Accumulation pattern of trace metals in *Spinacia oleracea* harvested from soil treated with urine in comparison with other soil amendments in Pretoria, South Africa

L. L. Mugivhisa · J. O. Olowoyo

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Abstract

**Purpose** The study assessed trace metal levels in the leaves, stalks and roots of *Spinacia oleracea* harvested from soil treated with urine in comparison to chemical fertilizers and biosolids.

**Methods** *Spinacia oleracea* seedlings were planted on soils pretreated with urine, chemical fertilizers and biosolids.

**Results** The soil treated with chemical fertilizers resulted in an increase of Cr, Pb, Cd, Cu, Ni and Sb while there was an increase in the concentrations of Zn and Hg in the soil treated with urine. The soil with no amendments recorded higher mean values of As and Mn, whereas the biosolids treatment did not show any increases of the trace metals in the soil. The concentration of Mn, Pb and Ni in the leaves and stalks of *S. oleracea* harvested from soil treated with urine were below the recommended limits for trace metals in edible plants as set by WHO even though the urine treatment recorded the highest concentration of Cd in the roots, stalks and leaves. The *S. oleracea* harvested from the soil treated with chemical fertilizers showed an accumulation Cu and Mn in the stalks and leaves while those harvested from soil treated with biosolids showed an accumulation of Cr, As, Zn and Ni in the stalks and Cr, Pb and Sb in the leaves and all trace metals in the roots except Cd and As. *S. oleracea* harvested from the soil with no amendments showed an accumulation of Cr, As, Zn and Ni in the stalks and Cr, Pb, Zn and Sb in the leaves. The transfer factor showed that Cd, Zn, Mn and Sn were translocated from the soil to the leaves even though the concentrations were below acceptable limits for human consumption.

**Conclusions** The study demonstrated that the use of urine as a soil amendment may not facilitate or increase the bioavailability of trace metals in the plant tissues.

**Keywords** Urine · Organic waste · Trace metals · Agriculture

Introduction

Agricultural lands in developing countries especially in sub-Saharan Africa are less productive as a result of recurrent droughts (Cofie et al. 2010). The soils in areas which are poor in plant nutrients and also over-utilized result in millions of people being exposed to shortage of food and diseases due to malnutrition (Egigu et al. 2014). Soils need to be fertilized and the demand for the synthetic chemical fertilizers has been escalating in order for the food demand to be addressed so that the high populations in developing countries can be supported. However, high prices of chemical fertilizers result in the reduction of production and yields mainly in developing countries where there are limitations as with regards to tools and inaccessibility of fertilizers (Sene et al. 2013). There has been a mission to explore new alternatives such as recycling of wastes like human urine to improve the fertility of the soil (Mnkeni et al. 2008).

The use of human urine as a valuable resource has been practised to improve the growth of mainly leafy vegetables in countries such as Germany, Sweden, USA, Mexico, Zimbabwe and Denmark even though there is a limitation of information in South Africa (Mnkeni et al. 2008). There
has been broad research carried out to compare the effectiveness of urine in fertilisation of crop in comparison of commercial fertilizers on a wide variety of cereals and vegetables such as cucumber (Heinonen-Tanski et al. 2007), pumpkin (Pradhan et al. 2009a), tomato (Pradhan et al. 2009b), red beet (Pradhan et al. 2010a), radish, potato, cauliflower (Pradhan et al. 2010b), cabbage (Mnkeni et al. 2005), spinach (Mnkeni et al. 2005), carrot (Mnkeni et al. 2008), beetroot (Mnkeni et al. 2008) and maize (Guzha et al. 2005). From all the research human urine was found to have compared equally favourable with the chemical fertilizers when both amendments were utilized at equal doses (Mnkeni et al. 2008).

