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Biostimulatory Influence of Biochar on Degradation of Petroleum Hydrocarbon Impacted Soil

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ABSTRACT

Soil pollution caused by petroleum hydrocarbon and its derivatives has become a grave global issue. Physico-chemical techniques are often expensive. However, bioremediation of petroleum hydrocarbon polluted soil is cost-effective. Therefore, the study was carried out to assess the biostimulatory influence of biochar on the degradation of petroleum hydrocarbon impacted soil in NNPC Depot, kano state. Soil samples were randomly collected from the polluted site to obtain a composite sample. About 400 g of the polluted soil was filled into pots and arranged in a 2*x*2 factorial experiment in a completely randomized design with three replications. Bone and wood char was at 2 levels (0 and 50 g/pot) each. Data were collected on the physicochemical properties (pH, TN, and Av. P) of the soil, Total Petroleum Hydrocarbon (TPH), and bacterial population. Data were analyzed using ANOVA at $\alpha_{0.05}$. Results obtained from the study show that biochar application significantly ($p<0.05$) enhanced TPH degradation and bacterial population in the polluted soil. However, Bone char significantly($p<0.05$) enhanced TPH degradation and bacterial population the most compared to wood char. Combined bone and wood char application resulted in significantly ($p<0.05$) lower residual TPH content in the polluted soil compared to using bone or wood char alone. Thus, bone and wood char should be used in the bioremediation of petroleum hydrocarbon impacted soils.

INTRODUCTION

Soil pollution due to petroleum and its derivatives has turned into a serious global issue. Petroleum hydrocarbons are found in nature and are used as the primary source of energy for both households and businesses. According to Erdogan and Karaka (2011); Bijay *et al.* (2012); Ofoegbu *et al.* (2014), vandalization of oil infrastructures, corrosion of old oil facilities and uncontrolled spilling in petroleum processing plants and storage tanks are causes of crude oil spillage. Crude oil, according to Erdogan and Karaka (2011), contains several hazardous compounds in slightly high fixations thus, it is physically, synthetically, and biologically damaging to the soil. It is spilled on the ground surface, where it slowly seeps into the soil resulting in soil fertility depletion. Environmental deterioration, groundwater contamination, biodiversity loss, and the threat to natural environmental sustainability are some of the effects of crude oil pollution (Bijay *et al.,* 2012; Ofoegbu *et al.,* 2014). Naturally, crude oil persists in soil far more than other carbon sources namely proteins and carbs, which take weeks to break down, and it lasts much longer under extreme conditions such as drought (Ali *et al.,* 2020).

The buildup of contaminants in animals and plant tissue due to soil pollution with petroleum hydrocarbons may result in mortality and mutations due to substantial harm of internal structure (Alvarez and Vogel, 2011). Physicochemical methods such as soil washing, solidification, vapor extraction, thermal desorption, etc. used in the treatment of petroleum hydrocarbon polluted soils have been proposed (Frick *et al.,* 1999; Dadrasnia *et al.*, 2015). However, these methods have some drawbacks as they are expensive and have limited local applications (Dadrasnia *et al*., 2015). Additionally, they do not usually result in the complete destruction of the contaminants. Bioremediation, on the other hand, which is the use of living creatures, most notably microbes, to break down pollutants in the surroundings into less hazardous forms, has shown promising results and it is economically and ecologically friendly (Nkereuwem *et al*., 2020). It degrades or detoxifies pollutants that are harmful to human health and/or the surrounding using naturally occurring microbes or plants through biostimulation (addition of nutrients/amendments) of the indigenous populations or bioaugmentation with oxidizing microorganisms (Adeleye *et al.,* 2019).

Many elements, such as nutrients, pH, temperature, moisture, oxygen, soil characteristics, and the presence of contaminants, might inhibit petroleum hydrocarbon decay in soil (Adeleye *et al.,* 2019). Biochar, according to Xiao *et al.* (2014), is carbon-rich charcoal produced by pyrolysis (thermal decomposition) of organic biomass or agricultural wastes and used as a soil amendment. It is made up of varying quantities of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), Sulphur (S), and ash. It's mostly used to increase soil nutrient content and sequester carbon from the atmosphere (Lehmann, 2009). When put to the soil, biochar has been shown to provide a number of environmental benefits, including increased soil fertility, improved plant growth, and the breakdown of pollutants (Novak *et al.,* 2010).

