Growth and nutritional composition of *Spinacia oleracea* L. harvested from soil treated with urine in comparison with other organic and inorganic soil amendments

L. L. Mugivhisa* and J. O. Olowoyo
Department of Biology, Sefako Makgatho Health Sciences University, Pretoria, South Africa
*Correspondence authour; Email: Liziwe.Mugivhisa@smu.ac.za

Abstract

The challenge of feeding the ever growing population is largely dependent on using the limited land and improving soil fertility. The study compared the effectiveness of using human urine as a source of soil nutrients in comparison with the commonly used soil amendments [chemical fertilizers (NPK) and biosolids] on the growth and nutritional composition of *Spinacia oleracea*. An enclosed plot was treated with different soil amendments before the introduction of *Spinacia oleracea* seedlings. After harvesting, the results showed a significant difference ($P < 0.05$) in the mean total biomass for the leaves of the plants harvested from all the plots and the values ranged from 1322.77 ± 0.88 g – 5528.28 ± 3.47 g. The differences in the result obtained for the mean leaf areas were also significant ($P < 0.05$) and with values ranging from 366.57 ± 10.9 cm^2 – 945.24 ± 31.3 cm^2. The concentrations of total N from the leaves followed the trend NPK > Urine > Control > Biosolids. The total P from the leaves showed equal amount for urine and NPK, followed by biosolids and the control having the least values. The levels of Ca recorded from the leaves followed the order biosolids > urine > NPK > control. In general, the study showed that the urine used as a soil amendment for this study competed favorably well with other amendments.

Introduction

In Africa, about half of the population live in rural areas and are directly dependent on subsistence farming for food and income (Bationo & Buerkert, 2001). With the increasing high cost of chemical fertilizers and the resulting effect of environmental pollution, recycling of wastes such as urine could be of great help (Kutu *et al.*, 2010; Maftouna *et al.,* 2006). In order to address the problem of high cost of chemical fertilizers facing both the commercial and subsistence farmers, the use of different types of organic amendments which may contain substantial amounts of soil nutrients that can supply the nutrients required for growth of plants in soils has been introduced (Uddin *et al.,* 2012). Roy *et al.,* (2014) pointed out that organic manures do not only increase the fertility of the soil but also improve the physical and chemical properties of the soil. Large scale urine use for commercial farming which involves application of urine which has been collected from a number of people/ households and then transported for the application to large areas of agriculture is possible as demonstrated in Sweden where there was collection of urine into large-scale storage slurry tanks (Richert *et al.,* 2010). However large-scale urine use involves storage of collected urine for a much longer period of time of more than three months so that proper hygienization can be achieved (Richert *et al.*, 2010).

One of the currently used organic fertilizers is human urine (Mnkeni *et al.,* 2008; Tidaker *et al.,* 2007; Kutu *et al.*, 2010). Human urine has already been used to successfully fertilize soil where maize (Ghuzha *et al.,* 2005), tomato (Mnkeni et al. 2008), wheat (Tidaker *et al.,* 2007), cucumber (Heinonen-Taski *et al.*, 2007) and cabbage (Pradhan *et al.,* 2007) were planted. According to Upreti *et al.* (2011), the use of urine for agricultural purposes can be as effective as the chemical fertilizers and hence can be used as an efficient source of nutrients for plants. However, the purpose of the present study is to introduce the organic fertilizers (urine) to local subsistence farmers in South Africa who cannot afford to buy chemical fertilizers and vegetables due to affordability. These subsistence farmers are being encouraged to make use of the land available in their backyards to grow their own vegetables using the freely available urine which is produced by all households including even the poorest households.

Plant macronutrients which have been reported to be excreted through human urine per person per year include 0.7–1.0 kg P, 2.5–4.3 kg N and 0.9–1.0 kg K (Sene, 2013). Fresh human urine which has not been diluted has been found to contain elements such as phosphorus 0.20–0.21 g/l, nitrogen 0.9–1.1 g/l, sulphur 0.17–0.22 g/l, potassium 0.9–1.1 g/l, magnesium 1.5–1.6 mg/l and calcium 13.0–16.0 mg/l. Other plant micronutrients such as zinc, copper, boron and iron have also been detected in fresh human urine (Sene, 2013).

