Application of human urine as liquid fertilizer in agriculture

by

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Engineering

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ABSTRACT

Human urine fraction contains the major plants nutrients in domestic wastewater, with 80% of the nitrogen (N), 55% of the phosphorus (P) and 60 % of the potassium (K). Thus, urine has a potential to be reused in agriculture as a liquid fertilizer in order to replace industrial fertilizer, especially in marginal area of developing countries, where accessibility of fertilizer for the farmers when needed in currently a great concerns. Therefore in recent years, human urine has been compared successfully with commercial fertilizer, using diverse types of vegetables and cereals. However, urine contains some salts, pathogens, pharmaceuticals and hormones. Concerning salts issues too much urine application in agriculture land may cause accumulation of several ions in soil. Among these ions, excess of sodium (Na) in soil, inhibits plant growth; while excess of N can built up in plant tissue and affect negatively amount of sugar and vitamin in vegetables, causing therefore health and taste issues to consumer. Subsequently, to address salts issues and provide a better understanding of urine application in agriculture, this present research work was carried out with these following specific objectives 1) evaluate when and how often-human urine should be applied in agriculture as liquid fertilizer 2) examine the effects of uncontrolled application of human urine dose in agriculture 3) examine the effects of continuous application of uncontrolled human urine application dose in agriculture. From this research it was suggested that human urine application at one time before seedling is not effective for plant growth. Besides, application of adequate human urine volume should be based on plant N requirement, and management of salts from urine is required even in the adequate urine volume application for its sustainable reuse in agriculture.

Chapter 1
This chapter describes the advantages and disadvantages of using human urine as liquid fertilizer in agriculture, summarizes the state of the art regarding its reuse in agriculture and then outlines the different objectives of this present research.

Chapter 2
This chapter addresses when and how often-human urine should be applied agriculture as liquid fertilizer. Thus, pot test was conducted in greenhouse using synthetic urine, spinach and sandy soil as media. Seven treatments, those apply same amount of urine in different pattern and control (total 8 cases) were set to know adequate urine application way. From this research, the results revealed that, human urine application at one time before cultivation is not effective for plant growth and production, because of high ions intensity in low saline soil and leaching of nutrients from soil (N= 38.3 %, P= 9.1 %).

Chapter 3
This chapter examines the effects of extra human urine volume application in plant and soil. Thus, pot test was conducted in greenhouse using synthetic urine, Komatsuna and sandy soil. 34.86ml of urine (U-Vo) which contained 140mg-N and corresponded to N-based standard requirement for Komatsuna, 69.72ml of urine (U-2Vo), 104.58ml of urine (U-3V_0), 58.82ml of modified Hoagland's solution (V-H) which contained 140mg-N as positive control and non-fertilizer (C) were applied into different pots (1/10000a). The results show that, application of extra urine volumes in the range of 2-3 higher than plant requirement had no inhibition effect on plant growth and production in one time cultivation, since treated soils, were far to be saline when considering the references values given by FAO, 1998 (EC<2mS/cm; SAR< 13). However, urine fertilization caused nutritional imbalance (low plant K/Na ratio), which was severe in extra urine volumes application (U-2Vo and U-3Vo). Besides, excess amount of urine volume increased nitrogen content in plant tissues.

Furthermore, application of triple volume of urine (U-3Vo) (U-3Vo) caused an accumulation of nitrogen and sodium in soil and promoted high N lost (>24 %) from soil. While, adequate urine application (U-Vo) caused a low N lost (<18%) from soil and no accumulation of N was observed, but more than 50 % of the total sodium applied through urine and irrigation water remained in soil after one time cultivation. Therefore, application of adequate human urine volume based on plant N requirement might be a better option for its sustainable reuse in agriculture.

Chapter 4

This chapter examines the effects of continuous application of extra human urine volume on plant and soil through continuous cultivation. Thus, the experiment was pursued using same soil, same plant (Komatsuna) and similar rate of fertilizer as designed in the 1st cultivation and then 2nd and 3rd times cultivation were performed. The results revealed that continuous application of extra urine volume in the range of 2-3 times higher than plant requirement had no inhibition effects on plant growth and yield after three times cultivations, since treated soils EC were still in the none soil saline zone [0-2 mS/cm] given by FAO (1998); and more than 40 % of the total Na applied through urine and irrigation water was removed by plant from soil in all urine treatments and might probably contributed to mitigate Na ion accumulation in soil. However, urine causes nutritional imbalance, but this phenomenon was not accelerated under three time cultivations with applications of extra urine volumes (U-2Vo and U-3Vo). Continuous application of double and triple volumes of urine increased nitrogen contents and plants decreased K/Na ratio. Moreover, accumulation of nitrogen and sodium occurred in triples volumes urine application (U-3Vo). Therefore, adequate application of human urine based on nitrogen (N) requirement is a suitable way for its sustainable reuse in agriculture, since no accumulation of nitrogen in soil and no increase of N in plant tissues, but plant K/Na ratio decreases compared to the positive control, modified Hoagland's solution (V-H). Furthermore, from one cultivation to another, soil EC increased in all urine treatments including, and about 60 % of the total Na applied through urine and irrigation water when considering 1st, 2nd and 3rd cultivation, remained in soil in urine treatments (U-Vo, U-2Vo and U-3Vo). Therefore, management of salts from urine is required even in the adequate urine volume application when urine is continuously used in the farmland as a liquid fertilizer.

Chapter 5, Conclusion and recommendations

This chapter summarized the major findings of this research work and proposes some recommendations for a sustainable reuse of urine in agriculture.

LIST OF PUBLICATIONS

Dissertation submitted for the degree

I. Title:

Application of human urine as liquid fertilizer in agriculture
（尿の農業利用に関する研究）

II. Published papers

1. Adequate human urine application pattern for agriculture. International Research Journal of Agricultural Science and Soil Science, Vol. 2 (1), pp. 038-045, 2012.

2. Effects of extra human urine volume application in plant and soil. International Research Journal of Agricultural Science and Soil Science, 3 (6), pp.182-191, 2013a

3. Effects of Continuous application of extra human urine volume on plant and soil. International Journal of Agricultural Science and Research, Vol.3 (3), pp.75-90, 2013b.

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Chapter 1

INTRODUCTION

1.1. INTRODUCTION

The global NPK fertilizer demand is increasing through the years and is basically affected by population and economic growth, agriculture productions, price and governances (FAO, 2008). The increased of fertilizer demand leads ineluctably to the instability of its price. However, in the mid-2008, this global nutrients consumption has decreased drastically due to mainly the economic crisis, and therefore the phosphate rock commodity price has increased up to 700 % from 2007 to 2008 (FAO, 2009). High fertilizer price causes low yields and less agricultural productions, especially in marginal area of developing, where tools are limited and accessibility of fertilizer when needed is currently a great concern. More concerning is the global peak of phosphorus production which is forecasted to occur around 2030; and once the maximum reached the production will drop down and seeing a widening gap between supply and demand (Minemaker, 2008 in Cordell, 2008). At the same time, it is already well known that, in domestic wastewater, human urine fraction contains the major plants nutrients, with approximately 80% of the nitrogen (N), 55% of the phosphorus (P) and 60 % of the potassium (K) (Kirchmann and Petterson, 1995; Jonsson et al. 2000). Subsequently, to mitigate the reliance on commercial fertilizer, and built a sustainable society based on sound resource recycling and low carbon society, link agriculture and sanitation, is an holistic strategy for waste management, food security and food production. Therefore, On-site Wastewater Differentiable Treatment Systems (OWDTS), which is based on the concept "don't collect" and "don't mixed" is a promising decentralized treatments system adapted for both develop and developing countries because of its low cost, no energy requirement and easy to operate and maintain (Lopez et al. 2002). At the household level faces, urine and grey water are properly separated and then treated before reusing in the farmland for sustainable agricultural production (Figure 1.1.)

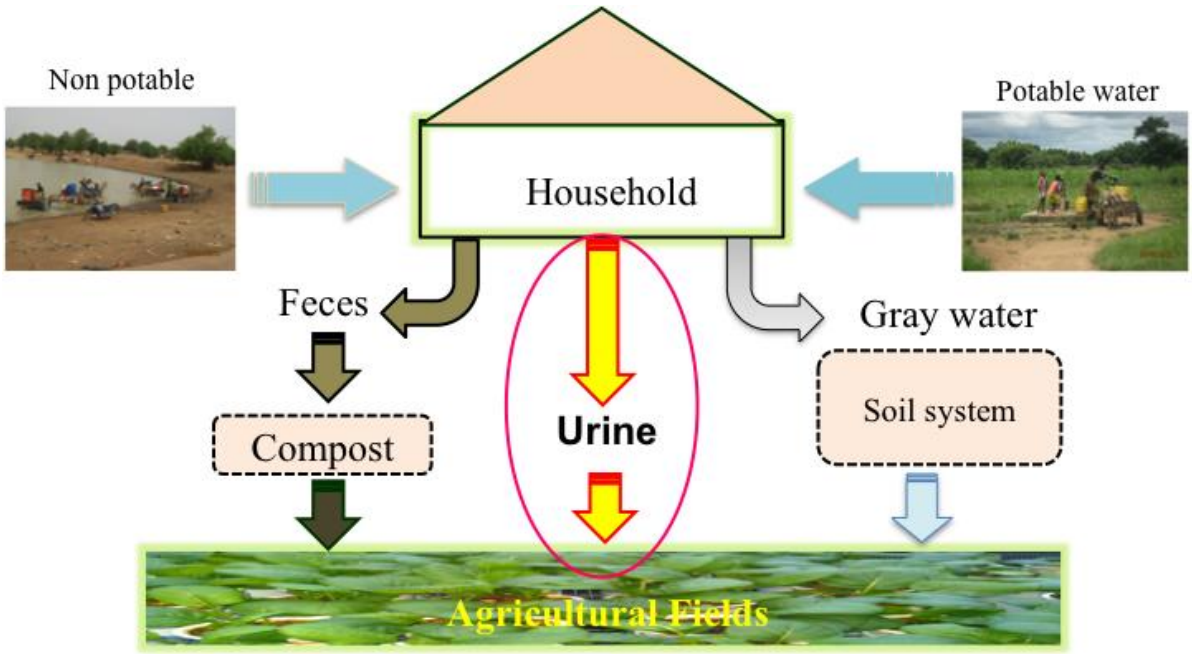

Figure 1.1. On-site Wastewater Differentiable Treatment (OWDTS) Concept.

1.2. CHARACTERISTIC OF HUMAN URINE

1.2.1. Human urine positive sides:

Human urine contains the largest proportion of plant nutrients in the household wastewater fractions and represents only 1 % of the total volume of wastewater (Figure1.2.). Physiological measurements indicate that the amount of plant macronutrients excreted via urine per person and year has been measured at 2.5-4.3 Kg-N, 0.7-1.0 Kg-P, and 0.9-1.0 Kg-K (Geigy Scientific tables, 1981; Guyton 1986; Hallmann, 1960). In undiluted fresh human urine, the following elements were found, nitrogen 7-9 g/L, phosphorus 0.20-0.21 g/L, potassium 0.9-1.1g/L, sulphur 0.17-0.22g/L, Calcium 13-16 mg/L and Magnesium 1.5-1.6 mg/L. Some plant micronutrients, i.e. copper, zinc, iron and boron were also found in fresh urine at levels of micrograms per litres (Kirschmann and Perttersson, 1995). Moreover, the nutrients in urine are readily available to crop, since the major proportion (90-100% of NPK contents in urine) is present in inorganic form (Kirshmann and Pettersson, 1995). Therefore, the use of human urine in agricultural field presents a high potential in the viewpoint of agronomic value and represent a novel way of nutrients recycling since, the main task of sanitation besides the highest hygiene standards is to keep the soil fertile (Otterpohl et al., 1998).

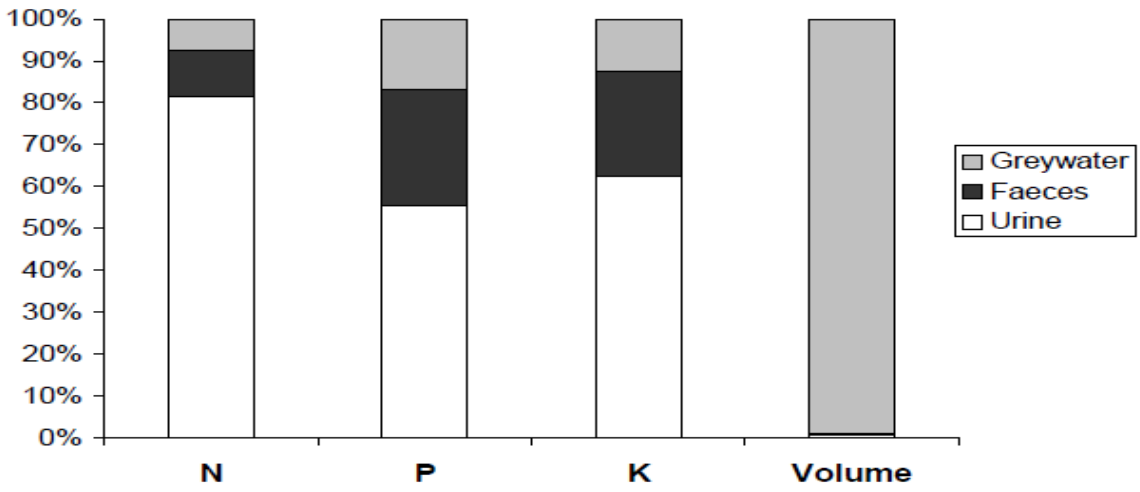

Figure 1.2. Content of major plant nutrient and volume in domestic wastewater (source Evaluation of microbial heath risks associated with the reuse of source-separated human urine; doctoral thesis by Höglund, 2001).

Furthermore, besides the macro and micro nutrients found in human urine, it is fundamental also to bear in mind that urine contains some harmful elements, which need to be addressed or investigated carefully for its sustainable reuse in arable land.

1.2.2. Human urine negative sides

Human urine contains some harmful substances namely salts (Krichmann and Perttersson 1995; Mnkeni et al., 2005, 2008), pathogens (Höglund, 2001; Pradhan et al., 2007, 2009; Heinonen-Tanski, 2007), and pharmaceuticals (Winker et al. 2008, 2010).

• **Salts**

Human urine contains a various salts and the most predominant among them is sodium chloride. The concentration of sodium (Na) and chlorine (Cl) in undiluted fresh human urine analysed by Kirshmann and Pettersson (1995) were amounting 0.94-0.98 g/L and 2.3-2.5 g/L respectively. While a value of 2.34 g/L of sodium content in urine was recently reported in the literature (Pradhan et al. 2010). In Niger, sodium contents in source-separated urine collection were in the range of 2.9-3.5 g/L (Dagerskog and Bonzi, 2010). Moreover, the evaluation of human urine as source of nutrients for selected vegetables in South Africa (Mekeni et al., 2008) showed a high Na (0.90 %) contents in bulk urine compared to N (0.74%) and P (0.029%) but slightly lower than K (1.62%). Consequently, applications of human urine as liquid fertilizer in agriculture may have potential to accumulate sodium (Na) ions in soil and eventually be detrimental for plant growth and production, especially in dry land when urine is planned to be reused at several years' scales. Besides, it has been reported earlier that salts including chlorides may be toxic to some plants (Holliman, 1998) and consequently there has been concern about the toxicity of human urine (Höglund, 2001).

• **Pathogens**

In healthy individual, urine is sterile in the bladder (Höglund, 2001). However, in general pure human urine contains very few enteric microorganisms (Heinonen-Tanski, 2007). The pathogens traditionally known to be excreted in human urine are Salmonella typhi, Salmonella paratyphi, and Shistosoma haematobium. Infections by S. typhi, and S. paratyphi only cause excretion in urine during the phase of typhoid and paratyphoid fevers when bacteria are disseminated in the blood (Feachem et al. 1983). These infections occur rarely in developed world (Lewis- Jones and Winkler 1991), while cause about 16 million cases per year in developing countries (Feachem et al, 1983). The Shistosomiasis (Bilharziasis) is one of the major human parasites infections mainly occurring in Africa and caused by Shistosoma haemomatobium; and theirs eggs are excreted in urine sometimes during the hold life of the host (Feachem et al, 1983, Höglund, 2001). Nevertheless, it is widely known, that many microorganisms die off during the hygienisation (simple storage) of human urine. The storage of urine in tropical developing countries may not be necessary for a long term (<3 months), even though storage for 6 month is recommended in the Nordic countries (Jönsson et al., 2004); because the heat (>20°C), U-V radiation and the increase of pH up to 9 caused by urea hydrolysis might be beneficial for the inactivation of pathogenic microorganisms in urine in the tropical world (Höglund, 2001; Heinonen-Tanski, 2007). Besides, the risk of microbial contamination is diminished by the fact that pathogenic microbes do not survive in soil for a long time (Feachem, 1983). Therefore, the reuse of urine in tropical area seems to present low risk of pathogen contamination. However, furthers investigation are required to elucidate this negative side of urine (public heath issues) in the standpoint of sustainability when is reuse in at large scale at the farmland of tropical developing countries.

• **Pharmaceuticals**

Human urine contains pharmaceuticals residues even after prolonged storage of urine as a treatment step (Winker, 2008). Thereby, the reuse of human urine is associated with a risk of transfer of pharmaceutical residues to the agricultures fields. About 70 % of pharmaceuticals taken in, are excreted in urine and is accounting for 50 % of the ecotoxicological risk (Lienert et al., 2007a, 2007b). Little is known in the fate of pharmaceuticals (anti-malarial drug, antibiotics and so fourth) present in urine regarding their accumulations in soil, transfer in ground water and uptake in plants. Nevertheless it has been reported recently that,

pharmaceuticals, which are polar substances and hardily biodegradables, have potential to be uptake by plants and eventually enter into human food chain (Winker, 2008). While, strictly speaking the environmental fate of pharmaceuticals and their effect on humans, animals and microorganisms is still unknown and remained a big challenge, which needs to be addressed urgently for a sustainable reuse of human urine in agriculture. Therefore, furthers research are required to provide a greater knowledge for this modern pollution problem with respect to their occurrence their distribution in the environment and what effects they have on organisms when these organisms are exposed to low levels of pharmaceutical compounds (Pal et al. 2010).

