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Bioaccumulation Potentials of *Momordica charantia* L. Medicinal Plant Grown in Lead Polluted Soil under Organic Fertilizer Amendment

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Abstract

This study investigated bioaccumulation factors and translocation factors of lead (Pb) by *M. charantia* so as to ascertain the bioaccumulation potentials of this medicinal plant. The elemental deposition of Pb were also assessed in order to compare the concentration of Pb present in plant tissues with the maximum permissible limits of 10 ppm recommended by WHO (1998, 2007). The experiment was a factorial combination of one heavy metal (Pb) at five levels of concentrations (0, 200, 400, 800 and 1,000 ppm) in a completely randomized design, replicated three times, with one medicinal plant species and two levels (0 and 9.4 g) of organic fertilizer (OBD- Plus). The seedlings were transplanted from nursery to experimental pots at the rate of one seedling per pot and grown for 10 weeks, after which the plants were harvested and dried for the analysis of Pb concentration both in soil and plant tissues using Atomic Absorption Spectrophotometry (AAS). The highest bioaccumulation factor for roots was 0.42 under fertilizer amendment, whereas the highest bioaccumulation factor for shoots was 0.26 under the same treatment. The highest transfer factor of *M. charantia* was 0.6. The results showed that *M. charantia* is a good phytostabilizer of Pb component. The highest lead deposition in the roots, which was 40% higher than in the shoots concentration, was above the safe limit; therefore this might pose health risks to human if consumed as herbal medicine.

Keywords: Atomic Absorption Spectrophotometer, bioaccumulation factor, Pb, phytoextraction, phytostabilizer, translocation factor

Introduction

Since the dawn of history, man has relied so much on medicinal plants for health and food needs (Nwachukwu *et al.*, 2010). The traditional uses of medicinal plants for curing and preventing illnesses, including the promotion of both physical and spiritual wellbeing among human beings have become paramount (Idu and Onyibe, 2007). Medicinal herbs are consumed all over the world. The use of medicinal herbs to relieve and treat many human diseases is increasing around the world due to their mild features and low side effects (Yap *et al.*, 2010). A World Health Organization (WHO, 2007) survey indicated that about 70-80% of the world population relies on non-conventional medicine, mainly of herbal sources, in their primary health care. This report revealed that medicinal plants and their trace elements play an important role in the treatment of diseases (Chan, 2003).

Heavy metals are known to have the ability to bio-accumulate and thus disrupt functions of vital organs and glands in the human body, affecting brain, kidney and liver (Suranjana and Minas, 2009). Medicinal plants can be contaminated by these heavy metals via roots uptake or by direct deposition of contaminants from the atmosphere onto plant surfaces (Dzomba *et al.*, 2012). WHO (2005) recommended that herbal drugs should not be used without qualitative and quantitative analysis of their heavy metal contents. Moreover, medicinal plants which form the raw materials for finished products must be checked for the presence of different contaminant such as heavy metals, pesticides, fungi and micro-organisms.

In an attempt to control pollution of medical plants by heavy metal, WHO regulated maximum permissible limits of heavy metals like arsenic, cadmium and lead to 0.1, 0.3, 10 ppm, respectively (WHO, 1998, 2007). Ingestion of high concentration of heavy metals such as chromium, cadmium and lead can cause reduced growth rate, hepatic and renal impairment (Blowes, 2002).

According to Khan *et al.* (2008), medicinal herbs are easily contaminated during growth, development and processing. This shows that soil environment in which medicinal plants grow is one of the major pathway through which heavy metal components enters into the plants tissues and thus into food chain.

