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Screening Of *Phyllanthus Muellerianus* For The Purpose Of Phytoremediation Of Lead In Enyigba Lead Mine Derelict, Ebonyi State, Nigeria

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Abstract: *Samples of Phyllanthus Muellerianus were collected from Enyigba mines in Ebonyi State alongside the soil samples. Different concentration in mg/kg of Zn, Pb, As, Cd, Ni and Cr were determined using Atomic Absorption Spectrophotometer (ASS) technique. The result showed the mean concentration of heavy metals to be Zn (11.40 mg/kg), As (2.40 mg/kg), Cd (0.30 mg/kg), Cr (1.93 mg/kg), Ni (14.88 mg/kg) and Pb (2.60 mg/kg). The concentration of the heavy metals in the soil were found to be in the order top soil > mid soil > sub soil which showed that the origin of the metal is anthropogenic in nature. The mean pH of the soil was 6.68 while the organic matter was 1.48 which hiders the availability of Pb in the soil. Translocation factor (TF > 1) was observed for Cd and Pb and Bioaccumulation factor (BAF > 1) was observed for Pb. This shows that this weed could be used as phytoremediation agent in lead and cadmium polluted environment.*
Keywords: *Phyllanthus Muellerianus, Enyigba mines, Phytoremediation and Lead.*

I. Introduction

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at high concentrations. Heavy metals such as cadmium (Cd), arsenic (As), chromium (Cr), thallium (Ti) and lead (Pb) are present in the earth crust for centuries and they cannot be degraded nor destroyed rather they move round in cycle and back to earth crust again. Some heavy metals are essential to maintain the human body metabolisms but in trace quantity (John, 2002). Heavy metals can get into the human body by exposure of industrial and consumer waste or even from acidic rain. Certain metals such as Cu, Zn, Ni are essential trace metals for majority of plants as they are contained in many plants enzymes. Some metal toxicity can be reduced by the uptake of certain plants to some extent. However low level of toxic metals such as Cd, Cr and Hg can affect human health regardless whether they are present in substance people ingest or in materials people get in contact with (Hogan, 2010). The effects of metallic pollutants depends on many factors such as environmental condition, pH of metal, organic substances, fertilizer etc. The uptake of some metals by plants could show its usefulness in phytoremediation due to its high translocation and bioaccumulation factors (Yanqun *et al.*, 2005). Plants are known to have unlimited uses ranging from food, decoration, shelter, fuel, clothing and medicines. Plants that grow in unwanted areas are referred to as weeds and at times, they may be benefical. Weed is a plant that is not desired within a certain context. The term *weed* is a subjective one, without any classification value, since a plant that *is* a weed in one context is *not* a weed when growing where it belongs or is wanted (Janick, 1979). *Phyllanthus* is the largest genus in the flowering plant family phyllanthaceae numbering from 750 to 1200. The aim of this work therefore is to determine the concentration of heavy metals in *phyllanthus muellerianu* collected from Enyigba lead and zinc mines and to compare the values in the plant and leaves and also to compare the values of these plant and soil.

II. Materials and Method

Study Area

This research work was carried out in Enyigba in Ebonyi state. The study area, Abakaliki, is in the mid of the South Eastern, Nigeria (latitudes 4°20' and 7°00'N and longitudes 5°25' and 9°35'). The Enyigba lead – zinc mine is located about 14 Km South of Abakaliki with sparse rural population of mainly farmers. The prevailing climate conditions are high rainfall, high temperatures, high atmospheric humidity and precipitation usually exceeding evapotranspiration for more than half the year.

Sample collection

Fresh plant and soil samples were collected from lead and zinc deposited mine Enyigba, Ebonyi State on the 15th of November, 2014. The samples were kept in polythene bag (black) to avoid rapid dehydration while kept the soil samples were stored in samples containers and labelled; top soil (0-30), mid soil (30-60) and deep sub soil (60-90).

