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An exploratory study of the impact of commingled biochar on removal of total petroleum hydrocarbon (TPH) from crude oil polluted soil

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An experimental scrutinization of bioremediation of crude oil polluted soil using furnace pyrolyzed comingled-biochar containing poultry litter, pine wood and rice straw char made at different proportions was carried out in the present study. The experiment was performed in five stages which include soil investigation, biochar production, characterization, and total petroleum hydrocarbon (TPH) remediation via green house. The purpose of this investigation is to evaluate the impact of biochar blend on TPH removal. The result showed that the efficiency of bioremediation was affected significantly by the acidic soil. Therefore, the commingled biochar seems promising in remediation of crude oil polluted soil and addition to soil nutrient. The highest TPH removal (46.74) was found in experimental run 12, which also had the highest level of independent variables (15 g of poultry litter-PL, 6 g of rice straw-RS, and 3 g of pine wood-PW char). This suggests that PL is more effective in the biochar mix than RS and PW. However, the efficiency of biochar-blended cleanup of soil varied depending on the biochar source and pyrolysis process as captured in the design of experiment using response surface methodology (RSM) *via* design expert. Biochar blend application to soils allows the development of microbial communities which are particularly important for nutrient cycling which leads to bio-stimulation enhancing the removal of TPH.

Key words: Biochar blend; furnace pyrolysis; bioremediation; crude oil polluted soil; experiment.

INTRODUCTION

Oil spills have occurred as a result of old infrastructure and poor maintenance on the side of industries (Vidal, 2014). According to the Nigerian government, 7,000 spills of crude oil took place between 1970 and 2000 (Vidal, 2014). Additionally, weathering can encourage soil pore blockage, which can lead to long-term consequences such soil death and decreased biota bioactivity and pollutant degradation (Lominchar et al., 2018). As a

result, an efficient cleanup method is required to satisfy the needs of the impacted areas within the Niger-Delta Region (Amnesty International, 2009). Even though numerous methods for cleaning up soil contaminated by crude oil have been suggested, there is still a need for efficient, environmentally acceptable methods for removing hydrocarbons. Several researchers recommended bioremediation, which is an effective, affordable, and environmentally sound method (Wu et al., 2016). However, studies have shown that adding biochar to soil can be a successful technique for the simultaneous remediation, generation of bioenergy, long-term carbon sequestration, and enhancement of soil quality (Su et al., 2016). Previous research has shown that biochar has a stronger ability to lower total and bioavailable Polycyclic-Aromatic Hydrocarbons (PAHs) concentrations in multicomponent polluted soils than green waste compost (Beesley et al., 2010). For example, the total and bioavailable PAHs are reduced by 31.8 and 34.1%, respectively, in polluted soils amended with biochar (Gomez-Eyles et al., 2011).

However, the percentage of reduction varied based on the pyrolysis settings, feedstock type, particle size and application rate of the biochar, contact time, and soil and organic pollutant characteristics. According to several research, biochar can significantly lower freely dissolved PAH concentrations (Kumari et al., 2014; Oleszczuk et al., 2014b). In order to reduce PAH bioaccumulation in turnip, Khan et al. (2015) discovered that different types of biochar (5%) were most effective in the following order: peanut shell biochar (84%) > soybean straw biochar (70%) > rice straw biochar (55%) > sewage sludge biochar (36%). However, the removal processes are often governed by the interactions of these pollutants with different attributes of biochar (Tan et al., 2015). Kong et al. (2018) used sawdust and wheat straw biochar synthesized at 300 and 500°C for remediation petroleum polluted soil. Despite its potential benefits, biochar has not been utilized on a large scale yet as well as combination of different biomasses. The primary cause is related to variations in the material characteristics from the biomass sources such as surface area (SA), aromaticity, cation exchange capacity (CEC), pH, nutritional contents, and porosity (Spokas, 2010). Furthermore, the properties of biochar made from various pyrolysis processes and feedstock types vary significantly, which has an impact on bioremediation. In view of this, the methods to solve some of the difficulties faced by traditional pyrolysis were taken into consideration. For example, in continuous-mode, furnace pyrolyzer is a device that consists of a cylindrical oven that is maintained at a consistent temperature (Ndukwu and Horsfall, 2020). However, the advantage of furnace pyrolysis is the temperature uniformity with high efficiency and energy saving potential (Batista et al., 2018).

To the best of our knowledge, the use of comingled biochar for amendment of crude oil-polluted soil is a new application. Therefore, this article uses an experimental technique to look into the impact of a biochar blend from furnace pyrolysis on TPH removal from crude oil-polluted soil.

MATERIALS AND METHODS

Description of study area

A sample of crude oil polluted soil from the Niger Delta Region was collected at Kpuite in Tai Local Government Area (LGA) of Rivers State, Nigeria. Kpuite is a town located within latitude 4°43'37" N and longitude 7°17'4" E (Available from: https://dailypost.ng/2013/10/10/oil-spill-hits-ogoni-again/, accessed on June 21, 2022). Tai LGA has estimated population of 194,732 people, with the majority of residents belonging to the Ogoni ethnic group (Okon and Ogba, 2018). It has a total area of 159 km^2, an average temperature of 25°C with significant crude oil and natural gas deposits and is home to a number of oil mining companies. It is recognized for growing a variety of crops such as vegetables, bananas, plantains, and cassava, thus farming is thriving there as well. Figure 1 depicts a map of the research area.

Soil sampling and characterization

The crude oil polluted soil samples were collected using soil auger at a depth of 30 cm in Kpuite, homogenized and then poured into a sack bag. The physicochemical properties analyzed include pH, nitrogen, potassium, moisture content, temperature, electric conductivity, cation-exchange capacity and phosphorus content. In addition, the total petroleum hydrocarbon (TPH) and microbes present in the polluted soil were also determined.

Determination of soil pH

From the evenly mixed soil sample in the sack bag, 10 g sieved (5 mm) and air-dried soil was weighed and placed into a 50 ml beaker, followed by addition of 25 ml distilled water. The liquid was manually stirred with a glass rod for 30 min before being allowed to settle for 1 h. A pH meter with electrode (Kent EIL 7055) was placed in the polluted soil sample to determine the pH.

