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Study of Phytoremediation Potential of Fluted Pumpkin (*Telfairia occidentalis*) for Soil Contaminated with Heavy Metals

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Authors' contributions

The work was carried out in collaboration between all authors. Author DMF designed the study. Author HAA wrote the first draft of the manuscript. All authors managed the analysis, read and approved the manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate the phytoremediation potential of fluted pumpkin (*Telfairia occidentalis*) for some heavy metals (Cd, Cu, Pb, Cr and Co) in soil.
Study Design: The experimental design was based on the assumption of homogeneity of points where the experimental soils were collected.
Place and Duration of Study: Department of Chemistry, Nigerian Defence Academy (NDA) Kaduna, Nigeria, between January 2011 and April 2012.
Methodology: Surface soil (0 -20 cm) taken from various points of the department were used for the experiment. Pumpkin seeds were planted in polythene bags containing 1.5 kg of the soil which was contaminated with the metals of interest. The experiment consisted of six (6) contamination treatments and a control. These treatments were 5 mg kg^{-1}, 20 mg kg^{-1}, 75 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1} and 220 mg kg^{-1} of each metal. The soil and the pumpkin tissues (roots, stems and leaves) were analyzed for the metals, eight (8) weeks after planting, through atomic absorption spectroscopic (AAS) method. The physical and chemical characteristics of the soil were also determined.
Results: The plant's shoot length, 8 weeks after planting, showed that *Telfairia occidentalis* grew better on uncontaminated (control) soil. Generally, the trend showed that the higher the metals level in the soil the shorter was the plant shoot length. The

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metals (Pb and Co) accumulated more in the shoot than in the roots (Translocation factor- TF > 1) and also than in the soil (Bioaccumlation factor- BF >1). Copper and chromium on the other hand accumulated more in the root and in the soil than in the shoot (both the TF and BF values are less than one).
Conclusion: The plant (pumpkin) can tolerate and survive high metals level in soil. It can be used as phytoremediator of soil contaminated with Pb and Co and to a lesser extent Cd.

Keywords: Phytoremediation; pumpkin; soil heavy metals.

1. INTRODUCTION

Intensive development of industry of transport and technology has contributed to the rise in heavy metals content in soils and crops to a level hazardous to population inhabiting the affected regions. High level of metals exerts a negative influence on the development of plants, their use of nutrients and metabolism [1]. The increasing use of wide variety of heavy metals in industries and agriculture has caused a serious concern of environmental pollution [2].

Remediation of heavy metal-contaminated sites is particularly challenging because unlike organic contaminants which are oxidised to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation and are toxic and their total concentration in soils persists for a long time after their introduction [3-5]. Conventional soil remediation techniques involves excavating, detoxification, soil washing with chemicals, and/or destruction of contaminant physically or chemically, as a result, the contaminant undergo stabilization, solidification, immobilization, incineration or destruction. These methods, however, often exchange one problem for another [6]. Plant based bioremediation technologies have been collectively termed phytoremediation, this refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soils and ground water [6]. An alternative agriculture which expands the use of plants well beyond food and fiber is beginning to change plant biology, as plant-based biotechnologies have been recently developed that take advantage of the ability of plant roots to absorb or secrete various substances' [7]. The process where the plant roots absorb and translocate the contaminants to the shoot is termed phytoextraction while the process in which the roots secrete various substances to remove, detoxify or immobilize the contaminants is termed phytostablization. Thus plant phytoremediate polluted environment through phytoextraction or phytostablization. This technology offers a low cost method for soil remediation and some extracted metals may be recycled for value [8].

Johnson et al. [9] studied two species of Sunflower (*Tithonia diversifolia* and *Helianthus annus*) and investigated their potential to remove heavy metals from contaminated soil in Abeokuta, Nigeria. The contaminants were added as lead nitrate ($Pb(NO_3)_2$) and zinc nitrate ($Zn(NO_3)_2$) at 400 mg kg^{-1}. Their results showed that *T. diversifolia* and *Helianthus annus* mopped up substantial concentration of Pb and Zn in the above ground biomass compared to the concentration in the roots and that the efficiency of these plants in cleaning the contaminated soil was at the early stage of growth (4 weeks after planting). Field study was conducted on nine crops, including pumpkin, for accumulation and distribution of Cd and Pb in their organs [10]. The results showed that field pumpkin accumulated most of the Cd and Pb in the leaves than in the roots, stems or fruits. The results also indicated phytoremediation ability of the pumpkin for Pb (TF = 1.68). In a similar study elsewhere, Azime et al. [11] reported that cadmium concentrations in edible parts of fluted pumpkin were less than U.S. EPA standards for agriculture and human beings. Apajobi et al. [12] carried out a research in a non-oil prospecting area of Delta state, Nigeria and reported that lead (Pb) level in leaf of *Telfairia occidentalis* (mean value range of 4.300 – 5.800mg kg^{-1}) is much less than the approved standard limit set by World Health Organization (WHO). They finally concluded that the plant grown in the area is safe for human consumption. In fact, the ideal type of plant that can serve as phytoremediator is a specie that creates a large biomass, grows quickly, has an extensive root system and can be easily cultivated and harvested [13].

