

# Adequate human urine application pattern for agriculture

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The objective of this study was to evaluate when and how often human urine should be applied in soil as fertilizer solution. Thus, pot test using spinach and synthetic urine was conducted in greenhouse. Seven treatments, those apply same amount of urine in different pattern and control (total 8 cases) were set to know adequate urine application way. Plant height at 35 cultivation days and fresh and dry weight of plant after harvest were significantly lower in 100% urine applied in one time before seedling (a) and control (h) than no urine applied before seedling and then every 2 days application after outbreak of plants (g). Moreover, phosphorus level content in treatment (g) ( $1.6 \pm 0.2$  mg/g plant DW) was statistically higher than treatment (a) and control (h), both same level ( $0.5 \text{ mg/g plant DW}$ ). The N/P ratio in (a) ( $95.2 \pm 24.7$ ) was significantly different than (g) ( $28.4 \pm 3.5$ ). However, in all treatments was observed N/P > 16, which means phosphorus limitation. Therefore one possible reason for the low growth in (a) might be the availability of phosphorus. Besides, leached nitrogen in (a) (38.3 %) was higher than others treatments. Hence another possible reason might be leached nitrogen. Our results shows clearly that urine application at one time before seedling is not effective for plant growth. Furthermore urine application at frequent intervals might be a better way to promote plant growth.

**Keywords:** Human urine, pattern, fertilizer effect, *Spinacea oleracea*.

## INTRODUCTION

On site wastewater differentiable treatment is a promising decentralized treatment 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 likewise nitrogen,

phosphorus and potassium and has a potential to be reuse in agriculture (Krichmann et al., 1995; Höglund, 2001; Dagerskog et al., 2010). Many 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; 2010a, 2010b). 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

**Table 1.** Sandy soil Properties and Nutrients Contents before Cultivation

parameter	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.

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.

## MATERIALS AND METHOD

### 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 is low organic matter, was used as media to analyse nutrient (nitrogen and phosphorus) distribution in plant, soil and leached water. Physical and chemical properties of the soil were showed in Table1 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. 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 design to 29 ml 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.

### Urine Application Ways

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

### Growth and Harvesting

The cultivation period of spinach was designed at 70 days

**Table 2.** Soil Properties after Cultivation, N = 3 in each treatment

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

Arithmetic mean values  $\pm$  standard deviation (SD)

after sowing likewise Nishihara et al. (2001). However the duration was shorten 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  $-30^{\circ}\text{C}$ . Chemical properties (pH and electrical conductivity (EC)) in soil after harvest were showed in Table 2.

### Soil, Plant and Leached Water Analysis

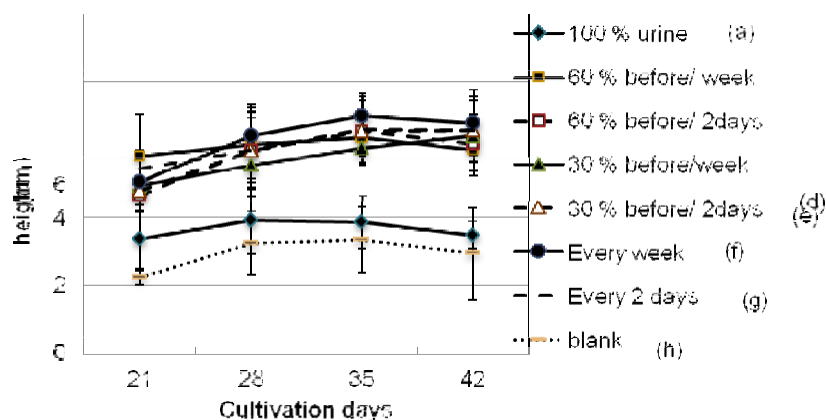
Total nitrogen and total carbon in soil was determined by Sumigraph NC-220F (Sumika Chemical Analysis Service, Japan) apparatus. Regarding total phosphorus in soil, soil samples were digested with nitrate-perchloric acid digestion method and then measured by ascorbic acid method using spectrophotometer 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 phos-

phorus 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.