Determination of nitrogen

A 0.0001g of soil sample was placed into a Kjeldahl digestion flask. The weight of sample contained 0.1 *N* acid and between 14 – 56 mg of nitrogen. The sample weight taken was less than 2.2 g. To the flask 0.7 g of HgO was added with 25 mL of H_2SO_4 and 15 g of K_2SO_4. The flask was placed in an inclined position and gradually heated until frothing stopped, then quickly boiled, causing the condensate to develop around halfway up the flask's neck. The solution was boiled for 2 h until it became transparent, and then 50 mL of standard acid was added. The receiving flask was then filled with 6 drops of indicator solution. The absorbing mixture was cooled to room temperature before adding about 200mL of water and cooling the flask contents. The total nitrogen in soil was calculated from Equation 1:

$$\text{Total nitrogen (\%)} = \left(\left(\frac{(A - B) \times N \times 0.01401}{C} \right) \times 100 \right)$$

(1)

Where A is the standard NaOH solution needed for blank titration (mL), B is the standard NaOH solution necessary for sample final
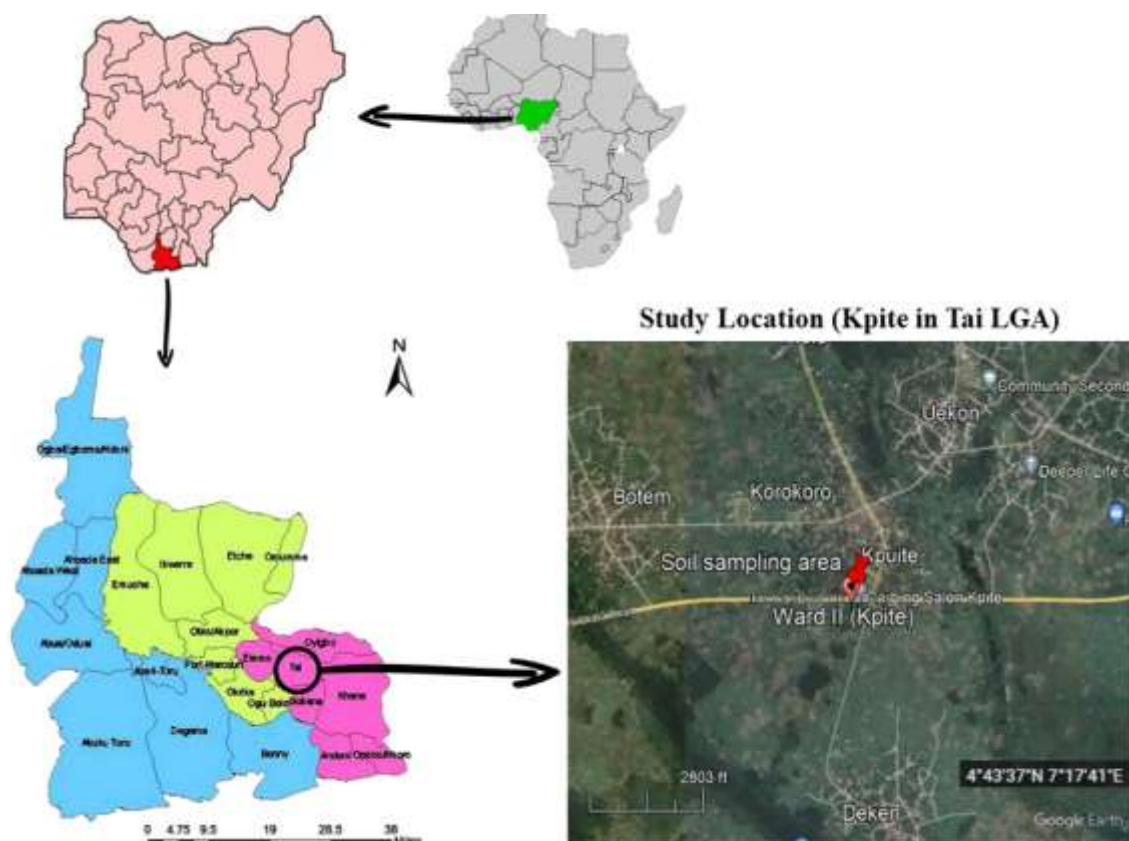
Figure 1. Map of study area showing the soil sampling point.
Source: Google Map, 2023

volume of around 10 mL. The material was chilled and diluted without being allowed to dry. Then the total phosphorus content was determined with acid wash water in wash tubes.

Determination potassium

A 125 mL Erlenmeyer flask was filled with a 2.5 g sample of air-dried polluted soil and then mixed with 50 mL of the 0.10 regular hydrochloric acid extraction solution before shaken automatically for 15 min. A 50 mL Erlenmeyer flask was used to fill the soil suspension after it has been filtered through Whatman No. 12 folded filter paper. Potassium concentration was measured in the soil solution extract without further dilution using a Beckman Model B Flame Spectrophotometer.

Then percentage transmittance of each potassium standard reference solution and the soil solution extract was measured. Referring to a calibration curve created by mapping the readings for percent transmittance against the potassium concentrations of the five reference solutions, the amount of potassium in the soil solution extract was calculated.

Determination of moisture content

The gravimetric method with oven drying was the technique used to determine the water content of the crude oil polluted soil. The soil sample was collected in a moisture-can and the wet weight recorded. It was then dried in an oven at 105°C for 24 to 48 h

before being reweighed. The amount of water lost was then computed as a percentage of the dry soil's bulk.

Determination of Cation-Exchange-Capacity (CEC)

3 g of 1 mm air-dried soil sample was placed in a 250 ml Erlenmeyer flask, and 100 ml of 1 N NH$_4$OAC (pH = 7.0) solution was added. The flask was shaken thoroughly by hand and allowed to stand overnight (cover the flask mouth with parafilm). The mixture was filtered with light suction using a Bchner funnel and no. 2 filter paper and then poured into a clean flask. A small (25 ml) portion was added at a time. The presence of Ca^{2+} was checked, and the vacuum closed. Then the funnel was lifted carefully out of flask, 3 drops of filtrate was transferred from the funnel end into a test tube, with additional 3 drops 1 N NH$_4$Cl, 3 drops 1:1 NH$_4$OH, and 3 drops 10% ammonium oxalate. No precipitate indicates the completion of filtering.

The soil was filtered with light suction using 200 mL 1 N NH$_4$Cl followed by 100 mL 0.25 N NH$_4$Cl. In addition, it was washed with 200 ml of isopropyl alcohol, following a small (25 mL) portion at a time. Then 10 drops of the filtrate and 10 drops of 0.1 N AgNO$_3$ were added to a clean test tube. At a time when the chloride is no longer present, the collection flask was emptied and cleaned while the filtrate was discarded. In addition, the soil was filtered with 300 mL of 10% NaCl (in 6 portions). The filtrate was kept in a clean bottle for CEC determination. Then 20 mL of the filtrate was transferred into a microkjeldahl flask, a spoon of MgO powder was added. In addition, 40 mL of the solution was distilled and added into 5 ml of 2% H$_3$BO$_3$. Finally, the boric acid solution was titrated

with standard H_2SO_4 (0.01 N). The CEC of the soil was calculated from Equation 2:

$$CEC \left(in\ meq. \frac{1}{100g}.\ soil \right) = V \times 0.001N \times \left(\frac{300mL}{20mL} \right) \times 100g. \left(\frac{1}{Ms} \right) \quad (2)$$

where, V is the volume of 0.001 N H_2SO_4 spent for titration, in ml, 300 mL is the total volume of 10% NaCl, used to substitute the NH^{4+}, 20 mL is the volume of filtrate used for distillation and Ms is the weight of the soil sample used.

Determination of Total Petroleum Hydrocarbon (TPH)

TPH analyses are defined by the following methods of soil sample collection, extraction, cleanup, separation, and quantification:

Soil extraction

An amber glass bottle was filled with 10 g of soil sample. The soil sample in the glass bottle was also mixed with anhydrous sodium sulphate (Na2SO4) according to USEPA procedure 8015C. The sample was then swirled. Na2SO4 was added to the soil sample to draw out the moisture. The soil sample was given 300 g/ml of a surrogate (1-chlorooctadecane) standard. After adding 30 ml of dichloromethane (DCM) to the sample as an isolating solvent, the bottle carrying the soil sample was tightly corked and moved to a manual shaker. Details of this method can be found in the report of Alinnor and Nwachukwu (2013).

