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Single and mixed chelants-assisted phytoextraction of heavy metals in municipal waste dump soil by castor

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Abstract. The phytoextraction of some toxic heavy metals from municipal waste dump soil by castor plant (Ricinus communis) was tested under natural and single or mixed chelant-assisted scenarios in pot microcosms. A sandy loam with total metal contents (mg/kg): Cd (84.5), Cu (114.5), Ni (70.3), Pb (57.8), and Zn (117.5), was sampled from an active dumpsite in Calabar, Nigeria and used for the study. Castor (small seed variety) was grown under natural phytoextraction or single/binary chelant (citric acid, oxalic acid, and EDTA) applications (5-20 mmol/kg soil) for 63 days. Castor exhibited no visual phytotoxic symptoms with typically sigmoid growth profiles at the applied chelant doses. Growth rates, however, decelerated with increase in chelant dose. Post-harvest biomass yields were higher under chelant application than for natural phytoextraction. Both root and shoot metal concentrations (mg/kg) increased quasilinearly and significantly ($p \leq 0.05$) with increase in chelant dose, furnishing maximum levels as: Cd (55.6 and 20.9), Cu (89.5 and 58.4), Ni (49.8 and 19.6), Pb (32.1 and 12.1), and Zn (99.5 and 46.6). Ranges of translocation factors, root and shoot bioaccumulation factors were 0.21-3.49, 0.01-0.89 and 0.01-0.51, respectively. Overall, the binary chelant treatments were less toxic for *R*. communis growth and enhanced metal accumulation in shoots to a greater extent than the single chelant scenarios, but more so when EDTA was present in the binary combination. This suggests that the mixed chelants could be considered as alternative treatments for enhanced phytoextraction and revegetation of degraded waste dump soils.

Keywords: waste dump soil; heavy metals; Ricinus communis; phytoextraction; mixed chelants

1. Introduction

The disposal of high amounts of solid wastes generated from urban areas has been a serious environmental problem over the years, and is a major growing concern especially in the developing countries of the world (Abdus-Salam 2009). Huge active waste dumps are commonplace sights within residential and industrial areas, and on shoulders of minor and major roads in most cities in developing countries, probably due to an inadequate regulatory framework and enforcement system (Elaigwu *et al*. 2007, Obasi *et al*. 2012). In Nigeria, the waste dumps are frequently scavenged for reusable and recyclable materials or put to agricultural use by the urban populace in a bid to augment personal incomes and offset food insecurity occasioned by incessant

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rural-urban drift (Wuana *et al*. 2012).

Aside the biological hazards (bacteria, helianthus, viruses, protozoa, micro-fauna) and physical risks (sharp objects, psychosocial discrimination, insecurity and land tenure problems) commonly associated with the waste dumps, they also bear mixed chemical hazards (heavy metals, nitrogenous compounds, phosphorus compounds, minerals, pesticides, petrochemicals and other organic contaminants) which are eventually released to the receiving soil and groundwater (Hope 2006, Nabulo *et al.* 2008). Unlike most organic contaminants which are ultimately degraded by microbial action, the heavy metals, as chemical hazards, are not susceptible to microbial or chemical degradation, and so, their total inputs in soils can persist for a long time after their initial introduction (Gisbert *et al*. 2003, Kirpichtchikova *et al*. 2006, Mahmood 2010). Heavy metals that are most commonly found in contaminated soils (but not necessarily listed in order of abundance) are arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), nickel (Ni), and zinc (Zn) (Wuana and Okieimen 2011). Even though, soils are naturally able to limit the toxic effects of the heavy metals; at elevated levels this natural ability is impaired (Gismera *et al*. 2004, Fawzy 2008), and the heavy metals may pose risks to humans and the ecosystem through direct ingestion or contact with contaminated soil, the food chain, drinking contaminated groundwater, reduction in food quality via phytotoxicity, reduction in land usability for agricultural production leading to food insecurity and land tenure problems (McLaughlin *et al*. 2000, Ling *et al*. 2007). In order to mitigate the associated risks, the scientific community has continued to search for low-cost and ecologically sustainable technologies to cleanup soils contaminated by the heavy metals.

Phytoremediation is frequently listed among the best demonstrated available environmentally less-invasive *in situ* technologies regarded as primary remedies for restoring the quality and functionality of soils disturbed by toxic heavy metals (Pedron and Petruzzelli 2011, Wuana and Mbasugh 2013). Of the many approaches to phytoremediation, phytoextraction (phytoaccumulation, phytoabsorption, or phytosequestration) in which, plants are used to remove metals from soils, transport and concentrate them in above-ground biomass has been advocated by numerous authors owing to its cost-effectiveness, among other favourable factors (Padmavathiamma and Li 2007, Karczewska *et al*. 2009, Saraswat *et al*. 2009). As a general rule for this purpose, native species are preferred to exotic plants, which can be invasive and endanger the balance of the ecosystem (Kimenyu *et al*. 2009). Furthermore, a plant for use in phytoextraction is expected to (i) be heavy-metal tolerant, (ii) grow rapidly with a high biomass yield per hectare, (iii) have high metal accumulating ability in the foliar parts, (iv) have a profuse root system, (v) possess a high bioaccumulation factor, (vi) be adaptive to prevailing environmental and climatic conditions, (vii) be resistant to pathogens and pests, (viii) be easy to cultivate and harvest, and (ix) be repulsive to herbivores to avoid food chain contamination (Scragg 2006, Jadia and Fulekar 2008, Wuana and Okieimen 2011, Ali *et al*. 2013).

The castor plant (*Ricinus communis* L.) is a fast growing, non-edible oil producing plant from the spurge family (*Euphorbiaceae*). In addition to the many industrial uses of castor oil and other medicinal uses of the vegetative parts (Joshi *et al*. 2004, Mahmud *et al*. 2006, Rajkumar and Freitas 2008, Worbs *et al*. 2011, Pooja *et al*. 2013, Salihu *et al*. 2014, Warra 2015); recent reports have indicated that *R. communis* can be a multi-tasking for use in phytoremediation of soils contaminated by toxic heavy metals and carbon abatement technology due to its relatively high growth rate, profuse root system, prolific biomass yield, metal tolerance and metal accumulation and high carbon fixation (Mahmud *et al*. 2006, Rajkumar and Freitas 2008, Shi and Cai 2009, Vamerali *et al*. 2010, Huang *et al*. 2011, Bosiacki *et al*. 2013, Olivares *et al*. 2013, Miniño *et al*. 2014, Zhang *et al*. 2014).

During phytoextraction, the chemical additives such as organic and inorganic chelants usually applied to enhance the phytoextraction process and the desired remedial endpoints are plant-metal-site-specific (Gunawardana *et al.* 2011). Additionally, the chelants possess varying chemical affinities for different metals, and so the presence of metal mixtures with their synergistic or antagonistic interactions may impair the beneficial effects of the chelants (do Nascimento *et al.* 2006). Thus, chelant-assisted phytoextraction may encounter limitations in mixed metal contaminated sites as the effect of chelant on metal complex formation would vary depending on the nature and composition of the soil solution. Chelant combinations with sufficiently differing formation constants for the metals in the solution are necessary for selective complexation of one metal in the presence of the others (Gunawardana *et al.* 2011). Currently, metal phytoextraction literature is dominated by single-chelant applications. Consequently, in this study, pot experiments were designed to evaluate the influence of single and binary chelant applications on the phytoextraction of Cd, Cu, Ni, Pb, and Zn in a municipal waste dump soil by *R. communis*.

2. Materials and methods

2.1 Ricinus communis seeds, chemicals and apparatus

Ricinus communis seeds (small seed variety) were obtained from the Department of Plant Breeding and Seed Science, University of Agriculture, Makurdi, Nigeria. Citric acid monohydrate ($C_5H_8O_7{\cdot}H_2O$), MW=201.14, ≥99.0%); oxalic acid ($C_2H_2O_4$, MW=90.03, ≥99.0%) and the disodium salt of ethylenediaminetetraacetatic acid ($Na_2EDTA{\cdot}2H_2O$, MW=372.2, ≥99.0%) all of Sigma-Aldrich patent were used as organic chelants. The following apparatuses were used: hand trowel, polythene bags**,** mortar and pestle, 2 mm sieve, glassware (borosilicate, Pyrex), weighing balance (Gallenkamp 80), pH meter (Fisher Hydrus 300 model), an electric heater, atomic absorption spectrophotometer, AAS (Phoenix-986, Biotech Engineering Management Co. Ltd., UK).