1.3. HUMAN URINE REUSES IN AGRICULTURE: THE STATE OF THE ART

Since human urine contains valuables plant nutrients, extensive research work has been recently conducted to compare urine with commercial fertilizer using diverse type of vegetables and cereals - barley (Kirshmann and Pettersson, 1995), weat (Jönsson et al., 2004), maize (Guzha et al. 2005; Kassa et al., 2010), spinach (Mnkeni et al., 2005), cabbage (Mnkeni et al., 2005; Pradhan et al. 2007; 2010b) cucumber (Heinonen-Tanski et al., 2007), carrot (Mnkeni et al., 2008), beetroot (Mnkeni et al., 2008), pumpkin (Pradhan et al. 2009a), tomato (Pradhan et al., 2009b), red beet (Pradhan et al., 2010a), cauliflower (Pradhan et al., 2010b), broadleaf mustard (Pradhan et al., 2010b), potato (Pradhan et al., 2010b) and radish (Pradhan et al., 2010b). From all these trials, human urine achieved equal fertilizer value to industrial fertilizer when both were used at a similar dose. Therefore, these studies have highlighted the positive aspect of urine reuse in the arable land as liquid fertilizer. However, focusing on urine application, sometimes a 1/4 of the total volume of urine required for cultivation was applied 3 days before planting (Cabbage, cauliflower, broadleaf mustard) ; and in the other hand no application urine in prior to planting was performed (cucumber, pumpkin, reed beet and potato). Besides, Jönsson et al (2004) reported that in crop cultivation normal strategy of fertilizer application is once or twice per growing season, and thus proposed if urine is applied only once this should be normally carried out prior to or at the time of sowing/planting. Thereby, it has not been clearly addressed when and how-often human urine should be applied in agricultural farmland for a sustainable food production. Furthermore, since urine contains also some salts, especially a high sodium chlorides, its reuse in agriculture for long term view is also current unknown ; and further research on urine and salinity would be welcome to avoid long term problems (Dagerskog and Bonzi, 2010). Too much human urine application in agricultural land may cause soil salinity, sodium (Na) and nitrogen (N) accumulation in soil. Mnkeni et al., (2005) observed a depressed growth of spinach and cabbage in one time cultivation at high application rate of human urine caused by an increase of salinity of the treated soils. Excess of sodium in soil stressed plant, by altering the water uptake in the root zone, causing ions specific toxicities and interfering with completive nutrients which so called nutritional imbalance (Dasgan et al., 2002; Asano et al. 2007; Franzen, 2007; Rosen et al., 2008; Lee, 2012). Whiles, high concentration of nitrogen in soil can build up in plant tissues and affect negatively amount of sugar and vitamins in vegetables causing therefore health and taste issues to consumers (Brady and Weil 1996; Turan and Sevimli, 2005). Furthermore, Kirchmannn and Pettersson (1995) reported that N lost from soil is promoted in high rate of human urine application. Therefore, how much human urine should be use in agriculture and how do continuous application of overzealous urine volume affect plant and soil need to be addressed in order to provide a sound vision of urine application in agriculture.

1.4. RESEARCH OBJECTIVES

The overall objective of this thesis was to investigate and propose an adequate application of human urine as a liquid fertilizer in agriculture.

The specific objectives of the present investigations were:

- ♣ Evaluate when and how often-human urine should be applied in agriculture as fertilizer.
- ♣ Examine the effects of uncontrolled human urine application dose in agriculture.
- ♣ Examine the effects of continuous application of uncontrolled human urine dose in agriculture.

1.5. SYNTHETIC URINE COMPOSITIONS

The composition of human urine fluctuates from one person to another and depends mainly on diet, climate, physical activity, time of the days and body size (Heinomen- Tanski et al., 2007; Pradhan et al., 2010b). Moreover the thermodynamic and the kinetics of precipitation in synthetic urine do not differ from real urine (Ronteltap et al., 2003). Therefore, considering this latter fact and to have more general and precise effect of human urine application in agriculture as liquid fertilizer, synthetic urine (Wilschnach et al. 2007) was used in this thesis. The composition of the synthetic urine is shown in the Table 1.1.

Table 1.1. Composition of synthetic urine used in the research work

Sr#	Component		(g/L)	nM
1	$CaCl_2.H_2O$		0.65	4.4
2	$MgCl_2.6H_2O$		0.65	3.2
3	NaCl		4.60	78.7
4	$NaSO_4$		2.30	16.2
5	Na_3citrate. $2H_2O$		0.65	2.6
6	Na_2-COO_2		0.02	0.15
7	KH_2PO_4		4.2	30.9
8	KCl		1.60	21.5
9	NH_4Cl		1.00	18.7
10	NH_2CONH_2	(urea)	25.0	417
11	$C_4H_7N_3O$	(creatinine)	1.10	9.7

Source: Wilsenach et al. 2007

1.6. CONCLUSION

Human urine contains large amount of valuables plant nutrients such as nitrogen (N), phosphorus (P) and potassium (K) and can replace industrial fertilizer. However, urine contains also some harmful substances likewise salts, pathogens and pharmaceuticals. Regarding salts, an overzealous application of human urine may have potential to increase soil salinity, accumulate salts (sodium) in soil and eventually affect adversely plant growth

and production. Therefore, to address these salts issues from urine and provide a better understanding of urine application in agriculture for a sustainable food production, this present following questions are the core of our research and are discussed profoundly in this thesis.

- ♦ When and how often human urine should be applied in agriculture as liquid fertilizer ?
- ♦ How much urine should be used in agriculture as liquid fertilizer ?
- ♦ How do continuous applications of urine affect soil characteristics and plant growth ?

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Chapter 2

ADEQUATE HUMAN URINE APPLICATION PATTERN FOR AGRICULTURE

2.1. INTRODUCTION

On site wastewater differentiable treatment is a promising decentralized sanitation system that is based on the concept **" Don't collect"** and **"Don't mix"**. At household level, faeces, urine, lower-load graywater and higher-load graywater are properly separated and treated. Thus, this new sanitary approach system provides many advantages mainly recovery and recycles of nutrients, control of micro pollutants and pathogens and reduction of wastewater flow (Lopez et al., 2002). Regarding recovery and recycle of nutrients, it is well known that human urine contains some macronutrients such as nitrogen, phosphorus and potassium and has a potential to be reuse in agriculture as a liquid fertilizer (Krichmann et al., 1995; Höglund, 2001; Dagerskog et al., 2010). Subsequently, extensive research works have been recently done to compare human urine and industrial fertilizer with cucumber, pumpking, red beet, potato, radish cabbage, cauliflower and broadleaf mustard (Heinomen-Tanski et al., 2007; Pradhan et al., 2007; 2009; 2010). Sometimes, 1/4 of the total urine volume required for cultivation was applied 3 days before planting (cabbage, cauliflower mustard etc.) and in other hand no application in prior to planting (cucumber, pumpking, red beet, potato etc.). In addition, Jönsson et al. (2004) reported that in crop cultivation, normal strategy of fertilizer application is once or twice per growing season and proposed if urine is applied only once, this should normally be carried out prior to or at the time of sowing/planting Thereby it has not been previously well addressed when and how often urine should be applied in soil as fertilizer. Therefore it seemed reasonable to study human urine finding a better way for its application in soil. Pot test with Spinach (*Spinacia oleracea*) was conducted and then observed, plant growth and fertility effect under different urine application ways.

2.2. MATERIAL AND METHOD

2.2.1. Pot experiments

The experiment was conducted in greenhouse located in Hokkaido University in Japan (43°04′11.6″N, 141°20′22.4″E) from March to June 2011. Two compartments closed pot system was used in the present study to analyse leached water. The inside of plastic pot, with diameter and height respectively 12.4cm and 10.2 cm, was covered in its bottom part by 150 μm nylon mesh to prevent root expansion. The external one was a wagnel pot (1/10000a size), which allowed collecting leached water weekly. Washed sandy soil, which had low organic matter, was used as media to analyse nutrients (nitrogen and phosphorus) distribution in plant, soil and leached water. Physical and chemical properties of the soil were showed in Table 2.1, and the method was described as bellow. Within each experimental pot, six spinach seeds were germinated in petri dish with filter paper and incubated at 25°C for 2 weeks and then sown. After outbreak of plant (2 weeks after seedling), all pots were uniformed to 2 plants. Spinach was selected in this experiment due to its worldwide distribution, high marketability and high nutrients requirement (Burt, 2006). Synthetic urine (Wilsenach et al., 2007) was used in this work to have more general and precise effect of urine since human urine fluctuates composition fluctuates from person to another. Based on the total nitrogen required to grow Spinach (NPK 112-157-168 Kg/ha) recommended by Kirkwyland et al. (2010), the composition of synthetic urine and the pot system size, the total volume of synthetic urine

was designed to 29 ml and containing the following dose NPK 112.0-9.8-18.9 mg/pot. In practical, however, diluted (1/3) synthetic urine was performed. About 440 ml of irrigation water was applied in each pot in the 1st week. About 560 ml, 560 ml, 560 ml, 700 ml, 620 ml and 520 ml of irrigation was applied in the 2nd, 3rd, 4th, 5th, 6th and 7th week, respectively. Totally 3.96 l of irrigation water was used per pot during all the cultivation period.

Table 2.1. Sandy soil Properties and Nutrients Contents before Cultivation

Parameters	**Soil before cultivation**
total N (mg/g dry weight)	0.21 ± 0.00
total P (mg/g dry weight)	0.02 ± 0.00
total C (mg/g dry weight)	1.01 ± 0.02
pH	5.43 ± 0.18
EC (μS/cm)	49.5 ± 8.9
Porosity	0.31 ± 0.01
Water holding capacity (mL/100g dry weight)	24.31± 2.58
Water drainage (mL/s)	0.90 ± 0.02
Moisture content (%)	0.31 ± 0.07

Arithmetic mean values ± standard deviation (SD), (N= 3). The porosity, water holding capacity, water drainage and moisture contents were determine as describe Horisawa et al., 1999.

2.2.2. Urine application ways

Total 7 treatments were set to know better a way of urine application in soil (a) 100% of urine equivalent to 29 ml was applied before cultivation; 60% of urine was applied before cultivation and then remained urine (40%) was regularly applied (b) every week (c) every 2 days after 15th day sowing corresponding to the total emerged of plants in all pots; In similarly manner, 30% was applied before cultivation and then (d) every week (e) every 2 days; no urine applied before cultivation and then (f) every week (g) every 2 days. Totally 52 experimental pots were used for 7 treatments replicated 7 each and 1 treatment without urine as control negative replicated 3 times

2.2.3. Growth and harvesting

The cultivation period of Spinach was designed for 70 days (Nishihara et al., 2001). However, the duration was shortened to 49 days do to weak plant growth. Therefore, the total input of nitrogen and phosphorus at the end of cultivation was (a) 112, 9.8 (b) 98, 7.8 (c) 94.8, 7.5 (d) 90, 7.1 (e) 89.2, 7.1 (f) 78.8, 6.3 and (g) 73.9, 5.9 mg/pot respectively. Plants heights were measured weekly (from 1 week after urine started to be applied) and leaves area were measured after harvesting. In the plant height measurement, highest leaf in pot was selected. In the leaf area measurement, well growth 3 pots per treatment were selected, and then, 2 biggest external leaves were measured by paper copy method. Furthermore, fresh weight of shoots and roots were weighted separately lyophilized shoots and roots were weighted as dry weight and then stored at –30°C. Chemical properties (pH and electrical conductivity (EC) in soil after harvests were showed in Table 2.

2.2.4. Soil, plant and leached water analysis

Total nitrogen and total carbon in soil was determined by Sumigraph NC-220F (Sumika Chemical Analysis Service, Japan) apparatus. Regarding total phosphorus in soil, soil samples

were digested with nitrate-perchloric acid digestion method and then measured by ascorbic acid method using spectrophotometer at 880 nm (Standard Method, 1989). pH and EC in soil were determined by 1:2.5 dilution method and 1:5 dilution method, respectively. Measurements of porosity, water holding capacity and water drainage were followed by Horisawa et al. (1999). In the case of plant, shoot and root were separately analyzed. Dried shoot and root were grinded by mortar and muddler. Total nitrogen and total phosphorus in powdered plant were measured by same method with soil analysis described as above. In leached water, total nitrogen and total phosphorus were analysed by Hach method 8190. All data in the results were analysed with 3 samples in each treatment and its mean value and standard error were showed in results. The statistical analysis of each data was analysed with Stat View software version 5.0.

Table 2.2. Soil properties after cultivation, N = 3 in each treatment.

treatment	**pH**	**EC (µS/cm)**
100 % urine	6.11 ± 0.13	173.0 ± 25.0
60 % before / week	6.12 ± 0.16	161.2 ± 20.9
60 % before / 2 days	6.28 ± 0.05	137.1 ± 29.5
30 % before / week	6.34 ± 0.08	156.5 ± 5.4
30 % before / 2days	6.22 ± 0.07	147.9 ± 17.2
every week	6.53 ± 0.24	151.7 ± 2.0
every 2 days	6.35 ± 0.18	146.6 ± 12.2
control	5.47 ± 0.30	86.0 ± 6.4

Arithmetic mean values ± standard deviation (SD)

2.3. RESULTS

2.3.1. Plant growth

Weekly plant height measurement is represented in the Figure 2.1 and shows change in time course positively until 35 days after cultivation, before starting to decrease slightly, attesting therefore maximum growth stage of the Spinach. The heights at 35 days were (a) 3.9±0.8, (b) 6.4±0.6, (c) 6.6±0.9, (d) 6.1±1.4, (e) 6.6±1.0, (f) 7.0±0.6, (g) 6.6±1.0 and (h) 3.3±1.0 cm, respectively. Urine application at one time before seeding (a) was in the same level with the control (h) and significantly lower than (b), (c), (d), (e), (f) and (g) (Turkey-Kramer test, $p<0.05$).

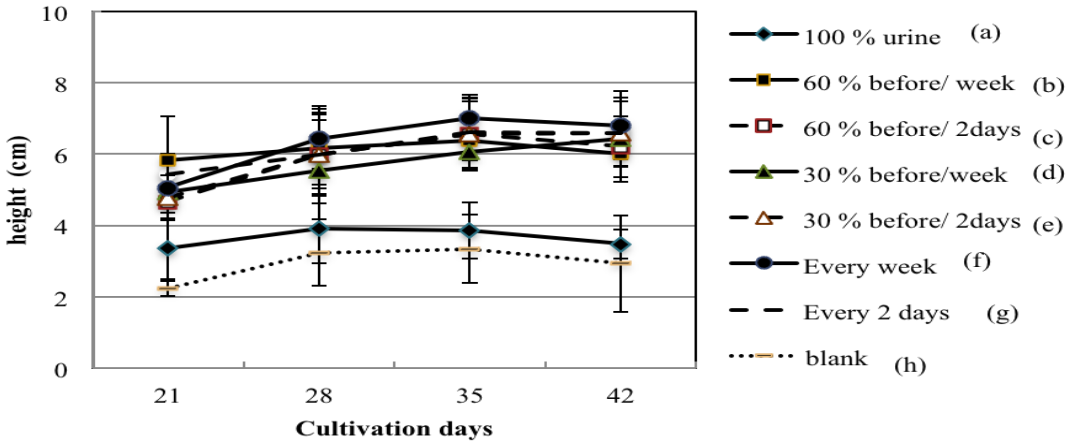

Figure 2.1. Growth rate of Spinach plant height from different way of urine application in sandy soil (arithmetic means ± SD); N=4 in each treatment.

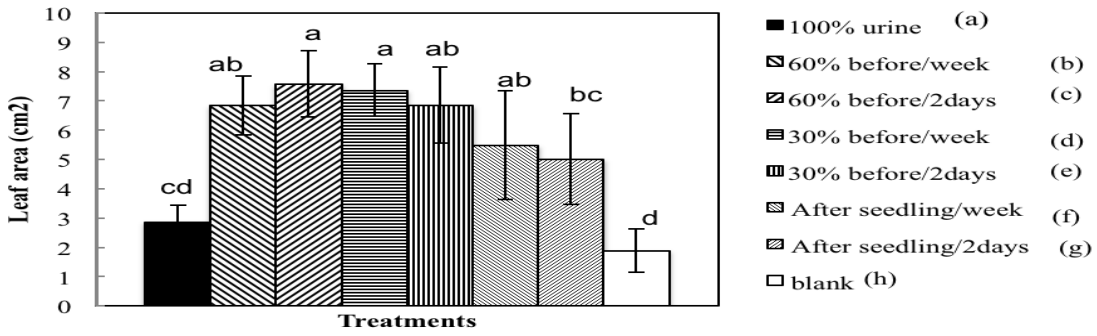

Figure 2.2 Leaf area measurement of spinach plant after cultivation; in each pot the 2 biggest external leaves were selected for 3 pots per treatment (arithmetic means ± SD); different alphabetical letter showed significant difference (Turkey-Kramer, p<0.05).

Similarly to plant height, leave areas after cultivation in (a) was in the same level with that of control (h) and significantly lower than that of (b), (c), (d), (e), and (f) (Turkey-Kramer test, $p<0.05$) (Figure 2.2). Their values were (a) 2.9±0.6, (b) 6.8±1.0, (c) 7.6±1.1, (d) 7.4±0.9, (e) 6.8±1.3, (f) 5.5±1.9, (g) 5.0±1.6 and (h) 1.9±0.7 cm^2, respectively. Furthermore, shoot dry biomass of (a) was same level with the control (h) but, both were significantly lower than treatments (c) and (g) (Turkey-Kramer test, $p<0.05$) (Table 2.3). In the case of root dry weight, root dry biomass of (a) and the control (h) were also in the same level, but both significantly lower than treatment (b). Therefore, from these results, it was clearly observed a low plant growth and production in 100% urine (a) applied at one time before cultivation (a). Besides, there is tendency that frequent urine application provided high growth of plant shoot.

Table 2.3. Fresh and Dry weight of Spinach (g/pot) from different way of urine application

	Shoot		**Root**	
Treatment	FW	DW	FW	DW
100% urine	1.24±0.36b	0.18±0.04b	0.21±0.07b	0.05±0.02b
60%before/week	3.62±0.54a	0.79±0.23ab	2.15±0.56a	0.35±0.13a
60%before/2days	3.27±0.79ab	0.89±0.21a	1.17±1.01ab	0.28±0.06ab
30%before/week	2.96±0.80ab	0.60±0.32ab	1.23±0.97ab	0.24±0.04ab
30%before/2days	3.45±0.61a	0.68±0.36ab	1.38±0.97ab	0.23±0.11ab
every week	2.99±1.19ab	0.56±0.14ab	0.71±0.55ab	0.22±0.16ab
every 2 days	4.64±0.96a	0.80±0.22a	1.59±0.84ab	0.23±0.11ab
control	1.17±0.42b	0.16±0.09b	0.25±0.20b	0.07±0.05b

Arithmetic mean values ± standard deviation (SD); different alphabetical letter showed significant difference (Turkey-Kramer test, $p<0.05$)

2.3.2. Nutrients effects

To confirm nutrients effect in all treatment, nitrogen and phosphorus content in plant shoot was measured (Table 2.4). In the case of nitrogen, no significant difference was observed urine treatment (a)-(g), although control (h) (35.3±1.3 mg/g plant DW) was significantly lower level than (a), (d), (f) and (g) (46.9±2.9, 46.1±2.5, 47.6±1.2 and 46.5±5.6 mg/g plant DW, respectively) (Turkey-Kramer test, $p<0.05$). In the case of phosphorus, plant shoot

concentration in (a) (0.5±0.1 mg/g plant DW) was same level with control (h), but both were significantly lower than treatment (g) (1.6±0.2 mg/g plant DW) (Turkey-Kramer test, $p<0.05$). N/P ratio, which is one indicator of nutrient balance in plant, showed high value (about 25-100) in all treatment (Table 2.4). The value of (a) (95.2±24.7) was significantly higher than that of (g) (28.4±3.5) (Turkey-Kramer test, $p<0.05$).

