JEAS

Journal of Engineering and Applied Sciences 6 (2010) 1-7

JOURNAL OF ENGINEERING
AND
APPLIED SCIENCES

Chemical fractionation and plant uptake of heavy metals in chromated copper arsenate (cca) contaminated soil amended with ethylenediamine tetraacetic acid (edta)

F.E. Okieimen, [1]D.U. Okuonghae, G.O. Tsetimi

Center for Geoenvironmental and Climate Change Adaptation Research, University of Benin, Benin City
[1] Department of Mathematics, University of Benin, Benin City

Abstract

Contamination of soils by heavy metals is of considerable concern with respect to heath risk, phytotoxity, long term effects on soil fertility and depreciation of land. Of particular concern is the widespread low-to-medium levels of pollution of agricultural and cultivated land and land with high environmental sensitivity index, for which conventional physicochemical methods of remediation are generally unsuitable. Innovative technologies, such as phytoremediation, has been proposed as low-cost, ecofriendly, less-intensive remediation technique for soils contaminated with heavy metals. This study examined the phytoextraction capacity of maize plant for As, Cr and Cu in CCA contaminated soil amended with EDTA. Maize seedlings grown on CCA contaminated soil (M_{Total} 426.0 $mg.kg^{-1}$, As 31.70 $mg.kg^{-1}$, Cr 24.1 $mg.kg^{-1}$ and Cu 152.9 $mg.kg^{-1}$) samples were treated with 100ml of EDTA solutions (0, 20, 40, 60, 80 and 100mM) 15 days after germination. The maize plants were harvested 5days after EDTA application and the levels of As, Cr and Cu in the roots and shoots of the plant were determined by Atomic Absorption Spectrophotometry (AAS). It was found that EDTA application markedly enhanced As, Cr and Cu extraction by maize, with transfer constants and translocation factors of metals in the amended soil several orders of magnitude higher than the corresponding values in unamended soil. A mathematical model was proposed to show the relationship between the amount of the metal mobilized from the contaminated soil matrix following EDTA application with plant uptake parameters and indices.

1. Introduction

Contamination of soil and sediments with organic and inorganic pollutants is a by-product of industrialization and urbanization. Although a small portion of heavy metals in soils is derived from natural processes, a much higher proportion originates from anthropogenic sources such as mining, smelting, use of mineral fertilizers and pesticides, sewage sludge and animal husbandry and manure application, etc (Bolan *et al*; 2004)

Conventional clean up technologies (*ex situ* and *in situ*) such as excavation and dumping, containment methods (e.g. vitrification, stabilization) soil washing/flushing, are generally costly to be used to restore contaminated soils and are often harmful to the normal properties (e.g. texture and organic matter) of the soil (Holden, 1989; Smith *et al.* 1995). Phytoremediation, the use of green plants to remove pollutants from the environment or render them harmless (Raskin *et al*., 1997) with its low cost and environmental friendly nature, has received much attention in the last several years (Salt *et al*., 1998; Garbisu and Alkorta, 2001). The success of phytoremediation process, whereby metals are effectively removed from the soil is dependent on an adequate yield of plants and on the efficient transfer of metals from roots to shoots. The main drawback of phytoremediation is the low mobility and bioavailability of heavy metals in most soils, particularly in neutral to alkaline soils. Even the use of heavy metal hyperaccumulating plant such as *Thalpsi, Urtica, Chenopodium, Alyssum*, etc is limited due to their slow growth rate and low biomass yields (Kaysar *et al*., 2000). Therefore, most of the more recent studies have focused on the use of fast growing crops with high biomass yield (e.g. maize plant) combined with chemical alteration of the mobility and bioavailability of the metals (Cooper *et al.,* 1999; Shen *et al*., 2002; Luo *et al*., 2005). An increase in heavy metal mobility can be achieved by adding chelating agents capable of solubilising and complexing heavy metals into the soil solution as well as promote metal translocation from the roots to the harvestable parts of the soil with transpiration as the driving force (Huang *et al*., 1997; Komarek *et al*., 2007; Wu *et al*., 1999). A number of

soil amendments have been suggested for use in enhancing phytoextraction but the aminopolycarboxylic acids, particularly, ethylene-diaminetetraacetate (EDTA) has received most attention (Blaylock and Huang, 2000; Jiang *et al*., 2003).

