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Use of Human Urine Fertilizer in Cultivation of Cabbage (*Brassica oleracea*)—Impacts on Chemical, Microbial, and Flavor Quality

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Human urine was used as a fertilizer in cabbage cultivation and compared with industrial fertilizer and nonfertilizer treatments. Urine achieved equal fertilizer value to industrial fertilizer when both were used at a dose of 180 kg N/ha. Growth, biomass, and levels of chloride were slightly higher in urine-fertilized cabbage than with industrial-fertilized cabbage but clearly differed from nonfertilized. Insect damage was lower in urine-fertilized than in industrial-fertilized plots but more extensive than in nonfertilized plots. Microbiological quality of urine-fertilized cabbage and sauerkraut made from the cabbage was similar to that in the other fertilized cabbages. Furthermore, the level of glucosinolates and the taste of sauerkrauts were similar in cabbages from all three fertilization treatments. Our results show that human urine could be used as a fertilizer for cabbage and does not pose any significant hygienic threats or leave any distinctive flavor in food products.

KEYWORDS: Cabbage; fertilizer; glucosinolates; nitrogen; sanitation; taste; urine

1. INTRODUCTION

Human urine is a natural resource produced by every household. In human excreta, urine contains mostly nitrogen (N), phosphorus (P), and potassium (K) (*1*). Urine has a fertilizer value of N/P/K 18:2:5 (*2*), and for urine mixed with flush water, the ratio can be N/P/K/S 15:1:3:1 (*3*). Each individual produces 1–1.5 L of urine per day, the chemical composition of which depends on his/her feeding habits, the amount of drinking water consumed, physical activities, body size, and environmental factors (*4*). In general, pure human urine contains very few enteric microorganisms (*5*). The nutrient content present in human urine may mean it can be a good fertilizer for plants. This may be increasingly important in the future, with population growth and the corresponding increased demand for food and demand to save water and energy.

Urine is produced after filtration of the blood in the kidneys, and therefore, it contains low-molecular-weight compounds, since proteins are not filtered. About 75–90% of N is excreted as urea, the remainder being in the form of either ammonium or creatinine (*6*). Urea is rapidly degraded by urease to ammonium and water, which may elevate pH values up to a pH of 9; this can also reduce the bacterial population, although ammonia evaporation is also higher at higher pH values.

The urea/ammonium in urine and urea/ammonium in artificial fertilizers are similar; that is, 90–100% of urine N is in the form of either urea or ammonium, as has been verified in fertilizing experiments (*7, 8*). The P and K contents in urine are almost totally (95–100%) in an inorganic form (*6*). These ions are directly plant-available; for example, the phosphate plant availability from urine has been demonstrated to be as good as that of chemical phosphate (*7*). Urine has been successfully used to fertilize barley (*8*) and cucumbers (*5*). The growth and yields were as good as could be obtained with mineral fertilization. The use of urine fertilizer is not very common, but it is increasing in some areas of Finland.

The main objectives of this study were to evaluate the use of urine fertilizer on (1) growth and pest-resistance of a crop plant, (2) chemical and microbial quality of the crop, and (3) flavor quality of a vegetable food product prepared with natural lactic acid fermentation. We wanted to compare the effects of fertilization with urine and conventional mineral fertilization on these properties. Cabbage was selected for this work because of its worldwide distribution and its high N requirement. Cabbage can be cultivated in domestic gardens, and it can be preserved by turning it into sauerkraut. Leaves can also be used for animal fodder. The leaves of the cabbage lie close to the ground and therefore microbial contamination can be a major problem (*9*).

Glucosinolates (GLSs) were selected for the evaluation of chemical flavor compounds. These are sulphur- and glucose-containing compounds mainly found in plants of the *Brassicaceae* family. GLSs are divided into three groups according to the amino acid from which they are derived: aromatic, indolyl, and alkenyl GLS. The chemical structure of GLSs can vary,

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Table 1. Applied Amounts of Nutrients/Plant during Whole Cultivation Time

applied amount of nutrients/plants	industrial fertilizer (g)	urine fertilizer (L)
N	10.2	10.2
P	6.8	0.7
K	19.2	3.9
total fertilizer applied	113	11

but they always possess a sugar compound, sulfur, and nitrogen (*10*). The breakdown products of GLS are biologically active compounds that may be toxic or volatile; therefore, these compounds can influence crop flavor and human health, as well as pest populations. The glucosinolate contents in cabbage have been found to be influenced by different fertilizer treatments (*11*), and therefore, this study also compared the glucosinolate contents of cabbage fertilized with urine and with other fertilizer treatments.