In this study, the potential of fluted pumpkin (*Telfairia occidentalis*) for phytoremediation of soil contaminated with Cd, Cu, Pb, Cr and Co has been investigated. The plant, popularly known as Ugu in Nigeria is a perennial vegetable. It belongs to the cucurbitaceace family and is grown for its leave and seeds, which are very nutritious. The leaves are rich in Mg, Fe and fibers which are used as food supplements and in the management of severe anemia in some African pregnant women [14]. The vegetable is a fast grower which can easily be cultivated and harvested. Harvesting of shoots, up to 50 cm long, can begin one month after germination. The plant features and less number of work done for its phytoremediation potentials [10], which were limited to few metals, made this research to choose the plant for the investigation.

2. MATERIALS AND METHOD

2.1 Sample Preparation

The study was carried out at NDA, permanent site Kaduna, Nigeria. Soils samples (0 – 20 cm depth) from the site were taken mixed thoroughly to obtain a homogenous soil mass from which 1.5 kg was weighed (each) into 21 black polythene bags. Different concentrations (5 mg kg^{-1}, 20 mg kg^{-1}, 75 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1} and 220 mg kg^{-1}) of each metal (Cd, Cu, Pb, Cr and Co) were prepared. A volume of $50cm^3$ of a particular concentration for each of the metals were mixed to give a total of $250cm^3$ which was then used to pollute the soil. A set of three bags were used for a particular soil treatment in order to have a replicate analysis, this gave a total of six sets (i.e. 18 bags) for the mixed metals treatment. Additional set that will make the total to be 7 sets (i.e. 21 bags) was treated with 250 cm^3 of deionised water alone to serve as control. The pumpkin seeds were then planted in these soils. The work was carried out between January 2011 and April 2012.

At two weeks and 4 weeks i.e.14 and 28 days after planting (DAP), the shoot length was measured using a meter rule from the base of the plant to the apex or youngest leaf [15] and leaf samples were removed. At harvest i.e. 8 weeks (56 DAP), the shoot length were recorded again and the plant from each bag was uprooted from the soil. The plants were then separated into roots, stems and leaves. The length of the roots was also measured. The plant's tissues at 8 weeks (56 DAP) were washed separately first, with running tap water and rinsed with deionised water. The plant materials i.e. all tissue samples (leaves, stems and roots) were air dried and then oven-dried at $80^{\circ}C$ for 16 hours [16,17].

2.2 Plant and Soil Analysis

Plant sample digestion was carried out by the method described by Hornwitz [18]. The oven dried plant tissues were ground to powder and 0.5 g were weighed into a 50 cm^3 beakers.

Concentrated Nitric acid (5.0 cm^3) was added and the beakers heated on a hot plate in a fume cupboard to a small volume. Concentrated Perchloric acid (5.0 cm^3) was added and then boiled again for few minutes after which 15.0 cm^3 of deionised water was also added, and allowed to cool to room temperature. The whole mixture was then transferred to a 100 cm^3 volumetric flask and made up to the mark with deionised water. These solutions were placed in plastic sample bottles to await analysis. A blank was prepared using same procedure excluding the plant sample.

Physical and chemical characteristics of the soil were determined before the treatment. Such parameters as soil texture, cation exchange capacity (CEC), soil pH, electrical conductivity (EC), organic matter (OM), organic carbon, base saturation, bulk density and percent porosity were determined. The soil texture was determined by the Bouyoucos hydrometer method). Soil pH and EC were measured on 1:2 extract (Soil:Water). Cation Exchange Capacity (CEC) was determined as described [19].

Soil samples from each Polyethylene bag was collected, air-dried for 2 weeks, ground into powder and sieved though a 2 mm mesh sieve [20]. Powdered soil sample (1.0 g) was accurately weighed and placed in a beaker. The tri-acid digestion procedure described by Allen et al. [21] was used in the soil sample digestion: These include a mixture of concentrated nitric acid (HNO_3), concentrated H_2SO_4 and Perchloric acid ($HClO_4$) in the ratio of 5:1:1. The acid mixture (20 cm^3) was added to the soil sample in a beaker and heated on a hot plate at 80°C to a small volume. The sample after cooling was filtered into a 100 cm^3 volumetric flask and made up to the mark with deionised water. The digest was then transferred into plastic sample bottles to await analysis. A blank digestion was also carried out in the same way as above excluding the soil sample.

