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EDTA-assisted Phytoextraction of Heavy Metals by *Amaranthus hybridus* Cultivated on Soil Collected from Selected Dumpsites in Ekiti State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author EEA managed the literature searches, analyses of the study performed the spectroscopy analysis and author OSA managed the experimental process and author SSA identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to asses the effectiveness of EDTA phytoextraction of heavy metals by Amaranthus hybridus cultivated on selected dumpsites, with a view to applying the plant in environmental restoration.
Study Design: It is an analytical study.
Place and Duration of Study: This study was conducted at Ekiti State University Ado Ekiti, Nigeria between November 2010 and June 2012.
Methodology: Topsoil (0-15cm) samples were randomly collected on selected dumpsites. The pH and organic matter content of soil were determined prior to plant cultivation. Sequential extraction of heavy metal from soil samples were conducted using modified Tessier's procedure and acid digestion to obtain the distribution pattern of metals in soil samples. The heavy metals concentration inddiferent sections of plant with or without EDTA application was determined using

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Flame Atomic Absorption Spectrophotometer, leading to the calculation of phytoextraction efficiency.
Results: The results revealed the mean pH and organic matter content torange from 6.12±0.08-6.56±0.14 and 5.40±0.02-5.84±0.15% respectively; these values were observed to be higher on dumpsites when compared with control sites. Application of synthetic chelate effectively increased the mobility of heavy metals from root to shoot of the plants and as a result of this, concentrations of heavy metals in the shoots of plants were higher in the experiment than control with concentrations of Cd (139.3, 130.0, 126.0 and 123.8 mg/kg; experiment,46.9, 44.2, 37.9 and 23.5 mg/kg; control), Cr (60.5, 59.2, 56.0 and 53.0; experiment, 24.4, 18.2, 16.6 and 16.6 mg/kg; control), Cu (189.3, 180.6,176.0 and 173.8; experiment, 69.0, 66.2, 59.9 and 44.5 mg/kg; control), Pb (227.2, 228.8, 296.3 and 278.7; experiment, 110.1, 104.8, 82.4 and 78.2 mg/kg; control) and Zn (148.0, 129.2, 121.0 and 116.4; experiment, 68.2, 63.0, 58.0 and 51.8 mg/kg; control) at Aba Egbira, Atikankan, Igbehin and Moshood street dumpsites respectively . Bioaccumulation factor (BF), translocation factor (TF) and remediation ratio (RR) were obtained to be greater than one for Cd, Cu and Pb, which showed that these metals were translocated in the plant's shoot and as a result, *Amaranthus hybridus* is effective in chelant-assisted phytoextraction.
Conclusion: Therefore, the use of *Amaranthus hybridus* is advocated as a candidate plant for restoring dumpsites polluted with heavy metals.

Keywords: Amarantus; heavy; metals; effect; phytoextraction; dumpsites.

1. INTRODUCTION

Concern has been expressed with regard to the accumulation of toxic heavy metals such as cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr), mercury (Hg) and lead (Pb) and their impact on both human health and the environment [1]. Ecosystem has been exposed to heavy metals through consumption of contaminated crops and vegetables grown on dumpsites or by drinking of water that percolated through such soils [2]. Contamination of soils with toxic metals has often resulted from human activities; especially those related to mining, industrial emmisions, disposal or leakages of industrial waste, application of sewage sludge to agricultural soils, and fertilizer and pesticide use [3,4]. The government and public need to be aware of the implications of polluted or contaminated environment on human health, thereby causing increasing interest amongst the scientists on the development of appropriate technologies to remediate contaminated sites [5].

Therefore, the cleanup of heavy metal contaminated soils is emergent and imperative. Phytoremediation was considered as an alternative cleanup method by using ornamental plants and grasses to remove, destroy, or sequester hazardous contaminants from environmental media, such as soil, water and air [6,7,8]. It is an emerging technique as a cost-effective, environmentally friendly, and technically applicable in situ, making it preferable to other chemical or mechanical techniques [7,9].

At present, there are two strategies of phytoextraction: (1) continuous phytoextraction which depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle (e.g., hyper accumulators), and (2) chelate-enhanced phytoextraction based on the application of chelating agents to the soil to enhance metal uptake by plants [8,10,11]. Hyperaccumulators are capable of accumulating large amount of trace elements including nickel (Ni), arsenic (As), Zn, Cd, and Pb in their above-ground tissues without any toxic symptoms [12,13].The effectiveness of a candidate plant to extract heavy metals from root to shoot transport system and tolerate very high concentration of such metals provides hyperaccumulators with potential detoxification capacity. The slow growth and small size of nearly 400 known hyperacumulators have limited their applications [14]. Several researchers have screened fast-growing, high-biomass accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots [15,16,17].

