

Phytoextraction and Bioaccumulation Potentials of *Ipomoea asarifolia* (Desr.) Roem. & Schult on Chromium-polluted Soils

ABSTRACT

Once deposited, heavy metals accumulate in soils and water systems over time due to their stability, representing an important environmental pollution problem with many potential adverse effects on health and food safety. The present study investigated the phytoextraction and bioaccumulation potentials of *Ipomoea asarifolia* (Ginger-leaf morning-glory) on soils experimentally-polluted with Chromium (Cr). The plant was grown on soils amended with 0, 1500, 2000, and 2500 mg Chromium (VI) chloride (CrCl₆) salt, mixed with small portions of soil and made up to 3kg salt/soil mixtures each. These were thoroughly homogenised and applied into 4 separate polythene-pots, labelled A, B, C and D respectively. Sample A contains 3kg unpolluted soil which served as the control. The concentrations of Cr applied were therefore; 0, 102.20, 134.85 and 167.76 mg/kg in the soils A-D respectively, and each concentration level was made in triplicates. The sorption and bioaccumulation of Cr in the plant's roots, stems and leaves, over three harvesting phases of the study period was analysed using Atomic absorption spectrophotometry (AAS). The results revealed that *I. asarifolia* accumulated a total biomass of 0.12±0.03, 93.81±0.88, 118.45 ±0.87 and 155.63±3.18 mg/kg Cr from the soils A-D respectively, indicating that the plant has an exciting potential for bioaccumulation of Cr. The two Transportation Indices (TI); Roots Transportation Index (RTI) and Shoot Transportation Index (STI) for uptake of Cr into the plant's stems and leaves respectively, were all found to be greater than one (TI>1), an indication of the plant's phytoextraction potential for Cr. The results obtained therefore, suggest that *I. asarifolia* could be effective in phytoremediation of Chromium-polluted soils.

Keywords: Chromium, pollution, phytoextraction, bioaccumulation, *Ipomoea asarifolia*.

Introduction

Heavy metals can build-up in the environment through natural and artificial sources. Once introduced, they remain in the soils and water systems for many years due to their stability (Nordberg *et al.*, 2005). Heavy metals do not degrade like organic (carbon-based) molecules, with the exceptions of mercury (Hg) and selenium

(Se) which can be transformed and volatilized by microorganisms (Tomsett *et al.*, 1992). Normally, agricultural soils contain low background levels of heavy metals, but industrial activities or by-products, and other soil amendment practices add small amounts of the heavy metals to soils, increasing their natural levels and causing their

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build up over time (Jayakumar *et al.*, 2013). Contamination of soils with the heavy metals could result from many other sources, such as mine tailings, disposal of high metal wastes, leaded gasoline and paints, sewage sludges, petrochemicals spillage etc (Rahman *et al.*, 2013).

Chromium (Cr), cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), copper (Cu) and zinc (Zn) are the most common heavy metal pollutants of soils (Türkdogan *et al.*, 2003). Some heavy metals, such as Cobalt (Co), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Vanadium (Vn), Cu and Zn though considered as a group of inorganic chemical hazards, are beneficial microelements required in minute quantities by organisms for proper growth and wellbeing, while others particularly Cd, As, Hg and Pb are toxic and considered as 'cumulative poisons' because they do not have any known beneficial effect in humans (Trichopoulos *et al.*, 1997). Chromium (VI) compounds are known human carcinogens, whilst chromium (III) is considered as an essential element. The maximum permissible limits of Cr in agricultural soils established by World Health Organization is 100 mg/kg (WHO, 1998).

Phytoextraction process uses plants to eliminate heavy metal contamination from soils without destroying the structure, texture and fertility of the soil. It is the most common mechanism of phytoremediation and involves bioaccumulation of metals in the roots and shoots (stems and leaves) of phytoremediation plants, which are later harvested and incinerated (McGrath and Zhao, 2003; Divya *et al.*, 2012). 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 cheap and provides possibility of bio-recovery of metals (Marques *et al.*, 2009). Hyperaccumulation plants could accumulate high concentrations of metals in their shoot biomass even when these metals are

present at low concentrations in the bulk soil. This has been observed in both field and laboratory studies (Baker and Whiting, 2002).

