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Phytoextraction Ability of Two Ornamental Plant Species

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Abstract: This study investigated heavy metal uptake ability of *Azadiracta indica* and *Polyalthia longifolia* in soil enhanced with different levels of cow dung. A Complete Randomized Design was adopted. Two (2kg) of homogenous soil composite was weighed using a weighing balance (Setra 80S, USA) calibrated into 50 planting bags of height 8cm, diameter 14cm and surface area of 0.095 m^2. The planting bags were arranged into 4 groups of 5 replications and group 5 with 5 replications was filled with the reference. Cow dung concentration of 100g, 200g and 300g was weighed into group 1, 2 and 3 respectively while group 4 and 5 with 0g of cow dung stands as control (polluted soil) and double control (referenced soil). After two weeks of post remediation, the entire experiment was divided into set A and B as seedlings of *Azadiracta indica* and *Polyalthia longifolia* were transplanted from the nursery unit into set A and B respectively. The addition of different levels of cow dung amendments enhanced metal availability and mobility in the biomass of the studied plant species. It also caused a decrease in soil pH and conductivity and triggered increase in the concentration of some soil essential nutrient such as potassium, phosphorus and nitrogen. Translocation factor and bioaccumulation factor was greater than 1 was observed for test plant grown in treated soil of various levels, this showed that metals were more concentrated in plant than soil. *Azadiracta indica* and *Polyalthia* had the potential to accumulate cadmium, lead and zinc in their biomass when grown in cow dung amended soil. The study therefore recommends that 200g cow dung amendment had the potential to enhance the bioavailability and mobility of Zn, Cd and Pb in shoot and root of *Azadiracta indica* and *Polyalthia longifolia*

Keywords: Phytoextraction; *Azadiracta indica; Polyalthia longifolia* cow dung, heavy metals; phytoextraction; soil chemical properties.

1. Introduction

Heavy metals are natural constituent of soil and its existence is within the permissible limits set by environmental regulatory bodies. High incidence of heavy metal in the environment results from various anthropogenic activities and hence expose the environment to metal toxicity [1]. Heavy metals are element with atomic number greater than 20 and density greater than 5gcm^{-3}.Some of these metals (Al, Zn and Fe) finds it expression in metabolic processes of living things and they are used at a minute concentration. Heavy metals are detrimental at high concentrations, these metals interfere with the physiological and biological processes of living organisms and result in excess expression of reactive oxygen species which is capable of causing death[2]. Heavy metals are recalcitrant in the environment and this nature makes them non degradable waste hence they accumulate along food chain [3]. Since heavy metals complete its cycle in food chain it now an issues facing the underdeveloped and developed countries. Conventional metals removal methods adopted over the years involves the use of sophisticated equipment's which are very expensive and the outcome of this exercise are not commensurate with amount and time invested [4]. Report has shown that soil restoration using conventional methods cause more damage to the physical (bulk density, porosity, particle density and soil structure), chemical (nutrient composition, pH and conductivity) and biological properties (flora and micro fauna) of soil and the tendency of exposing these heavy metals to other unpolluted areas is also a challenge [5,6]. Bioremediation approach which involves the use of plant suitable for the restoration of heavy metals impacted site is phytoextraction. Phytoextraction is part of phytoremediation strategy by which plant accumulate pollutant in its harvestable tissues. The concept of phytoextraction depends on two strategies such as natural phytoextraction (hyperaccumulators) and induced phytoextraction (use of EDTA, NTA, amendment and genetically modified plants). Report has shown that most plant species used in phytoextraction processes are edible crop either as a food source for man or animals and these metals can find its way to food chain through grazing pathway of animals [2].

2. Motivation of the Study

The use of ornamental plants should be seen as an alternative in addressing this pitfall since most ornamental plants are not food sources both for man and animals. Ornamental plants are plant grown for decorative purposes and they are of different shapes, sizes and color. The use of ornamental plants is highly encouraged since they are suitable to a broad array of climate with desired morphological and physiological characteristics [7]. This use of ornamental plants is very necessary and its regular practice can turn a polluted environment into an ecotourism and hence increase in aesthetic and economic benefits is achieved [8].

