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Lead Accumulation and Distribution at Cellular level in Native Plants Growing on Battery Wastes Contaminated Sites in Ibadan, Nigeria

Ayotunde A. Adeosun[1], Sifau A. Adejumo[1*] and Prashant Srivastava[2]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.
[2]Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), University of Newcastle, ATC Building, Callaghan, NSW 2308, Australia.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAA helped in collating the data, performed the laboratory and statistical analyses. Author SAA designed the study, wrote the protocols, wrote the manuscript, managed the analyses of the study and supervised the study. Author PS contributed to literature search, discussion and reviewed the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Effective phytorextraction depends on the identification of fast growing plants that can tolerate and accumulate high concentration of metals in their tissue. This study was conducted to identify potential lead hyperaccumulators among the native plant species growing on two abandoned lead-acid battery waste-contaminated sites.
Methodology: Plant samples were collected in triplicates from these sites. Pb accumulation in different parts, translocation (TF) and bioaccumulation factors (BCF) as well as cellular distribution of Pb among the cell organelles (cell wall, mitochondria, plastids, nucleus and soluble fraction) were determined.
Results: Among the plant species (*Gomphrena celosioides, Sporobolus pyramidalis, Imperata cylindrica, Chromolaena odorata, Cynodon. dactylon, Rhynchospora corymbosa* and *Eleusine*

**Corresponding author: E-mail: nikade_05@yahoo.com;*

indica) found on these sites, *G. celosioides* had the highest Pb concentration (12, 657 mg/kg) in its shoot and the highest BCF (18.66) and TF (25.62) while others had TF and BCF values that were less than 1. *S. pyramidalis* and *E. indica* had lower Pb accumulation. Pb was mostly accumulated in the cell wall and there was an increase in the production of phenolics, flavonoids and chlorophyll. **Conclusion:** *G. celosioides* can therefore be described as a Pb hyperaccumulator and its Pb is mostly accumulated in the cell wall.

Keywords: Environmental contamination; phytotoxicity; phytoremediation; metallophyte; translocation; antioxidant.

1. INTRODUCTION

Abiotic stress factors generally pose serious threats to agricultural production and food security [1]. Soil contamination with heavy metals is most worrisome as it has become a global problem particularly in areas with high anthropogenic pressures. It has resulted in agricultural yield reduction by reducing the amount of cultivable lands and increasing soil degradation and phytotoxicity [2,3]. Soil and water contamination by heavy metals causes bioaccumulation in plants thereby threatening human health through food chain [4]. Excessive metal accumulation in plants also results in oxidative stress due to production of reactive oxygen species [5,6,7,8]. Lead (Pb), in particular is most toxic because of its hazardous health effects on both animal and man [9,10]. The accumulation of Pb, through its mutagenic ability, may also cause DNA damage and have carcinogenic effects on human [11,10,8].

Various physical and chemical methods are being employed for remediation of contaminated sites based on soil properties, such as adsorption and desorption [12,13,14]. Conventional remediation methods such as soil washing and flushing, excavation and reburial [15,16] as well as chemical remediation methods [17] and compost remediation [18] are some of the methods employed for recovery of heavy metal contaminated substrates. However, the prohibitive cost of some of these methods, their environmental implications and effectiveness in total removal of these contaminants makes them unacceptable. Hence, there is a need for the development of sustainable and environmentally-friendly method for clean-up of Pb-contaminated sites.

Focus is now on the use of plants to mop up heavy metals, a process known as phytoextraction [19-23]. It is environmentally-friendly and is fast gaining attention worldwide as a clean-up strategy. The technique employs plants to extract heavy metals from contaminated media [24,16]. The successful application of phytoextraction technique however, demands that the plant species to be used must have the ability to accumulate high metal concentration in the above ground tissue, grow fast, able to tolerate and reproduce under the toxic conditions and produce high biomass [25,15,26]. These unique properties of being able to accumulate and tolerate high concentration of heavy metals in the above-ground tissues are the attributes of plant species known as hyperaccumulators [27]. Whereas most plant species are sensitive to the contaminated conditions, the hyperaccumulators have evolved the ability to survive, reproduce in toxic environments and have adapted to the condition of high metal concentrations [16,28]. Therefore, for successful phytoremediation, the first and foremost step is identification of a hyperaccumulator that has already adapted to a particular contaminated site under specific environmental conditions [28]. This is a prerequisite for the effective application of this technology [29].

