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Influence of Groundnut and Rice Activated Charcoals Addition on Oil Polluted Soil and Mobility of Heavy Metals in the Root and Shoot of *Vigna unguiculata* L. Walp in the Tropical Rainforest

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Authors' contributions

This work was carried out in collaboration among all authors. Author OOE conceived and designed the experiments, author AKL analyzed the data, wrote and proofread the scripts, author JAJ carried out the experiment and analyzed the data under the supervision of authors OOE and AKL. All authors read and approved the final manuscript.

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ABSTRACT

In Nigeria, spent oil destroys crops, contaminate farmland, aquaculture and drinking water. It also alters the soil properties, leads to deficit of water and oxygen, shortages of soil nutrients like N and P, adsorbs to soil particles and reduces porosity and aeration of soil. The study aims to determine the potential of groundnut hull (GAC) and rice husk (RAC) activated charcoal in biodegradation of spent lubrication oil in soil with reduction in mobility and bioaccumulation of heavy metals in the tissues of the cultivated cowpea (*Vigna unguiculata* L. Walp) plants. Experimental units were set up in a screen house containing cowpea seedlings planted in different soil treatments: polluted, unpolluted as well as RAC and GAC remediated polluted soil, each in 5 replicates. Mobility and bioaccumulation of the heavy metals in the root, shoot and seeds of the cowpea were analyzed. Cd, Cr and Pb were below detectable limit (BDL), Cu was 0.21 and Zn was 0.26 mg/kg in unpolluted soil while the levels of heavy metals were elevated in spent oil polluted soil: Cd 0.1, Cr 0.25, Pb 0.12, Cu

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0.7 and Zn 0.63 mg/kg. RAC treatments significantly decreased the mobility and bioaccumulation of the heavy metals in the root ($F_{3, 14}$ = 19.338, *Sig* = 0.00 ($p < 0.05$) so also, GAC significantly decreased the heavy metals mobilization and bioaccumulated in the cowpea roots ($F_{3, 14}$ = 91.224, Sig = 0.000 ($p < 0.05$). RAC and GAC are potential effective and low cost phytoremediation materials.

Keywords: Heavy metals; bioaccumulation; pollution; spent engine oil; cowpea; activated charcoal.

1. INTRODUCTION

Contamination of soil by heavy metals and metalloids is usually by accumulation of heavy metal wastes discharged from industrial disposal, spillage of petrochemicals, leaded gasoline and paints, mine tailings, application of fertilizers, animal manures, sewage sludge, pesticides, waste water irrigation, coal combustion residues and atmospheric deposition [1,2]. The heavy metals form an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) [3]. Accumulation of these heavy metals usually take place in soils, because they have a very long half-life so they can accumulate and retain in the living organisms [4,5]. The heavy metal contamination of soil has been of widespread concern [6]. Urban soil is highly influenced by anthropogenic factors via industrial processes and economic activities which lead to changes and degradation in air, soil and water properties [7]. The accumulation of organic and inorganic matter by direct and indirect source is evident in urban soil [8,9]. Heavy metals are dangerous to human health because the intake rate is relatively higher compared to its excretion rate [10], the heavy metals are easily inhaled, ingested and dermally contacted and by absorption by living organisms most especially plants [11]. Used motor oil contains heavy metals and polycyclic aromatic hydrocarbons (PAHs) that could contribute to chronic hazards such as mutagenicity and carcinogenicity [12,13], the danger of toxic metals is further aggravated by their persistence in the environment [14].

The used and unused engine oil contain heavy metals, although, more in the used engine oil [15]. The increase inflow of new and used automobiles into the Nigerian markets had resulted in cumulative increase in automobile servicing, repairs and workshops activities across the cities. The automobile used oil contains oxidation products, sediments, water and metallic particles due to wear and tears of machineries, organic and inorganic chemicals from oil additives and metals that are present in fuel and transferred to the crankshaft during combustion [16]. The variations in the substances found in used engine oil depend on the brands and types of engine used, the mechanical condition of the engine, the automobile sources and the number of kilometers driven before changing the oil [17,18]. Nigeria accounts for more than 87 million litres of spent lubricants discharge annually [19].

Improper disposal of the used engine oil impacts on the ecosystem, destroy crops, farmland, and aquaculture such as fish, periwinkles and drinking water [17], adsorb to the soil particles, reduces porosity and therefore reduces aeration of soil because it is less viscous than unused oil [20]. It alters the soil properties, leads to water and oxygen deficit as well as shortage of soil nutrient like nitrogen and phosphorus [21]. Adequate attention has not been given to proper disposal of used engine oil [19], its pollution is more widespread than crude oil pollution [22]. The heavy metals contents of the used oil bioaccumulate in animals and plants, in plant it leads to chlorosis, weak growth, poor yield, reduced nutrient uptake, disorder in metabolism and reduced ability to fix molecular nitrogen [23].