However, the use of these species for phytoremediation on a commercial scale is limited due to its low biomass production and slow growth rate. In order to compensate for the low metal accumulation, many researches have been conducted using synthetic chelators such as Ethylene Diamine Tetraacetic Acid (EDTA), Ethyle Glycol Tetraacetic Acid (EGTA) and other syhnthetic chelators to enhance the mobility and bioavailability of heavy metals in soils and increase phytoextraction efficiency [18,19]. Another limitation is how these heavy metals will be treated after phytoextraction; hence, phytomining is proposed for the total removal of these heavy metals from the ecosystem. EDTA is probably the most efficient chelate at increasing the concentration of various metals in above ground plant tissues [17,20,21]. Its high efficiency relies on solubilization of poorly available metals in soil (e.g Pb, Cr, Cu), followed by a largely passive accumulation of metal complexes in plant shoots through the transpiration stream [22,23].

Amaranthus hybridus is a popular nutritious leafy vegetable crop, widely consumed in Nigeria and some part of Africa [24]. The plant grows naturally on dumpsites and as a result it was selected for this study. Research on the genus Amaranthus include *Amaranthus tricolor* and *Amaranthus retroflexus* which were used for the uptake of cadmium, mercury, zinc and copper; and *Amaranthus spinosus* that was used for the accumulation of cadmium, zinc and iron [7,25,26]. This work was carried out to assess the effect of EDTA on phytoextraction of heavy metals by *Amaranthus hybridus* cultivated on dumpsites found in selected locations in Ekiti state, Nigeria, with a view to suggesting the technology for treatment of polluted sites.

2. MATERIALS AND METHODS

2.1 Soil Preparation and Experimental Procedure

Top soil (0-15 cm) was collected in November, 2010 from four dumpsites namely: Aba Egbira (7°37^{1}N), Atikankan (5°13^{1}E) at Ado Ekiti and Igbehin street (6°30^{1}N), Moshood road (8º36^{1}E) at Ikere Ekiti, Ekiti state, Nigeria. Control samples were collected 200m away from each dumpsite. The volume of domestic waste on these dumpsites has increased over the years as a result of the increased in economic activities since the creation of the state in 1996. These soil samples were randomly collected on each dumpsite to make a total of eight soil samples (two from each dumpsite). The soil sample were air dried , thoroughly mixed by a mechanical mixer and passed through a 4 mm metal sieve to remove fiber and non soil particulate. These soil parameters: pH range, organic matter content range and heavy metals concentration were determined prior to planting. pH and organic matter content (loss on ignition) was determined according to Hong and Teresa [27]. The soil samples were transferred into plastic pots to conduct green house experiment.

2.2 Pot-culture Experiment

Plastics pots of 15 cm high and 20 cm wide were filled with 5 kg of soil that had been previously sieved using a sieve with 4 mm mesh size. The uniform seedlings of *Amaranthus hybridus* obtained from a farm in Ado Ekiti after proper identification by a plant scientist in Ekiti State University herbarium were planted on pots marked experiment (with EDTA) and control (without EDTA). The experiment was spiked with 1.0 g/kg of EDTA at preflowering, flowering and maturity stages after planting according to Sun et al. [28]. The plant seedlings were cultivated 11th April, 2011 and harvested 8th July, 2011, atfer maturity.These plants were cultivated in green house and no fertilizer was added. The soil samples were watered to 75% waterholding capacity and this level was maintained throughout plant’s life span. A petri dish was placed under each pot to collect potential leachates which were immediately added to each pot to prevent loss of nutrients and target heavy metals. The plant dry weight was determined after harvesting and drying.

2.3 Sequential Extraction of Heavy Metals

The sequential extraction of heavy metals was carried out by method prescribed by Tessier et al. [29], modified by Campanella et al. [30]. The metal in aqueous phase of soil was extracted with 45 mL of 1M ammonium acetate at pH 5 with acetic acid under stirring for 24 hours. The exchangeable fraction was determined through extraction with 22.5 mL of hydroxylammonium chloride (1M) and 22.5 mL acetic acid (25%), with stirring at room temperature. Metal adsorbed on inorganic soil constituent was extracted with 12.5 mL of 0.1M HCl and stirring for 24 hours. Those associated with organic matter was treated with 12.5 ml of 0.5 M NaOH and stirring for 24 hours, later dried under IR lamp at 60°c and then digested with 4 ml of 65% HNO_3 and 2 mL of 40% HF in a microwave oven. The metals at the mixed solids phase were extracted using 12.5 mL of 8M HNO_3 and digested for 3 hours at 80°c. Lastly, the residual solid was digested with 4 ml of oxidising mixture (HNO_3: HCl) and 6 ml HF in Teflon recepient put in microwave oven. The sample was also shaken with 5.6g HBO_3 to avoid silica evaporation and diluted to 100 mL by deionized water.

