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PHYTOREMEDIATION POTENTIAL OF *Ipomoea asarifolia* ON LEAD-POLLUTED SOILS

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ABSTRACT
This study evaluated the potential of Ipomoea asarifolia to remediate lead (Pb) polluted soils. The plant was grown on soils amended with varying levels of Pb in different polythene-pots and atomic absorption spectrophotometry (AAS) was used to analyse the accumulation of Pb in roots, stems and leaves of the plant within three harvesting phases of the study period. The results revealed that the plant accumulated a total biomass of 308.13mg, 392.07mg and 482.21mg Pb from 328.24 ± 2.33mg/kg, 433.03 ± 0.59mg/kg and 537.25 ± 0.92mg/kg Pb-polluted soils respectively. The Transportation Indices for Pb translocation to the different parts pf the plant showed that I. asarifolia has both RTI and STI of less than 1 (TI<1) for Pb, indicating that the plant has both phytoaccumulation and phytostabilisation potential for Pb in soils polluted with the heavy metal. The results therefore, suggest that the plant could have potential for phytoremediation of Pb-polluted environments.
Key words: Phytoremediation, pollution, Ipomoea asarifolia, heavy metal, lead

INTRODUCTION

Heavy metals are elements with metallic properties (e.g. conductivity and ductility) and atomic masses greater than 20. They constitute an ill-defined group of inorganic chemical hazards. Although Metals are natural components in soil, contamination results from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Rahman *et al*., 2013). Contamination of soils with heavy metals may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, food chain (soil→plant→human or soil→plant→animal→human), drinking of contaminated ground water, reduction in food quality via phytotoxicity and reduction in land usability for agricultural production, causing food insecurity and land tenure problems (Nor *et al*., 2012). The most common heavy metal contaminants are Cd, Cr, Hg, Pb, Cu, Zn, and As (Türkdogan *et al*., 2003). Some heavy metals, such as Co, Cu, Fe, Mn, Mo, Ni, V and Zn are required in minute quantities by organisms, while others, particularly As, Cd, Hg and Pb do not have any beneficial effect on organisms and are thus regarded as the “main threats” since they are very harmful to both plants and animals.

Lead is among the heavy metals most commonly found at contaminated sites. It enters the body system through air, water and food (Divrikli *et al*., 2003). Pb is a serious cumulative body poison, which can affect almost every organ and system in the body. Long-term exposure to high levels of Pb can cause severe damage to the brain and/or kidneys, and ultimately leads to death (Nikolic and Sokolovic, 2004). To safeguard public health, effective approaches are necessary to clean up soils polluted by heavy metals. Some of the conventional technologies for remediation of heavy metal polluted sites include immobilization, soil washing, and phytoremediation (Petra *et al*., 2012).

Phytoremediation is an aspect of bioremediation that uses plants for the treatment of metals-polluted sites. Naidu and Harter (1998) defined phytoremediation as an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. It is suitable when the pollutants cover a wide area of land and when the heavy metals are within the plant’s root zones (Marques *et al*., 2009). This idea of using metal-accumulating plants to remove heavy metals and other pollutants was first introduced in 1983, but the concept has been implemented for the past 300 years on waste-water discharges (Chaney and Malik, 2003).

Phytoremediation is receiving attention lately as an innovative and cost-effective alternative to the more established treatment technologies used at hazardous waste sites. Its advantages include: maintaining the biological activity and physical structure of soils, its inexpensive and it provides the possibility of biorecovery of the metals. Heavy-metals polluted soils can be phytoremediated via different mechanisms, including phytoextraction, phytostabilization, phytovolatilization, phytostimulation, phytodegradation and rhizofilteration, and the first two are particularly the focus of this work.

The ideal plant species for phytoextraction are those possessing the ability to accumulate and tolerate high concentrations of metals in harvestable tissue, with a rapid growth rate (Guisson, 2007). Accumulation of metals by plants is governed by their growth rate and ability to translocate the metals to the above-the-ground tissues (Labanowski *et al*., 2008). Large variety of plant species have been tested for their phytoremediation capacities for various metals. Examples of these plants are *Brassica* sp., clover (*Trifolium pratense* L.), panikum (*Panicum antidotal*), *Salix populas*, and *Nicotiana* sp. (Marques *et al.,* 2009).