Table 2. 4. Concentration of nitrogen and phosphorus in Spinach shoot after cultivation

Treatments	**N (mg/g plant DW)**	**P (mg/g plant DW)**	**N/P ratio**
100 % urine	46.9±2.9a	0.5±0.1b	95.2±24.7a
60%before/week	43.9±4.2ab	1.2±0.4ab	41.3±14.6ab
60% before/2days	41.0±1.0ab	1.1±0.2ab	37.9±6.4ab
30%before/week	46.1±2.5a	1.0±0.5ab	57.6±38.9ab
30%before/2days	42.8±3.5ab	1.0±0.4ab	49.4±27.8ab
every week	47.6±1.2a	1.0±0.1ab	47.6±7.2ab
every 2 days	46.5±5.6a	1.6±0.2a	28.4±3.5b
control (no urine)	35.3±1.3b	0.5±0.1b	74.8±22.3ab

Arithmetic means ± SD; different alphabetical letter showed significant difference (Turkey-Kramer test, $p<0.05$).

2.3.3. Nutrients distribution

Nitrogen distribution in shoot of Spinach, root of Spinach, soil and leached water is given in Figure 2.3. The distribution in (a) was shoot 7.6%, root 1.1%, soil 35.9%, leached water 38.3% and unknown part 17.1%. In the contrast, the distribution in (g) was shoot 49.3%, root 8.8%, soil 39.8%, leached water 0.1% and unknown part 2.0%. Nitrogen distribution ratio of shoot part in (a) was significantly lower than that in other treatments and the ratio of leached water in (a) was higher than that in other treatment. Similarly to nitrogen distribution, phosphorus distribution ratio in (a) was lower in shoot part (1.0%) and higher in leached water (9.1%) than those in other treatments (Figure 4). Phosphorus distribution ratio of shoot part in (g) was higher (22.2%) and the ratio of leached water was lower (1.0%). In all treatments, about 80% of phosphorus was remained in soil.

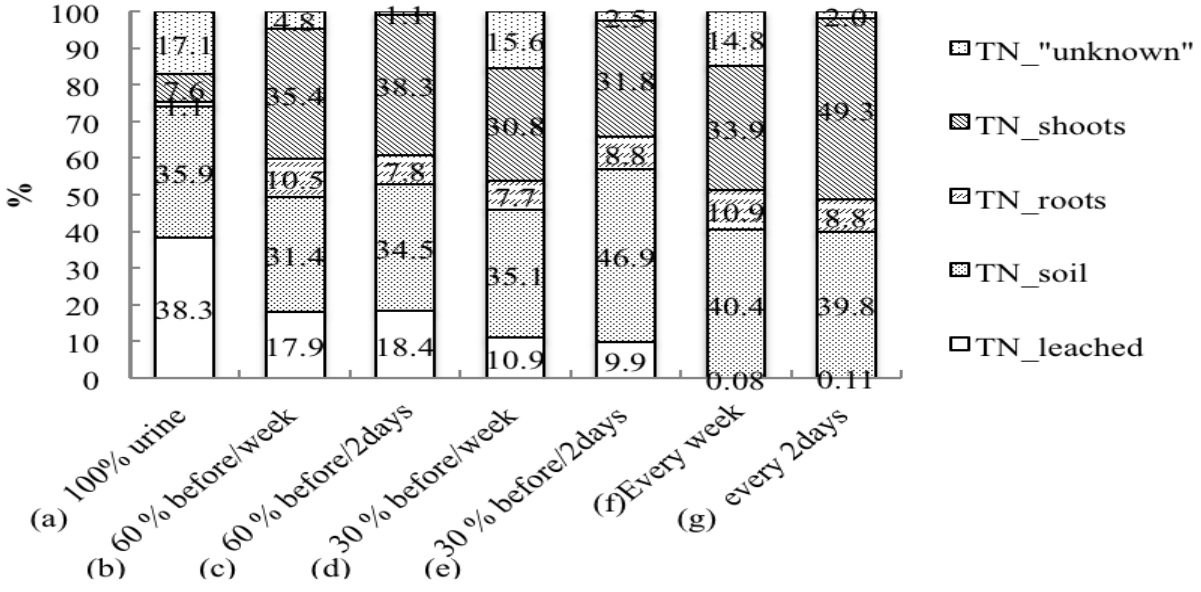

Figure 2.3. Nitrogen balance in each urine treatment after cultivation (N= 3).

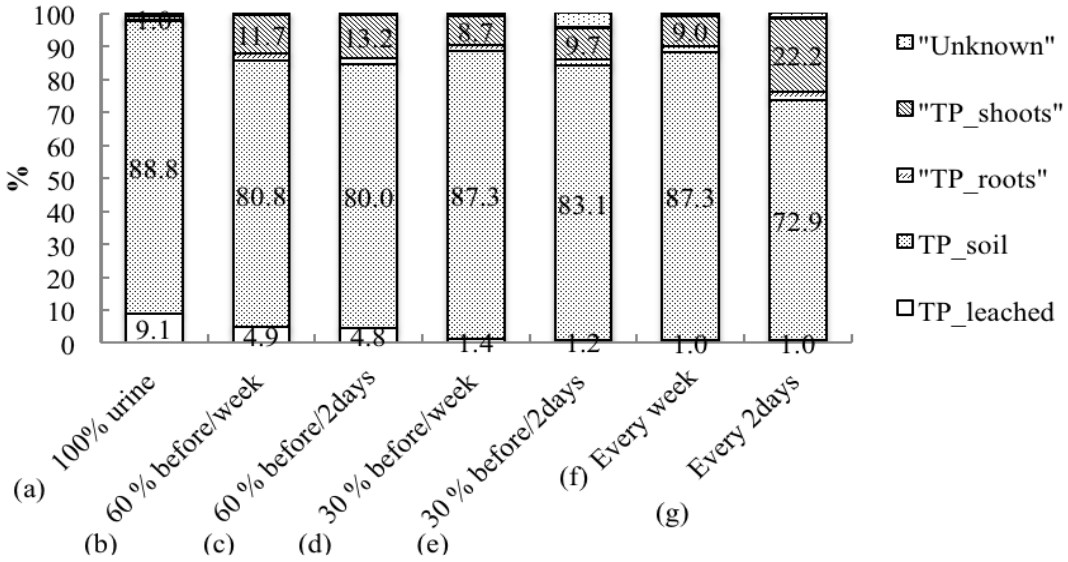

Figure 2.4. Phosphorus balance (%) in each urine treatment after cultivation (N= 3)

2.4. DISCUSSIONS

In the present study, low growth of spinach in 100% urine applied at one time before seeding (a) and high growth in no urine applied before every 2 day (g) were observed. One reason of the difference might be phosphorus availability. It has been known that high N/P ratio (N/P > 16) in shoot indicates phosphorus limitation, generally speaking (Peter et al., 2004). In the present results, therefore, all spinach would suffer from phosphorus limitation. Especially phosphorus limitation in (a) was severe (N/P = 95.2 ± 24.7) and that in (g) was moderate (N/P = 28.4 ± 3.5). This limitation leads us to consider that phosphorus availability might affect spinach growth in the present study. This consideration was also supported by a correlation between phosphorus content in shoot (Table 2. 4) and shoot dry weight (Table 2. 3). It has been reported that phosphorus in urine was mainly existed as phosphate ion (Krichmann and Pettersson, 1995) and the phosphate was easy to make an immobile form with Fe and Al under acidic soil condition (Schachtman et al., 1998) such as present study (Table 2. 2). The low phosphorus content in plant shoot of 100% urine might be associated to a low root activity compared to others treatments since, our original soil had low ions intensity and received a lot of ions in one time. Thus, that might cause some damages in the root zone, and thereafter provided low plant growth and production in 100 % urine application. Furthermore, frequent urine application such as treatment (g) (every 2 days urine application with no application before cultivation) caused high phosphorus uptake by Spinach, since available phosphorus was supplied frequently. In the present study, it was also observed that leached nitrogen was high ratio in 100% urine applied at one time before cultivation (a). This phenomenon was also considered as another possible reason for the low plant growth and production in (a), since nitrogen is major factor of plant growth. Pernilla et al. (2007) found relatively low amount of leached N (6.3%) in clay soil by using stored urine as fertilizer for Wheat. One reason might be the difference in physical structure of soil. Because sandy soil used in the present study was characterized as low water holding capacity (Hijikata et al.,

2011). Second reason might be nitrogen form in synthetic urine and biological activity in soil. It has been known that urea in urine was changed to ammonium in storage urine (Müllegger et al., 2010). Retention capacity of ammonium form is higher than that of urea in soil due to the cation exchange capacity (CEC) of soil, although sandy soil has been regarded as low CEC. Generally speaking, urea applied in soil is easy to degrade to ammonium by enzyme from soil microorganism. However, activity of soil microorganism in the present study might be low in the early stage of cultivation, because our soil was washed and air dried before cultivation. Therefore, if we consider the urine reuse in low water holding capacity, low CEC and low activity of soil microorganisms such as dry land agriculture (FAO, 2008), it was suggested that 100% urine applied at one time before seeding (a) is not adequate way and frequent urine application might be better from the view of nitrogen leaching. Spinach growth in all treatment in the present study was relatively lower than that of reference (Nishihara et al., 2001). One reason might be that Spinach is sensitive to acid condition (Burt, 2006); and second reason might be unbalance of nitrogen and phosphorus in urine as mentioned above. This unbalance is in agreement with previous studies (Heinomen-Tanski et al., 2007; Pradhan et al., 2009), which have shown that phosphorus in urine is low compared to chemical fertilizer. In urine reuse for agriculture, therefore, it is better to supplement with ash (heinomen-Tanski et al., 2007) or use with compost (Hijikata et al., 2011), which has the ability to supply phosphorus and soil modification.

2.5. CONCLUSION

From this investigation, it was concluded that urine application at one time before cultivation gave low Spinach growth, low phosphorus availability and high leached of nitrogen from soil. Furthermore, no urine applied before cultivation and every 2 days application after the plant emerged, showed relatively well plant growth but also high uptake of nitrogen and phosphorus. Thereafter, human urine application at frequent interval gave relatively favourable condition for spinach growth under sandy soil condition. However, P limitation in urine was palpably manifest in this work. Consequently urine used as fertilizer with ash and compost might be a better vision to promote high plants yield in sandy soil.

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Chapter 3

EFFECTS OF EXTRA HUMAN URINE VOLUME APPLICATION IN PLANT AND SOIL

3.1. INTRODUCTION

The world fertilizer demand has increased throughout years and is affected basically by factors such as population and economic growth, agriculture production, prices and governance policies (FAO, 2008). This increase of fertilizer demand leads ineluctably to the instability of its price and the overuse of our resources especially phosphorus and potassium which are finite and non-renewable one (Roberts and Stewart, 2002). To mitigate this demand of fertilizer and build a sustainable society based on resources recycling and low carbon society, links agriculture and sanitation is one idea, because the main task of sanitation besides highest the hygienic standards is to keep the soil fertile (Otterpohl et al., 1998). Consequently, in recent year, source separation of black water has spurred a strong interest for both developed and developing world and is highlighted on the On-site Wastewater Differential Treatment System (OWDTS) and Ecological Sanitation (EcoSan), which are a low cost sanitation system, easy to implement, operate and maintain. At the household level, faeces, urine, and graywater are properly separated and treated. The merits of this approach are mainly recovering and recycling nutrients, controlling micro pollutants and pathogens and reducing wastewater flower (Otterpohl et al., 1997, 1998; Lopez et al., 2002). In this system, source-separated urine means that 60-90 % of the plant nutrients nitrogen (N), phosphorus (P) and potassium (K) ingested can be retrieved in solution, and therefore has a potential to be reuse in agriculture as liquid fertilizer (Krichmann and Pettersson, 1995). Thus, urine has been compared successfully with commercial chemical fertilizer using many types of vegetables and crops (Heinomen-Tanski et al. 2007; Pradhan et al., 2007, 2009, 2010a; Mnkeni et al., 2008). However, it contains some salts (Krichmann and Pettersson, 1995; Mnkeni et al., 2005, 2008), pathogens (Höglund 2001; Pradhan et al., 2007), and pharmaceuticals (Winker et al., 2008, 2010). Concerning salts issues, too much volume of human urine applied on agricultural land as fertilizer, may cause simultaneously excess of sodium in soil and eventually in plant. Sodium inhibits plant growth since disrupting the water uptake in the root, dispersing soil particles, restricting root growth and/or interfering with the uptake of competitive nutrients (Asano et al. 2007; Franzen, 2007; Rosen et al., 2008; Lee, 2012). In other hand, excess of nitrogen can affect negatively amount of sugar and vitamins in vegetables and build up in the plant tissues causing therefore health and taste issues to consumers (Brady and Weil 1996; Turan and Sevimli 2005). Therefore, the objective of this study was to investigate the effect of extra urine volume application in plant and soil. Pot test with Komatsuna (*Brassica rapa var.peruviridis*) was conducted in greenhouse and then observed, plant growth and nutrients effect in both plant and soil under different urine volumes applications.

3.2. MATERIELS AND METHOD

3.2.1. Pot experiments

Pot test was conducted in the greenhouse at Hokkaido University, Japan (43°04′11.6″N, 141°20′22.4″E) from August 6 to September 9 of 2011. Inside the greenhouse, the following temperature range 19-23°C min. (31-43°C max.), humidity range 27-58% min. (61-88% max.), monthly mean global radiation range 7.2-21.8 MJ/m^2 and sunshine duration range 1.2-10.3 h were recorded. Two compartments closed pot system was used in this experiment to

analyse leached water. The inside compartment in the system was for cultivation, with a plastic pot (volume 0.7 L, top diameter 12.4 cm, bottom diameter 8.5 cm and height 10.2 cm) and its bottom was covered by 150 μm nylon mesh to prevent root expansion and allowing water passing through. The external compartment was for collecting leached water, with a Wagner pot (cylindrical shape, diameter 11.0 cm and height 15.0 cm, defined as 1/10000 a size) (Figure 3.1).

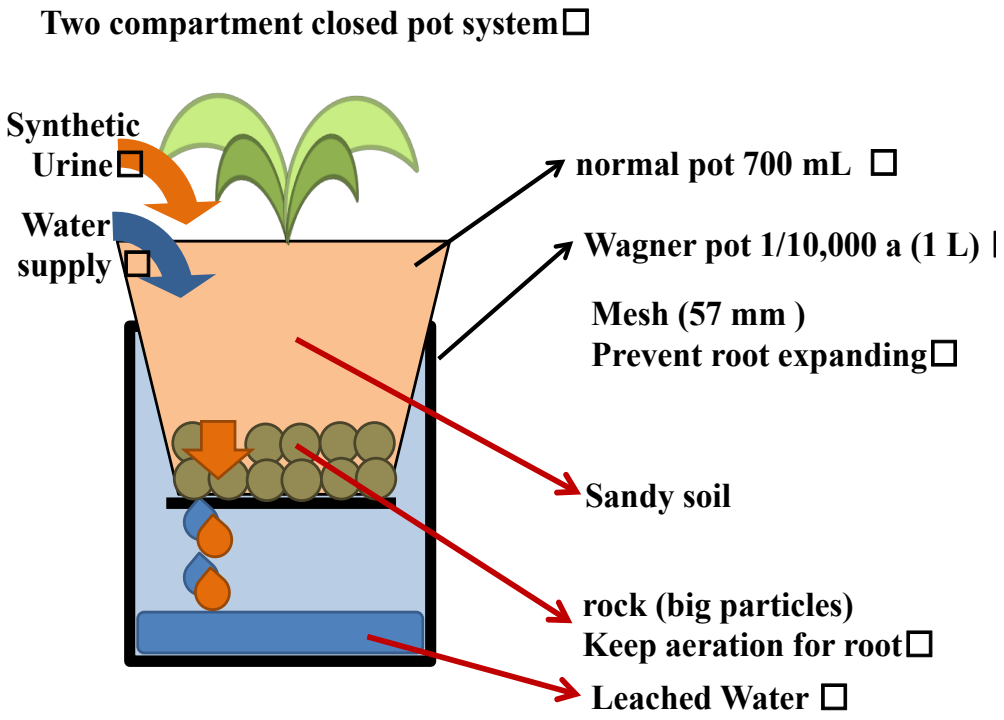

Figure 3.1. Two compartments closed pot system used in the experiment.

Washed sandy soil mixed with small gravels (6:3 soil:gravel ratio) to keep good aeration of soil and facilitate root development was set as media (0.84-Kg soil pot). Initially, the soil pH was 5.4 and therefore before cultivation, the soil was limed by adding 2g of CaMg $(CO_3)_2$ in each pot as recommended by Fujiwara and Narimatsu (2006). The physical and chemical characteristics of the soil are shown in the Table 3.1. Komatsuna (*Brassica rapa var. peruviridis*), a leafy vegetable commonly consumed in Japan, was selected in this work because it presents high growth rate (Thao et al., 2008) and can be harvested in short term (35 days) after sowing in warm climates (Widjajanto et al., 2003). Within each experimental pot, six Komatsuna seeds were germinated in petri dish with filter paper and incubated at 25°C for 1 day and then sown. Seven days after seedling emerged, the seedlings were reduced to two plants per pot.

Table 3.1. Physical and chemical properties of bulk soil before cultivation.

Parameters	**Original sandy soil**
pH	7.50±0.09
EC (μS/cm)	49.4±4.70
total C (mg/g DW)	1.38±0.17
total N (mg/g DW)	0.18±0.01
total P (mg/g DW)	0.28±0.03
K (mg/g DW)	1.91±0.36
Na (mg/g DW)	0.60±0.04

Mean values ± standard deviation (SD) with 5 replications;
"Na" means total sodium extracted with nitrate-perchloric acid digestion.

