

Full Length Research Paper

Chelate-assisted phytoextraction of metals from chromated copper arsenate (CCA) contaminated soil

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This study examined the effect of citric acid and ethylenediaminetetraacetic acid (EDTA) application on As, Cr and Cu phytoextraction of maize (*Zea mays* L.) plant in chromated copper arsenate (CCA) contaminated soil. The soluble and available metal pools in the contaminated soil were determined to be 6.20 ± 0.12 mg/kg and 9.80 ± 0.39 mg/kg As; 34.70 ± 1.10 mg/kg and 69.80 ± 1.44 mg/kg Cr and 18.40 ± 0.70 mg/kg and 46.30 ± 1.16 mg/kg Cu respectively, while the pseudo-total metal contents were 31.70 ± 0.29 mg/kg As, 241.40 ± 1.28 mg/kg Cr and 152.90 ± 1.82 mg/kg Cu. Maize seedlings grown on contaminated soil samples were treated with 100 ml of citric acid and EDTA solutions of various concentrations (0, 2, 4, 6, 8 and 10 mM), 15 days after germination. The plants were harvested five days after amendment application and the levels of As, Cr and Cu in the roots and shoots (mg/kg dw) were determined by AAS. Post-harvest mobilization and redistribution of As, Cr and Cu in the soil samples were examined using the BCR sequential extraction method. The total levels of metal uptake (root + shoot) from the unamended soil sample were 6.8 mg/kg As, 5.7 mg/kg Cr and 38.8 mg/kg Cu representing 35, 9 and 37%, respectively, of the potentially available metals. It was found that citric acid and EDTA application markedly enhanced As, Cr and Cu extraction by maize plant, with uptake varying in the order Cu > Cr > As.

Key words: Phytoextraction, citric acid, ethylenediaminetetraacetic acid (EDTA), heavy metals, chromated copper arsenate, maize plant.

INTRODUCTION

Phytoremediation, the use of plants for the containment and/or absorption of xenobiotics from soil and water offers an economic, ecofriendly and non-invasive alternative remediation technology for heavy metal(s) contaminated soils. The success of phytoremediation technology whereby metals are effectively removed from soil is dependent on adequate plant yield and on efficient transfer of metals from plant roots to shoots (Evangelou et al., 2007). Some plants such as *Thalpsi*, *Urtica*, *Chenopodium*, *Polygonum*, *Sachalasse*, *Alyssum* etc, which are known metal hyperaccumulators have shown the ability to extract, accumulate and tolerate high levels of heavy metals (McGrath and Zhao, 2003). However, most of these plants are innately slow-growing and have small biomass and these tend to limit their application in remediation of heavy metals contaminated soils (Mulligan

et al., 2001). More recent research efforts in phytoextraction have focused on fast growing crop species such as maize, tobacco, etc, which though are not metal hyperaccumulating, have high biomass yields (Robinson et al., 2000; Luo et al., 2005; Komarek et al., 2004; Meers et al., 2005; Tandy et al., 2006). For example, Komarek et al. (2004) compared phytoextraction efficiency of maize (*Zea mays*) to hybrid poplar (*Polulus nigra* × *Populus maximoviczika*) after application of EDTA to Pb contaminated soils and reported that phytoextraction efficiency was pH dependent: maize exhibited better results than poplar in more acidic (pH ≈4) soils, while poplar proved more efficient at near-neutral pH (about 6).

However, in many soils, only a fraction of heavy metals is readily available for plant uptake, thus limiting the level of metal uptake and the practical field application of phytoextraction. To increase metal availability and extend practical field application of phytoextraction in the remediation of soils contaminated with heavy metals, the use of complexing agents such as amino polycarboxylic

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acids for example ethylenediaminetetraacetic acid (EDTA), chelating organic acids (for example citric acid) have been used to desorb metals from soil matrix into soil solution to facilitate uptake by plants (Wu et al., 1999; Blaylock and Huang, 2000; Jiang et al., 2003).

The objectives of this study were to examine the effects of citric acid and EDTA application on the phytoextraction of As, Cr and Cu by maize (*Zea mays* L.) plant in CCA contaminated soil and on the mobilization and redistribution of the metals in post-harvest maize soil fractions.

MATERIALS AND METHODS

Soil samples were collected from ten locations within the premises of an active wood treatment factory located in the south-west peri-urban area in Benin City, Nigeria, bounded by latitude 6°06' and 6°30' N and longitude 5°30' and 5°45' E in the geomorphic unit referred to as the Benin low-lands. The samples were pooled, sieved through a 2 mm screen and air-dried.

The physico-chemical properties and As, Cr and Cu levels in the soil sample are given in Table 1 (Uwumarongie and Okieimen, 2010). Analar grade chemicals viz: acetic acid, ammonium acetate, citric acid, hydroxylamine hydrochloride and EDTA were obtained from BDH Ltd. and used without further purification.

Determination of water soluble and bioavailable metal fractions in the contaminated soil

The water soluble fractions of As, Cr and Cu in the soil samples were determined by agitating 5 g-portion of the soil in 25 mL of distilled water for 6 h followed by centrifugation at 1500 rpm. The amount of the metals in the supernatant determined by AAS (Buck Scientific VGF model 210A) is reported as the water soluble fractions. The bioavailable fractions of As, Cr and Cu in the soil was determined by extraction of an aliquot of the soil sample with 0.01 M CaCl₂ solution following the method described by Oliver et al. (1999).

Pot experiments

Air-dried soil samples (1 kg) were placed in plastic pots and maintained at 60% field water capacity by adding deionised water. Four grains of viable maize were sown in each pot. Fifteen days after germination, sub-samples of the pots were treated with 100 mL of 0, 20, 40, 80 and 100 mM solutions of citric acid and EDTA. Amendments application was performed by applying the solution to the top of the pots. Post-germination treatment as opposed to pre-sow treatment was adopted to preclude phytotoxic growth depressions (Meers et al., 2004).

