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Root-shoot partitioning of copper, chromium and zinc in *Lycopersicon esculentum* and *Amaranthus hybridus* grown in cement-polluted soil

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Abstract

Tomato (*Lycopersicon esculentum*) and amaranth (*Amaranthus hybridus*) were grown on cement-polluted soil in a greenhouse under ambient conditions and natural photoperiod. Plants were harvested two months after planting and their roots and above ground parts were cleaned and separated. Heavy metals (Cu, Zn and Cr) in roots, shoots and soil samples were determined by atomic absorption spectrophotometry. There was no significant difference in total accumulation of Cu and Zn in biomass between the two vegetables. However, significant differences in their partitioning were observed. Both vegetables exhibited large transfer of Cu and Zn from soil to roots, but translocation of Cu to above-ground parts was significantly reduced for both species. Translocation of Zn to above-ground parts in amaranth plants was relatively high, but it was restricted in tomato. Cr uptake was significantly restricted within roots of tomato whereas roots of amaranth did not accumulate Cr. These findings showed that both tomato and amaranth have a potential to absorb excessive Cu and Zn into roots, but tranlocation of Cu to above-ground parts occurred only in amaranth.

Key words: amaranth, bioaccumulation, heavy metals, soil pollution, tomato, transfer factor, translocation index.

Introduction

Heavy metal contamination of food is one of the most important aspects of food safety and quality assurance (Sharma et al. 2009). Vegetables represent a rich source of vitamins, minerals, and fibers for human diet, and they also have beneficial antioxidative effects. However, vegetables grown in contaminated soils can accumulate relatively high amounts of heavy metals (Sharma et al. 2006; Marshall et al. 2007; Sharma et al. 2007). Therefore, heavy metal contamination of vegetables should not be underestimated.

Excessive accumulation of heavy metals in agricultural land may result in soil contamination and increased heavy metal uptake by crops, leading to deleterious effects on food quality and safety (Garcia, Millan 1998). Food chain contamination is one of the important pathways for the entry of these toxic metals into the human body (Ma et al. 2006). Therefore, dietary intake of heavy metal through contaminated vegetables may pose health risk to humans (Singh et al. 2010). Excessive bioaccumulations of toxic heavy metals in vegetables may render dietary nutrients unavailable to humans or induce unsolicited health problems that may be unnoticed for some time.

In many cities in the developing countries, like Nigeria, unavailability of suitable land for farming leads to conversion of hazardous and contaminated places such as road verges, banks of drainage channels, dumpsites, old mines and land surrounding industrial facilities to vegetable gardens to meet local demand for fresh vegetables. The Lafarge-Cement WAPCO factory is the largest cement factory in Nigeria, with two production plants sited in Sagamu and Ewekoro, southwestern Nigeria with total production capacity of 4.5 million metric tonnes (Lafarge-WAPCO, 2011; Ogunbileje et al. 2013). Gbadebo and Bankole (2007) reported substantial amounts of heavy metals in air and dust around the Lafarge-Cement WAPCO, Sagamu. Also, a recent study by Ogunkunle and Fatoba (2013) revealed that the soil surrounding the Sagamu cement factory was highly polluted with Pb, Cu, Cr and Cd, with concentration levels exceeding the international standard limits. Soil pollution due to heavy metals may threaten human health via effects on the quality of food produced, from both surface and underground water, but also on its effect on air quality, especially when pollutant-laden particles originating from soil are resuspended by wind (Addo et al. 2012).

Monitoring and assessment of heavy metal concentrations in vegetables from market sites have been carried out in some developed and developing countries. Therefore, this study was aimed at assessing the uptake of heavy metals from natural contaminated soil by amaranth and tomato and their tissue partitioning, with regard to health implications of excess accumulation of heavy metals in edible parts.

Materials and methods

Experimental setup

Contaminated soil used for the vegetation experiment was collected 50 m from the western fence of the Lafarge-Cement WAPCO factory, Sagamu, southwestern Nigeria at a depth of 0 to 15 cm. Control soil was collected from the experimental farm at the University of Ilorin, Ilorin, Nigeria. The soil samples were collected in bulk in two big polyethylene bags and brought to the University of Ilorin, Nigeria. The soil was later throughly mixed to establish homogeneity.

