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Surendra K. Pradhan

Yield and Quality of Vegetables Fertilized with Human Urine and Wood Ash

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EASTERN FINLAND

AUTHOR: SURENDRA K PRADHAN

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6

Academic Dissertation

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Author's address: University of Eastern Finland
Department of Environmental Science
P.O.Box 1627
70211 KUOPIO
FINLAND
email: surendra.pradhan@uef.fi

Supervisors: Docent Helvi Heinonen-Tanski, Ph.D.
University of Eastern Finland
Department of Environmental Science
P.O.Box 1627
70211 KUOPIO
FINLAND
email: helvi.heinonentanski@uef.fi

Professor Jarmo Holopainen, Ph.D.
University of Eastern Finland
Department of Environmental Science
P.O.Box 1627
70211 KUOPIO
FINLAND
email: jarmo.holopainen@uef.fi

Reviewers: Professor Blanca Elena Jiménez Cisneros, Ph.D.
Instituto de Ingenieria UNAM
Ciudad Universitaria, Mexico
email: bjimenezc@iingen.unam.mx

Professor Pearson Mnkeni, Ph.D.
Department of Agronomy, University of Fort Hare, Alice,
South Africa
email: pmnkeni@ufh.ac.za

Opponent: Professor Sirpa Kurppa, Ph.D.
MTT, Agrifood Finland
Email: sirpa.kurppa@mtt.fi

ABSTRACT

On the one hand, demand of food and safe drinking water is increasing with population growth and on the other hand, there is more and more environmental contamination due to unmanaged sanitation. Open defecation and raw wastewater are the potential sources of microbial and chemical contaminant in environment. These environmental pollutants can be reduced by adopting the idea of eco-toilet (urine separating toilet).

This study evaluated the fertilizer value of urine with / without wood ash in the cultivation of different vegetables. The yield and chemical and microbial quality of the vegetables fertilized with urine with or without ash were compared with the vegetables from mineral fertilizer and without fertilization. Cabbage (*Brassica campestris*) and pumpkin (*Cucurbita maxima*) were cultivated with manipulation of N as treatments provided from either mineral fertilizer or urine fertilizer. Similarly, tomato (*Solanum lycopersicum*) and red beet (*Beta vulgaris*) were cultivated with manipulation of N and P as treatments; mineral fertilizer, urine + ash and urine fertilizer. There was a control treatment (without fertilization) in all the experimental studies. In addition to these experimental studies, a questionnaire study has been done on people's awareness about urine and ash fertilizer in six communities from Central Nepal.

This study showed that the growth rate and yield production of plants fertilized with urine with or without ash fertilizer was always significantly higher compared to those obtained without fertilization. Mineral and urine fertilizer produced similar amounts of cabbage, tomatoes and red beet. Urine + ash fertilizer produced less yield (tomato) in the pot experiment but it produced more yield (red beet) in the field experiment compared to only urine fertilizer.

There were no indicator microbes detected in tomatoes but a few indicator microbes were detected in cabbage and pumpkin. Similarly, a few indicator microbes were also detected in mineral and urine + ash fertilized red beet roots. The cabbage and red beet from all treatments had similar amounts of nitrate but this was lower in pumpkin and tomato from urine fertilizer compared to the other treatments. Urine fertilized tomato contained a significantly higher amount of β-carotene compared to that in non-fertilized tomatoes. The fertilized soil always had a higher total-N content compared to the soil before cultivation. In the red beet experiment the soil pH and conductivity was increased in the wood ash supplemented soil in comparison to other treatments.

Our questionnaire study showed that the majority of the participants were not aware about eco-toilet, fertilization with urine or the fertilizer value of wood ash but this was dependent on education. The majority of the participants expressed interested to use urine fertilizer since it is a free fertilizer and mineral fertilizer was not in their immediate locality and agricultural yields were declining with the time.

In conclusion, urine fertilizer with or without ash, can be used as a substitute for mineral fertilizer in vegetable production, to increase the yield biomass, to improve the reported nutrient quality and to improve the soil potential, without chemical and microbial risks. Meanwhile, on awareness program about this concept needs to be organized in different parts of the world which might help people to adopt this practice.

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CAB Thesaurus: fertilization; fertilizers; urine; wood ash; cultivation; vegetables; cabbages; *Brassica campestris*; pumpkins; *Cucurbita maxima*; tomatoes; *Lycopersicon esculentum*; beetroots; *Beta vulgaris*; yields; yield increases; growth rate; crop quality; chemical properties; flavour; sugar content; protein content; β-carotene; lycopene; glucosinolates; microbial contamination; sanitation; hygiene; nutrients; nitrogen; phosphorus; soil pH; conductivity; awareness; questionnaires; Nepal

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Surendra K Pradhan

Abbreviations

ADI	acceptable dietary intake
C	carbon
Ca^{2+}	calcium
$CaCO_3$	calcium carbonate
CAO	calcium oxide
Cd	cadmium
Cl^-	chloride
$CO(NH_2)_2$	Urea
Cr	chromium
Cu	copper
DAD	diode array detector
DW	dry weight
FAAS	flame atomic absorbance spectrometry
FW	fresh weight
HCO_3^-	hydrogen carbonate
HPLC	high pressure liquid chromatography
INGO	international non-governmental organization
K^+	potassium
K_2SO_4	potassium sulphate
K_2CO_3	potassium carbonate
Mg^{2+}	magnesium
$MgNH_4PO_4\ 6H_2O$	struvite
MS	mass spectrometry
N	nitrogen
Na^+	sodium ion
NGO	non-governmental organization
$NH_3(aq)$	ammonia liquid
$NH_3(g)$	ammonia gas
NH_4-N	ammonium nitrogen
Ni	nickel
NMR	nuclear magnetic resonance
NO_3-N	nitrate nitrogen
NO_2-N	nitrite nitrogen
OH^-	hydroxide ion
OM	organic matter
P	phosphorus
Pb	lead
WHC	water holding capacity
Zn	zinc

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are to referred in the text by their chapter numbers.

CHAPTER 2. 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.

CHAPTER 3. Pradhan, S.K., Pitkänen, S., Heinonen-Tanski, H. (2009) Fertilizer value of urine in pumpkin (*Cucurbita maxima*) cultivation. Journal of Agriculture and Food Science. 18: 57–68.

CHAPTER 4. 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 Agriculture and Food Chemistry. 57(16): 7612–7617.

CHAPTER 5. Pradhan, S.K., Weisell, J., Holopainen, J.K., 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. (DOI:10.1021/jf9029157)

CHAPTER 6. Pradhan, S.K., Heinonen-Tanski, H. (2009) Knowledge and awareness of eco-sanitation in central Nepal: a questionnaire survey. Environment, Development and Sustainability. DOI 10.1007/s10668-009-9220-5

CONTENTS

CHAPTER 1: General Introduction....15
1.1. Importance of the study- use of urine and ash fertilizer....17
1.2. Eco-sanitation……………....17
1.3. Urine fertilizer....21
1.4. Quality of urine....22
1.5. Quality of fruits and vegetables....24
1.6. Eco-sanitation in community level……………....27
1.7. Nutrients recycle………....28
1.8. Obstacles....29
1.9. Wood ash fertilizer....29
1.10. Soil quality....30
1.11. Aim of the study....30
References....32

CHAPTER 2: Use of human urine fertilizer in cultivation of cabbage (*Brassica oleracea*) – impacts on chemical, microbial and flavor quality....41

CHAPTER 3: Fertilizer value of human urine in pumpkin (*Cucurbita maxima*) cultivation 51

CHAPTER 4: Stored human urine supplemented with wood ash as fertilizer in tomato (*Solanum lycopersicum*) cultivation and its impacts on fruit yield and quality 65

CHAPTER 5: Human urine and wood ash as plant nutrients for red beet (*Beta vulgaris*) cultivation: impacts on yield quality....73

CHAPTER 6: Knowledge and awareness of eco-sanitation in central Nepal - a questionnaire survey....81

CHAPTER 7: General Discussion....97
7.1. Urine and ash quality....99
7.2. Growth and yield Production....99
7.3. Microbial quality....101
7.4. Chemical quality....102
7.5. Residual nutrients in soil....103
7.6. Acceptance of urine fertilizer....105
7.7. Conclusions....106
References....107

CHAPTER 1

GENERAL INTRODUCTION

General Introduction

1.1. IMPORTANCE OF THE STUDY-USE OF URINE AND ASH AS FERTILIZER

Safe pure water and food availability will be two of the main issues dominating this century. On the one hand, the availability of water and food may be reduced due to global warming (reducing rain fall or increasing sea level or flood in different part of the world), drought, population growth, bio-fuel production etc. On the other hand, water sources, food and soil can become contaminated with enteric microbes, N (nitrogen) and P (phosphorus) due to unmanaged sanitation and this is the potential source of many diseases and environmental pollution. It is important to reduce the environmental burden but equally important to increase the food production. It is believed that the eco-sanitation especially the separating eco-toilet (Figure 1) is one good way to reduce water contamination, improve environmental hygiene and also to increase the food production. Human urine contains a high amount of plant nutrients, N, P and potassium (K^+) (Schouw et al. 2002, Heinonen-Tanski et al. 2007, Pradhan et al. 2007, 2009a, 2009b). Similarly, wood ash contains high amounts of P, K^+, Ca^{2+} (calcium) and Mg^{2+} (magnesium) (Erich et al. 1991, Moyin-Jesu 2006 and 2007, Huotari et al. 2008, Pradhan et al. 2009a). These elements present in urine and wood ash could be used as fertilizer for plant cultivation. There is still debate about the bioavailability of the nutrient content in urine and wood ash, and quality of the products from urine fertilizer. The nutrients present in urine and wood ash might response differently in different cultivation environment and there is not much scientific information to answer this issue. These controversial issues encouraged us to conduct this study and as far as we are aware, this is the first time that the use of urine with ash in plant cultivation has been addressed.

