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PHYTOEXTRACTION OF HEAVY METALS BY *Ricinus communis* IN SOIL AMENDED WITH CHELANTS AND POULTRY MANURE

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Received: April 15, 2019 **Accepted:** August 17, 2019

Abstract: Pot experiments were designed to investigate the response of *R. communis* to heavy metals phytoextraction tested under single or mixed chelants and poultry manure assisted scenarios. This was achieved when a moderately contaminated sandy loamy soil was stressed with a 500 mgkg^{-1} conjointly metal (Cd, Cu, Pb), blended with; single or mixed chelant (EDTA and oxalic acid) and poultry manure. After growth, the maximum heights and leaf breadths of *R. communis* were; 5.01-69.0 and 1.90-38.4 cm, with plants typically luxuriant and greenish. pH, bulk density, electric conductivity, organic carbon, of the parent soil were determined. The results revealed that pH (6.21), (9.30 cmol Kg^{-1}), BD (1210 kg/m^3), OM (8.63 %) were found in the parent soil used. Pseudo-total metal concentration (mgkg^{-1}) of the metals (Cd, Cu and Pb) were; 10.2, 8.93 and 5.22, respectively. Maximum tissue metal concentrations in the various potting media were: soil-metal-PM-EDTA+OX (436.0-490.6 mg Cd/kg; 453.2-475.5 mg Cu/kg; 452.3-469.8 mg Pb/kg. Maximum values for Cd, Cu and Pb root bioaccumulation factor (RB$_f$), shoot bioaccumulation factor (SB$_f$), and translocation factor (T$_f$) were; 0.68, 0.29 and 0.42; 0.67, 0.28 and 0.42; and 0.66, 0.27 and 0.41, respectively. Pot experiments revealed high metal transferabilities with no apparent phytotoxic symptoms in *R. communis* at the doses applied, suggesting some degree of tolerance to the metals. 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.

Keywords: Heavy metals, *Ricinus communis*, phytoextraction, poultry manure, chelants

Introduction

The pollution of soil is a crucial matter that has attracted considerable public attention over the past few decades (Ahmadpour *et al*., 2012). This is because pollution is a threat for the survival of mankind and the most important dispute of our era (Wang *et al.*, 2004). Geological and anthropogenic activities are sources of heavy metal contamination. Sources of anthropogenic metal contamination include industrial effluents, fuel production, mining, smelting processes, military operations, small-scale industries (including battery production, metal products, metal smelting and cable coating industries), brick kilns and coal combustion (Dembitsky, 2003). 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).

The mobilization of heavy metals by man through extraction from ores, and also the disposal of municipal sewage and agricultural practices have led to the release of these elements into the environment.

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). Phytoextraction of metals offers benefits such as; decontamination of land, exploitation of more widely-dispersed resources of surface metals and recovery of metals from ore bodies with low metal concentration. Phytoextraction has emerged as a promising, cost-effective alternative to theconventional engineering-based remediation methods.It is a relatively recent technology and is perceived efficient, novel, eco-friendly, and solar-driven technology with good public acceptance. It is a method which, plants are used to remove metals from soils, transport and concentrate them in above-ground biomass (Padmavathiamma and Loretta 2007). Phytoremediation is an area of active current research. New efficient metal hyperaccumulators are being explored for applications in phytoremediation and phytomining. Plants to be used for phytoextraction should have: (a) tolerance to high concentrations metals, (b) high metal-accumulation capability, (c) heavy biomass, (d) ability to grow fast and a (e) profuse root system (f) Tolerance to the toxic effects of the target heavy metals. (g) Resistance to pathogens and pests. (h) Repulsion to herbivores to avoid food chain contamination. The success of phytoextraction depends especially on the plant's ability (a) to accumulate biomass rapidly, and (b) to store large quantities of the up taken metals in the shoot tissue (McGrath, 1998; Blaylock and Huang, 2000)

Chelate-enhanced, phytoextraction is based on the fact that the application of organic and inorganic chelating agents to the soil to significantly enhance metal accumulation by plants (Garbisu and Alkorta, 2001). The formation of chelates prevents precipitation and sorption of the metals, thereby maintaining their availability for plant uptake (Salt *et al.*, 1995). 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). The solubilization of heavy metals through inorganic agents relies mainly on desorption (Bru¨mmer *et al.*, 1986). Heavy metal solubility in soils is mainly controlled by the soil reaction (pH), the amount and kind of sorption sites, and the total amount of heavy metals in the soil (Hornburg and Bru¨mmer, 1993; Gray *et al.*, 1999).

