



Full Length Research Paper

Effects of extra human urine volume application in plant and soil

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Abstract

Although it has been known that human urine could be reused as nutrients for plant, too much application may cause an accumulation of sodium (Na) and/or nitrogen (N) in soil and eventually inhibit plant growth. A study was carried out to evaluate the effects of extra urine application in plant and soil. Thus, pot test was conducted in greenhouse using synthetic urine, Komatsuna and sandy soil. 34.86 ml of synthetic urine (U-V₀) which contained 140 mg-N and corresponded to N-based standard requirement for Komatsuna, 69.72 ml of synthetic urine (U-2V₀), 104.58 ml of synthetic urine (U-3V₀), 58.82 ml of modified Hoagland solution (V-H) which contained 140 mg-N as positive control and non-fertilizer (C) were applied into different pots (1/10000a). The results indicate that U-2V₀ and U-3V₀ had no inhibition effect in plant height, shoot and root dry biomasses after one time cultivation, since low amount of Na was applied through urine in soil and Komatsuna is salt moderately plant. However, low K/Na ratio in plant shoot and root, defined as a primary salt stress, was observed in all urine treatments and this phenomenon was especially accentuated in U-3V₀. Furthermore, the “unknown-N” calculated from N-balance before and after cultivation could be N-volatilization from pot and was higher in U-2V₀ (28.75%) and U-3V₀ (24.37%) compared to U-V₀ (17.1%). The results suggest that, application of adequate human urine volume based on plant N requirement is a better option for its reuse.

Keywords: Urine reuse, adverse effect, salt, stress, nitrogen lost, Komatsuna.

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

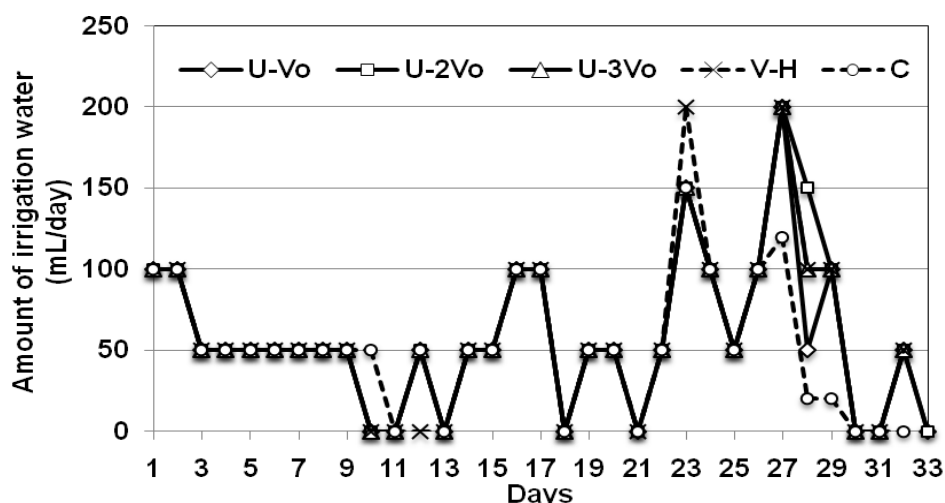


Figure1. Change in time course of irrigation water applied in pot during Komatsuna cultivation in sandy soil using 3 different urines Volumes treatments (U-Vo, U-2Vo and U-3Vo), 1 modified Hoagland's solution (V-H) and 1 non fertilizer

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.

MATERIALS AND METHOD

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/m² 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). Washed sandy soil mixed with small gravels (6:3 soils: 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 (CO₃)₂ in each pot as recommended by Fujiwara and Narimatsu (2006). The physical and chemical characteristics of the soil are shown in the Table 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.

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

Table 1. Physical and chemical properties of bulk soil before cultivation

Parameters	Sandy soil
pH	7.50±0.09
EC ($\mu\text{S}/\text{cm}$)	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 \pm standard deviation (SD) with 5 replications; "Na" means total sodium extracted with nitrate-perchloric acid digestion

