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ARTICLE

Human Urine and Wood Ash as Plant Nutrients for Red Beet (*Beta vulgaris*) Cultivation: Impacts on Yield Quality

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The objective of this study was to evaluate the effect of human urine and wood ash fertilization on the yield and quality of red beet by measuring the microbial, nutrient, and antioxidant (betanin) content of the roots. Red beets were fertilized with 133 kg of N/ha as mineral fertilizer, urine and ash, and only urine with no fertilizer as a control. The mineral-fertilized plants and urine- and ash-fertilized plants also received 89 kg of P/ha. Urine and ash and only urine fertilizer produced 1720 and 656 kg/ha more root biomass, respectively, versus what was obtained from the mineral fertilizer. Few fecal coliforms and coliphage were detected in mineral-fertilized and urine- and ash-fertilized red beet roots. The protein and betanin contents in red beet roots were similar in all treatments. In conclusion, this study revealed that urine with or without ash can increase the yield of red beet and furthermore the microbial quality and chemical quality were similar to the situation in mineral-fertilized products.

KEYWORDS: Betanin; glucose; nitrogen; red beet; urine; wood ash

INTRODUCTION

Human urine contains large amounts of nitrogen (N), phosphorus (P), and potassium (K^+) (*1*), and furthermore, these elements are 90−100% plant available (*2*). In general, pure human urine contains very few enteric microorganisms (*3*). Urine has been successfully used to fertilize barley (*4*), maize (*5*), cucumber (*3*), cabbage (*6*), and tomato (*7*). Similarly, wood ash contains a large amount of P, K^+, calcium (Ca^{2+}), and magnesium (Mg^{2+}) which can be used as fertilizer (*7*−*12*). Very few studies have examined the combined use of urine and wood ash in fertilizing nutritious plants (*7*).

Therefore, it seemed reasonable to study the fertilizer value of human urine with or without wood ash in cultivation of different plants because of (1) their different fertilizer demands, (2) the variation in the nutrient content in urine, and (3) the bioavailability of the nutrient content in these resources in different soil types. In many societies, urine and wood ash can be available as waste and their fertilizer value would be important; today many poor people cultivate soil without being able to afford any fertilization products. Better yields may become increasingly important as humankind strives to meet the increased demands for food. Similarly, it is very important to reduce water contamination, save energy, and also reduce greenhouse gas emissions by using renewable sources like wood.

The main objectives of this study were, therefore, to evaluate the use of urine and ash fertilizer with respect to (1) the growth and yield production of red beet, (2) the chemical and microbial quality of the red beet root, and (3) the flavor characteristics of the red beet roots. Our study hypothesis was that red beet root production and their microbial and chemical quality would be similar if the red beet fertilized with urine, urine and ash, or mineral fertilizer and would be significantly higher than what could be achieved without fertilization. Red beet was selected for this work because it is a root vegetable and it could be suspected of being responsive to the soil nutrients and possibly microbiological risk.

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MATERIALS AND METHODS

Urine Collection and Hygiene. The used urine had been collected in 2007 from several eco-toilets (where urine is collected separately from feces) in private homes around Tampere, Finland. The levels of *Salmonella* spp., fecal coliforms, clostridia, enterococci, and coliphages were determined from the urine solution, and these analyses were done with the standard methods as described by Pradhan et al. (*7*).

The nutrient contents of the urine (total N, NH_4^+ N, NO_3^- N, NO_2^- N, total P, PO_4^+ P, and K^+) were analyzed with the standard methods as described by Pradhan et al. (*7*), and the Na^+ content was analyzed by FAAS (*13*).

Wood Ash Collection and Nutrients. Birch tree (*Betula* sp.) wood ash was collected from the furnace of a household in Nilsiä, Finland. The collected ash was sieved and used within 2−3 months of collection. The nutrient contents of the wood ash (P, K^+, Ca^{2+}, and Mg^{2+}) were analyzed as described by Pradhan et al. (*7*), and the Na^+ content was analyzed by the same method that was used for urine analysis.