The maize plants were harvested five days after application of the amendments by cutting the shoots 0.5 cm above the surface of the soil, and the roots were carefully removed. The roots were steeped in 0.01 M CaCl₂ for 30 min to remove any exogenous metals and were thereafter washed free of salt solution. The roots and shoots were washed and rinsed thoroughly with deionised water and were thereafter dried at 70 °C until constant weight. The dried plant materials were ground using agate mill.

Subsamples of the ground shoots (200 mg) and roots (100 mg) were digested in a mixture of concentrated HNO₃ and HClO₃ (4:1 by volume) and the As, Cr and Cu in the digestate solutions were determined by AAS. Reagent blank and analytical duplicates were used to ensure accuracy and precision of analyses. The data

reported in this paper are the mean values of triplicate determinations.

Heavy metal fractionation in the contaminated soil

The heavy metal fractionation in the contaminated soil sample was determined by the BCR (European Communities Bureau of Reference) sequential procedure (Golia et al., 2007; Tokalioglu et al., 2006). The protocol operationally defined the metal distribution into the following pools: B₁, extractable; B₂, reducible; B₃, organic bound and; R, residual fractions. The results given in Table 2 indicate that As is fairly evenly distributed among the operationally defined pools, while relatively larger proportions of Cr and Cu are associated with the intransigent soil phases

RESULTS AND DISCUSSION

Physico-chemical properties of contaminated soil

Textural analysis showed a preponderance of sand fraction (73.10%) followed by clay (24.80%) and silt (2.10%) thus classifying the soil as sandy loam. Although sandy soils are known to have poor retention capacity of water and metals, the relatively large proportion of clay (24.80%) in the contaminated soil sample suggests that the soil will drain poorly with implications for potential deleterious impact of retained pollutants on environmental receptors.

The acidic pH 5.92 of the soil is generally within the range for soil in the region. Soil pH plays a major function in the sorption of heavy metals as it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates. It also influences ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay edges (Tokalioglu et al., 2006). The soil had moderate organic matter content (2.15%) and relatively high cation exchange capacity (CEC) (47.84 meq/100 g). CEC measures the ability of soils to allow for easy exchange of cations between its surface and solutions. The relatively high level of clay and CEC indicate low permeability and leachability of metals in the soil.

Contamination status of the soil

The pseudototal levels of As, Cr and Cu given in Table 1 were used to estimate their intervention levels in the contaminated soil via the Department of Petroleum Resources (DPR, 2002) method which considers the organic matter and clay contents of the soil. Intervention levels of metal contaminants in soil give indications of quality for which the functionality of the soil for human, animal and plant lives is considered to be impaired. The intervention levels of As, Cr and Cu are given in Table 3. The results show that the pseudo-total levels of As, Cr and Cu are markedly higher than the intervention values

Table 1. Physico-chemical properties of CCA contaminated soil.

Physico-chemical property	Value
pH	5.92 ± 0.10
Clay (%)	24.80 ± 0.00
Silt (%)	2.10 ± 0.00
Sand (%)	73.10 ± 0.00
Nitrogen (%)	0.34 ± 0.08
Carbon (%)	1.22 ± 0.30
Organic matter (%)	2.15 ± 0.40
Phosphorus (mg/kg)	44.74 ± 3.73
Calcium (meq/100 g)	5.68 ± 0.40
Magnesium (meq/100 g)	1.96 ± 0.30
Sodium (meq/100 g)	0.19 ± 0.10
Potassium (meq/100 g)	0.57 ± 0.10
CEC (meq/100 g)	48.74 ± 0.10
As (mg/kg)	31.70 ± 2.90
Cr (mg/kg)	241.40 ± 12.80
Cu (mg/kg)	152.90 ± 18.20

Table 2. BCR sequential fractionation of As, Cr and Cu in CCA contaminated soil.

Step	Extractant	As (%)	Cr %	Cu %
B ₁ - Extractable	40 mL of 0.1 M CH ₃ COOH, 16 h at room temperature	23.13	18.98	20.36
B ₂ - Reducible	40 mL of 0.5 M NH ₂ OH.HCl (pH 2) 16 h at room temperature	24.15	21.84	23.35
B ₃ - Organic-bound	10 ml of 8.8 M H ₂ O ₂ , 1 h at room temperature; then 1 h at 85 °C; cool, add 50 mL of 1 M CH ₃ COONH ₄ (pH 2) 16 h at room temperature	25.17	25.44	25.78
R - Residual	Aqua regia digestion (21 mL conc. HCl + 7 mL conc. HNO ₃) 16 h at 180 °C	27.55	33.74	30.51

Table 3. Contamination status of soil sample from active wood treatment site.

Metal	Intervention value (mg.kg ⁻¹)	Contamination factor (M _{contam} /M _{ref})	Contamination/Pollution index (M _{Target})
As	8.81 ± 1.26	90.57 ^a ; 21.13 ^b	1.09
Cr	90.52 ± 9.28	689.71 ^a ; 2.41 ^b	2.41
Cu	76.51 ± 6.08	16.09 ^a ; 3.06 ^b	4.25
C _D = degree of contamination = ΣC _f		796.39 ^a ; 26.60 ^b	

C_f = M_{contam}/M_{ref}; a is with reference to the control soil sample (0.35 mg.kg⁻¹ As, 0.35 mg.kg⁻¹ Cr and 9.50 mg.kg⁻¹ Cu) and b is with reference to uncontaminated soil (1.50 mg.kg⁻¹ As, 100.0 mg.kg⁻¹ Cr and 50.0 mg.kg⁻¹ Cu (Sparks, 2000).

and these values correspond to moderate (with respect to As) and high (with respect to Cr and Cu) levels of contamination of the soil (Uwumarongie and Okieimen, 2010).

