Research Paper

Effect of human urine application on cabbage production and soil characteristics

Yitayal Addis Alemayehu, Assefa Adane Demoz, Mekonnen Amberber Degefu, Getachew Dagnew Gebreeyessus and Sintayehu Fetene Demessie

ABSTRACT

The aim of this study was to examine the effect of human urine on soil quality and salinity through repeated applications, and compare it with the effects of synthetic fertilizer and non-fertilized treatments. Six different fertilizer mixtures were applied to pots of head cabbage: 100% tap water, 100% urine, 1:1 urine and tap water, 1:2 urine and tap water, 1:3 urine and tap water, and synthetic fertilizer. The study design was completely randomized design (CRD) with three replications of pot-based experiments. The growth and yield parameters of producing head cabbage (*Brassica oleracea*) among treatments were compared. Soil residual test of the optimum treatment was also conducted for the optimum treatment to examine the effect on its characteristics. The optimum yield was obtained from the application of 1:3 of urine and water, and comparable to the synthetic fertilizer ($F = 21.78$; $p = 0.964$). The difference in the electrical conductivity of soil was statistically significant ($F = 2.324$; $p = 0.049$) after three rounds of applications which should be considered during urine fertilizer utilization. Generally, urine contains nutrients, which can substitute synthetic fertilizer, at a dilution factor of three. However, education should be delivered to enhance public acceptance and to create awareness on urine collection, storage and application for sustainable utilization.

Key words | cabbage, human urine, nutrients, pH, salinity, yield

Yitayal Addis Alemayehu (corresponding author)
Assefa Adane Demoz
Mekonnen Amberber Degefu
Getachew Dagnew Gebreeyessus
Sintayehu Fetene Demessie
Kotebe Metropolitan University,
Addis Ababa,
Ethiopia
E-mail: *yituaddis@gmail.com*

INTRODUCTION

Global agriculture depends largely on synthetic plant nutrients to increase and enhance productivity (Simha *et al.* 2016). The increasing price of nutrients has aggravated the cost of food items. In the midst of these dual problems, there is the possibility of farmers producing food while contributing to the orderliness of the environment. This can be achieved by recycling human and animal excreta, part of which, human urine, is becoming a concern (Mihelcic *et al.* 2011).

Urine contains most of the essential plant macronutrients: nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) and micronutrients: boron (B), copper (Cu) and zinc (Zn) excreted by human beings, and all are found in plant available forms (Richert *et al.* 2010). However, the phosphorus/nitrogen and potassium/nitrogen ratios are slightly lower than synthetic fertilizers and the need of many crops due to the higher nitrogen content in the urine compared to P and K. Yet, it can still be applied as a well-balanced N-rich fertilizer (Jönsson *et al.* 2004; Pradhan *et al.* 2010; Richert *et al.* 2010), together with other macro and micro plant nutrients.

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Urine may also contain pathogens such as viruses (JC polyomavirus, human adenovirus, rotavirus, hepatitis A virus and norovirus GII) and bacteria (*Aeromonas* spp., *Clostridium perfringens* and *Shigella* spp.) (Bischel *et al.* 2015a, 2015b; Lahr *et al.* 2016) or be contaminated with urinary tract viruses (Goetsch *et al.* 2018) and faeces (Schönning *et al.* 2002), which have the potential to be transferred to soil and vegetables. As well, the perceptions and attitudes of people, and cultural settings, may cause problems in using urine as a fertilizer among farmers and consumers (Lamichhane & Babcock 2013; Wilde *et al.* 2019).

Despite the pathogen content and cultural problems, different mechanisms have been reported for nutrient recovery from urine. The simplest way reported for P recovery is the precipitation of struvite ($MgNH_4PO_4{\cdot}6H_2O$) from source-separated urine. The P content of struvite varies depending on the recovery process. For example, Krähenbühl *et al.* (2016) reported about 60%, and up to 98% of human excreted phosphorus is reported by de Boer *et al.* (2018). However, the process can precipitate only 3% of N in struvite (Pronk & Koné 2009), and about 97% of N is lost. Jagtap & Boyer (2018) reported 99% N, 91% P, and 80% K recovery using an integrated, multi-process approach of struvite precipitation, NH_3 stripping and evaporation. In the processes, the fate of secondary and micro-nutrients has not been reported, which may be discarded along with other substances in the supernatant. In addition, there may be a lack of economic and technological feasibility for larger-volume applications. Therefore, storage, dilution and direct application can rather minimize nutrient loss, and be taken as a sustainable and a circular solution to the issues of sanitation, water and food security.

Pradhan *et al.* (2007) and Chrispim *et al.* (2017) reported the experimental results from the application of urine as a nutrient source for cabbage, corn and lettuce production. However, the effect on soil has not been adequately considered, and the comparison is made only for non-fertilized treatments in the case of Chrispim *et al.* (2017). Studies also indicated that storage of urine for at least six months is required to kill pathogens in it (Pradhan *et al.* 2007, 2009a, 2009b, 2010; Viskari *et al.* 2018). In Bischel *et al.* (2015a), detection of viruses (JC polyomavirus and adenovirus) and bacteria (*Aeromonas* spp., *C. perfringens* and *Shigella* spp.) after four months of storage has been reported. In such cases, storage for a longer period may be required as the length of storage time is dependent on the type of pathogen in urine and storage temperature. For example, it can be stored for 1 week at a temperature of 19 °C, 2 weeks at 28 °C (Pradhan *et al.* 2011), one month at 20.5 °C (Amoah *et al.* 2017; Pandorf *et al.* 2019), two months at 27 °C (Akpan-Idiok *et al.* 2012) and 11 months at 25 °C (Jana *et al.* 2012). During storage, urine becomes hydrolyzed and its pH increases, which can result in ammonia volatilization if it is not stored in tightly closed containers (Tilley *et al.* 2008).

