Accumulation of multiple heavy metals in plants grown on soil treated with sewage sludge for more than 50 years presents health risks and an opportunity for phyto-remediation

Awonke Mbangi[1]*, Pardon Muchaonyerwa[1] and Rebecca Zengeni[1]

[1]*School of Agriculture, Earth and Environmental Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa*

ABSTRACT

Long-term application of sewage leads to heavy metal accumulation in soils, causing serious risks to plants, humans, animals and the environment, and phytoremediation could be essential. A study was conducted to determine the concentration of heavy metals in self-seeding vegetables, amaranthus (*Amaranthus dubius*), tomato (*Solanum lycopersicum*), black nightshade (*Solanum nigrum*), *Rumex pulcher* and turf grass, grown on land treated with sewage sludge for over 50 years. A pot experiment was conducted to determine phytoremediation potential of Indian mustard (*Brassica juncea*), lucern (*Medicago sativa*), vetch (*Vicia sativa*), rape (*Brassica napus*) and ryegrass (*Lolium perenne*), using the same soil. Another pot experiment was conducted to determine effects on tissue metal composition of Indian mustard of adding increasing concentrations of EDTA. All the self-seeding vegetables had tissue Zn, Cu, Cr, Ni, Cd and Pb concentrations higher than toxicity thresholds. Turf grass tissue had higher concentrations of all the metals than all the self-seeding vegetables growing on the soil. Indian mustard and rape had the highest biomass and tissue concentration of most of the metals studied. Addition of EDTA to the soil drastically increased uptake of Zn, Cu, Cd and Pb but not Cr and Ni. The findings of this study imply that self-seeding vegetables and turf grass growing on the polluted soils pose serious health risks and that Indian mustard, and to some extent rape, have potential for phytoremediation, especially if grown on the soil treated with EDTA.

Keywords: heavy metals, phytoremediation, risk, sewage sludge, turf grass, uptake

INTRODUCTION

Disposal of sewage sludge presents challenges because of pathogenic organisms (e.g. *Escherichia coli*) as well as heavy metals like cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) (Korboulewsky et al., 2002). When the sludge is applied on soil, the metals accumulate in the soil until they reach hazardous levels, with potential of leaching to ground water (Wuana and Okieimen, 2011). Environmental effects of heavy metals depend on their chemical forms and bioavailability, which depend on soil factors such as texture, pH, redox potential and soil organic matter content (Sybhashini and Swamy, 2013; Wuana and Okieiman, 2011; Barazani et al., 2004). For example, Cd becomes more soluble and bioavailable at low pH (< 6.5), increasing uptake and incorporation into plant tissues and presenting risks to health of humans and animals that feed on the plants. At high pH (> 7.5) these metals are less soluble as they precipitate out of solution. As a result of the risks posed by heavy metals to plants, animals and humans, there is a need to understand effects of long-term sludge application on metal concentrations in soil and plant tissue (Williams and Brown, 2011; Lone et al., 2008). Species with particularly high metal uptake ratios could be considered for phyto-remediation (Tangahu et al., 2011).

Over 50 years of land application of sewage sludge by the Darvill Waste Water Works (DWWW), situated east of Pietermaritzburg, has resulted in soil pollution by metals, including Zn, Cu, Cr, Ni, Cd and Pb (Mdlambuzi, 2014). The self-seeding edible plants on metal-polluted sites pose a risk to the surrounding community, who feed on the vegetables. Some of these plants, like amaranthus, grow naturally in the area, while others, like tomatoes, grow from seeds emanating from the sewage sludge applied on the land over time. The high organic material in the soil and water, as supplied by the irrigated sludge, makes these plants flourish, and the poor residents of the surrounding communities take advantage of this.

In a study by Bempah et al. (2012) and Maleki and Zarasvand (2008), heavy metal limits approved by FAO/WHO and Codex Alimentarius Commission are used to determine risk associated with consumption of plants contaminated by heavy metals. The Joint FAO/WHO Expert Committee on Food Additives (1999) and Codex Alimentarius Commission (1991) give the following limits: Cd – 0.2 $mg{\cdot}kg^{-1}$, Cu – 40 $mg{\cdot}kg^{-1}$, Zn – 60 $mg{\cdot}kg^{-1}$, Cr – 2.3 $mg{\cdot}kg^{-1}$, Ni – 30 $mg{\cdot}kg^{-1}$ and Pb – 0.3 $mg{\cdot}kg^{-1}$. Consumption of plants containing heavy metal levels above the limits will have detrimental effects on humans or animals. There is a need to understand the level of risks the communities are exposed to through the consumption of vegetables that spontaneously grow on the site. Besides phytoremediation, alternative uses of the land without posing major risks to human health could also be tested.

A private company, Duzi Turf, produces turf-grass on the polluted soil, at DWWW, for sale as instant lawn. Turf grass accumulates large quantities of heavy metals (Onder et al., 2007; Qu et al., 2003). It is essential to understand the levels of metals exported to consumers' yards, both as part of turf-grass tissue and soil associated with the roots, to determine the extent of cross-contamination through transferring of the soil. In addition to understanding the risks posed, there is a need to devise cost-effective ways for remediation of the polluted

* To whom all correspondence should be addressed.
e-mail: awonkembangi15@gmail.com

Received 12 July 2017; accepted in revised form 18 September 2018

http://dx.doi.org/10.4314/wsa.v44i4.06
Available on website http://www.wrc.org.za
ISSN 1816-7950 (Online) = Water SA Vol. 44 No. 4 October 2018

site. Phytoremediation, the use of plants to extract, contain or immobilize contaminants, could be a cheaper alternative to reclaim heavy metal polluted soil at DWWW. Plants that can rapidly grow at high density and accumulate heavy metals in tissue are suitable for phytoremediation (Gosh and Singh, 2005).

