Effects of *Blighia sapida* derived Biochar Amendment on Mobility and Bioavailability of Heavy Metals in Lead-Acid Battery Contaminated Soil

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ABSTRACT

This study was carried out to investigate the effects of *Blighia sapida* derived Biochar on the mobility, bioavailability and toxicity of Heavy metals in a lead-acid battery contaminated soil. Soil samples were collected from three different lead-acid battery charging workshops in Benin city, Edo state, Nigeria using systematic grid sampling method to form a composite sample. The *Blighia sapida* (ukpe) hardwood was crushed to dust and pyrolysed in a microwave furnace at 350°C for 1 hour 20 minutes using slow pyrolysis condition. The lead-acid battery contaminated soil, *Blighia sapida* (ukpe) hardwood derived biochar were characterized using standard methods. The lead-acid battery contaminated soils were amended with the *Blighia sapida* hardwood derived biochar (5%, 10%, 15%, 20%). The geochemical fractions of heavy metals were determined using modified European Community Bureau of Reference (BCR) three-step sequential extraction procedures and total heavy metal content with the aid of Atomic Absorption Spectrophotometer (AAS) VGP 210 model. The total heavy metals in the lead-acid battery contaminated soil was 127.50 mg/kg Pb, 0.32 mg/kg Cr, 0.03 mg/kg Cd, 0.18 mg/kg Ni and 135.00 mg/kg Fe. The total heavy metal content in the *Blighia sapida* (ukpe) hardwood derived biochar was found to be 0.23 mg/kg Pb, 0.18 Cr, 0.01 mg/kg Cd, 0.01 mg/kg Ni and 0.43 mg/kg Fe. The results obtained from the pot experiment showed that the heavy metal content in lead-acid battery contaminated soil amended with *Blighia sapida* hardwood derived biochar were found to decrease as the percentage of amender increases (5%>10%>15%>20%). From the fractionation process, the bioavailable metals were 30 mg/kg Pb, 0.08 mg/kg Cr, 0.11 mg/kg mg/kg Ni and 0.19 mg/kg Fe from the fraction that is water soluble, mobile and adsorbed to carbonate while Cd was below detection limit of the instrument used. The mobility factors of the metals were 23.97% Pb, 26.67% Cr, 68.75% Ni and 0.15% Fe, with Ni being more mobile and bioavailable to plant. The mobility factors and bioavailability indices of the metals were found to reduce as the percentage of amender reduces. The result obtained from this study indicates that *Blighia sapida* (ukpe) hardwood derived biochar was effective in reducing the mobility, bioavailability and toxicity of heavy metals in lead-acid battery polluted soil.

Keywords: *Blighia sapida*, bioavailability, biochar, heavy metals, mobility, toxicity

INTRODUCTION

Soil pollution is a global problem and occurs when the concentration of an element or compound in soil exceeds a natural background threshold value (Chapman, 2007). It can also be seen as the presence of any element or compound either through direct or indirect exposure, which causes a toxic response to bioata or human, resulting in unacceptable environmental risks world-wide (Mench *et al*., Beesley *et al*., 2011). Although small amounts of contaminants in soil are derive from natural processes, a much higher proportion comes from anthropogenic activities, industrialization, urbanization and related activities (Okieimen *et al*., 2014).

Biochar is the porous, low density, carbon-rich pyrolysis of biomass (Okieimen and Okuo, 2014). The large surface area and cation

exchange capacity determined to a large extent by source materials and pyrolysis temperature enables sequestration of both inorganic and organic contaminants, reducing pollutant mobility when applied to contaminated soils (Okieimen and Okuo, 2014). Carbon rich amendments, such as biochar are increasingly receiving attention as soil amendment because they cannot only mitigate climate change by sequestering carbon from atmosphere into soil (Morris, 2006; Lehmann, 2007) but also improve soil moisture and nutrients retention (Lehmann *et al*., 2011) hence increasing crop productivity. These diverse potential benefit, together with the fact that biochar produced from a range of biomass can potentially be relatively cost-effective and environmentally beneficial tool for environmental remediation have stimulated increasing research interest in soil application of

biochar (Tang *et al.*, 2013). Attention has been to biochar as a soil conditioning properties and benefits to physico-chemical characteristics (Beesley *et al.*, 2010).

The major inorganic contaminants in soil are heavy metals. Heavy metal contaminants in soil decrease the soil quality, affect detrimentally the quality of the biomass produced and impair aquatic environment.

Soil polluted with lead-acid batteries has become a great danger to humans and other living organisms as it has seriously endanger their food chains, as such, a global problem. The inorganic contaminants (Heavy metals) in the soil come from the bioavailable form.

These heavy metals have the highest concentration at which diverse health effect cannot be seen in the exposed receptor. Maximum acceptable level of metals is the allowable concentration of the heavy metal in soil. The concentration threshold values recommended by various agencies (ISO/WHO/ DIN/BSI/EURO/EPA) are presented in Table 1.