However, majority of known hyperaccumulators reported are from temperate regions while few were reported for the tropical environment [30]. Unlike other parts of the world, in Africa, the study on identification of natural/native hyperaccumulator is scanty. Few metallophytes (metal tolerant and/or hyperaccumulator plants) species have been reported. More importantly, Pb is also one of the least studied toxic metals in relation to phytoextraction. Of about 450 plant species reported worldwide for phytoextraction, few of them are Pb hyperaccumulators while majority are for nickel and zinc. Knowledge on the application of phytoextraction strategy for the remediation of Pb-contaminated soils is therefore lacking [28,31]. This has been identified as a limiting factor in studying and understanding the molecular and genetic mechanisms involved in Pb tolerance [28]. There is a need therefore to undertake a study of the plant species adapted to Pb contaminated sites and their ability to accumulate and tolerate high concentrations of metal (Pb) in their tissues. Though previous

reports have confirmed the ability of metal tolerant plants in accumulating high amount of heavy metals in their tissues [32,33] yet the individual tolerance mechanisms also need to be understood.

For instance, tolerance in plants involves enzymatic and non-enzymatic strategies as well as coordination of complex physiological and biochemical processes [34-36]. Upregulation of some antioxidant defense system to counteract the deleterious effects of high metal concentrations in the tissue have also been reported [37-40,7]. Similarly, under toxic metal conditions plants are able to tolerate heavy metals through osmotic adjustment and sequestration with different antioxidants like cysteine-rich peptides, proline, glycine betaine, glutathione and other biochemical compounds [34]. Plants growing on the contaminated soil also make use of cellular compartmentalization or the exclusion principle [40] apart from anti-oxidation for metal tolerance and survival mechanism [41,42,39,43,44,45,7,8].

Identification of natural Pb hyperaccumulator from the metalliferous sites and determination of their tolerance mechanisms will help the development of an effective phytoremediation. Once the uptake, translocation, and detoxification mechanisms in hyperaccumulating plants are identified, it will also lead to improved applicability of the phytoremediation technology in Nigeria. More importantly, for bioengineering process, these plants will also be a source of genetic resources for development of transgenic plants with high biomass, increased tolerance and efficient metal (Pb) removal. This study was carried out to [1] determine the heavy metal (Pb) accumulating ability of different plant species growing on heavy metal contaminated sites as well as their translocation to above ground parts [2] investigate the distribution of metal (Pb) among different cell organelles.

2. MATERIALS AND METHODS

2.1 Site Description

The contaminated and abandoned lead acid battery wastes dumpsites located at Kumapayi (Egbeda Local Government Area) and Lalupon (Lagelu Local Government Area) in Ibadan, Oyo State, Nigeria were used for the study. These sites are characterized by high metal contents most especially Pb [32,18]. Lalupon in Lagelu Local Government Area lies between longitude 7°28[1] N and latitude 4°04[1] E while Kumapayi is between longitude 7°24' N and latitude 4°00′E. The total soil lead concentrations on these sites as previously reported by Ogundiran [17] and Adejumo et al. [18] averaged 124,000 and 139,500 mg/kg of lead for Lalupon and Kumamayi sites respectively. Physico-chemical parameters of both sites showed that the sites are slightly acidic and low in carbon content [Adejumo et al. [18]. The area occupied by the wastes had some sections completely devoid of plants while some had plants growing on it which is a reflection of their ability to cope with the stress induced by high metal concentration in the soil (Fig. 1).

2.2 Plant Sampling and Analysis

Plant samples were collected in triplicates from the contaminated sites with the aid of hand trowels. Individual plants were carefully uprooted and the adhering rhizopheric soils carefully removed by washing under tap water using soft brush and later rinsed with distilled water to ensure total removal of the soil. The shoots were also washed gently in distilled water to remove all the solid particles and dust adhering to them. The plant samples were then partitioned into two components; roots and shoots, oven-dried at 80°C for 48 hrs and ground into fine powder using milling machine. The shoot and root metal concentrations were determined following the method described by Ogundiran and Osibanjo [39]. Specific quantity (0.5 g) was then taken from each sample for ashing at 600°C for 12 hours. Thereafter, the ash was washed with 10ml of 2 M HNO_3 and analysis of the plant samples for heavy metals was carried out using Atomic Absorption Spectrophotometer (AAS) (VGP210 BUCK Scientific Model. Chicago, Illinois, USA).

2.3 Soil sampling and Analysis

The rhizopheric soils from different plant roots were also collected and analysed separately for total Pb concentration. Metal analysis of the samples was carried out in triplicates by acid digestion (2 M Nitric acid) of 1 g of soil in plastic centrifuge tubes and boiled for2 hours at 100°C in water bath. The tubes were stirred at 20 minutes intervals. The digested samples were then filtered and analysed for Pb using Atomic Absorption Spectrophotometer (AAS) (VGP210 BUCK Scientific Model. Chicago, Illinois, USA).

A B

Fig. 1. Heavy metal contaminated sites in Lalupon [A] and Kumapayi [B] showing the native weeds along the edges

2.4 Bioaccumulation and Translocation Factors

After the determination of the lead concentration in the shoot, root and soil, the translocation and bioaccumulation factors for each plant species were calculated. Translocation factor is defined as ratio of the metal concentration in the shoot to that of root. Bioaccumulation factor is defined as the total metal concentration in the plant [shoot and root] divided by the metal concentrations in the soil [46].