3.2.2. Fertilizer treatments

Synthetic urine (Wilsenach et al., 2007) was used in this work to obtain more general and precise effects of extra urine volume application in plant and soil, because the composition of urine fluctuates from one person to another and depends mainly on diet and physical activity (Pradhan et al., 2010b). The synthetic urine contained 12.05 g/L of nitrogen, 0.96 g/L of phosphorus, 2.04 g/L of potassium and 2.84 g/L of sodium. Based on the total nitrogen required to grow Komatsuna (140 kg/ha) recommended by Fujiwara et al. (2006), the composition of synthetic urine and the pot system size, diluted (1/3) synthetic urine volume was designed as U-V_0 (34.86 ml), and containing the following dose of N (140 mg/pot), P (11.12 mg/pot), K (23.73 mg/pot) and Na (33.05 mg/pot). In other term U-Vo was defined as the adequate urine volume required for 35 days cultivation of Komatsuna. To know the effects of extra human urine volume on plant and soil, 3 different urine treatments volumes [U-Vo, U-2Vo (NPK 280-22.24-47.46, Na 66.1 mg/pot) and U-3Vo (NPK 420-33.36-71.19, Na 99.15 mg/pot)] were set in one hand. In the other hand, 1 modified Hoagland's solution volume [V-H (NPK 140-9.41-31.53, Na 0.0 mg/pot)] and 1 nonfertilizer treatment [C (NPK 0-0-0, Na 0.0 mg/pot] were set respectively, as positive (same N with plant requirement and without minors compounds except KH_2PO_4) and negative controls. Totally, 25 experimental pots were run in this experiment, with 4 fertilizer treatments and 1 control (5 cases replicated 5 times each). All fertilizers were regularly applied just after the seedlings reduced to two plants per pot at days 7, 14, 21, and 28 after sowing as recommended by Sene et al. (2012).

3.2.3. Irrigation water, Growth and Harvesting

Tap water was used as irrigation water. Before the experiment, the theoretical daily amount of water needed for irrigation (24.6 ml/pot), was determined by multiplying the crop coefficient of Komatsuna in the development stage (Kc=0.60) and the potential evapotranspiration (ETo=4.1 mm/day) and the pot size system (1/10000a). The ETo value was computed by using ETo Calculator version 3.1 (FAO, 2009), and the input data (solar radiation, temperatures max and min, humidity max and min) were derived from Sapporo weather station 47412 (Japan) for the cover period of August 6 to September of 2010. In practical, the theoretical daily irrigation was modified to 50 ml/pot. However, based on the daily observation, the modified daily irrigation water was slightly modified during the cultivation period (Figure 3.1 and Figure 3. 2).

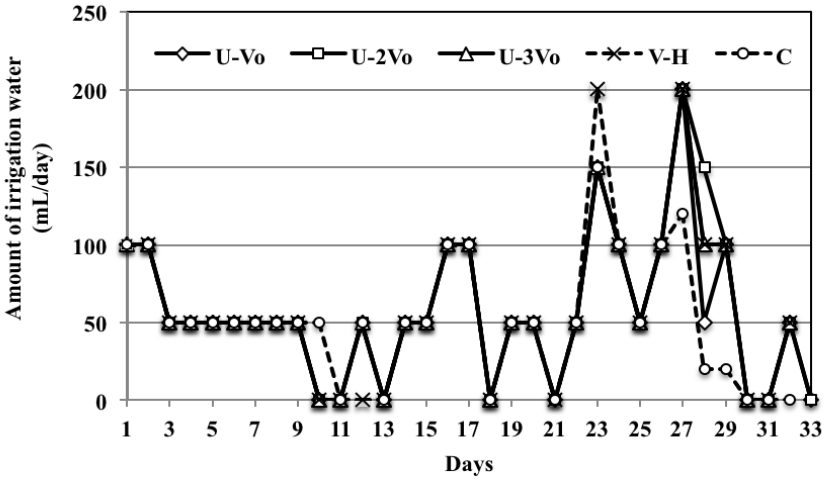

Figure 3. 1. Change in time course of irrigation water applied in pot during Kotmatsuna cultivation in sandy soil using 3 different urines Volumes treatments (U-Vo, U-2Vo and U-3Vo), 1 modified Hoagland's solution (V-H) and 1 nonfertilizer (C).

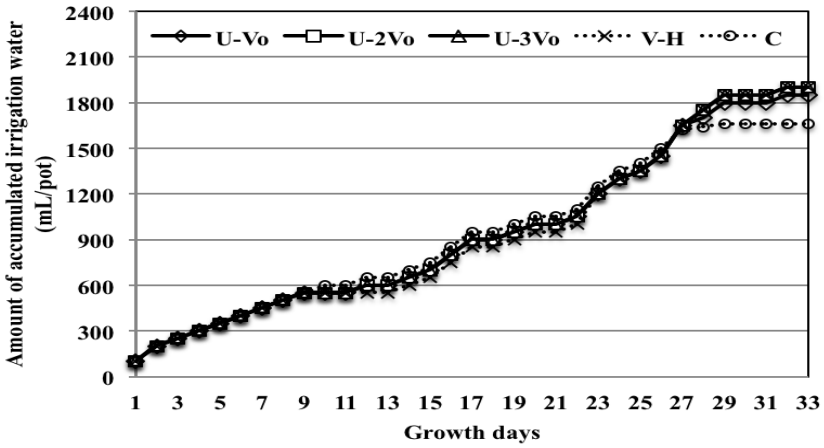

Figure 3. 2. Total amount of irrigation water accumulated in pot (0.84 Kg-soil).

Table 3.2. Total irrigation water volume and total applied amount of N, P and Na derived from irrigation water during the pot experiment

Treatments	**Total volume** L/pot/ 33days	**Total N** mg/pot/33days	**Total P** mg/pot/33days	**Total Na** mg/pot/33 days
U-Vo	1.85	8.66	0.30	53.78
U-2Vo	1.95	9.13	0.31	56.79
U-3Vo	1.90	8.89	0.30	55.23
V-H	1.90	8.89	0.30	55.23
C	1.66	7.77	0.27	48.26

U-V_0, urine volume V_0 applied; U-$2V_0$, urine volume $2V_0$ applied; U-$3V_0$, urine volume $3V_0$ applied; V-H, modified Hoagland's solution applied; C, control.

Table 3.3. Total leached water volume and total amount of N, P and Na derived from leached water during pot experiment (N=5).

Treatments	**Leached water** Volume (mL)	**Total N leached** (mg/pot)	**Total P leached** (mg/pot)	**Total Na leached** (mg/pot)
U-V_0	51.40±3.85	0.25±0.01	0.01±0.00	0.22±0.04
U-$2V_0$	50.56±6.27	0.06±0.04	0.02±0.01	0.27±0.10
U-$3V_0$	49.16±7.82	0.08±0.01	0.01±0.00	0.18±0.07
V-H	50.46±4.77	0.09±0.01	0.02±0.00	0.18±0.05
C	70.47±4.75	0.70±0.13	0.02±0.01	2.08±2.71

Mean values ± standard deviation (SD). U-V_0, urine volume V_0 applied; U-$2V_0$, urine volume $2V_0$ applied; U- $3V_0$, urine volume $3V_0$ applied; V-H, modified Hoagland's solution applied; C, control.

3.2.4. Chemical analysis

Total nitrogen and total carbon in soil was determined by Sumigraph NC-220F (Sumika Chemical Analysis Service, Japan). Regarding total phosphorus in soil, soil samples were digested with nitrate-perchloric acid digestion method and then measured by ascorbic acid method using spectrophotometer at 880 nm (Standard Method, 1989). pH and electrical conductivity (EC) in soil were determined by 1:2.5 dilution method and 1:5 dilution method, respectively with specific electrochemical probes. Measurement of sodium, in soil was done by using Plasma Atomic Emission Spectrometer, ICPE-9000 (SHIMADZU Chemical Analysis Service, Japan) after nitrate-perchloric acid disgestion. The SAR, which is the ratio of sodium, calcium and magnesium cations, was determined after water extracted from soil samples, filtered (0.45 □m pore size, mixed cellulosic ester, ADVANTEC, Japan) and then measured the cations concentration with ICPE-9000 apparatus. In the case of plants, dried shoot and root were separately grinded by mortar and muddler prior to nutrients analysis. Total nitrogen, total phosphorus, sodium, calcium, magnesium and potassium were measured by same method with soil as described above. In leached water, total N and total P were analysed by Hach Method 10071 and 8190 respectively. Sodium in leached water and irrigation water was determined by ICPE-9000 apparatus after filtration (0.45 □m pore size, mixed cellulosic ester, ADVANTEC, Japan).

3.2.5. Statistical analysis

All data in the results were analysed with 5 samples for each treatment and its mean value and standard error were shown in all results. The statistical analysis of each data was analysed with Stat View software version 5.0 by means of a one-way analysis of variance (ANOVA) combined with Turkey-Kramer's post-hoc test to determine the differences among treatments. In the case of none parametric data, Kruskal-Wallis test was conducted to confirm the significant difference.

3.3. RESULTS

3.3.1. Effect of extra urine application volume on plant growth and dry biomass production

Komatsuna plants grew well in all treatments except control. The weekly plant height measurement for urine treatments U-V_0, U-2V_0 and U-3V_0 and modified Hoagland's solution, V-H was not statistically different among them (ANOVA, p>0.05) but all were significantly higher than the control C at 21 and 28 days after sowing (Turkey-Kramer, p<0.05) (Figure 3.3).

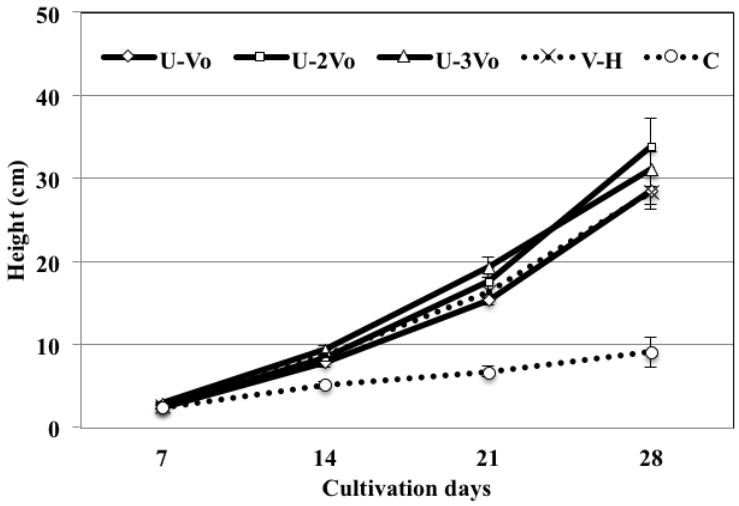

Figure 3.3. Change of Komatsuna height during cultivation in sandy soil using 3 different urine treatments (U-Vo, U-2Vo and U-3Vo), 1 modified Hoagland's solution (V-H) and 1 nonfertilizer (C).

Table 3.4. Komatsuna dry biomass obtained from different urine volume applications in sandy soil; N=5 in each treatment.

Treatments	**Shoot dry weight** (g/pot)	**Root dry weight** (g/pot)
U-Vo	2.38±0.23a	0.54±0.14a
U-2Vo	2.49±0.88a	0.42±0.19a
U-3Vo	3.10±0.43a	0.45±0.17a
V-H	2.71±0.33a	0.60±0.07a
C	0.34±0.21b	0.16±0.10b

Means ± SD; different letters indicates significant difference (Turkey-Kramer test, p<0.05).

Similarly to plant height, shoot dry weight (g/pot) obtained after cultivation (Table 3.4) showed that urine treatments U-V_0, U-2V_0 and U-3V_0 and modified Hoagland's solution, V-H did not have any significant difference among them. Similar observations were also found in root dry biomass. Therefore, from these results it was observed that urine volume application had no inhibition effects on plant height growth, shoot and root dry biomasses under our current "experimental conditions".

Table 3.5. Effect of urine volume application on N, P, Na, Ca, Mg and K concentrations (mg/g dry plant) in Komatsuna shoots cultivated in sandy soil ; N=5 in each treatment.

Treatment	Shoot						
	N	**P**	**Na**	**Ca**	**Mg**	**K**	**K/Na**
U-Vo	42.45±2.33c	0.66±0.19ab	14.28±1.22b	19.87±1.45a	5.25±0.30a	41.56±4.76a	2.91±0.11a
U-2Vo	63.75±6.22b	0.93±0.34a	20.60±2.47a	18.53±1.30a	4.94±0.40a	28.40±3.72b	1.40±0.07b
U-3Vo	80.40±1.83a	0.96±0.22a	17.75±1.16a	14.31±1.05b	3.77±0.30b	24.75±1.81b	1.40±0.15b
V-H	31.74±2.43d	0.49±0.14b	5.08±1.21c	23.03±3.74	6.03±0.83	50.03±8.94	9.97±0.72
C	10.45±1.73e	0.34±0.12b	1.92±0.34d	16.28±2.54	4.85±0.53	22.31±4.70	11.59±0.88

Means ± SD; different letters indicates significant difference (Turkey-Kramer test, $p<0.05$). In the case of Ca ($p=0.0037$), Mg ($p=0.003$), K ($p=0.0011$) and K/Na ($p= 0.0003$) parameters, all 5 treatments included were significantly different in non-parametric test (Kruskal-Wallis).

Table 3.6. Effect of urine volume application on N, P, Na, Ca, Mg and K concentrations (mg/g dry plant) in Komatsuna roots cultivated in sandy soil ; N=5 in each treatment.

Treatments	Root						
	N	**P**	**Na**	**Ca**	**Mg**	**K**	**K/Na**
U-Vo	23.22±1.26b	0.47±0.19	12.82±1.34b	9.91±1.45b	2.64±0.32	8.19±1.34a	0.64±0.12a
U-2Vo	32.78±2.22a	0.61±0.43	16.38±1.39a	17.02±2.21a	2.40±0.23	4.68±0.74b	0.28±0.02b
U-3Vo	32.65±4.09a	1.03±0.25	14.88±2.64a	13.56±3.06a	2.92±0.82	3.61±0.59c	0.11±0.05c
V-H	19.58±2.10b	0.46±0.27	8.82±1.17c	23.00±2.53	3.75±0.20	13.92±1.20	1.59±0.08
C	11.93±1.23c	0.47±0.36	2.34±0.36d	11.22±0.80	3.93±0.42	15.88±1.58	5.44±0.51

Means ± SD; different letters indicates significant difference (Turkey-Kramer test, $p<0.05$). In the case of Ca ($p=0.0007$), Mg ($p=0.005$), K ($p=0.0002$) and K/Na ($p= 0.0001$) parameters, all 5 treatments included were significantly different in non-parametric test (Kruskal-Wallis).

3.3.2. Chemical contents in plant shoot and root and remained in soil after cultivation

The chemical contents in plant shoot and root were summarized in the Table 3.5 and Table 3.6 Concentration of nitrogen in plant shoot increased significantly (Turkey-Kramer, $p<0.05$) with increasing urine volume application in pot. Roots nitrogen contents were significantly higher in U-$2V_0$ and U-$3V_0$ (both in same level) than U-V_0 treatment. In the case of phosphorus, no significant difference was observed in plant shoot and root for urine treatments U-V_0, U-$2V_0$ and U-$3V_0$. Na concentrations in Komatsuna shoot and root were significantly lower in treatment U-V_0 than others urine conditions (U-$2V_0$ and U-$3V_0$, both same level) but all of them were obviously higher than modified Hoagland's solution (V-H). In contrast, the concentrations of K in Komatsuna shoot and root for U-$2V_0$ and U-$3V_0$ (both in same level) were statistically lower than U-V_0; while modified Hoagland's solution, V-H was significantly higher than all urine conditions. Similarly to K, the ratios of K/Na in plant shoot and root for urine treatments U-$2V_0$ and U-$3V_0$ decreased significantly in comparison with the basic urine volume requirement to cultivate Komatsuna, U-V_0. Nitrogen concentration in cultivated soil was statistically higher in treatment U-$3V_0$ than U-V_0, U-$2V_0$ and V-H, which were in the same level (Table 3.7). Phosphorus concentrations in remained had no significant effect with urine volume application. Concentrations of Na in soil after cultivation were in the same level for all treatments. However, soil SAR value in urine treatment U-$3V_0$ were significantly higher than others conditions.

Table 3.7. Soils physical and chemical properties after Komatsuna cultivation in sandy soil; N=5 in each treatment.

Treatment	**pH**	**EC** (μS/cm)	**Total N** (mg/g dry)	**Total P** (mg/g dry)	**Total Na** (mg/g dry)	**SAR value**
U-V_0	7.60±0.10b	61.0±4.9	0.16±0.02b	0.24±0.04	0.70±0.05	0.46±0.04b
U-$2V_0$	7.69±0.17ab	88.8±37.7	0.17±0.01b	0.25±0.01	0.72±0.05	0.52±0.06b
U-$3V_0$	7.75±0.08ab	180.3±23.1	0.22±0.01a	0.29±0.03	0.76±0.03	0.72±0.08a
V-H	7.85±0.06a	96.8±9.3	0.14±0.02bc	0.24±0.03	0.68±0.04	0.34±0.01
C	7.62±0.10b	88.2±7.0	0.12±0.01c	0.20±0.03	0.67±0.07	0.38±0.03

Mean values ± standard deviation (SD); different letters indicates significant difference (Turkey-Kramer test, $p<0.05$). For EC ($p=0.001$), Total P ($p=0.003$) and SAR ($p=0.003$) parameters, all 5 treatments included were significantly different in non-parametric test (Kruskal-Wallis).

Table 3.8. Nitrogen distribution in each treatment after cultivation (N=5).

N distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	148.90	148.90	148.90	148.90
Urine	140	280	420	140
Irrigation water	8.66	9.13	8.89	8.89
Seeds	0.74	0.74	0.74	0.74
Total input	298.30 (100%)	438.77 (100%)	578.53 (100%)	298.53 (100%)
Total Output				
Shoot	100.85±7.74 (33.81%)	157.51±52.67 (35.90%)	240.78±37.06 (41.62%)	85.65±9.92 (28.69%)
Root	12.57±3.68 (4.21%)	13.62±5.09 (3.10%)	14.56±5.58 (2.52%)	11.73±2.28 (3.93%)
Soil-remained	133.62±13.83 (44.79%)	143.82±11.48 (32.78%)	182.1±8.60 (31.48%)	121.53±14.64 (40.71%)
Leached	0.25±0.01 (0.08%)	0.06±0.04 (0.01%)	0.08±0.01 (0.01%)	0.09±0.01 (0.03%)
"Unknown"	51.01 (17.10%)	123.75 (28.20%)	141.01 (24.37%)	79.53 (26.64%)

Mean values ± standard deviation (SD); Original Soil means soil before cultivation.