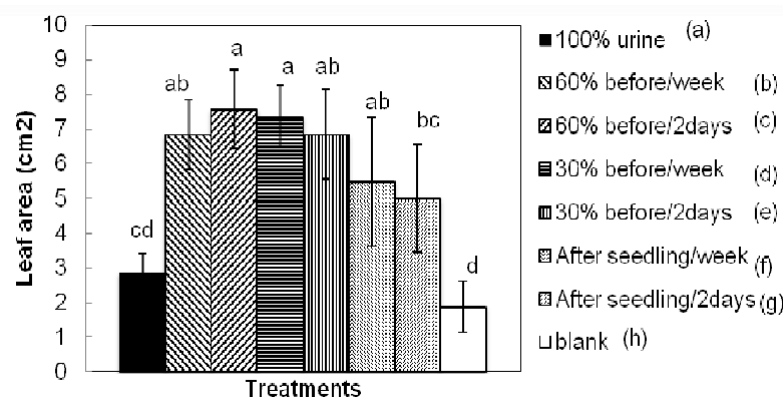
## RESULTS

### Plant Growth

Weekly plant height measurement is represented in the figure 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)  $3.9 \pm 0.8$ , (b)  $6.4 \pm 0.6$ , (c)  $6.6 \pm 0.9$ , (d)  $6.1 \pm 1.4$ , (e)  $6.6 \pm 1.0$ , (f)  $7.0 \pm 0.6$ , (g)  $6.6 \pm 1.0$  and (h)  $3.3 \pm 1.0$  cm, respectively. Urine application at one time before seeding (a) was same level with control (h) and significantly lower than (b), (c), (d), (e), (f) and (g) (Turkey-Kramer test,  $p < 0.05$ ). Similarly to plant height, leave areas after cultivation in (a) was 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). Their values were (a)  $2.9 \pm 0.6$ , (b)  $6.8 \pm 1.0$ , (c)  $7.6 \pm 1.1$ , (d)  $7.4 \pm 0.9$ , (e)  $6.8 \pm 1.3$ , (f)  $5.5 \pm 1.9$ , (g)  $5.0 \pm 1.6$  and (h)  $1.9 \pm 0.7$   $\text{cm}^2$ , respectively. Furthermore, Shoot dry weight of (a) was same level with that of control (h) and significantly lighter than that of (c) and (g) (Turkey-Kramer test,  $p < 0.05$ ) (Table 3). From these results, the low growth in the case of 100% urine applied



**Figure 1.** Growth rate of spinach plant height from different way of urine application in sandy soil (arithmetic means  $\pm$  SD); N=4 in each treatment



**Figure 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  $\pm$  SD); different alphabetical letter showed significant difference (Turkey-Kramer method,  $p < 0.05$ )

at one time before seedling (a) was clearly observed. Besides, there is tendency that frequent urine application provided high growth of plant shoot.

### Nutrients Effects

To confirm nutrient effect in all treatment, nitrogen and phosphorus content in shoot part was measured (Table 4). In the case of nitrogen, no significant difference was

observed during urine applied treatment (a)-(g), although control (h) ( $35.3 \pm 1.3$  mg/g plant DW) was significantly lower level than (a), (d), (f) and (g) ( $46.9 \pm 2.9$ ,  $46.1 \pm 2.5$ ,  $47.6 \pm 1.2$  and  $46.5 \pm 5.6$  mg/g plant DW, respectively) (Turkey-Kramer test,  $p < 0.05$ ). In the case of phosphorus, on the other hands, the content of (a) ( $0.5 \pm 0.1$  mg/g plant DW) was same level with control and significantly lower than that of (g) ( $1.6 \pm 0.2$  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

**Table 3.** Fresh and Dry weight of spinach (g/pot) from different urine treatments

Treatment	Shoot		Root	
	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  $\pm$  standard deviation (SD); different alphabetical letter showed significant difference (Turkey-Kramer test,  $p < 0.05$ )

**Table 4.** Concentration of nitrogen and phosphorus in shoots part of spinach after cultivation

Treatment	N	P	N/P
	(mg/g plant DW)	(mg/g plant DW)	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  $\pm$  SD; different alphabetical letter showed significant difference (Turkey-Kramer test,  $p < 0.05$ )

treatment (Table 4). The value of (a) (95.2±24.7) was significantly higher than that of (g) (28.4±3.5) (Turkey-Kramer test,  $p < 0.05$ ).

