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# Phytoremediation of Heavy Metals Using Some Selected Leguminous Crops

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author GIA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HON, VSN and ECO managed the analyses of the study. Author ECO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The phytoremediation of heavy metals (cadmium, chromium, copper and lead) using *Phaseolus vulgaris* and *Arachis hypogaea* were investigated using standard techniques. Heavy metal polluted soil samples were collected from Crush Rock Industries Ishiagu, Ebonyi State and heavy metal free soil samples (used as control) were obtained from Ebonyi State Ministry of Agriculture, Ishiagu Station. The seeds of the two plants were collected from the Enugu State Ministry of Agriculture. The experimental setup consists of 4 contaminated potted soils each of *P. vulgaris* and *A. hypogaea*. Another 4 potted soils not contaminated with heavy metals served as control. Soil analysis was carried out prior to planting. The polluted soil sample had slightly acidic pH (pH was 6.34±0.29), higher Cation Exchange Capacity (21.80±0.33), higher Cd (25.18±0.34), Cr (10.20±0.21), Cu (28.54±0.49) and Pb (9.92±0.36) levels but lesser soil organic carbon (0.87±0.10). After the duration of 62 days the plants were harvested, their leaves and roots were digested and subjected to further experimental tests [determination of Metal concentration, transfer factor and bioaccumulation factor (BAF)]. *A. hypogaea* showed highest Cd translocation factor 1.63±0.08, TF<1 was observed in all the plants examined for Cr. *A. hypogaea* showed the highest BAF for Cd, (1.16±0.08). BAF<1 was observed for Cr and Cu in the two plants examined. The BAF

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of *A. hypogaea* was higher than that of *P. vulgaris*. Plants from the control showed zero to very minute concentration of heavy metals in their tissues. Although the study plants are food crops, they can also play a role in the phytoremediation of some heavy metals.

*Keywords: Heavy metal; Phaseolus vulgaris; Arachis hypogaea; phytoremediation; bioaccumulation.*

## 1. INTRODUCTION

Heavy metals are serious environmental pollutants. Environmental pollution by heavy metals has increased due to the influence of industrial development [1,2]. According to Nriagu [3], about 90% of anthropogenic emissions of heavy metals have occurred over the years. It is now well recognized that human activities lead to a substantial accumulation of heavy metals in soils on a global scale. Several methods have been used for removing the pollutants from the contaminated environments. Soils that are contaminated with heavy metals can be treated by acid leaching, soil washing, physical or mechanical separation of the contaminant, electro-chemical treatment, electrokinetics, chemical treatment, thermal or pyrometalurgical separation, or biochemical processes [4,5]. Remediation techniques that can be used for removing heavy metals from contaminated ground water include extraction and treatment with activated carbon adsorption, air stripping [6], chemical, biological, biochemical and biosorptive treatment technologies [5].

The use of some of these remediation techniques requires a high cost [7], a long time [6], logistical problems [8] and a technical complexity [9]. Therefore, an alternative solution is needed for heavy metals removal from the environment. Bioremediation is an innovative and promising technology available for removal of heavy metals and recovery of the heavy metals in polluted water and lands [10]. Scientists and engineers have been investigating the ability of living plants as a remediation alternative. This technology involves the extraction of metals by plant roots and the translocation thereof to shoots [11]. The root and shoots are subsequently harvested to remove the contaminants from the soil. Phytoremediation is one of bioremediation techniques that can be used as an alternative solution for heavy metal remediation process. The phytoremediation of metals is a cost-effective, efficient, environment- and eco-friendly ‘green’ technology based on the use of metal-accumulating plants to remove toxic metals, including radionuclides as well as organic pollutants from contaminated soils and water [9]. Considering the scope of phytoremediation and the limited researches on the use of food crops in the remediation of heavy metal contaminated soil, it is therefore necessary to investigate the potential of some legumes (*Phaseolus vulgaris* and *Arachis hypogaea*) in remediating heavy metals from contaminated soils. The principal goal of this research is to assess the translocation and bioaccumulation factor of these study crops.