Despite several studies on nutrient content and recovery technologies, few studies have reported the frequency of application and the effect of repeated doses on pH and salinity of soil after production. The purpose of this study was, therefore, to experimentally determine the most effective dilution factor of source-separated urine and the impacts on soil characteristics over repeated applications during head cabbage production.

METHODS

Experimental design and treatments

The treatments consisted of different concentrations of urine as a fertilizer: T_0 (negative control, none fertilized (100% tap water)), T_1 (100% urine), T_2 (1:1 urine and water), T_3 (1:2 urine and water), T_4 (1:3 urine and water) and T_5 (synthetic urea mixed with compound fertilizer as a positive control (urea + NPK (19:19:19)) (Table 1). A dilution factor up to three was specified, as dilution more than this could cause flooding and waterlog seeds in an attempt to satisfy the nutrient requirements of cabbage (Liu *et al.* 2017). The experiment was laid out as a completely randomized design (CRD) with three replications (Figure 1) with 10 L plastic pots arranged in a greenhouse having an area of 12 m^2 (3 m × 4 m). Both the urine and synthetic fertilizer application were performed based on fertilizer application guidelines reported by Onduso (2011) and Richert *et al.* (2010). Each pot was spaced at 50 cm, both vertically and horizontally. All the pots (T_0–T_5) were administered tap water, and different concentrations of urine/synthetic fertilizer (Table 1). Five cabbage seeds were planted in

Table 1 | Nutrient application rate and intervention frequency

Treatment	Combination (urine:water)	Total volume (L)/pot	Calculated N, P, K mass (g)	Application phases/pot	Watering/pot/week
T_0 (−Control)	0:1	15	0	30	3
T_1	1:0	1.5	4.29, 0.3, 1.9	3	3
T_2	1:1	0.75	2.15, 0.13, 0.96	3	3
T_3	1:2	0.36	1.07, 0.07, 0.46	3	3
T_4	1:3	0.31	1.00, 0.06, 0.40	3	3
T_5 (+ Control)	Urea + NPK (19:19:19) (45 g +5 g = 50)	NA	2.3, 0.95, 0.95	3	3

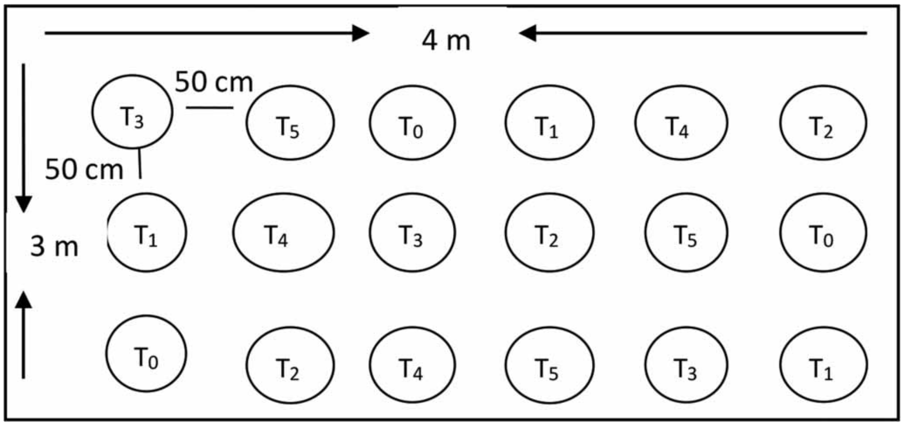

Figure 1 | Experimental design employed in the study.

each pot to observe the germination capacity, after which, only one shoot was allowed to grow. Once the optimum production had been identified (T_4), the experiment was repeated twice more (three trials in total over 18 months) to examine the soil pH and salt concentration change. For the other treatments, measurements were taken only for one round of application (six months). Cabbage was selected for the experiment as it is easy to measure the growth and yield parameters, commonly produced around the study area, and has a growth period of five to six months.

Urine collection, pre-analysis and application setup

The urine used was collected from students at Kotebe Metropolitan University (KMU) compound in 25 L containers (jerry cans) in five selected toilets for 3 days. A total of 125 L of urine was stored for about three months (21 March 2018–23 June 2018) in the temperature range of 19 °C to 22 °C. Storage is used to raise the pH and kill potential pathogens in urine (Lahr *et al.* 2016; Goetsch *et al.* 2018) or when it is contaminated with faecal matter (Pradhan *et al.* 2011; Amoah *et al.* 2017; Pandorf *et al.* 2019). A storage time of less than one month is reported as adequate to mitigate risks associated with even urinary tract virus (Goetsch *et al.* 2018). It is also used to raise the ammonia concentration for further stripping or precipitation (Zamora *et al.* 2017; Jagtap & Boyer 2018; Wei *et al.* 2018; Nagy *et al.* 2019). In the current study, urine was stored for the former reason, and for appropriate dilution. Fresh urine was sampled for pH and total ammoniacal nitrogen (TAN), and the stored urine was taken to the laboratory for chemical and nutrient analysis. The type and physico-chemical characteristics of the soil which was used for cultivation had been measured prior to urine application. Then, 18 pots were filled with 10 kg of the soil having the same physico-chemical characteristics, and prepared for the six treatments: T_0–T_5. Treatments were applied prior to sowing, at 2 weeks and two months after sowing (Richert et al. 2010). To avoid odour, foliar burning and ammonia loss, the urine was covered by the soil immediately after application (Pradhan et al. 2010). The total nutrient application rates and intervention frequency are indicated in Table 1.

