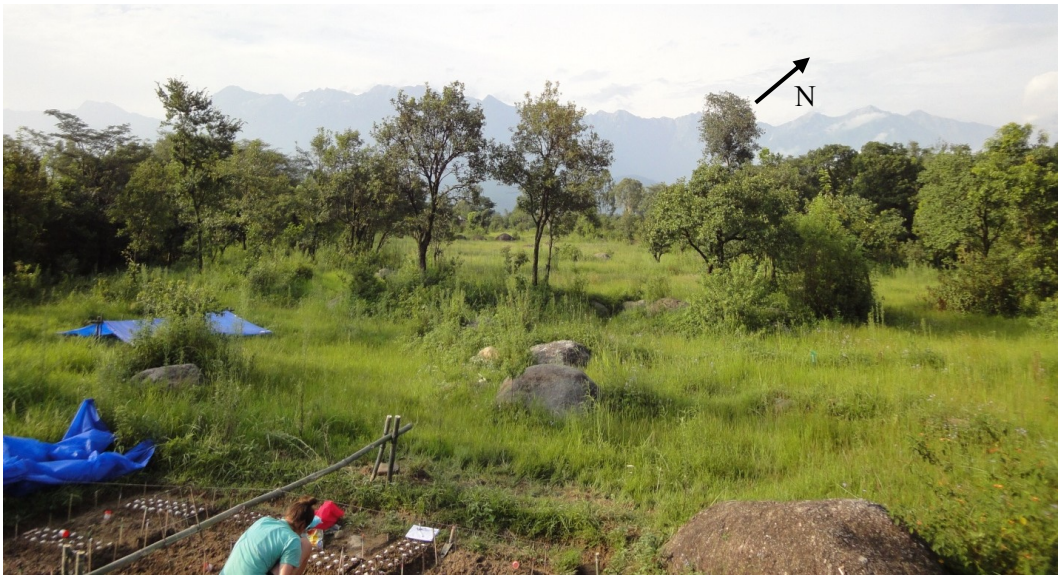

Figure 4.4 – Image of the spinach field trial location
Image of Block 1 (center) and Block 2 (left) and Block 3 (not shown) was to the right. Each block was in a separate terrace: Block 1 was the driest, Block 2 had the highest elevation, and Block 3 was the lowest elevation and wettest.

The blocks were replicated three times in space (Blocks 1, 2 and 3) for two growing periods (Period 1: August 28th – October 1st, 2011, and Period 2: September 29th – November 5th, 2011). The experiment was conducted in a terraced rice paddy field and left fallowed for over ten years; the local farmers harvested the grass or allowed cattle to graze the area, and burned once a year. The blocks could not be side by side in the field as the terraces were too small, and therefore were placed in separate terraces separated by a few meters (Figure 4.4). For each block, an area of 4 m by 2 m (8 m^2) was manually ploughed, with a hoe and a three-pronged tool, 15 cm deep, and all surface vegetation and the majority of the root mass was removed. A 30 cm wide ditch was excavated around each 8 m^2 block to divert the water flow from the higher terraces through two drains into the lower terraces. The blocks were divided into 16 experimental units of 0.3 m by 0.8 m (0.24 m^2) rectangular beds (Figure 4.3), separated by 20 cm wide ditches to limit the flooding, increase the drainage, increase air circulation, and function as a walking path. Two surface soil samples were taken from each block to measure organic content, nitrogen, phosphate, potassium, pH and EC levels (measured at the Agricultural University of Himachal Pradesh, India).

Run 1 had two blocks (1 and 2) and Run 2 had three blocks (1, 2 and 3). For Run 2, the soil of the Blocks 1 and 2 was tilled again to remove the clumps of clay formed by moisture (5 cm deep); the rain cover was kept in place after the first harvest so that the raised beds were not compromised with the heavy rainfall from the monsoons. During Run 3, Blocks 1 and 2 received 75% of the application rate of fertilizer in Table 4.4 as the plants were not grown for a typical full period of 55 days during Run 1 (Zvalo, 2008). Block 3 received 100% of the fertilizer application levels as it was not part of Run 1. Run 2 (Blocks 1, 2, and 3) was sown at the beginning of the regular season for spinach in Himachal Pradesh, India (Viswanathan et al., 2011).

Block 1 was the driest, having the fewest higher terraces draining into it. It was the first to be tilled and had the most root mass remaining at the end of tillage compared to Blocks 2 and 3. The raised beds were approximately 10 cm high. The block's beds were level in height and the ditches between beds rarely filled with water. The closest tree was 5 m away and approximately 6 m tall, giving minimal shade. Block 1 had several rocks and boulders surrounding it and one submerged boulder within the perimeter (surface area 50 by 20 cm). The bed of treatment mineral fertilizer, Year 3, was rotated by 90° and the

ditches of the surrounding raised beds were not as wide in order to make room for the boulder.

During heavy rainfall (Appendix 4.1 and Appendix 4.2), the terraces above Block 2 drained into the block, causing it to flood (Figure 4.5). The raised beds of Block 2 were not as uniform as Block 1; the plants on the east side were exposed to higher water levels than the west side. The raised beds were 8 to 13 cm in height. Human urine Year 1 was rotated 90° as the block was not a perfect square.

Figure 4.5 – Image of Block 2 during preparation
Blocks 1 and 2 flooded repeatedly during preparation

Block 3 was the largest, lowest, and initially the wettest. The surrounding terraces drained into this block, and extra time was put into building deeper ditches to limit flooding. The heights of the raised beds were not uniform, with the east side at 6 to 10 cm in height, and the west side at 10 to 16 cm in height. The tarpaulin was kept over the block for three weeks before planting to limit the rainfall and decrease the moisture content of the soil.

4.3.3 Fertilizer application

The soil was manually tilled, 3 cm deep, before and after the application of fertilizers, to incorporate the nutrients evenly. For the human urine treatment, undiluted urine was

applied based on a concentration of 6 g N L^{-1} of urine (Table 4.4). The 10 L containers were mixed together to have equal distribution of nutrients for all blocks. Before application, the containers were rocked back and forth to mix any product that had settled. The mineral fertilizer treatments used 46-0-0 (urea), 0-52-34 (phosphorus and potassium) and 0-0-60 (potassium) fertilizers. The total phosphate applied for each simulated year was 82.9, 248.8, 414.6, 580.5, 746.3 kg P ha^{-1}, for treatment year 1, 3, 5, 7, and 9, respectively. The total potassium for each year was 199.2, 597.7, 996.2, 13.94.7 and 1793.1 kg K ha^{-1}, respectively. For the combination treatment, urine was the nitrogen fertilizer and the same levels of phosphate and potassium were applied based on the mineral fertilizer treatment. All the fertilizers were applied on the same day (for dates of fertilizer application, planting and harvest, see Table 4.5). A 80 cm x 30 cm metal frame divided into 24 even squares (10 cm x 10 cm) was used to apply the fertilizer equally and to plant the seeds at proper spacing. After applying the fertilizer, the blocks were left exposed to 3mm of rain to moisten the soil, dissolve the fertilizer, and decrease germination injury (Ramoliya et al., 2003).

Table 4.4 – The fertilizer application rates for Simulated Years 1 through 9
The four fertilizer treatments were human urine, mineral fertilizer, combination (human urine and mineral fertilizer: phosphate and potassium), and the control (no fertilizer applied).

Simulated Year	Human	Mineral fertilizer[y]			Combination		
	Urine	46-0-0	0-52-34	0-0-60	Urine [z]	0-52-34 [y]	0-0-60 [y]
1	19.8	260.9	365.4	192.9	19.8	354.5	152.3
3	59.3	782.6	1096.2	578.8	59.3	1063.6	456.9
5	98.8	1304.3	1827.0	964.7	98.8	1772.6	761.4
7	138.3	1826.1	2557.9	1350.5	138.3	2481.6	1066.0
9	177.9	2347.8	3288.7	1736.4	177.9	3190.7	1370.6
Control	0	0	0	0	0	0	0

Note: 1 kg/ha = 1 mg/0.01 m^2 and 1 m^3 ha^{-1} = 1 ml/0.01 m^2
[z] units: m^3 ha^{-1}
[Y] units: kg ha^{-1}

Farmers may apply N fertilizer more than once during the growing season to maintain ideal concentrations as N volatilizes and leaches away (Chapter 2). For this experiment, one application was used based on the recommendations that the last urine application should be 30 days before harvest for edible crops (Schönning et al., 2004).

4.3.4 Spinach seed

Spinach NBR-Priya seeds were sourced from Noble Seeds Pvt. Ltd., Delhi, India and were guaranteed to have a minimum germination rate of 60%. The germination rate was tested by spreading the seeds onto a moist towel in an unsealed container. The inside of the container was maintained at 25^{o}C and kept in a dark room. The emergence and moisture of the towel was checked daily. In the field, the spinach seeds were over-sown (>3 per 10 cm x 10 cm area, 1.5 cm deep and covered lightly with soil) and later thinned to 40 plants per raised bed after emergence (Table 4.5). Every third day, leaf count, emergence, and visual assessment for any signs of stress was recorded for all plants. Each block was weeded every three days. The final emergence rate was recorded on day 14 and was calculated based on the thinned rate of on 72 plants.

4.3.5 Harvesting

The blocks were harvested in the morning (Table 4.5). The entire plant was pulled out of the soil where the shoot was cut from the root, and the shoot was placed into a labelled paper bag.

Table 4.5 – The spinach field trial dates
The dates of applying fertilizer, planting and harvesting for each block of the two growth periods, Run 1 and Run 2.

	Run	Fertilizer applied	Planted	Harvested
Block 1	1[z]	Aug 27	Aug 28	Sep 17
Block 2	1	Aug 29	Aug 30	Oct 1
Block 1	2	Oct 3	Oct 5	Nov 5
Block 2	2	Oct 4	Oct 5	Nov 5
Block 3	2	Sep 28	Sep 29	Oct 30

[z] Run 1 for Block 1 was 20 days, all other blocks grew for 32 days

4.3.6 Laboratory analysis

The treatments were analysed for biomass yield (fresh and dried), final length (bottom of stem to tip of the longest leaf), the chemical composition in the leaves, and the chemical composition of the soil. The shoot length (from the cut shoot to the tip of the longest leaf), fresh mass and dry mass were recorded on AGN 503-PO balance (AXIS LCGC, Paigah Colony, Hyderabad, India). The shoots were oven dried at 45^{o}C for 4 days in a Hot Air Oven Digital (Sunline, New Delhi, India) at the Agricultural University of Himachal Pradesh, India. The soil analysis was performed by the Agricultural University of Himachal Pradesh, India (Table 4.6). The dried plants were transported to McGill

University for chemical analysis of N-P-K, Na, Ca, Mn and Mg concentrations the method described in Parkinson (1975) using Lachat Instruments (QuickChem Method 13-115-01-1-B and 13-107-06-2-A, Lachat Instruments, 6645 West Mill Road, Milwaukee, WI 53218 USA).

Table 4.6 – Methodology used at the Agricultural University of Himachal Pradesh

Parameter	Methodology
Texture	International pipette method by Piper (1966)
pH & EC	Soil to water, 1:2.5
Organic content	Walkely and Black (1934)
Nitrogen	Alkaline permanganate method by Subbiah and Asija (1956)
Phosphate	0.5 m sodium bicarbonate, pH 8.5 by Olsen et al. (1954)
Potassium	Ammonium acetate, pH 7.0 by Black (1965)
Water holding capacity	Weight/weight basis method by Singh (1980)

4.3.7 Statistical analysis

For the statistical analysis, six plants were randomly chosen from each simulated year (six being the minimum number of plants which survived from all levels and blocks). The field experiment was a randomized complete block design (RCBD) and analyzed in an ANOVA for biomass and a MANOVA for soils and emergence rate using SAS 9.2 software (SAS Institute Inc., Cary, NC). The biomass and survival rate were analysed with an ANOVA. The effects of the treatments were computed by pairwise comparisons with the Duncan multiple range tests at alpha 0.05. The correlation between measured parameters was computed using the CORR procedure in SAS. A significant correlation was described by type: positive/negative; and by strength: weak (<0.3), moderate (0.3-0.7) and strong (>0.7). The power of the experiment was calculated in SAS with the calculated standard deviation and sample number. The standard deviation was calculated from the coefficient variance and the mean.

4.4 Results

4.4.1 Human urine

During storage, the human urine pH stabilized from an average of 6.3 to 9 and white precipitates formed on the bottom of the container. Further analysis of the urine for elements such as the nutrient concentration (N, P, K) was not possible in the rural location of Arla, Himachal Pradesh, India.

4.4.2 Initial soil characteristics

The initial physical (Table 4.7) and chemical composition (Table 4.8) of the soil are summarized below and were analyzed at the Agricultural University of Himachal Pradesh, India. The soil was a sandy loam with a high organic content (10.4%).

Table 4.7 – The physical composition of the soil
Surface soil in Arla, Kangra District, Himachal Pradesh, India. See Table 4.6 for test methods.

Clay (%)	12.5
Silt (%)	27.5
Sand (%)	57.5
Bulk density (Mg m^{-3})	1.1
Particle density (Mg m^{-3})	2.2
Water holding capacity	61.0
Porosity (%)	49.0

Table 4.8 – The chemical composition of the soil
The average chemical composition of the surface soil of Blocks 1, 2 and 3. The bracketed numbers were the ideal range supplied by the Agricultural University of Himachal Pradesh with the results. See Table 4.6 for test methods.

Organic content (g kg^{-1})	10.4	(5-10)
Nitrogen (kg ha^{-1})	535.3	(280-560)
Phosphorus (kg ha^{-1})	27.9	(10-25)
Potassium (kg ha^{-1})	70.2	(118-280)
EC_{SE} (dS m^{-1})	0.072	(< 2.0)
pH_{SE}	5.15	(6.5-7.5)

4.4.3 Power of the experiment

The plant parameters and survival rates were analyzed in an ANOVA. The soil parameters and emergence rate were calculated in a MANOVA. For the plant tissues, the magnesium, calcium, and manganese concentrations were below the power of 0.80, which were accepted, as these were not the main parameters of concern. Of the soil parameters, the nitrogen had a test power of ≤ 0.80 (Table 4.9).

Table 4.9 – The power of the experiment
The measured plant parameters (including the survival rate) were analyzed in an ANOVA. The measured soil parameters and the emergence rates were analyzed in a MANOVA. The power of the test was computed by calculating the standard deviation from the coefficient of variance and the mean (SAS 9.2, 2009); a power ≥ 0.8 was accepted.

PLANTS	Standard deviation	Power	N	Significant	Hypothesis
Survival (%)	0.159	0.988	48	Yes	Rejected
Fresh biomass (kg ha^{-1})	428.2	0.998	48	Yes	Rejected
Dry biomass (g $plant^{-1}$)	0.071	>0.999	48	Yes	Rejected
Shoot Length (cm)	3.887	0.997	48	Yes	Rejected
Number of leaves	0.704	>0.999	48	Yes	Rejected
Nitrogen (g kg^{-1})	3.384	0.994	48	Yes	Rejected
Phosphate (g kg^{-1})	1.049	>0.999	48	Yes	Rejected
Potassium (g kg^{-1})	10.65	0.85	48	No	Accepted
Sodium (g kg^{-1})	4.904	>0.999	48	Yes	Rejected
Magnesium (g kg^{-1})	1.797	0.526	48	No	Accepted
Calcium (g kg^{-1})	3.168	0.528	48	No	Accepted
Manganese (g kg^{-1})	0.098	0.391	48	No	Accepted
SOILS					
Emergence (%)	0.212	>0.999	80	Yes	Rejected
EC_{SE} (dS m^{-1})	0.958	>0.999	80	Yes	Rejected
pH_{SE}	0.558	0.922	80	Yes	Accepted
Nitrogen (kg ha^{-1})	321.3	0.772	80	No	Accepted
Phosphate (kg ha^{-1})	151.6	0.999	80	Yes	Rejected
Potassium (kg ha^{-1})	1699	0.995	80	Yes	Rejected

4.4.4 Emergence and survival rates

The tested germination rate of the spinach seed was 72%. The field emergence, as recorded on day 14, decreased through the simulated years. For Block 2, during Run 1, the emergence was only measured for 12 days as on day 14 it was raining and no measurement could be taken. The MANOVA model and treatment effects were significant ($P \leq 0.0001$) for the emergence rates (Table 4.10). The mineral fertilizer at Years 5 (40.6%), 7 (38.3%) and 9 (26.7%) and the combination fertilizer at Year 9 (43.6%) had significantly lower emergence rates to the human urine treatments (Year 5, 84.2%; Year 7, 83.3%; Year 9, 82.2%) and to the control (88.1%) (Figure 4.6).

Table 4.10 – Emergence and survival rates of spinach

The four fertilizer treatments were human urine, mineral fertilizer, combination and the control at simulated years (kg N ha^{-1}): 1 (120), 3 (360), 5 (600), 7 (840), and 9 (1080) with the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$ for each simulated year. Different letter indicates statistical significant difference within the simulated year.