3.3.3. Distribution of Na, total N and total P in soil, plant and leached water.

Nitrogen distribution in Komatsuna shoot, Komatsuna root, soil and leached water is given in the Table 3.8. The distribution of nitrogen showed that plants nitrogen shoot in urine treatments U-V_0 (100.85mg/pot), U-$2V_0$ (157.51mg/pot) and U-$3V_0$ (240.78mg/pot) increased with increasing urine volumes application in the experimental pot. Concerning soil data, the amount of nitrogen (mg/pot) remained in soil after cultivation in U-Vo (133.62) and U-2Vo (143.82) were lower than that in the original soil (148.90**)**; but N remained in soil in treatment U-3Vo (182.1) was high compared to the original soil. Besides, the "Unknown" parts of nitrogen in urine treatment U-$2V_0$ (123.75mg/pot) and U-$3V_0$ (141.01mg/pot) were higher compared to the adequate urine volume application, U-V_0 (51.01mg/pot). Similarly to nitrogen distribution ratio in plant shoot, Na distribution ratio in shoot increased with increasing urine volume application in soil (Table 3.9). However, more than 96% of the total amount of Na applied in pot remained in soil after cultivation; and based on the total input of Na through urine and irrigation water, the amount of Na uptake by plants (shoot and root) and remained in soil after cultivation were 46.84 %, 53.16 % (U-Vo); 48.41%, 51.59 % (U-2Vo); and 43.14 %, 56.86% (U-3Vo), respectively. The "unknown" parts of Na for all treatment showed negative values, which were included in their respective error bars.

Table 3.9. Sodium (Na) distribution in each treatment after cultivation (N=5).

Na distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	508.47	508.47	508.47	508.47
Urine	33.05	66.10	99.15	0.00
Irrigation	53.78	56.69	55.23	55.23
Seeds	0.002	0.002	0.002	0.002
Total Input	595.30 (100%)	631.26 (100%)	662.86 (100%)	563.71 (100%)
Total output				
Shoot	33.90±3.02 (5.69%)	56.19±6.24 (8.90%)	60.04±6.80 (9.06%)	13.56±2.65 (2.41%)
Root	6.77±1.39 (1.14%)	7.16±3.70 (1.13%)	7.50±0.88 (0.98%)	5.21±0.37 (0.92%)
Soil-remained	590.20±39.10 (99.14%)	610.51±43.72 (96.71%)	636.74±25.38 (96.06%)	573.61±34.96 (101.76%)
Leached	0.22±0.04 (0.04%)	0.27±0.10 (0.04%)	0.18±0.07 (0.03%)	0.18±0.05 (0.03%)
"Unknown"	-35.01 (-6.01%)	-42.88 (-6.79%)	-40.62 (-6.13%)	-28.86 (-5.12%)

Mean values ± standard deviation (SD); Original Soil means soil before cultivation.

3.4. DISCUSSIONS

3.4.1. Effect of extra urine volume application in plant dry biomass

In this present study it was observed that extra human urine application volume did not show any specific difference on Komatsuna plant height, shoot dry weight and root dry weight. One possible reason might be, a salt sensitivity of the plant specie. It has been reported that extra urine application caused an inhibition effect of spinach and cabbage (both, moderately salts sensitive) dry biomass at 800 mg-N/Kg soil levels (Mnkeni et al., 2005) and carrot (salt sensitive) biomass and yield at 400 mg-N/Kg soil level, whereas no depressed effect was found in beetroot (salt tolerant) at the similar level (Mnkeni et al., 2008). In the case of our study, Komatsuna, one family of cabbage, is defined as salt moderately sensitive and the highest urine application (U-$3V_0$) was 500 mg-N/Kg soil, lower than spinach and cabbage cases (both, moderately salts sensitive) but slightly in the same level with carrot, salt sensitive plant. Another possible reason could be, a low Na applied through urine volume application in our experimental conditions. Especially, soil EC in the present study was approximately 10 times lower than that given in the literatures. It was pointed out that soil whose EC is below 4 mS/cm is considered to be non-saline soil (FAO, 1997). Besides, it has been reported that SAR value below 13 is desirable level of sodium in soil and may not affect plant growth (Davis et al., 2007). Considering this EC and SAR references values, soils in urine treatments U-V_0, U-$2V_0$ and U-$3V_0$ were far to be saline (Table 3.7). Thus, the low level of sodium in soil may probably justify the none inhibition effects of urine volume application observed in Komatsuna growth, shoot and root dry matters.

3.4.2. Effect of sodium from extra urine volume application in plant and soil

It was observed, a decrease uptake of K and an increase uptake of Na, in plant shoot and root for all urine treatments compared to modified Hoagland's solution (V-H); and remarkably, the ratio of K/Na was significantly declined in three-time fold the basic urine volume application (U-3V_0) (Table 3.5, Table 3.6). These findings support a result of low K^+ and high Na^+ uptake in red beed with urine cultivation (Pradhan et al., 2010a) and high level of residual K^+ in urine fertilized soil after tomato cultivation (Pradhan et al., 2009). One possible explanation for the phenomena could be the substitution of K^+ by Na^+, which is well known as the primary effects of plant responses to salinity and leads to nutritional imbalances (Alfocea et al., 1996; Gorham et al., 1997; Dasgan et al., 2002; Tuna et al., 2007). In the present study, the ratio of K/Na in plant was negatively correlated with SAR value in soil (Table 3.7). The SAR value increased with the increasing urine volume application and was significantly higher in U-3V_0 than U-V_0 and U-2V_0. Thus, Komatsuna plants fertilized with urine were moderately stressed and this stress was acute where a high volume of urine (U-3V_0) was applied. Hence, further urine volumes applications may have a potential to accentuate this competition of nutrients and then to affect negatively Komatsuna growth. Additionally, among all urine treatments more than 96 % of the Na applied in pot remained in soil after cultivation (Table 3.8); and considering only, the total Na application through urine and irrigation water, more than 50% of remained soil after one time cultivation. Therefore, continuous application of extra human urine volume through many times cultivations has a potential to increase accumulated sodium in soil and then affects subsequently vegetables growth and yields.

3.4.3. Effect of nitrogen from extra urine volume application in plant and soil

It was observed that excess of urine volume application affected nitrogen concentration in plant shoot and root and this finding was clearly shown in shoot part (Table 3.5). This agrees with the observations of Mnkeni et al. (2005; 2008), who have reported an increase of nitrogen concentration in plants tissues of spinach, cabbage, carrot and beetroot by increasing human urine application rate in experimental pot. Unfortunately, the level of nitrate in Komatsuna could not be studied in this work, since when excess of N is applied to the soil, nitrate concentration in vegetables (cabbage, Carrot, Okra) is often high and its metabolites may cause public health issues (Turan and Sevimli, 2005; Mubashir et al., 2010). Subsequently, care should be taken regarding this high nitrogen contents in shoot by using more urine volume than plant requirement. Similarly to nitrogen concentration in shoot, the nitrogen uptake rate in plant shoot, increased with the increasing urine volume application (Table 8). Furthermore, it was also observed from the N-balance, a none accumulation of nitrogen remained soil after cultivation compared to the original soil in adequate (U-Vo) and double (U-2Vo) application of urine volumes. Therefore, adequate human urine application did not accumulate nitrogen in soil. One probable reason might be the remarkably observed "Unknown" part of nitrogen which shown high values where excess volumes of urine were applied (Table 8). One possible explanation for this "Unknown" of nitrogen might be volatilization, because nitrogen in urine is applied in urea form, which is easy to degrade by enzyme from soil microorganism to ammonium and then converted to ammonia gas. This process occurs more slowly in acidic soil than in alkaline one (Staines et al., 2011). Moreover, it has been known that this process is faster in soil where urea concentration is high and soil pH included in the range of 6.5-9.5 (Cabrera et al., 1991) and at the pH 7.5, the concentration of ammonia at equilibrium is higher enough that much of N can be lost to the atmosphere in a few days (On-Farm, 2008). So considering, the high amount of urea applied in soil (U-2V_0 and U-3V_0) and our soil pH (Table 3.7), it was suggested that extra urine volume application

in soil (U-2V_0 and U-3V_0) is not adequate way and application of urine volume based on the plant requirement (U-V_0) might be better in the view point of volatilization. The nitrogen loss from human urine fertilized soil has been showed as 6-7% in a report of Kirshmann and Pettersson al. (1995) and was smaller than those obtained in the present results (17-28%). One reason might be that our pot test was conducted in summer where the maximum temperature in greenhouse reached 43°C and it is well documented that ammonia volatilisation is high in warm temperature (Russelle, 1996; On-Farm, 2008) and increasing temperature markedly increased ammonia volatilization (Ernest and Massey, 1960). Because, high temperature caused high rate of ammonia volatilization and warm soil cannot hold as much ammonia gas (Jones et al. 2007; 2013).

3.4.4. Effect of phosphorus from extra urine volume application in plant and soil

Excess of urine volume application at the level of 2-3 times higher than plant volume requirement have no effect on plant phosphorus contents in shoots and roots. Likewise in plant shoot and root, phosphorus concentration in remained soil was not affected by extra urine volume application. This might be due to the low phosphorus contents in human urine (Pradhan et al., 2009; Sene et al., 2012).

3.5. CONCLUSIONS

From these results, it was concluded that application of human urine volume in the range of 2-3 times higher than plant requirement had no inhibition effects on Komatsuna growth, shoot biomass and root biomass in one time cultivation, since treated soils, were far to be saline when considering the references values given by FAO, 1998 (EC< 4 mS/cm; SAR< 13). However, application of extra urine volume (U-2Vo & U-3Vo) increases nitrogen concentration in plant tissues. Besides, human urine application causes nutritional imbalance (substitution uptake of K^+ by Na^+), which is severe in double (U-2Vo) and triple (U-3Vo) of urine volume applications. In the case of soil, more about 50 % the total sodium applied through urine and irrigation in all treatments remained in soil after one time cultivation. Subsequently, furthers applications of urine may have a potential to increase accumulated sodium ion in soil, and thereafter affect adversely plant growth and production. Furthermore, applications of double (U-2Vo) and triple (U-3Vo) volumes of urine cause accumulation of nitrogen soil and also increase its loss from soil. Therefore, we suggest that application of adequate amount of urine based on plant nitrogen requirement might be a better option for its sustainable use in agriculture.

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Chapter 4

EFFECTS OF CONTINUOUS APPLICATION OF EXTRA HUMAN URINE VOLUME ON PLANT AND SOIL

4.1. INTRODUCTION

High price of fertilizer causes lower yields and less production, especially in marginal area of developing countries, where tools are limited and accessibility of fertilizer when needed is currently a great concern. More concerning is the global peak in phosphorus production, which is estimated to occur around 2030, and after the maximum reached, production will drop year upon year, seeing a widening gap between demand and supply (Cordell, 2008). At the meantime, it is widely known that in domestic wastewater, urine fraction contains approximately 80 % of the nitrogen, 55 % of the phosphorus and 60 % of the potassium (Höglund, 2001). Therefore, source separation of black water, which diverts urine and faeces, is a holistic approach to reduce the reliance on chemical fertilizer for a sustainable food production, by allowing to recycle plants nutrients in urine to the soil. Thus, in recent years numerous research work have shown that, human urine has a potential to substitute industrial fertilizer due to its large amount of nitrogen (N), phosphorus (P) and potassium (K) contents (Krichmann and Pettersson, 1995; Heinomen-Tanski et al. 2007; Pradhan et al., 2007, 2009a, 2009b, 2010a). However, urine contents some salts (Krichmann and Pettersson, 1995; Mnkni et al., 2005, 2008), pathogens (Höglund 2001, Pradhan et al., 2007), and pharmaceuticals (Winker et al., 2008; 2010). Subsequently, for the long-term view related to a sustainable agricultural production, uncontrolled urine application in agriculture land, may cause simultaneously soil salinity, sodium (Na) accumulation and nitrogen accumulation in soil. It has been reported that extra urine application inhibited a growth of cabbage, spinach and carrot accompanied with increase of soil EC (Mnkeni et al., 2005; 2008). Furthermore, sodium is contained in the urine at the level of 1-9 g/L (Krichmann and Pettersson, 1995; Mnkeni et al., 2008; Pradhan et al., 2010b). The excess of sodium accumulation in soil have a potential to be detrimental to plant growth, by disrupting the water uptake in the root zone, interfering with completive nutrients and/or dispersing soil particles as a result of breaking down soil aggregates (Asano, 2007; Franzan, 2007; Allotey et al., 2008; Lee, 2012). Regarding to nitrogen, additionally, high volume of urine application increases nitrogen concentration in plant tissues (Mnkeni et al., 2008). The high concentration of nitrogen in soil might affect negatively amount of sugar and vitamins in vegetables and build up in the plant tissues causing therefore health and taste issues to consumers (Brady and Weil 1996; Turan and Sevimli 2005). It has been known that nitrogen lost from soil during cultivation was promoted in urine fertilization (Kirchmann and Pettersson, 1995) and in high rate of urine application (Sene et al., 2013a). Thereby, it has not been addressed the effects of continuous application of excess urine volume on plant and soil through continuous cultivations. Therefore the objective of this study was to investigate the effects of continuous application of extra urine volume in plant and soil. Pot test with Komatsuna (*Brassica rapa var.peruviridis*), was conducted in greenhouse for continuous cultivations, and then observed, plant growth and nutrients effects in both plant and soil under continuous application of different urine volumes.

4.2. Material and Methods

4.2.1. Pot experiments

The experiments were carried out during three successive cultivation periods (August-September 2011, May-June 2012 and July-August 2012) in the greenhouse at Hokkaido University, Japan (43°04′11.6″N, 141°20′22.4″E). The recorded climate data in each cultivation period are summarized in the Table 4. 1. Two compartments closed pot system (both in plastic) was used in this experiment to analyse leached water. To prevent root expansion, the inside pot (with volume 0.7 L, top diameter 12.4 cm, bottom diameter 8.5 cm and height 10.2 cm) was covered in its bottom part by 150 μm nylon mesh. The external one was a Wagner pot (cylindrical shape, diameter 11.0 cm and height 15.0 cm, defined as 1/10000a size), which had allowed collecting leached water per week. Washed sandy soil mixed with small gravels (6:3 soil:gravel ratio) to keep good aeration of soil and to facilitate root development, were set as media (0.84-Kg soil pot). Before starting the first cultivation, soil pH was 5.4, and therefore, it was limed by adding 2g of CaMg $(CO_3)_2$ per pot as recommended Fujiwara and Narimatsu (2006). The physical and chemical characteristics of limed soil (original soil) are shown in the Table 2. Komatsuna (*Brassica rapa var. peruviridis*), a leafy vegetable commonly consumed in Japan, was selected in this work, because, it is resistant to diseases for continuous cultivation (Fjellstrom and Williams, 1997; Morikawa and Saigusa, 2001) and presents high growth rate (Thao et al., 2008). Within each experimental pot, six Komatsuna seeds were germinated in petri dish with filter paper and incubated at 25°C for 1 day and then sown. Seven days after seedling emerged, the seedlings were reduced to two plants per pot. This procedure was repeated at the beginning of the second and third cultivation each.

Table 4.1: Climate data inside the greenhouse during the 3 successive cultivation periods

Cultivation periods	Temperature (°C)	Humidity (%)	Sunshine duration (h)	Solar Radiation (MJ/m^2)
August-September 2011	18.8-42.9	27.3-88.5	4.6-5.8	7.2-21.8
May-June 2012	12.9-37.7	16.0-72.0	4.7-6.8	13.1-27.3
July-August 2012	17.3-42.4	17.7-77.7	4.3-6.0	11.1-25.1

The sunshine duration (h) and solar radiation (MJ/m^2) were obtained from Sapporo Weather Station No 47412 (Lat 43°03.6'N Lon 141°19.7'E), while the temperature and Humidity were recorded inside the greenhouse using a wireless Thermometer and Humidity Sensor.

Table 4.2: Physical and chemical properties of bulk soil before cultivation.

Parameters	**Original sandy soil**
pH	7.50±0.09
EC (μS/cm)	49.4±4.70
total C (mg/g DW)	1.38±0.17
total N (mg/g DW)	0.18±0.01
total P (mg/g DW)	0.28±0.03
K (mg/g DW)	1.91±0.36
Na (mg/g DW)	0.60±0.04
SAR value	0.21±0.30

Mean values ± standard deviation (SD) with 5 replications; "Na" means total sodium extracted with nitrate-perchloric acid digestion.

4.2.2. Fertilizer treatments

Synthetic urine (Wilsenach et al., 2007) was used in this research work to obtain more general and precise effects of continuous application of extra human urine in plant and soil, because the composition of urine fluctuates from one person to another and depends mainly on diet, climate, physical activity, time of the days and body size (Heinomen- Tanski et al., 2007; Pradhan et al., 2010b). The synthetic urine contained 12.05g/L of nitrogen, 0.96g/L of phosphorus, 2.04g/L of potassium and 2.84g/L of sodium. Based on the total nitrogen required to grow Komatsuna (140 kg/ha) recommended by Fujiwara et al. (2006), the composition of synthetic urine and the pot system size, diluted (1/3) synthetic urine volume was designed as U-Vo (34.84 ml) and containing the following dose of N (140mg/pot), P (11.12 mg/pot), K (23.73mg/pot) and Na (33.05 mg/pot). To know the effects of continuous application of extra human urine volume in plant and soil, 3 different urine treatments volumes [U-Vo, U-2Vo (NPK 280-22.24-47.46, Na 66.1 mg/pot) and U-3Vo (NPK 420-33.36-71.19, Na 99.15 mg/pot)] were set in one hand. In the other hand, 1 modified Hoagland's solution volume [V-H (NPK 140-9.41-31.53, Na 0.0 mg/pot)] and 1 nonfertilizer treatment [N-C (NPK 0-0-0, Na 0.0 mg/pot)] were set as positive (same N with plant requirement and without minors compounds except KH_2PO_4) and negative controls, respectively. Totally, 25 experimental pots were carried out corresponding to 5 treatments replicated 5 times each. Komatsuna were continuously cultivated three times (35 days each) using the same soil and the similar rate of fertilizer in each cultivation period. All fertilizers were regularly applied just after the seedlings reduced to two plants per pot at days 7, 14, 21, and 28 after sowing as recommended by Sene et al. (2012).

