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Full Length Research Article

REMEDIATION OF CRUDE OIL CONTAMINATED SOIL WITH LOCALLY FORMULATED BIOREMEDIATION AGENT

F.E. Ugoma[1], U.J.J. Ijah[1], O.P. Abioye[1] and I.O. Musa[2]

[1]Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria
[2]Department of Microbiology, Skyline University Nigeria, Kano State, Nigeria

*Corresponding Author Email Address: innocentmusa0011@gmail.com Phone: +2347031990685

ABSTRACT
Hydrocarbon pollution is one of the major environmental challenges facing the Niger Delta region of Nigeria, and over the years, various methods and strategies have been suggested to be used in tackling these problem. This research was aimed at restoring crude oil contaminated soil with locally formulated bioremediation agent (coded LOFBA). The local bioremediation agent (LOFBA) formulated consisted of cow dung, chicken droppings and periwinkle shells. Four soil treatments (unpolluted soil, polluted soil with crude oil, polluted soil remediated with LOFBA and polluted soil remediated with NPK) were setup using completely randomized blocked design (CRBD). The microbial isolates were identified on the basis of cultural, morphological and biochemical characteristics. Physicochemical properties of the soil (pH, total nitrogen, sulphate, phosphorus, total organic carbon, moisture, exchangeable cations, heavy metals) were estimated using standard procedures while total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAH) were determined using the gas chromatography-mass spectroscopy (GCMS). The LOFBA had pH 5.6, high calcium (47.325%), Nitrogen (1.49%), phosphorus (0.26%), electrical conductivity (194.81µmho/cm) and high counts of bacteria and fungi. The microbial isolates identified were *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Acinetobacter aceti, Corynebacterium diphtheriae, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Soil remediated with LOFBA showed a significantly ($p < 0.05$) higher bacterial counts total nitrogen and exchangeable ions (K, Mg, Na and Ca) than other treatments. Heptadecane, pristine, octadecane, eicosane, herieicosane and hentriacontane were more highly degraded in LOFBA remediated soil than NPK remediated soil. Naphthalene was the only PAH present in all soil samples after six months although LOFBA remediated soil had the lowest concentration. Besides, Acenaphylylene was detected in crude oil contaminated soil and soil remediated with NPK while none was detected in soil remediated with LOFBA. The results also revealed that total petroleum hydrocarbon (TPH) from unamended soil decreased from 21.33 mg/kg to 16.61 mg/kg (22.13% degradation), from 15.18 mg/kg to 3.03 mg/kg (80.04% degradation) in LOFBA remediated soil while that of NPK remediated soil decreased from 18.70 mg/kg to 7.97 mg/kg (57.38% degradation) after six months. The results indicate that the locally formulated bioremediation agent (LOFBA) enhanced the recovery of the oil contaminated soil better than NPK fertilizer. LOFBA is therefore, recommended for oil spill remediation in the tropic.

Keywords: Restoration, oil spills, soil, Bioremediation agents.

INTRODUCTION
Crude oil is extremely complex mixture of a wide variety of low and high molecular weight hydrocarbons. This complex mixture contains saturated alkanes, branched alkanes, alkenes, naphthenes (homo-cyclics and hetero-cyclics) and aromatics (Abha and Singh, 2012). Crude oil also contains heavy metals and much of the heavy metal content of crude oil is associated with pyrrolic structures known as porphyrins (El-Sabagh *et al*., 2016). The high demand for, and use of petroleum and its products worldwide has made crude oil contamination a global problem with serious health and environmental impact (Alvarez *et al*., 2011; Musa *et al*., 2021). Oil spills occur due to factors such as corrosion of flow line and pipelines, over flow in tanks, hose failure, over pressure, rupture or burst of flow lines and pipelines, maintenance and operator error, sabotage and oil well blowout (Ijah and Antai, 2003a; Akpan *et al*., 2013; Ikhumetse *et al*., 2022; Musa *et al*., 2022).
In the Niger Delta of Nigeria, oil pollution and gas flaring have affected both land and water bodies. The impacts include loss in the productive capacity of soil, with implications on living organisms and economically on the people in the polluted area, and consequently, high poverty rate and unemployment (Akpan *et al*., 2013; Ikhumetse *et al*, 2022). Oil spill events in many countries outside Nigeria, especially in more technologically advanced countries attract rapid and adequate response for clean-up and remediation actions. On the contrary, in most developing countries including Nigeria, prolonged delays in the reclamation of oil impacted surfaces are experienced with adverse effects on the environment and agriculture. Hence remediation efforts on oil impacted areas in Nigeria should be geared towards the restoration of land to arable status. Remediation by chemical and thermal technologies are not environmentally friendly. Thus bioremediation is favored. Bioremediation is the use of biological processes to degrade, break down, transform or essentially remove contaminants or impairment quality from soil and water (Nnaemeka and Iyiegbu, 2015). Bioremediation entails the use of microorganisms or their products (bioaugmentation), nutrients (biostimulation), plants (phytoremediation), to reclaim crude oil contaminated environment (Nnaemeka and Iyiegbu, 2015). he use of chemical fertilizer is being replaced by the use of organic wastes due to inherent advantages. They are cheap to obtain and contain Nitrogen and Phosporus which are crucial elements in crude oil biodegradation (Ijah and Antai, 2003b; Idowu *et al*., 2020). The use of organic nutrients such as chicken droppings, cow dung, melon shells, cassava peels, soybeans waste and periwinkle shells, in oil spill bioremediation help to check environmental pollution problem created by the accumulation of these wastes in the environment (Ijah *et al*., 2013). Considering the adverse effects of oil spillage on the socio economic life and the environment of the oil bearing