Sampling

The soil sample was taken from 3 m × 4 m (12 m^2) of land dug to 30 cm deep after the upper cover had been removed. The soil was then mixed and triplicate samples (each of 2 kg) were taken to the laboratory of Ethiopian Construction Design and Supervision Works Corporation. The same type of soil was used for the vegetable production, and another triplicate sample was taken to a laboratory to observe the effect of urine application on the soil's physico-chemical properties after production. In addition, triplicate samples of cabbage from the optimum treatment and the controls were taken for analysis to measure the nutrient uptake of the head cabbage. For urine, triplicate samples of 100 mL were taken at different storage times (every week until the pH remained constant) to the laboratory for analysis.

Physico-chemical analysis

The pH (at 1:2.5 soil and water ratio) and EC (at 1:5 soil and water ratio) were measured by a CP-505 pH meter employing the procedures in APHA 4500-H^+B conductivity cell potentiometric method (APHA 2005). The moisture content and organic carbon of soil samples were determined by gravimetric and Walkley Black method, respectively, and texture class by hydrometer following the procedures in APHA 2540G. The Kjeldahl method for total nitrogen (Pearson 1976), and the ammonium acetate method for potassium were employed, respectively. A DTPA extraction method was used for the determination of manganese, copper, iron and zinc (Aşkın et al. 2017). For phosphorus determination, the Olsen method was applied (Iatrou et al. 2014). The available sulfur was analysed following KH_2PO_4 extraction and turbidimetric methods (Crosland et al. 2001). Soluble salts (Na, K, Ca, Mg, Cl^-, HCO_3^-, CO_3^{2-}) were determined using volumetric and SO_4^{2-} by turbidimetric methods (APHA 2005). All the analyses were performed in the Ethiopian Construction Design and Supervision Works Corporation's laboratory.

For the urine samples, APHA (2005) methods were also employed for the determination of the following parameters: total and available P were determined by ascorbic acid method, and Na^+ and K^+ were determined by flame photometric method. Titrimetric method was employed for Ca^{2+} and Mg^{2+} determinations. Cl^- and SO_4^{2-} were determined by Mohr argentometric and turbidimetric methods, respectively. Micro-nutrients: 1, 10-phenothroline, periodate oxidation, bicinchoninate and zincon methods were used for Fe, Mn, Cu and Zn, respectively. For TN and NH_4^+-N determination, the Kjeldahl and Nessler methods were employed, respectively. The pH and EC were measured with the same method employed for the soil and the cabbage. pH and NH_4^+-N were determined both for fresh and stored urine.

Growth and yield measurements

Plant growth parameters (morphological characters) were measured and observed as per the growth nature of the plant every 2 weeks, whereas biomass estimation (fresh weight) was performed at the end of the experiment. The plant germination capacity, germination time, height (from base to tip of the longer leaf), leaf area (length × centre width) and number of leaves were determined by the methods used in Mohana et al. (2011) and Amoah et al. (2017). For the purpose, five seeds were sown in each pot in triplicate, and only one seed per pot was treated after germination time and germination index had been measured (others removed from each pot). The measurement height of cabbage, number and area of leaves were performed every 2 weeks until cabbage head formation. The germination capacity was calculated by using Equation (1) reported by Fessehazion et al. (2014) as:

$$Germination\ Capacity\ (GC) = \frac{No.\ of\ seeds\ germinated}{Total\ no.\ of\ seeds} \times 100 \quad (1)$$

The nutrient uptake and recovery efficiency of the treatments were determined by using Equations (2) and (3) applied by Amoah *et al.* (2017) as:

$$\text{Nutrient uptake}\left(\frac{g}{pot}\right) = \frac{\text{Nutrient content} \times \text{Sample dry weight (g/pot)}}{100} \quad (2)$$

$$\text{Nitrogen Recovery Efficiency} = \frac{\text{N uptake from fertilized pot}\left(\frac{g\,N}{pot}\right) - \text{from non-fertilized pot}}{\text{Total nitrogen applied}} \quad (3)$$

Statistical analysis

The collected data were analysed using Origin Pro 9.0 software. The mean difference among treatments of urine and the controls were tested using one-way analysis of variance (ANOVA), and pair-wise significant differences were determined using a Tukey multiple comparison test. The results were considered statistically at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of storage time on pH of urine

The nitrogen content and type in water, wastewater and urine is highly dependent on pH. Since the pH is variable, storing urine until the pH becomes constant is essential. For sustainable management and utilization, the pH value should be known before application to land, otherwise it could negatively affect the soil properties. In this study, the pH of fresh urine was 6.18 ± 0.05; however, it increased to 9 and remained almost constant at 9.18 ± 0.01 after three months of storage, as indicated in Figure 2. The nitrogen in fresh urine is fixed in the form of urea ($CO(NH_2)_2$) (85%), and as total ammonia ($NH_3 + NH_4^+$) (5%) (Udert *et al.* 2006).

