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Full Length Research Paper

Zinc uptake by vegetables: Effects of soil type and sewage sludge

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Studies were carried out to investigate how sludge applied to 3 soil types to improve the yield of carrots (*Daucus carota*) and spinach (*Spinacea oleracea*) would affect the amount of Zn taken up by these vegetables. A 3 year old (type 1 sludge) and a 3 month old (type 2 sludge) sludge were applied to a vertisol, an arenosol, a chromic/calcic luvisol and a ferric luvisol at (v/v, %) ratios of 0:100, 5:95, 10:90, 20:80, and 40:60 sludge : soil. Spinach and carrots were grown on these soil-sludge mixtures for 9 and 13 weeks, respectively, after which the concentration of Zn in both was determined. Sludge application resulted in an increase in total Kjeldahl nitrogen (TKN), available phosphorus, organic matter content, (OM) and cation exchange capacity (CEC) of soils. Percentage increase varied with soil type but ranged from 900 - 3600%, 700 - 3000%, 60 - 300% and 9 - 600% for TKN, available phosphorus, OM, and CEC, respectively. Sludge application also increased the fresh weight of spinach by up to 31% and carrots by up to 10%, these increases also varied with type of soil on which vegetable was grown. Spinach accumulated more Zn than carrots. Carrots and spinach grown on the arenosol had the highest mean concentration of Zn with values of 131.58 mg/kg and 86.33 mg/kg, respectively. Soil type, sludge age and sludge application rate may not individually affect the amount of Zn accumulated by these vegetables, but they could interact to increase the uptake.

Key words: Bioavailability, vertisol, luvisol, arenosol, *Spinacea oleracea*, *Daucus carota*.

INTRODUCTION

Heavy metals in agricultural soils are of environmental concern because of their potential to bioaccumulate along the food chain and their capability to cause harm to plants, humans and other animals. The application of sewage sludge to soil has been documented as a major source of heavy metals including Zn to agricultural soils (Alloway, 1995; Berti and Jacobs, 1998; Sloan et al. 1997; Tlustoš et al., 2001; Wong et al., 2001). Zinc is necessary for enzymes and enzymatic function and is therefore a constituent of about 100 enzymes in both plants and animals. It is also required for protein synthesis, carbohydrate metabolism and is a constituent of insulin and semen. In plants, Zn is essential for growth because it controls the synthesis of indoleacetic acid, which regulates plant growth.

Though Zn is essential for both plants and animals, it could become toxic at high concentrations. Excessive uptake of Zn by plants causes stunting of shoot, curling and rolling of young leaves, death of leaf tips and chlorosis (Rout and Das, 2003). High Zn intake in humans could cause nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, headaches (Panel on Micronutrients, 2001) and inhibition of Cu absorption which sometimes produce Cu deficiency and associated anemia (Broun et al. 1990; Willis et al. 2005). Growing vegetables including spinach (*Spinacea oleracea*) and carrots (*Daucus carota*) on soils amended with sewage sludge containing a high concentration of Zn could result in the accumulation of Zn by these vegetables (Zhou et al., 2005; Fytianos et al., 2001; Badaway and El-Motaium, 2002; Samse-Peterson et al., 2002; Wong et al.

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2001; and Bunzl et al., 2001). The amount of Zn accumulated by any vegetable grown on sludge-amended soil is influenced by several factors including the bioavailability of Zn in the sludge-amended soil, the type of soil on which the sludge is applied, the make up of the sludge, as well as the rate at which it is applied to the soils (Golia et al., 2008; Jung, 2008; Islam et al., 2007; Khairiah et al., 2004; Parkpian et al., 2002; Richards et al., 2000; Logan et al., 1997; Merrington et al., 2003).

Soil orders vary in their properties depending on the parent material from which they were derived, the climatic condition under which weathering has taken place, and anthropogenic activities. Luvisols for example are generally slightly acidic with low organic matter (OM) content. Vertisols on the other hand are characterized by a high OM and clay content, high cation exchange capacity (CEC) and the presence of swelling clays (Brady and Weil, 1999). Arenosols usually have a high sand content, low CEC and OM content. These differences in properties among soil orders may influence the soil's response to the addition of sludge and eventually, to the bioavailability and uptake of Zn by vegetables grown on these soils when amended with sludge. The uptake of Zn by vegetables grown on the sludge-amended soil may also depend on the sludge composition.