4.2.3. Irrigation water, Growth and Harvesting

Tap water was used as irrigation water. Before the first cultivation, the theoretical daily amount of water needed for irrigation (24.6ml/pot), was determined by multiplying the crop coefficient of Komatsuna in the development stage (Kc=0.60) and the potential evapotranspiration (ETo=4.1 mm/day) and the pot size system (1/10000a). The ETo value was computed by using ETo Calculator version 3.1 (FAO, 2009), and the input data (solar radiation, temperatures max and min, humidity max and min) were derived from Sapporo Weather Station No 47412 (Lat 43°03.6'N Lon 141°19.7'E, Japan) for the cover period of August 6 to September of 2010. In practical, the theoretical daily irrigation was modified to 50 ml/pot. However, based on the daily observation, the modified daily irrigation water was slightly modified during all cultivation periods. The total volumes of water applied in

experimental pot during the 1st, 2nd and 3rd cultivation period and theirs chemical contents are shown in Table 4.3.

Table 4.3. Total irrigation volume and total applied amount of N, P and Na derived from irrigation water during each cultivation periods.

	Total volume L/pot	**Total N** mg/pot	**Total P** mg/pot	**Total Na** mg/pot
	1st cultivation period			
Treatments				
U-Vo	1.85	8.66	0.30	53.78
U-2Vo	1.95	9.13	0.31	56.79
U-3Vo	1.90	8.89	0.30	55.23
V-H	1.90	8.89	0.30	55.23
N-C	1.66	7.77	0.27	48.26
	2nd cultivation period			
Treatments				
U-Vo	1.97	9.22	0.32	57.27
U-2Vo	1.97	9.22	0.32	57.27
U-3Vo	1.97	9.22	0.32	57.27
V-H	1.97	9.22	0.32	57.27
N-C	1.70	7.96	0.27	49.42
	3rd cultivation period			
Treatments				
U-Vo	1.35	6.32	0.22	39.24
U-2Vo	1.35	6.32	0.22	39.24
U-3Vo	1.30	6.08	0.21	37.79
V-H	1.35	6.32	0.22	39.24
N-C	0.95	4.45	0.15	27.62
	1st cultivation + 2nd cultivation + 3rd Cultivation			
Treatments				
U-Vo	5.17	24.20	0.84	150.29
U-2Vo	5.27	24.66	0.85	149.04
U-3Vo	5.17	24.20	0.84	150.29
V-H	5.22	24.43	0.84	151.75
N-C	4.31	20.17	0.70	125.29

1st cultivation (33days), 2nd cultivation (34 days) and 3rd cultivation (34 days); U-Vo, urine volume Vo applied; U-2Vo, urine volume 2Vo applied; U-3Vo, urine volume 3Vo applied; modified Hoagland's solution applied; N-C, non-fertilizer control.

The application of irrigation water was done by a graduated pet bottle (50 mL between two graduations where the total volume was 0.5 L) equipped with a plastic shower head, which had allowed spraying water uniformly inside the pot. The total volumes of water leached from the pots after each cultivation time and theirs chemicals contents are shown in the Table 4. Plants heights were measured every week, and from 1 week after sowing. In the plant height measurement, the highest leaf in each pot was selected. At 35 days after sowing, shoots and roots were separately harvested. The fresh samples were lyophilized and weighted as dry weight and then stored at -30 °C.

Table 4.4. Total leached water volume and total amount of N, P and Na derived from leached water during pot experiment

	Leached water Volume (mL)	**Total N leached** (mg/pot)	**Total P leached** (mg/pot)	**Total Na leached** (mg/pot)
	1st cultivation period			
Treatments				
U-Vo	51.40±3.85	0.25±0.01	0.01±0.00	0.22±0.04
U-2Vo	50.56±6.27	0.06±0.04	0.02±0.01	0.27±0.10
U-3Vo	49.16±7.82	0.08±0.01	0.01±0.00	0.18±0.07
V-H	50.46±4.77	0.09±0.01	0.02±0.00	0.18±0.05
C	70.47±4.75	0.70±0.13	0.02±0.01	2.08±2.71
	3rd cultivation period			
Treatments				
U-Vo	50	0.31±0.53	0.01±0.00	0.26±0.24
U-2Vo	50	0.02±0.02	0.01±0.00	0.11±0.05
U-3Vo	50	0.03±0.02	0.00±0.00	0.09±0.02
V-H	50	0.08±0.13	0.01±0.00	0.13±0.08
C	50	0.01±0.01	0.02±0.01	0.07±0.01

Mean values ± standard deviation (SD). In the 2nd and 3rd cultivations no water was leached from the pot but after the 3rd cultivation the bottom part of all pots was washed with 10% HCL using 50 ml volume per pot.

4.2.4. Chemical analysis

Total nitrogen and total carbon in soil was determined by Sumigraph NC-220F (Sumika Chemical Analysis Service, Japan). Regarding total phosphorus in soil, soil samples were digested with nitrate-perchloric acid digestion method and then measured by ascorbic acid method using spectrophotometer at 880 nm (Standard Method, 1989). pH and electrical conductivity (EC) in soil were determined by 1:2.5 dilution method and 1:5 dilution method, respectively with specific electrochemical probes. Measurement of sodium, in soil was done by using Plasma Atomic Emission Spectrometer, ICPE-9000 (SHIMADZU Chemical Analysis Service, Japan) after nitrate-perchloric acid disgestion. The SAR, which is the ratio of sodium, calcium and magnesium cations, was determined after water extracted from soil samples, filtered (0.45 μm pore size, mixed cellulosic ester, ADVANTEC, Japan) and then measured the cations concentration with ICPE-9000. In the case of plants, dried shoot and root were separately grinded by mortar and muddler prior to nutrients analysis. Total nitrogen, total phosphorus, sodium, calcium, magnesium and potassium were measured by same method with soil as described above. In leached water, total N and total P were analysed by Hach Method 10071 and 8190 respectively. Sodium in leached water and irrigation water was determined by ICPE-9000 apparatus after filtration (0.45 μm pore size, mixed cellulosic ester, ADVANTEC, Japan).

4.2.5. Statistical analysis

All data in the results were analysed with 5 samples for each treatment and its mean value and standard error were shown in results. The statistical analysis of each data was analysed with SPSS software version 21 by means of a one-way analysis of variance (ANOVA). Means were separated by Turkey-Kramer's post-hoc test (honestly significant difference, $p<0.05$) or Dunnett's multiple range test (least-significant difference, $p<0.05$).

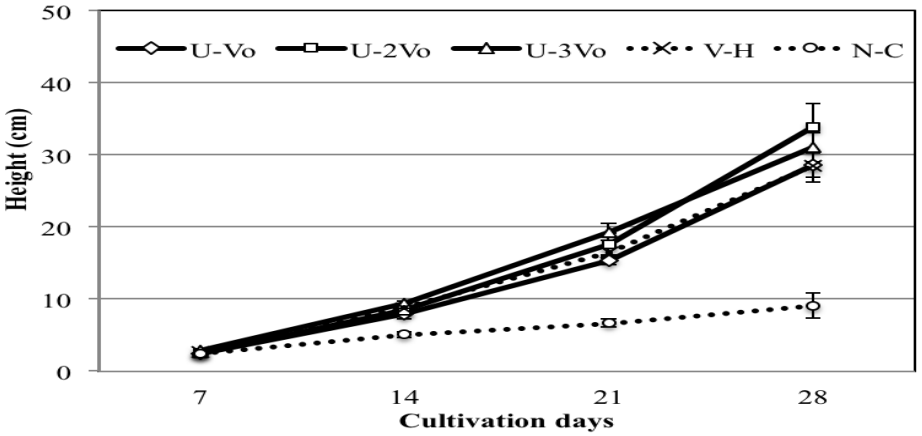

Figure 4.1. Change in time course of Komatsuna height during the 1st cultivation.

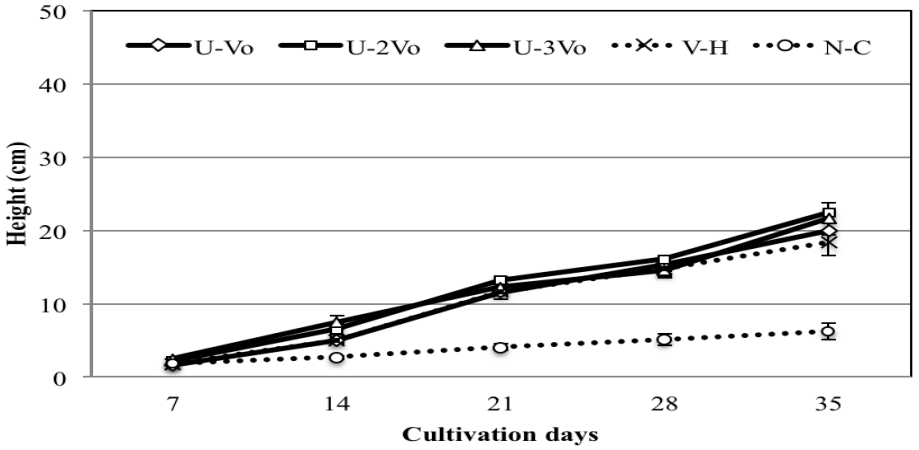

Figure 4.2. Change in time course of Komatsuna height during the 2nd cultivation.

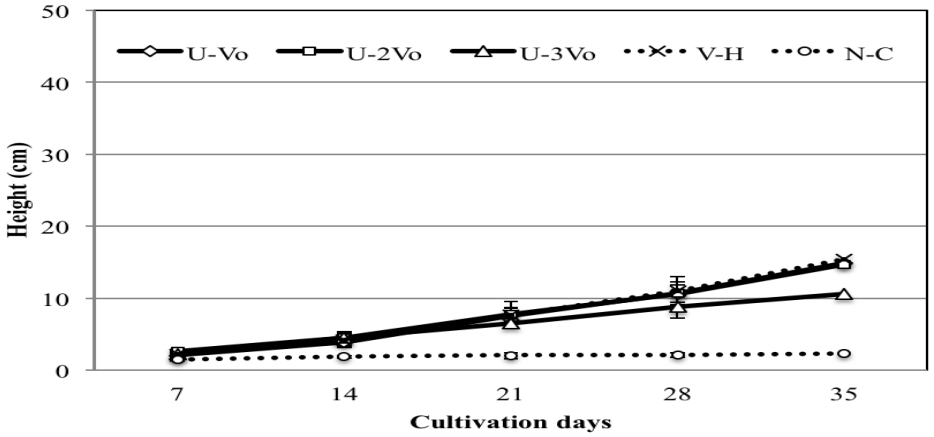

Figure 4.3. Change in time course of Komatsuna height during the 3rd cultivation.

4.3. RESULTS

4.3.1. Effects of continuous application of extra urine volume on plant growth and dry biomass

Weekly Komatsuna heights measured during the 1st, 2nd, and 3rd cultivation period are given in Figure 4.1, Figure 4.2 and Figure 4.3, respectively. In each of the cultivation period, the plant height measurement for urine treatments U-Vo, U-2Vo and U-3Vo and modified Hoagland's solution, V-H were not statistically different among them (ANOVA, $p>0.05$), but all were significantly higher than the N-C from the day 21 to 35. Similarly to plant height, Komatsuna shoot dry weight obtained after the 1st cultivation (Table 5) did not differ significantly among all treatments compared to the N-C. In the 2nd cultivation step, shoot dry weight for all treatments were statistically different among them except U-Vo and V-H (both, same level), and the highest values were observed in the highest rate of urine application (U-2Vo and U-3Vo). Concerning, the 3rd cultivation, shoot dry biomass production in urine treatments U-Vo, U-2Vo and U-3Vo and V-H did not show any significant different among them. Furthermore, root dry matter productions in all set conditions were not statistically different among them, except the N-C in the 1st, 2nd and 3rd cultivation. Therefore from these results, it was observed that continuous application of extra urine volume had no depressed growth on plant height, shoot and root dry weights in our specific experimental system.

Table 4.5. Komatsuna dry biomass obtained from different urine volume application in sand soil for continuous cultivation; N=5 in each treatment.

Parameters	**Shoot dry weight (g/pot)**	**Root dry weight (g/pot)**
	1st cultivation period	
Treatments		
U-V_0	2.38±0.23a	0.54±0.14a
U-2V_0	2.49±0.88a	0.42±0.19a
U-3V_0	3.10±0.43a	0.45±0.17a
V-H	2.71±0.33a	0.60±0.07a
N-C	0.34±0.21b	0.16±0.10b
	2nd cultivation period	
Treatments		
U-V_0	2.97±0.32c	0.58±0.11x
U-2V_0	3.61±0.72b	0.64±0.10x
U-3V_0	4.82±0.41a	0.58±0.06x
V-H	3.21±0.38c	0.66±0.12x
N-C	0.22±0.07d	0.04±0.03y
	3rd cultivation period	
Treatments		
U-V_0	1.44±0.28a	0.34±0.07x
U-2V_0	1.92±0.60a	0.31±0.09x
U-3V_0	1.30±0.62a	0.25±0.21x
V-H	1.71±0.35a	0.36±0.08x
N-C	0.09±0.05b	0.04±0.03y

Means ± SD; different letters within a column (a, b, c, d) indicates honestly significant difference (Turkey-Kramer test, $p<0.05$); different letters within a column (x, y) indicate at least-significant difference (Dunnett T3 test, $p<0.05$

4.3.2. Chemical contents in plant shoot and root and soils properties after the 1st, 2nd and 3rdcultivation

Chemical contents in plant shoot and root for the 1st, 2nd and 3rd cultivation are shown in the Table 4.6 and Table 4.7, respectively. Concentrations of nitrogen in plant shoot and root increased significantly (Turkey-Kramer, $p<0.05$) with increasing extra urine volume application in each cultivation period. In all fertilized conditions, nitrogen concentrations in Komatsuna shoot obtained after the 2nd cultivation period were lower compared to theirs respective in the 1st and 3rd cultivations (Table 4.6). Phosphorus concentrations in plant shoot and root did not show any specific change with urine treatments U-Vo, U-2Vo and U-3Vo in the 1st, 2nd and 3rd cultivation. Sodium contents in Komatsuna shoot and root were obviously low in V-H and N-C in comparison with all set urine conditions (U-Vo, U-2Vo and U-3Vo) in the 1st, 2nd and 3rd cultivation. In contrast, K contents in Komatsuna shoot were significantly higher in V-H than U-Vo, U-2Vo, and U-3Vo in the 1st, 2nd and 3rd cultivation. Similarly to K in plant shoot, the ratios of K/Na and Ca/Na in Komatsuna shoot and root after each cultivation period were significantly higher in all modified Hoagland's solution in comparison to others conditions. Moreover, K/Na ratio decreased from the 1st to 2nd cultivation in all treatments including, but from the 2nd to 3rd cultivation no acceleration of this trend were observed.

Table 4.6. Effect of continuous application of extra urine volume on N, P, Na, Ca, Mg and K concentrations (mg/g-dry plant) in Komatsuna shoots cultivated in sandy soil; N=5 in each treatment.

Parameters	N	P	Na	Ca	Mg	K	K/Na	Ca/Na
	Shoot- 1st cultivation							
Treatment								
U-V_0	42.45±2.33c	0.66±0.19ab	14.28±1.22c	19.87±1.45a	5.25±0.30ab	41.56±4.76a	2.91±0.11w	1.39±0.08x
U-2V_0	63.75±6.22b	0.93±0.34a	20.60±2.47a	18.53±1.30a	4.94±0.40b	28.40±3.72b	1.40±0.07x	0.92±0.09y
U-3V_0	80.40±1.83a	0.96±0.22ac	17.75±1.16b	14.31±1.05b	3.77±0.30c	24.75±1.81b	1.40±0.15x	0.81±0.03y
V-H	31.74±2.43d	0.49±0.14bc	5.08±1.21d	23.03±3.74a	6.03±0.83a	50.03±8.94a	9.97±0.72v	4.61±0.53w
N-C	10.45±1.73e	0.34±0.12b	1.92±0.34e	16.28±2.54ab	4.85±0.53b	22.31±4.70b	11.59±0.88v	8.54±0.80v
	Shoot -2nd cultivation							
Treatment								
U-V_0	27.22±1.96c	0.41±0.03	12.70±1.35b	18.49±1.49b	5.98±0.37	13.77±1.23b	1.10±0.18wx	1.47±0.24c
U-2V_0	48.84±2.59b	0.42±0.05	16.75±2.11a	20.45±2.46ab	6.50±0.55	12.42±1.30b	0.75±0.14x	1.24±0.25c
U-3V_0	57.26±2.72a	0.41±0.03	15.67±1.08a	23.58±0.61a	6.99±0.48	14.56±1.44b	0.93±0.08x	1.51±0.10c
V-H	25.99±2.77c	0.37±0.05	4.24±0.49c	17.83±1.27b	6.43±0.81	24.93±2.35a	5.94±0.82v	4.27±0.73b
N-C	13.64±2.44d	0.33±0.55	3.83±1.24c	19.15±4.79b	7.50±2.16	11.59±1.73b	3.28±1.08w	5.10±0.39a
	Shoot -3rd cultivation							
Treatment								
U-V_0	44.74±1.91c	0.47±0.23	10.27±0.89b	26.46±1.69	8.79±0.25	14.97±1.64w	1.46±0.17w	2.60±0.36w
U-2V_0	54.79±3.76b	0.45±0.27	11.83±1.34b	25.52±2.96	8.86±0.74	16.76±1.41w	1.44±0.25w	2.20±0.54wx
U-3V_0	81.18±4.08a	0.30±0.22	18.94±1.69a	26.41±2.42	9.97±0.98	13.08±3.74wx	0.70±0.21x	1.40±0.17x
V-H	43.82±5.57c	0.40±0.14	5.09±0.30c	25.50±2.68	8.44±0.41	31.45±2.10v	6.20±0.50v	5.02±0.43v
N-C	14.36±2.74d	0.20±0.07	5.55±2.53c	24.25±2.45	8.00±1.26	9.62±1.43x	1.98±0.79w	4.87±1.45vw

Means ± SD; different letters within a column (a, b, c, d, e) indicates honestly significant difference (Turkey-Kramer test, $p<0.05$); different letters within a column (v, w, x, y, z) indicates at least-significant difference (Dunnett T3 test, $p<0.05$).

Table 4.7. Effect of continuous application of extra urine volume application on N, P, Na, Ca, Mg and K concentrations (mg/g-dry plant) in Komatsuna roots cultivated in sandy soil ; N=5 in each treatment.