The experiment was carried out in the greenhouse of the Department of Plant Biology, University of Ilorin, Nigeria, with 12 pots containing 1 kg of the sieved soil in each plastic container. Six containers contained the cement-polluted soil while the remaining six containers contained the Control soil. Seeds of *Lycopersicon esculentum* L. (cv. Roma VF) and *Amaranthus hybridus* L. (cv. Purple hybrid) were sown in plastic containers containing 1 kg of sieved contaminated or control soil. Six containers per treatment were used for each crop species. They were arranged in a complete randomized block design. Two weeks after planting plants were thinned to three seedlings per container. Plants were left to grow for two months with regular watering with borehole water under natural photoperiod and ambient conditions.

After two months (before flowering) plants were harvested, separated into roots and shoots (stem and leaves together), and washed with tap water to remove soil. Plant samples were properly tagged and oven-dried at 80 °C for 2 h.

Chemical analysis

Samples of the cement-polluted soil used for the experiment were air-dried under room temperature and sieved through a 2-mm pore sieve to obtain a fine particle size (Ullrich et al. 1999). One gram of the soil sample was weighed into a 250 mL glass conical flask using an electronic weighing balance (Mettler Toledo PL203) and 10 mL of concentrated HNO_3 (70%; Sigma-Aldrich Corporation, USA) was added. The mixture was boiled gently for 40 min to oxidize all easily oxidizable matter. After cooling, 5 mL of 70% $HClO_4$ (Sigma-Aldrich Corp, USA) was added and the mixture was boiled gently until dense white fumes appeared (AOAC 1990; Hseu 2004). The solution was cooled, filtered using Whatman No. 42 filter paper and transferred into a 50 mL volumetric flask and diluted to a 25 mL mark with deionized water. The exchangeable fractions were extracted from the soil with 8 mL of 0.5 M $MgCl_2$ at pH 7.0 for 40 min in a test tube with continuous agitation using a motorized shaker JK VXR S17 (Tessier et al. 1979; Li et al. 1995; Chojnacka et al. 2005). The supernatant was filtered using Whatman No. 42 filter paper into a 50 mL volumetric flask and diluted to a 25 mL mark with deionized water. pH was determined by dissolving 1 g of the air-dried soil in 2.5 mL of deionized water and shaken until homogeneity was reached. A glass electrode pH meter (PHS-3C Model) was used to measure pH of the soil sample in water with a 1:2.5 soil to water solution (Shukla 2009). Soil organic matter content was determined using the ignition method of Reddy et al. (2009).

The harvested plant samples were oven-dried for 2 h at 80 °C and 0.25 g of each of the plant samples was weighed using an electronic weighing balance (Mettler Toledo PL203) into a 250 ml glass conical flask and 3.5 mL of concentrated HNO_3 (70%, Sigma-Aldrich Corp, USA) was added. The mixture was boiled gently for 15 min to oxidize all organic matter. After cooling, 1.5 mL of 70% $HClO_4$ (Sigma-Aldrich Corp, Germany) was added and the mixture was boiled gently until dense white fumes appeared which showed the end of digestion (Hseu 2004). The solution was cooled, filtered using Whatman No. 42 and transferred into a 50 mL volumetric flask and diluted to a 25 mL mark of the flask with deionized water. Digestion of plant parts were made in triplicate and all the digestates (soil, plant and extractable) were then analyzed in duplicate for copper, zinc and chromium by atomic absorption spectrophotometry (Buck Scientific 210 VGP). Quality control was ensured by digestion of replicates and the use of blanks to exclude introduction of impurity through reagents.

Determination of transfer factor and translocation index

Transfer factor (TF) was calculated as TF = C_{tp} / C_{ts}, where C_{tp} is the metal concentration in plant roots (mg kg^{-1} dry mass) and C_{ts} is the bioavailable metal concentration in the corresponding soil (mg kg^{-1} dry mass; Chojnacka et al. 2005; Prabu 2009). Translocation index (T_i) was determined as the ratio of metal content in shoots (both stem and leaves) (mg kg^{-1} dry mass) to that of the metal content of roots (mg kg^{-1} dry mass; Baker 1981; Ghosh, Singh 2005).