Sludge composition varies from one wastewater treatment plant to the other depending on the sludge treatment and stabilization processes employed and the nature of the wastewater received. Most sewage sludge will usually contain microbes, OM, N, P, Cu, Mg and Mn, which affect the bioavailability of Zn in soils (Krebs at al., 1998; Wong et al., 2001). The bioavailability of Zn in sludge- amended soil is also affected by the rate at which the sludge is applied. Sludge application rate (SAR) may have an influence on the uptake of Zn by vegetables through its influence on the amount of Zn, OM and other sludge components added to the soil. Ngole (2007) reported an increase in Zn bioavailability with increase in SAR in 3 soil types amended with sewage sludge. It is not known whether this increased bioavailability is translated to an increase in the amount of Zn taken up by carrots and spinach grown on these sludge-amended soils.

Uptake of Zn from sludge-amended soil may vary with vegetables. Spinach, carrots, lettuce, raddish and zucchini have been reported to accumulate Zn in their tissues (Zhou et al., 2005). Carrots and spinach are 2 vegetables that are widely consumed in Botswana. Due to its semi-arid climate, arable agriculture in Botswana has not been very profitable. As a result, only 20% of the national vegetable demand is produced locally (Bok et al., 2006). Arable agriculture is mainly practiced on luvisols and vertisol which constitute only 5 and 0.6% respectively of the soil cover in Botswana. 71% of the soil cover in the country is arenosols which are usually characterized by high weight percent sand, low CEC and very low phosphorus concentration. Improving the productivity of the arenosols, vertisols and luvisols could result in improved yield of these vegetables. Sludge application is one way that could be used to improve these soil properties but there are concerns regarding the accumulation of heavy metals including Zn by these vegetables. This study was designed to determine how sludge application rate, soil type and sludge age would affect the amount of Zn accumulated by spinach (a leafy vegetable) and carrot (a root vegetable) grown on sludge-amended soils in Botswana. The results would be of significance to other regions with similar soil types and climatic conditions.

MATERIALS AND METHODS

Study area

The soil types used in the study were vertisol from Pandamatenga (located between latitudes 18°25′S and 18°40′S and between longitudes 25°05′E and 25°47′E), luvisols from Barolong farms (located between latitudes 25°30′S and 25°45′S and between longitudes 25°00′E and 25°45′E) and Tuli Block area (located between latitudes 22°12′S and 24°00′S and longitudes 27°00′E and 29°15′E) and arenosol from Mmamabula (located at latitudes 28°34′S and longitude 26°34′E) all in Botswana. Whereas the luvisols from Barolong were calcic and chromic in nature, those from Tuli block area were mainly ferric. Soils from Barolong farms, Tuli block and Pandamatenga areas were chosen because these are the main agricultural regions in Botswana. The soil cover in Mmamabula is dominated by arenosols, which make up 71% of the soil coverage in Botswana (Soil Mapping and advisory Services Botswana, 1990). Their response to sludge application is therefore of importance to the agricultural sector.

Sludge and soil sampling

A 3 year old (type 1) and a 3 months old (type 2) anaerobically-stabilized- air-dried sewage sludge were randomly collected from the respective piles of sludge at a municipal waste water treatment plant in Botswana. Each sludge type was thoroughly mixed manually to form a homogenous sample which was representative of the particular sludge type. The 4 soil types, chromic/calcic luvisol (luvisol1), ferric luvisol (luvisol2), arenosol and vertisol were randomly collected from the respective areas around Botswana. A composite of each soil type was then prepared. The soil composites and sludge samples were characterized for their Zn content. The properties of both the soil composites and sludge used are indicated in Table 1. Detailed properties of the soils are reported by Ngole et al. (2006).

Whereas the 2 luvisols had a loamy sand texture, the textures of the arenosol and vertisol were sand and clayey loam respectively. The pH, cation exchange capacity, organic matter content, total kjeldahl nitrogen (TKN) and available phosphorus of the soils also varied (Table 1).

Experimental design

Each sludge type was separately mixed with each soil type using volume per volume percent (v/v %) ratios of 0:100, 5:95, 10:90, 20:80, and 40:60 sludge: soil. The 0:100 sludge-soil mixture of each soil type served as the control for each set of soil-sludge mixtures.

Table 1. Properties of soils and sludge used in the study.

