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Assessment of the Phytoremediation Potential of Heavy Metal Contaminated Soil Using Vigna Unguiculata L. (Walp)

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Abstract: Phytoremediation is a plant-based approach involving use of plants to extract and remove elemental pollutants or lower their bioavailability in soil. Thus, this study aimed at assessing the phytoremediation potential of Vigna unguiculata grown in heavy metal contaminated soil. Mature seeds of V. unguiculata were obtained from local farmers in Ojo-Lagos, Nigeria; heavy metal contaminated, and control soils were obtained from Iba and LASU Botanical Garden, Ojo-Lagos respectively. Physiochemical analyses of soil samples were done before and after transplanting. Nurseries were made and one seedling was transplanted into 5 buckets each for control and contaminated soils respectively. Growth parameters- stem height, stem girth, leaf length, and so on were measured. Heavy metal analysis was done using standard analytical procedures. Metal transfer factors and bioaccumulation potential were also studied. Data collected were analyzed using mean-standard deviation. Soil physiochemical parameters and heavy metals analyzed showed reduction in most of the metals studied before and after soil analyses. Results showed the transfer factors for Zn (0.07 mg/kg), Fe (6.72mg/kg), Mn (1.33 mg/kg), As (1.00mg/kg), Pb (0.19mg/kg), Cd (0.007mg/kg) while the bioaccumulation potential of Zn (0.08 mg/kg), Fe (3.86mg/kg), Mn (1.09mg/kg) As (1.00mg/kg), Pb (0.20mg/kg), Cd (0.001mg/kg). Also, the result revealed the metal uptake rate of Fe (21.75%), Cd (0.94%), Zn (1.40%), Pb (84.80%) and Mn (0.11%). It could be concluded that V. unguiculata reduces heavy metals in soil, had high transfer factors, bioaccumulation, and uptake rates. This study recommends that V. unguiculata could be used for phytoremediation of heavy metal environmental contaminated soils.

Keywords: Heavy metals, Phytoremediation, Bioaccumulation, Vigna unguiculata

Introduction

Air, water, and soil pollution are characterized as "undesirable changes in physical, chemical, and biological characteristics" (Yuvaraj & Mahendran, 2018) that have negative effects on human health, ecosystem health, economic development, quality of life, and cultural artefacts. Pollutants are a common cause of this, as they can have a negative impact on people's health, quality of life, possessions, and environment (Ogun et al., 2023). An increasing risk to human and environmental health may result from soil pollution in recent decades. Human activities are the primary contributors to soil contamination, which can lead to an alarming buildup of toxins (Cachada et al., 2018; Ogun et al., 2023) in the ground. Both naturally occurring and man-made sources of contaminants (organic and inorganic compounds) are included in the definition of "soil pollution" (Ogun et al., 2023) used here. Lack of clearly described monitoring factors and indicators may make soil quality monitoring a challenging process. However, as the world's population rises, so do the problems that threaten soil quality and the requirement for maintaining soil fertility over the long term (Cachada et al., 2018).

The term "heavy metal" is used to describe a class of chemical elements with large atomic weights, atomic numbers, and densities. Cadmium, Mercury, Lead, Arsenic, Zinc, Copper, Nickel, and Chromium are all examples of heavy metals/metalloids that are commonly encountered. Anthropogenic and natural sources of these heavy metals/metalloids include oil and gas wastewater, agricultural phosphate fertiliser use, sewage sludge, metal mining/smelting, pesticide application, electroplating, and fossil fuel combustion (Muradoglu et al., 2015). Since heavy metals cannot be broken down chemically or physically, they remain in the soil for a very long time and constitute a serious hazard to the environment (Suman et al., 2018). Several metals are typically applied to agricultural soils alongside organic and mineral fertilizers (Zwolak et al., 2019). Plant protection products are yet another potential source of the metals. Soil characteristics are important in determining the bioavailability of heavy metals, which affects their movement through the soil and subsequent uptake by plants (Zwolak et al., 2019). There is a need for remediation since these metal concentrations in soil have become a threat to plant safety and food security.