Results

Bioavailable metal concentrations that were extracted with $MgCl_2$ from control and cement-polluted soil are presented in Table 1. Extractable Cu content was significantly higher in the cement-polluted soil than in control soil. Cr concentration in the two soils were very small and statistically did not differ. In contrast, substantial quantity of Zn was extracted from both soil samples in similar concentration.

Cement-polluted soil had significantly higher total concentration of both Cu and Cr in comparison to control soil (Table 1). However, while the total Zn concentration was higher in the plluted soil, the difference was not

Table 1. Bioavailable ($MgCl_2$-extractable) and total metal concentrations (mg kg^{-1}), pH and organic matter content of control and cement-polluted soil used for plant cultivation. *, significant difference between control and cement-polluted soil (P < 0.05)

Parameter	Metals	Cement-polluted soil	Control soil	t-value	P-value
$MgCl_2$-extractable metals	Cu	19.17 ± 0.953	0.028 ± 0.003	34.730*	0.001
	Cr	0.025±0.000	0.037 ± 0.018	1.000	0.500
	Zn	17.88 ± 4.240	17.91 ± 1.018	0.013	0.991
Total metals	Cu	22.34 ± 10.413	2.21 ± 0.226	1.316*	0.039
	Cr	17.87 ± 1.838	10.70 ± 1.294	5.248*	0.016
	Zn	33.84 ± 14.452	25.86 ± 10.168	0.724	0.523
pH		5.83± 0.901	7.14 ± 0.010	4.750*	
Organic matter (%)		4.43 ± 2.15	0.67 ± 0.115	3.02*	

statistically significant. In addition, cement-polluted soil was significantly more acidic and had significantly higher organic matter content.

Root tissues of *L. esculentum* plants grown in cement-polluted soil accumulated all heavy metals in significantly higher concentrations (more than three-, two- and three-fold, for Cu, Cr and Zn, respectively) in comparison to shoot tissues (Table 2). Similarly, four-fold concentration of Cu was found in root tissues of *A. hybridus* in comparison to shoot tissues. In contrast, shoot tissues accumulated significantly higher concentration of Zn than that in roots. Cr concentration in both parts of *A. hybridus* plants was below the detection limit. Comparing the two plants, L. esculentum accumulated higher concentration of Cu in both roots and shoots, and higher Zn level in roots, while Zn shoot concentration was higher in *A. hybridus*.

Metal transfer factors for all measured heavy metals were higher in *L. esculentum* than in *A. hybridus*, indicating a higher uptake rate of respective ions (Fig. 1A). The transfer factor for Zn in *L. esculentum* was the highest, follwed by those of Cr and Cu, showing extremely active Zn uptake in tomato roots.

Generally, root-shoot translocation of all measured heavy metals was low (< 0.5) for the two studied plants, except for Zn in amaranth, as indicated by translocation indexes (Fig. 1B). *A. hybridus* had a larger translocation index value for Zn (< 2.5) than did *L. esculentum*. However, the translocation index for Cu was extremely low and similar for the plant species. Translocation of Cr for *L. esculentum* was also relatively low.

Discussion

The total concentrations of recovered heavy metals in soil were considerably below the threshold limits for agricultural soils as set by the CCME (2001) for the European Union, USA, Canada and the UK. However, it has been argued that the international threshold limits may not be applicable to weathered tropical soils, which are predominantly sandy in nature and have relatively low cation or metal retention capacity (Agbenin et al. 2009). However, this may not be true for the southwest zone of Nigeria, which is classified as a humid tropical zone with high rainfall, evapotranspiration and humidity. Also, the soil is ferralitic in the study area, in contrast to the northern part of Nigeria, which is predominantly sandy.

The study indicated high bioavailability of Cu and Zn in the cement-polluted soil used in the experiment, in relation to the total Cu and Zn. concentration This high bioavailability of Cu and Zn may be due to the low soil pH, which can increase solubility (Hess, Schmid 2002). There have been reports that low pH increases solubility of Cu in soil, making it phyto-bioavailable and reducing complexation with organic matter and chemo-sorption on oxides and silicate clays (Bolan et al. 2003). The organic matter content of soil can favour bioavailability of Zn, making it more abundant for uptake by vegetables, as metals associated with organic matter have high mobility due to decomposition and oxidation of organic matter with time (Salomons 1995). McLean and Bledsoe (1992) reported