2. MATERIALS AND METHODS

2.1. Plant Materials and Plantation. Cabbage (*Brassica oleracea* L., var. Castello F1) commercial seedlings were planted in the research garden of the University of Kuopio (62.9° N and 27.7° E) on May 23, 2006. The total cultivation area was 63 m^2. The area was divided into 12 plots measuring 4.5 m^2 each. A 25 cm (total 9 m^2) narrow protective strip was used between each of the treatment plots. The soil of the cultivated plots was inorganic silty clay loam, and the area was used as a grass field in the previous year. The plot experiment was designed as a Latin square model for three different treatments: urine fertilizer, industrial fertilizer, and no fertilizer, all with four replicates. Eight cabbage plants were cultivated in each plot in a zigzag form with the plants situated 60 cm from each other. Each plot had two rows, four plants situated in each row, and the distance between two rows was about 55 cm.

2.2. Fertilizer Treatments. Urine-fertilized and mineral-fertilized plots were fertilized as recommended for cabbage by Oregon State University (*12*) at a dose of 180 kg N/ha (**Table 1**). Urine fertilizer (0.93:0.063:0.36 N/P/K) was arranged so that the applications were on the following cultivations days: 7, 29, 40, 50, 64, and 71, with the volume of urine as 1.4, 1.6, 2.0, 2.0, 2.0, and 1.9 L/plant, respectively. The urine was applied with a measuring beaker and sprinkled about 30 cm around the plant. The soil surface was scratched before and after application of the urine fertilizer so that the liquid could be better absorbed.

In a similar manner, commercial (here called industrial) fertilizer [Puutarhan Y1, 9:6:17 N/P/K, which also contained Ca (1%), Mg (2%), S (10%), Cu (0.1%), Fe (0.1%), Mn (0.7%), Mo (0.01%), Zn (0.1%), and Se (0.001%)] was applied on days 7, 40, 50, 64, and 71 with the provided doses being 37.5, 15, 20, 20, and 20 g/plant, respectively. Industrial fertilizer was applied about 30 cm around the plants and mixed by scratching the soil.

Initially, the soil pH was 6.4, and thus the soil on the ninth day of cultivation was limed with 30×10^3 kg/ha of dolomite lime (20% Ca and 5% Mg by Juuan Dolomiittikalkki, Finland), and a soil pH of pH 7.2 was recorded 1 week later.

2.3. Urine Collection and Hygiene. The urine used in this study had been collected during the previous winter from several ecotoilets (where urine is collected separately from other excreta by making partition in the same pan for urine and excreta) in Västanfjärd, western Finland, from private homes, where it had been diluted with toilet flush water. The stored urine was analyzed for microbiological properties. Faecal coliforms, clostridia, enterococci, and the coliphage viruses were determined from the mixture and sediment sample. Faecal coliforms were determined by the SFS 4088 (*13*) standard plate count method on MFC agar and incubated at 44 °C for 24 h. Enterococci were cultured in bile esculin azide agar and incubated at 37 °C for 48 h (*14*). Clostridia were determined with sulphite–iron agar after heat treatment and anaerobic incubation at 37 °C for 48 h (*15*) by the standard plate-counted method. After incubation, bacterial colonies were counted and microbial numbers calculated as CFU/mL. Coliphages were determined by the ISO method (*16*) with two *Escherichia coli* hosts, that is, ATCC 13706 and *E. coli* ATCC 15597, on THG agar by a double-layer method and incubated at 37 °C for 24 h; plaques were counted on the next day (**Table 2**). The nutrient content of the urine was analyzed by Sydvästra Finlands vatten- och miljöundersökning, Turku, Finland, according to the SFS standard methods, which correspond to the APHA methods (*17*).

Table 2. Indicator Microorganisms in Urine Solution and Sediment (CFU/mL and PFU/mL for Bacteria and the Coliphage Viruses, Respectively)

microorganisms	solution	sediment	Finnish guideline value for microbes in compost (*35*)
faecal coliforms	Idl	Idl	<1000 CFU/g
enterococci	Idl	Idl	NG
clostridia	5	9	NG
coliphages host *E.coli* ATCC 13706	3	8	NG
coliphages host *E.coli* ATCC 15597	1	Idl	NG

[a] Idl = less than detection limit; detection limit = 1 CFU/mL and 1 PFU/mL. NG = not given.

2.4. Climate and Irrigation. The precipitation was 36 mm, 27 mm, 30 mm, and 33 mm with average temperatures of 9.9, 16.1, 17.8, and 17.7 °C in May, June, July, and August, respectively. In this respect, the summer was an average of 2.6 °C warmer than the normal summers in Finland, that is, when compared with the observational period of the years 1971–2006 of the Finnish Meteorological Institute (*18*).