Vegetables play a crucial part in the human diet since they are derivatives of a balanced diet made up of proteins, carbohydrates, minerals, vitamins and trace elements (Mohod, 2015; Chary *et al.,* 2008). *Spinacia oleraceae* has been utilized as a staple food both in the cooked and raw forms and is regarded as a protective supplementary vegetable in South Africa and all over the world (Lion and Olowoyo, 2013). The vegetable grows throughout the whole year (Zeka *et al.,* 2014) and is a Nitrogen hungry crop which has a short growth cycle and grows throughout the whole year (Kutu *et al.,* 2010). *Spinacia oleracea* has a maturation period of about six weeks and may produce a large mass of fresh leaves in a minimum period of 40 days (Nemadodzi, 2015).

According to Duncker & Matsebe (2008), even though the South African government has made food security a priority and it is sufficient in the production of food, 1.5 million children are estimated to be suffering from malnutrition with 14 million people being vulnerable to food security. In South Africa, the Department of Agriculture, Forestry and Fisheries (DAFF) has been given an agricultural mandate to improve the production of crops in particular at household level by 2% in order to achieve the food security at household level (Wilkinson *et al.,* 2010).

About 70% of the poor in South Africa reside in the rural areas where the economy is not adequately lively and have to rely on subsistence farming and production of food usually in poor soils (Duncker & Matsebe, 2008). The people in the rural areas have small backyard gardens but are however unable to grow vegetables because of the financial constraints. The prices of the chemical fertilizers for improving the soil fertility and the commercial vegetables for food are usually expensive for the majority of the poor unemployed people in the rural settings as well as in the settlement areas around major cities.

The introduction of the use of urine for agricultural purposes is still a new subject in South Africa and there is still limited information on the introduction of human urine as a source of nutrients (Mnkeni *et al*., 2008). The introduction of urine from the ECOSAN toilets needed to be encouraged once there was formulation of appropriate guidelines in South Africa (Mnkeni *et al.,* 2008). According to Mugivhisa and Olowoyo (2015), there was willingness of people (69.9% of the respondents [74.3% females, 69.9% students, 75.0% (27–36) age group] to change their attitudes and unwillingness to use urine for the cultivation of crops if they were better informed about the safety of human urine use for agricultural purposes. According to a study by Benoit (2012) on the individual's perception and the potential of urine as a fertilizer in Ethekwini, South Africa, it was concluded that there was willingness by the participants to learn more about the use of urine as a fertilizer and to see the application of urine as a fertilizer in practice.

In order to improve the cultivation, production and availability of the widely consumed vegetable in South Africa using urine as a cheap source of soil nutrient, the present study was set out to compare the effectiveness of using human urine as a source of soil nutrients in comparison with the commonly used soil amendments [chemical fertilizers (NPK) and biosolids] on the growth rate and nutritional composition of *Spinacia oleracea* harvested from soil treated with human urine and other organic and inorganic soil amendments.

Materials and methods

Experimental design

The study was conducted at the production unit of the Sefako Makgatho Health Sciences University at Molotlegi Street in Ga-Rankuwa, Pretoria in South Africa about 37 km north of Pretoria (https: //en.wikipedia.org/wiki/Ga-Rankuwa). The study area is at an altitude of 1 339.0 m above sea level with the coordinates 25° 442 S, 28° 44 E. The climate in the study area is a mixture of extreme hot and extreme rains in summer, followed by warm winters and autumns with the average annual temperature of 17.3 ^{0}C (63.2 ^{0}F) and the total annual precipitation of 732 mm (28.8 inches) (https://www.pretoria. climatemps. com/).

The production unit is an area within the campus (Farm Center) where animals such as horses and goats are also kept. The area which was used for the study was fenced off so as to prevent the entry of the animals into the area. The area for conducting the semi-field trials was within the fenced off area. No farming has been carried out on the area previously and the areas was covered in grass which had to be cleared before cultivating the test crops in the semi-field trials.