A number of plants that can accumulate heavy metals to concentrations greater than 1 000 mg·kg^{-1} (hyper-accumulators) exist (Malik and Biswas, 2012). These plants have been found to accumulate metals including Zn, Cu, Cr, Ni, Cd and Pb (Dell'amico et al., 2008; Wu et al., 2006; Sheng and Xia, 2006; Belimov et al., 2005). Most of these plants, however, are known to have small shoot and root growth and an absence of commercially available seeds. This has encouraged researchers to not only study the hyper-accumulators, in their quest to phyto-remediate polluted soils, but also to look at field crops that produce a lot of biomass and can withstand toxicity of heavy metals, and accumulate them at greater amounts than ordinary plants, though not high enough to be classified as hyper-accumulators (Vamerali et al., 2010). Vamerali et al. (2010) gives another set of limits that differ from those given previously. These limits give toxicity threshold levels of heavy metals in plants, above which ordinary plants do not survive. Plants able to withstand such high levels have great potential in phytoremediation. These thresholds limits are: Zn – 150 mg·kg^{-1}, Cu – 15 mg·kg^{-1}, Cr – 2 mg·kg^{-1}, Ni – 20 mg·kg^{-1}, Cd – 5 mg·kg^{-1} and Pb – 20 mg·kg^{-1}. While most phytoremediation work has focused on one or two heavy metals at a time, the area at DWWW is polluted with multiple heavy metals. There is a need to test a number of plant species for their ability to accumulate multiple heavy metals from a polluted soil. Besides testing different plant species, attempts have been made to manipulate the soil, including adding chelating agents to mobilise the metals in soil to increase uptake by plants.

Some chelating agents (ligands), including ethylenediaminetetraacetic acid (EDTA), form more than one strong bond to a metal atom and effectively prevent the atom from reacting with any other substance and thus enable it to remain in solution (Oxtoby et al., 2015; Tan, 2010). Soil application of EDTA was shown to significantly increase uptake of Zn by barley, Indian mustard and oats (Ebbs and Kochian, 1998), while the same effects were reported for Pb (Wu et al., 2004). Soil application of EDTA could improve the potential of some plants for phytoremediation through increased uptake of multiple metals from polluted soil. The objective of this study was to determine the effects of long-term application of sewage sludge on concentrations of heavy metals in tissue of self-seeding plants, in relation to their risks to human health. Another objective was to determine the effects of selected plant species and soil addition of EDTA on uptake of multiple heavy metals in relation to phytoremediation.

MATERIALS AND METHODS

Study site

The study was carried out at Darvill Waste Water Works (DWWW) (29.60°S; 30.43°E; altitude 596 m amsl) on the eastern boundary of the city of Pietermaritzburg in KwaZulu-Natal Province, South Africa. Over 70% of the 78-ha land has been irrigated with sewage sludge using a sprinkler system for more than 50 years. A portion of the land is under commercial turf grass production, while self-seeding plants, including vegetables, overgrow the remaining area.

Tissue metal concentrations of self-seeding plants and turf grass on the polluted soil

Sampling self-seeding plants and turf grass at DWWW

Self-seeding plants were sampled using a 1 m^2 quadrant on three different parts of the land, for each plant species. On each of the sampling points the quadrant was placed and all of the plants within the borders of the quadrant were sampled and a composite sample was obtained for which analyses were done. The plants were rinsed with distilled water, oven-dried at 65°C to constant weight and ground to < 0.5 mm using a Fritsch Pulverisette mortar grinder before analysis. Commercial turf grass grown by a private company, Duzi Turf, on parts of the land was purchased. The turf grass was also sampled using 3 replicates of the 1 m^2 quadrant, with the top 5 cm of the soil attached to the roots. The soil was separated from the rooting system and the turf was dried to constant weight and ground before analysis. The soil was air-dried and sieved before analysis.

Analysis of plant tissue metal concentrations

The plant shoot samples were digested at 120°C with nitric and perchloric acid mixture (4:1 ratio) as described by Hseu (2004). The digests were analysed for heavy metals using the 720 Varian inductively coupled plasma optical emission spectrometer (ICP-OES). Tissue metal concentrations were compared with toxicity thresholds (Bempah et al. 2012). Concentrations above the limits cause serious harm to animals and humans consuming the plants.

Phytoremediation potential of selected plant species

Soil

The soil used in the two pot trials was from land treated with sewage sludge for over 50 years (at DWWW). The soil was sampled from 28 points (0–30 cm depth) using a spade, and mixed to form one composite sample. A control soil with no history of sewage sludge (upslope from the irrigated area) was sampled in the same way. Before use in the pot trials, the soils were air-dried, sieved (< 2 mm), and analysed for physico-chemical properties and heavy metal concentrations. The analysis included soil pH (1M KCl), extractable phosphorus (P), exchangeable calcium (Ca), magnesium (Mg), potassium (K), using methods of the Non-Affiliated Soil Analysis Work Committee (1990). Total carbon (C) and nitrogen (N) were determined with TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator. For heavy metal analysis, the soil samples were digested following the EPA 3051 method (US EPA, 1998) using the MARS 5 Microwave Accelerated Reaction System (CEM Corporation, USA) and analysed for Cd, Cr, Cu, Ni, Pb and Zn using the 720 Varian inductively coupled plasma optical emission spectrometer (ICP-OES). The characteristics of the soil used are given in Table 1.

Total trigger values were 200, 120, 350, 150, 3.0 and 100 mg·kg^{-1} for Zn, Cu, Cr, Ni, Cd and Pb, respectively. Maximum permissible limits were 700, 375, 450, 200, 5 and 150 mg·kg^{-1} for Zn, Cu, Cr, Ni, Cd and Pb, respectively (Herselman and Moodley, 2009).

