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Evaluation of the potential of agricultural wastes-cattle manure and poultry manure for bioremediation of crude oil-contaminated soil

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ABSTRACT

The Niger Delta region of Nigeria suffers from petroleum pollution, which affects ecosystem functioning and human health, which necessitates finding sustainable remediation options that utilize local resources. In this work, cattle manure (CM) and poultry manure (PM), which are primarily utilized as biofertilizer for agricultural purposes, were utilized to bioremediate crude oil-contaminated soil on a laboratory scale. In addition to being readily accessible, CM and PM are also sustainable bioresources that are host to a wide variety of microflora that can be used for bioaugmentation. At the end of the 1.5 month study, the impact of the amendments on speciated total petroleum hydrocarbons (STPH) in the range of nC₁₀-nC₄₀ was evaluated. A significantly higher STPH degradation of 36% in PM-amended soil was observed compared to CM-amended soil (23%); and only 1% degradation in enhanced by natural attenuation soil (RENA). The pre-dominant aliphatic fractions in the samples analyzed were nC₁₆-nC₃₅. In comparison to the CM amendment option, PM amendments achieved better bioremediation of these fractions. Moreover, the effect of biowaste ratio amendment to the contaminated soil showed that the ratio 1:1 (w/w) for both bioamendments performed better than the ratio 1:2 (w/w), suggesting that the higher the amount of amendment to contaminated soil, the more effective the bioremediation. The results of this study demonstrate the potential of PM as a sustainable, affordable, and local bioremediation technique for recovering soil contaminated by crude oil in the Niger Delta.

KEYWORDS



Amendments; animal biowaste; animal manure; bioremediation; crude oil-contaminated soil; total petroleum hydrocarbon

Introduction

Hydrocarbons pollution has endangered wildlife, altering water and soil chemistry due to the release of various environmental pollutant (dos Santos and Maranhão 2018). Petroleum hydrocarbons pollution in the environment comes from various sources including crude oil exploration and exploitation, transportation, leakage from tanks, pipeline vandalism, and sabotage, among others (Aisien, Aisien, and Oboh 2015; Lim, Lau, and Poh 2016). The operation of local and illegal oil refineries in the Niger Delta region of Nigeria is a major cause of oil pollution on the land. In the region, a kpo fire occurs when illegal oil operatives burn crude oil at bunkering or dump sites to refine petroleum products.

According to the Nigerian National Petroleum Corporation (NNPC), in 2013 alone, the company reported over 2000 pipeline breaks, with 181.7 million tons of crude oil product lost, worth about N21.5 billion, and 34 fire incidents due to illegal oil refining. Oil spills and waste from illegal kpo fire operations are contaminating soil and negatively impacting flora, fauna, and ecosystem of the region.

In the Niger Delta region of Nigeria, the United Nations Environment Programme (UNEP) in 2011 reported that in Ogoniland alone, more than 69 sites were severely polluted with crude oil (concentrations >139,000 mg/kg) affecting the environmental matrix- soil (agriculture), air, and water quality standards and posing a grave human health

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threat. Consequently, UNEP projected at least 1 billion US dollar for the 10 years of the 30 years cleanup plan for crude oil-polluted sites in Ogoniland in the Niger Delta. Thus, there is an urgent need to clean-up these polluted sites. However, Nigeria lacks the funding capacity and expertise, like most developed nations to address all contaminated sites accordingly (Sam, Coulon, and Prpich 2016). This has greatly affected agricultural activities in the region since soil-dependent microorganisms have been adversely affected, thus, there is severe persistent food insecurity in the region. Though Nigeria relies on remediation enhanced by natural attenuation (RENA) for petroleum hydrocarbon contaminated land sites after cleanup, this approach for soil remediation is very slow since RENA depends on indigenous soil microorganisms mainly to degrade hydrocarbons. In addition, RENA approach cannot effectively address the crude oil pollution issues in the Niger Delta region since spilled oil has penetrated a considerable depth down the top soil thus causing groundwater contamination (UNEP 2011). Therefore, there is a need for remediation approaches that could harness local resources that are often overlooked and/or abandoned in Nigeria, since funding for more expensive remediation is limited.