Both the digested (plant and soil) samples were analysed for the heavy metals used in polluting the soil. The concentrations of the metals were determined using Atomic Absorption Spectrometry (Buck Scientific 210VGP).

2.2.1 Calibration curves

Calibration curves of absorbance against concentration for each of Cd, Cu, Pb, Cr and Co were prepared. The curves yielded good linearity and this implied that the instrument responded very well to the standard analyte of interest and therefore, would respond to the analyte in the samples. The calibration curves were used for the determination of the metal concentrations in samples. The actual concentration of the metal in soil and plant samples were determined as follows:

$X \text{ (mg)} = (V_{ss} \times C_{cal}) / 1000$
X = amount of metal in digested solution
V_{ss} = volume of sample solution
$C_{cal.}$ = concentration of metal in sample solution from the calibration curve

Amount of metal in soil and plant samples ($mg\ kg^{-1}$) = (Xmg × 1000) / Wt(g)
Wt = weight of sample digested

3. RESULTS AND DISCUSSION

The physico-chemical parameters of the experimental soil were presented on Table 1. These parameters are important soil properties that affect metal mobility in the soil. The moderate pH value of the soil is an indication that most of the soil's Pb, Cd and Cu are in insoluble solid forms [22] and the soil is optimal for the growth of most plants [23]. The high organic matter content of the soil means less availability of Cd for plant uptake [24]. The low organic carbon content of the soil indicated that there was less activities in the soil.

Table 1. Physicochemical parameters of soil in the study area

Silt (%)	22
Sand (%)	59
Clay (%)	19
pH	6.77
Conductivity (mS cm^{1})	0.21
Organic Carbon (%)	0.54
Organic Matter	9.36
Base Saturation (%)	73.75
Bulk Density (g cm^{-1})	1.55
Porosity (%)	38
Oil & Grease (mg L^{-1})	0.04
Moisture Content (%)	5
Magnesium (g kg^{-1})	2.6
Calcium (g kg^{-1})	4.4
Potassium (g kg^{-1})	1.15

The plant shoot length which is a growth parameter was measured at 2, 4 and 8 weeks using a meter rule from the base of the plant to the apex or youngest leaf [15]. The length of the root was also taken at harvest (8 weeks after planting).

The results were presented on Table 2. The plant grown on control and 5 mg/kg treatment grew longer and greener than those grown on the other treatments. The shoot length at 8 weeks after planting showed a general pattern of the higher the soil metals concentration the shorter the length. This implied that the metals concentration had a negative effect on the plant's growth. However, despite the effect, the plant was able to tolerate and survive the polluted soil condition.

Table 3 presents the metals concentration in various plant's tissues (roots, stems and leaves) at harvest. The results obtained for Pb corroborate that of Sekara et al. [10] in the sense that the plant's shoot (stems and leaves) contained more Pb than the roots. This situation was the same for the soil treatments but not for the control. At some pollution conditions (75 mg kg^{-1} and 100 mg kg^{-1}), cadmium distribution in the various plant organs (tissues) were in agreement with the report of Sekara et al. [10]. Under these conditions, the level of Cd in the plant leaves was higher than in the roots and the stems. Cobalt (at 20 mg kg^{-1}, 75 mg kg^{-1}, 100 mg kg^{-1} and 200 mg kg^{-1} soil treatments); cadmium (at 5 mg kg^{-1}, 75 mg kg^{-1} and 100 mg kg^{-1}); copper (at 5 mg kg^{-1} and 200 mg kg^{-1}) and chromium (at 5 mg kg^{-1} only) showed similar pattern as lead (Pb). Their levels in the plant shoot (at the above respective stated condition(s) of soil treatment were higher than in the roots. This means that the metals at indicated soil treatments were mostly translocated to the shoot after absorption by the roots from the soil.