Soil cleanup

A glass column was used to clean the sample. As part of the column preparation process, glass cotton was placed into the column. DCM was used to dissolve silica gel, which was then added to the column as slurry. "After the addition of anhydrous Na2SO4, pentane was added to the column. In a beaker, a concentrated sample extract was mixed with cyclohexane and put onto the column. The sample extract was eluted with pentane, and the eluted material was collected in a beaker under the column. Following elution, the column was washed with DCM. The eluted sample was placed in a fume closet at room temperature overnight to allow for evaporation."

TPH separation and detection

TPH was extracted and identified in soil samples using an Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) equipment (USEPA method 8015C). The concentrated sample eluted from the column was then injected into the GC vial in the amount of 3 µl. The GC's micro-syringe was cleaned three times with blank DCM before taking the sample for analysis. After that, the sample was utilized to rinse the micro-syringe once more. The sample was then injected into the column to separate the chemicals in the sample. Following separation, the compounds were passed through a flame ionization detector. FID is used to identify the compounds in the sample. TPH concentration was determined in milligrams per kilogram using a particular chromatogram.

Determination of microbes present in soil

Total heterotrophic bacteria (THB): Microbial analysis of heterotrophic bacteria was accomplished by weighing ten grams

(10 g) of crude oil polluted soil sample with an analytical balance (Metter weighing balance PB3002 Switzerland) and combining with 90 ml of sterile distilled water to form the stock suspension. A 10-fold dilution in stages of the soil sample was performed. 1 ml of the diluted sample was then plated on nutritional agar for bacteria and potato dextrose agar for fungi count using the pour plate method. Nutrient agar plates were incubated at 37°C for 24 h and PDA plates at 282°C for 72 h. After incubation, discrete colonies of culture were counted on potato dextrose and nutrient agar plates and the unit was expressed in cfu/g.

Hydrocarbon utilizing fungi (HUF) and hydrocarbon utilizing bacteria (HUB)

Ten grams of soil samples (contaminated) was serially diluted in 90 ml of sterile distilled water. An aliquot portion (0.1 ml) from dilution 105 was inoculated on pre-sterilized surface dried nutrient agar medium and uniformly spread to obtain discrete and countable colonies (Cheesbrough, 2000). In the Bushnelli Haas agar (BHA) medium supplemented with crude oil, a comparable quantity of the 103 was inoculated (vapour phase method). The plates inoculated with the suspension from the dilutions were incubated at room temperature for 24 - 48 h hydrocarbon degrading bacteria and fungi.

The vapour-phase inoculation method was used for Bushnelli Haas Agar (BHA). The procedure involved covering the lid of the petri dish with sterile filter paper that had been moistened with crude oil. The colonies were counted after incubation using standard methods (Cheesbrough, 2000).

Determination of soil temperature

A thermocouple temperature probe was used to determine the temperature of the soil sample.

Determination of Electric Conductivity (EC)

This experiment was carried out using a two-electrode conductivity meter. A 100 g of the soil sample was measured into a beaker and the conductivity meter was turned on. The electrode of the conductivity meter was immersed into the sample. The reading of the conductivity was displayed on the screen of the conductivity meter and the result was recorded. The electrode was then removed from the beaker containing the sample and rinsed with distilled water. This same procedure was repeated for other samples collected and the results recorded.

Soil textural analysis

A 50 g of crude oil polluted soil sample was measured, dried at room temperature, grounded with wooden roller and sieved through 2 mm mesh. The particle size distribution was ascertained by using Bouyoucos hydrometer method according to Gee and Bauder (1986) and Okon and Ogba (2018).

Procedure for biochar production

Collection of biomasses

Pine wood (PW) were obtained from the Timber Saw-Mill at Mile 2 Diobu, Port Harcourt, poultry litter (PL) was collected from a poultry farm at Igwuruta, Rivers State, while rice straw (RS) was collected from a rice farm at Abakaliki, Ebony State all in Nigeria. The feedstocks were collected at different locations due to availability

Figure 2. The biomass samples (a) pine wood (b) poultry litter and (c) rice straw.
Source: Authors

and accessibility. The feedstocks (PW), (RS) and (PL) were washed and dried separately at 60°C for 24 h to fully remove the water. RS and PL were pulverized to pass through a 10-mesh (2 mm) sieve in order to reduce their initial particle size while PW feedstock was hammer-milled and pelletized to approximately 6 mm at a wood processing plant. PW treatments were converted into cylindrical pellets by removing the pure components and total moisture content with deionized H_2O and pelletizing with a pellet mill fitted with a 6-mm die and roller set. The processed feedstocks were then separated and stored in separate bins and the biomass sample is presented in Figure 2.

Furnace assisted pyrolysis of biomass

The biochar samples were created from various feedstocks at pyrolysis temperatures ranging from 400 to 500°C and at different residence time using an Electric Vulcan furnace A130 model with a gas expeller (rice straw (RS) = 400°C for 1 h., poultry litter (PL) = 500°C for 1 h 30 min, pine wood pellets (PW) = 500°C for 2 h). Furnace assisted pyrolysis was selected due to some advantages such as temperature uniformity and high efficiency and energy saving (Allyson, 2011).

The function of the air expeller is to remove flue gases during pyrolysis. The feedstock was stored in a stainless-steel tube reactor that was tightly closed using the closing caps on both ends. The closing cap features a gas intake on one end and a vent for pyrolytic fumes on the other. The electric furnace was set to the specified temperature during pyrolysis. After completion of the residence time, the pyrolyzed samples were cooled down to room temperature, stored and labeled as RS-BC-400, PL-BC-500, and PW-BC-500 according to pyrolysis temperature and residence time. The pyrolysis experiment was done in batches since the furnace is very small. After pyrolysis, the PL and RS biochars were passed through a 2-mm screen, and the material that remained on the sieve is known as "pellets." A portion of the pellets were ground such that they could pass through a 0.42-mm sieve; this particle size is known as "dust." Pyrolysis residence time was between 1 to 2 h for the selected feedstock. In order to characterize, biochars were ground to <0.30 mm. The electric furnace used for the pyrolysis is shown in Figure 3. The sample of biochar produced from the Vulcan furnace is presented in Figure 4.

Biochar characterization

After shaking for 2 h at 200 rpm, the pH of each ground biochar sample was determined using a 1:2 (v/v) biochar/deionized H2O mixture. The ash, C, and N contents of biochars on an oven dry weight basis was assessed at Giolee Global Resources Laboratory at Stadium Road, Port Harcourt, Rivers State, Nigeria utilizing the American Society for Testing and Materials (ASTM) D1372

and 3176 standard combustion procedures (ASTM, 2006). The P and K contents were measured on an oven dry weight basis using Environmental Protection Agency (EPA) method 3052, an oven-assisted acid digestion procedure (US EPA, 1996), and quantified using an inductively coupled plasma mass spectrometer, as described by Novak et al. (2009).