Table 2. Distribution of heavy metals (mg kg^{-1}) in different parts of tomato and amaranth plants grown on cement-polluted soil, in comparison to FAO/WHO limits. *, significant difference between roots and shoots (P < 0.05)

Plant species	Metals	Roots	Shoots (stem + leaves)	t-value	P-value	FAO/WHO limit
Lycopersicon esculentus	Cu	171.1 ± 43.3	52.3 ± 49.8	3.072*	0.037	40
	Cr	0.280 ± 0.021	0.125 ± 0.006	12.483*	0.001	2.3
	Zn	606.8 ± 83.0	192.2 ± 45.9	8.337*	0.001	60
Amaranthus hybridus	Cu	144.1 ±0.6	36.5 ± 5.7	24.169*	0.026	40
	Cr	0.00	0.00	-	-	2.3
	Zn	209.4 ± 1.1	587.4 ± 141.3	3.784*	0.034	60

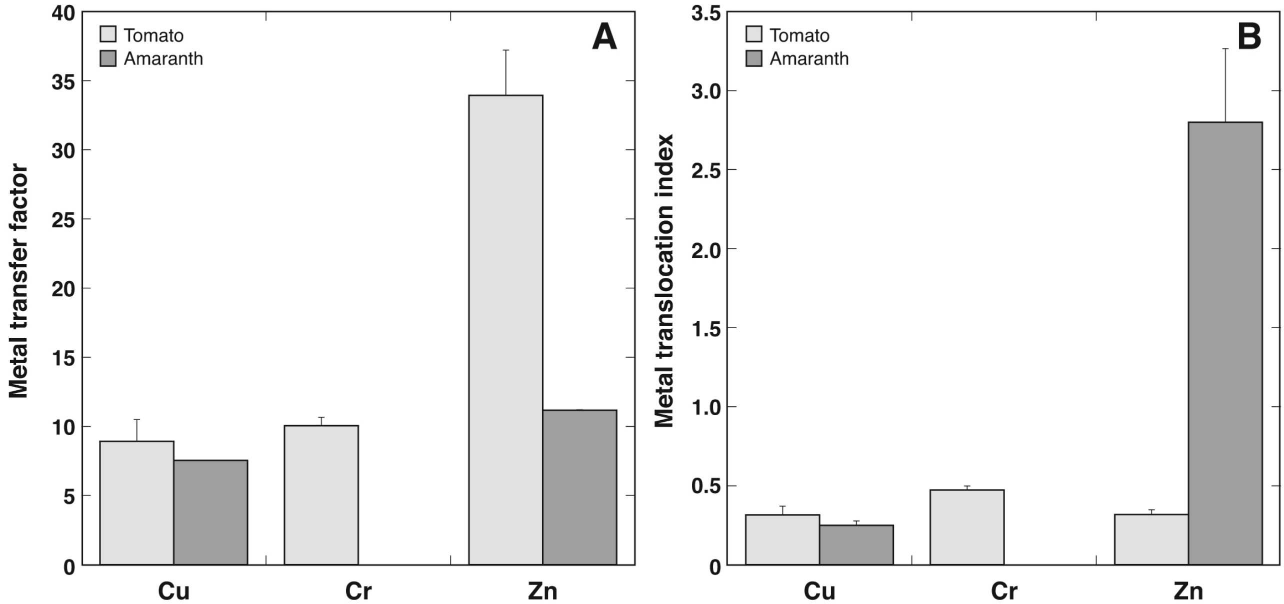

Fig. 1. Metal transfer factors (A) and metal translocation indexes (B) in *Lycopersicon esculentum* and *Amaranthus hybridus* plants grown on cement-polluted soil

that high mobility of Cu in soil can be attributed to its high complexing nature with soluble soil organic matter, which leads to easy leaching from the soil. Amrhein et al. (1992) also reported increased mobility of Cu in the presence of dissolved organic matter, which was consistent with the result of this study. The relatively low organic matter content of the soil can not explain the high bioavailability of Cu; thus, high acidity of the soil is likely the explanation for this.

The high concentrations of Cu and Zn in *A. hybridus* were similar to the concentrations reported by Romer and Keller (2001), Mattina et al. (2003), Nafiu (2010) and Ondo et al. (2012), who showed that amaranths intensively absorbed and conducted Zn into the shoot. Ogundiran and Osibanjo (2008) also reported Zn concentration more than 1000 mg kg^{-1} for plants grown on contaminated soil in south-western Nigeria but the reported Zn concentrations in the present study were below 1000 mg kg^{-1}. Concentration of Zn in *L. esculentum* was comparable to reported tomato metal uptake by Shilev and Babrikov (2005) from industrial polluted soil, but the recorded Cu concentration was lower than their results.