TABLE 1 Selected properties of soils used in the study (mean ± standard error)		
Parameter	**Polluted soil**	**Control soil**
pH(KCl)	5.9 ± 0.09	5.0 ± 0.04
Total C (%)	16.0 ± 0.10	2.2 ± 0.21
Total N (%)	1.1 ± 0.18	0.2 ± 0.01
P (mg·kg^{-1})	223.6 ± 33.1	10.1 ± 2.07
K (cmol(+)·kg^{-1})	2.0 ± 0.28	0.3 ± 0.02
Ca (cmol(+)·kg^{-1})	29.2 ± 2.43	4.4 ± 0.06
Mg (cmol(+)·kg^{-1})	5.6 ± 1.40	2.2 ± 0.04
Zinc (Zn)	792 ± 45.4	183 ± 13.6
Copper (Cu)	264 ± 15.5	20 ± 1.1
Chromium Cr)	898 ± 152.4	38 ± 5.2
Nickel (Ni)	188 ± 11.5	63 ± 10.0
Cadmium (Cd)	7.4 ± 0.36	1.9 ± 1.25
Lead (Pb)	221 ± 36.2	74 ± 20.9

Testing phytoremediation potential of selected plant species

The pot experiment was conducted under glasshouse conditions at the University of KwaZulu-Natal (UKZN) in Pietermaritzburg. The experiment was laid out in a randomized complete block design with 5 plant species and 2 soil pollution levels (polluted and control), replicated 3 times. The plant species used were Indian mustard (*Brassica juncea*), lucerne (*Medicago sativa*), grazing vetch (*Vicia sativa*), rape (*Brassica napus*) and ryegrass (*Lolium perenne*). Pots with an inner diameter of 20 cm and height of 17 cm were filled with 3 kg of soil. Plant seeds were sown at optimum rates. Lucerne and vetch were sown at 25 kg·ha^{-1}, mustard and rape at 6 kg·ha^{-1}, and rye at 30 kg·ha^{-1}.

Fertilizer was added to the control soil before planting, at recommended rates following a soil test, so that the plants could achieve optimum growth. The polluted soil had sufficient phosphorus (P) and potassium (K) for all the plants. Limestone ammonium nitrate was applied at 100 kg·ha^{-1} N for Indian mustard and rape, and at 160 kg·ha^{-1} N for ryegrass, for both the polluted soil and the control. No N was applied on lucerne and vetch. Phosphorus and potassium were applied on the control soil, at 200 kg·ha^{-1} P (single superphosphate) and 225 kg·ha^{-1} K (potassium sulphate) for mustard and rape, 45 kg·ha^{-1} P and 31 kg·ha^{-1} K for ryegrass and at 43 kg·ha^{-1} P and 175 kg·ha^{-1} K for lucerne and vetch. The locations of the pots in each block were rotated periodically to ensure uniform light intensity to all pots. Plants were watered with distilled water, to replenish water loss through evapotranspiration. Weeds were removed manually during the duration of the experiment.

After 6 weeks shoots were harvested by cutting with scissors at the soil surface. The plant shoots were cleaned immediately after harvesting by rinsing in distilled water. The shoots were oven dried at 70°C to constant weight to determine dry matter yield, and ground to < 2 mm before analysis for heavy metals with ICP-OES after microwave digestion.

Testing effects of soil addition of EDTA on phytoremediation potential of Indian mustard

A follow-up pot experiment was conducted to determine the effect of addition of EDTA on heavy metal uptake and phytoremediation potential of Indian mustard. This plant was selected because it accumulated the greatest dry matter in the previous pot experiment. Indian mustard seeds were grown in 12 pots with 3 kg soil, without fertiliser addition. The plants were grown for 4 weeks in order to allow them to establish before addition of EDTA at 0, 3, 6 and 10 mmol·kg^{-1} of soil with each rate replicated 3 times. The plants were grown for a further 2 weeks before harvesting, drying, grinding and analysis of tissue Zn, Cu, Cr, Ni, Cd and Pb, as described for the first pot trial.

Statistical analysis and data handling

A one-way analysis of variance (ANOVA) was carried out using Genstat 14th edition to determine the effects of type of self-seeding plant on shoot tissue heavy metal concentrations. A two-way ANOVA was conducted to determine the effects of soil pollution and plant type on tissue metal composition for the pot trial. Mean separation was done using the least significant difference (LSD) at $p = 0.05$. Means and standard errors were calculated for the metal concentration in tissue of turf grass and in associated soil. The means were then compared with maximum permissible limits for plants and soils.

RESULTS

Heavy metal concentration in self-seeding plants and turf grass on polluted soil

Self-seeding plants

All the metals, except Cu, were above their maximum permissible limits in plants growing on land treated with sludge (Table 2). Metal concentrations were not significantly different among all plants, except Pb and Cd, which were different in tomato and *S. nigrum* for Pb as well as *Rumex* and *S. nigrum* for Cd, respectively. The highest concentrations of all the metals were in *Amaranthus* tissue with the exception of Cd. The lowest concentrations for Zn, Cu and Ni were in *S. nigrum*, and the lowest concentrations for Cr and Pb were in tomato. Tissue of *Amaranthus* sold at the market (harvested from study site) had 94.8, 33.5, 48.8, 100.6, 0.8 and 2.9 mg·kg^{-1} of Zn Cu, Cr, Ni, Cd and Pb, which were all above their MPL except Cu.