Since the growing season was very dry, the cabbage plants were irrigated for 2 h every second day during the first month, for 3 h every second day during the second month and for 2 h every day in the third month of cultivation. No irrigation was applied on rainy days. The precipitation rate was 2 mm/h during the irrigation period.

2.5. Growth and Harvesting. The area of the largest leaf of each plant was measured on every seventh day to determine the growth rate of the plants. Insect damage was monitored on a numerical scale of 0–5 to assess leaf damage (*19*). The values on this scale were as follows: 0 = no damage; 1 = minor feeding damage; 2 = damage on outer leaves, found only after careful inspection; 3 = minor but clear damage on leaves; 4 = clear damage in outer and inner leaves; 5 = damage completely through the outer leaf to the inner leaves. Insects were identified after observing their presence on the damaged plant parts and captured with a sweep net for further identification.

The cabbages were harvested on the 89th day of cultivation (seedling plantation), and they were weighed separately for total biomass and commercial biomass. Outer dirty or broken leaves were removed to obtain the commercial biomass, and the head circle of the commercial products was measured.

2.6. Microbial Analyses. The commercial product of cabbage was analyzed for microbiological hygiene and nutrients content. Thus, faecal coliforms, clostridia, enterococci, and coliphages were determined by the same hygiene analysis as used for urine, and total coliforms were determined by the SFS 3016 (*20*) standard plate count method on m-Les Endo agar with 24 h of incubation at 37 °C.

2.7. Nutrient Analyses. The cabbages were dried at 60 °C for 4 days to analyze mineral contents. N, P, K, Ca, Mg, S, Fe, B, Cu, Mn, and Zn were analyzed by a commercial laboratory (Viljavuuspalvelu Oy Mikkeli, Finland). For N analysis, the Kjeldahl method (*21*) was used, while inductively coupled plasma–atomic emission spectrometry was used for the analysis of other nutrients (*22*).

Dry cabbages were milled, and then 0.5 g of the milled samples were mixed with 100 mL of ion-free water (18.2 MΩ) and heated at 50 °C for 30 min in a water bath, and 5 mL of this solution was placed into a vial with a syringe filter, and this was then frozen until analyzed. The samples assayed were for NO_3^-, NO_2^-, and Cl^- using ion chromatography (Dionex DX-120, AS40 Automated sampler, Dionex Corporation, U.S.A.).

2.8. Glucosinolate Analyses. The samples were collected from each fresh cabbage from each treatment plot, and the glucosinolates were

Table 3. Main Chemical Parameters in Used Urine Solution

parameters	quantity mg/L except pH and dry matter
pH	8.6
dry matter	<1 %
chloride	440
BOD_7	180
total P	63
suspended P	61
total N	930
ammonium N	940
nitrate + nitrite N	<0.5
potassium	360

analyzed from four pooled plot replicates of all treatments. The samples were transferred to liquid N and stored at –80 °C until analysis. They were freeze-dried and homogenized with a mortar and pestle, and an approximately 300 mg sample was weighed into test tubes. The GLS analysis was based on the method reported in the Official Journal of the European Communities (*23*) and by Loivamäki et al. (*10*). The GLSs were extracted and subsequently desulfated with purified sulfatase (*Helix pomatia* type H1) overnight. The desulfo-GLSs were eluted from columns with 0.5 mL of water on the following morning. The desulfo-GLSs were separated using high-performance liquid chromatography (Hewlett-Packard series 1050, 1040 M series II detection system; Hewlett-Packard, Waldbronn, Germany, and Waters, Milford, MA) using a SunFire C_{18} reversed-phase (3.5 μm, 2.1 × 100 mm Waters) column and a gradient from 0% acetonitrile (ACN) to 25% ACN in 45 min (30 °C, flow rate 0.2 mL min^{-1}). GLSs were detected at a wavelength of 229 nm. Individual GLSs were identified by comparing the retention times of samples with the retention times of reference samples. The quantities of the GLSs identified were calculated by using the peak area of each compound and the peak area and concentration of the internal standard (sinigrin). The result was multiplied with the equivalent response factor of each desulfoglucosinolate as provided in the Official Journal of the European Communities (*23*).

2.9. Sauerkraut Preparation. A total of 2.8 kg of cabbage heads taken from two to four cabbage plants from each experimental plot was used to make one jar from each experimental plot. This was achieved by chopping fresh cabbage into small pieces and pouring it gradually into a glass pot, pressing it gently with a wooden pestle. Marine salt (3%) was added layer-by-layer during the preparation. All 12 pots were closed with air-tight lids, which allow gas to get out but not in, and they were kept in a dark room at room temperature to allow fermentation. After 18 days of fermentation, the pH had decreased to around 3.7– 4.4. The sauerkraut was stored at +5 °C and analyzed for microbiological hygiene and nitrate and nitrite contents in a similar manner as that of the raw cabbage plants.