Ipomoea asarifolia (ginger-leaf morning-glory) is a tropical, succulent perennial plant of the family *convolvulaceae* and genus *Ipomoea*. It is a common weed found throughout West Africa, low-lying and creeping along the ground in valleys, streams and river banks. Its local names in Nigeria include; 'Duman kada' in Hausa, 'Gboro ayaba' in Yoruba and 'Ndukwu ohia' in Igbo languages (Jegade *et al.*, 2009). The plant has ornamental value and is used 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). To safeguard public health, cost-effective approaches for cleaning-up soils polluted with heavy metals are necessary. This study therefore, investigated the capacity of *I. asarifolia* to sorb and accumulate Cr from amended soils as a natural and environment-friendly soil restoration approach. The plant has advantage over other phytoremediation plants in that it's drought-resistant, succulent, extensively-leafy and hardly eaten by farm animals, thus eliminating the fear of food chain contamination and highly limiting toxicity to the ecosystem.

Materials and Methods

Amendment of Soils with Chromium (VI) chloride (CrCl₆)

The study was conducted in the Biological Garden of Usmanu Danfodiyo University Sokoto, Nigeria from April 30, 2017 through July 8, 2017. Soil sample was collected from the surrounding area of Biochemistry Department of the University. Experimental soil amendment procedures were adopted from McGrath and Zhao (2003). CrCl₆ salt was ground and varying quantities; 0, 1500, 2000 and 2500 mg were mixed with small portions 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 which served as the control. Therefore, the concentrations of Cr were; 0, 102.20, 134.85 and 167.76 mg//kg in the soil samples A-D respectively, and each concentration level was made in triplicates. The mixtures were left uncovered at ambient temperature for five days to allow the metal to stabilise and speciate before commencement of the experiment.

Analysis of Physicochemical Properties of the Soil

The physicochemical properties of the prepared soil samples were analyzed by standard analytical procedures. The pH was determined using pH meter, particle sizes were determined using hydrometer following soil suspension with distilled, deionized water and 50ml of sodium hexametaphosphate reagent. 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

The seedlings of *I. asarifolia* growing wild around sports area of permanent site, Usmanu Danfodiyo University Sokoto, Nigeria 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 for record purposes.

Phytoextraction and Bioaccumulation of Cr from Cr-amended Soils

The collected seedlings of *I. asarifolia* were carefully transplanted in each of the prepared soil samples A-D and allowed to grow for 70 days. The plants were periodically irrigated with distilled, deionized water ensuring that no drainage path was allowed to prevent leaching out of the metal. The plants were harvested in three phases during the

period. Three (3) plants were carefully uprooted from each concentration level and the control on days 30 (Phase 1), 50 (Phase 2) and 70 (Phase 3) after transplanting. The removed plants were gently washed with deionized water to remove the remaining soil and the parts (roots, leaves and stems) carefully separated, spread over filter papers and air-dried. The dried parts were ground to powder with a mortar and pestle, sieved through a 2 mm-mesh sieve to obtain fine particles. These were put into clean plastic bags and stored in the laboratory at 4-6°C until required for the analysis.

Determination of Chromium Concentrations in the Soil and Plant's Parts

Exactly 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 HClO₄, HNO₃ and H₂SO₄ respectively on a hot plate. Following completion of the digestion, samples were cooled, made up to 25 ml with distilled and deionized water, and filtered using Whatman filter paper in volumetric flasks. Exactly 20 ml of the filtrates were used to determine the concentrations of Cr using Atomic Absorption Spectrophotometer (Model: AA-6300). For the plant's roots, stems and leaves, 500mg of powdered fractions of each were digested with 10ml of HClO₄ and HNO₃ mixture (1:3) on a hot plate. The resulting clear solutions were made up to a mark of 25ml in volumetric flasks with deionized water and subsequently filtered using Whatman filter papers. Exactly 20ml of the filtrates were analysed for Cr using Atomic Absorption Spectrophotometer (Blaylock and Huang, 1999).