Plant Materials and Plantation. Red beet (*Beta vulgaris* var. Rubia) commercial seeds (Hammenhögs) were sown in outdoor plots in the University of Kuopio research garden (62° 53′ 39″ N and 27° 37′ 17″ E) on June 5, 2008. The total cultivation area was 72.4 m^2 and was divided

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Table 1. Soil Properties and Nutrient Contents of Cultivated Soil before and after Cultivation[a]

parameter	soil before cultivation	soil after cultivation with different fertilizer treatments				F^b	P^b
		none	mineral	urine and ash	urine		
total N (g)	1.75 ± 0.77	2.19 ± 0.35	2.42 ± 0.32	2.45 ± 0.21	2.58 ± 0.37		NS[e]
NO_3^- N (mg)	8.58 ± 3.54	1.89 ± 0.45	2.54 ± 0.23	4.06 ± 1.52	4.37 ± 2.47		NS[e]
NO_2^- N (mg)	7.11 ± 3.28	LDL[c]	LDL[c]	LDL[c]	LDL[c]		
NH_4^+ N (mg)	1.42 ± 0.11	0.82 ± 0.27	0.84 ± 0.41	0.99 ± 0.25	0.94 ± 0.20		NS[e]
Cl^- (mg)	3.18 ± 1.16	2.20 ± 0.20	3.04 ± 0.34	2.78 ± 0.70	2.54 ± 0.67		NS[e]
P (mg)	124.9 ± 36.3	196.8 ± 27.7	161.8 ± 56.3	179.3 ± 27.2	230.9 ± 111.7		NS[e]
Na^+ (g)	ND[d]	0.42 ± 0.06	0.40 ± 0.06	0.43 ± 0.04	0.42 ± 0.04		NS[e]
K^+ (g)	5.90 ± 1.31	5.93 ± 0.38	6.25 ± 0.41	6.24 ± 0.94	5.92 ± 1.11		NS[e]
Ca^{2+} (g)	7.28 ± 1.25	7.47 ± 0.82	6.85 ± 0.72	7.56 ± 0.97	6.96 ± 1.09		NS[e]
Mg^{2+} (g)	7.80 ± 1.12	7.65 ± 0.32	8.56 ± 0.19	8.62 ± 1.13	7.88 ± 0.56		
OM %	12.0 ± 1.0	ND[d]	ND[d]	ND[d]	ND[d]		
pH	7.34 ± 0.02	7.41 ± 0.08 a	7.17 ± 0.19 a	7.85 ± 0.21 b	7.13 ± 0.16 a	16.264	0.0001
conductivity (μS/cm)	ND[d]	69.1 ± 11.8 a	76.7 ± 0.1 a	153.4 ± 42.7 b	78.0 ± 13.1 a	11.204	0.001

[a] The result is presented as kilograms of dry weight [arithmetic mean (AM) ± standard deviation (SD); $N = 6$ in each treatment for soil after cultivation]. [b] The F and P values were from ANOVA with values for the comparison between the treatments only for the soil after cultivation. [c] Lower than the detection limit; detection limit for NO_3^- N of 0.6 mg/kg of dry weight. [d] Not determined. [e] Not significant.

Table 2. Chemical and Physical Parameters of Urine and Wood Ash and Applied Amounts of Nutrients and Fertilizers per Plot during the Entire Cultivation

parameter	in urine (g/L)	in wood ash (g/kg)	mineral fertilizer	urine and ash fertilizer	urine fertilizer
total N (g)	8.36	ND[a]	15 (133 kg/ha)	14.9 (14.9 + NK[b])	14.9
NO_3^- N (g)	0.01	ND[a]			
NH_4^+ N (g)	8.57	ND[a]			
total P (g)	0.7	36	10 (89 kg/ha)	10 (1.2 + 8.8)	1.2
PO_4^- P (g)	2.03	ND[a]			
K^+ (g)	2	137	28.4 (252 kg/ha)	37.2 (3.7 + 33.5)	3.7
Ca^{2+} (g)	ND[a]	216	NK[b]	52.9 (NK[b] + 52.9)	ND[a]
Mg^{2+} (g)	ND[a]	47	3.34 (30 kg/ha)	11.0 (NK[b] + 11.0)	ND[a]
Cl^- (g)	3.03	ND[a]	NK[b]	5.54	5.54
Na^+ (g)	2.34	ND[a]	NK[b]	4.28	4.28
pH	9.2	11.14			
conductivity (mS/cm)	47.2	7.9			
total fertilizer applied			167 g	1820 mL + 245 g	1820 mL