## 2. MATERIALS AND METHODS

The seeds of *P. vulgaris* and *A. hypogaea* were purchased from the Enugu State Ministry of Agriculture and were all authenticated by Prof. J.C. Okafor, a renowned Taxonomist of “Fame Agricultural Center, Independence Layout, Enugu”. A voucher specimen was kept for references. The soil surface was cleared with a hand trowel to a depth of approximately 10 cm to 20 cm before the soil sample was collected using a stainless hand trowel. About 10 kg of sub soil (15 cm) was collected from Ebonyi State Ministry of Agriculture, Ishiagu Station. About 10 kg of heavy metal polluted sub-soil was also collected from Crush Rock Industry, Ishiagu Ebonyi State. The physicochemical properties of the soil including the pH were determined. The soil samples were subjected to analysis to determine the extent of heavy metal contamination.

Soils were introduced into 12 units of 7 L capacity experimental pots which were segregated into 2 groups (A and B). Group A contains the heavy metal polluted soil, while group B contains the unpolluted soil serving as a control. 8 pots were in Group A containing the heavy metal polluted soil, and *P. vulgaris* was cultivated in 4 of these pots while *A. hypogaea* was cultivated in the remaining 4 pots, while Group B served as the control containing 4 pots with unpolluted soil and each of the two plants cultivated in separate pots. The experiment lasted for a total of 62 days.

### 2.1 Digestion and Analysis of Soil Samples

Soil samples were digested prior to planting using dry-ash method according to Akio and

Johannes [12]. One gram of the representative soil sample was weighed into a porcelain crucible and heated on a heating mantle to volatilize all organic matter. One millilitre of concentrated nitric acid was added and evaporated to dryness using a heating mantle. The sample was introduced into a muffle furnace and ash at 450°C for 4 hr. After ashing, the dish was removed from the muffle furnace and 25 ml of aqueous hydrochloric acid solution (1:1) was used to wash out the sample into a 100 mL beaker. The solution was heated gently for 30 minutes for complete dissolution. The solution was allowed to cool and filtered into a 100 mL volumetric flask. The digest was made up to the mark using distilled water. The metals in the sample were determined [13] using a Hatch Model DR 300 Spectra Atomic Absorption Spectrophotometer.

### 2.2 Digestion and Analysis of Plant Samples

Plant samples were digested according to the method of Food Safety and Standards Authority of India [14]. One gram of each of the test sample was weighed into 100 mL beaker. Concentrated hydrochloric acid and nitric acid were added to the weighed samples in the ratio 3:1 volume by volume, that is 30 mL of hydrochloric acid and 10 mL of nitric acid to each sample. 10 drops of hydrogen peroxide was added to each of the sample. Hydrogen peroxide increases the complexing properties of the mineral acids. Each of the preparation was heated on a laboratory hot plate in fume cupboard. Heating was continued until sample digests completely. Each digest was diluted with 50 mL of distilled water and filtered into a 100 mL volumetric flask using Whatman filter paper. The filtrate was made up to the mark with distilled water. Metals were determined [13] using Atomic Adsorption Spectrophotometer.

### 2.3 Determination of Bioaccumulation Factor (BAF)

The Bioaccumulation factor (BAF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (according to Ghosh and Singh [15]) and was calculated using the formula.

$$\text{BAF} = \frac{Metal\ concentration\ in\ plant\ tissue}{Initial\ concentration\ of\ metal\ in\ substrate\ (Soil)}$$

### 2.4 Determination of Transfer Factor

Translocation factor (TF) is the plant's ability to translocate heavy metal from the root to harvestable aerial part. When TF>1, it indicates a preferential partitioning of metals from soil to root and from root to shoot respectively. Mathematically, TF is expressed as

$$\text{TF} = \frac{Heavy\ Metal\ Concentration\ in\ Shoot}{Heavy\ Metal\ Concentration\ in\ Root}$$

### 2.5 Plant Growth Parameters

Data collection started two weeks after sprouting. Growth parameters recorded at different stages of crop growth and development were: Plant height (cm), Stem girth (cm), Leaf area ($cm^2$), Number of leaves and Root length (cm). Height of the plant (cm) was recorded on a weekly basis, using a tape rule. The height was measured from the base of the stem to the tip of the leaf. Number of leaves was also counted every week to know the mean number of leaves that the crop produced. Stem girth reading was taken using a tape rule. The area of the leaf was gotten by adding the leaf length to the leaf width and multiplying by 0.75.