During storage, the urea was almost completely hydrolysed after three-month storage time in the temperature range of 19–22 °C, and the NH_4^+ concentration reached $2,683 \pm 31$ mg/L and was 392 ± 67 for the fresh urine. The increase in pH during storage is attributed to the increase in the NH_4^+ concentration. This is also supported by Wei *et al.* (2018), as they reported a change in pH from 4.2 ±

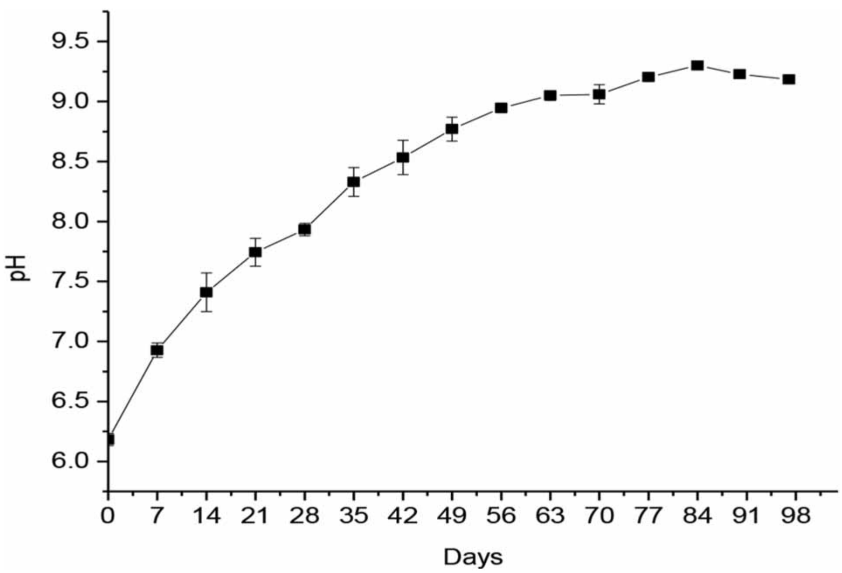

Figure 2 | Effect of storage time on pH of human urine.

0.3 to 8.9 ± 0.1 and NH_4^+ concentration from 443 ± 78 mg/L to 5,615 ± 266 mg/L during hydrolysis at 18–25 °C for about three months. Pandorf *et al.* (2019) reported that urine can be hydrolysed after a one-month storage time in the temperature range of 14 °C–27 °C.

Different reports have supported storage of urine in order to kill pathogens in it or in case contaminated with faecal matter (Pradhan *et al.* 2007; Chrispim *et al.* 2017). If microorganisms are found in urine, they usually die and do not pose any threat for further utilization of urine as soil fertilizer (Heinonen-Tanski *et al.* 2007; Nagy & Zseni 2017). However, storage for a longer period (more than six months) is recommended to minimize risks of pathogen transfer at room temperature (Jana *et al.* 2012). Storage is also important to maintain a constant pH for appropriate dilution and application. The pH value after 7 days of storage remained alkaline, which could be due to high ammonia production, and that could alter the pH of soil which originally was about 6 (Table 2), and limit growth if it is applied without dilution/neutralization. Therefore, either it should be diluted or mixed with other substances with pH below 7.

Nutrients and other characteristics in urine and soil

The results of the nutrient and salt content of urine and soil are indicated in Table 2. The concentration of the total nitrogen in stored urine was 2,862 mg/L, but the nitrate and nitrite contents were about 0.65 mg/L, together very low, indicating that the major nitrogen component at higher pH is ammonia. The urine contained salts (expressed as EC, Cl^- and SO_4^{2-} contents) which could affect the soil characteristics. It also contained considerable micro-nutrients (Fe, Zn, Cu and Mn) suitable for crop and vegetable growth.

The soil used for cabbage production was Vertisol, clayey in texture and slightly acidic. Its nutrient composition indicated that the soil should be supplemented with nutrients for cabbage production.

Table 2 | Characteristics of stored human urine and soil before application ($N = 3$)

Stored human urine		**Soil characteristics**	
Parameter	**Mean ± SD**	**Parameter**	**Mean ± SD**
pH	9.18 ± 0.21	Sand (%)	20.21 ± 0.12
EC (mS/cm)	19.81 ± 1.47	Clay (%)	56.69 ± 2.14
		Silt (%)	23.10 ± 2.09
TN (mg L^{-1})	2,862 ± 63	Texture class	Clay
NH_4^+ (mg L^{-1})	2,683 ± 31	pH	6.22 ± 0.05
NO_3^- (mg/L)	0.58 ± 0.13	EC (mS cm^{-1})	0.25 ± 0.02
NO_2^- (mg L^{-1})	0.07 ± 0.02	MC (%)	4.75 ± 0.14
TP (mg L^{-1})	178.36 ± 9.22	SOC (%)	1.24 ± 0.10
K^+ (mg L^{-1})	1,276 ± 43	TN (%)	0.10 ± 0.07
Ca (mg L^{-1})	20.47 ± 2.69	Available P (mg kg^{-1} of soil)	22.48 ± 5.94
Mg (mg L^{-1})	18.45 ± 2.78	Available K (mg kg^{-1} of soil)	1,008 ± 21
Na (mg L^{-1})	793.09 ± 18.93	Available S (mg kg^{-1} of soil)	28.99 ± 5.56
Cl^- (mg L^{-1})	1,002 ± 22	Fe (mg kg^{-1} of soil)	17.75 ± 1.35
SO_4^{2-} (mg L^{-1})	491.72 ± 18.10	Cu (mg kg^{-1} of soil)	1.63 ± 3.23
Fe (μg L^{-1})	357.78 ± 4.71	Zn (mg kg^{-1} of soil)	2.77 ± 0.30
Cu (μg L^{-1})	7.30 ± 1.30	Mn (mg kg^{-1} of soil)	26.42 ± 0.14
Zn (μg L^{-1})	265.36 ± 5.74		
Mn (μg L^{-1})	8.62 ± 1.11		

SOC, soil organic carbon; TN, total nitrogen; MC, moisture content.