[a] Not determined. [b] Not known.

into 24 experimental plots each (1.5 m × 2.25 m) 1.13 m^2 in area with 50 cm (total of 45.4 m^2) with narrow protecting strips between the different plots, and 12 seeds were sown in each plot at a spacing of ~20 cm. The cultivated soil was clay loam, and its chemical properties are listed in **Table 1**. This cultivated area had been used for cultivation of pumpkin in 2007. The experimental area was designed as a Latin square model for four different treatments, i.e., no fertilizer (control), mineral fertilizer, urine and ash fertilizer, and urine fertilizer, all with six replicates. The 10 tallest plants from each of six rows par treatment were used for growth indicator analysis since many nonfertilized red beets were too small for other types of analysis.

Fertilizer Treatments. Mineral-fertilized and urine and ash-fertilized plots were treated at doses of 133 kg of N/ha and 89 kg of P/ha, but the plots fertilized with only urine received less P since urine had a lower P content than N content (**Table 2**). Mineral fertilizer [Puutarhan Y1, 9% N, 6% P, and 17% K (6.5% NH_4^+ −N and 2.5 % NO_3^- −N, NO_2 −N) which also contained 2% Mg, 10% S, 0.05% B, 0.1% Cu, 0.1% Fe, 0.7% Mn, 0.01% Mo, 0.1% Zn, and 0.001% Se] was applied on cultivations days 1, 18, 28, and 36. This fertilizer was applied at a dose of 41.7 g/plot at each time. Mineral fertilizer was applied on the soil ~20 cm on both sides of the rows and mixed by tilling the soil.

Urine fertilizer (8.36 g of N/L, 0.7 g of P/L, and 2 g of K/L) (**Table 2**) was applied on the same day as mineral fertilizer at a rate of 455 mL/plot. The urine was applied with a measuring beaker and spread on the soil ~20 cm on either side of the rows. The soil surface was tilled before and after application of urine fertilizer so that the liquid would be better absorbed. Wood ash (37 g of P/kg and 137 g of K/kg) (**Table 2**) was applied on the third day after each interval of use of urine fertilizer, and it was applied as a dose of 61.05 g/plot at each interval. Wood ash was applied around the plants and mixed by tilling the soil. The soil from nonfertilized plots was also tilled at the same time and in a similar manner.

Climate and Irrigation. The levels of precipitation during the cultivation period in June, July, and August were 116, 116, and 85 mm, respectively; the average temperatures were 13.6, 15.6, and 13.2 °C, respectively (*14*), and the average amounts of sunlight per day were 19.9, 18.7, and 15.8 h, respectively (*15*). Since the precipitation was very frequent during the cultivation period, there was rarely any need for irrigation.

Growth and Harvesting. The height of plants (above ground parts) was measured, and the number of leaves was counted from each plant on every seventh day to determine the growth rate of the plants. Plants were harvested 84 days after cultivation (i.e., from seedling plantation). The total biomass and root biomass were weighed separately.

Microbial and Nutrient Analyses of Beet Root. The red beet roots were washed with sterilized water, peeled, and mixed with sterilized water to make a solution mixture for microbiological hygiene analysis. Thus, levels of *Salmonella* spp., fecal coliforms, clostridia, enterococci, and coliphages were determined following the same methods that were used for urine analysis.