The direct utilisation of human urine as a fertilizer for agricultural purposes is controversial and associated with problems with respect to hygiene, transport, storage and spreading (Lindt et al. 2000). Some of the harmful substances which are present in urine include pathogens, salts and pharmaceuticals (Sene et al. 2013). However, the urine of a healthy individual is considered sterile in the bladder even though as it passes through different types of dermal tissues, bacteria are added to it resulting in less than ten thousand bacteria per ml being excreted with urine (Hoglund et al. 2002). Urine is also considered sterile unless it has been cross-contaminated by faeces (Mnkeni et al. 2008). The majority of the agents such as bacteria, protozoa, viruses and helminthes in excreta which cause diseases are usually shed in faeces rather than in urine (Hoglund et al. 2002). As a result, the health risks which are associated with the consumption of urine for agricultural purposes have been found to be insignificant (Upreti et al. 2011). The pathogens which cause venereal diseases can also be excreted through urine but there is no indication of whether they have a potential to thrive outside the human body and if this would be of any health significance (Hoglund et al. 2002). The drying of soil which results from evaporation from the plants can result in the decreased chances of survival of these pathogenic agents in the soil (Heinonen-Tanski and Wijk-Sijbesma, Heinonen-Tanski and Van Wijk-Sijbesma 2005). The risk of contamination by the microbes is reduced since microbes which are pathogenic are unable to survive in the soil for a long period of time (Sene et al. 2013).

Storage of human urine for a couple of weeks results in a great reduction of the number of enteric microbes and this renders urine a safer fertilizer compared with animal manure which requires more than 6 months for decomposition (Egigu et al. 2014). The microorganisms die during the hygienization step of simple urine storage even though in Nordic countries the storage of urine might need to be longer and be for a period of more than 6 months (Sene et al. 2013). There has also been an introduction of a set of guidelines by the Vietnamese government for effective and proper composting of excreta before its consumption so as to limit and reduce the risk of infections by the helminths (Jensen et al. 2007).

The levels of heavy metals in urine have been found to be low and to be 50–1000 times lower than in soils or composts produced in gardens. However, the trace metals levels have been found to be 100–500 times more abundant in urine than in rainwater (Kirchmann and Petterson 1995). According to a study by Paschal et al. (1998), from the 13 trace metals which were measured in the urine of United States residents eight of the analytes were present at detectable concentrations. Trace metals which are of great concern are those which exist almost everywhere in the environment such as Cr, Cu, Zn, Fe, Mn and Pb. The heavy metals receive significant interest throughout the world because of their effects which are toxic, teratogenic and mutagenic even at low concentration levels (Mohod 2015).

Anthropogenic activities such as the utilisation of soil amendments and fertilizers in high-production agriculture contribute to the accumulation of trace metals in the soils (Kidd et al. 2007). The avenue of the entry of trace elements into the human food chain is mainly through the uptake of these trace elements from the soil especially by leafy vegetables (Dingkwoet et al. 2013). The essential nutrients like Fe, Co, Ni, Mn and Co have low permissible limits in living organisms (Salawu et al. 2015); however, the deficiency or excess of these trace elements could cause several disorders (U wah et al. 2011).

The increasing levels of heavy metals in the environment have been attributed to agricultural practices such as organic and inorganic fertilizer supplements (Agrawal et al. 2007). Heavy metals in the soil can be translocated to different parts of the plants through the uptake by the roots. The uptake and accumulation of the trace metals by the different parts of the plants is dependent on the concentrations and the forms of the heavy metals which are available in the soil. The accumulation of the heavy metals can result in the alteration of the physico-chemical properties of the soil and also result in the plants becoming toxic and the food chain being contaminated (Agrawal et al. 2007). Leafy vegetables like Spinacia oleracea have been found to result in increased translocation of trace metals to the parts of the plant which are above the ground resulting in higher levels of heavy metals in the leafy parts (Agrawal et al. 2007).

Spinacia oleracea is one of the most common and popular green leafy vegetable crops used in South Africa as a source of iron (Olowoyo et al. 2011). It grows throughout the whole year with a maturation period of about six weeks and above, hence producing a large mass of fresh leaves in a minimum period of time (Zeka et al. 2014). The study
assessed the accumulation of trace metal levels in *S. oleracea* harvested from soil treated with urine in comparison to the other commonly used amendments (chemical fertilizers and biosolids).

**Materials and methods**

**Experimental design**

The study was conducted at the production unit of the Sefako Makgatho Health Sciences University. The size of the plot was 5.0 m by 8.45 m. It was separated into four beds measuring 3.45 m by 0.55 m each. The four beds representing different treatments were separated with an interspace of 0.6 m. The plot was chosen to emulate conditions which occur in the real world with regards to agricultural practices.