2.10. Flavor Testing. Triangle and ordinal taste testing of the sauerkrauts made from differently fertilized cabbage was conducted with a panel of 20 individuals; the ability of panel participants to recognize basic tastes (sweet, sour, salty, and bitter) had been pretested according to the procedure recommended by Meilgard et al. (*24*). The panel consisted of 14 women and 6 men aged from 22 to 60 years, mainly university and polytechnic students and staff. The tasting sessions were organized in a test kitchen with the tasters being served three sauerkraut samples marked with blind code numbers twice in the triangle taste test. For the differentiation test, two sauerkrauts were tasted, one plate served with a sample made from cabbage fertilized with urine and the other two plates served with samples that were mineral fertilized. At the next session, they were supplied with sauerkraut samples made from cabbage fertilized with urine or sauerkraut made from cabbage with no urine fertilizer. The tasters had to determine which sample was different.

In the ordinal test, the tasters were served three sauerkraut samples from all different fertilizers labelled with blind codes, and they were asked to evaluate which sample they preferred. The tasters could drink water between tests and taste the produce as many times as they wished.

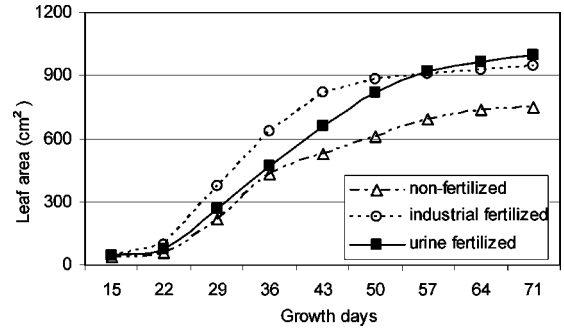

Figure 1. Leaf area development of cabbage plants with different fertilizer treatments during cultivation days (from seedling plantation; $N = 4$).

They could also utilize the possible differences in the texture or color of sauerkraut. The test method and the analysis of data have been described in detail by Holopainen et al. (*9*).

2.11. Statistical Analyses. All the data were tested for normality before statistical analysis; the data from biomass, head circle, microbial, and chemical analyses were analyzed with the SPSS 14.0 for Windows statistical package by means of a one-way analysis of variance (ANOVA) combined with Tukey's post-hoc test. The data from microbial analysis were log-transformed for statistical analysis. The data from field experiments and glucosinolate analysis were analyzed by means of the Kruskal–Wallis H test and the correlation between parameters by the Pearson correlation test (with a two-tailed test of significance).

3. RESULTS

3.1. Urine Quality. The smell of the urine used was mild. Very few indicator microorganisms were detected in the hygiene analysis of the urine (**Table 2**). The physical and chemical parameters of the urine are given in **Table 3**.

3.2. Plant Growth. The plants in all the treatments grew well. The cabbage growth on both fertilized and nonfertilized plots was normal or gradual. The color of leaves in the nonfertilized cabbages was paler than that of the others according to visual estimation. One cabbage plant in the industrial-fertilized plot was multiheaded (probably bug damage), and one plant from the urine-fertilized group died prior to harvest time. The growth rate of industrial-fertilized cabbage plots was slightly better than that of the urine- and nonfertilized cabbage plots on day 22 of the trial. Urine-fertilized plants were growing faster at trial day 36; the rate of growth remained higher than that of the nonfertilized cabbage since that time point. The total growth rate of urine-fertilized cabbages was slightly higher than that of the mineral-fertilized plants during the final three trial weeks, but the difference did not achieve statistical significance (**Figure 1**).

The growths of the fertilized cabbages were more rapid; the heads started to develop from trial day 45; thus, the harvesting was too late for both fertilized cabbages, but it was optimal for the nonfertilized cabbages. The total biomass and the commercial biomass and head circle of the urine-fertilized cabbage plots were all slightly higher than those in the industrial-fertilized cabbage plots but did not differ from that of nonfertilized cabbage plots (**Table 4**). Urine-fertilized cabbages were significantly ($P < 0.05$) larger compared to nonfertilized cabbages but did not differ from industrial-fertilized cabbages. If the data were analyzed according to individual plants, the results would appear as follows: total biomass ($F = 10.7$, $P < 0.001$; $N =$ 31–32), commercial biomass ($F = 10$, $P < 0.001$; $N = 21$–23), and head circle ($F = 11.4$, $P < 0.001$; $N = 21$–23). Total biomass, commercial biomass, and the head circle of urine-fertilized cabbages were all significantly larger than the corre-