The need for remediation of spent oil polluted soil is growing daily because there are so many factors that hinder the degradation of the spent oil in the soil. Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process [24,25]. Positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated by some authors [26,27]. Soil remediation practices are energy-intensive or require large areas of land (land farming, soil vapor extraction, thermal desorption, etc.) and many depend on the introduction of inorganic fertilizers which rely on energy-intensive synthesis and mining of non-renewable resources, such as phosphorus [28,29]. There are many natural plants and animal materials that have been proposed as useful in bioremediation practices such as wood residues [30], animal dungs [31] which can be co-composted with horse manure [32] and brewery's spent grain waste [33]. Lemieux [30] claimed ramial chipped wood remediated polluted soil has increased fertility because its chemical composition has rich ratio of polysaccharides to proteins (C:N) varying between 50:1 and 175:1. Moreno-Caselles *et al*. [31] claimed animal manure has high levels of nutrients such as N, P and K and Kirchmann and Ewnetu [32] claimed when co-composting with horse manure the concentration of paraffin molecules in the oil can be reduced within 110 days, by at least 80% and could reduce the petroleum residues by 93% within 50 days.

Cowpea (*Vigna unguiculata*) is a good biomarker of heavy metal pollutants in the soil, the plants root can absorb heavy metals in the soil [34]. This crop is commonly used as a cover crop to suppress weeds [35] and to control erosion, it restores fertility in poor soil and the hay obtained from cowpea plays a particularly vital role in feeding animals during the dry season [36,37]. 5.4 million tons of dried cowpeas are produced worldwide, Africa production is about 5.2 million tons and Nigeria is the largest producer and consumer in Africa and accounts for 61% of production in Africa and 58% worldwide [38]. Cowpea cultivation requires good and well-drained soil. The plant may not be grown as the first crop on lands that have been in fallow for more than 10 years like in the forest zone because of its ability to fix molecular nitrogen as leguminous plants making the land too fertile for its normal growth leading to low yield [29]. However, cowpea should be rotated with other crops, e.g., maize, after one or two successive cropping seasons because of its nitrogen fixing property [29]. Again, Cowpea root can absorb heavy metals in the soil most especially where the soil is contaminated with heavy metals [34]. The heavy metals are taken up by the plant roots and the plant develops chlorosis, weak plant growth, yield reduction, reduced nutrient uptake, disorders in metabolism and reduced ability to fix molecular nitrogen [29].

The study is aimed at investigating the effects of spent engine oil on soil properties, the remediation potential of groundnut hull (GAC) and rice husk activated charcoals (RAC) on the polluted soil and compare the growth parameters of cowpea (*Vigna unguiculate*) cultivated in the remediated and unremediated soil.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out on the field site at the Federal University of Technology Akure, Ondo State Nigeria (FUTA), Longitude 070 17´29´´N and Latitude 050 08´45´´E; environmental conditions of 28 ± 2°C, Relative humidity 78 ± 5% and 13 hL: 11 hD photoperiod in the screen house behind the School of Science (FUTA).

2.2 Sources of Experimental Materials

Groundnut hull and rice husk were obtained from farmers in Akure; and spent engine oil used was obtained from Automobile Workshop around the University. Seeds of cowpea were obtained from International Institute of Tropical Agricultural (IITA) Ibadan, through the Let Farm Manager Akure; and topsoil was obtained within the University compound and sieved with a 2 mm mesh.

2.3 Carbonization of Agro-wastes and Activation of the Carbon

Activated charcoal was prepared by a chemical method. The agro-wastes were washed thoroughly, air dried and oven dried at 160°C for 2hours. 2kg of the samples were carbonized and grounded into powder separately with electric blender, 900 gram each was soaked in water for 24hours, thereafter it was drained and impregnated with 30% H_2SO_4 solution for 24hours and drained. The samples were dried using electric oven at temperature of 160°C for 2 hours and then activated using a muffle furnace at a temperature of 700°C for 2hours. The activated carbons produced were allowed to cool before adding distilled water, thereafter it was dried at temperature of 120°C for 2hours and stored in air tight plastic bottles carefully labeled [39,40].

2.4 Determination of the Properties of the Activated Charcoal

2.4.1 pH and conductivity

2g of sample was weighed into 100ml beaker and 4ml of distilled water was added in a ratio of

1:2. The mixture was shaken and allowed to stand for 30minutes after which the pH and conductivity were analyzed using Elico pH meter (model L1 -120) and conductivity meter (model M-180) respectively.

2.4.2 Bulk density

Bulk Density was determined by putting 2g of samples into 10ml measuring cylinder and weighed (W_1), it was gently tapped to eliminate air space in the measuring cylinder and the volume was noted. Then, the new mass of the sample and measuring cylinder were recorded (W_2). Both the volume and mass of the sample were determined.