Heavy metals can be grouped as essential and non-essential based on their role in biological systems; hence, it is necessary to take remediation measures to prevent heavy metals from entering terrestrial, atmospheric, and aquatic environments, and mitigate the contaminated land (Gerhardt et al., 2017). There are varieties of remediation approaches that have been developed to reclaim heavy metal-contaminated soil. These measures are mainly based on mechanical or physio-chemical techniques, such as soil incineration, excavation and landfill, soil washing, solidification, and electric field application (Sheoran et al., 2011; Cristaldi et al., 2017; Wang et al., 2017).

In phytoremediation, plants are used to either completely remove or significantly reduce the bioavailability of elemental contaminants in soil (Berti & Cunningham, 2000; Padmavathiamma & Li, 2007). Even at low quantities, ionic substances in the soil are absorbed by plants through their root systems. By establishing a rhizosphere ecosystem, plants can reclaim damaged soil and stabilise soil fertility by accumulating heavy metals and regulating their bioavailability (Cristaldi et al., 2017; Wang et al., 2017; Jacob et al., 2018). The incorporation of organic and inorganic materials affects plant metal mobility and assimilation. Metal bioavailability to plants may also be influenced by the age of the soil, as suggested by several studies (Padmavathiamma & Li, 2007; Zaid et al., 2020). Absorption of metals also varies among plant species, with both soil conditions and plant type playing a role. Metal concentrations were also shown to vary considerably depending on the specific plant tissue they were measured in, the plant species they belonged to, and even the variety within the same species (Zulfiqar et al., 2019; Zwolak et al., 2019).

The medium-sized, edible bean of the *Vigna unguiclata* legume is grown in many parts of the world. It is a cowpea subspecies that was domesticated in Africa and is now grown across the World (Herniter et al., 2019). This vining plant emits compounds from its roots that beckon the nitrogen-fixing bacterium rhizobia. Nodules, which look swolen on the roots, protect the rhizobia and provide a source of carbon. In return, they receive a useful, stable form of nitrogen. *V. unguilata* can replenish soil nutrients by releasing bacteria into the soil just before death, (Lindström & Mousavi, 2020). Since heavy metals are harmful to both plants and humans, this study evaluates the viability of utilizing *Vigna unguiculata* L. (Walp) to phytoremediate soil contaminated with these metals.

Materials and Methods

Collection of Soil Sample and Experiment Site

Contaminated soil samples were taken from the area of Iba in Lagos, Nigeria, which is famous for its extensive anthropogenic activity. Debris was removed from the sampling area, and then the top 15 centimeters of soil were removed. Soil samples were collected in a polyethylene bag, then combined and mixed to create a single representative sample. Soil used in the control group was obtained from the Botanical Garden at Lagos State University in Ojo, Nigeria. The research took place in the Botanical Garden on the campus of Lagos State University in Ojo, Lagos State, Nigeria.

Soil Sample Preparation and Digestion

The contaminated and the garden soil samples were taken to the laboratory for heavy metal analysis before planting to check the level of metal in the soil. The several methods used for the metal analysis followed the procedures employed by Oladele et al. (2016).

Experimental Design and Treatments

Ten (10) buckets received five kilogrammes (5kg) of soil each that had been well mixed. The bases of the buckets were pierced to prevent water logging and to improve soil aeration. The 10 buckets were divided into two groups of five each: control (garden soil that had not been treated), and contaminated soil, which contained five buckets. A Completely Randomised Design (CRD) was used to plan the experiment.

Nursery Practice

Vigna unguiculata seeds were planted in the nursery in a bowl of loamy soil that had been well ground up and treated with manure. Then, using a sprayer, this was watered often. The germination time of the seeds was 3 days, and 2 weeks of establishment in the nursery were given to the seedlings before they were transplanted.

Transplanting

The seedlings were transplanted into buckets filled with 4 kg of soil at the rate of one-plants-per-bucket which makes up ten stands for ten buckets. The buckets were perforated at the base to avoid water logging and to increase the soil aeration. The buckets were arranged in two major groups as follows: Garden soil (control), -5buckets, contaminated soil-5buckets. Weeding was then carried out manually on a weekly basis.