2.5 Determination of Sub-cellular Fractions

For this purpose, *G. celosioides, C. odorata*, *C. dactylon* and *I. cylindrica* were separated into root, stem and leaf while other species (*E. indica, R. corymbosa* and *S. pyramidalis)* could only be separated into shoot and root. As described by Zeng et al. [40], fresh samples (1 g each) of plant roots, stems and leaves were weighed and homogenized in a medium containing 0.25 mM sucrose, 50 mM Tris - HCl (pH 7.5) and 1mM ascorbic acid at 4°C. Cells were separated by gradient centrifugation technique at 4°C into five different fractions (Cell walls, plastids, nuclei, mitochondria, ribosomes and soluble materials). Firstly, for cell wall fraction, the homogenate was strained through a nylon cloth (80 µm) and the residue on the nylon cloth was designated as the cell wall fraction (FI), mainly containing cell walls and cell wall debris. The filtrate was centrifuged at 1500 g for 10min (root sample 2500 g for 20 min) and the pellet/residue was designed as plastid fraction (FII). The supernatant of the first centrifugation step was then centrifuged at 5000 g for 20 minutes, and the pellet/residue was designed as nucleus fraction (FIII). The supernatant of the second centrifugation step was finally centrifuged at 15,000 g for 30 minutes; and the pellet/residue was taken as the mitochondria fraction (FIV) while the last supernatant was considered as soluble fraction (FV). The different cell fractions were oven-dried at 70°C to constant weight, ashed at 500°C for 12 hours and dissolved in 10 ml of HNO_3 in distilled water (1:1v/v), then the concentration of Pb in each sub-cellular fraction for the roots and shoots were determined for each plant species using atomic absorption spectrophotometer (AAS) (VGP210 BUCK Scientific Model. Chicago, Illinois, USA).

2.6 Statistical Analysis

Data collected were analysed using analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. RESULTS

3.1 Plant Species Identified from Different Sites, Their Tissue Lead (Pb) Contents and Soil Pb Concentrations

Seven different plant species were identified at Lalupon contaminated site namely: *S. pyramidalis, E. indica, C. dactylon, G. celosioides, R. corymbosa, I. cylindrica and C. odorata* whereas five were identified on Kumapayi site (*E. indica, C. dactylon, G. celosioides, I. cylindrica* and *C. odorata)*. Though the number of species identified on these sites varied, yet the fact that the same types of plant species were growing on these sites suggested that these species were associated with Pb contamination. The brief description of these

plant species are as given in Table 1 and Fig. 2. There were differences in the lead concentrations found in the shoot, root and rhizospheric soil of each plant species. In the shoot, the highest lead concentration was found in *G. celosioides* (12,125 mg/kg) and the lowest in *E. indica* (242 mg/kg). For the root, the highest lead concentration was found in *C. dactylon* (1710 mg/kg) and the lowest was in *E. indica* (234 mg/kg). For the rhizospheric soil, the highest lead concentration was found in the soil collected from the root of *E. indica* (40,600 mg/kg) while the lowest was found in that of *G. celosioides* (697 mg/kg). *G. celosioides* had the highest bio-accumulation and translocation factor with values 25.62 and 18.66, respectively followed by *R. corymbosa*. The lowest value of bio-accumulation and translocation factor was however found in *S. pyramidalis* which are 0.273 and 0.03 respectively (Table 2).

Similarly, for the plant samples collected from Kumapayi, the highest lead concentration was found in *G. celosioides* shoot (930 mg/kg) followed by *C. odorata* (317.0 mg/kg) and *S. pyramidalis* (267.0 mg/kg). The lowest values were recorded in *I. cylindrical* (122.5 mg/kg) and *R. corymbosa* (124 mg/kg). For the root, the highest lead concentration was found in *R. corymbosa* (496 mg/kg) and the lowest in *I. cylindrica* (129 mg/kg). Generally, higher lead concentration was found in the rhizospheric soils collected from Lalupon site compared to those of Kumapayi. The rhizopheric lead concentrations in Kumapayi soil were 650, 1720, 1025, 1090 and 1750 mg/kg for *G. celosioides*, *S. pyramidalis, I. cylindrica C. odorata* and *R. corymbosa* respectively. *G. celosioides* as observed in Lalupon also had the highest bio-accumulation and translocation factors of 1.26 and 2.6, respectively followed by *R. corymbosa* of 0.25 and 0.4, respectively. The lowest value was however found in *S. pyramidalis* which are 0.03 and 0.27 respectively (Table 3).