	Simulated year				
Emergence (%)	1	3	5	7	9
Control	88.1% +/- 5.0%	88.1% +/- 5.0%	88.1% [a] +/- 5.0%	88.1% [a] +/- 5.0%	88.1% [a] +/- 5.0%
Human urine	91.9% +/- 3.5%	86.4% +/- 13.3%	84.2% [a] +/- 17.3%	83.3% [a] +/- 10.9%	82.2% [a] +/- 24.0%
Mineral fertilizer	82.8% +/- 17.2%	58.9% +/- 26.8%	40.6% [b] +/- 34.5%	38.3% [b] +/- 36.8%	26.7% [b] +/- 31.3%
Combination	90.3% +/- 6.9%	83.1% +/- 10.4%	75.8% [a] +/- 17.1%	77.5% [a] +/- 18.6%	43.6% [b] +/- 22.8%
Survival (%)					
Control	97.5% +/- 4.3%	97.5% +/- 4.3%	97.5% [a] +/- 4.3%	97.5% [a] +/- 4.3%	97.5% [a] +/- 4.3%
Human urine	99.2% +/- 1.4%	99.2% +/- 1.4%	98.3% [a] +/- 1.4%	98.3% [a] +/- 1.4%	88.3% [ab] +/- 12.6%
Mineral fertilizer	93.3% +/- 6.3%	78.3% +/- 7.6%	63.3% [b] +/- 29.0%	60.0% [b] +/- 36.8%	47.5% [c] +/- 28.8%
Combination	96.7% +/- 3.8%	90.0% +/- 13.2%	80.8% [ab] +/- 5.2%	79.2% [ab] +/- 12.3%	59.2% [bc] +/- 25.3%

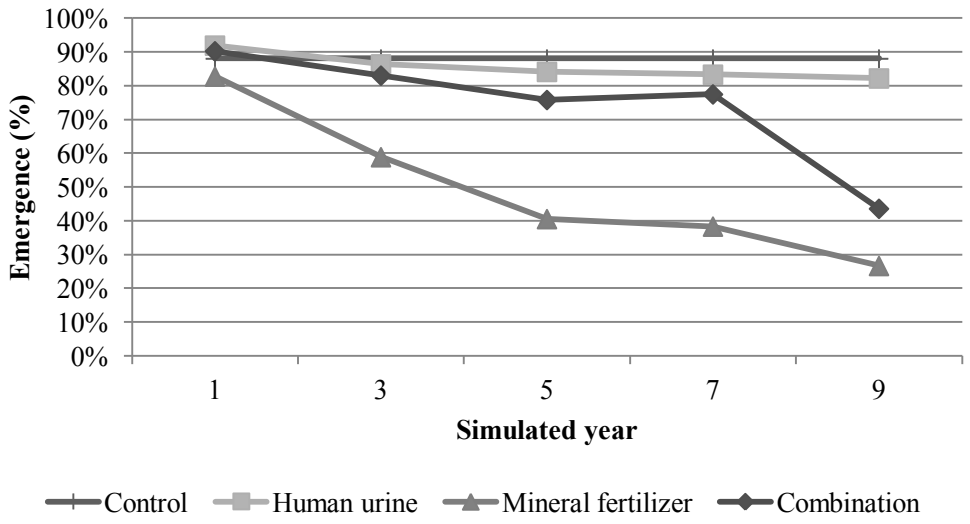

Figure 4.6 – The average emergence rates (%)
The four treatments (human urine, mineral fertilizer, combination, and the control) at Years 1 through 9. Refer to Table 4.10 for standard deviation and significant difference.

The survival rate ANOVA model (P = 0.002) and the treatment effects (P = 0.002) were significant for the second growth season (Table 4.10). The treatment units were thinned to 40 plants, this was the maximum survival rate. The mineral fertilizer treatment at Years 5 (63.3%), 7 (60.0%) and 9 (47.5%), had survival rates significantly lower to the control (97.7%) and to the human urine treatment (Figure 4.7). The combination fertilizer treatment at Year 9 (59.7%) was significantly lower to the control.

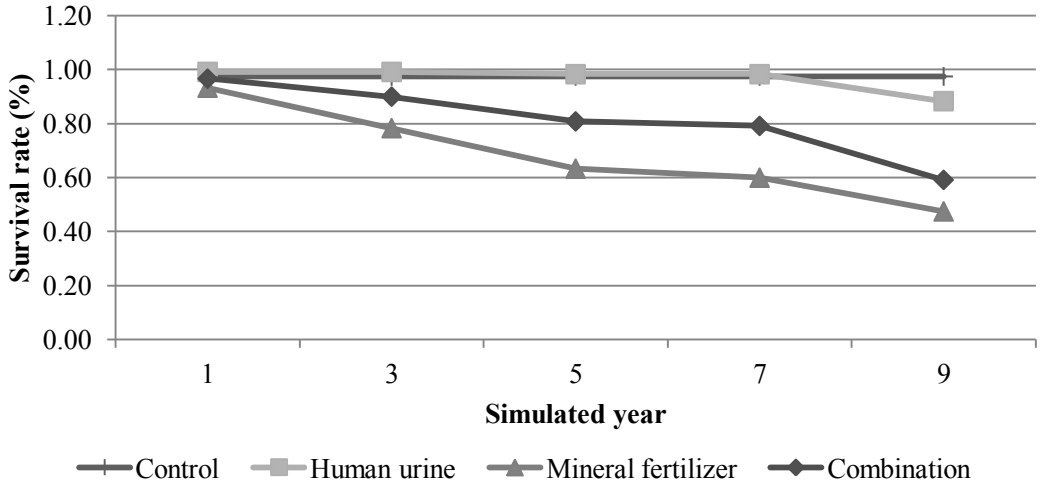

Figure 4.7 – The average survival rate of spinach
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control (hidden, 97.5%)) at Years 1 through 9. Refer to Table 4.10 for standard deviation and significant difference.

4.4.5 Soil chemical analysis

The EC_{SE} MANOVA model and the treatment effects were significant (both $P < 0.0001$) (Table 4.11). Treatments had significantly higher EC_{SE} compared to the control (0.40 dS m^{-1}): mineral fertilizer at Years 3 (2.10 dS m^{-1}), 5 (2.39 dS m^{-1}), 7 (2.63 dS m^{-1}), and 9 (4.15 dS m^{-1}); combination fertilizer at Year 5 (2.31 dS m^{-1}), 7 (2.63 dS m^{-1}), and 9 (4.04 dS m^{-1}) (Figure 4.8). None of the human urine conductivities were significantly different to the control, nor were the treatments above the threshold of 2.0 dS m^{-1} (Steppuhn et al., 2005). At Year 9, the human urine EC_{SE} (1.40 dS m^{-1}) was significantly lower than the mineral fertilizer and the combination fertilizer treatments. None of the soil samples reached an EC_{50} of 8.2 dS m^{-1}.

Table 4.11 – Analyzed soil components

The EC_{SE}, pH_{SE}, N, P, K of the soil from the four fertilizer treatments, human urine, mineral fertilizer, combination and the control, at simulated years (kg N ha^{-1}): 1 (120), 3 (360), 5 (600), 7 (840), and 9 (1080) with the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$ for each simulated year. Different letter indicates statistical significant difference within the simulated year.

	Simulated year														
EC_{SE} (dS m^{-1})	1			3			5			7			9		
Control	0.40	+/-	0.48	0.40 ^{b}	+/-	0.48	0.40 ^{b}	+/-	0.48	0.40 ^{b}	+/-	0.48	0.40 ^{b}	+/-	0.48
Human urine	1.03	+/-	0.73	1.20^{ab}	+/-	0.79	1.24^{ab}	+/-	0.96	1.64^{ab}	+/-	0.89	1.42 ^{b}	+/-	1.11
Mineral fertilizer	0.40	+/-	0.32	2.10 ^{a}	+/-	1.50	2.39 ^{a}	+/-	1.90	2.63 ^{a}	+/-	1.16	4.15 ^{a}	+/-	1.04
Combination	0.63	+/-	0.25	1.20^{ab}	+/-	0.69	2.31 ^{a}	+/-	1.59	2.63 ^{a}	+/-	2.12	4.04 ^{b}	+/-	1.40
pH_{SE}															
Control	4.65	+/-	0.40	4.65	+/-	0.40	4.65	+/-	0.40	4.65	+/-	0.40	4.65	+/-	0.40
Human urine	4.21	+/-	0.11	4.15	+/-	0.33	4.21	+/-	0.11	4.49	+/-	0.45	4.65	+/-	0.72
Mineral fertilizer	4.43	+/-	0.23	4.53	+/-	0.70	5.01	+/-	0.91	4.95	+/-	0.84	5.35	+/-	0.92
Combination	4.49	+/-	0.17	4.47	+/-	0.47	4.67	+/-	0.64	5.01	+/-	0.63	5.17	+/-	0.70
Nitrogen (kg ha^{-1})															
Control	338.80	+/-	62.85	338.80	+/-	62.85	338.80	+/-	62.85	338.80 ^{b}	+/-	62.85	338.80 ^{b}	+/-	62.85
Human urine	420.40	+/-	192.26	529.20	+/-	259.69	608.60	+/-	385.30	654.40^{ab}	+/-	236.09	701.00^{ab}	+/-	309.33
Mineral fertilizer	492.40	+/-	194.61	609.60	+/-	333.17	681.00	+/-	372.24	781.20^{ab}	+/-	474.66	1015.60 ^{a}	+/-	538.44
Combination	691.40	+/-	773.59	497.40	+/-	349.26	632.40	+/-	407.81	833.00 ^{a}	+/-	607.54	845.20 ^{a}	+/-	535.80
Phosphate (kg ha^{-1})															
Control	4.00	+/-	3.00	4.00	+/-	3.00	4.00 ^{c}	+/-	3.00	4.00 ^{c}	+/-	3.00	4.00 ^{c}	+/-	3.00
Human urine	19.82	+/-	24.56	10.82	+/-	10.52	14.62^{cb}	+/-	12.31	26.20^{bc}	+/-	17.91	135.80^{bc}	+/-	196.82
Mineral fertilizer	86.40	+/-	76.94	149.74	+/-	143.75	239.24^{ab}	+/-	140.63	326.10 ^{a}	+/-	211.50	347.70^{ab}	+/-	281.80
Combination	85.00	+/-	53.17	174.20	+/-	98.93	255.02 ^{a}	+/-	180.99	229.46^{ab}	+/-	219.90	394.22 ^{a}	+/-	255.44
Potassium (kg ha^{-1})															
Control	117.80	+/-	47.74	117.80	+/-	47.74	117.80 ^{b}	+/-	47.74	117.80 ^{b}	+/-	47.74	117.80 ^{b}	+/-	47.74
Human urine	1199.80	+/-	1426.37	247.20	+/-	153.60	1556.60^{ab}	+/-	3074.79	820.00^{ab}	+/-	1099.82	891.40 ^{b}	+/-	1156.20
Mineral fertilizer	841.60	+/-	729.33	2140.60	+/-	1263.05	3092.20 ^{a}	+/-	2613.43	3295.00 ^{a}	+/-	2491.77	4786.80 ^{a}	+/-	2526.84
Combination	937.80	+/-	786.35	1348.80	+/-	1847.63	2340.00^{ab}	+/-	2530.12	3071.20 ^{a}	+/-	2995.69	3727.00 ^{a}	+/-	1871.75

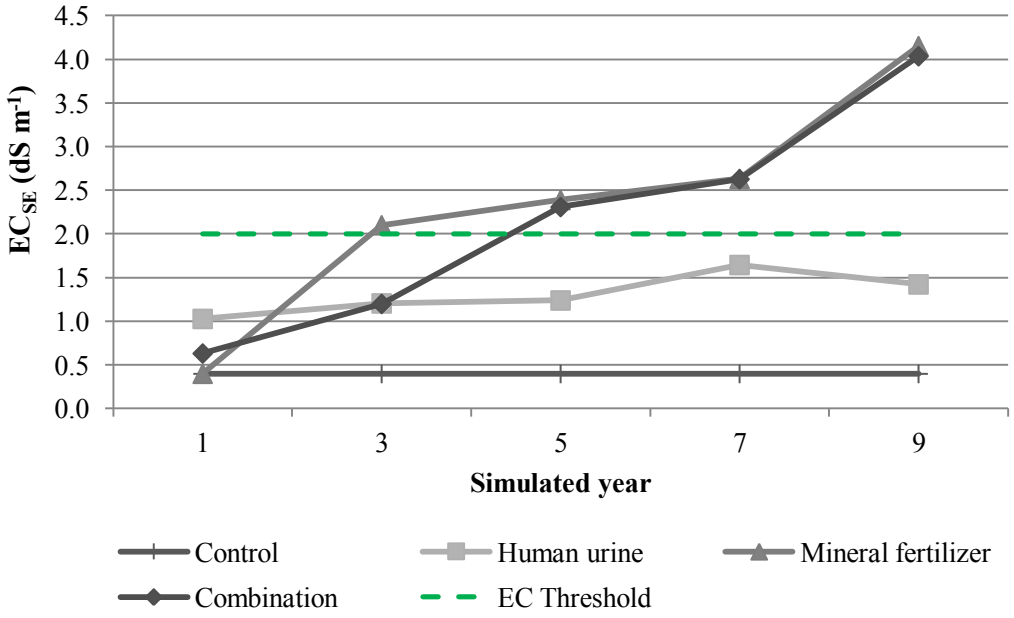

Figure 4.8 – The average EC_{SE} of the soil
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control (hidden, 0.40 dS m^{-1})) at Years 1 through 9. The red line, at 2 dS m^{-1}, is the maximum EC_{SE} threshold for spinach (Steppuhn et al., 2005). Refer to Table 4.11 for standard deviation and significant difference.

The MANOVA model for the pH_{SE} of the soil samples (P = 0.0102) and the treatment effects (P = 0.0277) were significant, though none of the treatments were significantly different to the control (Table 4.11). There is no significant difference between treatments in any given year. All treatments were acidic, with a range from 4.15 to 5.35 (Figure 4.9).

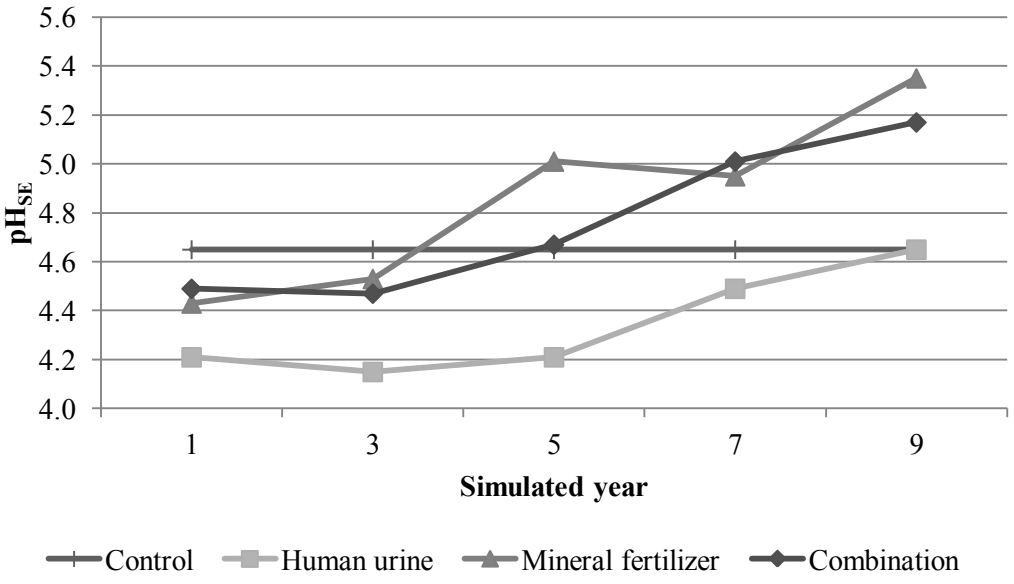

Figure 4.9 – The average pH_{SE} of the soil
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.11 for standard deviation.

The MANOVA model for the nitrogen concentration ($P < 0.0001$) in the soil was significant and the treatment effects ($P = 0.161$) was not significant (Table 4.11). The concentration of nitrogen increased linearly with the increase of the fertilizer rate (Figure 4.10).

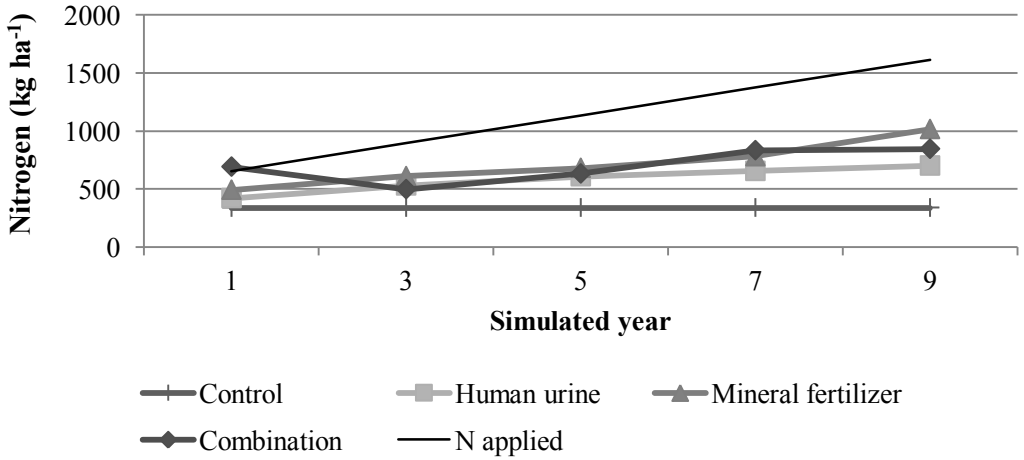

Figure 4.10 – The average nitrogen concentration in the soil
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) for Years 1 through 9. N applied is the mass of nitrogen applied to the soil, starting at the original soil concentration of 535.3 kg N ha^{-1}. Refer to Table 4.11 for standard deviation and significant difference.

The phosphate MANOVA model (P = 0.0002) and the treatment effects (P = 0.0001) were significant (Table 4.11). At Years 5, 7 and 9, the mineral fertilizer (239.24 kg P ha^{-1}, 326.10 kg P ha^{-1}, and 347.70 kg P ha^{-1}, respectively) and the combination fertilizer treatments (255.02 kg P ha^{-1}, 229.46 kg P ha^{-1}, and 394.22 kg P ha^{-1}, respectively) were significantly higher than the control (4.00 kg P ha^{-1}). No human urine phosphate soil concentrations were significantly different to the control. At Year 5, the human urine treatment (14.62 kg P ha^{-1}) was significantly lower to the combination treatment. At Year 7, the human urine treatment (26.20 kg P ha^{-1}) was significantly lower to the mineral fertilizer treatment. At Year 9, the human urine treatment (135.80 kg P ha^{-1}) was significantly lower to the combination treatment (Figure 4.11).