Germination period and germination capacity

The nutrient-deprived treatment (the negative control) and synthetic fertilizer applied treatment (positive control) germinated faster (within 3 days) than urine-treated pots (4–7 days) (Table 3). For urine received treatments (T_1–T_4), the germination period increased as dilution was decreased, and reached 7 days for non-diluted urine applied pots. The same is true for the amount of germination per pot. Out of the five cabbage seeds sown in each pot of each treatment and replica, only three of them germinated for non-diluted urine treatments, which was likely due to difficulty in adapting to the increase in NH_3 concentration in the soil while taking up the nutrients. The treatments that were diluted more than 50% and the positive control germinated completely (Table 3).

Although the germination time was relatively longer, diluted urine-treated treatments, especially 1:2 and 1:3 ratios, were almost comparable with the growth of the cabbage treated with synthetic fertilizer. This is contrary to the report by Pradhan *et al.* (2007), which concluded that urine-fertilized plants may germinate and grow more rapidly, so the plants can be harvested earlier, thus making more efficient use of the land. In this study, urine-fertilized cabbage took longer periods to germinate, but provided yield almost in the same period as other treatments.

Growth results

The growth parameters: number and area of cabbage leaves and height of the cabbage were measured for 75 days, until head formation began. Except for treatments T_0 and T_1 (which took about 90 days), others started head formation after 70 days. This could be explained by the importance of nitrogen for leaf growth and faster production. The cabbage grown with diluted urine was deep green, while the colour of leaves for the unfertilized cabbage was lighter green than the others which displayed colours between these two extremes.

Table 3 | Germination capacity of cabbage for each of the treatments ($N = 3$)

Treatment	Germination period (days)	Number of seeds germinated	Germination capacity (%)
T_0 (− Control)	3 ± 0.58	4 ± 0.00	80
T_1 (1:0)	7 ± 1.53	3 ± 0.58	60
T_2 (1:1)	5 ± 0.00	4 ± 0.00	80
T_3 (1:2)	4 ± 0.00	5 ± 0.58	100
T_4 (1:3)	4 ± 0.58	5 ± 0.00	100
T_5 (+ control)	3 ± 0.58	5 ± 0.00	100

As indicated in Figure 3, the growth of all treatments had comparable rates by the 15th day of cultivation. During this period, the T_3 and the positive control had almost the same growth. At 60 days of cultivation, the growth of T_3 was comparable with the synthetic fertilizer treatment. The numbers of leaves on cabbages applied with diluted urine were more than the non-fertilized and undiluted urine treatments after 75 days. The fewer numbers of leaves of T_1 (undiluted urine) was expected as the pH of applied urine had been about 9, which could limit growth due to NH_3 toxicity. Generally, the numbers of leaves of treatments T_3 and T_4 were comparable with the positive control (synthetic fertilizer) after 60 days.

Within the first 2 weeks, the height of cabbage in treatments T_4 and the positive control was observed to be the shortest, but reached 28.4 ± 1.22 cm and 29.5 ± 2.00 cm, respectively, becoming the tallest after two months. The reason could be the addition of lower nitrogen before planting which was later adjusted by adding more urine for the second round after 15 days. The one-way ANOVA test indicated that the lengths of diluted urine applied treatments differed significantly from the non-diluted urine and the negative control ($F = 26.31$, $p = 4.48E\text{-}6$). This indicated that addition of diluted urine resulted in better growth in the height of head cabbage relative to nutrient-deprived treatment (negative control). The treatment that received three times diluted urine (T_4) was the longest to be compared with the positive control. If length only is considered, all treatments that received diluted urine were not significantly different to one another.

With regard to area of leaves, narrowed leaf was observed within the first 2 weeks, but dramatically increased and became wider after the addition of the second round of urine and synthetic fertilizer application. This could be due to the application of more nitrogen as it supports vigorous vegetative growth and the dark green colour of cabbage, as reported in Semuli (2005). Statistically, treatments T_3 and T_4 provided no significantly different results with that administered with synthetic fertilizer (positive control) ($F = 2.32$,