## 3. RESULTS

### 3.1 Analysis of Soil Samples

The results for the analysis of the experimental soil samples are shown in Table 1. The table revealed that most of the measured parameters varied considerably in both soil samples. The pH for the unpolluted soil sample (control) was slightly neutral 7.41 while that of the polluted soil sample was slightly acidic 6.34. The unpolluted soil sample showed higher soil organic carbon of 12.12%. The levels of N, P and K were higher in the polluted soil samples (0.05%, 9.10% and 8.8% respectively). The sand, silt, and gravel percentages of the unpolluted soil samples were higher than the percentages observed in the polluted soil samples (63.90%, 15.30 and 5.05% respectively).

### 3.2 Heavy Metal Concentrations in the Plant Tissues

The concentrations of the heavy metals in the leaves and roots of *Phaseolus vulgaris* and *Arachis hypogea* are summarized in the Table 2. The table revealed the various concentrations of heavy metals in the root and leaves of the two study plants (*P. vulgaris* and *A. hypogaea*). For plant samples from polluted soil, it was observed that the concentrations of Cd were higher in the leaves of *P. vulgaris* (15.07 mg/kg) and *A. hypogaea* (18.12 mg/kg) compared to the roots (10.04 and 11.09 mg/kg respectively). The levels of Cd observed in all the plant parts were above the WHO maximum allowable limit (0.10 mg/kg). The concentrations of Cr were higher in the roots than the leaves of all the plant samples. The concentration of Cu in the leaves of *P. vulgaris* and *A. hypogaea*, were 13.54 and 14.65 mg/kg respectively. While the concentrations of Cu in the roots of *P. vulgaris* and *A. hypogaea*, were: 9.27 and 9.23 mg/kg respectively. The concentrations of Cu observed in all the plant parts were below the WHO maximum allowable concentration (73.00 mg/kg). The concentrations of Pb in the leaves and roots of the plant samples were observed as follow; *P. vulgaris* (leaves 4.11 mg/kg, root 3.05 mg/kg), *A. hypogaea* (leaves 6.23 mg/kg, root 4.17 mg/kg). All concentrations of Pb observed in the plant parts were above WHO maximum allowable limit (0.30 mg/kg). In contrast to Cr, Cd, Cu and Pb concentrations which were higher in the leaves than the roots of the two plants. The concentrations of Cr observed in all the plant parts were higher than the WHO maximum allowable limit (0.05 mg/kg).

Most of the heavy metals analyzed such as Cd and Cr were not observed in the plant tissues of *P. vulgaris* and *A. hypogaea* from the unpolluted soil (Table 2). Again, Cu and Pb were not observed in the leaves of these two plants (*P. vulgaris* and *A. hypogaea*) from the unpolluted soil, but were observed in the root samples at very minute concentrations (0.01 mg/kg). The concentrations of all the heavy metals analyzed for all plant samples were below WHO maximum allowable concentration.

**Table 1. Characteristics of the soil used for experiment**

| Parameters | Unpolluted soil | Polluted soil |
|---|---|---|
| pH-$H_2O$ | 7.41±0.15 | 6.34±0.29 |
| CEC (meq/100 g soil) | 11.20±0.10 | 21.80±0.33 |
| Organic carbon (% wt) | 12.12±0.21 | 0.87±0.10 |
| Nitrogen (% wt) | 0.02±0.00 | 0.05±0.00 |
| Phosphorus (% wt) | 4.4±0.12 | 9.10±0.20 |
| Potassium (ppm) | 3.2±0.15 | 8.80±0.42 |
| Sand (% wt) | 63.90±0.43 | 28.90±0.31 |
| Silt (% wt) | 15.30±0.22 | 12.00±0.28 |
| Gravel (% wt) | 5.05±0.11 | 2.12±0.30 |
| Clay (% wt) | 19.00±0.47 | 21.32±0.38 |
| Cadmium, Cd (ppm) | 1.00±0.00 | 25.18±0.34 |
| Chromium, Cr (ppm) | 0.50±0.00 | 10.20±0.21 |
| Copper, Cu (ppm) | 2.50±0.02 | 28.54±0.49 |
| Lead, Pb (ppm) | 2.85±0.05 | 9.92±0.36 |