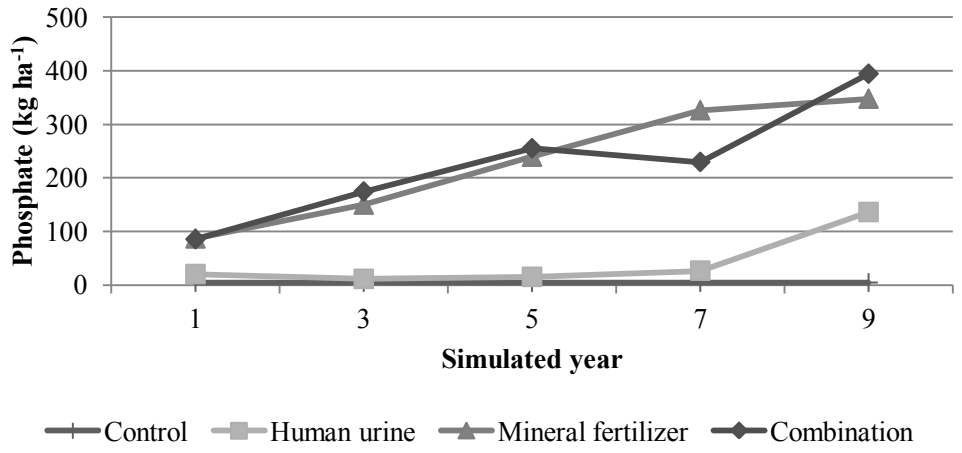

Figure 4.11 – The average phosphate concentration in the soil
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) for Years 1 through 9. Refer to Table 4.11 for standard deviation and significant difference.

The ANOVA model of concentration of potassium in the soil (P < 0.0001) and the treatment effects (P = 0.0006) were significant (Table 4.11). The mineral fertilizer and combination fertilizer treatments increased linearly as the fertilizer rate increased, while the human urine treatment remained low (Figure 4.12). The mineral fertilizer at Years 5 (3092.20 kg K ha^{-1}), 7 (3295.00 kg K ha^{-1}) and 9 (4786.80 kg K ha^{-1}) and the combination fertilizer at Years 7 (3071.20 kg K ha^{-1}) and 9 (3727.00 kg K ha^{-1}) had potassium concentrations significantly higher to the control (117.80 kg K ha^{-1}). None of the human urine treatments were significantly different to the control. At Year 9, the human urine treatment (891.40 kg K ha^{-1}) was significantly lower to the two other treatments.

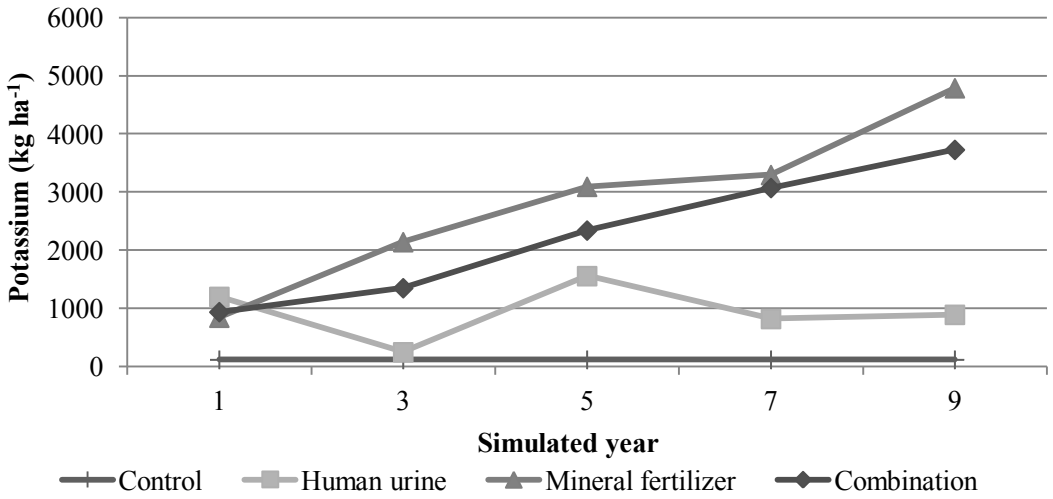

Figure 4.12 – The average potassium concentration in the soil
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) for Years 1 through 9. Refer to Table 4.11 for standard deviation and significant difference.

4.4.6 Biomass physical and chemical analysis

The biomass produced from Run 1 was too small to be analyzed, as the individual shoots could not register on the balance of 0.001g accuracy, and the collected biomass from each treatment was too small to be chemically analyzed. The average dry biomass per plant for the levels for Run 2 was large enough for analysis. The following results are thus from Run 2. The physical properties are summarized in Table 4.12. The concentrations of elements are summarized in Table 4.13.

The fresh biomass was calculated based on the total mass produced per 0.01 m^2 area. The fresh mass ANOVA model and treatment effects were significant (both P = 0.0003). The control (51 kg ha^{-1}) was significantly lower compared to the human urine at Years 7 (890 kg ha^{-1}) and 9 (1479 kg ha^{-1}); mineral fertilizer at Year 5 (949 kg ha^{-1}); and all of the combination fertilizer treatments (Figure 4.13). The combination fertilizer treatment overall produced a higher harvest and peaked in production at Year 3 (1769 kg ha^{-1}). Mineral fertilizer fresh biomass production peaked at Year 5 (949 Kg ha^{-1}). The human urine treatment was significantly higher to the mineral fertilizer treatment (215 kg ha^{-1}) at Year 9 where human urine produced its highest yield.

Table 4.12 – Spinach biomass production for Run 2

The measured biomass components from the four fertilizer treatments, human urine, mineral fertilizer, combination and the control, at simulated years (kg N ha^{-1}): 1 (120), 3 (360), 5 (600), 7 (840), and 9 (1080) with the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$ for each simulated year. Different letter indicates statistical significant difference within the simulated year.

	Simulated year											
Fresh mass (kg ha^{-1})	**1**			**3**			**5**			**7**			**9**		
Control	51 ^{b}	+/-	52	51 ^{b}	+/-	52	51 ^{b}	+/-	52	51 ^{c}	+/-	52	51 ^{c}	+/-	52
Human urine	307 ^{b}	+/-	193	619 ^{b}	+/-	317	647^{ab}	+/-	498	890^{ab}	+/-	103	1479 ^{a}	+/-	972
Mineral fertilizer	843^{ab}	+/-	213	776 ^{b}	+/-	450	949 ^{a}	+/-	856	424^{bc}	+/-	316	215^{bc}	+/-	251
Combination	1143 ^{a}	+/-	384	1769 ^{a}	+/-	86	1464 ^{a}	+/-	547	1688 ^{a}	+/-	327	1022^{ab}	+/-	985
Dry mass (g $plant^{-1}$)															
Control	0.03 ^{b}	+/-	0.02	0.03 ^{c}	+/-	0.02	0.03 ^{c}	+/-	0.02	0.03 ^{c}	+/-	0.02	0.03 ^{b}	+/-	0.02
Human urine	0.13^{ab}	+/-	0.03	0.21^{bc}	+/-	0.08	0.25 ^{b}	+/-	0.15	0.28^{ab}	+/-	0.09	0.34 ^{a}	+/-	0.18
Mineral fertilizer	0.14^{ab}	+/-	0.04	0.22 ^{b}	+/-	0.11	0.31^{ab}	+/-	0.09	0.15^{bc}	+/-	0.07	0.09 ^{b}	+/-	0.06
Combination	0.26 ^{a}	+/-	0.13	0.41 ^{a}	+/-	0.12	0.48 ^{a}	+/-	0.11	0.38 ^{a}	+/-	0.07	0.31 ^{a}	+/-	0.13
Shoot length (cm)															
Control	2.87 ^{c}	+/-	0.36	2.87 ^{b}	+/-	0.36	2.87 ^{b}	+/-	0.36	2.87 ^{c}	+/-	0.36	2.87 ^{c}	+/-	0.36
Human urine	9.88 ^{a}	+/-	2.85	13.02 ^{a}	+/-	3.87	14.03 ^{a}	+/-	4.64	22.06 ^{a}	+/-	12.42	18.27 ^{a}	+/-	3.77
Mineral fertilizer	13.64 ^{a}	+/-	0.98	14.75 ^{a}	+/-	2.08	15.76 ^{a}	+/-	1.17	12.38 ^{b}	+/-	2.07	9.84 ^{b}	+/-	2.95
Combination	15.11 ^{a}	+/-	3.40	18.41 ^{a}	+/-	2.53	18.21 ^{a}	+/-	3.33	18.80^{ab}	+/-	0.43	15.38^{ab}	+/-	2.40
# leaves (per plant)															
Control	2.72 ^{b}	+/-	0.10	2.72 ^{b}	+/-	0.10	2.72 ^{c}	+/-	0.10	2.72 ^{c}	+/-	0.10	2.72 ^{b}	+/-	0.10
Human urine	5.50 ^{a}	+/-	0.33	6.28 ^{a}	+/-	1.08	6.17 ^{b}	+/-	1.42	6.78 ^{ab}	+/-	0.25	6.89 ^{a}	+/-	0.51
Mineral fertilizer	6.72 ^{a}	+/-	0.59	6.83 ^{a}	+/-	0.76	7.50 ^{ab}	+/-	1.01	6.50 ^{b}	+/-	0.76	6.11 ^{a}	+/-	0.67
Combination	6.67 ^{a}	+/-	0.58	7.50 ^{a}	+/-	1.01	7.72 ^{a}	+/-	0.42	7.94 ^{a}	+/-	0.10	7.22 ^{a}	+/-	0.69

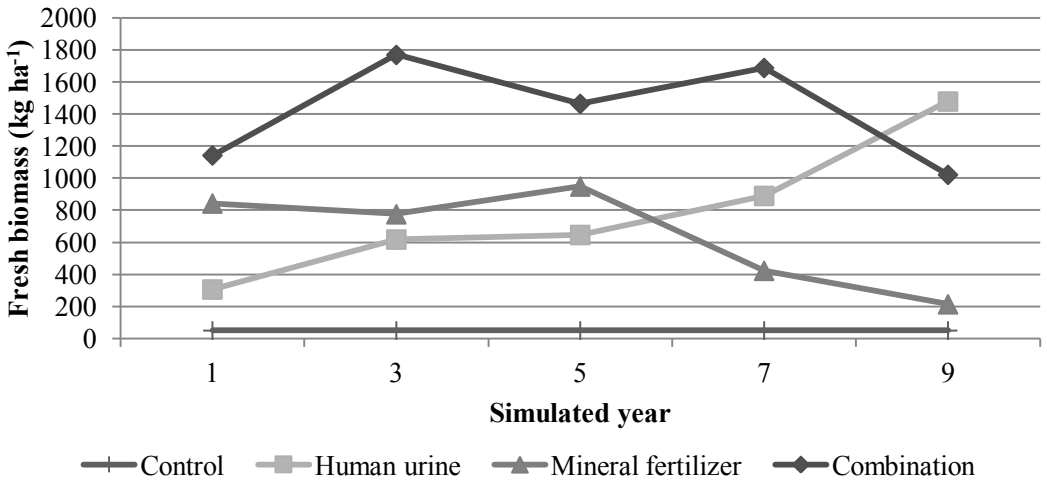

Figure 4.13 – The fresh biomass produced (kg ha^{-1})
The four treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.12 for standard deviation and significant difference.

The average dry mass per plant ANOVA model and the treatment effects (both P = 0.0001) were significant (Table 4.12). The control at 0.03 g plant^{-1} had the lowest yield, and the combination treatment at Year 5 had the highest yield at 0.482 g plant^{-1} (Figure 4.14). The average yield of the human urine treatments increased linearly as the fertilizer application levels increased. Human urine at Year 5 (0.25 g plant^{-1}), 7 (0.28 g plant^{-1}) and 9 (0.34 g plant^{-1}) were significantly higher to the control. All of the combination treatments simulated years were significantly higher to the control. The mineral fertilizer (0.13 g plant^{-1}) and combination fertilizer (0.48 g plant^{-1}) treatments experienced a quadratic response with a local maximum at Year 5.The human urine and mineral fertilizer treatments were not significantly different for Year 1 through 7. At Year 9, the human urine treatment (0.34 g plant^{-1}) was significantly higher than the mineral fertilizer treatment (0.09 g plant^{-1}).

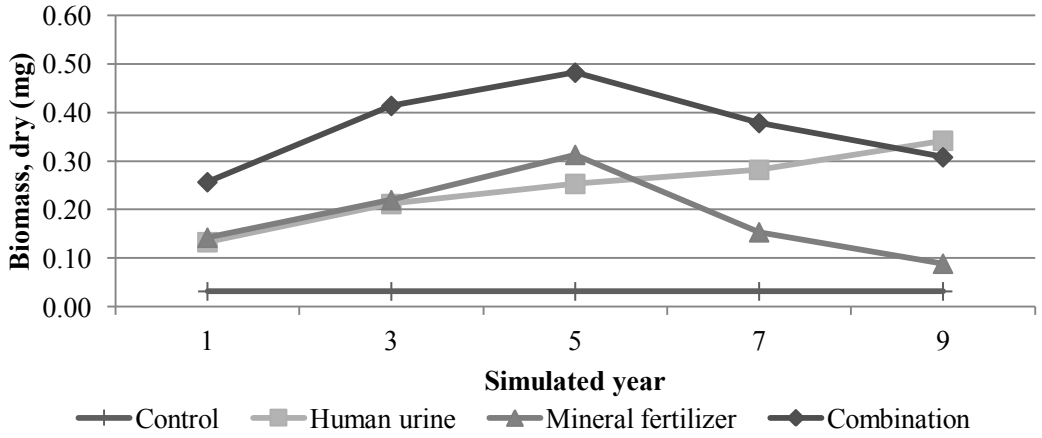

Figure 4.14 – The average dry biomass per plant
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.12 for standard deviation and significant difference.

The shoot lengths of the spinach had a significant ANOVA model and treatment effects (both P = 0.0005) for Run 2 (Table 4.12). All treatments were significantly longer than the control (2.87 cm). Human urine treatment at Year 7 had the longest shoot (22.06 cm) (Figure 4.15). At Years 7 and 9, the human urine treatment (22.06 cm and 18.27 cm, respectively) was significantly longer than the mineral fertilizer treatments (12.38 cm and 9.84 cm, respectively).

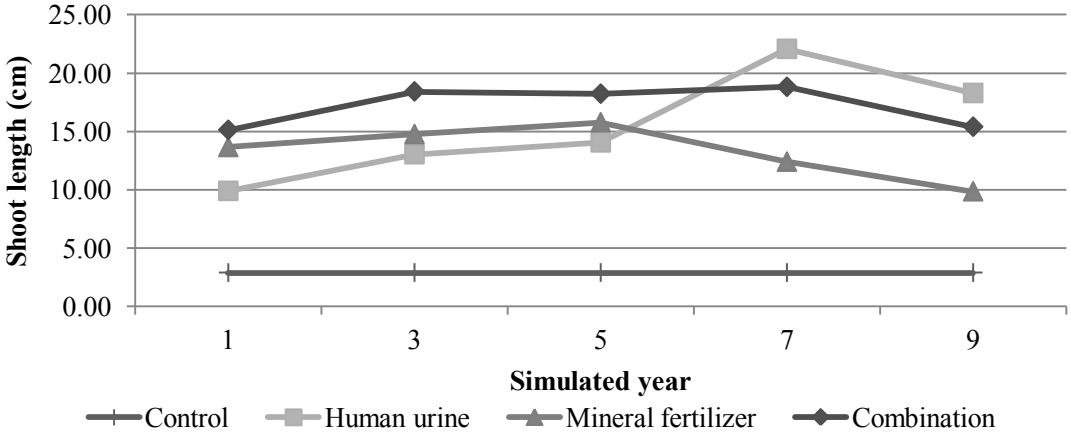

Figure 4.15 – The average spinach shoot length
The four treatments (human urine, mineral fertilizer, combination and the control) for Years 1 through 9. Refer to Table 4.12 for standard deviation and significant difference.

The ANOVA model and the treatment effects were significant (both $P < 0.001$) for the average number of leaves per plant (Table 4.12). All treatments had significantly more leaves than the control (2.72 leaves plant^{-1}). The human urine and mineral fertilizer treatments were not significantly different at any year. The combination treatment at Year 7 had the most leaves (7.94 leaves plant^{-1}) (Figure 4.16).

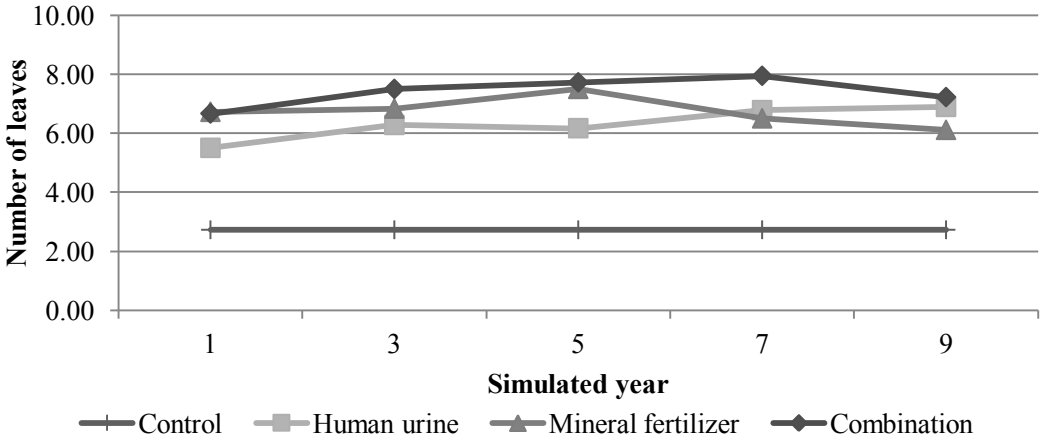

Figure 4.16 – The average number of leaves per spinach plant
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.12 for standard deviation and significant difference.