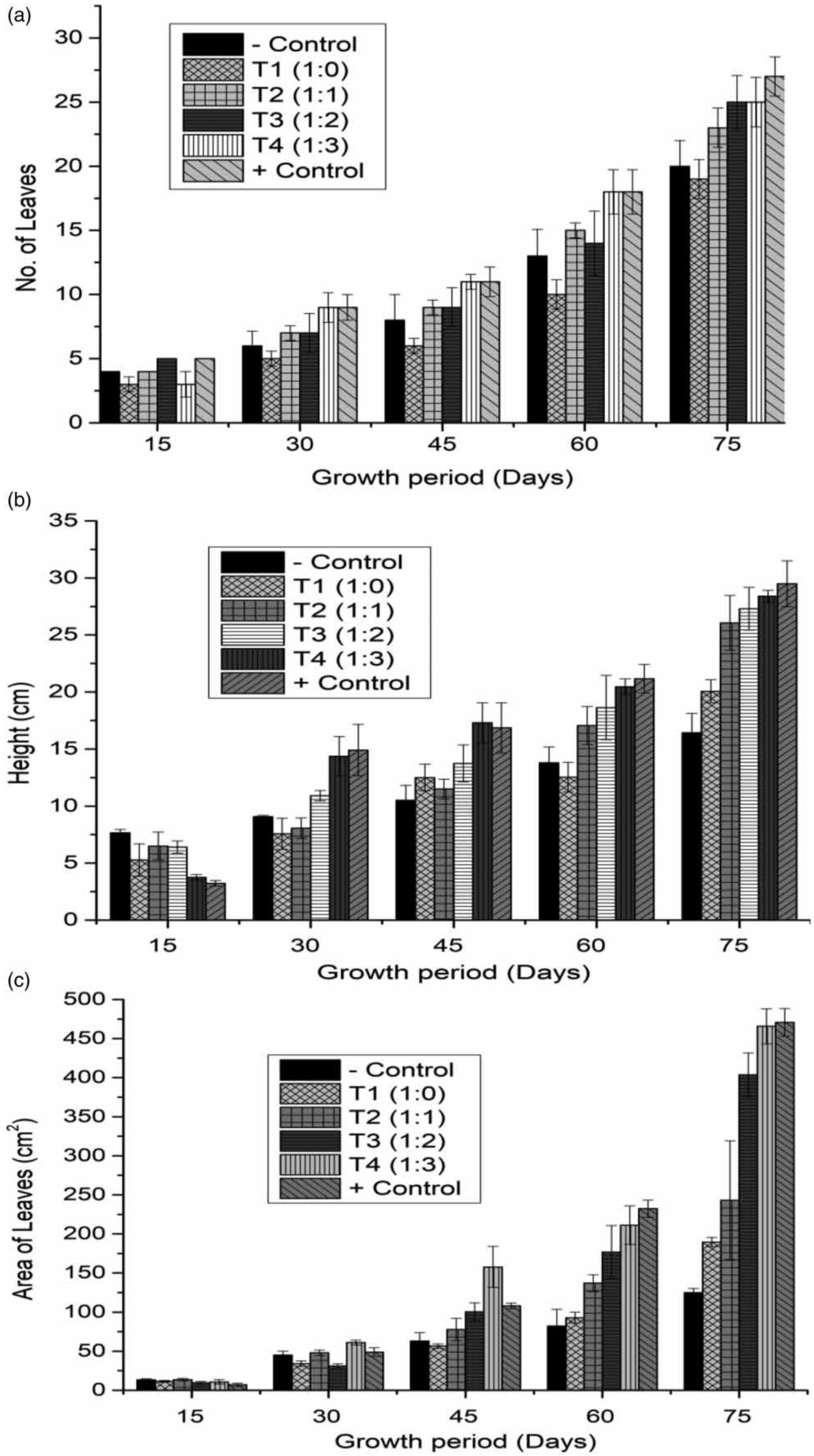

Figure 3 | Number of leaves (a), height (b) and area of leaf (c) of cabbage with different growth periods.

$p = 0.24$). Therefore, to produce leafy vegetables like head cabbage, land application with two times or three times diluted urine could be important.

Generally, the growth rate (expressed as number and area of leaves, and height of cabbage) of treatments T_3 and T_4 differed significantly from others except with that of the positive control (the synthetic fertilizer). The total growth rate of T_4 was slightly higher than T_3 and slightly lower than the synthetic fertilizer, but not significantly different. This implies that T_4 (urine diluted three-fold) provided the optimum production and shall be applied at this concentration for vegetables. The difference of growth rates among the treatments could be attributed to the difference in the nutrient supplement, pH and salinity of the urine. The result of this study is contrary to the one reported by AdeOluwa & Cofie (2012), which shows a 40% increment in vegetable productivity than synthetic fertilizer. It is also shown that plant growth and leaf production was significantly high for urine-applied cultivation of both corn and lettuce (Chrispim *et al.* 2017), which is also contrary to the current study. The difference in the results of the studies could be due to a difference in soil type, soil quality, urine storage and nutrient application rates.

Yield results

Head weight and circumference of head cabbage produced in the treatments were measured as a yield indicator that can be compared both with the negative and positive controls. The undiluted urine application (T_1) provided the lowest production, even lower than the non-fertilized treatment (T_0) (Table 4), which may be due to NH_3 toxicity manifested by the increase in the pH of urine after hydrolysis.

The yield results of diluted urine treatments (T_2–T_4) were better than each of the non-diluted (T_1) and nutrient-deprived (T_0) treatments. T_3 and T_4 had values of head weight which is not significantly different ($F = 21.78$, $p = 0.16$) from synthetic fertilizer. This indicates that urine should be diluted in order to get more production as cabbage requires a pH ranging from 5.5 to 6.5 for higher production (TNAU 2016). This is supported by Pandorf *et al.* (2019), who claimed that the application of diluted urine significantly increases the yield compared to non-fertilized treatments and it is also comparable with synthetic

Table 4 | Yield results of cabbage production for the treatments ($N = 3$)

Treatment	Head weight (fresh wt (kg)	Circumference (cm)
T_0 (0:1)	0.76 ± 0.10a	44.57 ± 1.33a
T_1 (1:0)	0.67 ± 0.08a	40.77 ± 1.80a
T_2 (1:1)	0.95 ± 0.07a	45.78 ± 0.97a
T_3 (1:2)	1.18 ± 0.06b	56.55 ± 2.41b
T_4 (1:3)	1.47 ± 0.08b	62.85 ± 1.08b
T_5 (Synthetic fertilizer)	1.54 ± 0.11b	66.46 ± 1.12b

Different letters in columns show statistical difference at 0.05 significant levels.

fertilizer for snap bean and turnip productivity. Other findings also showed that the use of urine fertilizer increases the biomass of the cabbage head, and it is expected to have similar effects on other crops too (Pradhan *et al.* 2007). The difference in yield between treatments may also be due to the differences in the nutrient, pH and salt supplements. Although the fertilizing value of urine is comparable to synthetic fertilizer, urine-treated cabbage may contain more microorganisms compared to synthetic fertilizer treated cabbage. Pradhan *et al.* (2007) and AdeOluwa & Cofie (2012) reported the presence of indicator microorganisms on cabbage and amaranthus, respectively. Thus, ingestion of raw vegetables cultivated with urine may result in undesirable consequences on consumers.

