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Phytoremediation of Cadmium-Polluted Soils with *Ipomoea asarifolia* (Desr.) Roem. & Schult

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ABSTRACT: Phytoremediation is an alternative method for restoring soils polluted with heavy metals which is cost-effective and environment-friendly. The present study evaluated the potential of *Ipomoea asarifolia* to remediate soils experimentally-amended with Cadmium. The plant was grown on soils amended with 0, 1500, 2000, and 2500 mg $CdCl_2$ salt. The salt was mixed with small portions of the soils and made upto 3kg salt/soil mixtures each. These were applied into 4 separate polythene-pots labelled; A, B, C and D respectively. Sample A containing 3kg non-amended soil (without Cd) served as the control. The concentrations of Cd applied to the soils were therefore; 0, 306.61, 408.82 and 511.02 mg//kg soils in the samples A-D respectively. Atomic absorption spectrophotometry (AAS) was used to analyse the bioaccumulation of Cd in the plant's parts, over three harvesting phases of the study period. The results revealed that *I. asarifolia* is a good phytoaccumulator as it accumulated a total biomass of 0.23 ± 0.63, 272.85 ± 1.99, 377.40 ± 0.63 and 459.48 ± 0.60 mg/kg Cd from the amended soils A-D respectively. The Transportation Indices; RTI and STI for translocation of Cd to the plant's stems and leaves were both greater than 1 (TI >1), indicating that the plant has a phytoextraction potential for Cadmium. These results therefore, suggest that *I. asarifolia* could be effective in phytoremediation of Cadmium-polluted environments.

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Chemical elements with metallic properties, such as conductivity and ductility having atomic masses greater than 20 are termed heavy metals. Heavy metals can contaminate the general environment through many routes. Following deposition from natural and artificial sources, they accumulate in soils and water systems for many years due to their stability. Thus, representing an important environmental pollution problem with its potential adverse effects on food safety and health (Nordberg *et al*., 2005).

Contamination of soils with the heavy metals results from the industrial wastes, 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). They do not degrade like organic molecules, once introduced into, heavy metals remain in the environment. The only exceptions are Hg and Se which can be transformed and volatilized by microorganisms (Tomsett *et al*., 1992).

Cadmium is among the highly toxic heavy metals, chronic exposure to even its lower levels causes its build-up in the kidneys leading to kidney and lung damage, fragile bones, hypertension, cardiovascular disease, arthritis, diabetes, anaemia, cancer, cirrhosis, reduced fertility, hypoglycemia and strokes (Cui *et al*., 2004; Singh *et al*., 2011). The exact safe levels of heavy metals in soil is not clear. As a result, there is a wide discrepancy among different countries regarding the exact safe value of each heavy metal in the soil. For example, the maximum permissible level of Cd in soil set out by EU, UK and USA standards are 3.0, 1.8 and 3.0 mg/kg respectively (EAA, 2007). To safeguard public health, effective approaches to clean up soils polluted by heavy metals are therefore, necessary.

Elimination of heavy metals from the contaminated environment by conventional remediation methods is not only difficult, but expensive and time-consuming, especially when it involves large areas of soil. Remediation can be done *in situ* (on-site), or *ex situ* (removed and treated off-site), and both approaches are extremely expensive (Kabata-Pendias and Pendias 1989). *In-situ* methods are used to remediate soil without excavation of the contaminated site. These involve technologies that remove or transform the metals by immobilization to reduce their bioavailability and ease separation from the bulk soils (Igwe and Abia, 2006). *Ex-situ* methods involve removal of the contaminated soil for restoration on or off-site, and returning the restored soil back to the site. The conventional *ex-situ* methods rely on excavation, detoxification and/or destruction of the metal physically or chemically by stabilization, solidification, immobilization or incineration (Petra *et al*., 2012; Jeanna and Henry, 2000).

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.

Lately, phytoremediation is receiving attention 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, it is inexpensive and provides the possibility of biorecovery of the metals. Heavy-metals polluted soils can be phytoremediated via different mechanisms, including phytoextraction, phytoaccumulation, phytostabilization, phytovolatilization, phytodegradation and rhizofilteration (Chaney and Malik, 2003). The first two are particularly the focus of this work.

Ipomoea asarifolia (salsa or ginger-leaf morning-glory) is a tropical, globrous succulent perennial plant in the convolvulaceae family of the genus *Ipomoea*. It is a common weed of hydromorphic soils, low-lying and long-trailing the ground in inland valleys, streams and river banks, found throughout West Africa.

In Nigeria, its common names include 'Duman kada', 'Gboro ayaba' and 'Ndukwu ohia' in Hausa, Yoruba and Igbo languages respectively (Jegede *et al*., 2009). The plant has ornamental value and is important in dune fixation. Its toxicity in cattle, sheep and goats has been demonstrated experimentally under natural conditions and therefore hardly eaten by farm animals (Lombi *et al.,* 2009). This study investigated the phytoremediation potential of *I. asarifolia* in Cd-polluted soils.