Table 1: Critical limit or maximum allowable concentration of heavy metals in soil in several countries for protection or all land uses (IAEA, 2004)

Countries	Critical Limit (mg/kg)				
	Pb	Cd	Ni	Cr	Fe
Demark	40	0.3	10	50	
Finland	38	0.3	40	80	
Netherland	85	0.8	35	100	
Switzerland	50	0.8	50	75	
Ireland	50	1.0	3.0	100	
Canada	25	0.5	2.0	0.2	
Eastern Europe (This include Russia, Ukraine, Maldova and Belarus)	32	2.0	85	90	
NESREA (2009)	40	3.0	70	100	N.L.
(National Environmental Standards and Regulations Enforcement Agency)					

(N.L = Not listed)

This implies that the critical limit or maximum allowable concentration of heavy metals in soil in several countries mentioned above for protection of all land use, range as follows: 25 – 85mg/kg for lead (Pb); 0.3 – 3.0 mg/kg for cadmium (Cd); 2 – 85mg/kg for Nickel (Ni) and 0.2 – 130 mg/kg for chromium (Cr).Some of these heavy metals over the years have proven to be carcinogenic to human, and also has negative impacts on the soil biota. Although several researches have been carried out on the effect of biochar on the bioavailability, mobility and toxicity of inorganic contaminants, there is need to monitor the bioavailable form of these metals, its mobility and toxicity with the aim to reduce their presence in the soil by treating the lead-acid battery contaminated soil with *Blighia sapida* (Ukpe as called by the Binis) hardwood derived biochar which are readily available in Benin city, Edo state, Nigeria.

MATERIALS AND METHODS
Biochar Preparation

The *Blighia sapida* hard wood (Ukpe) used for the preparation of biochar was obtained from the Saw Mill (Plate 1) located in Akpata, Egor community in Egor local government area of Edo state, Nigeria. The hard wood was pyrolysed at 350°C for I hour twenty minutes. The char produced was ground and sieves to less than 2mm of size.

Plate 1: *Blighia sapida* **hard wood (Ukpe***)* **being processed at the Saw Mill**

Soil Sampling

The soil samples were collected from three (3) different lead battery charging shops located within Benin City (Plate 2). Systematic grid sampling method was used to collect soil samples at the depth of 0-15cm, and the sample were mixed together to form a composite sample. The soil was air-dried for one (1) week, sieved to pass through <2mm mesh sieve, and any debris were removed.

Plate 2: Lead Battery Contaminated Sampling Site

Maize grain

The maize grain (*zea mays* L.) used for the study was obtained from Edo state ministry of Agriculture, Sapele road, Benin city, Edo state, Nigeria.

Acid Digestion of Soil Sample

The potentially bioavailable Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Iron (Fe) in the soil sample and the biomass was extracted by acid digestion using modified Tessier, (1979) method (10ml HNO_3: 1.0ml acid, $HClO_4$). This method is as follows;

1.0g of dry-ground sample (soil) was weighed; 10mL of 10:1 nitric acid and perchloric acid ($HNO_3/HClO_4$) were added in kjeldahl digestion flask. The sample was heated in a fume chamber to evaporate the organic components, until white fumes of perchloric acid appeared.The flask was cooled, filtered using Whatman no.42 filter. The filtrate digest was made up to mark of 100ml standard volumetric flask with de-ionized water and stored in a refrigerator until analysis. The heavy metal content (Pb, Cd, Cr, Ni and Fe) were determined using Atomic Absorption Spectrophotometer (AAS), Buck Scientific Model 210VGP in Chemistry Department, University of Benin, Benin City.

Soil pH Determination

Twenty grams of each aggregate soil sample were weighed into a 50mL beaker and 20mL of distilled water was added. The soil/water mixtures (1:1 ratio) were allowed to stand for 30 minutes and stirred occasionally with a glass rod. The Suntex pH meter was standardized using buffer 4 and 7. The electrodes were rinsed with distilled water and subsequently immersed into the soil/water mixture and the he pH was finally recorded. (Anegbe *et al*., 2014).

Particle size analysis - Hydrometer method

Fifty grams of soil sample was weighed into a 250mL beaker, 100mL of distilled water and 10mL of concentrated H_2O_2 were added to the soil. The content of the beaker was heated until frothing stopped. The mixture was cooled and transferred into shaking bottles. 20mL of sodium hexametaphosphate solution was added and the mixture was shaken for one hour. The suspension was transferred to one litre sedimentation cylinder and brought to mark with distilled water. A plunger was used to agitate the suspension. The hydrometer was then lowered into the suspension and its reading was taken after 40 seconds. The temperature was noted. The first reading, R_1, gave the percent clay and silt. The suspension was allowed to stand for 2 hours before the hydrometer reading was taken again. The second reading, R_2, gave the percent clay.

Calculations:

$$\%(Clay + Silt) = \frac{(R_1 + 0.3X \times 100)}{W(g)} \qquad (1)$$

$$\%Clay = \frac{(R_2 + 0.3X \times 100)}{W(g)} \qquad (2)$$

Where; R_1 = Hydrometer reading after 40 seconds
R_2 = Hydrometer reading after 2 hours
X = Degree rise above calibration point of hydrometer
0.3 = Correction factor for every degree rise above the calibration temperature of the hydrometer
W = Weight of soil sample (50g)

$$\%Sand = 100 - (Clay + Silt)\% \qquad (3)$$

$$\%Silt = 100 - (Sand + Clay)\% \qquad (4)$$

(Asagbe *et al*., 2007).

Determination Total Organic Matter and Organic Carbon

Total Organic Carbon was determined using direct estimation of organic matter by Loss-on-Ignition (LOI) using method adopted by Storer, (1984). This method is outlined below;

the porcelain crucible was heated for one hour at $375^{\circ}C$, and was allowed to cool in the open to about $150^{0}C$, and then placed in desiccators to cool for 30 minutes before weighing. About 5g of oven dried samples were placed into each crucible. The crucibles containing the samples were place in muffle furnace at room temperature and heated slowly to $375\pm5^{\circ}C$. The temperature was maintained at $375\pm5^{\circ}C$ overnight (about 16 hours). The furnace was turned off and the temperature was allowed to drop to about $150^{\circ}C$ and the crucible was removed and placed in a desiccator for 30 minutes after which the crucible was weighed to the nearest milligram.