3.2 Cellular Distribution of Pb in Different Plant Species Collected from Contaminated Site

Generally, the highest Pb concentration was found in the cell wall (with small variations) irrespective of the plant's parts and species. *G. celosioides* had the highest Pb concentration in the cell wall (FI) of its leaf which is 74% of the total Pb in the leaf. Cell wall Pb concentration was about 43% in the stem (FI=43%) and the lowest in the leaf mitochondria and soluble fractions (F4=0% and F5 = 3%). However, the trend changed in the root with the highest Pb concentration found in the root plastid (F2) and amounting to 80% of the total Pb followed by those of the nucleus (F3 = 9%) and cell wall (F1 = 7%). The lowest concentrations were also found in the mitochondrion and soluble fractions (0 and 4% respectively). Stem also had 42% of its total Pb concentration in the plastid fraction. The total Pb concentration in all these organelles

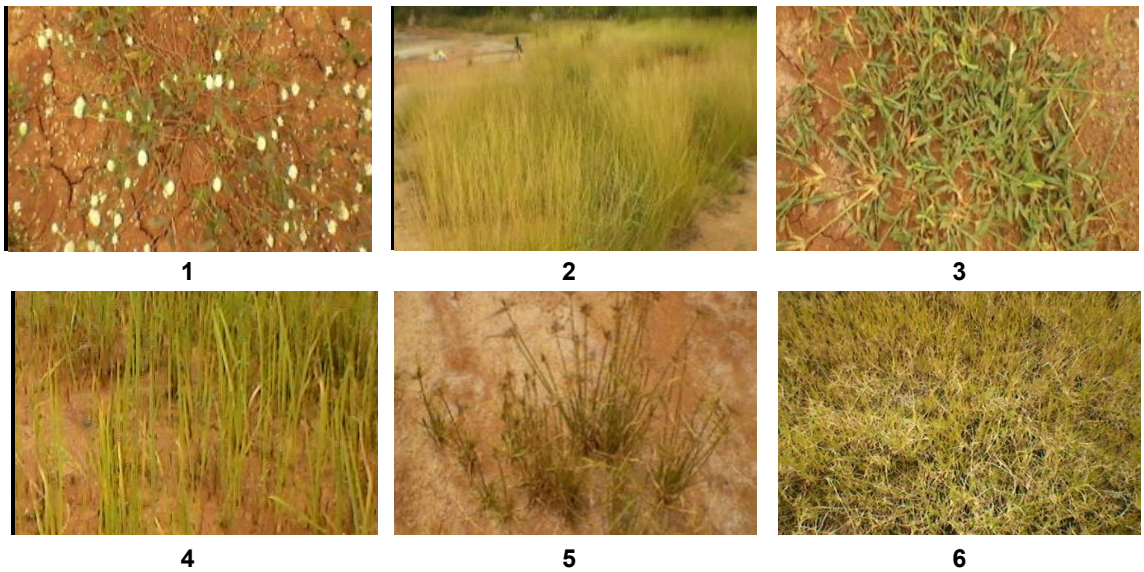

Fig. 2. The native weeds growing on Heavy metal (Pb) contaminated sites in Lalupon and Kumapayi 1.*G. celosioides*, 2. *S. pyramidalis, 3. E. indica, 4 I. cylindrica,* 5. *R. corymbosa* and 6. *C. dactylon*

Table 1. Brief description of the plant species identified at contaminated sites

Name of plant	Common name	Family	Distribution	Description
G. celosioides	Prostrate Globe-Amaranth	Amaranthaceae	Native of South America but widely distributed in the tropics	Much branched, mat forming prostrate plant with whitish to pink colour flower, hairy leaves of 3-4cm long, short petioles and deep taproot
C. odorata (L.) R.M. King & H. Rob	Siam weed	Asteraceae	Widely distributed but Native of North America. It is considered as one of the world most invasive weeds	Triangular leaf with pitchfork three-vein
R. corymbosa (L.) Britton matamat	Sedge	Cyperaceae	Grows in coastal swamps. Widely distributed in Coast South to Brunswick Heads, Africa	Graminoid, Stout shortly rhizomatous perennial
S. pyramidalis	Cat's tail dropseed	Poaceae	Common in Australia and widely distributed in tropical and Sub-tropical environment	An upright and long head grass of 90-200 cm tall with large dense tussock
C. dactylon (L) Pers	Bermudagrass	Poaceae	Widely distributed in tropical and subtropical as well as some temperate regions but originated in Turkey and Pakistan	Stoloniferous perennial mostly with rhizomes of 40-50 cm in clay and 70-80 cm in sand soil. 10-40 cm tall
I. cylindrical (L.) P. Beauv	Speargrass in W/Africa and Asia or Cogongrass in America	Poaceae	Native to Asia but occurs in a wide range of habitat and widely distributed in tropical and subtropical zones as well as some warm parts of temperate regions	It is stemless perennial and has extensive rhizomes with slender, sharp pointed, linear lanceolate blady leaves of 1.5 m tall
E. indica	Goosegrss or Wiregrass	Poaceae	Common in warm climates, accidentally introduced into North America from Eurasia and found in roadsides, fields, and waste areas. It is a typical weed of disturbed urban areas.	An annual plant about ½–2' tall, tufted at the base with multiple green, glabrous, flattened culms and alternate leaves.