Table 4. Average Plant Biomass (kg) and Head Circle (cm) of the Cabbages[a]

	fertilization treatments				
growth parameters	none	industrial	urine	F values	P values
total biomass	3.1 ± 1.0	4.3 ± 0.7	4.7 ± 1.6	2.346	0.151
commercial biomass	2.0 ± 0.8	3.3 ± 0.8	3.5 ± 1.8	2.536	0.134
head circle	57.8 ± 6.0	68.8 ± 6.0	71.4 ± 11.8	3.406	0.079

[a] Mean ± standard deviations. F and P values from one way ANOVA analysis ($N = 4$). The mean value was transformed to $\log_{10}$ for statistical analysis ($P = 0.05$).

Table 5. Indicator microorganisms per one gram of cabbage commercial biomass as $\log_{10}$ mean ± standard deviation ($N = 4$)[a]

	fertilization treatment		
microorganisms	none	industrial	urine
total coliforms	3.9 ± 0.2	3.9 ± 0.2	4.0 ± 0.4
faecal coliforms	2.0 ± 0.3	1.8 ± 0.2	2.3 ± 0.7
enterococci	3.0 ± 0.3 a	2.2 ± 0.4 b	2.9 ± 0.3 a
clostridia	Idl	Idl	Idl
coliphages host *E.coli* ATCC 13706	Idl	Idl	Idl
coliphages host *E.coli* ATCC 15597	Idl	Idl	Idl

[a] The samples were analyzed from each plot. Idl = less than detection level, detection level = 10 CFU/g and 10 PFU/g; the means indicated with the same letter within a row do not differ statistically significantly ($P < 0.05$). Mean separation results were derived by one-way ANOVA with Tukey's result for enterococci: nonfertilized with urine fertilized ($P = 0.971$), industrial fertilized with urine fertilized ($P = 0.027$), and nonfertilized with industrial fertilized ($P = 0.019$).

sponding values in nonfertilized cabbages, $P < 0.001$, 0.004, and 0.001, respectively. The commercial product biomass was comparatively lower in all treatments because of the need to remove layers of damaged outer leaves. The outer leaves of the larger cabbages were more extensively damaged; about 30% of the data from the commercial biomass and for the head circle have been excluded from the results.

3.3. Insect Damage. Diamondback moths and larvae (*Plutella xylostella* L.), flea beetles (*Phyllotreta undulata* L.), and mustard beetles (*Phaedon cochleariae* Fabr.) were the insects which were detected doing damage to the cabbage plants. Insect damage was marginally significantly positively correlated ($r = 0.73$) with the growth rate of cabbage ($P < 0.001$). Insect damage declined after 57 days of the trial, and the plant growth rate was also slower at that time.

3.4. Hygienic Quality of the Crop Plants. Some indicator microorganisms were found in all fertilized cabbages (**Table 5**); among them, enterococci was statistically ($F = 9.32$; $P = 0.013$) higher in nonfertilized and urine-fertilized cabbage than in industrial-fertilized cabbage. The NO_3^- contents in urine-fertilized cabbages did not differ significantly from those of the other treatments. The NO_2^- contents were less than the detection limit for all treatments, whereas the Cl^- contents were significantly ($F = 4.7$; $P = 0.037$) higher in urine-fertilized compared to nonfertilized cabbages, but the urine-fertilized cabbages did not differ from industrial-fertilized cabbages in this respect (**Table 6**). Nitrate and chloride contents in the cabbages correlated significantly positively to each other ($r = 0.757$; $P = 0.004$). P, K, Ca, Mg, S, Fe, B, Cu, and Zn (data not shown) contents were similar in all fertilized cabbages, but the Mn contents were slightly different ($P < 0.05$); that is,the Mn concentrations were 0.8, 1.0, and 0.5 mg/kg in urine-fertilized, mineral-fertilized, and nonfertilized cabbages, respectively.

3.5. Glucosinolates. There was no difference detected in any of the treatments in terms of the GLS concentration. The aromatic-type GLS (gluconasturtin) was the predominant compound in all treatments. Indolyl and alkenyl compound contents were similar in all treatments; in absolute terms, the concentrations of indolyl and alkenyl were slightly lower than those of the aromatic compound (**Table 7**).