The aim of this experiment is to compare the phytoextraction ability of two ornamental plant species in grown in an enhanced heavy metal polluted soil.

3. Materials and Methods

A soil suspected of heavy metal pollution was collected at 0-20cm depth using a soil auger at an abandoned metals scraps dumpsite alongside an uncontaminated soil at a fallow farmland beside Geology Department, Rivers State University Port Harcourt, Nigeria. The collected soils were analyzed to ascertain selected heavy metal concentration level and nutrient quantity present. The analyzed homogenous soil composite was weighed using a weighing balance (Setra 80S, USA) calibrated in kilogram, 2kg each into 50 planting bags of height 8cm, diameter 14cm and surface area of 0.095 m^2. Adopting a Randomized Complete Block Design, the planting bags containing soil was then arranged 4 groups of 5 replication each for heavy metal pollutant soil and group 5 with 5 replications was filled with the reference soil (uncontaminated). Fresh cow dungs obtained from Faculty of Agriculture cattle farm Rivers State University was processed mechanically and sieve through 2 mm wire mesh to obtain a fine composite of cow dung after drying for 4 weeks. Various concentrations of 100g, 200g and 300g of the processed cow dung was weighed into group 1, 2 and 3 respectively while group 4 and 5 with 0g of cow dung represented the control (polluted soil) and double control (referenced soil). The cow dung treated soil were mechanically mixed to obtain a homogenized enhanced soil then was allowed to stand for 2 weeks for post remediation treatment. The entire experiment was divided into set A and B and seeding of *Azadiracta indica* and *Polyalthia longifolia* were transplanted from the nursery unit into set A and B respectively. This experiment was conducted in an open roofed space and was monitored for 2 months. Watering was done using 50cl distilled water and weeding was done when necessary. After 2 months, plant species were carefully removed from the planting bags by cutting the planting bags open and the plant was removed and dipped into a bucket of water to remove the remaining soil particles. The plants roots were separated from shoot with a sharp blade and plant part with soil collected were labeled in accordance with treatment application and transported to research laboratory for analysis. The plant parts were analyzed for Cd and Pb concentration while the soil were analyzed for Cd, Pb and soil chemical properties.

Table 1 Soil physicochemical composition

S/N	Parameter	Unpolluted	Polluted
1	pH	5.2	8.49
2	Conductivity (us/cm)	65	119
3	Zn (mg/kg)	4.93	220.3
4	Pb (mg/kg)	13.6	289.5
5	Cd (mg/kg)	1.0	35.6
6	Nitrate (mg/kg)	75.6	145
7	Phosphorus (mg/kg)	1.0	0.12
8	Calcium (mg/kg)	110	120
9	Magnesium (mg/kg)	210	232
10	Bulk density	1.5	1.0
11	Particle density	5.8	5.0
12	Porosity	2	0.1
13	K (mg/kg)	49.3	12

Table 2. Nutrient composition of cow dung

S/N	**Parameter**	cow dung
1	pH	8.1
2	Ash %	42
3	Zn (mg/kg)	1.0
4	Pb (mg/kg)	2.3
5	Cd (mg/kg)	1.2
6	Nitrogen (mg/kg)	0.76
7	Phosphorus (mg/kg)	2.23
8	Potassium (mg/kg)	7.43