2.2 Description of study area

Calabar is a city bound by the spatial coordinates 4°15′-5°15′N and 8°15′-8°25′E in south-eastern Nigeria. The city is the capital territory of Cross-River State and receives an annual rainfall of about 254 mm and a mean annual temperature and relative humidity of about 26.8°C and 84.6%, respectively. The vegetation cover is characterized by mangrove and rain forest ecosystems which form part of the rich fauna and flora of the area. The city is a socio-economic centre due to its thriving tourism potentials and fast developing sea port. The influx of visitors to this city during the annual carnival parade increases human population and industrial activities leading to the generation of huge amounts of solid and liquid wastes (Afangideh *et al.* 2011). Other specific activities responsible for heavy solid waste generation are flour mills, detergents, food processing, oil bunkering, tanneries, fertilizer blending, metal works, automobiles, auto-mechanic activities, financial institutions, and abattoirs (Ekwere *et al*. 2014). The waste so generated is disposed at the only approved waste dump which, though hitherto located far away from the municipality, has been encroached upon by the rapidly expanding city.

2.3 Soil sampling, pre-treatment and characterization

Surface soils are the first locus of input of metals where they tend to accumulate on a relatively long term basis (Krishna and Grovil 2007, Abenchi *et al*. 2010). In this study, five surface (0-20 cm) soil samples were randomly collected with the aid of a chrome-plated hand trowel at five different points at an active waste dump in urban Calabar into polythene bags and taken to the laboratory. The samples were further air-dried, ground, sieved to give <2 mm particle size and composited. Standard operating procedures were used to test soil properties: pH (1:25 soil/water ratio), textural analysis, bulk density, moisture content, organic matter, cation exchange capacity. Available Cd, Cu, Ni, Pb, and Zn were extracted by diethylenetriaminepentaacetic acid (DTPA), followed by atomic absorption spectrophotomertic (AAS) analysis, while pseudototal Cd, Cu, Ni, Pb and Zn were determined by digestion with aqua regia (HCl-HNO_3) followed by metal assay by AAS.

2.4 Phytoextraction experiments with Ricinus communis

Dried and sieved soil samples (3 kg) were placed in polythene bags (4.5 L). Three seeds of *R. communis*, previously cold treated (10^{o}C) for 12 h according to Wuana *et al*. (2013) to break dormancy, were sown in each pot. A week after germination, the seedlings were thinned to one and the set ups treated with 100 mL of citric acid (CA), oxalic acid (OA) or ethylenediaminetetraacetic acid (EDTA) as single or binary (CA+OA, CA+EDTA and OA+EDTA) solutions in deionized water at different doses (mmol chelant/kg soil): 0, 5, 10, 15 and 20. The 0 mmol chelant/kg soil treatment served as control set ups (natural phytoextraction); while the higher doses represented the chelant-assisted scenarios. The solutions were sprayed at the top of the pots and placed in a completely randomized design. Surface irrigation with deionized water was adopted to water the plants during growth and no fertilizers were applied. Night and day cycles were naturally obtained by maintaining the pots in an open area. The plants were monitored weekly for changes in appearance (colour), height and leaf breadth. Prior to harvest, the plants were left without watering for 1 day and then, carefully uprooted from the soils after the 63rd day, separated into roots and shoots, rinsed with deionized water, and dried at 110°C for 72 h. Post-harvest fresh and dry biomass were recorded for roots and shoots.

2.5 Plant biomass digestion and heavy assay in Ricinus communis

One (1.0) gram of dried and ground plant biomass was digested with a mixture of 16 mL of 65% v/v HNO_3 and 8 mL of 35% v/v H_2O_2. The mixture was heated almost to dryness. After evaporation, 16 mL of concentrated HNO_3 and 8 mL of concentrated H_2O_2 was added to the residue and heated until a clear digest appeared. The total digestion time was ≈3 h at 130^{o}C. The digest was made up to the 100 mL with deionized water. The digest was analyzed by AAS for Cd (228.8 nm), Cu (324.7 nm), Ni (232.0 nm), Pb (217.0 nm), and Zn (213.9 nm). Prior to analysis, Standard metal solutions were used to calibrate the AAS machine. The instrument settings and operational conditions were done in accordance with the manufacturer's specifications. Tissue (root and shoot) metal concentrations (mg/kg dry weight plant biomass) were then, got by converting corresponding metal concentrations in the plant digest (expressed in mg/L) using the appropriate mass balance relationship.

2.5 Quality control/assurance and statistical treatment of data

Analytical grade chemicals were used to prepare standard solutions and reagents. All glassware and plastics were washed with deionized water, rinsed with (1+1) HNO_3 and finally with deionized water. Procedural blank samples were subjected to similar treatments using the same amounts of reagents. In all cases, measurements were performed in triplicate. Graphical work was performed using the Microsoft Excel® software. One-sample t-tests were used to test the significance of differences within individual treatments; while analysis of variance (ANOVA) was used to test differences for all investigated variables during the experiment between treatments and controls at the 5% probability level ($p \leq 0.05$) by means of the SPSS 17.0 (SPSS, Chicago, Ill.) statistical package.

3. Results and discussion

3.1 Physicochemical properties of the study soil

Some physicochemical properties of the composite waste dump soil collected for the study are recorded in Table 1. The soil is preponderantly acidic (pH 5.2) and is composed of 78.9% sand, 13.2% silt and 7.8% clay, qualifying it as a sandy loam. The soil pH, coupled with the texture suggests the potential leachability of soil heavy metals to lower profiles. The other attributes are, bulk density (1.79 Mg/m^3), organic matter (9.3%), cation exchange capacity (8.65 cmol/kg). Pseudototal metal concentrations (mg/kg) were: Cd (84.5), Cu (114.5), Ni (70.3), Pb (57.8), Zn (117.5). Apart from Pb, the pseudototal metal contents of the waste dump soil obtained in this study are well above the levels reported for other surface soils subjected to other land uses within Calabar (Ekwere *et al*. 2014). The available metals (mg/kg) were: Cd (62.40), Cu (99.80), Ni (66.10), Pb (52.10), and Zn (110.30) implying that 73.8%, 87.2%, 94.0%, 90.1%, and 93.9% of their respective pseudototal concentrations were potentially available for plant uptake. With the exception of Zn, pseudototal metal concentrations in the soil were higher than their corresponding critical levels (mg/kg): Cd (1.4), Cu (63.0), Ni/Pb (50.0), and Zn (200) for agricultural soils (CCME 2007). The critical metal concentration is the range of values above which toxicity is considered to be possible.

In order to understand the degree of contamination of the site by the metals and attendant potential ecological risks, single-metal contamination factors, (C_f) and comprehensive potential ecological risk index (*RI*) were calculated based on the foregoing critical values using the approach of Hakanson (1980). The C_f values: Cd (60.34), Cu (1.82), Ni (1.41), Pb (1.16), and Zn (0.59) indicate that the site is highly contaminated with Cd, moderately contaminated with Cu, Ni and Pb; but slightly contaminated with Zn. The *RI* value of 1833.14 suggests a very high potential biotoxicity from the metals (with Cd posing the greatest risk, 98.78%) to soil fauna and flora and eventually to humans through the food chain (soil-plant-human) since the waste dumps are prone to urban agriculture.