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local communities and environs, the contaminated soil should be remediated with materials that are readily available and less expensive. Over the years the local waste materials used in this study for oil spill bioremediation were singly used in the laboratory and in the environment and they proved promising for bioremediation of crude oil polluted soil (Ijah and Antai, 2003b; Ijah and Ndana, 2003, Ijah *et al*., 2013; Idowu and Ijah, 2017; Idowu *et al*., 2020; Yahemba *et al*., 2022). It is therefore necessary to formulate these waste materials into a bioremediation agent and use in simulated oil spills in the field. The present study focused on the use of locally formulated bioremediation agent to restore oil contaminated soil.

MATERIALS AND METHODS

Description of Study Area

Simulated oil spill study was conducted in an uncontaminated environment in Ahoada East Local Government Area, Rivers State, Nigeria. Ahoada East is located northwest of Port Harcourt with its coordinate N 5°04'13" E 6°38'35. Its vegetation is mainly a high dense rain forest. The rainy season in the area is from April to November and dry season is from December to March. The area has a mean annual rainfall of 250 mm and average annual temperature of 26^{o}C (Ehirim and Nwankwo, 2010). Thus the occupations of the people are mainly farming, fishing, and hunting. Ahoada East Local Government Area has experienced several oil spills (Sweetcrude Report, 2016).

Collection and Processing of Samples

Crude oil free soil for the study was collected from a farm land in Ahoada East Local Government Area, Rivers State, Nigeria and transported in polythene bags to the site of experiment, stones, particles and plastics were removed from the soil before use.
Bonny light crude oil (BLC) used for the study was collected from Shell Petroleum
Development Company of Nigeria Limited (SPDC), Port Harcourt, Rivers State, Nigeria in clean jerry cans and transported to the experimental site. Periwinkle shells used for the study were collected from Nembe waterside in Port Harcourt, Rivers State, Nigeria in polythene bags and transported to the experimental site. The samples were incinerated to ash for ease of grinding into fine powder. The cow dung used for the study was collected from Central Abattoir, Port Harcourt and transported using plastics bucket to the experimental site. The samples were sun dried for 3 days before use. The chicken droppings used for the study were collected from poultry house (Battery cage system) in Ahoada, Rivers State, Nigeria in clean polythene bags and transported to the experimental site. The samples were sun dried for 3 days before use. The inorganic fertilizer used was NPK and was collected from Agricultural Development Project (ADP) in Ahoada East Local Government Area, Rivers State, Nigeria and transported to the experimental site.

Experimental Design and Treatment

The experimental design used was completely randomized block design (CRBD) and consisted of four treatment options, in plastic buckets, each bucket, containing 3kg of soil. The buckets were perforated to allow water drain out. The treatment options in duplicates included uncontaminated soil (control 1), crude oil contaminated soil (control 2), crude oil contaminated soil with locally formulated bioremediation agent (Coded LOFBA) formulated by combining varying amounts of periwinkle shells, cow dung and chicken droppings), and crude oil contaminated soil with inorganic fertilizer (NPK). Each bucket was polluted with two litres of crude oil except for the uncontaminated soil (control 1) and mixed properly. It was then allowed for two weeks to ensure proper seepage of the crude into the soil. After two weeks, 1kg of the LOFBA or NPK fertilizer was introduced into the crude oil polluted soil and mixed properly. A total of fifty six buckets were used. The moisture regime of the soil was maintained by adding water to the treatments at interval of 2 days. The experiments were exposed to the open field conditions for six months (Idowu and Ijah, 2017). The physicochemical and microbiological properties of the oil free soil and the locally formulated bioremediation agent (LOFBA) are presented in Table 1.