Soil Pre-treatment and Analysis

After drying, the samples was finely grounded using a plastic pestle and mortar and was sieved until the finest particles were obtained. 0.5g of the sieved samples was weighed using a weighing balance into a 250ml. 2.0ml of aqua regia (HNO_3 + 3HCl) and 5.0ml of perchloric acid ($HClO_4$) was added. After digestion of the samples, Atomic Absorption Spectroscopy (VGP210 Bulk Scientific model) was used to analyze for Zn, As, Cd, Cr, Ni and Pb. In addition, from the soil samples, the pH, percentage of Organic Matter and percentages of sand, silt, and clay were also determined using Orion 920A pH meter; Walkley & Black method and Hydrometer method respectively.

Plant Pre-treatment and Analysis

The plant samples were immediately taken to the department of Applied Biology, Ebonyi State University, Abakaliki for proper identification and labelling. The roots, stems and leaves samples were washed carefully with distilled water to remove all soils, dusts and unwanted particles on the surface of the samples, while the soil samples were spread on a clean plastic tray and air dry until no trace of water was found in it to avoid interference during analysis. 1.0g of the powdered sample was weighed into a conical and 10 cm^3 of the digestion mixture (a mixture of sulphuric acid, perchloric acid and nitric acid in ratio 1:4:40 by volume) was added and left to stand overnight. The content was first heated at 70 °C for 40 minutes and then the heat was increased to 120 °C until the colour of the mixture became clear with appearance of white fumes which shows the completion of digestion process (Audu and Lawal, 2005). The digest was diluted with 10 cm^3 of water and boiled for 15 minutes, cooled and transferred into 50 cm^3 volumetric flasks. The sample solution was then filtered through a filter paper into a screw capped polyethylene bottle and stored for heavy metal determination using Absorption Spectroscopy (VGP210 Bulk Scientific model). Stock standard solutions of Zn, As, Cd, Cr, Ni and Pb containing 1000ppm of each metal were prepared by dissolving weighed amounts of suitable anhydrous analytical grade salts in distilled water.

Statistical analysis

Each analysis was done in triplicates to ensure reproducibility and minimize error. Data were expressed as means with standard deviation and were subjected to one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) multiple range test ($P \leq 0.05$) was used to evaluate differences between means of treatments and plant species.

III. Results And Discussion

Tables 1 shows the concentration of heavy metals in the top soil, mid soil and sub soil and in the roots, leaves and stems of *phyllanthus muellerianus.* Table 2 shows the soil properties of Enyigba lead mine derelict while Tables 4 and 5 show the Translocation and Bioaccumulation factors of heavy metals in *phyllanthus muellerianus*

Table 1: The Concentration of Heavy Metals in Soil and Plant Samples

Parameter Conc. (mg/kg)						
Metals	Zn	As	Cd	Cr	Ni	Pb
Top Soil	56.02 ± 6.22	3.21 ± 0.22	0.48 ± 1.02	2.00 ± 0.81	16.20 ± 1.41	2.60 ± 0.82
Mid Soil	34.12 ± 2.00	2.01 ± 0.21	0.48 ± 1.02	2.00 ± 0.81	16.12 ± 1.40	2.60 ± 0.82
Sub Soil	12.44 ± 1.28	1.98 ± 0.20	0.31 ± 1.00	1.80 ± 0.40	12. 32 ± 1.20	2.62 ± 0.84
Roots	10.12 ± 1.20	1.80 ± 0.16	0.30 ± 0.90	1.56 ± 0.31	6.89 ± 0.54	2.60 ± 0.18
Stems	8.05 ± 0.88	1.50 ± 0.12	0.32 ± 0.71	1.31 ± 0.11	6.38 ± 0.48	2.64 ± 0.08
Leaves	6.11 ± 0.52	0.50 ± 0.08	0.32 ± 0.82	1.31 ± 0.11	6.38 ± 0.48	1.80 ± 0.22

Table 2: Soil Properties

Properties	(n = 3)
Sand (%)	60.22 ± 5.2
Silt (%)	8.02 ± 0.7
Clay (%)	34.60 ± 4.1
Organic Matter (%)	1.48 ± 0.6
pH	6.68± 0.6