3.2 Growth attributes and post-harvest biomass of Ricinus communis

The most common visual evidence of metal toxicity is the attenuation in plant growth variables with increasing metal availability in soil (Reichman 2002). Depending on their chemical nature,

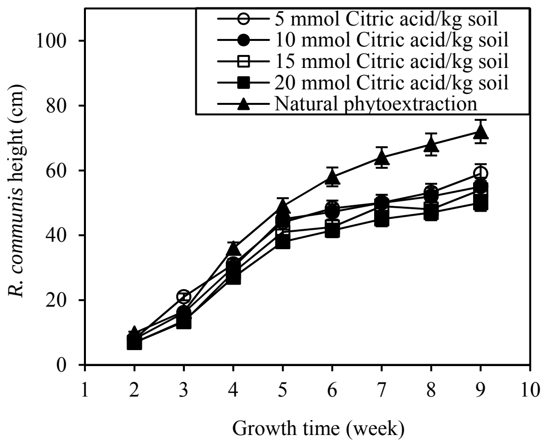
Table 1 Selected physicochemical properties of waste dump soil used for the study*

Property	Value
pH (H_2O)	5.20±0.03
Moisture content (%)	28.36±1.26
Bulk density (Mg/m^3)	1.79±0.03
Sand (%)	78.93±2.10
Silt (%)	13.23±1.00
Clay (%)	7.84±0.55
Textural classification	Sandy loam
Organic matter (%)	9.30±0.06
Cation exchange capacity (cmol/kg)	8.65±1.22
Available metals (mg/kg)	
Cd	62.40±0.05
Cu	99.80±1.13
Ni	66.10±0.00
Pb	52.10±0.15
Zn	110.30±0.26
Pseudototal metals (mg/kg)	
Cd	84.50±0.00
Cu	114.50±1.30
Ni	70.30±2.00
Pb	57.8±1.70
Zn	117.50±2.50

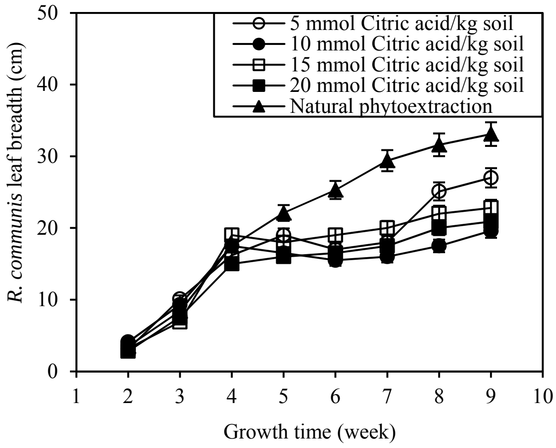
*Mean of triplicate measurements ± standard deviation

chelating agents are able to solubilize hitherto bound metal species in soil, bringing them into the soil solution phase, thereby rendering them more available for plant uptake. Consequently, this study employed increasing chelant (CA, OA, EDTA, CA+OA, CA+EDTA, CA+OA) doses to check their effects on the growth attributes of *R. communis* by weekly monitoring for changes in plant appearance, height and leaf breadth. Post-harvest root and shoot biomass (wet and dry) yields were also determined after 9 weeks of growth. *R. communis* exhibited no visual phytotoxic symptoms during the experimental period. The plants were typically greenish and luxuriant with essentially sigmoid growth profiles (Figs. 1 and 2); i.e., plant heights and leaf breadths increased slowly in the first two weeks, followed by a sharp increase up to the fifth week and then retardation beyond this, especially in soils receiving chelant treatments. Changes in the growth parameters with time were statistically significant ($p<0.05$) for all the treatments. Growth rates were fast enough and appeared to follow an approximate order: 0 mmol/kg (i.e., control) >5 (mmol/kg) >10 (mmol/kg) >15 (mmol/kg) >20 (mmol/kg). Aside being a favourable attribute in metal phytoextraction, the fast growth rate of *R. communis* has been shown to be advantageous as a green route to carbon abatement whereby high levels of atmospheric carbon (IV) oxide are sequestrated and fixed as carbon in the aerial biomass and roots (Vanaja *et al*. 2008).

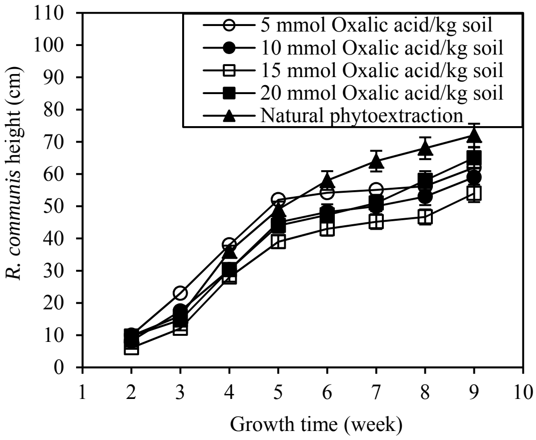
Under natural phytoextraction, ranges of *R. communis* height and leaf breadth were 9.8-72.0 and 3.5-33.1 cm, respectively (Figs. 1 and 2). Huang *et al*. (2011) reported an average height of 54.0 cm in soil containing 3.22 mg/kg Cd after *R. communis* grew for 8 weeks under natural phytoextraction. Most breeding programs have searched genotypes with short height (less than 1.5 m), height of primary raceme between 20 and 40 cm, less than 150 days for harvesting.



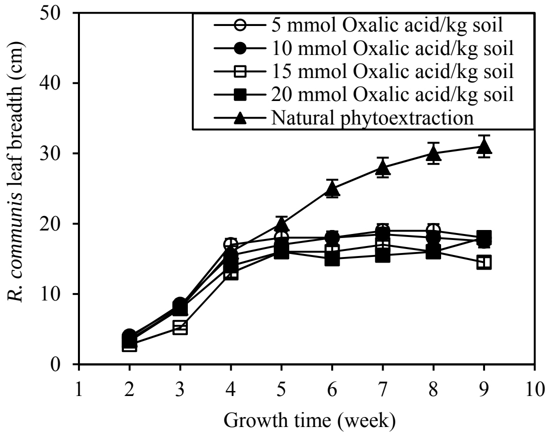
(a) *R. communis* height



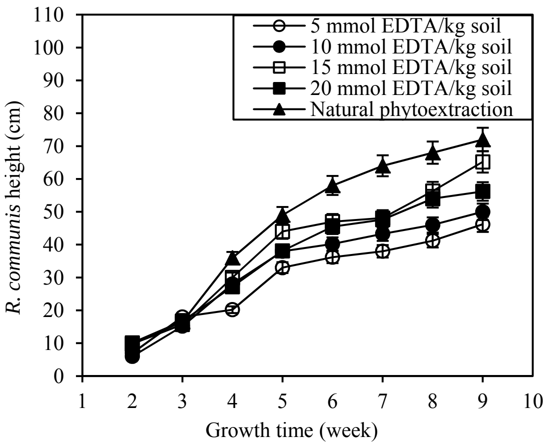
(a) *R. communis* leaf breadth



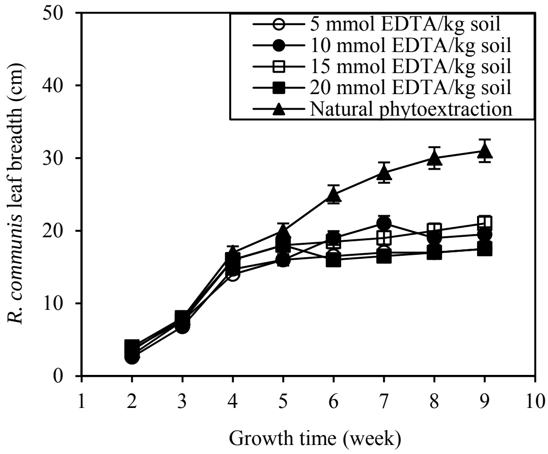
(a) *R. communis* height



(b) *R. communis* leaf breadth

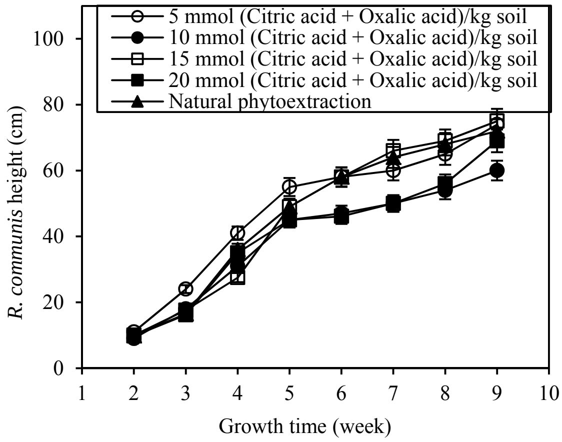


(a) *R. communis* height

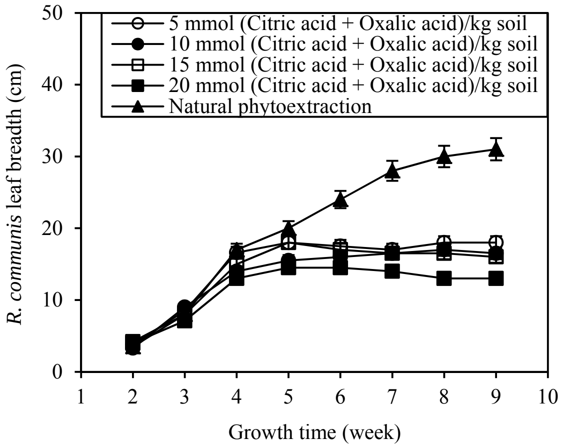


(b) *R. communis* leaf breadth

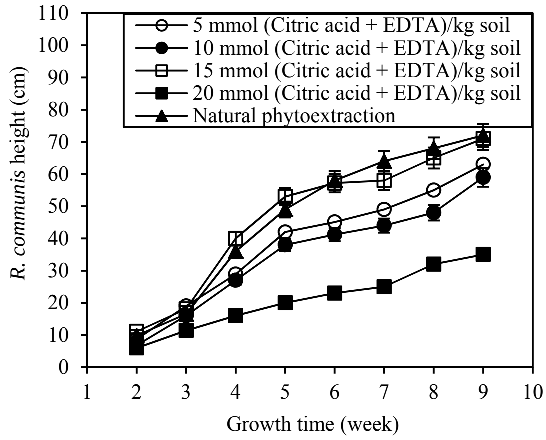
Fig. 1 Growth profiles of *R.communis* in waste dump soil under different doses of single chelant treatments