**Soil amendments collection**

For urine collection, consent was sought from the male students. A 25 L plastic container was placed in the male students’ toilet and students were asked to urinate in it. The collected urine was then stored for a period of 3 months in a sealed plastic container 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 collected from a sewage treatment area on campus. Chemical fertilizers (NPK) were purchased from a registered trading nursery.

**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 and each amendment was applied into separate beds. The soil with no amendment did not receive any treatment. From the literature, urine was used in liquid form and fertilizers were used in solid forms (Ranasinghe et al. 2016; Akpan-Idiok et al. 2012). The idea is to check whether urine will perform as a cheap alternative source of nutrients to soil. From all the studies conducted and that are available to us, litres of urine were varied and compared to commercially available fertilizers (Ranasinghe et al. 2016; Akpan-Idiok et al. 2012). The majority of the plant nutrients which are present in human excreta are found in urine. An estimate from the data from five countries (India, Haiti, Uganda and South Africa) which was made was that each person produces about 5 kg of elemental NPK in excreta in a year with about 4 kg being in the urine and 1 kg in faeces (Winblad and Simpson-Hebert 2004). The application of all the amendments was done only once before sowing the seedlings throughout the study. An equal total number of 40 seedlings of *S. oleracea* purchased from a registered marketer were transplanted into each bed a week after the pre-treatment with the soil amendments. All plants were watered in the morning and late in the afternoon on a daily basis. The plants were harvested eight weeks after planting.

**Soil and plant analysis**

*Spinacia oleracea* harvested from soil treated with the amendments was washed and separated into roots, stalks and leaves. The separated plant samples were oven dried at 70 °C for 48 h and then ground with an aid of a mortar and pestle to achieve homogeneity of the samples. The soil samples which were treated with the amendments were collected after harvesting of *S. oleracea*. All soil samples were air dried, ground with pestle and mortar and sieved with a 2.0 mm sieve (Germer et al. 2011).

The pH of the soil was determined in distilled water using the 1:12 soil water suspension and in calcium chloride (CaCl₂) using 1:12 soil CaCl₂ suspension and measured with an aid of a pH metre (Germer et al. 2011). About 0.5 g of the ground soil samples was added with 2.0 ml of HClO₄, 2.0 ml of HCl, 8.0 ml HHNO₃ and 2.0 ml HF. The solutions resulting from a mixture of the soil and the acids were then analysed for trace metals with an aid of the Inductively Coupled Plasma Mass Spectrometry (ICP–MS) for the determinations of the trace metals concentrations in the soil samples. From the ground plant samples, 0.2 g of each of the different parts was digested with the acids using 2.0 ml HCl, 2.0 ml HF, 5.0 ml HNO₃ and 1.0 ml HClO₄. The solutions which resulted from the solutions of the plant samples and the acids were then analysed for levels of trace metals using ICP-MS.

All the apparatuses which were used for homogenization of the soil and plant samples were cleaned with distilled water so that cross contamination could be avoided. For quality assurance purposes, the blanks for the soil and plant samples were separately prepared and the Certified Reference Materials (CRM) for plant and soil samples were done (Lion and Olowoyo 2013). The analyses were also carried out in triplicate (Olowoyo et al. 2015). The CRM 482 was used along with the samples for the purpose of quality assurance.
Statistical analysis

Analysis of variance (ANOVA) and Student’s t test were used to determine whether the differences obtained from the mean concentrations of trace metals measured in *S. oleracea* harvested from the soil treated with different amendments were significant.

Transfer factor (TF)

The transfer factor (TF) was calculated as the concentration ratio of the trace metals in the stalks and leaves of *S. oleracea* to the concentration of the trace metals in the soil. The TF index was calculated with an aid of the relationship as described in Sithole et al. (2016).

In the stalks, \( TF = \frac{C_{\text{stalk}}}{C_{\text{soil}}} \) and in the leaves, \( TF = \frac{C_{\text{leaf}}}{C_{\text{soil}}} \) with TF representing the transfer factor of *S. oleracea*, \( C_{\text{stalk}} \) and \( C_{\text{leaf}} \) representing the concentration of the trace metal in the stalks and leaves of *S. oleracea*, respectively, and \( C_{\text{soil}} \) representing the concentration of the trace metal in the soil before the cultivation of *S. oleracea*. TF > 1 indicates that *S. oleracea* is enriched with the elements from the soil (accumulator), whereas TF < 1 means that *S. oleracea* is an excluder of the trace metals from the soil.

