PHYTOEXTRACTION TRIALS OF CADMIUM AND LEAD CONTAMINATED SOIL USING THREE WEED SPECIES

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ABSTRACT

Study on the phytoextraction of cadmium (Cd) and lead (Pb) artificially contaminated soil using 3 weed species (Ageratum conyzoides, Syndrella nodiflora and Cleome rutidosperma) was carried out at the Centre for Ecological Studies, University of Port Harcourt. A Randomized Complete Block Design consisting of 2 sets of contamination treatments designated as Cd(T) and Pb(T) with 3 phytoextraction applications for each set. Cd(T) and Pb(T) were contaminated with 100 mg of Cd and Pb in 4 kg soil respectively. There was also a control with pollution but no phytoextraction application for each set. After 13 weeks of phytoextraction trials, results showed percentage reductions in soil Cd in Ageratum conyzoides (35.8%), Syndrella nodiflora (45.1%) and Cleome rutidosperma (55.3%); and Pb was Ageratum conyzoides (68.7%), Syndrella nodiflora (27.3%) and Cleome rutidosperma (24.8%). The plant concentration factor (PCF) for Cd was Ageratum conyzoides (0.39), Syndrella nodiflora (0.73) and Cleome rutidosperma (1.15) and; Pb was Ageratum conyzoides (1.96), Syndrella nodiflora (0.32) and Cleome rutidosperma (0.31). The mobilization ratio for Cd was Ageratum conyzoides (0.91), Syndrella nodiflora (0.88) and Cleome rutidosperma (0.90) and; Pb was Ageratum conyzoides (1.06), Syndrella nodiflora (0.77) and Cleome rutidosperma (0.57). Therefore, Cleome rutidosperma and Ageratum conyzoides are good phytoextraction plants for Cd and Pb respectively. Also Ageratum conyzoides can be regarded as accumulator for Pb with mobilization ratio > 1 while Cleome rutidosperma and Syndrella nodiflora are Cd and Pb excluder plants.

Key words: weed species, contamination, heavy metals, phytoextraction, *Ageratum conyzoides*, soil.

INTRODUCTION

The rise in industrialization and consumption of industrial-processed products has led to increase contamination of the environment with toxic substances such as heavy metals. McGrath *et al.* (2001) reported that heavy metals affected over

1,400,000 sites in Europe. Heavy metal pollution can be defined as the introduction of heavy metals into the environment in quantities that causes alteration in the physical, chemical and biological systems of the environment with detrimental effects on microorganisms, plants, animals and man.

Heavy metals are not totally biologically insignificant as most of them are needed for normal biological functioning as micronutrients; but when the quantities exceeded the normal requirement, they become pollutants. Beyersmann and Hartwig, (2008) observed that the dose and mode of exposure, oxidation state and complex form may make some metals that are indispensable to plant to be toxic. The most important characteristic of heavy metals as pollutants is that they cannot undergo biodegradation and persist environment for many years but can also bioaccumulate and biomagnify in living organisms. Heavy metals get into the environment through human activities such refining; mining, electroplating, municipal wastes and burning (knox et al., 1999; Gisbert et al., 2003; Liu et al., 2005).

Cadmium (Cd) and lead (Pb) are heavy biological metals with no known importance. Cd as a non-redox metal is highly phytotoxic. It induces lipid profile changes (Quariti, 1997). It also affects an enzyme (H⁺-ATPase) associated with membranes (Fodor et al., 1995); and photosynthesis (Siedlecka and Baszynsky, 1993). Pb contamination exists in an insoluble form in the upper surface of the soil and it is highly immobile subsequently absorbed by plants animals via food chain. Sources of Pb contamination include paints, municipal sewage, and burning of leaded gasoline (Gisbert et al. 2003)

Following the ecological consequences associated with heavy metals pollution, remediation of contaminated environment becomes inevitable. Since natural attenuation may not be applicable due to non biodegradability of these heavy metals;