was, however, more in the leaf than in other plant parts. The cellular distribution of Pb in the root and shoot of *S. pyramidalis* showed that the root which had the highest Pb concentration also had the highest Pb concentration in the cell wall (FI=53%) compared to other cell fractions and was higher than that of the shoot (FI=25%). The Pb concentration in the plastid fraction of the shoot (FII= 42%) was higher than that of other fractions including that of the cell wall. However, the Pb concentration in the cell wall was more than that of the nucleus (FIII = 19%), mitochondrion (FIV = 3%) and soluble fractions (FV= 11%) of the shoot and the same for the root. In the case of *I. cylindrica*, the highest Pb concentrations were also found in the root cell wall (FI=50%). The concentration in the root cell wall fraction was more than that of the stem (FI = 9%) and leaf (FI = 25%). Mitochondrion and soluble fractions also had the lowest concentrations as observed in other plant species. Meanwhile, the *I. cylindrica* stem unlike what was recorded for *G. celosioides* and *S. pyramidalis*, had the highest concentration in the nucleus fraction (FIII=60%) followed by that of the plastid fraction (FII = 19%). The root and stem of *C. odorata* had the highest Pb percentages in the cell wall (53 and 40%) compared to the concentrations in the other fractions and the lowest was in the soluble fraction. The highest concentration was, however, found in the plastid fraction of the leaf

(FII = 52%), followed by that of the mitochondrion fraction (FIV= 24%) and the lowest was also in the soluble fraction (FV=4%). For each fraction except for the plastid fraction, the concentration in the root was more than that in other plant parts. The first three fractions had the highest lead concentrations in the root. For *E. indica*, the highest concentration was found in the cell wall of both root and shoot (Root: 97% and shoot: 72%). The concentration in the cell wall and other fractions were however more in the root than the shoot. Except for the cell wall fraction, the lead concentrations in other fractions of both the shoot and root were small. The same trend was observed in *R. corymbosa* having the highest concentration in root cell wall (FI = 97%) and shoot cell wall (79%). The lowest was found in the mitochondrion of both root and shoot (Table 4 and Fig. 3).

4. DISCUSSION

Lead concentrations on the two sites characterized them as extremely contaminated sites [47,48] as previously reported by Ogundiran and Osibanjo [32] and Adejumo et al. [18]. However, in this study, higher concentrations of lead were found in the rhizospheric soil, shoot and root of plants collected from Lalupon and were more than those of Kumapayi. This probably could be attributed to the fact that the Pb content in Lalupon site [which was the original Government approved dump-site for these lead slag wastes] is still very high unlike Kumapayi site which is now surrounded by residential buildings. The dumping of different household wastes on this site could have reduced or transformed Pb into non-available form; hence lower Pb concentration compared to that of Lalupon. The concentration of Pb in plant was also more in plant species collected from Lalupon than those of Kumapayi in accordance with differences in Pb concentrations [49]. Meanwhile, variations observed in the heavy metal concentrations of different plant species was an indication of different behaviors displayed by different plant species under extreme metal toxicity. Whereas some plant species accumulate most of the heavy metals inside the root, others accumulate high concentration of contaminants in the above-ground tissues. The metal content of rhizospheric soil around each plant species also explains the nature of different plant species and differentiates them as hyperaccumulator, excluder or tolerant plants [50.51]. Accumulation and exclusion have been viewed as the two fundamental mechanisms by which plants respond to high level of heavy metals in the soil [52]. Those plant species that use the exclusion principle are able to restrict the uptake and the upward movement of Pb in their tissues while those in the group called hyperaccumulators have evolved the strategy to translocate and store the metals preferably in the shoot at a concentration which is usually above the soil concentration [53,54]. Hyperaccumulation is therefore described as the opposite of metal exclusion. In plants that accumulate high concentration of heavy metals [hyperaccumulators], shoot to root quotient greater than one are commonly reported while shoot to root quotient less than one characterizes heavy metal excluders [27]. Based on this understanding, *G. celosioides* from Lalupon with high metal concentration in the shoot and small concentration in the root and soil coupled with high bioaccumulation and translocation factors can be described as an hyperaccumulator [27,28]. *E. indica* on its own is a type of plant known as excluder [55] with lowest concentration of Pb in the shoot and root coupled with highest concentration of Pb in the rhizospheric soil. *S. pyramidalis* and *I. cylindrica* can also be grouped under this category because of high Pb concentrations in their rhizospheric soils coupled with the bioaccumulation factor which was less than one. Similarly, on Kumapayi site, *G.* also fulfilled the criteria of a hyperaccumulator with the highest values for both bioaccumulation and translocation factors while *S. pyramidalis, C. dactylon, E. indica, I. cylindrica* and *C. odorata* can also be grouped as Pb excluders. These results affirm other reported species of *Gomphrena* known as *G. clausseni* which was described as a novel metallophyte extremely tolerant to high zinc and cadmium exposure [33].

