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Assessing the potential of *Ageratum conyzoides* for the phytoremediation of lead-polluted soils

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ABSTRACT

Remediation of heavy metal contaminated soil has been a serious challenge to most developing nations of the world, because the conventional remediation strategy for heavy metal-contaminated soils involves excavation, though effective but extremely costly and disruptive. In this study, *Ageratum conyzoides* was investigated for its potential to remediate lead-polluted soil both naturally and chelate-induced forms under green house conditions. Pot experiments were conducted in nursery beds using lead spiked soils. Varing concentrations of lead salt solution were used. The plant was grown both directly on the soil and on soils modified with ethylenediaminetetraacetic acid (EDTA) and oxalic acid. The concentration of lead absorbed by the plants in the unmodified soil gradually increased with time. The maximum concentration absorbed in the root over a four-week study period was 37.12 mg/kg, while that of the shoot was 36.33 mg/kg. This increased significantly in the EDTA and oxalic acid-modified soils with maximum values (Root: 84.98.mg/kg, Shoot: 85.19 mg/kg) and (Root: 81.89 mg/kg, Shoot: 80.16 mg/kg), respectively. The plant growth was not affected by the increase in concentration of lead absorbed by the roots and shoots, when compared with the control, which implies that Ageratum *conyzoides* exhibited tolerance for lead-contaminated soils. The transfer factor ranged from 0.62 - 0.95 in unammended lead-contaminated soil; 0.75 - 1.04 and 0.84 - 0.98 in soils amended with chelating agents (EDTA and oxalic acid, respectively). Thus EDTA and oxalic acid can be used to enhance the absorption of lead from contaminated soil by *A. conyzoides*.

INTRODUCTION

Heavy metal pollution of soil is a significant problem of environmental concern throughout the world. The toxicity of heavy metals to humans, animals, microorganisms and plants (Wagner, 1993; Gaetke and Chow, 2003; Hernandez-Ochoa et al., 2005; Quartacci et al., 2005; Bodar et al., 2006; Fotakis and Timbrell, 2006) have made heavy metal remediation from the environment a subject of keen research interest. Among the various heavy metals of concern, lead (Pb) is of particular interest because of its toxicity concerns amongst which include its ability to impair cognitive ability in children and adults, with children particularly more vulnerable (ATSDR, 2007).

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Deposition of lead in soil through emissions from industrial production processes; automobiles using leaded fuel petrol; smoke and dust emissions of coal and gas-fired power stations; the laying of lead sheets by roofers as well as the use of paints and anti-rust agents; and mining and smelting of metalliferous ore have been reported (Gulson et al., 1995; EPA, 1998; Wuana and Okieimen, 2011; NASEM, 2017; Adeyi and Babalola, 2017). In Nigeria, mining activities have contributed significantly to environmental burden of Pb in some parts of the country in recent time (Udiba et al., 2019). Considering that Pb concentrates in the upper layers of soil due to its low solubility in soil solution implies that exposure to Pb in Pb-contaminated soils may be high thereby necessitating the need for its removal.

Remediation by conventional methods which include soil

Figure 1. Picture of *A. conizoides* showing the fibrous root and flowered shoot.

excavation, transport, treatment and disposal are quite expensive (Blaylock et al., 1997; Cooper et al., 1999). In this regard, phytoremediation has been put forward as a promising, environment-friendly, alternative remediation technology for the remediation of heavy metals, including Pb, from contaminated soils (Salt et al., 1998).

Phytoremediation involves the use of plants with metal-hyper-accumulating ability to remove metals from soil solution through the roots with subsequent transfer to over-ground parts of the plant (shoot) where they can be easily harvested (Baker et al., 1994; Brown et al., 1994). It can be natural or chemically assisted with soil amendments using EDTA, diethylenetriaminepentaacetic acid (DTPA) and low molecular weight organic acids like citric and oxalic acids. Phytoremediation of Pb from contaminated soils is marred by the low bioavailability of Pb in soil solution attributable to its complexation with organic matter, sorption on oxides and clays or precipitation as carbonates, hydroxides and phosphates (Lim et al., 2004). This has necessitated the use of the soil amendments to improve Pb availability in soil solution. For example, EDTA has been noted as an effective chelating agent for the solubilization of soil-bound Pb (Blaylock et al., 1997; Huang et al., 1997; Kos and Le tan, 2003; Alkorta et al., 2004; Hong and Jiang, 2005). It has been shown to dissolve Pb adsorbed to soil particles (Means et al., 1978) and enhance uptake and translocation from roots to shoots (Nascimento et al., 2006). Despite the performance of EDTA in solubilizing Pb in contaminated soils for plant absorption, investigations are still on-going for other amendments due to the stability of EDTA and its metal complex in the environment.

