Using soil heavy metal enrichment and mobility factors to determine potential uptake by vegetables

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ABSTRACT

This study investigated copper (Cu) and lead (Pb) enrichment factor (EF) and mobility factor (MF) as possible indicators of their uptake by spinach (*Spinaceae oleraceae*) and carrots (*Daucus carota*) grown on a sludge-amended luvisol (SAL). Sewage sludge was applied to luvisol at different rates and spinach and carrots planted. Enrichment of Cu and Pb in SAL was determined, and values regressed with those of Cu and Pb concentrations in spinach and carrots. Concentration of Cu and Pb in vegetables was calculated using the regression model obtained, and calculated values compared with actual values. Pb MF were higher than Cu MF but Cu and Pb EF_{soil} values were < 3.0, indicating minor enrichment from sludge addition. EF had 10% reliability in predicting Cu and Pb uptake in vegetables. MF was more than 70% reliable in predicting carrot Cu uptake and spinach Pb uptake. EF and MF are not effective as predictors of heavy metal uptake by vegetables. The role of other soil components including root exudates and by-products from microbial activities should also be investigated.

Keywords: bioavailability; luvisol; sludge application; regression model

Sludge is used to amend soils in several countries (Logan et al. 1996, Tlustoš et al. 2000) but it is also a major source of heavy metals including Cu and Pb in soils (Tlustoš et al. 2000, Ngole 2007). Cu is a vital component of many enzymes and proteins needed for plant growth but excess Cu in plants may cause reduced growth, chlorosis and root malformation (Nicholls and Mal 2003). Pb has no physiological role in plants. In excess, it disturbs electron transfer reactions, affecting mitochondrial respiration and photosynthesis (Xiong et al. 2006). The possibility of plants accumulating these metals from sludge-amended soils (SAS) has resulted in countries setting guidelines on concentrations of heavy metals in sludge for agricultural use. The guidelines consider total concentration of heavy metals in sludge but this may only indicate the risk to which plants are exposed (Chimbari et al. 2001). Reliable prediction of possible heavy metals uptake by vegetables from SAS will guide decisions regarding sewage sludge use in agriculture.

Different parameters were used to investigate metal uptake by plants grown on SAS. Mingorance et al. (2007) and Yoon et al. (2006) used biconcentration factor (BF) (ratio of metal concentration in plant to metal concentration in soil) to investigate heavy metal uptake by plants. Badaway and El-Motaium (2001) used MF (ratio of an element in the mobile fractions (exchangeable + CO_3^{2-}) to that in the inert fractions (reducible + oxidizable + residual). Methods used to characterize heavy metal input in soils also vary. Mingorance et al. (2007) and Mtanga and Machiwa (2007) used EF (relative abundance of a chemical element in soil compared to the relative abundance in a local control site). Manzoor et al. (2006), Franco-Uría et al. (2009) and Dragovic et al. (2008) used multivariate analyses. In this study, Cu and Pb, MF and EF in SAL were used to predict their uptake by spinach and carrots. The uptake predictive reliability of the two factors was determined.

MATERIAL AND METHODS

Sampling of soil and sludge. 60 luvisol samples were collected at depths of 0–50 cm (rooting zone of most crops) from Barolong Farms (25°30'S, 25°45'S and 25°00'E, 25°45'E) Botswana, and homogenized to form a composite representative soil sample for

Table 1. Some properties of luvisol and sludge used

Properties	Luvisol	Sludge	Units
Sand	85.25	–	wt %
Silt	11.99	–	
Clay	5.76	–	
pH H_2O	6.80	5.70	–
Electrical conductivity	211.00	4700.00	µs/cm
Cation exchange capacity	7.70	39.02	cmolc/kg soil
Exch K	1.17	1.40	
Exch Ca	3.9	1.27	
Exch Mg	2.15	1.14	
Exch Na	0.59	1.34	
Organic matter	1.88	23.10	%
TKN	2.26	4.40	
NH_4-N	314.20	612.20	mg/kg
NO_3-N	207.10	546.20	
P	33.60	7319.00	
Cu	18.01	119.5	
Pb	51.9	295.5	

Exch – exchangeable. Modified after Ngole et al. (2006)

the area. A three-year old sludge generated through activated-sludge method of wastewater treatment, anaerobically digested, and air-dried, was sampled from different spots on a sludge pile at a wastewater treatment plant in Botswana. Though the sludge underwent no further treatment, periodic aeration may have occurred when sludge pile was shifted to create space for younger sludge. Sludge samples were homogenized to form a representative sample of the pile.