The average cabbage weight per head for treatments T_3 and T_4 (1.18–1.47 kg) was higher than the weight surveyed in Addis Ababa 02 market in Ethiopia (0.72–1.45 kg). This is contrary to the field study conducted in Ghana where 0.6–0.8 kg is reported, considerably lower than the market (1.1–1.2 kg) (Amoah *et al.* 2017). Although there was no statistically significant difference between T_3 and T_4 regarding head weight ($F = 21.78$, $p = 0.16$), they differ in circumference, thus it can be deduced that the 1:3 combination is better for optimum yield. A similar study carried out in Zimbabwe reported that yields of vegetables and maize irrigated with urine at a dilution factor of three were the highest (Morgan 2004).

Nutrient uptake and N-recovery efficiency

The nutrient contents of produced cabbage in each of the treatments were not statistically different from one another

($F = 1.03$, $p = 0.45$). Treatments T_1 to T_4 (diluted and non-diluted urine) provided comparable nutrient content with that of the synthetic fertilizer (T_5) (Table 5). This indicates that the soil needs to be supplemented with nutrients before the cultivation of cabbage or other crops.

However, the nitrogen recovery efficiency of T_4 was significantly different from other treatments ($F = 12.01$, $p = 0.03$), indicating dilution requirement of the soil before application. Generally, estimates of overall efficiency of applied fertilizer have been reported to be about or lower than 50% for N, less than 10% for P, and about 40% for K (Baligar *et al.* 2001). T_4 of the current study exceeded this figure a little, which could show comparable plant availability of urine nutrients with the synthetic fertilizer.

Effect of repeated application of urine on selected soil characteristics

The effect of urine application on soil pH, nutrients and salinity was tested on the treatment that yielded optimum production (T_4). It was found that the pH of the soil used for head cabbage production remained almost constant after three rounds of urine application. This supports the idea that stored human urine should be diluted or mixed with lower pH liquids before application. The result also showed no significant difference for most of the parameters MC, SOC, TN, Fe and Cu, even after three rounds of application (Table 6). Both the statistically unchanged result of TN and the significant decrease in K concentration

Table 5 | Nutrient content, uptake and N-recovery efficiency of head cabbage ($N = 3$)

Treatment	Nutrient content (%)			Nutrient up take (g/pot)			N-recovery efficiency (%)
	N	P	K	N	P	K	
T_0	0.20 ± 0.02a	0.11 ± 0.01	1.27 ± 0.12	0.29 ± 0.02	0.16 ± 0.03	1.41 ± 0.21	Control
T_1	0.38 ± 0.03b	0.29 ± 0.05	1.33 ± 0.02	0.51 ± 0.03	0.38 ± 0.04	1.83 ± 0.21	5.13 ± 1.03a
T_2	0.35 ± 0.04b	0.27 ± 0.02	1.66 ± 0.05	0.65 ± 0.03	0.51 ± 0.02	3.18 ± 0.21	16.74 ± 2.76b
T_3	0.32 ± 0.05b	0.25 ± 0.01	1.57 ± 0.04	0.71 ± 0.04	0.57 ± 0.06	3.79 ± 0.41	41.12 ± 4.63c
T_4	0.30 ± 0.04b	0.19 ± 0.03	1.43 ± 0.04	0.84 ± 0.03	0.56 ± 0.05	4.16 ± 0.81	56.00 ± 5.96d
T_5	0.36 ± 0.03b	0.15 ± 0.01	1.38 ± 0.04	1.07 ± 0.04	0.48 ± 0.01	4.30 ± 0.86	34.35 ± 4.21c

Different letters in numbers show statistical difference at 95% confidence interval.

Table 6 | Effect of repeated urine application on soil characteristics ($N = 3$)

Parameter	Values (mean ± SD)			
	Before application	1st trial	2nd trial	3rd trial
pH-H_2O (1:2.5)	6.22 ± 0.05a	6.33 ± 0.08a	6.38 ± 0.07a	6.41 ± 0.09a
MC (%)	4.75 ± 0.14a	4.64 ± 0.19a	4.26 ± 0.21a	3.84 ± 0.17a
SOC (%)	1.54 ± 0.10a	1.94 ± 0.17a	2.13 ± 0.16a	2.37 ± 0.18a
TN (%)	0.17 ± 0.07a	0.14 ± 0.03a	0.14 ± 0.05a	0.11 ± 0.03a
Av. P (mg P_2O_5/kg soil)	22.48 ± 5.94a	27.68 ± 6.88a	37.75 ± 6.42b	50.49 ± 8.75c
Av. K (mg K_2O/kg soil)	1,007.67 ± 21.28a	871.75 ± 17.94b	643.25 ± 15.68c	470.49 ± 12.54d
Fe (mg/kg of soil)	18.75 ± 1.35a	21.69 ± 2.98a	22.48 ± 3.13a	24.70 ± 3.18a
Cu (mg/kg of soil)	1.63 ± 0.23a	1.58 ± 0.23a	1.39 ± 0.41a	1.18 ± 0.21a
Zn (mg/kg of soil)	2.77 ± 0.30a	3.42 ± 0.56b	3.79 ± 0.67b	4.25 ± 0.71b
Mn (mg/kg of soil)	262.42 ± 28.14a	284.74 ± 27.13a	328.84 ± 31.15b	373.50 ± 31.21c

Different letters in rows show statistical difference at 0.05 significance level.