Considerable efforts have been dedicated to developing approaches for the remediation of petroleum hydrocarbon (PHC)-contaminated soils, apart from remediation enhanced by natural attenuation RENA (Akpokodje and Uguru 2019; Chao, Daoji, and Huixue 2020; Ofoegbu, Momoh, and Nwaogazie 2015). Some remediation approaches to PHC contamination in soil are costly and consume a lot of energy including incineration, soil washing, and soil vapor extraction (Kujat 1999; Lim, Lau, and Poh 2016). In 2009, the United State Environmental Protection Agency (US EPA) Office of Land and Emergency Management established a plan on Principles for Greener Cleanups. One principal approach to achieve these goals is bioremediation; which requires the application of plants, microbes, and other soil inhabitants to breakdown, remove and/or mitigate contaminants (Akpokodje and Uguru 2019; Chawla et al. 2013; Cook and Hesterberg 2013; dos Santos and Maranhão 2018).

Bioremediation provides the potential to remediate site while enhancing soil properties that support soil functions to enhance plant growth, as well as provides a positive esthetic for the surrounding human population (Chawla et al. 2013; Slegers 2010). Thus, bioaugmentation can be initiated using bovine dung (BD), cattle manure (CM), and poultry manure (PM), which are organic biowastes expelled by domestic livestock, including cattle, cows, chickens, and turkeys. In this study, cattle dung, poultry litter, and the microflora associated with them are explored in terms of their applications within agriculture, biotechnology, and environmental remediation. Additionally, a broad spectrum of microorganisms found in both manures contribute to the bioaugmentation and enhancement of soil biogeochemical processes (Behera and Ray 2021). On this basis, both CM and PM can be viewed as a biore-source for sustainable bioremediation of crude oil contaminated land in Nigeria. Typically, cattle excrete residue (CM) is made up of moisture (80%), undigested plant residues (14.4%), and microbes (5.6%) (Behera and Ray 2021). CM is the remnant of plant matter that has passed through the digestive tract of the cattle. CM and PM, respectively, are livestock excreta and litter that can be easily accessed, are sustainable, and have a variety of micro-organisms that can be used for bioremediation. Hence, bioremediation of crude oil polluted sites becomes cost-effective as a result of their utilization for bioaugmentation, making it 80–90% cheaper than engineering systems, which would encourage its widespread deployment (Chen et al. 2015; Megharaj et al. 2011; Singh et al. 2017; Stephenson and Black 2014). However, bioremediation techniques can be also affected by soil nutrients, pH, temperature, moisture, oxygen, soil properties, and concentration of contaminant (Ghazali et al. 2004; Sabaté, Viñas, and Solanas 2004; Semple, Reid, and Fermor 2001). The critical elements to effective implementation of remediation approach includes: control of optimum nutrient, and control of environmental conditions-oxygen, moisture, temperature, and pH to promote the survival and growth of soil microorganisms. To date, petroleum-contaminated soils have been remedied using a variety of biowastes, as shown in Table 1.

Table 1. Soil parameter results against amendments for previous studies.

Soil parameter	Amendment/percent degradation				References
TPH 90-day experiment (mg/kg)	Control (Co) Deg: 53%	Biochar (B) Deg: 53%	Compost (C) Deg: 60%	B + C Deg: 62%	Olivia, Rachel, and Melissa 2019
TPH 99-day experiment (mg/kg)	PKHA/CD Deg: 64%	PKHA Deg: 59%	NPK Deg: 77%	Cow manure Deg: 69%	Ofoegbu, Momoh, and Nwaogazie 2015
TPH 28-day experiment (mg/kg)	Co Deg: 32%	Goat manure Deg: 87%	Poultry manure Deg: 79%	Cow manure Deg: 71%	Obiakalaje et al. 2015

PKHA: palm kernel ash; CD: cow dung; NPK: nitrogen, phosphorus, and potassium; Deg: hydrocarbon degradation.