The concentration of nitrogen in the dry biomass (Figure 4.17) increased linearly as the fertilizer application rates increased. The ANOVA model for nitrogen concentration ($P < 0.0001$) and the treatment effects ($P = 0.008$) were significant (Table 4.13). All treatments were significantly higher than the control (21.53 g N kg^{-1}). None of the treatments were significantly different to each other through Years 1 to 9. The highest concentration of nitrogen was in the human urine treatment, Year 9 (37.83 g N kg^{-1}).

Table 4.13 – Nutrient concentrations in spinach tissue for Run 2.
The chemical analysis of N, P, K, Na, Mg, Ca, and Mn for the four fertilizer treatments, human urine, mineral fertilizer, combination and the control, at simulated years (kg N ha^{-1}): 1 (120), 3 (360), 5 (600), 7 (840), and 9 (1080) with the standard deviation. Mean separation by Duncan's multiple range test at $P \leq 0.05$ for each simulated year. Different letter indicates statistical significant difference within the simulated year. The potassium, calcium, magnesium, and manganese ANOVA models were not significant.

	Simulated year									
Nitrogen (g kg^{-1})	**1**		**3**		**5**		**7**		**9**	
Control	21.53 [b]	+/- 2.83	21.53 [b]	+/- 2.83	21.53 [b]	+/- 2.83	21.53 [b]	+/- 2.83	21.53 [b]	+/- 2.83
Human urine	31.96 [a]	+/- 5.62	34.40 [a]	+/- 5.66	32.90 [a]	+/- 3.63	33.94 [a]	+/- 4.96	37.83 [a]	+/- 4.57
Mineral fertilizer	29.92 [a]	+/- 1.22	33.36 [a]	+/- 4.33	34.35 [a]	+/- 5.65	34.39 [a]	+/- 1.66	36.03 [a]	+/- 5.65
Combination	30.24 [a]	+/- 1.34	34.01 [a]	+/- 6.77	35.98 [a]	+/- 5.89	35.88 [a]	+/- 4.02	36.74 [a]	+/- 7.87
Phosphate (g kg^{-1})										
Control	0.62 [b]	+/- 0.07	0.62 [b]	+/- 0.07	0.62 [b]	+/- 0.07	0.62 [b]	+/- 0.07	0.62 [b]	+/- 0.07
Human urine	1.21 [b]	+/- 0.59	1.89 [b]	+/- 1.20	1.53 [b]	+/- 0.73	1.70 [b]	+/- 0.81	1.62 [b]	+/- 0.14
Mineral fertilizer	3.04 [a]	+/- 1.05	3.39 [ab]	+/- 0.49	3.65 [ab]	+/- 0.60	5.53 [a]	+/- 0.65	4.24 [a]	+/- 0.44
Combination	4.36 [a]	+/- 1.82	4.09 [a]	+/- 1.24	3.23 [a]	+/- 0.90	3.82 [a]	+/- 0.91	5.28 [a]	+/- 2.92
Potassium (g kg^{-1})										
Control	22.90	+/- 1.22	22.90	+/- 1.22	22.90	+/- 1.22	22.90	+/- 1.22	22.90	+/- 1.22
Human urine	32.60	+/- 15.01	27.65	+/- 13.23	16.73	+/- 2.15	27.88	+/- 12.02	43.27	+/- 9.48
Mineral fertilizer	25.88	+/- 7.99	45.15	+/- 9.20	36.71	+/- 8.84	28.35	+/- 17.30	31.81	+/- 19.43
Combination	21.69	+/- 5.56	19.42	+/- 4.42	26.71	+/- 13.92	16.92	+/- 1.69	18.31	+/- 1.14
Sodium (g kg^{-1})										
Control	16.96 [b]	+/- 7.56	16.96 [a]	+/- 7.56	16.96 [b]	+/- 7.56	16.96 [b]	+/- 7.56	16.96 [bc]	+/- 7.56
Human urine	28.77 [a]	+/- 9.82	23.77 [a]	+/- 7.29	27.83 [a]	+/- 6.00	31.83 [a]	+/- 3.13	29.08 [a]	+/- 3.57
Mineral fertilizer	8.08 [b]	+/- 4.59	7.71 [b]	+/- 4.75	10.04 [b]	+/- 1.28	6.71 [c]	+/- 3.00	7.63 [c]	+/- 1.16
Combination	13.65 [b]	+/- 3.10	15.65 [ab]	+/- 3.86	14.46 [b]	+/- 3.56	17.40 [b]	+/- 5.13	23.50 [ab]	+/- 7.93

(Table 4.13 Continued)	Simulated year									
Magnesium (g kg^{-1})	**1**		**3**		**5**		**7**		**9**	
Control	7.19	+/- 1.41								
Human urine	5.94	+/- 2.01	5.83	+/- 3.66	4.98	+/- 1.70	5.08	+/- 0.59	4.63	+/- 1.07
Mineral fertilizer	8.13	+/- 2.81	6.56	+/- 2.14	8.13	+/- 1.97	6.77	+/- 1.88	6.40	+/- 1.43
Combination	7.63	+/- 1.35	6.08	+/- 0.85	5.19	+/- 1.19	6.15	+/- 0.82	6.46	+/- 1.16
Calcium (g kg^{-1})										
Control	10.44	+/- 3.47								
Human urine	16.13	+/- 4.01	16.21	+/- 6.93	12.40	+/- 4.17	10.27	+/- 2.44	12.67	+/- 1.79
Mineral fertilizer	11.94	+/- 1.96	14.33	+/- 5.83	11.21	+/- 1.36	14.08	+/- 2.15	15.33	+/- 3.26
Combination	12.50	+/- 1.75	11.15	+/- 4.23	12.21	+/- 3.25	13.29	+/- 3.04	11.21	+/- 1.86
Manganese (g kg^{-1})										
Control	0.28	+/- 0.09								
Human urine	0.31	+/- 0.13	0.24	+/- 0.13	0.23	+/- 0.10	0.28	+/- 0.05	0.19	+/- 0.04
Mineral fertilizer	0.35	+/- 0.05	0.31	+/- 0.08	0.30	+/- 0.06	0.27	+/- 0.09	0.28	+/- 0.03
Combination	0.41	+/- 0.17	0.34	+/- 0.15	0.29	+/- 0.17	0.33	+/- 0.09	0.30	+/- 0.05

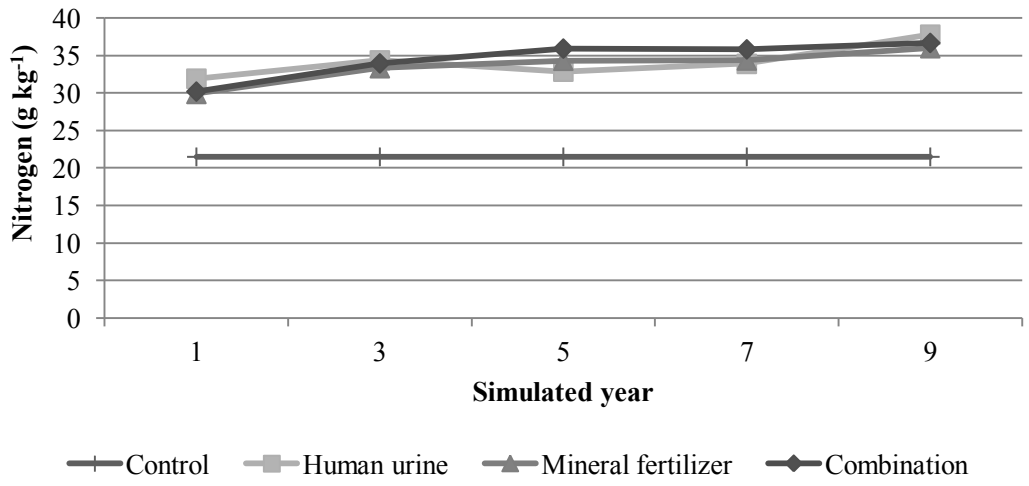

Figure 4.17 – The average nitrogen concentration in spinach tissue
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.13 for standard deviation and significant difference.

The ANOVA model for the concentration of phosphate in the biomass (Figure 4.18) and the treatments effects were significant (both $P < 0.0001$) during Run 2 (Table 4.13). Human urine at all years was not significantly different to the control (0.62 g P kg^{-1}). The mineral fertilizer at Year 7 had the highest concentration (5.53 g P kg^{-1}). The combination fertilizer experienced an inverse quadratic response with a local minimum at Year 5 (3.23 g P kg^{-1}).

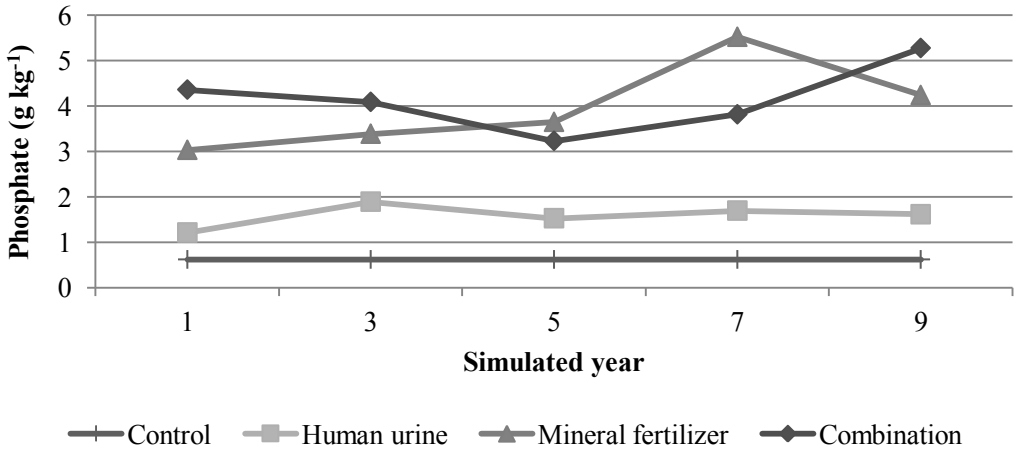

Figure 4.18 – The average phosphate concentration in spinach tissue
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.13 for standard deviation and significant difference.

The ANOVA model of the concentration of sodium in the biomass (Figure 4.19) and the treatment effects were significant (both $P < 0.0001$) during Run 2 (Table 4.13). Human urine treatments at all years contained the five highest sodium concentrations, Year 7 had the highest concentration (31.83 g Na kg^{-1}). Mineral fertilizer had the five lowest sodium concentrations, Year 7 was the lowest (6.71 g Na kg^{-1}), which was significantly lower to the control (16.96 g Na kg^{-1}).

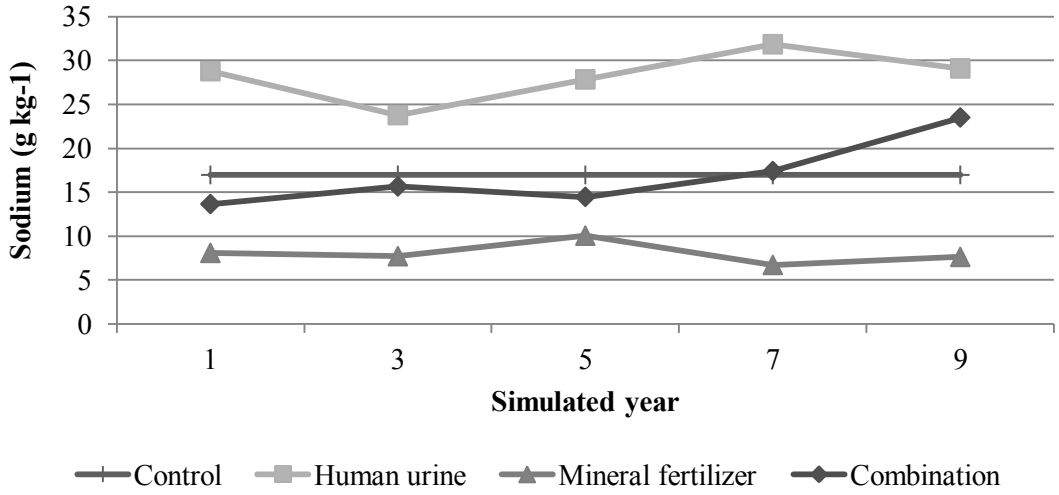

Figure 4.19 – The average sodium concentration in spinach tissue
The four fertilizer treatments (human urine, mineral fertilizer, combination and the control) at Years 1 through 9. Refer to Table 4.13 for standard deviation and significant difference.

The following photographs (Figure 4.20 to Figure 4.25) are of the spinach plants in the raised soil beds one day before harvest. There were no signs of disease, but pest damage was observed for all treatments.

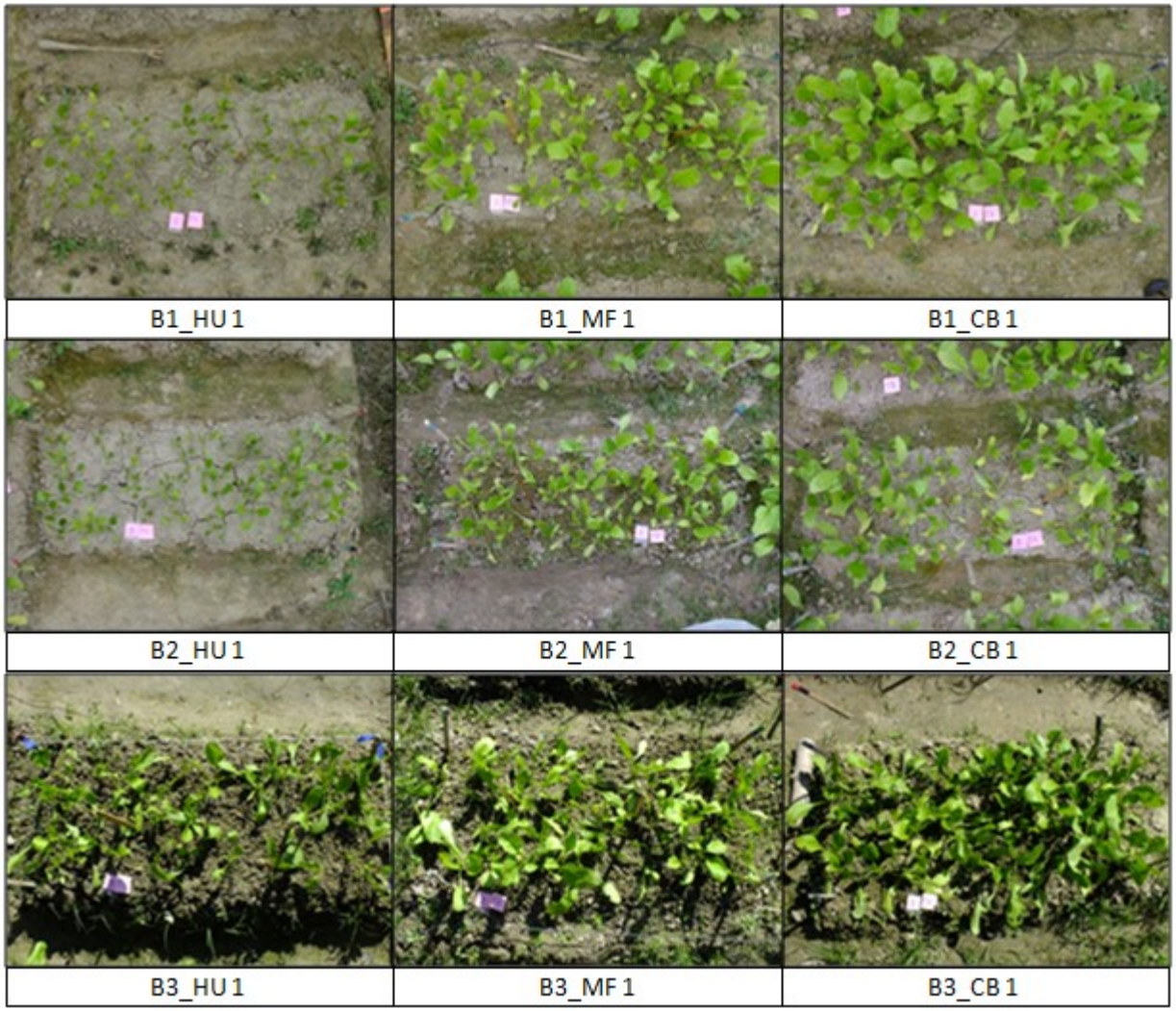

Figure 4.20 – Visual presentation of the spinach plants at Simulated Year 1
For three treatments: human urine (HU); mineral fertilizer (MF); and combination (CB) for each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3).