This study examined the effect of EDTA application on the mobilization of As, Cr and Cu from chromated copper arsenate (CCA) contaminated soil and their subsequent uptake by maize plant. CCA is one of the most widely used chemical preservative for wood against fungal attack and decay, although it is banned in many countries and restricted in many applications. A water-borne wood preservative, CCA is composed of oxides of chromium (35-65%), copper (16-45%) and arsenic (18-20%). The Cu and As components are the active preservative ingredients, while Cr serves as a fixing agent, binding CCA components of the wood frequently used in decks, fences, utility poles, etc. The permissible CCA retention levels in treated wood depends on the area of application of the wood product: low retention values (typically 4kg.m^{-3}) for plywood, lumber and wood meant for above-ground applications, medium (4-10kg.m^{-3}) for load bearing components (e.g. piling structural poles and columns; and high (13-40kg.m^{-3}) for wood components used in foundation in salt water applications. Inadvertent spillage of CCA, indiscriminate discharge of wood waste materials, exposure of treated wood, etc, may lead to elevated levels of As, Cr and Cu, in the vicinity of wood treatment facility. Previous studies carried out by Uwumarongie and coworkers (Uwumarongie and Okieimen, 2007; Uwumarongie *et al*., 2008; Uwumarongie, 2009) showed that the soil in the vicinity of an active wood treatment site in Benin is very severely contaminated or slightly polluted with potential of impacting a nearby surface water resource (Ogba River). The need to develop innovative remediation technology for the soil in the wood treatment provided the impetus for this and previous other studies in our laboratory (Anoduadi *et al*., 2009; Uwumarongie and Okieimen, 2010; Okieimen *et al*., 2010).

2. Materials and methods

Contaminated soil sample was obtained from an active wood treatment site in the South-western part of Benin City. The physicochemical properties and the As, Cr and Cu levels in the soil sample are given in Table 1, while its pollution status is given in Table 2.

Table 1
Physico-chemical properties of CCA contaminated soil

Properties	
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/100g)	5.68 ± 0.40
Magnesium (meq/100g)	1.96 ± 0.30
Sodium (meq/100g)	0.19 ± 0.10
Potassium (meq/100g)	0.57 ± 0. 10
CEC (meq/100g)	48.74 ± 0.10

Values are means ± SD, n = 3

Table 2
Contamination status of soil sample

Metal	Soluble fraction (mg kg^{-1})	Available fraction (mg kg^{-1})	Psuedototal (mg kg^{-1})	C_f
As	6.20 ± 1.20	9.80 ± 3.90	31.70 ± 2.90	90.57[a]
Cr	34.70 ± 11.00	69.80 ± 14.40	241.40 ± 12.80	689.71[a]
Cu	18.40 ± 7.00	46.30 ± 11.50	152.90 ± 18.20	16.09[a]
C_D = degree of contamination ΣC_f				796.39[a]

$\Sigma C_f = M_{contam}/M_{ref.}$, a is with reference to control soil sample (0.35mg kg^{-1} As, 0.35mg kg^{-1} Cr and 9.50mg kg^{-1} Cu)

Air dried contaminated samples (1kg) were placed in plastic pots and maintained at 60% field water capacity with deionised water and sown with four grains of maize. Fifteen days after germination, subsets of the pots were treated with 100ml of 0, 20, 40, 60 80 and 100mM EDTA solution. Post-germination treatment as different from pre-sow treatment was adopted to preclude possible phytoxic growth depressions (Meers *et al*., 2004). The maize plants were harvested five days after EDTA application by cutting the shoots 0.5cm above the surface of the soil and the roots were carefully removed from the soil. The roots were steeped

in 0.01M $CaCl_2$ solution for 30min to remove metals on the surface of the roots (exogeneous M_{exo}) and thereafter were washed and rinsed thoroughly with deionised water. The amounts of As, Cr and Cu in the extractant was determined by AAS reported as exogeneous metals in the roots. The roots and shoots were washed with deionised water and dried at 70°C for 48h and ground. The ground roots and shoots subsamples were digested with HNO_3:$HClO_3$ (4:1 v/v) and the amounts of As, Cr and Cu in the digestate were determined by AAS. The levels of As, Cr and Cu in the roots subsamples are reported as endogeneous (M_{endo}) metal contents. The effect of EDTA application on the distribution pattern of As, Cr and Cu in the contaminated soil was determined by the sequential extraction method proposed by the European Communities Bureau Reference (BCR) as described by Golia *et al* (2007) and summarized in Table 3. Reagent blank and analytical duplicates were used to ensure accuracy and precision of analysis. The data reported are average values of triplicate determinations.