Table 1: Physicochemical and microbiological properties of soil and locally formulated bioremediation agent (LOFBA) used

Parameters	Soil	LOFBA
pH	7.7	5.6
EC (µmho/cm)	127.32	194.81
Moisture (mg/kg)	0.37	NA
TOC (%)	2.28	9.43
Total Nitrogen (%)	0.17	1.49
Sulphate (mg/kg)	1.3	0.2
Phosphate (mg/kg)	0.09	0.04
Phosphorus (mg/kg)	5.8	0.26
Nickel (mg/kg)	0.413	0.466
Vanadium (mg/kg)	0.823	0.641
Lead (mg/kg)	<0.001	0.391
Chromium (mg/kg)	<0.001	<0.001
Na (mg/kg)	6.876	3.420
Mg (mg/kg)	4.912	1.374
K (mg/kg)	5.208	1.609
Ca (mg/kg)	10.723	1621.325
TAHB (CFU/g)	5.48×10^4	8.74×10^3
TAHF (CFU/g)	3.93×10^3	6.27×10^2
Sand (%)	87.21	NA
Silt (%)	1.47	NA
Clay (%)	16.32	NA
Textural class	Sandy loam	NA

KEY: C- Electrical conductivity, TAHB- Total aerobic heterotrophic bacteria TF-Total fungi, µmho/cm micro - ohms per centimeter, mg/kg - milligramme per kilogramme, CFU/g-colony forming units per gramme, NA – not applicable

Enumeration of total aerobic heterotrophic bacteria

Soil samples were withdrawn every month for microbiological analysis for a total period of six months. Total aerobic heterotrophic bacteria (TAHB) were enumerated by the spread plate method on Nutrient agar (NA). Soil suspensions were prepared by 10 fold

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serial dilutions with 1g of soil, using normal saline as diluent. Zero point one milliliter (0.1 mL) aliquots of appropriate dilutions were spread on duplicate plates of sterile nutrient agar. The plates were incubated at 28°C ± 2°C for 48 hours (Ijah *et al*., 2017). Colonies that formed during this incubation period were counted and expressed as colony forming unit per gramme of soil (CFU/g).

Enumeration of hydrocarbon utilizing bacteria

The enumeration of hydrocarbon utilizing bacteria (HUB) was done using the vapour phase method **(**Chikere *et al*., 2009**)**. Appropriate dilutions of the samples withdrawn from the four treatment conditions were inoculated into modified mineral salt medium. The medium components were 0.42g $MgS0_4 \cdot 7H_2O$, 0.29g KCl, 0.83g KH_2PO_4, 0.42g $NaNO_3$, 1.25g Na_2HPO_4, 10g NaCl, 250mg Amphotericin B and 20 g Agar powder (Oxoid, Basingstoke, Hants, United Kingdom). These were weighed out and hydrated in 1000 mL of sterile distilled water in an Erlenmeyer flask. The medium was sterilized by autoclaving at 121°C, 15Psi for 15minutes before dispensing into sterile Petri dishes. The mineral salt agar (MSA) was inoculated with appropriate dilutions of the soil samples. Filter paper (Whatman No 1) was saturated with Bonny light crude oil and the crude oil impregnated papers were aseptically placed onto the covers and the Petri dishes inverted. The hydrocarbon saturated filter papers supplied hydrocarbon by vapour-phase transfer to the inoculum. The plates were incubated at 28°C ± 2°C for 7days and colonies were counted from duplicate plates and mean values were recorded in colony forming units per gramme (CFU/g).

Characterization and Identification of Bacterial Isolates

The characterization of bacteria was based on Gram's stain reaction, morphological characteristics and biochemical tests. The biochemical tests included indole, urease, oxidase production, motility, nitrate reduction, starch hydrolysis, catalase, fermentation of carbohydrates, citrate utilization and spore formation (Cheesbrough, 2000). The bacterial isolates were identified by comparing their characteristics with those of known taxa using Bergy's Manual of Determinative Bacteriology (Holt *et al*., 1999).

Determination of Total Petroleum Hydrocarbon (TPH)

Total petroleum hydrocarbon was determined using dichloromethane as described by Okparanma and Mouazen (2013). Twenty grammes of dried soil samples were weighed into 100 mL conical flask and 20g of activated anhydrous sodium sulphate and 20 mL of Dichloromethane were gently added into the flask containing the test soil sample. This was allowed to stand for 1 hour and then filtered into 50 mL conical flask. The procedure was repeated for the residual soil until a colourless solution was obtained. The equipment prior to analysis was calibrated with 5 level multi-state n-alkane mixtures to obtain calibration graphs for the individual analyte. The extracts were analyzed by gas chromatography (using Hp Agilent 7890A Gas Chromatography Agilent technologies, Berkshire, United Kingdom) equipped with two flame ionization detector (FID) detectors, an Agilent 7890A auto sampler and 5 capillary column (15 m x 0.25 mm) with a nominal film thickness of 0.25 µm, splitless injection method (all in batch). Injection volume was 1 µL and injection temperature gas program was started at 40°C and raised by 10°C/minute until 300°C was maintained for 8 minutes. Helium was used as a carrier gas (2 mL/min). The column was held at 35°C for 15minutes. Real values of TPH were calculated as product of raw data on FID Table or graph and dilution factor used for each sample:

$$\text{TPH (mg/kg)} = \frac{\text{Instrument reading x total volume of extract}}{\text{Weight of sample}}$$