Castor (*Ricinus communis* L.) is one of the oldest cultivated oil crops in the world (Ruwanthi, 2012). The castor oil plant (*Ricinus communis*) is a species of flowering plant in the spurge family, Euphorbiaceae. Castor bean (*Ricinus communis* L.) has been extensively exploited for their heavy metal tolerance and phytoremediation potential (Adhikari and Kumar, 2012). 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 (Rajkumar and Freitas, 2008; Shi and Cai, 2009; Huang *et al*., 2011; Miniño *et al.,* 2014; Zhang *et al*., 2014; Wuana *et al.,* 2016).

The present study was aimed at assessing the growth potential and response of *Ricinus communis* under the influence of poultry manure and chelants (single and binary) in soils with Cd, Cu and Pb.

Materials and Methods

Chemicals and apparatus used for the study included cadmium nitrate, coppers sulphate, lead nitrate, oxalic acid and disodium salt of ethylenediaminetetraacetatic acid. Allorganic chelants used were of Sigma-Aldrich patent. Atomic absorption spectrophotometer (Buck Scientific Model 2006A, Norwalk, Connecticut, US).

Description of study area

This study was carried out on a parent soil sample collected from the extended Judges' quarters layout sited in Makurdi, north-central of Nigeria. It is located at 7^o 48'N and longitude 8^o 37' E in the lower Benue River Basin, Annual rainfall is 1200–1650 mm distributed between March/April and October/November, followed by a marked dry season (of up to 4 months). The ranges of daily maximum and minimum temperatures are 30–34^{o}C and 22–24^{o}C, respectively, during the rainy season and 33–37^{o}C and 18–24^{o}C, respectively, in the dry season. Daily global irradiation and mean hours of insulation are, respectively, 314–433 cal cm^2 day$^{–1}$ and 4.00–7.74 h (Sha'Ato *et al*., 2002).

Soil sampling, characterization, artificial contamination and treatment with chelant and/or manure

In this study five surface (0-20 cm) soil samples were randomly collected using a chrome-plated trowel. The soil samples were air dried, ground, sieved to <2-mm particle size, composited, and stored in polythene bags as the parent soil (PS). A composite sample of poultry manure (PM) collected from a poultry farm in Makurdi and was kept for two weeks to age. The physicochemical properties of the parent soil and the raw poultry manure used as an amendment were determined as described by Wuana *et al*. (2012). Pseudototal Cd, Cu, and Pb contents were determined by digestion with aqua regia (HCl–HNO_3) which was subsequently followed by metal assay using AAS.

In order to study the effect(s) of metal dose on *R. communis* growth, the parent soil was spiked with a solution (500 mg/kg) conjointly dose of Cd, Cu, and Pb and a poultry manure (15% w/w). A solution of 100 mL increasing doses (mmol chelant/kg soil): 0, 5, 10, 15 of disodium salt of EDTA and/or sodium oxalate solutions was added over a thin layer the 5.0-kg portions of the metal-manure-spiked soil, to furnish a soil-metal-poultry manure-EDTA, soil-metal-poultry manure-sodium oxalate and, soil-metal-poultry manure-EDTA and sodium oxalate potting media. Each treatment was performed in triplicate and incubated for 2 weeks at ambient temperature to simulate field conditions. Sub-samples of the amended soils were digested and pseudototal Cd, Cu, and Pb contents determined by AAS analysis.