Various authors have investigated the hyper-accumulating ability of many plants for the phytoremediation of Pb contaminated soils (Blaylock et al., 1997; Tu and Shelley, 2002; Wang et al., 2003; O'Connor et al., 2003; Bennet et al., 2003, Aiyesanmi et al., 2012). Furtherance to the on-going screening of indigenous weeds in Nigeria for their phytoremediating ability of heavy metals, the present study investigated the potential of *Ageratum conyzoides* for the phytoremediation of Pb-contaminated soils. *A. conyzoides* is an erect herbaceous annual fast growing plant with large biomass, lot of fibrous roots, and strong growth adaptability to different ecological conditions (Baker, 1965; Ming, 1999) (Figure 1). Species of the plants are mostly found in Southeastern North America, Central America, Caribbean, Mexico, and several countries in tropical and sub-tropical regions, including Brazil and Nigeria (Baker, 1965; Ming, 1999).

MATERIALS AND METHODS

Soil sampling and characterization

Soil samples used for the pot experiments were obtained from a site within the premises of the Federal University of Technology, Akure, Nigeria. Thereafter, the soil was air-dried for few days and then sieved through a 2 mm mesh sieve. The sieved soil sample was then subjected to the

following physico-chemical parameters: Soil pH which was determined in a mixture of soil and deionized water (1:2, w/v) with a glass electrode. Soil particle size was measured using the hydrometer method (Sheldrick and Wang 1993). Total nitrogen was determined using the Kjeldhal method (Margesin and Schinner, 2005). Organic carbon and organic matter content were determined by Walkey-Black wet oxidation approach (Schulte, 1995). Cation exchange capacity (CEC) was determined as the summation of total exchangeable base and exchangeable acidity. The exchangeable acidity was determined by the KCl extraction method (McLean, 1965), while exchangeable bases (Ca, Mg, K and Na) were determined by the neutral ammonium acetate extraction method. Amounts of exchangeable Ca and Mg were determined by EDTA titration, while Na and K were determined using flame emission spectrophotometer. Total Phosphorus was determined colorimetrically (Margesin and Schinner, 2005). The background Pb concentration of the soil was determined by flame Atomic Absorption Spectro-photometer (Buck scientific 210 VGP) after the soil had been digested with a mixture of concentrated HCl, HNO_3 and HF (3:1:1, v/v/v) (Aiyesanmi et al., 2012).

Pot experiments

The experiments were carried out following the methods described by Aiyesanmi et al. (2012). The air-dried and sieved soil samples (2 kg) were weighed into plastic pots. Seedlings of *A. conyzoides* were obtained from the same source where the soil samples were collected, trans-planted into the pots and allowed to stabilize for one week. Upon plant stabilization in the soil, 150 ml of 50, 100, 200, 500 and 1000 mg/L of lead ion solutions prepared from lead nitrate ($Pb(NO_3)_2$ were introduced in duplicates into 10 plastic pots containing the seedlings without chelating agents. These translated to Pb concentration (mg/kg) of 3.75, 7.50, 15.0, 37.50 and 75.00 respectively in the soils. Another set of ten pots (duplicates of each contaminant concentration) were further treated with 100 ml of 100 mg/L EDTA, as chelating agent. Similarly, another set of ten pots were treated with 100 ml of 100 mg/L of oxalic acid as chelating agent. The thirty pots described so far in addition to five control pots (without Pb contamination and amendment) were used to monitor Pb uptake in the plants after a week of contamination and amendment application. Additional sets of pots sufficient for three more weeks (35 pots per week) were prepared to monitor the plant uptake of Pb for a total period of four weeks.

A total of 140 pots were used in this study. The pots were placed in a screen-house where they were exposed to about 12 h of daylight throughout the period of 4 weeks of this study. The pots were mildly watered with distilled water every 48 h to avoid flooding, but sufficient to keep the plant growing.