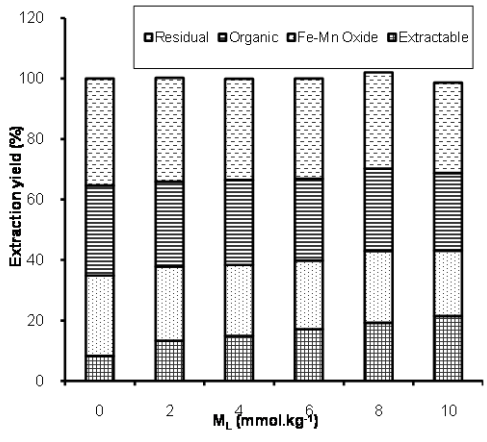
Table 3
BCR sequential extraction procedure used for heavy metal speciation

Step	Metal pools	Extractant	Agitation time
B_1	Extractable	40mL of 0.11M CH_3COOH	16h at room temperature
B_2	Reducible	40mL of 0.5M $NH_2OH.HCl$ (pH 2)	16h at room temperature
B_3	Organic-bound	10ml of 8.8M H_2O_2	1h at room temperature then 1h at 85°C
		Cool + 50mL of 1M CH_3COONH_4 (pH 2)	16h at room temperature
R	Residual	Aqua regia digestion (21mL concentration HCl + 7mL concentration HNO_3)	16h at 180°C

3. Results and discussion

3.1. Effect of EDTA application on metal mobility/availability

Chemical fractionation of the contaminated soil using the BCR sequential extraction method revealed the effect of EDTA application on the distribution pattern of As, Cr and Cu among the operationally defined pools: exchangeable, carbonate-bound, Fe-Mn-oxides bound, organic matter complexed and residual fractions as shown in Fig 1(a-c). The results show that EDTA application enhanced the exchangeable metal pools; generally by more than 100% and was accompanied by reduction in the less mobile metal fractions: Fe-Mn oxide bound, organic matter complexed and residual pools of As, Cr and Cu in the contaminated. The observed enhanced mobilization of the metals, with values of M_f/M_f^o metals (where M_f and M_f^o are the values of relative mobility indices of the metals in EDTA amended an unamended soil samples respectively) of about 2.0 and varying in the order As > Cu > Cr.



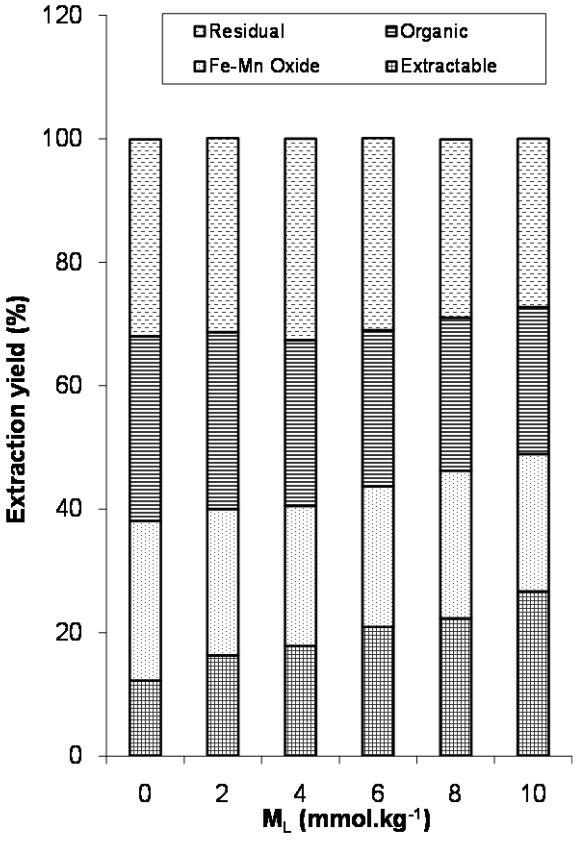


Fig.1. Distribution patterns of (a)As, (b) Cr and (c) Cu in CCA contaminated soil amended with EDTA.

3.2. Metal uptake by maize in CCA contaminated soil

Table 4 gives the effect of EDTA application on metal accumulation in maize plant grown on CCA contaminated soil.