Pot experiments with R. commmunis

Three seeds of *R. communis*, previously cold treated (10^{o}C) for 12 h according to Wuana *et al*. (2013) to break dormancy, synchronize germination prior to pot experiments (Revathi *et al.,* 2010). Pots were placed in a completely randomized design. Five seeds were sown in each pot (volume = 5000 mL) of 5.0 kg soil and a week after germination, the seedlings were thinned to three. Surface irrigation with deionized water was employed to water the plants during growth and no fertilizers were applied. Night and day sequence was naturally obtained by maintaining the pots in an open area. The plants were monitored for twelve (12) weeks for changes in appearance (colour), height, and leaf breadth. Prior to harvest, plants were left without watering for one (1) day. They plants were carefully uprootedfrom the soils after the 90th day, separated into roots and shoots, rinsed with deionised water, and dried at 110^{o}C for 72 h. The concentrations of Cd, Cu and Pb (mg kg^{-1}dw of *R. communis* biomass) was determined by HNO_3–H_2O_2 digestion (Nolan *et al*., 2005) followed by atomic absorption spectrophotometric measurements.

Plant biomass digestion and heavy assay in Ricinus communis

A one (1) gram dried and ground *R. communis* biomass sample was digested with a mixture of 4.0 mL of 65% v/v HNO_3 and 2.0 mL of 35% v/v H_2O_2. After evaporation, 4.0 mL of concentrated HNO_3 and 2.0 mL of concentrated H_2O_2 were added to the residue and heated until a clear digest appeared. The digestion time of 3 h at 130^{o}C was employed. The digest was made up to the 5.0 mL with 1.0 M HNO_3 (Wuana *et al.,* 2013). The concentrations for Cd, Cu, and Pb were then estimated using Atomic Absorption Spectrophotometer (AAS).

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 deionised water, soaked (1 + 1)HNO_3 overnight and finally rinsed with deionised water. Procedural blank samples were subjected to similar treatments using the same amounts of reagents. In all cases, measurements were performed in triplicate. Analysis of variance (ANOVA) was used to test differences for all investigated variables during the experiment between treatments and controls at 5% probability level ($p \leq 0.05$) by means of SPSS 19.0 (IBM statistics) package.

Results and Discussion

Physicochemical properties of the study soiland manures

Table 1 presents some physicochemical attributes of the parent soil; manure used for the study and the metal-stressed and manure-amended composite soil collected. The parent soil and raw poultry manure are slightly acidic pH (6.21 and 6.49), therefore mobility and bioavailability of metals may not be effectively enhanced (Alkorta *et al.,* 2004) as metal solubility of Cd, Cu and Pb tends to increase at lower pH and decrease at higher pH values (Rieuwerts *et al.,* 1998). The addition of manure is expected to play indifferently a major role either in bioavailability or immobilization process of the concentration of metals (Prasad *et al*., 2003). The bulk density, 1210 kg m$^{–3}$ suggested a good available water capacity, soil porosity and plant nutrient availability. Texturally, the percent of sand, silt and clay in the soil were 78.3, 13.7 and 8.33 %, patterning the soil as sandy loam.Soil organic matter and poultry manure values were 8.63 and 33.4%. Organic matter is a key for sorbing phase of metals and also plays a role in the retention of metals by soil solids, thus, decreasing mobility and bioavailability. Cation exchange capacity (CEC) is one of the important parameters in determining metal availability. The CEC for the soil and poultry manure were low (slightly saline) and this may enhance metal uptake. Therefore, organic amendments contained low levels of Cd, Cu and Pb which is suitable for use as ameliorants (Thomas and Dauda, 2015). Plant available Cd, Cu and Pb, in the soil were 7.20, 6.93 and 3.22 mg kg-1; while the pseudototal Cd, Cu and Pb contents, were 10.2, 8.93, and 5.22 mg kg^{-1} (Table 1). Values obtained were lower than their corresponding upper critical levels, defined as the range of values above which toxicity is considered to be possible (Maiz *et al*., 2000; Wuana *et al*., 2012) indicating that the parent soil was relatively uncontaminated in terms of Cd, Cu and Pb. Pseudototal Cd, Cu and Pb in poultry were 10.6, 2.40 and 40.3 mg kg$^{–1}$, respectively. Manure amendments decreased the plant available Cd, Cu and Pb in the soil indicating the ability of the manures to bind the metals in the soil. Manure could sequester heavy metals and enhance fixation and immobilization in soil is recommended provided it has been allowed to age sufficiently. Therefore, soil pH, bulk density coupled with the texture suggests the potential leachability of soil heavy metals to lower profiles which makes it suitable for plant metal extraction (Wuana *et al*., 2016).