The size of the plot was 10.0 m by 16.9 m and separated into two sub-plots measuring

5.0 m by 8.45 m. Each sub-plot was separated into four beds measuring 3.45 m by 0.55 m each and separated by an interspace of 0.6 m. The soil used in the experiment was found to be sandy loam. The plot was chosen to emulate conditions of how subsistence farmers would normally arrange their plots and carry out the plantation of the crops such as *Spinacia oleracea* in their backyard gardens.

Soil amendments collection. For the collection of the urine samples consent was sought from the male students of Sefako Makgatho Health Sciences University. A 25.0 L plastic container was placed in the male students' toilet and students were asked to urinate in it. The collected urine was then stored in a sealed plastic container for a period of 3 months in order for the possible losses of ammonia to be minimised (Kutu et al., 2010). Male students were chosen so as to exclude the interferences of hormones and contraceptives which could be present in the urine samples of female students. The dry biosolids were made up of the treated sewage from the flushing toilets at Sefako Makgatho Health Sciences University and the neighbouring teaching hospital. The dry biosolids were collected from a sewage treatment area on campus after being dried and stored for a period of three months. Chemical fertilizers (NPK) were purchased from a registered trading Plantland Kwikery Nursery in Akasia in Pretoria North and manufactured at Omina Fertilizers in Johannesburg in South Africa.

*Treatments***.** Equal and comparable amounts of soil amendments [human urine, chemical fertilizers (2:3:2 NPK fertilizers) and biosolids] were added to each of the four beds representing each group of the amendments and thoroughly mixed with the soil. The treatments / amendments were 5.0 L of urine, 5.0 kg chemical fertilizers (NPK) and 5.0 kg biosolids each applied into separate beds. An equal number of 40 seedlings of *Spinacia oleracea* which were transplanted per amendment / treatment and control were purchased from a registered marketer, Plantland Kwikery Nursery in Akasia in Pretoria North were transplanted in each bed a week after the pre-treatment with the soil amendments. The seedlings were six weeks old post sprouting from the seeds. The plants were watered twice on a daily basis in the morning at 08 H00 and in the afternoon at 16 H00. The application of all the amendments was done only once before sowing the seedlings throughout the study. The plants were harvested eight weeks after planting.

Spinacia oleracea growth parameters measurements

Growth rate in the form of leaf length, leaf width, leaf numbers, stalk girth and leaf area were recorded (Blanco and Folegatti 2003) from two weeks after planting and they were all taken every 7 days at the same time so as to exclude bias. The leaf area (LA) was calculated from the width (W) and length (L) measurements (Blanco and Folegatti, 2003). The stalk girth was measured using a 15cm Mitutoyo Vernier calipers with a precision of 0.02 mm. At harvest, the *Spinacia oleracea* plants were uprooted and soil particles were washed off with distilled water so as to exclude a bias of inaccurate weight measurements. The plants were separated into roots, stems and leaves and their respective weights to represent the biomass, were then determined.

Plant analysis of nutrients

The separated plant samples were oven dried at 70 ^{o}C to a constant weight and then ground with an aid of a mortar and pestle to achieve homogeneity of the samples (Mnkeni *et al.*, 2008). Apparatuses which were used for homogenization were cleaned with distilled water so that cross contamination could be avoided. The total carbon % and total nitrogen % in the separated parts of *Spinacia oleracea* were determined with a Carlo Erba NA 1500 C/N/S Analyzer using a dry oxidation method known as the Dumas method (Jimenez and Ladha, 1993). For Ca, Mg, P, K, Na, Fe, Cu, Mn, Na and Zn the determinations were made with an aid of the multi-element and a sequential instrument, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) manufactured by Agilent. About 0.5 g of the samples was digested with 7.0 ml concentrated HNO_3 and 3.0 ml $HClO_4$ at temperatures up to 180 ^{0}C and brought to a 100 ml volume flask (Zasoski and Burau, 1977). For quality assurance purposes, the blanks for the plant samples were separately prepared (Lion and Olowoyo, 2013). Certified Reference Materials (CRM) 482 were prepared using the same procedure as the plant and soil samples so as to test for the accuracy of the used method. Each of the soil and plant samples and the resulting solutions were then analyzed for the nutrient contents using ICP – MS.