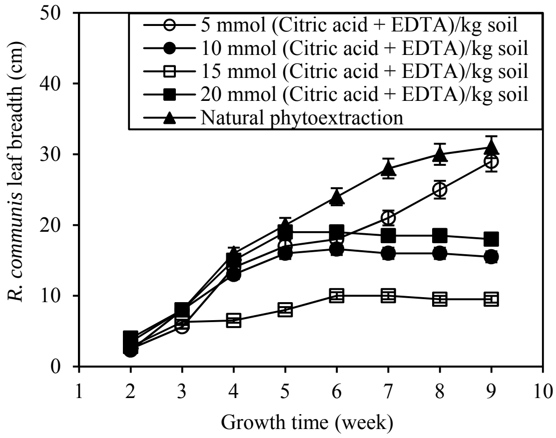
(a) *R. communis* height



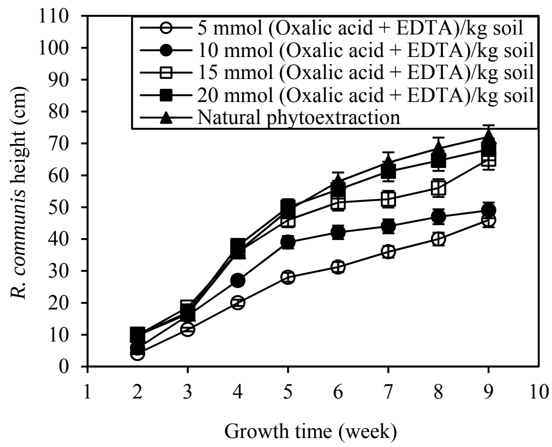
(a) *R. communis* leaf breadth



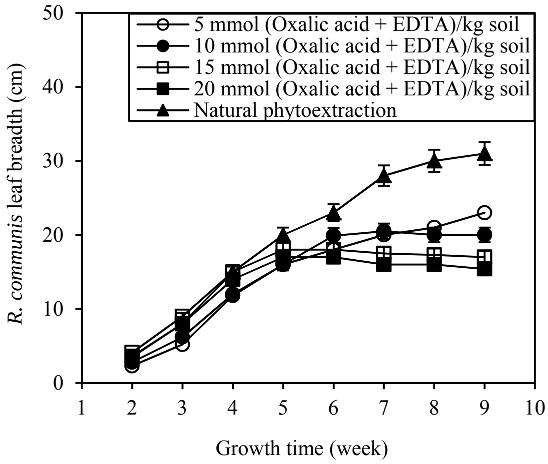
(a) *R. communis* height



(b) *R. communis* leaf breadth



(a) *R. communis* height



(b) *R. communis* leaf breadth

Fig. 2 Growth profiles of *R.communis* in waste dump soil under different doses of binary chelant treatments

In the single chelant-assisted scenarios, ranges of *R. communis* heights and leaf breadths for CA, OA, EDTA were 8.0-59.0 cm and 3.1-27.0 cm; 6.1-65.0 cm and 2.8-19.0; 15.1-65.2 cm and 2.6-19.5 cm, respectively. The binary chelant-assisted scenarios, CA+EDTA, CA+OA and OA+EDTA, recorded *R. communis* heights and leaf breadths in the ranges 9.0-75.0 cm and 3.3-17.5 cm; 6.0-63.0 cm and 2.5-19.0; 4.1-68.1 cm and 2.3-20.5 cm, respectively.

The effectiveness of phytoextraction as a remedial option for metal contaminated soils greatly depends on an adequate plant biomass yield in addition to a high tissue metal accumulation, among other favourable factors. The root, being the first plant part exposed to soil metals is expected to be robust enough. In this study, it was observed that the roots of *R. communis* spread extensively and penetrated the entire soil core in the pots during the experiments with mean root weights accounting for 27-30% of the total fresh plant biomass. A massive and prolific root system offers the advantage of increasing root contact with the soil solution leading to enhanced metal absorption by the plant. This could probably be seen as one of the important advantages of *R. communis* over other herbaceous plants for possible phytoextraction applications in heavy metal contaminated soils (Huang *et al*. 2011).

Average wet and dry root biomass yields (g/plant) during natural phytoextraction were: 18.1 and 5.5, respectively. In pots with single chelant treatments, root wet biomass ranges were: (23.6-56.6); (28.3-48.2); and (23.3-51.0) for CA, OA, and EDTA-treated soils, respectively; while corresponding dry biomass ranges were: (7.1-18.7); (8.5-13.5); and (7.0-15.3). For the binary chelant treatments, wet biomass yields were: (23.1-36.1); (29.2-35.7); and (19.3-34.6), for CA+OA, CA+EDTA, and OA+EDTA scenarios, respectively; whereas corresponding dry biomass yields were: (6.7-11.8); (8.8-11.0); and (6.4-9.7).

Shoot biomass yields represented 70-73% of the total plant biomass, and were higher under chelant-assistance than natural phytoextraction, possibly due to increased nutrient availability following chelant application. Soil nutrient availability is considered among the most important factors influencing plant biomass production (Reddy and Matcha 2010, Chatzistathis and Therios 2013). Apart from enhancing nutrient availability and high biomass production, chelating agents such as EDTA, CA and OA are able to increase metal availability in soil leading to increase metal absorption by roots for onward translocation to shoot, and hence efficiency of the phytoextraction process (Miniño *et al*. 2014).

Average wet and dry biomass yields (g/plant) were 60.2 and 13.7, respectively under natural phytoextraction. In CA, OA, and EDTA-treated soils, wet shoot biomass ranges were (84.3-176.7), (90.6-172.1), and (72.8-164.4), respectively. Corresponding dry biomass yields were: (18.5-38.4), (20.1-31.5), and (14.7-30.8). In the CA+OA, CA+EDTA, and OA+EDTA-treated soils, *R. communis* wet and dry biomass (g/plant) ranged as 79.5-124.3 and 16.0-24.5; 98.1-123.2 and 13.1-26.0; and 65.3-123.7 and 14.8-24.6, respectively. In all cases, wet biomass yields were generally higher than corresponding dry yields. On the whole, wet and dry plant biomass did not vary significantly (p>0.05) with increase in chelant dose. Observed differences in root and shoot biomass were, however, significant (p<0.05) for all the treatments.