The contamination status of the soil measured in terms of contamination factor, C_f, a ratio of the metal concentration in the contaminated soil to that in uncontaminated soil, and degree of contamination, C_D, is given in Table 3. The values of these contamination indices classify the soil as moderate – to – highly contaminated (Hakanson, 1980). On the basis of the C/P index and the

total levels of As, Cr and Cu in the contaminated soil sample, the soil in the wood preservation site may be classified as slightly contaminated with respect to As, moderately polluted with respect to Cr and severely polluted with respect to Cu.

Contaminant solubility in soil solution and mobility are increasingly being used as key indicators of potential risk to environmental receptors. Table 4 gives the water-soluble, bioavailable and mobile pools of As, Cr and Cu in the contaminated soil. The mobile pool of the metals is

Table 4. Soluble, available and mobile pools of As, Cr and Cu in CCA contaminated soil.

Metal	Soluble fraction (mg.kg ⁻¹)	Available fraction (mg.kg ⁻¹)	Mobile fraction (mg.kg ⁻¹)
As	6.20 ± 0.12	9.80 ± 0.39	6.80 ± 0.27
Cr	34.70 ± 1.10	69.80 ± 1.44	43.20 ± 1.63
Cu	18.40 ± 0.70	46.30 ± 1.15	29.30 ± 0.80

given as the B₁ fraction from the BCR sequential extraction scheme.

These results when compared with the data in Table 3 suggest that with the exception of Cr for which the available fraction exceeds the regulated target value, the contaminated soil in the wood preservation site may not present imminent risk to environmental receptors. However, the relatively low levels of soluble and available forms of the metals may be bioaccumulated and become associated with long-term deleterious effects on human and environmental receptors.

Metal accumulation by maize plant

Figure 1 shows the amount of As, Cr and Cu in the roots of maize plant. It can be seen that: (i) citric acid and EDTA application markedly enhanced the concentrations of the metals in the roots; (ii) that the enhancements in metal uptake in EDTA amended soils were more marked than for citric acid amended soils and increased with increased level of amendment application and; (iii) that the amounts of the metals taken up by the plant varied in the order Cu > Cr > As. The results indicate that at relatively high levels of amendment application, more than the bioavailable pool of As and Cu were taken up in the roots of maize with the apparent order of the levels of metal uptake being Cu > Cr > As and did not correlate with the bioavailable (0.01 M CaCl₂ extractable) pool of the metals in the contaminated soil i.e. Cr > Cu > As. Copper being an essential element to plants may have been taken up by the plant actively, while As and Cr which would exhibit phytotoxic effects at relatively lower concentrations than Cu, may have being taken up by the plant *via* a passive mechanism.

The processes involved in chelate-assisted phytoextraction include: (a) desorption/dissolution of metal from the soil matrix; (b) transport to the roots by diffusion and mass flux; (c) adsorption and uptake by roots and; (d) transfer to xylem and translocation to shoots. The predominant theory for metal-chelate uptake is the split-uptake (free metal ion) mechanism, by which only free metals are absorbed by plant roots (Marschner et al., 1986; Samet et al., 2001).

According to Schowanek et al. (1997), complexing agents may be divided into three categories depending on their metal complex formation constant: weak (for example, zeolites, polycarboxylate and citrate); moderate

and; high (for example EDTA). It would therefore be expected that metal complexes formed with EDTA will less readily yield the free metal ion (the form in which it was thought that plant uptake of metals mainly occurred) than metal-citrate complexes; and should result to lower enhancement in metal uptake. The results of more recent studies suggest that metal-chelate uptake occurs simultaneously with free metal ion by plant roots in chelate-assisted phytoextraction (Wenger et al., 2008).

Figure 2 shows the amounts of As, Cr and Cu in the shoot of maize plant. The results show that as with the roots, the amount of As, Cr and Cu accumulated in the maize shoots were similarly enhanced by citric acid and EDTA application. In comparison with metal accumulation in maize shoots in the unamended soil sample, the increase in the amounts of the metals accumulated in maize shoots in the amended soils were generally about the same order of magnitude (about 5-fold for As, 11-fold and 13-fold for Cr and 7-fold and 8-fold for Cu in citric acid and EDTA amended soils, respectively). The relatively small increase in metal accumulation in the plant shoots may suggest a low phytotoxic threshold of the plant for containment and that severally cropping cycles may be required to effectively phytoremediate the contaminated soil.

Metal transfer coefficient

Soil-to-plant transfer ratio is an important component of phytoextraction. For an amendment to be considered effective in enhancing phytoextraction of metals, it must not only enhance mobilization of metals from soil matrix into soil solution, it should in addition facilitate metal uptake by plant roots and their translocation into plant shoots. Metal transfer coefficients, T_c, given as the ratio of the metal concentration in plant shoots to the pseudototal concentration in soil obtained for As, Cr and Cu are shown in Figures 3 and 4 given in for citric acid and EDTA amended soils, respectively. It can be seen from the results that: (a) amendment application markedly enhanced the values of T_c and; (b) the values of T_c are generally about the same order of magnitude in citric acid and EDTA amended soils. It has been suggested that values of T_c based on the potentially available fraction of metals rather than on the pseudototal amounts, may provide a more reliable assessment of the effectiveness of amendment application in phytoextraction (Okieimen et

