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Comparative Studies on The Effects of Biochar and Cow Dung Amendments on the Mobility, Bioavailability and Toxicity of Heavy Metals in Lead-Acid Contaminated Soil

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Abstract

This study compared the heavy metals remediation potency of biochar and cow dung in soils contaminated by lead-acid batteries. Soil samples were gathered from three different battery charging shops in Benin City at the depth of 0-15cm using systematic grid sampling method. Hardwood was pyrolyzed at 350°C for 80minutes and cow dung was air dried and pound into powder. The soil was characterized using standard methods, and was fractionated using modified European Community Bureau of Reference (BCR) three-step sequential extraction procedure while the concentration of heavy metals was determined using Atomic Absorption Spectrophotometer (AAS). The total metal concentration in the lead-acid contaminated soil was 127.50mg/kg Pb, 0.32mg/kg Cr, 0.03mg/kg Cd, 0.18mg/kg Ni and 135.00mg/kg Fe. The contaminated soil was remediated with biochar and as well with cow dung at 5%, 10%, 15% and 20%. The soil was also amended using biochar-cow dung mixture (1:1, 2:1 and 1:2). The outcome revealed that the metal content in lead-acid contaminated soil remediated with biochar and cow dung were found to generally decrease as the percentage of amenders increases, with biochar being further efficient in lowering the levels of heavy metals in the contaminated soil. However, biochar when mixed with cow dung at 2:1 ratio at 20% amendment gives a better result than when used separately. Pot experiment was designed and the amounts of heavy metals bioavailable to plant were determined. The mobility factors and bioavailability indices of the metals reduced as the percentage of amenders' increases. The result obtained from this study revealed that although biochar and cow dung were effective amenders, biochar was more potent in lowering the mobility, bioavailability and toxicity of heavy metals in lead-acid contaminated soil. Biochar when mixed with cow dung at 2:1 ratio gave the best result in remediation of lead-acid contaminated soil.

Keywords: Bioavailability, Biochar, Cow dung, Mobility, Toxicity

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INTRODUCTION

Soil pollution is the availability of toxic substances in soil to the level that its poses a risk to living organisms including humans. It is the presence of any compound in soil either through direct or indirect exposure, resulting to a toxic response to biota or human, leading to an intolerable environmental risks world-wide (Beesley *et al*., 2011).

Soil polluted with lead-acid batteries has become a great danger to humans and other living organisms as it has seriously endangered their food chains, as such, a global problem. The inorganic contaminants (Heavy metals) in soils emanate from the bioavailable forms (Jacob *et al*., 2022).

Biochar is the porous, low density, carbon-rich of pyrolysis of biomass (Okieimen and Okuo, 2014). The large surface area and cation exchange capacity gotten to a large extent by source materials and pyrolysis temperature, with higher temperature promoting structure evolution and enhancing surface area of biochar, enables sequestration of both inorganic and organic contaminants, lowering contaminant mobility when applied to contaminated soils (Okieimen and Okuo, 2014). These different possible benefit, together with the fact that biochar gotten from different biomass can likely be remarkably cost-effective and environmentally useful tool for environmental remediation have stimulated more research interest in soil application of biochar (Tang *et al*., 2013). Lately, interests have been drawn to biochar as a soil remediating substance and it advantages to physico-chemical properties (Beesley *et al*., 2010).

Heavy metals over the years have proven to be carcinogenic to humans, and also have negative impacts on the soil biota and as well the food chain (Jacob *et al*., 2022). Many researchers have worked differently using these amenders, and have reported that they are effective in soil remediation. This research therefore seeks to compare the potentials of hardwood-derived biochar and cow dung in remediating lead-acid contaminated soil, and as well examined the effects of the mixture of both amenders in remediating lead-acid contaminated soil.

METHODOLOGY

Biochar Preparation

The hardwood sawdust used to prepare biochar was obtained from the Saw Mill located in Akpata, Benin city, Edo state, Nigeria. The hardwood sawdust was pyrolysed at 350°C for 1 hour twenty minutes. The biochar gotten was ground and sieved to below 2 mm of size.

Soil Sampling

The soil samples were picked from three (3) different lead-battery charging shops located within Benin City at the depth of 0-15 cm. Systematic grid sampling method was adopted during the sampling. The soil was air-dried for one (1) week, sieved to <2mm and any debris were removed.

Acid Digestion of Soil Sample

The amount of Lead, Cadmium, Chromium , Nickel and Iron in the soil sample/biomass were extracted by acid digestion using modified Tessier, *et al*., (1979) and the heavy metal concentration (Pb, Cd, Cr, Ni and Fe) were determined using AAS.

Soil pH Determination

The pH of the soil was determined using the method described by Anegbe *et al.,* (2014).