Statistical analysis

One way Analysis of Variance (ANOVA) was used to determine the significant differences in the mean values and standard deviation obtained for all the parameters recorded from the *Spinacia oleracea* harvested from the soil treated with the different amendments used in the study.

Results and discussion

Spinacia oleracea growth parameters

The results for the growth parameters (mean leaf length, mean leaf width, mean leaf area, the number of leaves, stalk girth and mean biomass) are presented in Table 1. The results of the study indicated that there were no significant differences in the mean leaf length and the mean leaf width of *Spinacia oleracea* grown in soil treated with different amendments ($P < 0.05$). The values of mean leaf length and mean width recorded for *Spinacia oleracea* harvested from soil treated with chemical fertilizers were 35.94 ± 2.55 cm and 23.77 ± 6.13 cm respectively while they were 35.94 ± 2.55 cm for mean leaf length and 22.14 ± 6.54 cm for mean width for *Spinacia oleracea* harvested from soil treated with urine. The values of mean leaf length were 24.91 ± 3.89 cm and 24.87 ± 2.47 cm for *Spinacia oleracea* harvested from soil treated with biosolids and control respectively. The values for the mean leaf width were 14.60 ± 3.90 cm and 14.59 ± 3.61 for the Spinacia oleracea harvested from soil treated with biosolids and control respectively. The differences in the results obtained for the mean leaf areas were also significant ($P <$ 0.05) and with values ranging from 366.57 ± 10.9 cm^2–945.24 ± 31.3 cm^2. Similarly the biomass of leaves and stalks was found to be significantly higher in *Spinacia oleracea* harvested from soil treated with chemical fertilizers compared to the other

TABLE 1

The plant nutrients in Spinacia oleracea grown in soils treated with different amendments

Treatments	*Mean leaf length (cm)**	*Mean leaf width (cm)**	*Mean leaf area* (cm^2)**	*Total no. of leaves***	*Stem girth (mm)***	*Biomass* Leaves**	Stalk**	Roots**
Urine	35.94 ± 2.55	22.14 ± 6.54	713.94 ± 153.81	278	3.80 ± 0.42	4070.66 ± 4.31	3539.21 ± 1.83	857.59 ± 0.74
NPK	39.04 ± 5.66	23.77 ± 6.13	945.24 ± 313.95	248	2.98 ± 0.43	5528.28 ± 3.47	3682.38 ± 2.00	769.86 ± 0.47
Biosolids	24.91 ± 3.89	14.60 ± 3.90	370.17 ± 153.02	246	2.70 ± 0.76	2164.16 ± 3.30	1332.07 ± 0.45	650.19 ± 1.16
Control	24.87 ± 2.47	14.59 ± 3.61	366.57 ± 109.04	1.55	1.84 ± 0.32	1322.77 ± 0.88	668.27 ± 0.85	369.70 ± 0.19

Values followed by * are not significantly different and ** are significantly different

amendments and the control ($P < 0.05$). The results are in agreement with the findings of Pradhan *et al.* (2009) where the total biomass of pumpkin fruit used in the study was higher in plants harvested from soil treated with mineral fertilizers than plants harvested from soil fertilized with urine or the control.

It is possible from the present study that the N in the urine might have been lost due to volatilization in the form of ammonia during the treatment and storage stages. During application of urine to the soil, there is rapid decomposition of urea into carbon dioxide and ammonia which is then rapidly lost into the atmosphere by volatilization (Egigu *et al.,* 2014). However the N content in the present study was not analysed after collection and before application. The collected urine was stored in a sealed plastic container for a period of three months and immediately covered by the soil after it had been applied in order for the possible losses of ammonia.