Measurement of Growth and Yield Parameters

Plant height was measured from the ground level to the growth point with a meter rule in centimeters and the observation recorded for each treatment. Also, the numbers of branches were counted, and the stem girth measured using vernier caliper. The total number of leaves per plant were counted for each treatment and recorded. The measurements were taken in an interval of 2 weeks from the day the plant was transplanted. The leaf area was determined using Oluwole *et al*. (2019) formula:

$$\text{Leaf Area} = 0.853 + (L \times B) \times 8.7440.$$

Pre-treatment Methods for Plant Samples for Heavy Metal Analysis

The plant samples' leaves, stems, and roots are cleaned separately by gently washing with distilled water. The heavy metal concentrations in the leaves, stem, and root were then measured after each sample was subsampled, air-dried in the lab for three days at room temperature, and then subjected to further ashing processing. A porcelain crucible containing 5g of each dry sample was weighed, and it was then thoroughly ashed at 5500C for 4 hours in a muffle furnace. A 5 ml solution of diluted (1:1) nitric acid was then added to the residue after it had cooled. In 25 cc of distilled water, the mixture was diluted. Filter paper Whatman #1 was used to remove the solution. In order to identify the metals, the filtrate was retained.

Heavy Metals Analysis

The solution produced by dry ashing the plant sample at 550°C and dissolving the ash in distilled deionized water in a flask was used to analyse the heavy metal components of the sample. With the use of an atomic absorption spectrophotometer (Buck Scientific Model 200A), all the metals (Mn, Cu, Zn, Pb, and Cd) were examined. For soil samples, the same process was repeated (Oluwole et al., 2020).

Determination of Transfer Factor (TF)

The transfer of metal from the soil to the plant body is described by the term "heavy metal transfer factor" (TF), which is also known as "accumulation factor AF" or "bioconcentration factor BCF." According to Rashid et al. (2016), this was computed by dividing the heavy metal content in the plant by the corresponding concentration in the soil.

Transfer Factor (TF) = Conc. HM in aerial part of the plant / Conc. HM in the Soil

Determination of the Multiplication Coefficient (MC)/Bioconcentration Factor

The concentration of heavy metals absorbed by the plants in relation to their concentration in the soil was calculated using the Multiplication Coefficient (MC)/Bioconcentration Factor (BCF). Yoon et al. (2006) similarly used the equation: to determine the BCF.

Bioconcentration Factor (BCF) = Conc. HM in the roots / Conc. HM in the Soil

Determination of the Remediation/Metal Uptake Rate

The quantity of heavy metal that the plant sample removed from the contaminated soil, abstracted from it, or accumulated was determined as follows: R = quantity of metal lost by the soil or gained by the plant represented as a percentage of original amount. i.e.

$$\text{Rate } (\%) = \frac{C^{nth\ week} - C^{oth\ week}}{C^{oth\ week}} \times \frac{100}{1}$$

Where, $C^{nth\ week}$ and $C^{\text{oth week}}$ are concentrations of the heavy metals in the soil or plant sample at time (weeks) = „n“ and „0“ respectively.

Statistical Analysis

All data collected were in triplicates and these were analyzed by means±standard deviation using SPSS version 18.

Results

Phytoremediation Potential of *Vigna unguiculata* Growth in Heavy Metal Contaminated Soil

a. Stem Height

Table 1 shows the phytoremediation potential of *Vigna unguiculata* stem height in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest stem height compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 6^{th} week after transplant.

b. Leaf Length

Table 2 shows the phytoremediation potential of *Vigna unguiculata* leaf lenght in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest leaf length compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 4^{th} week after transplant (Table 2).

c. Leaf Breadth

Table 3 shows the phytoremediation potential of *Vigna unguiculata* leaf breadth in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest leaf breadth compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 4^{th} week after transplant (Table 3).