phytoremediation has emerged as a viable alternative for the removal of these contaminants from soil or water. Phytoremediation is the use of higher plants removal/reduction in-situ contaminants from soil or water. This technology is based on the premise that plants possess the ability to degrade, contain or transfer contaminants (Cunningham et al., 1996) thus, making the contaminated environment less toxic or free from the contaminants. Phytoextraction (a form of phytoremediation) is containment approach adopted by plants to remove contaminants (mostly metals) from the soil or water and accumulate them in their above-ground biomass. These plants demonstrate high level of tolerance without showing metal toxicity syndrome (Cardwell et al., 2002). Certain factors such as soil conditions, plant species and the heavy metal type may affect the accumulation of metals by plants (Barman et al. 2001; Spinoza-Quinones et al., 2005). More than 500 species of plants have been proven with demonstrated ability to hyperaccumulate heavy metals (Kramer, 2010). On the other hand, some plants reduce the heavy metal content in the soil and maintain the metal content (concentration) in the shoot at low irrespective of the external concentration of the metal. These set of plants are called metal excluder plants. Polyalthia longifolia has been identified as a typical Pb excluder plant (Tanee and Amadi, 2016).

Despite advances made in this technology (phytoremediation) in the developed world, little is been done in developing countries like Nigeria where pollution problem is on the increase. Secondly, many breakthroughs in the field of phytoremediation has been associated with the use of foreign plant

species but not much work has been done in Nigeria especially in the Niger delta using the indigenous plant species despite the rich biodiversity, hence the thrust of this study.

Ageratum conyzoides, L (commonly called goat weed), Syndrella nodiflora (L) Gartn (common name: Nodeweed) and Cleome rutidosperma, DC (common name: spider plant or purple cleome) are herbaceous weeds commonly found on agricultural areas, roadsides and planted forests. The use of these plants in this study will provide a clue on good uses of these plants because of their abundance. It will also widen our scope of plants that can be used in phytoremediation (phytoextraction) of heavy metal contaminated soil.

MATERIALS AND METHODS Experimental site

The study undertaken at the experimental field Centre at the for Ecological Studies, University of Port Harcourt located within the tropical rain forest belt of Nigeria on Latitude 4° 65` N and Longitude 7° 5` E. The area experiences two distinct seasons (dry and rainy). The area is also noted for high temperature and high relative humidity, which are characteristics of the equatorial region. The experiment was set up under transparent shade to allow light penetration but prevent direct rainfall into the set-ups.

Sources of materials and preparation

Loamy soil was collected at a depth of 0 – 10 cm (surface soil) from a fallow land beside Department of Gas Engineering, University of Port Harcourt. The soil was collected in bulk, homogenized properly and taken to the experimental site for bagging. The bags used were obtained from Agricultural Development Programme

(ADP), Rumudomaya, Port Harcourt. The bags were not perforated so as to prevent leaching of the contaminants from the bags. 4 kg of soil was weighed into each bag.

The heavy metals used were obtained in their salt form from Bernaco Enterprises Nig. Ltd. Port Harcourt. CdSO₄. 8/3H₂O and Pb(NO₃)₂ were used to obtained Cd and Pb respectively. 10 g of each metal salt solution was dissolved in 1 litre of distilled water to obtain 6.256 g/l and 4.4394 g/l for Pb and Cd respectively as its equivalent of 100 mg concentration.

The 3 weed species used for the phytoextraction Ageratum were conyzoides,L. Syndrella nodiflora; (L) Gaertn. and Cleome rutidosperma, DC. Young seedlings of these weed species were collected from the vicinity of the Centre for Ecological Studies. They were raised in nursery for two weeks before transplanting into the experimental pots (bags). Seedlings of equal size, vigour and age were used to ensure similar conditions.

Experimental design

The Randomized Complete Block Design (RCBD) was used for the experiment. This consist of 2 sets (blocks) designated as Cd(T) and Pb(T). Each set comprises 20 bags (plots). Each bag of Cd(T) was contaminated with 100 mg of Cd while each bag in Pb(T) set was contaminated with 100 mg of Pb. The set-up was allowed to stand for one week. After the one week, each of Cd(T) and Pb(T) was separated into 4 groups of 5 replicates designated as Cd (T₁, T_2 , T_3 , T_4) and Pb (T_a , T_b , T_c , T_d). Three (3) seedlings each of Ageratum conyzoides L, Syndrella nodiflora, (L) Gaertn. and Cleome rutidosperma, DC were transplanted into T₁ and T_a; T₂ and T_b; T₃ and T_c respectively. T₄ and T_d received no planting (control). The experiment was monitored for 13 weeks. Watering was done three times a week with 300 ml of distilled water per plot. The experimental plots were devoid of weeds by handpicking any visible weeds.