The washed and peeled red beet roots were lyophilized at −60 °C for 4 days and milled to make a powder which was used in all the chemical analyses conducted in this study. Total N, P, K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_3^-, NO_2^-, Cl^-, and soluble sugar analysis was performed as described by Pradhan et al. (*7*).

Betanin Analysis. Approximately 500 mg of milled red beet root was taken into a test tube, and 10 mL of deionized water was added and homogenized for 1 min. This homogenized mixture was centrifuged for 10 min (1500 rpm), and the clear supernatant was collected. The residual portion was re-extracted with 10 mL of water, and both of these samples were pooled to be assayed by HPLC (high-pressure liquid chromatography). The betanin concentration was quantified by comparison to an

Table 3. Presence of Some Enteric Microbes in the Urine Solution and Red Beet Roots[a]

		red beet (root)			
microorganism	urine solution	no fertilizer	mineral	urine and ash	urine
Salmonella spp.	none/20 mL	ND[b]/25 g	ND[b]/25 g	ND[b]/25 g	ND[b]/25 g
fecal coliforms	none/30 mL	LDL[c]	27 ± 36	14 ± 21	LDL[c]
enterococci	LDL[c]	LDL[c]	LDL[c]	LDL[c]	LDL[c]
clostridia	LDL[c]	LDL[c]	LDL[c]	LDL[c]	LDL[c]
coliphages host *E. coli* ATCC 13706	96 ± 16	LDL[c]	LDL[c]	LDL[c]	LDL[c]
coliphages host *E. coli* ATCC 15597	LDL[c]	LDL[c]	5 ± 9	26 ± 51	LDL[c]

[a] Numbers ± SD [colony forming units (CFU) per milliliter or plaque forming units (PFU) per milliliter of urine and colony forming units per gram or plaque forming units per gram of red beet root] (N = 6 for red beet root). [b] Not detected; detection limits for all microbes (except *Salmonella*) were 10 CFU/g or 10 PFU/g of red beet root. [c] Lower than the detection limit.

external standard of betanin, and it was isolated for this purpose from the sample extracts as described by Kujala et al. (*16*). In the isolation procedure, the injection volume was 200 μL and the flow rate was 1.0 mL/min. The isolated solutes were identified by MS (mass spectrometry) and NMR (nuclear magnetic resonance).

HPLC Analysis. Betanin was analyzed in an HPLC system consisting of an HP 1090 LC autosampler with a DAD (diode array detector). The column used was a Bischoff RP-18 column [250 mm × 4.0 mm (inside diameter), Purospher, 5 μm]. Two solvents, acetonitrile (A) and formic acid with water (0.4:99.6, v/v) (B), were used. The injection volume was 20 μL, and the flow rate was 1.0 mL/min. In the analysis of betanin, the elution profile was 100% B from 0 to 5 min and 0 to 13% A in B from 5 to 35 min (linear gradient), and detection at 538 nm.

MS. The molecular formula of the betanin compound was confirmed by HRMS (high-resonance mass spectrometry) measurements by using a QSTAR XL hybrid quadrupole TOF instrument (Applied Biosystems, Foster City, CA) in the positive electrospray ionization mode. HRMS: calculated for $[M]^+$ $C_{24}H_{27}N_2O_{13}$ 551.1513, found 551.1514. This analysis has been described previously by Kujala et al. (*16*) and Wybraniec (*17*).

NMR Analysis. The molecular structure of the betanin was confirmed via NMR (*16*, *18*). The ^1H NMR spectra were recorded using a Bruker AVANCE III 500 spectrometer equipped with a 5 mm inverse probe operating at 500.36 MHz. The spectra were recorded at 300 K in D_2O and CD_3OD. One-dimensional proton spectra were recorded with a standard protocol utilizing a 30° flip angle, a pulse recycle time of 8.5 s, and a spectral width of 5 kHz consisting of 64K data points. Spectra were zero-filled to 128K with a 0.3 Hz exponential weighting applied prior to Fourier transformation. $^1H-^{13}C$ gradient-enhanced heteronuclear single-quantum correlation (two-dimensional HSQC) experiments were conducted in the phase-sensitive mode using sensitivity improvement and adiabatic pulses for inversion and refocusing with gradients in back-inept. The data matrix was 256 × 1K, and the spectral width was 5 kHz for protons and 20 kHz for carbon. An evolution time $1/(4J_{CH})$ of 1.72 ms was used. For each FID, 32 transients were accumulated. A pure squared cosine window function was applied in both dimensions prior to Fourier transformation.