Accumulation of metals in plants is highly dependent on their availability in soil (Rahimi *et al.*, 2013). These plants can uptake the heavy metals that are found in soil, water or air (Mclanghin *et al.*, 1999). Soil to plant transfer is one of the key processes of human exposure to heavy metals through the food chain. Heavy metals uptake from soil via the root and direct deposition of contaminants from the atmosphere on plant surfaces can lead to plant contamination by heavy metals (Zhuang *et al.*, 2009). In addition, accumulation of these metals in the edible parts of medical plants has potential health

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implication on humans and animals (Lokhande and Sathe, 2003). Heavy metals are the most harmful and insidious pollutants because of their non-biodegradable nature and their potential to cause adverse effects at certain levels of exposure and absorption (Sharma and Chhetri, 2005). Once the heavy metals get accumulated as contaminants, they can neither be destroyed nor altered by chemical or physical means (Kabata-Pendias, 2010). Since there is an inextricable link between the plants and the soil environment, there is a great need to monitor the bioaccumulation potentials of medicinal plants as they grow on polluted soils (Dada and Awotoye, 2013).

The use of synthetic chelates has been shown to dramatically stimulate the potential for Pb accumulation in plants. Compounds such as EDTA, DTPA (Diethylene triamine pentaacetate) and low molecular weight organic acid such as oxalic acid and citric acid, prevent Pb precipitation and keep the metals as soluble chelate-Pb complexes available for uptake into roots and transport within plants. Ethylene diaminetetraacetate (EDTA) is probably the chelating agent that is most efficient at increasing the solubility of heavy metals in soil solutions from the solid phase (Blaylock *et al.*, 1997; Huang *et al.*, 1997). EDTA application solubilizes about 80% of the total soil metal, thereby making them available for phytoextraction. Adding organic fertilizer could improve soil structure, and then promote desorption of Pb out from the soil surface, thus enhance its bioavailability. Zhang (2009) found that continuously adding organic fertilizer would inhibit the activity of Pb, Hg and Cd. This is because continuously adding the organic fertilizer increases the soil organic matter and thus inhibit the activity of metals.

One of the greatest concerns of human health is caused by Pb contamination. Lead (Pb) is a major anthropogenic pollutant and has accumulated in different terrestrial and aquatic ecosystems (Verma *et al.*, 2003). In year 2000, EPA (Environmental Protection Agency) enlisted lead as a potential carcinogenic element in nature. Inhalation and ingestion are the two major routes of exposure, and the effects from both are the same (Henry, 2000). Lead accumulates in the body, which may lead to poisoning or even death. The gastro-intestinal tract, kidneys and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk of impaired development, lower IQ, shortened attention span, hyperactivity and metal deterioration, with children under the age of six being at a more substantial risk (Henry, 2000). Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia and weakness of joints when exposed to Pb (Henry, 2000).

In this study, the medicinal plant *Momordica charantia* L. was studied for its curative effects. *Momordica charantia* L. is commonly known as bitter melon, papilla, bitter gourd, salsamino, corrila, karela, hanzal, assorossie, ampalaya, nigauri, goya, pare, kho gua, sora, balsam apple, pear or balsamina, and several other common names (Taylor, 2002). In South-West Nigeria, it is called "Ejirin" (Sofowora, 1993). It is an economically important medicinal plant belonging to the family *Cucurbetaceae*. The immature fruits are eaten as vegetables and are a good source of vitamin C, vitamin A, phosphorus and iron (Sultana and Bari, 2003; Paul *et al.*, 2009). The bitter flavour is due to the alkaloid momordicine produced in its fruits and leaves (Paul and Raychandhuri, 2010). Fruits and seeds of bittergourd possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, antitumor and antidiabetic property (Taylor, 2002). The fruits and leaves of *M. charantia* have medicinal potential in treatment of many diseases such as piles, leprosy, jaundice, diabetes, snake-bite and have also been found to have vermifuge and antioxidant properties (Jonathan *et al.*, 2012). It also contains cytotoxic proteins (ribosome inactivating) such as momorcharin and momordin (Santhi *et al.*, 2011). In many parts of the world, especially poor countries, this may be one of the therapies available to treat diabetic patients.

Hence, the essence of this study was designed to: (1) investigate the bioaccumulation factor of lead contamination in *Momordica charantia;* (2) determine the translocation factors of Pb in the medicinal plant under experiment; and (3) compare the concentration of the Pb accumulated by the plant with the recommended concentration accepted by the international organizations.