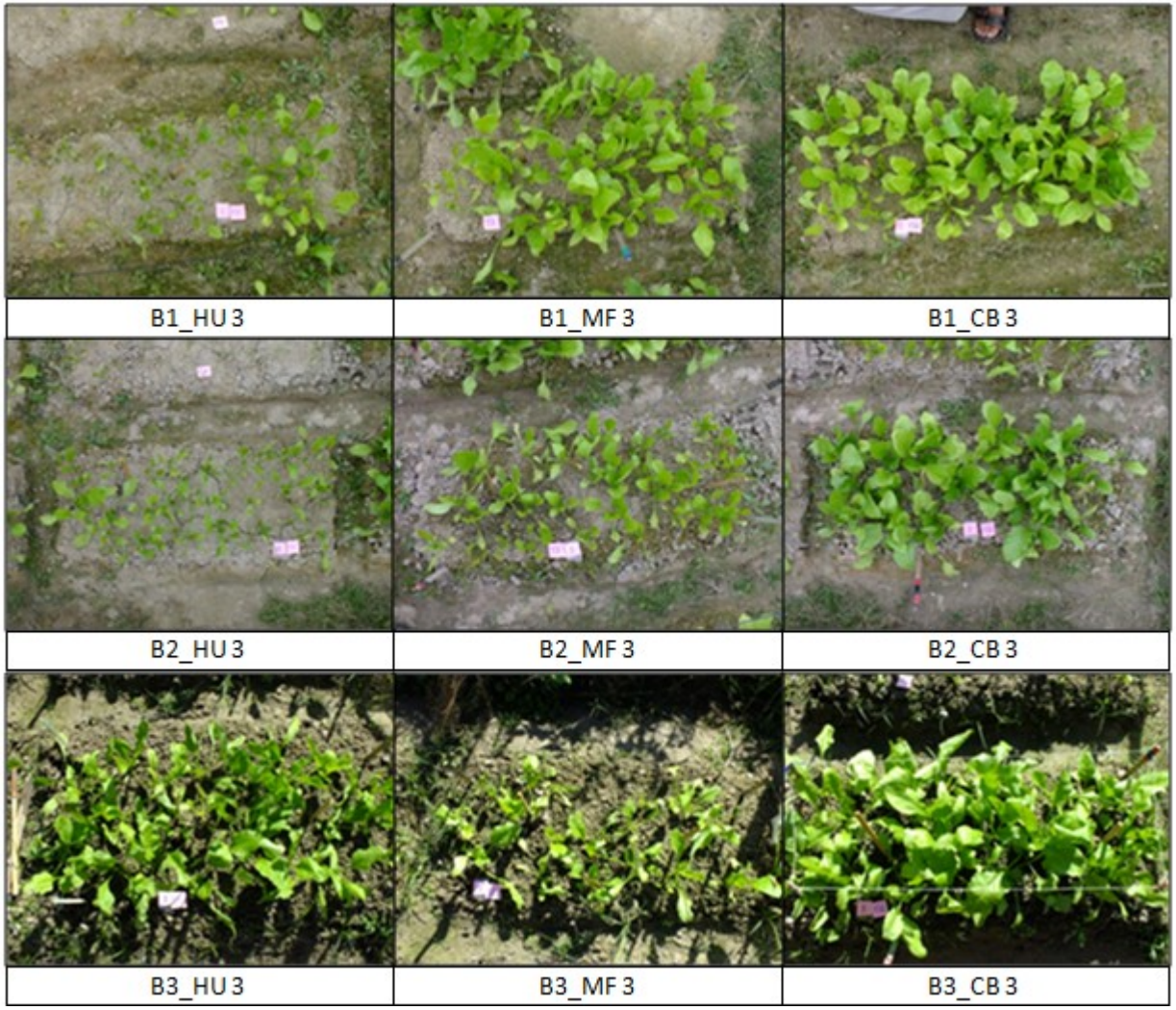

Figure 4.21 – Visual presentation of the spinach plants at Simulated Year 3
Tor three treatments: human urine (HU); mineral fertilizer (MF); and combination (CB) for each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3).

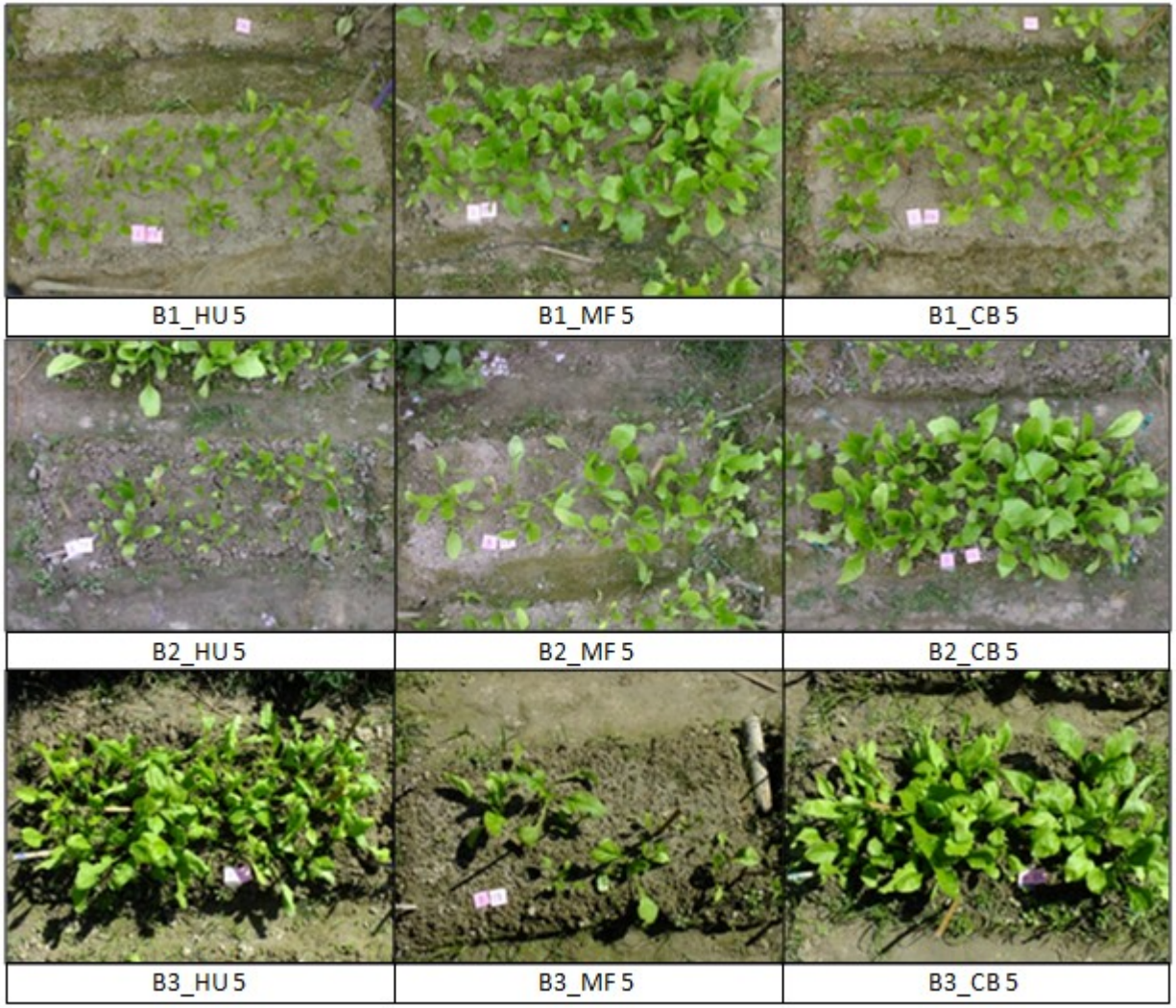

Figure 4.22 – Visual presentation of the spinach plants at Simulated Year 5
For the three treatments: human urine (HU); mineral fertilizer (MF); and combination (CB) for each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3).

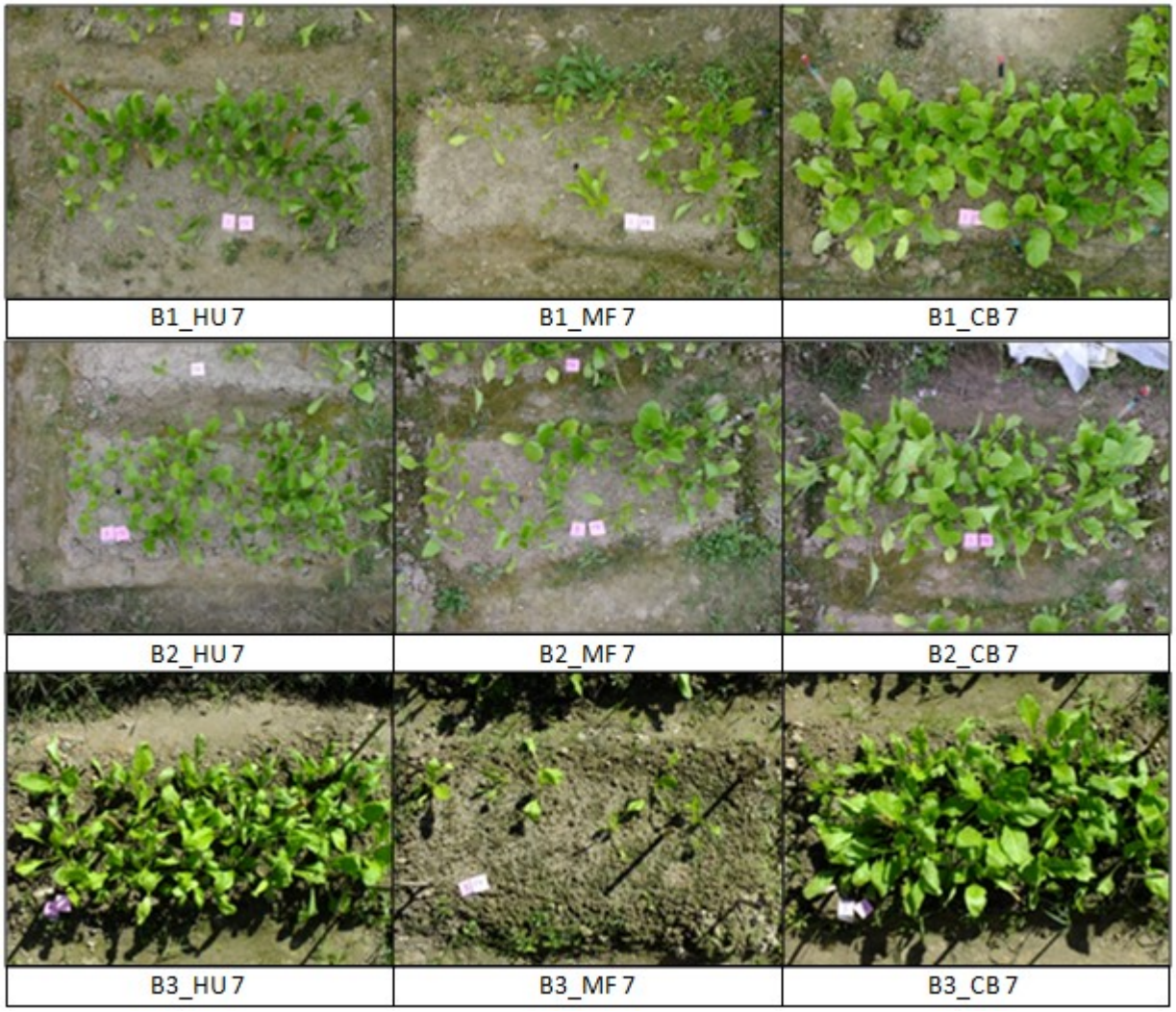

Figure 4.23 – Visual presentation of the spinach plants at Simulated Year 7
For the three treatments: human urine (HU); mineral fertilizer (MF); and combination (CB) for each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3).

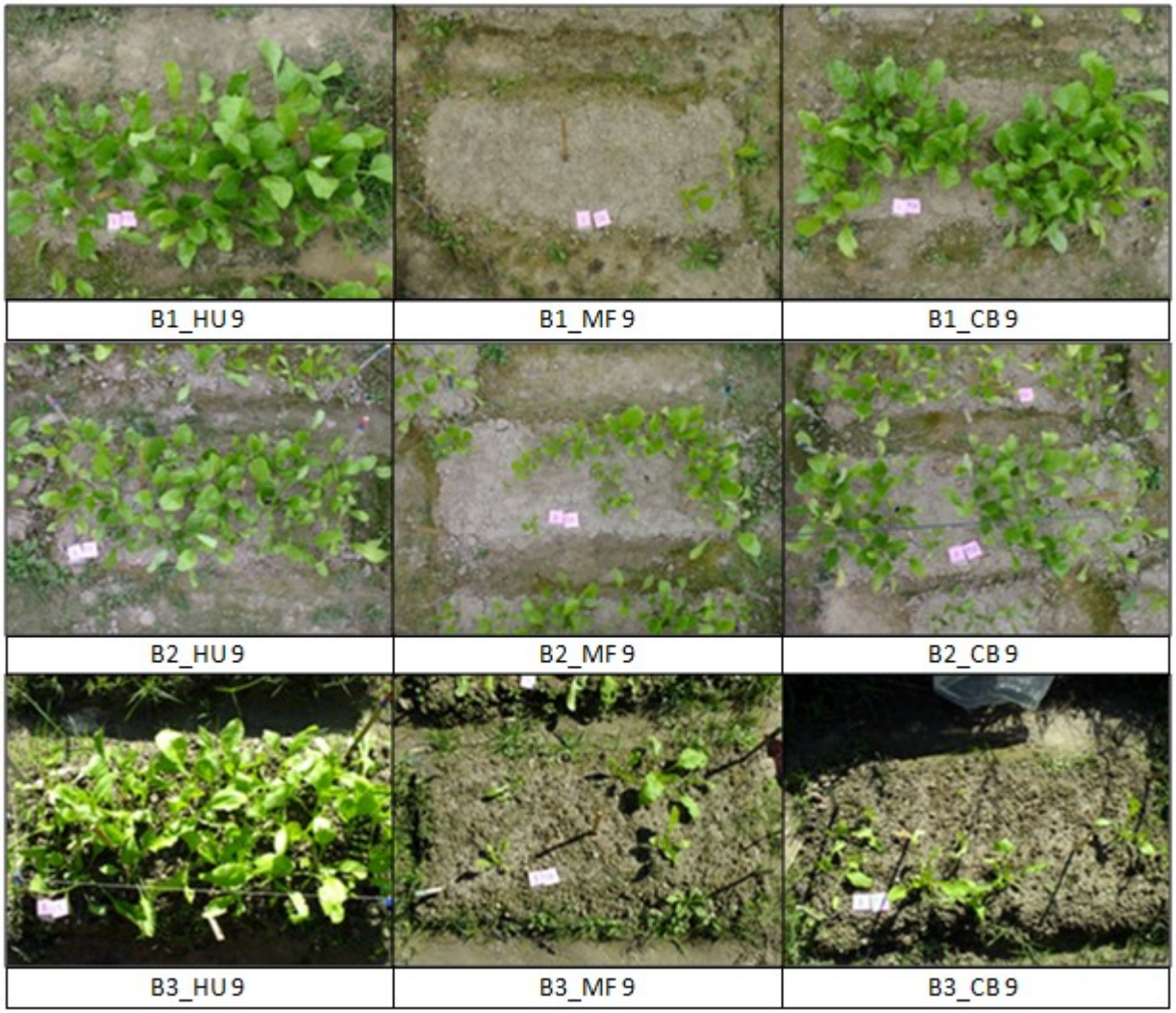

Figure 4.24 – Visual presentation of the spinach plants at Simulated Year 9
For the three treatments: human urine (HU); mineral fertilizer (MF); and combination (CB) for each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3).

Figure 4.25 – Visual presentation of the Control spinach plants
For each block: Block 1 (B1), Block 2 (B2) and Block 3 (B3) there were 40 plants, but the leaves were so small it was hard to see the plants in the images; the bottom image was an enlargement of the control from Block 3.

4.5 Discussion

The hypothesis that the spinach seedlings of the three fertilizer treatments (human urine, mineral fertilizer, and combination) would be equivalent in biomass production at each simulated year not correct as the human urine (Year 9) and combination (Year 3 and 7) treatments produced significantly higher yield than the mineral fertilizer. The second part of the hypothesis for fresh and dry biomass production of each fertilizer treatment compared to the control was not correct as at each level, at least one treatment was not significantly higher than the control (typically the mineral fertilizer treatment). For the shoot length and the number of leaves, all the treatments were significantly higher than the control. The second hypothesis (EC of the soil would increase and the human urine and combination treatments would have higher soil values than the mineral fertilizer treatments) was not correct as the human urine EC values did not change significantly and it was the combination and mineral fertilizer treatments that increased about the 2 dS m^{-1} threshold for spinach (Steppuhn et al., 2005). And the third hypothesis (pH of the soil for the three fertilizer treatments will decrease at equivalent rates at increased simulated years) was in rejected as the pH values did not change significantly for any treatment.

The initial physical and chemical soil sample results were not received until after Run 1, and fertilizer applications rates were not based on the initial measurements of the soil. The physical components of the soil, sandy loam, were ideal for spinach (Zvalo et al., 2008). The soil bulk density was low resulting in acceptable water availability and no limitation on root growth (Brady et al., 2002). The wet conditions of the soil due to the monsoon rains could have potentially decreased oxygen availability to the roots, but with the high water holding capacity and the low bulk density of the soil, oxygen and water availability should not have been a limiting factor (Brady et al., 2002). The low particle density of the soil was determined to be due to the high organic content (typical range for mineral soil is 2.60 to 2.75 Mg m^{-3}) (Brady et al., 2002), as the soil samples did have roots remaining from the grass.

The initial chemical analysis of the soil determined that the soil pH was not ideal for spinach as it was acidic, but the low soil EC_{SE} was ideal for this experiment. Human urine and fertilizers in general contain salts which increase the salinity and EC of the soil with repeated use. The low EC was most likely due to the monsoon seasons with the

heavy rains stripping the salts from the soil. The nutrient level of nitrogen and potassium was low and would also have been impacted by the monsoon rains. The initial high soil phosphate concentration most likely occurred due to the grazing cattle and local monkeys excreting on the soil.

The leaves from Run 1of Block 1 were too small for proper measurement, either mass or chemical analysis. Run 1 was started during the monsoon season and before the typical cultivation season for spinach (in mid-September); the environmental conditions were very wet (673.5 mm of rain in 35 days) and hot (average 26.4^{o}C, max 38 ^{o}C) for spinach. During Run 1 for Block 2, the growth period was extended to 32 days to compensate for these non-ideal conditions. Still, the total mass harvested was too small for chemical analysis.

For Run 2 , the spinach growth rates were adequate for chemical analysis at McGill University (the procedure at McGill University required 0.16 g of dry mass to complete all the analysis while the Agricultural University required 1 g per chemical analysis). By the end of Run 2, there was less rain (129.7 mm in 35 days) and the raised beds were beginning to dry particularly, Blocks 1 and 2 (Figure 4.20 to Figure 4.25). The plants did not show visible signs of water stress and were not irrigated.