Results and discussion

From all the trace metals examined, Zinc (Zn) showed the highest mean concentration in the roots, stalks and leaves followed by Manganese (Mn) and then Copper (Cu) (Tables 1, 2, 3). The concentration of Zn in the roots, stalks and leaves ranged from 135.60 ± 8.45 to 2423.00 ± 222.09 mg/kg. The leaves of *S. oleracea* harvested from soil treated with chemical fertilizers showed the highest concentration of Zn while the stalks harvested from soil treated with chemical fertilizers showed the highest concentration of Zn while the stalks harvested from soil treated with urine showed the least concentration with no significant difference (\( p < 0.05 \)). The order of the Zn concentration in *S. oleracea* was leaves > roots > stalks for all the amendments and this is in agreement with the study conducted by Farooq et al. (2008) where *S. oleracea* stalks recorded the least concentration of Zn. Even though the concentration of Zn in the stalks harvested from soil treated with urine was below the recommended value of 200 µg/g (Alia et al. 2015; Jung 2008) the leaves harvested from the soil treated with urine was above this recommended value.

Zn is important for the normal growth of the plant and for the development and growth in human beings. Zn is essentially required by plants in minute quantities (25–150 µg/g) in dry tissue (Jung 2008) and the level of Zn in agricultural products should be below 200 µg/g and the

### Table 1

| Treatments | Cr (mg/kg)* | Pb (mg/kg)* | Cd (mg/kg)** | As (mg/kg)* | Zn (mg/kg)* | Cu (mg/kg)* | Mn (mg/kg)* | Ni (mg/kg)* | Sb (mg/kg)* | Hg (mg/kg)* |
|------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Urine      | 2.48 ± 0.30 | 0.95 ± 0.080 | 1.14 ± 0.12  | 0.57 ± 0.018 | 3.28 ± 0.010 | 3.28 ± 0.010 | 4.53 ± 0.013 | 4.53 ± 0.013 | 4.53 ± 0.013 | 4.53 ± 0.013 |
| Chemical fertilizers | 1.82 ± 0.21 | 0.83 ± 0.076 | 0.44 ± 0.034 | 0.35 ± 0.018  | 2.18 ± 0.010 | 2.18 ± 0.010 | 3.00 ± 0.013 | 3.00 ± 0.013 | 3.00 ± 0.013 | 3.00 ± 0.013 |
| Biosolids | 4.54 ± 0.12 | 3.79 ± 0.117 | 0.21 ± 0.013  | 0.35 ± 0.018  | 4.54 ± 0.12 | 4.54 ± 0.12 | 5.00 ± 0.013 | 5.00 ± 0.013 | 5.00 ± 0.013 | 5.00 ± 0.013 |
| Soil with no amendment | 1.36 ± 0.071 | 0.87 ± 0.016 | 0.10 ± 0.006  | 0.12 ± 0.014  | 1.36 ± 0.071 | 1.36 ± 0.071 | 1.50 ± 0.013 | 1.50 ± 0.013 | 1.50 ± 0.013 | 1.50 ± 0.013 |

Values: mean ± SD

* Not significantly different ** Significantly different

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### Table 2  Trace metals in the stalks of *Spinacia oleracea* harvested from soil treated with different soil amendments

| Treatments               | Cr (mg/kg)* | Pb (mg/kg)* | Cd (mg/kg)** | As (mg/kg)* | Zn (mg/kg)* | Cu (mg/kg)* | Mn (mg/kg)* | Ni (mg/kg)* | Sb (mg/kg)* | Hg (mg/kg)* |
|--------------------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Urine                    | 0.65 ± 0.031| 0.47 ± 0.030| 0.68 ± 0.088| 0.059 ± 0.051| 135.60 ± 8.448| 5.043 ± 0.216| 37.74 ± 2.293| 1.50 ± 0.026| 0.017 ± 0.0027| 0.054 ± 0.013 |
| Chemical fertilizers     | 1.56 ± 0.128| 0.42 ± 0.031| 0.23 ± 0.025| 0.061 ± 0.003| 366.5 ± 19.695| 7.44 ± 0.444| 80.58 ± 4.078| 3.09 ± 0.215| 0.013 ± 0.005 | 0.048 ± 0.017 |
| Biosolids                | 1.46 ± 0.051| 0.31 ± 0.020| 0.094 ± 0.014| 0.014 ± 0.037 | 195.8 ± 7.805| 6.63 ± 0.201| 42.64 ± 1.638| 2.53 ± 0.759 | 0.047 ± 0.006 | 0.033 ± 0.011 |
| Soil with no amendment   | 1.80 ± 0.053| 0.38 ± 0.013| 0.15 ± 0.005| 0.13 ± 0.023 | 885.10 ± 18.762| 6.44 ± 0.167| 30.09 ± 0.570| 3.35 ± 0.090 | 0.019 ± 0.006 | 0.038 ± 0.14 |