Sample Analysis

Soil samples collected after were contamination but before transplanting of the weeds and at the end of the experiment (13 week after planting). Soil sample from each treatment/bag was collected and taken to the Laboratory for determination of heavy metals (Cd and Pb) concentration. The phytoextraction plants were carefully harvested from the soil without damaging the roots. The shoot of each plant was separated from the root by cutting and were taken to the Laboratory for Cd and Pb contents analyses.

Cd and Pb in the samples (Soil and plants) were determined by Atomic Absorption Spectrophometry (varian spectral AA-250 plus) after digestion on an electrothermal plate for 15 minutes using perchloric and nitric acids.

Plant concentration factor (PCF) also known as Transfer factor and; Mobilization ratio (MR) or translocation factor were also calculated according to the methods of Augustynowicz *et al.* (2014) and Ye *et al.* (2014) respectively using the following formulae

 $PCF = \frac{concentration of metal in plant}{concentration of metal in soil}$

 $MR = \frac{concentration\ of\ metal\ in\ shoot}{concentration\ of\ metal\ in\ root}$

Statistical Analysis

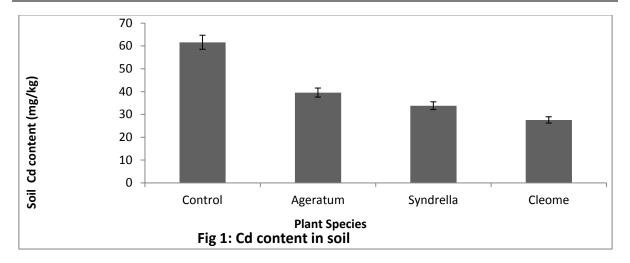
Mean and standard error mean (SEM) were calculated from the data obtained. Analysis of variance and Least Significance Difference (LSD) were used to separate means using SPSS data analysis version 20.

RESULTS

Results showed varying degree of heavy metal reduction in soil using the three plant species as phytoextraction species. There was also variation in the accumulation of the heavy metals by the three plant species.

Significant (p=0.05) reduction in soil Cd was observed in phytoextracted with the 3 species as compared with the control. That is, the control recorded the higher Cd content than the remediated soil (Fig. 1). Highest soil Cd reduction (53.30%) was observed in *Cleome* sp phytoextracted soil followed by *Syndrella nodiflora* (45.12%) while *Ageratum conyzoides* recorded the least (35.83%).

In the Pb contaminated soil, significant reductions were also observed in the soil phytoextracted with the 3 weed species as compared with the control (no phytoextraction). Highest reduction was recorded at Ageratum convzoides phytoextracted soil (68.73%) while there was no significant difference (p=0.05) between reductions in Syndrella nodiflora (27.29%)and Cleome rutidosperma (24.79%)remediated (Fig. soils



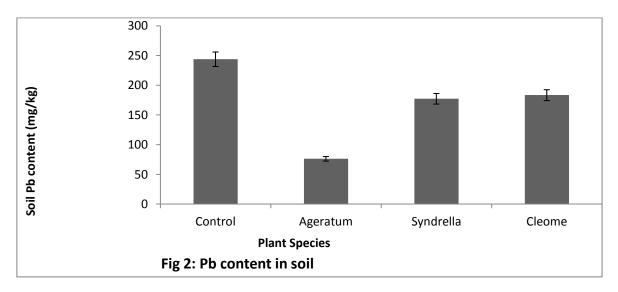
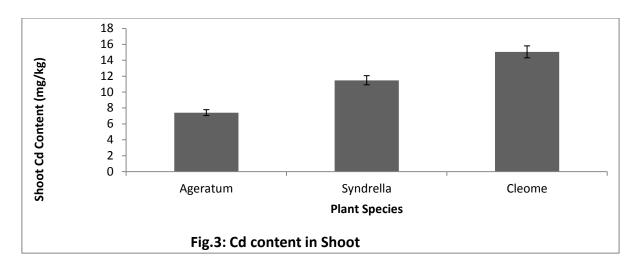
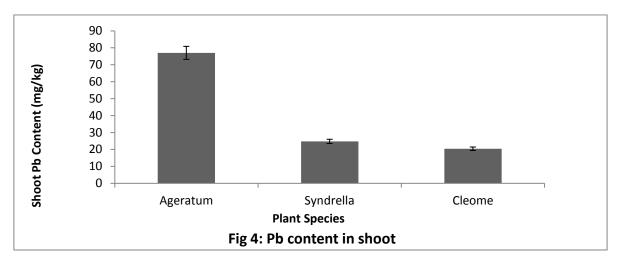