Table 1: Selected physicochemical properties of parent soil and poultry manure

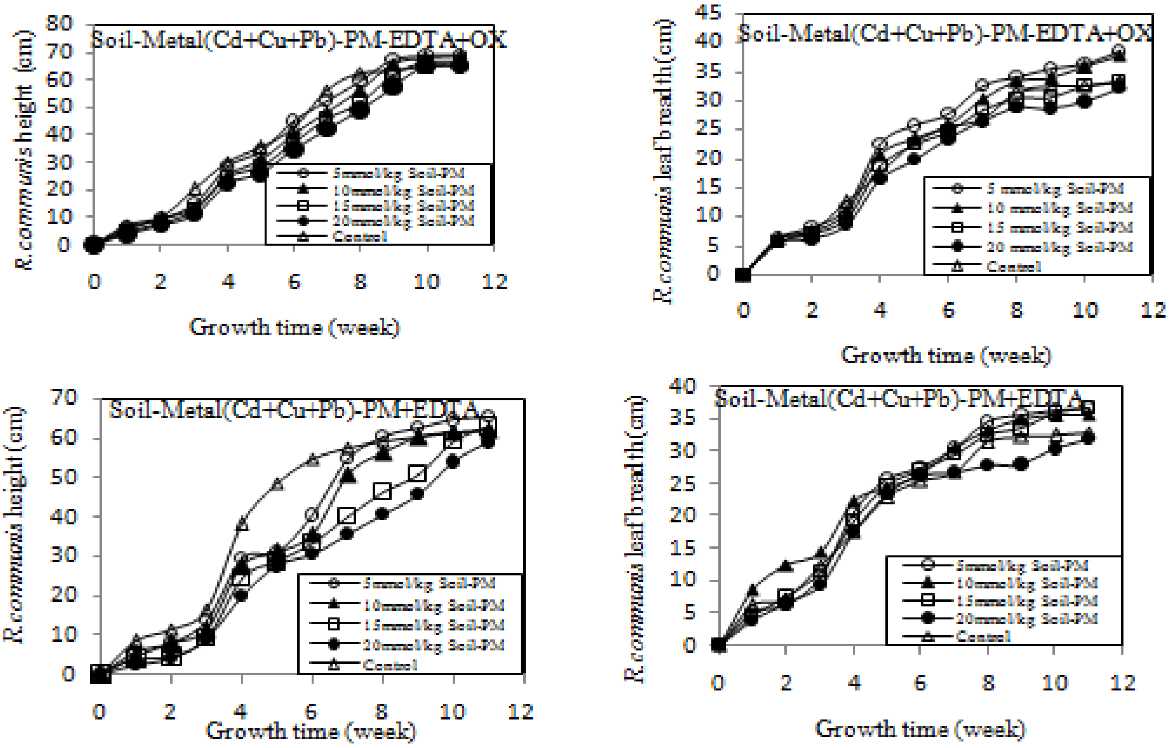
Property	Parent soil	Poultry manure
pH$_{(s)}$	6.21 ± 0.01	6.49±0.02
Organic Matter (%)	8.63 ± 0.03	33.4±0.21
Soil Bulk Density (kg/m^3)	1210 ± 1.00	
Cation exchange capacity(cmol Kg^{-1})	9.30 ± 0.01	
Sand (%)	78.3 ± 0.15	
Silt (%)	13.7 ± 0.03	
Clay (%)	8.33 ± 0.44	
Textural classification	Sandy loam	
Available metals (mg kg^{-1})		
Cd	7.20 ± 0.65	6.6 ±0.53
Cu	6.93 ±0.11	0.89±0.29
Pb	3.22 ±0.31	28.3±0.44
Pseudo-total metal concentration (mg kg^{-1})		
Cd	10.2±0.12	10.6±0.22
Cu	8.93 ±0.50	2.40±0.41
Pb	5.22 ±0.20	40.3±0.01

*Mean of triplicate determinations ± standard deviation

Growth attributes of Ricinus communis at increasing doses of chelants

In this study, changes in the growth attributes (heights and leaf breadths), were investigated by pot experiments in soil stressed with Cd, Cu and Pb with increasing doses of chelants (EDTA and/or OX) with poultry manure treatments.