1.2. ECO-SANITATION

Sanitation means to sanitize hazardous waste to reduce the associated health and environmental risks. In eco-sanitation, the wastes are sanitized and also recycled or reused properly if possible. Therefore eco-sanitation is an interesting concept and an important way to solve the sanitation issue since it confers multiple benefits. The most common sanitation system in the world is the pit latrine which provides on-site sanitation for 20% of the population in developing countries (WHO 2007, Esrey et al. 1998). Although a pit toilet is better than open defecation in protecting the population from infectious diseases, it still may contaminate the groundwater due to the transport of pathogens originating from fecal deposits and it can cause nitrate leaching (Lagerstedt et al. 1994). If the fecal matter is kept dry, especially if its pH can be elevated by adding lime or ash, enteric pathogens will be inactivated (Esrey et al. 1998, Carlander and Westrell 1999). Therefore, the eco-toilet is a good way to solve these problems.

In general, an eco-toilet means a low energy consuming toilet. There are many kinds of eco-toilet on the market but we will focus on the urine diverting toilet, where urine and fecal matter are collected separately (Figure 1). With this technology people can obtain urine as a free fertilizer and the remaining fecal matter will be easier to hygienize/compost. The use of eco-toilet is also eco-efficient which means more goods produced by the recycling of waste resources and it also reduces pollution. Water is limited in many areas of the world and in this respect, the eco-toilet could be beneficial because it does not need water or can be maintained with very little water which will allow the fecal matter to dry as a way to reduce the survival of enteric microbes (Esrey et al. 1998). Although the concept of eco-toilet is different from the conventional toilet, the idea of eco-toilet can be made very simply as the installation of a can only for urine collection next to the already existing toilet pan (Figure 1C). The collected urine is safer and easier to use as a fertilizer even in short time interval compared to animal manure which needs more than 6 months to be composted. Fertilizer needs might be higher in tropical countries where often more than 3 crops can be cultivated around the year.

Figure 1. Different types of separating eco-toilets

Diarrheal disease is the third leading cause of death from infectious diseases (WHO 2008). It is known that many people from third world do not have their own toilet, as more than 50 % of the people from third world are out of sanitation facilities (UNICEF 2008a) so it can be assumed that the idea about eco-toilet may encourage them to build their own toilet which would have multiple benefits. In the eco-toilet the urine is collected and stored for a few weeks to reduce the number of enteric microbes (Chandran et al. 2009) and used as fertilizer to increase the yield (Pradhan et al. 2007, 2009a). Fertilizers are becoming expensive (Hargrove, 2008) and they may not affordable for many people in many impoverished areas of Asia and Africa or may not be available in many remote villages. Therefore urine as a free fertilizer could be a good solution for use in these situations. Agricultural land in tropical countries is over-cultivated which required high amount of fertilizer. Farmers from many tropical countries are not aware about the level of recommended fertilizer for different plants which means the soil potential may become reduced. As will be shown, cultivation of different vegetables by using urine with or without ash fertilizer not only increases food yield but also improve the soil potential (Pradhan et al. 2009a). Fertilizer is not only an important means to increase the yield, it is also a way to improve the chemical quality of the products. Many children from developing countries suffer from vitamin-A deficiency. This can be avoided by using fertilizer to grow vegetables and fruits rich with carotenoids, especially β-carotene (Pradhan et al. 2009a).

Although there is not much scientific information about the incidence/violence related with toilet there are many cases reported in local newspapers (UNICEF, 2006). A toilet in the house could be very important for people, especially important for women who often limit their eating and drinking to ensure that they only need to urinate or defecate at night so when they are unobserved. They suffer from lack of privacy and dignity and need to walk long distances to find a suitable place for defecation if they do not have suitable household toilet facilities (Voices, UN-Habitat). However, open defecation at night or in the bush means that they are vulnerable to the risk of bitten by snakes, other animals, sexual harassment and assault or other accidents (UNICEF 2008).

Table 1. Sanitation coverage percentage reported by the WHO/UNICEF for some countries (WHO 2008). (NG = not given)

Countries	Households in urban / rural	Primary school / Secondary school
Ghana	15/6	
Kazakhstan	97/98	NG/25
Madagascar	18/10	
Mongolia	64/31	
Nepal	45/24	40/40
Uganda	29/34	
Vietnam	88/56	12/NG

The lack of a sanitation system is a major issue for environmental hygiene and the use of eco-toilet is a good solution. The demand of fertilizer increases with demand for food for the growing population. A large amount of fertilizer is needed to increase the yield of cereals or other products. About five times more cereal protein is needed to produce meat protein through farming (Kawashima et al. 1997). Since fertilizer is a very important means to increase the food and mineral fertilizer is becoming expensive (Hargrove, 2008) but it is possible that this problem can be partly solved by using urine fertilizer. Human urine is a natural resource, which is available in every household, containing high levels of NPK (Schouw et al. 2002). The use of urine fertilizer could 1) increase crop yields, 2) reduce the water and food contamination, 3) reduce the amount of fecal contaminated wastewater and 4) decrease the environmental pollution by reducing energy consumption, as 1.4–1.8 liter of diesel fuel is needed to produce 1 kg N fertilizer and 0.2 liter diesel is needed for the transportation of 1 kg N fertilizer (Bhat et al. 1994).

Manufacture of industrial fertilizer. Commercial urea production began in the 1920s after the development of the Haber-Bosch process (Smil 2001). In the late 1960s, urea represented only about 5% of the world's nitrogen but this has escalated and it is now estimated to account for more than half of world nitrogen fertilizer. The use of urea may reach 70% of total nitrogen production by the end of the next decade

(Figure 2) but this prediction may not be same for all of this time period. The global shift toward the use of urea as a fertilizer has started because it is less explosive than ammonium and nitrate when stored and it is more stable and cost effective to transport than other forms of reactive nitrogen. However, there are no significant differences in plant uptake efficiency between ammonium bases fertilizer and urea fertilizer (Smith and Chalk 2005).

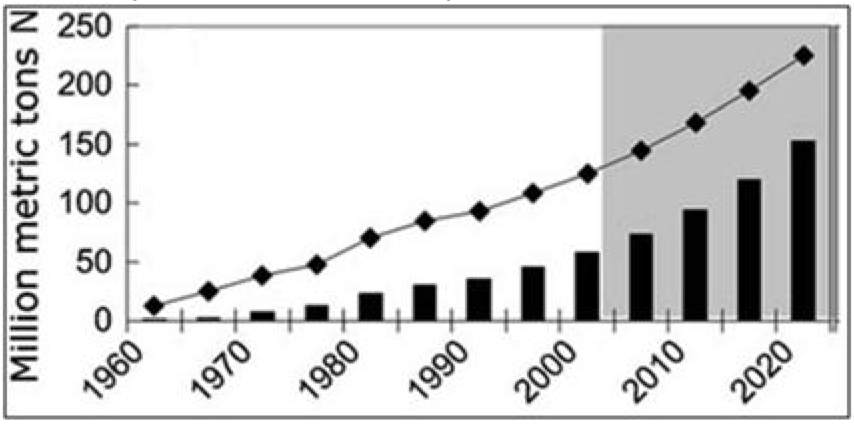

Figure 2. The global consumption (million metric tons of N) of total synthetic nitrogen fertilizers (solid line) and urea consumption (solid bars) since 1960. The shaded area is the predicted data. This figure is taken from Gilbert et al. (2006).