Chromium Transportation Indices (TI)

The efficiency of Cr sorption and translocation to the various parts of *I. asarifolia* was evaluated by determining the two Transportation Indices (RTI and STI), as described by Clunel *et al.*, 2011. Root Transportation Index (RTI) measures transport of the metal from roots to stems and was calculated as the ratio of Cr concentration in the stems to that in

the roots, while Shoot Transportation Index (STI) measures transport of the metal from stems to leaves and was calculated as the ratio of Cr concentration in the leaves to that in the stems.

Statistical Analysis

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

Results

The results of the study were summarized in Tables 1-2 and Figures 1-3. Table 1 showed the physicochemical characteristics of the soil samples used in the study. The results revealed a pH of 6.04 and 6.30, and organic matter contents of 8.86 and 9.06 for the Cr-amended and non-amended soils respectively, indicating that both soils were slightly acidic with slightly high organic matter contents. The textural composition of the soil was silt-clay-sandy in the proportions: 47.26%, 29.67% and 28.42% for non-amended and 46.98%, 31.35% and 26.35% for Cr-amended. The application of CrCl_6 salt to the soil did not significantly alter these properties, making it suitable for the plant's growth. The Cr-amended soils had increased concentrations of Cr proportional to the amounts of the CrCl_6 salt added and were greater than 100 mg/kg WHO maximum permissible levels in soils (WHO, 1998). This clearly indicated that the soils were experimentally-amended with the heavy metal.

Cr-sorption capacity of *I. asarifolia* and its bioaccumulation into the plant's part within the three harvesting phases of the study period were presented in Table 2 and Figures 1-3. These results showed that the total biomass of Cr in the plant parts was 0.12 ± 0.03 , 93.81 ± 0.88 , 118.45 ± 0.87 and 155.63 ± 3.18 mg/kg Cr from the soil samples A-D

respectively. Thus, there was significant ($p < 0.05$) increase in the total Cr biomass with increasing Cr-concentrations in the soil. In contrast, the capacity to transport Cr through the plant's parts measured by the Transportation Indices (RTI and STI), presented in Table 2 revealed that both indices decreased with increasing concentration of Cr in the soil, but the values were greater than 1 ($\text{TI} > 1$); RTI: 1.94, 2.30, 2.01 and 1.68; STI: 0.59, 1.61, 1.44 and 1.20 for the Cr-concentration levels A-D respectively. These results indicate that *I. asarifolia* has a great phytoextraction potential. The bioaccumulation of Cr in the plant's parts increases significantly ($p < 0.05$) in the order: leaves>stems>roots, implying that the plant has accumulated higher amounts of Cr in its leaves than stems and roots, signifying that *I. asarifolia* is a promising bioaccumulator of Cr.

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

Physical properties	Non-amended soil	Cr-amended soil
Clay (%)	29.67	32.22
Sand (%)	28.42	26.66
Silt (%)	47.26	39.69
pH	6.04	6.50
OM (%)	8.86	8.60

Table 2: Concentration of Cr in the Parts of *I. asarifolia* and the Transportation Indices

Initial Conc. in Soils (mg/kg)	Total Cr in Plant's Parts (mg/kg)				Transportation Indices	
	Roots	Stems	Leaves	Total Biomass	RTI	STI
1.99	0.36	0.70	0.41	1.47	1.94	0.59
102.20	13.39	30.77	49.65	93.81	2.30	1.61
134.85	20.11	40.36	57.98	118.45	2.01	1.44
167.76	33.11	55.72	66.80	155.63	1.68	1.20

Values were expressed as mean ±SEM (n=3)