R. communis plants were monitored weekly for changes in height and leaf breadth within the time stretch of 12 weeks and results illustrated in Fig. 1. At the elapse of the 90-day pot study, Soil-Metal-PM-EDTA+OX of *R. communis* height and leaf breadth were 6.23-69.0 and 4.31-38.4 cm, while that of Soil-Metal-PM-EDTA and Soil-Metal-PM+OX were; 5.91-66.5 and 4.89-36.9 cm; 5.23-65.7 and 3.96-34.4 cm. The plants were typically greenish and luxuriant with essentially sigmoid growth profiles; 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 Soil-Metal-PM-EDTA+OX treatments (Fig. 1). Chelant dose is seen to affect the growth rate 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).In all, the binary treatment of Soil-Metal-PM-EDTA+OX media recorded the greatest heights and leaf breadths. This could be as the result of the combine effect of chelants (EDTA and OX) where EDTA, with a large coordinating sphere complex would be quantitatively formed, leading to greater metal solubilisation and increased nutrient availability for *R. communis* (Wuana *et al*., 2016), while the oxalic acid solubilizes metal, (Mench and Martin, 1991; Nigam *et al.*, 2001) and mobilizes mineral nutrients (Zhang *et al.*, 1989; Jones *et al.*, 1996) thereby influencing the plant growth (Reddy and Matcha, 2010; Chatzistathis and Therios, 2013). Interestingly, poultry manure enhanced the growth attributes of *R. communis*, thus, justifying its frequent land application by farmers. 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; Wuana *et al*., 2016). Changes in the growth parameters with time were statistically significant (p<0.05) for all the treatments. The growth profile correlated with chelant dose and the amendment type. Growth was in the order of Soil-Metal-PM-EDTA+OX> Soil-Metal-PM-EDTA> Soil-Metal-PM-OX. Observed differences in *R. communis* heights and leaf breadths were significant (p<0.05) within and between individual potting media. However, a visual assessment of *R. communis* in the study did not show signs of phytotoxic symptoms which could alter photosynthetic order with a consequent effect on stomata or mesophyll cells in which both photochemical and biochemical reactions could be affected (Kosobrukhov *et al*., 2004).



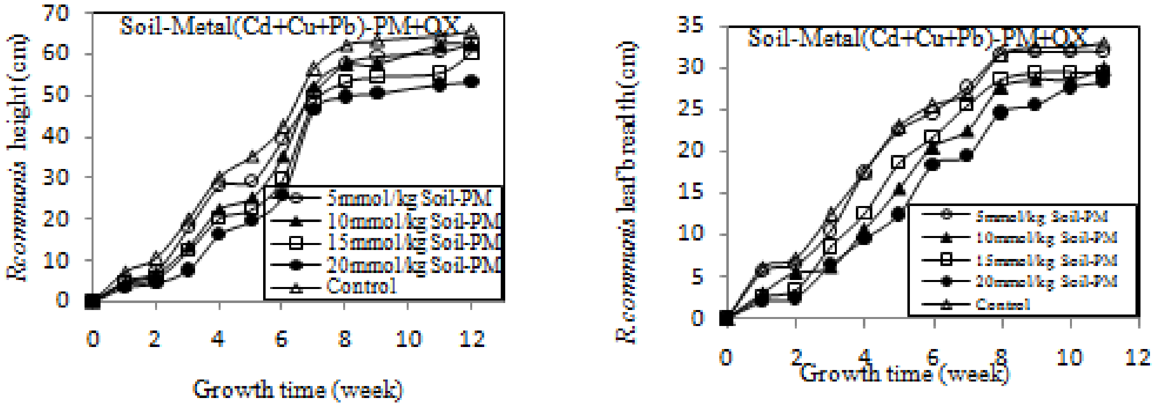


Fig. 1: Growth profile of *R. communis* in metal-poultry manure-chelant(s) amended soil