Flavor Testing. The flavor taste test was conducted only for the mineral-fertilized, urine- and ash-fertilized, and only urine-fertilized red beet roots due to the limited amount of red beet roots in the nonfertilized treatment. Triangle and ordinal taste testing of the boiled peeled chopped red beet roots from differently fertilized plots was conducted with a panel of 17 individuals; the ability of panel participants to recognize basic tastes (sweet, sour, salty, and bitter) had been pretested according to the procedure recommended by Meilgaard et al. (*19*). The tasting session was conducted as described by Pradhan et al. (*6*).

Soil Analysis. Basic physical and chemical properties, pH, conductivity, organic matter (OM) content, and total NO_3^-−N, NO_2^-−N, NH_4^+−N, P, K^+, Na^+, Ca^{2+}, and Mg^{2+} contents of the soil were analyzed before and after red beet cultivation. Soil samples were taken at a depth of 15 cm from the cultivated plots and sieved. The sieved fresh soil was used for pH, conductivity, NO_3^-−N, NO_2^-−N, Cl^-, and NH_4^+−N analysis. The sieved soil was air-dried at 60 °C for 24 h and used for OM content analysis. The air-dried soil was milled in a coffee grinder and used for total N, P, K^+, Na^+, Ca^{2+}, and Mg^{2+} analysis as described by Pradhan et al. (*7*).

Statistical Analyses. The basic statistical parameters of raw data were calculated with MS Excel to characterize the overall features of the data sets. The raw and transformed data were subjected to a normality test prior to the other statistical analyses. The data from growth rate, total biomass, roots biomass, and microbial and chemical analysis were analyzed with SPSS 14.0 for Windows Statistical Package by one-way ANOVA combined with Tukey's post hoc test and repeated measurement test. The correlation between parameters was analyzed by Spearman's correlation test (with a two-tailed test of significance).

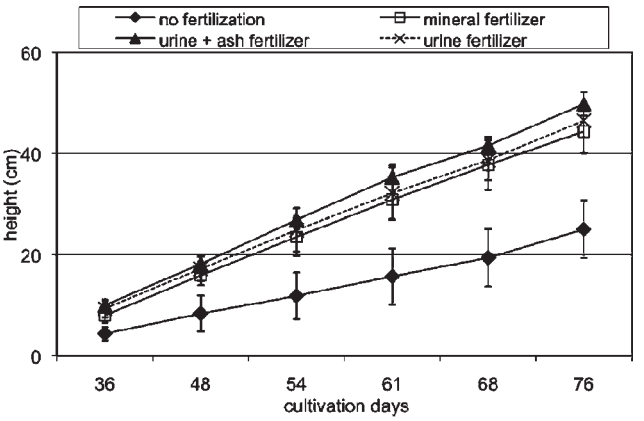

Figure 1. Growth rate of red beet arable plant height from different fertilizers (arithmetic mean with SD) (N = 6 in each treatment).

RESULTS AND DISCUSSION

Study Significance and Urine Quality. This study has tried to build a bridge between sanitation and agriculture. In other words, we tried to exploit the fertilizer value of human urine (*3*−*7*) and wood ash (*7*−*12*) as a fertilizer resource instead of viewing these products simply as waste. Here, the red beets were cultivated with urine and wood ash fertilizer. The odor of the urine used was very strong because of the high concentration of ammonium (**Table 2**). The high concentration of NH_4^+ N increases the pH of the solution which is an important factor in reducing the level of enteric microbes (*20*, *21*). However, few enteric microbes were detected from the used urine (**Table 3**).