There was considerable uptake of Cu and Zn by roots of *L. esculentum* and *A. hybridus* showing that these two vegetables have large transfer potential from soil and affinity to bioaccumulate Cu and Zn in their roots. Garba et al. (2012) reported that a transfer factor less than 1.0 indicates high metal transfer and bioaccumulation, while Klocke et al. (1984) indicated that the a transfer factor below 0.5 represents a low metal transfer rate and low bioaccumulation potential. The relatively high transfer factor in thic study could be due to the effect of low pH of the soil, as high soil pH can stabilize soil toxic elements resulting in decreased leaching of toxic elements (Li et al. 2005; Zheng et al. 2012). Another possible reason could be synergistic complementation of both Cu and Zn in uptake by root hairs. Ondo et al. (2012) reported that Cu and Zn addition as supplement to soil increased accumulation of these trace metals in roots of *Amaranthus cruentus*. However, the large difference between root and shoot concentrations of Cu in the vegetables showed that the plants have restricted internal transport of metals from roots towards aerial parts, as reported by Dahmani-Muller et al. (2000). Cu might be immobilized in root cells of the two vegetables, as indicated by the low translocation index, which is consistent with the reported translocation index below 1.0 for *Amaranthus spinosus* (Olowoyo et al. 2011). According to Baker (1981), a translocation index less than 1 characterizes the plant as heavy metal excluder. The results of the present study indicated that *L. esculentum*

Table 3. International threshold values in different regulatory systems of heavy metal concentrations (mg kg–1) in soils. Source: CCME (2001)

Metal	EU	USA	Canada	UK
Cu	140	80 – 200	170	63
Cr	180	400	1500	64
Zn	300	200 – 300	1400	200

and *A. hybridus* can beconsidered as excluders of Cu and Cr. Most plants have been reported to have typical ability to accumulate Cu in roots without a proportional transport into aerial parts, due to complexation and sequestration in cellular structures (e.g. vacuoles) of root tissues (MacFarlane, Burchett 2000; Singh, Sinha 2005; Kidd et al. 2007).

The results of this study indicate that *A. hybridus* had greater ability to transport Zn to aerial parts than did *L. esculentum*, shown by a higher translocation index. It seems that *L. esculentum* has a mechanism to sequester/detoxify excess Zn in the vacuoles of root cells, whereas *A. hybridus* lacks this mechanism. *A. hybridus* was an accumulator of Zn, as indicated by high amounts of Zn accumulated in the aerial parts compared to the root concentration (root/shoot ratio > 1). Baker (1981) reported that the Zn root/shoot ratio for *Thlapsi* spp and *Minuartia verna* was > 1, characterizing these plants as metal accumulators whereas *Armeria maritima* and *Silene vulgaris* had a ratio < 1 suggesting exclusion. There have been reports that Zn, being extremely soluble and mobile, has very high transport affinity from soil to shoots of plants, thereby making its assimilation easy by plants leading to phytotoxicity (Brun et al. 2001; Rout, Nafiu 2010).

The distribution of these heavy metals in *L. esculentum* and *A. hybridus* showed that these vegetables are tolerant to Cu and Zn, but the mechanism of their tolerance differed, which may not be due to a physiological attribute but a syndrome of adaptation at the cellular level (Ernst 1975). Baker (1981) stressed that the mechanisms of tolerance of both excluder and accumulator species are largely internal, in that there is active detoxification of metal ions. The difference lies in the sites of detoxification, in which excluders' site of detoxification is largely in the roots while it is the shoot for the accumulators (Baker 1981).

In conclusion, this study showed that vegetables like amaranths and tomatoes have the potential to transfer Cu and Zn in large amounts from a bioavailable pool in the soil, probably due to their synergistic effects on root accumulation. The large translocation potential of Zn to aerial parts by *A. hybridus* might be due to the nutritional composition needs of this leafy vegetable. *L. esculentum* also had a significant transfer potential but translocation to aerial parts was restricted.

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