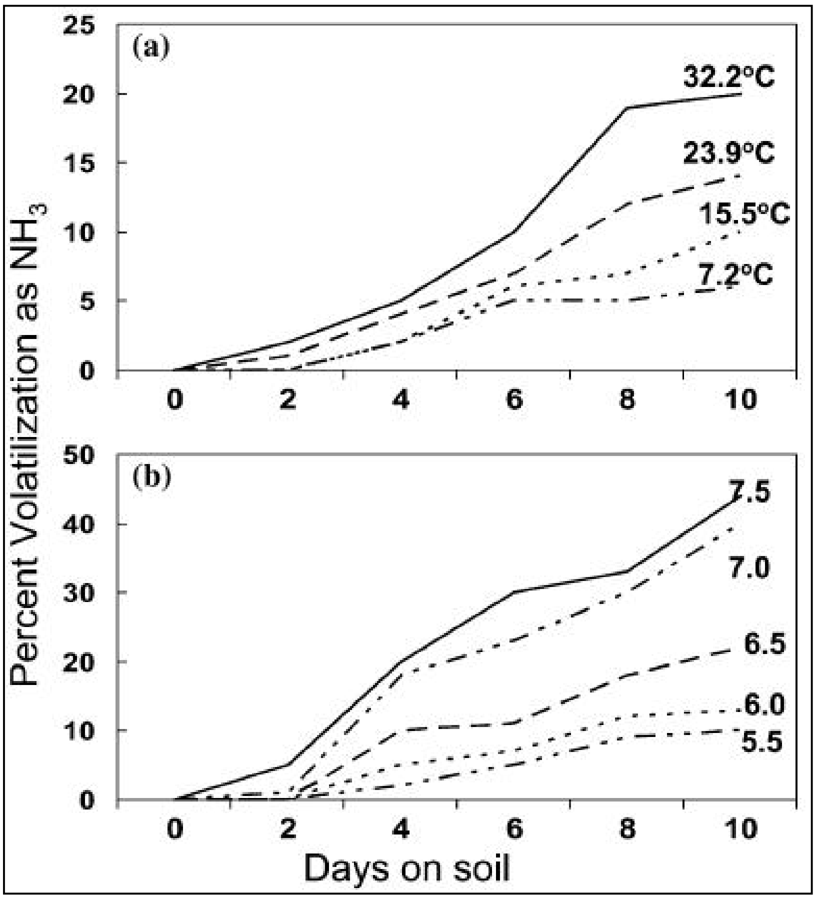

Figure 3. The percent of surface-applied urea fertilizer to soils that volatilizes as ammonia as a function of days on bare soil in North Dakota. (a) Response pattern with variable soil temperatures; (b) response pattern with variable soil pH. This figure is taken from Gilbert et al (2006)

1.3 URINE FERTILIZER

The urine is filtered by the kidneys and contains only low molecular weight substances. The pH of urine during excretion is normally around 6, but can vary in a range between 4.5 and 8.2 (Lentner et al. 1981). About 75–90% of N is excreted in urine as urea ($CO(NH_2)_2$) with the remainder mainly as ammonium and creatinine (Lentner et al. 1981). Urea quickly degrades to ammonia and carbon dioxide by urease, and the hydroxide ions produced normally will increase the pH to about 9.3, usually this happens within hours (Vinnerås et al. 1999, Jönsson et al. 2000). This process can be similar if urea fertilizer (urine/synthesized) is applied in soil but the duration of this process can vary from a few days to a few weeks.

Hydrolysis of urea (Hanaeus et al. 1996).
Ammonium and bicarbonate are produced and pH becomes increased:

$$CO(NH_2)_2 + 3H_20 \leftrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Ammonium is in equilibrium with dissolved ammonia:

$$NH_4^+ + OH^- \leftrightarrow NH_3\,(aq) + H_2O \quad (2)$$

The pKa-value for the equilibrium is 9.3 at 25 ^oC.
Dissolved ammonia is in equilibrium with gaseous ammonia:

$$NH_3\,(aq) \leftrightarrow NH_3\,(g) \quad (3)$$

Ammonium is a directly plant-available N fertilizer and urea and ammonium are two of the most widely used industrial N fertilizers in the world (Overdahl et al. 1991). Many crops prefer nitrate or ammonium and the ammonium applied to arable soil is transformed to nitrate within a few weeks. The plant availability of urine N is based on its urea or ammonium content. This is to be expected, as 90–100% of N in urine is found as urea or as ammonium, as verified in fertilization experiments (Kirchman & Pettersson 1995, Richert Stintzing et al. 2001). A loss of nitrogen as NH_3 and toxicity to plants can occur during urine management and application as fertilizer (Blouin 1979). Ammonia evaporation can be minimized by immediate mixing of urine with top soil (Rodhe et al. 2004). The nitrogen content in human urine exist in the form of ammonium bicarbonate solution and N crop uptake is less from urine (42%) than in the case with ammonium nitrate (53%) which indicates that around 6–7% N is being lost by volatilization (Kirchmann and Pettersson 1995). High pH and temperature increase the loss of ammonia (Figure 3) and thus it is important that care is taken during the application of urine fertilizer i.e. it should be applied in the evening or there should be irrigation after application of urine. The loss of nitrogen through ammonia evaporation during storage and spreading can be reduced by 10–20 % with better management and good agricultural practice (Johansson 2000, Richert Stintzing et al. 2001, Jönsson et al. 2004).

Rock phosphate is the raw material for the commercial P fertilizer. The shortage of raw material for P has increased its price and its use in agriculture is on the wane. In general, plants take up P from the soil in an inorganic form (Marschner 1995) but a considerable fraction of the soil P (50–80% of the total) is present in organic compounds (Turner et al. 2002), which are unavailable to plants unless mineralization takes place. P limits plant growth, requiring large inputs of phosphate fertilizer in agricultural systems. On the other hand, if there are unmanaged sources of high levels of phosphorus, this element can leach to lakes, rivers, and other bodies of water (Correl 1998, Uusi-Kämppä and Heinonen-Tanski 2008). The P present in the urine is 95–100% inorganic in the form of phosphate ions (Lentner et al. 1981) and these ions are directly plant-available (Kirchmann & Pettersson 1995).

Similarly, K^+ is also a very important element for plants since this element especially increases the growth of the plant root (Mullins et al. 1994). In urine K^+ is present as ions which are directly plant-available. This is the same form of potassium as supplied in chemical fertilizers (Kirchmann & Pettersson 1995). Cl^- is also an important element for plants although its roles in plants are controversial (Hind et al. 1969). The high concentration of Cl^- found in human urine can be also typical in cow and pig urine (Kemppainen 1989).

Urine fertilizer and its chemical products. The collected urine can be used for N and P extraction by formation of struvite ($MgNH_4PO_4\ 6H_2O$). This is one technical option to reduce the volume of the urine and this technology is attractive because it is a good slow-release crystal fertilizer and free of pharmaceutical residues (>98% removal) (Johnston and Richards 2003, Escher et al. 2006) but this technology is expensive and may not be easy to achieve at the household level. Similarly, another technical option is to stop hydrolysis of urea by the addition of acid so that the N loss as ammonia during application can be reduced (Hellström et al. 1999).

1.4 QUALITY OF URINE

Microbial quality. In general, pure human urine contains very few enteric microorganisms (Heinonen-Tanski et al. 2007) if there is no fecal contamination or in a healthy person, urine is sterile when it is excreted from body (Palmquist and Jöhanssone 2003). The microbial quality of collected urine depends on the possible route of contamination which could be either by excretion of pathogens in urine or by fecal contamination. Theoretically, urine could contain *Salmonella typhi* and some other *Salmonella* bacteria if this bacterium group were present in the blood in the acute phase, *Mycobacterium tuberculosis* if the tuberculosis bacteria had infected in kidneys, bilharzias causing parasites, *Schistosoma haematobium* (Feachem et al. 1983, Obeng et al. 2008). However, enteric indicator microbes are known to be destroyed with increasing pH during the storage time period (Chandran et al. 2009). In addition, pathogens can not survive in soil for a longer time (Table 2). Höglund et al. (2002) and WHO (2006) has recommended that the urine should be stored for 6 months at 20 ^{o}C before use as a fertilizer for these hygienic reasons. The guidelines for the use of fertilizer are; for fecal coliforms <1000 CFU/g compost in Finland, (Guidelines for microorganisms) and for *E. coli* <10^5/100 ml grey-water (WHO 2006).

Table 2. Survival times of pathogens in soil at 20–30 °C (Feachem et al. 1983) in urine (WHO 2006) and, inactivation of Ascars suum, egg in urine (Nordin et al. 2009). (NG = not given)

Organisms		Survival time in soil (days)	90% reduction in urine at 4 °C/20 °C (days)
Viruses	Enteroviruses	<100 (usually <20)	
	Rotavirus	NG	172/35
	S. typhimurium (phage 28B)	NG	1466/71
Bacteria	Faecal coliforms	<70 (usually <20)	NG
	Salmonella spp	<70 (usually <20)	NG
	Vibrio cholera	<20 (usually <10)	NG
	Gram negative	NG	1/1
	Gram positive	NG	30/5
Protozoa	*Entamoeba histolytica cysts*	<20 (usually <10)	NG
	Cryptosporidium oocysts	>12 months	NG
	Cryptosporidium parvum	NG	29/5
Helminth	*Ascaris suum* egg		10 (inactivate at 34 °C)

Chemical quality. Human urine contains a high amount of N and an adequate level of P and K^+. Collectively, the chemical content in urine depends on the diet of the individuals. The N contents could be lower in urine collected from vegetarians or consumers with a low protein diet. The meat consumption rate is lower in people in many developing countries, especially in Africa and South Asia (Andrew and Speedy 2003) so that the N contents may be lower in urine collected from developing areas. Similarly, the greater amount of sweating which occurs in tropical countries increases the dermal nitrogen excretion and thus it decreases the renal nitrogen excretion to urine (Takahashi et al. 1985). In addition to NPK, sufficient amount of Na^+ and Cl^- are also present in urine (Table 3).