(The bulk density of the activated carbon (volumetric density) = mass of many particles of the activated carbon divided by the total volume they occupy) using the following formula

$$\text{Bulk Density} = \frac{W_2 - W_1}{\text{Volume of sample}} \quad (g/cm^3)$$

2.4.3 Ash Content

Ash Content (%) was determined by weighing 2g of sample into an empty pre-weighed crucible. The crucible was transferred into an electric furnace at 550°C for 6hours, after which it was removed from furnace and allowed to cool in a desiccator. The crucible was weighed again and the difference in mass was calculated.

2.5 Bioremediation and Control Experimental Set Up

7kg of soil thoroughly mixed together, was weighed with weighing scale (model no: R-1409/01511) into 7 litres plastic pot (24 ×22×16cm) perforated 3-5 holes at the base. This was repeated into 40 plastic pots. 5 of the plastic pots with unpolluted soil were labeled as control I while the remaining 35 plastic pots with soil were polluted with 30mls of spent engine oil mixed thoroughly with the aid of hand trowel. Activated charcoal in the concentrations of 20, 30 and 40g of each GAC and RAC were prepared in five replicates and each added and mixed up with the soil in 30 plastic pots and clearly label accordingly. The remaining 5 pots containing polluted soil were left without treatments as control II. The experimental units were arranged in a completely randomized design inside the screen house and left for one week before planting three cowpea seeds in each. In each soil treatment, after the cowpea had germinated, the heavy metal contents were examined periodically for each vegetative stage of the culture (root, shoot and seed) and the soil, in order to determine the periods of greatest metal translocation.

2.6 Preparation of Samples for Heavy Metals Analysis

During the period of planting, one seedling from each treatment replicate was uprooted at the vegetative stage of development with its soil, the samples were labeled and analyzed for heavy metal concentrations. The concentrations were determined by aqua regia method, as modified by Salt et al. [41]. 0.5g of air-dried, pre-sieved (with < 2 mm mesh) soil samples were digested with 15ml of aqua reagent (mixtures of concentrated HCl and concentrated HNO_3) (Analar grade). The mixtures were left overnight in the digestion block without heating under the switch-on fume cupboard. The following day, they were heated for 2 hours to 140°C, gradually increasing the temperature to control foaming. Distilled water was added to cool the digestates and then filtered with Whatman No. 542 filter paper and topped up to 50 ml with distilled water. The total metal concentration extracts were analyzed for Cr, Cd, Ni, Pb and Zn using flame atomic absorption spectroscopy with Atomic Absorption Spectrophotometer (AAS) (Model 210/211 VGP) (manufactured by Buck Scientific, USA). Spectroanalyses were done with flame AAS runs on SOLAAR software system version 5.28. Standards were made up in 2.7 M HCl/0.5 M HNO_3 (10 ml HCl and 3.5 ml HNO_3 per 100 ml) [42].

2.7 Statistical Analysis

Data collected were analyzed using paired Sample t-test to compare and correlate the means of heavy metal levels in unpolluted soil / spent oil polluted soil and RAC / GAC remediated polluted soil and the bioaccumulated levels of heavy metals in the root, shoot and seed of cowpea planted in each experimental soil units. ANOVA with Duncan's New Multiple Range Tests (DNMRT) were used to separate the means of heavy metal levels using the Least Significant Difference (LSD) test at 95% for 20, 30 and 40g of Activated Charcoal of remediating agents RAC20, RAC30 and RAC40 (soil remediated with rice husk) and GAC20, GAC30 and GAC40 (soil remediated with groundnut hull).

3. RESULTS

3.1 Evaluations of Heavy Metals in the Cowpea Seed, Unpolluted Soil and Spent Oil Pollution

The results of the analyses of heavy metals in the (Control I) unpolluted soil showed Cd, Cr and Pb were below detectable limit (BDL) while Cu was 0.21 and Zn was 0.26 mg/kg (Fig. 1). While in (Control II) untreated spent oil polluted soil, the levels of heavy metals were elevated: Cd 0.1, Cr 0.25, Pb 0.12, Cu 0.7 and Zn 0.63 mg/kg (Fig. 1). The levels of heavy metals in the cowpea planted as biomarkers were as follow Cd 0.01, Cr 0.01, Pb 0.1, Cu 0.15 and Zn 0.81 mg/kg (Fig. 1).