Fig. 3 shows the accumulation of Cd in the shoots of the three plants (*Ageratum conyzoides, Syndrella nodiflora* and *Cleome rutidosperma*) used in the phytoextraction trial. *Cleome rutidosperma* recorded the highest accumulation of Cd in the shoot as compared with the other 2 plants. The least accumulation was observed in *Ageratum*

conyzoides (p=0.05). Also the accumulations of Pb in the shoots of the 3 phytoextracted plants were significantly different. Ageratum conyzoides accumulated the highest Pb in the shoot while Syndrella nodiflora recorded the least accumulation (Fig. 4).

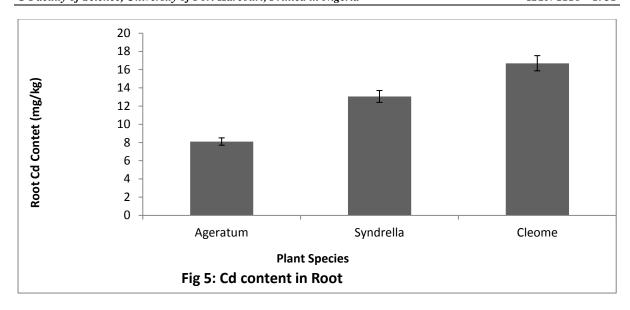
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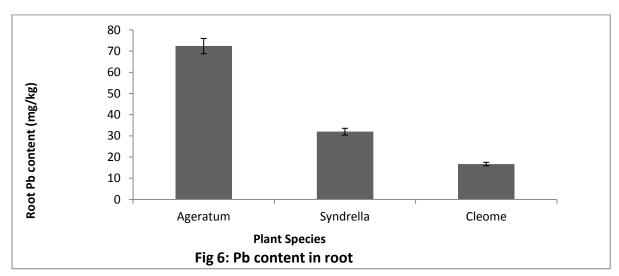




The accumulations of the heavy metals (Cd and Pb) in the roots of the 3 phytoextraction plants are as shown in Fig. 5 and 6. Result showed that the root of *Cleome rutidosperma* absorbed and accumulated more Cd than the roots of *Ageratum conyzoides* and *Syndrella nodiflora*, while *Syndrella nodiflora* accumulated more than

Ageratum conyzoides (Fig. 5). The concentration of Pb in the roots was highest in Ageratum conyzoides followed by Syndrella nodiflora and Cleome rutidosperma; but there was no significant (p=0.05) difference in the Pb concentration in roots between Syndrella nodiflora and Cleome rutidosperma (Fig. 6).





The plant concentration factor (PCF) or Transfer factor of Cd contaminated soil for the 3 plants showed that *Ageratum conyzoides* and *Syndrella nodiflora* had PCF values of less than one (1) with the exception of *Cleome rutidosperma* which had PCF value greater than 1. In Pb contaminated soil, it was only *Ageratum conyzoides* that recorded a PCF value greater than 1 while the PCF of the other 2 plants were less than 1(Table 1).

Table 2 shows the Mobilization Ratio (MR) or Translocation Factor in the 3 phytoextraction plants for the two metals. Results showed that it is only *Ageratum conyzoides* that recorded a mobilization ratio of value greater than 1 in Pb contaminated soil. All other plants showed Mobilization ratio (MR) of less than 1 for the two heavy metals (Cd and Pb) polluted soil.

Table 1. Plant Concentration Factor (PCF) or Transfer factors of the three weed species

Weed species	Plant Concentration Factor (PCF)	
	Pb	Cd
Ageratum conyzoides	1.96	0.39
Cleome rutidosperma	0.31	1.15
Syndrella nodiflora	0.32	0.73

Table 2. Mobilization Ratio or Translocation factor of the three weed species

Weed species	Mobilization Ratio (MR)		
	Pb	Cd	
Ageratum conyzoides	1.06	0.91	
Cleome rutidosperma	0.57	0.90	
Syndrella nodiflora	0.77	0.88	

DISCUSSION

Plants have different mechanisms of tolerating and accumulating metals in their biomass leading to the concept of hyperaccumulators, accumulators and excluders. In hyperaccumulators, metals are accumulated in the above-ground parts (shoot and leaf) of the plant (0.01- 1%) of the dry weight (Kramer, 2010; van der Ent et al., 2013) while in excluders, the metal level of the shoot is kept at constant low level irrespective of the external concentration level of the metal. Briat and Lebrun (1999) identified three major principles that are involved in metal tolerance in plants. These are (1) regulating metal uptake by root of plants by preventing overload (2) creating a mechanisms for the maintenance of non toxic forms of metal at the intracellular level and (3) Detoxification of oxygen radicals by the production of antioxidant enzymes.