d. Leaf Girth

Table 4 shows the phtoremediation potential of *Vigna unguiculata* leaf girth in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest leaf girth compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, there was no regression in the leaf girth of *Vigna unguiculata* in control soil (Table 4).

e. Leaf Petiole

Table 5 shows the phytoremediation potential of *Vigna unguiculata* leaf petiole in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest leaf petiole compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 4^{th} week after transplant (Table 5).

f. Number of Leaflet

Table 6 shows the phytoremediation potential of *Vigna unguiculata* number of leaflets in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest number of leaflets compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 6^{th} week after transplant.

g. Leaf Area

Table 7 shows the phytoremediation potential of *Vigna unguiculata* leaf area in heavy metal contaminated soil. *Vigna unguiculata* in control soil has the highest leaf area compared to those in contaminated soil at the end of the 8^{th}week after transplant. However, *Vigna unguiculata* in contaminated soil was increasing but started regressing after the 6^{th} week after transplant.

Table 1. Phytoremediation potential of *vigna unguiculata* stem height in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	12.0±3.05	12.30±1.04	18.2±1.08	18.0±0.55
Control soil	16.2±3.82	23.7±0.87	25.0±1.11	26.2±1.00

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 2. phytoremediation potential of *vigna unguiculata* leaf length in heavy metal polluted soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	3.5±0.47	5.8±1.85	5.1±1.74	3.0±1.73
Control soil	7.5±0.78	8.8±1.26	10.0±0.26	10.1±1.61

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 3. Phytoremediation potential of *vigna unguiculata* leaf breadth in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	3.0±0.10	3.1±0.85	2.6±0.57	2.0±1.15
Control soil	4.0±0.40	5.2±0.61	5.5±0.67	6.0±0.50

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 4. Phytoremediation potential of *vigna unguiculata* leaf girth in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	0.5±0.06	0.8±0.15	1.1±0.10	1.2±0.06
Control soil	0.5±0.10	0.9±0.06	1.3±0.06	1.5±0.06

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 5. Phytoremediation potential of *vigna unguiculata* leaf petiole in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	3.8±0.36	4.2±0.67	4.1±2.65	1.0±0
Control soil	6.2±0.55	6.8±0.65	13.3±2.04	7.8±1.26

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 6. Phytoremediation potential of *vigna unguiculata* number of leaflet in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	7.5±1.15	3.7±1.15	4.0±1.73	2.0±3.46
Control soil	8.0±0.0	12.3±3.51	25.0±10	36.7±16.04

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 7. Phytoremediation potential of *vigna unguiculata* leaf area in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	92.67±17.71	158.07±84.92	116.80±45.76	53.32±30.29
Control soil	263.17±51.46	400.98±104.02	455.54±71.66	462.54±63.44

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Physiochemical Analysis of the Soil before and after Remediation

Table 9 shows the physiochemical and heavy metal analysis of the soil samples used before and after remediation. Thus, the result revealed that the heavy metals such as As, Cd, Fe, Pd, Ni, Mn, Zn and so on decreased progressively in soil after remediation in both control and polluted soils respectively (Table 9).

Heavy Metal Transfer Factors of *Vigna Unguiculata* (White Beans) Grown in Contaminated Soil

Figure 2 shows the heavy metal transfer factor phytoremediation potential of *Vigna unguiculata*. The result revealed that iron (6.72) metal had the highest transfer factor followed by manganese (1.33), arsenic (1), lead (0.19), cadmium (0.001), had the least metal transfer factor.

Heavy Metal Bioconcentration Factors of *Vigna Unguiculata* Grown in Contaminated Soil

Figure 3 shows the heavy metal bioconcentration factors phytoremediation potential of *Vigna unguiculata*. The result revealed that iron (3.86) metal had the highest transfer factor followed by manganese (1.09), arsenic (1.00), lead (0.20), cadmium (0.001), had the least metal transfer factor.