The human urine treatments had no significant changes to the soil chemistry. The EC_{SE} did not exceed the threshold of 2.0 dS m^{-1} (Steppuhn et al., 2005) at any of the human urine simulated years and were not significantly higher to the control. The mineral fertilizer, Years 3, 7 and 9, and combination treatments, Years 5, 7 and 9, surpassed the EC_{SE} threshold, but there was no correlation to the lower emergence rates. All of the pH soil samples values were below the ideal range for spinach and none of the values were significantly different neither to the control nor to the initial soil samples. At such low readings, manganese could have accumulated to toxic levels and other elements would not be available, limiting the use of pH to assess sensitivity (Power et al., 1997).

The high organic carbon to nitrogen ratio of 32:1 (based on bulk density and depth of 0.15 m) in the soil meant that the bioavailable nitrogen could have been limited due to immobilization by bacteria (Camberato, 2011). The bioavailability of nitrogen was not significantly impacted as the only treatment deficient in nitrogen, < 30 g kg^{-1}, was the

control; all other tissue samples were sufficient (within the range of 30-45 g kg^{-1}) (Hochmuth et al., 2004). The increase in yield and number of leaves of the spinach plants had a positive moderate correlation to the increased application of nitrogen, which is similar to previous studies (Ahmadil et al., 2010, Jones et al., 2005). The MANOVA model for the concentration of nitrogen in the soil was not significant; the ANOVA model for the plant tissue was significant with the control being significantly lower to all other treatments. This supported the assumption that the collected human urine contained 6 g N L^{-1} was correct.

Though the spinach tissues samples were sufficient in nitrogen, the leaves were a light green; particularly visible in Blocks 1 and 2 for Years 1, 3 and 5. All of the tissue samples were deficient in magnesium (<10 g kg^{-1}) (Hochmuth et al., 2004). As magnesium deficiency symptoms are similar to nitrogen deficiency symptoms (Zvalo et al., 2008), the plants may have been showing signs of magnesium deficiency. The magnesium levels of the soils were not analyzed and it is unknown if the soils were deficient. The acidity of the soil meant the aluminium and hydrogen ions could have displaced the magnesium, accelerating leaching and decreasing availability (Camberato et al., 2011). All the tissue samples, including the control, were high in calcium (>10 g kg^{-1}) indicating the soils are naturally high in calcium (Hochmuth et al., 2004).

The low phosphate levels in the human urine soil samples denoted that additional phosphate sources are required to reach the optimal fertilization levels required by the plants (Heinonen-Tanski et al., 2007). The concentrations of phosphate in the soil samples were mirrored in the tissue analysis. The phosphate tissue samples for human urine (for all years) and the control were deficient: < 3 g kg^{-1} (Hochmuth et al., 2004). The phosphate tissue samples from the mineral fertilizer and combination treatment were sufficient (3.0-5.0 g kg^{-1}) with two treatments > 5.0 g kg^{-1}: mineral fertilizer, Year 7, and combination, Year 9, (Hochmuth et al., 2004).

Though the phosphate concentrations in the tissues of the human urine treatments were deficient, they were not statistically lower to the mineral fertilizer treatment, except at Year 9, indicating that the phosphate present in human urine was available to the crops (Kirchmann et al., 1995). This could have been due to three reasons. First, the sodium from the urine could have reacted with phosphorus to create monosodium phosphate and

disodium phosphate; these forms are easily absorbed by the plants (Power et al., 1997). Second, the higher temperatures in India would have also increased the available inorganic phosphorus, as for every increase of 15^{o}C there is up to 33% available inorganic phosphorus from increased microbial activity (Power et al., 1997, Sharpley, 2011). Third, mineralization of organic phosphorus is typically higher in the tropics with distinct wet and dry seasons (Sharpley, 2011). The sodium tissue concentrations still had a moderate negative correlation with phosphate and can be explained due to the human urine treatments having significantly lower phosphate fertilizer applied while receiving significantly higher sodium concentrations.

The majority of the treatments, including the control, were deficient in potassium < 30 g kg^{-1} (Hochmuth et al., 2004). The potassium concentrations in the plant tissue for human urine, Year 9, and mineral fertilizer, Year 3, were high (> 40 g kg^{-1}) (Hochmuth et al., 2004). All but three of the treatments had deficient potassium concentrations (< 30 g kg^{-1}) (Hochmuth et al., 2004), while the three with sufficient potassium concentrations (> 30-40 < g kg^{-1}) were Year 1 for human urine, and Years 5 and 9 for mineral fertilizer. The potassium deficiency was not visually evident as none of the plants had browning at the tips of the leaves (Zvalo et al., 2008).

Sodium had a positive moderate correlation with the survival rates, as human urine had the highest tissue concentration of sodium and the highest survival rates. Overall, the survival rates of spinach were higher with the human urine treatment (92.6%) and the control (90.0%) than the mineral fertilizer (68.3 %) and combination treatments (81.6%). The higher survival rate in the human urine treatments did not result in higher yields. At Year 9, the survival rates for the mineral fertilizer and the combination decreased by > 13%, while the EC_{SE} had increased by > 1.4 dS m^{-1} from Year 7. The relationship between the potassium (low) and sodium (high) explains the differences in the EC_{SE} levels between treatments and the lack of visual potassium deficiencies. The correlation between the concentration of potassium in the soil and the EC_{SE} was positive and moderate. This was due to the EC_{SE} levels of both the mineral fertilizer and combination treatments increasing as the potassium level increased. The human urine treatment was low in potassium and the EC_{SE} levels remained low despite the human urine having a high EC_{SE} (19 dS m^{-1} (Putnam, 1971)). This EC_{SE} result indicates that the potassium applied was the driving factor for the increase. The lower EC_{SE} of the human urine

treatment soil samples was due to the higher plant up-take of sodium, as seen in an earlier study by Mnkeni, (2008). We believe that the spinach plants were able to compensate for the low potassium levels by utilizing the sodium from the urine. The spinach plants were able to substitute potassium for sodium, when potassium was limited (Subbarao et al., 2003). Sodium has been used as a potassium fertilizer replacement for greenhouse grown spinach as the leafy crop can accumulate sodium without negatively affecting the quality (Subbarao et al., 2003). The mineral fertilizer treatment had the lowest sodium tissue concentrations while overall having the highest potassium tissue concentrations, but there was no correlation between the sodium and potassium tissue concentrations.

The sodium tissue concentration of the control was not significantly different to any of the mineral fertilizer and combination treatments, but was still relatively high. A possible explanation for the high control concentration could be that the plants were handled several times with bare hands and any sodium presence on the researchers' fingers may have transferred to the plant, but we do not think this can explain all of the increase in sodium (the remainder remains unaccounted for). As for the lower combination reading for the human urine, potentially there was some chemical reaction with the mineral fertilizer in the soil that decreased the uptake of sodium in the plants. The available soil analysis tests were limited at the Agricultural University of Himachal Pradesh and the local government soil laboratories and the concentration of sodium in the soil was not tested.

4.5.1 Complications encountered

The wet clay in the soil was difficult to till and it clumped together. Breaking the clumps was futile as the clumps reformed each time there was more moisture. The clumps caused difficulty in incorporating the fertilizer evenly. Some plants were delayed during emergence as the shoots weaved through or around the clumps. The soil beds were uneven with the clumps, and leaves that touched the clumps would either rot with the moisture or burn from the fertilizer. The high heat and high relative humidity also caused the fertilizer granules to clump together making accurate measurements and disbursement difficult. The granular fertilizer, in particular urea, melted in the sun and was incorporated as quickly as possible to decrease volatilization of the nutrients. While applying the human urine some drained out the side of the soil bed as the soil was too moist to adsorb more moisture. The soil beds of Block 3 were made wider and were covered for a longer

duration to dry the soil, and less human urine leaked out the sides of the raised beds compared to Blocks 1 and 2.

Pests were a major issue and bed nets were not placed on the blocks the first day of seeding. During Run 1, Block 1 was covered by a net 15 days after planting. To treat Block 2 equally, a net was placed on the plants only at night until day 15, so they received an equal exposure of sunlight. For Run 2, the nets were on at all times and there was less pest damage. There was one incident of vandalism to the weather station and the bed nets, causing a loss of data in the weather collection and light damage to a few plants.

The month of August had 22% more rain than the 20 year average (Table 4.2); this was confirmed by the local farmers who said typically by mid-August the rains had tapered off and planting would begin. As the rain persisted, the yields from Run 1 were affected negatively. The rain shelters protected the plants from the heavy rains, but there was still some flooding of the blocks. The tarp was only 50 cm above the ground which limited the air flow and caused some heat stress to the plants. The plants received limited sunlight due to the rain shelter and the continuous rain. Many of the plants were smaller than what would have been ideal for farmers to use or sell. Run 1 was planted early in the season and the reduction in yield was expected. Run 2 began in the typical spinach season for Himachal Pradesh, India. Due to time constraints with the Indian Research Visa, I did not have the time for longer growth periods. The plants could not mature to 55 days, decreasing the fresh mass yield and the results were not ideal to compare with other published data that grew the plants for a longer season (Ahmadil et al., 2010, Boese et al., 1990).

The harvesting of Blocks 1 and 2 during Run 2 was performed on the same day, starting at 03h00. The University closed at 17h00, and 1300 plants had to be harvested and weighed by this time. Harvesting started with Block 1 and the plants were covered with dew. As the sun rose and harvesting continued to Block 2, much of the dew had evaporated; changing the moisture content of the plants. Block 3 was harvested on a holiday (unknown to the household where the researchers were staying) and the school was closed. The length of the plants from Block 3 were measured on the day of harvest and stored in a refrigerator until the following day. There was a loss of moisture content before they were weighed, but the bigger issue was the delay of getting them in the oven.

Blocks 1 and 2 were harvested three days after Block 3. Due to time restrictions (visa expired), some bags were dried over a hot plate on the day of departure. Ultimately, the dry plants were weighed at McGill University.

The combination treatment received more phosphate and potassium than the mineral fertilizer treatment; the combination treatment received the same mass of 0-52-34 and 0-0-60 as the mineral fertilizer treatment plus the phosphate and potassium from the urine applied. The lack of significant difference between the mineral fertilizer and combination treatments in the concentration of phosphate in the soil indicates that additional phosphate from the human urine did not add a significant amount.

For this analysis to have been thorough, the sodium adsorption ratio (SAR) of the soils should have been measured as this value is a better representation of the adverse effects of sodium (Subbarao et al., 2003). No testing facilities were located at the Agricultural University of Himachal Pradesh that could perform this test. However, it can be determined that the soils were not sodic as the EC_{SE} was predominately below 4.0 dS m^{-1} and the pH_{SE} was well below 8.5 (Power et al., 1997).

4.5.2 General findings and optimal application rate

The general guideline of applying the volume of urine produced by one person per day per meter squared was similar to the volume of human urine applied in Year 1 (1.9 L m^{-2}). Year 1 for human urine and mineral fertilizer treatments were not statistically significantly different to the control, though the fresh mass of the fertilized treatments were at least six times higher. The combination treatment, Year 1, was significantly higher than the control and over 22 times greater in fresh biomass produced per meter squared. At no point did the human urine treated plants produce a significantly lower biomass than the mineral fertilizer treated plants. This indicated two things: 1) the general guideline was accurate for being equivalent to the mineral fertilizer; and 2) for optimal biomass production, human urine should be used in conjunction with phosphate and potassium fertilizers.

The excessive fertilizer application rates negatively impacted the plants in the mineral fertilizer and combination treatments, but not the plants in the human urine treatments. Though overall, the fertilizer treatments (human urine, mineral and combination) had no yield loss compared to the control, even for the treatments with significantly lower

survival rates (mineral fertilizer, Years 5, 7 and 9, and the combination treatments, Year 9). The negative impacts on the biomass production rate for the mineral fertilizer treatments started at Year 5, but at Year 3, the EC_{SE} was already above 2.0 dS m^{-1}. The fresh and dry mass from Years 1 and 2 of the mineral fertilizer were not significantly different, but the EC_{SE} of Year 1 was significantly lower – confirming that the optimal fertilizer application rates for mineral fertilizer was Year 1. Overall, the combination treated plants produced the highest biomass but had negative impacts on the biomass production rates from Year 5 through 9 and when the EC_{SE} was greater than 2.0 dS m^{-1}. The optimal fertilizer application rate for the combination treatment was Year 3, with the peak fresh biomass and the EC_{SE} below 2.0 dS m^{-1}. The human urine biomass production continued to increase through the simulated years and the EC_{SE} remained below 2.0 dS m^{-1}, the optimal fertilizer application rate was Year 9.

The volume of human urine required to fertilize one ha with human urine at the observed optimal application rate would be 178 m^3. There would be problems with leaching as the water holding capacity of the soil would be surpassed and leaching nutrients could cause damage to the environment (Chapter 2). An average household of 4.34 people would produce a maximum of 2.4 m^3 of urine in one year. Human urine could be used as a supplement to other fertilizers, used on a smaller scale or used in an urban setting (2.4 m^3 of urine could fertilize a 1225 m^2 plot land at the Year 1 application rate). As human urine is high in N but lower in P and K, it would be good fertilizer where there are already high concentrations of phosphate in the soil (Heinonen-Tanski et al., 2007), or in combination with animal manure, as animal manure is high and phosphate and potassium (Tidaker et al., 2005). The household and surrounding community would benefit from EcoSan through the decrease of open defecation, the reduced risk of contaminating drinking water sources, and cheaper sources of nutrients (Höglund, 2001).

Nutrients and salts are removed from the soil with every harvest, and from leaching during heavy rains (monsoons), therefore salt accumulation may be limited and additional fertilizers would be required for maintaining crop yields (Heinonen-Tanski et al., 2007). India predominantly uses nitrogen fertilizers which make the adoption of human urine as a fertilizer an easier task as it is equivalent to synthetic sources, especially areas that struggle to afford manufactured fertilizer. Adoption would be easier if or when the government opens the market on the price of nitrogen fertilizer. Otherwise the start-up

cost for storage tanks is not a good investment. Rural areas with poor roads and poor access to markets may benefit most from a urine-diverting EcoSan system. Farmers tend to live close to their land and it may be easier to transport humane urine to the fields than from a faraway market. To decrease the cost of storage, minimal storage times with multiple nitrogen dressing could be used. A cost-benefit analysis of the cost of using human urine compared to mineral fertilizer in India would be ideal.

The inconvenience of not having a more accurate balance turned into a positive outcome for developing general guidelines for human urine as a fertilizer. The lack of significant difference between the mineral fertilizer and combination treatments indicated that the mass of phosphate and potassium applied from human urine was irrelevant. This assumption simplifies calculations for required P-K application rates as the state's recommended fertilizer application rates can be followed. This would simplify procedures and be less confusing at the time of implementing an EcoSan system in a new household or community.

With minimal effort, the risk to health from using human urine as a fertilizer was reduced. Wearing a mask while handling the urine and washing hands afterwards decreased the chance of urine-to-oral and hand-to-oral accidental transmission. The food-to-oral risk was also low as the majority of spinach consumed was well cooked. There was however high risk of hand-to-oral and food-to-oral contamination of raw food in the kitchens due to poor food preparation hygiene: cutting boards and counter tops were not washed with hot water and soap.

4.6 Conclusion

In order for EcoSan projects to be successful, plant yield has to increase, sanitation levels have to rise, profits need to increase and labour should remain in the same range as current levels. To achieve this, specific crop guidelines, such as optimal fertilizer application rates are required (Winblad et al., 2004). This study confirmed that human urine can be used as an equivalent fertilizer to N-P-K. There was no significant difference between nitrogen concentrations in the plant tissue for the three fertilizer treatments (the control was significantly lower). The phosphate concentrations in the plant tissue from the control and the human urine were not significantly different and the mineral and combination treatments were significantly higher than the other two. The general

guidelines of applying the volume of urine produced by one person per day per meter squared produced higher spinach biomass than the control, but produced significantly higher biomass in combination with phosphate and potassium.

The spinach plants used sodium to compensate for the low concentration of potassium in the human urine treatments. This was a particularly interesting observation as it not only supported previous studies with spinach, but demonstrated that crops with the same ability to compensate will perform well with EcoSan systems.

EcoSan closes the nutrient cycle by using human urine as a fertilizer, and decreases the dependency on mineral fertilizer. This process is beneficial, when implemented with success, for communities in two ways: 1) the waste nutrients from the excrements are better managed; and 2) farmers have access to an alternative to the more costly mineral fertilizer. By transforming human urine into a tradable good, such as fertilizer, these nutrients will be better managed and will decrease the negative impacts on the environment. There are limitations in the large volumes of human urine required can cause difficulty with storage and application. Research development to find options to decrease the volume of the urine and still maintaining nitrogen levels would be ideal to promote urine-diverting EcoSan system.

4.7 References

AHMADIL, H., AKBARPOUR, V., DASHTI, F. & SHOJAEIAN, A. 2010. Effect of different levels of nitrogen fertilizer on yield, nitrate accumulation and several quantitative attributes of five Iranian spinach accessions. *American-Eurasian Journal of Agriculture and Environmental Sciences,* 8**,** 468-473.

BOESE, S. R. & HUNER, N. P. A. 1990. Effect of Growth Temperature and Temperature Shifts on Spinach Leaf Morphology and Photosynthesis. *Plant Physiology,* 94**,** 1830-1836.

BRADY, N. C. & WEIL, R. R. 2002. *The Nature and Properties of Soils,* Upper Saddle River, New Jersey, Prentice Hall.

CAMBERATO, J. J. 2011. Bioavailability of nitrogen. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

CAMBERATO, J. J. & PAN, W. L. 2011. Bioavailability of calicum, magnesium, sulphur, and silicon. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

HEINONEN-TANSKI, H., SJÖBLOM, A., FABRITIUS, H. & KARINEN, P. 2007. Pure human urine is a good fertilizer for cucumbers. *Bioresource Technology,* 98**,** 214-217.