Table 2. Soil treatments, shoot length at various ages and root length at harvest

Soil treatment (mg kg^{-1})	Shoot heights (cm)			Root length at 8weeks
	2 weeks	4 weeks	8 weeks	
5	30	119	126	28
20	14	75	98	22
75	17	43	80	24
100	21	78	91	21
200	40	71	88	17
220	26	63	82	24
Control	44	106	161	34

Table 3. Heavy metal concentration in plants parts at harvest

Soil treatments (mg kg^{-1})	Plant parts at 8wks	Metal concentrations(mg/kg)				
		Cd	Cu	Pb	Cr	Co
5	L	5.6 ±0.00	2.0±0.00	3.2±0.01	3.8±0.01	1.0±0.01
	S	4.0 ±0.06	12.0±0.01	5.0±0.03	0.8±0.00	0.4±0.00
	R	7.2 ±0.03	6.0±0.04	3.4±0.02	1.4±0.04	1.6±0.06
20	L	1.6 ±0.02	4.8±0.02	0.8±0.01	1.2±0.05	1.2±0.00
	S	5.4 ±0.07	6.8±0.04	4.4±0.01	1.6±0.00	1.0±0.00
	R	13.2 ±0.04	16.8±0.07	1.8±0.01	4.4±0.03	0.8±0.04
75	L	18.0 ±0.02	9.0±0.01	2.0±0.01	2.0±0.01	1.0±0.00
	S	17.4 ±0.07	6.4±0.03	1.0±0.00	1.4±0.01	0.8±0.03
	R	10.6± 0.03	15.6±0.02	2.0±0.03	13.8±0.06	1.2±0.00
100	L	7.0 ±0.04	6.4±0.06	2.6±0.05	0.8±0.02	1.4±0.03
	S	5.6 ±0.00	7.0±0.01	1.8±0.08	1.6±0.04	2.2±0.05
	R	3.0 ±0.01	18.2±0.01	3.8±0.00	3.4±0.01	1.8±0.01
200	L	8.8 ±0.03	8.8±0.02	1.0±0.00	1.2±0.00	1.0±0.01
	S	12.2 ±0.01	10.2±0.02	5.4±0.02	1.4±0.00	1.8±0.00
	R	24.4 ±0.08	14.0±0.03	3.8±0.00	2.4±0.08	2.0±0.00
220	L	3.6 ±0.03	10.0±0.08	1.8±0.01	0.8±0.00	0.6±0.02
	S	2.0 ±0.00	7.8±0.02	5.2±0.06	1.4±0.02	0.6±0.03
	R	57.4 ±0.20	23.6±0.04	2.6±0.04	5.4±0.03	1.8±0.02
Control	L	0.4 ±0.00	0.4±0.02	0.2±0.03	0.6±0.01	0.2±0.00
	S	0.6 ±0.00	2.2±0.01	1.0±0.01	0.4±0.00	0.4±0.01
	R	6.4 ± 0.05	3.0±0.01	3.0±0.04	2.2±0.07	0.8±0.03

The metal concentrations in the soil after harvesting the plants were shown in Table 4. Copper has the highest soil concentration in all the treatments including the control followed by Cr. Cobalt has the least concentration even at the higher metal treatments of 200 mg kg^{-1} and 220 mg kg^{-1}. This implied that the soil's Cu and Cr were probably in insoluble forms to be available for plant absorption and subsequent translocation to the shoot.

Table 4. Heavy metal concentration in soil

Soil treatments ($mg\ kg^{-1}$)	Metal concentrations (mg/kg)				
	Cd	Cu	Pb	Cr	Co
5	4.6±0.04	16.0±0.04	0.8±0.01	5.4±0.01	0.6±0.03
20	5.8±0.01	18.0±0.07	2.6±0.04	8.4±0.00	6.0±0.01
75	4.2±0.04	20.0±0.10	2.6±0.04	10.2±0.04	0.6±0.01
100	8.4±0.03	25.6±0.06	3.6±0.04	22.8±0.03	0.6±0.00
200	39.8±0.04	51.0±0.10	5.0±0.07	25.8±0.04	2.8±0.04
220	22.6±0.05	37.8±0.06	5.8±0.08	26.8±0.10	2.0±0.01
Control	0.8±0.03	12.0±0.04	4.4±0.01	4.6±0.04	1.0±0.03

3.1 Translocation Factor

The translocation factor (TF) for metals within a plant was expressed by the ratio of Cshoot/Croot to show metal translocation properties from roots to shoots [25-28]. The translocation factor (TF), the ratio of shoot to root, indicates internal metal transportation. The TF values for this work are presented on Table 5. It can be seen that at different treatments the TF values for the metals are greater than one while at other treatments we have TF<1. Translocation factor greater than one shows that metals were effectively translocated to the shoot from root [25,26,28]. The maximum rate of TF was recorded for Cd at 100 $mg\ kg^{-1}$ treatment (4.20) followed by Cd at 75 $mg\ kg^{-1}$ (3.34), while the lowest is for Cd at 220 $mg\ kg^{-1}$ (0.10) followed by Cr at 75 $mg\ kg^{-1}$ (0.25).