3.6. Hygienic Quality of the Sauerkraut. The microbial quality of the sauerkraut samples was good; total coliforms, faecal coliforms, clostridia, and coliphages (somatic and RNA-coliphage) were not detected in any samples (detection limits: 10 CFU and PFU/g). Enterococci were detected with the geometric mean and geometric standard deviation being 228 ± 150 CFU/g in the industrial fertilized and 123 ± 225 CFU/g in the nonfertilized cabbages, but the levels were too low to be detected in the urine-fertilized cabbages in all parallel plot samples ($N = 4$). The NO_3^- contents in the sauerkraut made from all fertilized cabbages were low and relatively lower than that in fresh cabbages, but nevertheless the NO_3^- content was higher ($F = 17.77$; $P = 0.002$) in sauerkraut made from urine-fertilized cabbage compared to sauerkraut made from industrial- and nonfertilized cabbages. The NO_2^- contents were also lower but slightly higher in sauerkraut compared to the NO_2^-content of fresh cabbage (**Table 6**).

3.7. Flavor Quality of the Sauerkraut. In the taste assessment test, 10 out of the 20 panelists could taste a difference between the sauerkraut made from cabbage fertilized with urine and that from the cabbage fertilized with industrial fertilizer, but this was not statistically significant ($P > 0.05$). In contrast, 13 of 20 panelists could differentiate the sauerkrauts made from urine and nonfertilized cabbage, this difference being statistically different ($P < 0.01$) in data for the triangle taste test (*25*). The panelists did not prefer any particular samples, and all sauerkraut samples were evaluated as good by the tasters, since seven preferred the sauerkraut made from urine-fertilized cabbage, five preferred sauerkraut made from industrial-fertilized cabbage, and eight preferred sauerkraut made from nonfertilized cabbage.

4. DISCUSSION

The growth of cabbage yields in this study indicates that urine could be used as a good fertilizer for cabbage and could represent a feasible alternative to industrial fertilizers. The initial growth rate of cabbage plants in industrial-fertilized plots was higher than that in urine-fertilized plots because of the larger amount of fertilizer applied at the beginning, but later, the growth rate was better in urine-fertilized plots when equal amounts of fertilizer were applied. The growth rate of urine-fertilized cabbage could have been better if an equal amount of fertilizer had been applied, the amounts at each interval from the very beginning. Although the initial growth rate of industrial-fertilized cabbages was better, their growth ceased after a certain midpoint through the trial possibly because of N deficiency, whereas the urine-fertilized cabbages continued to grow; this is similar to the situation with cucumber growth (*5*). It is possible that the level of available N for plants could remain for a longer time in urine fertilization than in the case with the industrial fertilization or perhaps the availability to the plants is simply better since urine is already in a liquid form.

The outer leaves of the cabbage head were damaged during harvesting due to the dry and hot weather of summer; many

Table 6. NO_3^-, NO_2^-, and Cl^- mg/kg Contents in Different Types of Fertilized Cabbage and Sauerkraut, Mean ± Standard Deviation ($N = 4$)[a]

nutrient contents		fertilization treatments: none	industrial	urine	F value	P value
fresh cabbage	NO_3^-	270 ± 200	320 ± 270	480 ± 110	1.18	0.351
	NO_2^-	ldl	ldl	ldl	NA	NA
	Cl^-	73 ± 37 a	100 ± 65 ab	170 ± 27 b	4.69	0.040
sauerkraut	NO_3^-	10 ± 7 a	9 ± 2 a	210 ± 93 b	17.77	0.001
	NO_2^-	22 ± 2	25 ± 2	24 ± 6	0.853	0.458

[a] Each sample was prepared from plants of one experimental plot. ldl = less than detection level, i.e., <8 mg/kg. NA = not analyzed. The means indicated with the same letter within a row do not differ statistically significantly ($P < 0.05$). Mean separation results were derived by one-way ANOVA with Tukey's results for (i) Cl^- in fresh cabbage: none with industrial ($P = 0.672$), none with urine ($P = 0.037$), industrial with urine ($P = 0.143$); (ii) for NO_3 in sauerkraut: none with industrial ($P = 0.999$), none with urine ($P = 0.002$), industrial with urine ($P = 0.143$).

Table 7. Mean Concentration of Glucosinolates [GLS; μmol/g dw (Dry Weight); ± SD] in Cabbage from Different Fertilizer Treatments ($N = 4$)[a]