3.3 Heavy metal accumulation by Ricinus communis

The effect of natural phytoextraction and single or binary chelant applications at increasing doses in soil on the accumulation of Cd, Cu, Ni, Pb, and Zn in *R. communis* was evaluated by measuring root and shoot metal concentrations (Figs. 4 and 5). Metal transferability to *R. communis* was also measured by calculating the dimensionless parameters, (i) root

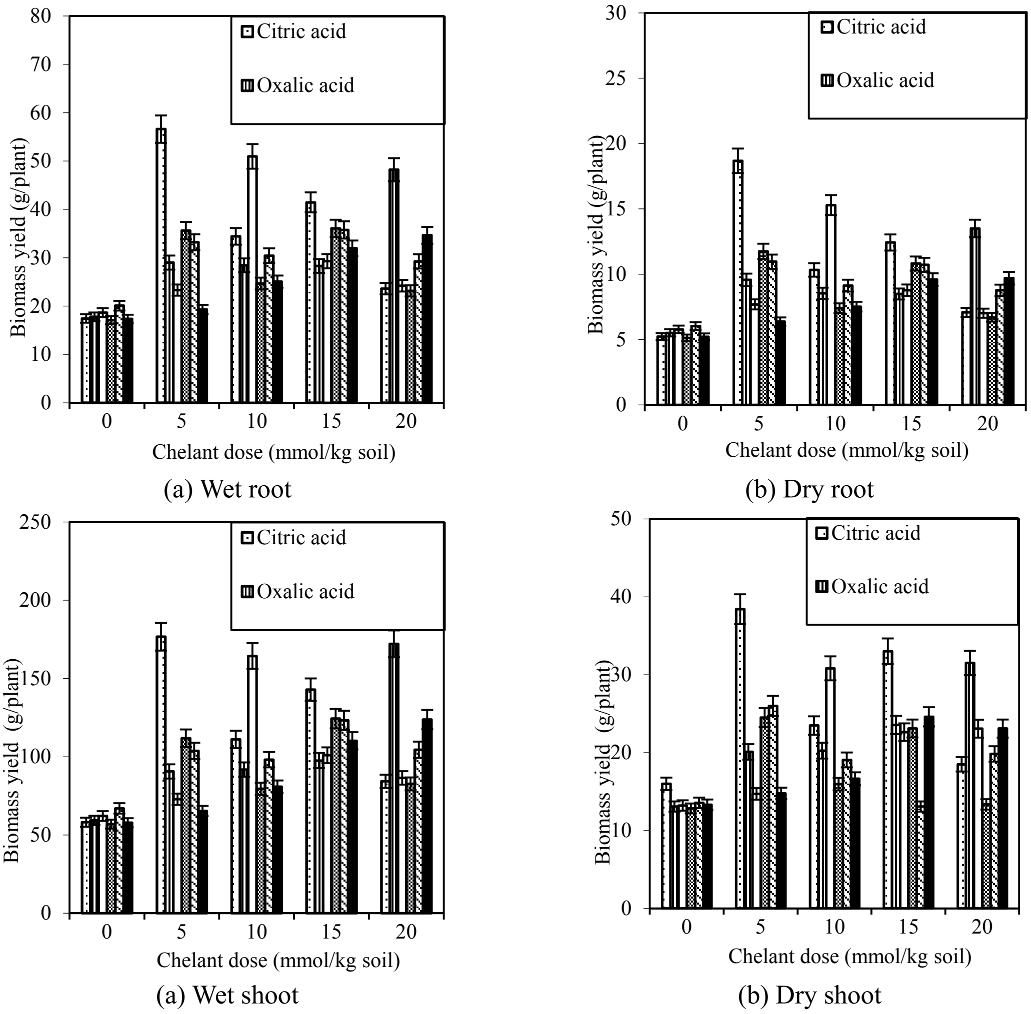


Fig. 3 Post-harvest *R. communis* biomass yields (after 63 days of growth) in heavy metals contaminated waste dump soil under different doses of single and binary chelant treatments

bioaccumulation factor (RB_f), (ii) shoot bioaccumulation factor (SB_f), and translocation factor (T_f) with the aid of Eqs. (1)-(3)

$$RB_f = \frac{C_{M(root)}}{C_{M(soil)}} \tag{1}$$

$$SB_f = \frac{C_{M(shoot)}}{C_{M(soil)}} \tag{2}$$

$$T_f = \frac{C_{M(shoot)}}{C_{M(root)}} \tag{3}$$

where $C_{M(\mathrm{root})}$ and $C_{M(\mathrm{shoot})}$ are the respective metal concentrations (mg/kg) in dry root biomass and dry shoot biomass, and $C_{M(\mathrm{soil})}$ is the pseudototal metal concentration (mg/kg) in the waste dump soil before *R. comunnis* growth.

3.3.1 Cadmium, Nickel and Lead

Plots of tissue metal concentrations in *R. communis* versus chelant dose showed similar profiles for Cd, Ni and Pb. Under natural phytoextraction (setup without chelant addition), mean root concentrations of Cd, Ni and Pb were 1.2, 0.6 and 2.5 mg/kg. Corresponding shoot concentrations were 0.9, 0.4 and 1.5 mg/kg which furnished RB_f, SB_f and T_f respectively as Cd (0.01, 0.01 and 0.75); Ni (0.01, 0.01 and 0.67); and Pb (0.04, 0.03 and 0.60). Upon chelant application, tissue Cd, Ni and Pb concentrations, as well as RB_fs and SB_fs increased quasilinearly and significantly ($p \leq 0.05$) with increase in chelant dose for most treatments; somewhat furnishing plateaux at very high chelant doses. For T_f, average values approximately decreased with increase in chelant dose. Except for a few treatments in which EDTA was present, root and shoot Cd, Ni and Pb concentrations did not correlate well with chelant doses. For all treatments, root Cd, Ni and Pb concentrations were higher than corresponding shoot concentrations. Maximum root metal concentrations were 55.6, 49.8 and 32.1 mg/kg for Cd, Ni and Pb, respectively. These concentrations were equivalent to 46.3-, 83.2-, and 12.8-fold increase, respectively relative to the natural phytoextraction scenario. Shoots concentrations were 20.9, 15.1 and 12.1 mg/kg for Cd, Ni and, and Pb, respectively, representing 23-, 37.8, and 8.1-fold increase over natural phytoextraction. Depending on the nature of treatment, the concentrations of the three metals in the roots appeared to follow an approximate order: OA+EDTA>CA+EDTA>EDTA>CA+OA>OA>CA. In the shoot the order was CA+OA>OA>CA>OA+EDTA>CA+EDTA>EDTA. The transfer coefficients i.e., Cd (RB_f=0.09-0.66; SB_f=0.16-0.23; T_f=0.23-2.45); Ni (RB_f=0.09-0.28; SB_f=0.22-0.87; T_f=0.22-0.99); Pb (RB_f=0.08-0.56; SB_f=0.10-0.21; T_f=0.34-2.07) followed about the same sequence. The values of the transfer coefficients increased in the order Cd>Ni>Pb, suggesting that Cd was most available for plant uptake (Li *et al*. 2012).

It seemed that the presence of EDTA (whether as single application or binary combination with CA or OA) enhanced Cd, Ni and Pb accumulation in roots to a greater extent than in shoot.

This is probably due to the greater ability of EDTA to raise metal concentrations in soil solutions (do Nascimento *et al*. 2006, Cutright *et al*. 2010, Zhang *et al*. 2014), rendering them more available for absorption by the roots than does CA or OA. This superlative ability of EDTA can be explained on the basis of stoichiometry and equilibria of complex formation. EDTA is hexadentate; while oxalate and citrate are bidentate and tridentate, respectively (at the operating soil pH>5). For the divalent Cd, Cu, Ni, Pb, and Zn ions, the stoichiometric ratio of metal:EDTA in the normal octahedral complexes is always 1:1; whereas this ratio is 1:3 for the metal:oxalate and 1:2 for metal:citrate octahedral complexes. Therefore, in the presence of fixed pseudototal concentration of a given metal and chelant (single or binary) dose in soil, more of the metal:EDTA complex would be quantitatively formed, leading to greater metal solubilisation and enhanced absorption by *R.communis* roots than the metal:oxalate and metal:citrate complexes would do. This observation is also supported by the ranking of stability constants, log K for the respective metal complexes: [Cd-EDTA=16.46, Cu-EDTA=18.80, Ni-EDTA=18.62, Pb-EDTA=18.04, Zn-EDTA=16.50]>[Cd-OA=3.89, Cu-OA=6.30, Ni-OA=5.16, Pb-OA=6.50, Zn-OA=4.87]>[Cd-CA=3.74, Cu-CA=6.10, Ni-CA=4.80, Pb-CA=4.08, Zn-CA=4.50] (Smith and Martell 1976).