3.1 Determination of Parameters

Soil pH and conductivity were determined electronically using a glass electrode pH meter (PHS. 25 Model) and conductivity meter (HANNA HI Series) respectively. Plant sample was first rinsed with distilled water and oven dried at 100°C for 48 hours. The dried plant sample was grounded to fine powder analysis for Zn, Pb and Cd was done after digestion using BUCK scientific 200A model of Atomic Absorption Spectrophotometer (AAS). The soil was also dried and sieved through a 2mm sieve to remove coarse soil particles before analysis. The determination of soil selected heavy metals was by Spectrophotometer method (AAS) (BUCK scientific 200A model) after digestion using a hot plate for 20 minutes with 1:2:2 ratio of perchloric, nitric and sulphuric acids (API-RP-45, 2005). Soil phosphorus and nitrogen content were determined by Oxidation, Ascorbic acid and Kjedahl methods respectively (Stewart *et al*., 1974). Potassium content was determined using atomic absorption spectrometer (AAS) through digestion method. Soil bulk and particle density were carried out according to the method of Blake and Hartage(1986), while porosity was determined by calculation method expressed as: % Ø = (1- BD/PD) x 100. Where Ø = porosity, BD = bulk density and PD = particle density.

Pollution Load Index. The degree of soil pollution for each metal was measured using pollution load index (PLI) technique which depends on soil metal concentrations. $PLI = C_{soil}/ C_{reference}$

Transfer/Bio accumulation factor. Metal concentration in the extracts of soils and plants. It was expressed using the formula of (Malik *et al*., 2010). C_{plant}/C_{soil}.

Translocation Factor Translocation factor (TF) or mobilization ratio (Gupta *et al*., 2008) this was expressed as: TF=Concentration of metal in plant shoots/Concentration of metal in Plant roots

3.2 Data Analysis and Presentation The data generated (means and standard error of mean) was estimated using the Statistical Analysis System (SAS). Data were analyzed using Analysis of Variance (PROC ANOVA) procedures (SAS Institute Inc, 2002). The Least significant difference were calculated and the means were separated according to the procedures of the Duncan's New Multiple Range Test (DNMRT).

4. Results and Discussion

4.1 Results

Table 3 contains the result of Pb, Cd and Zn concentration in plant parts of *Azadiracta indica.* Increase in accumulation of Pb in shoot and root of plant was recorded in *Azadiracta indica* grown in amended soil containing different levels of cow dung treatment. *Azadiracta indica* grown in 300g cow dung treatment showed the highest increment in Pb and Cd shoot concentration while least in Shoot Pb and Cd accumulation was recorded for plant grown in polluted control soil with 0g amendment. Highest increment in root Pb accumulation was found in plant grown in 100g cow dung amendment. Highest in shoot Zn accumulation was for *Azadiracta indica* plant grown 200g cow dung and there was significant different between and within treatment at (p=0.05).Highest accumulation of Pb in shoot and root was found in *Azadiracta indica* grown in 300 and 100g cow dung amendment respectively. In Table 4, an increase in accumulation of Zn was highest in *Polyalthia longifolia* grown in 300g for shoot and root respectively and Pb was found highest in shoot of *Polyalthia longifolia* grown in 300g cow dung and 100g cow dung for root accumulation. The accumulation of Cd recorded an increase in 200g and 0g (polluted un-treated soil) in shoot and root respectively. There was significant difference between and within treatment application at (p=0.05).In Table 5 and 6, it was observed that highest increase in the concentration of Pb, Cd and Zn in soil after phytoextraction process was found in the control polluted soil (0g un-treated soil) while the least was found in 200g cow dung amended soil phytoextracted with *Azadiracta indica*and decrease in Pb and Cd was found in 200g cow dung treated soil and 100g for Zn soil concentration in *Polyalthia longifolia* treated soil.

Figure 1-4: showed the effect of different concentration of amendment treatment on soil conductivity, pH, nitrogen content, phosphorus and potassium in soil phytoextracted with *Polyalthia longifolia* and *Azadiracta indica*. Decrease in conductivity level was found in amended polluted soil and the highest increment in conductivity, pH and nitrogen content was found in polluted control soil (0g treated soil) as shown in fig 1-3. There was significant difference between and within treatment at (p=0.05). Figure 4 and 5 showed an increase in phosphorus and potassium in treated soil of various concentration of cow dung manure. Highest increament in soil P and K content was found in *Azadiracta indica* phytoextracted soil with 100g, 200g and 300g cow dung treatment and least decrease was recorded in soil phytoextracted with *Polyalthia longifolia.*