Values: mean ± SD  
* Not significantly different  
** Significantly different

### Table 3  Trace metals in the leaves of *Spinacia oleracea* harvested from soil treated with different soil amendments

| Treatments               | Cr (mg/kg)* | Pb (mg/kg)* | Cd (mg/kg)** | As (mg/kg)* | Zn (mg/kg)* | Cu (mg/kg)* | Mn (mg/kg)* | Ni (mg/kg)* | Sb (mg/kg)* | Hg (mg/kg)* |
|--------------------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Urine                    | 1.59 ± 0.082| 0.44 ± 0.011| 2.42 ± 0.098| 0.052 ± 0.008| 764.90 ± 10.151| 16.90 ± 0.14| 330.20 ± 4.038| 7.73 ± 0.26 | 0.019 ± 0.006 | 0.097 ± 0.018 |
| Chemical fertilizers     | 1.64 ± 0.20 | 1.00 ± 0.076| 0.77 ± 0.067| 0.096 ± 0.008| 2423.00 ± 222.086| 18.40 ± 1.69| 446.0 ± 16.147| 5.62 ± 0.420| 0.023 ± 0.003 | 0.091 ± 0.018 |
| Biosolids                | 1.42 ± 0.18 | 0.50 ± 0.00058| 0.18 ± 0.008| 0.098 ± 0.032| 590.50 ± 15.849| 12.63 ± 0.422| 87.46 ± 3.484| 4.92 ± 0.113 | 0.018 ± 0.002 | 0.069 ± 0.013 |
| Soil with no amendment   | 1.98 ± 0.056| 6.52 ± 0.32 | 0.60 ± 0.053| 0.043 ± 0.023 | 1072.00 ± 58.501| 14.20 ± 0.607| 81.67 ± 4.175| 3.58 ± 0.238 | 0.031 ± 0.006 | 0.058 ± 0.016 |

Values: mean ± SD  
* Not significantly different  
** Significantly different
dietary intake of Zn should not be above 150 μg/g (Alia et al. 2015; Jung 2008). In humans, excess amounts of Zn may cause metal poisoning and retardation of growth especially in young children (Sithole et al. 2016) and in plants it may inhibit plant growth, cause damage and chlorosis in leaves resulting in a reduction in chlorophyll (Alia et al. 2015).

Mn concentration varied between 30.09 ± 0.57 and 446.00 ± 46.15 mg/kg. The leaves of S. oleracea harvested from the soil treated with chemical fertilizers showed the highest concentrations of Mn while the least concentration was in the roots harvested from the soil with no amendment. The concentration of Mn in S. oleracea harvested from the soil treated with urine and other amendments was lower than the permissible limit of 500 mg/kg as recommended by WHO (2001). According to Dingkwoet et al. (2013), S. oleracea has been shown to be an excellent source of Mn in food. In plants, Mn plays a role in the splitting of water molecules which are essential for photosynthesis (Alia et al. 2015). In the human body, Mn is important for the manufacturing of enzymes even though in excess it has a negative impact on the enzyme activity, translocation, absorption and consumption of other mineral elements resulting in oxidative stress (Lion and Olowoyo 2013).