Determination of Polycyclic aromatic hydrocarbons (PAHS)

The PAHs in the soil samples were determined using the gas chromatography mass spectroscopy (GCMS). The equipment prior to analysis was calibrated with 5 level 17 priority PAH mixtures to obtain calibration graphs for the individual analytes. Five grams of soil sample was weighed into a beaker and 10 mL of Dichloromethane was added and stirred properly for 10 minutes to ensure proper extraction and fractionated through a silica gel column to obtain the PAH fraction and concentrated to 1 mL. The samples were analysed by injecting 3mL of the sample extracts into the GCMS as described for TPH analysis (American Society for Testing and Materials, ASTM, 2005).

Determination of Rates of Biodegradation of Crude Oil in Soil

The hydrocarbon content of the soil samples collected from the unamended and remediated soils was determined gravimetrically by diethyl ether cold extraction method of Adesodun and Mbagwu (2008). Ten grammes (10g) of the soil was weighed into 250 ml flask and 50mL of diethyl ether was added, and shaken for 30 minutes in an orbital shaker. The diethyl ether extract was filtered with Whatman No. 1 filter paper, 1mL of the liquid phase of the extract was diluted with 9mL of diethyl ether and was measured at 420nm wavelength using spectrophotometer. The residual crude oil in the soil was estimated with standard curve derived from fresh crude oil diluted with diethyl ether (Ijah and Ndana, 2003; Adesodun and Mbagwu, 2008).

Statistical Analysis

All data generated were subjected to statistical package for Social Sciences (SPSS) using two way analysis of variance (ANOVA) to compare the means of the various treatments to establish significant relationship at ($p \geq .05$).

RESULTS

Bacterial counts in remediated soil

Figure 1 shows the counts of total aerobic heterotrophic bacteria (TAHB) in crude oil contaminated and remediated soils at different time intervals. The results revealed a progressive increase in TAHB counts with increasing time although, decreased counts were observed after the 5th and 6th month for crude oil contaminated soil and crude oil contaminated soil remediated with locally formulated bioremediation agent (LOFBA) and NPK fertilizer (Figure 1). Crude oil contaminated soil had the least counts (3.14±013 x 10 cfu/g) at the start of the experiment while the highest counts (8.91±0.09 x 10 CFU/g) was observed in crude oil contaminated soil remediated with LOFBA. Crude oil contaminated soil treated LOFBA had significantly ($p \leq 0.05$) higher counts of total aerobic heterotrophic bacteria than the NPK fertilizer treated soil. In all sampling months significantly ($p \leq 0.05$) lower microbial counts were observed in soil contaminated with crude oil except at the start of the experiment where the counts were comparable with those obtained in crude oil contaminated soil remediated with NPK (Fig 1).

Hydrocarbon utilizing bacterial (HUB) counts in crude oil contaminated soil, LOFBA treated soil and oil polluted soil remediated with fertilizer (NPK) are presented in Figure 2. The results revealed a progressive increase in bacterial counts with

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increase in time for crude oil contaminated soil remediated with LOFBA from the start of the experiment (1.21±0.03 x 10^4 CFU/g) through the 5th month (4.41±0.03 x 10^4 CFU/g), with a decline (3.94±0.06 x 10^4 CFU/g) at the 6th month. Crude oil contaminated soil and NPK fertilizer treated soil had progressive counts until the 3rd month. Crude oil contaminated soil, LOFBA remediated soil and soil treated with NPK fertilizer had peak HUB counts of 2.24±0.05 x 10^4 CFU/g , 4.41±0.03 x 10^4 CFU/g and 2.73±0.14 x 10^4 CFU/g respectively at the 5th month. LOFBA remediated soil had a significantly ($p \leq 0.05$) higher HUB counts in all sampling months compared to other soil treatments.

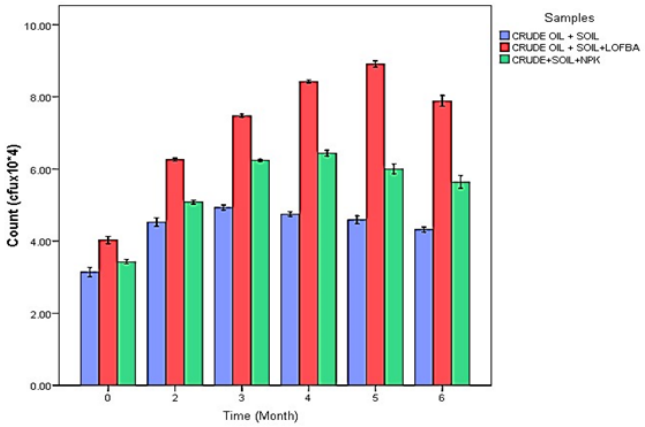

Fig.I: Counts of total aerobic heterotrophic bacteria in soil amended with locally formulated bioremediation agents