Soil Amendment and Treatment

The soil sample already prepared was homogeneously and thoroughly hand mixed with hardwood derived biochar at 5, 10, 15 and 20 percent amendments. The mixing was done on a volume basis taking note of their different densities (0.3 gml^{-1} for biochar as repoted by Beesley *et al*., (2010). The remediation process was stabilized by saturating the soils with deionized water every 3 days in the laboratory until 40 days. After 40 days, the mixture was air dried and kept for analysis (Jacob *et al*., 2022).

Particle size analysis

The particle size analysis was carried out to determine the percentage of clay, silk and sand in the sample. Hydrometer method was adopted as described in Asagbe *et al*., (2007).

Determination of Soil Organic Matter (SOM), Soil Organic Carbon (SOC) and soil Nitrogen (N)

SOM and SOC were determined using Loss on Ignition (LOI) method adopted by Storer, (1984) as described by Radojevic and Bashkin, (1999). While soil nitrogen was determined using Micro-Kjeldahl digestion method described in Jacob *et al.,* (2022).

Determination of Phosphorus and Cation Exchange Capacity (CEC)

Phosphorus was determined using method described by Bray and Kurtz, (1945) while CEC was determined through method described in Nottidge *et al*., (2005)

Sequential chemical fractionation of heavy metals

Pb, Cd, Ni, Cr and Fe in soil were fractionated using the modified three-step BCR sequential extraction procedure as described in Jacob *et al.,* (2022)

Pot experiment

Pot experiment was employed in this study. Maize seeds were carefully sown in cylindrical pots, each containing 1000 g of soil. Ten maize grains were sown per pot and they were irrigated with deionized water at 3 days interval. Night and day cycle were naturally obtained by maintaining the pots in a green house. The grown plants were harvested after twenty-one (21) days and were analyzed for any toxicity (Jacob *et al.,* 2022).

Heavy Metals Characterization of Grown Maize Plant

The amount of heavy metals presence in the grown maize plants and their bioavailability indices were determined using the modified method of Moreno *et al.,* (1997).

Mobility Factor (M_f)

Since fraction B_1 represent the first three fractions in the modified Tessier *et al*., (1973), that is; water or weak acid soluble, exchangeable and carbonate bound (Kabala and Singh, 2001), the relative index of metal mobility called mobility factor (M_f) is a measure of the potential mobility (Salbu *et al*., 1998: Kabala and Singh, 2001); was calculated from the BCR 3-steps sequential extraction data using the expression:

$$M_f = \left(\frac{B_1}{B_1 + B_2 + B_3 + R}\right) \times 100$$

RESULTS AND DISCUSSION

Characterization of the contaminated soil (control) and soil amended with Biochar

The physico-chemical properties of lead-acid battery contaminated soil (control) and amended soils were determined (Table 1). The organic matter present in the contaminated soil was low (3.93%), indicating that the soil has an increased metal mobility as well as bioavailability (Jacob *et al*., 2022).

The pH of the lead-acid contaminated soil is 5.86. Hazelton and Murphy (2007) reported that metal bioavailability is somewhat high when pH is less than 6.5. Increase in soil pH leads to decrease in metals mobility, and the chemical activity is lowest when soil pH is close to neutral 7.0.

Low Cation Exchange Capacity (CEC) implies the soil has a low resistance to change in soil Chemistry caused by anthropogenic activities. The value of CEC of lead-acid contaminated soil is 1.39 cmol/kg. The value is rated as high. CEC is linked to the soil ability to desorbed heavy metals. The higher the CEC value, the more exchange site availability on soil minerals for metal retention (Barry *et al*., 1995).

Table 1 also confirmed that the soil structural classifications are sandy-laom with the use of soil textural triangle for determining soil textural class from percentage of sand, silt and clay. In sandy soil, organic matters disintegrate rapidly compared to fine-textured soils due to greater amount of oxygen available for decomposition in sandy soils which is the case with the lead-acid contaminated soil. Easy decomposition of organic matter brings about increased mobility and bioavailability of trace metals which have formed soluble organic complexes in the soil (Jacob *et al*., 2022). The soil CEC increase with percentage clay and organic matters. Also the pH buffering ability of a soil is mainly base on the amount of organic matter and clay present. (Milford, 1997). This shows that the use of hardwood derived biochar will help to increase the loamy nature by increasing the organic matters of the amended soils.
The electrical conductivity of the lead battery contaminated soil is 1928 µs/cm. This value indicates the presence of salts. However, the implication of this high value of electrical conductivity in lead-acid contaminated soil is due to its high quantity of sand with low quantity of clay.

The pH, TOC, N, TOM, EC and CEC values increase as the percentage of 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 biochar's adsorption strength is high and will be able to decrease the bioavailability level of heavy metals in soils.