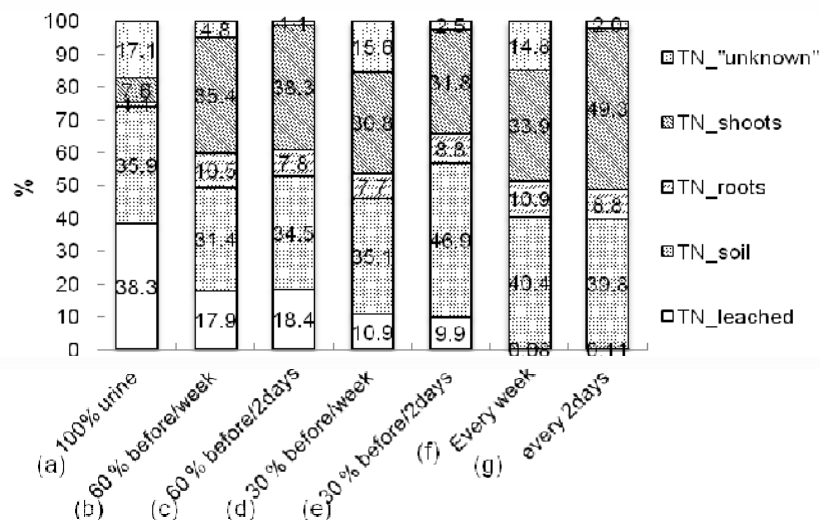
### Nutrients Distribution

Nitrogen distribution in shoot of spinach, root of spinach, soil and leached water is given in figure 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 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

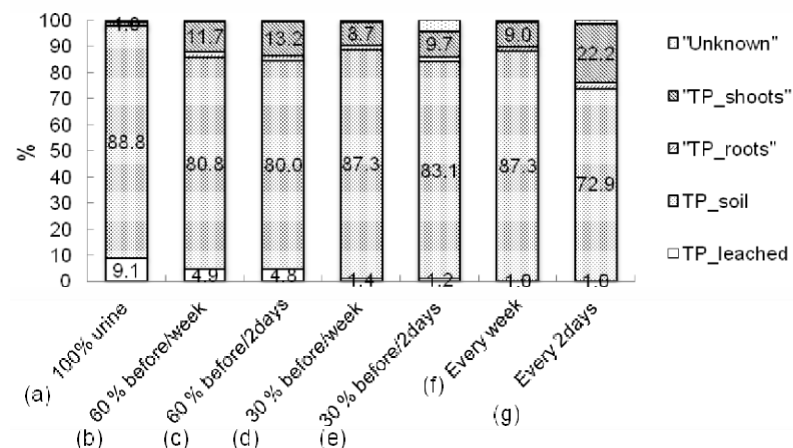
distribution ratio of shoot part in (g) was higher (22.2%) and the ratio of leached water was lower (1.0%). In all treatments, about 80% of phosphorus was reminded in soil.

### DISCUSSION

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 ( $N/P = 95.2 \pm 24.7$ ) and that in (g) was moderate ( $N/P = 28.4 \pm 3.5$ ). This limitation leads us to consider that



**Figure 3.** Nitrogen balance in each urine treatment after cultivation (N= 3 pots per treatment).



**Figure 4.** Phosphorus balance (%) in each urine treatment after cultivation (N= 3 pots per treatment)

phosphorus availability might affect spinach growth in the present study. This consideration was also supported by a correlation between phosphorus content in shoot (Table 4) and shoot dry weight (Table 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). Therefore, it was considered that frequent urine application such as treatment (g) (every 2 days urine application with no application before

cultivation) causes high phosphorus uptake by spinach, since available phosphorus was supplied frequently.

In the present study, it was observed that leached nitrogen was high ratio in 100% urine applied at one time before seeding (a). This phenomenon was also considered as one possible reason of low growth 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 an agreement with previous studies used other vegetables (Heinonen-Tanski et al., 2007; Pradhan et al., 2009), which showed that phosphorus in urine is low compared to chemical fertilizer. In urine reuse for agriculture, therefore, it is better to supplement with ash (Heinonen-Tanski et al., 2007) or use with compost (Hijikata et al., 2011) that have the ability to supply phosphorus and soil modification.

## CONCLUSION

From this investigation, it was concluded that urine application at one time before seedling gave low spinach growth, hence low phosphorus availability and high leached nitrogen. Furthermore, no urine applied before seedling and every 2 days after outbreak of the plant showed well growth but also high uptake of Nitrogen and Phosphorus. Thereafter, human urine applied with 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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