*Results are in mean±SE*

*The heavy metals analysed (Cd, Cr, Cu and Pb) were far higher in polluted soil sample (Cd 25.18 mg/kg, Cr 10.20 mg/kg, Cu 28.54 mg/kg, Pb 9.92 mg/kg) than the unpolluted*

**Table 2. Heavy metal concentration in the leaves and root of the two study plants**

| Soil sample | Plant | Plant organs | Cd (mg/kg) | Cr (mg/kg) | Cu (mg/kg) | Pb (mg/kg) |
|---|---|---|---|---|---|---|
| Polluted soil | *P. vulgaris* | Leaf | 15.07±0.05 | 4.01±0.00 | 13.54±0.12 | 4.11±0.08 |
| | | Root | 10.04±0.00 | 5.05±0.02 | 9.27±0.10 | 3.05±0.01 |
| | *A. hypogaea* | Leaf | 18.12±0.10 | 4.01±0.00 | 14.65±0.25 | 6.23±0.10 |
| | | Root | 11.09±0.01 | 5.05±0.01 | 9.23±0.10 | 4.17±0.10 |
| Unpolluted soil (Control) | *P. vulgaris* | Leaf | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | | Root | 0.01±0.00 | 0.02±0.00 | 0.01±0.00 | 0.01±0.00 |
| | *A. hypogaea* | Leaf | 0.00±0.00 | 0.00±0.00 | 0.02±0.00 | 0.00±0.00 |
| | | Root | 0.01±0.00 | 0.01±0.00 | 0.01±0.00 | 0.01±0.00 |
| **WHO/FAO MAL** | | | **0.10** | **0.05** | **73.00** | **0.30** |

*MAL – Maximum allowed limit. Results are in mean±SE*

**Table 3. Translocation factor of heavy metals in *P. vulgaris* and *A. hypogaea***

| Soil sample | Plant | Cd (mg/kg) | Cr (mg/kg) | Cu (mg/kg) | Pb (mg/kg) |
|---|---|---|---|---|---|
| Polluted soil | *P. vulgaris* | 1.50±0.05 | 0.79±0.00 | 1.46±0.10 | 1.35±0.10 |
| | *A. hypogaea* | 1.63±0.08 | 0.79±0.01 | 1.59±0.25 | 1.49±0.10 |
| Unpolluted soil | *P. vulgaris* | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| (Control) | *A. hypogaea* | 0.00±0.00 | 0.00±0.00 | 2.00±0.10 | 0.00±0.00 |

*Results are in mean±SE*

**Table 4 BAF of heavy metals in *P. vulgaris* and *A. hypogaea* grown in polluted soil**

| Soil sample | Plant | Cd (mg/kg) | Cr (mg/kg) | Cu (mg/kg) | Pb (mg/kg) |
|---|---|---|---|---|---|
| Polluted soil | *P. vulgaris* | 1.00±0.02[a] | 0.89±0.00[ab] | 0.80±0.05[ab] | 0.72±0.04[a] |
| | *A. hypogaea* | 1.16±0.08[b] | 0.89±0.01[ab] | 0.83±0.01[ab] | 1.05±0.10[b] |

*Results are in mean±SE. Same letter across a column is not significantly different at 0.05 level probability*

Table 2 revealed higher concentrations of heavy metals in the leaf samples than in the root samples of the two plant samples. Cu was the most abundant heavy metals observed in the plant tissue sample, while Cr was the least heavy metal observed in the plant tissue samples. Cr concentrations in the root samples were higher than Cr concentrations in the leaf samples of the two plants investigated as happened in contaminated soil.