3.2 Properties of Groundnut Hull and Rice Husk Activated Charcoal and the Soil used

Activated charcoal is pure carbon, odourless and tasteless powder processed under high temperature in a vacuum and treated chemically to raise its ability to adsorb various gases and particles. The properties of activated charcoal vary, the Groundnut hull activated charcoal (GAC) had pH value of 7.35 and Rice husk activated charcoal (RAC) has 6.06 (Table 1). The conductivities of GAC and RAC were 485 and 260µS/cm respectively. Similarly, bulk densities were 0.48g/cm^3, 0.53g/cm^3 for GAC and RAC respectively while rice husk had a higher ash content of 59.47% compared to groundnut husk with 3.91% (Table 1).

3.3 Evaluation of Heavy Metals (mg/kg) Mobility and Bioaccumulation

3.3.1 Evaluation of heavy metals (mg/kg) mobility and bioaccumulation in Cowpea root at the end of the experiment

At the end of the experiment, the levels of the heavy metals bio-accumulated in the root of the cowpea planted in all the experimental soil units were analyzed. In the roots of the cowpea planted in both the unpolluted and spent oil polluted soil, the levels of the heavy metals bio-accumulated were significantly different (*Sig* = 0.004 (p < 0.05). The level of Zn in the cowpea root in unpolluted soil was 0.19 ± 0.006 against 0.61 ± 0.005 mg/kg in spent oil polluted soil; Cu in unpolluted soil was 0.08 ± 0.006 against 0.17 ± 0.006 mg/kg in polluted soil and Cd level in unpolluted soil was 0.08 ± 0.00 against 0.19 ± 0.006 mg/kg in polluted soil. Similarly, in all the remediated spent engine oil polluted soil with both RAC and GAC, decrease in the levels of the heavy metals' bio-accumulated in the root were observed, the decrease in mean levels were significantly different (*Sig* = 0.000 (p < 0.05). ANOVA showed with the increased concentrations of the RAC treatments, the decrease in the mean levels of heavy metals compared were significantly different ($F_{3,\ 14}$ = 19.338, *Sig* = 0.00 ($p < 0.05$) so also, with the increased concentrations of GAC treatments, the decrease in the mean levels of heavy metals when compared were significantly different ($F_{3,\ 14}$ = 91.224, Sig = 0.000 ($p < 0.05$) (Fig. 1). GAC40 and RAC40 were most effective activated charcoal concentrations treatments for remediation of spent engine oil polluted soil, the two agents reduced all the heavy metals bio-accumulated in the cowpea roots except Zn (Fig. 2). The reduction in the heavy metals bio-accumulated by the root with RAC40 was significantly higher than in GAC40 (p < 0.05).

3.3.2 Evaluation of heavy metals (mg/kg) mobility and bioaccumulation in Cowpea shoot at the end of the experiment

After 12 weeks, the shoot of the cowpea planted in the soil treatments were examined for the levels of the heavy metals bio-accumulated, record showed in both the unpolluted and spent oil polluted soil, the mobility and bioaccumulation of heavy metals in the shoot were significantly different (t = -4.042, *df* = 4, Sig = 0.016 (p < 0.05). The bioaccumulated level of Zn in the cowpea shoot in unpolluted soil was 0.45 ± 0.006 against 0.47 ± 0.006 mg/kg in spent oil polluted soil; Cu in the cowpea shoot in unpolluted soil was 0.00 against 0.17 ± 0.005 mg/kg in polluted soil; and Pb level in the cowpea shoot in unpolluted soil was 0.00 against 0.15 ± 0.00 mg/kg in polluted soil. Similarly, in all the remediated spent engine oil polluted soil using RAC or GAC, there was a reduction in the mobility and bioaccumulation of heavy metals in the cowpea shoot, the two treatments were not significantly different (t = -1.096, *df* = 14, Sig = 0.292 ($p > 0.05$). ANOVA showed both RAC and GAC treatments decreased the mobility and availability of heavy metals in the cowpea shoots significantly. Again, with increased concentrations of the RAC treatments, there was corresponding significant decrease in the mobility and bioaccumulation of heavy metals in the shoots ($F_{3, 14}$ = 46.601, *P* = 0.000 ($p < 0.05$) so also, with the increased concentrations of GAC treatments, the was corresponding significant decrease in the mobility and bioaccumulation of heavy metals ($F_{3, 14}$ = 9.024, *P* = 0.002 ($p < 0.05$) (Fig. 3). However, GAC20, GAC30 and RAC40 were considered most effective activated charcoal concentrations for remediation of spent engine oil polluted soil, the three agents reduced greatly the mobility and bioaccumulation of all the heavy metals in the cowpea shoots except the mean levels of Zn, this level was considerably reduced with GAC30 and RAC40 treatments (Fig. 3). The reduction in the heavy metals' mobility and bio-accumulation in the shoot with RAC40 treatment was significantly higher than with GAC40 treatment (p < 0.0).