Table 3. Pb, Cdand Zn concentration in shoot and root of *Azadiracta indica.*

		Pb		**Cd**		**Zn**	
S/N	Treatments	Root	Shoot	Root	Shoot	Root	Shoot
1	100g	68.5±1.3[a]	196±5.4[ab]	4±0.06[b]	16.5±2.3[a]	69±2.0[b]	156±8.3[c]
2	200g	43.9±1.05[b]	171.3±8.3[b]	3.8±0.01[bc]	16.92±0.03[a]	56±4.2[d]	189±12.4[a]
3	300g	6.0±0.03c	201±3.4[a]	5.2±0.1[b]	17.9±0.3[a]	67±3.7[c]	177±3.0[b]
4	*0g pol	62.0±0.01[a]	20±0.31[c]	8±0.01[a]	3.1±2.0[b]	209.3±5[a]	26.8±1.0[d]
5	**0g Unpo	6.0±0.2[c]	5.0±0.01[d]	1.0±0.002[c]	2.0±0.001[b]	4.2±0.02[e]	3.2±0.001[e]
LSD		0.35	2.89	1.77	0.42	2.10	2.56

- Polluted control soil (0g amendment) ** unpolluted control (0g amendment)

Table 4. Pb, Cd and Zn concentration in shoot and root of *Polyalthia longifolia*

		Pb		**Cd**		**Zn**	
S/N	Treatments	Root	Shoot	Root	Shoot	Root	Shoot
1	100g	53.0±0.02[a]	108±5.4[b]	2.8±0.003[c]	15.2±0.1[a]	45±2.4[b]	148±10.21[b]
2	200g	48.0±1.5[b]	100.2.3±8.3[b]	3.2±0.001[c]	15.72±0.02[a]	43±2.0[b]	159±8.9[b]
3	300g	18.0±0.3[d]	216±3.4[a]	4.01±0.02[b]	14.5±0.34[a]	49±3.7[b]	167±6.8[a]
4	0g polluted	26.0±0.1[c]	45±0.31[c]	10.0±1.2[a]	3.10±0.001[b]	179.3±8.0[a]	15.6±1.5[c]
5	0g Unpo	8.0±0.02[e]	11.0±0.01[d]	1.0±0.0002[d]	1.03±0.001[c]	3.22±0.1[c]	1.2±0.003[d]
LSD		0.76	3.24	1.56	1.67	2.75	2.87

Table 5 and 6 respectively showed the effects of amendment of various concentration on the selected soil metals after phytoextraction with *Azadiracta indica* and *Polyalthia longifolia*. The highest increment in Cd, Pb and Zn after phytoextraction with *Azadiracta indica* was found in unamended polluted soil while the least decrease in soil metals was found in 200g cowdung amended soil. Cowdung application of different concentrations also showed a slight decrease in soil metal and there was significant different between and within treatment application and metal reduction at (P=0.05).In table 6, the highest increment in the studied soil metals was recorded in polluted untreated soil while the least decrease for soil Pb and Cd was recorded in 200g cowdung treated soil and with 100g amendment showed a decrease in Zn concentration phytoextracted with*Polyalthia longifolia*.

Table 5: Concentration of Cd, Pd and Zn in enhanced soil phytoextracted with *Azadiracta indica.*

Selected Metals	**100g**	**200g**	**300g**	**Polluted cont**	**Unpolluted contro**
Pb	65±0.001[c]	60.8±0.002[c]	74±0.02[b]	200±12.67[a]	7.67±0.01[d]
Cd	10.4±0.05[b]	9.5±0.002[c]	11±2.1[b]	28.7±3.6[a]	0.3±0.0002[d]
Zn	98±9.45[b]	95.6±2.5[b]	87±0.02[c]	186±10.5[a]	2.2±0.001[d]

*Mg/kg

Table 6: Concentration of Cd, Pd and Zn in enhanced soil phytoextracted with *Polyalthia longifolia*