Table 8. Phytoremediation potential of *vigna unguiculata* leaf area in heavy metal contaminated soil

Parameters	2 WAT	4 WAT	6 WAT	8 WAT
Contaminated soil	92.67±17.71	158.07±84.92	116.80±45.76	53.32±30.29
Control soil	263.17±51.46	400.98±104.02	455.54±71.66	462.54±63.44

WAT-Weeks after transplant; Values are presented as Mean±Standard Deviation

Table 9. Physiochemical analysis of the soil before and after remediation

Parameters	Con. Before	Con. After	Ctd. Before	Ctd. After
pH (at 25°C)	5.80	6.21	1.95	5.90
Electrical Conductivity (uS/cm)	109.2		20340.0	
Total Organic Carbon (%)	25.81	18.47	15.46	17.92
Total Organic Matter (%)	44.40	31.72	26.60	30.78
Phosphate (mg/kg, PO43-)	37.02	25.23	80.09	40.56
Total Nitrogen (mg/kg)	123.70	1560.15	63.6	61.23
Total Petroleum Hydrocarbons (mg/kg)	0.05	0.03	28099.0	10000
Arsenic (mg/kg)	0.05	0.03	45.07	0.05
Cadmium (mg/kg)	50.07	43.24	83.70	51.44
Iron (mg/kg)	40.48	22.50	201.03	32.55
Lead (mg/kg)	0.07	0.05	42.14	0.32
Manganese (mg/kg)	41.91	20.10	44.78	37.26
Mercury (mg/kg)	0.05	0.03	0.05	0.001
Nickel (mg/kg)	30.70	20.70	49.75	30.64
Zinc (mg/kg)	10.24	14.21	99.59	33.40

Con. Before and After = control soil sample used before and after planting; Ctd: Before and After = contaminated soil sample used before and after planting.

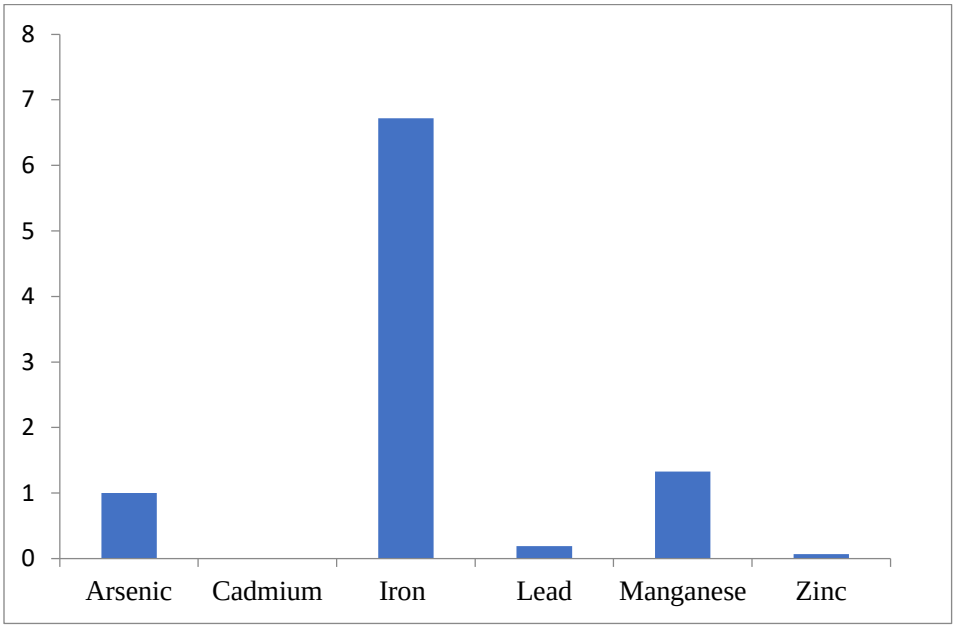

Figure 1. Heavy metal transfer factors of *vigna unguiculata* grown in contaminated soil