The number of leaves of *Spinacia oleracea* as shown in Table 1 was significantly higher in *Spinacia oleracea* harvested from soil treated with urine than the number of leaves of *Spinacia oleracea* harvested from the soil treated with other amendments and the control. The result of the increased number of leaves in *Spinacia oleracea* harvested from soil treated with urine in the present study is comparable with the result from Starzak *et al.* (2006) where parameters such as number of leaves, number of fruit (peppers), numbers of flowers in petunia and spinach plants were found to be significantly higher in plants that were harvested from soil treated with urine as compared to chemical fertilizers. In the present study, it could be that the granules of the chemical fertilizers had still not completely dissolved in the soil.

The nutrients in Spinacia oleracea

Percent levels of total N, total Carbon and Tissue P in the leaves, stalks and roots of *Spinacia oleracea* harvested from the soil treated with different amendments are represented in Fig. 1, 2 and 3 respectively. The highest mean value for total N in the leaves, stalks and roots was recorded from *Spinacia oleracea* harvested from soil treated with the chemical fertilizers compared to all the other treatments (Fig. 1). The values of total N in the leaves of *Spinacia oleracea* were more than the total N in the stems and stalks of *Spinacia oleracea* harvested from soil treated with different amendments. Sene *et al.* (2013) noted in a separate study that the levels of total N in the shoot of the plant increase significantly whenever there is an increase in the volume of inorganic fertilizer applied. The increase in the level of total N observed from the plants especially from those harvested from inorganic fertilizers used in this study may be due to volatilization of N since the N in the urine is applied in the form of urea which can easily be degraded into ammonium and then changed into the ammonia gas by the enzyme from the soil microorganisms (Sene *et al.,* 2013). In the present study, the highest values for total N in the leaves, stalks and roots could have been recorded from *Spinacia oleracea* harvested from the chemical fertilizers since the study was carried out in March and harvested in May which are hot months in the South African climate. According to Sene *et al.* (2013), increases in temperature might result in higher rates of volatilization of ammonia since warmer soils are unable to hold a lot of ammonia gas.

The levels for total carbon in the leaves, stalks and roots of *Spinacia oleracea* harvested from the soil treated with different amendments are shown in Fig. 2. The trend in the concentrations of total C from the leaves were Urine > NPK > Biosolids > Control and the differences obtained from the different plant tissues for each of the treatments were not significant ($P < 0.05$). There was little or no variation between the levels of total C in *Spinacia oleracea* harvested in soils treated with the amendments. The results of the present study are not comparable with those of He *et al.* (2015) where the total C concentrations in the stems and roots were significantly higher than the total C in the leaves of Xeric species (*Reaumuria soongorica)* which were determined in relation to varying climatic, geographical and soil conditions (He *et al.,* 2015). The trend observed in the present study might be due to the fact that the total C was measured after harvest when there was no need for extra carbon which is mainly used during the growth stages and contributes to the leaf biomass.

The levels of total P from different parts of *Spinacia oleracea* harvested from soil treated with different amendments are shown in Fig. 3. From the leaves of *Spinacia oleracea*, the highest mean values of total P were recorded in soil treated with chemical fertilizers, followed by those harvested from soil treated with urine, while the lowest mean value of total P was recorded from plants harvested from the soil treated with the biosolids (Fig. 3). The levels of total P in human urine have been