2.4 Plant and Soil Analysis

The plants were immersed in 0.01M HCl solution to remove any external heavy metals [31] and rinsed with deionized water for 1 min. Subsequently, the plants were separated into parts: roots, stem, fruits and leaf. After that, they were dried at 100℃ for 10 min, subsequently at 70℃ in an oven to complete dryness. The plants and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). Heavy metals concentration was determined using flame atomic absorption spectrophotometer (Perkin Elmer, model 306).

2.5 Data Analysis

All treatments were replicated three times for control and experiment. The statistical analyzes were conducted using Analysis of Variance (ANOVA) procedures and mean separated by Duncan's Multiple Range Test (DMRT) using SPSS 15.0. Significant difference was determined between multiple treatments by (LSD) test. Bioaccumulation factor (BF), the ratio of contaminant concentration in plant to that in soil; Translocation Factor (TF), the quotient of contaminant concentration in shoot to roots; and Remediation Ratio (RR) were calculated. The remediation ratio was calculated according to this equation:

$$RR\ (\%) = \frac{M_{shoot} \times W_{shoot}}{M_{soil} \times W_{soil}} X\ 100(\%)$$

Where Mshoot is concentration of metals in the shoots of plants (mg/kg), Wshoot is the plant dry plant shoot (g); Msoil is concentration metals in soil measured in each pot (mg/kg) Wsoil is the mass of soil in the pot (g). The RR reflects the amount of metals extracted by a plant from soil, which indicate phytoextraction efficiency under chelant-induced experiments. Ratio of heavy of heavy metals in shoot of plant in chelants-assisted accumulation was compared with non-chelants assisted accumulation as: M_{shoot} (Exp.)/M_{shoot}(Cont.).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Parameters of Soil and Plant Dry Weight Production

The results of physicochemical analyses of soil and dry weight production of plant after harvest are presented in Table 1. Results obtained from pH determination revealed that dumpsites have higher pH values (6.12±0.08-6.56±0.14) when compared to control sites (5.40±0.02-5.84±0.15).

The pH of soil is a dynamic quality that can have a tremendous effect on the ability of plants to grow and thrive in it. Soil pH affects the uptake of essential nutrients by plant, soil microbial activity as well as health of plant in general [32].

The organic matter content of dumpsites; Aba Egbira (AB), Atikankan (AT), Igbehin street (IG) and Moshood road (MO) was found to be 7.8±0.12, 6.02±0.11, 5.6±0.10 and 3.6±0.14 respectively, while at control sites located 200 m away from each of the dumpsites lesser values (6.2±0.13, 5.80±0.12, 4.8±0.17 and 2.56±0.11) was observed for each of the dumpsites respectively.

Organic matter content of soil is an indication of vast array of carbon compound in soil. It is usually created by plants, microbes and other organisms, these compound plays varieties of roles in nutrient, water and biological cycles. For example, soil organic matter is known to increase the nutrient holding capacity of soil. It also acts as pool of nutrients for plant [33].

Table 1. physicochemical parameters of soil and dry weight production of plant cultivated on selected dumpsites

Dumpsite	pH		OMC (%)		Weight (g)		% Red
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	
AB	6.41±0.12	5.84±0.15	7.8±0.12	6.2±0.13	2.58	1.62	37.21
AT	6.56±0.14	5.72±0.14	6.02±0.11	5.80±0.12	2.42	1.58	34.71
IG	6.22±0.12	5.64±0.20	5.60±0.10	4.80±0.17	2.34	1.62	30.77
MO	6.12±0.08	5.40±0.02	3.60±0.14	2.56±0.11	2.18	1.52	30.28

AB: Aba Egbira; AT: Atikankan; IG: Igbehin; MO: Moshood; OMC: Organic Matter Content Red: Percentage reduction in weight; Exp: Experiment; Cont: Control

In addition, the application of EDTA to the plants caused a reduction on their biomass production to the levels of 37.21, 34.71, 30.77 and 30.28 %, in Aba Egbira, Atikankan, Igbehin street and Moshood road dumpsites respectively, when compared to the plants without EDTA Table 1. There were symptoms of phytotoxicity at earlier stage of EDTA application, with visible yellow leaves of plants, which disapper on germination. Clistenes et al. [34] reported that synthetic chalates have shown high effectiveness in heavy metals mobility and accumulation in the shoot of plants.