Determination of soil and sludge properties. Selected properties of homogenized luvisol and sludge were determined. Their pH and electrical conductivity were determined in a 1:2.5 soil: water suspension. Wet combustion and ascorbic acid reduction methods were used in organic matter (OM) and phosphorus (P) determination, respectively (van Reeuwijk 2002). Plant available nitrogen was extracted with KCl (Csuros 1997) whereas H_2SO_4 and Se were used to digest samples for total Kjeldahl nitrogen (TKN). Cation exchange capacity and exchangeable K, Na, Ca and Mg were determined using CH_3COONH_4 extraction technique (van Reeuwijk 2002). Instrumentation used included Schimadzu 1601 UV-Visible Spectrophotometer (P), Gerhardt Kjeldtherm digestion system, Turbosorg scrub unit and Vapodest 30 distilling (TKN, NO_3-N, and NH_4-N) and Varian 220 FS flame atomic absorption spectrometer (FAAS, California, USA) (exchangeable K, Na, Ca and Mg, Cu and Pb). Properties of homogenized luvisol and sludge used are indicated in Table 1.

Greenhouse experiment. Composites of sludge and luvisol were mixed at volume per volume percent (v/v %) ratios of 0:100 (control), 5:95, 10:90, 20:80, and 40:60 sludge: luvisol and were allowed to mature for three months. Cu and Pb concentrations in the different fractions of SAL were determined as described by Tessier et al. (1979) (Table 2). Each mixture was sieved with a 4 mm sieve (Hammer and Keller 2002) and transferred into ten plant pots each measuring 25 cm in diameter (6750 cm^3). Pots were moved into a greenhouse and allowed to acclimatize for three days. Spinach and carrot seeds were then planted directly on potted SAL. Five pots were used for growing spinach and five for carrots. During growth, greenhouse temperature was maintained at 20°C and each pot received 500 ml of tap wa-

Table 2. Sequential extraction procedure used to determine metals in different fractions

Stage	Fraction	Reagents	Comments
1	exchangeable	1 mol/dm^3 $MgCl_2$	suspension shaken for 1 h at 25°C
2	carbonate	1 mol/dm^3 CH_3COONa adjusted to pH 5 with CH_3COOH	suspension shaken for 5 h at 25°C
3	reducible	0.04 mol/dm^3 $NH_2OH.HCl$ in 25% v/v CH_3COOH	suspension heated in water bath for 6 h at 96 ± 3°C with occasional agitation
4	oxidizeable	3 ml of 0.02 mol/dm^3 HNO_3 and 5 ml of 30% H_2O_2 adjusted to pH 2 with HNO_3 + 3.2 mol/dm^3 CH_3COONH_4 in 20% v/v HNO_3	mixture heated at 85 ± 2°C for 2 h in water bath with intermittent agitation. Mixture again heated at 85 ± 2°C for 3 h
5	residual	3 HCl + HNO_3	residue was evaporated to near dryness in water bath at 110°C

At the end of each stage, suspension was centrifuged at 3000 rpm for 45 min. Supernatant was decanted and stored for analyses. Residue was washed with 8 ml of ultra pure water and again centrifuged. Second supernatant was discarded ter every 48 h. No other fertilizer was applied to SAL. Carrots and spinach were grown for 13 and 9 weeks, respectively. Edible leaves of spinach and roots of carrots were harvested, washed and rinsed with tap and distilled water, respectively, to remove soil particles and patted dry. Plants were then oven-dried at 70°C for 72 h, ground and used for Cu and Pb analyses (Su and Wong 2004).

Chemical analyses. Fractionation of Cu and Pb was done as indicated in Table 2. Samples from each mixture were also digested with aqua regia (3 HCl + HNO_3) to determine concentrations of Cu, Pb and Al (aluminum). Al was used as a reference lithogenic element to normalize concentrations of Cu and Pb in luvisol (Mingorance et al. 2007, Mtanga and Machiwa 2007). Al concentration was determined only in the aqua regia extract whereas Cu and Pb concentrations were determined in all extracts. Dilution of extract was done only for Al determination. Cu and Pb concentrations in spinach and carrots were determined in 1 g of oven-dried sample that was ashed for 6 h at 500–550°C (Almás and Singh 2001) and then digested with concentrated HNO_3 (Martínez et al. 2003). The FAAS was used for analyses.