Though, the general belief is that Pb has poor mobility yet different assertions have been made on the solubility of Pb. Metal uptake is said to be a function of the root architecture and rhizopheric constituents [56]. It has been found that pH, nature and presence of different chemicals in the rhizospheric environment are the factors that determine the solubility of Pb. Uptake of and tolerance to Pb, therefore depend on root system and conditions. Some roots have been reported to be capable of producing exudates that will either aid or prevent the solubility of metal [44]. Previously, it was reported that majority of metal hyperaccumulators are able to secret some chemical substances into the soil through their roots which help in solubilizing metals thereby facilitating their uptake [57,56]. Some however

secrete substances that are capable of binding and precipitating metals in the solution or contaminated media.

The mechanism responsible for the restriction of metal uptake and translocation in these plants is not yet understood and were not tested in this study. However, *E. indica* which behaved as metal excluder might probably be secreting substances into the rizospheric soil which results in the reduced Pb uptake [58]. The lower concentration of Pb in the root and shoot of *E. indica* under high Pb concentration further suggests a mechanism of extreme metal avoidance reported in some tolerant plants by which uptake of metal by root and transport from root to shoot are restricted [59,57]. Pb-induced callose deposition has also been reported in the rhizodermis of plants under Pb stress. The callose has been reported to inhibit cell-to-cell transport [56]. The formation of Fe and Mn plaques on roots surface as rightly observed by Hansel et al. [60] on rice may provide a means of attenuation and external exclusion of metals. These plaques have been reported to increase the sequestration of Pb on the root surface and in the rhizosphere, providing a means of external exclusion of soil Pb [60].

It has been reported that these metaliferous plant species employ different strategies to be able to withstand the toxic environment. Among these, metal chelation and sequestration into the vacuole or excretion to the apoplast have been described as important mechanisms for metal tolerance by hyperaccumulators [59,61]. Some have evolved the strategy of translocating and storing metals in higher concentration in the shoot cell wall [53,62] thereby preventing the toxic ions from the most sensitive organelles through compartmentalization [37]. All the cell organelles, such as nucleus, mitochondria and chloroplasts are the key components of a plant cell which contribute to the survival of cell. The cell wall is however the main component and the first barrier against metal stress. It consists of dead tissues like lignin, cellulose and hemicellulose and so, lowers physiological activities. To overcome stress condition, hypertolerant plants have evolved physiological strategies which they employ to remove the toxic ions from the most sensitive subcellular parts, such as the cytosol which is also classified as soluble fraction and other organelles that are directly involved in the metabolic activities and concentrate it in the cell wall or vacuoles [37]. This restriction to the cell wall and sequestration in the vacuoles are the mechanisms reported to be widely used by hypertolerant species to reduce internal metal concentration and bioavailability [37,63.26].

Table 2. Lead accumulation in the root and shoot of plant species collected from Lalupon contaminated site

Name of plant	Shoot mg/kg	Root mg/kg	Soil mg/kg	Translocation factor	Bioaccumulation factor
G. celosioides	12125.0^{aA}	473.2^{fB}	697.0^{f}	25.6^{a}	18.7^{a}
C. odorata	960.0^{dA}	928.0^{dA}	$19,150.0^{e}$	1.0^{c}	0.1^{d}
R. corymbosa	2720.0^{bA}	2505.0^{aB}	495.0^{g}	1.1^{c}	1.2^{b}
S. pyramidalis	242.0^{fB}	887.5^{eA}	$38,250.0^{b}$	0.3^{d}	0.1^{d}
C. dactylon	1237.5^{cB}	1710.0^{bA}	$37,450.0^{c}$	0.7^{b}	0.1^{d}
I. cylindrica	288^{eB}	940.0^{cA}	19450.0^{d}	0.3^{d}	0.1^{d}
E. indica	156^{gB}	234.0^{gA}	40600.0^{a}	0.7^{b}	0.5^{c}

Means followed by the same alphabet are not significantly different from each other while those followed by different alphabet are significantly different at P< 0.05 using DMRT. The comparison among the plant species on the same column were indicated by a, b, c, d and e while that of root and shoot for each plant species were A and B on each row

Table 3. Lead accumulation in the root and shoot of plant species collected from Kumapayi contaminated site