3.2 Distribution of Heavy Metals in Different Phases of Soil

The results of sequential extraction of heavy metals of soil collected from selected dumpsites in Ekiti State are shown in Fig. 1. The results revealed that EDTA was an efficient soil ammendment as heavy metals were distributed more in non-residual phase than residual phase of soil, as those metals found in non-residual phase are probably more bioavailable. Since more of these metals are available in non-residual phases of soil, the tendencies for their mobility and bioavailability would increase.

Sequential extraction procedure according to Camparalla et al. [30] classified heavy metals sources as anthropogenic and lithogenic. Also, the ease of extraction of these metals is dependent on how strongly bound or dissolved in aqeous phase, inorganic constituents, organic matter and as secondary minerals. The most easily soluble phase is considered to be the most bioavailable while the last fraction is the least bio-available [35].

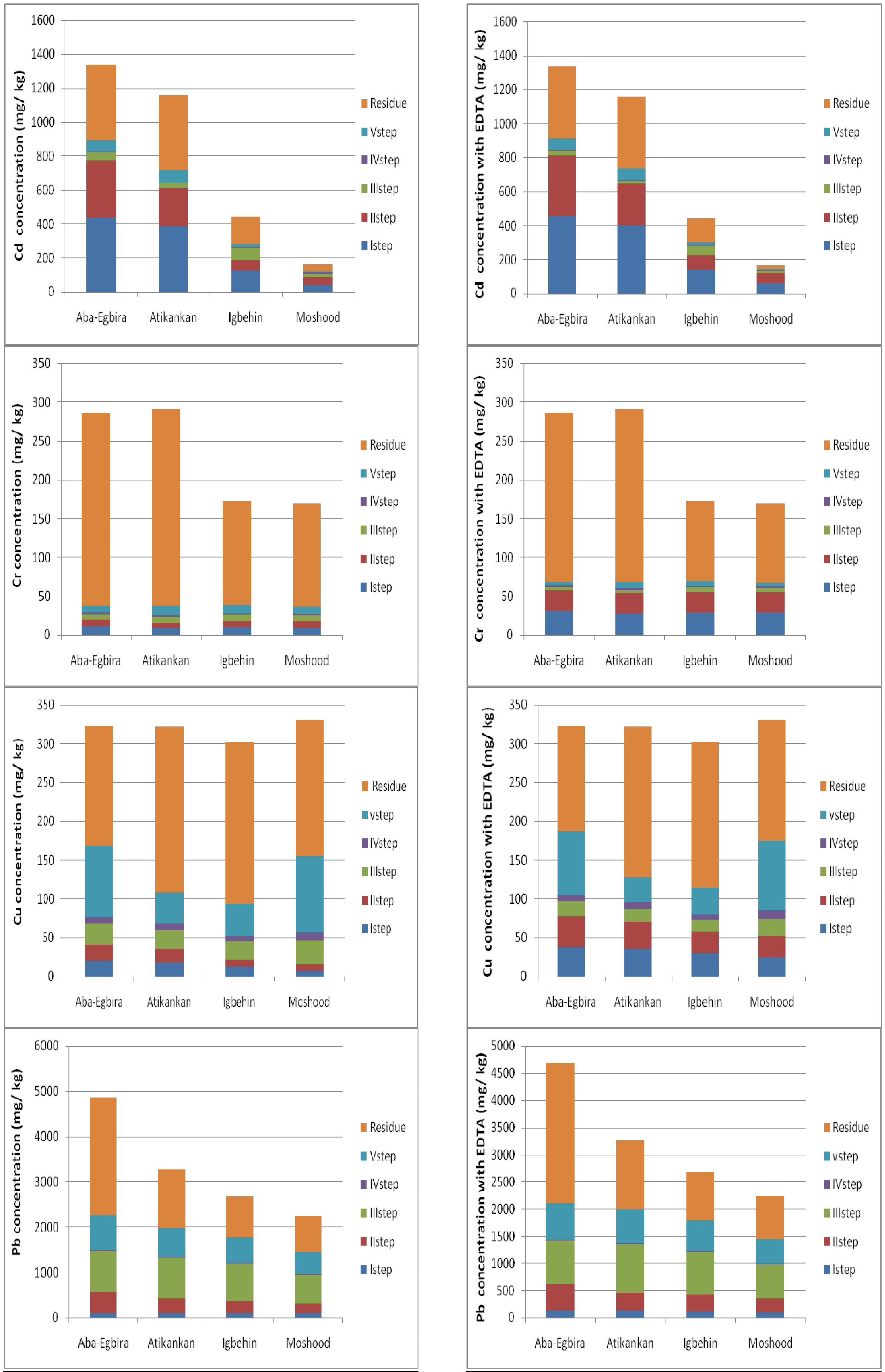
3.3 The Influence of EDTA on Heavy Metals Uptake and Accumulation