Table 4
Effect of EDTA application on As, Cr and Cu uptake by maize from CCA contaminated soil. Values are means ± SD (n = 3)

Level of citric acid application (mmol.kg^{-1} soil)	Metal uptake by maize plant (mg.kg^{-1} dw)								
	Roots						Shoots		
	Exogeneous			Endogeneous					
	As	Cr	Cu	As	Cr	Cu	As	Cr	Cu
0	0.60±0.00	1.10±0.30	1.00±0.00	5.90±1.00	4.40±0.80	36.10±1.10	0.90±0.00	1.30±0.40	2.70±0.30
2	0.90±0.03	1.90±0.40	1.70±0.40	8.90±1.80	11.80±1.00	42.80±1.10	1.60±0.20	3.80±0.80	4.90±0.81
4	1.10±0.02	2.70±0.06	2.80±1.00	9.40±0.42	19.90±0.95	49.80±1.20	2.3±0.60	6.20±0.40	5.50±0.20
6	1.40±0.04	3.90±0.70	3.90±0.50	9.80±0.64	27.10±1.40	54.30±1.44	3.0±1.00	8.70±0.28	9.40±0.33
8	1.60±0.05	4.40±0.50	4.70±0.80	10.90±1.60	37.30±2.70	60.40±2.00	3.80±1.00	11.40±0.82	16.20±0.48
10	2.00±0.04	6.00±0.04	6.80±0.09	11.60±0.93	42.80±2.00	66.40±2.88	4.70±1.10	16.90±0.31	22.80±0.50

3.2.1. Metal accumulation in the roots of maize plant

The results in Table 4 show that the total levels of As, Cr and Cu in the roots system of maize plant grown on the CCA contaminated soil for 20days varied in the order Cu > Cr > As. It can be seen that EDTA application markedly enhanced the uptake of the metals by the roots system of maize plant. The amounts of the metals absorbed by maize plant roots (endogeneous) are markedly higher than the corresponding amounts adhering to surface of the roots (exogeneous) as shown in Fig. 2 and represent more than 100% available As, 60% available Cr and 100% available Cu. These results ($1 < M_{endo}/M_{exo} \geq 10$) indicate that metals are not accumulated to a large extent on the surface of the roots of maize plant and that transport and flux of the metals to roots' rhizosphere may be the factor regulating uptake of the metals by the roots of maize plant.

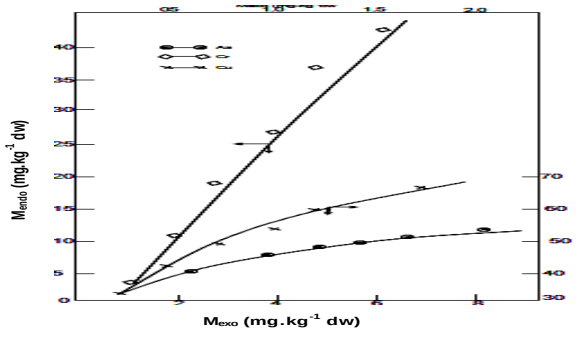


Fig. 2. Relationship between amounts of metals adhering to roots (M_{exo}) and the amounts of absorbed by the roots (M_{endo}) of maize plant grown on CCA contaminated soil amended with EDTA.

3.2.2. Metal accumulation in the shoots of maize plant

Table 4 gives the amounts of As, Cr and Cu accumulated in the shoots of maize plant grown on CCA contaminated soil for 20days. It can be seen that EDTA application markedly enhanced the amounts of the metals in maize shoots, 5-fold for As, 13-fold for Cr and 8-fold for Cu. Soil-to-plant transfer ratio (amount of metal in the shoots, M_{shoots}, to the pseudototal amount in the soil) is an important aspect of phytoextraction. The effect of EDTA on the transfer ratio (coefficient, TC) of As, Cr and Cu from the CCA contaminated soil to maize plant is shown in Fig 3. It would be seen from these result that EDTA markedly increased the amounts of transfer of the metals from the soil to the maize shoots with up to 30% of available As, and about 20% each of available Cr and Cu transferred from the soil matrix into maize biomass within the short duration (20 dys) of this study.

Translocation factor, TF, the ratio of amount of metal in plant shoots (M_{shoots}) to amount in the roots (M_{endo}). Fig. 4 shows the variation of M_{shoots} with M_{endo} in maize plant grown on EDTA amended CCA contaminated soil. It can be seen that the values of M_{shoots} are disproportionately smaller than the values of M_{endo}: with less than 40% of M_{endo} translocated to the plant shoots. Adsorption by carboxylic groups and other cation exchanging moieties within apoplasmic cell wall of the roots reportedly contribute to the net metal accumulation in the roots of plants (Huang *et al*. 1989) growing on heavy metal contaminated soil.