Table 1: Physico-chemical Properties of lead-acid battery contaminated Soil (used as control) and soil amended with biochar.

Parameters	0% control	5% Amendment	10% Amendment	15% Amendment	20% Amendment
Clay (%)	11.10	-	-	-	-
Silt (%)	6.90	-	-	-	-
Sand (%)	82.00	-	-	-	-
pH	5.86	6.20	6.25	6.34	6.40
TOC (%)	2.28	4.24	8.41	12.44	16.71
N (%)	1.15	1.15	1.17	1.18	1.21
TOM (%)	3.93	7.31	14.50	21.47	28.81
EC (µs/cm)	1928	2.40	2.38	2.33	2.20
Na (mg/kg)	4.30	4.80	5.00	5.10	5.81
K (mg/kg)	150.00	150.10	151.00	151.10	151.40

Ca (mg/kg)	195.00	198.00	200.01	202.00	202.90
Mg (mg/kg)	0.91	1.08	1.10	1.14	1.18
CEC(cmol/kg)	1.39	1.40	1.42	1.43	1.44
P (mg/kg)	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

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

In B_1 fraction in Table 2, the value of lead, chromium, nickel and iron were 30.00 mg/kg, 0.08 mg/kg, 0.11 mg/kg and 0.19 mg/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 detection level.

In the B_2 fraction, the value of lead, chromium, cadmium, nickel and iron were 51.02 mg/kg, 0.14 mg/kg, 0.01 mg/kg, 0.01 mg/kg and 54.80 mg/kg respectively. This means that 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 is not mobile and hence not easily available to plants (Abu-Kukati, 2001).

In the B_3 fraction, the amount of lead, chromium, nickel and iron was 18.02 mg/kg, 0.07 mg/kg, 0.04 mg/kg and 0.14 mg/kg respectively while cadmium was below detection level. This is an indication that these metals can be bound by different forms of organic matter, living organisms, and attached on mineral particles through bioaccumulation (Joseph *et al.*, 2010). Deterioration of these materials by oxidation results to a liberation of soluble metals. In this fraction, metals without manipulation are strongly bound and not mobile or bioavailable to plants.

In the residual fraction, the value of lead, chromium and iron was 26.00 mg/kg, 0.01 mg/kg and 75.52 mg/kg respectively, while cadmium and nickel were below detection level. Here metals are tightly bonded inside the crystal structure of the minerals making up the soil. The metals in this fraction are not mobile and not available at any condition for plant uptake. The mobility factor of these metals follows this order; Ni>Cr>Pb>Fe.

Table 2: 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	BDL	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	BDL	0.04	0.14
Residual (mg/kg)	26.10	0.01	BDL	BDL	75.52
M_f (%)	23.97	26.67	0.00	68.75	0.15

BDL = below detection 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

Mobility Factor of Metals in Lead-acid Battery Contaminated Soil

This result shows that lead (Pb) content in the Fraction that is Water soluble, Mobile and adsorbed to Carbonate exceeded the maximum permissible limit of heavy metals in soil in Nigeria, while chromium and nickel content were within the maximum permissible concentration of heavy metals in soil in Nigeria set by NESREA (2009). The heavy metal content for cadmium was below the range. This implies that the lead-acid battery contaminated soil was heavily polluted with regards to lead in which their mobile and bioavailable content exceeded the maximum permissible concentration for all land use.