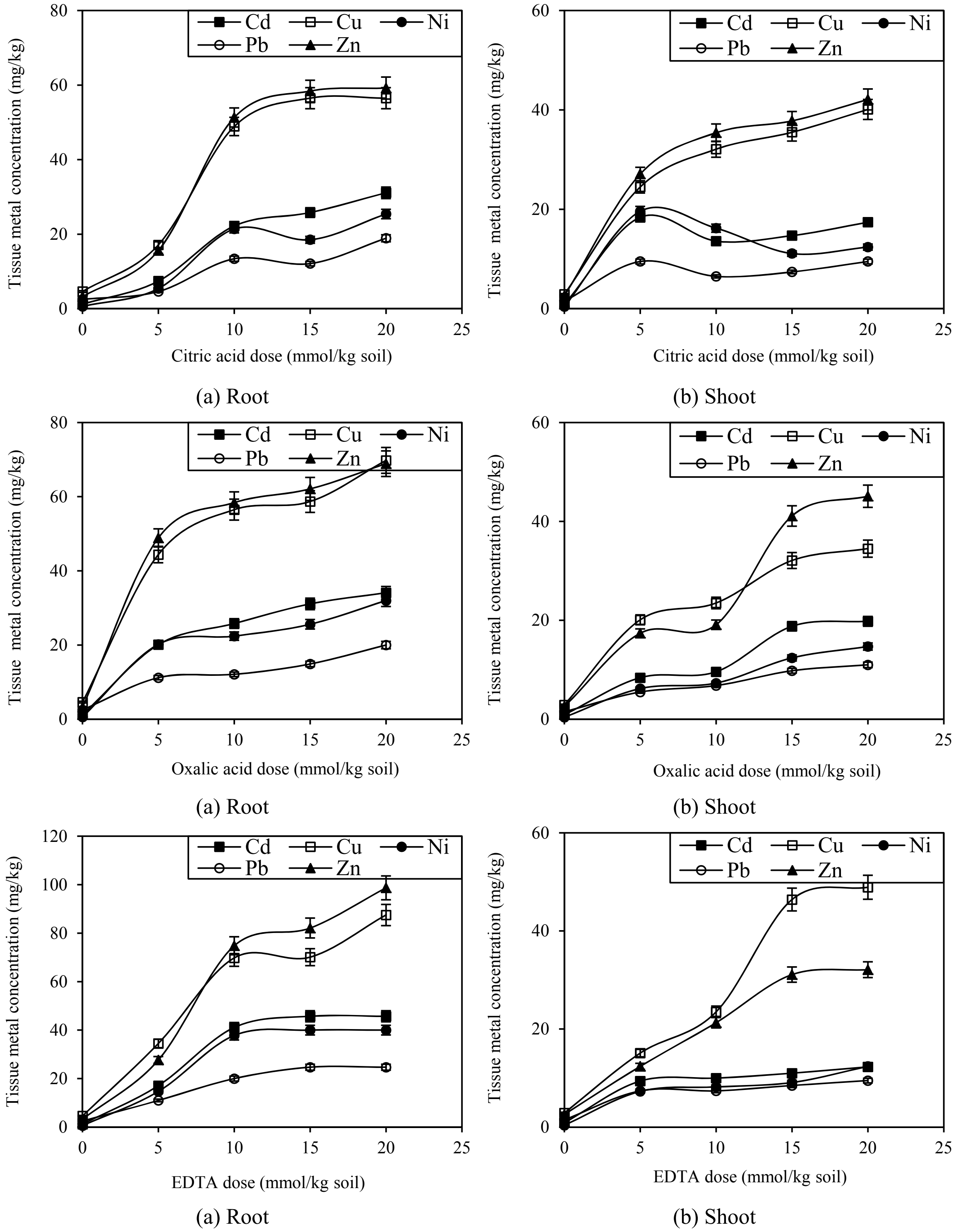
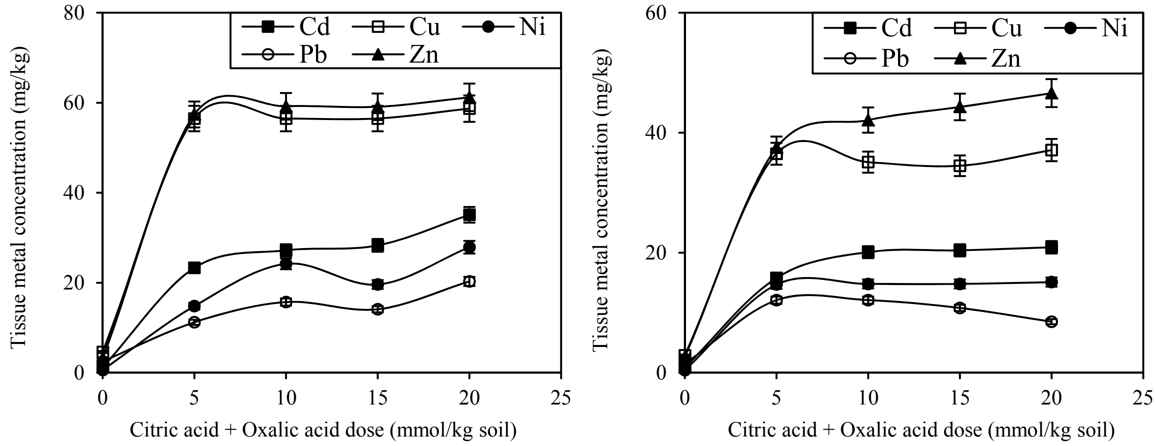
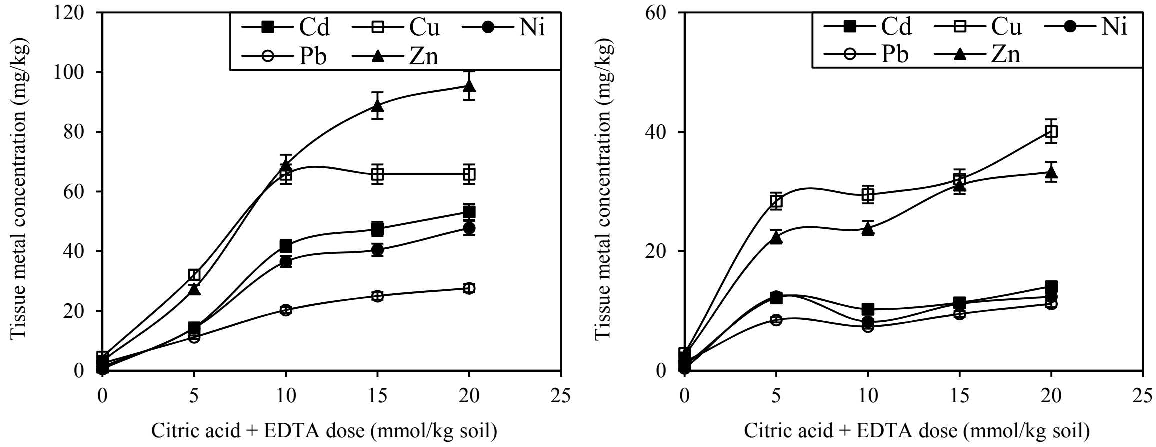


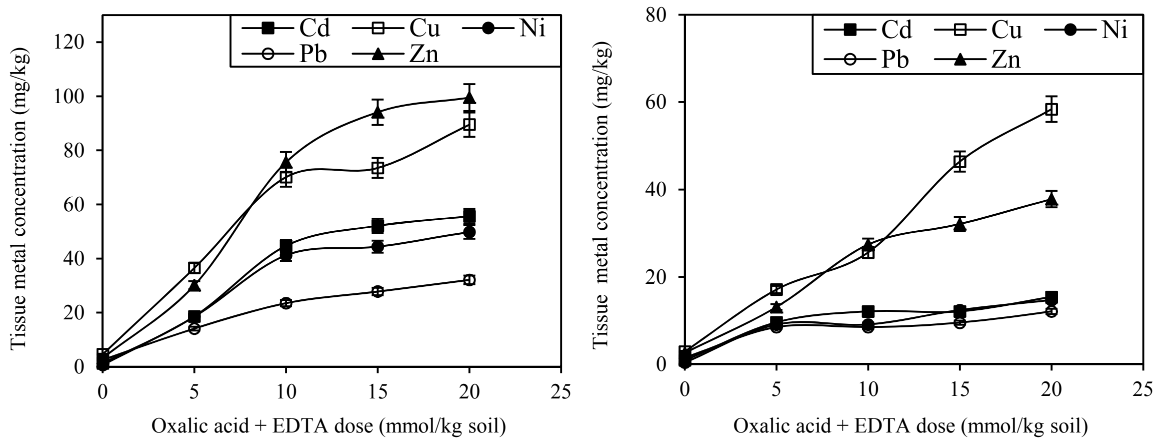
Fig. 4 Tissue (root and shoot) metal concentrations in *R.communis* grown (for 63 days) in waste dump soil under different doses of single chelant treatments



(a) Root (b) Shoot



(a) Root (b) Shoot



(a) Root (b) Shoot

Fig. 5 Tissue metal concentrations in *R.communis* grown (for 63 days) in waste dump soil under different doses of binary chelant treatments

Metal translocation from root to the shoot, on the other hand, depends on the size of metal-chelant coordination sphere. The metal-EDTA coordination sphere being larger than that of metal-CA or metal-OA, the latter can more easily overcome the various biophysical barriers across cell membranes and long distance root-to-shoot translocation (Emamverdian *et al*. 2015).