Calculation;

$$\text{Loss on Ignition}\left(\%\right) = \frac{W_o - W_s}{W_o} \times \frac{100}{1} \quad (5)$$

Where W_o is the weight of oven-dried sample (g) and W_s is the weight of sample after ignition (g).

$\%\text{organic matter in soil} = \%\text{organic carbon} \times 1.724$
Where 1.724 = factor for the conversion of soil organic matter (SOM) to organic carbon (SOC).

SOM contains approximately 58% carbon; therefore, a factor of 1.724 can be used to convert SOM to SOC (Radojevic and Bashkin, 1999).

Determination of soil Nitrogen (Micro-Kjeldahl Digestion)

A 0.2g of finely ground soil was weighed into Kjeldahl digestion flask and one tablet of kjeldahl catalyst (one tablet of Sodium Sulphate containing 0.05g selenium) and 4.0mL was added of conc. H_2SO_4. The mixture was shaken very well to ensure the complete mixing of the soil and catalyst. The flask was placed on the heater and digested until a clear mixture was obtained. The mixture was cooled until it was just warm to touch. Then, 10mL of distilled water was added. Care was taken to ensure that the mixture in the flask was not solidified before addition of water. The mixture was filtered through Whatman No. 42 filter paper into 100mL volumetric flask and made up to mark, and 5mL of the filtrate was measure into a 50mL flask. Then after, a 2.5mL of the alkaline phenol and 2.5mL of Sodium Hypochlorite (bleach) were added and shaken well until colour developed, and was made up to 25mL by volume. The reading was taken through the use of a colorimetre at 630nm. The standard was prepared using the same method but without the use of the digested filtrate.

Percentage Nitrogen was calculated using equation (6).

$$\%N = \frac{IR \times S_r \times C_v \times D_v \times 10^{-6} \times 100 \times Cf}{W_s \times A_t} \quad (6)$$

Where;
IR = Instrument reading
S_r = Reciprocal of slope
C_v = Colour volume
D_v = Digest volume
W_s = Weight of sample
A_t = Aliquot taken
Cf = Correction factor

Determination of Phosphorus

A 5g of soil was weighed into a polypropylene bottle and 40mL of the extracting solution was added and stoppered. The mixture was shaken manually for one minute and filtered with Whatman No.1 filter paper. The clear supernatant was kept for phosphorus determination (Bray and Kurtz, 1945).

Cation Exchange Capacity

A 5g of soil sample was weighed into 250mL polypropylene bottle and 100mL of 1M NH_4OAC solution was added and stoppered. The mixture was shaken for 30minutes in a mechanical shaker (Heldoph) at 200rpm for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper. The concentrations of K^+, Na^+, Mg^{2+} and Ca^{2+} in the extract were determined using flame photometer. The equipment was standardized using various concentrations of the metals ranging from 2-20ppm prepared from analytical grade reagents (Sigma, BDH and Buck Scientific). The summation of the various cations was reported as cation exchange capacity (Nottidge *et al*., 2005).

Sequential chemical fractionation of heavy metals in soil

Pb, Cr, Ni, Cd and Fe in soil were fractionated using the modified European Community Bureau of Reference (BCR) three-step sequential extraction method.

This method involves the following steps:

(i) B_1 – extraction to recover the fraction that is mobile, soluble in water or weak acids, or adsorbed to soil carbonates.

(ii) B_2 – extraction to recover the fraction bound to iron and manganese oxides.

(iii) B_3 – extraction to recover the fraction bound to organic matter and sulphides.

The extractions were performed by shaking 1.0g portion o the pre-treated soil sample oven dried at $105^{0}C$ for 2 hours in a mechanical shaker according to the steps described below:

Step 1 (B_1): A 40mL of solution A (0.11M acetic acid solution) was added to 1.0g of

pretreated soil in a 100mL centrifuge tube and extracted by shaking at 30rpm for 16hours at ambient temperature (overnight). No delay occurred between the addition of the extractant solution and the beginning of the shaking. The extract was separated from the soil residue by centrifugation, and decantation of the supernatant liquid into a polyethylene container. The container was stoppered and stored in refrigerator until analysis. The residue was washed with 20mL distilled water, shaking for 15 minutes and centrifuging for 20 minutes. The liquid was carefully decant off and discard, while the solid residue was retained for step 2.

Step 2 (B₂): A 40mL of solution B (975mL of 0.5M hydroxylammonium hydrochloride plus 25mL of 2M nitric acid solution) was added to the residue from step 1 in the centrifuge tube and extracted by shaking for 16 hours at ambient temperature (overnight). No delay occurred between the addition of the extractant solution and the beginning of the shaking. The liquid supernatant was separated from the solid residue by centrifugation and decantation as in step 1. The liquid supernatant was retained in a stoppered polyethylene tube as before, for analysis. The residue was washed by adding 20mL of distilled water, shaking for 15 minutes and centrifuged for 20 minutes. The liquid supernatant was decanted and discarded taking care to avoid discarding any solid residue. The residue was retained for step 3.