TABLE 2 Mean concentrations of heavy metals in plants growing in the study area						
Species	**Metal concentration (mg·kg^{-1})**					
#MPL	**Zn 60**	**Cu 40**	**Cr 2.3**	**Ni 30**	**Cd 0.2**	**Pb 0.3**
Rumex	79.7	14.0	43.9	96.4	0.7	2.2
Amaranthus	94.8	33.5	48.8	100.6	0.8	2.9
Tomato	64.0	17.9	27.4	98.8	1.2	0.8
S. nigrum	53.1	10.0	31.4	85.5	1.4	3.2
$LSD_{0.05}$	61.0	22.4	22.4	27.5	0.59	1.5

#MPL = maximum permissible limit (Bempah et al., 2012; Vamerali et al., 2010 for Ni)

TABLE 3
Heavy metal concentrations (mean ± standard error) in turf grass and associated soil

Metal	Concentration (mg·kg^{-1})			
	Turf tissue	*MPL	Root-associated soil	#MPL
Zn	414.7 ± 12.6	150	470.3 ± 31.1	700
Cu	66.5 ± 8.5	40	87.4 ± 4.5	375
Cr	403.7 ± 31.5	2	563.1 ± 51.7	450
Ni	197.3 ± 22.7	15	105.1 ± 6.12	200
Cd	2.0 ± 0.7	5	4.9 ± 0.5	5
Pb	20.7 ± 2.1	10	68.5 ± 19.2	150

*Vamerali et al., 2010; #Herselman and Moodley, 2009

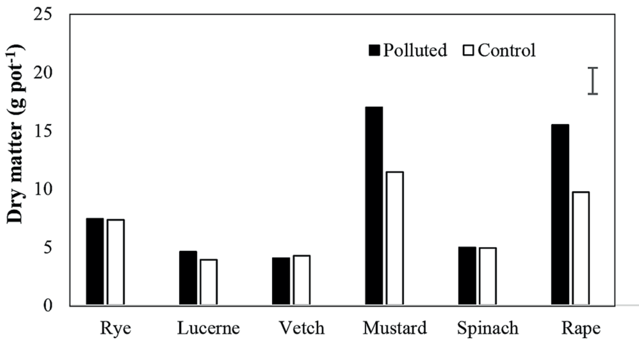

Figure 1
Shoot dry matter of plants grown on soil after long-term application of sewage sludge. The error bar represents least significant difference at $p < 0.05$

Turf grass and soil associated with roots

Concentration of Cr, Cu, Ni, Pb and Zn in the turf grass tissue was higher than their respective MPL (Table 3). Cr and Ni were the most highly concentrated, with Ni 13 times its MPL and Cr 200 times its MPL. The concentration of Cd was well below the limit. Concentration of Cr on soils associated with roots of turf grass was higher than the MPL (Table 3). Concentrations of Cu, Ni, Zn and Pb in the root-associated soil were lower than the MPL.

Phytoremediation potential of selected plants grown on polluted soil and effects of EDTA

Dry matter and metal concentrations of selected plants

Mustard and rape had higher dry matter on the polluted soil than the control, while there were no differences for the other plants (Fig. 1). Mustard and rape had higher dry matter than all the other plants on both polluted and control soils (Fig. 1).

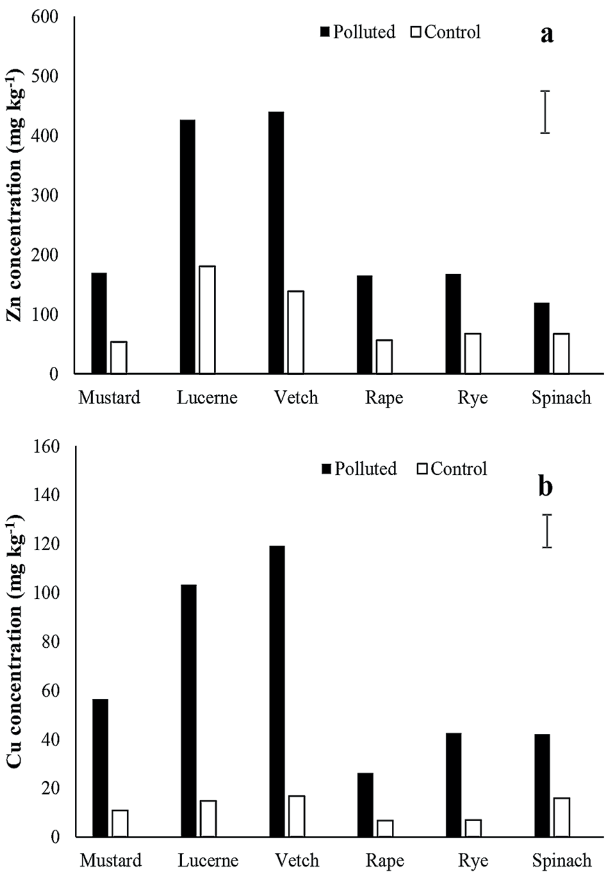

Figure 2
Tissue Zn (a) and Cu (b) concentrations of plants grown on soil after long-term application of sewage sludge. The error bar represents least significant difference at $p < 0.05$. *Toxicity threshold; 150 and 15 mg·kg*$^{-1}$ *for Zn and Cu, respectively (Vamerali et al., 2010).*

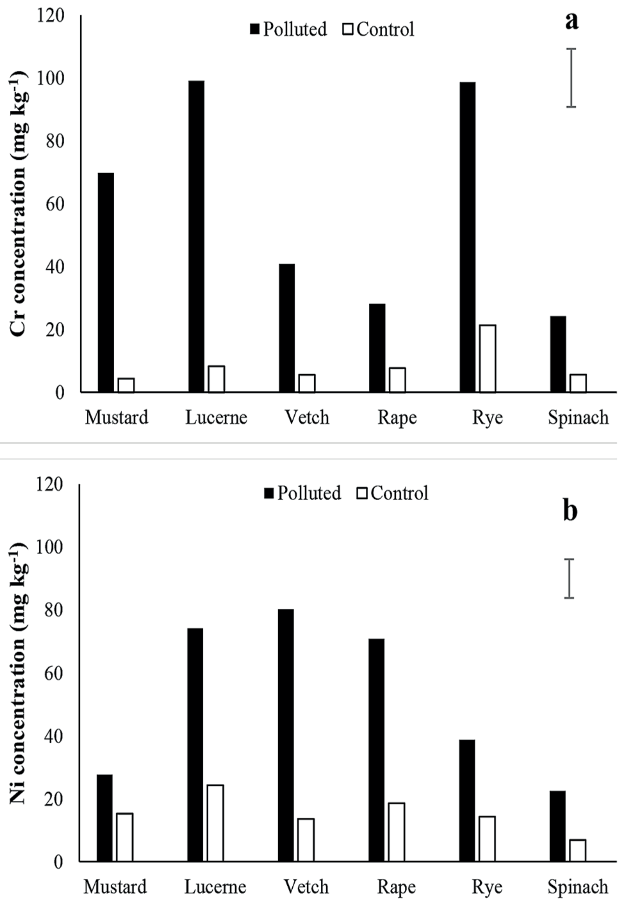

Figure 3
Tissue Cr (a) and Ni (b) concentrations of plants grown on soil after long-term application of sewage sludge. The error bar represents least significant difference at $p < 0.05$. *Toxicity threshold; 2 and 20 mg·kg*$^{-1}$ *for Cr and Ni, respectively (Vamerali et al., 2010).*