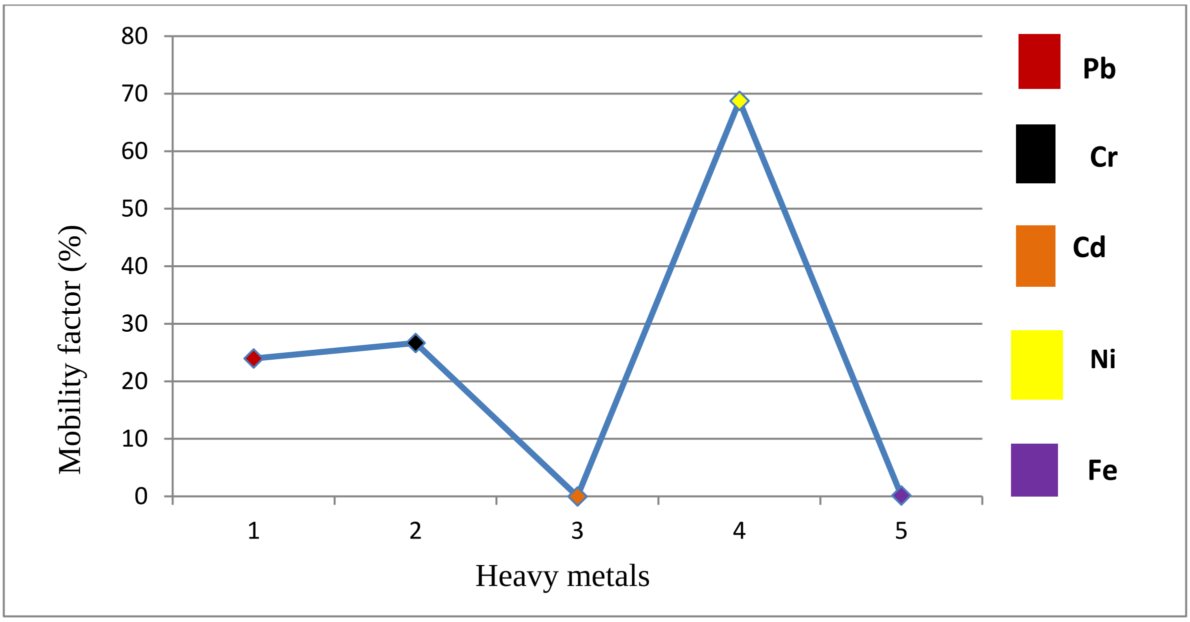

Figure 1. Mobility Factor of Metals in Lead-acid Battery Contaminated Soil

Table 3: B_1 (Fraction that is Water soluble, Mobile and adsorbed to Carbonate) of lead-acid battery Contaminated Soil Compared to Maximum permissible Concentration (M.P.C) of Heavy Metals in Soils by NESREA (2009) for All Land Use.

Fraction	Lead (mg/kg)	Chromium (mg/kg)	Cadmium (mg/kg)	Nickel (mg/kg)
B_1	30.00	0.08	BDL	0.11
M.P.C.	10.00	100.00	3.00	70

BDL = Below Detectable Level

Total Heavy metals concentration in the contaminated soil and Soil remediated using Biochar.

From Table 4, the data revealed that the amount of heavy metals in the lead-acid battery contaminated soil reduced gradually as the percentage of biochar amendment increases (5%>10%>15%>20%), which is an indication that the hardwood derived biochar is a good adsorbent with a high metal immobilizing ability.

Table 4: Total Heavy metal concentration in lead-acid battery contaminated soil, Biochar and Soil amended with Biochar.

Heavy Metals (mg/kg)	0% Control	5% amendment	10% amendment	15% amendment	20% amendment
Lead	127.50	127.08	127.01	126.00	124.10
Chromium	0.32	0.30	0.28	0.24	0.18
Cadmium	0.03	0.01	BDL	BDL	BDL
Nickel	0.18	0.16	0.10	0.03	0.01
Iron	135.00	135.00	134.02	131.00	130.01

BDL = Below Detectable Level and B = Biochar

Geochemical Fractions of Heavy Metals (mg/kg) in soil amended with biochar.

The results from Table 5 revealed that of a total of 127.08 of lead, 0.30 of chromium and 135 of iron found in the contaminated soil, only a fraction of 25 lead, 0.08 chromium and 0.12 iron were mobile and bioavailable when amended with 5% biochar, while cadmium and nickel were not bioavailable. 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 mobility factor was equally found to reduce as the percentage of biochar amendment increases.

Table 5: Geochemical Fractions of Heavy Metals (mg/kg) in lead-acid battery contaminated soil amended with biochar.

Fractions	Metals	5%	10%	15%	20%	M.A.L (NESREA 2009)
B_1	Pb	25.00	23.80	23.00	20.01	10.00
	Cr	0.08	0.02	BDL	BDL	100.00
	Cd	BDL	BDL	BDL	BDL	3.00
	Ni	BDL	BDL	BDL	BDL	70.00
	Fe	0.12	0.10	0.10	0.02	-
B_2	Pb	76.01	75.00	72.05	70.02	-
	Cr	0.04	0.01	BDL	BDL	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	BDL	-
	Fe	38.00	35.02	32.10	30.00	-
B_3	Pb	15.20	16.20	14.00	11.20	-
	Cr	0.06	0.02	BDL	BDL	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	BDL	-
	Fe	0.84	0.70	0.65	0.44	-
Residual	Pb	10.01	11.20	16.90	24.80	-
	Cr	0.08	0.20	0.18	0.08	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	0.02	0.03	BDL	BDL	-
	Fe	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 Detection 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.P.L =Maximum Permissible Limit, M_f = Mobility factor.

Total Heavy Metals (mg/kg) in Maize Plant and their Bioavailability Indices from Amended lead-acid Contaminated Soil.