HOCHMUTH, G., MAYNARD, D., VAVRINA, C., HANLON, E. & SIMONNE, E. 2004. Plant Tissue Analysis and Interpretation for Vegetable Crops in Florida. Gainesville, FL: Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

HÖGLUND, C. 2001. *Evaluation of microbial health risks associated with the reuse of source-separated human urine.* Doctoral, Royal Institute of Technology.

JONES, C. L., MANESS, N. O., STONE, M. L., SOLIE, J. B. & ZAVODNY, D. 2005. Variable rate nitrogen application on row crop spinach. *American Society for Agriculture and Engineering Annual International Meeting.* Tampa, Florida.

KIRCHMANN, H. & PETTERSSON, S. 1995. Human urine - Chemical composition and fertilizer use efficiency. *Fertilizer Research,* 40**,** 149-154.

POWER, J. F. & PRASAD, R. 1997. *Soil Fertility Management for Sustainable Agriculture,* Boca Raton, FL, CRC Press.

PUTNAM, D. F. 1971. Composition and concentrative properties of human urine. *National Aeronautics and Space Administration Contractor Report.* Huntington Beach, California: McDonnell Douglas Astronautics Company - Western Division.

SHARPLEY, A. 2011. Phosphorus availability. *In:* HUANG, P. M., LI, Y. & SUMMER, M. E. (eds.) *Handbook of Soil Science: Resource Management and Environmental Impacts.* 2 ed. Boca Raton, FL: CRC Press.

STEPPUHN, H., GENUCHTEN, M. T. V. & GRIEVE, C. M. 2005. Root-Zone Salinity: II. Indices for Tolerance in Agricultural Crops. *Crop Science Society of America,* 45**,** 221-232.

SUBBARAO, G. V., ITO, O., BERRY, W. L. & WHEELER, R. M. 2003. Sodium—A Functional Plant Nutrient. *Critical Reviews in Plant Sciences,* 22**,** 391-416.

TIDAKER, P., MATTSSON, B. & JONSSON, H. 2005. Environmental impact of wheat production using human urine and mineral fertilisers - a scenario study. *Journal of Cleaner Production,* 15**,** 52-62.

WINBLAD, U., HEBERT, M., CALVERT, P. & MORGAN, P. 2004. *Ecological sanitation*, Stockholm environment institute.

ZVALO, D. V. & RESPONDEK, A. 2008. Spinach. *In:* AGRAPOINT (ed.) *Vegetable Crops Production Guide for Nova Scotia.* Atlantic Provinces Agricultural Services Coordinating Committee.

4.8 Appendix

Appendix 4.1 - The daily rainfall and temperature at the experimental station for Run 1. The actual rainfall was the total amount of rain.

	Rain Fall (mm)					
Date (2011)	Block 1	Block 2	Block 3	Actual	Temp (^{o}C)	%RH
28-Aug	3	0	0	4	30	
29-Aug	1	0	0	13	24	80
30-Aug	0	0	0	14	35	61
31-Aug	4	0	0	60	30	66
1-Sep	1	1	0	77	28	78
2-Sep	0	0	0	9	24	85
3-Sep	0.5	0.5	0	14	33	65
4-Sep	2	2	0	6	35	72
5-Sep	1	1	0	4	28	75
6-Sep	3	9	0	60	32	76
7-Sep	0	0.5	0	29	16	-
8-Sep	0	0	0	10	18	-
9-Sep	1	1	0	38	20	-
10-Sep	0	0	0	13	24	-
11-Sep	0	0	0	1	24	-
12-Sep	0.5	0.5	0	6	22	-
13-Sep	1	1	0	66	24	-
14-Sep	1	0.5	0	4	26	-
15-Sep	0	0	0	0	27	-
16-Sep	0.5	0.5	0	11	24	-
17-Sep	1.5	1	0	9	24	-
18-Sep	0	0	0	7	20	-
19-Sep	0	8	0	41	26	-
20-Sep	0	4	0	36	25	-
21-Sep	0	0	0	1	18	-
22-Sep	0	0	0	24	30	-
23-Sep	0	0	0	0	30	-
24-Sep	0	0	0	0	30	-
25-Sep	0	0	0	20		-
26-Sep	0	0.5	0	1	30	-
27-Sep	0	0	0	14	30	-
28-Sep	0	0	0	26	28	-
29-Sep	0	0.5	1	1	27	-
30-Sep	0	0	0	0	24	-
1-Oct	0	0	0	0	31	64
Average	0.6	0.9	0.03	19.1	26.4	72.2
Total	21.0	31.5	1.0	673.5		

Appendix 4.2 - The daily rainfall and temperature at the experimental station for Run 2. The actual rainfall was the total amount of rain.

	Rain Fall (mm)					
Date (2011)	Block 1	Block 2	Block 3	Actual	Temp (°C)	%RH
2-Oct	0	0	0	1	29	70
3-Oct	0	0	3	31	31	67
4-Oct	0	0	0	8	-	-
5-Oct	0	0	0	0	30	65
6-Oct	0.5	0	0.5	0.5	31	58
7-Oct	0	0	0	0	27	74
8-Oct	3	3	3	3	26	75
9-Oct	8	8	8	12	27	73
10-Oct	0	0	0	22	24	79
11-Oct	0.25	0.25	0.25	10	24	75
12-Oct	0	0	0	0	30	61
13-Oct	0	0	0	0	26	63
14-Oct	0	0	0	0	30	55
15-Oct	0	0	0	0	28	62
16-Oct	0.2	0.2	0.2	1	28	60
17-Oct	0	0	0	0	29	71
18-Oct	0	0	0	0	30	66
19-Oct	0	0	0	0	30	56
20-Oct	0	0	0	0	28	54
21-Oct	6	6	6	6	29	61
22-Oct	1.5	1.5	1.5	23	28	60
23-Oct	0	0	0	0	29	53
24-Oct	1	1	1	8.5	29	67
25-Oct	1	1	1	5	23	79
26-Oct	0	0	0	0.25	28	72
27-Oct	0	0	0	0	26	55
28-Oct	1	1	1	1	23	70
29-Oct	0	0	0	0.5	26	73
30-Oct	0	0	0	0	-	-
31-Oct	0	0	0	0	-	-
1-Nov	0	0	0	0	-	-
2-Nov	0	0	0	0	27	51
3-Nov	0	0	0	0	28	43
4-Nov	0	0	0	0	28	48
5-Nov	0	0		0	27	54
6-Nov	0	0	0	0	27	58
7-Nov	0	0	0	0	24	56
Average	0.64	0.63	0.75	3.71	27.7	63.2
Total	22.45	21.95	24.45	129.70		

Connecting Statement

In Chapter 4, the experiment was influenced by the local setting and many challenges were faced during the set-up, the harvest and the analysis of collected samples. Some of the challenges were inherent to research and exacerbated by the setting while other issues were encountered directly because of being located in northern rural India. Chapter 5 highlights the reasons for selecting India as the location for the experiment and the issues that aroused from this decision.

5 Performing research in a developing/emerging country: a guide to communicating and working in rural Northern India

5.1 Introduction

Globalization creates many opportunities to travel to distant countries for work such as foreign aid and investment, opportunities which often highlight the differences between cultures. For my Master's research at McGill University, Montreal, Quebec, Canada, I travelled to northern India and was faced with hurdles at each step of the project. Some hurdles were similar challenges I would have faced here; others were unique to my location in India. The objective of this chapter is to highlight some of the stumbling blocks of working in a foreign developing country such as India with focus on the challenge of operating a field project in a rural area and of working with human resources.

I selected India because of the dire need for financially and environmentally sustainable methods of waste management (outlined in Chapters 1 and 2). India presents great opportunities to implement innovative solutions to replace the meagre or lacking infrastructure. Improving sanitation is at the forefront of the government's priorities (SACOSAN 2008). As explained in previous chapters, there is also a need for environmentally sustainable fertilizers to maintain crop production rates. Several organizations and individuals are dedicated to finding ecological sanitation (EcoSan) solutions in India (SACOSAN 2008), including Professor Vijay Raghavan from the Indian Institute of Technology, who is researching the use human urine as a fertilizer.

A formal partnership was initiated between myself and Professor Vijay Raghavanm, and, through this partnership, funding for travel and research expenses was secured from the Indo-Canadian Shastri-Institute (ICSI) and a research visa was approved. Visas have become more difficult to obtain since the 2010 terrorism incidents, as India has changed the application process for and duration of visas: the date a research visa is issued by the embassy is the day one can enter India. The research visa issued in Canada could take up to two months to process or as short as two weeks, and the return flight must be booked within the given time of the visa in order to enter the country. The requested duration (maximum one year) is not necessarily approved and cannot be extended without supporting documents from the Indian partnership.

Before departing to India, the formal partnership with the Indian Institute of Technology was dissolved due to the institution's request for $3000US in fees for the partnership. This sum was not covered by my fellowship with ICSI, and I had received no grant for my work in Canada. There were no Memorandums of Understanding (a formal declaration of partnership) between our universities. Attempts were made to have me labelled as an employee with the Indian Institute of Technology, but without success. With one week before departure, I was left with no official partnership.

The research visa required registration with the Foreign Registration Office within two weeks of arrival in India. The registration procedure requires proof of residence in India (such as a lease or electricity bill) and proof of collaboration with an institute. I was able to use documents I already possessed, but would not be to extend the duration of the research visa, which meant I had only five months. The only other option would have been to leave the country and return as a tourist, which ICSI would not have approved. The failed partnership deprived my research of technical support for developing an applicable methodology for my chosen area, and there was little information in English available online about the typical agricultural practices, such as the recommended fertilizer application rate and how and when people typically seed. It also left me without lodging arrangements.

I planned to depart for India despite these obstacles. Days before departure, through random acquaintances, an unofficial partnership was made with Dawson College (Montreal, Quebec, Canada) which had a group of volunteers travelling to Palampur, Himachal Pradesh, with Volunteering India. It was through this group that I was provided with a source of human urine and acquired access to land (along with affordable, clean, and safe lodging for the duration of my stay).

By not being associated with a university, I did not have access to a large human body from which to collect urine. My plan had been to collect urine in the main washrooms of my dormitory. As I had to start collection as soon as possible due to my restricted visa duration, I did not have the time to make arrangements with a local school. Instead I acquired permission from the Ethics Board at McGill University to collect urine from the Dawson volunteers. The volunteers were consuming a typical India vegetarian diet and I

assumed the nutrient concentrations would not significantly differ from those of the local citizens.

While collecting urine, attempts were made to acquire access to a section of land. Contact was made with a female professor (who wished to remain anonymous) who was performing studies with cattle urine at the Agricultural University of Himachal Pradesh. She was reluctant to help, and through discussions with various people it became apparent that I would not receive help unless I had an official partnership with the university – and possibly not without living on campus. This partnership would also not guarantee access to a plot of land on campus. Without a formal partnership, I could not obtain information about standard agricultural practices in the area. After one failed partnership and a bad experience with the bureaucracy of visa application, I was reluctant to rely on this partnership with no guarantee of access to land. Finding a plot of land in the vicinity of my lodging with Volunteering India was difficult, as the majority was being used for rice paddies and the farmers spoke no English.

After two weeks of fruitless searching, the regional manager of Volunteering India, Padam (Bobby) Dev Singh, offered me the use of his land. Mr. Singh had monitored my attempts to source things for myself, allowing me the opportunity to learn about the surrounding area and its transportation system. The offered land was a terraced rice paddy left fallow, located in Arla, Himachal Pradesh, a ten-minute walk and 20-minute bus ride from the town of Palampur. With the human urine collected and land secured, I began preparing the experimental plots.

5.2 Obtaining materials & equipment

Resourcefulness and imagination was required to set-up an experimental station with minimal inputs while living in a rural location. The fallowed fields were overgrown with grass and shrubs, requiring tilling and irrigation (Figure 5.1). Fertilizer and seed availability is dependent on the time of year. Midway through the monsoon season, they were no longer in demand. Due to the limitations of the supply chain, it took three villages and many days of searching to find spinach seeds and N-P-K fertilizers. Spinach seeds were selected for the experiment for two reasons: my initial greenhouse experiment with spinach demonstrated no significant impact on plant emergence with synthetic human urine as fertilizer, and spinach plants mature more quickly compared to other

available seeds (such as wheat, okra and squash). I did not have time for two plant species trials.

Figure 5.1 – Example of the tilling required for preparing the blocks for planting

Palampur's main market was full of shops supplying everyday needs, but farming supplies were harder to locate. I soon learned that many vendors tended to be general distributors, displaying random items and storing many more. A rake and a hoe were difficult to find without knowing Hindi – some retail business owners spoke a little English, but not the assistant staff. The handmade farming tools produced in the area proved to be superior and cheaper than mass-produced, imported tools. Some parts were purchased separately, and handles were shaved down manually to fit (Figure 5.2). My pre-assembled and elegant rake broke on the first day of use. Later, a local welder made a new handle from rebar that was purchased for a quarter of the cost and was superior in quality, lasting till the end of the project. Overall, there were few options for tools and most were of poor quality.

Figure 5.2 – The tools used to till the blocks
The handles of the hoes were sanded by hand to have the heads fit. The three-pronged rake broke on the first day.

Materials for setting up a weather station were available: non-digital thermometers with min/max indicators, electric thermo/relative humidity meters with probes, and rain gauges. The thermometers had an accuracy of +/- 4^{o}C. The rain gauge capacity of 40 mm/m^2 needed to be emptied at least twice a day during periods of heavy rain (Figure 5.3). A plastic bucket, painted white and punctured with holes, was placed upside-down on a seven-foot-tall bamboo stick to isolate the thermometers; the probe of the digital thermometer remained in the bucket, while the display unit was removed each night (Figure 5.3). The rains were so frequent that a makeshift rain shelter was pitched out of plastic to keep my writing equipment dry. Personal protective equipment (face masks, protective goggles, and gloves for sanitation and manual labour) was not readily available.

Figure 5.3 – The weather station (left) and the rain gage (right)

Plastic bags are typically used for transporting soil samples. India had an unexpected law: a ban on plastic bags. This was due to the lack of a garbage collection system; cows often ate discarded bags and became ill. For field research this impacted the ability to collect samples. Often Universities used cloth or paper bags to collect samples, cherishing the few plastic bags that remain. Paper bags were easily found, but, because of manufacturing inconsistencies, the weight of the bags varied significantly and had to be weighed individually.

Electricity was widely accessible but not reliable, impacting laboratory equipment as ovens and balances could not be used. Outages were common, with frequencies depending on the area: outages occurred for several hours daily in poorer areas; richer areas experienced a few outages a week, for only a few minutes. Building wiring was often exposed and care had to be taken to switch off the supply while plugging and unplugging appliances in order to reduce surge damage. The outages were most common when raining, when I was not working in the field. This made trips into town inconvenient as random shops would close in heavy rains and I could not access faster internet or print documents. There were also floods and mudslides, causing roads to be closed.

Like most sponsored International projects, I was required to track my expenses. Unfortunately, most vendors did not have official receipts and simply scratched out receipts onto scrap paper with no name or date. Vendors only accepted cash in the Indian rupee ($0.021 USD to 1 rupee was the average exchange rate in 2011). ATMs were available (credit cards only) at larger town centers, but were not always reliable: often out of cash, out of service, or out due to power failures, especially during festivities.

5.3 Accessing laboratory facilities

Acquiring technical support from the Agricultural University of Himachal Pradesh was slightly more bureaucratic than in Canada. The University provides the service of soil sample analyses for NPK, EC, and pH regularly to farmers, but all relationships require approval of the head of the department. I required a letter from myself, from my supervisor in Canada, and a copy of my passport and visa in order to receive approval.

Communicating with the staff at the University was difficult. The professors, who acted as consultants, and the head of the Department of the Soil Sciences, spoke intermediate English; the staff, including the accountant and the technicians, spoke little to no English. This challenge was accentuated when there was not another student present to aid with translating.

University staff typically work Monday to Saturday from 10 AM to 5 PM. Technicians and consultants often have other commitments, such as field work. The professors and the head of the department were frequently out of office, and setting appointments was futile: rarely were they present at the given time and date, even though I was a paying client. The national government acknowledged the many celebrations in Hinduism with numerous government holidays throughout the year. Banks, schools, and many vendors were inconsistently closed during the holidays. The numerous holidays disrupted experiments as the whole university shut down (even the ovens, which were also turned off at night).

Analyses were often not completed by the agreed-upon date, possibly due to university holidays and the frequent power failures. The university's laboratory equipment tended to be old, used, and in limited supply. Precise equipment, such as a milligram balance, was unavailable at the university (Chapter 4). Picking up analysis results required the

technician, the professor, the accountant, and the head of the department to be present in order to sign forms. The professors communicated on a need-to-know basis, and would often leave me in the office >30 minutes with no explanation. Being a polite Canadian only got me so far. To keep to agreed-upon deadlines, a more aggressive approach was required. It was a frustratingly slow process, which seemed typical for Himachal Pradesh and the rest of India.

5.4 Using transportation

My experimental station was located approximately two hrs, by public transit, from the Agricultural University of Himachal Pradesh, making taxies were the easiest way to transport samples to the university. There were two types of taxis in Palampur: auto-rickshaws and automobile taxis. Auto-rickshaws were cheaper than automobile taxis but slower, with less room for tall people and luggage. Auto-rickshaws were not metered, and a price had to be negotiated before departing. The automobile taxis were also not metered and varied in quality and size. Most taxi drivers did not speak English and typically attempted to overcharge foreigners. Taxis without yellow license plates were unofficial; hiring private taxis through friends was cheaper, but unofficial drivers may be pulled over by traffic police.