### 3.3 Translocation Factor of Heavy Metals

The translocation factors of the heavy metals (Cd, Cr, Cu and Pb) for *P. vulgaris* and *A. hypogaea* are presented in Table 3. The table revealed the translocation factor of Cd for *P. vulgaris* and *A. hypogaea* to be greater than 1 (1.50 and 1.63 respectively). TF<1 was observed for Cr in the two plant samples, while TF>1 was also observed for Cu and Pb in the two plant samples.

For unpolluted soil sample, TF equals zero in the two plant samples for Cd, Cr and Pb. *A. hypogaea* showed TF = 2.00 for Cu (Table 3).

### 3.4 Bioaccumulation Factor of Heavy Metals

The results on bioaccumulation factor (BAF) of heavy metals in *P. vulgaris* and *A. hypogaea* grown in polluted soil are shown in Table 4.

It was observed that some of the BAF of the plants examined were greater than 1 for Cd and Pb. The BAF of Cd was highest in *A. hypogaea* (1.16) than *P. vulgaris* (1.00), similarly to Pb (1.05). *P. vulgaris* and *A. hypogaea* showed similar BAF values for Cr (0.89).

## 4. DISCUSSION

The present study investigated the phytoremediation potentials of some legumes (*P. vulgaris* and *A. hypogaea*) on soil polluted with heavy metals (Cd, Cr, Cu and Pb). Among the heavy metals investigated, Cr, Cu and Pb were observed to be below the Dutch standard maximum allowed limits (Cr 100.00 mg/kg, Cu 36.00 mg/kg and Pb 85.00 mg/kg) while Cd concentrations was above Dutch standard maximum allowed limit (Cd 0.80 mg/kg). The concentrations of all the heavy metals observed in the unpolluted soil sample were below Dutch standard maximum allowed limits.

The soil pH in the study was observed to be slightly alkaline (7.41±0.15) in unpolluted soil sample and slightly acidic (6.34±0.29) in polluted soil sample. This is because of the presence of heavy metals in the polluted soil sample. Soil pH specifically affects plant nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions they undergo. The optimum pH range for most plants is between 5.5 and 7.5. The effect of pH on heavy metal availability to plant has been reportedly researched and it is accepted that as pH decreases, the solubility of cationic forms of metals in the soil solution increases and therefore, they become more available to plants [16]. Soil pH is one of the factors which influence the bioavailability and transport of heavy metals in the soil and according to Cheng [17], heavy metal mobility decreases with increasing soil pH.

The phytoremediation capacity of a plant is highly dependent on the translocation factor and bioaccumulation factor of the plant. Translocation factor is the plant's ability to translocate heavy metal from the root to harvestable aerial part.

When TF>1 is obtained, it indicates a preferential partitioning of metals from soil to root and from root to shoot. Bioaccumulation factor of metals is used to determine the quantity of heavy metals that is absorbed by the plant from the soil. It is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil [15]. The study investigated the TF and BAF of the four study plants. The TF of the legumes (*P. vulgaris* and *A. hypogaea*) for Cd, Cu and Pb were greater than 1 (TF>1). The BAF values of *A. hypogaea* for Cd and Pb were greater than 1 (BAF>1) but was less than 1 for Cr. The BAF values of *P. vulgaris* were less than 1 for Cr, Cu and Pb but equal to 1 for Cd.

## 5. CONCLUSION

The study revealed that the two plant samples were efficient in absorbing and translocating some of the heavy metals (Cadmium, Copper, and Lead). The metal mostly accumulated by the study plants was cadmium. Translocation factor greater than one (TF>1) was observed in plants cultivated in Cd, Cu, and Pb contaminated soil. Chromium concentration was very low in the plant tissues and the TF observed in the samples cultivated in Cr contaminated soil was less than one (TF<1). *Arachis hypogaea* and *Phaseolus vulgaris* were good bio-accumulators of Pb. Amongst the two food crop studied, *A. hypogaea* was more suitable in phytoremediation of heavy metals.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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