Table 2: Heavy-metal concentration in *R. communis* biomass, Q_P (mg kg^{-1}); soil total metal, Q_T (mg kg^{-1}); plant-available metals (calcium chloride–extracted), Q_e (mg kg^{-1}); pH and OM (%) of poultry manure amended soil treated with different doses of chelants

Amendment Type	Dose (mmolkg^{-1})	Q_P	Q_e	Q_T	pH	OM
Cd-PM EDTA+OX	5	436.0±0.41	480.1±0.31	525.3±0.33	5.80±0.12	8.71±0.14
	10	455.7±0.21	480.5±0.91	525.3±0.21	5.70±0.30	11.5±0.22
	15	477.9±0.11	481.3±0.17	518.8±0.19	5.51±0.15	13.80±0.34
	20	490.6±0.31	483.5±0.10	512.4±0.81	5.40±0.00	14.0±0.25
Cu-PM EDTA+OX	5	453.2±0.07	473.4±0.38	508.4±0.32	6.33±0.10	9.32±0.14
	10	461.7±0.54	478.1±0.11	511.7±0.11	6.38±0.34	10.7±0.30
	15	470.4±0.24	480.0±0.12	511.1±0.52	5.82±0.41	12.2±0.54
	20	475.5±0.23	480.5±0.08	502.7±0.45	5.79±0.32	13.9±0.00
Pb-PM EDTA+OX	5	452.3±0.56	471.2±0.40	522.8±0.22	6.27±0.00	8.14±0.42
	10	459.1±0.31	471.3±0.64	513.6±0.14	5.87±0.19	8.14±0.30
	15	461.9±0.41	480.5±0.66	505.9±0.36	6.01±0.20	11.2±0.11
	20	469.8±0.50	495.8±0.50	511.3±0.24	5.77±0.34	12.8±0.31
Cd-PM EDTA	5	409.1±0.14	455.2±0.54	530.7±0.18	6.59±0.31	10.2±0.21
	10	416.9±0.31	458.6±0.36	524.6±0.10	6.50±0.14	12.5±0.22
	15	427.3±0.40	457.3±0.64	516.2±0.11	6.35±0.34	12.9±0.40
	20	448.6±0.12	460.1±0.20	510.2±0.23	6.00±0.11	13.7±0.35
Cu-PM EDTA	5	312.6±0.41	461.2±0.66	529.6±0.23	6.80±0.12	10.2±0.24
	10	335.9±0.51	468.3±0.85	530.4±0.11	6.72±0.31	10.4±0.14
	15	388.8±0.20	467.6±0.01	524.2±0.87	6.50±0.20	13.7±0.40
	20	415.0±0.00	470.0±0.22	519.2±0.31	6.45±0.42	15.6±0.31
Pb-PM EDTA	5	268.6±0.60	471.2±0.64	557.7±0.21	6.82±0.45	9.88±0.14
	10	313.1±0.31	473.8±0.48	549.6±0.34	6.74±0.10	10.0±0.00
	15	374.1±0.41	475.2±0.51	539.9±0.40	6.21±0.25	13.2±0.21
	20	414.1±0.71	473.8±0.12	531.1±0.12	5.91±0.14	15.2±0.13
Cd-PM OX	5	283.5±0.04	480.1±0.34	592.9±0.54	6.99±0.41	8.4±0.33
	10	310.8±0.42	471.2±0.40	581.6±0.71	6.89±0.30	10.6±0.25
	15	356.3±0.71	473.0±0.22	580.0±0.25	6.62±0.13	12.5±0.44
	20	396.6±0.31	471.7±0.60	546.0±0.55	6.30±0.22	13.5±0.20
Cu-PM OX	5	283.5±0.26	461.2±0.24	569.7±0.18	6.72±0.10	10.2±0.42
	10	312.6±0.11	465.8±0.61	568.2±0.70	6.70±0.34	9.1±0.34
	15	345.2±0.51	466.0±0.21	566.8±0.82	6.66±0.25	11.5±0.11
	20	364.6±0.64	465.2±0.68	535.4±0.34	6.41±0.00	12.8±0.20
Pb-PM OX	5	279.9±0.30	471.2±0.42	588.6±0.41	6.24±0.12	11.2±0.14
	10	320.3±0.52	473.4±0.66	583.8±0.64	6.55±0.21	10.1±0.42
	15	361.2±0.00	471.0±0.14	577.4±0.31	5.98±0.11	12.1±0.32
	20	388.6±0.42	473.8±0.51	589.2±0.42	6.89±0.33	15.0±0.15