The plant *Ipomea asarifolia* (Convolvulaceae), is a globrous succulent perennial plant trailing on the ground. It is a common weed of hydromorphic soils, low lying and inland valleys, streams and river banks, and is found throughout West Africa. In Nigeria, its common names include Duman kada (in Hausa), Ndukwu ohia (in Igbo), and Gboro ayaba (in Yoruba) (Jegede *et al*., 2009). The plant has ornamental value and is important in dune fixation. Its toxicity has been demonstrated experimentally and under natural conditions, in cattle, sheep and goats (Lombi *et al.,* 2009). However, its phytoremediation potential is yet to be reported. This study therefore, aimed at investigating the feasibility of *Ipomea asarifolia* to accumulate Pb from Pb-polluted soils, as well as its capacity to translocate the metal through its roots, stems, and leaves.

MATERIALS AND METHODS

Soil amendment with Lead (Soil pollution)

Soil sample was obtained around Biochemistry Department of Usmanu Danfodiyo University Sokoto, experimental soil amendment procedures were adopted from McGrath and Zhao (2003). 1500mg, 2000mg, and 2500mg of Lead Nitrate ($Pb(NO_3)_2$) was applied into 3kg ground soils in 3 separate polythene-pots and mixed well, 1 polythene-pot containing 3kg unamended soil (without lead) was kept as control. Therefore, the concentrations of Pb applied to the soils were 312.99mg/kg, 418.32mg/kg and 521.65mg/kg and 0mg\kg soil respectively, and each level was replicated ×3. To avoid leaching out of the metal, no drainage pathway was allowed.

Determination of chemical and physical nature of the soils

Samples of the prepared soils were analyzed for their chemical and physical characteristics. Soil pH was determined using pH metre, soil particle size was determined using hydrometre after making soil suspension with distilled water and 50 ml of sodium hexametaphosphate reagent while soil organic matter content was determined by titration with 0.5 M Ferrous sulphate solution, as described Meira *et al*., 2008.

Plant collection, identification and authentication

Seedlings of *Ipomoea asarifolia* plant growing wild around Usmanu Danfodiyo University, Sokoto were collected, identified and authenticated in the herbarium of the Botany Unit, Department of Biological Sciences of the University. A specimen with voucher number UDUH/ANS/0140 was kept in the herbarium.

Phytoremediation studies on the Pb-amended soils

Seedlings of *I. asarifolia* were carefully transplanted in each of the prepared soil pot as described above and grown for 70 days with periodic irrigation with deionized water.

Plant's harvest

The plants were harvested in three phases. Three plants were carefully harvested from each pb-concentration level and the control on days 30 (Phase 1), 50 (Phase 2) and 70 (Phase 3) of transplanting. The plants were gently washed with deionized water to remove the remaining soil. The roots, leaves and stems were separated carefully, spread over filter papers and completely air dried. The dried parts were ground to powder and stored for analysis.

Determination of Pb concentrations

Prior to analysis, soil and plant samples were digested. 1g each of the ground soil samples was weighed into a 50ml beaker and digested with a mixture of 4ml, 10ml and 2ml each of concentrated $HClO_4$, HNO_3 and H_2SO_4 respectively on a hot plate. The samples were then cooled, made up to 25 ml and filtered in volumetric flasks. 20ml of the filtrates were used to determine the concentrations of Pb using Atomic Absorption Spectrophotometer.

For the plant's roots, stems and leaves, 500mg of powdered fractions of each were digested with 10ml of $HClO_4$ and HNO_3 mixture (1:3) on a hot plate. The resulting clear solutions were made up to a mark in 25ml volumetric flasks with deionized water and subsequently filtered using Whatman filter papers. 20ml of the filtrates were analyzed for Pb using Atomic Absorption Spectrophotometer (Blaylock and Huang, 1999).

Transportation Indices (TI)

Two Transportation Indices (TI) were used to evaluate the efficiency of the Pb transport through the plant's organs. Transportation Index from roots to stems (RTI) was calculated as the ratio of Pb concentration in the stems to that in the roots. Transportation Index from the stems to leaves (STI) was calculated as the ratio of Pb concentration in the leaves to that in the stems as described by Clunel *et al.*, 2011.