glucosinolates	fertilization treatments: none	industrial	urine
indolyl GLS	*0.65 ± 0.06*	*0.67 ± 0.12*	*0.66 ± 0.13*
neoglucobrassicin	0.04 ± 0.05	0.07 ± 0.08	0.08 ± 0.12
4-hydroxyglucobrassicin	0.04 ± 0.01	0.04 ± 0.03	0.08 ± 0.06
glucobrassicin	0.56 ± 0.23	0.40 ± 0.10	0.51 ± 0.19
4-methoxyglucobrassicin	0.08 ± 0.04	0.07 ± 0.06	0.11 ± 0.07
alkenyl GLS	*0.72 ± 0.28*	*0.58 ± 0.21*	*0.78 ± 0.17*
glucoalyssin	0.00 ± 0.00	0.02 ± 0.03	0.02 ± 0.04
gluconapin	0.16 ± 0.05	0.21 ± 0.05	0.15 ± 0.10
glucoraphanin	0.07 ± 0.01	0.08 ± 0.04	0.12 ± 0.07
glucobrassicanapin	0.02 ± 0.01	0.03 ± 0.05	0.04 ± 0.05
glucoerucin	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.04
gluconapoleiferin	0.03 ± 0.00	0.03 ± 0.03	0.06 ± 0.04
progoitrin	0.34 ± 0.03	0.29 ± 0.15	0.24 ± 0.08
aromatic GLS			
gluconasturtin	*1.48 ± 0.46*	*1.82 ± 1.29*	*2.24 ± 1.43*
total glucosinolates	2.85 ± 0.67	3.07 ± 1.53	3.69 ± 1.62

[a] Individual compounds groups are in italics.

outer broken leaves needed to be removed to achieve commercial product standards. The total biomass and commercial biomass were slightly higher in urine-fertilized cabbages. These plants were dark-colored, compact-headed, and slightly larger than industrial-fertilized plants and were clearly different from the nonfertilized plants. The total biomass of the nonfertilized plants could have had been even lower if the cultivation land had been of poorer quality. On the other hand, there was more extensive leaf damage in the larger cabbages.

The chemical composition of urine depends on many factors. The consumption of protein-rich food increases N in urine, but this is usually expensive in developing countries, and therefore it would be predicted that N contents in urine would be lower in developing countries than in the developed countries (*26, 27*). Nitrogen loss by sweating due to high temperatures and physical labor in tropical countries may also reduce the N content in urine. In the urine used in this trial, the N was almost exclusively in the ammonium form, although it is claimed that ammonification can be in equilibrium with urea (*28*).

Only very few microorganisms were detected in the urine, and they were present at a similar level as reported earlier (*5, 29*). Microbial numbers were higher in sediment than in the solution, as demonstrated (*5*). Urine can become contaminated with pathogens from small particles of feces during collection, but when the pH increases to the alkaline state (pH 9), the numbers of enteric microbes become reduced. Microbial reduction can happen more rapidly in tropical countries because of high temperatures. A storage time of 6 months under outdoor conditions has been recommended for urine in Sweden because of hygienic aspects (*26, 27*). This may not be necessary, and in fact, it might be impracticable in South Asian regions where people live in close proximity to each other and there is no space to store urine in small domestic plots. Furthermore, the indigenous population does not have money to invest in building large urine storage tanks.

The few pathogens in urine fertilizer actually applied into the soil are not a high risk for agricultural production, since those pathogens are unable to gain access to the agricultural consumer products. An EU guideline for *Escherichia coli* in precut fruits and vegetables is <1000 CFU/g (*30*). This study showed that the microbiological qualities of urine-fertilized cabbage plots and sauerkraut made from these plants were similar to the industrial- and nonfertilized cabbage plots and sauerkrauts made from these types of plants. Some indicator microbes were detected in all fertilized cabbages, but those are more probably attributed to other factors, for example, contamination by birds, insects, and so forth. Furthermore, the use of pure urine fertilizer in an agricultural field will cause less of a problem than open-field defecation which is a common practice in poor area of developing countries; thus, microbes from feces can be transported to watercourses during rain fall and flooding. Nevertheless, urine fertilizer needs to be used with care to reduce any possible risks; it should never be applied directly to any parts of the plants, since, in addition to possible microbial contamination, plain urine can physically damage many plants (*26*).

In the cultivation of cabbage, urine fertilizer can be provided until the beginning of head development or about 45 days of cultivation. The time elapsing between fertilization and harvesting should be more than 25 days so that N uptake can be optimized, and this may also improve the microbiological quality, as reported by Jönsson et al. (*26*), although the microbial qualities were similar in our study when the final application of urine fertilizer was done only for the 18 days before harvesting.

Granular industrial fertilizer can remain undissolved, lying in the upper layer of dry soil, for example, during the dry season, if there is insufficient irrigation. Urine is a soluble liquid fertilizer, which may mean that N is more rapidly available and effective even in the dry season, and it is also easier to apply. The application of urine fertilizer at different intervals can represent one way to reduce N losses via ammonia evaporation; this may be more important in tropical regions where there can be rather high N losses from the soil due to the high temperatures.