Table 3: Translocation Factors of heavy metals in *phyllanthus muellerianus*

Metals	Zn	As	Cd	Cr	Ni	Pb
Roots	10.12	1.80	0.30	1.56	6.89	2.62
Stems	8.05	1.50	0.32	1.31	6.38	2.64
$TF = C_{stem} / C_{root}$	0.79	0.83	1.07	0.84	0.93	1.01

Table 4: Bioaccumulation Factors (BAF) of Heavy Metals in *Phyllanthus Muellerianus*

Bioaccumulation Factors						
Metals	Zn	As	Cd	Cr	Ni	Pb
Mean Soil Concentration	11.40	2.40	0.42	1.93	14.88	2.60
Roots Concentrations	10.12	1.80	0.30	1.56	6.89	2.62
BAF = C_{root} / C_{soil}	0.88	0.75	0.71	0.89	0.46	1.01

IV. Discussion

Table 1 showed that the concentration of the heavy metals in the soil were found to be in the order top soil > mid soil > sub soil. Higher concentration of metals in top soil than in the mid or sub soil shows that the source of the metals in the environment is from anthropogenic origin.

Table 2 shows the soil characteristics by their organic matter, sand, silt and clay contents. The percentage of organic matter content is 1.48 % in Enyigba mine. The extent of soil pollution with heavy metals and subsequent uptake by crops depend on organic matter content among other factors. Usually, organic matter content increases with decrease in pH and an increase in metal concentrations (Adhikari *et al.,* 2004). The organic matter content serves as complexing agent for heavy metals (Brady and Wiel, 1999). Some metals such as Pb have low solubility at pH range of 5.5 – 7.5 which is normal for most mineral soils. Soils with pH around 6.68 have lesser availability of heavy metals such as Pb, Cu, Mn and Zn because they are less soluble at about this pH (Assuncao, 2003). Organic matter and pH values have been reported to independently and associatively influence the concentrations of heavy metals in soils (Nweke *et al.,* 2008). Hence for phytoremediation to take place, soil amendment must be conducted using chemicals that will make these metals available.

From Table 3, bioaccumulation factor (BAF) greater than one was observed for Pb in *phyllanthus muellerianus*. For a plant to be an efficient phytoremediation tool in the contaminated soil, the BAF have to be higher than one (Brown *et al.,* 1994). This means that *phyllanthus muellerianus* can be used as a good phytoremediation agent. Most researchers believe that plants for phytoremediation should accumulate more metals in the roots than in the shoot (Salt *et al.,* 1998). This is due to the mobilization of the protective mechanisms of plants, which inhibits the transport to further tissues and organs (Nwoko and Egunobi, 2002). Some authors, all the same, have reported higher cadmium content in shoot than in roots of the studied plants (Roosens *et al.,* 2003)

From Table 4, translocation factor (TF) of Cd and Pb are greater than one which suggest that *phyllanthus muellerianus* has the ability to absorb and contain Cd and Pb. Translocation factors is an index that shows the ability of a metal to move from one part of the plant to another and is often employed for plants roots and stems (Yanqun *et al.,* 2005). TF >1 suggests that Cd could be effectively translocated from the roots to the shoots. A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots, characterized by the TF being greater than one (Zhao *et al.,* 2006). Since the result showed TF >1, therefore, *phyllanthus muellerianus* could be labelled as Cd and Pb hyperaccumulator (Baker and Brooks *et al.,* 1989). This suggests that *phyllanthus muellerianus* could be used for phytoremediation of Cd and Pb.

V. Conclusion

Based on values of BAF > 1, *phyllanthus muellerianus* has the potential to be a hyperaccumulator of Pb. Similarly TF > 1 values for Pb and Cd shows that *phyllanthus muellerianus* has the ability to be used as phytoremediation agent in cleaning up led and cadmium contaminated land such as Enigba lead-zinc derelict in Ebonyi state, Nigeria.

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