Statistical Analysis

Statistical analysis was performed using IBM SPSS v20 software and one-way analysis of variance (ANOVA) was used to analyze the data. Differences were considered significant within 5% confidence interval ($p \leq 0.05$) and the values were denoted by superscripts.

RESULTS

The physical nature of the soils used in the study is presented in Table 1. The results showed that both the amended and non-amended soils were slightly acidic (pH 6.04 and 6.30 respectively) with slightly high organic matter contents of 8.86 and 9.06 respectively. The textural composition of the soil was silty clay sand in proportion: 47.26%, 29.67% and 28.42% for non-amended and 46.98%, 31.35% and 26.35% for amended soils. The application of $Pb(NO_3)_2$ salt to the soil did not significantly change these properties, making it suitable for plant's growth. The Pb uptake capacities of *I. asarifolia* and its translocation to various organs of the plant during the three harvesting phases of the study period presented in Figures 1-3 and Table 2 showed that the total biomass of Pb in the plant was 308.13mg, 392.03mg and 482.21mg for the three Pb-concentration levels respectively. The total biomass therefore, increased with increase in pb-concentration, corresponding to the metal uptake by the plant. Accumulation in the plant's organs also increased significantly ($P<0.05$) in the order: roots>stems>leaves, parallel to the increase in the metal's availability in the soils.

The Transportation Indices (TI) of Pb through the plant's parts, presented in Table 2 revealed that the two Transportation Indices; RTI and STI also increased with increase in the soil availability of the metal and were both less than 1 (TI<1). The values for RTI = 0.58, 0.59 and 0.64 and STI = 0.71, 0.87 and 0.85 observed for the three Pb concentration levels respectively, implies that *I. asarifolia* accumulates Pb in its roots higher than in the other organs, and this root confinement could be attributed to the relatively large ion size of Pb.

Table 1: Physicochemical and textural properties of soil used in the study.

Physical properties	Non-amended soils	Pb-amended soils
Clay (%)	29.67	31.35
Sand (%)	28.42	26.35
Silt (%)	47.26	46.98
pH	6.04±0.30	6.30±1.10
OM (%)	8.86±3.90	9.06±3.60

The data showed that the texture of the soil is silty clay sand, the pH is slightly acidic and organic matter (OM) content is slightly high in both soils. Amendment of the soil with $Pb(NO_3)_2$ did not significantly change these physicochemical properties. The values are expressed as means ± SEM (n=3)

Table 3: Total Pb uptake by different organs of *I. asarifolia* and TI of the roots (RTI) and stems (STI).

Initial conc. in soils (mg/kg)	**Total Pb in plant's organs (mg)**				**Transportation Indices**	
	Roots	**Stems**	**Leaves**	**Biomass**	**RTI**	**STI**
328.24	154.63	89.77	63.73	308.13	0.58	0.71
433.03	186.07	109.95	96.05	392.07	0.59	0.87
537.25	221.08	140.80	120.33	482.21	0.64	0.85
16.23	8.30	2.64	1.27	12.21	0.32	0.48

The data showed both the total biomass of Pb in the plant parts and the transportation indices (TI) increased with increasing availability of the metal in the soils. Higher concentration of Pb however, accumulated within the plant's roots more than all other parts. RTI and STI and STI are found to be less than 1, suggesting potential of *I. asarifolia* for phytoaccumulation and stabilization of the heavy metal.