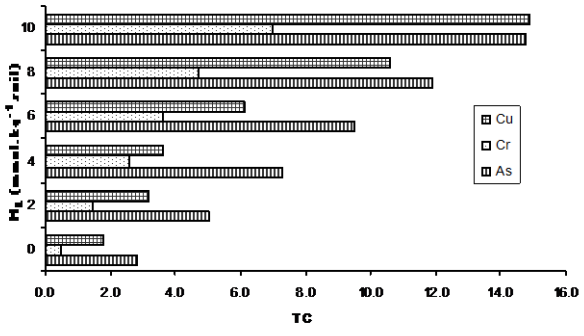


Fig. 3. Transfer coefficients of As, Cr and Cu to maize plant grown on CCA contaminated soil amended with EDTA.

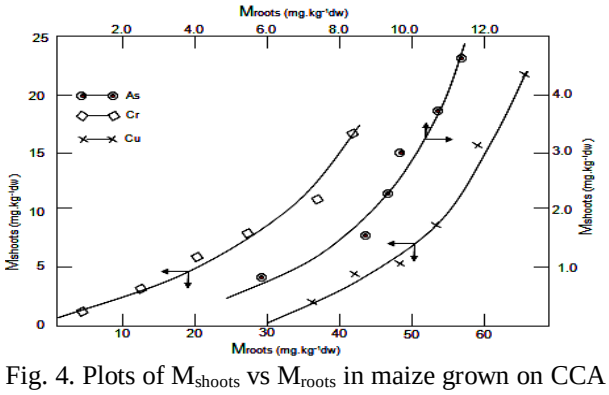


Fig. 4. Plots of M_{shoots} vs M_{roots} in maize grown on CCA contaminated soil amended with EDTA.

In chelate assisted phytoextraction of metals, it is important that in addition to mobilizing metals from

soil matrix to the root zone, the chelant assists in the effective translocation of the metals to the growing parts (shoots) of the plant (Huang et al. 1989). Relatively high metal concentration in the root zone could damage the roots and inhibit plant growth. Figure 5 shows that EDTA application markedly improved the translocation of As, Cr and Cu from the roots to the shoots of maize plant grown on CCA contaminated soil by as much as 12-fold.

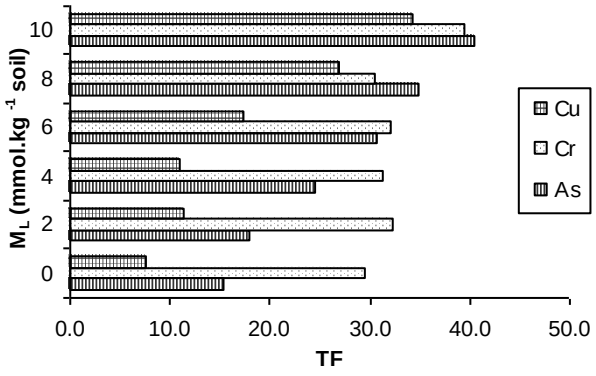


Fig. 5. Translocation factor of As, Cr and Cu from roots to shoots of maize plant grown on CCA contaminated soil amended with EDTA.

3.3. Mathematical model

Metal uptake by plants can be studied in many different ways. In most studies metal fractions in soil are directly related to the metal uptake by the above ground plant parts (Hough *et al.* 2005). To understand where and how metal speciation has its effect on metal uptake, metal uptake process can be divided into the following steps (Kalis *et al*., 2007): (a) metal content of the soil, (b) metal concentration in soil solution (c) metal adsorption on surface of roots (M_{exo}), metal uptake by the roots (M_{endo}) and metal uptake by the shoots (M_{shoots}).

Under the same soil conditions, the amount of metals taken up by plants depends largely on the conentration of the metal in soil solution. Therefore the addition of complexing agents (chelants) to contaminated soil should increase the amount of the metal available in soil solution for plant uptake. In the case of EDTA application to CCA contaminated soil, increase in the levels of metals in soil would occur through the formation of EDTA complexes of As, Cr and Cu. It should therefore be possible to define a relationship between the increased amounts of metals in soil solution, measured in terms of relative mobility factor, M_f, and metal uptake parameters such as M_{exo}, M_{endo} and M_{shoots} and metal uptake indices such as TC and TF.

Equation (I) shows a mathematical model that describes the relationship between the level of amendment appli-
cation (M_L) and the mobility factor (M_f):

$$\frac{dm_f}{dm_L} = b + 2cm_L - \frac{d}{e^{1+m_L}} \quad (1)$$

where b, c, and d are constants, which significantly depends on the metals.
Solving for m_f in equation (1) (by integration) yields the final solution:

$$m_f = bm_L + cm_L^2 + \frac{d}{e^{1+m_L}} + a, \quad (2)$$

where a is a constant.