Growth and Biomass. The growth rates of the red beet plants from all fertilizer treatments were normal considering the cultivation time (84 days) in the cold Nordic climate except for the nonfertilized red beet plants and roots which were very small. The growth rates, total biomasses, and root biomasses of the mineral-fertilized, urine and ash-fertilized, and urine-fertilized plants were not statistically different ($P > 0.05$), but all were significantly higher ($P < 0.05$) than those observed in nonfertilized plants. The trend toward a high growth rate (**Figure 1**) and biomasses (**Table 4**) of red beet was observed in urine- and ash-fertilized plants followed by urine-fertilized plants and mineral-fertilized plants. The size of the nonfertilized red beets was very small with hardly any edible parts. Similar results were reported for cabbage (*6*), tomato (*7*), cucumber (*3*), wheat (*22*), and

Table 4. Rates of Production of Red Beets[a] with Different Fertilizer Treatments [tons per hectare (AM ± SD) (N = 6)]

measurement	no treatment	mineral	urine and ash	urine	F	P
total biomass FW	3.30 ± 2.78 a	16.20 ± 5.67 b	20.54 ± 2.97 b	17.80 ± 4.14 b	21.350	0.0001
root biomass FW	1.29 ± 1.10 a	6.44 ± 2.51 b	8.16 ± 1.55 b	7.10 ± 1.78 b	17.129	0.0001
root biomass dry weight	0.16 ± 0.14 a	0.78 ± 0.31 b	0.92 ± 0.17 b	0.78 ± 0.20 b	15.159	0.0001
root biomass/plant (g of FW)	26.8 ± 22.8 a	134.2 ± 52.4 b	170.1 ± 32.4 bc	218.6 ± 72.2 c	21.350	0.0001

[a] The red beet biomass is calculated as 48000 plants/ha.

Table 5. Nutrient Contents in Red Beet Roots (values presented as per kilogram of dry weight) (AM ± SD) (N = 6)[a]

chemical	no treatment	mineral	urine and ash	urine	F[d]	P[d]
NO_3^- (g)	22.9 ± 5.4	37.2 ± 18.8	34.4 ± 12.8	30.0 ± 12.3		NS[b]
NO_3^- (mg/kg of FW)	2967 ± 705	4527 ± 2289	3799 ± 1413	3312 ± 1355		NS[b]
NO_2^- (mg)[c]	LDL	LDL	LDL	LDL		
Cl^- (g)	2.66 ± 1.06 a	2.51 ± 0.75 a	6.38 ± 1.77 b	4.33 ± 0.96 ab	10.166	0.001
total P (mg)	95.8 ± 14.3	89.3 ± 37.4	89.4 ± 21.2	89.3 ± 23.1		NS[b]
Na^+ (g)	2.04 ± 0.70 a	1.79 ± 0.66 a	3.61 ± 0.74 ab	5.55 ± 2.37 b	9.496	0.0001
K^+ (g)	29.9 ± 5.0 ab	34.3 ± 4.9 b	39.4 ± 6.8 b	20.6 ± 6.4 a	10.820	0.0001
Ca^{2+} (g)	0.64 ± 0.13	0.78 ± 0.08	0.87 ± 0.10	0.78 ± 0.14		NS[b]
Mg^{2+} (g)	1.09 ± 0.30	1.22 ± 0.22	1.51 ± 0.45	1.55 ± 0.42		NS[b]

[a] The NO_3^- contents are also presented on a FW basis. [b] Not significant. [c] Detection limit for NO_2^- N of 125 mg/kg of FW of red beet root. LDL, lower than the detection limit. [d] The F and P values were from an ANOVA.