Pharmaceutical residues and hormones in urine. Pharmaceuticals are a very diverse group of chemicals and they vary with respect to types, derivatives and amounts from year to year. Although most antibiotics are used for the treatment of infections in humans and animals, a significant quantity of these are also used as a feed supplement to promote growth of food animals. As much as 90% of some antibiotics can be excreted to urine as the parent compound (Phillips et al. 2004, Kumar et al. 2005). Goodman et al. (2006) reported that as much as 10 % of administered Tricor (Fenofibrate) is excreted via urine without metabolism. Hence, the risks of pharmaceuticals in urine is a topic of interest especially as not every pharmaceutical is destroyed during storage (Butzen et al. 2005, Gajurel et al. 2007). Paracetamol (analgesic) for home medication has been detected in wastewater (Tidåker 2003). However, many antibiotics are photodegradable (Doll and Frimmel 2003) and many medicinal compounds are degraded by soil microbial activity (Badalucco et al. 1994, Winker 2009). Thus it can be assumed that any possible medicinal residues in urine fertilizer would be degraded after urine application in soil. The medicinal residues in fertilizer applied to soil could theoretically be taken up by vegetation but only in a few cases and at low concentrations depending on the individual medicines (Winker 2009).

Endocrine-disrupting chemicals (EDCs) or hormones are worrying chemical compounds in wastewater and which are also excreted via urine. Although the effect of estrogen in the environment is not clearly understood, it has been argued that they pose a possible environmental risk (Teske et al. 2008). Elevated estrogen levels have been detected in soil as well as groundwater and surface water adjacent to agricultural fields fertilized with animal waste (Hanselman et al. 2003). On the other hand, EDS is also present in animal manure which has been in use as fertilizer for many centuries.

In wastewater treatment ozonation is applied to specifically inactivate the biological activities of pharmaceuticals and hormones (Huber et al. 2003) e.g. ozonation (1.1 g/L) removed 99% of hormones in urine (Escher et al. 2006). However, recent studies indicate that photodegradation of estrogenic compounds by natural solar radiation is a plausible degradation pathway in both sea water (Zuo et al. 2006) and river water (Lin and Reinhard 2005). Several natural and synthetic estrogenic hormones, e.g. 17α-estradiol, estrone (Colucci et al. 2001), and 17β-ethynylestradiol (Colucci and Topp 2001), were found to degrade rapidly in soil. Biodegradation is an important process in the environment and this can be influenced by pH, soil type, temperature and microbes (Gavalchin and Katz 1994, Hildebrand et al. 2006). Xuan et al. (2008) reported that the low pH (>2 pH) in the soil might prevent degradation of hormones due to slow microbial activity at such a pH. Degradation of chemicals also depends on the structure of the molecules such as aromatic compounds with sulphate and halogen groups are degraded more slowly than those without moieties (Jones et al. 2005). The concentrations of some substances in urine might also be affected by degradation during storage of the urine (Winker et al. 2008). However, possible plant uptake of medicinal residues and hormones will need to be studied in detail, on the other hand, urine from the healthy person in an ordinary family may contain so little pharmaceutical residues that the risks may be acceptable (Winkler 2009).

Moreover, human urine is almost free of cadmium and a total of thirteen other heavy metals (Palmquist et al. 2003). The heavy metal content in human urine is 50–100 times lower than that generally present in Swedish soil (Anderson 1977) or in garden compost (Kirchmann and Widén 1994).

1.5 QUALITY OF FRUITS AND VEGETABLES

Microbial quality. Raw fruits and vegetables have been known to serve as vehicles for infectious disease for a long time. The bacterial contamination can take place during both pre- and post-harvest periods, however, raw vegetables in the market were found to be more contaminated with total coliforms and *E. coli* than the vegetables in the agricultural field (Thi Van Ha et al. 2008) this might be due to an unhygienic post harvesting environment. In fact, vegetable crops produced in a natural environment cannot be expected to remain free from microbial contamination (Sagoo et al. 2003) and the processing stages of fresh-cut products, such as handling, cutting, shredding, slicing and grating, are all potential sources of contamination, which could further increase the microbial load. Pradhan et al. (2007) detected a few microbes in cabbage but there was no difference in the microbial quality of urine fertilized cabbage compared with mineral fertilized and control cabbage. Although there is always a risk of microbial contamination in field-grown fruits and vegetables via soil, air and water, the risk is higher in leaf vegetables, root vegetables and vegetables with a rough surface such as cauliflower or broccoli compared to smooth surfaced fruit and vegetables.

Chemical quality. Contamination by different heavy metals is known to be a risk factor related with vegetables. The NO_3^- (nitrate) and NO_2^- (nitrite) contents in vegetables are another major concern. In addition to these common chemicals present in vegetables, there are large amounts of other chemical compounds present in different types of vegetables. Protein, carbohydrates, fats, vitamins and minerals are the main components which should be included in our diet as sources of energy and if we are to increase food production with urine fertilizer it is important that these dietary components are available to poor people. A few other chemical compounds, which were examined in this study, are described in brief;

Protein. Protein is a very important chemical component of our diet. The main protein sources in diet are often meat, egg, fish and milk products and the production and consumption of protein in the world are represented by the nitrogen balance (Kawashima et al. 1997). The use of fertilizer has increased the amount of protein in canola (Brennan and Bolland 2007) and in wheat (Ayoub et al. 1995) which appears to be similar in other food crops. Increasing of agricultural products or plant biomass will indirectly increase the availability of animal protein if it is used for animal feed. In this circumstance, urine fertilizer may help to make more protein available in their diet, even for poor people.

Glucosinolates. The glucosinolates are a class of aromatic compounds that contain a side chain (R-group) and D-glucopyranose as β-thioglucoside attached to carbon atom no. 0 in (Z)-N-hydroximine sulfate esters (Olsen and Sorensen 1981). The glucosinolates and their breakdown products are important aroma and flavor compound in *Brassica* vegetables (MacLeod 1976). In general, N and S fertilizer does not affect the level of glucosinolates in broccoli (Rosa et al. 2006) and this may well be similar in plants other than *Brassica*. There is epidemiological evidence to support the possibility that glucosinolates breakdown products derived from *Brassica* vegetables may protect against human cancers, especially those of the gastrointestinal tract and lung (Johnson 2002).

Lycopene and **β**-carotene. Lycopene is a bright red carotenoid pigment and phytochemical found in tomatoes and many other red fruits and vegetables. Carotenoids are synthesized by both plants and micro-organisms and are widely found in the environment, being involved in the color pigmentation of many flowers, fruits and vegetables. Lycopene is the most common carotenoid found in the human body and is one of the most potent carotenoid antioxidants. It has been demonstrated to have possible anti cancer activities and is involved in the modulation of intercellular communication and alterations in intracellular signaling pathways (Stahl and Seis 1996). Fanasca et al. (2006) described that a high level of K^+ in fertilizer solution could increase the lycopene content in tomato. Trudel and Ozbun (1971) have reported a 40% increase in the lycopene concentration when the potassium concentration in the nutrient solution has been increased from 0 to 8 mM, whereas a 26% decline in the β-carotene concentration has been observed. Similarly, Taber et al. (2008) reported that K^+ fertilizer could increase the lycopene concentration in tomato (cultivar, Florida hybrid). The β-carotene content seemed to increase with an increased nitrogen supply; though there is some controversy about the impact of nitrogen supply on the lycopene content (Colla et al. 2003, Dumas et al. 2003).

Betanin. Betalains are the nitrogenous vacuolar pigments of 13 families within the plant kingdom also accumulating in some members of the Basidiomycetes (Clement and Mabry 1996). Betanin is a red glycosidic food dye obtained from red beet. The beets can serve as a natural organic food coloring if the beetroots themselves have been grown organically. The main food pigments found in the common red beet (*Beta vulgaris)* are the betalains, water soluble compounds from a class of the flavanoid molecules found in certain plant species. Betalains are a source of antioxidants and are susceptible to color changes depending on their environment. The betalains in plant extracts possess good antioxidant properties and recently, there has been a renewal of scientific interest in the functional properties of these plants (Cao et al. 1996). Betanin is a specific betacyanin and is the most prominent pigment in the red beet root since it accounts for between 75% – 90% of the total visible color.

Nitrate and nitrite. The concentrations of NO_3^- and NO_2^- in vegetables have been the focus of attention for a long time. Both NO_3^- and NO_2^- are widely present in human and animal foodstuffs, both as intentional additives and as undesirable contaminants. In general, leaf, stem and flower vegetables are rich in NO_3^- (Kenny et al. 1975). The application of nitrogenous fertilizers leads to accumulation of NO_3^- in a wide range of plant species (Barker et al. 1971). Most of the nitrogen (N) in plants is taken up initially in the NO_3^- form. However, vegetables which accumulate very high concentrations of NO_3^- may also contain significant amounts of NO_2^-. This high NO_2^- concentration arises mainly from microbial reduction of nitrate (Wright and Davidson 1964). A diet with high level of nitrate and nitrite can be a problem especially for infants (blue baby syndrome). The acceptable daily intake for the NO_3^- ion is 3.65 mg / kg of body weight (equivalent to 219 mg / day for a 60 kg person) (EU 1995).