Results showed that the 3 weed species differ in their ability to absorb and accumulate Pb and Cd in their biomass and subsequent translocation into their aboveground parts. *Cleome* sp and *Ageratum* sp

significantly reduced more than 50% of Cd and Pb respectively, from the contaminated soil while Syndrella sp had less than 50% reduction for the two metals. This is an indication of the plants preference for accumulation of different metals. In other words, a plant may effectively accumulate one metal but inefficient for another metal. The observed variation in their accumulation ability might be due to combination of factors such as plant species and plant traits; and other physicochemical, physiological and morphological mechanisms of metal retention in the plant tissues (Favas et al., 2016). This goes to show that the ability of the 3 plants to manipulate the rhizosphere for acquisition of metals and other nutrients from the soil differs. Other factors such as pH, temperature, the cation exchange capacity (CEC), and organic matter content of the soil may also contribute to the differential absorption (Msaky and Calvet, 1990; Salim et al., 1993).

The absorption and subsequent accumulation of metals by the plants has been shown to be dependent upon the

degree of metal contamination in the soil (Caldwell et al., 2012). The size and type of root system have also been observed as one of the causes of differential absorption of metals by plants (Prasad, 2007; Nakbanpote et al., 2016). For instance, plants with fibrous adventitious roots covered with numerous root hairs may create a high surface area for absorption than plant without root characteristics such (Nakbanpote et al., 2016). Thus, it may be deduced that the structure and architecture of root systems have important role to play in plant adaptation to water, nutrients and metals absorption/accumulation (Staňová et al., 2012).

Results showed differences in metal concentrations in the shoot and root of the 3 plant (weed) species. Cleome sp showed highest concentration of Cd and Ageratum sp showed highest concentration of Pb in both their roots and shoots. This showed that Cleome sp and Ageratum sp possess Cd and Pb metals accumulating characteristics, respectively, than Syndrella sp suggesting the action of physiological barriers. Liao et al. (2000) observed that Xylem sap pH and redox potential can affect the types, amount and movement of metals ions in the xylem while Clemens et al. (2002) report that membrane transport proteins mediated this xylem loading process.

Excluder and accumulator plants can be empirically distinguished on the basis of the Plant concentration factor (PCF) (also known as Transfer factor or bioconcentration factor) Augustynowicz *et al.*, 2014; Chen *et al.*, 2015; Trotta *et al.*, 2006; Zhuang *et al.*, 2007), and of the translocation factor (TF) or Mobilization ratio (MR) (Rahman *et al.*, 2014; Ye *et al.*, 2014) Results showed that *Ageratum* sp had

a Transfer factor of 1.96 and Translocation factor of 1.06 for Pb while the other plants (*Cleome* sp and *Syndrella* sp) had values < 1. Plant with PCF and Translocation factor > 1 can be regarded as accumulator while those < 1 can be called excluder plants (Macnair, 2003; Trotta *et al.*, 2006). Hence, *Cleome rutidosperma* and *Syndrella nodiflora* can be classified as Cd and Pb excluder plants while *Ageratum conyzoides* can be regarded as Pb accumulator plant.

The results from the study have demonstrated the ability of the 3 tested weed species (Cleome rutidosperma, Syndrella nodiflora and Ageratum conyzoides) their Pb and in accumulations. Significant reductions in both Cd and Pb were recorded in the contaminated soil as compared with the phytoremediation). control (no The reduction gradient was as follows: Cd: Cleome rutidosperma (55.3%) > Syndrella nodiflora (45.1%) > Ageratum conyzoides (35.8%) and; Pb was Ageratum conyzoides (68.7%) > Syndrella nodiflora (27.3%) > Cleome rutidosperma (24.8%). Thus, Ageratum convzoides and Cleome rutidosperma were found to be effective phytoextraction plants especially in Pb and Cd polluted soil, respectively. On the basis of their PCF and MR, Ageratum conyzoides can be classified as accumulator plant while Cleome rutidosperma and Syndrella nodiflora as excluder plants.

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