Plant harvest and analysis

The plants on contaminated soil, chelate-amended contaminated soil and control were harvested from the pots on a weekly basis. The plants were carefully uprooted by broken up the soils and were thorughly washed with tap water and later with deionized water. The roots and overground parts (shoots) were separated, oven-dried at 70°C for 24 h, weighed and ground into fine powder. About 0.5 g aliquot of plant powder further dried to constant was ashed in a muffle furnace at 500°C for 4 h. The ash was then dissolved in 20 ml 0.5 M HCl, filtered using Whatman No. 1 filter paper and the filtrate was then diluted to 50 ml with deionized water and analysed for Pb using flame Atomic Absorption Spectrophotometer (Buck Scientific, 210 VGP) (Aiyesanmi et al., 2012).

Quality control

All reagents used in the study were of analytical grade (BDH Laboratory supplies, Poole, England). Certified Reference Soil (SOIL-7) obtained from Internation Atomic Energy Agency was analysed along with the soil sample. Values obtained are compared with the expected certified values in Table 1 under results and discussion section. Recovery study was done on the plant samples for method validation. The recovery study involved spiking known concentrations of Pb ion solution into aliquot sample of ground plant samples taken randomly from three different treatments and ashed along with the test plant samples. The percentage recovery was calculated based on the concentration of Pb in the original plant samples vis a vis the concentrations in the spiked sample. Recovery of lead from the spiked samples was between 93 to 102 percent.

RESULTS AND DISCUSSION

Physicochemical properties of soil sample

The results of analysis of the soil sample used in the pot experiment for this study had nitrogen content of 0.37%, potassium (0.45 cmol/kg), organic matter (3.84%) and phosphorus (12.03 mg/kg) (Table 2), suggesting that it will favourably support the growth of the plant (FAO, 1990). The pH value of the soil was 6.30 indicating that the soil is weakly acidic. Under pH conditions (pH<5.5), metal cations are more mobile, while at higher pH metals tend to sorb to mineral surfaces (GWRTAC, 1997). At a pH of 6.30, the mobility of the lead ion in the soil may be low(Harter, 1983), thereby necessitating the need for soil amendments.

The particle size distribution in the soil was shown to be 51% sand, 31% clay and 18% silt, indicating that the soil used for the study was sandy clay loam according to

Table 1. Lead concentration in soil with observed and certified values for IAEA Soil 7 – CRM.

Sample	Concentration (mg/kg)
Soil (untreated)	3.33
CRM Observed value[a]	65.20 ± 1.15
CRM Certified values[b]	55.0 – 71.0

[a], Mean of triplicate determinations; [b], neutron activated analysis, AAS, fluorimetry, emission spectroscopy, colorimetry, volumetry, with or without separation and sample pre-treatment and preconcentration steps (Analytical Quality Control Service – IAEA, Austria).

Table 2. Physicochemical parameters of soil sample.

Parameter	pH	OM (%)	CEC (cmol/kg)	K (cmol/kg)	P (mg/kg)	N (%)	Texture (%)		
							Sand	Clay	Silt
Values	6.3±0.02	4.85±0.16	13.36±0.11	0.45±0.12	0.03	0.37	51	31	18

classification based on textural triangle. A sandy soil with little organic matter content will have a very low cation exchange capacity (Mengel, 2011). The CEC value of the soil is low enough to favour the desorption of cations into the soil solution, although soil pH remain a more dominant factor in determining desorption and bioavailability of Pb in soil solution.

Lead levels in experimental soils

The background lead concentration was 3.33 mg/kg which falls within the permissible limit of 85 – 200 mg/kg Pb in soils (DPR, 2002; Kabata-Pendias, 1995). This shows that the soil sample is not contaminated thereby making it suitable for the spiking of Pb in this study. The method validation study using SOIL-7 showed 94.5% conformance (Table 1). Thus, additively, the effective concentrationss of lead in the experimental soils at different levels of contaminations were 7.08, 10.83, 18.33, 40.83 and 78.33 mg/kg.