Concentrations of both Zn and Cu (Figs 2a and b) were higher in the plants grown in the contaminated soil compared to the control. Lucerne and vetch had the highest concentration of both Zn and Cu for both soils.

Cr and Ni (Fig. 3) were higher in the plants grown in the contaminated soils than in the control. Cr concentrations were above the limit in all plants grown on both soils. Ni was also above the limit in the polluted soil for all plants. Lucerne, vetch and rape had the highest Ni concentration (Fig. 3b).

Rape and mustard had the highest Cd concentration (Fig. 4a). The plants grown in the control soil had lower concentrations of Cd with respect to the limit. Pb concentration was highest in vetch and rye (Fig. 4b). Tissue Pb concentrations were below the threshold for all the plants on the control soil.

Dry matter and tissue metal concentrations of Indian mustard grown with EDTA

Mustard dry matter yield declined when EDTA was added to the soil, relative to the control soil (Table 4). There was no significant difference in dry matter between the 3 and 6 mmol·kg^{-1} treatments. The 10 mmol·kg^{-1} EDTA concentration resulted in death of the plants. Tissue concentrations of Pb, Cd and Zn increased and Cr decreased with increase in EDTA application rate (Table 4). Tissue Cu at 3 mmol·kg^{-1} EDTA was higher than the control, while Ni did not respond to EDTA application.

DISCUSSION

The high soil concentrations of the metals (Table 1) explain their extremely high tissue concentrations, except Cu, in all the plants sampled from the site. The elevated levels of heavy metals in soils is a result of long-term application of sewage sludge, an assertion which is supported by several other researchers (Madyiwa et al., 2004; Mapanda et al., 2005; Katanda et al., 2007; Bergkvistet al., 2003). A survey done by Snyman et al. (2004) showed that 61% and 44% of sludges surveyed in South Africa exceeded the limits for Ni and Zn, with 35% exceeding the limits for at least two metals. The lower soil Cu than the MPL explains the tissue levels for Cu that were lower than the toxicity threshold. While Ni concentrations in the soil approached the MPL, the tissue composition of the plants growing on the site exceeded the toxicity threshold for Ni. The high metal concentrations were bioavailable at the relatively low soil pH (5.9), resulting in greater uptake by plants (Wuana and Okieman, 2012). The extremely high tissue Zn, Cr, Ni, Cd and Pb in all four plants growing on the site present a major health risk to people consuming these vegetables. Indigenous vegetables are popular with local communities and their consumption is being encouraged in South Africa due to their high nutritive value, including protein, minerals and vitamins (Mnkeni et al., 2007). Consumption of indigenous vegetables with extremely high levels of heavy metals from DWWW therefore poses health risks.

Amaranthus poses the greatest risk for the introduction of these metals to the food chain as it has the highest demand (as indicated by its sale at the market) and it is the most consumed plant by local communities. For example, the elevated concentrations of Cd in the edible parts of *S. nigrum* and *Amaranthus* could result in its accumulation in the kidney and liver resulting in hypertension and cardiovascular diseases, causing renal damage and osteoporosis in humans (Jarup et al., 1998). While the accumulation of the heavy metals in *Amaranthus*, *Rumex*, *S. nigrum* and tomato may pose a risk to human and animal health, these plants may have potential to

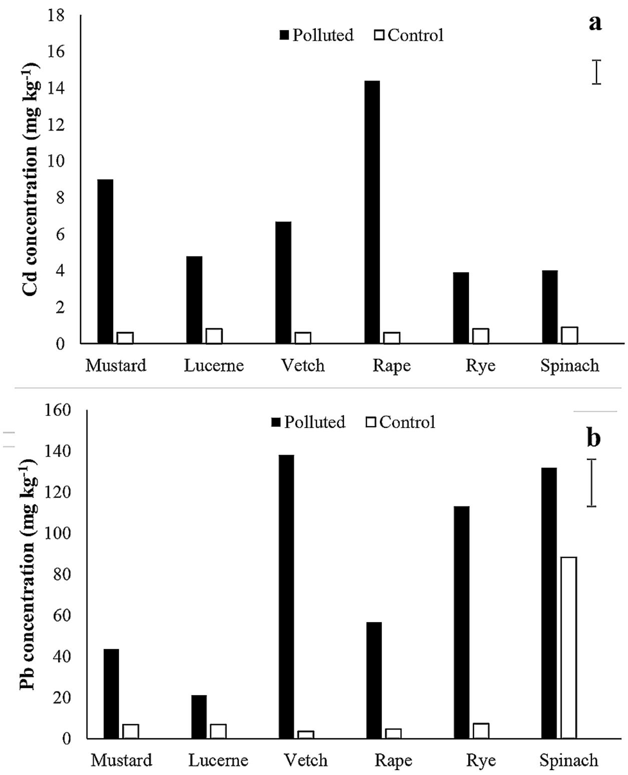

Figure 4

Tissue Cd (a) and Pb (b) concentrations of plants grown on soil after long-term application of sewage sludge. The error bar represents least significant difference at $p < 0.05$. *Toxicity threshold; 2 and 20 mg·kg^{-1} for Cd and Pb, respectively (Vamerali et al., 2010)*