Table 6. Chemical Contents of Red Beet Roots (grams per kilogram of dry weight) (AM ± SD) (N = 6)

chemical	no treatment	mineral	urine and ash	urine	F[a]	P[a]
protein	100.9 ± 16.2 a	126.5 ± 10.7 ab	149.0 ± 23.5 b	155.9 ± 24.9 b	8.364	0.001
D-glucose	4.18 ± 1.11	2.68 ± 2.28	3.64 ± 1.36	6.41 ± 2.00	3.096	0.059
sucrose	577.2 ± 24.4 b	567.9 ± 21.2 b	522.7 ± 35.7 ab	467.2 ± 86.4 a	4.678	0.017
D-fructose	0.28 ± 0.35	0.85 ± 0.68	1.00 ± 1.07	1.50 ± 0.91		NS
betanin	32.5 ± 6.9	27.2 ± 4.2	27.8 ± 8.8	24.6 ± 5.4		NS

[a] The F and P values were obtained via an ANOVA.

maize (*23*). In fact, the urine- and ash-fertilized biomass was somewhat better, though statistically not significant, than that from the mineral and urine fertilizer treatments, possibly because of the additional nutrients present in wood ash (*24*) and the better availability of nutrients in urine (*25*). These assumptions are supported by the work of Fenn et al. (*26*) which showed that the use of Ca^{2+} with ammonium fertilizer increased the absorption of ammonium in red beet and also increased the total biomass. Thus, urine and ash fertilizer and urine fertilizer produced 1720 and 656 kg/ha more edible red beet roots biomass than the mineral fertilizer, respectively.

Microbial Quality of Red Beet Roots. There were no enteric microbes detected in urine-fertilized red beet roots, but a few enteric microbes were detected in mineral-fertilized and urine- and ash-fertilized red beet roots (**Table 3**). These microorganisms might be due to soil contamination (*27*) as reported for spinach (*28*) or cabbage (*6*, *28*), and they may have been deposited by birds and other animals. However, it is always important to spread the urine fertilizer around the plants and avoid applying it directly onto any parts of plants. It is also important that the urine fertilizer should be terminated at least 1 month before harvesting to avoid any possible risk of crop contamination (*25*).

Chemical Quality of Red Beet Roots. This study showed that the NO_3^-, NO_2^-, P, Ca^{2+}, and Mg^{2+} contents of red beet roots from all fertilizer treatments were very similar compared to the contents of the other mineral nutrients (**Table 5**). The result for NO_3^- content is supported by the result presented for cabbage (*6*). The NO_3^- contents in red beet roots were positively correlated with Ca^{2+} (r = 0.568; P = 0.028) and Mg^{2+} (r = 0.456; P = 0.050) content, and this might be due to cations stimulating nitrate uptake as reported for maize (*29*). With respect to the mineral nutrients, the Na^+ and Cl^- contents were higher in urine and ash fertilized and urine-fertilized red beet roots than the corresponding levels in red beet roots from other treatments. This finding may due to the fact that the urine contained a large amount of Na^+ and Cl^-. A similar result was observed for Cl^- in cabbage (*6*) and in tomato (*7*). However, the amounts of Na^+ (40 ± 8 and 61 ± 26 mg/100 kg of FW) and Cl^- (70 ± 20 and 48 ± 11 mg/100 g of FW) present in urine and ash-fertilized and urine-fertilized red beet roots were similar as reported by Souci et al. (*30*) (i.e., 86 mg of Na^+/g of FW and 100 mg of Cl^-/100 g of FW) and the U.S. Department of Agriculture (*31*) (i.e., 78 mg of Na^+/100 g of FW) for red beet roots. The K^+ content was lower in urine-fertilized red beet root than in the other fertilizer treatments; this might be due to the lower K^+ or high Na^+ contents in urine fertilizer treatments. This result is supported by findings of work with tomato (*32*) and sugar beet (*33*) which showed that some plants may preferentially take up Na^+ instead of K^+. This can be attributed to cation competition; i.e., the uptake of Na^+ is strongly influenced by the level of exchangeable K^+ and Na^+ in the soil (*33*).