Sugars. Sucrose, lactose, and fructose are the main compounds in the group of soluble sugars. Human taste buds interpret their flavor as sweet. Sugar as a basic food carbohydrate primarily comes from sugar cane and from sugar beet, but also appears in fruits as well as in many other sources. Disaccharides occur most commonly as sucrose which is created from one glucose and one fructose molecules. Barik (2003) reported that the N and K fertilizer can increase the yield of sugar in sugar beet. An increase of yield increases the availability of carbohydrate which is the main source of energy for human and animals.

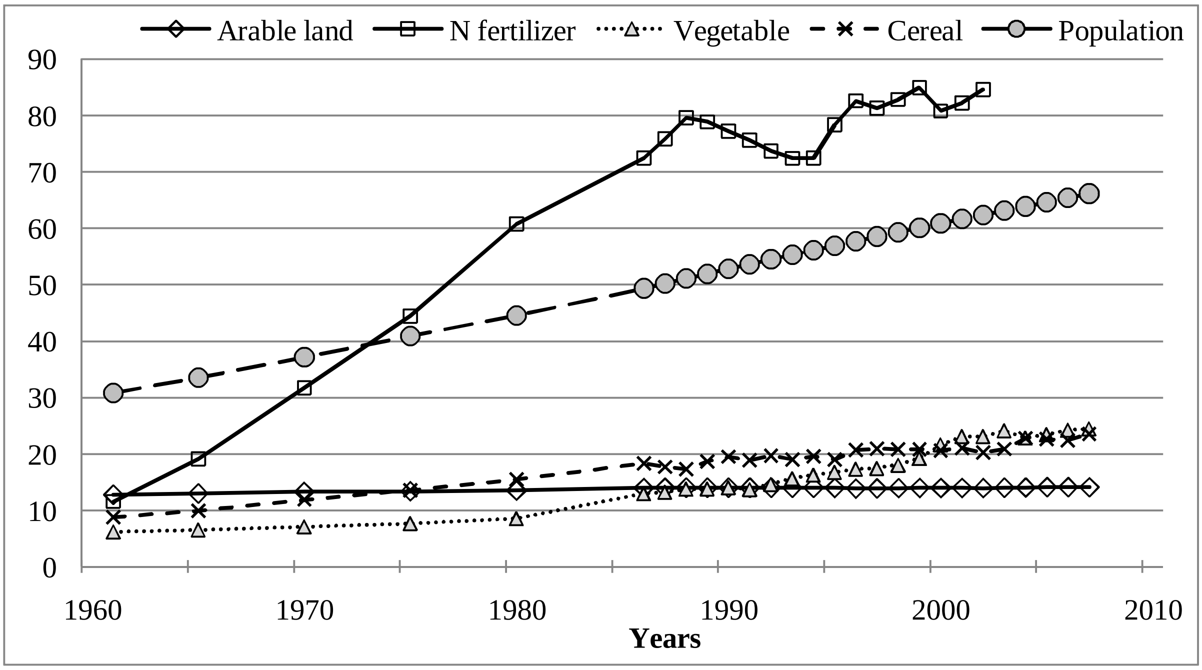

Figure 4. The increase of the world population, arable land area for farming and nitrogen fertilizer consumption since 1961. (Sources: FAOSTAT and census IDB). Arable land (10^8 ha), N fertilizer consumption (10^6 ton), vegetable production (10^7 ton), cereal production (10^8 ton) and population (10^8).

1.6 ECO-SANITATION AT THE COMMUNITY LEVEL

In global terms, about 4 billion cases of diarrhea occur annually and of these, it is estimated that 88% are attributable to unsafe water, and inadequate sanitation and hygiene (WHO 2007). About 1.8 million people die every year from diarrheal diseases, the vast majority being children <5 years (WHO 2007). WHO has estimated that 94% of the diarrheal cases could be prevented by increased availability of clean water through improved sanitation and hygiene.

Direct disposal of untreated domestic wastewater to the nearest water body such as a river, pond or lake is common in many developing countries e.g. reported by Rutkowski et al. (2007) in Kathmandu metropolitan area. Similarly, the use of water for irrigation from rivers or streams carrying sewage (IWMI 2003) is on the increase in the developing countries which can pose a potential risk for many diseases. Since the installation and upkeep of wastewater treatment plants are very expensive in many developing countries, they may not be affordable. Thus, the domestic wastewater quality needs to be improved by using different inexpensive way to protect the nearest water sources.

Very few studies into eco-sanitation have been carried out in developing countries (Regmi 2005, Pradhan et al. 2009c). Since eco-sanitation and the eco-toilet are novel concepts, it is very important that people should receive valid information about these topics and it is also equally important to evaluate their true opinions, their current awareness and prejudices. As Pradhan et al. (2009c) concluded it is very important to demonstrate this concept in practical ways so that the people, many of whom were uneducated, from indigenous society will better understand its benefit.

Many small farmers in third World countries have limited access to chemical fertilizers. In some areas of the developing world, chemical fertilizers are not readily available due to the lack of transportation e.g. mountainous regions in south Asia (Pradhan et al. 2009c). In some countries, the prices of the fertilizer have risen sharply as government subsidies have been removed in response to the economic crisis as well as increasing the price for transportation and this issue is reflected in a reduction in fertilizer use which will influence the crop yields production in negative way (Raussen 1998). The price of food is increasing due to the increase in the cost of fertilizer and limitations in the amount of arable land (Figure 4). Furthermore, the price of food is on the increase since crops are being diverted to bio-fuel production, the trend likely to continue in the future (Mitchell 2008).

The eco-toilet is one good way to reduce environmental contamination. If people can accept that human urine can be used as a fertilizer, poverty rates in developing countries could be reduced to a certain extent. Increasing the availability of vegetables would decrease their cost and promote the consumption of vegetables which is necessary to improve the dietary status, and to reduce non-communicable disease (WHO 2007) especially improving the health of children and mothers.

1.7 NUTRIENTS RECYCLE

Recycling of materials is essential in a sustainable environment and some elements such as C (carbon), N and P are important for the function of a healthy ecosystem (Winblad 1996). The manufacture of commercial fertilizers which are intensively used in agriculture consumes fossil energy and mineral resources (Hodge et al. 1994). If it continuous as its current level, than it is estimated that phosphate stores will become scarce in 10 years, and economically exploitable reserves are estimated to last for a further 100 years at the most (Steen 1998). Some nutrients like phosphorous accumulate in the soil for longer periods, and phosphorus leakage is insidious but dependent upon agricultural methods, plant uptake and irrigation.

Table 3. Nutrient contents (g/L) and pH values of human urine.

N	P	K^+	Cl^-	Na^+	pH	References
1.79-	0.21-	0.87-	2.5-			Kirchmann and Pettersson (1995)
2.61	0.2	1.15	2.24			
2.8	0.2	1.2				Heinonen-Tanski et al. (2006)
0.93	0.06	0.4	0.44		8.6	Pradhan et al. (2007)
8.2	0.7	2.1	3.03	2.34	11.14	Pradhan et al. (2009a)
7.4	0.29	16.2	NG	9		Mnkeni et al. (2008)

Table 4. Nutrient contents (g/kg) and pH values of ashes from different materials.

Ashes from	P	K^+	Ca^{2+}	Mg^{2+}	pH	References
Wood	14	41.3	317.4	22.5		Etiegni et al. (1991)
Wood	6.9	28.6	109.4	16.2		Huang et al. (1993)
Wood	9	43.6	168	11.1		Steenari et al. (1997)
Wood	36	137	216	47	11.1	Pradhan et al. (2009a)
Paper and pulp	1.8	10.3	94.9	6.5		Ohno and Erich (1990)
Paper and pulp	3	13.3	120	7.73		Muse & Mitchell (1995)
Pulp	6	34	211	19		Patterson et al. (2004)
Pine tree	15	54.6				Erich (1991)

Table 5. The concentrations of heavy metals in different manures used as fertilizers (µg/L or µg/kg)

Materials	Cu	Zn	Cr	Ni	Pb	Cd	References
Human urine	67	30	7	5	1	0	Jönsson et al. (2004)
Cattle manure	5220	26640	684	630	184	23	Jönsson et al. (2004)

1.8 OBSTACLES

Introducing eco-toilets in communities where the people have a high environmental awareness and willingness to make the effort to recycle in these situations the main problem is the reuse of anthropogenic nutrients on arable land. Storage, transport and spreading are the key issues for use of urine especially if it were to be organized on a large scale. It is important that the whole system for collection, storage and handling of human urine needs to be constructed to minimize nutrient losses. Experiences from handling and storage of animal manure have revealed that nitrogen losses can be as high as 30–77% (Misselbrook et al. 2004, Parkinson et al. 2004), and phosphorus losses around 4–30% (Kirchmann 1998, Parkinson et al. 2004). However, the issues may be quite different in the Third World. Different cultures may not accept the use of human urine as a fertilizer, especially for edible fruits and vegetables, and it may be especially difficult to introduce such a novel idea in many traditional societies. Another obstacle might be hygienic concerns; if the people are illiterate it may be difficult to teach them how to reduce possible enteric contamination during urine collection.