The solution to (1) (given in (2)) with given initial conditions for the three metal can be seen in Figure 6. The dots are results from the actual experiments while the curves are solutions from (1) for the three metals, As, Cr and Cu. Clearly we can see a very good fit to the data. Hence equation (1) can be used to determining the rate at which M_f changes with respect to M_L.

An interesting feature of Figure 6 is that for some points on the curves, we will have the same gradient especially when $M_L \geq 2$. This shows that the rate at which the M_f changes with varying values of M_L for all three metals is not significantly different, the M_f for all three metals increases at the same rate with an increase in M_L.

Let Y represents some specific properties being investigated i.e. M_{exo}, M_{endo}, M_{shoots}, transfer coefficient (TC) and Translocation factor (TF):

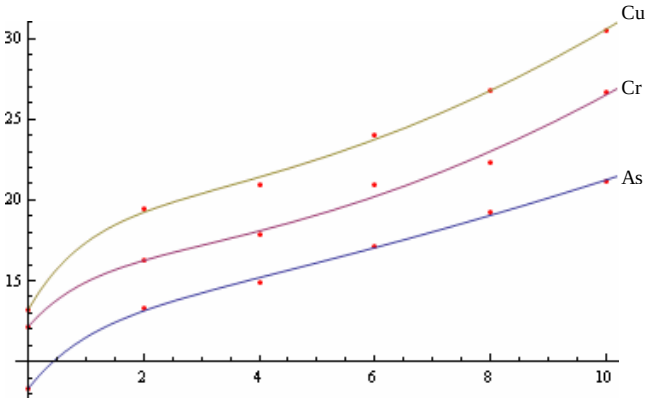


Fig.6. Plot of M_L against M_f for As, Cr and Cu.

A fitting mathematical model that shows the relationship between Y and the mobility factor, M_f can be seen in equation 2:

$$\frac{dY}{dm_f} = a_2 + 2c_2m_f \quad (3)$$

where a_2 and c_2 are constants which significantly depend on the metals and the specific property, Y.

The solution to equation (3) is given by

$$Y = b_2 + a_2m_f + c_2m^2_f \quad (4)$$

where b_2 is a constant

The solution to (3) (given in equation (4)), with given initial conditions holds for $M_f \geq 8$ (This is so since $M_L = 0$ values of $M_f \geq 8$).

Figures 7-11 show the plots of M_f against the different properties for Y, for all three metals. The dots are results from actual experiments while the curves are the solution to (3) (using (4)) . Again we find good fits for the data using the solutions to (3). With the solution to (3), one can find the amount of metal uptake for all three metals, with respect to the specific properties, as we vary M_f.

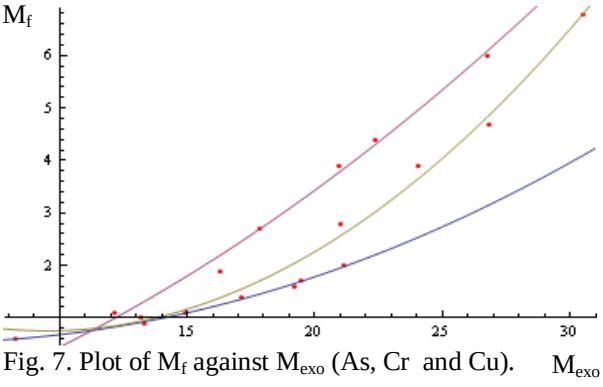


Fig. 7. Plot of M_f against M_{exo} (As, Cr and Cu).

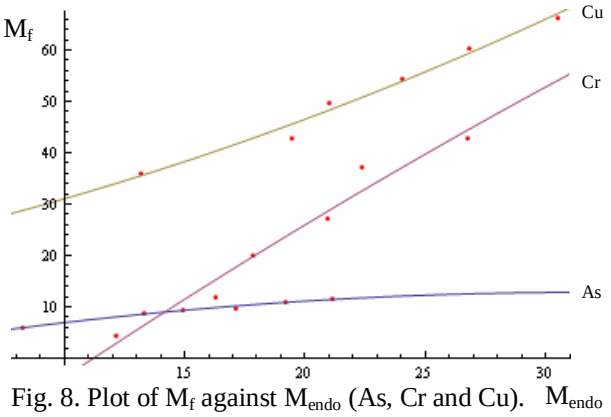


Fig. 8. Plot of M_f against M_{endo} (As, Cr and Cu).