Selected Metals	**100g**	**200g**	**300g**	**Polluted cont**	**Unpolluted contro**
Pb	75±1.8[c]	65±2.01[d]	100±8.56[b]	195.6±17.4[a]	4.2±0.2[e]
Cd	8.42±0.3[c]	7.8±0.01[c]	10.5±0.1[b]	30.65±6.0[a]	1.3±0.001[d]
Zn	100.64±12.6[c]	108.4±17.8[b]	96.97±8.9[d]	175±19.01[a]	1.8±0.01[e]

Result in Fig1-3 showed the effect of different levels of cowdung amendment on soil physicochemical properties phytoextracted with *Azadiracta indica* and *Polyalthia longifolia*. Decrease in conductivity, pH and nitrogen content was recorded in amended polluted soil across treatment option. Highest increment in soil conductivity, pH and nitrogen content was reported in polluted soil with 0g treatment option phytoextracted with *Azadiracta indica* and *Polyalthia longifolia*. There was significant difference between plant species and treatment application at (p=0.05).

Fig 4 and 5 showed an increase in phosphorus and potassium in cowdung treated soil phytoextracted with *Azadiracta indica* and *Polyalthia longifolia*. Highest increment in soil K and P was found in 100g, 200g and 300g cowdung treated soil while the least was recorded in polluted untreated soil.

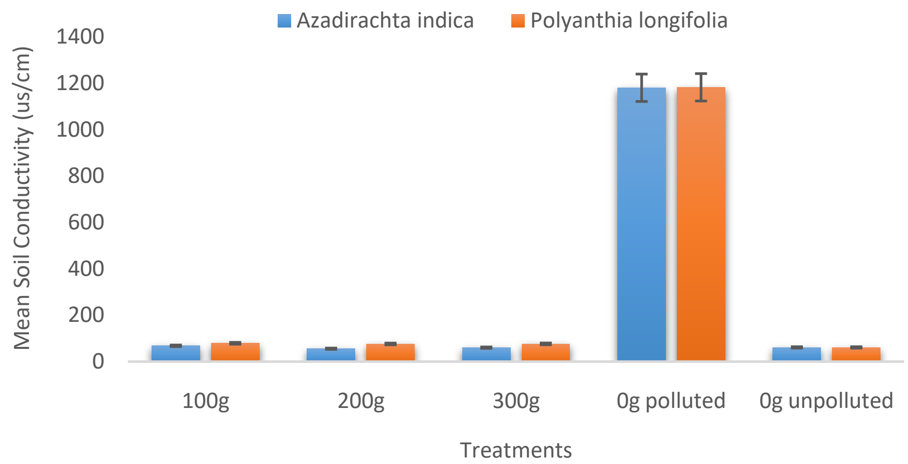

Fig 1 Effects of different concentration of amendments on conductivity in *Azadiracta indica* and *Polyanthia longifolia* phytoextrcted soil

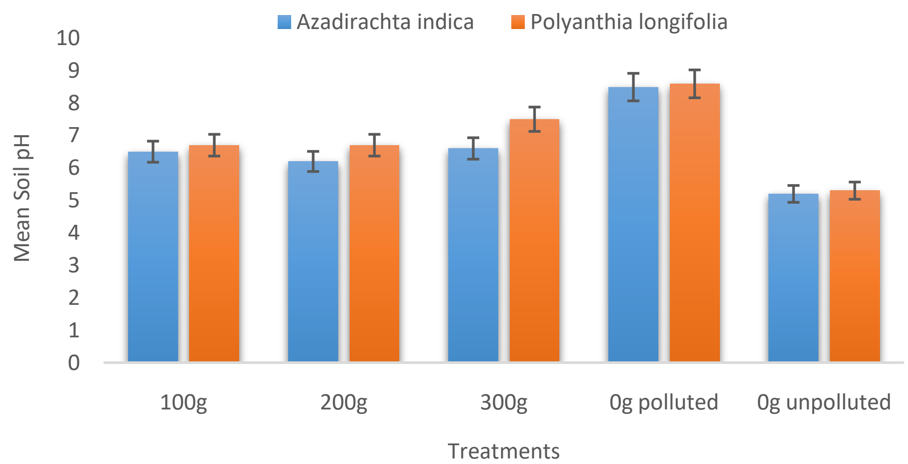

Fig 2. Effects of different concentration of amendments on pH in *Azadiracta indica* and *Polyanthia longifolia* phytoextrcted soil