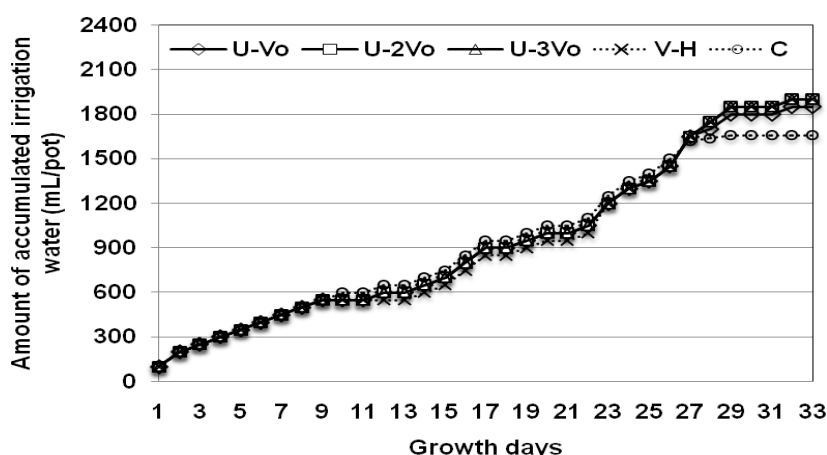


Figure 2. Total amount of irrigation water accumulated in pot (0.84 Kg-soil) during Komatsuna cultivation using 3 different urine treatments (U-Vo, U-2Vo and U-3Vo) and 1 modified Hoagland's solution (V-H) and 1 Nonfertilizer (C)

of synthetic urine and the pot system size, diluted (1/3) synthetic urine volume was designed as U-V₀ (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). To know the effects of extra human urine volume on plant and soil, 3 different urine treatments volumes [U-V₀, U-2V₀ (NPK 280-22.24-47.46, Na 66.1 mg/pot) and U-3V₀ (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 non fertilizer 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 KH₂PO₄) 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).

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 ($K_c=0.60$) and the potential evapotranspiration ($E_{To}=4.1$ mm/day) and the pot size system (1/10000a). The E_{To} value was computed by using E_{To} 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 1 and Figure 2). 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

Table 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-V ₀	1.85	8.66	0.30	53.78
U-2V ₀	1.95	9.13	0.31	56.79
U-3V ₀	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-V₀, urine volume V₀ applied; U-2V₀, urine volume 2V₀ applied; U-3V₀, urine volume 3V₀ applied; V-H, modified Hoagland's solution applied; C, control

Table 3. Total leached water volume and total amount of N, P and Na derived from leached water during pot experiment

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-V₀, urine volume V₀ applied; U-2V₀, urine volume 2V₀ applied; U-3V₀, urine volume 3V₀ applied; V-H, modified Hoagland's solution applied; C, control

with a plastic shower head which had allowed spraying water uniformly inside the pot. The total volumes of water applied in pot during the whole cultivation period in each treatment is presented in the Figure were 1.85 (U-V₀), 1.95 (U-2V₀), 1.90 (U-3V₀), 1.90 (U-V₀), and 1.66 L (C). In contrast, 51.4 (U-V₀), 50.56 (U-2V₀), 49.16 (U-3V₀), 50.46 (V-H) and 70.47 mL (C) were leached from experimental pots. The all leached water was collected from the external compartment and then analysed the chemical parameters (chemical contents in applied irrigation water and leached from pot are shown Table 2 and Table 3, respectively). 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, Komatsuna shoots and roots were separately harvested. The fresh samples were lyophilized and weighted as dry weight and then stored at -30 °C.

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 digestion. 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 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 µm pore size, mixed cellulosic ester, ADVANTEC, Japan).

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

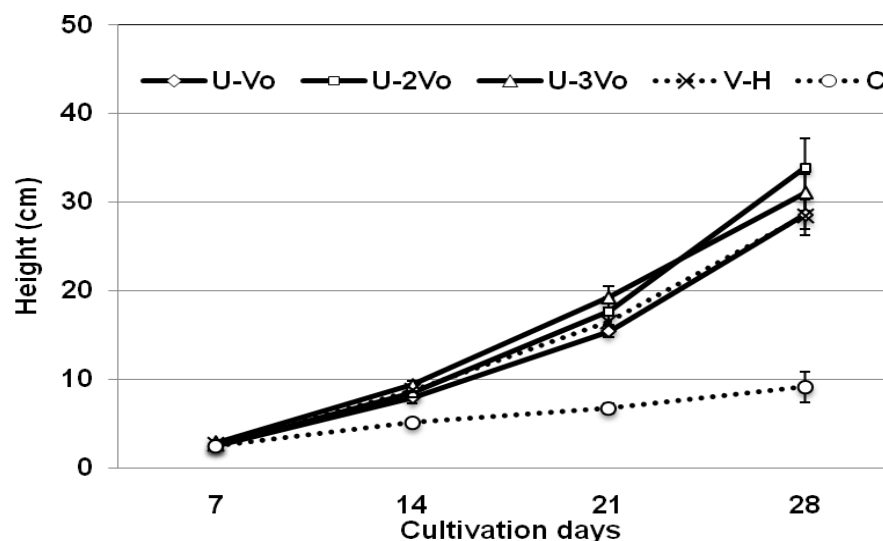


Figure 3. Change of Komatsuna height during cultivation in sandy soil using 3 different urine treatments (U-V₀, U-2V₀ and U-3V₀), 1 modified Hoagland's solution (V-H) and 1 non fertilizer (C)

Table 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-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
C	0.34±0.21b	0.16±0.10b

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

determine the differences among treatments. In the case of none parametric data, Kruskal-Wallis test was conducted to confirm the significant difference.