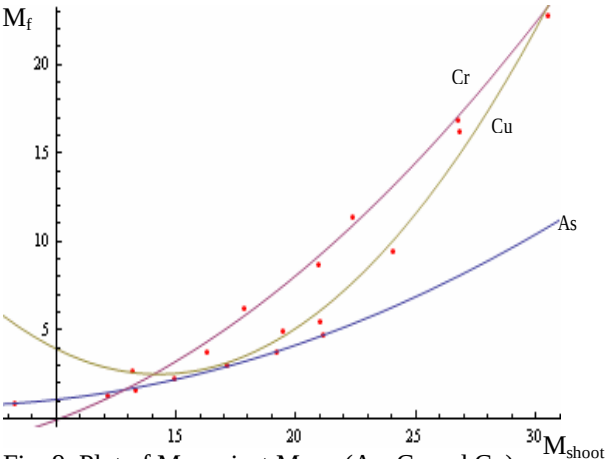


Fig. 9. Plot of M_f against M_{shoot} (As, Cr and Cu).

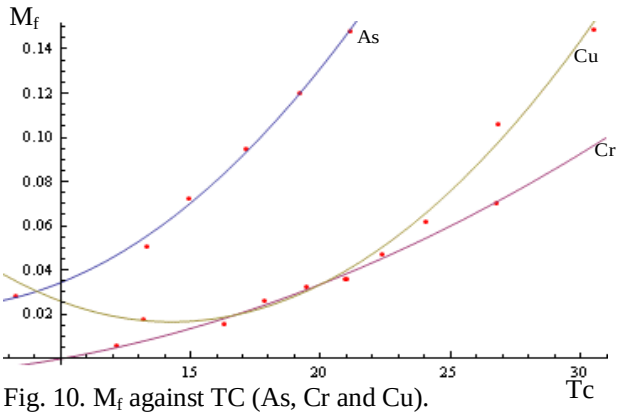


Fig. 10. M_f against TC (As, Cr and Cu).

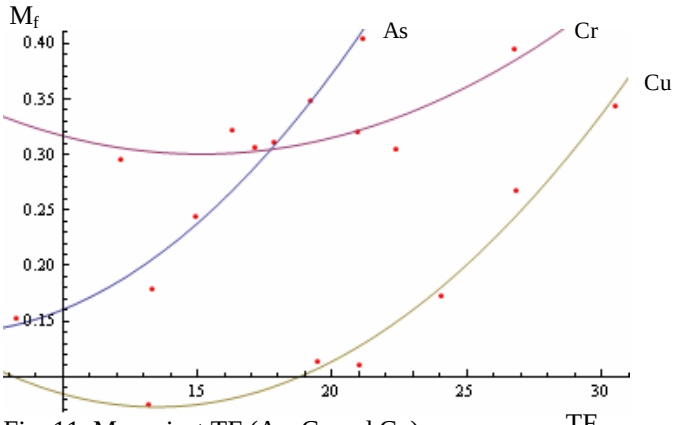


Fig. 11. M_f against TF (As, Cr and Cu).

4. Conclusion

This study examined the effect of EDTA application on the mobilization and plant (maize) uptake of As, Cr and Cu from CCA contaminated soil. The results obtained show that metal mobilization transfer and translocation to maize shoots were markedly enhanced by EDTA application to the contaminated soil. A mathematical model based on differential equation was developed to correlate the extent of metal mobilization through EDTA application to metal uptake parameters and indices.

References

Anoduadi, C.O., Okenwa, B.L., Okieimen, F.E., Tyowua, A.T., Uwumarongie-Ilori, E.G., 2009. Metal immobilization in CCA contaminated soil using laterite and termite mound soil. Evaluation by chemical fractionation. Nigerian Journal of Applied Science, 27, 77-87.

Blaylock, M.J., Huang, J.W., 2000. Phytoextraction of heavy metals. In: Phytoremediation of Toxic metals: Using Plants to Clean Up the Environment (Raskin, I., Ensley, B.D. Eds). John Wiley & Sons, New York.

Bolan, N.S., Adriano, D.C., Mahmairaja, S., 2004. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Critical Reviews in Environmental Science and Technology, 34, 291-338.

Cooper, E.M., Sims, J.T., Cunningham, J.W., Huang, J.W., Berti, W.R., 1999. Chelate-assisted phytoextraction of lead from contaminated soils. Journal of Environmental Quality, **28**, 1709-1719.