Bioremediation experiment design

The bioremediation experiment was designed using Box-Behnken Design (BBD) applying response surface methodology (RSM) in Design-Expert 13. RSM is a popular method for studying a process in which the response of interest is affected by different variables, with the goal of optimizing the response (Olatunji et al., 2021). It is a helpful tool because it allows for the evaluation of the effects of several factors and their interactions on one or more response variables (Olatunji et al., 2022). The parameters that affect the process (Poultry litter biochar, Rice straw biochar and Pine wood biochar all measured in grams) are called the independent variables, while the response (TPH) is called dependent variable. The BBD technique in RSM was used in the design due to the number of independent variables. This technique accepts a minimum of three independent variables, which in this study are pine wood biochar, poultry dropping biochar and rice straw biochar. The BBD technique is known for very high level of accuracy when used for predictions. In adopting RSM, selection of contributing parameters, their levels and proper experimental design are essential. RSM is a collection of methods for building empirical studies of the connections involving a response and a number of input parameters.

Since PL char, RS biochar, and PW biochar (g) are all quantifiable independent variables. It is presumptive that the independent variables are continuous and subject to minor experimental error. Usually a second-order model is utilized to find a suitable approximation for the functional relationship between independent variables and the response surface (Olatunji et al., 2021b). This is expressed in Equation 3:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i{}^2 + \sum_i \sum_j \beta_{ij} X_i X_j + \varepsilon$$

(3)

Where: ε is the random error.

The experimental range and design values are presented in Tables 1 and 2.

Bioremediation of crude oil polluted soil

400 g of crude oil polluted sample was placed in different plastic bottles, labelled 1 to 17 and control. Biochars (PW, PL and RS),
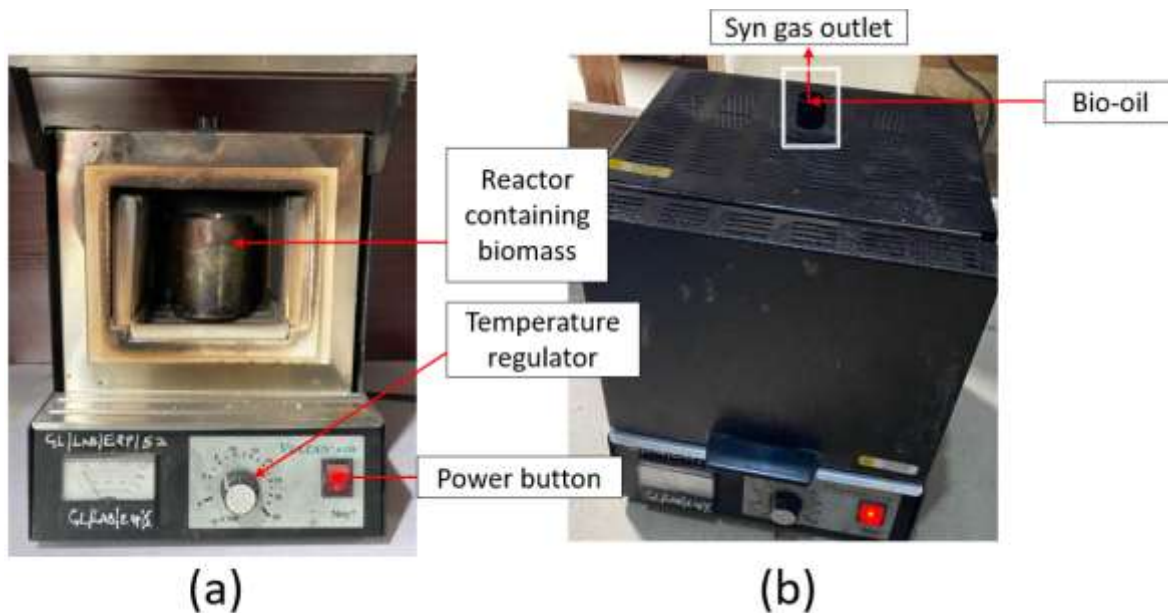
Figure 3. The "Vulcan Furnace A-130" used for biomass pyrolysis (a) open (b) closed.
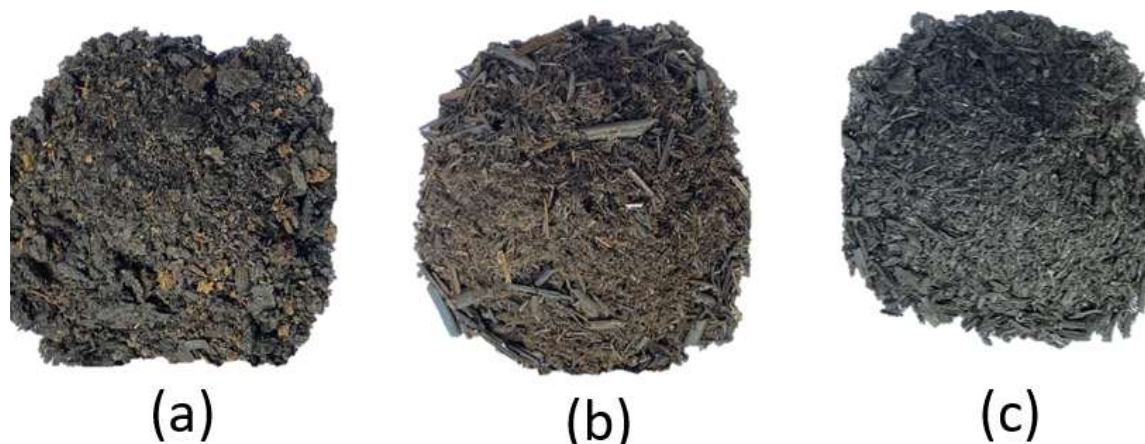Source: Authors

Figure 4. Biochar produced (a) poultry litter (b) rice straw and (c) pine wood.
Source: Authors

were added to each of the crude oil polluted soil in appropriate proportions as indicated on RSM experimental design. The soil was mixed twice weekly to provide sufficient aeration and moistened by the addition of water twice every week to adjust the water holding capacity throughout the experimental period. In the control sample water was also added twice a week except biochar according to Agarry and Ogunleye (2012a).

The plastic bottles were then incubated at room temperature (varied from 27 to 33°C) and kept in a wooden greenhouse made with dimensions; Length =140 cm, Width =55 cm, and Depth =110 cm as shown in Figure 3. Each plastic bottle (diameter = 15 cm and height = 8 cm) was labelled (SS+BCB) and the mixture was rigorously stirred to ensure nutrients and bacteria homogeneity. In order to keep the soil salinity at tolerable levels, plastic saucers were used to prevent loss of water from beneath the bottle. Each

bottle's crude oil-contaminated soil was added with varying quantities of PL (5 - 15g), RS (2 - 6g), and PW (2 - 4g) as specified on the RSM experimental design (Table 1). The range was selected according to the reports of Agarry and Ogunleye (2012b). In addition, 400g of the crude oil polluted soil was collected from the homogenized portion and used as control sample. In the control sample biochar was not added. The green house used for the experiment is presented in Figure 5.

In total, 17 microcosms and a control sample was set-up and left to bioremediate for 30 days. All microcosms were mixed manually twice per week to enhance oxygenation and kept moist during the 30-day experimental period.

Efficiency of crude oil removal was assessed after 30-days by measuring the total petroleum hydrocarbon content (TPH) of remediated soil.

Table 1. Variable levels and the experimental limit.