Given the negative impacts of crude oil pollution on soil organisms and plants, as well as the implications for food security and environmental safety, the quest for a better alternative method of treating petroleum hydrocarbon impacted soil that is cheap and readily available becomes imperative. The objectives of the present study were to 1) assess the efficacy of Bone-char and Wood-char in degrading Total Petroleum Hydrocarbon (TPH) content in petroleum hydrocarbon polluted soil and 2) determine the combinatorial effect of Bone-char and Wood-char in the degradation of TPH in petroleum hydrocarbon impacted soil.

MATERIALS AND METHODS

Study area and location

This research was conducted using petroleum hydrocarbon polluted soil samples from Kano Depot of Nigeria National Petroleum Company (NNPC)/Pipelines and Products Marketing Company (PPMC), Kano State, Nigeria. Kano State is made up of 44 local government areas with Kano Municipal as the capital city. NNPC/PPMC Depot is in Tarauni local government area and it is located on latitude 8°57'55.76" north and longitude 11°96'91.38" east.

Soil sample collection and preparation

Soil samples collection was done as described by Nkereuwem *et al*. (2010). Topsoil (0-20 cm) samples were randomly collected, air-dried, sifted using a two-millimeter sifter. The sifted samples were then packed inside polyethylene bags.

Biochar production

The bone and wood chars were produced by pyrolysis of cattle bone and wood at the Department of Soil Science Laboratory, Federal University Dutse, Dutse, Jigawa State, Nigeria, using the procedure described by Onokebhagbe *et al.* (2018). The products of the pyrolysis were then sieved using a 2mm sieve. This was done to ensure uniformity in particle size. The biochar was chemically analyzed using the procedure outlined by Beesley *et al.* (2010).

Design of the experiment

It was a two-factor factorial experiment with four treatment combinations: Bone-char at two-level (0 and 50 g/pot) and Wood-char at two-level (0 and 50 g/pot). This was laid out in a completely randomized design and replicated 3 times.

Factors:

1. Bone char at 2 levels (B=bone char)
   a. Without bone char (B_1- 0 g/pot)
   b. With bone char (B_2-50 g/pot)
2. Wood char at 2 levels (W= wood char)
   a. Without wood char (W_1- 0 g/pot)
   b. With wood char (W_2- 50 g/pot)

The treatments are listed thus: B_1W_1, B_1W_2, B_2W_1, B_2W_2

Biodegradation Assay/pot trial

About 400 g of the polluted soil was weighed into twelve (12) experimental pots. Thereafter, biochar (bone and wood char) was applied to the potting soil at 0 and 50 g/pot, respectively. The biochar was mixed thoroughly with the soil for even distribution. Moisture was maintained through the application of sterile distilled water (25 mL) twice a week (Abioye *et al.,* 2012). The experiment lasted for 8 weeks.

Data collection

The TPH accumulation in the soil as well as selected physicochemical properties of the soil were determined at the end of the experiment. The bacterial colony count and identification were also carried out.

Analysis of the soil sample's physical and chemical features

Particle size determination was calculated using the hydrometer procedure as outlined by Bouyoucous (1951), and the soil pH was obtained using the procedure outlined by Udoh and Ogunwale (1986). Walkley-Black's modified technique as described by Nelson and Sommers (1996), was used to calculate the organic carbon. The micro Kjeldahl digestion and distillation technique, as reported by Udo and Ogunwale (1986), and the Bray P 1 procedure as outlined by Bray and Kurtz (1945), were used to determine the total nitrogen and accessible phosphorous concentrations of the soil. Potassium, sodium, calcium and magnesium were calculated using the procedure outlined by Jackson (1958); calcium and magnesium were measured with an AAS, whereas potassium and sodium were measured with flame photometry. The effective cation exchange capacity was evaluated using the technique outlined by Juo *et al*. (1976), whereas the procedure outlined by Mclean (1982), was used to measure the exchangeable acidity.

Microbial enumeration of bacteria and biochemical test

The number of viable bacteria was estimated using the plate count technique (Ochei and Kolhatkar, 2008) while a biochemical test was done as described by Barrow and Feltham (1993).

The total petroleum hydrocarbon (TPH)

Total Petroleum Hydrocarbon (TPH) was determined using the US EPA 1850C method described by USEPA (2003).

Data analysis

Proc. GLM of GenStat version 17 was used to perform analysis of variance (ANOVA) on all data obtained, and significant means were separated using appropriate post-hoc methods.