Plant tolerance to Pb contamination, EDTA and oxalic acid amendment

Visual assessment of the response of *A. conyzoides* to different concentrations of lead was monitored throughout the four weeks of the experiment. There was no plant death recorded, for both contamination and amendment treatments in all plants on soil contaminated with 7.08 mg/kg of Pb. The length of the roots ranged between 8-10 cm with average of seventeen (17) fibrous roots, while the shoots length ranged from 36 to 45 cm with an average of eighteen (14) leaves. The length of the leaves ranged between 2 and 8 cm with widths of between 0.80 and 4 cm. The observed features of the biomass were not significantly ($p>0.05$) different from what were recorded with control plants. However, at the highest concentration of 78.33 mg/kg Pb contamination, yellowing of the leaves was observed in some plant after the first ten (10) days and this symptom later disappeared. The yellowing is attributed to phytotoxicity as the plants were later analysed as having the highest uptake of Pb. The disappearance of this yellowing effect could be as a result of the plant adopting a detoxification method by exudation of metals in order to survive (Lasat, 2000).

Lead uptake into plant parts

Uptake from untreated, oxalic acid-treated and EDTA-treated soil

The uptake of Pb into plant parts: root and shoot, as shown in Tables 3 and 4 was observed to increase with increasing contaminant concentration. The plant exhibited a moderate uptake of Pb from contaminated soils with or without chelating agent amendment. On the unamended Pb-contaminated soil, the highest uptake of 37.12±0.06 and 36.33±0.06 mg/kg in the roots and shoots respectively were recorded at week 4 on 78.33 mg/kg Pb-contaminated soil. These values could increase with time as the plant has the characteristic of growing for some months before it withers. Earlier study on the uptake of Pb into the roots and shoots of some plants showed *Talinum triangulare* with 45.45 and 40.55 mg/kg respectively; *Chromolaena odorata* (103.7 and 55.68 mg/kg) and *Synedrella nodiflora*

Table 3. Uptake of Pb (mg/kg) into roots of *A. conyzoides*.

Soil amendment	Concentration of Pb (mg/kg) in plant root				
	A	B	C	D	E
Week 1					
Un-amended	2.55^{e}±0.12	7.85^{e}±0.02	9.04^{e}±0.33	9.53^{e}±0.11	21.63^{e}±0.02
Oxalic-amended	4.35^{d}±0.12	9.65^{d}±0.23	10.8^{e}±0.12	16.25^{d}±0.19	32.26^{d}±0.20
EDTA-amended	6.25^{c}±0.10	10.65^{d}±0.22	12.84^{d}±0.06	18.25^{c}±0.09	36.24^{d}±0.21
Week 2					
Un-amended	3.10^{e}±0.02	8.50^{e}±0.02	9.33^{e}±0.02	9.15^{e}±0.04	23.70^{e}±0.01
Oxalic-amended	6.25^{c}±0.06	11.36^{d}±0.22	15.26^{c} ±0.1	20.06^{c}±0.23	45.18^{c}±0.14
EDTA-amended	7.45 ^{b}±0.04	13.53^{c}±0.45	17.36 ^{c} ±0.08	22.06^{c}±0.13	48.28 ^{c}±0.09
Week 3					
Un-amended	7.20^{b}±0.06	8.79^{e}±0.13	9.67^{e}±0.06	11.84^{e}±0.08	29.51^{d}±0.02
Oxalic-amended	7.36^{b}±0.02	13.35 ^{c}±0.31	18.42^{b} ±0.10	28.54^{b}±0.18	60.88^{b}±0.20
EDTA-amended	8.34 ^{a}±0.02	15.24^{c} ±0.32	20.39 ^{b} ±0.13	29.54^{b}±0.15	62.64^{b}±0.23
Week 4					
Un-amended	8.51^{a}±0.10	8.06^{e}±0.01	11.55^{d}±0.04	19.60^{a}±0.01	37.12^{d}±0.06
Oxalic-amended	8.18^{b}±0.03	18.26^{b}±0.12	25.50^{a}±0.21	36.14^{a}±0.11	81.89^{a}±0.13
EDTA-amended	9.15 ^{a}±0.03	22.45^{a}±0.11	28.52^{a}±0.12	38.14 ^{a}±0.06	84.98 ^{a}±0.12

Values are represented in mean±standard deviation. The values with different lower cases (a-e) down the colum are significantly different (p<0.05).**A,** Test soil with 7.08 mg/kg Pb; **B,** test soil with 10.83 mg/kg Pb; **C,** test soil with 18.33 mg/kg Pb; **D,** test soil with 40.83 mg/kg Pb; `E, test soil with 78.33 mg/kg Pb.