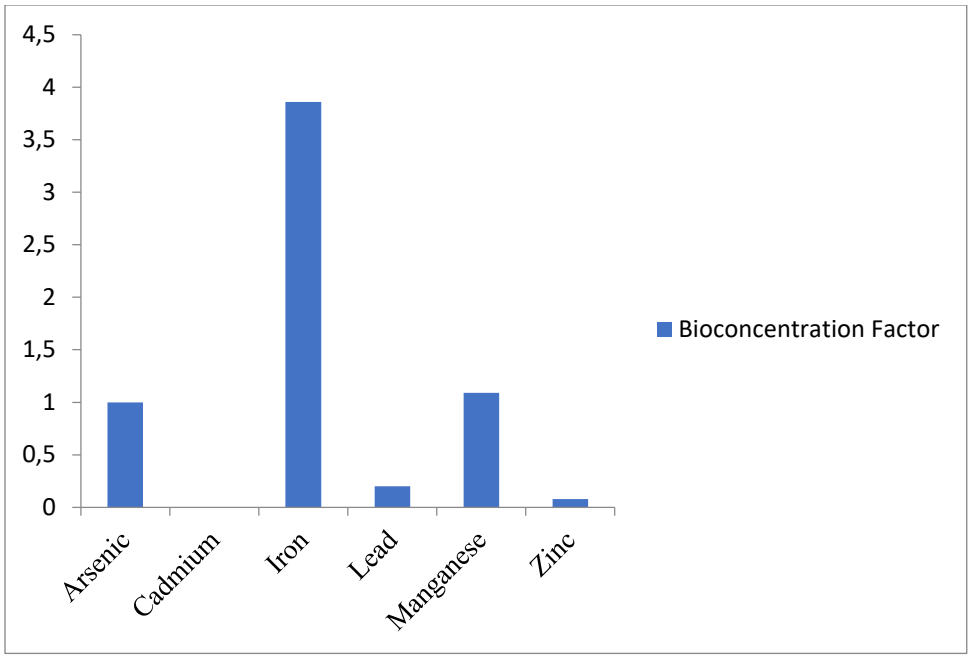

Figure 2. Heavy metal bioconcentration factors of *vigna unguiculata* grown in contaminated soil

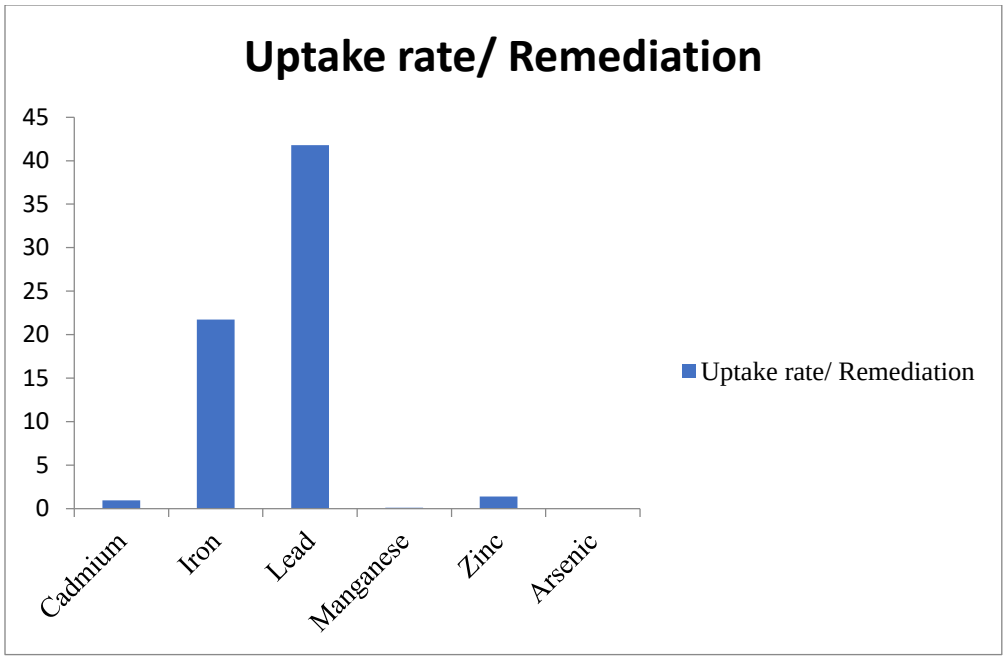

Figure 3. Heavy metal remediation/uptake rate of *vigna unguiculata* grown in contaminated soil