Factors	High level	Medium level	Low level
Poultry litter, grams [A]	15	10	5
Rice straw, grams [B]	6	4	2
Pinewood, grams [C]	4	3	2

Source: Authors

Table 2. Full-factorial BBD for the three independent variables.

Experimental Runs	Poultry litter, grams [A]	Rice straw, grams [B]	Pinewood, grams [C]
1	10	2	2
2	15	2	3
3	10	6	2
4	10	4	3
5	10	4	3
6	15	4	4
7	5	6	3
8	10	4	3
9	10	6	4
10	5	2	3
11	10	4	3
12	15	6	3
13	10	4	3
14	5	4	2
15	10	2	4
16	15	4	2
17	5	4	4
Control	-	-	-

Source: Authors

Total Petroleum Hydrocarbon (TPH) analysis

After the bioremediation period of 30 days, 20 grams of each soil sample was obtained from the bulk mixture and dried at room temperature for 72 h for TPH analysis using FLUORAT-02 analyzer via fluorometric method. The extraction solvent for the TPH was hexane and 460 nm-wavelength absorbance measurements were made with a UV-visible spectrophotometer. 5 g of the soil was placed in 200 ml beaker and 150 ml of toluene was added. The mixture was stirred continuously for 30 min, left to stand in a fume cupboard for 2 h and then filtered using Whatman No 42-filter paper. The residue, (soil), was allowed to dry in an oven at 50°C. TPH concentrations were then measured from a calibration curve prepared by plotting measured absorbance at different initial concentrations of TPH against each other. The percentage of total petroleum hydrocarbons degraded was measured after 30 days using Equation 5:

$$\%\text{TPH removal} = \frac{C_0 - C_f}{C_0} \times 100$$

Where C_0 = initial TPH concentration in soil (g/Kg) and C_f = final concentration of TPH in bioremediated soil (g/Kg).

RESULTS AND DISCUSSION

Physicochemical properties of soil

The results of the physicochemical properties are presented in Tables 3 and 4. From the results, it was observed that the electrical conductivity (EC) value is 73.4 µS/cm. This indicates that the polluted soil is non-saline, as the electrical conductivity (EC) is below 4000 µS/cm (Miller and Donahue, 1995) and does not exceed the critical value of 2000 µS/cm (Miller and Donahue, 1995; Okon and Ogba, 2018). This indicates that the soil does not have salinity problem prior to remediation. Qin et al. (2012) suggested that salinity had great impact on bioremediation of petroleum hydrocarbon. High salinity suppresses the growth of microbes which is capable of limiting the rate of biodegradation (Ebadi et al., 2018).

Figure 5. Greenhouse used for bioremediation experiment.
Source: Authors

Table 3. Physical and chemical features of soil contaminated by crude oil.

Parameter	Value
pH	4.720
Temperature (^{o}C)	28.50
Moisture Content (%)	21.49
Electrical Conductivity ($\mu S/cm$)	73.40
Cation Exchange Capacity (meq/100 g)	6.200
Total Nitrogen (%)	0.690
Potassium (%)	0.279
Total phosphorus (%)	21.02

Source: Authors

Table 4. Soil textural classification prior to remediation.

Parameter	Value (%)
Sand	62.81
Silt	20.59
Clay	16.60

Source: Authors

Despite this, the most readily available type of nitrogen in soil is ammonium. However, as shown in Table 3, the total nitrogen concentration of the polluted soil sample is 0.690%. When compared to the medium range of 0.10 to 0.45% (Brady and Weil, 1996) for soils in the research area, this figure is considered high. The crude oil polluted soil had an acidic pH (4.72), a temperature of 28.5 (°C), a moisture content of 21.49 (%), a cation exchange capacity of 6.2 (meq/100g), and potassium, total nitrogen, and available phosphorus concentrations of 0.279, 0.69, and 21%, indicating that soil nutrition was deficient and imbalanced. The texture of the sand, silt, and clay fractions varied in the study area.

However, the texture of the soil determines how much water can be held in it, how readily it can be tilled, how much aeration it receives, and how fertile it is (FPDD, 1990). Sand generated on loosely consolidated coastal plain sand and sandstones contributes to the soil's high sand content. But the crude oil spill's influence which increased substantially the percentage of sand had a negative impact on the soil quality in the impacted areas. This is due to possible high oil drainage into the lower horizon of the soil, which results in an aeration issue as oil accumulates in the air pores and obstructs the easy movement of nutrients to the soil.

Bacteria and fungi in soil degrading hydrocarbons prior to remediation

Table 5 displays the findings of the total petroleum hydrocarbon-degrading bacteria and fungi in the crude oil-polluted sample. It was discovered that complete heterotrophic and hydrocarbon-using bacteria available are 5.8 (10^{3} cfu/g) and 5.7 (10^{2} cfu/g) respectively. This

Table 5. Soil microbial analysis.

Microbe	Value
Total Heterotrophic Bacteria (10^3 cfu/g)	5.8
Total Heterotrophic Fungi Count (10^2 cfu/g)	1.3
Hydrocarbon Utilizing Bacteria (10^2 cfu/g)	5.7
Hydrocarbon Utilizing Fungi (10^2 cfu/g)	0.3

Source: Authors

indicates a promising bioremediation process if bacteria are stimulated by biochar. However, hydrocarbon utilizing fungi are between 0.3 - 1.3 (10^2 cfu/g). The variation in cfu/g from 0.3 to 5.8 cfu/g of the different species indicates a higher content of hydrocarbon utilizing bacteria (HUF) and can be attributed to the high level of moisture in the polluted soil.

Total heterotrophic and hydrocarbon utilizing bacteria are 5.8 (10^3 cfu/g) and 5.7 (10^2 cfu/g) respectively (Table 5). This indicates a promising bioremediation process if bacteria are stimulated by biochar. However, hydrocarbon utilizing fungi are between 0.3 and 1.3 (10^2 cfu/g).

Soil total petroleum hydrocarbon (TPH) content

The initial TPH concentration of the crude oil polluted soil is given as 1405 mg/kg. This value indicates a significant level of pollution in the study area.

Physicochemical characteristics of biomass

The results of the physicochemical analysis of raw biomass before pyrolysis are presented in Table 6. It was observed that the initial pH and moisture content of pinewood (PW) char was 3.53 and 12.33 (%) respectively. This shows that PW biomass is acidic which may not be favorable for bioremediation of acidic soil with pH. Also, PL had acidic pH of 4.92 while rice straw (RS) has a higher pH of 7.22. However, the pH of PW and PL are expected to increase after pyrolysis and also considering the blend biochar from poultry litter and rice straw biomasses (Figure 6).

It was observed that the pH of pine wood increased from 3.53 to 3.6, still indicating an acidic condition, while the pH of poultry litter increased significantly from 4.92 to 7.16, and the pH of rice straw char increased from 7.22 to 8.23. Consequently, the pH of the biochar is now favorable for bioremediation. However, their nitrogen is still low ranging from 0.12 to 0.15% for all three biochars. The ash content of pine wood, rice straw and poultry litter char were 18.96, 5.48 and 25.6% respectively. This shows that the physicochemical properties of biochar are affected by pyrolysis temperature, and residence time.

According to Chatterjee et al. (2020), increasing the pyrolysis temperature led to greater C and ash contents, lower N contents, and higher pore volume and micro surface area.