(Table 6) indicate that head cabbage takes up these nutrients from the urine rather than the soil reserves. This is also reported in Atanasova *et al.* (2007), who stated the decrease of N and K residuals after the harvest of head cabbage crop in comparison with the initial soil reserves indicated complete absorption of the nutrients from supplied fertilizers.

The available phosphorus and zinc increased in the soil after the first round and manganese after the second round in soil reserves. This may signal a need for the rotation of crop type each year for nutrient absorption and optimum production.

The most common negative effects reported for human urine is an accumulation of salts in the soil. For example, Mnkeni *et al.* (2008) reported that soil electrical conductivity (EC) increased with urine application up to 4.64 and 13.35 mS cm^{-1}, under beetroot and carrot, respectively. In this study, it was increased by 0.55 mS cm^{-1} after three rounds of application. The difference was statistically significant in this round in comparison with the EC of soil before head cabbage production ($t = 4.56$, $p = 0.05$). A study conducted on urine-treated maize production showed similar effects on soil salinity (Kassa *et al.* 2018). However, other findings showed the application of urine, even up to three times more urine than the plant requirement, has no effects on soil salinity (Sene *et al.* 2019).

High levels of Na is undesirable in soils and plants as it inhibits the uptake of desired nutrients like N and K for plant growth (Jagtap & Boyer 2018). Soluble sodium and chloride content of the soil were significantly changed ($t = 4.34$, $p = 0.049$; $t = 12.23$, $p = 0.007$, respectively) after the third round application of diluted urine in 1:3 ratios (Table 7). In addition, the sum of cations and anions indicated a significant difference after the second round application ($t = 3.56$, $p = 0.07$; $t = 3.50$, $p = 0.07$). Therefore, diluted urine can be applied for a consecutive three rounds, but needs soil treatment or the use of salt tolerant vegetables like tomato and broccoli. As well, rotation of different cabbage species in each round may be important in order to increase the absorption of nutrients and ions so that soil salinity could be decreased (Shahbaz *et al.* 2012).

In general, the results showed that human urine compared well with synthetic fertilizer as a source of N, P and K for vegetables, but optimum rates depend on the sensitivity of the crops to soil salinity. This should be monitored when human urine is regularly used for fertilizing crops as reported by Mnkeni *et al.* (2008) for beetroot and carrot production.

CONCLUSION

The aim of this study was to examine the effect of human urine on improving soil quality and producing vegetables during three rounds of application to exhaustively observe

Table 7 | Effect of three-fold diluted urine on the salt content of soil ($N = 3$)

	Values (mean ± SD)			
Parameter (1:5 extract except EC)	**Before application**	**1st round**	**2nd round**	**3rd round**
EC (mS/cm)(1:2.5)	0.25 ± 0.02a	0.32 ± 0.04a	0.52 ± 0.04a	0.80 ± 0.07b
Sol. Na (meq/L)	0.8 ± 0.06a	1.21 ± 0.13a	1.43 ± 0.16a	1.93 ± 0.19b
Sol. K (meq/L)	0.39 ± 0.02a	0.33 ± 0.01a	0.29 ± 0.02a	0.25 ± 0.02a
Sol. Ca (meq/L)	2.26 ± 0.18a	2.31 ± 0.14a	2.37 ± 0.18a	2.52 ± 0.19a
Sol. Mg (meq/L)	0.7 ± 0.02a	0.97 ± 0.06a	1.14 ± 0.09b	1.26 ± 0.13b
Sum of cations	4.15 ± 1.13a	4.82 ± 1.16a	5.23 ± 1.16b	5.96 ± 1.17b
CO_3^{2-} (meq/L)	Trace	Trace	Trace	Trace
HCO_3^- (meq/L)	1.05 ± 0.12a	1.09 ± 0.13a	1.15 ± 0.13a	1.19 ± 0.14a
Cl^- (meq/L)	1.24 ± 0.13a	1.73 ± 0.18b	2.29 ± 0.18b	3.39 ± 1.12c
SO_4^{2-} (meq/L)	0.93 ± 0.07a	0.96 ± 0.08a	0.98 ± 0.07a	1.1 ± 0.08a
Sum of anions	3.22 ± 1.18a	4.18 ± 1.21a	5.02 ± 1.26b	5.68 ± 1.19b

Different letters in numbers show statistical difference at 95% confidence interval.

salt concentrations. Based on the findings, human urine contains nutrients that can substitute synthetic fertilizer for vegetable production. Storage of the urine increased the pH up to 9.1, and ammoniacal nitrogen to 2,683 ± 31 mg L^{-1} until 70 days at room temperature. After 70 days, it remains almost constant, to be diluted and applied. Application of urine at a dilution factor of three provided the optimum yield of head cabbage. In this combination, the N-use efficiency is also reached at its optimum point. Significant difference was observed in soil electrical conductivity (salt concentration) after three consecutive production seasons. Therefore, not more than a two-round use of urine for cabbage is recommended in order to prevent salinity problems. Mixing human urine with low pH and low salt content substrates could be vital, which is a research area for the future.

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