Discussion

In this study, the phytoremediation potential of *Vigna unguiculata* cultivated in contaminated heavy metal polluted soil were studied and compared to those in the control soil. It was observed that the *Vigna unguiculata* cultivated in the control soil performed well morphologically compared to those in contaminated soil (Tables 1-7). This is due to the presence of higher hydrocarbon in the contaminated soil (Table 8). It has been reported that soil rich in hydrocarbon creates a barrier for absorption of water and reduces aeration i.e., it doesn't allow the free flow of water

into the soil, and it blocks oxygen from getting through the soil. The finding of study agrees with the work of Anyalogbu et al. (2017) who documented reduction in morphometric parameters such as plant height, leaf length, leaf breadth and so on in studies on phytoremediation potential of *Talinum triangulare* in heavy metal and hydrocarbon contaminated soil.

From the result of the physiochemical analysis of the soil, there was a reduction in the heavy metals-before planting the values were: Arsenic (45.07), Cadmium (83.70), Zinc (99.59), Iron (201.03), Lead (42.14) and Manganese (44.78); and values after planting reduced: Arsenic (0.05), Cadmium (51.44), Zinc (33.40), Iron (32.50), Lead (0.32) and Manganese (37.26). These reductions in physiochemical analysis of heavy metals observed agreed with the findings of Puga et al., (2015) and Oladele et al. (2018), who observed similar results using V. unguilata. This reduction has been attributed to translocation metals from the soil to roots via root hairs. More so, in this study, it could be documented that Iron has the highest transfer factor of 6.72mg/kg followed by Manganese with 1.33mg/kg, Arsenic with 1mg/kg, Lead with 0.19mg/kg, Zinc with 0.07mg/kg, and cadmium with 0.007mg/kg (Fig.1). From this, the highest heavy metal that is moveable and moves faster from the soil into the plant is iron. This study concurs with the findings of Matijevi et al. (2014). This was due to metal specific properties and plant affinities for metal mobilization (Cristaldi et al., 2017; Wang et al., 2017).

Furthermore, in this study, iron has the highest heavy metal bioaccumulation potential of 3.86mg/kg followed by Manganese with 1.09mg/kg, Arsenic with 1.00mg/kg, Lead 0.20mg/kg, Zinc 0.08mg/kg and Cadmium 0.00mg/kg (Fig. 2). Moreover, in this study, Lead has the highest uptake rate/remediation with 841.80% followed by Iron with 21.75%, Zinc 1.40%, Cadmium 0.94%, Manganese 0.11%, and Arsenic 0.00% (Fig. 3). These results are in consonance with the findings of Anyalogbu et al. (2017), who reported higher bioaccumulation and uptake rate of heavy metals in Talinum triangulare cultivated in heavy metal contaminated soil. This could be attributes to metal specific properties and plant affinities for metal mobilization and soil properties (Cristaldi et al., 2017; Wang et al., 2017).

Conclusion

From this study, it could be concluded that *Vigna unguiculata* has the potential of phyto-remediating heavy metal contaminated soil. This is evident in this study as contaminated soil reduces the growth (leaf length, stem girth, stem height, leaf breadth, leaf area and petiole) of *Vigna unguicutulata.* It also revealed the reduction of heavy metals in soils before and after through physiochemical analyses; iron has the highest transfer factor and bioaccumulation in the *Vigna unguicutulata*; and however, iron metal was the highest remediated by *Vigna unguicutulata*. It could be recommended that *Vigna unguicutulata* exhibited phytoremediation potentials, which are good for reduction of environmental contaminants, but the plant used could be a determinant when it was exposed to prolonged heavy metal contamination. Plants with high amount of heavy metal concentrations are toxic to human and other living organisms when ingested into the body; thus, should be avoided.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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