1.9 WOOD ASH FERTILIZER

Wood ash is a by-product of energy production from wood-burning in industries and households. It contains a high amount of Ca^{2+} in the form of $CaCO_3$ and CaO, K^+ (in the form of K_2SO_4 and K_2CO_3) and significant levels of micronutrients (Gorecka et al. 2006) (Table 4). Ash may be considered as a valuable soil conditioner but it does not contain the N i.e. N:P:K values are 0:1:3. Therefore, in order to achieve adequate fertilization, additional supplementation with an N fertilizer would be necessary (Gorecka et al. 2006). The plant availability of the P and K^+ supplemented via wood ash is of interest from an agronomic point of view. The use of wood ash in agriculture would not only increase plant yields, but would also decrease soil acidity as ash contains a large amount of Ca^{2+}.

Agronomic benefits resulting from land application by products of the pulp and paper industries such as biosolids from effluent treatment systems or wood ash from energy systems have been widely studied in Europe (Karsisto 1979), the United States (Vance 1996, Mitchell and Black 1997), and more recently Canada (Lickacz 2002). Applications of ash at levels less than 50 t/ha in greenhouse and field studies have increased the yields of oats and beans (Krejsl and Scanlon 1996), wheat (Etiegni et al. 1991, Huang et al. 1993), bean, barley and alfalfa (Meyers and Kopecky 1998) and barley (Petterson et al. 2004) as well as other forage crops (Naylor and Schmidt 1989, Muse and Mitchell 1995, Meyers and Kopecky 1998). Although wood ash does not contain N, the application of wood ash may promote mineralization of the considerable reserves of soil organic N, and thus over the long-term improve the availability of N for tree growth (Saarsalmi et al. 2006). Unmanaged disposal of wood ash can pollute water sources with P which might be a problem for ecosystems (Tulonen et al. 2002). In addition, disposal of wood ash regularly in the same place might elevate the soil pH. There are reports that the microbial biomass or microbial activities increases if wood ash applied to Nordic soil, thus being due to an increase in the DOC (dissolved organic carbon) and pH (Jokinen et al. 2006).

1.10 SOIL QUALITY

The natural soil varies according to its physical and chemical properties. The yield potential of arable soil also depends on the history of the soil use. The soil organic matter content is one of the main indicators of the soil potential (Jimenez et al. 2002). Today, in many places soil potential is decreasing due to unmanaged cultivation and excessive use of different chemical fertilizers, herbicides, insecticides as well as environmental changes. In general terms, unmanaged tropical soils treated with limited amounts of manure based fertilizer without liming can be assumed to be acidic (Rasiaha et al. 2004). Similarly, the soil in the Nordic countries may also be acidic as Lindroos et al (1995) reported a pH value of about 4–5 in different parts of Finland. The acidic soil could be treated with wood ash to elevate soil pH as well as improving the soil nutrients. As Pradhan et al. (2009a) reported that soil nitrogen levels increased in cultivated soil compared to the same soil before its cultivation. Similarly, Gong et al. (2008) reported that the application of organic manure and mineral fertilizer increased crop residue, TOC and culturable microorganisms in soil. Therefore, it is very important to appreciate that the use of fertilizer can improve soil potential and urine and ash fertilizer could be an important way to improve the soil quality such as, organic matter, total nitrogen and pH.

1.11 AIMS OF THE STUDY

We were encouraged to conduct this study because urine and wood ash contain high amounts of plant nutrients though most often they are viewed simply as waste. The aims of the thesis were:

1) to evaluate the fertilizer value of human urine in cultivation of cabbage (*Brassica oleraceae* Var. Castello), and pumpkin (*Cucurbita maxima* Var. Kokken)

2) to evaluate urine fertilizer with and without wood ash supplementation in the cultivation of tomato (*Solanum lycopersicum* Var. Sparta) and red beet (*Beta vulgaris* Var. Rubia)
3) to study the acceptance and attitudes about urine and ash fertilizer.

These study plants were selected because it represents, leaf, vine, fruit and root vegetables and many of them are commonly cultivated in all over the world and it also included outdoor and indoor cultivation. In addition, one aim of this study was to analyze the growth rate and, microbiological and chemical quality of the agricultural products obtained from urine and urine + ash fertilization and to compare these values with those in agricultural products from mineral fertilizer and control (no fertilization). Urine was used mainly as the source of N and the wood ash was used for supplementing with P and K^+ arranged on the basis of fertilizer recommendations for these plant species.

Urine might be not easily accepted as a fertilizer in many societies especially if it is applied on edible plants. Many people are suspicious about the quality of the urine fertilized products and this study tried to address these questions. Therefore the acceptance and attitudes about urine fertilizer in a Nepalese population have been studied in six communities from central Nepal. In addition, use of wood ash as a supplement with urine fertilizer was hypothesized to represent a good solution since additional use of ash fertilizer complements the eco-sanitation issue. This study showed that urine, with or without wood ash can be used as a fertilizer in the place of commercial fertilizer in the plants examined here.

The research questions

1) Does urine with or without wood ash fertilizer produce a significantly higher yield than the situation without fertilization?
2) Does urine fertilizer produce a similar amount of yield as can be produced from mineral fertilizer?
3) Is the chemical and microbiological quality and the taste of the urine fertilized agricultural products similar to the products treated with mineral fertilizer?
4) Does farmers accept human urine as a fertilizer for crops production in different communities?

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CHAPTER 7.

General Discussion

General Discussion

7.1 Urine and ash quality

Several studies have tried to explore the fertilizer value of human urine (Richert Stintzing et al. 2001, Guzha et al. 2005, Heinonen-Tanski et al. 2007) and wood ash (Meyers and Kopecky, 1998, Erich et al. 1991, Patterson et al. 2004, Merino et al. 2006, Huotari et al. 2008) in vegetable cultivation since urine is often seen as waste. In this study, the experimental plants were cultivated by using urine and urine + wood ash fertilizer and the crops were compared with those grown with mineral fertilizer and without fertilizer. The urine used was collected from two different sources. The urine collected from Västanfjärd contained low NPK since it had been diluted with toilet flushing water whereas the urine collected from Tampere contained high NPK. The odor of the high NPK content urine was very strong due to the high concentration of the NH_4^+–N or ammonia. Although the concentration of NPK was different in the urine collected from different places, the urine pH and the microbial quality was rather similar in both sources. One finding was that the elevation of pH in stored urine reduced the enteric microbes even in the urine containing less NPK (Höglund et al. 2002, Chandran et al. 2009) and this might be helpful information as the N content in urine can vary due to many factors. However, a few enteric microbes were detected in used urine and this might have been due to cross contamination in the separating pan. These few enteric microbes present in urine fertilizer could be anticipated to be destroyed in soil or not to survive for a long time in soil (Feachem et al. 1983) and in rhizosphere (Walker et al. 2004).

Wood ash contains a high amount of plant nutrients but it does not contain nitrogen (Table 4). The birch tree (*Betula* sp.) wood ash was collected from Nilsiä, Finland and used as a supplement of P and K^+ in combination with urine fertilizer. Wood ash has been used as fertilizer in many countries (Martikainen 1985) though it is often considered as waste in many communities (Chapter 6).

7.2 Growth and yield production

Urine fertilizer contained a relatively high amount of N compared to P and K^+ (Table 3). The amount of applied N was similar in all fertilizer treatments except for the control or no fertilization. N is primarily responsible for the vegetative growth of plants (Bi et al. 2003). In general, the growth rates of the urine fertilized plants were higher or at least similar to that obtained with mineral fertilizer in field cultivation (Chapters 2, 3 and 5) in conformation of our working hypothesis. The growth rate of urine fertilized plants was lower in the pot experiment in greenhouse cultivation (Chapter 4) but this might be due to some loss of ammonium N from urine fertilized pots in the greenhouse with its higher room temperature (Rodhe et al. 2004). However, ammonia loss can be reduced by 1) applying urine fertilizer in the evening, 2) if possible, irrigating after the use of urine fertilizer and 4) application of urine fertilizer close to the soil by using long nozzle can.

In addition, the growth rate of the urine and urine + ash fertilized plants were always higher than non-fertilized plants (Chapters 2 – 5). It was clearly seen that the fertilized plants produce significantly higher amounts of biomass compared to those with no fertilization. The root biomass of red beet with ash supplement was comparatively higher than that obtained with the other treatments, this might be due to presence of P and K^+ in ash. Mullins et al. (1994) reported that the application of K^+ increased the root biomass/root area of cotton plants. Increasing of root area/biomass might be important to secure plants more strongly to protect them against wind or landslides and furthermore, more roots would be predicted to absorb nutrients and water from deep layers which might be very important for plants growing in arid areas.