Materials and Methods

Study area

The study was carried out under a screen house in the Biological Gardens of Obafemi Awolowo University campus, Ile -Ife, Nigeria. The town Ile-Ife lies within latitude 7^0 30' N – 7^0 35' N and longitude 4^0 30' – $4^0$35' E; the latitude of the study area is $7^0$31' N and longitude is $4^0$31' E. Osun state is geographically located in moderately hot, humid tropical climatic zone of South-western Nigeria. There are two distinct seasons in the area, namely the rainy season, which lasts from March to October, and the dry season, which last for the rest of the year, that is October to March. The mean value for temperature is about 30°C during the dry season, while during rainy season, low temperatures are experienced between July and August with the minimum temperature as low as 24°C.

Collection of materials

Samples of top soils were randomly collected from the depth of 0-20 cm where anthropogenic activities were minimal. The soil samples were air dried for a week. The air dried soil samples were sieved using 2 mm mesh gauze to remove debris and stones. Seven-liter plastic pots of 23 cm diameter were used for the experiment. In order to allow aeration, the plastic pots were perforated at the base using soldering iron.

Viable (correctly identified) seeds of *M. charantia* were obtained from the wild.

Experimental design

The experiment was a factorial combination of one heavy metal (Pb) at five levels of concentrations (0, 200, 400, 800 and 1,000 ppm) laid in a completely randomized design and replicated three times, with one medicinal plant and two levels (0 and 9.4 g) of organic fertilizer (OBD-Plus) at the rates of 0 kg and 40 kg per hectare.

Pre-planting operations

The physical and chemical parameters of the soil samples used for the experiment were determined using standard methods.

A nursery bed was prepared for *Momordica charantia* and the seeds were planted at 2 cm depth. The nursery bed was watered to field capacity.

Preparation of lead solution and the amendment

Heavy metal used in the experiment was Lead (Pb) and Lead (11) nitrate {Pb $(NO_3)_2$} salt served as the source of the lead (Pb) used. A known weight of Lead (11) nitrate with respect to each concentration of 0, 200, 400, 800 and 1,000 ppm was dispensed into one litre of volumetric flask, allowed to cool and shaked vigorously. The resultant solution was dispensed in a sterile bottle, allow to set and ready for use. Organic fertilizer OBD-Plus with nitrogen (N) content of 0.95% was used to augment the soil at the concentration of 0 and 9.4 g at the rate of 0 kg and 40 kg respectively per hectare.

Pollution of soil

Each pot was labeled using permanent maker with respect to the concentration of heavy metal and fertilizer applied. Plastic trays were placed under each pot for the collection of excess water to prevent loss of pollutants. After the application of pollutant, the pots were left for one week to allow for equilibration. Thereafter, organic fertilizer was applied to augment the soil.

Transplanting into polluted soil

Two weeks after germination of the seeds in the nursery bed, the seedlings with a good growth and uniform height were selected and transplanted to each experimental pot at the rate of one seedling per pot. Wetting was done every day to field capacity.

Post planting operations

Harvesting

All the plants were carefully harvested and soil samples were taking from each pot. Plant roots were rinsed under running tap water, while water droplets were removed using blotting papers. Each plant was separated into root and shoot.

Laboratory analysis

Soil heavy metal analysis

Soil extracted for heavy metals analysis was carried out using the IITA (1982) methodology. 10 g of each soil sample were placed in a conical flask. One hundred milliliters of a mixture of 10 ml HNO_3, 5 ml HCL, made up to 250 ml with distilled water were added to each soil sample. This was shaken for 30 minutes on a reciprocal shaker and filtered through Whatman filter paper. Analysis of the soil extract for Pb was carried out using Spectronic 20 Absorption spectrophotometer.