The total heavy metal in maize plant and their bioavailability indices from the amended lead-acid contaminated soil using biochar is shown in Table 6. Before the amendment, the bioavailability indices of the heavy metals were 19.69% Pb; 12.50% Cr; 38.89% Ni and 0.09% Fe. But after remediation, the bioavailability indices reduced to only 9.32% Pb while Cr, Ni, and Fe became unavailable. This is an indication that the biochar was active in bringing down the bioavailability of heavy metals in the soil to the plants. The biochar brought about an increase in stabilized organic matters and a decrease in the dissolved organic contents hence lowering the bioavailability of the heavy metals. Violante and Giranfreda, (2000), reported that biochar forms complexes of deprotonated multidentate organic acid and the deprotonated multidentate organic acid are familiar with forming 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% amendments respectively.

Table 6: Total Heavy Metals (mg/kg) in Maize Plant and their Bioavailability Indices from Amended lead-acid battery Contaminated Soil.

Levels of amendment (%) w/w	Parameters	Pb	Cr	Cd	Ni	Fe
0 (Control)	Total heavy metal in plant	25.10	0.04	BDL	0.07	0.12
	Bioavailability indices (%)	19.69	12.50	-	38.89	0.09
5	Total heavy metal in plant	17.50	0.04	BDL	BDL	0.09
	Bioavailability indices (%)	13.78	13.33	-	-	0.07
10	Total heavy metal in plant	14.36	BDL	BDL	BDL	0.07
	Bioavailability indices (%)	11.30	-	-	-	0.05
15	Total heavy metal in plant	13.54	BDL	BDL	BDL	0.06
	Bioavailability indices (%)	10.75	-	-	-	0.05
20	Total heavy metal in plant	11.56	BDL	BDL	BDL	BDL
	Bioavailability indices (%)	9.32	-	-	-	-

BDL = Below Detectable Level

Characterization of the soil amended with cow dung

The results (Table 7) shows that the values of pH, TOC, TOM, EC, CEC and N are directly proportional to the percentage of cow dung in the amended soil, with the soil amended with 20% cow dung having the highest values. This result is an indication that cow dung when applied to the soil as an amender will bring about an increase in the growth of plants which correspond with Sohi *et al.,* 2009 and Urunmatsoma *et al.,* 2010 results.

This is as well in agreement with the fact that Cow dung boost the organic matter content of the amended lead-acid battery contaminated soil because of its very high organic matter content (Urunmatsoma *et al., 2010*).

Table 7: Physico-chemical properties Soil Amended with cow dung

Parameters	0% control	5% Amendment	10% Amendment	15% Amendment	20% Amendment
Clay (%)	11.10	-	-	-	-
Silt (%)	6.90	-	-	-	-

Sand (%)	82.00	-	-	-	-
pH	5.86	6.31	6.42	6.54	7.01
TOC (%)	2.28	3.89	7.58	11.20	14.86
N (%)	1.15	1.33	1.82	1.84	2.08
TOM (%)	3.93	6.71	13.07	19.31	25.62
EC (µs/cm)	1928	2.31	2.24	2.08	2.01
Na (mg/kg)	4.30	4.38	5.00	5.31	5.60
K (mg/kg)	150.00	151.10	152.20	153.00	155.01
Ca (mg/kg)	195.00	201.30	221.01	238.00	251.80
Mg (mg/kg)	0.91	1.20	1.30	1.38	1.55
CEC (cmol/kg)	1.39	1.42	1.53	1.62	1.69
P (mg/kg)	20.38	25.00	28.10	32.20	38.00

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

Total Heavy metal concentration in lead-acid contaminated soil amended with Cow dung.
From table 8, it is revealed that the heavy metals concentration in the lead-acid contaminated soil reduced as the percentage of Cow dung amendment increases (5%>10%>15%>20%). This shows that Cow dung has the ability to reduce metal mobility and bioavailability in soil.

Table 8: Total Heavy metal concentration in lead battery contaminated soil, Cow dung and Cow dung amended soil.

Parameters (mg/kg)	0% Control	5% amendment	10% amendment	15% amendment	20% amendment
Lead	127.50	127.01	126.80	125.30	124.06
Chromium	0.32	0.25	0.20	0.19	0.15
Cadmium	0.03	0.01	BDL	BDL	BDL
Nickel	0.18	0.14	0.09	0.02	BDL
Iron	135.00	136.03	124.01	121.09	104.00

BDL = Below Detectable Level

Geochemical Fractions of Heavy Metals (mg/kg) in lead-acid contaminated soil amended with cow dung.
Here (Table 9), of a total of 127.01mg/kg of lead, 0.25mg/kg of chromium and 136.03mg/kg of iron found in lead-acid battery contaminated soil amended with 5% cow dung, only a fraction of 25.10mg/kg lead, 0.12mg/kg chromium and 0.21mg/kg iron were bioavailable and mobile. And, of a total 0.01mg/kg cadmium and 0.14mg/kg nickel found in lead-acid battery contaminated soil amended with 5% cow dung, none were mobile and bioavailable in the soil. The mobility and bioavailability of the heavy metals were as well found to decrease as the percentage of cow dung 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 cow dung amendment increases. The mobility factor was equally found in this trend; 5%>10%>15%>20%.