Name of plant	Shoot[mg/kg]	Root[mg/kg]	Soil [mg/kg]	Translocation factor	Bioaccumulation factor
G. celosioides	930^{aA}	473.2^{bB}	650^{e}	1.26^{a}	2.6^{a}
S. pyramidalis	267^{dB}	411^{cA}	1720^{b}	0.65^{d}	0.4^{c}
I. cylindrica	122.5^{eB}	129^{eA}	1025^{d}	0.95^{c}	0.3^{d}
C. odorata	317^{cA}	268^{dB}	1090^{c}	1.18^{b}	0.5^{b}
R. corymbosa	124^{eB}	496^{aA}	1750^{a}	0.25^{e}	0.4^{c}

Means followed by the same alphabet are not significantly different from each other while those followed by different alphabet are significantly different at P< 0.05 using DMRT. The comparison among the plant species on the same column were indicated by a, b, c, d and e while that of root and shoot for each plant species were A and B on each row

Table 4. Cellular distribution of Pb in different plant species collected from contaminated site

Plant Species	Plant parts	F1	FII	FIII	FIV	FV
				Pb (µg/g FW)		
G. celosioides	Root	1070.33^{c}	13121.33^{a}	1422.33^{b}	65.40^{e}	618.76^{d}
	Stem	1039.53^{a}	1026.00^{a}	184.43^{b}	92.53^{c}	94.47^{c}
	Leaf	18531.07^{a}	1415.14^{c}	4306.51^{b}	95.33^{e}	770.53^{d}
S. pyramidalis	Root	1876.50^{a}	1467.00^{b}	114.75^{c}	49.50^{d}	45.00^{d}
	Shoot	153.23^{b}	256.50^{a}	154.75^{b}	22.50^{d}	67.50^{c}
I. cylindrical	Root	2526.70^{a}	2108.20^{a}	112.57^{c}	209.25^{b}	72.00^{c}
	Stem	114.75^{c}	238.40^{b}	746.67^{a}	90.03^{d}	64.33^{e}
	Leaf	141.75^{b}	182.25^{a}	112.47^{c}	69.75^{d}	69.74^{d}
C. odorata	Root	902.24^{a}	598.53^{b}	123.75^{c}	45.00^{d}	22.50^{e}
	Stem	769.47^{b}	92.25^{d}	953.00^{a}	102.40^{c}	2.25^{e}
	Leaf	67.53^{c}	301.23^{a}	45.00^{d}	137.25^{b}	19.50^{e}
E. indica	Root	13776.75^{a}	166.50^{b}	143.75^{c}	79.77^{d}	22.60^{e}
	Shoot	1127.25^{a}	92.25^{c}	73.60^{d}	69.75^{d}	206.67^{b}
R. corymbosa	Root	10367.50^{a}	166.50^{b}	146.70^{b}	22.60^{d}	69.76^{c}
	Shoot	1217.25^{a}	72.80^{c}	73.60^{c}	70.50^{c}	106.50^{b}
C. dactylon	Root	1127.25^{a}	92.25^{c}	74.50^{d}	79.75^{d}	213.67^{b}
	Shoot	241.75^{b}	282.25^{a}	122.48^{c}	81.75^{d}	79.74^{d}

F1 = cell wall, FII = plastid fraction, FIII = nucleus fraction, FIV = mitochondria fraction, FV= soluble fraction.
The alphabets indicate significant differences among the various cell fractions for each plant part [P<0.05]