Angelova *et al*. (2016) have reported SB_f and T_f values >1 for Cd and Pb in *R. communis* under natural phytoextraction. SB_f and T_f values of 1.03 and 1.73 have also been reported for Pb in *R. communis* under EDTA treatment or citric acid at 5.10 mmol/kg (Miniño *et al*. 2014). Root and shoot metal concentrations, as well as calculated transfer coefficients, recorded in this study indicate that *R. communis* may not necessarily be an accumulator of Cd, Ni nor Pb. However, this primary colonizing plant can be well suited to cope with the local toxic soil conditions and can help to mitigate the bioavailability of these metals in contaminated soil (Oliveras *et al*. 2013). Owing to this ability, the plant could a useful candidate for revegetation of degraded lands because of the ability of these plants to absorb heavy metal from a contaminated soil (Jaya and Jayaputra 2014).

3.3.2 Copper and Zinc

R. communis was able to naturally phytoextract and accumulate 4.6 and 3.2 mg/kg of Cu and Zn, respectively in roots. Corresponding shoot concentrations were 2.9 and 2.6 mg/kg furnishing RB_f, SB_f and T_f, respectively for Cu as 0.75, 0.03 and 0.03; and for Zn as 0.02, 0.03 and 0.81 respectively. For both Cu and Zn, chelant application raised those parameters quasilinearly and significantly ($p \leq 0.05$) with increase in chelant dose for all treatments; except T_fs for which, a converse trend was observed. Cu and Zn essentially exhibited similar profiles for tissue metal-chelant dose plots and for most treatments; tissue metal concentrations were well correlated with chelant doses. Root Cu (17.1-89.5 mg/kg) and Zn (15.6-99.5 mg/kg) were greater than corresponding shoot concentrations (15.1-58.4 mg/kg) and (12.4-46.6 mg/kg**)**. Since these tissue metal concentrations were recorded for *R. communis* grown for approximately two months, it is expected that the plant will be able to extract and accumulate higher amounts of the metals if allowed to stay longer absorbing in the soil (do Nascimento *et al*. 2006). Depending on the nature of treatment, tissue Cu and Zn concentrations and transfer coefficients were enhanced in an approximate order: OA+EDTA>CA+EDTA>EDTA>OA>CA+OA>CA.

The presence of EDTA appeared to enhance Cu and Zn accumulation in roots to a greater extent than in shoot. The differential in the size of metal-complex coordination sphere for EDTA, CA and OA can be adduced as a plausible reason for this observation as is the case with Cd, Ni and Pb uptake. Overall, transfer coefficients indicated that *R. communis* accumulated Zn (RB_f=0.13-0.85; SB_f=0.15-0.40; T_f=0.33-1.00) to an extent comparable to Cu (RB_f=0.15-0.78; SB_f=0.21-0.51; T_f=0.34-0.88). This may be due to the comparable radii of the divalent ions (Cu=0.72 Å; Zn=0.74 Å) and log K values (Cu-EDTA=18.80; Zn-EDTA=16.50).

4. Conclusions

The study has demonstrated that CA, OA and EDTA treatments enhanced *R. communis* biomass in the waste dump soil. Tissue (root and shoot) heavy metal concentrations (mg/kg) increased quasilinearly and significantly with increase in chelant dose, furnishing maximum levels as: Cd (55.6 and 20.9), Cu (89.5 and 58.4), Ni (49.8 and 19.6), Pb (32.1 and 12.1), and Zn (99.5 and 46.6). Ranges of root bioaccumulation factors, shoot bioaccumulation factors and root-to-shoot translocation factors were 0.01-0.89, 0.01-0.51, and 0.21-3.49, respectively. Overall, the binary chelant treatments were less toxic for *R. communis* growth and enhanced metal accumulation in shoots to a greater extent than the single chelant scenarios, but more so when EDTA was present in the binary combination. This suggests that the mixed chelants could be considered as alternative treatments for enhanced phytoextraction and revegetation of degraded waste dump soils.

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References

Abdus-Salam, N. (2009), "Assessment of heavy metals pollution in dumpsites in Ilorin metropolis", *Ethiopian J. Envir. Stud. Manage.*, **2**(2), 92-99.

Abenchi, E.S., Okunola, O.J., Zubairu, S.M.J., Usman, A.A. and Apene, E. (2010), "Evaluation of heavy metals in roadside soils of major streets in Jos Metropolis", *Niger. J. Envir. Chem. Ecotoxicol.*, **2**(6), 98-102.

Afangideh, A.I., Njar, G.N., Ewa, E.E., Eli, H.D. and Iwarra, A.I. (2011), "Assessment of water quality status of borehole in calabar south local government area, Cross River State", *Int. J. Biosci.*, **1**(5), 71-76.

Ali, H., Khan, E. and Sajad, M.A. (2013), "Phytoremediation of heavy metals-Concepts and applications", *Chemosphere*, **91**(7), 869-881.

Angelova, V., Perifanova-Nemska, M. and Ivanov, K. (2016), "Potential of Castor Bean (*Ricinus communis* L.) for phytoremediation of soils contaminated with heavy metals", *Int. J. Envir. Ecol. Engr.*, **3**(5), 5-10.

Bosiacki, M., Kleiber, T. and Kaczmarek, J. (2013), "Evaluation of suitability of *Amaranthus caudatus* L. and *Ricinus communis* L. in phytoextraction of cadmium and lead from contaminated substrates", *Archiv.Envir.Protec.*, **39**(3), 47-59.

Canadian Council of Ministers of the Environment, CCME (2007), *Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health*, Publication No. 1299. Available at http://www.ccme.ca.

Chatzistathis, T. and Therios, I. (2013), "How soil nutrient availability influences plant biomass and how biomass stimulation alleviates heavy metal toxicity in soils: The cases of nutrient use efficient genotypes and phytoremediators, respectively", Eds., Miodrag, Darko, Matovic, Biomass Now-Cultivation and Utilization, doi: dx.doi.org/10.5772/53594.

Cutright, T., Gunda, N. and Kurt, F. (2010), "Simultaneous hyperaccumulation of multiple heavy metals by *Helianthus Annuus* grown in a contaminated sandy-loam soil", *Int. J. Phytorem.*, **12**(6), 562-573.

do Nascimento, C.W.A., Amarasiriwardena, D. and Xing, B.S. (2006), "Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil", *Envir. Pollut.*, **140**(1), 114-123.

Ekwere, A.S., Ekwere, S.J., Ephraim, B.E. and Ugbaja, A.N. (2014), "Distribution of heavy metals in urban soils; a case study of Calabar area, south-eastern Nigeria", *Geosciences*, **4**(1), 23-28.

Elaigwu, S.E., Ajibola, V.O. and Folaranmi, F.M. (2007), "Studies on the impact of municipal waste dumps on surrounding soil and air quality of cities in northern Nigeria", *J. Appl. Sci.*, **7**(3), 421-425.

Emamverdian, A., Ding, Y., Mokhberdoran, F. and Xie, Y. (2015), "Heavy metal stress and some mechanisms of plant defense response", *Scientific World J.*, 1-18.

Fawzy, E.M. (2008), "Soil remediation using in-situ immobilization techniques", *Chem. Ecol.*, **24**(2), 147-156.

Gisbert, C., Ros, R., de Haro, A., Walker, D.J., Pilar-Bernal, M., Serrano, R. and Avino, J.N. (2003), "A plant genetically modified that accumulates Pb is especially promising for phytoremediation", *Biochem. Biophys. Res. Commun.*, **303**(2), 440-445.

Gismera, M.J., Lacal, J., da Silver, P., Garcia, R., Sevilla, M.T. and Procopio, J.R. (2004), "Study of metal fractionation in river sediments. A comparison between kinetic and sequential extraction procedures", *Environ. Pollut.*, **127**(2), 175-182.

Gunawardana, B., Singhal, N. and Johnson, A. (2011), "Effects of amendments on copper, cadmium, and lead phytoextraction by *Lolium perenne* from multiple-metal contaminated solution", *Int. J. Phytorem.*, **13**(3), 215-232.

Hakanson, L. (1980), "An ecological risk index aquatic pollution control. A sedimentological approach", *Water Res.*, **14**(8), 975-1001.