Properties	Soil types				Sludge types	
	Luvisol 1	Luvisol 2	Arenosol	Vertisol	Type 1	Type 2
Sand (wt %)	85.25	83.33	95.97	34.12	-	-
Silt (wt %)	11.99	11.85	2.29	37.28	-	-
Clay (wt %)	5.76	4.81	1.75	28.60	-	-
Water retention capacity (%)	43.8	32.8	20.7	51.1	-	-
pH	6.80	6.54	4.96	7.07	5.7	7.4
Cation exchange capacity ($cmol_c$/kg soil)	7.70	5.10	0.30	72.70	39.02	39.52
Organic matter content (%)	1.88	1.83	0.80	2.30	23.1	32.4
Olsen P (mg/kg)	33.6	19.51	4.45	52.15	7319.0	9306.0
Total Kjeldahl nitrogen (%)	2.26	2.23	1.03	2.54	4.4	5.5
Zn (mg/kg)	41.30	27.0	9.60	116.8	400.00	341.00

Each of the mixtures were individually passed through a sieve with a mesh size of 4 mm (Hammer and Keller, 2002) and then transferred into 10 different plant pots in a greenhouse. 5 of the 10 pots were reserved for the growth of spinach and the other 5, for the growth of carrots. Whereas carrots were grown for 13 weeks, spinach was grown for 9 weeks. At time of harvest, the fresh weight of the spinach and carrots were recorded.

Determination of Zn in carrots and spinach

To determine the concentration of Zn in the vegetables, the leaves of the spinach and the edible roots of the carrots were washed and rinsed in distilled water and dried in an oven at 70°C until a constant weight was attained (Ye et al., 1998). 1 g of the dried residue of each sample was then weighed into a porcelain crucible and placed in a muffle furnace and the temperature of the furnace gradually increased to between 500 and 550°C over a period of 2 h and maintained for 6 h (Almăs and Singh, 2001). The ashed samples were then digested with concentrated HNO3 (Martínez et al., 2003; Logan et al., 1997) and analyzed for Zn using a Spectra AA Varian 220 FS flame atomic absorption spectrometer (FAAS) with deuterium correction and equipped with a graphite furnace component (GTA 10).

Statistical analyses

The growth of vegetables was carried out twice using freshly mixed soils and sludge collected from the same areas and piles respectively. The values reported are therefore means of the 2 growth cycles. Analyses of variance (ANOVA) as described by Mead et al. (1996) was employed in this study to determine the effect of soil type, sludge age and SAR on Zn uptake by both vegetables. The International Rice Research Institute Statistical Software Package (IRRISTAT) version 4.4 was used to compute ANOVA. Using the same software package, Tukey's test was carried out to determine the significance of treatment means at P = 0.05. The amount of Zn accumulated by the vegetables was determined by calculating the concentration factor (CF). Concentration factor according to Yoon et al. (2006), Samse-Peterson et al. (2002), Bunzl et al. (2001) and Fytianos et al. (2001) is the ratio of the concentration of an element in a plant to that in the soil on which the plant is grown (Equation 1).

$$CF = \frac{\text{Concentration of metal in the vegetable}}{\text{Concentration of metal in the soil}} \quad (1)$$

The relationship between Zn concentration in vegetables and fresh weight was determined using Pearson correlation coefficient.

RESULTS

Effect of sludge application on some soil properties and vegetable yield

Application of sludge to the different soils affected their pH, water retention capacity (WRC), cation exchange capacity (CEC), organic matter (OM) content, available P and TKN. The pH of all four soil types were reduced by sludge addition whereas WRC and CEC were increased (Figure 1). These effects were more visible in arenosol followed by the luvisols and then the vertisol (Figure 1). No difference was observed with sludge age. Sludge application significantly increased the OM, TKN and available P content of the soils.

These effects were also more obvious in the arenosol than in the other soil types (Figure 2). The difference between sludge types was also insignificant (Figure 2). The improvement of these soil parameters as a result of sludge application was reflected in the fresh weight (FW) of spinach and carrots (Figure 3). Spinach responded more to the application of sludge than carrots.

Uptake of Zn by vegetables

The background concentration values of Zn was highest in the vertisol (116.80 mg/kg) and lowest in the arenosol (9.60 mg/kg soil). Luvisol 1 and 2 had background concentrations of 41.30 and 27.0 mg/kg Zn respectively. Zinc concentration in type 1 sludge was higher than in type 2 sludge (Table 1). The concentrations of Zn in spinach grown on the different soils with and without sludge varied as indicated in Figure 4. Spinach grown on the sludge-amended and unamended vertisol had the lowest concentrations of Zn while those grown on both the sludge-amended and unamended arenosol had the