Bioremediation experimental result

The result of a 30-day bioremediation experiment carried out in a wood green house with different biochar blend making 17 experimental runs and 1 control are presented in Table 6. The experimental analysis was based on the reduction of TPH content of the polluted soil and pH. A significant reduction of TPH was observed for all the different proportions of blended biochar after the 30-days period. However, pH of all the different proportions was within the range of 6.26 to 6.91.

## The interaction effect of the commingled biochar on TPH removal

In Figure 7a, the 2D contour and 3D surface plot shows the effect of interaction between PL char and RS char (g). This plot demonstrates that both PL char and RS char have positive mutual impact on the biodegradation of TPH. Similarly, in Figure 7b, the contour plot indicates that mutual but negative effect of TPH removal. However, at a fixed weight (g) of RS char, it was observed that increase in weight (g) of PL biochar resulted in higher TPH degradation. This is an indication that PL biochar had a significant and positive impact on TPH removal more than RS char, whereas PW biochar resulted in negative effect due it its physicochemical properties such as low pH (3.60). Although, PL and RS biochar had higher pH values 7.16 and 8.23 which caused the significant and positive effect in the remediation process.
In Figure 7c, the 2D contour plot illustrates the interaction effect between PW char and RS char (g) on the degradation process. The plot indicates a negative effect without mutual influence between PW and RS char on the degradation process. Moreover, as PW char increases, it is observed that the decrease in the weight (g) of RS char leads to lower TPH reductions. It was revealed that the highest TPH (46.74) removal occurred in experimental run 12 which shows the highest level of independent variables such as 15 g of PL, 6 g of RS and 3 g of PW char respectively. This indicates that PL in the biochar mix is more effective than RS and PW.

Ducey et al. (2015) utilized feedstocks in four ratios (100% pine chip; 80:20 mixture of pine chip to poultry litter; 50:50 mixture of pine chip to poultry litter; 100% poultry litter) prior to pyrolysis and soil amendment as a biochar product. Their results demonstrated significant shifts in microbial community composition in response to biochar amendment, the effects of which were greatest with 100% poultry litter biochar. This agrees with the

Table 6. The independent variables and residual TPH after 30-days bioremediation period.

Experimental runs	PL biochar (g)	RS biochar (g)	PW biochar (g)	TPH (%)
1	10	2	2	39.90
2	15	2	3	43.93
3	10	6	2	38.07
4	10	4	3	35.55
5	10	4	3	35.30
6	15	4	4	42.77
7	5	6	3	35.44
8	10	4	3	34.56
9	10	6	4	36.04
10	5	2	3	33.25
11	10	4	3	39.33
12	15	6	3	46.74
13	10	4	3	41.01
14	5	4	2	26.38
15	10	2	4	32.17
16	15	4	2	38.59
17	5	4	4	27.91
Control	-	-	-	*11.30*

Source: Authors
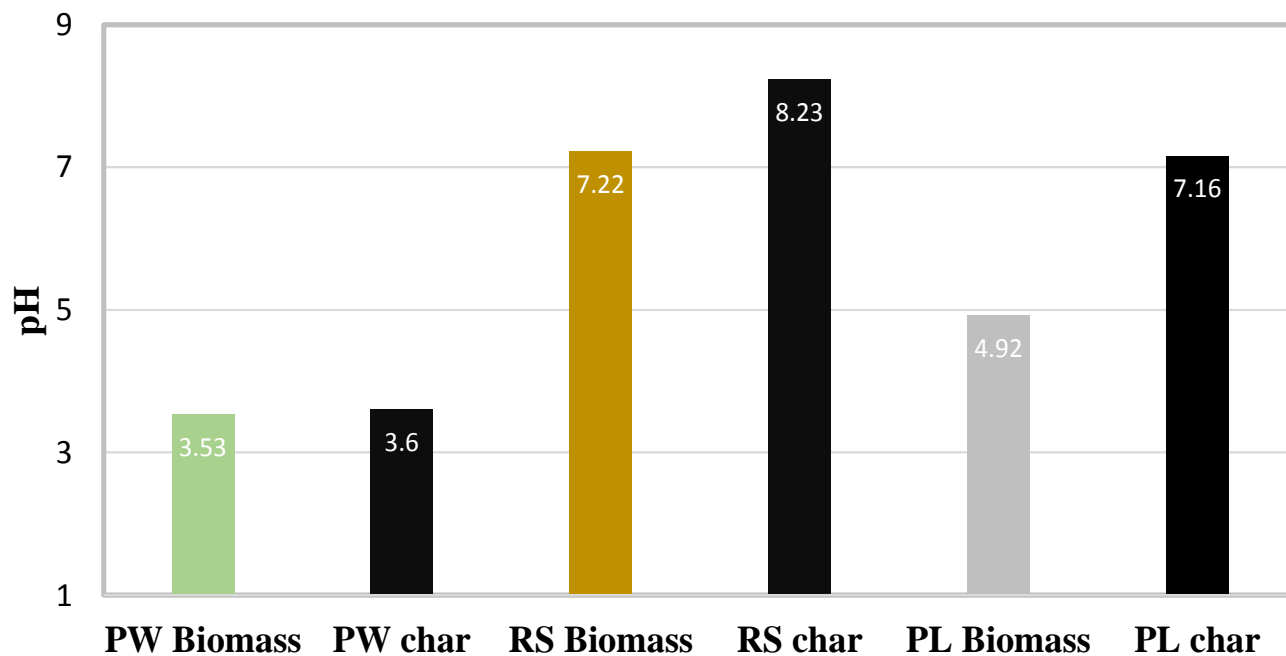
Figure 6. Variation pH of biomasses and biochar produced via furnace assisted pyrolysis.
Source: Authors