MATERIALS AND METHODS

Soil amendment with Cadmium Chloride (soil pollution): Soil sample was obtained from the surroundings of Biochemistry Department, Usmanu Danfodiyo University Sokoto. Experimental soil amendment procedures were adopted from McGrath and Zhao (2003). Cadmium Chloride was ground and varying quantities of 0, 1500, 2000, and 2500 mg $CdCl_2$ salt were mixed with a small portion of the soil and made up to 3kg metal/soil mixtures. The contents were mixed-well for even distribution of the salts in the soils and applied into 4 separate polythene-pots labelled A, B, C and D respectively. Sample A contains 3kg non-amended soil (without Cd) and served as the control. Therefore, the concentrations of Cd applied to the soils were 0, 306.61, 408.82 and 511.02 mg Cd/kg soils in the samples A-D respectively and each treatment level was replicated ×3. The suspensions were left uncovered at ambient temperature for five days to allow the metal to stabilise and speciate before the commencement of the experiment.

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 0.5M Ferrous Sulphate solution, as described by Meira *et al*., 2008.

Plant collection, identification and authentication: Seedlings of *I. asarifolia* plant growing wild in the permanent site, 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 Cd-amended soils: Seedlings of *I. asarifolia* were carefully transplanted in each of the prepared soil pots as described above and grown for 70 days with periodic irrigation with deionized water. To avoid leaching out of the metal, no drainage pathway was allowed.

Plant's Harvest: The plants were harvested in three phases. Three plants were carefully removed from each Cd-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 carefully separated, spread over filter papers and completely air dried. The dried parts were ground to powder and stored until required for analysis.

Determination of Cd 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 Cd 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 analysed for Cd using Atomic Absorption Spectrophotometer (Blaylock and Huang, 1999).

Transportation Indices (TI): Two Transportation Indices (TI) were used to evaluate the efficiency of the Cd absorption and transport through the plant's organs. Transportation Index from roots to stems (RTI) was calculated as the ratio of Cd concentration in the stems to that in the roots. Transportation Index from the stems to leaves (STI) was calculated as the ratio of Cd 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 AND DISCUSSION

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) and with slightly high organic matter contents of 8.86 and 9.06 respectively. The textural composition of the soil was silty-clay-sand in the proportion: 47.26%, 29.67% and 28.42% for non-amended and 46.98%, 31.35% and 26.35% for Cd-amended soils. The application of ($CdCl_2$) salt to the soil did not significantly change these properties, making it suitable for plant's growth. The Cd uptake capacity 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 Cd in the plant was 0.23 ± 0.63, 272.85 ± 1.99, 377.40 ± 0.63 and 459.48 ± 0.60 mg/kg Cd from the Cd-concentration levels A-D respectively. Thus, the total Cd biomass increased with increasing Cd-concentration in the soil. Cd-accumulation in the plant's organs also increased significantly ($P<0.05$) in the order: leaves > stems > roots, parallel to the increase in the metal's availability in the soils.

The Transportation Indices (TI) of Cd translocation through the plant's parts, presented in Table 2 revealed that both RTI and STI decreased with increase in the availability of the metal in the soils and both have values greater than 1 (TI >1), suggesting that *I. asarifolia* is a promising phytoextracting plant. The values for RTI = 0.00, 1.51, 1.44 and 1.17 and STI = 0.00, 1.61, 1.45 and 1.22 observed for the four Cd concentration levels respectively, implies that *I. asarifolia* accumulated Cd in its leaves more than the other organs. This high accumulation of Cd in the plant's leaves could be its mechanism of avoiding the toxic effects of the metal in its stems.

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

Property	Non-amended soils	Cd-amended soils
Clay (%)	29.67	33.26
Sand (%)	28.42	30.42
Silt (%)	47.26	40.22
pH	6.04	6.40
OM (%)	8.86	8.11

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 $CdCl_2$ salt did not significantly changed these physicochemical properties. The results showed that the total biomass of Cd in the plant's parts increased while the transportation indices (TI) decreased with increasing concentration of $CdCl_2$ salt in the soils. Higher amounts of Cd accumulated within the plant's leaves more than all other parts. The values for RTI and STI were both greater than 1 (TI >1), suggesting that *I. asarifolia* has the potential for phytoextraction of the heavy metal. Values are expressed as means ± SEM, (n=3), ND = Not detected. The current study investigated the potential of *I. asarifolia* for phytoremediation of soils experimentally-amended with varying quantities of Cadmium Chloride ($CdCl_2$) salt and the results express the plant's capacity to take up and accumulate appreciable amounts of Cd in its parts, especially leaves.