These animal biowastes were found to be useful in bioremediation of crude oil-contaminated agricultural soil for enhanced remediation. However, none of these studies assessed the effectiveness of amendments against RENA; and limited studies on the effect of quantity of amendment on contaminant immobilization. These biowastes are reservoir of several microorganisms capable of breaking down hydrocarbons while producing a wide range of metabolites, which are highly beneficial to nature. The use of biowaste (compost) has been shown to improve the amount of soil organic carbon, the availability of nutrients, the pH of the soil, and the water retention capacity (Kästner and Miltner 2016; Wu et al. 2017). Agegnehu, Srivastava, and Bird (2017) reported on the potential of biowaste to enhance the sorption of contaminants to the newly introduced organic amendment and reduce sorption to the parent soil. According to Kästner and Miltner (2016) and Sayara, Sarrà, and Sánchez (2010), biowaste increases soil microbial diversity to quicken the remediation process. There is also evidence that biowaste increases nutrient availability in soil (Chen et al. 2015). Therefore, more quantity of amendment would likely enhance the remediation efficiency. There is a need to further explore low-cost, sustainable, and environmentally friendly remediation methods for crude oil-contaminated sites in the Niger Delta region of Nigeria. The application of animal biowastes are non-toxic, cost-effective, and sustainable. Utilizing biowaste to remediate crude oil-contaminated lands in the Niger Delta region of Nigeria will reduce their environmental footprint, improve solid waste management, and consequently provide reclaim fertile land for agricultural purposes. The use of animal biowaste as a novel source of microbes for bioremediation is the focus of this study. As a result, this study aims to assess the effectiveness of cattle manure

(CM) and poultry manure (PM) in removing speciated total petroleum hydrocarbons (STPH) from soils contaminated with crude oil at laboratory-scale. Also, analyze the effects of amendment quantity on remediation, and evaluate the effects of amendment against RENA.

Materials and methods

Study area and sample collection

Five kilograms of bulk surface soil sample (0–20 cm depth) was collected with a shovel on 20 March 2021 from the Niger Delta University Research Farm, Bayelsa State, Nigeria. The soil is sandy loam with ~ 737 g/kg (sand), 141 g/kg (clay), and 122 g/kg (silt). The soil physicochemical properties of the soil are as follows: pH 4.8 (acidic), organic matter content (41.6 g/kg), organic carbon (21.7 g/kg), nitrogen (1.98 g/kg), and bulk density (1.45 g/cm³). The soil sample had an average moisture content of 24.3 wt%. To maintain the soil's field moist state, a Ziploc bag was used to collect and store it tightly at room temperature. Poultry manure (PM) and cattle manure (CM) were collected from Niger Delta University poultry and cattle rearing farms, respectively. The fresh CM has an average moisture content of 78.12 ± 2.03 wt%, whereas the PM has an average moisture content of 73.81 ± 1.02 wt%. The PM and CM samples were subjected to a 4-day air-drying procedure at 21 °C in the laboratory. This aging method allowed the manures to maintain roughly 36 wt% of moisture and served as a catalyst for the composting process in preparation for bioaugmentation. The CD microflora contains a broad range of microbes comprising of about 60 species of bacteria, such as *Bacillus* sp., *Lactobacillus* spp., *Citrobacter koseri*, *E. aerogenes*, *Escherichia coli*, *Pseudomonas* sp., *Corynebacterium* spp., *Paenibacillus flaviporus*, *Acinetobacter* spp., etc. fungal, such as

Aspergillus, *Vericosporium* spp., *Trichoderma*, *Rhizopus stolonifera*, *Blastomyces* sp., *Fusarium* sp., *Pleurofragmium* sp., *Trichoderma harzianum*, etc. One hundred species of protozoa and yeasts, such as *Saccharomyces*, *Sporobolomyces*, *Trichosporon*, *Candida* sp. (Gupta, Aneja, and Rana 2016; Bhatt and Maheshwari 2019; Behera and Ray 2021). Likewise, the PM microflora includes *E. coli*, *Salmonella* spp., *Bacillus*, *Campylobacter* spp., *Penicillium*, *Aspergillus*, *Fusarium*, *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Flavobacterium*, etc. (Fries et al. 2005).

The crude oil sample used in this study was obtained from the Warri Refining & Petrochemicals Company (WRPC) Ltd., Delta State, Nigeria. The following are the physical and chemical characteristics of the crude oil: nitrogen (0.1 wt%), moisture content (0.01 wt%), sulfur content (0.12 wt%), salt content (0.11 wt%), and American Petroleum Institute (API) gravity (36.2°). Based on the gas chromatography chromatogram, the crude oil sample's saturated hydrocarbon molecular composition fraction showed that n-alkanes range from nC₈ to nC₄₀.