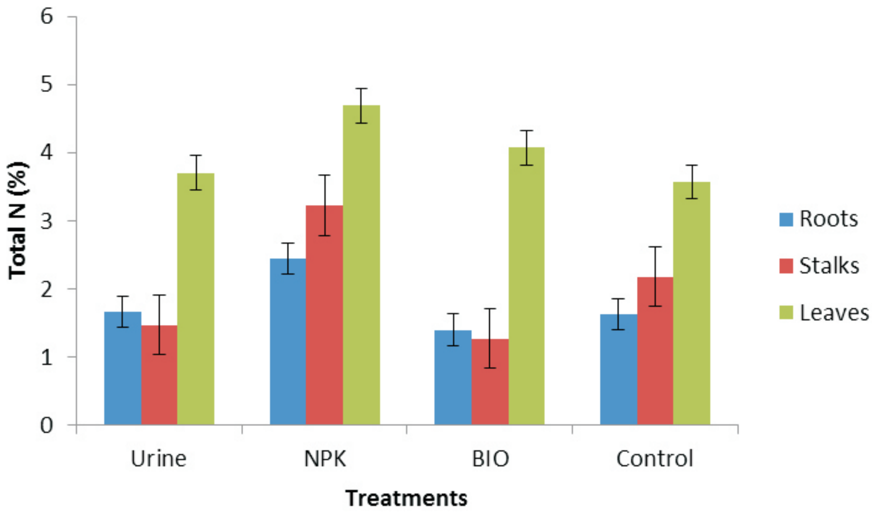

Fig. 1. Levels of Total N (%) in the roots, stalks and leaves of *Spinacia oleracea* harvested from soil treated with different treatments.

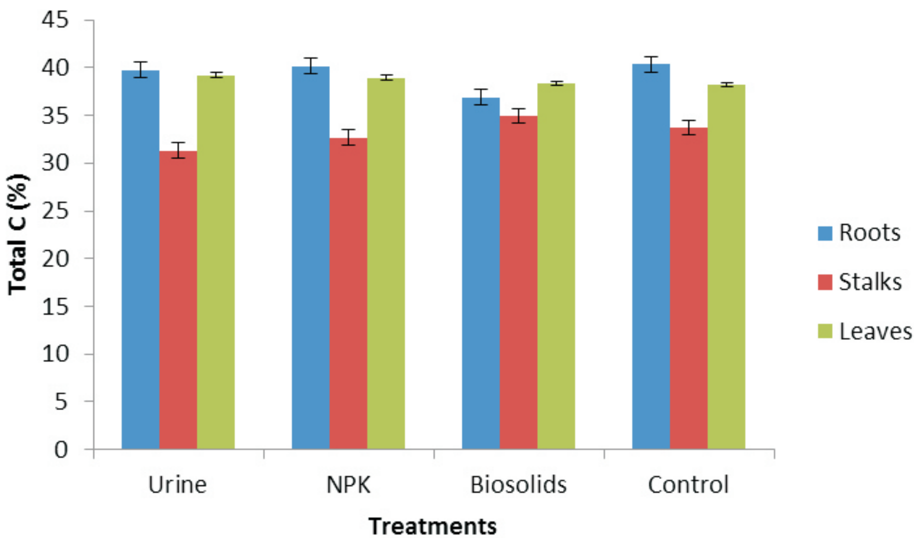

Fig. 2. Levels of Total C (%) in the roots, stalks and leaves of *Spinacia oleracea* harvested from soil treated with different treatments.

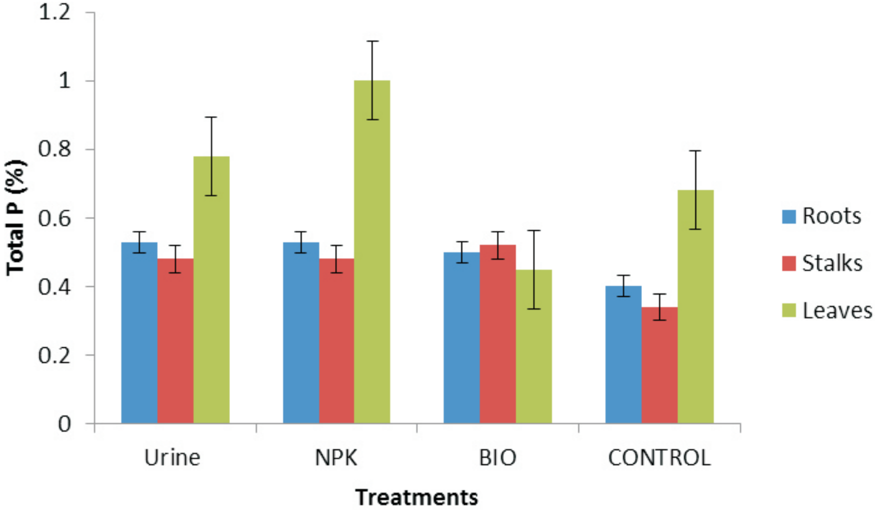

Fig. 3. Levels of Total P (%) in the roots, stalks and leaves of *Spinacia oleracea* harvested from soil treated with different treatments.