Table 2 presents the concentration of heavy metals in tissues of *Amaranthus hybridus* with and without EDTA treatment in the four dumpsites considered. The results showed that the concentrations of heavy metals were higher with addition of EDTA (1g/kg) than without EDTA (control), particularly with concentration of Cd (139.3, 130.0, 126.0 and 123.8 mg/kg; experiment, 46.9, 44.2, 37.9 and 23.5 mg/kg; control), Cr (60.5, 59.2, 56.0 and 53.0; experiment, 24.4, 18.2, 16.6 and 16.6 mg/kg; control), Cu (189.3, 180.6,176.0 and 173.8; experiment, 69.0, 66.2, 59.9 and 44.5 mg/kg; control), Pb (227.2, 228.8, 296.3 and 278.7; experiment, 110.1,104.8, 82.4 and 78.2 mg/kg; control) and Zn (148.0, 129.2, 121.0 and 116.4; experiment, 68.2, 63.0, 58.0 and 51.8 mg/kg; control) at Aba Egbira, Atikankan, Igbehin and Moshood street dumpsites respectively. These results are similar to those obtained by Yue-bing et al. [36] while examining the role of EDTA and CA on heavy metals phytoextraction in hyperaccumulator *Sedum alfredii*. Concentrations of heavy metals (Cd, Cr, Pb, Zn, and Cu) were also found to increase in the order leaf > stem > root >fruits in both control and experiments as well as in all dumpsites under consideration. *A. hybridus* was found to have accumulated considerably high concentration of Cd, Cr, Pb, Cu and Zn in their harvestable sections.

3.4 Remediation Efficiency

The bioaccumulation factor (BF) and translocation factor (TF) were used to evaluate the effectiveness of plant in metal accumulation and translocation [37,38]. As listed in Table 3, the BF and TF values of Cd, Cr, Cu, Pb and Zn increased with the application of EDTA relative to control. The BFs and TFs of Cd, Cu and Pb were all greater than 1.0 in the treatment of 1g/kg EDTA, indicating high capability of Cd, Cu and Pb uptake and transport by *A. hybridus* [36]. The phytoextraction of plants depends not only on heavy metal concentration in above-ground biomass, but largely on the biomass yield of the plants [39].

The RR is a reflection of the concentration of metals accumulated by a plant from soil, which is an indication of phytoextraction efficiency. In addition to this, the plant gave excellent performance in remediating soil polluted with Cd, Cu and Pb as BF, TF and RR values were ≥1. The greater the values of BF, TF and RR the more the plant could be useful as hyperaccumulators. *A. hybridus* has been found to grow naturally on various soils including dumpsites, which was the reason for high plant biomass even when 1g/kg EDTA was applied at different germination stages. Similar results were obtained for *Amaranthus dubius* as a hyperaccumulator of As, Cr, Cu, Pb and Hg [40]. This may be because *A. hyridus* and *A. dubius* belong to the same family.


Cd concentration (mg/ kg)
Cd concentration with EDTA (mg/ kg)
Cr concentration (mg/ kg)
Cr concentration with EDTA (mg/ kg)
Cu concentration (mg/ kg)
Cu concentration with EDTA (mg/ kg)
Pb concentration (mg/ kg)
Pb concentration with EDTA (mg/ kg)
Aba-Egbira
Atikankan
Igbehin
Moshood
Residue
Vstep
IVstep
IIIstep
IIstep
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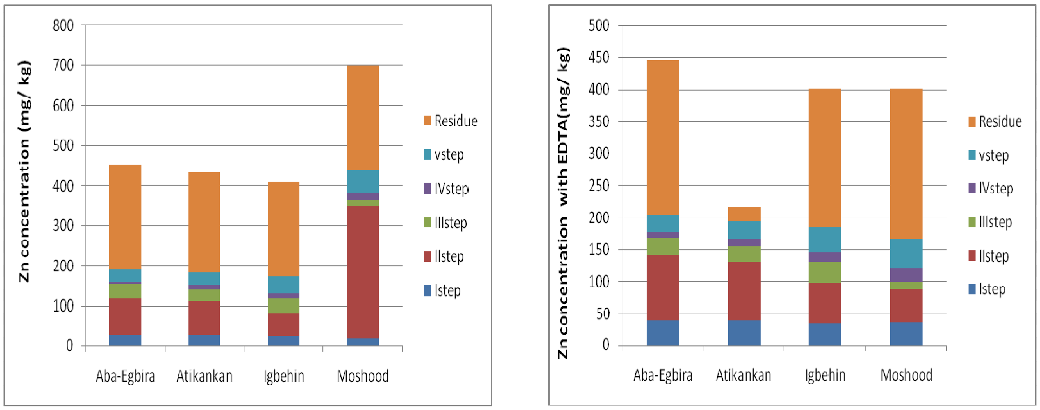