Mesocosm experiment

The 5 kg bulk soil sample was divided equally into five portions and labeled A, B, C, D, and E (1 kg each). Each sample was spiked with 80 ml of Nigerian crude oil (equivalent to 67,600 mg oil/kg soil) allowed to equilibrate at room temperature ($T = 21^\circ\text{C}$) for 48 h. The initial concentration of crude oil in soil is 67,600 (oil mg/kg soil). Oil-contaminated soil was blended with amendment manure in a tray and left at ambient temperature under aerobic conditions. One sample (A) was kept as control (soil without amendment) until the end of the 1.5 month experiment, while amendments were added to the remaining four samples (B, C, D, and E). The ratios of CP or PM amendments to soil contaminated by crude oil were 1:1 (w/w) or 1:2 (w/w). After mixing thoroughly, all mesocosm experiments were kept at ambient conditions for 1.5 months. Aeration (oxygen supply level) and soil moisture were maintained by blending samples once a week with a spatula and sprinkling about 100 ml of water every week. Following a sampling of

each mesocosm at the end of the 1.5-month trial, soil samples (10 g each) were submitted for hydrocarbon analysis in the range of nC₈-nC₄₀ using Speciated Total Petroleum Hydrocarbon (STPH) analysis method at the Integrated Scientific and Engineering Solutions Laboratory in Port Harcourt, Rivers State, Nigeria.

Gas chromatography and hydrocarbon analysis

The STPH analysis applied USEPA 8015D-Non-halogenated organics using gas chromatography-flame ionization detector (GC-FID). A 10 g soil sample weighed into a clean extraction bottle and dried with anhydrous sodium sulfate. This was followed by adding 20–40 ml of dichloromethane and shake for 1 h in a shaker. The extract was then allowed to settle for 20 min. The sample was carefully filtered through glass funnel fitted with glass wool and sodium sulfate into a clean beaker rinsed with methylene chloride. Sample extract was concentrated using a rotary evaporator. The initial calibration was the external standard method. The concentrations of the aliphatic standards are 10, 20, 30, 40, and 50 mg/ml, respectively. Calibrations were done using Agilent 7890 A GC/FID. The column used was 100% dimethylpolysiloxane phase (Agilent J&W DB-5 column), 30 m, 0.32 mm id, 0.25 μm phase. Helium was used as the carrier gas. The injection temperature, injection solvent, and injection volume are 300 °C, dichloromethane, and 2 μL , respectively. The injection mode was splitless. The oven temperature was initially 65 °C (hold for 2.0 min), then increased to 15 °C/min up to 320 °C (hold 10 min). The components of the calibration are as follows: n-Octane (nC₈) to n-Tetraoctane (nC₄₀). The GC automatically calculates the concentration by the formula:

$$\text{Concentration (mg/kg)} = (X_s \times V_t \times D) / (V_i \times W_s)$$

Where X_s denotes the calculated mass of the analyte (μg), V_t the total volume of the concentrated extract (μL), D the dilution factor, V_i the volume of extract injected, and W_s the weight of sample extracted (g).

Statistical analysis

Single-factor analysis of variance (ANOVA) was carried out on soils treated with cattle manure (CM) and poultry manure (PM) to check if any correlation exists between them. If a statistically significant test result ($p \leq 0.05$) is obtained, then that indicates that the test hypothesis is to be rejected or is false. This suggests that there is just a 5% possibility of the outcomes happening. Hence, there is no difference in mean between both groups under the null hypothesis being true.

Results and discussion

Comparison of hydrocarbon degradation rates among different amendments

The effect of cattle manure (CM) amendment to crude oil-contaminated soil ratio (1:1 or 1:2 w/w) was analyzed for STPH. The concentration of STPH reduction and percent degradation were higher when a 1:1 (w/w) ratio was used as displayed in Table 2. This implies that the quantity of amendment enhanced degradation of STPH by 36 and 27% for PM amendment to contaminated soil ratio of 1:1 (w/w) and 1:2 (w/w), respectively. A similar trend was observed in the case of CM amendment option. That is, 23 and 15% biodegradation of hydrocarbons were observed for contaminated soil ratio of 1:1 (w/w) and 1:2 (w/w) respectively. In contrast, the hydrocarbon concentration of sample (A) used as an experimental control (soil without amendment) did not change. Notably, the hydrocarbon degradation is greater when the weight of the manure amendment and the contaminated soil mass are equal than when the manure amendment is applied at 50% to the contaminated soil. Just like many other taxonomic genera, both bacteria and fungi found in the microflora of CM and PM are heterotrophic, meaning they can use hydrocarbons