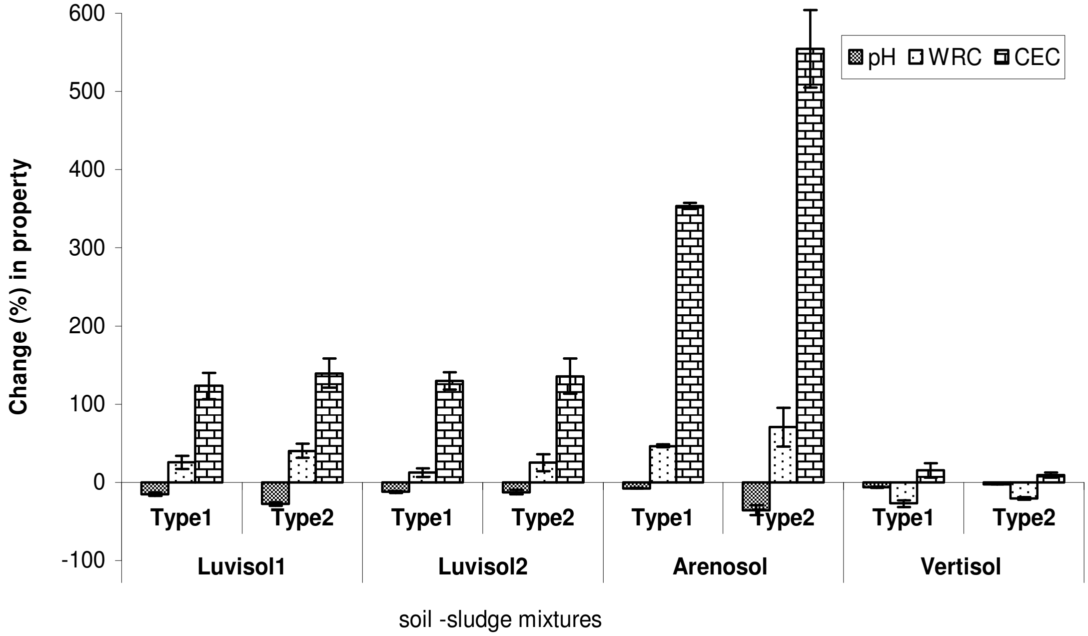

Figure 1. Effect of sludge type on the pH, EC and CEC of the different soil types (Error bars indicate standard deviation).

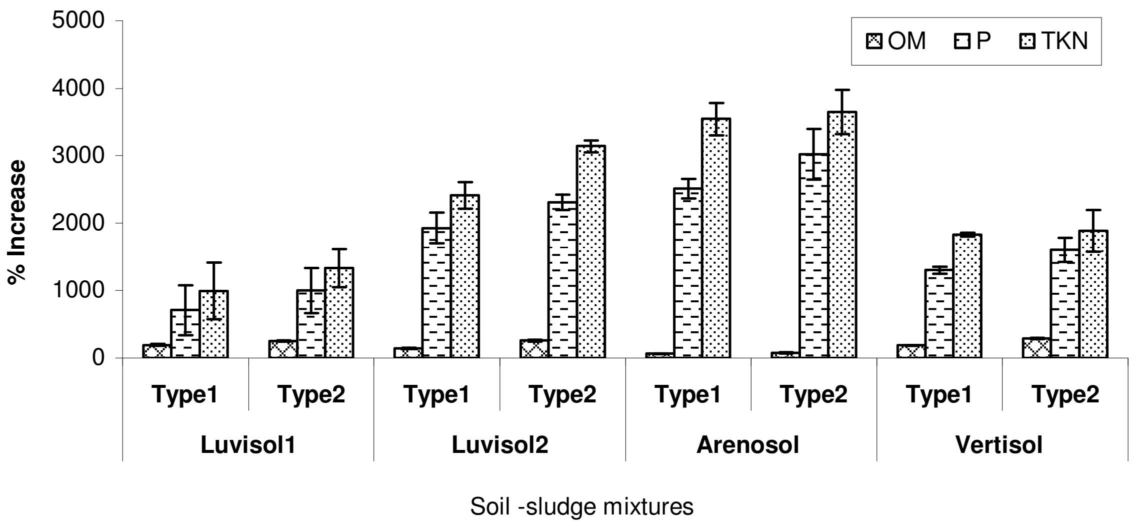

Figure 2. Effect of sludge type on OM, available P and TKN of the different soil types (Error bars indicate standard deviation).

highest Zn concentration (Figure 4). Sludge addition increased the concentration of Zn in spinach as indicated in Figure 4. The concentration of Zn in spinach grown on the different soils before and after sludge addition increased in the order spinach grown on arenosol > spinach grown on the luvisol 1 = spinach grown on the luvisol 2 > spinach grown on the vertisol (Figure 4).

The highest concentration of Zn in carrots grown on the unamended soil was obtained from those grown on the arenosol and the lowest from those grown on the vertisol (Figure 5). Except for carrots grown on luvisol 1 where Zn concentration was increased, sludge addition resulted in a decrease in the concentration of Zn in carrots (Figure 5). The concentration of Zn in carrots grown on the un-amended soil increased in the order carrots grown on arenosol > carrots grown on the luvisol 1 > carrots grown on the luvisol 2 > carrots grown on the vertisol. Sludge application altered this pattern to carrots grown on luvisol1 > carrots grown on the luvisol 2 > carrots grown on the arenosol > carrots grown on the vertisol (Figure 5).