Urine fertilized plants always produced a higher biomass than non-fertilized plants (Chapters 2–5). Total yield biomass, fruit yield biomass and plant biomass of the mineral, urine + ash and urine fertilized plants were similar to each other (Chapters 2, 4 and 5). A similar result has been reported for cucumber (Heinonen-Tanski et al. 2007), wheat (Tidåker et al. 2007) and maize (Mnkeni et al. 2008). In general, growth rate and the total biomass of urine fertilized cabbage and red beet were slightly higher (not significantly) than that achieved with mineral fertilizer (Chapters 2 and 5) and the possible reason might be due to availability of NPK in liquid urine fertilizer. The biomass of red beet obtained after urine + ash fertilizer treatments was comparatively higher than obtained with other treatments (Chapter 5). This might be due to the additional nutrients (P, K, Ca^{2+} and Mg^{2+}) present in the wood ash (McDonald et al. 1994, Mullins et al. 1994). This proposal is supported also by a previous study which showed that the use of Ca^{2+} with ammonium fertilizer increased the absorption of ammonium in red beet (Fenn et al. 1994).

Urine + ash fertilized plant produced a significantly lower tomato fruit yield than that from mineral fertilized plants (Chapter 4) this might be due to loss of N at high temperature and increasing soil pH. In addition, total biomass of urine + ash fertilized plants (above ground) were higher than that obtained with urine fertilizer (Chapter 4) and this was also higher than mineral fertilizer (Chapter 5). This might be due to the additional nutrients supplement with ash (Fenn et al. 1994). Similarly, urine fertilizer produced 9% more cabbage and 10% more red beet than it could be achieved from mineral fertilizer as well as 52% more cabbage, 43% more pumpkin fruit, 420% more tomato fruit and 440% more red beet than it could be achieved without fertilization. However, the use of wood ash to increase the soil pH raises the risk of losing N through ammonium volatilization (Figure 3) therefore it is very important that the ash should be used at least three days prior or after urine application. In addition volatilization of ammonium nitrogen from applied urine is also depends on pH of the soil, i.e. volatilization could be higher in alkaline soil compared to acidic soil.

Although urine contains high amounts of N which increases the vegetative growth of plants (Bi et al. 2003), the reproductive growth can be limited by other nutrients such as P and K^+ which are comparatively low in urine. The flower development and fruit production was significantly lower in urine fertilized plants compared to the situation in mineral fertilized plants (Chapter 3). Similarly, mineral fertilized tomato produced significantly more flowers and fruits compared to urine + ash and slightly more ($P>0.05$) than urine fertilized plants (Chapter 4). This may be traced to the lack of P or / and K^+ meaning that the plants might take up Na^+ instead of K^+ as reported by Kaya et al. (2007) in tomato plants. This result is supported also by the findings that lower K^+ uptake has been shown to reduce the number of flowers and fruits (Reidel et al. 2004, Read et al. 2006). In order to increase the P and K^+ availability, and thereby possibly the number of fruits, it is recommended that urine fertilized crops should also be supplied with other fertilizers such as wood ash.

7.3 Microbial quality

The microbial quality of the urine fertilized agricultural products was similar with the products obtained from mineral fertilizer. A few enteric indicator microbes were detected in the plants irrespective if they were non-fertilized, mineral, urine + ash and urine fertilized products but the frequency was rather similar (Chapters 2, 3 and 5). The presence of these microorganisms might be due to soil contamination as reported by Gibbs et al. (1997) and Rai and Tripathi (2007). The field vegetables can be easily contaminated with enteric microbes because of different environmental factors such as insects, other animals, soil, manures, water, harvesting equipment and human handling (Machado et al. 2006). On the other hand, enteric microbes present in environment especially in soil do not survive for a long time (Feachem et al. 1983). The risk of contamination is higher if the vegetables grow nearest to the soil surface compared to those above the ground (Chapters 2–5) and as similarly report is presented by (Gibbs et al. 1997, Machado et al. 2006, Rai and Tripathi 2007).

Animal manure has been the most common fertilizer used since centuries and it frequently contains enteric microorganisms (Pell 1997, Kudva et al. 1998, Lau and Ingham 2001). The untreated manure can pose a risk of contamination or it can be a potential source for many diseases. The animal manure, municipal sludge and urine fertilizer needs to be treated by different methods to hygienize them before application to the field (US Department of Agriculture Food Safety 1998). Spreading of fertilizer and planting is the vulnerable time for pathogen entry into the food chain (Ingham et al. 2004, Machado et al. 2006). However, it is always important to use / apply urine fertilizer around the plants, not directly on any parts of the plants to avoid burning. It is also important that the use of urine fertilizer should be stopped at least 1 month before harvesting to avoid any possible risk of crop contamination. It is recommended that the urine fertilizer should be applied in the early growth of the crops since this is optimal for both microbial hygiene and the high nutrients requirement during the growth of the plant.

7.4 Chemical quality

The chemical quality of agricultural products is a major issue all over the world. The nitrate contents in urine fertilized products were either similar (Chapters 2, 3 and 5) or less (Chapter 4) than that present in mineral fertilized products. The low nitrate in urine fertilized products (Chapter 4) might be due to the higher Cl^- content in urine fertilizer; this being supported by the report of Muraka et al. (1973) that uptake of NO_3^- can be blocked competitively by Cl^-. The nitrate uptake and distribution in crops is important both for environmental reasons and for the quality of agricultural products. The residual nitrate, i.e. not taken up by crops, may potentially contribute to water contamination (Gastal et al. 2002, Wang et al. 2002). On the other hand, high nitrate uptake may accumulate high amount of nitrate in plants, especially in vegetables. Nitrate accumulation in plants is a major concern, and this is known to be a problem in many crops (Vander Boon et al. 1990). In fact, the potential adverse health effects of nitrate are mediated via its metabolite, nitrite (EFSA 2008) which can be the cause of blue baby syndrome, intestinal disorders, or it can even cause cancer, particularly gastric tumor (Mensinga et al. 2003).

With respect to the mineral nutrients the Cl^- content in urine fertilized products (Chapters 2–5) was higher than was the case with the other treatments. Similarly, the Na^+ content was higher in urine fertilized red beet roots compared to mineral fertilized red beet roots (Chapter 5). This finding can be explained by the fact that urine contains high amount of Na^+ and Cl^-. The K^+ content was lower in urine fertilized tomato and red beet root compared to other fertilizer treatments (Chapters 4 and 5) this might be due to lower K^+ or high Na^+ contents in urine fertilizer. In support of the claims of Tuna et al. (2007) and Milford et al. (2008) that plants can take up Na^+ instead of K^+ due to cation competition. It is important to do further study to find the ways to improve the K^+ quality of urine fertilized products. Fruit and vegetables rich in potassium are important for conserving bone mineral (Frassetto et al. 2001) and have other beneficial health effects (He and MacGregor 2008).

The result of our study showed that the protein content in urine fertilized products is either similar (Chapters 3 and 5) or higher (Chapter 4) than that in mineral fertilized products. The protein content was higher in fertilized products compared to non-fertilized products (Chapters 4 and 5) as reported also by Brennan et al. (2007) for canola and Hoffmann (2005) for sugar beet. This might reflect the importance of N for protein synthesis. Brennan et al. (2007) reported that N application could increase the numbers of seeds and the protein content in canola. Although vegetables are not the main sources of protein, this result highlights the fact that the impact of urine fertilizer not only to increase the biomass but it is also important for improving the yield quality.

The D-glucose and D-fructose contents were similar in urine and mineral fertilized products (Chapters 3, 4 and 5). The sucrose content was higher in mineral and urine + ash fertilized red beet roots compared to urine fertilized red beet roots (Chapter 5) this might be due to the lower amount of P, K^+ or other nutrients in urine fertilizer. This speculation is supported by the report of Draycott and Durrant (1976) which claimed that the sugar content in beet was influenced by the amount of P applied.

The total glucosinolates content in urine fertilized cabbages was similar as that achieved with mineral fertilized cabbages and there is a similar result reported by Rosa et al. (2006) for broccoli. It is assumed that the glucosinolates are sensitive to NaCl (Rosa et al. 2006) but they were not influenced by the level of NaCl present in urine fertilizer.

The lycopene content was similar in tomato fruits (Chapter 4) after all kinds of fertilization and it was not influenced by supplement of P and K^+ (Oke et al. 2005 and Fontes et al. 2000). The β-carotene level was significantly greater in tomato fruits with urine fertilizer compared to non-fertilized tomato fruits as reported by Kopsell et al. (2007a and b). The concentrations of lycopene and β-carotene may vary due to the influence of temperature and the intensity of light as has been reported for cherry tomato (Heinonen et al. 1989, Zanfini et al. 2007). This finding indicates that the fertilizer is important not only to increase the yields but also to increase the amounts of very important chemical compounds such as the carotenes. The β-carotene content in fruits should be as high as possible from a public health stand-point since this compound is a precursor of vitamin-A. There might be many other fruits and vegetables which need fertilization to improve their chemical qualities.

The amount of betanin was similar in red beet root obtained from all fertilizer treatments (Chapter 5). This result suggests that the antioxidant quality of agricultural products fertilized with urine would be similar as those produced with mineral fertilizer. In addition, the size of the urine and mineral fertilized red beet root was similar therefore the total amount of betanin obtained with these fertilizers was similar and higher than that determined in non-fertilized red beet root.