Urine-fertilized cabbage plants achieved their maximum growth earlier than other cabbages; that is, the cultivation time may be shorter in urine-fertilized cabbages. If the cultivation times were to be shorter, then the land use could be more effective. This could be another benefit to indigenous populations with very small plots of land. This result will need to be

confirmed with other plants like lettuce, broccoli, cauliflower, and radishes.

More than 160 cabbages could be cultivated in 90 m^2 land plots, all being fertilized with the urine collected by one individual in one year, assuming a urine excretion rate of 1.5 L/day with the urine containing 2.9 g N/L, as is the case in developing countries according to Jönsson et al. (*26*). In other words, the production could be 752 kg of cabbage, which is 5.1 times higher than the recommendation for consumption of fruits and vegetables/person/year (*31*). According to the present results, it would be possible to obtain 64 kg more cabbage from urine-fertilized plots than from industrial fertilized plots and 256 kg more than from nonfertilized plots.

In this study, the contents of total glucosinolates from urine-fertilized cabbage heads did not differ from the other treatments. A similar result was presented by Rosa et al. (*11*); in that study, the application of fertilizer containing N and S did not affect the level of glucosinolates in broccoli. It was noted that glucosinolates were sensitive to salt alone but not to NPK fertilizer with a salt mixture, and thus our results with cabbage suggest that a similar situation may exist with other *Brassica* plants. Reddy et al. (*32*) observed that elevated CO_2 levels could affect the ratio of GLS groups in cabbage and oil seed rape foliage in a cultivar-dependent manner. In our study, the type of fertilization had no impact on the GLS contents.

The nitrate and nitrite levels were low in all cabbages compared to the concentration of 784 mg/kg of nitrate reported from fresh cabbage by Silicano et al. (*33*). The acceptable daily intake for the nitrate ion is 3.65 mg/kg of body weight (equivalent to 219 mg/day for a 60 kg person) (*34*). The nitrogen content of cabbage plants affects the performance of the specialist insect pest of *Brassica* plants (*32*), and we found that there was a good correlation between the extent of insect damage and the cabbage growth rate, and at the final harvest, growth reduction attributable to the pest insect was not detectable. Insect damage was more severe in the industrial-fertilized cabbages, which had a faster initial growth than the urine-fertilized cabbages. This probably reflects the preference of many insects, for example, the diamondback moth, flea beetle, and mustard beetle, to feed and reproduce on industrial NPK-fertilized cabbage plants.

All sauerkrauts were evaluated as tasting good, which might be because of the similar GLS and other nutrients contents, but there is no strong evidence about how GLS and nutrient contents can affect the taste. We did not analyze the sugar content, and this might have explained why some individuals preferred the sauerkraut made from nonfertilized plants and why they were not so satisfied with the urine-fertilized plants, which also had an elevated NO_3^- content. There may be several factors which could influence the taste, that is, the head size, head age, and texture, and in those respects, the urine- and industrial-fertilized cabbages were different from the nonfertilized cabbage, and these might also modify the taste of the sauerkraut. The chloride contents were higher in urine-fertilized cabbages than the others, and this might also have influenced the outcome if the taste assessment had been conducted with fresh cabbage.

In summary, this study demonstrated for cabbage cultivation that the fertilizer value of human urine is as good as industrial fertilizer in terms of its chemical contents, and furthermore, its hygienic quality can also be guaranteed. The use of urine fertilizer increased the biomass of the cabbage, and it is anticipated that the situation will be similar for other agricultural products. Urine-fertilized plants may grow more rapidly, so the plants can be harvested earlier, thus making more efficient use of the land. The use of urine fertilizer could reduce the demand for industrial fertilizer to some extent, which would reduce the environmental pollution released during fertilizer manufacture and transportation.

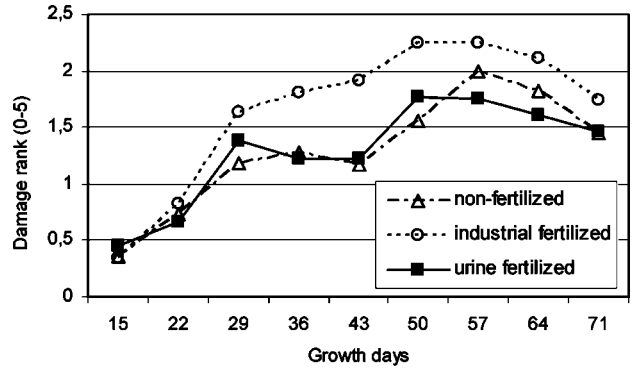

Figure 2. Insect damage of cabbages with different treatments at different days of cultivation (from seedling plantation; $N = 4$).

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