Protein, Soluble Sugars, and Betanin. The protein contents in red beet roots were similar to values reported by Sauci et al. (*30*), i.e., 10.5 g/kg of FW, and by the U.S. Department of Agriculture (*31*), i.e., 16 g/kg of FW. The protein content was higher in fertilized red beet roots compared to control (**Table 6**), as reported previously for sugar beet (*34*), canola (*35*), and tomato (*7*). Furthermore, protein contents in red beet roots were positively correlated with Mg^{2+} (r = 0.699; P = 0.0001), Ca^{2+} (r = 0.698; P < 0.0001), and Na^+ (r = 0.719; P < 0.0001) concentrations. This emphasized the importance of fertilization; i.e., human urine can be considered as an alternative fertilizer not only to increase the biomass but also to improve the yield quality. Sucrose was the dominant compound among soluble sugars. The sucrose content

in urine-fertilized red beet roots was lower ($P = 0.025-0.31$) compared to that in nonfertilized and mineral-fertilized red beet roots (**Table 6**), but these values were close to those presented by Souci et al. (*30*), i.e., 61−89 g/kg of FW. The sucrose concentration was negatively correlated with that of Mg^{2+} ($r = -0.527$; $P = 0.020$) and Na^+ ($r = -0.801$; $P < 0.0001$), although a previous study in potato claimed that the sucrose content was not influenced by any fertilizer treatment (*36*).

The amount of betanin was similar in red beet roots from all fertilizer treatments (**Table 6**). However, the yield of betanin per hectare was higher in fertilized plots since the red beet yields were much higher in the fertilized plot. The betalains in plant extracts have good antioxidant properties, and recently, there has been renewed scientific interest in the functional properties of these plants (*37*−*39*).

Flavor Quality. In the taste assessment test, there were no taste differences between mineral-fertilized red beet roots and urine-fertilized and urine- and ash-fertilized red beet roots ($P < 0.05$) (*40*). Of 17 panelists, five preferred the mineral-fertilized and another five the urine-fertilized red beet roots and seven preferred the urine and ash-fertilized red beet roots. Therefore, there was no type of red beet root that was statistically preferred over the other (*40*), which was also observed for tomato (*7*).

Residual Nutrients in Soil. The residual nutrient, total NO_3^-−N, NO_2^-−N, Cl^-, and NH_4^+−N, P, K^+, Cl^-, Na^+, Ca^{2+}, and Mg^{2+}, contents were similar in the soil after different treatments (**Table 1**). The total N was elevated in the soil after treatments compared to the situation before cultivation which could be due to the presence of some organic residues in soil as reported by Pradhan et al. (*7*) in tomato cultivation. Although the Na^+ and Cl^- contents were higher with urine fertilizer, their contents were similar in the soil applied with mineral fertilizer and control treatments which might be due to the high water solubility of Na^+ and Cl^- combining to form salt which could have been washed out with rainwater. Similarly, the levels of Ca^{2+} and Mg^{2+} were also higher in wood ash, but this did not increase the level of these elements in soil after cultivation, a result in agreement with results reported by Pradhan et al. (*7*). However, the soil pH and conductivity were increased after the application of wood ash fertilizer, but it has been reported to be neutralized within 12 months (*41*).

In conclusion, this study supports our main hypothesis; i.e., the applied N amount was similar in all fertilizer treatments, and thus, the growth rate, total biomass, and root biomass of the red beet were also similar with all fertilizer treatments and higher than in the unfertilized situation. This study showed that urine and ash fertilizer and the urine fertilizer could produce 27% and 10% more red beet root biomass, respectively, than the mineral fertilization. In addition, nitrate, protein, D-glucose, D-fructose, and betanin concentrations in red beet roots from urine with or without ash and mineral fertilizer were similar to each other. We conclude that human urine, with or without ash, could be used as a fertilizer for red beet cultivation and produce a similar or even larger amount of red beet biomass. Similar results could be expected in plants of other species from the same family. We recommend that the urine should be used at least 3 days before ash application to avoid possible ammonia evaporation due to the increasing pH. More research is needed to clarify how different nutrient contents of urine and ash can improve the cultivation of valuable crop plants.

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