Heavy metal analysis in plant samples

1.0 g of the powdered sample was weighed into a conical flask in triplicate. 10 cm^3 of the digestion mixture (a mixture of sulphuric acid, perchloric acid and nitric acid in ratio 1:4:40 by volume) was added and left to stand overnight. Thereafter, the flask was heated at 70 °C for about 40 minutes and then the heat was increased to 120 °C. The mixture turned black after a while and the digestion was complete when the solution became clear with appearance of white fumes (Audu and Lawal, 2005). The digest was diluted with 10 cm^3 of water and boiled for 15 minutes. This was then allowed to cool, transferred into 50 cm^3 volumetric flasks and diluted to the mark with water. The sample solution was then filtered through a filter paper into a screw capped polyethylene bottle and stored for heavy metal determination using Spectronic 20 Atomic Absorption Spectrophotometer with a digital read out system.

Statistical analysis

Separate analysis of variance for the heavy metal concentration at different treatments and mean separation by Fisher's LSD at P < 0.05 were calculated. All analyses were carried out using SAS version 9.2.

Bioaccumulation factor and Translocation factor

$$\text{Bioaccumulation factor for roots} = \frac{\text{Concentration of heavy metal in root}}{\text{Concentration of heavy metal in soil}}$$

$$\text{Bioaccumulation factor for shoots} = \frac{\text{Concentration of heavy metal in shoot}}{\text{Concentration of heavy metal in soil}}$$

$$\text{Bioaccumulation factor for the whole plant} = \frac{\text{Concentration of heavy metal in plant}}{\text{Concentration of heavy metal in soil}}$$

$$\text{Transfer factor} = \frac{\text{Concentration of heavy metal in shoot}}{\text{Concentration of heavy metal in root}}$$

Results and Discussion

Physical and chemical properties of the soil used in the experiment

The physical and chemical characteristics of the soil used within the screen house study were presented in Table 1. The textural classification of the soil was loamy sand. The pH in 1:1 soil to water was 5.8 for the topsoil, indicating a slightly acidic soil condition. The organic carbon content of the soil was 40.10 g/kg, while nitrogen, phosphorus and potassium values were 3.20 g/kg, 10.4 mg/kg and 2,605 mg/kg respectively. The calcium (Ca) and magnesium (Mg) contents were 31.0 mg/kg and 2,432.5 mg/kg respectively, with lead concentration of 0.098 ppm.

Bioaccumulation factor (BCF) of Momordica charantia with and without fertilizer application

The bioaccumulation factors for *M. charantia* were shown in Table 2. The bioaccumulation factor for shoots of *M. charantia* (BCFsh) without fertilizer application ranged between 0.01-0.18, while under fertilizer application the BCFsh ranged between 0.03 to 0.26, with 44% increase when compared with the control. Across the various pollution strengths, as the level of lead concentration increased, the BCFsh in the root and shoot increased also. Although, the highest shoots' bioaccumulation factor was reported in *M. charantia* treated with organic fertilizer.

The bioaccumulation factor for roots of *M. charantia* (BCFr) ranged between 0.03-0.32 without fertilizer application and between 0.02-0.42 under fertilizer application (Table 2). Similarly, as the level of lead concentration increased, the bioaccumulation factor for roots increased for both treatments, with and without fertilizer application. The bioaccumulation factor for roots was higher under fertilizer application.

The bioaccumulation factor for the whole plant (BCFplt) ranged between 0.04-0.50 without fertilizer application and between 0.02-0.68 under fertilizer application (Table 2). The bioaccumulation factor for the whole plant also increased as the level of lead concentration increased, with the highest value recorded under fertilizer application.