findings of this research work were soil sample remediated with high amounts of PL char showed high % TPH removal (Table 6). Saeed et al. (2021) found that the soil analysis showed a crude oil degradation efficiency of 34% for biochar derived from a single biomass.
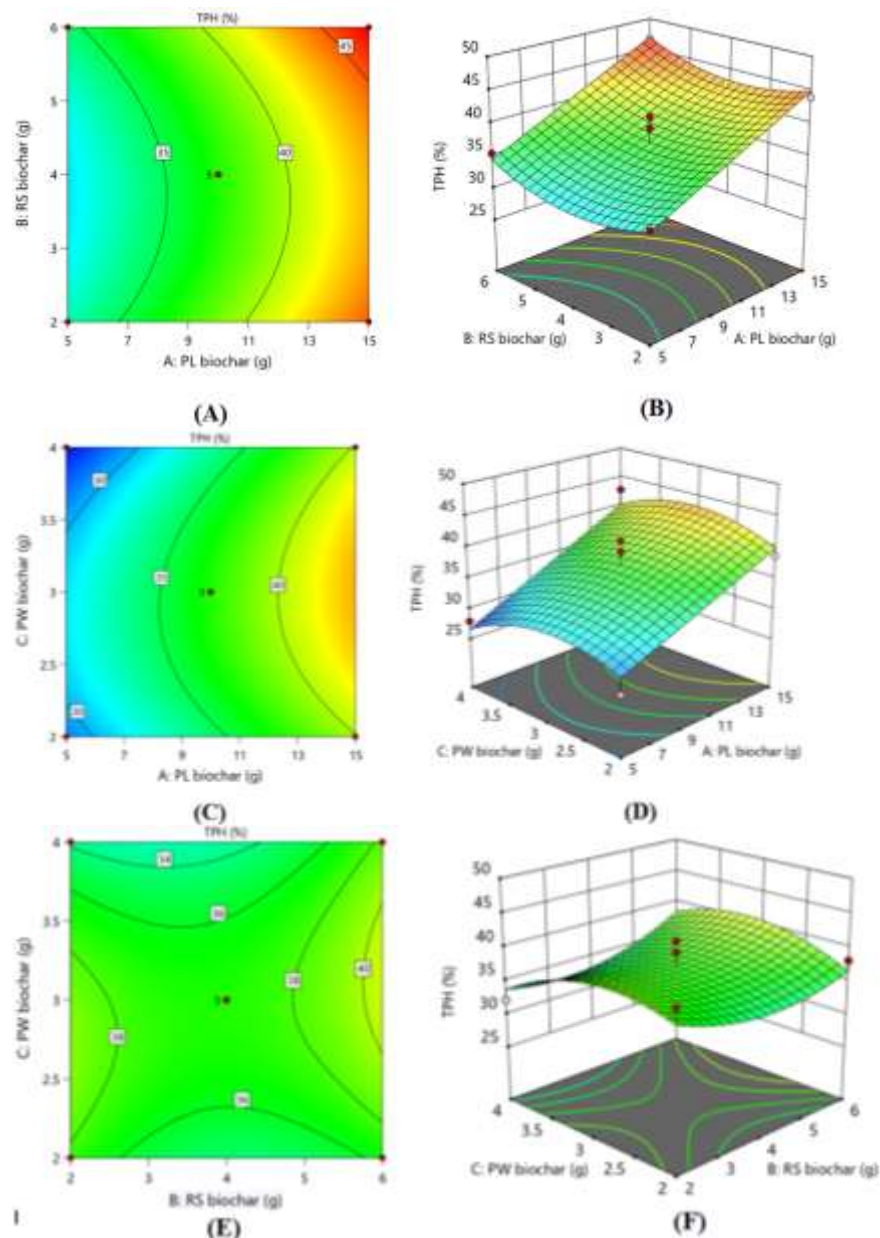
Figure 7. 2D contour and 3D surface plot (A – B) effect of PL biochar and RS biochar (C – D) effect of PL biochar and PW biochar (E – F) effect of RS biochar and PW biochar on TPH degradation.
Source: Authors

Therefore, the biochar blend seems promising for the remediation of crude oil-polluted soil and as an addition to soil nutrients. The effectiveness of biochar-facilitated soil remediation was case specific, changing with the biochar source, amendment rate as captured in the design of experiment using response surface methodology (RSM) *via* design expert. Biochar blend application to soils allows the development of microbial communities (that is, mycorrhizal fungi) which are particularly important for nutrient cycling (Lambers et al.,

2008) which leads to bio-stimulation enhancing the removal of TPH.

Biochar's toxicity to the environment and human health

While biochar is made from bio-based materials, it is essential to note that hazardous chemicals from feedstock or created products during pyrolysis still exist

and pose environmental concerns (Kusmierz and Oleszczuk, 2014a). Organic fractions such as polyaromatic hydrocarbons and volatile organic chemicals, as well as inorganic heavy metal fractions and persistent free radicals, are among the harmful substances (Zheng et al., 2018). As a result, this section highlights the significance of assessing biochar risk evaluation from the perspectives of the pyrolysis process, feedstock, and potential dangers in biochar handling, as well as the methods utilized in risk evaluation. The chemical, physical, and structural properties of biochar are influenced by feedstock parameters and pyrolysis time and temperature, which are critical in understanding biochar functionality. However, biochar use has been associated to several soil application concerns, such as biochar being poisonous, enabling greenhouse gas emissions, reducing pesticide efficiency, and affecting soil bacteria (Ndirangu et al., 2019). Potential hazards result from pyrolysis conditions promoting the formation of certain traits and functional groups as well as contaminated feedstock. Through the food chain, these created hazardous chemicals are a threat to human health. Ndirangu et al. (2019) asserts that determining the toxicity levels is the first stage in the risk management of hazardous biochar; however, this step was not completed in the current study due to financial and time restraints. However, it can be taken into account in future research for evaluating the biochar blend's toxicity.

Proposed approach to promote the recovery of soils attacked by hydrocarbons

Due to its wide availability of the requisite biomass, sustainability, cost-effectiveness, high efficiency, significant internal surface area, and ideal physicochemical qualities such as pH, nutrient, etc., biochar mix has shown a good potential to treat crude oil-polluted soil. However, utilizing biochar blends may increase soil fertility, while reducing TPH level and recycle agricultural waste (Zahed et al., 2021). The usage of biochar blended from various feedstocks is still relatively new, nevertheless. Since the biochar blend would have a balance in the mixture of the properties (for example, the pH of RS char is 8.23, PW char is 3.6, and PL char 7.16) it has been proved to be successful in the remediation of TPH from acidic soil (pH = 4.72). The goal is to increase the remediation efficiency. As a result, the biochar blend evaluated in this study is strongly recommended to aid in the recovery of soils impacted by hydrocarbons.

Conclusion

The remediation of soil with biochar-blend has been demonstrated to have an effect not only on the soil physicochemical properties such as pH, but also in the removal TPH at a fast rate. The blend (PW, PL and RS biochar) is suitable for acidic soil. This comes in the form of increased soil aggregates with concomitantly increased water retention capabilities, improved soil pH levels, as well as increased available nutrients. It was observed that PW char did not perform very well due its low pH (3.6). In further studies it can be removed in the biochar blend, while considering more of PL and RS char.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agarry SE, Ogunleye OO (2012a). Factorial Designs Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Weathered Bonny Light Crude Oil through Bio-stimulation and Bioaugmentation Strategy. Journal of Environmental Protection 3(8):748-759.

Agarry SE, Ogunleye OO (2012b). Box-Behnken design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using bio-stimulation strategy. http://www.journal-ijeee.com/content/3/1/31

Alinnor IJ, Nwachukwu MA (2013). Determination of total petroleum hydrocarbon in soil and groundwater samples in some communities in Rivers State, Nigeria. Journal of Environmental Chemistry and Ecotoxicology 5(11):292-297.

Allyson S (2011). Biochar Production for Carbon Sequestration, A Major Qualifying Project Submitted to the faculty of Worcester Polytechnic Institute In partial fulfillment of the requirements for the Degree of Bachelor of Science in Chemical Engineering, Worcester Polytechnic Institute (WPI) in Shanghai, China.

Amnesty International (2009). Oil spill in Niger-Delta, Available from: https://www.amnesty.org/en/latest/news/2018/03/niger-delta-oil-spills-decoders/

Batista EMCC, Shultz J, Matos TTS, Fornari MR, Ferreira TM, Szpoganicz B, De-Freitas RA, Mangrich AS (2018). Effect of surface and porosity of biochar on water holding capacity aiming indirectly at preservation of the Amazon biome. Scientific Reports 8:10677.

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL (2010). Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multielement polluted soil. Environmental Pollution 158(6):2282-2287.