Hope, B.K. (2006), "An examination of ecological risk assessment and management practices", *Envir. Int.*, **32**(8), 983-995.

Huang, H., Yu, N., Wang, L., Gupta, D.K., He, Z., Wang, K., Zhu, Z., Yan, Y., Li, T. and Yang, X. (2011), "The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil", *Bioresour. Technol.*, **102**(23),11034-11038.

Jadia, C.D. and Fulekar, M.H. (2008), "Phytotoxicity and remediation of heavy metals by fibrous root grass (sorghum)", *J. Appl. Biosci.*, **10**(1), 491-499.

Jaya, I.K.D. and Jayaputra, N. (2014), "The potential of intercropping food crops and energy crop to improve productivity of a degraded agriculture land in arid tropics", *J. Degraded Mining Lands Manage.*, **1**(3), 111-116.

Joshi M., Waghmare S., Chougule P. and Kanase, A. (2004), "Extract of *Ricinus communis* leaves mediated alterations in liver and kidney functions against single dose of CCL", *J. Ecophysiol. Occupation. Hlth.*, **4**(34), 169-173.

Karczewska, A., Gałka, B., Kabała, C., Szopka, K., Kocan, K. and Dziamba, K. (2009), "Effects of various chelators on the uptake of Cu, Pb, Zn and Fe by maize and Indian mustard from silty loam soil polluted by the emissions from copper smelter", *Frenesius Envir. Bull.*, **18**(10a), 1967-1974.

Kimenyu, P.N., Oyaro, N., Chacha, J.S. and Tsanuo, M.K. (2009), "The potential of *Commelina bengalensis*, *Amaranthus hybridus*, *Zea mays* for phytoremediation of heavy metals from contaminated soils", *Sains Malaysiana*, **38**(1), 61-68.

Kirpichtchikova, T.A., Manceau, A., Spadini, L. Panfili, F., Marcus, M.A. and Jacquet, T. (2006), "Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling", *Geochimica et Cosmochimica Acta*, **70**, 2163-2190.

Krishna, A.K. and Govil, P.K. (2007), "Soil contamination due to heavy metals from an industrial area of Surat, Gujarat, Western India", *Envir. Monit. Assess.*, **124**(1-3), 263-275.

Li, J., Baker, A.J.M., Ye, Z., Wang, H. and Shu, W. (2012), "Phytoextraction of Cd contaminated soils:current status and future challenges", *Critical Rev. Envir. Sci. Technol.*, **42**(20), 2113-2152.

Ling, W., Shen, Q., Gao, Y., Gu, X. and Yang, Z. (2007), "Use of bentonite to control the release of copper from contaminated soils", *Aus. J. Soil Res.*, **45**(8), 618-623.

Mahmood, T. (2010), "Phytoextraction of heavy metals - the process and scope for remediation of contaminated soils", *Soil Envir.* **29**(2), 91-109.

Mahmud, R., Inoue, N., Kasajima, S., Kat, M., Shaheenj, R., Miah, M.A.M. and Rahman, M.S. (2006), "Response of common buckwheat and castor oil plant against different levels of soil arsenic concentration: a comparative study", *Fagopyrum*, **23**, 45-51.

McLaughlin, M.J., Zarcinas, B.A., Stevens, D.P. and Cook, N. (2000), "Soil testing for heavy metals", *Commun. Soil Sci. Plant Anal.*, **31**(11-14), 1661-1700.

Miniño, H., Rendina, A., Barros, M.J., Bursztyn, A., de los Ríos, A., Wassner, D. and de Iorio, A.F. (2014), "Use of organic ligands in lead Phytoextraction by Castor bean (Ricinus communis L.)", *Augmdomus*, **6**, 66-80.

Nabulo, G., Oryem Origa, H., Nasinyama, G.W. and Cole, D. (2008), "Assessment of Zn, Cu, Pb and Ni contamination in wetland soils and plants in the Lake Victoria basin", *Int. J. Envir. Sci. Tech.*, **5**(1), 65-74.

Obasi, N.A., Akubugwo, E.I., Ugbogu, O.C. and Otuchristian, G. (2012), "Assessment of physico-chemical properties and heavy metals bioavailability in dumpsites along Enugu-Port Harcourt expressways, south-east Nigeria", *Asian J. Appl. Sci.*, **5**(6), 342-356.

Olivares, A.R., Carrillo-González, R., González-Chávez, M.D.A. and Hernández, R.M. S. (2013), "Potential of castor bean (*Ricinus communis* L.) for phytoremediation of mine tailings and oil production", *J. Envir. Manage.*, **114**, 316-323.

Padmavathiamma, P.K. and Li, L.Y. (2007), "Phytoremediation technology: Hyper-accumulation metals in plants", *Water Air Soil Pollut.*, **184**(1-4), 105-126.

Pedron, F. and Petruzzelli, G. (2011), "Green remediation strategies to improve the quality of contaminated soils", *Chem. Ecol.*, **27**, 89-95.

Pooja S.J., Namita G., Anil, K.M. and Karuna, S. (2014), "New anti-inflammatory triterpene from the root of Ricinus communis", *Natural Product Res.*, **28**(5), 306-311.

Rajkumar, M. and Freitas, H. (2008), "Influence of metal resistant-plant growth promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals", *Chemosphere*, **71**(5), 834-842.

Reddy, K.R. and Matcha, S.K. (2010), "Quantifying nitrogen effects on castor bean (*Ricinus communis* L.) development, growth and photosynthesis", *Indus. Crops Prod.*, **31**(1), 185-191.

Reichman, S.M. (2002), "The responses of plants to metal toxicity: a reviewfocusing on copper, manganese and zinc", *Australian Min. Energy Envir. Found.*, **14**, 1-14.

Salihu, B.Z., Gana, A.K. and Apuyor, B.O. (2014), "Castor Oil Plant (*Ricinus communis* L.): botany, ecology and uses", *Int. J. Sci. Res.*, **3**(5), 1333-1341.

Saraswat, S. and Rai, J.P.N. (2009), "Phytoextraction potential of six plant species grown in multimetal contaminated soil", *Chem Ecol.*, **25**(1), 1-11.

Scragg, A. (2006), *Environmental Biotechnology* (2nd edition), Oxford University Press, Oxford, UK.

Shi, G.R. and Cai, Q.S. (2009), "Cadmium tolerance and accumulation in eight potential energy crops", *Biotechnol. Adv.*, **27**(5), 555-561.

Smith, R.M. and Martell, A.E. (1976), *Critical Stability Constants*, Vol. 1-4, Plenum Press, New York.

Vamerali, T., Bandiera, M. and Mosca, G. (2010), "Field crops for phytoremediation of metal-contaminated land-A review", *Envir. Chem. Lett.*, **8**(1), 1-17.

Vanaja, M., Jyothi, M., Ratnakumar, P., Vagheera, P., Reddy, P.R., Lakshmi, N.J., Yadav, S.K., Maheshwari, M. and Venkateswarlu, B. (2008), "Growth and yield responses of castor bean (*Ricinus communis* L.) to two enhanced CO_2 levels", *Plant Soil Envir.*, **54**(1), 38-46.

Warra, A.A. (2015), "Castor seed oil and its potential cosmetic and pharmaceutical applications, achieves of scientific research", **1**(1), 17-22.

Worbs, S., Köhler, K., Pauly, D., Avondet, M., Schaer, M., Dorner, M.B. and Dorner, B.G. (2011), "*Ricinus communis* intoxications in human and veterinary medicine-A summary of real cases", *Toxins*, **3**(10), 1332-1372.

Wuana, R.A, Adie, P.A. and Asegh, I.N. (2012), "Seasonal variation in bioavailability of some toxic metals in waste dump soils of Makurdi, North-Central Nigeria", *J. Biodivers. Envir. Sci.*, **2**(11), 7-17.

Wuana, R.A. and Mbasugh, P.A. (2013), "Response of roselle *(Hibiscus sabdariffa)* to heavy metals contamination in soils with different organic fertilizations", *Chem. Ecol.*, **29**(5), 437-447.

Wuana, R.A. and Okieimen, F.E. (2011), "Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation", *ISRN Ecology*, 1-20.

Zhang, H., Guo, Q., Yang, J., Chen, T., Zhu,G., Peters, M., Wei, R., Tian, L., Wang, C., Tan, D., Ma, J., Wang, G. and Wan, Y. (2014), "Cadmium accumulation and tolerance of two castor cultivars in relation to antioxidant systems", *J. Envir. Sci.*, **26**(10), 2048-2055.

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