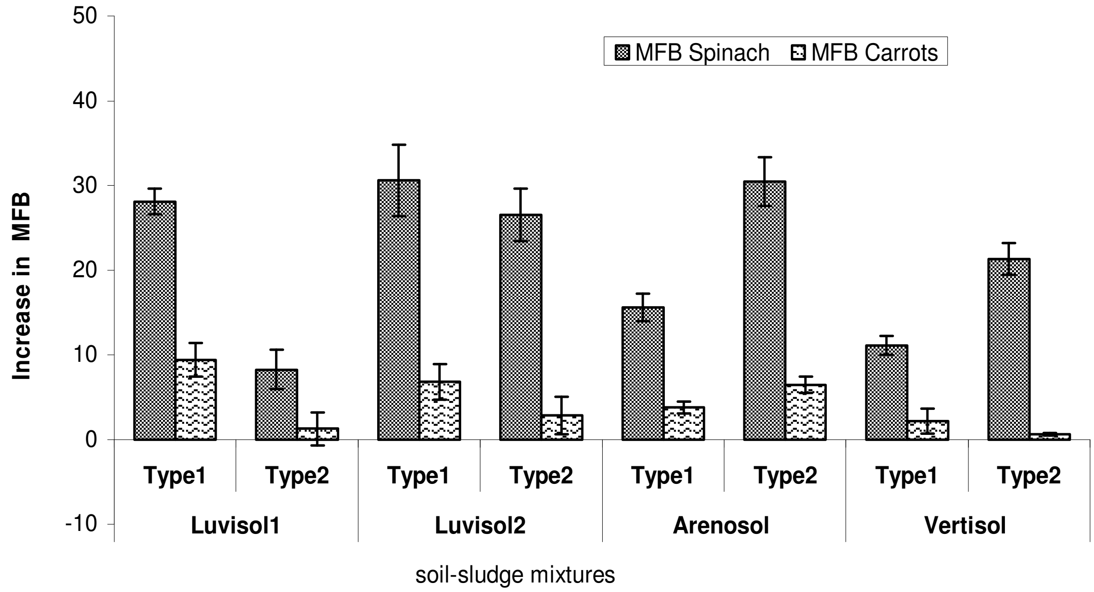

Figure 3. Effects of sludge application on the mean fresh biomass (MFB) of carrots and spinach (Error bars indicate standard deviation).

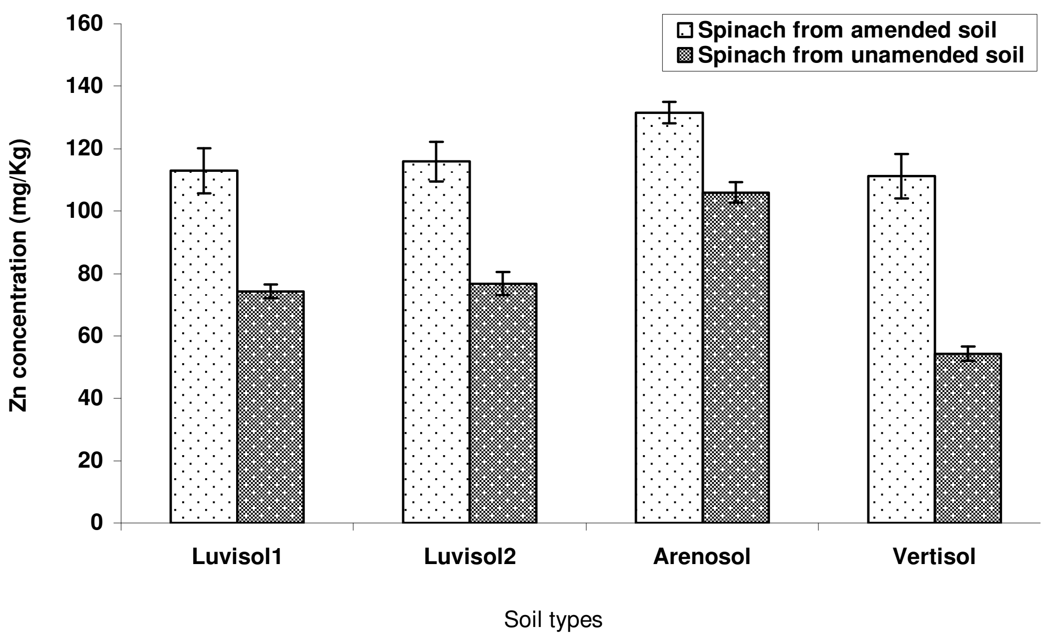

Figure 4. Concentration of Zn in spinach grown on the different soils with and without sludge (Error bars indicate standard deviation).