Fig. 1. Chemical speciation of Cd, Cr, Cu, Pb and Zn in soil collected from selected dumpsites

Table 2. Concentration of heavy metals in the tissues of *Amarantus hybridus* withand without EDTA treatment (mg/kg) on selected dumpsites

	Root		Stem		Leaf		Seed	
Cd	**Exp.**	**Cont.**	**Exp.**	**Cont.**	**Exp.**	**Cont.**	**Exp.**	**Cont.**
AB.	68.0a	46.0a	42.1a	10.1a	85.0a	30.0a	12.2a	6.8a
AT.	64.0a	42.0a	38.0ab	10.0a	82.0ab	28.0a	10.0ab	6.2ab
IG.	60.0a	41.2a	36.0ab	9.8a	80.0ab	22.0ab	10.0ab	6.1ab
MO.	58.0a	40.0a	36.0ab	6.2ab	78.0ab	12.8b	9.8b	4.5b
Cr								
AB.	16.0a	7.6a	14.4a	5.8a	26.0a	10.6a	20.1a	8.0a
AT.	16.0a	7.4a	14.0a	3.2ab	26.0a	7.0ab	19.2ab	8.0a
IG.	15.1ab	7.0ab	12.8ab	3.0ab	24.2ab	7.2ab	19.0ab	6.4ab
MO.	15.0ab	7.0ab	10.2b	3.0ab	23.8ab	6.8b	19.0ab	6.8ab
Cu								
AB.	88.0a	56.0a	62.1a	20.1a	105.0a	40.1a	22.2a	8.8a
AT.	84.0ab	52.0ab	58.0b	20.0a	102.0a	38.0a	20.6ab	8.2ab
IG.	80.0b	51.0ab	56.0b	19.8ab	100.0ab	32.0ab	20.0ab	8.1ab
MO.	78.0c	50.0ab	56.0b	16.2ab	98.0b	22.8b	19.8b	5.5c
Pb								
AB.	68.5a	26.2a	11.2a	48.1a	120.0a	36.0a	96.0a	26.0a
AT.	62.5ab	24.2a	12.8ab	42.8ab	120.0a	36.0a	96.0a	26.0a
IG.	50.4ab	20.2ab	98.2ab	30.4b	118.0ab	32.0ab	80.1ab	20.0ab
MO	48.2c	19.8c	86.6b	28.2c	112.1ab	31.0ab	80.0ab	19.0c
Zn								
AB.	36.0a	12.1a	26.0a	10.0a	96.0a	42.1a	26.0a	16.1a
AT.	30.1a	11.2ab	25.2b	10.0a	82.0ab	36.8ab	22.0ab	16.2a
IG.	28.0ab	10.1ab	24.0ab	9.8ab	76.0b	32.1b	21.0ab	16.1a
MO.	27.0ab	10.0ab	24.0ab	9.6ab	72.2c	26.2c	20.2ab	16.0a

Values followed by different letters differ at p<0.05 (LSD test)

The plant experienced yellowing of leaves at pre-flowering stage whem 1g/kg EDTA was applied but a great increse in biomass levels was observed at other stages, this may be due to phytotoxity as heavy metals are immobilzed within the area covered by roots of plant by EDTA [41]. This observation was not noticed in the control experiments. The concentration of heavy metals in diferent tissues of plants in the four dumpsites did not vary significantly at p<0.05 (LSD test), showing that the plant characteristic did not have much change in each dumpsite. Also, there was a gradual reduction in the BF, TF and RR values in this order Moshood road< Igbehin street< Atikankan<Aba Egbira.

Table 3. BF, TF and RR values of heavy metals in *Amarantus hybridus*

		BF		TF		RR	
		EXP	**CON**	**EXP**	**CON**	**EXP**	**CON**
Cd	AB	1.1	0.6	2.0	1.0	1.2	0.6
	AT	1.2	0.8	2.0	1.1	1.3	0.7
	IG	1.1	0.6	2.0	0.9	1.1	0.6
	MO	1.1	0.5	2.1	0.5	1.1	0.6
Cr	AB	0.4	0.1	0.4	0.2	0.4	0.1
	AT	0.8	0.2	0.6	0.5	0.7	0.2
	IG	0.3	0.1	0.4	0.4	0.3	0.1
	MO	0.2	0.1	0.5	0.4	0.2	0.1
Cu	AB	1.6	0.9	2.2	1.2	1.7	0.9
	AT	1.5	0.8	2.1	1.3	1.6	0.6
	IG	1.2	0.8	2.2	1.2	1.3	0.8
	MO	1.1	0.7	2.2	0.9	1.2	0.7
Pb	AB	1.6	0.8	1.7	0.8	1.5	0.9
	AT	1.5	0.9	1.5	0.7	1.4	0.9
	IG	1.2	0.7	1.8	0.8	1.3	0.8
	MO	1.0	0.6	1.8	0.9	1.1	0.8
Zn	AB	0.8	0.2	0.4	0.2	0.9	0.2
	AT	0.7	0.1	0.3	0.2	0.7	0.1
	IG	0.6	0.1	0.3	0.1	0.7	0.1
	MO	0.6	0.1	0.3	0.1	0.7	0.1