TABLE 4
Dry matter and shoot tissue metal concentrations of mustard grown on polluted soil amended with increasing concentrations of EDTA

Parameter	EDTA concentration (mmol·kg^{-1})			LSD	Limit#
	0.0	3.0	6.0		
Dry matter (g·pot^{-1})	17.0	11.2	10.6	4.9	–
Zinc (mg·kg^{-1})	169.1	490.6	781.6	20.7	150
Copper (mg·kg^{-1})	56.4	138.7	87.8	76.3	15
Chromium (mg·kg^{-1})	69.8	46.3	25.6	35.9	2
Nickel (mg·kg^{-1})	27.7	31.2	47.1	20.0	20
Cadmium (mg·kg^{-1})	9.0	102.6	172.0	68.3	2
Lead (mg·kg^{-1})	43.7	285.9	745.3	67.0	20

#Vamerali et al. (2010)

remediate the pollution. Deliberate efforts could be made to grow these plants for high biomass production to maximise uptake of the metals. The pot experiment showed that tissue metal concentrations were all above the toxicity thresholds.

The high metal concentrations in the polluted soil, which were readily available at pH 5.9, explain the higher tissue metal concentrations in all the plants than for the control. The higher tissue Cd concentration in mustard and rape than in the other plants could be explained by high soil concentrations and

http://dx.doi.org/10.4314/wsa.v44i4.06
Available on website http://www.wrc.org.za
ISSN 1816-7950 (Online) = Water SA Vol. 44 No. 4 October 2018

preferential uptake by plants of the Brassicaceae family. The Brassicaceae family is well known for accumulating a wide range of heavy metals especially Cd and Zn (Babula et al., 2012). Although all the metals occurred at high concentrations in the soil, the highest tissue concentrations of Zn, Cu and Ni occurred in grazing vetch and lucerne (legumes), of Cr in lucerne and ryegrass, and of Pb in grazing vetch and ryegrass. These results indicated that no single plant could accumulate all the metals at the highest tissue concentrations. However, tissue metal concentrations did not follow the same trend as dry matter yield, where mustard and rape (Brasiccas) accumulated the greatest and legumes (lucerne and grazing vetch) had the least.

Based on dry matter, Zn uptake was 2 871, 2 545, 1 776, 1 956 and 830 $\mu g \cdot pot^{-1}$ for mustard, rape, vetch, lucerne and ryegrass, respectively. Copper uptake was 958 for mustard, while those of the other plants were < 500 $\mu g \cdot pot^{-1}$. In mustard and rape Cd uptake levels were 153 and 223 $\mu g \cdot pot^{-1}$, respectively, with < 30 $\mu g \cdot pot^{-1}$ for all other plants. Lead uptake levels were 742 and 875 $\mu g \cdot pot^{-1}$for mustard and rape, with < 600 $\mu g \cdot pot^{-1}$ for all other plants. While all other plants took up < 500 $\mu g \cdot pot^{-1}$ Ni, rape had 1 096 $\mu g \cdot pot^{-1}$ Ni. Based on the uptake (product of dry matter and tissue composition), mustard and rape (Brasiccas) took up the highest levels of all metals than the other plants. However, mustard took up more Zn, Cu and Cr and less Ni, Cd and Pb than rape. The high uptake of all the metals by at least one Brassica (mustard or rape), indicates that these plants have the highest potential for phytoremediation. For effective remediation of the Darvill site, these two plants could be grown as bicultures (mixtures). The high biomass accumulation of these plants in a mixture could further increase their effectiveness. However, using these plants presents major risks as they are edible vegetables, and, if consumed, could cause serious health risks. The site needs to be effectively secured if this approach is used. Besides crop mixtures the addition of EDTA could significantly increase the accumulation of the metal by individual plants.

While addition of EDTA at 3 and 6 $mmol \cdot kg^{-1}$ decreased dry matter by 34 and 38%, respectively, these rates increased tissue Zn, Cu, Cd and Pb composition more than for any other plant tested without EDTA addition. The increases of tissue Zn, Pb, and Cd concentration with increase in EDTA concentration were in agreement with Ebbs and Kochian (1998), Wu et al. (2004), Marques et al. (2009) and Farid et al. (2013) for plants including barley, Indian mustard, oats, and *S. nigrum*. Chelating agents like EDTA have been demonstrated to improve the uptake of metals like Pb, Cd and Zn, and at certain levels of concentration these can greatly affect the growth of plants (Ghani, 2010).

The lack of response of Ni and decrease of Cr in tissue could be explained by poor translocation of the complexes from the roots to the shoots. Chen and Cutright (2001) reported higher Cr in the root tissue than the shoots of sunflower (*Heliantus annuus*), when EDTA was used. The decline in dry matter with EDTA addition could be because of osmotic effects and metal toxicity, causing disruptions in the normal functioning of enzymes in plants (Cheng, 2003). Although dry matter decreased, 3 $mmol \cdot kg^{-1}$ EDTA increased uptake of Zn, Cu, Cd and Pb by mustard by 1.9, 1.6, 7.5 and 4.3 times, respectively. The respective increases at 6 $mmol \cdot kg^{-1}$ EDTA, were 2.8, 1, 11.9 and 10.6 times. The extremely high tissue content increases the effectiveness of remediation. However, if this vegetable is consumed, it poses serious health risks. The success of indigenous and exotic vegetables and pasture plants for phytoremediation, therefore, depends on securing the site from local communities who could harvest and consume the vegetables, or graze their animals. Plants that are not edible, and have other value, like turf-grass, could be ideal. Turf grass has the greatest potential of all the plants growing on the study site.