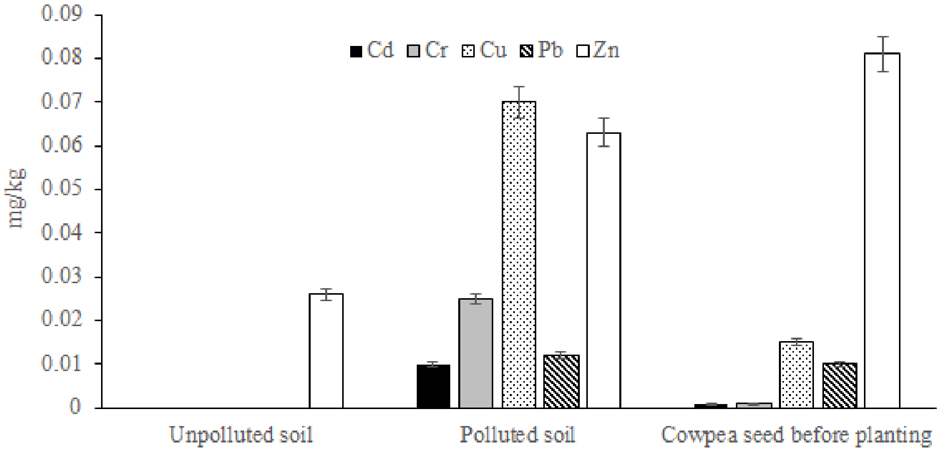

Fig. 1. Levels of heavy metals (mg/kg) in the soil, soil with treated oil and Cowpea seed before the experiment

Table 1. Characterization of Activated Charcoal

Activated charcoal	pH	Conductivities (µS/cm)	Bulk density (g/cm^3)	Ash Content (%)
Groundnut Hull	7.35	485	0.48	3.91
Rice Husk	6.06	260	0.53	59.47

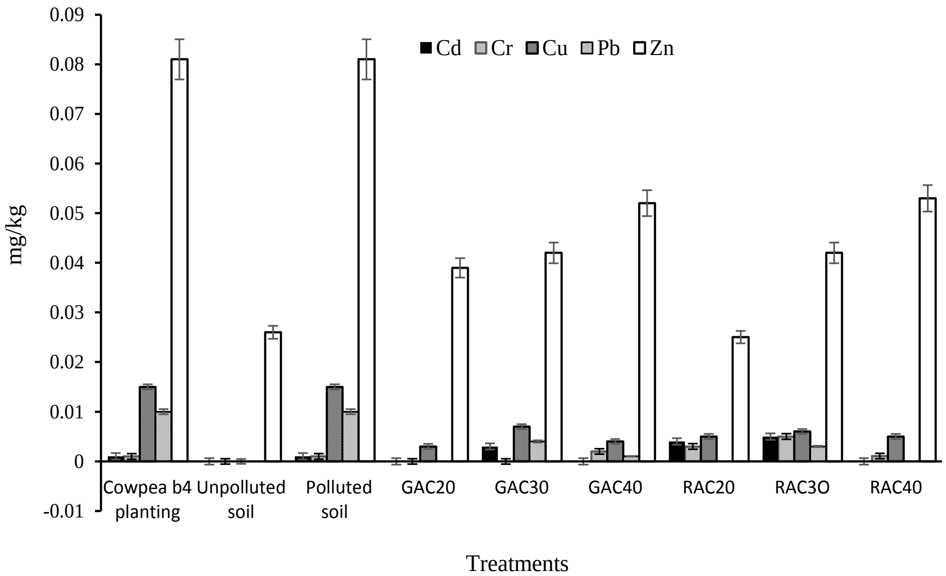

Fig. 2. Mobility and bioaccumulation of heavy metals (mg/kg) in the Cowpea root in different soil treatments

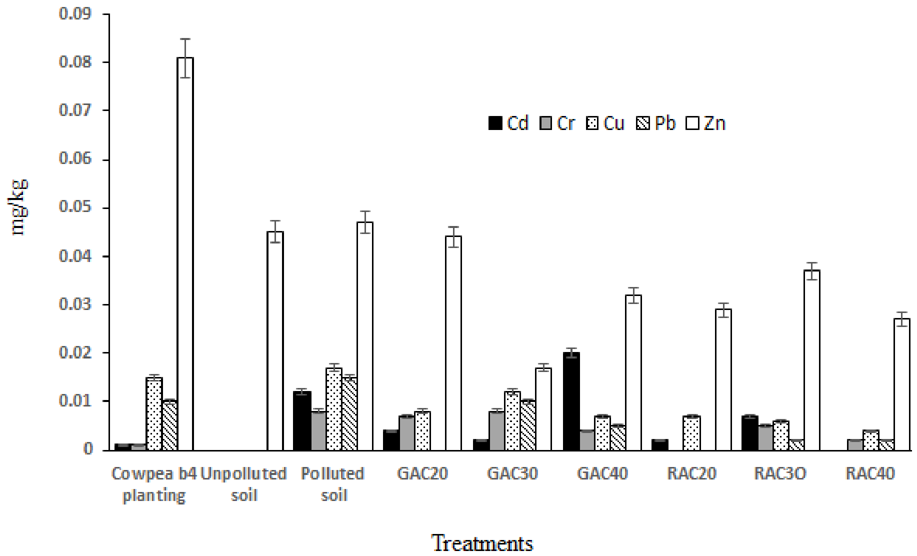

Fig. 3. Mobility and bioaccumulation of heavy metals (mg/kg) in the Cowpea shoot in different soil treatments