Table 4. Uptake of Pb (mg/kg) into shoots of *A. conyzoides.*

Soil amendment	Concentration of Pb (mg/kg) in Plant Shoot				
	A	B	C	D	E
Week 1					
Un-amended	1.60^{e}±0.04	5.90^{e}±0.15	7.34^{e}±0.05	7.80^{e}±0.03	12.40^{e}±0.02
Oxalic-amended	3.86^{d}±0.02	9.33^{d}±0.12	10.45^{d}±0.14	15.21^{d}±0.13	30.37^{d}±0.21
EDTA-amended	5.86 ^{c}±0.02	9.63^{d}±0.12	11.45^{d}±0.04	16.67^{d}±0.14	33.34^{d}±0.11
Week 2					
Un-amended	2.75^{d}±0.02	7.40^{e}±0.09	8.14^{e}±0.02	8.93^{e}±0.06	18.65^{e}±0.12
Oxalic-amended	5.96^{c}±0.12	10.80^{c} ±0.03	14.46^{c}±0.18	18.22^{c}±0.16	43.38^{c}±0.22
EDTA-amended	6.96 ^{a}±0.12	11.80 ^{c}±0.03	16.46^{c}±0.12	20.22^{c}±0.24	49.98 ^{c}±0.13
Week 3					
Un-amended	6.25^{b}±0.03	7.61^{e}±0.13	7.87^{e}±0.11	9.15^{e}±0.12	9.15^{e}±0.12
Oxalic-amended	6.32^{b}±0.26	12.97^{c} ±0.05	17.23^{b}±0.32	27.36^{b}±0.12	58.46^{b} ±0.16
EDTA-amended	6.32 ^{b}±0.26	14.97^{b} ±0.05	18.23^{b}±0.34	27.36^{b}±0.34	63.33^{b} ±0.15
Week 4					
Un-amended	7.13^{a}±0.02	6.95^{d}±0.21	7.15^{e}±0.01	16.50^{b}±0.01	36.33^{d}±0.06
Oxalic-amended	6.89^{a}±0.24	16.22^{b} ±0.19	24.06^{a}±0.19	34.91^{a}±0.12	80.16^{e}±0.13
EDTA-amended	6.89^{a}±0.24	18.22^{a} ±0.19	26.06^{a}±0.18	30.91^{a}±0.12	85.19^{a}±0.23

Values are represented in mean±standard deviation. The values with different lower cases (a-e) down the colum are significantly different (p<0.05). **A,** Test soil with 7.08 mg/kg Pb; **B,** test soil with 10.83 mg/kg Pb; **C,** test soil with 18.33 mg/kg Pb; **D,** test soil with 40.83 mg/kg Pb; `E, test soil with 78.33 mg/kg Pb.

(309.5 and 127.1 mg/kg) by the fourth week of planting without EDTA enhancement treatement (Aiyesanmi et al.,2012). The uptake of Pb by *A. Conyzoides* in this studyshowed significantly (p<0.05) low uptake values compared with *C. odorata* and *S. nodiflora*, while the results are comparable with that of *T. triangulare*.