These current findings demonstrate that urine fertilizer can represent an alternative to mineral fertilizer and also emphasizes the importance of fertilizer use to increase the nutritional value of the vegetables. Hopefully, this result will motivate people to use urine and ash fertilizer to increase crop yield and the nutritional value of their agricultural products as many people cultivate their fields and gardens without any fertilizer. Although we have not studied the quality of many other vegetables it can be postulated that the results would be similar.

7.5 Residual nutrients in soil

In many places, the soil potential is declining due to unmanaged cultivation, overuse, cultivation without fertilizer, land erosion, wash-out of nutrition, drought etc. Organic matter (OM) is important in maintaining several soil properties (Gregorich et al. 1994). Manure is a good organic fertilizer to improve soil potential but urine and wood ash fertilizer also achieved improvement in the soil potential. Many of the analyzed residual nutrients contents were similar in the soil after all the fertilization experiment (Chapters 3, 4 and 5). The OM (Chapter 4) and total-N (Chapters 4 and 5) were slightly increased in all fertilized and non-fertilized soil after cultivation compared to the situation in the soil before cultivation and this might be due to the presence of some organic residues or other microorganism colonizing in the soil.

Although the Na^+ and Cl^- contents were higher in urine fertilizer their contents were similar in the soil treated with mineral fertilizer and no fertilization in outdoor field cultivation (Chapters 3 and 5). This result might be due to the high water solubility of Na^+ and Cl^- which form salt which can be washed out with water in field cultivation. The Cl^- content was higher in pot soil after cultivation i.e. in this case the salt could not washout of the soil in pot cultivation. Schwarz et al. (2002) reported that 1.25 to 8.75 dS/m EC (electric conductivity) did not affect leaf photosynthesis and dry matter but the increase in EC did reduce the leaf area. The NaCl content in urine is dependent on diet and different plants might have different salinity tolerance ranges but the relatively low amount of NaCl applied with urine fertilizer would not pose a risk since NaCl is a highly water soluble salt.

The level of residual P was greater in urine + ash fertilized soil compared to urine fertilized soil in the pot experiment (Chapter 4) but there was no P increase in urine + ash fertilized soil in field cultivation (Chapter 5). This might be because the P was applied with ash which could not neutralized in small amount of soil in the pot but it neutralized or mixed well in the field soil. However, it will be important to study the long term use of urine and ash fertilizer in soil because if the residual P increases this could leach into water sources. The level of residual K^+ was greater in urine fertilized pot soil and this may be due to the fact that plants might take up Na^+ instead of K^+ as reported by Tuna et al. (2007). This explanation may also explain the lower K^+ contents in urine fertilized tomato fruits (Chapter 4). The use of wood ash in soil could also be beneficial as it may mineralize the soil organic N (Saarsalmi et al. 2006).

Similarly, the level of Ca^{2+} and Mg^{2+} were higher when ash was added but it did not increase the concentration of these elements in soil after the cultivation. The mineral fertilized soil had high conductivity in pot cultivation (Chapter 4) this might be due to high residual NO_3^-–N (Stamatiadis et al. 1999). On the other hand, urine + ash fertilized soil had high pH and conductivity in field cultivation (Chapter 5) which might be due to the high Ca^{2+} content in wood ash. However, conductivity and pH are known to be normalized within 12 months in loamy soil in Italy (Perucci et al. 2008).

Furthermore, it is important to be aware of the soil pH before the wood ash should be used in the field since the ash is alkaline. In general, the unmanaged tropical soils which have been treated with limited manure fertilizer without liming can be assumed to be acidic (Rasiaha et al. 2004) and therefore the use of ash in tropical soil would not pose a risk. The use of wood ash in agriculture confers many benefits as Weber et al. (1985) reported that the wood ash supplement (10 tons ha^{-1}) increased peat soil pH from 4.6 to 5.5 and increased all microbial activities, resulting in increased mineralization and improve N availability. They also reported that the potential N_2 fixation (C_2H_2 reduction) was increased by the ash treatment.

7.6 Acceptance of urine fertilizer

After these successful experimental studies, people's opinions about this idea have been studied. A cross-sectional study was carried out in six small communities in Nepal (Chapter 6). The result of our study showed that knowledge about eco-sanitation and urine fertilizer was mainly dependent on education (Chapter 6). The majority of the participants were willing to use urine fertilizer and the acceptance of urine fertilizer was higher in farmers compared to other professions. The possible reasons could be that poor farmers own very small plots of land and they must produce as much food as possible. Surprisingly, many farmers who did not know about urine fertilizer were interested to use urine as a fertilizer. This might be because they realized that fertilizer was important but they could not afford to buy commercial products as reported by Manandhar et al. (2004). Regmi (2005) from Nepal also reported that majority of the dry toilet users responded that the dry toilet was better than the traditional pit toilet.

There are many kinds of eco-toilets on the market but they need to be modified according to the locality, culture and still be affordable. It is believed that the high acceptance rate in Nepal might be due to the fact that the eco-toilet option was very simple and affordable (Chapter 6). Medilanski et al. (2006) reported that the people were interested in a simple affordable eco-toilet which would be easier to maintain rather than a sophisticated expensive alternative. The people who are aware about urine fertilizer not necessarily are interested in using urine as a fertilizer in home plots (Chapter 6) This emphasizes the importance of discussing and convincing the people about this concept as presented by Lienert and Larsen (2006).

In many countries, wood ash is used as a fertilizer (Martikainen 1985) but this was not the case in Nepal (Chapter 6) and the situation may well be similar in many other developing countries. Some people use ash as a detergent for cleaning dishes but very few people in Nepal were aware about the fertilizer value of wood ash (Chapter 6) which is probably true in other developing countries. One possible reason for not using of ash fertilizer was ignorance about the fertilizer value of ash (Chapter 6). Another possible reason for not using the ash fertilizer might be that it does not contain N and nitrogen is the main component of fertilizer, responsible for the visible result on plant growth.

Many households in the developing world do not have a toilet and in that way the situation is similar to Nepal (Chapter 6). This study carries the message that "the construction of toilet is an investment not expenditure" which might encourage people to build a toilet. The multiple benefits of eco-toilet presented in our study might be helpful to the people, NGOs and INGOs who are working on the field of sanitation. Implementation of this idea increases the quality and quantity of the food and also improves the public health so people do not need to lose their working or school days and in this way improve the standard of living. However, it is important to appreciate people's opinion about acceptance of urine fertilizer applied to edible plants. There may be less resistance to the use of urine fertilizer in non-edible plants such as trees, flowers or fodder for animals. Increasing of fodder or plant biomass is not only important for cattle farming but it can protect against landslides and it can be considered as a carbon sink.

7.7 Conclusions

This study showed that the fertilizer value of urine with or without ash can be very similar to that obtained with mineral fertilizer and it carries no microbial and chemical risks on the crop products. Our result also showed that the fertilizer not only increased the biomass production it also improved the chemical quality of the products. Soil potential was improved in cultivated soil compared to the situation prior to cultivation. Urine with or without wood ash, could increase the food production by 43–440% in comparison to the situation without fertilization and it could also improve the environmental health. This technology could be important and useful for populations in rural, peri-urban and urban areas especially in the developing countries where mineral fertilizers are expensive and sometime even unavailable. This concept will also be useful in Finnish or European rural areas (especially in Eastern Europe) that are not connected to central wastewater treatment system. To a certain extent the urine collected can be used in cultivated backyards, roof top gardens etc. in urban and peri urban areas. On a large scale, a municipality would needs to have a policy to use the collected urine in parks, alleys to river banks, near agricultural field or forests etc. Similarly, use a system where by the N and P could be extracted by making struvite from large volume of urine collected.

However, the possible long term effect of urine and ash fertilizer in different soil types, the effect of NaCl in urine on the uptake of other nutrients by different plants and the possible risk of pharmaceutical residues and hormone in urine fertilized plant products all need to be studied in more detail.

The findings of this study demonstrated the fertilizer value of urine but work will be needed to make this technology more acceptable to the general public. Since human excreta are not generally viewed as fertilizer, there may be resistance to using human urine as a fertilizer. The eco-toilet currently available on the market may not be acceptable in all societies. This emphasizes the importance of modifying the system so that it is appropriate for local circumstances. In this way, people will be encouraged to change their sanitary habit so that it becomes eco-sanitation. This change can only happen voluntarily, it can not be forced on the population by decree. The population has to realize that urine fertilization is not only acceptable but also effective.

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Surendra K. Pradhan

Yield and Quality of Vegetables Fertilized with Human Urine and Wood Ash

This doctoral dissertation contributes to the knowledge about yield and microbial and chemical quality of the vegetables produced by using urine with or without wood ash fertilizer. Urine with or without wood ash fertilizer produced similar amount of vegetable biomass which can be produced by mineral fertilizer and the quality of the products were also similar to the products from mineral fertilizer. However, possible risk of pharmaceuticals residue in urine has been a question of this work.

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