3.3.3 Evaluation of heavy metals (mg/kg) mobility and bioaccumulation in Cowpea seeds at the end of the experiment

The mobility and availability of heavy metals in the seeds of the cowpea seeds harvested from the unpolluted soil, polluted soil and remediated polluted soil at the end of the experiment revealed the following; After 12 weeks, heavy metals movement and bio-accumulation in the seeds of the cowpea planted in both the unpolluted and spent oil polluted soil were significantly different (t = -4.071, *df* = 4, Sig = 0.015 ($p < 0.05$) The mobility and availability of Zn in the cowpea seeds in unpolluted soil was 0.74 ± 0.006 against 1.33 ± 0.006 mg/kg in spent oil polluted soil; Cr in unpolluted soil was 0.01 ± 0.006 against 0.19 ± 0.004 mg/kg in polluted soil and Cu level in unpolluted soil was 0.01 ± 0.003 against 0.71 ± 0.006 mg/kg in polluted soil. Similarly, in all the remediated spent engine oil polluted soil using RAC or GAC, there was a reduction in the mobility and bioaccumulation of heavy metals in the seeds, this decreased mean levels were significantly different (t = 3.828, *df* = 14, Sig = 0.002 ($p < 0.05$). ANOVA showed RAC treatments decreased the mobility and availability of heavy metals in the cowpea seeds significantly and similarly, GAC treatments also decreased the levels of heavy metals mobility and availability significantly. ANOVA showed at different concentrations of the RAC treatments, the mobility and availability of heavy metals compared were significantly different ($F_{3,14}$ = 9.080, P = 0.002 ($p < 0.05$) so also, with changes in the concentrations of GAC treatments, the mobility and availability of heavy metals compared were significantly different ($F_{3,14}$ = 10.17, P = 0.001 ($p < 0.05$) (Table 2). GAC40 and RAC40 in the spent engine oil polluted soil reduced the mobility and availability of heavy metals in the cowpea seeds (Fig. 4). However, the reduction in the mobility and bio-accumulation of the heavy metal by the seeds with RAC40 was significantly higher than in GAC40 ($p < 0.05$) (Fig. 4).

3.4 Discussion

This study has found used motor oil as environmentally damaging substance when washed off into cultivated areas. The used oil contains a number of chemical components from engine wears which includes iron, steel, copper, lead, zinc, barium, cadmium, sulfur and ash. These contents might have both short-term and long-term effects which could be injurious to plants growth and may be a potential threat to food web [43]. The used oil contaminated soils usually undergo changes in physical, chemical and microbiological properties that often result in buildup of essential organic (C, N) and non-essential inorganic (Pb, Zn, Fe, Co, Cu) elements in the soil which will eventually translocate into plant tissues [44]. Some of these elements were the heavy metals investigated in this study. Therefore, it becomes imperative that spent oil polluted agricultural soil is renewed and;

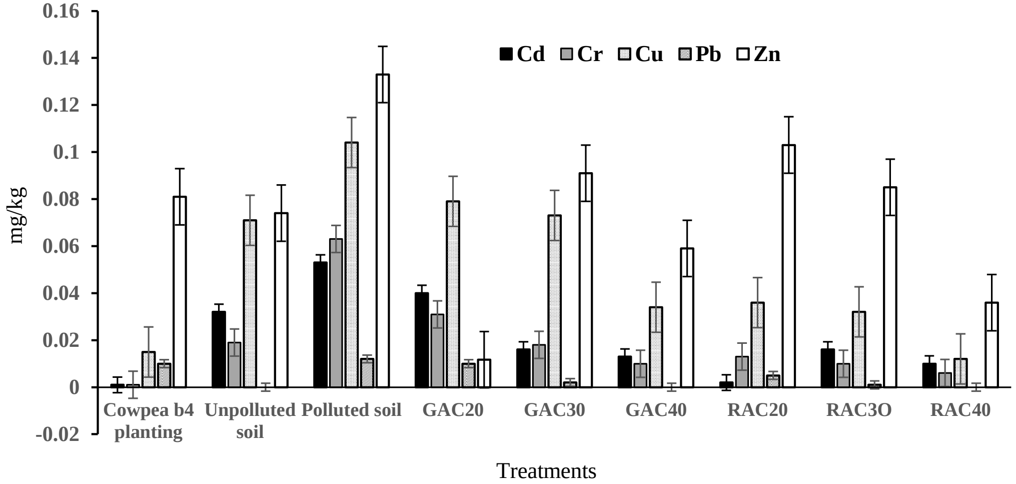

Fig. 4. Mobility and bioaccumulation of heavy metals (mg/kg) in the Cowpea seeds in different soil treatments after 12 weeks

