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ORIGINAL RESEARCH

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[1] · J. O. Olowoyo[1]

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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

✉ L. L. Mugivhisa
Liziwe.Mugivhisa@smu.ac.za

[1] Department of Biology, Sefako Makgatho Health Sciences University, Medunsa Campus, P.O Box 139, Pretoria 0204, South Africa

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 compositing 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 (Uwah 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-Hébert 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 grinded with an aid of a mortar and a 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, grinded 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 ($CaCl_2$) using 1:12 soil $CaCl_2$ 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 $HClO_4$, 2.0 ml of HCl, 8.0 ml $HHNO_3$ 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 HNO_3 and 1.0 ml $HClO_4$. 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, $TF = C_{stalk}/C_{soil}$ and in the leaves, $TF = C_{leaves}/C_{soil}$ with TF representing the transfer factor of *S. oleracea*, C_{stalk} and C_{leaf} representing the concentration of the trace metal in the stalks and leaves of *S. oleracea*, respectively, and C_{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 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 Trace metals in the roots 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	2.48 ± 0.30	0.95 ± 0.080	1.14 ± 0.12	0.57 ± 0.108	328.70 ± 12.80	17.22 ± 0.98	111.60 ± 11.086	9.12 ± 0.901	0.058 ± 0.009	0.32 ± 0.018
Chemical fertilizers	1.82 ± 0.21	0.83 ± 0.076	0.44 ± 0.034	0.095 ± 0.013	450.10 ± 45.38	15,43 ± 0.954	146.00 ± 13.106	7.155 ± 0.742	0.059 ± 0.00252	0.29 ± 0.017
Biosolids	4.54 ± 0.12	3.78 ± 0.117	0.21 ± 0.013	0.35 ± 0.094	573.7 ± 20.353	30.9 ± 0.988	259.0 ± 8.085	14.82 ± 0.200	0.24 ± 0.009	1.13 ± 0.017
Soil with no amendment	1.36 ± 0.071	0.87 ± 0.016	0.10 ± 0.006	0.12 ± 0.114	321.80 ± 4.652	9.62 ± 0.0225	64.56 ± 1.015	3.36 ± 0.17	0.031 ± 0.006	0.154 ± 0.024

Values: mean ± SD

* Not significantly different

** Significantly different

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 ± 46.147	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 $CaCl_2$ 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 have not 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

Table 4 Trace metals of soil samples treated with different soil amendments for cultivation of *Spinacia oleracea*

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	114.83 ± 2.58	13.41 ± 0.05	0.068 ± 0.012	3.53 ± 0.05	102.43 ± 0.29	33.47 ± 0.29	209.13 ± 3.15	49.02 ± 0.72	0.0077 ± 0.0015	0.286 ± 0.013
Chemical fertilizers	123.67 ± 1.93	14.77 ± 3.96	0.074 ± 0.008	3.96 ± 0.07	92.71 ± 1.46	37.21 ± 0.32	177.4 ± 2.10	55.39 ± 0.88	0.0097 ± 0.0012	0.236 ± 0.024
Biosolids	113.97 ± 0.70	14.04 ± 0.18	0.074 ± 0.007	3.73 ± 0.33	84.83 ± 0.66	34.48 ± 0.60	215.20 ± 3.71	52.34 ± 0.56	0.0087 ± 0.0015	0.169 ± 0.037
Soil with no amendment	91.27 ± 1.41	11.60 ± 0.09	0.08 ± 0.010	4.60 ± 0.42	70.85 ± 1.50	27.93 ± 0.12	218.33 ± 1.19	47.8 ± 0.29	0.0073 ± 0.0060	0.174 ± 0.012

Values: mean ± SD

* Not significantly different

** Significantly different

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

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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References

Agrawal SB, Singh A, Sharma RK, Agrawal M (2007) Bioaccumulation of heavy metals in vegetables: a threat to human health. Terrestrial and Aquatic Environmental Toxicology @ 2007 Global Science Books

Akpan-Idiok AU, Udo IA, Braide EI (2012) The use of human urine as an organic fertilizer in the production of okra (*Abelmoschus esculentus*) in South Eastern Nigeria. Resour Conserv Recy 62:14–20

Alia N, Sardar K, Said M, Salma K, Sadia A, Sadaf S, Toqeer A, Miklas S (2015) Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. Int J Environ Res Public Health 12:7400–7416