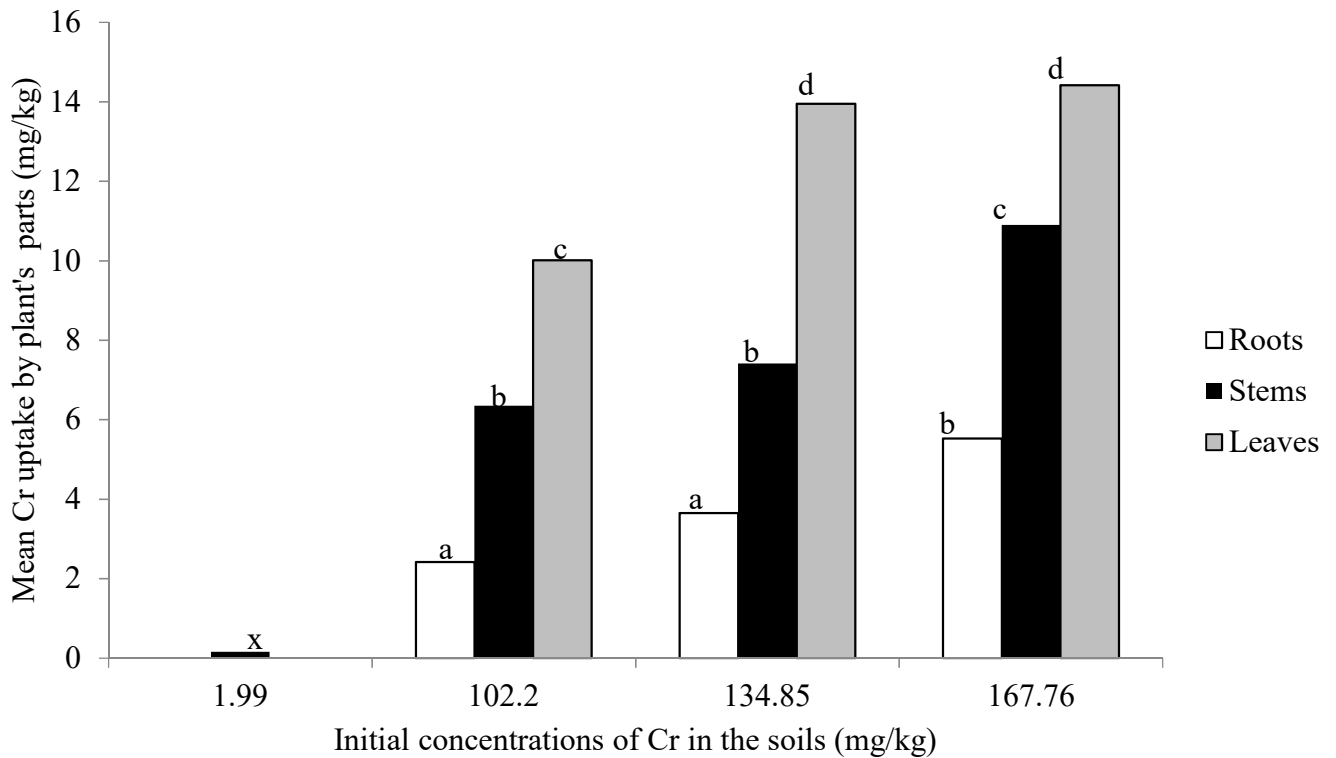


Figure 1: Concentration of Cr in different parts of *I. asarifolia* after 30 days across the samples. The values are significantly different ($p < 0.05$) where superscripts located on top of the bars are different.