as sources of carbon and energy for growth. Some of the identified petroleum degraders found in CM and PM include *Bacillus* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Arthobacter* sp., *Enterobacter* sp., *Aspergillus* sp., *Mucor* sp., and *Trichoderma* sp. Therefore, the more manure amendments, the higher the population of heterotrophic microorganisms, implying a higher degradation of petroleum. While this preliminary study does not examine the microstructure of the microbe, it is worth doing so in the future. In the contaminated soils, biowaste PM and CM inoculate the environment with a diversity of microorganisms by seeding the soil with microbes for bioaugmentation. Through degradation, these microbes used the hydrocarbons as a carbon source for their metabolism. These results agree with the findings of Oyedele and Amoo (2014) in remediating soil artificially contaminated with crude oil using CM. The authors reported that the percent of TPH degradation was 90, 94, and 97% for 400, 500, and 600 g of CM amendment, respectively. They concluded that CM may have contained hydrocarbon utilizing microbes to enhance hydrocarbon degradation in soil. Similarly, 1:1 (w/w) ratio performed better than 1:2 (w/w) ratio for poultry manure (PM) to crude oil contaminated soil ratio as shown in Table 2. This also confirms the effect of the quantity of amendment on STPH degradation in soil. Thus, based on the microbes present in PM and CM, crude oil-contaminated soil can be bioremediated. Overall, PM performed better than the CM amendment in remediating crude oil contaminated soil. This was demonstrated in both amendment ratios. However, this was not true in previous studies. For example, Obiakalaje et al. (2015) assessed the remediation potential of goat manure (MG), PM, and CM for soil TPH. The authors concluded that GM performed better (87.1%) than PM (70.7%), followed by CM (32.1%). In another study, Agarry, Aremu, and Aworanti (2013) reported that PM performed (73%) better than GM (50%). The reported differences could be due heterogeneity of soils and oil and possible interactions between the soils amendment and the soil constituents (Knaebel et al. 1994). None of these studies investigated the type of microbes and contents in the

Table 2. A Summary showing and comparing CM and PM ratio used and extent of STPH degradation.

Ratio (w/w)	Amendment	Concentration (mg/kg)/Extent of degradation %
1:1	CM	51981/23
1:2	CM	56896/15
1:1	PM	42978/36
1:2	PM	48789/27

CM: cattle manure; PM: poultry manure; STPH: speciated total petroleum hydrocarbons.

amendment; which might play a role in petroleum hydrocarbon degradation in soil. Therefore, further study should investigate these. However, oil-contaminated soils can be bioremediated by using PM and CM due to abundant macro and micronutrients, which provide optimal conditions for microbial growth and proliferation.

Biodegradation of the aliphatic fractions were also investigated. No fractions with carbon chain below than nC_{10} was detected from the samples analyzed. This may be that all fractions less nC_{10} volatilized during the experiment because of their light weights. The pre-dominant aliphatic fractions were nC_{16} - nC_{35} throughout the samples. In terms of the effectiveness of bioremediation, again, PM amendment performed better than the CM amendment (Table 3). This also confirms the biodegradation results of the STPH shown in Table 2. There are a wide variety of microorganisms found in the PM and CM, including *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Alcaligenes* spp., all of which use crude oil as their only carbon source. The results therefore demonstrate that both CM and PM biowaste act as microbial seeders, providing microorganisms capable of degrading aliphatic hydrocarbons in crude oil-contaminated soil.

ANOVA with a single-factor (p -value) was used to measure the extent of correlation between TPH concentrations and amendment CM and PM after bioremediation based on 0.05 significance threshold stated in the section Statistical analysis. A p -value of ~ 0.06 was found between CM and PM. In this case, the null hypothesis is supported, indicating that the means of CM and PM are similar. This also demonstrates a moderate correlation between the amendments and validates the different amendments' levels of

bioremediation. These results suggest that animal biowastes (i.e., CM and PM) can introduce a variety of microorganisms into crude oil-contaminated soil, accelerating the hydrocarbon breakdown process by utilizing them as carbon sources during metabolism under aerobic conditions. Intracellular enzymes play a major role in the biodegradation of the crude oil hydrocarbons by the host microbes within the manures. It has been reported that the biodegradation of petroleum hydrocarbons by microbes follows four stages: (1) emulsification by surfactants secreted by microorganisms, (2) adsorption of emulsified petroleum hydrocarbon on the cell membrane of the microbes, (3) endocytosis, and (4) enzymatic reaction degrading the hydrocarbon molecule (Li, Li, and Qu 2019). There are several factors that determine the degree of bioremediation, including oxygen levels, temperatures, pH levels, the presence of water, soil moisture, and the type and number of organisms present. In the next phase of the study, the effect of these variables on bioremediation levels will be examined in more detail.