Cofie O, Adeoti A, Nkansah-Boadu F, Awuah E (2010) Farmers' perception and economic benefits of excreta use in Southern Ghana. Resour Conserv Recy 55:161–166

Dingkwoet DJ, Danladi SM, Gabriel MS (2013) Comparative study of some heavy and trace metals in selected vegetables from four local government areas of Plateau State, Nigeria. J Environ Sci Toxicol Food Technol 6:86–93

Egigu MC, Melak B, Kebede A, Muthuswamy M (2014) Use of human urine as fertilizer for vegetation cultivation. Int J Agric Innov Res (IJAIR) 3(1):254–258

Farooq M, Anwar F, Rashid U (2008) Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrial area. Pak J Bot 40:2099–2106

Germer J, Addai S, Sauerborn J (2011) Response of grain sorghum to fertilization with human urine. Field Crop Res 122:234–241

Guzha E, Nhape I, Rockstrom J (2005) An assessment of the effects of human faeces and urine on maize production and water productivity. Phys Chem Earth 30:840–845

Heinonen-Tanski H, Van Wijk-Sijbesma C (2005) Human excreta for plant production. Bioresour Technol 36:403–411

Heinonen-Tanski H, Sjoblom A, Fabritus H, Karinen P (2007) Pure human urine is a good fertilizer for cucumbers. Bioresour Technol 98:214–217

Hoglund C, Ashbolt N, Stenstrom TA, Svensson L (2002) Viral persistence in source-separated human urine. Adv Environ Res 6:265–275

Iqbal MA, Chaudhary MN, Zaib S, Imran M, Ali K, Iqbal A (2011) Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb) in agricultural soils and spring seasonal plants, irrigated by industrial waste water. J Environ Tech Manage 2(1):1–9

Jensen PKM, Phuc PD, Knudsen LG, Dalsgaard A, Konradsen F (2007) Hygiene versus fertilizer: the use of human excreta in agriculture-a Vietnamese example. Int J Hyg Environ Health 211:432–439

Jung MC (2008) Heavy metal concentration in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W Mine. Sensors (Basel) 8:2413–2423

Kidd PS, Domìnguez-Rodrìguez MJ, Monterroso C (2007) Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere 66:1458–1467

Kirchmann H, Petterson S (1995) Human urine—chemical composition and fertiliser use efficiency. Fert Res 40:149–154

Kutu FR, Muchaonyerwa P, Mnkeni PNS (2010) Complementary nutrient effects of separately collected human faeces and urine on the yield and nutrient uptake of (*Spinacia oleracea*). Waste Manag Res 29:532–539

Lindt B, Ban Z, Byde'n S (2000) Nutrient recovery from human urine by struvite crystallization with ammonia adsorption on zeolite and Wollastonite. Bioresour Technol 73:169–174

Lion GN, Olowoyo JO (2013) Population health risk due to dietary intake of toxic heavy metals from *Spinacia oleracea* harvested from soils collected in and around Tshwane, South Africa. S Afr J Bot 88:178–182

Mnkeni PNS, Austin A, Kutu FR (2005) Preliminary studies on the evaluation of human urine as a source of nutrients for vegetables in the Eastern Cape Province, South Africa. In: Ecological Sanitation: A Sustainable Integrated Solution. Proceedings of the 3rd International Ecological Sanitation Conference, Durban, South Africa, 23–26 May 2005, pp 418–426. http://conference2005.ecosan.org/papes/mnkeni.etal.pdf

Mnkeni PNS, Kutu FR, Muchaonyerwa P (2008) Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. Waste Manag Res 26:132–139

Mohod CV (2015) A review on the concentration of the heavy metals in vegetable samples like spinach and tomato grown near the area of Amba Nalla of Amravati City. Int J Innov Res Sci, Eng Technol. doi:10.15680/IJIRSET.2015.0405019

Olowoyo JO, Okedeyi OO, Mkolo NM, Lion GN, Mdakane STR (2011) Uptake and translocation of heavy metals by medicinal plants around a waste dump site in Pretoria, South Africa. S Afr J Bot 78:116–121

Olowoyo JO, Mugivhisa LL, Busa NG (2015) Trace metals in soil and plants around a cement factory in Pretoria. South Africa. Pol J Environ Stud 24(5):2087–2093