Table 9: Geochemical Fractions of Heavy Metals (mg/kg) in lead-acid battery contaminated soil amended with cow dung.

Fractions	Metals	5%	10%	15%	20%	M.P.L (NESREA)
	Pb	25.10	22.00	21.90	20.01	10.00
	Cr	0.12	0.10	0.07	0.03	100.00
B_1	Cd	BDL	BDL	BDL	BDL	3.00
	Ni	BDL	BDL	BDL	BDL	70.00

	Fe	0.21	0.10	0.06	0.10	-
B_2	Pb	75.08	75.10	73.00	69.80	-
	Cr	0.04	0.02	BDL	BDL	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	BDL	-
	Fe	37.80	36.10	31.08	29.70	-
B_3	Pb	15.10	15.10	14.20	12.80	-
	Cr	0.05	0.03	0.01	BDL	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	BDL	-
	Fe	0.81	0.78	0.64	0.38	-
Residual	Pb	11.01	11.01	11.50	19.20	-
	Cr	0.02	0.02	0.09	0.10	-
	Cd	BDL	BDL	BDL	BDL	-
	Ni	0.06	0.07	BDL	BDL	-
	Fe	92.50	84.01	87.35	71.50	-
M_f (%)	Pb	19.87	17.86	18.16	16.43	-
	Cr	54.55	58.82	41.18	23.08	-
	Cd	0.00	0.00	0.00	0.00	-
	Ni	0.00	0.00	0.00	0.00	-
	Fe	0.16	0.08	0.05	0.10	-

M.A.L. = Maximum permissible Limit, Mf = Mobility factor and B.D.L. = Below Detectable Level

Total Heavy Metals (mg/kg) in Maize Plant grown on amended soil and their Bioavailability Indices.

Heavy metals content in maize plant and their bioavailability indices from the amended lead-acid contaminated soil using cow dung are shown in Table 10. At 5% of lead-acid battery contaminated soil/biochar mixture, the bioavailability indices reduced from 19.69% Pb; 12.50%; 38.89% Ni and 0.09% Fe to 14.46% Pb; 32.00% Cr and 0.12% Fe. At 10% soil/ cow dung mixture, the bioavailability indices reduced to 14.20% Pb; 1.20% Cr and 0.06% Fe. At 15% soil/cow dung mixture, the bioavailability indices reduced to 12.93% Pb; 10.53% Cr and 0.02% Fe. At 20% soil/cow dung mixture, the bioavailability indices reduced to only 10.88% Pb. This is an indication that cow dung was useful in bringing-down the bioavailability of heavy metals in the soil to plants. At least, for lead (Pb) as a major contaminant in the soil in question, there was a gradual reduction in bioavailability indexes as we moved from 5 – 20%. In order words, the effectiveness of cow dung in remediating lead-acid contaminated soil follows this order; 20%>15%>10%>5%.

Numerous studies have shown that the application of cow dung to soil has the potential of raising the pH of the soil. At high pH, most of the toxic metals are precipitated out of solution thus decreasing their mobility and bioavailability (Mbagwu, 1989; Gadepalle *et al.*, 2007; Lehmann, 2003).

Table 10: Total Heavy Metals (mg/kg) in Maize Plant and their Bioavailability Indices from Amended lead-acid battery Contaminated Soil.

Cow dung /soil mixture (%) w/w	Parameters	Pb	Cr	Cd	Ni	Fe
0 (Control)	Total heavy metal in plant	25.10	0.04	BDL	0.07	0.12
	Bioavailability indexes (%)	19.69	12.50	-	38.89	0.09
5	Total heavy metal in plant	18.36	0.08	BDL	BDL	0.16
	Bioavailability indices (%)	14.46	32.00	-	-	0.08

10	Total heavy metal in plant	18.00	0.06	BDL	BDL	0.07
	Bioavailability indices (%)	14.20	1.20	-	-	0.06
15	Total heavy metal in plant	16.20	0.02	BDL	BDL	0.02
	Bioavailability indices (%)	12.93	10.53	-	-	0.02
20	Total heavy metal in plant	13.50	BDL	BDL	BDL	BDL
	Bioavailability indices (%)	10.88	-	-	-	-

Characterization of the soil remediated with biochar-cow dung mixture

This mixture was done using the highest level of amendments (20%) for each amender. From the characterization (Table 11), the pH of Biochar-Cow dung (B+C) mixture at the ratio of 1:1, 2:1 and 1:2 are 6.90, 6.70 and 7.00 respectively. A pH near neutral will results in micro-nutrient cations being soluble enough to meet the plant essential requirements without becoming toxic. The increase in pH brought about by the amendments helps to bind the metals and also increase nutrients in the soil if the results of the 20% amendment are compared with 1:1, 2:1 and 1:2 ratios.