found to be much lower compared to the levels in chemical fertilizers. As a result other supplements such as ash and compost have been found to work well with human urine in the ability to supply P (Sene, 2013)

In the roots and stalks of *Spinacia oleracea* the levels of total P in *Spinacia oleracea* treated with the urine and the chemical fertilizers were similar. Like chemical fertilizers, urine is a source of fast acting nutrients of plants which can work quickly to provide nourishment for plants (Kvarnstrom *et al.,* 2006). The results are comparable to those of Sene *et al.* (2013) where excess of urine application did not have any significant effect on the levels of P in the leaves.

The mean concentration levels of potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) in the roots, stalks and leaves of *Spinacia oleracea* grown in soil treated with different amendments are shown in Table 2. The maximum concentrations of Ca with the value 1.27 ± 1.05 mg/kg were recorded from the leaves of *Spinacia oleracea* harvested from soil treated with urine. The lowest concentration of Ca (0.26 ± 0.01 mg/kg) was recorded from the leaves of *Spinacia oleracea* harvested from soil treated with chemical fertilizers. These results of the present study correspond with the results of Akpan -Idiok et al. (2012) where mineral fertilizers were found to lack Ca since their levels in okra leaves which were grown in soil treated with mineral fertilizers were found to be much lower in comparison with the levels in *Spinacia oleracea* grown in soil treated with urine

The values recorded for Na from *Spinacea oleracea* harvested from soil treated with urine were 0.24 ± 0.03 mg/kg (leaf), 2.51 ± 0.89 mg/kg (stem) and 1.49 ± 0.19 mg/kg (roots). The maximum concentration of Na with the value 2.51 ± 0.89 mg/kg was recorded in the stalks of *Spinacia oleracea* harvested from soil treated with urine. The values recorded from the different parts of the plants from soil treated with urine were more than those recorded for *Spinacia oleracea* harvested from soil treated with other amendments in this study. Mnkeni *et al.* (2008) had earlier reported significantly higher Na levels in leaf tissue of selected vegetables and this was due to application of urine.

In Senecal-Smith (2013) also, the concentrations of Na in *Spinacia oleracea* tissue from the soils treated with human urine were significantly higher than in the control and all the mineral fertilizer treatments. According to Mnkeni *et al.* (2008), the utilization of high rates of human urine can contribute to the salinization of the soil and high accumulation of Na in tissues of plants. However, there was a different trend with regards to the concentration levels of K and Mg which were found to be highest in leaves of *Spinacia oleracea* treated with biosolids compared to the other amendments and the control. According to Akpan -Idiok *et al.* (2012) human urine is an alkaline fertilizer which has reasonable quantities of nutrients such as P, N, K, Mg, Na and Ca whereas the mineral fertilizer was found to be lacking Mg, Ca and other essential micro-nutrients which have been found to be in abundant quantities in human urine by several authors.

Conclusion

The results from the study indicated that urine performed favorably well and better than the biosolids treatment and the control as a soil amendment even though it was out performed by chemical fertilizers (NPK) with respect to the mean leaf length and mean leaf width. However, with respect of the number of leaves, urine performed better than all the other amendments and the control resulting in more number of leaves in a bunch compared to the number of leaves with other treatments. Urine contributed to elevated levels of total C, Na and Ca in *Spinacia oleracea* compared to the chemical fertilizers and biosolids. It may be necessary to carry out a similar research for other leafy and fruity vegetables in order to ascertain the probability of using urine as a soil amendment.

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