The concentration of Copper (Cu) in the roots, stalks and leaves was 5.04 ± 0.22–30.9 ± 0.99 mg/kg. The roots of S. oleracea harvested from soil treated with biosolids showed the highest concentration of Cu while stalks of S. oleracea harvested from soil treated with urine showed the least concentration of Cu. There were no significant differences in the concentration of Cu (p < 0.05). The decreasing trend of the concentration of Cu for all amendments was leaves > roots > stalks. The concentration of Cu in the stalks of S. oleracea harvested from the soil treated with urine was below the critical limit of 10.00 μg/g as set by WHO (Sani et al. 2011; Iqbal et al. 2011), whereas the concentration of Cu in the leaves harvested from soil treated with urine was above the recommended value. The result of Cu concentration in the edible parts (stalks and leaves) in the present study are much lower compared to the level of 37.62 mg/kg in S. oleracea harvested from soil under agricultural activities reported in Agrawal et al. (2007). Cu is an essential cofactor for most metalloproteins and plays a role in the manufacturing of enzymes which work as antioxidants and excess amounts of Cu may result in the malfunctioning of enzymes (Singh and Taneja 2010).

The concentration of Cr ranged from 0.65 ± 0.03 to 4.54 ± 0.12 mg/kg (Tables 1, 2, 3). The highest concentration of Cr was present in the roots of S. oleracea harvested from soil treated with biosolids, whereas the least concentration of Cr was found in the stalks harvested from soil treated with urine. The concentration of Cr in the leaves harvested from soil treated with urine was above the recommended level of 1.30 mg/kg by WHO (2001) while the concentration in the stalks harvested from soil treated with urine was lower than the recommended value. In humans and animals, Cr is considered to be important for the glucose and protein metabolic processes and it also prevents the increase of the triglycerides and cholesterol. In plants, it promotes growth and in low concentrations it increases plant yield (Peralta-Videa et al. 2009). The translocation and absorption of Cr is mainly altered by the chelating agents, pH and OM of the soil (Peralta-Videa et al. 2009).

The concentration of lead (Pb) in the roots, stalks and leaves of S. oleracea ranged from 0.31 ± 0.02 to 6.52 ± 0.32 mg/kg (Tables 1, 2, 3). The highest concentration of Pb was recorded from the leaves of S. oleracea harvested from the soil with no amendment while the least concentration was recorded from the stalks of S. oleracea harvested from soil treated with biosolids. There was no significant difference in the concentration of Pb (p < 0.05). The concentration of Pb in the roots, stalks and leaves of S. oleracea harvested from soil treated with urine was below the recommended level of 2.00 μg/g for edible portions of vegetables as set by WHO (Sani et al. 2011; Iqbal et al. 2011). Among the trace metals, Pb is the most highly toxic trace metal to people through the food chain since it is persistent and does not have any biological value (Tang et al. 2015).

Cadmium (Cd) concentration in the leaves, stalks and roots varied between 0.09 ± 0.014 and 2.42 ± 0.098 mg/kg. The leaves of S. oleracea harvested from soil treated with urine showed the significantly highest concentration of Cd (p < 0.05) which was also higher than and the permissible limit of 0.02 mg/kg in plants (Iqbal et al. 2011) while the least concentration of Cd was in the stalks of S. oleracea harvested from soil treated with biosolids. The concentration range of Cd in the present study exceeded the recommended concentration of Cd in edible vegetables which should range between 0.05 and 0.9 μg/g (Jung 2008). Exposure to high concentrations of Cd result in decreased rate of growth, hypertension, anaemia, poor mineralization of bones and damage to the renal tubules (Iqbal et al. 2011). In the present study, the result of the range of the concentration of Cd was also slightly higher than the concentration range of 0.02 ± 0.00–0.28 ± 0.02 μg/g in Lion and Olowoyo (2013) where S. oleracea was harvested from waste dump sites. The concentration of Nickel (Ni) ranged from 1.50 ± 0.026 to 14.82 ± 0.20 mg/kg. The highest concentration of Ni was present in the roots harvested from soil treated with biosolids while the least concentration was in the stalks harvested from soil treated with urine. The
concentration of Ni in the roots, stalks and leaves harvested from soil treated with urine were below the recommended limit of 10.0 mg/kg. Ni is a trace metal which occurs only at very low concentrations in the environment and it is essential in small quantities even though it can be dangerous when the maximum tolerable amounts are exceeded (Wuana and Okieimen 2011) resulting in blindness, increased level of sugar in the blood and cholesterol in the serum (Iqbal et al. 2011).