Effect of sludge age on the uptake of Zn by vegetables

Mean Zn concentration was higher in carrots grown on soils with type 2 sludge (83.99 mg/kg), than in those grown on soils with type 1 sludge (70.30 mg/kg). Spinach grown on soils with type 1 sludge on the other hand had higher mean concentration of Zn (121.06 mg/kg) than those grown on soils with type 2 sludge (114.77 mg/kg). These differences were however insignificant ($P > 0.05$)

Effect of sludge application rate on amount of Zn accumulated by vegetables

The amount of Zn taken up by spinach from the different

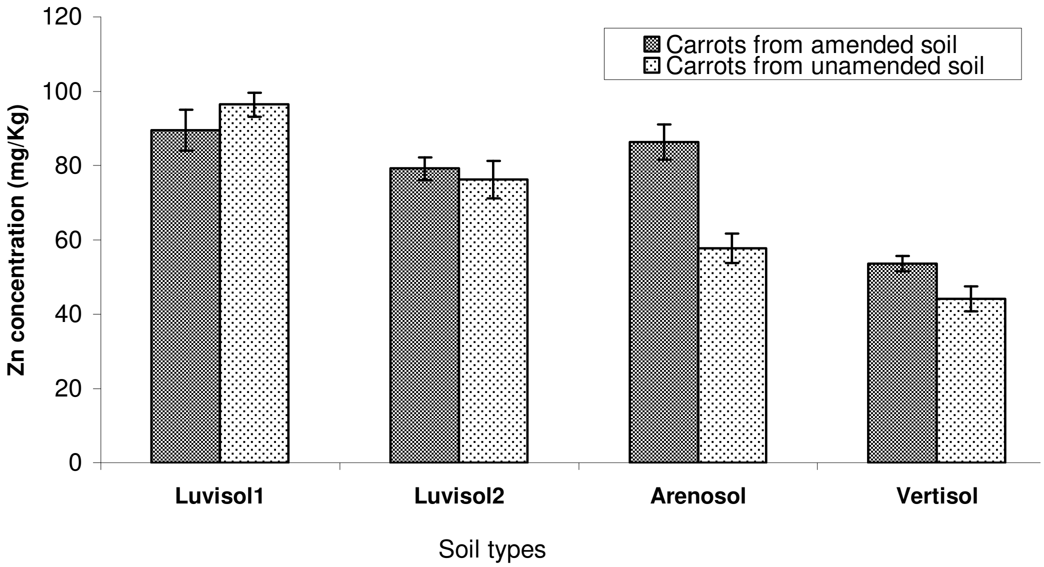

Figure 5. Concentration of Zn in carrots grown on the different soils with and without sludge (Error bars indicate standard deviation).

sludge-amended soils was increased by the addition of sludge at an SAR of 5% (v/v), but further increase in SAR had no effect on the amount of Zn taken up by spinach from the various mixtures (Figure 6). Highest mean concentration of Zn was obtained at an SAR of 20% for spinach (Figure 6). In carrots, the concentration of Zn increased with increase in SAR but these increases are insignificant ($P > 0.05$). Zinc concentration increased with increase in SAR in carrots grown on the vertisol and both luvisols but the concentration of Zn in carrots grown on the arenosol increased up to an SAR of 10% and decreased with further increase in SAR (Figure 6).

DISCUSSION

Effects of soil type, sludge age and SAR on Zn concentration in vegetables

The pattern obtained for Zn accumulation by vegetables grown on the sludge-amended soil was similar to those grown on the soils without sludge even though, the background concentration of Zn in the soils varied. Though type 1 sludge had a higher concentration of Zn than type 2 sludge, sludge type did not affect the concentration of Zn in carrots and spinach probably because the Zn may have been adsorbed into the organic matter. This is justified by the fact that the labile fraction of Zn in type1 sludge (125 mg/kg) was less than that in type 2 sludge (172.5) (Ngole et al., 2006). Increasing SAR increased the amount of sludge-borne Zn added to the soils but this did not have any influence on Zn concentration in carrots. Increase in SAR however affected Zn concentration in spinach. Differences observed in Zn uptake with SAR in spinach and carrots could be attributed to the species of vegetable. Studies by Tlustoš et al. (2001) and Fytianos et al. (2001) have reported increased uptake of Zn by spinach compared to other vegetables. In addition, spinach generally requires a higher concentration of Zn than carrots for healthy growth. An explanation is thus advanced for the higher concentration of Zn in spinach compared to carrots.