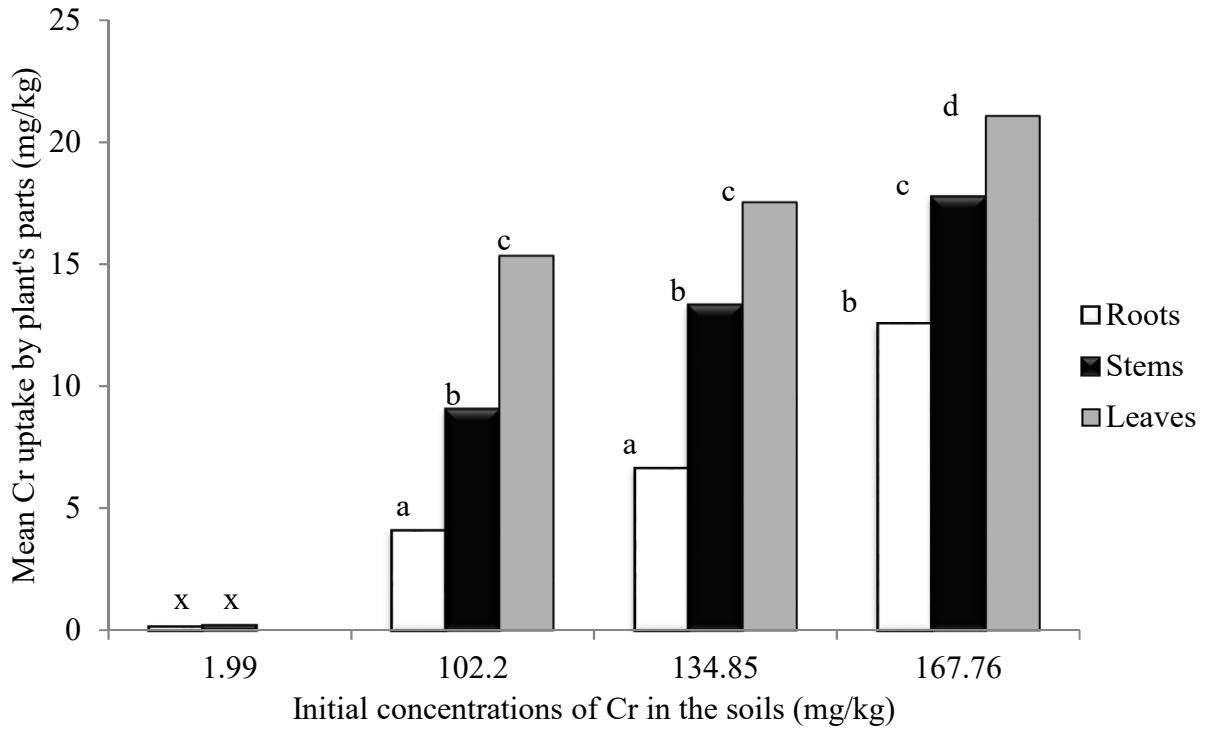


Figure 2: Concentration of Cr in different parts of *I. asarifolia* after 50 days across the samples. The values are significantly different ($p < 0.05$) where superscripts located on top of the bars are different.