Analyses of data. Experiment was repeated twice with each sample analyzed in duplicate. Cu and Pb MF in SAL were determined as in Equation 1 (Badaway and El-Motaium 2001).

$$MF = \frac{S_1 + S_2}{S_3 + S_4 + S_5} \quad (1)$$

Where: S_1, S_2, S_3, S_4, and S_5 – metal concentration in exchangeable, carbonate, reducible, oxidizable, and residual fractions of SAL, respectively.

Cu and Pb EF for luvisol (EF_{soil}) were calculated as in Equation 2 (Mingorance et al. 2007, Šmuc et al. 2009).

$$EF_{soil} = \frac{(M/Al)\ \text{sludge – amended soil}}{(M/Al)\ \text{control soil samples}} \quad (2)$$

Where: M and Al – concentration of metal and aluminum, respectively.

Copper and Pb EF in vegetables (EF_{plant}) were calculated as in Equation 3 (Mingorance et al. 2007)

$$EF_{plant} = \frac{M\ \text{(plant grown on sludge amended soil)}}{M\ \text{(plant grown on control soil)}} \quad (3)$$

Where: M – concentration of metal.

EF_{soil} was correlated with EF_{plant} to determine the relationship between metal enrichment in SAL and that in plants. Cu and Pb EF_{soil} and MF were each regressed with Cu and Pb concentrations in spinach and carrot. Regression model with the highest R^2 value was used to calculate concentrations of Cu and Pb in vegetables. Calculated values were compared with actual values to determine reliability of factors in predicting Cu and Pb uptake from SAL. Differences between actual and calculated Cu and Pb concentrations were assessed using the Student *t*-test. All analyses were done using SPSS 17.0.

RESULTS AND DISCUSSION

Effect of sludge addition on mobility of Cu and Pb. Cu concentration in SAL increased with sludge application rate (SAR) (Figure 1) with Cu EF_{soil} being 0.58, 0.99, 1.1 and 1.1 at SAR of 5%, 10%, 20% and 40%, respectively. EF values < 3.0 indicate minor enrichment (Cheng et al. 2001). With all Cu EF values being < 3.0, enrichment of Cu in SAL was minor. Cu concentration in sludge was higher than in luvisol (Table 1) explaining the increase in Cu EF_{soil} with SAR. Cu concentration in SAL increased with SAR but its bioavailability reduced (Table 3). Ščančar et al. (2001) also reported low availability of Cu in similar studies. The increase in Cu concentration in S_3, S_4 and S_5 fractions with SAR (Figure 1) may explain the decrease in Cu mobility. Increased OM in SAL with SAR (data not shown) would have increased the Cu-binding capacity of the

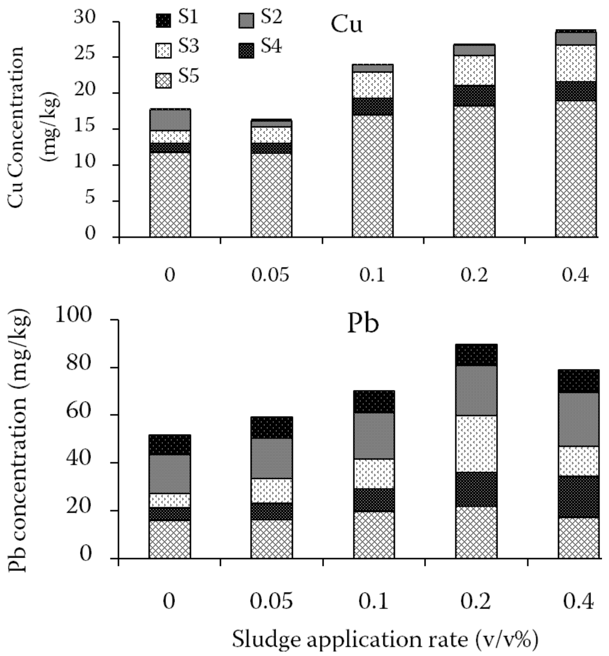

Figure 1. Concentrations of Cu and Pb in different fractions of sludge-amended luvisol