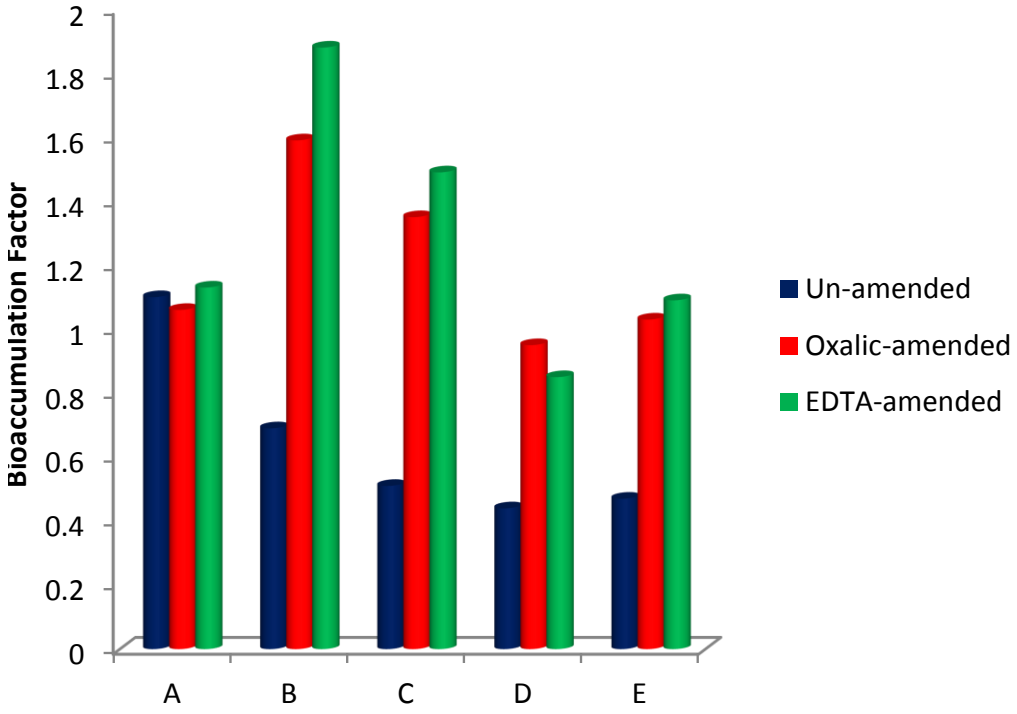

Figure 2. Bioaccumulation factor of Pb in *A. conyzoides* on Pb-contaminated soils.

The effects of treating the soil with chelating agents: Oxalic acid and EDTA (Tables 3 and 4), showed significant ($p<0.05$) increase in the uptake of Pb into the roots and shoots of the plants on the EDTA and oxalic acid-ammended contaminanted soils over those on unammended soil. An increase of 120 and 127% were recorded in the root uptake of Pb for *A. conyzoides* on oxalic acid and EDTA ammended soils respectively compared to those on unammended soil. Similarly, 122 and 136% increase in shoot uptake of Pb was observed on oxalic acid and EDTA ammended soils respectively. In most cases of root and shoot uptake of Pb by *A. conyzoides*, higher uptake recorded in EDTA-ammended soil compared to oxalic acid ammendment was in agreement with what has earlier been reported by some authors (Blaylock et al., 1997; Huang et al., 1997; Kos and Le tan, 2003; Alkorta et al., 2004; Hong and Jiang, 2005; Nascimento et al., 2006; Ebrahimi, 2014). In all these, EDTA was reported to be the most potent soil amending agent for metal availability in soil.

Bioaccumulation factor (BF)

One of the important features for a plant to be applicable in the phytoremediation of heavy metals is its ability to concentrate and retain the metal in its parts. Bioaccumulation factor, defined as the ratio of metal concentration in the plant to the soil provides such information. The average BF of the plant based on the initial concentration of Pb in contaminated soils across the various contaminant concentrations is presented in Figure 2. The BF of the study plant on untreated soil ranged from 0.16 – 0.73 across the various contaminant concentration and also increased with increasing contaminant concentration. The BF for the study plant on Oxalic acid and EDTA amended soils ranged from 0.22 – 1.53 and 0.25- 1.60 respectively.

It can be observed that the BF increased as contaminant concentration increases. The closeness in BF values for EDTA and oxalic acid amended soils mean that both amendment have similar enhancement capacity for lead uptake by *A. conyzoides*, even though EDTA has been reported as a potent amendment agent over other enhancing agents (Huang et al., 1997; Kos and Le tan, 2003; Alkorta et al., 2004; Hong and Jiang, 2005, Nascimento et al., 2006; Ebrahimi, 2014). Also, the similarity in performance suggests that, although EDTA might have performed better in terms of increasing the bioavailability of Pb in soil solution, the increase may not necessarily translate into increase in uptake by the plants if the plant does not have the potential to hyperaccumulate. In a metal-contaminated site in Florida, Yoon et al. (2006) found that *Gentiana pennelliana* (Wire grass) had the highest BF for Pb (BF = 11) among the 36 plants screened, while Kim et al. (2003) reported BFs for Pb between 4 and