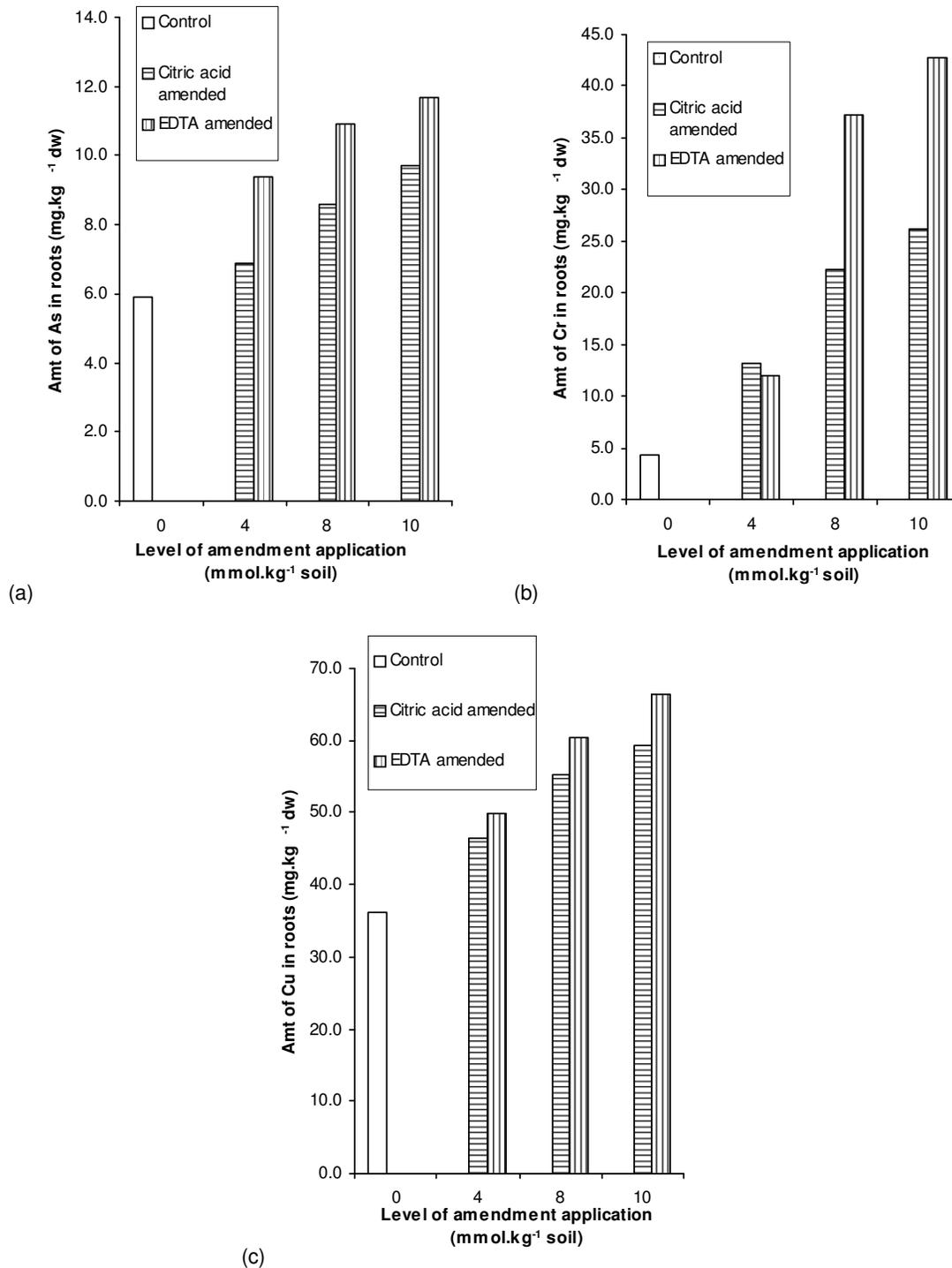


Figure 1. Amount of metals: (a) As, (b) Cr and, (c) Cu in the roots of maize (*Zea mays* L.) grown on CCA contaminated soil amended with citric acid and EDTA.

al., 2010).

Metal translocation factor

Translocation factor, (TF) defined as the ratio of a metal

(loid) concentration in plant shoots to that in the roots, may be used to evaluate the effectiveness of a chelating agent in enhancing the capacity of plant to transfer metals from roots to shoots. The effect of citric acid and EDTA application on the values of TF of As, Cr and Cu in maize plant is shown in Figures 5 and 6. The results