The organic matter content of the amendment with 1:1, 2:1 1:2 ratios were found to be 7.50%, 15.69% and 11.55% respectively. This shows a drastic increment from the value of organic matter found in the parent soil (3.93%). 2:1 ratio amendment has the highest TOM (15.69%), N (1.45%) and TOC (9.10%), as well as CEC value of 1.48 cmol/kg. This shows that hardwood derived biochar when mixed with Cow dung at 2:1 ratio serves as a better adsorbent for the removal of heavy metals in lead-acid battery contaminated soil than when mixed at 1:1, 1:2 and when used alone. The effectiveness of these adsorbents follows this trend; **2:1> 1:2 > 1:1.**

Table 11: Physico-chemical Properties of lead-acid battery contaminated Soil amended with different ratios Biochar and Cow dung.

Parameters	0% Control	B+C at 1:1 ratio (20% Amend.)	B+C at 2:1 ratio (20% Amend.)	B+C at 1:2 ratio (20% Amend.)
Clay (%)	11.10	-	-	-
Silt (%)	6.90	-	-	-
Sand (%)	82.00	-	-	-
pH	5.86	6.90	6.70	7.00
TOC (%)	2.28	4.40	9.10	6.70
N (%)	1.15	1.30	1.45	1.38
TOM (%)	3.93	7.59	15.69	11.55
EC (µs/cm)	1928.00	2.09	2.05	2.20
Na (mg/kg)	4.30	4.90	5.41	5.35
K (mg/kg)	150.00	151.00	156.01	153.30
Ca (mg/kg)	195.00	200.51	210.00	208.40
Mg (mg/kg)	0.91	1.01	1.10	1.10
CEC (cmol/kg)	1.39	1.42	1.48	1.47
P (mg/kg)	20.38	1044.10	1074.20	1012.00

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

Determination of the total heavy metal content in soils amended with Biochar-Cow dung (B+C) mixtures.

The total heavy metal concentration in lead-acid contaminated soil amended with Biochar-Cow dung mixture (Table 12) ranges from 0.01 mg/kg to 121.02 mg/kg for 1:1 ratio with Cd having value below detection level, 0.22 mg/kg to 122.01 mg/kg for 1:2 with Cd and Ni having values below detection level, and 0.01 mg/kg to 122.01 mg/kg for 2:1 ratio with Cd having value below detection level. These result shows that Biochar when mixed with Cow dung at 2:1 ratio is more effective in immobilising (adsorbing) heavy metals in the soil than 1:1 and 1:2 ratios. This may be due to the large surface area of the used biochar.

Table 12: Total Heavy metal concentration in lead battery contaminated soil and soil amended with Biochar-Cow dung (B+C) mixture.

Parameters (mg/kg)	0% Control	B + C at 1:1 ratio (20% amend.)	B + C at 2:1 ratio (20% amend.)	B + C at 1:2 ratio (20% amend.)
Lead	127.50	121.02	122.01	122.01
Chromium	0.32	0.27	0.22	0.29
Cadmium	0.03	BDL	BDL	BDL
Nickel	0.18	0.01	BDL	0.01
Iron	135.00	98.00	92.00	99.80

B = Biochar, C = Cow dung and BDL = Below Detectable Level

Geochemical Fractions of Heavy Metals (mg/kg) in lead-acid battery contaminated soil amended with biochar-cow dung mixture at different ratios.

The fractionalization of the contaminated soil amended with 20% biochar-cow dung mixture at 1:1, 2:1 and 1:2 ratios gives the result shown in Table 13.

Table 13 shows that 20.00, 18.20 and 20.20 of lead were mobile and bioavailable for plant uptake in 1:1, 2:1 and 1:2 ratio respectively. This indicated that the mixture of biochar & cow dung at 2:1 ratio was more effective in remediating lead-acid battery contaminated soil than 1:1 and 1:2 ratios. This is probably due to the high TOM and TOC values found in the hardwood derived biochar. The mobility factors were found to be this order; 2:1>1:1>1:2.

This improvement is attributed to Biochar which provides a recalcitrant soil carbon pool, which is carbon negative and it is highly stable with age and an increase in organic materials (Sohi *et al.,* 2009). This organic matter seems to have the highest ability for sorption of trace elements in cationic form, which bring about an increase in the amount of insoluble organic complex compound formed with the heavy metals, thus immobilizing the heavy metal (Violante *et al.,* 2010; Peng *et al.,* 2009). 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.