Occurrence of bacterial isolates

The bacteria isolated from the uncontaminated soil, crude oil contaminated soil and remediated soil samples were identified as species of *Bacillus*, *Acinetobacter, Escherichia, Aerococcus, Staphylococcus, Corynebacterium, Pseudomonas, Cellulomonas and Streptococcus*. The results showed that *Bacillus subtilis, Bacillus megaterium, Acinetobacter aceti*, *Corynebacterium diphtheriae, Pseudomonas aeruginosa, Bacillus cereus* and *Streptococcus pyogenes* were present in the four treatments (Table 1). Only LOFBA and NPK fertilizer remediated soils had *Aerococcus viridians* and *Bacillus sphaericus* while *Micrococcus varians, E. coli, A.viridans, B.sphaericus, Bacillus pumidus and Staphylococcus aureus* were absent in the oil contaminated soil sample (Table 1). *Staphylococcus aureus* was found in uncontaminated soil and NPK remediated soil while *Cellulomonas flavigena* was found in the crude oil contaminated as well as LOFBA remediated soils (Table 1). It was observed that crude oil contaminated soil remediated with LOFBA had a wider diversity of bacteria than other treatment.

Table 2: Occurrence of bacterial isolates in the various soil treatments

Bacteria	Unpolluted Control soil	Soil +Crude oil	Soil + Crude oil + LOFBA	Soil + Crude oil+ NPK
Aerococcus viridans	-	-	+	+
Bacillus sphaericus	-	-	+	+
Bacillus subtilis	+	+	+	+
Bacillus megaterium	+	+	+	+
Acinetobacter aceti	+	+	+	+
Staphylococcus aureus	+	-	-	+
Corynebacterium diphtheriae	+	+	+	+
Micrococcus luteus	-	+	+	-
Pseudomonas aeruginosa	+	+	+	+
Bacillus cereus	+	+	+	+
Bacillus pumilus	+	-	+	+
Micrococcus varians	+	-	+	+
Cellulomonas flavigena	-	+	+	-
Streptococcus pyogenes	+	+	+	+
Escherichia coli	+	-	+	+

+: Present, -: Absent, LOFBA: Locally formulated bioremediation agent

Total petroleum hydrocarbon (TPH) in crude oil and soil samples

Table 3 presents the concentrations of the various hydrocarbons present in crude oil, residual crude oil from contaminated soil and crude oil contaminated soils remediated with LOFBA and NPK fertilizer. The hydrocarbons included short (≤ C9), medium (C_{10} - C_{24}), long (> C_{24}) hydrocarbon chains, pristine and phytane. The hydrocarbon Tetracosane (C_{24}) had the highest concentration (41410.74 mg/kg) while the lowest was Pentatriacontane (C_{35}) (330.98 mg/kg) in crude oil. In crude oil contaminated soil, heptadecane had the highest concentration (2375.60 mg/kg) while undecane and octadecane had the lowest content (0.35 mg/kg). Soils remediated with LOFBA and NPK fertilizer revealed that tetradecane (C_{14}) and heptadecane (C_{17}) had high concentrations (20.93 and 166.53 mg/kg respectively) while nonadecane and tridecane had the least concentrations (0.17 and 0.28 mg/kg respectively). However, the remediated soils revealed reduced concentrations for most of the petroleum hydrocarbons. The results also showed a lower concentration (1.53 mg/kg) of dodecane in contaminated soil compared to the remediated soils. Heptadecane, pristane, octadecane, eicosane, heneicosane, hentriacontane were observed to have lower concentrations in LOFBA than NPK remediated soils while the reverse was revealed for tetradecane and nonacosane (Table 3). Generally, in crude oil contaminated soil and in remediated soils, the concentrations of the hydrocarbons detected decreased tremendously (Table 3).

Polycyclic Aromatic Hydrocarbon (PAH) Biodegradation

The polycyclic aromatic hydrocarbons (PAH) in crude oil (undegraded) and in residual crude oil from the soil treatments are presented in Table 4 The results revealed that naphthalene had the

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highest concentration (11.08 mg/kg) whereas Benz [a] anthracene and Benz [a] pyrene and pyrene had 0.02 mg/kg each which is the lowest concentration in Bonny light crude oil (BLC) analysed. There was an observable decrease in the concentration of some polycyclic compounds in crude oil contaminated soil while some were not detected (Table 4). It was observed that crude oil contaminated soils treated with LOFBA and NPK had almost all the polycyclic compounds removed except naphthalene which was present in both treatments in highly reduced amounts (0.10 and 0.15 mg/kg respectively) and acenaphthylene (0.15 mg/kg) which was detected only in NPK amended soil (Table 4).