Table 2. Levels of heavy metals (mg/kg) in the seeds of cowpea planted in the different treated soil

Sample codes(seeds)	Cu (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Cowpea Seeds Planted	0.015	0.001	0.001	0.01	0.081
Unpolluted soil	0.032±0.003^{d}	0.019±0.006^{d}	0.071±0.009^{d}	0.0	0.074±0.006^{c}
Polluted soil	0.053±.006^{f}	0.063±.006^{f}	0.104±0.009^{f}	0.012±0.006^{e}	0.133±0.006^{h}
GAC20	0.041±0.003^{e}	0.031±0.009^{e}	0.079±0.006^{e}	0.010±0.006^{d}	0.117±0.006^{g}
GAC30	0.016±0.006^{c}	0.019±0.001^{d}	0.072±0.003^{d}	0.020±.006^{b}	0.091±0.012^{e}
GAC40	0.013±0.006^{b}	0.010±0.006^{b}	0.034±0.006^{b}	0.00	0.059±0.006^{b}
RAC20	0.021±0.033^{d}	0.013±0.003^{c}	0.036±0.003^{c}	0.005±0.00^{c}	0.103±0.006^{f}
RAC3O	0.016±0.006^{c}	0.010±0.006^{b}	0.032±0.012^{b}	0.001±0.003^{b}	0.085±0.007^{d}
RAC40	0.010±0.003^{a}	0.006±0.00^{a}	0.012±0.00^{a}	0.00	0.036±0.006^{a}

The means ± standard error represent three (5) replicates. Means having the same alphabet down the column are not significantly different from one another using Duncan's New Multiple Range Test (DNMRT) at $p < 0.05$. RAC20, RAC30 and RAC40 = soil remediated with 20, 30 and 40g Activated Charcoal of rice husk; GAC20, GAC30 and GAC40 = soil remediated with 20, 30 and 40g Activated Charcoal of groundnut hull; control II = untreated spent oil polluted soil and control I = unpolluted soil.

using a natural and environmentally friendly restoration technique such as activated charcoal formed from rice husk and groundnut hull which from our findings could be potentials for bioremediation technique. Remediation helps to convert soil contaminants into a substance with lower toxicity, and also in immobilization, soil washing, chemical and photochemical reduction and soil flushing [45].

A detailed comparison of our data with those of other studies revealed a good agreement that rice husk [46] and groundnut hull [47] activated charcoals shared almost the same properties, they have adsorption, which is the binding of organic and inorganic compounds to a surface and absorbency which makes the two biological amendments capable of degrading or detoxifying spent oil pollutants and enrich the soils. There was a high ash content in RAC compared to GAC with low ash content, however, the two agents still possess a higher ash contents than the contaminated farm soil. Ash contents play a significant role, it represents the incombustible and inorganic residue which are made up of carbon, nitrogen and some percentage of phosphates, it also defines the alkalinity and raises the pH levels in garden soil and makes it a good soil for plants to thrive. The rich Carbon, Nitrogen and Phosphorus components of RAC and GAC make it important nutrients constituents for effective biodegradation of used oil contaminants. When spent lubricant oil is added to soil, it reduces its nitrogen and available phosphorus contents [48]. The low carbon and nitrogen ratio found in the oil polluted soil signifies a low capacity for microbial degradation of the oil contaminants [49]. Nitrogen is the most common limiting nutrient, when it is not available or very little in quantity, the microorganisms in the soil cannot biodegrade the spent oil contaminants Schulten and Schnitzer [50]. Nitrogen and Phosphorus are important nutrients for hydrocarbon-utilizing bacteria that biodegrade the oil contaminants in the soil environments Kim et al. [51], Okoh [52] and Abioye et al. [53]. Therefore, addition of GAC and RAC as amendment agents for the oil polluted soil is in contexts of its ash constituent. This ash contents could also influence the microbe-moderated nitrogen cycle for effective biodegradation Kim et al. [51] and Abioye et al. [53]. Lemieux [30], observed ramial chipped wood used in phytoremediation increased the soil fertility and remediation of contaminants in the soil as a result of its chemical composition viz-a viz the rich ratio of polysaccharides to proteins (C:N) which varies between 50:1 and 175:1 as compared to using woodchips from stem wood which has a C:N ratio of 400:1 to 600:1 under the same conditions.