Table 2: Total Pb uptake by different organs of *I. asarifolia* and TI of the roots (RTI) and stems (STI).

Initial Conc. in Soils (mg/kg)	Cd Concentration in Plant's Organs (mg)				Transportation Indices	
	Roots	Stems	Leaves	Total Biomass	RTI (Roots)	STI (Stems)
0.61	ND	ND	0.23	0.23 ± 0.63	0	0
307.11	55.15	83.51	134.19	272.85 ± 1.99	1.51	1.61
409.22	92.13	133.05	152.22	377.40 ± 0.63	1.44	1.45
511.62	127.39	149.43	182.66	459.48 ± 0.60	1.17	1.22

At high concentrations and when mobile in the environment, heavy metals can move between media (e.g. soil to water) and taken up by plants. 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 slightly acidic pH. The application of Cd to the soils had no significant effect on its pH (Table 1). This could be attributed to the low production of hydroxyl ions by the reaction between the $CdCl_2$ and the soil elements. This agrees with the report by Jieng-feng *et al*., (2009) in soils experimentally-polluted with $CdCl_2$ and ($Pb(NO_3)_2$).

In general, interaction of metal with soil matrix is central to its phytoremediation by plants, because the activity of metals is reduced by their sorption to the soil particles (Chaney and Malik, 2003). Desorption of metals from soil into solution is enhanced in acidic soils due to H^+ competition for the binding sites. Soil pH therefore, affects not only the bioavailabilty, but also metal uptake into the plant's roots. This effect however, appears to be metal specific, for example; Zn uptake into the roots of *T. caerulescens* showed little pH dependence, whereas as uptake of Cd, Cr and Mn depended highly on the soil acidity (Chaney and Malik, 2003). Organic matter contents were slightly elevated in all the soil samples (Table 1). The elevation may probably be due to the plants litter deposited on the surface of the soils over the years (Jieng-feng *et al*., 2009). Soil pH and organic matter contents (humic acids, fulvic acids, polysaccharides and organic acids) affects the solubility of trace elements in them (Chaney and Malik, 2003). The Cd-amended soils had high concentrations of Cd proportional to the amounts of the salt added which were higher than their maximum acceptable levels in soils (WHO, 1998), a clear indication that the soils were experimentally amended with the heavy metal.

Toleration of high concentrations of Cd by *I. asarifolia* throughout the study period indicates that the plant had mechanisms to avoid the toxic effects of the metal in the soils in which it grows. Many other phytoremediation plants with mechanisms against toxicity of heavy metals in their surroundings have been reported (Jarup, 2003; Li *et al*., 2007). Contrary to this, some studies reported symptoms of leaf chlorosis and yellowishness in the plants grown in high doses of Cd in artificially polluted soils (Chen *et al.,* 2004; Marques *et al*., 2009). The results from the study showed that *I. asarifolia* accumulated high amounts of Cd in its various organs (biomass) especially in the leaves proportional to the metal's concentrations in the soils (Table 2). The maximum permissible limits of Cd in plants recommended by WHO (1998) is 0.02 mg/kg.

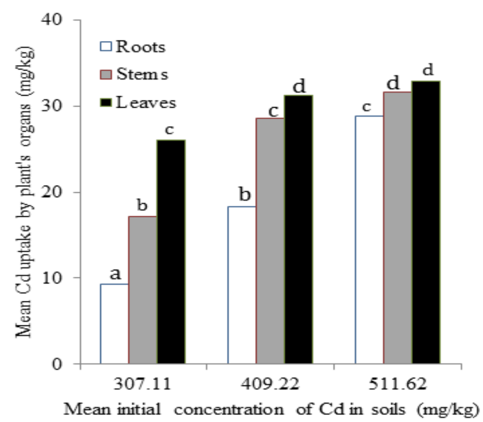


Fig 1: Cd uptake into different organs of *I. asarifolia* from Cd-amended and control soils 30 days (phase 1) after transplanting. Cd accumulated in the different parts of the plant in the order: leaves > stems > roots for all the concentration levels, which is significantly different (P<0.05) where superscripts located on top of the bars are different.

The translocation of the metals through the plant's organs was equally examined. The results obtained in all the harvesting periods as presented in Figures 1-3 showed that uptake and distribution of Cd differ significantly ($P<0.05$) among the roots, stems and leaves of the plant in an order: leaves > stems > roots proportional to the Cd concentrations in the soils. This trend could be attributed to the bioavailability of the metal in the soils which allowed for increasing rate of uptake by *I. asarifolia* (Rahman *et al*., 2013). Several studies have reported similar effect in other phytoremediation plants (Chaney and Oliver, 1996; Salt *et al*., 1998; Jeanna and Henry, 2000; Lombi *et al.,* 2009; Marques *et* al., 2009). In all the harvesting periods, the Translocation Index of the Cd from roots to stems of the plant (RTI) and from stems to leaves of the plant (STI) decreased with increased Cd bioavailability in the soils (Tables 2). This could possibly mean that the plant's phytoextraction ability for Cd may reduce with increased concentrations of the metals in polluted soils. The high roots-to-stems and stems-to-leaves translocation of any metal in the plant indicates that such plant has potential to be used in the phytoextraction of the metal (Salt *et al.,* 1998). The accumulation of higher concentrations of Cd in the leaves of the plant could be one of its mechanisms of avoiding the toxic effects of the metals in its stems, this correlated with observation reported by Marques *et al*. (2009) that accumulation of higher amounts of heavy metal(s) in the aerial or easily harvested parts is a feature of the of plants suitable for phytoextraction.