Heavy metal accumulation by Ricinus communis

The phytoavailability of Cd, Cu and Pb to *R. communis* in response to manure and increasing levels of chelants amendment in soil was evaluated. A measure of Cd, Cu and Pb transferability to *R. communis* were calculated using the following relationship;

$$MAF=\frac{Metal\ concentration\ in\ R.\ communis\ tissue}{Pseudototal\ metal\ concentration\ in\ soil} \quad (1)$$

$$RB_f=\frac{Metal\ concentration\ in\ root\ of\ R.communis\ tissue}{Pseudototal\ metal\ concentration\ in\ soil} \quad (2)$$

$$SB_f=\frac{Metal\ concentration\ in\ shoot\ of\ R.communis\ tissue}{Pseudototal\ metal\ concentration\ in\ soil} \quad (3)$$

$$T_f=\frac{Metal\ concentration\ in\ shoot\ of\ R.communis\ tissue}{Metal\ concentration\ in\ root\ of\ R.communis\ tissue} \quad (4)$$

(i) metal accumulation factor (MAF), (ii) root bioaccumulation factor (RB_f), (iii) shoot bioaccumulation factor (SB_f), and translocation factor (T_f).

Phytoextraction can be estimated by the rate of metal accumulation and biomass production (Liang, 2009). Under Soil-Metal-PM-EDTA+OXphytoextraction, mean tissue concentrations of Cd, Cu and Pb were; 467.5, 461.8, and 464.0 mg/kg. While their respective mean pseudototal metal concentration were; 478.0, 481.4, and 479.7 mg/kg. This, however,furnished a MAF of 0.97, 0.95, and 0.96 for Cd, Cu and Pb, respectively. The uptake of Cd by the *R. communis* was higher than that of Cu and Pb. This result is consistent with the result reported by Alloway (1995) where, in his submission, averred that Cd, because of the tendency for being more mobile in the soil, is more available for plants uptake than other metals, including Pb. The concentration cadmium uptake is likely mediated through transporters or channels for other divalent ions as reported by (Cosio *et al.*, 2004). The heavy metal cadmium was depleted from the contaminated soil, suggesting absorption of cadmium metal by plant.

Root mean metal concentration for Soil-Metal-PM-EDTA+OX amended by Cd, Cu and Pb were 326.1, 321.5 and Pb 318.5 mg/kg and were greater than their corresponding mean shoot concentrations 136.8, 134.1, and 132.2 mg/kg. This however, furnished a RB_f, SB_f and T_f of (0.68, 0.29 and 0.42); (0.67,0.28 and 0.42); and (0.66,0.27 and 0.41) for Cd, Cu and Pb respectively. Metal concentrations as well as RB_f, SB_f and T_f were observed to significantly ($p \leq 0.05$) increase with increasing chelant doses.

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). The bioaccumulation of Cd, Cu and Pb were different among treatments. The metal contents in the plant tissues varied. Reason envisioned might be a function of the physiological factor of the plant, metal level uptake from solution and the xylem translocation from root to the aerial. Observed differences between the potting media were, however, not significant ($p > 0.05$) for soil-metal-EDTA, soil-metal-OX scenarios. However, the metal concentrations in the tissue of plant appeared to follow an approximate order: EDTA+OX>EDTA>OX.