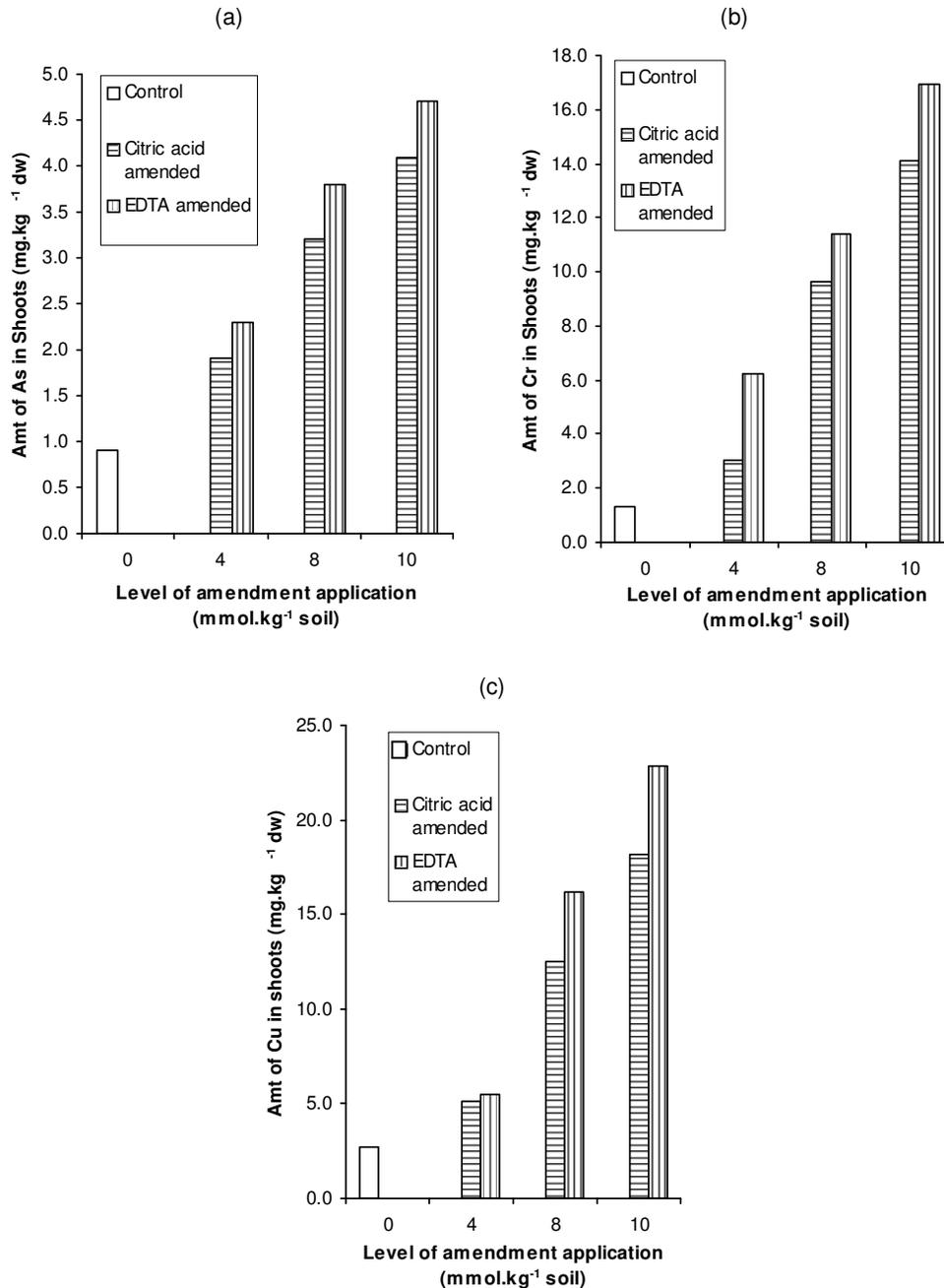


Figure 2. Amount of metals: (a) As, (b) Cr and, (c) Cu in the shoots of maize (*Zea mays* L) grown on CCA contaminated soil amended with citric acid and EDTA.

show marked improvements in the translocation of As, Cr and Cu from the roots of maize plants in the CCA contaminated soil by the application of citric acid and EDTA, with increases in the values of TF generally 100% higher than the corresponding values for contaminated soil. The substantial increase in the values of TF is consistent with the report of Luo et al. (2005) for EDTA and EDSS enhanced metal phytoextraction of metals from contaminated soil. As with transfer coefficient, the levels of improvement in metal translocation in citric acid

amended soils are about the same order of magnitude but are somewhat lower than in EDTA amended soil.

The results in Figures 5 and 6 indicate that less than 40% of the metals absorbed in the roots of the maize plant in the unamended soil was translocated to the shoot. Adsorption by carboxylic groups and other cationic exchanging moieties within the apoplasmic cell wall reportedly contributed to net accumulation in plant roots (Huang and van Steveninck, 1989) and may explain the disproportionate amounts of the metals in the shoots

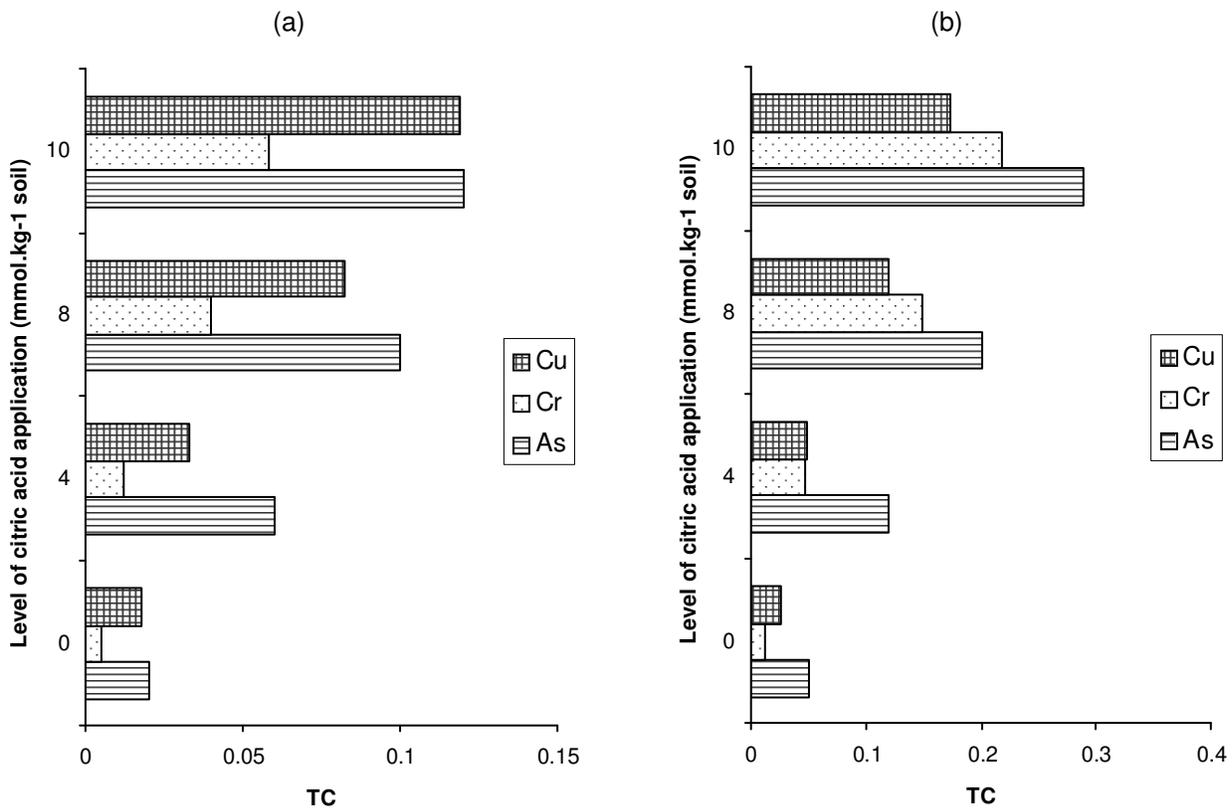


Figure 3. Effect of citric acid application on transfer coefficients of As, Cr and Cu with reference to: (a) pseudototal metal levels and; (b) plant available metal pools to maize in CCA contaminated soil.