Table 13: Geochemical Fractions of Heavy Metals (mg/kg) in lead-acid battery contaminated soil amended with biochar-cow dung mixture at different ratios.

Fractions	Metals	B + C at 1:1 ratio (20% amend.)	B + C at 2:1 ratio (20% amend.)	B + C at 1:2 ratio (20% amend.)	M.P.L (NESREA)
	Pb	20.00	18.20	20.20	10.00
	Cr	0.02	BDL	0.01	100.00
B_1	Cd	BDL	BDL	BDL	3.00
	Ni	BDL	BDL	BDL	70.00
	Fe	0.12	0.08	0.10	-
	Pb	70.00	68.00	69.00	-
	Cr	BDL	BDL	BDL	-
B_2	Cd	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	-
	Fe	30.00	28.10	30.10	-
	Pb	13.00	10.20	14.10	-

B_3	Cr	BDL	BDL	BDL	-
	Cd	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	-
	Fe	0.40	0.32	0.41	-
Residual	Pb	17.01	24.80	17.03	-
	Cr	0.21	0.18	0.27	-
	Cd	BDL	BDL	BDL	-
	Ni	BDL	BDL	BDL	-
	Fe	62.00	62.01	68.00	-
M_f (%)	Pb	16.67	15.02	16.79	-
	Cr	8.70	0.00	3.47	-
	Cd	0.00	0.00	0.00	-
	Ni	0.00	0.00	0.00	-
	Fe	31.14	0.09	0.10	-

M.P.L. = Maximum Permissible Limit, M_f = Mobility factor, B = Biochar, C = Cow dung and BDL. = Below Detectable Level

Total Heavy Metals (mg/kg) in the cultivated Maize Plant and their Bioavailability Indices. The total heavy metals in maize plants and their bioavailability indices from the amended lead-acid battery contaminated soil using biochar/cow dung mixture follows the order; 1:1>1:2>2:1 ratio (Table 14). This is an indication that biochar when mixed with cow dung at the ratio of 2:1 are more effective in remediating lead-acid battery contaminated soil, followed by 1:2 and then 1:1.

Table 14: Total Heavy Metals (mg/kg) in Maize Plant and their Bioavailability Indices from Amended lead-acid battery Contaminated Soil.

Boichar/Cow dung /soil mixture (%) (w/w)	Parameters	Pb	Cr	Cd	Ni	Fe
0 (Control)	Total heavy metal in plant	25.10	0.04	BDL	0.07	0.12
	Bioavailability indices (%)	19.69	12.50	-	38.89	0.09
B + C at 1:1 ratio (20% amend.)	Total heavy metal in plant	12.20	BDL	BDL	BDL	BDL
	Bioavailability indices (%)	10.08	-	-	-	-
B + C at 1:2 ratio (20% amend.)	Total heavy metal in plant	11.40	BDL	BDL	BDL	BDL
	Bioavailability indexes (%)	9.34	-	-	-	-
B + C at 2:1 ratio (20% amend.)	Total heavy metal in plant	11.01	BDL	BDL	BDL	BDL
	Bioavailability indices (%)	9.02	-	-	-	-

CONCLUSION

This study disclosed that the lead-acid battery workshop and it's environ was contaminated with Pb, Cr, Cd, Ni and Fe, but only polluted by Pb. This study has also shown that hardwood derived biochar and cow dung are very useful remediating materials for lead-acid battery contaminated soil. They lowered the bioavailability of the studied heavy metals (Cr, Cd, Ni, Pb and Fe) considerably. The addition of biochar and cow dung increases pH, nitrogen, total organic carbon, organic matter, cation exchange capacity and phosphorus present in the contaminated soil. At 20% biochar amendment, the bioavailability index of these metals indicated that the only metal bioavailable to plant was Pb with 9.32%, and the same for 20% cow dung amendment with 10.88%. This revealed that hardwood-derived biochar was more active in immobilizing heavy metals in lead-acid battery contaminated soil than cow dung. Mixing the biochar with cow dung at 20% amendment revealed that biochar when mixed with cow dung at 2:1 ratio was more effective in immobilizing heavy metals in soil from lead-acid battery workshop than when mixed at 1:1 and 1:2 ratio. The order of bioavailability indexes is as follows: 1:1>1:2>2:1. There was a reduction in the bioavailability indexes of Pb from 16.69% (control) to 9.02 (20% amendment with 2:1 ratio of biochar-cow dung mixture). This reduction in available heavy metal in soil to plant will go a long way in reducing its availability to man and other animals, hence reducing the consequences associated with consuming plants contaminated with heavy metals in this study.

Although the application of hardwood derived biochar, cow dung and the mixture of both can be used to remediate soil polluted with lead-acid and can as well improve soil fertility, the mixture of biochar and cow dung at 2:1 ratio is recommended in this study.

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