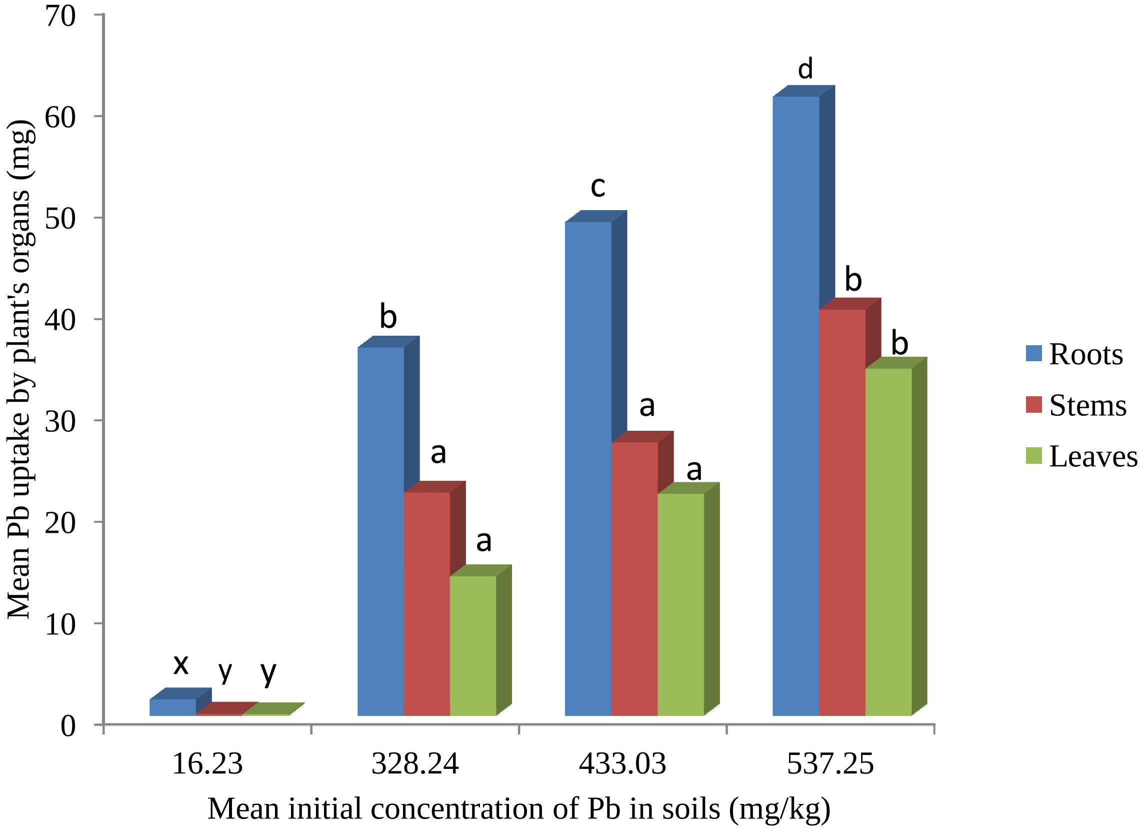

Figure 1: Pb uptake among different organs of *I. asarifolia* from Pb-amended and control soils after 30 days (phase 1) of the transplant. Pb accumulate among different parts of the plant in the order: roots>stems>leaves for all the concentration levels, which is significantly different ($P<0.05$) when superscripts located on top of the bars are different.

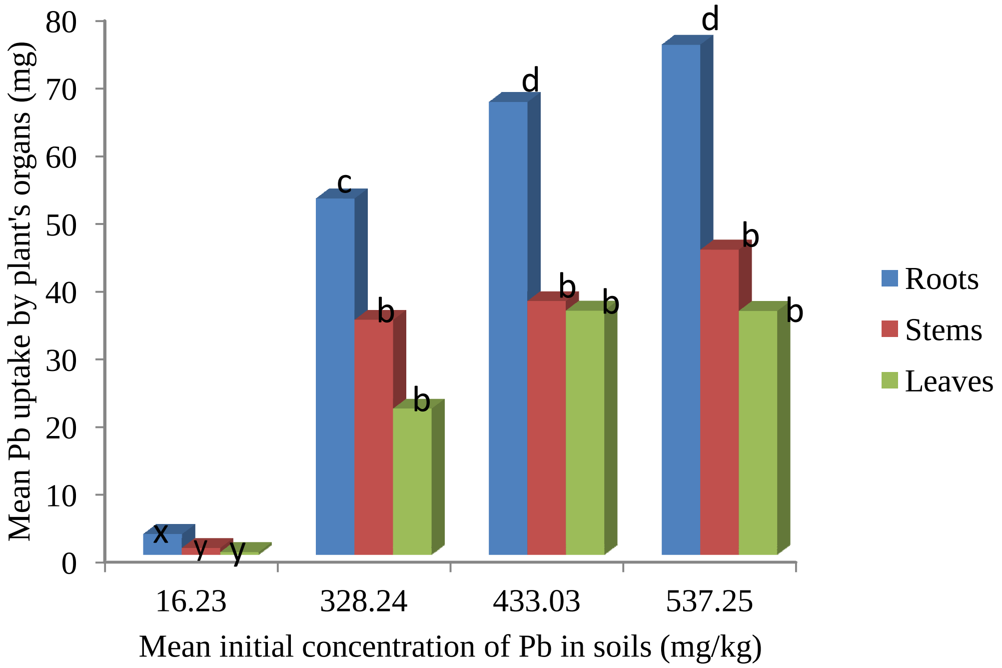

Figure 2: Pb uptake among different organs of *I. asarifolia* from Pb-amended and control soils after 50 days (phase 2) of the transplant. Pb accumulate among different parts of the plant in the order: roots>stems>leaves for all the concentration levels, which is significantly different (P<0.05) when superscripts located on top of the bars are different.