Garbisu, A.C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technology, **77**, 229-236.

Golia, E.E., Tsiropolous, N.G., Dimikrov, A., Mitsios, I., 2007. Distribution of heavy metals in agricultural soils in Greece using the modified BCR sequential extraction method. International Journal of Environmental Analytical Chemistry, 87(13-14), 1053-1063.

Holden, T., 1989. How to Select Hazardous Waste Treatment Technologies for Soils and Sludges: Alternative Technologies and Emerging Technologies. Noyes Data Corporation, Park Ridge, New Jersey.

Hough, R.L., Tye, A.M., Crout, N.M.J., McGrath, S.P., Zhang, P., Young, S.D., 2005. Evaluating a free ion activity model applied to metal uptake by Lolium perenne L. grown in contaminated soils. Plant Soil, **270**, 1-12.

Huang, C.X., van Steveninck, R.F.M., 1989. The role of particular pericycle cells in the apoplastic transport in root meristerm of barley. Journal of Plant Physiology, 133, 554-558.

Huang, J.W.W., Chen,J., Berti, W.R., Cunningham, S.S., 1997. Phytoremediation of lead-contaminated soil: role of synthetic chelates in lead phytoextraction. Environmental Science and Technology, **3**, 800-805.

Jiang, X.J., Luo, Y.M., Zhao, Q.G., Baker, A.J.M., Christie, P., Wong, M.H., 2003. Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. Chemosphere, 50, 813-818.

Kalis, E.J.J., Temminghoff, E.J.M., Viser, A., van Riemsdijk, W.H., 2007. Metal uptake by *Lolium perenne* in contaminated soil using a four-steps approach. Environmental Toxicology and Chemistry, 26,

Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H.R., Gupta, S.K., Schulin, R., 2000. Enhancement of phytoextraction of Zn, Cd and Cu from calcerous soil: the use of NTA and sulphur amendments. Environmental Science and Technology, 34, 1778-1783

Komarek, M., Tlustas, P., Szakova, J., Chrastny, V., Ettler, V., 2007. The use of maize and poplar in chelate-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere, 6**7**, 640-651.

Luo, C., Shen, Z.G., Li, X., 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cu with EDTA and EDSS. Chemosphere, 59, 1-11.

Meers, E., Ruttens, A., Hopgood, M., Samson, D., Tack, F.M.G., 2005. Comparison of EDTA and EDSS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere, 58, 1011-1022.

Okieimen, F.E., Tsetimi, G.O., Egbuchunam, T.O., 2010. The role of chelating agents in metal uptake and accumulation by maize (Zea mays L.) grown on CCA contaminated soil. Nigerian Journal of Applied Science, 28, 139-144.

Raskin, I., Smith, R.D., Salt, D.E., 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. Current Opinion in Biotechnology, **8**, 221-226.

Salt, D.E., Smith, R.D., Raskin, R., 1998. Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology, 49, 643-668.

Shen, Z.G., Li, X.D., Wang, C.C., Chen, H.M., Chua, H., 2002. Lead phytoextraction from contaminated soil with high-biomass plant species. Journal of Environmental Quality, **31**, 1893-1900.

Smith, L.A., Means, J.L., Chen, A., Alleman, B., Chapman, C.C., Tixier, J.S., Brauning Jr., S.E., Gavaskor, A.R., Royer, M.D., 1995. Remedial Options for Metals-Contaminated Sites. Lewis Publishers, New York.

Uwumarongie, E.G., 2009. Assessment of Heavy Metal Mobility in Chromated Copper Arsenate (CCA) contaminated soil. PhD thesis, University of Benin, Benin City.

Uwumarongie, E.G., Okieimen, F.E., 2007. Comparison of two sequential extraction schemes for metals in CCA contaminated soils. Nigerian Journal of Applied Science, **25**, 13-21.

Uwumarongie, E.G., Okieimen, F.E., 2010. Extractive decontamination of CCA contaminated soil using organic chelating acids. African Journal of Environmental Science and Technology, 4(9), 567-576.

Uwumarongie, E.G., Okieimen, F.E., Uwumarongie, O.H., 2008. Spatial and speciation of copper, chromium and arsenic in contaminated soil. Journal of the Chemical Society of Nigeria, 33(1), 112-121.

Wu, J., Hsu, F.C., Cunningham, S.D., 1999. Chelate-assisted pH phytoextraction: Pb availability, uptake and translocation constraints. Environmental Science and Technology, **33**, 1898-1904.