Zinc accumulation in vegetables

Spinach had higher CF values than carrots indicating higher uptake of Zn, thus confirming observations by other researchers (USDA 1997). Both spinach and carrots grown on the arenosol had higher CF values where as those grown on the vertisol had the lowest (Table 2).

Higher uptake of Zn by spinach and carrots grown on arenosol can be explained by the properties of the arenosol. It was characterized by high sand content (95.7%), low OM content (0.80%), low clay content (1.75%), low pH (4.9) and low CEC (0.3 cmolc/kg soil), which according to Jung (2008), Rout and Das (2003) and Hinesly et al. (1984) influence the bioavailability of heavy metals to plants. Though there was no significant individual influence of soil type, sludge age and sludge application rate on the amount of Zn accumulated, interactions between sludge type and SAR, sludge type and soil type and SAR and soil type significantly influenced Zn uptake in the vegetables ($P < 0.05$).

Addition of sludge resulted in a proportionate decrease in pH and increase in OM and CEC. Decomposition of OM is likely to have resulted in the production of fulvic and humic acids which usually form chelates and ligands with Zn, increasing its bioavailability. According to

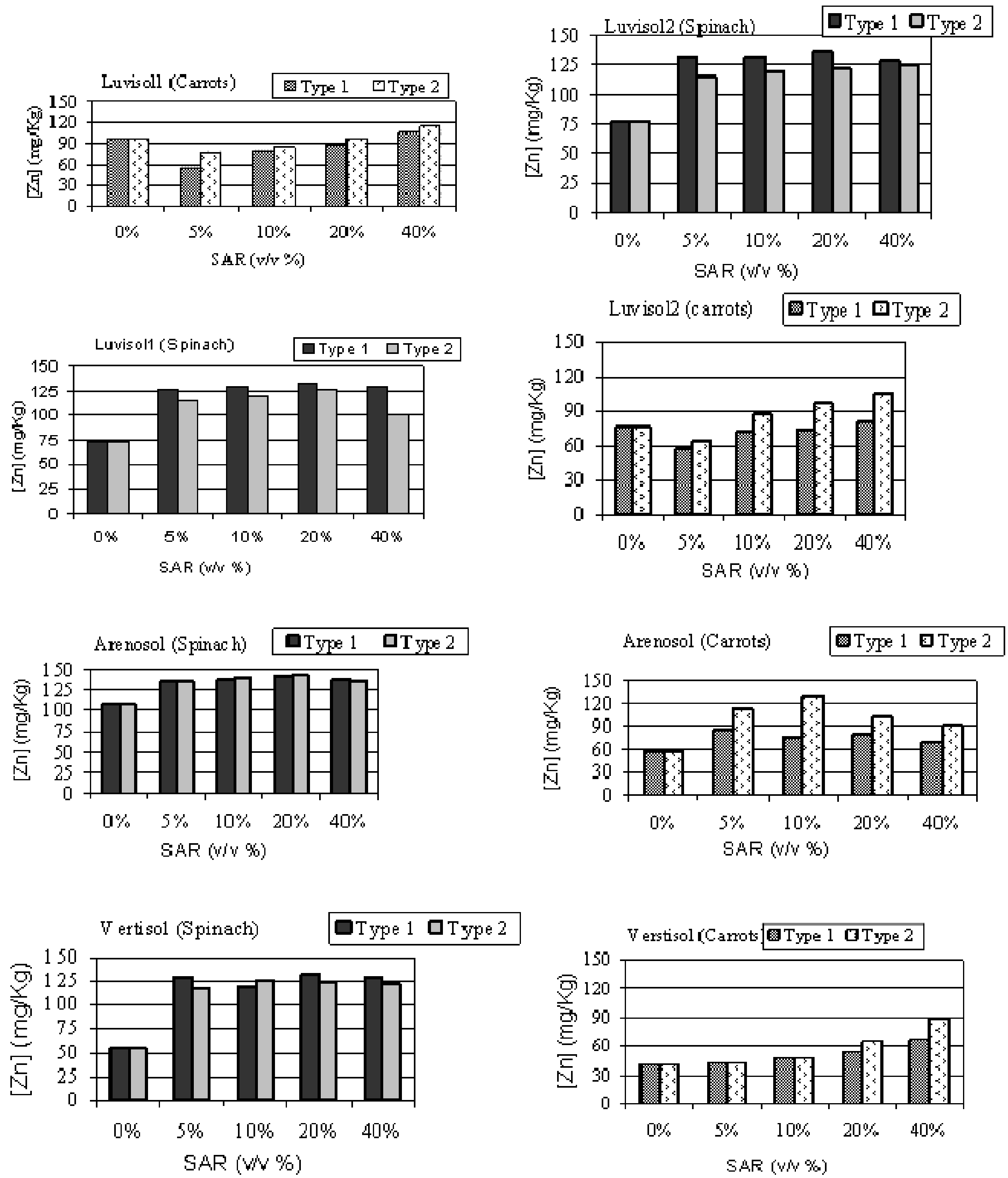

Figure 6. Concentration of zinc in spinach and carrots grown on the different soil-sludge mixtures.