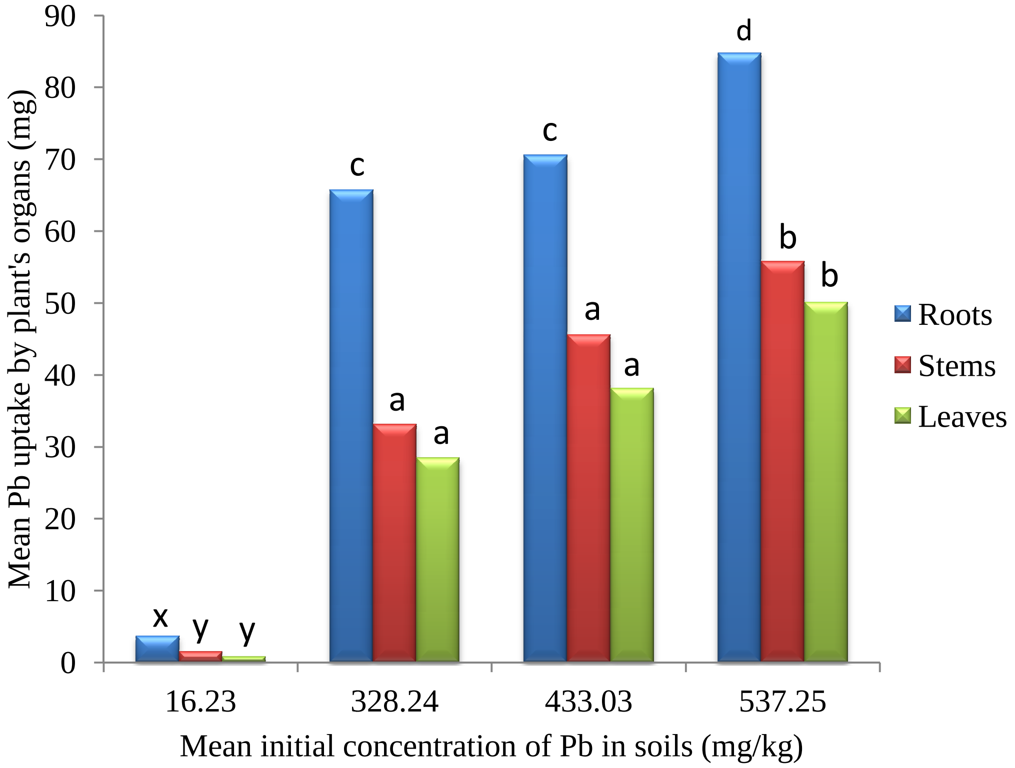

Figure 3: Pb uptake among different organs of *I. asarifolia* from Pb-amended and control soils after 70 days (phase 3) of the transplant. Pb accumulate among different parts of the plant in the order: roots>stems>leaves for all the concentration levels, which is significantly different (P<0.05) when superscripts located on top of the bars are different.

DISCUSSION

The physicochemical nature of the soils used in the study showed that the soil is suitable for plant's growth with the textural composition of silt clay sand and average pH values of 6.04±0.3, 6.4±1.10, for non-amended and Pb-amended soils, respectively (Table 1). The application of Pb in the soils at various degrees had no significant effect on the initial pH values of the soils. This could be attributed to the low production of hydroxyl ions as a result of the reaction between the salts and the soil elements. Jieng-feng *et al.* (2009) also reported similar observation in soils experimentally polluted with ($Pb(NO_3)_2$) and $CdCl_2$. Organic matter contents were also slightly elevated in all the soils. The elevated organic matter contents were probably due to plants litter deposited on the surface of the soils over the years (Jieng-feng *et al.*, 2009). The solubility of trace elements in soils depends on the soil pH and organic matter contents (humic acids, fulvic acids, polysaccharides and organic acids) (Chaney and Malik, 2003).