In the effective remediation of the soil contaminants with the RAC and GAC amendments, these activated charcoals improve the soil pH in combination with its high content of P and N in the ash. The pH values of the amendment compounds RAC (6.06) and GAC (7.35) falls within the range that could facilitate the growth of degradative micro-organisms while the two important elements N and P might stimulate the degrading microorganisms to initiate the remediation of crude oil polluted soil and in addition facilitate the synthesis of enzymes required to degrade the petroleum hydrocarbon which constitutes the spent oil contaminant. Similarly, the activated charcoal amendments increase the water retention capacity of the soil and its moisture. Brady and Weil, [54] observed that water retention is the function of the soil type and organic matter. The moisture content of the soil plays an important role in bioremediation by supporting microbial activities while limited water inhibits microbial degradation and at the same time excessive water fill pores and resist the diffusion of oxygen towards microorganisms [55]. Similarly, the soil pH buffering capacity [56,57] and moisture are factors necessary for bioremediation. Nwankwegu and Onwosi [56] and Prince [57] claimed that soil pH influences the microbial activities and they emphasized that low moisture percentage inhibits microbial activity while excessive moisture level, promotes hypoxic conditions which are less conducive for rapid biodegradation. Again, the polluted soil used in this experiment, is slightly acidic (6.23) while the activated charcoal RAC has a pH of 6.06 and GAC is 7.37, these agents can complement to provide a suitable alkaline condition for degradation.

Mobility and availability of heavy metals in polluted soil to the plant is governed by the interactions between microorganisms and the prevailing environmental conditions such as moisture, pH, temperature, aeration etc. as well as the physico-chemical interactions between the polluting compounds and the soil matrix [58]. Although, Khan et al. [59] and Ko et al. [60] were of the opinion that bioremediation in the laboratory and field are incompatible, unlike the small scale laboratory bioremediation carried out in this study, the dynamics of large-scale field bioremediation is difficult to control and regulate under abiotic conditions. The activated charcoal from rice husks and groundnut hulls agro wastes are organic wastes which were the sources of nutrients supplied to the degradative microbes present in the oil polluted soil, thus enabling complete degradation of the spent oil contaminants. Abioye et al. [53] reported similar observation with increase in mineralization of crude oil when amended with organic wastes. As the concentration of contaminant oil reduced, there was effective remediation of heavy metals and the growth parameters of plants improved [61]. Phytoremediation reduces the toxicity of heavy metals in plants by reducing the mobility and availability in the plant tissues [62].

In this study, cowpea (*Vigna unguiculata* [L.] Walp) was chosen because it is undoubtedly a strategic legume species for food security and health, originated in the African continent with excellent nutritional and nutraceutical properties and several agronomic, environmental and economic advantages which it contributes to food security and maintenance of environment [37]. The levels of heavy metals bio-accumulated in the root, shoot and seeds of the cowpea plants in unpolluted soil were low, high in spent oil polluted soil and very low in remediated polluted soil, the heavy metals bioaccumulations levels were significantly different. However, bioaccumulated level of heavy metals in the cowpea shoot and seeds were found to be lower than in the root. This differences in heavy metal bioaccumulation by the cowpea could be attributed to many factors: the quantity found present in the soil treatments, the heavy metal species and plant parts. This observation agreed with the claim by Alloway and Davies [63] and Grant and Dobbs [64], they reported that plant grown on soils possessing high level of heavy metal concentrations have increased heavy metal ion contents. Juste and Mench, [65] claimed the uptake of the heavy metal ions were influenced by both the metal species and plant parts while Itanna [66] was of the opinion that accumulation of the heavy metals in cowpea could also be found higher or lower when compared to other plants. They claimed heavy accumulation depends on the plant type, *V. unguiculata* had low accumulation of heavy metals in contrast with other leafy vegetables because leafy vegetables have high translocation rate and transpiration rate compared to other vegetables and also the transfer of metals from root to stem and further to the fruit (vegetable) is lower which results in low accumulation of heavy metals than in leafy vegetables.

4. CONCLUSION

In Nigeria, where our staple foods are predominantly crops and seeds, the people eating crops planted on spent engine oil contaminated soil might be exposed to heavy metals, the accumulation of which could pose serious health challenges. The reduction in the level of heavy metals in the soil and cowpea planted on spent oil polluted soil amended with RAC and GAC reaffirmed the effectiveness of the agro waste in bioremediation. These techniques are cost effective and are not toxic to the environment compared to other physical and chemical methods of remediation. Therefore, the activated charcoals of groundnut hull and rice husk are potential bioremediation agents of spent oil polluted soil, cheap and readily available.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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