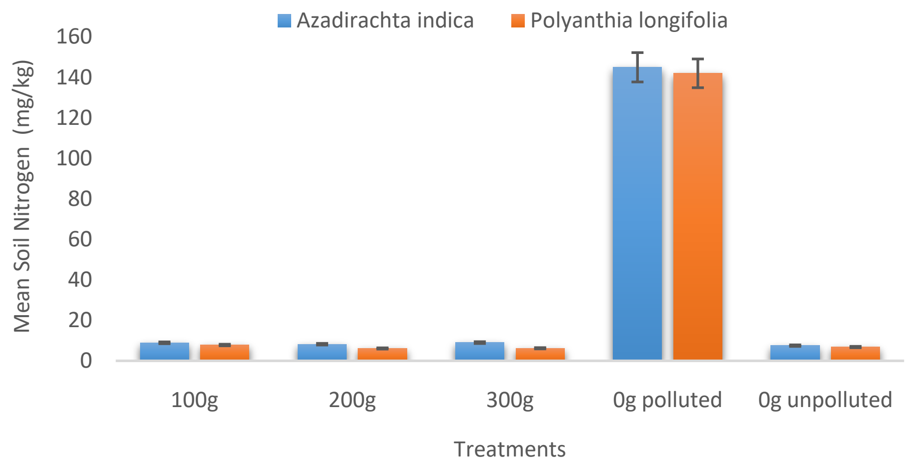

Fig 3. Effects of different concentration of amendments on nitrogen in *Azadiracta indica* and *Polyanthia longifolia*

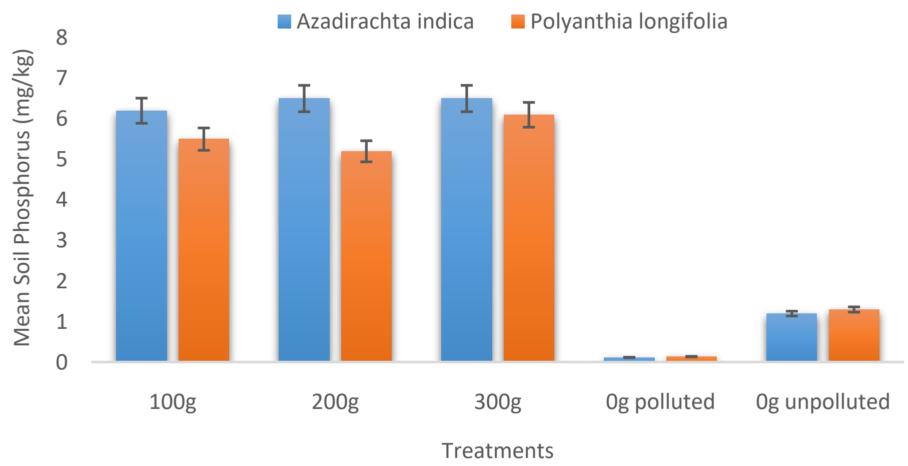

Fig 4. Effects of different concentration of amendments on potssium in *Azadiracta indica* and *Polyanthia longifolia*