The higher concentration of all heavy metals in turf grass suggests that turf grass has greater potential for removal of heavy metals from the site. Turf grass was able to accumulate a number of the heavy metals (Cr, Cu, Ni, Pb and Zn) to far above the normal toxicity threshold (Bempah et al., 2012; Vamerali et al., 2010), and this result was in agreement with Onder et al. (2007) and Qu et al. (2003). Tissue Cr, Cu, Ni, Pb and Zn were 211, 1.5, 11, 2 and 3 times the toxicity threshold limit, respectively. Except for Cr, all metals were lower than the MPL in the soil associated with the root system, further indicating the remediating effects of turf grass. The fast growth and strong regeneration capacity of turf grass, allowing it to be mowed many times annually, makes it highly suitable for phytoremediation. However, the sale of turf-grass for instant lawn, could transfer large quantities of metals to residential areas, golf courses and other sites as part of the tissue and soil associated with the root system. The level of pollution off-site may largely depend on the size of the land and the frequency of replacement of the lawn. Small properties receiving turf grass on a yearly basis would be in greater risk of accumulating these metals than sport fields or recreation grounds with large areas.

CONCLUSION

Long-term application of sewage sludge increased tissue Cr, Zn, Ni, Cd, Pb concentration of self-seeding vegetable plants to levels higher than the toxicity limits, posing health risks to local communities who consume the plants. While there were variations in dry matter and tissue metal composition of plants tested for phytoremediation, either Indian mustard and/or rape had the greatest uptake of each of the metals studied. Although addition of EDTA reduced dry matter of mustard, it increased heavy metal uptake by up to 12 times, depending on the metal and the concentration of EDTA. The high concentrations of the metals could pose a health risk if the vegetables are consumed, and security structures need to be established to limit access. Phytoremediation of the site could be better achieved with bicultures of Indian mustard and rape, and this practice needs to be tested under field conditions. Turf-grass tissue had between 2 and 211 times the toxicity threshold limit for the different heavy metals. The sale of turf-grass as instant lawn transfers large quantities of metals in plant tissue and on soil associated with the roots, posing a risk to human health. There is a need for deliberate efforts to increase biomass production of the indigenous vegetables growing at the site to contribute to phytoremediation. Frequent mowing of the turf grass could have significant rehabilitation effects, and needs to be tested. While EDTA application improves heavy metal uptake through increased mobility and bioavailability, the practice could also result in leaching of the metals and therefore needs to be studied under field conditions.

ACKNOWLEDGEMENT

The National Research Foundation (NRF) funded the research through a rated researcher incentive (GUN95948) and an MSc bursary for the first author.

REFERENCES

BABULA P, ADAM V, HAVEL L and KIZEK R (2012) Cadmium accumulation by plants of Brassicaceae family and its connection with their primary and secondary metabolism**.** In: Anjum NA, Ahmad I, Pereira ME, Duarte AC, Umar S, Khan NA (eds) *The Plant Family Brassicaceae: Contribution Towards Phytoremediation. Environmental Pollution 21*. 71–97. https://doi.org/10.1007/978-94-007-3913-0_3

BARAZANI O, SATHIYAMOORTHY P, MANANDHAR U, VULKAN R and GOLAN-GOLDHIRSH A (2004) Heavy metal accumulation by *Nicotiana glauca* Graham in a solid waste disposal site. *Chemosphere* **54** (7) 867–872. https://doi.org/10.1016/j.chemosphere.2003.10.005

BELIMOV AA, HONTZEAS N, SAFRONOVA VI, DEMCHINSKAYA S V, PILUZZA G, BULLITTA S and GLICK BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.* **37** (2) 241–250. https://doi.org/10.1016/j.soilbio.2004.07.033

BEMPAH CK, BOATENG J, ASOMANING J and ASABERE SB (2012) Heavy metals contamination in herbal plants from some Ghanaian markets. *J Microbiol. Biotechnol. Food Sci.* **2** (3) 886–896.

BERGKVIST P, JARVIS N, BERGGREN D and CARLGREN K (2003) Long-term effects of sewage sludge applications on soil properties, cadmium availability and distribution in arable soil. *Agric Ecosyst. Environ.* **97** (1) 167–179. https://doi.org/10.1016/S0167-8809(03)00121-X.

CHEN H and CUTRIGHT T (2001) EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere* **45** (1) 21–28. https://doi.org/10.1016/S0045-6535(01)00031-5

CODEX ALIMENTARIUS COMMISSION (1991) Joint FAO/WHO Food Standards Program, Nineteenth Session, 1–10 July 1991, Rome.

DELL'AMICO E, CAVALCA L and ANDREONI V (2008) Improvement of Brassica napus growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol. Biochem.* **40** (1) 74–84. https://doi.org/10.1016/j.soilbio.2007.06.024

EBBS SD and KOCHIAN LV (1998) Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). *Environ. Sci. Technol.* **32** (6) 802–806. https://doi.org/10.1021/es970698p

FARID M, SHAKOOR MB, EHSAN S, ALI S, ZUBAIR M and HANIF M (2013) Morphological, physiological and biochemical responses of different plant species to Cd stress. *Int. J. Chem. Biochem. Sci.* **3** 53–60.

GHANI A (2010) Toxic effects of heavy metals on plant growth and metal accumulation in maize (*Zea mays* L.). *Iranian Journal of Toxicology* **4** (3) 325–334.

GHOSH M and SINGH S (2005) A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J. Energ. Environ.* **6** (4) 18.

HERSELMAN JE and MOODLEY P (2009) Guidelines for the utilisation and disposal of wastewater sludge: Volume 4 of 5: Requirements for the beneficial use of sludge at high loading rates. WRC Report No. TT 350/09. Water Research Commission, Pretoria.

HSEU ZY (2004) Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresour. Technol.* **95** (1) 53–59. https://doi.org/10.1016/j.biortech.2004.02.008

JARUP L, BERGLUND M, ELINDER CG, NORDBERG G and VANTER M (1998) Health effects of cadmium exposure–a review of the literature and a risk estimate. *Scand. J. Work Environ. Health* **24** (1) 1–51.

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (1999) Toxicological evaluation of certain food additives. ILSI Press International Life Sciences Institute, Geneva.