RESULTS AND DISCUSSION

Table 1. Physical and Chemical Features of The Polluted Soil

Parameters	**Value**	
	Before Amendment	**After Amendment**
pH (H_2O)	**7.3**	**8.0**
Total Nitrogen (%)	1.5	2.55
Av. Phosphorus (mg/kg)	62	46
Electrical conductivity (µS/cm)	1.01	1.32
Exchangeable bases (cmol/kg)		
Calcium	4.1	3.9
Magnesium	1.9	1.4
Sodium	0.06	0.08
Potassium	0.34	0.35
Particle size (%)		
Sand	72	69
Silt	10	16
Clay	18	15
Textural class	Sandy loam	Sandy loam

The physical and chemical features of the polluted soil are listed in Table 1. The pH, total nitrogen, and Av. Phosphorus before and after biochar amendment are7.3, 1.5% and 62 mg/kg and 8.0, 2.55% and 46 mg/kg, respectively. The textural class of the soil is sandy loam.

Table 2. Selected Chemical Parameters of Biochars

Property	Bone char	Wood char
pH	7.5	8.9
Total Nitrogen (g/kg)	20.3	5.45
Total Phosphorus (g/kg)	54.1	1.70
Potassium (cmol/kg)	3.4	12.6

Selected chemical properties of the biochar (bone and wood chars) are presented in Table 2.

Table 3. Effects of Bone and Wood char on Total Petroleum Hydrocarbon (TPH) contents of polluted soil at 8 weeks

Treatments	**Total Petroleum Hydrocarbon (mg/kg)**
Bone char	
B1	324.4a
B2	149.3b
LSD	8.02
Wood char	
W1	283.2a
W2	190.4b
LSD	8.04

LSD=Least significant difference, B1=Bone char at 0 g/pot, B2= Bone char at 50 g/pot
W1=wood char at 0 g/pot, W2= wood char at 50 g/pot

Significantly lower (149.3 mg/kg) TPH volume of the polluted soil was obtained with bone char application (Table 3) compared to treatment with no bone char. Similarly, wood char application resulted in significantly lower (190.4 mg/kg) TPH quantity of the polluted soil compared to treatment with no wood char (Table 3).

Table 4. Interactions of Bone char with Wood char on Total Petroleum Hydrocarbon (TPH) contents of polluted soil

Bone char	**Wood char**	**Total petroleum hydrocarbon (mg/kg)**
B1	W1	400.9a
B1	W2	247.9b
B2	W1	165.6c
B2	W2	132.9d
	LSD	11.35

B1=Bone char at 0 g/pot, B2= Bone char at 50 g/pot
W1=wood char at 0 g/pot, W2= wood char at 50 g/pot

Combined application of bone char at 50 g/pot with wood char at 50 g/pot resulted in significantly ($p<0.05$) lower (132.9 mg/kg) residual total petroleum hydrocarbon quantity of the soil in comparison to the other treatments (Table 4). Significantly ($p<0.05$) higher (400.9 mg/kg) residual TPH content of the soil was gotten from the combination of 0 g/bag bone char with 0 g/pot wood char compared to those of 0 g/pot bone char with 50 g/pot wood char and 50 g/pot bone char with 0 g/pot wood char. Furthermore, combined application of 50 g/pot bone char with 0 g/pot wood char also resulted in significantly ($p<0.05$) lower (165.6 mg/kg) residual total petroleum hydrocarbon quantity of the soil in comparison to the combined application of 0 g/pot bone char with 50 g/pot wood char (Table 4).

Table 5. Effects of Bone and Wood char on Bacterial Colony Count (CFU/g soil) of petroleum hydrocarbon polluted soil

Treatments	Bacterial colony count (CFU/g soil)
Bone char	
B1	1.77
B2	3.43
LSD	1.28
Wood char	
W1	2.10
W2	3.10
LSD	NS

Bone char application at 50 g/pot resulted in significantly higher (3.43 CFU/g) bacterial colony count compared to 0 g/pot bone char (Table 5) while 50 and 0 g/pot wood char applications did not considerably differ although the use of 50 g/pot wood char yielded greater bacterial count (Table 5). The outcome of this research shows proliferation in culturable bacterial count. However, treatments amended with biochar had significantly higher bacterial counts.

Table 6. Bacteria identification according to Biochemical characteristics

Organisms	Coagulase	Catalase	Oxidase	Citrate utilization	Nitrite reduction	Urease
Streptoceous pyogeus	-	-	-	-	-	-
Staphylococcus epidermis	-	+	-	-	+	+
Pseudomonas aeruginosa	-	+	+	+	+	-

Note: + = positive; - = negative

From the biochemical characteristics test (Table 6), three organisms were identified namely *Streptoceous pyogeus*, *Staphylococcus epidermis,* and *Pseudomonas aeruginosa* (Table 6).