Step 3 (B₃): A 10mL of 8.8M of solution C (Hydroxide peroxide solution) was added carefully to small aliquots to avoid losses due to violent reaction to the residue in the centrifuge tube. The vessel was covered with a watch glass and digested at room temperature for 1 hour with occasional shaking. The digestion was continued for 1 hour at 85°C and volume reduced to a few mL. Thereafter, 50mL of solution D (1.0M ammonium acetate solution) was added to the cool moist residue and shaken for 16 hours at ambient temperature (overnight). No delay occurred between the addition of the extractant residue and the beginning of the shaking. The extract was separated from the solid residue by centrifugation and decantation in polyethylene tube as in step 1 and 2 stoppered and retained as before analysis using Atomic Absorption Spectrophotometer (AAS).

Residue (R): Aqua regia digestion was employed in the extraction of the residue fraction and the digest was kept for heavy metal assay.
The concentrations of Cd, Fe, Pb, Cr and Ni in every extract were measured using AAS.

Soil Amendment and Treatment

The soil sample already prepared was homogeneously and thoroughly hand mixed with *Bligha sapida* hardwood derived biochar at 5, 10, 15 & 20 percent amendment. The mixing was carried out on a volume basis on account for the various densities of the soil and amendment (0.3 gmL^{-1} for biochar as reported by Beesley *et al*., (2010).

Pots were equilibrated by saturating the soils with de-ionized water every 3 days in the laboratory until 40 days. After 40 days, the material in the pot was collected, air dried and kept for analysis.

Pot experiment

Pot experiment was adopted. Maize seeds were carefully sown in cylindrical pots, each containing 1000g of soil. Ten maize grains were sown per pot and surface irrigation with de-ionized water was adopted to water the plant growth at 3 days interval. Night and day cycles were naturally obtained by maintaining the pots in a greenhouse. The grown plant was harvested from the soil after 21 days. The plants were analyzed for any toxicity.

Heavy Metals Characterization of Grown Maize Plant

A 0.25g of the powered maize plant was weighed, and 5mL of the nitric-perchloric acid (3:1) mixture was added. The mixture was soaked overnight and was heated under reflux for one hour at 150°C. The temperature was raised to 235°C and heated until dense white fume occurred, and heating continued for another 2 hours. The extract was cool to about 100°C, and 1mL of 50% HCl was added, and heated until dense white fume occurred. The sample was washed with distilled water into a 100mL standard flask, and more distilled water was added to make up the mark. The heavy metals content was analyzed using Atomic absorption Spectrophotometer.

The bioavailability indices (Bi) were calculated using the modified method of Moreno *et al*., (1997);

$$\text{Bi} = \frac{mg\ heavy\ metal\ /kg\ total\ biomass\ \times 100}{Total\ Heavy\ metal\ content\ (mg\ /kg\ soil)} \quad (7)$$

Quality control and assurance

In order to ensure the accuracy and reliability of the results obtained, analytical grade reagents (BDH and Sigma) were used. All glasses and plastic were cleansed by acid (HCl), washed and rinsed thoroughly with distilled water. Buck scientific standard solution was used to calibrate the AAS. Blank determinations were subjected to similar extraction method using same amount of reagents. Matrix spikes were analyzed to determine the performance (precision and bias) of the analytical methods used and to determine whether matrix interferences exist.

RESULTS AND DISCUSSION
Physico-chemical Properties of *Blighia sapida* derived Biochar, lead-acid battery contaminated Soil and the Amended Soil.

The physico-chemical properties of the *Bligha sapida* wood biochar, lead-acid battery contaminated soil and amended soil were determined and the results are shown in table 2. From the characterization, the organic matter content of the lead-acid battery contaminated soil was low (3.93%) indicating that the soil might have low adsorption strength and an increased metal mobility and bioavailability, hence a low heavy metal immobilization capacity. The biochar has a very high organic matter (95.30%), an indication that its adsorption strength is high and will be able to reduce metal mobility and bioavailability, and hence, a strong ability to immobilize metals in soil (Peng *et al*., 2009; Udeigwe, 2010). This equally shows that the *bligha sapida* hardwood derived biochar has the ability to buffer against rapid changes in salinity, sodicity and pH. It also shows its ability to serves as energy and nutrient for micro-organisms and moderate extreme soil temperature.

The pH of the lead battery contaminated soil is 5.86 and that of the biochar is 6.96. Hazelton and Murphy (2007) reported that metal availability is relatively low when pH is around 6.5 to 7.0. The mobility of trace element is reduced with increasing soil pH because of the precipitation as insoluble hydroxides, carbonates and organic complexes, and the chemical activity is lowest when soil pH is close to neutral 7.0. The important factors controlling mobility and solubility of metal in soil are soil pH and the content of organic matter, clay minerals, oxy-hydroxides and other mineral with which it interacts (Heidary-Monfared, 2011).

From the Table 2, low cation exchange capacity (CEC) implies the soil has a low resistance to change in soil chemistry caused by land use. The value of cation exchange capacity of lead battery contaminated soil is 1.39cmol/kg and *Bligha sapida* hardwood biochar is 1.04cmol/kg are rated as high. CEC is the key parameter describing sorption and desorption of plant nutrients and contaminant in soil. CEC is directly related to the soil capacity of desorbing heavy metals. The greater the CEC value, the more exchange site

availability on soil minerals for metal retention (Barry and Kurtz, 1995). Cation exchange capacity being the capacity to hold and exchange cations, provides the buffering effect to changes in pH and available nutrients.