RESULTS

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-V₀, U-2V₀ and U-3V₀ 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). Similarly to plant height, shoot dry weight (g/pot) obtained after cultivation (Table 4) showed that urine treatments U-V₀, U-2V₀ and U-3V₀ 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".

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 5 and Table 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-2V₀ and U-3V₀ (both in same level) than U-V₀ treatment. In the case of phosphorus, no significant difference was observed in plant shoot and root for urine treatments U-V₀, U-2V₀ and U-3V₀. Na concentrations in

Table 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-V ₀	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-2V ₀	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-3V ₀	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 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

Treatment	Root						
	N	P	Na	Ca	Mg	K	K/Na
U-V ₀	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-2V ₀	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-3V ₀	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)

Table 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 values
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)

Komatsuna shoot and root were significantly lower in treatment U-V₀ than others urine conditions (U-2V₀ and U-3V₀, 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-2V₀ and U-3V₀ (both in same level) were statistically lower than U-V₀; 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-2V₀ and U-3V₀ decreased

significantly in comparison with the basic urine volume requirement to cultivate Komatsuna, U-V₀. Nitrogen concentration in cultivated soil was statistically higher in treatment U-3V₀ than U-V₀, U-2V₀ and V-H, which were in the same level (Table 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-3V₀ were significantly higher than others conditions.

Table 8. Nitrogen distribution in each treatment after cultivation (N=5)

N distribution (mg/pot)	U-V₀	U-2V₀	U-3V₀	V-H
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). Total input means existing amount of N from Original sandy soil (148.90±9.73mg/pot) plus N applied through fertilizer, plus N from irrigation water and N from Komatsuna seeds (0.74±0.01mg/pot)

Table 9. Sodium distribution in each treatment after cultivation (N=5)

Na distribution (mg/pot)	U-V₀	U-2V₀	U-3V₀	V-H
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). Total input means existing amount of Na in original sandy soil (508.47±32.85mg/pot) plus Na applied through urine and plus Na applied through irrigation water. In V-H, no Na was applied in pot

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 8. The distribution of nitrogen showed that plants nitrogen shoot in urine treatments U-V₀ (33.81%), U-2V₀ (35.90%) and U-3V₀ (41.62%) increased with increasing urine volume application in the experimental pot. The “Unknown” parts

of nitrogen in urine treatment U-2V₀ (28.20%) and U-3V₀ (24.37%) were higher compared to the basic urine volume application, U-V₀ (17.10%). Similarly to nitrogen distribution percentage in plant shoot, Na distribution ratio in shoot increased with increasing urine volume application in soil (Table 9). However, more than 96% of Na amount applied in pot remained in soil after cultivation. The “unknown” parts of Na for all treatment showed negative values, which were included in their

respective error bars.

DISCUSSION

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-3V₀) 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-V₀, U-2V₀ and U-3V₀ were far to be saline (Table 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.

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-3V₀) (Table 5, Table 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 7). The SAR value increased with the increasing urine volume application and was significantly higher in U-3V₀ than U-V₀ and U-2V₀. Thus, Komatsuna plants fertilized with urine were moderately stressed and this stress was acute where a high volume of urine (U-3V₀) 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 8). 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.

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 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 remarkably observed that the "Unknown" part of nitrogen 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-2V₀ and U-3V₀) and our soil pH (Table 7), it was suggested that extra urine volume application in soil (U-2V₀ and U-3V₀) is not adequate way and application of urine volume based on the plant requirement (U-V₀) 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).

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 urine volume application. This might be due to the low phosphorus contents in human urine (Pradhan et al., 2009; Sene et al., 2012).

CONCLUSIONS

From these results, it was concluded that 2-3 times higher application of human urine volume than plant requirement had no inhibition effects on Komatsuna growth, shoot biomass and root biomass in one time cultivation, since less amount of Na was input from urine in cultivation of Komatsuna salt moderately plant. However, urine uses as fertilizer in one time cultivation cause primary salt stress for plant (substitution uptake of K⁺ by Na⁺), which is more accentuated when 2-3 times extra urine volume is applied to soil. Besides continuous cultivation with excess application of urine has a potential to affect negatively plant growth, since more than 96 % of sodium applied in pots was remained in soil after one time cultivation. Furthermore, the extra applications of urine cause nitrogen loss from soil. Therefore, we suggest that it is better to apply an adequate amount of urine based on plant nitrogen requirement.

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