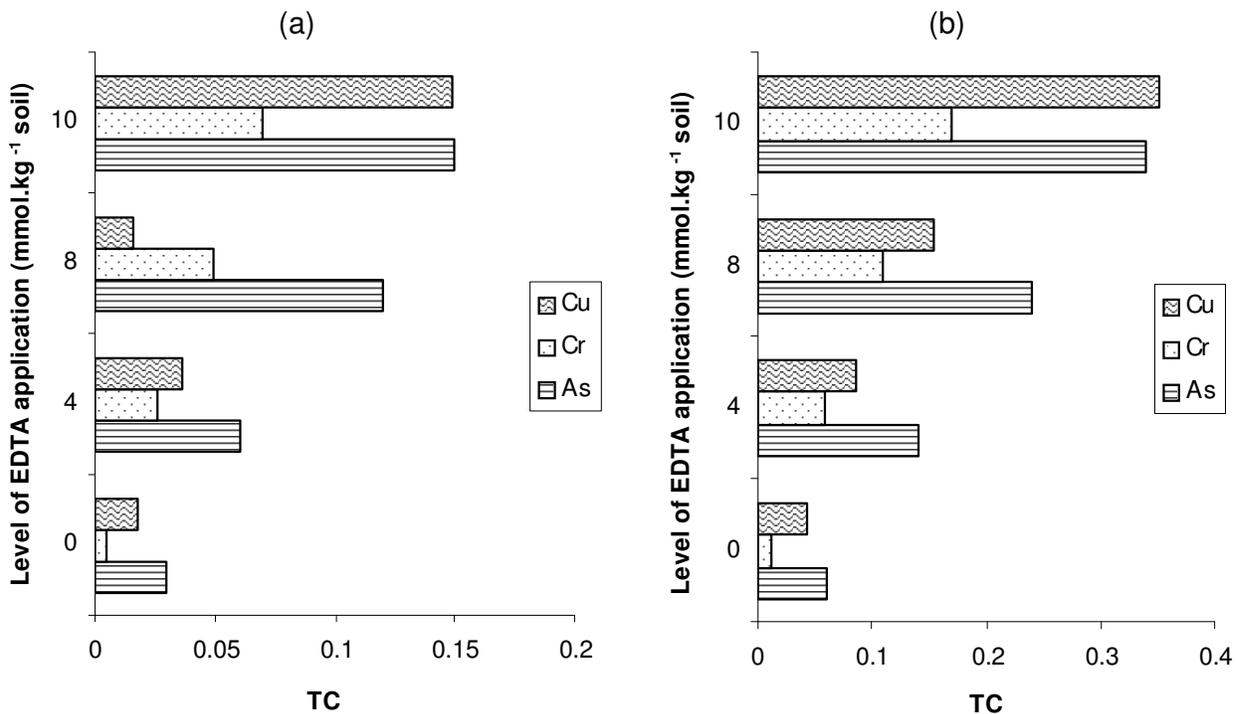


Figure 4. Effect of EDTA application on transfer coefficients of As, Cr and Cu with reference to: (a) pseudototal metal levels and; (b) plant available metal pools to maize in CCA contaminated soil.

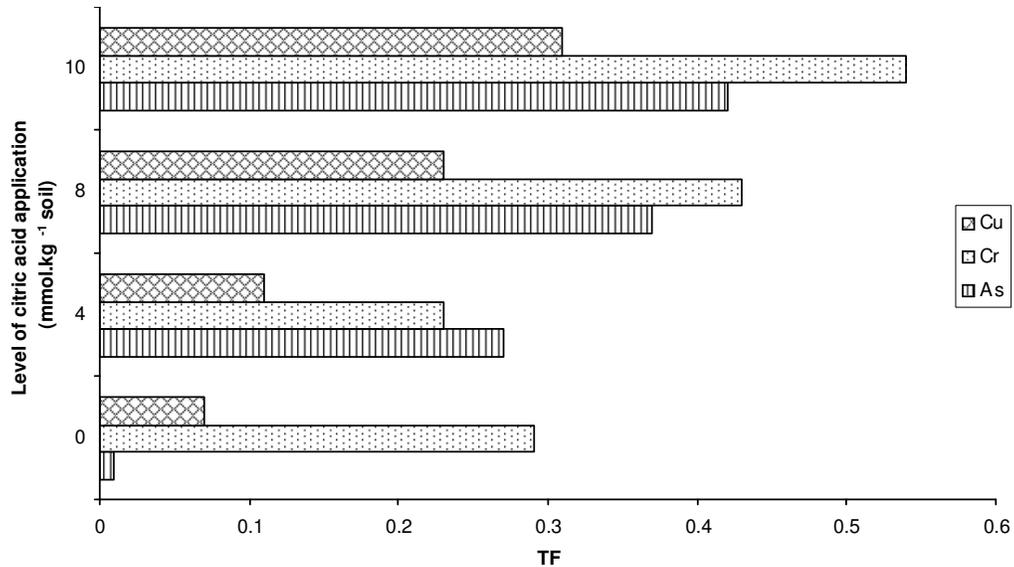


Figure 5. Effect of citric acid application on translocation factor of As, Cr and Cu from root to shoot of maize in CCA contaminated soil.