Parameters	N	P	Na	Ca	Mg	K	K/Na	Ca/Na
	Root – 1st cultivation							
Treatment								
U-V_0	23.22±1.26b	0.47±0.19	12.82±1.34w	9.91±1.45c	2.64±0.32c	8.19±1.34b	0.64±0.12x	0.79±0.20c
U-2V_0	32.78±2.22a	0.61±0.43	16.38±1.39v	17.02±2.21b	2.40±0.23c	4.68±0.74c	0.28±0.02y	1.04±0.13c
U-3V_0	32.65±4.09a	1.03±0.25	14.88±2.64vw	13.56±3.06bc	2.92±0.82bc	3.61±0.59d	0.11±0.05z	0.93±0.25c
V-H	19.58±2.10b	0.46±0.27	8.82±1.17x	23.00±2.53a	3.75±0.20ab	13.92±1.20a	1.59±0.08w	2.65±0.47b
N-C	11.93±1.23c	0.47±0.36	2.34±0.36y	11.22±0.80c	3.93±0.42a	15.88±1.58a	5.44±0.51v	3.87±0.55a
	Root -2nd cultivation							
Treatment								
U-V_0	23.24±0.74c	0.45±0.29	5.62±0.46b	34.67±6.57c	4.18±0.43w	5.29±1.21c	3.95±0.65w	6.20±1.24x
U-2V_0	28.17±0.77b	0.51±0.17	6.43±0.69ab	42.79±5.96c	4.13±0.22w	3.84±0.61c	2.46±0.29x	6.78±1.54x
U-3V_0	32.73±1.29a	0.48±0.36	6.97±1.04a	29.40±2.66c	3.71±0.23w	5.39±0.99c	3.22±0.62wx	4.30±0.81x
V-H	22.90±0.87c	0.30±0.16	3.29±0.70c	66.33±11.31b	5.69±0.72v	9.03±0.92b	11.76±2.30v	20.38±1.82w
N-C	12.19±2.04d	0.26±0.07	2.60±0.39c	92.69±11.89a	6.35±0.63v	13.36±2.17a	3.90±1.78w	35.78±5.62v
	Root -3rd cultivation							
Treatment								
U-V_0	24.25±1.56c	0.43±0.11	4.67±0.36a	46.55±11.56w	4.73±0.58vw	5.38±0.87w	1.15±0.13y	10.05±2.79vx
U-2V_0	34.15±1.12b	0.50±0.22	5.02±0.57a	24.22±5.70w	3.68±0.35wx	7.69±1.02v	1.53±0.09x	4.86±1.19x
U-3V_0	46.86±1.53a	0.40±0.19	5.64±0.93a	10.23±0.77x	3.09±0.17x	9.19±1.31v	1.65±0.19x	1.87±0.46y
V-H	24.03±1.45c	0.32±0.20	2.46±0.24b	72.20±6.73v	4.92±0.22v	8.16±0.68v	3.35±0.48w	29.73±4.89w
N-C	11.62±3.19d	0.25±0.03	1.31±0.45c	65.99±29.98vw	4.13±2.34vx	14.94±3.88v	12.34±4.11v	42.39±16.67vw

Means ± SD; different letters within a column (a, b, c, d, e) indicates honestly significant difference (Turkey-Kramer test, $p<0.05$); different letters within a column (v, w, x, y, z) indicates at least-significant difference (Dunnett T3, $p<0.05$).

Soil physicals and chemicals properties after each of the cultivation period are summarized in the Table 8. Nitrogen concentrations in remained soil after each cultivation increased significantly from the 1st and 3rd cultivations in extra urine volumes application (U-2Vo, U-3Vo) cases. While in the adequate urine volume application (U-Vo) and the modified Hoagland's solution (V-H), N remained in soil increased from the 1st to 2nd cultivation and then both of them decreased from the 2nd to the 3rd cultivation. Phosphorus concentration in soil after the 1st and 2nd cultivation in all urine treatments (U-Vo, U-2Vo and U-3Vo) did not show any statistical different among them, but in the 3rd cultivation, U-3Vo was significantly higher than other treatments. Concentrations of sodium in cultivated soil for U-Vo, U-2Vo and U-3Vo were not significantly different (Turkey-Kramer, $p<0.05$) in all cultivation periods except in the 3rd one, where urine treatments U-2Vo and U-3Vo were in the same level, but both were statistically greater than U-Vo. Soil electrical conductivity (EC) increased from the 1st cultivation to the 3rd one; and within each cultivation, soil EC increased significantly in urine conditions, and these increase were faster in extra urine volume application cases. Conversely, from the 1st to 3rd cultivation, soil pHs decreased in all urine treatments and were statistically different among them after the 2nd and 3rd cultivation; but, the decrease was faster in extra urine volumes applications (Table 8).

Table 4.8. Soils physical and chemical properties after different urine volume application in sandy soil for continuous cultivation of Komatsuna. N=5 in each treatment.

Parameter	**pH**	**EC (μS/cm)**	**Total N (mg/g-dry)**	**Total P (mg/g-dry)**	**Total Na (mg/g-dry)**	**SAR values**
	0 cultivation period					
Original Soil	7.50± 0.09	49.4±4.70	0.18±0.01	0.28±0.03	0.60±0.04	0.210±.03
	1st cultivation period					
treatment						
U-V_0	7.60±0.10b	61.0±4.9y	0.16±0.02b	0.24±0.04ab	0.70±0.05	0.46±0.04xy
U-$2V_0$	7.69±0.17ab	88.8±37.7xy	0.17±0.01b	0.25±0.01ab	0.72±0.05	0.52±0.06x
U-$3V_0$	7.75±0.08ab	180.3±23.1w	0.22±0.01a	0.29±0.03a	0.76±0.03	0.72±0.08w
V-H	7.85±0.06a	96.8±9.3x	0.14±0.02bc	0.24±0.03ab	0.68±0.04	0.34±0.01z
N-C	7.62± 0.10b	88.2±7.0x	0.12±0.01c	0.20±0.03b	0.67±0.07	0.38±0.03yz
	2nd cultivation period					
treatment						
U-V_0	7.58±0.15b	132.0±11.2c	0.20±0.01c	0.41±0.03a	0.73±0.06ab	0.61±0.08ab
U-$2V_0$	6.96±0.06c	200.8±43.6b	0.23±0.02b	0.42±0.05a	0.77±0.09a	0.55±0.04ab
U-$3V_0$	6.54±0.05d	372.6±40.3a	0.27±0.01a	0.41±0.03a	0.80±0.07a	0.64±0.06a
V-H	7.90±0.14a	158.2±20.4bc	0.20±0.01c	0.37±0.05ab	0.64±0.05b	0.49±0.09b
N-C	7.69±0.06b	132.1±19.6c	0.11±0.02d	0.33±0.05b	0.61±0.08b	0.54±0.03a
	3rd cultivation period					
treatment						
U-V_0	7.43±0.13b	223.4±41.2c	0.18±0.02y	0.44±0.03b	0.74±0.07b	0.73±0.04x
U-$2V_0$	6.78±0.18c	438.8±60.2b	0.26±0.03x	0.44±0.03b	0.80±0.04a	0.72±0.02x
U-$3V_0$	6.50±0.12d	868.0±83.0a	0.42±0.04w	0.51±0.03a	0.88±0.08a	0.94±0.06w
V-H	7.87±0.15a	294.6±44.4c	0.17±0.01y	0.39±0.05b	0.63±0.06c	0.51±0.02z
N-C	7.97±0.18a	229.8±29.4c	0.11±0.02z	0.29±0.02c	0.66±0.05c	0.63±0.03y

(Table 4.8. Contd.)

Means ± SD; different letters within a column (a, b, c, d) indicates honestly significant difference (Turkey-Kramer test, $p<0.05$); different letters within a column (w, x, y, z) indicates at least-significant difference (Dunnett T3 test, $p<0.05$).

4.3.1. Distribution of Na, N and P in soil, plant, leached water in the 1st, 2nd and 3rd cultivation

Nitrogen distribution in Komatsuna shoot, root, soil and leached water in the 1st, 2nd and 3rd cultivation is presented in the Table 4.9, Table 4.10 and Table 4.11, respectively.

Table 4.9. Nitrogen distribution in 1st step cultivation (N=5).

N distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	148.90	148.90	148.90	148.90
Urine	140	280	420	140
Irrigation water	8.66	9.13	8.89	8.89
Seeds	0.74	0.74	0.74	0.74
Total input	298.30 (100%)	438.77 (100%)	578.53 (100%)	298.53 (100%)
Total Output				
Shoot	100.85±7.74 (33.81%)	157.51±52.67 (35.90%)	240.78±37.06 (41.62%)	85.65±9.92 (28.69%)
Root	12.57±3.68 (4.21%)	13.62±5.09 (3.10%)	14.56±5.58 (2.52%)	11.73±2.28 (3.93%)
Soil-remained	133.62±13.83 (44.79%)	143.82±11.48 (32.78%)	182.1±8.60 (31.48%)	121.53±14.64 (40.71%)
Leached	0.25±0.01 (0.08%)	0.06±0.04 (0.01%)	0.08±0.01 (0.01%)	0.09±0.01 (0.03%)
"Unknown"	51.01 (17.10%)	123.75 (28.20%)	141.01 (24.37%)	79.53 (26.64%)

Original Soil means soil before started the 1st cultivation.

Table 4.10. Nitrogen distribution in the 2nd cultivation period (N=5).

N distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	133.62	143.82	182.10	121.53
Urine	140	280	420	140
Irrigation	9.22	9.22	9.22	9.22
Seeds	0.74	0.74	0.74	0.74
Total input	283.58 (100%)	433.78 (100%)	612.10 (100%)	241.49 (100%)
Total Output				
Shoot	80.47±5.30 (28.38%)	184.93±7.94 (42.63%)	275.57±20.21 (45.02%)	82.70±4.73 (30.46%)
Root	13.43±2.29 (4.74%)	18.03±2.33 (4.16%)	18.95±2.63 (3.10%)	15.06±3.06 (5.55%)
Soil- remained	160.52±10.03 (56.60%)	191.00±12.71 (44.03%)	225.01±12.27 (36.76%)	163.33±6.50 (60.16%)
Leached	0 (0%)	0 (0%)	0 (0%)	0 (0%)
"Unknown"	29.16 (10.28%)	39.82 (9.18%)	92.53 (15.12%)	10.40 (3.83%)

Original Soil means soil after the 1st time cultivation.

Table 4.11. Nitrogen distribution in 3rd step cultivation (N=5).

N distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	160.52	191.00	225.01	163.33
Urine	140	280	420	140
Irrigation	6.32	6.32	6.08	6.32
Seeds	0.74	0.74	0.74	0.74
Total input	307.58 (100%)	478.06 (100%)	651.83 (100%)	310.39 (100%)
Total output				
Shoot	64.14±10.75 (20.85%)	104.14±27.57 (21.78%)	103.53±46.27 (15.88%)	73.35±6.84 (23.63%)
Root	8.27±1.67 (2.69%)	10.72±3.39 (2.24%)	10.7±8.89 (1.64%)	8.73±2.33 (2.81%)
Soil-remained	138.15±18.96 (44.92%)	193.95±20.53 (40.57%)	323.96±20.99 (49.70%)	129.67±9.21 (41.78%)
Leached	0.31±0.53 (0.1%)	0.02±0.02 (0%)	0.03±0.02 (0%)	0.08±0.13 (0.03%)
"Unknown"	99.61 (31.44%)	172.13 (35.40%)	216.75 (32.77%)	101.46 (31.75%)

Original Soil means soil after 2nd time cultivation.

In each of fertilizer treatment (U-Vo, U-2Vo, U-3Vo and V-H), the amount of nitrogen (mg/pot) content in plant shoot and root increased from the 1st to 2nd cultivation, and then started to be decreased from the 2nd to the 3rd cultivation. Additionally, within each of the cultivation, the distribution ratio of nitrogen in Komatsuna shoot and root was constantly high in the extra urine volumes application cases (U-2Vo and U-3Vo). Nitrogen distribution ratio in remained soil, was higher in extra urine treatments after each cultivation, and increased from one cultivation to another, especially in the 3rd cultivation of U-3Vo. The amount of nitrogen remained in soil pots in the adequate urine volume (U-Vo) application and modified Hoagland's solution (V-H) was in the same level within each cultivation and both of them increased from the 1st to the 2nd cultivation, while decreased from the 2nd to 3rd cultivation. Besides, the "unknown" part of nitrogen in urine treatments (U-Vo, U-2Vo and U-3Vo) for the 1st (51.01, 123.75 and 141.01 mg/pot, respectively), 2nd (29.16, 39.82 and 92.53mg/pot) and 3rd cultivation (99.61, 172.13 and 216.75mg/pot), were lower in the adequate urine conditions (U-Vo) compared to the extra urine cases (U-2Vo and U-3Vo). Similarly to nitrogen distribution in plant shoot, Na distribution amount (mg/pot) in Komatsuna shoot increased with increasing the application extra urine volumes after each cultivation time. The amount of Na remained in soil was greater in urine treatments U-3Vo (636.74, 683.07 and 699.93 mg/pot,) compared to U-2Vo (610.51, 625.29 and 608.93 mg/pot) and U-Vo (590.20, 593.72 and 595.60 mg/pot) after the 1st, 2nd and 3rd cultivations, respectively (Table 12, Table 13 and Table 14).

Table 4.12. Na distribution in the 1st cultivation period (N=5).

Na distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	508.47	508.47	508.47	508.47
Urine	33.05	66.10	99.15	0.00
Irrigation	53.78	56.69	55.23	55.23
Seeds	0.002	0.002	0.002	0.002
Total Input	595.30 (100%)	631.26 (100%)	662.86 (100%)	563.71 (100%)
Total output				
Shoot	33.90±3.02 (5.69%)	56.19±6.24 (8.90%)	60.04±6.80 (9.06%)	13.56±2.65 (2.41%)
Root	6.77±1.39 (1.14%)	7.16±3.70 (1.13%)	7.50±0.88 (0.98%)	5.21±0.37 (0.92%)
Soil-remained	590.20±39.10 (99.14%)	610.51±43.72 (96.71%)	636.74±25.38 (96.06%)	573.61±34.96 (101.76%)
Leached	0.22±0.04 (0.04%)	0.27±0.10 (0.04%)	0.18±0.07 (0.03%)	0.18±0.05 (0.03%)
"Unknown"	-35.01 (-6.01%)	-42.88 (-6.79%)	-40.62 (-6.13%)	-28.86 (-5.12%)

Original Soil means soil before the 1st time cultivation.

Table 4.13. Na distribution in the 2nd cultivation period (N=5).

Na distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	590.20	610.51	636.74	573.61
Urine	33.05	66.10	99.15	0.00
Irrigation	57.27	57.27	57.27	57.27
Seeds	0.002	0.002	0.002	0.002
Total input	680.52	733.88	793.17	630.88
	(100%)	(100%)	(100%)	(100%)
Total output				
Shoot	37.67±4.99	66.23±13.83	77.98±7.38	13.59±2.10
	(5.54%)	(9.02%)	(9.83%)	(2.15%)
Root	3.28±0.80	4.18±1.08	4.06±1.02	2.19±0.68
	(0.48%)	(0.57%)	(0.51%)	(0.35%)
Soil-remained	593.72±52.39	625.29±81.87	683.07±50.07	537.29±38.60
	(87.24%)	(85.20%)	(86.12%)	(85.17%)
Leached	0	0	0	0
	(0%)	(0%)	(0%)	(0%)
"Unknown"	45.85	38.18	28.06	77.81
	(6.74%)	(5.20%)	(3.54%)	(12.33%)

Original Soil means soil after the 1st time cultivation.

Table 4.14. Na distribution in the 3rd step cultivation (N=5).

Na distribution (mg/pot)	**U-Vo**	**U-2Vo**	**U-3Vo**	**V-H**
Input				
Original Soil	593.72	625.29	683.07	537.29
Urine	33.05	66.10	99.15	0.00
Irrigation	39.24	39.24	37.79	39.24
Seeds	0.002	0.002	0.002	0.002
Total Input	666.01	730.63	820.01	576.53
	(100%)	(100%)	(100%)	(100%)
Total output				
Shoot	14.89±3.14	23.28±8.95	24.10±11.27	8.71±1.97
	(2.24%)	(3.19%)	(2.93%)	(1.51%)
Root	1.60±0.37	1.55±0.39	1.33±1.12	0.90±0.26
	(0.24%)	(0.21%)	(0.16%)	(0.16%)
Soil-remained	595.60±48.45	608.93±33.25	699.93±39.38	481.55±26.80
	(89.43%)	(83.34%)	(85.21%)	(83.53%)
Leached	0.26±0.24	0.11±0.05	0.09±0.02	0.13±0.08
	(0.04%)	(0.02%)	(0.01%)	(0.02%)
"Unknown"	53.66	96.76	96.01	85.24
	(8.06%)	(13.24%)	(11.69%)	(14.79%)

Original Soil means soil after the 2nd time cultivation.

4.4. DISCUSSIONS

4.4.1. Effects of continuous application of extra urine volume on plant growth and dry biomass

In this present study the results show that continuous application of extra urine volume did not cause any depressed growth on Komatsuna height, shoot dry biomass and root dry biomass after three times cultivation. One reason might be difference in original soil characteristic. It has been reported a depressed growth of Spinach and Cabbage (both, salt moderately plant) dry biomasses at higher application rate of human urine (800 mg-N/Kg-soil level) which has been caused by salinity (EC=18.8 mS/cm) of the treated soil in comparison to the set control (EC=2 mS/cm) (Mnkeni et al., 2005). In the case of our study, Komatsuna, one family of Cabbage, is defined as salt moderately plant and the highest urine application volume (U-3Vo) in the original soil (EC=0.05 mS/cm) from the 1st to the 3rd cultivation was theoretically, 1500 mg-N/Kg soil level, which was higher than that applied by Mnkeni et al. (2005), and treated soil was far to be saline (EC=0.84 mS/cm) compared to the soil salinity rating based on EC (0-2 mS/cm: none saline soil; 2-4 mS/cm: slightly saline; 4-8 mS/cm: moderately saline; 8-16 mS/cm : strongly saline ; >16 mS/cm : very strongly saline) given by FAO (1998). Therefore, considering this difference in original soil characteristic, it was suggested that, application of extra human urine volume in saline soil might accelerate plant inhibition more strongly; and its application in non-saline soil may delay increase of soil EC and eventually attenuate the adverse effects of saline soil on plant growth. On the other hands, soil EC was obviously increased during three times cultivation in all urine treatments of this present study (Table 4.8). Therefore, some salts management plans such as leaching requirement or cultivation of clean up plants should be applied even in the case of low EC soil, when urine is continuously reused in several years scale. Another possible explanation for the none inhibition effects of Komatsuna plant fertilized with the continuous application of extra urine volume could be attributed to the difference in urine nitrogen concentration in both different trials of urine reuse. Because, Mnkeni et al. (2005) used source-separated storage urine, while in our experimental conditions, fresh synthetic urine was used; and it is well known that, in storage urine, nitrogen concentration is low due to ammonia volatilisation (Goosse et al. 2009; Udert and Wachter, 2012). In our study case, nitrogen concentration was high, since applied in urea form. However, in both trials, urine application was based on urine N value. Therefore, high volume of urine was probably applied in Mnkeni et al. (2005), which implies that a high amount Na and others salts were imputed in soil. Furthermore shoot dry matter produced in fertilized treatments after the 2nd cultivation step (spring season) were high compared to those obtained in the 1st and 3rd cultivation (both, summer season) and might be due to the difference in climatic conditions, especially the lowest temperature recorded in spring (Table 4.1). Similar responses of increase in Komatsuna dry weight was observed by Acikgo and Altintas (2011) when Komatsuna was cultivated in late winter-early spring (cold season) compared to late autumn-early winter (cool season) in greenhouse conditions. Besides, after three times cultivations, plants growth in all treatments decreased drastically (Table 5 and Figure 3) in comparison to the other cultivations period. One reason might be the high temperature (max=42°C) and solar radiation (max=25.1 MJ/m^2) in the 3rd cultivation time, since 1st and 3rd cultivation were both conducted in summer (same temperature range) and the only differences resided in solar radiation which was low in the 1st time cultivation (21.8 MJ/m^2) (Table 4.1). Bosque et al. (2005) reported a similar significant reduction in plant height and yield of piquin pepper plant in high solar radiation (39.5 MJ/m^2) and temperature (30°C).