BF: Bioremediation Factor TF: Traslocation Factor RR: Remediation Ratio, AB: Aba Egbira AT: Atikankan IG: Igbehin Street MO: Moshhood Road

4. CONCLUSION

EDTA had positive effects on metals bioavalability in soil and largely enhanced metal uptake and accumulation in *A. hybridus.* The concentrations of Cd, Cr, Cu, Pb and Zn in harvestable parts of plants were significantly increased after the treatments of EDTA compared with those in control. However, there was reduction in growth of plants on the application of 1.0g/kg EDTA, chracterised by yellowing of leaves at preflowering stage. Because the enhancement of heavy metals uptake in plants could offset the reduction of dry biomass, the total heavy metals concentration in plants increased with the addition of chelators, especially for Cd, Cu and Pb absorption in shoots. It is necessary to futher investigate the use of different doses of EDTA for enhanced phytoextraction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gardea-Torresdey J, Peralta-Videa J, Montes M, De La Rosa G, Corral-Diaz B. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements. Biores. Technol., 2004;92:229-235.
2. McLaughlin MJ, Zarcinas BA, Stevens DP, Cook N. Soil testing for heavymetals. Commun. Soil Sci. Plant Anal. 2000;31(11-14):1661–1700.
3. Cunningham SD, Ow DW. Promises and prospects ofphytoremediation. Plant Physiology. 1996;110:715-719.
4. Ensley BD. Rationale for use of phytoremediation.In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of ToxicMetals e Using Plants to Clean Up the Environment. John Wiley & Sons, New York. 2000;3-11.
5. Bolan NS, Anderson KBG, Vogeler CWN. Solute interactions in soils in relation to bioavailability and remediation of the environment. 5th International Symposium ISMOM 2008 - November 24th - 28th. Pucon, Chile; 2008.
6. Chen H, Cutright T. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere. 2001;45:21–28.
7. Prasad MNV. Phytoremediation of metal-polluted ecosystems: hype for

commercialization. Russ. J. Plant Physiol. 2003;50:686–700.