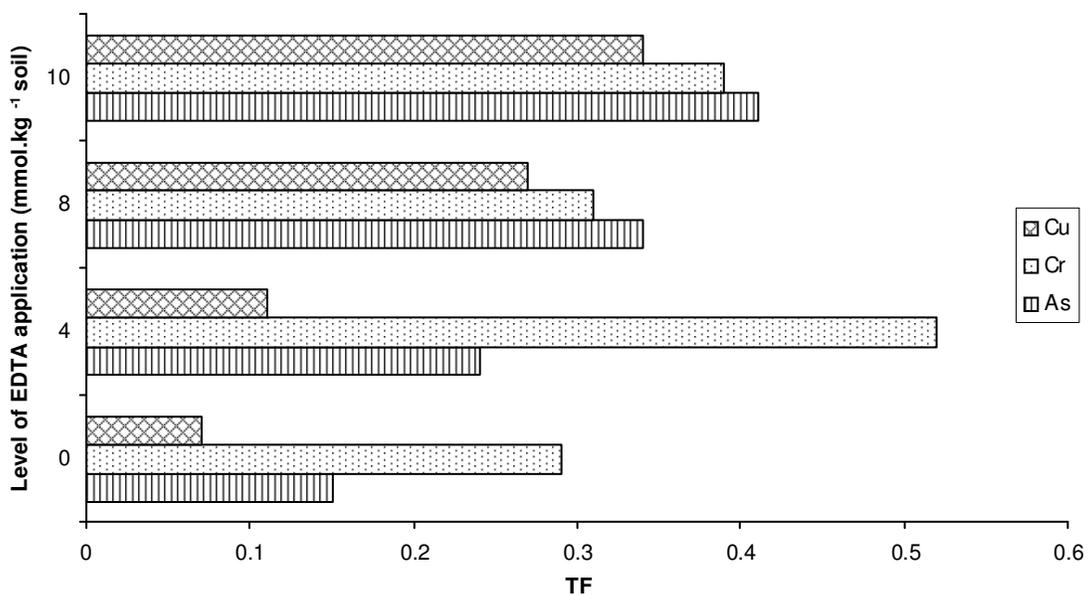


Figure 6. Effect of EDTA application on translocation factor of As, Cr and Cu from roots to shoots of maize in CCA contaminated soil.

relative to corresponding amounts in the roots of the maize plant grown on the CCA contaminated soil (Figures 5 and 6).

Post-harvest distribution of As, Cr and Cu in the contaminated soil

A major environmental concern in chelate-assisted phytoextraction of metals from contaminated soil is the

residual pools of mobilized metals which may not be assimilated by the plant. Prolonged mobilization of heavy metals after harvest is undesirable as the absence of an actively transpiring plant may result in percolation of mobilized metals with implications for ground water impactation. To examine these effects, BCR sequential extraction procedures were applied to the soil samples 3 days after harvesting the maize plants. The distribution patterns of As, Cr and Cu in the post harvest soil samples are shown in Figures 7 and 8 for citric acid and EDTA

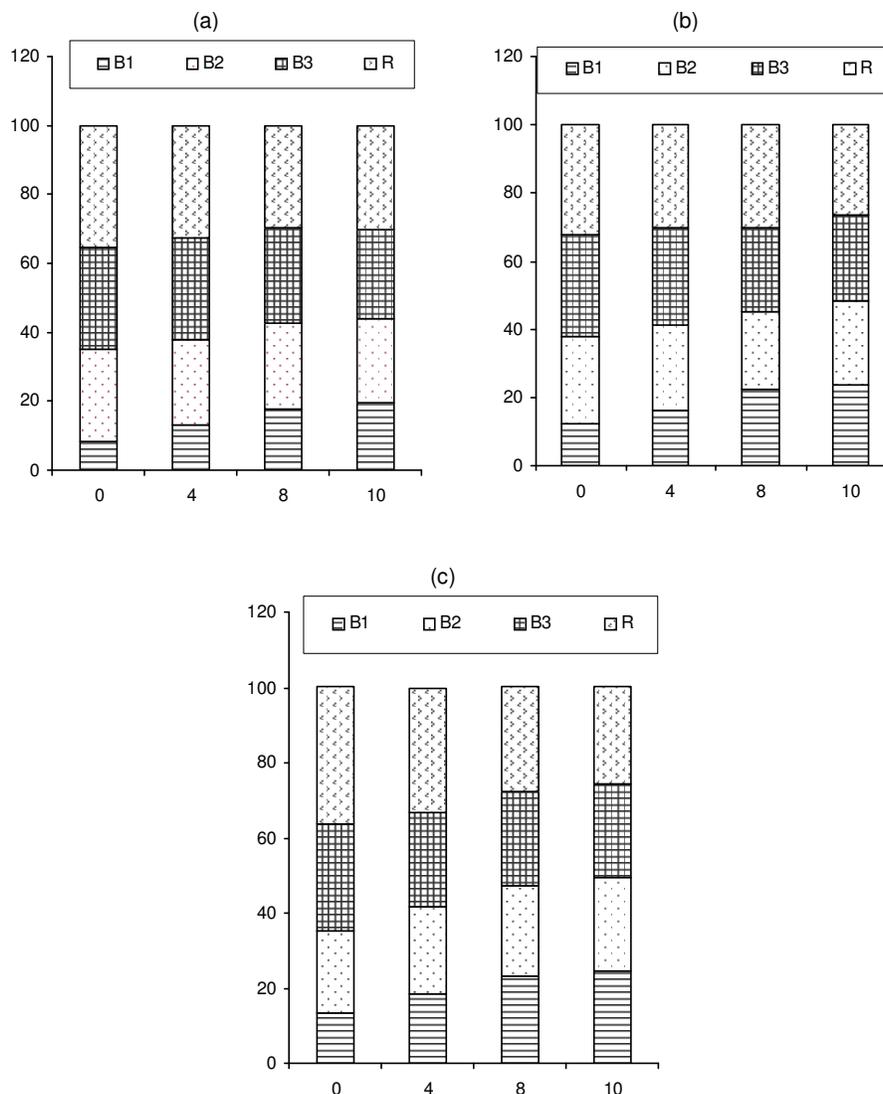


Figure 7. Metal mobilization and redistribution in post-harvest soil samples amended with citric acid (a) As, (b) Cr and (c) Cu.