All the metals except Co have TF>1 at the lowest treatment of 5 $mg\ kg^{-1}$ which indicates that translocation of metals (Cd, Cu, Pb and Cr) was effectively made from root to shoot. At the highest treatment which is 220 $mg\ kg^{-1}$ all metals with the exception of Pb has TF<1 which indicates that at this concentration, the metals (Cd, Cu, Cr and Co) accumulated by *Telfairia occidentalis* plant studied were largely retained in the roots. The root tissues accumulate significantly greater concentration of the metals than shoots indicating high plant availability of the metals as well as its limited mobility once inside the plant. All results for Pb showed TF>1 suggesting that Pb could be effectively translocated from the roots to the shoots. This observation is similar to [29,30] observations on *Solanum nigrum* plant.

Table 5. Translocation factor

Soil treatments ($mg\ kg^{-1}$)	Translocation factors				
	Cd	Cu	Pb	Cr	Co
5	1.33	2.33	2.41	3.29	0.88
20	0.53	0.69	2.89	0.64	2.75
75	3.34	0.99	1.50	0.25	1.50
100	4.20	0.74	1.16	0.71	2.00
200	0.86	1.36	1.68	1.08	1.40
220	0.10	0.75	2.69	0.41	0.67
Control	0.16	0.87	0.40	0.45	0.75

3.2 Bioaccumulation Factor

The bioaccumulation factor (BF) values are presented on Table 6. The BF value is calculated as the concentration of metal in the above ground part of the plant (shoot) divided by the concentration of the metal in the soil [31-33]. The factor (BF) is given as Cshoot/ Csoil where Cshoot and Csoil are metals concentration in the plant shoot (mg kg^{-1}) and soil (mg kg^{-1}), respectively. The BF represents the contaminant concentration in plants comparing with the environment concentration (in soil) [34]. For a plant to be efficient tool in the contaminated soil phytoremediation, the BF have to be higher than 1. BF>1 are indicative of metal accumulating in the above ground portion of a plant [35].

Table 6. Bioaccumulation factor

Soil treatments (mg kg^{-1})	Bioaccumulation factors				
	Cd	Cu	Pb	Cr	Co
5	2.09	0.88	10.25	0.85	2.33
20	1.21	0.64	2.00	0.33	0.37
75	8.43	0.77	1.15	0.33	3.00
100	1.50	0.52	1.22	0.11	6.00
200	0.53	0.37	1.28	0.25	1.00
220	0.25	0.47	1.21	0.08	0.60
Control	1.25	0.22	0.27	0.22	0.60

From Table 7, Cd and Co has BF>1 for all treatments except Cd at 200, 220 and Co at 20 and 220 mg kg^{-1}. Pb is the only metal that has BF>1 for all treatments with the overall highest BF at 5 mg kg^{-1} (10.25) suggesting considerable bioaccumulation of Pb from soils even in soils with relatively high Pb concentration. All Cu and Cr treatments have BF<1. At the highest treatment (220 mg kg^{-1}) all the metals expect Pb have BF <1. The control result gives BF <1 for all metals except for Cd (1.25), while the Pb and Cd only treatments also gives BF<1.

Table 7. One way ANOVA results on metals content in control and treated soils

Treatment (mg kg^{-1})	5	20	75	100	200	220
F	0.39	1.056	4.102	35.747	0.000	1.866
P	0.72	0.487	0.196	0.027		0.349

3.3 Data Analysis

The results for metals content in the plant leaves (Table 3) was analyzed statistically using one way ANOVA and the package used was SPSS 17.0. The results of the analysis were presented on Table 7. There was no significant difference between the mean metals content of the control and that of 5 mg kg^{-1}, 20 mg kg^{-1}, 75 mg kg^{-1}, and 220 mg kg^{-1} treated soils ($P > 0.05$). However, the difference in the mean metals content of control to that of 100 mg kg^{-1} and 200 mg kg^{-1} treated soils was statistically significant.

4. CONCLUSION

From the results obtained, it can be concluded that:

- Fluted pumpkin can tolerate and survive low (5 mg kg^{-1}) to high (220 mg kg^{-1}) metals concentrations in soil.
- The plant accumulate the Cu and Cr metals more in the roots than in the shoots, while Pb and Co metals were accumulated more in the shoots than in the roots.
- The plant can be used as an efficient phytoremediator for soil contaminated with Pb, Co and Cd (to a lesser extent).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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