A plant's ability to accumulate metals from soils can be estimated using the bioaccumulation factor (BCF), which is defined as the ratio of metal concentration in the plant to that in soil. In this experiment, under organic fertilizer application, the highest bioaccumulation factors in the root and shoot were 0.42 and 0.26 respectively. These result showed that lead accumulated in the roots more than in shoots. This report was in line with the findings of Parsa *et al.* (2007) and Cho-Ruk (2006). Also it is in

Table 1. Physical and chemical characteristics of soil used for the experiment

Characteristics	Value
pH in water (H_2O)	5.8
Organic carbon (g/kg)	4.10
Nitrogen (g/kg)	3.30
Clay	6.8%
Silt	4.0%
Sand	89%
Phosphorus (mg/kg)	10.4
Ca^{2+}	31.0
Mg^{2+} (mg/kg)	2432.5
K^{+}	2605
Na^{+}	272.5
Lead (ppm)	0.098

Table 2. Bioaccumulation and translocation factors for *Momordica charantia*

Lead concentration (ppm)		Bioaccumulation and translocation factor			
		BCFsh	BCFr	BCFplt	TF
FN	0	0.01	0.03	0.04	0.33
	200	0.06	0.16	0.23	0.33
	400	0.08	0.19	0.25	0.49
	800	0.17	0.28	0.45	0.55
	1000	0.18	0.32	0.50	0.60
F	0	0.01	0.02	0.02	0.09
	200	0.11	0.27	0.37	0.39
	400	0.12	0.23	0.34	0.52
	800	0.21	0.42	0.63	0.50
	1000	0.26	0.42	0.68	0.60

Legend: BCFsh- Bioaccumulation factor for shoot, BCFr- Bioaccumulation factor for root, BCFplt- Bioaccumulation factor for whole plant, TF- transfer factor FN- No fertilizer, F- Fertilizer

Table 3. Elemental deposition of lead (ppm) in the shoots of *Momordica charantia*

Lead concentration (ppm)		Concentration of lead (ppm) in *Momordica charantia*	
		Shoot	Roots
NF	0	0.00^{e}	0.00e
	200	9.42^{d}	28.36d
	400	23.61^{c}	48.63c
	800	98.00^{b}	179.20b
	1000	115.37^{a}	191.90a
F1	0	0.00^{e}	0.00e
	200	14.70^{d}	37.22d
	400	32.80^{c}	63.48c
	800	110.70^{b}	221.20b
	1000	151.00^{a}	251.30a

Legend: NF- No fertilizer, F- Fertilizer; Means with the same letters of superscript within the column are not significantly different at $P < 0.05$

agreement with the work of Mojiri (2011) who studied the potential of corn *(Zea mays)* for phytoremediation of soil contaminated with cadmium and lead, using EDTA for the bioavalability of heavy metals. Adejumo *et al.* (2011) studied in-situ remediation of heavy metal contaminated soil using Mexican sunflower (*Tithonia diversifolia*) and cassava waste as composts with favorable results. However, Wang *et al.* (2007) gave a contrary report when using *Bidens maximowicziana* to phytoremediate lead under EDTA-enhanced innovation. This may be due to the fact that *B. maximowicziana* have been reported to accumulate high concentration of lead in its tissues as a good hyperaccumulator of lead (Nie *et al.*, 2004).

Studies on Pb uptake in plants have demonstrated that roots have the ability to take up significant quantities of Pb, while simultaneously greatly restricting its translocation to above ground parts (Lane and Martins, 1977). Majority of lead is easily taken up by plants from the soil and accumulated in root, while only a small fraction is translocated upward to the shoots (Patra *et al.*, 2004). This may be because passive transport of ions occurred in the root which was directly exposed to the ionic environment in soil. This may extend to the endodermis and not beyond because of the casparian strip. According to Mojiri (2011), the capacity of soil to adsorb lead increases with increasing pH, cation exchange capacity (CEC), organic carbon content, soil/water Eh (redox potential) and phosphate levels. In natural setting, lead hyperaccumulation has not been documented. Some ways to induce Pb solubility are to decrease soil pH and lower its organic matter, because Pb binds to organic material in the soil (McBride, 1994; Sharma and Dubey, 2005).

The highest bioaccumulation factor of the whole plant obtained in the hereby experiment was 0.68 in *Momordica charantia*. None of the bioaccumulation factors was higher than 1, meaning that *Momordica charantia* is a good phytostabilizer of lead.