When samples were not being transported, the local buses were used. The bus drivers and bus conductors (those who collected the money) usually did not speak English, making explaining a destination tricky; often someone on the bus could translate. Some bus conductors also tried to overcharge foreigners. Bus stops were not typically marked; frequently bus drivers would drive past waiting passengers because they did not see them or did not think it beneficial to stop. Buses tended to be crowded and hot, with limited leg or head room for tall people, but were a cheap alternative to taxis (a Rs. 7 bus ride was equivalent to a Rs. 200 taxi ride). Buses to Palampur from Delhi were overnight, typically 11 hours. Tickets were approximately Rs. 770 for the deluxe bus (with air conditioning and reclining chairs), but was not comfortable.

5.5 Telecommunication

The cell phone was a worthwhile investment, as payphones were not available and land lines were uncommon (and often out of service). Cell phones could be purchased for as little as $25 Canadian (Rs.1200) and SIM cards at Rs. 25. Cell phones were a common

accessory and easy to purchase even in the smallest of towns making communicating with my supervisor in Canada relatively cheap. The various networks offered different charging rates for local, national and international calls, but were not always reliable, so many local citizens had two SIM cards for their phones. Recharging the phone with minutes was widely available at convenience stores (some companies were not as common, making it more difficult to recharge the minutes in rural areas) and some companies offered online services for recharge. I found IDEA to be the best for making calls to North America. To purchase a SIM card, documentation was required and, like a visa, it had become very strict since 2010's terrorist scare. A photocopy of a passport and visa and two passport size photos were required, along with a temporary address in India and local reference as a guarantee. Still, my SIM card was rejected, due to lacking my father's name on the application form.

Internet was required for communicating with my supervisor on methodologies and researching papers. Internet cafés were common, but can be hard to find due to poor signage. Speed, quality and cleanliness varied greatly, as did the price (Rs.15 to Rs. 50 per hour). Printing and photocopying were also available at most cafés (Rs. 2 to Rs.15 per page). To enhance communication, I purchased a USB modem, which provided Internet access at the speed of dial-up. The stick was relatively affordable: Rs.12700 for the initial purchase and Rs. 333 per month for unlimited access. The speed did improve from 2011 to 2012, but the signal was still too weak for Skype video and was significantly reduced when inside concrete buildings. Within a town's perimeter, there was typically a higher speed option for an extra premium. A data plan was purchased for my phone to facilitate Google maps for navigation.

5.6 Human resources

Due to time constrains, extra labour was required to help prepare the blocks, but was difficult to find. My lack of Hindi limited who I could communicate with and through informal conversations with shop owners in Arla, I learned two things about the difficulties in finding labour. First, those who I could communicate with, i.e. those with a high enough education to understand English, would not partake in manual labour, even if they did not have a job. Second, I was informed that that the state government provides stipends to those who cannot afford basic needs and that because of this there is a shortage of manual labourers. To meet the manual labour needs during the monsoon

season, migrant workers come from other states, such a Bihar, to work rice paddy fields and to pick tea.

However I was able to hire a labourer to aid with preparing the fields with the help of Bobby's family. Bobby's father owns a tea plantation and organized for an employee to come help me on a Sunday – their day off. Wilson arrived on time to help me in the fields and had no tools, food or water. It was the custom in this area that hired labour was not to come within the perimeter of the house of the employer nor use their washroom and the labour who I hired had to adhere to this practice. I was informed that hired labourers require constant supervision.

Field labour wage for eight hours ranged greatly by location, from as low as Rs. 70/80 a day for women/men in Ahmednagar, Maharashtra to Rs. 120/150/200 a day for children/women/men in Guntur, Andhra Pradesh (Viswanathan, Bhandari et al. 2011). Other states in India have a set minimum wage such as in Kozhikode, Kerala, where the average daily wage for men in March 2011 for ploughing was Rs. 510, for sowing was Rs. 365; and a woman's daily wage for weeding was Rs. 265 (Viswanathan, Bhandari et al. 2011). In the Kangra district of Himachal Pradesh, it was recommended by Bobby for me to pay Rs.200 for six hours of work; this was considered good pay.

I decided to pay Rs. 300 and provided food and water. Wilson was a hard worker and would have hired him again if the scheduling was not so difficult. For one, he was only available on Sunday, and two, he spoke no English. With no one else present to help with translation, it was very difficult to organize even a lunch break. I did not want to subject him to adding with the application of human urine without being able to properly explain why I am doing this (and I probably would have need McGill University's ethics approval).

5.7 Differences in cultures

As would be expected, there were vast cultural differences; some impacted me as a person living there and while others impacted my research. And some were at the intersection of both, for example, differences in how toilets are used and constructed. Indians are "washers" and use water to wash themselves after going to the toilet. Canadians are "wipers" and use toilet paper after going to the toilet. I attempted to be a

"washer" but, although it was difficult to locate toilet paper in the shops, it was still less challenging than adopting the practice of being a "washer." The India-style toilet is designed for squatting (Figure 5.4), which was the method used by my volunteers when donating their urine.

Figure 5.4 – Example of Indian-style squatting toilet

Indians prided themselves on appearance. Men wore pants as a status symbol, even when it was > 35°C outside; shorts were associated with farmers, who were lower in the caste system. Men would wear pants to the field only to remove them for work. I walked past other plots on the way to my field and at times would catch men quickly pulling up their pants as I passed. The women were always fully dressed in traditional clothing, exposing nothing above the calf or any shoulder or chest. I too wore long shorts to my field and removed them during my work to wear shorter, cooler shorts. By wearing traditional clothing in town, I attracted less attention and seemed not to be as much of a target for the foreign-person's-price.

The people I interacted with enjoyed discussing India politics and wanted to know how foreigners viewed their society. The caste system was said to no longer be prevalent, but to a foreigner from Canada it appeared to still be strong. The servants were not to use the washrooms within the household, but to practice open defecation in the trees beside the compound of where I lived. It was assumed that I had servants and cooks in Canada, as

most middle-income homes in India could afford the labour. As a foreign female researcher it was made clear that I was expected not to work alongside the hired labourer, but to supervise. To not work was a waste of time, so when someone came by I had to act like I was not working.

Communication was difficult not just because of my lack of Hindi skills, but also because of my lack of British lingo. With India being a former British colony, they used terms such as cybercafé and Photostat. This caused miscommunication even when speaking with a native person well-versed in English. Communication in India also varied from Canada in how formal people were with greetings. When speaking to a person considered above you, one would address them as Sir or Madam. Written proposals were formal and used vocabulary such as "kindly seek assistance" and ignoring such formalities would lead to insult and miscommunication. Such formalities were present throughout the culture but were particularly highlighted in the public sector.

The local women did not have the same liberties as foreign women, and generally worked in the home; this made it difficult to make friends with other women. It was easier to make friends with men, who were generally more outgoing and confident in speaking English, but in these and all of my interactions, I had to keep in mind what the cultural expectations were, especially as a woman.

5.8 Maintaining health and safety

My personal health and safety was a high priority to insure that I could continue working in the fields. The traffic was more intense in terms of recklessness and lack of law abiding drivers compared to Canada. There were no sidewalks and no paved shoulders on the narrow winding roads. At night, most of the town's stores would close by sunset and there were very few women out. The times I was late in returning back to my compound, I received anxious calls from Bobby and was offered car rides from random couples concerned for my safety. I generally felt safe in Himachal Pradesh and faced few issues with harassment. Some of the other foreign females I encountered who did not dress in traditional clothing and who seemed to have a less confident appearance (relative to my "don't fuck with me" face) were subjected to harassment from men on the buses and in the streets.

Himachal Pradesh was in a region with no malaria or dengue fever. However, there were scorpions (which could cause fever) (Figure 5.5), large spiders, poisonous snakes, and aggressive monkeys, all of which I had to be aware of while working alone in the fields.

Figure 5.5 – Examples of the arachnids seen in fields and buildings

The town of Palampur had a drinking-water treatment plant that consisted of a sedimentation tank with no disinfection, therefore bottled or filtered water was recommended, which is what I used. Food poisoning was a high possibility and did occur when "boil it, peel it, cook it or forget it" was not strictly followed. High turnover was the key to freshness, thus the street vendors with a lineup for fresh hot samosas or Indian burgers was safer than the more expensive, but empty, restaurants. Pharmacies were common and prescriptions were not required for the most common drugs, including treatment for colds, diarrhea, and ringworm. This was convenient as a doctor was not required to diagnose my ringworm which was easily treated with over-the-counter ointment.

5.9 Discussing and accessing toilets

People were always curious about what I was doing there, and I was not shy in sharing with them my work with using of human urine as a fertilizer. The general reaction was to laugh. In discussions about EcoSan with a variety of people (a woman running a daycare with no latrines; a few farmers; professors; a lawyer) made it clear that "only people of a low society would use such a toilet." Traditionally in the Hindu religion, the 'Untouchable' would clean human waste and general refuse, and it was made clear to me that the stigma has remained (Raghuram 2001; Ghose 2003). The people I spoke with stated that poorer states, like Bihar, would benefit from such a system, but not the people of Himachal Pradesh. The Hindu religion may have impacted their view on EcoSan, but there are still several projects of this nature throughout India being implemented despite this stigma (limited details on the success of the project) (SACOSAN, 2008, Winblad et al., 2004).

Finding a toilet in India was challenging. While in the field, I did what all other farmers do: pop a squat behind a tree. While commuting, women's public access to toilets was significantly less than men's; and when there was a women's toilet it would frequently be locked. Apparently the doors were locked due to men preferring the women's toilets to the unmaintained dirty men's toilets. When I would find a toilet, it was often was filthy (Figure 5.6) and difficult to use while wearing bagging Indian clothing and carrying a backpack.

Figure 5.6 – The cleanest of the public toilets at the Foreign Registration Office in Dharamsla, Himachal Pradesh, India

In addition to being unsanitary, the toilets were often not secure. For example, on a 12 hour overnight bus ride from Delhi to Palampur, we stopped for a short food and toilet break and I attempted to use the toilets that were inconveniently located at the back of the building. At least there was a light in the women's toilet. As I was trying to juggle a scarf, baggy pants and a backpack and not get them dirty while I squatted over the hole, I saw a man watching me through a "conveniently" placed hole in the wall. Being the sole foreigner among the few other women on the bus somewhere between Delhi and Palampur, all I could do was walk away with a full bladder.

The lack of access to toilets is an extra burden for women in their everyday lives (Government of India, 2006). With minimal privacy available, many women wait for dawn or dusk to urinate and defecate – leading to health issues, for instance urinary tract infections, chronic constipation and psychological stress (SACOSAN, 2008). Under these

conditions, the women's dignity and safety are at stake as they can endure harassment (verbal and physical) and often have to walk long distances in search of an appropriate location to defecate and even just to urinate (SACOSAN, 2008). The young females are also greatly impacted when they start menstruating and their school does not have adequate facilities for them, thus leading to a decrease of school enrolment (Government of India, 2006, SACOSAN, 2008).

During personal interactions with different communities, I observed that women appeared to be more aware of the necessity of improved sanitation behaviour as well as infrastructure. This has also been reported among women in rural areas of Kenya during Toilet Design Clinics (Salano, 2012). While it might seem effective to focus sanitation reform on women, in India men are typically the financial decision makers of the household, thus making changes in sanitation challenging to bring about.

5.10 Conclusion

Overall, the infrastructure in India was poor and many people lacked access to improved sanitation causing illness for locals and foreigners alike. The meager sanitation conditions highlighted the importance that sanitation projects have while my personal interactions reminded the importance of culturally acceptable solutions. The concept of EcoSan was seen as a poor person's solution. The lack of official partnership with a university was initially a setback, but in the end I was imbedded into the local community and gained a unique experience. Palampur, Himachal Pradesh, India was at times frustrating, and yet delightful and stimulating at the same time, such an experience would not have been gained by just working at the university. I achieved the goals of my experiment, even with the challenges. The quality of materials was poor and sourcing technical assistance was difficult, but I was able to make-do through being creative and resourceful with the materials at hand. Unfortunately, it is unknown if the soil analyses performed at the Agricultural University of Himachal Pradesh were completed to any standard, but the analysis of spinach tissue was a success. Many problems involved communications due to my lack of Hindi. The people of Palampur and surrounding areas spoke little English; a translator would have been a useful investment for portions of the project.

5.11 References

GOVERNMENT OF INDIA. Year. A movement towards total sanitation in India. *In:* South Asian Conference on Sanitation, 20-21 September 2006 2006 Islamabad, Pakistan. Government of India.

SACOSAN 2008. Sustaining the Sanitation Revolution - India Country Paper. *In:* DEPARTMENT OF DRINKING WATER SUPPLY & MINISTRY OF RURAL DEVELOPMENT (eds.) *SACOSAN III.* New Delhi: Rajiv Gandhi National Drinking Water Mission.

SALANO, G. 2012. *Toilet Design Clinics in Naivasha, Kenya* [Online]. WaterAid. Available: http://www.inclusivewash.org.au/Literature/Case%20Study%2004_Toilet%20Design%20Clinics%20in%20Naivasha%20Kenya.pdf [Accessed August 26 2012].

WINBLAD, U., HEBERT, M., CALVERT, P. & MORGAN, P. 2004. *Ecological sanitation*, Stockholm environment institute.

6 General conclusion

In order for ecological sanitation (EcoSan) projects to be successful, plant yields have to increase, sanitation levels have to rise, profits need to increase and labour should remain in the same range as current levels. To achieve this, specific crop guidelines, such as optimal fertilizer application rates are required. This thesis confirmed that human urine can be used as an equivalent to mineral fertilizers. In addition, the quantitative microbial risk assessment (QMRA) demonstrated that a urine-diverting EcoSan system used in areas prone to water-borne diseases would need specific handling and storage guidelines with recommended crops to decrease the food-to-oral disease risk.

Sensitivity analysis of synthetic human urine observed changes in the soil chemistry with the increased application of the fertilizers. The changes in pH and EC between the fertilizer treatments were significant at the higher fertilizer application levels (5 through 8) for both plant species. The spinach plants were not sensitive to the increase of electric conductivity of the soils while the mustard plants showed sensitivity; the mustard biomass production rates were not less than the control. During the 14-day trial, it was demonstrated that spinach was able to withstand significantly higher EC soil levels than were commonly reported. This result supports the theory that human urine as a fertilizer will have limited negative impacts on plant production and that different plant species will be impacted to varying degrees based on the ability of the plant to absorb the sodium in the urine.

In India, the field experiment of human urine as a fertilizer for spinach, confirmed that human urine was an equivalent fertilizer to mineral fertilizer based on the rate of biomass production. The general guidelines of applying the volume of urine produced by one person per day per meter squared produced six time higher fresh spinach biomass than the control; and when used in combination with mined phosphate and potassium, the production rate was 22.4 times greater than the control. The spinach fresh biomass continued to increase as the human urine application rate increased. There was no significant difference between nitrogen concentrations in the plant tissue for the three fertilizer treatments (the control was significantly lower). The phosphate concentrations in the plant tissue from the control and the human urine were not significantly different and the mineral and combination treatments were significantly higher than the other two. The optimal fertilizer application rate for Himachal Pradesh, India, was 59.3 m^3 of human

urine, resulting in 360 kg N, 570 kg of P_2O_5 and 720 kg of K_2O per ha per season which is higher than the current guidelines.

The acidification of the soil in the field experiment from human urine was not significantly different to the mineral fertilizer treatments. Existing fertilizer application practices use lime to buffer the pH of the soil and this practice may need to be applied to the use of human urine as a fertilizer based on our research. A follow-up field study is recommended to determine the rate of lime required based on the level of urine use. The trend of the increase in EC_{SE} of the soils from the sensitivity analysis as the concentration of human urine increased was no seen in the field experiment.

The sodium present in human urine was successfully taken up and used as a replacement for potassium by the spinach plants. Not all plant species have this ability to use sodium instead of potassium, thus guidelines required for EcoSan need to identify specific crops that have the ability of using sodium to compensate for low potassium levels. The elevated levels of sodium that can occur in the soil by using human urine can be managed by: rotation of application areas (within and between fields), regular monitoring of the soil salinity, and by flushing the soil with adequate water.

Urine has the potential to supply a significant portion of the global nitrogen fertilizer demand and more research into efficient scaling up of appropriate technologies is required to utilize this recycling of nutrients. A limitation of the urine-diverting EcoSan system is the volume of urine that is required for optimal plant production rates. This can cause logistical and financial challenges in collecting, storing and applying human urine. Some of the options for overcoming these challenges include using human urine in a continual application process (limiting storage requirements), promoting use for urban agriculture (decreasing transportation requirements), prioritizing use on high valued crops, and researching and developing economic ways of decreasing the volume without compromising the concentration of nitrogen. A cost-benefit-analysis is required to determine the point where using human urine is more economical than applying mineral fertilizers.

Overall, the infrastructure in India was poor and many people lacked access to improved sanitation causing illness for locals and foreigners alike. The meager sanitation

conditions highlighted the importance that sanitation projects have while my personal interactions demonstrated the importance of culturally acceptable solutions. The poor access to sanitation and high farming population of India makes it an ideal opportunity for promoting urine-diverting EcoSan, but India is faced with a third limitation: the cultural stigma of handling human waste. People who handle waste are typically seen to be lower in society, making people uncomfortable with the concept of EcoSan. Better marketing will be required for the EcoSan concept to be successful in penetrating the Indian market.

The Earth is running out of mineable minerals and nitrogen production is heavily dependent on fossil fuels, we need to return N-P-K that is contained in human waste back to the agricultural lands, if we want to continue at our current biomass production rates. This thesis demonstrated that urine can be used as an equivalent fertilizer to mineral fertilizer. Household and communities would benefit from the implementation of the EcoSan system by decreasing the disease burden from poor sanitation and by providing a free fertilizer source. A typical sized family in India (4.34 people) would produce enough urine to fertilize 1225 m^2 of plant production area every year. By transforming human urine into a tradable commodity, these nutrients will be better managed and will decrease the negative impacts on the environment. This study has shown that human urine can be part of the initial step towards transiting away from complete reliance on mineral fertilizer.