From the particle size characterization of the lead battery contaminated soil (Table 2), it shows that the soil structural classification are sandy loam with the use of soil textural triangle for determining soil textural class from percentage of silt, sand and clay. In sandy soil, organic matters breakdown faster than in fine-textured soils because of a higher amount of oxygen available for decomposition in the light textured sandy soils which is the case with the lead battery contaminated soil. Easy decomposition of organic matter leads to increased mobility and bioavailability of trace metals in the soil. The CEC of the soil increase with percentage clay and organic matters. Also the pH buffering capacity of a soil (its ability to resist pH change upon lime addition) is largely based on clay and organic matter content. This shows that the use of *Blighia sapida* wood derived biochar will help to increase the loamy nature that is an increase in organic matters of the amended lead-acid battery contaminated soil. This is in agreement with the fact that the *Blighia sapida* wood derived biochar increases the organic matter content of the amended lead-acid battery contaminated soil because of its very high organic matter content.

The electrical conductivity of the lead battery contaminated soil and *Blighia sapida* wood derived biochar are 1928μS/cm and 545μS/cm respectively. These values indicate the presence of salts in both. However, the implication of these high values of electrical conductivity in lead battery contaminated soil is because of it high quantity of sand and a low quantity of clay.

The pH, TOC, N, TOM, EC and CEC values increase as the percentage of *Blighia sapida* hardwood derived biochar increases in the amended soil, with soil amended with 20% biochar having the highest values of pH, TOC, N, TOM, EC and CEC. An indication that the *Blighia sapida* hardwood derived biochar's adsorption strength is high and will be able to reduce metal mobility and bioavailability, as its equally provide energy and nutrient for micro-organisms in the soil.

Table 2: Physico-chemical Properties of *Blighia sapida* derived Biochar, lead-acid battery contaminated Soil and the Amended Soil.

| Parameters	B	0% (contaminated soil as control)	5% Amendment	10% Amendment	15% Amendment	20% Amendment
Clay (%)	-	11.10	-	-	-	-
Silt (%)	-	6.90	-	-	-	-
Sand (%)	-	82.00	-	-	-	-
pH	6.96	5.86	6.20	6.25	6.34	6.40
TOC (%)	55.28	2.28	4.24	8.41	12.44	16.71
N (%)	1.08	1.15	1.15	1.17	1.18	1.21
TOM (%)	95.30	3.93	7.31	14.50	21.47	28.81
EC (µS/cm)	545	1928	2.40	2.38	2.33	2.20
Na (mg/kg)	3.70	4.30	4.80	5.00	5.10	5.81
K (mg/kg)	4.50	150.00	150.10	151.00	151.10	151.40
Ca (mg/kg)	200.00	195.00	198.00	200.01	202.00	202.90
Mg (mg/kg)	0.97	0.91	1.08	1.10	1.14	1.18
CEC (cmol/kg)	1.04	1.39	1.40	1.42	1.43	1.44
P (mg/kg)	4800.20	20.38	256.00	508.02	744.01	1004.07

TOC = Total Organic Carbon, **N** = Nitrogen, **TOM** = Total Organic Matter, **EC** = Electrical Conductivity, **CEC** = Cation Exchange Capacity, **B** = Biochar and **P** = Phosphorus

Total Heavy metal concentration in lead-acid battery contaminated soil, Biochar and Soil amended Biochar.

The total heavy metal content in the lead-acid battery contaminated soil shows that it contains 127.50mg/kg lead, 0.32mg/kg chromium, 0.03mg/kg cadmium, 0.18mg/kg nickel and 135mg/kg iron, while the *Blighia sapida* hardwood derived biochar has 0.23mg/kg lead, 0.18mg/kg chromium, 0.01mg/kg cadmium, 0.01mg/kg nickel and 0.43mg/kg iron. Comparing these values with NESREA permissible limits for heavy metal in soil (Table 1), it shows that the amount of lead was above NESREA (2009) permissible limit while that of chromium, cadmium and nickel were within the permissible limit.

From Figure 1, it is evident that the amount of heavy metals in the lead-acid battery contaminated soil reduced gradually as the percentage of biochar amendment increases (20%>15%>10%>5%). An indication that *Blighia sapida* hardwood derived biochar adsorption strength is high and has the ability to reduce metal mobility and bioavailability, and hence, have a strong ability to immobilize metals in soil.
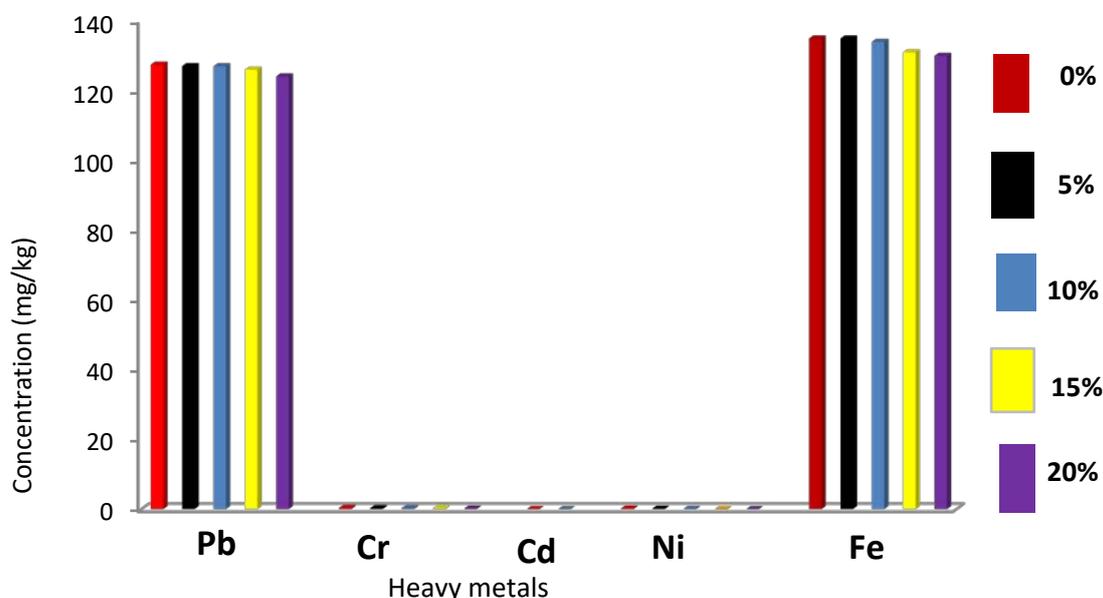
Figure 1: Total Heavy Metal Concentration in Lead-acid Battery Contaminated soil and Soil Amended with *Blighia sapida* Hardwood derived Biochar