Transfer factor

The transfer factors for *M. charantia* ranged from 0.31 without fertilizer application and 0.09-0.60 under fertilizer application as shown in Table 2. These results revealed that fertilizer amendment enhanced the mobility index of Pb from the root to the shoot of the medicinal plant.

Ability of plant to translocate metals from the roots to the shoots was measured using the transfer factor (TF). Enrichment occurs when a contaminant taken up by a plant is not degraded rapidly, resulting in an accumulation in the plant. The process of phytoextraction generally requires the translocation of heavy metals to easily harvestable plant parts, e.g. shoots. By comparing bioaccumulation factor and transfer factor, one can compare the ability of different plants in taking up metals from soil and translocating them to the shoots. Tolerant plants tend to restrict soil-root and root-shoot transfers, and therefore have much less accumulation in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass. Plants exhibiting transfer factor and particularly bioaccumulation factor values less than 1 are unsuitable for phytoextraction (Fitz and Wenzel, 2002), but they can be insidious if the belowground biomass that is phytostabilizing the metal components is consumed as source of herbal medicine (Dada and Awotoye, 2013).

The highest transfer factor of the experiment was 0.6, both without and with fertilizer application. All the transfer factors were less than 1, showing low translocation of Pb from their roots to shoots. This can be attributed to the effect of Pb toxicity. Tang *et al.* (2009) indicated that in *Arabis paniculata*, for the range of 9-267 μM of Pb concentration, when the transfer factor was below 1. Similarly, Aiyesanmi *et al.* (2012) recounted the effect of lead toxicity when studying lead accumulation effects in Siam weed (*Chromolaena odorata*), node weed (*Synedrella nodiflora*) and water leaf (*Talinum triangulare*) as potential phytoremediators.

Elemental depositions of lead (Pb) in M. charantia

Across the tested medicinal species, with and without fertilizer application, the bioaccumulation concentration of Pb in the roots and shoots of *M. charantia* increased as the level of Pb concentration increased in the soil as shown in Table 3. The Pb concentration in the shoots ranged between 0.00-115.37 ppm without fertilizer application and between 0.00-151.00 ppm under fertilizer application. Whereas the heavy metal deposition in the roots of *M. charantia* ranged between 0.00-191.90 ppm without fertilizer application and between 0.00-251.30 ppm under fertilizer application (Table 3). The roots of *M. charantia* had the highest bioaccumulation concentration of lead.

Momordica charantia showed high absorption and Pb uptake. Furthermore, all the tested plants showed a direct relationship between Pb-uptake and the level of lead contamination. This shows that at higher pollution level *M. charantia* has the phytoremediation ability to accumulate lead from the soil through passive transport especially under organic fertilizer inducement.

As anticipated from previous studies involving other species (Blaylock *et al*., 1997; Aiyesanni *et al*., 2012), higher absorption of Pb was recorded for plants grown on soil treated with organic fertilizer, because organic fertilizer had been known not to only to enhance the bioavailability of Pb, but also improved plant establishment and growth under pollution stress. This is because organic fertilizer serves as a good source of nitrogen and phosphorus nutrition for plants.

Comparing the lead concentration (9.42 ppm to 151.00 ppm) in the shoots with the recommended permissible limit of 10 ppm set by FAO/WHO (1976), the lead deposition in *Momordica charantia* were close and high above the safe limit recommended. Similarly, the highest lead deposition in the roots, which was 40% higher than in the shoots concentration, was also above the safe limit recommended by WHO (2007).

Conclusions

M. charantia as medicinal plant used in the current experiment showed a significantly high absorption of lead. It could also be observed that treatment of the soil with organic fertilizer enhanced the uptake of Pb in the plants by increasing the bioavailability of Pb in soil solution. The medicine plant exhibit characteristics of a phytostabilizer because the transfer factors were less than 1. This however, poses a health risk to human if consumed, since the levels of Pb were high in the shoots, which are the parts mostly used for herbal medicine.

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