KATANDA Y, MUSHONGA C, BANGANAYI F and NYAMANGARA J (2007) Effects of heavy metals contained in soil irrigated with a mixture of sewage sludge and effluent for thirty years on soil microbial biomass and plant growth. *Phys. Chemistry Earth Parts A/B/C* **32** (15) 1185–1194. https://doi.org/10.1016/j.pce.2007.07.016

KORBOULEWSKY N, DUPOUYET S and BONIN G (2002) Environmental risks of applying sewage sludge compost to vineyards. *J. Environ. Qual.* **31** (5) 1522–1527. https://doi.org/10.2134/jeq2002.1522

LONE MI, HE ZL, STOFFELLA PJ and YANG XE (2008) Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *J. Zhejiang Univ. Sci. B* **9** (3) 210–220. https://doi.org/10.1631/jzus.B0710633

MADYIWA S, CHIMBARI MJ and SCHUTTE F (2004) Lead and cadmium interactions in Cynodon nlemfuensis and sandy soil subjected to treated wastewater application under greenhouse conditions. *Phys. Chem. Earth Parts A/B/C* **29** (15) 1043–1048. https://doi.org/10.1016/j.pce.2004.08.006

MALEKI A and ZARASVAND MA (2008) Heavy metals in selected edible vegetables and estimation of their daily intake in Sanandaj, Iran. *Southeast Asian J. Trop. Med. Public Health* **39** (2) 335–40.

MALIK N and BISWAS AK (2012) Role of higher plants in remediation of metal contaminated sites. *Sci. Rev. Chem. Comm.* **2** (2) 141–147.

MAPANDA F, MANGWAYANA E, NYAMANGARA J and GILLER K (2005) The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agric. Ecosyst. Environ.* **107** (2) 151–165. https://doi.org/10.1016/j.agee.2004.11.005

MARQUES AP, MOREIRA H, RANGEL AO and CASTRO PM (2009) Arsenic, lead and nickel accumulation in Rubus ulmifolius growing in contaminated soil in Portugal. *J. Hazardous Mater.* **165** (1) 174–179. https://doi.org/10.1016/j.jhazmat.2008.09.102

MDLAMBUZI T (2014) Long-term effects of sewage sludge application on heavy metal distribution in a loam soil and indigenous vegetables. MSc thesis, University of KwaZulu Natal.

MNKENI AP, MASIKA P and MAPHAHA M (2007) Nutritional quality of vegetable and seed from different accessions of *Amaranthus* in South Africa. *Water SA* **33** (3) 377–380.

ONDER S, DURSUN S, GEZGIN S and DEMIRBAS A (2007) Determination of heavy metal pollution in grass and soil of city centre green areas (Konya, Turkey). *Pol. J. Environ. Stud.* **16** (1) 145–154.

OXTOBY DW, GILLIS HP and BUTLER LJ (2015) *Principles of Modern Chemistry*. Cengage Learning, Belmont.

QU R, LI D, DU R and QU R (2003) Lead uptake by roots of four turfgrass species in hydroponic cultures. *HortScience* **38** (4) 623–626.

RAHMAN MM, AZIRUN SM and BOYCE AN (2013) Enhanced accumulation of copper and lead in amaranth (*Amaranthus paniculatus*), Indian mustard (*Brassica Juncea*) and sunflower (*Helianthus annuus*). *PLoS ONE* **8** (5). https://doi.org/10.1371/journal.pone.0062941

SHENG XF and XIA JJ (2006) Improvement of rape (Brassica napus) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere* **64** (6) 1036–1042. https://doi.org/10.1016/j.chemosphere.2006.01.051

SNYMAN HG, HERSELMAN JE and KASSELMAN G (2004) A metal content survey of South African sewage sludge and an evaluation of analytical methods for their determination in sludge. WRC Report No. 1283/1/04. Water Research Commission, Pretoria.

SYBHASHINI V and SWAMY A (2013) Phytoremediation of cadmium and chromium from contaminated soils using *Physalis minima* Linn. *Am. Int. J. Res. Formal Appl. Nat. Sci.* **3** (1) 13–260.

TAN KH (2010) *Principles of Soil Chemistry*. CRC Press, Boca Raton.

TANGAHU BV, SHEIKH ABDULLAH SR, BASRI H, IDRIS M, ANUAR N and MUKHLISIN M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng.* **2011** 1–31. https://doi.org/10.1155/2011/939161

THE NON-AFFILIATED SOIL ANALYSIS WORK COMMITTEE (1990) Handbook of standard soil testing methods for advisory purposes. Soil Science Society of South Africa, Pretoria.

US EPA METHOD 3051A (1998) Microwave assisted acid digestion of sediments, sludge's, soils, and oils. *Test Methods for Evaluating Solid Waste* 1–30.

VAMERALI T, BANDIERA M and MOSCA G (2010) Field crops for phytoremediation of metal-contaminated land. A review. *Environ. Chem. Lett.* **8** (1) 1–17. https://doi.org/10.1007/s10311-009-0268-0

WILLIAMS DR and BROWN JD (2011) Living soil and sustainability education: Linking pedagogy and pedology. *J. Sustainability Educ.* **2** 1–18.

WU L, LUO Y, XING X and CHRISTIE P (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agric. Ecosyst. Environ.* **102** (3) 307–318. https://doi.org/10.1016/j.agee.2003.09.002

WU S C, CHEUNG KC, LUO YM and WONG MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by Brassica juncea. *Environ. Pollut.* **140** (1) 124–135. https://doi.org/10.1016/j.envpol.2005.06.023

WUANA RA and OKIEIMEN FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecol.* **2011** 1–20. https://doi.org/10.5402/2011/402647

http://dx.doi.org/10.4314/wsa.v44i4.06
Available on website http://www.wrc.org.za
ISSN 1816-7950 (Online) = Water SA Vol. 44 No. 4 October 2018