Conclusions

Besides destroying farmland and wildlife in the Niger Delta, crude oil pollution also contaminates surface and ground water, disrupts food chains, destroys ecosystems, destroys recreational activities, affects public health and safety, and destroys aquatic life. These environmental degradations are caused mostly by the illegal oil refining operation known as Kpofire in the region and oil spillages. Further research is needed to determine cost-effective and environmentally friendly remediation methods for crude oil-contaminated sites in

Table 3. Concentrations (mg/kg) of hydrocarbon fractions in contaminated soil, and bioremediated soils by cattle and poultry manures.

Sample		Hydrocarbons (mg/kg) fractions			
CS	Ali	nC_{10} - nC_{12}	nC_{12} - nC_{16}	nC_{16} - nC_{35}	nC_{35} - nC_{40}
		473.38	16611	95674	1922
CS + 0.5 kg_CM	Ali	nC_{10} - nC_{12}	nC_{12} - nC_{16}	nC_{16} - nC_{35}	nC_{35} - nC_{40}
		326.78	13036	77526	1929.9
CS + 1 kg_CM	Ali	nC_{10} - nC_{12}	nC_{12} - nC_{16}	nC_{16} - nC_{35}	nC_{35} - nC_{40}
		270.71	6182	18037	340.5
CS + 0.5 kg_PM	Ali	nC_{10} - nC_{12}	nC_{12} - nC_{16}	nC_{16} - nC_{35}	nC_{35} - nC_{40}
		13.93	308.59	1107.199	0.824
CS + 1 kg_PM	Ali	nC_{10} - nC_{12}	nC_{12} - nC_{16}	nC_{16} - nC_{35}	nC_{35} - nC_{40}
		11.25	280.44	919.21	0.465

CS: contaminated soil; CM: cattle manure; PM: poultry manure; Ali: aliphatic.

the Niger Delta region of Nigeria. This study evaluated the effectiveness of readily available agricultural wastes, such as cattle manure (CM), and poultry manure (PM) for the bioremediation of crude oil-contaminated soil in a 1.5 month experiment in the laboratory. More STPH degradation was observed in soil amended with PM. By seeding the crude oil-contaminated soil with biowaste PM and CM, the host microorganisms were capable of degrading hydrocarbons as their carbon source. The quantity of amendment in remediation was also investigated; and results show that the more the quantity of amendment, the faster the rate of degradation of STPH in soil. Aliphatic fraction (nC_{16} - nC_{35}), dominated in the samples analyzed. Again, with PM amendment, better bioremediation of the aliphatic fractions were achieved. Overall, PM amendment performed better than CM amendment. Single-factor ANOVA gave a p -value of 0.06 for CM and PM amended soils, which indicates significant correlation between the two amendment options. It is evident from the results that readily available, sustainable CM could provide a cost-effective bioremediation of petroleum contaminated lands.

Further research will include comparative study of a wide range of manures, including agricultural waste and sewage sludge, evaluating their bioaugmentation level with the goal of achieving bioremediation of petroleum hydrocarbon contaminated soils. The effect of oil concentration in the soil on biodegradation of hydrocarbons by the manure microflora. Following up on the project, a pilot trial will be conducted at a contaminated site in the Niger Delta region of Nigeria. Next, a techno-economic analysis of bioaugmentation of a petroleum contaminated land using animal manures will be conducted based on the experimental data from the field trial.

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Author contributions

Conceptualization: RKD and PPA; experimental: PPA, AF, RKD, and AH; data analysis: RKD; initial draft of paper: RKD, PPA, and AF; review: AH and RKD. All authors read and approved the final manuscript.

Disclosure statement

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Data availability statement

All data generated or analyzed during this study are included in this article.

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