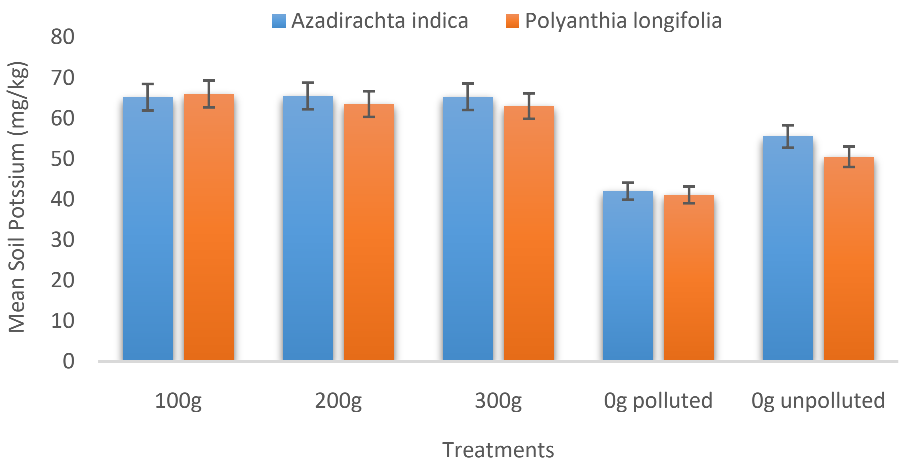

Fig 5. Effects of different concentration of amendments on potssium in *Azadiracta indica* and *Polyanthia longifolia*

The result in table 7 and 8 showed that accumulation of heavy metals was high in the studied plant species grown in amended soil. The accumulation of Cd and Zn was highest in the biomass of *Azadiracta indica* grown in 200g cowdung amendment and Pb was recorded more in shoot of *Azadiracta indica* grown in 300g cowdung amendment. In table 8, highest accumulation of Pb was recorded in shoot of *Polyalthia longifolia* grown in 300g cowdung amended soil while 200g cowdung soil treatment facilitated the uptake of Cd and Zn in shoot of *Polyalthia longifolia.*

Bioaccumulation factor as shown in table 9 and 10 showed that the studied metals were more concentrated in the plant biomass than soil

Table 7. Translocation Factor of heavy metal in soil phytoextracted with *Azadiracta indica*

Selected Metals	100g	200g	300g	Polluted cont	Unpolluted cont
Pb	2.84058	3.886364	33.5	0.322581	0.833333
Cd	4.125	4.25	3.6	0.375	2
Zn	2.26087	3.375	2.641791	0.138756	0.75

Table 8 Translocation Factor of soil phytoextracted with *Polyalthia longifolia*

Selected Metals	100g	200g	300g	Polluted cont	Unpolluted cont
Pb	2.037736	2.083333	12	1.730769	1.375
Cd	5.066667	5.333333	4	0.31	1.03
Zn	3.288889	3.697674	3.408163	0.089385	0.372671

Table 9 Bioaccumulation Factor of *Azadiracta indica*

Selected Metals	100g	200g	300g	Polluted cont	Unpolluted cont
Pb	0.915371	0.74266	0.715026	0.283247	0.808824
Cd	0.585714	0.6	0.657143	0.314286	0.3
Zn	1.119403	1.218905	1.21393	1.18408	1.419878

Table 10 Bioaccumulation Factor of *Polyalthia longifolia*

Selected Metals	100g	200g	300g	Polluted cont	Unpolluted cont
Pb	0.556131	0.511226	0.80829	0.24525	1.397059
Cd	0.52	0.542857	0.571429	0.374286	0.203
Zn	0.960199	1.004975	1.074627	0.970149	0.896552

Table 11 Pollution Load Index and Permissible limits of the studied heavy metals in soil