Geochemical Fractions of Heavy Metals in lead-acid battery Contaminated Soil.

In the B_1 fraction, the value of lead, chromium, nickel and iron were found to be 30.00mg/kg, 0.08mg/kg, 0.11mg/kg and 0.19mg/kg respectively. These values indicate that the metals are mobile and bioavailable to plants for uptake because they are weakly bound and adsorbed to carbonate, while cadmium was below detectable level.

In the B_2 fraction, the value of lead, chromium, cadmium, nickel and iron were found to be 51.02mg/kg, 0.14mg/kg, 0.01mg/kg, 0.01mg/kg and 54.80mg/kg respectively. Iron and manganese oxides which can be present between particles or coatings on particles are excellent substrates with large surface areas for absorbing trace metals. Under reducing conditions, Fe (III) and Mn (IV) could release adsorbed trace metals. In other words, this fraction is tightly held to the soil and it is affected or made available by manipulating the redox potential. In this fraction, the metals are not

mobile and hence not easily available to plants (Abu-Kukati, 2001).

In the B_3 fraction, the value of lead, chromium, nickel and iron were found to be 18.02mg/kg, 0.07mg/kg, 0.04mg/kg and 0.14mg/kg respectively while cadmium was below detectable level. This is an indication that these metals may be bound by various forms of organic matter, living organisms, and coating on mineral particles through complexation or bioaccumulation (Joseph *et al.*, 2010). Degradation of these substances by oxidation leads to a release of soluble metals. In this fraction, the metals outside redox potential manipulations are tightly bound, not mobile and are not available to plants for uptake.

In the residual fraction, the value of lead, chromium and iron were found to be 26.00mg/kg, 0.01mg/kg and 75.52mg/kg respectively, while cadmium and nickel were below detectable level. Here metals are firmly bonded within crystal structure of the minerals comprising the soil. The metals in this fraction are not mobile and not available at any condition for plant uptake.

Table 3: Geochemical Fractions of Heavy Metals in lead-acid battery Contaminated Soil.

Fractions	Lead	Chromium	Cadmium	Nickel	Iron
B_1(mg/kg)	30.00	0.08	B.D.L	0.11	0.19
B_2(mg/kg)	51.02	0.14	0.01	0.01	54.80
B_3(mg/kg)	18.02	0.07	B.D.L	0.04	0.14
Residual (mg/kg)	26.10	0.01	B.D.L	B.D.L	75.52
M_f(%)	23.97	26.67	0.00	68.75	0.15

B.D.L = below detectable level, **B_1**=Fraction that is Water soluble, Mobile and adsorbed to Carbonate, **B_2**=Fraction bound to iron and manganese oxides, **B_3**=Fraction bound to organic matter and sulphides, and **M_f** = Mobility factor
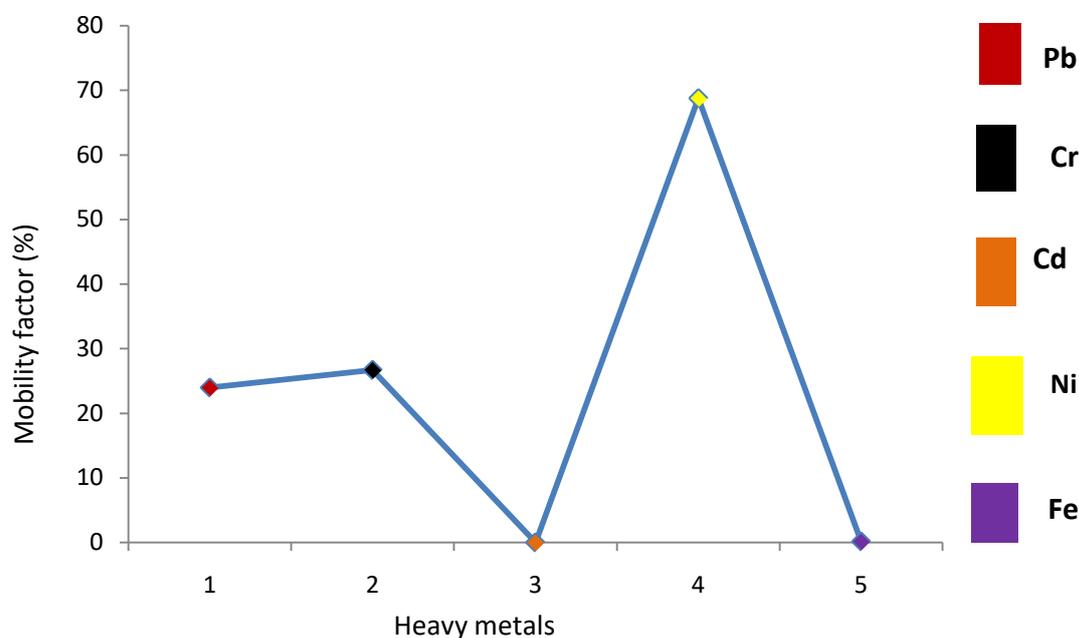
Figure 2: Mobility Factor of Metals in Lead-acid Battery Contaminated Soil