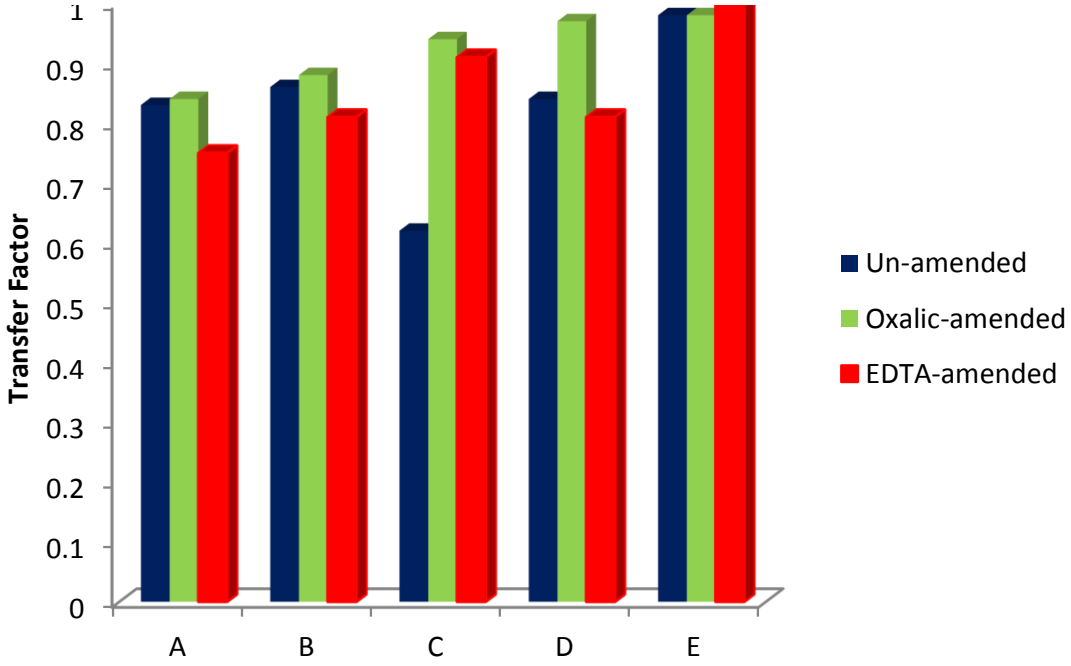

Figure 3. Transfer factor of Pb in *A. conyzoides* on Pb-contaminated soils.

58 in *Polygonium thunbergii*, suggesting that different plants have different ability to bioaccumulate Pb from contaminated soil.

Transfer factor (TF)

The transfer factor defined as ratio of metal concentration in shoots to that in roots is an index of translocation of metal from root to shoot. It can be observed in Figure 3 that the TF for *A. conyzoides* on Oxalic acid and EDTA-amended soils were higher than for the plant on unamended soil. The increase in TF was also concentration-dependent. The increase in TF in amended soils is in agreement with previous findings that addition of chelating agents enhance translocation of Pb from root to shoot (Vassil et al., 1998; Gleba et al., 1999, Farid et al., 2013). It has been reported that upon addition of EDTA to soils, it entered into the plant root in free form where it bound to metals and improved their mobility to overground parts (Farid et al., 2013). EDTA has the ability to disrupt cell membrane permeability and could therefore increase their permeability to solutes which must have contributed to more uptake of Pb (Chen and Cutright, 2001).

Conclusion

The ability of *A. conyzoides* for the phytoremediation of Pb-contaminated soil had been investigated using EDTA and oxalic acid as amendments. In the presence of Pb contamination, the plant exhibited a moderate uptake into roots and shoots without amendment, which increased significantly ($p<0.05$) on amendment of the soil with chelating agents. Despite the increased Pb uptake by the plants in the presence of Pb contamination and a further increase in uptake by amendment application, the efficacy of the plants falls below other hyperaccumulators of Pb. Also, considering the maximum concentration that was applied in the study, it is recommended that *A. conyzoides* can be applied to low Pb contaminated soil. This notwithstanding, the response of the plant to higher concentrations of Pb, effect of contact time (>4 weeks) on lead uptake, real field application to some Pb-contaminated sites and the potential of the plant to remove other heavy metals of environmental concern from contaminated or polluted soil need to be investigated.

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