Brady NC, Weil RR (1996). The Nature and Properties of Soils. Eleventh Edition, Prentice-Hall, Upper Saddle River, New York.

Chatterjee R, Sajjadi B, Chen WY, Mattern DL, Hammer N, Raman V, Dorris A (2020). Effect of Pyrolysis Temperature on Physicochemical Properties and Acoustic-Based Amination of Biochar for Efficient CO_2 Adsorption. Frontiers in Energy Research 8.

Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press UK, pp. 35-38.

Ducey TF, Novak JM, Johnson MG (2015). Effects of Biochar Blends on Microbial Community Composition in Two Coastal Plain Soils. Agriculture (Switzerland) 5(4):1060-1075.

Ebadi A, Khoshkholgh SNA, Olamaee M, Hashemi M, Ghorbani, NR (2018). Remediation of saline soils contaminated with crude oil using the halophyte Salicornia persica in conjunction with hydrocarbon-degrading bacteria. Journal of Environmental Management 219:260-268.

Fertilizer Procurement and Distribution Division (FPDD) (1990). Literature Review on Soil Fertility Investigations in Nigeria (in Five Volumes) Produced by the Federal Ministry of Agriculture and Natural Resources, Lagos. Federal Ministry of Agriculture and Natural Resources (FMANR) 6:133-159.

Gee GW, Bauder JW (1986). Particle-size analysis. In: Klute, I.I., Ed., Methods of Soil Analysis, Soil Science Society of America, Madison

pp. 383-412.

Gomez-Eyles JL, Sizmur T, Collins CD, Hodson ME (2011). Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environmental Pollution 159(2):616-622.

Google Maps (2023). Kpuite in TAI Local Government Area, Ogoni, Rivers State, Nigeria. Google Maps [online] Available through: https://dailypost.ng/2013/10/10/oil-spill-hits-ogoni-again/ [Accessed 21 June 2022].

Khan S, Waqas M, Ding FH, Shamshad I, Arp HPH, Li G (2015). The influence of various biochars on the bio-accessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (Brassica rapa L.). Journal of Hazardous Materials 300:243-253.

Kong F, Sun G, Liu Z (2018). Degradation of polycyclic aromatic hydrocarbons in soil mesocosms by microbial/plant bioaugmentation: performance and mechanism. Chemosphere 198:83-91.

Kpuite in Tai LGA. Available from: https://dailypost.ng/2013/10/10/oil-spill-hits-ogoni-again/, accessed on June 21, 2022.

Kumari KGID, Moldrup P, Paradelo M, Jonge LWD (2014). Phenanthrene sorption on biochar-amended soils: application rate, aging, and physicochemical properties of soil. Water, Air, and Soil Pollution 225(9):1-13.

Lambers H, Raven JA, Shaver GR, Smith SE (2008). Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23(2):95-103.

Lominchar MA, Lorenzo D, Romero A, Santos A (2018). Remediation of soil contaminated by PAHs and TPH using alkaline activated persulfate enhanced by surfactant addition at flow conditions. Journal of Chemical Technology and Biotechnology 93:1270-1278.

Miller RW, Donahue RL (1995). Soils in Our Environment. Prentice-Hall, London.

Ndirangu SM, Liu Y, Xu K, Song S (2019). Risk Evaluation of Pyrolyzed Biochar from Multiple Wastes. Hindawi Journal of Chemistry https://doi.org/10.1155/2019/4506314.

Ndukwu MC, Horsfall IT (2020). Prospects of Pyrolysis Process and Models in Bioenergy Generation: A Comprehensive Review. Polytechnica 3(1–2):43-53.

Novak JM, Busscher WJ, Laird DL, Ahmedna M, Watts DW, Niandou MAS (2009). Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science 174:105-112.

Okon IE, Ogba CO (2018). The Impacts of Crude Oil Exploitation on Soil in Some Parts of Ogoni Region, Rivers State, Southern Nigeria. Open Access Library Journal 5:4297.

Olatunji OM, Horsfall IT, Ubom EV (2021). Response surface optimization approach to predict the maximum %biodiesel yield via transesterification of esterified shea butter oil by utilizing bio-catalysts. Current Research in Green and Sustainable Chemistry 4:100167. https://doi.org/10.1016/j.crgsc.2021.100167

Olatunji OM, Itam DH, Akpan GE, Horsfall IT (2022). Predictive Modeling Coupled with Multiple Optimization Techniques for Assessing the Effect of Various Process Parameters on Oil and Pectin Extraction from Watermelon Rind. Process Integration and Optimization for Sustainability 6(3):765-779.

Oleszczuk P, Jośko I, Kuśmierz M, Futa B, Wielgosz E, Ligeza S, Pranagal J (2014a). Microbiological, biochemical and ecotoxicological evaluation of soils in the area of biochar production in relation to polycyclic aromatic hydrocarbon content. Geoderma 213:502-511.

Oleszczuk P, Zielińska A, Cornelissen G (2014b). Stabilization of sewage sludge by different biochars towards reducing freely dissolved polycyclic aromatic hydrocarbons (PAHs) content. Bioresource Technology 156:139-145.

Qin X, Tang JC, Li DS, Zhang QM (2012). Effect of salinity on the bioremediation of petroleum hydrocarbons in a saline-alkaline soil. Letters in Applied Microbiology 55(3):210-217.

Saeed M, Ilyas N, Jayachandran K, Gaffar S, Arshad M, Sheeraz-Ahmad M, Bibi F, Jeddi K, Hessini K (2021). Biostimulation potential of biochar for remediating the crude oil contaminated soil and plant growth. Saudi Journal of Biological Sciences 28(5):2667-2676.

Spokas KA (2010). Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Manager 1(2):289-303.

Su C, Zeng GM, Gong JL, Yang CP, Wan J, Hu L, Hua SS, Guo YY (2016). Impact of carbon nanotubes on the mobility of sulfonamide antibiotics in sediments in Xiangjiang River. RSC Advances 6:16941-16951

Tan X, Liu Y, Zeng G, Wang X, Hu X, Gu Y et al. (2015). Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere 125:70-85.

US EPA United States Environmental Protection Agency (1996). Microwave assisted acid digestion of siliceous and organically based matrices. In: Test methods for evaluating solid waste, physical/chemical methods. SW-846, US EPA, Washington. Available at http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3052.pdf.Accessed18 September 2012

Vidal J (2014). Shell ignored internal warnings over Nigeria oil spills, documents suggest. The Guardian. Available from: https://www.theguardian.com/global-development/2014/nov/13/shell-nigeria-oil-spills-amnesty-international

Wu M, Dick WA, Li W, Wang X, Yang Q, Wang T, Xu L, Zhang M, Chen L (2016). Bioaugmentation and bio-stimulation of hydrocarbon degradation and the microbial community in a petroleum contaminated soil International Biodeterioration and Biodegradation 107:158-164.

Zahed MA, Salehi S, Madadi R, Hejabi F (2021). Biochar as a sustainable product for remediation of petroleum contaminated soil, Current Research in Green and Sustainable Chemistry 4:100055.

Zheng H, Liu B, Liu G, Cai Z, Zhang C (2018). Potential toxic compounds in biochar: Knowledge gaps between biochar research and safety. In Biochar from Biomass and Waste: Fundamentals and Applications pp. 349-384.