Table 4: Polycyclic aromatic hydrocarbons (mg/kg) in crude oil extracted from crude oil contaminated and remediated soils after six months

Hydrocarbons	Crude oil	Crude oil + soil	Crude oil + soil + LOFBA	Crude oil +soil + NPK
Naphthalene	11.08	0.13	0.10	0.15
Acenaphthylene	0.37	0.02	N.D.	0.01
Flourene	0.21	0.02	N.D.	N.D.
Flouranthene	0.11	0.02	N.D	N.D.
Acenaphthene	0.09	N.D.	N.D.	N.D.
Phenanthrene	0.08	0.03	N.D.	N.D.
Anthracene	0.07	N.D.	N.D.	N.D.
Benz [b] flouranthene	0.05	N.D.	N.D.	N.D.
Benz [k] flouranthene	0.05	N.D.	N.D	N.D.
Benz [a] anthracene	0.02	0.01	N.D.	N.D.
Benz [a] pyrene	0.02	0.01	N.D.	N.D.
Pyrene	0.02	N.D.	N.D.	N.D.

N.D: Not Detected, LOFBA: Locally formulated bioremediation agent

Table 3: Hydrocarbons in residual crude oil extracted from contaminated and remediated soils after six months

Hydrocarbons	Treatments (mg/kg)			
	Crude oil	Crude oil + soil	Crude oil+ soil + LOFBA	Crude oil+ soil + NPK
Octane C_8	31753.95	0.35	2.97	0.39
Nonane C_9	27869.77	1.24	0.74	0.39
Decane C_{10}	28458.30	7.63	0.38	0.53
Undecane C_{11}	29538.01	0.35	0.60	0.85
Dodecane C_{12}	29055.15	1.53	23.21	44.46
Tridecane C_{13}	28589.87	70.67	2.91	0.28
Tetradecane C_{14}	966.67	60.70	20.93	2.87
Pentadecane C_{15}	27858.88	75.70	0.39	0.98
Hexadecane C_{16}	26167.14	116.15	0.43	3.41
Heptadecane C_{17}	35034.38	2375.60	0.26	166.53
Pristane	39101.37	102.35	0.24	11.70
Octadecane C_{18}	7565.99	1233.48	2.65	98.82
Phytane	7432.39	1211.70	0.24	3.08
Nonadecane C_{19}	23728.20	34.08	0.17	1.06
Eicosane C_{20}	26817.62	90.40	0.73	16.61
Heneicosane C_{21}	27298.55	1390.16	0.20	53.65
Docosane C_{22}	23590.80	1089.11	0.19	3.55
Tricosane C_{23}	23918.02	991.02	1.69	3.27
Tetracosane C_{24}	41410.74	1624.70	0.64	5.92
Pentacosane C_{25}	23138.02	893.31	0.22	0.98
Hexacosane C_{26}	20946.33	721.04	0.62	3.58
Heptacosane C_{27}	23517.58	739.11	0.31	3.23
Octacosane C_{28}	16996.14	583.41	0.96	2.32
Nonacosane C_{29}	16911.75	543.63	9.26	1.20
Triacontane C_{30}	11261.61	500.54	1.25	1.09
Hentriacontane C_{31}	15159.94	680.58	3.54	47.69
Dotriacontane C_{32}	6133.34	352.61	12.30	12.40
Tritriacontane C_{33}	5495.48	307.83	7.96	9.33
Tetratriacontane C_{34}	919.20	299.84	8.41	4.61
Pentratriacontane C_{35}	330.98	8.79	6.08	4.09

LOFBA **:** Locally formulated bioremediation agent

Bioremediation Rate for TPH in crude oil contaminated soil

Table 5 shows the residual total petroleum hydrocarbon (TPH) in the different soil treatments. The results showed that there was a time dependent decrease in total hydrocarbon content in the various soil treatments. The total petroleum hydrocarbon from unamended soil decreased from 21.33 mg/kg to 16.61 mg/kg (22.13
% reduction) while it decreased from 15.18 mg/kg to 3.03 mg/kg (80.04 % reduction) in LOFBA remediated soil after six month. Total petroleum hydrocarbon in NPK remediated soil decreased from 18.70 mg/kg to 7.97 mg/kg (57.38 % reduction) over the same period.

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Table 5: Bioremediation of total petroleum hydrocarbons (TPH) in remediated soils

	Weight of residual TPH (mg/kg)				
Soil treatments	**0**	**2**	**4**	**6**	***% reduction of crude oil content in soil within 6 months**
Soil + crude oil	21.33	18.68	17.24	16.61	22.13
Soil + crude oil + LOFBA	15.18	10.37	5.11	3.03	80.04
Soil + crude oil + NPK Fertilizer	18.70	15.05	9.54	7.97	57.38

*Weight of TPH start of experiment minus weight of residual TPH after six months divided by weight of TPH at the start of the experiment multiplied by 100.