8. Zhou QX, Song YF. Remediation of contaminated soils: Principles and Methods, Beijing: Science Press. 2004;489.
9. Lombi E, Zhao FJ, Dunham SJ, McGrath SP. Phytoremediation of heavy metal-contaminatedsoils: Natural hyperaccumulation versus chemically enhanced phytoextraction. J. Environ. Qual. 2001;30:1919–1926.
10. Garbisu C, Alkorta I. Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 2001;77(3):229–236.
11. Alkorta I, Hernández-Allica J, Becerril JM, Amezaga I, Albizu I, Onaindia M, Garbisu S. Chelate-enhanced phytoremediation of soils polluted with heavy metals. Rev. Environ. Sci. Biotechnol. 2004;3:55–70.
12. Baker AJM, Brooks RR. Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery. 1989;1:81–126.
13. Sun YB, Zhou QX, Ren LP. Growth responses of *Rorippa globosa* and its accumulation characteristics of Cd and as under the Cd–As combined pollution. Environ. Sci. 2007;28(6):1355–1360.
14. Salt D, Kramer U. Mechanisms of metal hyperaccumulation in plants. In: Raskin I; 2000.
15. Kumar P, Dushenkov S, Motto H, Raskin I. Phytoextraction: The use of plants to remove heavy metals from soil. Environ. Sci. Technol. 1995;29:1232-1238.
16. Salt D, Prince R, Pickering I, Raskin I. Mechanisms of Cadmium Mobility and Accumulation in Indian Mustard. Plant Physiol. 1995;109:1427-1433.
17. Huang J, Berti W, Cunningham S. Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol. 1997;31:800-805.
18. Chen YX, Lin Q, Luo YM, He YF, Zhen SJ, Yu YL, Tian GM, Wong MH. The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere. 2003;50:807-811.
19. Evangelou MWH, Ebel M, Schaeffer A. Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco (*Nicotiana tabacum)*. Chemosphere. 2006;63:996-1004.
20. Awokunmi EE, Asaolu SS, Ajayi OO, Adebayo OA. The role of EDTA on heavy metals phytoextraction by Jatropha gossypifolia grown on soil collected from dumpsites in Ekiti state, Nigeria. British J. of Envi. and Climate change. 2012;2(2):153-162.
21. Vassil AD, Kapulnik Y, Raskin I, Salt DE. The role of EDTA in lead transport and accumulation by Indian mustard.Plant Physiology. 1998;117:447-453.
22. Blaylock MJ, Salt DE, Dushenkov, Zhakarova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I. Enhanced accumulationof Pb in Indian mustard by soil-applied chelating agents.Environmental Science and Technology. 1997;31:860-865.
23. Sarret G, Vangrsosveld J, Manceau A, Musso M, D'Haen J, Menthonnex JJ, Hazeman JL. Accumulation forms of Zn and Pb in Phaseolus vulgaris in the presence and absence of EDTA. Environmental Science and Technology. 2001;35:2854-2859.
24. Odhav B, Beekrum S, Akula U, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J. Food Comp. Anal. 2007;20:430-435.
25. Jonnalagadda S, Nenzou G. Studies on arsenic rich mine dumps. II. The heavy element uptake by vegetation. J. Environ. Sci. Health, 32: 455-464. Kalac P, Svoboda L (2000). A review of trace element concentrations in edible mushrooms. Food Chem. 1997;69:273-281.
26. Bigaliev A, Boguspaev K, Znanburshin E. Phytoremediation potential of *Amaranthus* sp. for heavy metals contaminated soil of oil producing territory. Conference Proceeding In: 10th Annual International Petroleum Environmental Conference, Houston. al-Farabi Kazakh National University; 2003.
27. Hong C, Teresa C. EDTA and HEDTA effects on Cd, Cr and Ni uptake by *Helianthus annus*. Chemosphere. 2001;45:21-28.
28. Sun YB, Zhou QX, Wang L, Liu WT. The influence of different growth stages and dosage of EDTA on Cd uptake and accumulation in Cd-hyperaccumulator

(*Solanium nigrum L.*). Bull. Environ. Contam. Toxicol. 2009;82:348-353.
29. Tessier A, Campbell PGC, Bisson M. Sequential extraction procedurefor speciation of particulate trace metals. Anal. Chem. 1979;51,844-851.
30. Campanella L, Dorazio D, Pentronio BM, Pietrantonio E. Proposal for a metal \speciation study in sediments. Anal. Chemica Acta. 1995;309:387-393.
31. Aldrich M, Gardea-Torresdey JL, Peralta-Videa JR, Parsons JG. Uptake and reduction of Cr (VI) to Cr (III) by mesquite (*Prosopis spp*): Chromate-plant interaction inhydroponics and solid media studied using XAS. Environ. Sci. Technol. 2003;37:1859-1864.
32. Autum SW, Angel JS, Chaney RL, Delorme T, Mcintosh M. Soil biology and biochemistry. 2006;38:1451-1461.
33. Fagbote EO, Olanipekun EO. International Research Journal of Biotechnology. 2011;2(9):198-212.
34. Clistenes WA, Dula A, Baoshan X. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ. Pollution. 2006;140:114-123.
35. Li X, Thormton I. Chemical partitioning of trace and major elements in soil contaminated by mining and smelting activities. App. Geochem. 2001;16:1693-1706.
36. Yue-bing S, Qixing Z, Jing A, Wei-tao L, Rui L. Chelator-enhanced phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewaterwith the hyperaccumulator plant (*Sedum alfredii* Hance). Geoderma. 2009;150:105-112.
37. Sun LN, Yan XB, Wang WQ, Ma L, Chen S. Spatial distribution of Cd and Cu in soils in Shenyang Zhangshi Irrigation Area (SZIA), China. J. Zhejiang Univ. Sci. B. 2008;9(3):271–278.
38. Sun YB, Zhou QX, Diao CY. Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator Solanum nigrum L. Bioresour. Technol. 2008;99:1103–1110.
39. Komárek M, Tlustoš P, Száková J, Chrastny V. The use of poplar during at woyear induced phytoextraction of metals from contaminated agricultural soils. Environ. Pollut. 2008;151:27–38.
40. John JM, Himansu B, Bharti O. Bioaccumulation of Cr, Hg, As, Pb, Cu and Ni with the ability for hyperaccumulation by *Amaranthus dubius*. Afri. J. of Agric. Research. 2012;7(4):591-596.
41. Evangelou MWH, Ebel M, Schaeffer A. Chelate assisted phytoextraction of heavy metals from soils, Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere. 2007;68:989-1003.

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