The pH values of the soil treated with different amendments measured in water were slightly neutral to alkaline and ranged between 6.92 ± 0.067 and 7.31 ± 0.023 with a mean value of 7.16, whereas those measured in CaCl₂ were slightly acidic to sub-alkaline and ranged between 6.57 ± 0.038 and 7.03 ± 0.025 with a mean value of 6.80. From both methods of measurements, the soil with no amendment had the highest pH followed by the soil amended with biosolids and then the urine treatment soil with the least pH recorded for the soil treated with chemical fertilizers. According to Tang et al. (2015), pH is one of the most crucial factors which affect the availability of trace metals in the soil.

Table 4 shows the concentration of the trace metals in the soil treated with different amendments and used for cultivating *S. oleracea*. The concentrations of Cd, As and Hg were higher in the soil without the amendment compared to the soil which received the amendments and this could have been due to the nature of the soil and the *S. oleracea* harvested from the soil with no amendments might not have absorbed the trace metals from the soil.

The transfer factors (TF) of the stalks and leaves which were calculated from the concentration of the trace metals in the soil are shown in Tables 5 and 6, respectively. The leaves of *S. oleracea* harvested from soil treated with urine bioaccumulated Cd, Zn, Mn and Sb as the TF values of these trace metals were greater than 1. However, the stalks harvested from soil treated with urine did not bioaccumulate any of the trace metals indicating that the trace metals which were bioaccumulated in the leaves were absorbed from the soil by the roots and translocated to the leaves.

### Conclusion

The results from the study indicated that urine performed favourably well as a soil amendment in comparison with the other conventional soil amendments used in terms of the concentration of the trace metals in *S. oleracea*. The concentration of Mn, Pb and Ni in the leaves and stalks of *S. oleracea* harvested from soil treated with urine were below the recommended limits for edible vegetables as set out by WHO. The stalks harvested from soil
treated with urine showed the lowest concentrations of Cr, Zn, Cu and Ni while the leaves harvested from urine showed the least concentration of Pb compared to the stalks and leaves harvested from soil treated with other amendments. Even though the biosolids performed slightly better than urine and showed the least concentrations of Cr, Cd, Zn, Cu in the leaves and Pb, Cd and Hg in the stalks, the biosolids are not as easily available and affordable as human urine which is readily available at no cost in all the communities. It is hence concluded that the urine amendment can be recommended for the growing of *S. oleracea*. It is further recommended that the health effects of the Pb and Cd in the stalks and Cd, Ni and Hg in the leaves harvested from soil treated with urine which were present in highest concentrations compared to the stalks and leaves harvested from other amendments be further investigated.

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### Table 5 Translocation factors (TF) of *Spinacia oleracea* stalks harvested from soil treated with different soil amendments

| Treatments         | Cr  | Pb  | Cd  | As  | Zn  | Cu  | Mn  | Ni  | Sb  | Hg  |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Urine              | 0.06| 0.50| 0.60| 0.10| 0.41| 0.29| 0.34| 0.16| 0.29| 0.17|
| Chemical fertilizers| 0.86| 0.51| 0.54| 0.64| 0.81| 0.48| 0.55| 0.43| 0.22| 0.17|
| Biosolids          | 0.32| 0.08| 0.45| 0.04| 0.34| 0.21| 0.16| 0.17| 0.19| 0.03|
| Soil with no amendment | 1.35| 0.43| 1.42| 1.04| 2.75| 0.67| 0.47| 1.00| 0.61| 0.25|

### Table 6 Translocation factors (TF) of *Spinacia oleracea* leaves harvested from soil treated with different soil amendments

| Treatments         | Cr  | Pb  | Cd  | As  | Zn  | Cu  | Mn  | Ni  | Sb  | Hg  |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Urine              | 0.64| 0.47| 2.12| 0.09| 2.33| 0.98| 2.96| 0.85| 0.33| 0.30|
| Chemical fertilizers| 0.90| 1.20| 1.81| 1.01| 5.38| 1.19| 3.05| 0.79| 0.39| 0.31|
| Biosolids          | 0.31| 0.13| 0.85| 0.28| 1.03| 0.41| 0.34| 0.33| 0.07| 0.06|
| Soil with no amendment | 1.46| 7.47| 5.62| 0.35| 3.33| 1.48| 1.27| 1.07| 1.00| 0.38|
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