DISCUSSION

The ability of microorganisms to transform organic compounds depends on the ubiquity of microorganisms, their metabolic potential as well as their metabolic versatility (Newman and Banfield, 2002). The bacteria isolated from the soil samples in the present study were similar to those reported in the works of Van Hamme *et al.* (2003); and Riffaldi *et al.* (2006). In contrast to the study of Akpe *et al.* (2015), Gram positive bacteria were predominant in the present findings. Reports by Amadi *et al.* (2012) suggested species of *Bacillus* and *Pseudomonas* to be more predominant in crude oil polluted soil and this agrees with the findings in this work. The fact that more genera of bacteria were detected in LOFBA amended soil than other treatments suggest that both the soil and LOFBA contributed the organisms. This is an advantage in terms of oil bioremediation in the soil because the number and variety of microorganisms can influence oil biodegradation in the soil (Yahemba *et al*., 2022).

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and the amount of the hydrocarbons present (Das and Chandra, 2011). The decrease in concentration of hydrocarbon fractions in crude oil contaminated soil might have been due to natural attenuation, since the environment has mechanism for the breakdown of contaminants which enter them (Ogbuehi *et al.,* 2011). More so, it has been reported (Ijah and Antai, 2003a; Ijah and Ndana, 2003; Yahemba *et al*., 2022) as well as confirmed in this study that uncontaminated soil samples have some microorganisms capable of degrading crude oil. Some of the listed compounds in Table 3 may not only come from crude oil but may be end products of oil degradation. The decrease in concentrations of the various crude oil fractions recovered from LOFBA and NPK remediated soils is in agreement with the findings of Marquez-Rocha *et al.* (2001) and Hidayat and Tachibana (2012) that some microorganisms preferred short and long chain hydrocarbons while some preferred middle chain compounds. The variations may be due to factors such as microbial type available in the environment (Cooney *et al.,* 1985). Often times, petroleum hydrocarbon compounds bind to soil components, as such are difficult to be removed or degraded (Barathi and Vasudevan, 2001). The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes (Ulrici, 2000; Das and Chandra, 2011). However, the composition of the crude oil fractions may determine the degradation of total petroleum hydrocarbon in soils (Yemashova *et al*., 2007; Idowu and Ijah, 2017). The effective degradation of crude oil in LOFBA and NPK remediated soils as observed in this study may be due to the presence of some important nutrients such as nitrogen and phosphorus in these compounds.

The increase in concentration of the crude oil fraction dodecane (C_{12}) is not consistent with the result reported by Nozari *et al.* (2014) who investigated the effect of cometabolism on removal of dodecane by microbial consortium from soil in a slurry sequencing bioreactor. The authors reported that the rate of growth of bacterial consortium decreased with increased concentration of dodecane. Omotayo *et al*. (2011) in their study of degradation of aviation fuel by microorganisms isolated from tropical soil reported that dodecane, which is a liquid alkane, was selectively utilized by the strains poorly or not at all. The increase in concentration of dodecane in polluted soils remediated with LOFBA and NPK may be due to the toxicity of the hydrocarbon which inhibited the hydrocarbon degraders.

The absence of some polycyclic aromatic compounds in soil contaminated with crude oil supports the report that uncontaminated soils may contain microorganisms capable of degrading crude oil (Adams, 2015). Similarly, Kom -Regonne *et al.* (2012) reported that microorganisms capable of degrading PAHs naturally occur in the environment.

Contaminated soil remediated with LOFBA and NPK revealed better utilization of PAHs as most of the PAHs were not detected with the exception of naphthalene and acenaphthylene at very low concentrations (Table 4). However, crude oil polluted soil treated with LOFBA showed better degradative potential than NPK treated soil as only naphthalene was detected. It may be due to the fact that LOFBA provided a better growing environment for PAH degraders. Reports by several authors have revealed the degradative ability of microorganisms on naphthalene (Pathak *et al.,* 2009; Lin *et al.,* 2010; Kom-Regonne *et al.,* 2012; Odukoya and Lambert, 2015). However, the present findings showed that naphthalene was reduced but not completely degraded like some other PAH compounds. This could be traced to the phenomenon of surface adsorption/desorption effects of naphthalene on soil organic carbon and interstitial voids within the micro and sub-micro pores of the soil particle. Reports have also shown that less than the total amount of some aromatics such as naphthalene adsorbed onto soil particles are actually desorbed and released (Nkedi-Kizza *et al.,* 2006; Owabor and Agarry, 2009; Owabor *et al.,* 2010; Owabor *et al.,* 2011). This may therefore, limits their bioavailability for microbial degradation. Another possible reason may be due to the low concentration of heavy metals. Owabor *et al.* (2011) reported that bacteria and fungi were capable of degrading naphthalene in soil in the presence of the different concentrations of heavy metals in soil. Lower concentrations of heavy metals have been reported to slightly inhibit enzyme production (D'Annibale *et al.,* 2005, Baldrian, 2008). Hence heavy metal concentration may be implicated in naphthalene not being completely degraded. The type of degrading microorganisms may be another possible reason as fungi have been reported to degrade naphthalene better than bacteria. This is because fungal mycelia have a high surface area, which can maximize both mechanical and enzymatic

contacts with insoluble substrates (Barnett and Hunter, 1998; Potin *et al.,* 2004; Idowu and Ijah, 2017).