Paschal DC, Ting BG, Morrow JC (1998) Trace metals in urine of United States residents: reference range concentrations. Environ Res 76:53–59

Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J (2009) The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. Int J of Biochem Cell Biol 41:1665–1677

Pradhan SK, Pitkanen S, Heinonen-Tanski H (2009a) Fertilizer value of urine in pumpkin (*Cucurbita maxima* L.) cultivation. J Agric Food Sci 18:57–68

Pradhan SK, Holopainen JK, Weisell J, Heinonen-Tanski H (2009b) Stored human urine supplemented with wood ash as fertilizer in tomato (*Solanum lycopersicum*) cultivation and its impacts on fruit yield and quality. J Agric Food Chem 57:7612–7617

Pradhan SK, Holopainen JK, Weisell J, Heinonen-Tanski H (2010a) Human urine and wood ash as plant nutrients for red beet (*Beta vulgaris)* cultivation: impacts on yield quality. J Agric Food Chem 58:2034–2039

Pradhan SK, Piya RC, Heinonen-Tanski H (2010b) Eco-Sanitation and its benefits: an experimental demonstration program to raise awareness in central Nepal. Environ Dev Sustain. doi:10.1007/s10668-010-9273-5

Ranasinghe ESS, Karunarathne CLSM, Beneragama CK, Wijesooriya BGG (2016) Human urine as a low cost and effective nitrogen fertilizer for bean production. Procedia Food Sci 6:279–282

Salawu K, Barau MM, Mohammed D, Mikailu DA, Abdullahi BH, Uroko RI (2015) Determination of some selected heavy metals in spinach and irrigated water from Samaru area within Gusau Metropolis in Zamfara State. Nigeria. J Toxicol Environ Health Sci 7(8):76–80

Sani HA, Tsafe AI, Bagudo BU, Itodo AU (2011) Toxic metals uptake by Spinach (*Spinacia oleracea*) cultivated in Sokoto: a comparative study. Pak J Nutr (PJN) 10(6):572–576

Sene M, Ken NH, Ushijima K, Funamizu N (2013) Effects of extra human urine volume application in plant and soil. Glob J Pests Dis Crop Prot (GJPDCP) 1:051–060

Singh KB, Taneja SK (2010) Concentration of Zn, Cu and Mn in vegetables and meat foodstuff commonly available in Manipur: a North Eastern state of India. Electron J Environl Agric Food Chem 3:610–616

Sithole SC, Mugivhisa LL, Olowoyo JO (2016) Pattern and concentrations of trace metals in mushrooms harvested from trace metal-polluted soils in Pretoria, South Africa. S Afr J Bot. doi:10.1016/j.sajb.2016.08.010

Tang X, Li X, Liu MZ, Hashmi J, Xu, Brookes PC (2015) Effects of inorganic and organic amendments on the uptake of lead and trace elements by *Brassica chinensis* grown in acidic red soil. Chemosphere 119:177–183

Upreti HK, Shrestha P, Paudel P (2011) Effect of human urine as fertilizer on crop production. Agron J Nepal 2:168–172

Uwah EL, Ndahi NP, Abdulrahman FI, Ogugbuaja VO (2011) Heavy metal levels in Spinach (*Amaranthus caudatus*) and Lettuce (*Lactuca sativa*) grown in Maiduguri, Nigeria. J Environ Chem Ecotoxicol 3(10):71–78

Winblad U, Simpson-Hébert M (2004) Ecological Sanitation—revised and enlarged edition. Stockholm, Sweden: Stockholm Environmental Institute, pp. 14–22. Available at: http://docs.zetatalk.com/Sanitation/Ecological-Sanitation-2004.pdf. Accessed January 2008

World Health Organization (WHO) (2001) Toxicological Evaluation of Certain Food Additives. Joint FAO/WHO Expert Committee on Food Additives. Food Additive Series No. 683. World Health Organization, Geneva

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Network (ISRN). Ecology. doi:10.5402/2011/402647

Zeka N, Mero F, Skenderasi B, Gjanci S (2014) Effects of nitrogen sources and levels on yield and nutritive values of spinach (*Spinacia oleracea* L.). J Int Acad Res Multidiscip (IJARM) 2(2):327–337