Geochemical Fractions of Heavy Metals in *Blighia sapida* hardwood derived biochar

The metal contents in *Blighia sapida* hardwood derived biochar determined in the three fractions following the sequential extraction procedure are shown in Table 5.

In the B_1 fraction, the levels of lead, chromium, cadmium, nickel and iron were found to be 0.15mg/kg, 0.10mg/kg, 0.01mg/kg, 0.01mg/kg and 0.18mg/kg respectively. These values indicate that the metals are mobile and bioavailable to plants for uptake because they are weakly bound and adsorbed to carbonate. Comparing the values with the maximum allowable limit as approved by NESREA in Table 1, it is crystal clear that these values are within the maximum allowable limit. Hence, *Blighia sapida* hardwood derived biochar was not polluted with heavy metals, and can be used for remediation purposes.

In the B_2 fraction, the concentrations of lead, chromium and iron were found to be 0.03mg/kg, 0.05mg/kg and 0.20mg/kg respectively, cadmium and nickel were below detectable level. Hence, this fraction is not mobile and not easily available to plants.

In the B_3 fraction, the amounts of lead, chromium and iron were found to be 0.01mg/kg, 0.02mg/kg and 0.03mg/kg respectively, while cadmium and nickel were below detectable level. In this fraction, the metals outside redox potential manipulations are tightly bound, not mobile and are not available to plants for uptake.

In the residual fraction, only iron was detected to be 0.01mg/kg while lead, chromium, cadmium and nickel were below detectable level. The metals in this fraction are not mobile and not available at any condition for plant uptake.

Table 4: Geochemical Fractions of Heavy Metals in Ukpe hardwood (*Blighia sapida*) derived biochar

Fractions	Lead	Chromium	Cadmium	Nickel	Iron
B_1 (mg/kg)	0.15	0.10	0.01	0.01	0.18
B_2 (mg/kg)	0.03	0.05	B.D.L	B.D.L	0.20
B_3 (mg/kg)	0.01	0.02	B.D.L	B.D.L	0.20
Residual (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	0.01
M_f (%)	78.95	58.82	100	100	30.51

B.D.L = below detectable level, B_1=Fraction that is Water soluble, Mobile and adsorbed to Carbonate, B_2=Fraction bound to iron and manganese oxides, B_3=Fraction bound to organic matter and sulphides, and M_f = Mobility factor.

Geochemical Fractions of Heavy Metals in lead-acid battery contaminated soil amended with biochar.

The metal contents in lead-acid battery contaminated soil amended with *Blighia sapida* (Ukpe) hardwood derived biochar were determined through fractionation process as shown in Table 5. From the Table 5, it is evident that of a total of 127.08 mg/kg of lead, 0.30 mg/kg of chromium and 135 mg/kg of iron found in lead-acid battery contaminated soil amended with 5% biochar, only a fraction of 25mg/kg lead, 0.08 mg/kg chromium and 0.12 mg/kg iron were mobile and bioavailable.

Also, of a total 0.01 mg/kg cadmium and 0.16 mg/kg nickel found in lead-acid battery contaminated soil amended with 5% biochar, none were mobile and bioavailable for plant uptake. The mobility and bioavailability of the heavy metals were as well found to decrease as the percentage of biochar amendment increases (5% > 10% > 15% > 20%). The amount of heavy metals found in B_1, B_2 and B_3 fractions are found to decrease as the percentage of biochar amendment increases. The mobility factor was equally found to reduce as the percentage of biochar amendment increases (5% > 10% > 15% > 20%).

Table 5: Geochemical Fractions of Heavy Metals in lead-acid battery contaminated soil amended with biochar

Fractions	Metals	5%	10%	15%	20%	M.A.L (NESREA)
B_1	Pb (mg/kg)	25.00	23.80	23.00	20.01	10.00
	Cr (mg/kg)	0.08	0.02	B.D.L	B.D.L	100.00
	Cd (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	3.00
	Ni (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	70.00
	Fe (mg/kg)	0.12	0.10	0.10	0.02	-
B_2	Pb (mg/kg)	76.01	75.00	72.05	70.02	-
	Cr (mg/kg)	0.04	0.01	B.D.L	B.D.L	-
	Cd (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	-
	Ni (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	-
	Fe (mg/kg)	38.00	35.02	32.10	30.00	-
B_3	Pb (mg/kg)	15.20	16.20	14.00	11.20	-
	Cr (mg/kg)	0.06	0.02	B.D.L	B.D.L	-
	Cd (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	-
	Ni (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	-
	Fe (mg/kg)	0.84	0.70	0.65	0.44	-
Residual	Pb (mg/kg)	10.01	11.20	16.90	24.80	-
	Cr (mg/kg)	0.08	0.20	0.18	0.08	-
	Cd (mg/kg)	B.D.L	B.D.L	B.D.L	B.D.L	-
	Ni (mg/kg)	0.02	0.03	B.D.L	B.D.L	-
	Fe (mg/kg)	92.01	96.00	95.82	98.02	-
M_f	Pb (%)	19.81	18.86	18.26	15.88	-
	Cr (%)	30.77	8.00	0.00	0.00	-
	Cd (%)	0.00	0.00	0.00	0.00	-
	Ni (%)	0.00	0.00	0.00	0.00	-
	Fe (%)	0.09	0.08	0.08	0.02	-