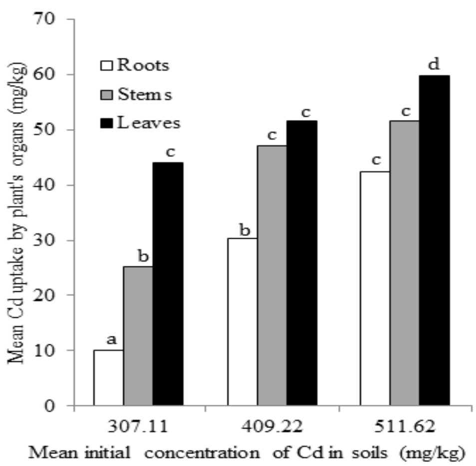
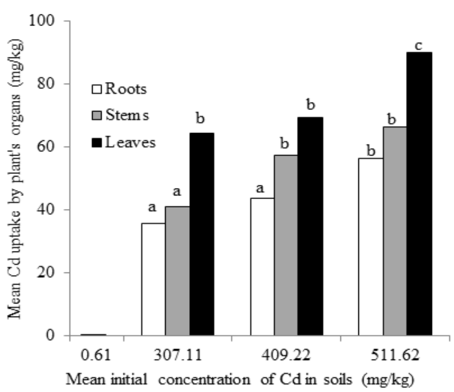


Fig 2: Cd uptake into different organs of *I. asarifolia* from Cd-amended and control soils 50 days (phase 2) after transplanting. Cd accumulated in the different parts of the plant in the order: leaves > stems > roots for all the concentration levels, which is significantly different ($P<0.05$) where superscripts located on top of the bars are different.



Fi 3: Cd uptake into different organs of *I. asarifolia* from Cd-amended and control soils 70 days (phase 3) after transplanting. Cd accumulated in the different parts of the plant in the order: leaves > stems > roots for all the concentration levels, which is significantly different ($P<0.05$) where superscripts located on top of the bars are different.

Phytoextraction is the most common mechanism of phytoremediation by plants through which they remove metal contaminants from the soil without altering the soil structure and/or fertility (Divya *et al.*, 2012). Phytoextractors accumulate heavy metals in their roots and shoots which can later be harvested and incinerated. Features that enable the plants for phytoextraction include rapid growth rate, extensive root system, high biomass and toleration of high amounts of heavy metals (Marques *et al*., 2009).

The translocation trend of Cd through the organs of *I. asarifolia* from different concentrations of the metals in soils was evaluated as the Transportation Indices (TI) of the metal. TI from roots to stems (RTI) is given by the ratio of Cd concentration in the stems to that in the roots. TI from the stems to leaves (STI) is obtained as the ratio of Cd concentration in the leaves to that in the stems (Clunel *et al*., 2011). Transportation Indices determine the efficiency of the plant to translocate metals from the root to the shoot, and from the shoot to the leaves (Ma *et al*., 2001). Plants with Transportation Indices less than one (TI<1) have the potential for phytostabilisation while those with Transportation Indices greater than one (TI>1) have the potential for phytoextraction (Labanowski *et al*., 2008). In all the harvesting periods, the TI of Cd is greater than one (TI>1), indicating that *I. asarifolia* has high potential for phytoextraction of Cd when grown in Cd-polluted soils. Furthermore, the accumulation of excessive concentration of Cd (>100mg/kg) in the plant's biomass showed that *I. asarifolia* is a good phytoaccumulator of Cd. The capacity to tolerate high amounts of heavy metals by plants leads to accumulation of the metal in the plant's harvestable parts; and which often poses a potential problem to the food chains (Cui *et al*., 2007). Fortunately, *I. asarifolia* is hardly eaten by farm animals so the fear of it contaminating the food chain when grown in sites polluted by Cd is highly limited.

Conclusions: From the results obtained in this study, it is apparent that *I. asarifolia* accumulated and resisted high concentrations of Cd in its biomass. It is therefore logical to conclude that the plant has promising potentials for phytoremediation of Cd-polluted soils with limited toxicity to the ecosystem. The plant could therefore, be effective for use in soil remediation programme.

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