Alloway (1995) decomposition of OM with age results in several products including fulvic acids which forms chelates with Zn. Studies by Barrow (1993) also indicated that organic ligands especially those formed by humic acids reduces the amounts of Zn adsorbed onto soil. These observations are further supported by results reported by Ngole (2007) where a shift in Zn from the less available to the more available fractions of different soils was observed in soils 90 days after sludge application. He et al. (2007) also reported a shift of Zn towards the

Table 2. Concentration factors of zinc in spinach and carrots grown on the different soil-sludge mixtures.

SAR (v/v %)	Zinc concentration factor of Spinach							
	Luvisol 1		Luvisol 2		Arenosol		Vertisol	
	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
0	1.80	1.80	2.84	2.84	11.03	11.03	0.46	0.46
5	2.69	2.38	3.05	3.79	4.44	3.08	0.68	0.82
10	1.73	1.50	1.95	2.87	3.72	2.97	0.57	0.75
20	1.06	1.16	1.48	1.43	1.79	1.84	0.50	0.57
40	0.70	0.56	1.04	0.84	0.93	0.75	0.42	0.46
	Zinc concentration factor of carrots							
SAR (v/v%)	Luvisol1		Luvisol2		Arenosol		Vertisol	
	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
0	2.33	2.33	2.82	2.82	6.00	6.00	0.35	0.35
5	1.18	1.61	1.35	2.13	2.81	2.61	0.22	0.30
10	1.06	1.06	1.07	2.10	2.07	2.78	0.22	0.28
20	0.70	0.89	0.81	1.14	1.00	1.31	0.20	0.30
40	0.57	0.64	0.66	0.72	0.47	0.51	0.22	0.34

exchangeable fraction in loamy soils mixed with sewage sludge confirming increased Zn mobility with sludge ageing.

The soil types varied in their properties including clay content, pH, OM content, and CEC (Table 1). Though the arenosol recorded the highest percentage increase in CEC (Figure 1), its overall CEC was still the lowest among the 4 soil types (data not shown) indicating a lower ability to adsorb cations. The vertisol had the highest background concentration of Zn but Zn accumulation in carrots and spinach grown on the vertisol was the lowest (Table 2). This observation can be justified by the higher OM and clay content of the vertisol (Table 1) which all decrease Zn mobility and bioavailability in soils (Rout and Das, 2003; Hinesly et al., 1984). The effects of sludge on soil pH, CEC and OM justifies the interactions between sludge type and SAR, sludge type and soil type and SAR and soil type on the uptake of Zn by both vegetables.

Relationship between fresh weight of vegetables and zinc uptake

Sludge addition also increased the amount of available P, and TKN of the soils and this was translated into an increase in the fresh weight of both spinach and carrots grown on the soils. Pearson's correlation was carried out between Zn concentration in the vegetables and their fresh weight to determine whether the amount of Zn accumulated by each vegetable was related to the fresh weight of the vegetables. Zinc concentration in spinach increased with increase in fresh weight ($r = 0.6$), with 40% ($R2 = 0.40$) of the increase in Zn in spinach being explained by increases in fresh weight. No relationship between Zn concentration in carrots and its fresh weight was observed ($r = 0.1$). Only 1% of the concentration of Zn in carrots was explained by its fresh weight ($R2 = 0.01$).

Conclusion

This study was designed to investigate how soil type, sludge type and SAR affect the amount of Zn accumulated by carrots and spinach grown on sludge amended soils. Sludge application significantly increased the concentrations of Zn in the 4 soil types (Ngole, 2007) but this increase was not reflected in the amount of Zn accumulated by the carrots and spinach. Zinc accumulation in spinach was influenced by SAR and paired interactions between SAR, sludge age and soil type. Sludge application rate, and sludge age individually had no influence on the amount of Zn absorbed by carrots but soil type and paired combinations between SAR, sludge age and soil type significantly influenced the amount of Zn accumulated by carrots. While sludge application significantly improved the yield of spinach and carrots grown on the soils, the uptake of Zn by spinach was significantly high. Though soil type, sludge age and SAR may not individually affect the amount of Zn taken up by these vegetables, they could interact to increase the uptake. Any vegetable production program that involves the use of sludge-amended arenosol, luvisols and vertisol should therefore consider the soil type, sludge age and SAR when monitoring uptake of heavy metals.

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