amended soils, respectively. The result show that amendment application increased post harvest mobile pools of the metals in the soil; As from $1.90 \pm 0.88 \text{ mg.kg}^{-1}$ in the unamended soil to $3.10 \pm 0.11 \text{ mg.kg}^{-1}$ and $3.70 \pm 0.17 \text{ mg.kg}^{-1}$ in EDTA – and citric acid amended soils, respectively; Cr from $26.30 \pm 1.11 \text{ mg.kg}^{-1}$ in the unamended soil to $44.60 \pm 2.10 \text{ mg.kg}^{-1}$ and $45.20 \pm 3.80 \text{ mg.kg}^{-1}$ in EDTA- and citric acid amended soils, respectively, and Cu from $16.90 \pm 1.00 \text{ mg.kg}^{-1}$ in the unamended soil to $17.60 \pm 3.50 \text{ mg.kg}^{-1}$ and $21.40 \pm 4.6 \text{ mg.kg}^{-1}$ in EDTA- and citric acid amended soils, respectively. Residual leachable pools of metal in assisted post-harvest, chelate-assisted phytoextraction soils are not uncommon (Schmidt, 2003; Lai and Chen, 2005) but the relatively high levels of leachable pools of As, Cr and Cu obtained in this study may be connected with the rather short duration of the study.

Conclusion

This study demonstrated that citric acid could be regarded as a good candidate chelate for environmentally safe phytoextraction of heavy metals in contaminated soils. Its enhancement of As, Cr and Cu uptake by maize and of the translocation of the metals from the roots to the shoots of the plant is comparable with that of EDTA. In the short duration of this study, increases in transfer coefficient and translocation factor following citric acid applications provide an impetus for further detailed studies on the capacity of maize to accumulate heavy metals in the presence of citric acid. Being biodegradable, increase in leachable metal pools in post-harvest, citric acid amended soil would present less environmental concern than the post-harvest leachable metal pools in EDTA-amended soil.

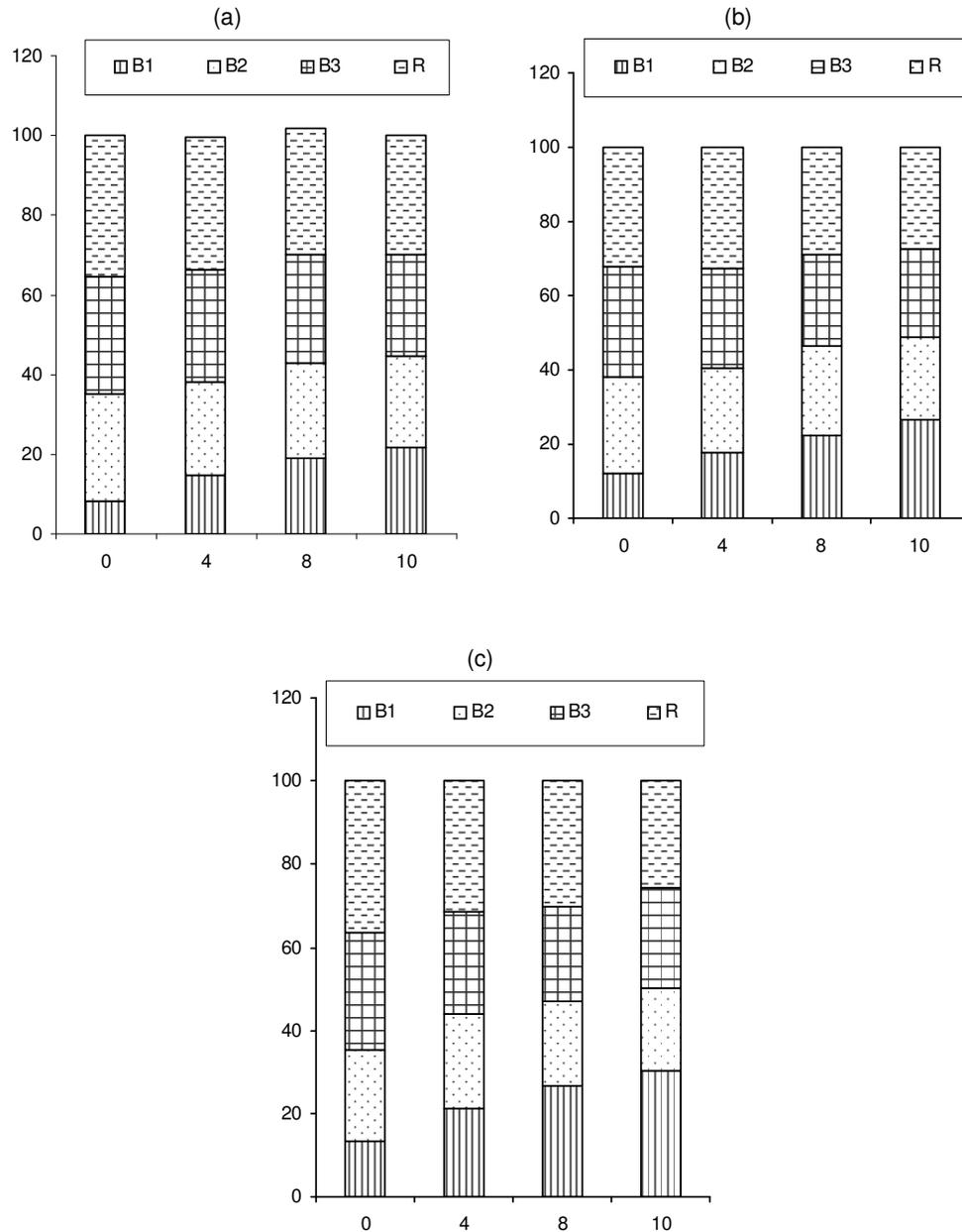


Figure 8. Metal mobilization and redistribution in post-harvest soil samples amended with EDTA (a) As, (b) Cr and (c) Cu.

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