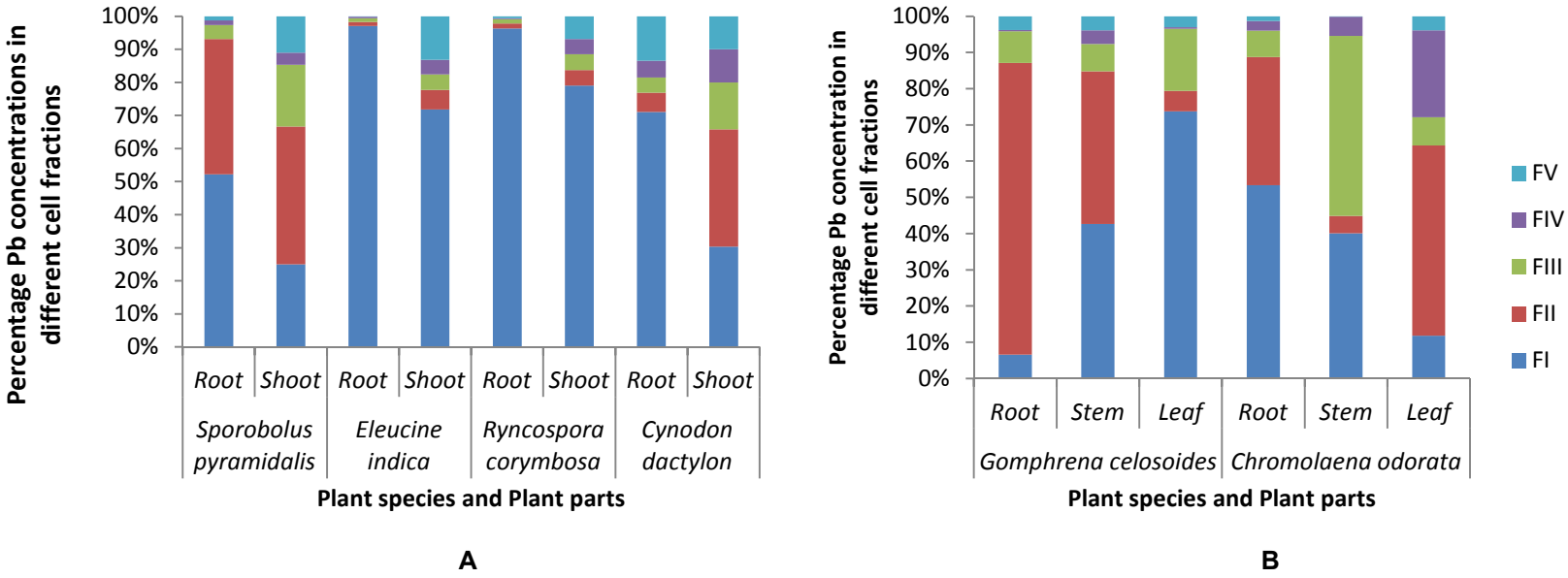

Fig. 3. Percentage Pb concentrations in different cell fractions and plant parts for different plant species sampled from contaminated sites

F1 = cell wall, FII = plastid fraction, FIII = nucleus fraction, FIV = mitochondria fraction, ~`FV= soluble fracti

In this study, the results of the cellular fractionation showed that the extraordinary tolerance mechanism adopted by these plants is confinement of the toxic metal (Pb) to the cell wall which was also a kind of exclusion principle at the cellular level. In all these plant species, the cell wall had highest Pb concentration compared to other organelles thereby preventing accumulation of lead in sensitive organelles like mitochondria and nucleus [64,37,65]. The plant cell wall is therefore the key organelle for the exclusion of metals in the plant [66]. The ability of *G. celosioides* to accumulate high concentration of Pb in the cell wall is an indication of hypertolerance ability of this species to Pb. Root cell wall with the highest Pb concentration is attributed to the ability of the root to form ligands with heavy metals. The cell wall has been reported to contain hydroxyl, carboxyl, aldehyde group, phosphate thiol and other functional groups which are capable of binding or reacting with these toxic metals thereby sequestering them [17,67].

However, the high Pb concentration found in the leaf plastids of some plant species from this contaminated site may be due to the fact that plastid is the main storage organelle in the plant. It plays a major role as the precursor of chloroplast which basically is mostly found in the leaf. This might also be a tolerance mechanism related to the binding of metals with macromolecules like organic acids which are located in the plastid fraction [68]. Accumulation of Pb in the leaf plastid fraction is therefore an important mechanism for Pb detoxification. Large amount of Cu has also been reportedly found in the cell wall and chloroplast of *Elsholtzia splendes* [68]. The Cu subcellular localization was in the order of chloroplast > cell wall > soluble fraction > other organelles. Pb is also capable of replacing divalent element like Mg^{2+} present in the chlorophyll pigment [10].

5. CONCLUSION

G. celosioides with the highest values for both bioaccumulation and translocation factors could be described as an hyperaccumulator while *E. indica, S. pyramidalis, C. dactylon* and *I. cylindrica* could be regarded as potential lead excluders/tolerant plants. The plants exhibiting TF and particularly BCF values less than one are unsuitable for phytoextraction because of limited ability of heavy metal accumulation and translocation by the plants. Plants that restrict soil–root and root–shoot transfers of heavy metals are classified as tolerant plants and therefore have less accumulation in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass. The advantage of this strategy over hyperaccumulation is the ability of metal-tolerant plant species to stabilize contaminants in soil thereby reducing the contaminant movement. Metals accumulated in the roots are considered relatively stable. It can also be concluded that restriction of Pb to the cell wall away from sensitive organelles is the most important mechanism being employed for detoxification of Pb by these plant species. Further studies are however required to fully understand the mechanisms for exclusion of *S. celosioides, E. indica* and hyperaccumulation of *G. celosioides*, respectively. These mechanisms if further studied can be used for the development of metal tolerant crops thereby preventing crop contamination by heavy metals. In other words, the mechanism of hyperaccumulation can be improved upon to enhance toxic metal uptake by hyperaccumulators as well as increasing the ability of food crops to take up more of essential nutrients most especially, the micronutrients. Judging from the results of this study, *G. celosioides* can be recommended for cleaning up of Pb contaminated site using phytoextraction technique.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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