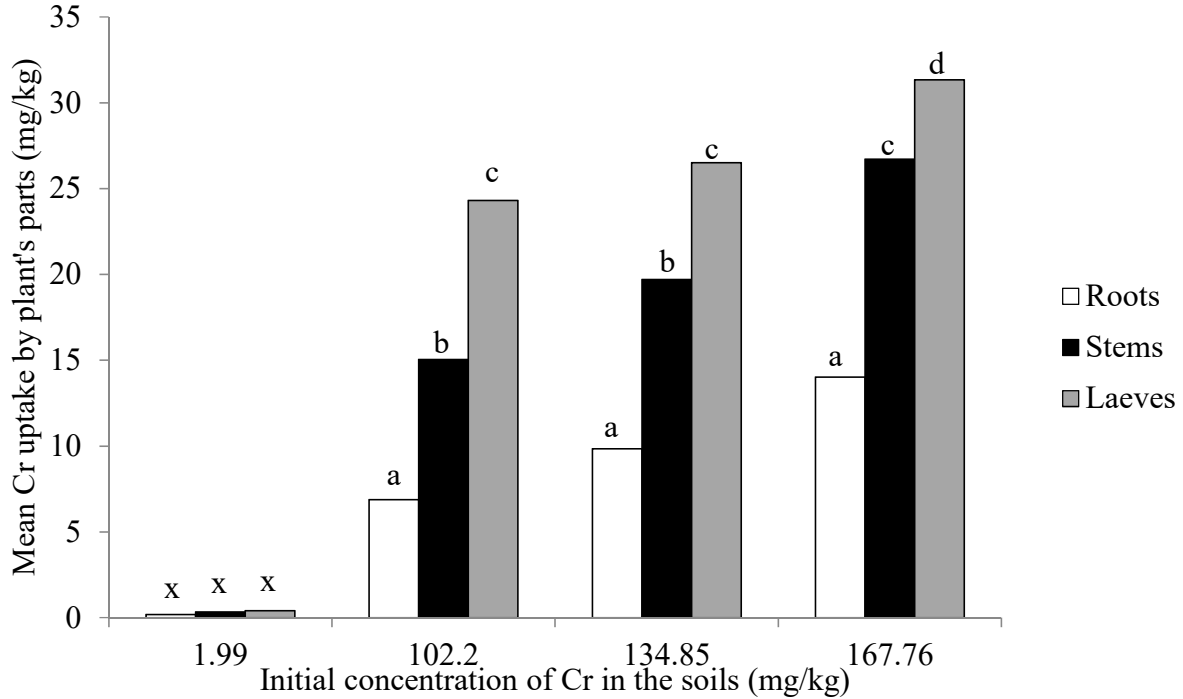


Figure 3: Concentration of Cr in different parts of *I. asarifolia* after 70 days across the samples. The values are significantly different ($p < 0.05$) where superscripts located on top of the bars are different.

Discussion

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. Soil remediation can be done *in situ* (on-site) without excavation of the contaminated soil, or *ex situ* (off-site) with removal of contaminated soil for restoration and returning the restored soil back to the site, both approaches are extremely expensive (Kabata-Pendias and Pendias, 1989). The *ex-situ* methods comprise excavation, detoxification and/or destruction of the metal physically or chemically by processes, such as stabilization, solidification, immobilization or incineration (Jeanna and Henry, 2000; Petra *et al.*, 2012).

Phytoremediation uses various plants to degrade, extract, contain, or immobilize contaminants from polluted sites. It is suitable when the pollutants cover a wide area and when they are within the root zones of the plants (Marques *et al.*, 2009). When in high concentrations and mobile in the environment, heavy metals can move between media (e.g. soil to water) and be sorbed by the plants. The most common mechanism of phytoremediation of heavy metals is phyto-extraction. Phytoextractors remove metal contaminants from the soil without altering the soil structure and/or fertility (Divya *et al.*, 2012). These plants can contain large amounts of heavy metals in their organs, due to their unique features, such as rapid growth rate, extensive root system,

high biomass and toleration of high amounts of metals (Marques *et al.*, 2009).

The potential of *I. asarifolia* for phytoextraction and bioaccumulation of Cr from soils experimentally-amended with varying quantities of Chromium (VI) chloride (CrCl_6) salt has been investigated in this study. The results obtained revealed the plant's capacity to sorb and bioaccumulate appreciable amounts of Cr in its parts, especially the leaves. The physicochemical characteristics of the soils used in the study showed that it is suitable for plant's growth having a textural composition of silt-clay-sand with slightly acidic pH and high organic matter content (Rahman *et al.*, 2013). The application of CrCl_6 to the soil has not significantly affected its pH (Table 1). This concurs with Jieng-feng *et al.*, (2009) who reported similar observation in soils experimentally-amended with CdCl_2 and $(\text{Pb}(\text{NO}_3)_2)$, and could be attributed to low hydroxyl ions production by the reaction between CrCl_6 and soil elements. Acidity enhances desorption of metals from soil into solution due to H^+ competition for the binding sites. Soil pH therefore, affects not only the bioavailability, but also rate of metal sorption into the plant's roots. In general, metal uptake by phytoremediation plants is facilitated by the interaction of the metal with soil matrix which reduces the activity of the metal (Chaney and Malik, 2003).