Table 3. Mobility faktor of Cu and Pb in sludge-amended luvisol

SAR (%)	Cu	Pb
0 (control)	0.21	0.90
5	0.07	0.76
10	0.05	0.69
20	0.07	0.50
40	0.09	0.68

luvisol resulting in increased Cu concentration in the S_4 fraction. Strong affinity of Cu for OM and high stability constants reported for OM-Cu complexes by Fuentes et al. (2008) supports this explanation. Pb concentration in SAL increased with SAR (Figure 1) with Pb EF_{soil} being 0.7, 1.0, 1.2, and 1.0 at SARs of 5%, 10%, 20% and 40%, respectively, which also indicate low enrichment. Pb bioavailability in SAL decreased slightly with increase in SAR (Table 3). Fractionation studies indicate that Pb will preferentially sorb into S_2, S_3, S_5 (Ma and Rao 1997) and S_4 fractions (Ščančar et al. 2001) depending on prevailing soil chemical environment. Binding of Pb in these fractions may reduce Pb concentration in the S_1 fraction, resulting in decreased Pb bioavailability.

Uptake of Cu and Pb by vegetables. Plant roots act as barriers in metal translocation from soil to plant which explains the lower concentrations of Cu and Pb in vegetables compared to SAL. Cu and Pb concentrations in spinach were higher than those in carrots (Figure 2) though carrot is a root vegetable in direct contact with the soil. Similar results were reported by Ščančar et al. (2001) and may indicate a selective uptake of Cu and Pb by spinach. Whereas Cu concentration in spinach increased with SAR, no change was observed in Cu concentration in carrots (Figure 2). Cu $EF_{spinach}$ indicated a slight increase in Cu uptake with SAR but no pattern was observed for $EF_{carrots}$ (Table 4). Pb $EF_{carrots}$ was higher than Pb $EF_{spinach}$, whereas Cu $EF_{spinach}$ was higher than those of carrots (Table 4). These differences may be explained by differences in species and selective

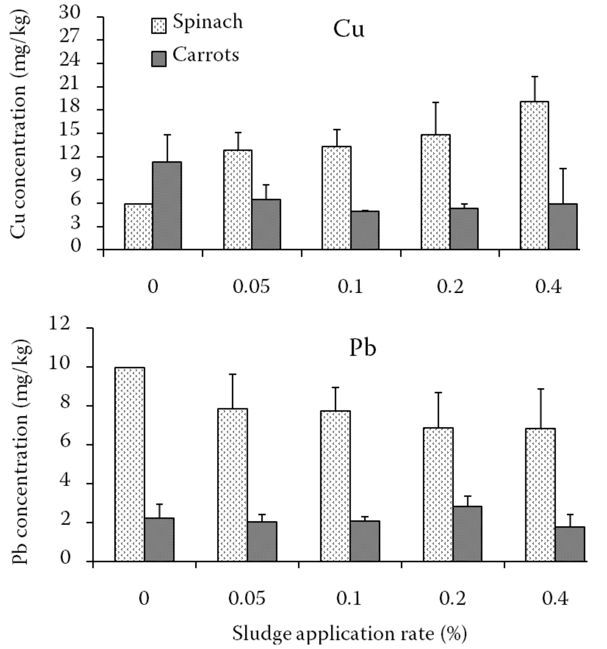

Figure 2. Concentration of Cu and Pb in spinach and carrots grown on sludge-amended luvisol

uptake of heavy metals from soil. Selective heavy metal uptake is exemplified; Pb concentration in spinach and carrots were lower than those of Cu (Figure 2) though Pb concentration and MF in SAL were higher.

Determining Cu and Pb uptake by carrots from Cu and Pb MF and EF. R^2-values obtained from regression between values of Cu MF and EF_{soil} and Cu concentration in spinach indicated variations in MF and EF of Cu in SAL accounted for ≤ 10% variation in spinach Cu uptake. Neither Cu MF nor EF_{soil} are reliable indicators of possible Cu uptake by spinach. Cu EF_{soil} was also irrelevant in determining Cu uptake by carrots (R^2 = 0.12). Low reliability of EF_{soil} in predicting vegetable Cu uptake is supported by the low correlation between Cu EF_{soil}, and Cu $EF_{spinach}$ (0.32), and $EF_{carrots}$ (–0.17). Variations in Cu MF explained 70% of variation in carrot Cu uptake. Differences between calculated and actual values of Cu concentration in carrots (Figure 3) were insignificant (P = 1.0). Pb EF_{soil} had