The findings of this study are consistent with those of Ikiogha *et al.* (2019), for tropical rainforest soils. This study's findings also support recent research by Nkereuwem *et al.* (2020 a and b), who found a rise in Total nitrogen in crude oil-contaminated soil treated with organic stimulants.

The enhanced TPH degradation observed in this research corroborates the results of Agarry *et al*. (2013), where a higher degradation rate constant due to biochar application in crude oil polluted soil was observed. The increased TPH degradation seen in this study could be due to the biochar used (bone and wood chars) acting as a bio-stimulant, enhancing nutrient availability, particularly phosphorus and micronutrients (Chan and Xu, 2009; Park *et al.,* 2011; Ikiogha *et al.,* 2019). These elements form the fundamental building elements of life, allowing microorganisms to flourish and generate the enzymes required to break down petroleum hydrocarbon pollutants. Despite the presence of microorganisms in polluted soil, their numbers may not be adequate to begin cleanup. Studies have shown that hydrocarbonoclastic bacteria require nitrogen, phosphorus, and carbon as building components, thus they must be stimulated in their growth and activities. Nitrogen is required for the synthesis of cellular proteins and cell walls, whereas P is required in the formation of nucleic acids, cell membranes, and adenosine triphosphate. Thus, bioremediation of polluted soil requires a sufficient provision of the above-mentioned nutrients, which are in turn utilized by hydrocarbonoclastic micro-organisms for their vigorous development and metabolousoperation (Adeleye *et al.,* 2018; Nkereuwem *et al.,* 2020). Nutrient deficiency in petroleum hydrocarbon polluted soils inhibit bio-remediation; nevertheless, soil hydrocarbonoclastic microbes usually gain from nutrients addition leading to improved bio-remediation of hydrocarbon impacted habitat (Beolchini *et al.,* 2010; Kauppi *et al.,* 2011).

The result of this study shows the combinatorial efficacy of bone and wood char in the degradation of TPH in petroleum hydrocarbon polluted soil. The optimum TPH degradation observed in the combined application of 50 g/pot bone char and 50 g/pot wood char could be linked to the synergistic effect of bone and wood char.

Similar outcomes were also recorded by Ugwoha *et al.* (2020), where they noted improved bio-remediation of crude-petroleum impacted soil through the combined application of pig droppings and bone char mixture. The results of this study also corroborate the findings of Ikiogha *et al.* (2019), where they reported a substantial decrease in TPH content of crude oil contaminated soil due to the combined application of NPK fertilizer and bone char. The reason for the observed reduction in TPH could be because biochar improves soil qualities such as water holding capacity, nutritional status, etc., and total microbial count as reported by Wang *et al*. (2017). Furthermore, due to the presence of pore spaces and a large surface area, biochar can provide superior support for microbial development (Wang *et al.*, 2017) thereby increasing petroleum hydrocarbon-degrading microbial communities.

This result confirms the outcome of Nwogu *et al.* (2015), where they noted an increase in the population of culturable hydrocarbon utilizing bacteria due to soil amendment. The increase in bacterial counts, according to Atlas and Bartha (1992), could be attributed to extra microbic communities, which employed intermediate commodities due to hydrocarbon degradation. The microbial counts recorded in this research conform with previous results by Ijah *et al.* (2003) and Nwogu *et al.* (2015). The bacterial genera isolated were *Streptococcus pyogens*, *Staphylococcus epidermis,* and *Pseudomonas aeruginosa*. These organisms have previously been identified by Bento *et al.* (2005); Onuoha (2013) and Nkereuwem *et al.* (2020), as microbes that utilize hydrocarbon during bio-remediation of crude oil impacted soils.

CONCLUSION

Biochar treatment considerably increases the bacteria population in the polluted soil, resulting in enhanced Total Petroleum Hydrocarbon (TPH) reduction, according to the findings of this study. Furthermore, the physicochemical properties of the soil were also influenced positively due to biochar application. All of the soil samples tested showed a reduction in Total Petroleum Hydrocarbons (TPH). However, when comparing treatments with and without biochar, the quantity removed was much higher with biochar application. Due to its considerable performance in TPH degradation as observed in this study, the application of biochar in bioremediation of petroleum hydrocarbon impacted soil is thus advocated.

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