4.4.2. Effects Na from continuous application of extra urine volume in plant and soil

In contrary to the obvious increased of soil EC, Na accumulation and SAR in soil were gradually increased from the 1st to the 3rd cultivation in this present study. The plants analysis showed that concentration of Na in plant shoots and roots (Table 4. 6 and Table 4. 7) and amount of Na uptake by plant (Table 4. 12, Table 4. 13 and Table 4. 14) increased with increasing the application of extra urine volume within each cultivation. The total amount of Na removed by plants from soil in all cultivation period including was amounting 98.11 (U-Vo), 158.59 (U-2Vo), 175.01 (U-3Vo) and 41.36 mg/pot (V-H), respectively. In case of U-2Vo and U-3Vo, particularly, the removal amount of Na reached more than 18 % of the total Na input (Original soil, seeds, urine and irrigation water) in pots from the 1st to 3rd cultivation; and considering only the total Na applied through urine and irrigation water, the removal amount of Na by plants from soil under three times cultivations reached more than 39 % in all urine treatments. Therefore, these amounts of Na removed by plant from soil, might probably contributed to mitigate Na accumulation in soil after three times cultivations with continuously application of extra urine volume in pots. In the other hand, it was observed a low uptake of K accompanied with a high uptake of Na in plant shoot for all urine treatments compared to modified Hoagland's solution after each of the cultivation step. This finding supports a result of low K^+ and high Na^+ uptake y reed beed in urine cultivation (Pradhan et al., 2010a), and high level of residual K^+ in urine treated soil after tomato cultivation (Pradhan et al., 2009b). This phenomenon might be due to the substitution of K^+ by Na^+, which is well known, as the primary effects of salts stressed plant to salinity and is also associated to low plant K/Na ratio (Alfocea et al., 1996; Gorham et al., 1997; Dasgan et al., 2002; Tuna et al., 2007). Besides, after each cultivation, Ca/Na ratio in Komatsuna shoot and root for all urine treatments were significantly lower than those obtained in V-H treatments. Interestingly, Tuna et al. (2007) have reported that high concentration of Na in the root zone inhibits uptake and transport of Ca, which causes subsequently salt stressed plant with a low Ca/Na ratio. In our study, the highest urine volumes applications conditions, especially U-3Vo, tended to lower K/Na and Ca/Na in comparison to others treatments during three times cultivations; but these ratios did not show any tendency in view point of continuous cultivation. Therefore, from these results, it was considered that plant stressed by Na was not accelerated under three times cultivations, with three times applications of extra urine volumes, even if, the SAR and Na accumulation in soil was gradually increased from the 1st to 3rd cultivation. Moreover, the results corroborate again (chapiter 3) that urine fertilization causes nutritional imbalance, since from the 1st to the 3rd cultivation, both ratios K/Na and Ca/Na in Komatsuna shoot and root were constantly high in the positive control (modified Hoagland's solution, V-H) in comparison to all urine treatments (U-Vo, U-2Vo and U-3Vo).

4.4.3. Effects of N from continuous application of extra urine volume in plant and soil

It was clearly observed that nitrogen concentration in plant shoot and root increased significantly in the highest urine dose applications after each cultivation compared to the adequate urine volume (U-Vo) and the positive control (V-H). Moreover, the concentration of nitrogen in Komatsuna shoot did not show any specific trend with continuous application of urine volume from the 1st to the 3rd cultivation (Table 4. 6 and Table 4. 7). Similar observations were also found in the total amount of nitrogen content in plant shoot (Table 4. 9; Table 4.10 and Table 4.11). Therefore, continuous application of urine volume did not accelerate N content in plant tissues, but its level was constantly high in the extra urine volume applications cases. The observed decreased of Komatsuna shoot N concentration in the 2nd cultivation period (spring season) of all fertilizer treatments compared to 1st and 3rd cultivation (both, summer season) might be associated to difference in climate conditions

(lowest temperatures, and highest solar radiation during the 2nd cultivation time) (Table 4.1), because nutrients contents in plants may be changed by the environment (Gent, 2002; Acikgo and Altintas, 2011). Moreover, it has been revealed that the difference in light intensities cause difference in plant transpiration rate and then resulting in different water and nitrogen absorption (Miyajima, 1994). Furthermore, from one cultivation to another, nitrogen concentration in remained soil for all fertilized treatments increased in the high rate of urine application in pots (Table 4. 8). The distribution of nitrogen shown also same tendency in cultivated soil through the different cultivations times (Table 4. 9, Table 4. 10, and Table 4. 11) with the high urine application treatments. Therefore, continuous application of excess amount of urine (U-2Vo and U-3Vo) caused an accumulation of nitrogen in soil under three times cultivations. Although, N in treated soil increased during three times cultivation, with the continuous application of extra urine volumes in pot, it was remarkably observed that the "Unknown" part of nitrogen was not increasing with the continuous cultivation (Table 4. 9, Table 4. 10 and Table 4. 11). Consequently, the N lost might be not associated to the effects of continuous application of extra urine in soil. Because, no specific tendency of N lost was clearly drawn out when considering the continuous cultivation pattern with continuous application of extra urine volumes in soil. Nevertheless N lost was constantly high in the highest urine application cases for all cultivation periods. These observations was likely to be related to the dissimilarities in climate conditions (Table 4.1) during the different cultivations period, since N lost was lower in the 2nd cultivation period (spring season, low temperatures) compared to the 1st and 3rd cultivation where both was conducted in same season (summer season, high temperatures).

4.4.4 Effects of pH from continuous application of extra urine volume in soil

It was observed that soil pH decreased in all urine treatments after the 2nd and 3rd cultivations, and this decrease was significantly faster in the highest urine applications rates (U-2Vo and U-3Vo) (Table 4.8). One explanation might be a high level of nitrate contents in the extra urine soil conditions after continuous cultivation of Komatsuna. It is widely known that nitrogen in urine is in urea form, which can be easily degraded by urease enzymes to ammonium and ammonia, and this process released one hydroxide ion (OH^-). Since our soil is well aerated, ammonium is nitrified (NO_3^-) by soil nitrifying bacteria and this reaction is accompanied by a release of two protons ions ($2H^+$). The uptake of nitrate by plant roots in the soil solution emits one hydroxide ion (OH^-). Globally, 2 protons and 2 hydroxides ions are released in soil, which mean the net effect of pH on the soil is small (Höglund, 2001). However, in the case of our experimental conditions, high urea concentration was applied in extra urine treatments soil and nitrate uptake by plant was a limited, since the uptake reached the plateau. Thereafter, no released of hydroxide ions from roots and more protons and/or nitrate remained in soil and subsequently causing soil acidification in extra urine treatments.

4.4.5. Effects of P from continuous application of extra urine volume in plant and soil

Continuous application of extra urine in the range of 2-3 times higher than plant requirement had no effect on phosphorus concentration in Komatsuna shoot and root after three times cultivations (Table 4.6 and Table 4.7). However similar observations were also found in soil after all cultivations step except in the 3rd one, where the highest urine application rate (U-3Vo) shown significant increased compared to others treatments (Table 8). These results confirm also the low level of phosphorus contents in human urine (Pradhan et al., 2009a, Sene et al., 2012) but long term used of urine may have potential to accumulate or improve soil phosphorus.

4.5. CONCLUSIONS

From this investigation, it was illustrated that continuous application of extra human urine volume in the range of 2-3 higher than plant requirement had no inhibition effect on Komatsuna growth, shoot dry biomass and root dry biomass after three times cultivations, since under three time cultivations, soil EC of all urine treated soils were lower than the soil salinity rating based on EC given by FAO (EC< 2mS/cm); and about 18% of the total amount of Na applied in the highest urine treatments (U-2Vo and U-3Vo) was removed by plant from soil, and the removal Na applied from urine and irrigation water reached more than 39 % in all urine fertilizer treatments. However, urine fertilization causes nutritional imbalance, but this phenomenon was not accelerated under three times cultivations with double (U-2Vo) and triple (U-3Vo) volumes urine application. Besides, N contents in plants tissues was not accelerated under three times cultivations, but its level was constantly high in the highest urine applications volumes. Furthermore, soil EC increased from the 1st to the 3rd cultivation in all set treatments and this increased was significantly high in the extra urine volumes application. Contrast to EC, pH decreased in extra urine conditions and might probably due to a high level of nitrate contents soil conditions after continuous cultivation. Moreover, continuous application of excess amount of urine (U-2Vo and U-3Vo) accumulated nitrogen and sodium in soil; while adequate urine volume (U-Vo) application causes no accumulation of N soil, but more than 60 % of Na applied through urine and irrigation water remained in soil after three times cultivations. Therefore, from these results we suggest that application of human urine volume should be based on plant N requirement, and management of salts from urine is required even in the adequate urine volume application for its sustainable reuse in agriculture.

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Chapter 5

CONCLUSION AND RECOMMENDATION OF HUMAN URINE REUSE IN AGRICULTURE

From this present research it is highlighted that, the following considerations concerning the proper use of human urine for a sustainable agricultural production should be adopted by farmers to promote green growth

When and how often human urine should be applied in agriculture?

- Human urine application in one time before seedling is not effective for plant growth and production due to mainly high ions intensity in low saline soil and high leached of nutrients from soil (N= 38.3 %, P= 9.1 %).

- Phosphorus (P) in human urine was palpably low in this research work (N/P ratio > 16 in Spinach shoots of all set urine treatments). Therefore, human urine controls with P-fertilizer such as industrial P_20_5 or P supplied through compost or ash might be a better way to promote sustainable agricultural production, especially for plant with high P demand.

How much urine should be applied in agriculture?

- Application of extra human urine in the range of 2-3 times higher than plant requirement does not affect plant growth and production in one time cultivation, since treated soils were far to be saline when considering the references values given by FAO, 1998 (EC<2mS/cm; SAR< 13).

- Human urine application causes nutritional imbalance (substitution of K^+ by Na^+, indicated by low K/Na ratio in plant), which is severe in double and triple volumes urine application (U-2Vo and U-3Vo).

- Application of excess amount of urine (U-2Vo and U-3Vo) in agricultural soil as a liquid fertilizer increases nitrogen content in plants tissues (> 35 % of input N moved to plant shoot). Therefore, particular attention should be taken concerning the high nitrogen contents in plant, when excess of urine is applied in arable land, due to eventual public heath issues.

- High rate of urine application (U-3Vo) caused an accumulation of nitrogen and sodium in soil and promoted high N lost (>24 %) from soil. While, adequate urine application (U-Vo) caused a low N lost (<18%) from soil and no accumulation of N was observed, but more than 50 % of the total sodium applied through urine and irrigation water remained in soil after one time cultivation.

- Thereafter, application of adequate human urine volume based on plant requirement might be a better option for its sustainable reuse in agriculture as liquid fertilizer.

How do continuous applications of human urine volume affect plant growth and soil characteristics?

- Continuous application of extra human urine in the range of 2-3 times higher than plant requirement had no adverse effects on plant growth and production after three times cultivations, because under three times cultivations, treated soils EC were still in the none soil saline zone [0-2 mS/cm] given by FAO, 1998; And more than 40 % of the total Na applied through urine and irrigation water was removed by plant from soil in all urine treatments and might contributed to mitigate Na ion accumulation in soil.

- Continuous applications of double and triple volumes of urine increase nitrogen content in plant tissues and decrease plant K/Na ratio.

- Accumulation of sodium (Na) and nitrogen (N) in soil occurred in triple volumes urine (U-3Vo) application.

- N lost from soil was not accelerated with continuous application of extra urine volume after 3 times cultivations, but was constantly high in extra urine (U-2Vo and U-3Vo) application volumes

- Continuous applications of urine increased soil EC, which was faster in excess amount of urine applications (U-2Vo and U-3Vo).

- Continuous applications of urine decreased soil pH, which was more pronounced in double and triple volumes of urine application.

- Therefore, adequate application of human urine based on nitrogen (N) requirement is a suitable way for its sustainable reuse in agriculture, since no accumulation of nitrogen in soil and no increase of N in plant tissues, but plant K/Na ratio decreases compared to the positive control, modified Hoagland's solution (V-H).

- Additionally to the increased of soil EC in all urine treatments including, about 60 % of the total Na applied through urine and irrigation water when considering 1^{st}, 2^{nd} and 3^{rd} cultivation, remained in soil in urine treatments (U-Vo, U-2Vo and U-3Vo). Therefore, management of salts from urine is required even in the adequate urine volume application when urine is continuously used in the farmland as a liquid fertilizer.

APPENDIX I: Composition of Modified Hoagland's Solution

Table 1.1. Modified Hoagland's Solution (Plant Nutrient Solution)

Component	*Stock solution*	*mL stock solution /1 L*
2M KNO_3	202.2 g/L	2.5 mL
2M $Ca(NO_3)_2 \times 4H_2O$	236g/0.5L	2.5
$Fe(SO_4) \times 7H_2O$	15g/L	1.5
2M $MgSO_4 \times 7H_2O$	493g/L	1
1M NH_4NO_3	80g/L	1
Minors		
1M KH_2PO_4	136g/L	0.5

1) Make up stock solutions and store in separate bottles with appropriate label.
2) Add each component to 800mL deionized water then fill to 1L.
3) After the solution is mixed, it is ready to water plants.

In this research we compared 2 liquids fertilizer synthetic urine with modified Hoagland's solution without minors cations except KH_2PO_4 because synthetic urine does not contents the exclude minors.

APPENDIX II: Effects of continuous application of extra human urine volumes on plant and soil - Phosphorus balance after 1st cultivation step, 2nd cultivation step and 3rd cultivations step:

Table 2. 1. Phosphorus distribution in the 1st cultivation (N=5)

P distribution (mg/pot)	U-Vo	U-2V0	U-3V0	V-H
Original Soil-0	236.21	236.21	236.21	236.21
Total Input	11.51	22.64	33.75	2.74
Input + Soil-0	247.72 (100%)	258.85 (100%)	269.96 (100%)	238.95 (100%)
Total output				
Shoot	1.59±0.51 (0.64%)	2.18±0.75 (0.84%)	2.85±0.85 (1.06%)	1.34±0.41 (0.56%)
Root	0.25±0.10 (0.10%)	0.23±0.12 (0.09%)	0.40±0.15 (0.15%)	0.27±0.13 (0.11%)
Soil-reminded	205.97±31.26 (83.15%)	214.03±12.00 (82.69%)	245.97±45.91 (91.11%)	205.01±24.70 (85.80%)
Leached	0.01±0.003 (0.004%)	0.015±0.005 (0.006%)	0.012±0.003 (0.004%)	0.016±0.002 (0.007%)
"Unknown"	39.91 (16.11%)	42.39 (16.38%)	20.73 (7.68%)	32.31 (13.52%)

Soil-0 means soil before 1st cultivation; Total input means Na input from urine plus Na input from irrigation water plus Na input from Komatsuna seed.

Table 2. 2. Phosphorus distribution in 2nd cultivation (N=5).

P distribution (mg/pot)	**U-V0**	**U-2V0**	**U-3V0**	**V-H**
Soil-1	205.97	214.03	245.97	205.01
Total Input	11.53	22.65	33.77	2.76
Input + Soil-1	217.50 (100%)	236.68 (100%)	279.74 (100%)	207.77 (100%)
Total output				
Shoot	1.54±0.64 (0.71%)	1.51±0.88 (0.64%)	2.71±1.32 (0.97%)	1.34±0.43 (0.64%)
Root	0.24±0.13 (0.11%)	0.33±0.11 (0.14%)	0.32±0.17 (0.11%)	0.20±0.13 (0.10%)
Soil-reminded	336.45±24.82 (154.69%)	343.83±29.99 (145.27%)	338.88±25.33 (121.14%)	299.52±45.19 (144.16%)
Leached	0 (0%)	0 (0%)	0 (0%)	0 (0%)
"Unknown"	-120.73	-108.99	-62.17	-62.17

Soil-1 means soil after 1st cultivation; Total input means P input from fertilizer plus P input from irrigation water plus P input from Komatsuna seed.

Table 2.3. Phosphorus distribution in 3rd cultivation (N=5)

P distribution (mg/pot)	U-V0	U-2V0	U-3V0	V-H
Soil-2	336.45	343.83	338.88	299.52
Total Input	12.05	23.18	34.29	3.28
Input + Soil-2	348.50 (100%)	367.01 (100%)	373.17 (100%)	302.80 (100%)
Total output				
Shoot	0.66±0.33 (0.19%)	0.87±0.52 (0.24%)	0.44±0.34 (0.12%)	0.67±0.21 (0.22%)
Root	0.15±0.04 (0.04%)	0.14±0.05 (0.04%)	0.11±0.11 (0.03%)	0.12±0.08 (0.04%)
Soil-reminded	333.30±18.45 (95.64%)	335.66±11.61 (91.46%)	405.49±13.44 (108.66%)	296.08±45.65 (97.78%)
Leached	0.01 (0%)	0.01 (0%)	0.00 (0%)	0.01 (0%)
"Unknown"	14.38 (4.13%)	30.33 (8.27%)	-32.87 (-8.81%)	5.92 (1.96%)

Soil-2 means soil after 2nd cultivation; Total input means P input from fertilizer plus P input from irrigation water plus P input from Komatsuna seed.Amount of Ca , Mg removed by plant shoot in the 1st , 2nd and 3rd cultivation