Table 4. Cu and Pb enrichment factor for spinach and carrots

SAR (%)	Cu		Pb	
	spinach	carrots	spinach	carrots
5	2.15	1.10	0.79	4.27
10	2.22	0.84	0.78	4.38
20	2.47	0.90	0.69	5.88
40	3.18	1.84	0.69	3.73

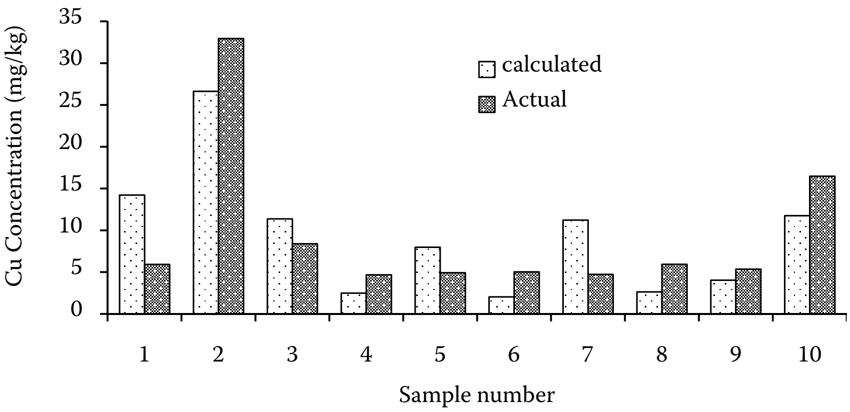

Figure 3. Calculated and actual values of Cu concentration in carrots

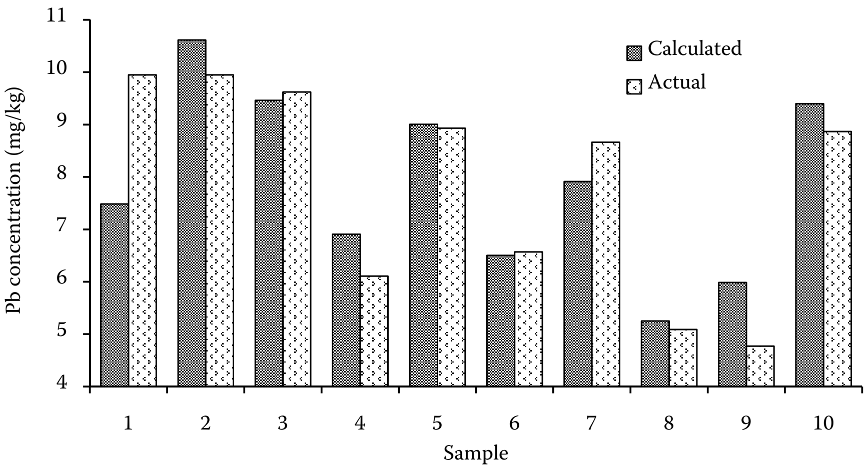

Figure 4. Calculated and actual values of Pb concentration in spinach

little influence on variations in carrot and spinach Pb uptake. No relationship was observed between Pb EF_{soil} and Pb $EF_{spinach}$ (r = –0.19), and $EF_{carrots}$ (0.46). Pb MF did not influence carrot Pb uptake but 74% of variations in spinach Pb uptake was accounted for by variations in Pb MF (R^2 = 0.74). Reliability of Pb MF as an indicator of spinach Pb uptake is depicted by similarities in calculated and actual values of spinach Pb concentration (Figure 4) (P = 0.9) (Figure 4).

Assessing potential heavy metal uptake by plants from sludge-amended soils. Plant heavy metal uptake varies with metal concentration and mobility in the growth medium and plant species in question. Results from this study indicate heavy metal enrichment and mobility in SAS are not the only factors influencing metal uptake from SAS by vegetables. SAS have high OM and microbial activities which may create redox conditions affecting metal availability. By-products from OM decomposition in SAS could also form complexes or chelates with heavy metals that may affect their bioavailability. In addition, roots produce exudates that influence activities around the rhizosphere. Dousset et al. (2001) reported increased availability of Cu, Mn, Pb, and Co due to phytosiderophores from plant roots. Prediction of heavy metal uptake by vegetables from SAS should therefore also, consider interactions between these components as they may influence heavy metal uptake by plants.

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