The rate of petroleum hydrocarbon breakdown in unamended contaminated soil (22.13%) was lower than that reported by Edwinwosu and Albert (2010) who showed that a 60% reduction of hydrocarbons in crude oil contaminated soil after 640 days was obtained. The high rate of biodegradation (80.04%) observed in contaminated soil remediated with LOFBA, agrees with the study of Abioye *et al*. (2009) who reported that 75% oil degradation was obtained on treatment of crude oil contaminated soil with melon shell. The observed variations in results may be due to the textural properties of the soil, availability of nutrients which may be drawn from the type of bioremediation agent used as well as the degree to which natural attenuation may be occurring (Lorestani, 2014; Ikuesan *et al*., 2015; Yuniati, 2017; Ezugwu *et al*., 2022).

The decline in the rate of crude oil degradation observed at the sixth month is in agreement with the findings of Chorom *et al.* (2010) who reported that petroleum degradation was decreased with increasing time. This observation also corresponds with the result of microbial growth. With increasing time, microbial growth and oil degradation decreased probably as a result of the presence of toxic components of oil and less availability of nutrients for the microbes (Ijah and Antai, 2003a, Ijah and Abioye, 2003; Yahemba *et al*., 2022). Sang- Hwan *et al*. (2007) reported a 42-51 % reduction in oil-polluted soil treated with fertilizer, whereas, only 18% of the hydrocarbon was eliminated in untreated soil. This correlates with the findings of the present study. The higher rate of crude oil degradation observed in LOFBA treated soil indicates that it is a better remediation agent than the conventional inorganic fertilizer (NPK). This may be due to the availability of nutrients to sustain the oil degrading microorganisms over time as lack of organic feeding matter as well as appropriate nutrients could deter the effectiveness of the oil degrading organisms (Ijah and Antai, 2003b; Ijah and Ndana, 2003; Auta *et al .,* 2014; Idowu *et al*., 2020).

The significantly high total aerobic heterotrophic bacterial (TAHB) counts observed in LOFBA remediated soil which increased with time till the fifth month (Table 4.) is in agreement with the study of Abioye *et al.* (2009) who reported a higher aerobic heterotrophic bacterial counts with increase in time in contaminated soil amended with melon shells. It also corroborates with the findings reported by Westlake *et al.,* (1978). However, the findings of the study by Ijah and Antai (2003b) do not agree with the report that crude oil polluted soil remediated with NPK had TAHB counts greater than crude oil contaminated soil, although lower than LOFBA treated soil. It may therefore, be argued that the remediating agent (LOFBA) had constituents which favoured the growth of the bacteria better or it remediated the contaminated soil better thereby reducing the chance of the microbes being negatively affected by the petroleum hydrocarbons. The variations in results observed with other studies may be due to the constituent compounds of the bioremediation agent engaged, the duration of the study, nature of the soil or the amount of oil involved in the study.

The hydrocarbon utilizing bacterial (HUB) counts were higher in LOFBA treated soil than the NPK treated soil. The results are similar to the report of Idowu *et al*. (2020). The authors remediated crude oil contaminated soil using organic wastes and found an increase in total hydrocarbon utilizing bacterial counts. The high counts of hydrocarbon utilizing bacteria in LOFBA amended soil may be due to the availability of appreciable quantity of nitrogen and phosphorus in lofba. These elements are vital as well as important in enhancing biodegradative activities of the microbes (Akpe *et al.,* 2015). This locally formulated bioremediation agent may also possess the bulking property like some agro-wastes which aid in loosening the compactness of soil, hence, creating room for proper aeration for bacteria present in the soil and in turn improving their metabolic activities in the contaminated soil (Abioye *et al.,* 2009; Akpe *et al.,* 2015; Ezugwu *et al*., 2022).

Conclusion

The Niger Delta of Nigeria has the petroleum wealth of Nigeria and as a consequence, experiences frequent oil spills which devastate agricultural land. This study successfully formulated a bioremediation agent (coded LOFBA) from local waste materials. The LOFBA was rich in nitrogen, phosphorus and calcium which are crucial elements in oil spill bioremediation. Besides, the bioremediation agent harboured a lot of crude oil degrading microorganisms. The study also revealed high bacterial counts in oil polluted soil remediated with LOFBA. LOFBA showed a better degradation of hydrocarbons, C8-C35 and polycyclic aromatic hydrocarbons than its NPK counterpart. After six month of bioremediation with LOFBA, the bioremediation agent caused 80.04% crude oil biodegradation while NPK fertilizer caused 57.38% crude oil petroleum degradation. The locally formulated bioremediation agent (LOFBA) is therefore recommended for oil spill bioremediation in the Niger Delta of Nigeria.

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