In this study, *I. asarifolia* tolerated and grew in high concentrations of Cr throughout the period, indicating that it may have evolved mechanisms

to avoid Cr toxicity in the soils. Several other phytoremediation plants with tolerance against toxicity of heavy metals in their surroundings have been reported (Jarup, 2003; Li *et al.*, 2007). In contrast, some symptoms of toxicity, such as chlorosis and yellowishness of leaves in the plants grown in high doses of Cr in artificially-polluted soils have been reported (Chen *et al.*, 2004; Marques *et al.*, 2009). The translocation of Cr through the plant's roots, stems and leaves in all the harvesting periods (Table 2, Figures 1-3) showed that concentrations of Cr differ significantly ($p < 0.05$) among the plant's parts in the order: leaves > stems > roots. This trend coupled with bioavailability of the metal in the soil allows for increased sorption of the metal by *I. asarifolia* (Rahman *et al.*, 2013). Similar trends in uptake of heavy metals by other phytoremediation plants have been reported (Chaney and Oliver, 1996; Salt *et al.*, 1998; Jeanna and Henry, 2000; Lombi *et al.*, 2009; Marques *et al.*, 2009).

The efficiency of the plant to translocate metals from the root to the shoot, and from the shoot to the leaves is determined by Transportation Indices (Ma *et al.*, 2001). If the TI is less than one ($TI < 1$), the plant has potential for phytostabilisation, whilst if the TI is greater than one ($TI > 1$) the plant is considered as a phytoextractor (Labanowski *et al.*, 2008). For all the three harvesting phases, the indices for Cr transport from roots to stems (RTI) and from stems to leaves (STI) are greater than one ($TI > 1$), suggesting that *I. asarifolia* has enormous potential for phytoextraction of Cr. The decrease in TI with increasing Cr concentration in

the soils observed (Table 2) could mean that the plant's capacity to extract Cr may diminish in soils polluted with excessive amounts of Cr.

Furthermore, the accumulation of large amounts of Cr ($> 100\text{mg/kg}$) in the plant's biomass showed that it is a good Cr bioaccumulator. Accumulation of high amount of Cr in the leaves could be the plant's mechanism of avoiding toxicity of the metals in its stems. This agreed with Marques *et al.* (2009) who reported that accumulation of excessive amounts of heavy metal(s) in the aerial or easily harvested parts is a feature of plants suitable for phytoextraction. The capacity to tolerate excessive amounts of heavy metals by plants leads to accumulation of the metal in the plant's harvestable parts; which often poses a potential threat to the food chains (Cui *et al.*, 2007). Consumption of heavy metal-contaminated foods causes depletion of certain essential nutrients in the body, leading to malnutrition-associated disabilities, intrauterine growth retardation, decreased immunological defense capability and impaired psycho-social behavior (Jarup, 2003; Arora *et al.*, 2008). Heavy metal ions can also form complexes with proteins, through the carboxylic acid ($-\text{COOH}$), amine ($-\text{NH}_2$) and thiol ($-\text{SH}$) groups altering the protein structure and producing modified proteins devoid of functional integrity. They could also inactivate important enzymes or form radicals that oxidize biological molecules resulting in malfunctioning or death of the cells (Neal and Guilarte, 2012).

Conclusion

In conclusion, the results of this study have shown that *I. asarifolia* phytoextracted and bio-accumulated considerable amounts of Cr from Cr-polluted soils. It is therefore, logical to conclude that the plant has a promising potential for phytoremediation and could be effective in restoration of Cr-polluted soils with limited toxicity to the ecosystem.

Recommendation

To unveil more potentials of *I. asarifolia* in soil remediation program, field trial experiments are hereby recommended which will provide insights on Cr uptake, transfer and distribution by the plant. Further research is also recommended to explore the kinetics of Cr sorption from the soil by the plant.

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