B.D.L = Below Detectable Level, **B_1**= Fraction that is Water soluble, Mobile and adsorbed to Carbonate, **B_2**=Fraction bound to iron and manganese oxides, **B_3**=Fraction bound to organic matter and sulphides, **M.A.L** =Maximum Allowable Limit, **M_f** = Mobility factor.

Total Heavy Metals in Maize Plant and their Bioavailability Indexes from Amended lead-acid battery Contaminated Soil.

Before the amendment at different percentage of lead-acid battery soil/biochar, the bioavailability indices of the heavy metals were 19.69% Pb; 12.50% Cr; 38.89% Ni and 0.09% Fe. At 5% lead-acid battery contaminated soil/biochar mixture, the bioavailability indices reduced to 13.78% Pb; 12.33% Cr and 0.07% Fe, while Ni was no more bioavailable. At 10% soil/biochar mixture, the bioavailability indexes reduced to 11.30% Pb and 0.05% Fe. At 15% soil/biochar mixture, the bioavailability indexes reduced to 10.75% Pb and 0.05% Fe. At 20% soil/biochar mixture, the bioavailability index reduced to only 9.32%. This shows that the bioavailability index reduces as the quantity of *bligha sapida* biochar increases (Table 6). This is an indication that the *Blighia sapida* hardwood derived biochar was effective in reducing the bioavailability of heavy metals in the soil to the plants. This observation was graphically represented in Figure 3.

The biochar brings about an increase in stabilized organic matters and a decrease in the dissolved organic contents hence reducing the bioavailability of the heavy metals. In addition, biochar possesses organic functional group on its surface and the negatively charged organic functional groups increases overtime during its oxidation in soil. The formation of surface functional groups and adsorption site on biochar influence its cation exchange capacity (CEC) and consequently the capacity of biochar amended soils to form stable insoluble metal-organic complexes with metal ions (Kumpiene *et al.*, 2008; Keiluweit *et al.,* 2010). Violante and Giranfreda, (2000), reported that biochar forms complexes of deprotonated multidentate organic acid and the deprotonated multi-dentate organic acid are known to form complexes with transition metals hence reducing their mobility in soil. However, lead-acid battery contaminated soil without amendment was found to be intoxicated with Pb as 25.10 mg/kg of Pb was absorbed by maize plant. 17.50 mg/kg, 14.36 mg/kg, 13.54 mg/kg and 11.56 mg/kg was equally absorbed by plant at 5%, 10%, 15% and 20% amendment respectively.

Table 6: Total Heavy Metals in Maize Plant and their Bioavailability Indexes from Amended lead-acid battery Contaminated Soil.

Biochar/soil mixture (%) w/w	Parameters	Pb (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Fe (mg/kg)
0 (contaminated soil as control)	Total heavy metal in plant (mg/kg)	25.10	0.04	BDL	0.07	0.12
	Bioavailability indexes (%)	19.69	12.50	0.00	38.89	0.09
5	Total heavy metal in plant (mg/kg)	17.50	0.04	BDL	BDL	0.09
	Bioavailability indexes (%)	13.78	13.33	0.00	0.00	0.07
10	Total heavy metal in plant (mg/kg)	14.36	BDL	BDL	BDL	0.07
	Bioavailability indexes (%)	11.30	0.00	0.00	0.00	0.05
15	Total heavy metal in plant (mg/kg)	13.54	BDL	BDL	BDL	0.06
	Bioavailability indexes (%)	10.75	0.00	0.00	0.00	0.05
20	Total heavy metal in plant (mg/kg)	11.56	BDL	BDL	BDL	BDL
	Bioavailability indexes (%)	9.32	0.00	0.00	0.00	0.00

CONCLUSION

In conclusion, this study provides a baseline data and information on the physico-chemical properties of *Blighia sapida* (Ukpe wood) hardwood derived biochar. It also revealed that *Blighia sapida* (Ukpe wood) hardwood derived biochar is a useful material for immobilizing heavy metals in soil and making it non-bioavailable to plant. Specifically demonstrated that *Blighia sapida* derived biochar is an effective amender for remediating soil polluted with lead-acid battery. High concentrations of *Blighia sapida* hardwood derived biochar were found to suppress the mobility and bioavailability of the heavy metals in lead-acid battery contaminated soil. Hence, the higher the quantity (concentration) of *Blighia sapida* hardwood derived biochar, the greater the quantity of heavy metals adsorbed from the contaminated soil. The results obtained in this study revealed that the soil from lead-acid battery workshop was contaminated with Pb, Cr, Cd, Ni and Fe, but only polluted by Pb. This research work has also shown that *Blighia sapida* (Ukpe wood) hardwood derived biochar is very useful remediating materials for lead-acid battery contaminated soil. It reduced the bioavailability of the studied heavy metals (Pb, Cr, Cd, Ni and Fe) considerably.

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