	Zn	Pb	Cd
PLI	55	20.6	35.6
WHO Standard	50	85	0.8

4.2 Discussion of Result

The negative effects of heavy metals contamination have attracted concern globally because of their persistence in the environment, their tendency to accumulate along the food chain and their toxicity, heavy metals are also capable of influencing soil physicochemical properties and essential plant nutrients negatively[9]. The addition of organic amendment (cow-dung) of various concentration's aided the various levels of absorption of Cd, Pb and Zn in the test plants. *Azadiracta indica* grown in amended soil containing different levels of cow dung treatment showed an increase in accumulation of Pb in shoot and root with 300g cow dung treatment showing the highest increment in Pb and Cd shoot concentration while least in shoot Pb and Cd accumulation was recorded for plant grown in polluted control soil with 0g amendment. Highest increment in root Pb accumulation was found in plant grown in 100g cow dung amendment. Highest in shoot Zn accumulation was also recorded in 200g cow dung and highest accumulation of Pb in shoot and root was found in *Azadiracta indica* grown in 300 and 100g cow dung amendment respectively. It was also observed that *Polyalthia longifolia* grown in 300g showed an increase in the accumulation of Zn shoot and root while Pb was found highest in shoot of *Polyalthia longifolia* grown in 300g and 100g cow dung for root. The variation in accumulation rate could be attributed to the type, level and tolerance ability and performance of the test plants might have influenced soil chemistry and nutrient content thereby increasing the availability of the selected heavy metals in solution phase. The organic amendment added during mineralization process by microorganisms released the potential nutrient embedded in the amendment and also influenced soil properties. This result corroborated with the findings of [10] who concluded in an experiment that heavy metal availability is controlled by plant internal and soil external factor, also adding that plants performed optimally when present in relevant conditioned soil. This result agreed with [11, 12]who also reported that addition of various levels of organic amendments is capable of increasing heavy metal mobility in rhizosphere soil thereby increasing accumulation in phytoextraction plants. The distinction found in heavy metal uptake ability between *Azadiracta indica* and *Polyalthia longifolia* could be credited to the internal attribute of the studied plants. This findings revealed that plants have different abilities in bioaccumulating heavy metals and also, its uptake rate in root and shoot varies,[5, 13, 14] at different period and similar environmental conditions and also reported that uptake and bioaccumulation of metals by plants and plant parts was plant species dependent. The decrease in the concentration of soil Cd in amended soil phytoextracted with *Azadiracta indica* and *Polyalthia longifolia* disagrees with [15] who reported that addition of organic amendment of various levels decreased the mobility of metals especially Cd which resulted in its bioavailability and translocation [16] report was contrary, suggesting that addition of organic amendment stabilizes contaminant in the soil thereby limiting there bioavailability. Soil pH plays a crucial role in nutrient and metals availability thereby influencing the efficacy of phytoextraction. Soil pH was generally reduced with various levels of cow dung treatment. This reduction may be attributed to the organic amendment added. The added amendment may have triggered microbial population and activity which might have influenced pH concentration during mineralization process. This finding corroborated with the report of [17] who reported that addition of organic amendment is capable of decreasing soil pH when acted upon by microorganisms. [18], showed that pH increase and decrease is a function of the type of organic amendment used, the microbial degradation process only make available the nutrient embedded in the amendment. Increase in soil phosphorus and potassium was also reported in cow dung amended soil. This observation could be attributed to the organic amendment added. The added amendment contain in it the essential nutrient need by plants which is made available during microbial decomposition process. This result corroborated with the findings of [14] who associated an increase in soil nutrient content to addition of organic amendment and concluded that these essential nutrients are made available during microbial decomposition process.[18] also reported an increase in soil nutrient in organic amended soil. The BF and TF ratio were calculated in order to evaluate the accumulation of elements in plants from soil to roots in order to ascertain the phytoremediation efficacy of the test plants in the presence of amendments. Bioaccumulation factor greater than 1 showed that the studied heavy metals were retained more in the plants grown with amendment than in unamended soils. Accumulation factor less than 1 indicated non-phytoaccumulation while greater than 1 showed phytoaccumulation. (by hyper accumulator). Plants with accumulation factor greater than 1 and those with accumulation factor less than 1 are classified as accumulators and excluders respectively [10]. The pollution load index showed that the studied metals were greater than the permissible limit of soil metals set by World Health Organization.

5. Conclusion

The tested plant species had the potential to reduce and accumulate heavy metals in their biomass showing no signs of toxicity effect. Results also revealed that the addition of different concentrations of cow dung significantly improved soil chemistry. *Azadiracta indica* and *Polyalthia longifolia* had the potential to accumulate cadmium, lead and zinc in their biomass when grown in cow dung amended soil. The study therefore recommends that 200g cow dung amendment can enhance the bioavailability and mobility of Zn, Cd and Pb in shoot and root of *Azadiracta indica* and *Polyalthia longifolia*

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