The Pb-amended soils had high concentrations of Pb (328.24±2.33mg/kg, 433.03±0.59 mg/kg, 537.25±0.92mg/kg) which were proportional to the amount of salts applied. These concentrations were higher than their maximum acceptable levels in soils (WHO, 1998) and a clear indication that the soils were experimentally amended with the heavy metal. The plant showed high level of tolerance to these concentrations throughout the study period. This is an indication that the plant has mechanisms to tolerate the presence of heavy metals in soils in which they grow and withstand their toxic effects. Many phytoremediation plants have also been reported as developing mechanism against toxicity of heavy metals in their surroundings (Jarup, 2003; Li *et al.*, 2007). However, this was unlike observations by some workers (Chen *et al.*, 2004; Marques *et al.*, 2009) that reported symptoms of leaf chlorosis and yellowishness in plants grown in high dosage of Cd and Pb in artificially polluted soils.

The results from the study showed that *I. asarifolia* has the capacity to accumulate Pb in its various organs proportional to the metals' concentrations in soils. As shown in Table 2, the total Pb biomass in the plant were 308.13mg, 392.07mg and 482.21mg from 328.24mg/kg, 433.03mg/kg and 537.25mg/kg Pb in polluted soils respectively (Table 2). High Pb accumulation by the plant may be attributed to its metal exclusion strategies (Peterson, 1983). This however, is contrary to Ross (1994) who reported that 30-300mg/kg of Cd and Pb are toxic to most plants. Meanwhile, the maximum permissible limits of Pb in plants as recommended by WHO (1998) is 2.0 mg/kg.

The translocation of the metals through the plant's organs was examined and presented in Figures 1-3, which showed that the uptake and distribution of Pb differ significantly ($P<0.05$) among the roots, stems and leaves of the plant in an increasing order: leaves>stems>roots and proportional to the Pb concentrations in the soils. The trend could be attributed to the availability of the metal in the soils. This concur with Rahman *et al.*, 2013, who reported that increase in availability of metals in soils increases their rate of uptake by phytoremediation plants. This effect has also been reported in several phytoremediation plants (Jeanna and Henry, 2000; Lombi *et al.*, 2009; Marques *et* al., 2009).

High amount of Pb is confined within the roots of *I. asarifolia,* an observation which could be attributed to the relatively large ion size of the metal. The implication of this is that Pb may not be easily translocated from the roots to stems and leaves of the plant when absorbed from the soils and therefore, the plant may not be suitable for phytoextraction of Pb from Pb-polluted soils. However, as reported by others; plants that have this attribute are suitable for phytostabilization of metals (Naidu and Harter, 1998; Chen *et al.*, 2004). In phytostabilization, plants reduce the mobility and availability of pollutants in their environment either by immobilizing or by preventing them from migration to other areas of the site (Meira *et al.,* 2008).

The translocation capacity of Pb through the organs of *I. asarifolia* from different concentrations in soils was evaluated as Transportation Indices (TI) of the metal. In all the harvesting periods, it was observed that translocation of the metal from roots to stems i.e. Root Transportation Index (RTI) and from stems to leaves of the plant i.e. Stems Transportation Index (STI) increased with increase in Pb availability in the soils. This is a further indication that the plant has the potential to withstand the toxic effects of Pb even at high concentrations. In all the harvest periods, the Transportation Indices of Pb (Table 2), were less than one ($TI<1$); an indication that the plant has the potential for phytostabilization of Pb. This is attributed to the plant's inefficiency to transport Pb from its roots to other organs (Divya *et al.* 2012).

Finally, since *Ipomoea asarifolia* accumulated in its organs, amounts of Pb that were much higher than those considered toxic for most plants and is hardly eaten by farm animals (Cui *et al.*, 2007), the fear of it contaminating the food chain when grown in Pb-polluted sites is avoided. The plant could therefore help prevent potential Pb toxicity in the ecosystem.

CONCLUSION

The results of the study have shown that *I. asarifolia* immobilized and resisted high concentrations of Pb in its various parts. This observation confirmed that the plant has potentials for both phytoaccumulation and phytostabilization of Pb from the polluted soils, and could therefore, be effective in phyremediation of Pb-polluted environments.

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