The concentrations (mg/kg) of Cd, Cu and Pb of *R. communis* grown in an array of Soil-Metal-PM-EDTA+OX, Soil-Metal-PM-EDTA, Soil-Metal-PM-OX perspective were also observed. In the Soil-Metal-PM-EDTA+OX potting scenario, tissue metal concentration varied from; 436.0-490.6 mgCdkg^{-1}, 453.2-475.5 mgCukg^{-1} and 452.3-469.8 mgPbkg^{-1}, respectively. For Soil-Metal-PM-EDTA, it varied from; 409.1-448.6 mgCdkg^{-1}, 312.5-415.5 mgCukg^{-1}, and 268.6-414.1 mgPbkg^{-1}. Whereas, for Soil-Metal-PM-OX, the tissue metal concentration varied from; 283.5-396.6 mgCdkg^{-1}, 283.5-364.6 mgCukg^{-1}, and 279.9 – 388.6 mgPbkg^{-1} (Table 2). *R. communis* showed distinct and higher accumulation at different chelant doses and of the amendment types. Tissue metal concentration is seen to be higher for binary chelant treatments than their corresponding single treatments. This could be probably due to the combined effect of the chelants as EDTA has the ability to raise metal concentrations in soil solutions (do Nascimento *et al.,* 2006; Zhang *et al*., 2014; Wuana *et al.,* 2016) and Low molecular-weight organic acids i.e. oxalic acid with its high influence in the acquisition of metals by either forming complexes with metal ions where after, are extracted or desorbed from soil components (Brown *et al*., 1994). Tissue metal concentration of treatments with EDTA was observed to have higher metal than OX. The metal-EDTA coordination sphere being larger than that of metal-OX, EDTA is hexadentate; while oxalate is bidentate (at the operating soil pH>5). For the divalent Cd, Cu, and, Pb, 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 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 would do (Wuana *et al*., 2016).

Higher concentration may lead to an increase in the contents of heavy metals in tissue compartments of plants but at the same time, it could damage the growth of plants, and consequently lead to decreased accumulation of metals (Zhixin *et al*., 2009). The criteria used for hyperaccumulation varies according the metal, ranging from 100 mgkg^{-1} dry mass for Cd to 1000 mgkg^{-1} for Cu, Co, Cr and Pb. These values exhibit a shoot-to-soil ratio of metal concentration and the factor for bioaccumulation is higher than 1 (Baker *et al.,* 1994).The accumulation of the selected metals varied by the nature of chelant and dosage used. Concentrations were enhanced in an approximate order: EDTA+OA>EDTA>OX. *R. communis* is a perennial plant, it is expected that the plant will be able to extract and accumulate higher amounts of the metals if allowed to stay longer in the soil (do Nascimento *et al.,* 2006). This however, indicate that *R. communis* is capable of removing the metals from the soil matrix and capable of translocating them from root to shoots.

Conclusion

The findings of this study have shown that the result for the physicochemical analysis of the parent soil were suitable for experimental ground. Growth of *R. communis* in the metals poultry and chelant study did not show phytotoxic symptoms and were essentially sigmoid. Poultry manure however enhanced the growth attributes. Tissue (root and shoot) of heavy metal concentrations (mg/kg) increased significantly with increased chelant doses and chelant amendment type. Altogether, the mixed chelant treatments showed enhanced metal accumulation in *R. communis* tissue to a greater extent than the single chelant scenarios, the single scenarios, EDTA showed more than oxalic acid. This suggests that the mixed chelants could be considered as alternative treatments for enhanced phytoextraction. In light of the above, the results provided ample evidence of *R. communis* as hyperaccumulator and could be very effective in the uptake of metals especially, in the presence of poultry manure and chelants.

Acknowledgment

We appreciate the assistance received from the staff of Chemistry laboratory, University of Agriculture, Makurdi during my laboratory work, especially Mr. Emmanuel Ukpoko for their valuable advice and technical support that has contributed to make this study a reality. Also is the staff of

Golden year limited, Port-Harcourt for carrying out Atomic Absorption Spectrometry (AAS) analysis.

Conflict of Interest

Authors declare that there is no conflict of interest in this study.

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