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Microbiological and Physicochemical Characteristics of Soil Contaminated With Crude Oil in Port Harcourt

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ABSTRACT

One of the many catastrophes produced by mankind throughout history is the environmental deterioration that results from oil spills during extraction, processing, transportation, and corrosion of pipelines or damage. This study is aimed at assessing the microbiological and physicochemical characteristics of soil contaminated with crude oil and of an uncontaminated soil. The microbiological analysis revealed that total heterotrophic bacteria counts for the uncontaminated soil and contaminated soil was $4.77\pm0.47\times10^6$CFU/g and $5.2\pm2.80\times10^8$CFU/g respectively, Total Fungal counts was $4.3\pm0.96\times10^4$ and $5.1\pm1.11\times10^3$SFU/g respectively, Hydrocarbon Utilizing Bacteria counts $3.4\pm0.10\times10^4$ and $3.9\pm0.59\times10^3$CFU/g respectively, and Hydrocarbon Utilizing Fungal counts was $2.8\pm0.59\times10^4$ and $3.0\pm0.53\times10^3$ SFU/g respectively for uncontaminated and contaminated soil. The bacterial isolates identified from the uncontaminated soil were *Serratia* spp, *Aeromonas* spp, *Micrococcus* spp. *Bacillus subtilis* while *Bacillus mycoides,* C*hryseobacterium* spp, *Bacillus thuringiensis, Pseudomonas* spp. and *Staphylococcus* sp. from the contaminated soil. The fungal isolates identified from uncontaminated soil include *Aspergillus* spp., *Trichoderma* spp., *Colletotrichum* spp, and *Aspergillus* spp, *Aspergillus* spp. *Penicillum* spp, *Fusarium* spp. *Aspergillus spp, Candida* spp. *Trichophyton* spp. *Alternaria* spp. and *Rhizopus* spp from contaminated soil. The physicochemical parameters before and after crude oil pollution revealed pH to be 5.63±0.03 and 5.83±0.02, temperature 27.47±0.35 and 27.80±0.10, moisture content 12.17±0.11 and 45.77±0.25, electrical conductivity 78.33±0.25µS/cm and 19.43±0.21µS/cm. Nitrogen 9.15±0.03mg/kg and 3.63±0.04mg/kg, phosphorus 6.29±0.04mg/kg and 35.37±0.25mg/kg, potassium 12.17±0.05mg/kg and 22.47±0.15mg/kg, TOC was 0.65±0.03% and 0.37±0.02%e, SOM 1.15±0.03% and 0.69±0.01% for uncontaminated and contaminated soil respectively. It can be concluded that oil-degrading microorganisms are abundant in the soil contaminated with crude oil. This can be exploited for large oil-spill clean-up campaigns.

Keywords: Crude oil contaminated soil, *Bacillus subtilis*, *Pseudomonas* species, *Aspergillus*, *Penicillum* spp, physicochemical.

Introduction

All components of the ecosystem are seriously threatened by oil spills. Soil pollution is a result of the frequent spilling of crude oil and its refined products during the extraction, transportation, storage, and distribution processes (Chikere *et al.,* 2019). The physical, chemical, and biological characteristics of soil are changed when crude oil is incorporated into it. This has an impact on agricultural crops, particularly those that are less tolerant to oil, and soil fertility. The decomposition of soil organic matter, the production of humus, the cycling of nutrients, and the stimulation of plant development are all significantly influenced by soil microorganisms, mostly bacteria and fungi.

Hydrocarbons have an indirect or direct impact on soil microorganisms after entering the soil. Petroleum use indirectly raises soil surface temperatures, alters the amount of organic matter in the soil, disrupts the oxygen and water cycles, and reduces the availability of nutrients. The structure and operation of soil microbial communities are therefore altered by these changes (Fan *et al.,* 2014; Labud *et al.,* 2007).

Petroleum hydrocarbons can directly harm soil community members by disrupting membranes in general, damaging membrane functions, halting growth, and lysing cells (Lăzăroaie, 2010; Zahir, *et al.,* 2006).

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The growth encouragement of microbial communities that can breakdown or tolerate hydrocarbons is another direct effect of petroleum put into the soil. Although these organisms are present in all soils, it has been observed that their abundance and breakdown patterns rely on the physicochemical properties of the soil and its origin (Chikere *et al.,* 2019; Cho *et al.,* 2015). Petroleum hydrocarbons are known to be harmful to microorganisms. The number of microorganisms in a soil sample decreases as a result of crude oil contamination. According to Okoye and Okunrobo (2014), an oil leak may have had an impact on the decrease in soil microorganisms in the contaminated region due to the extreme acidity/alkalinity caused by the oil, many soil bacteria' behaviors are altered, and this might occasionally result in their eventual death. From one source to another, the makeup of crude oil, a mixture of tens of thousands of organic components, might differ. Accordingly, different sources may experience different repercussions from a crude oil spill. The spill's environment will have an impact on the specifics of any potential biological harm, though. Physical and chemical characteristics of soil can be affected by oil contamination. By lowering the amount of Phosphorus that is accessible and raising the pH of the soil, oil depletes the soil's fertility, which may hasten the effects of alkalescency on the wetlands in the semi-arid region (Wang *et al.,* 2013). In comparison to nearby control sites, hydrocarbon-contaminated soils frequently have a higher daily maximum surface temperature (Aislabie *et al.,* 2004). According to Townsend *et al*. (2003), Labud *et al.* (2007), and Sutton *et al.* (2013), oil typically promotes an anaerobic environment in soil by burying soil particles, preventing air diffusion in the soil pores, and impacting soil microbial populations. According to Lin and Mendelssohn (2012), severe crude oil contamination can result in the full extinction of marsh vegetation.

Additionally, compared to pristine areas, soils contaminated with crude oil are hydrophobic (Quyum *et al.,* 2002; Aislabie and 2004). In addition to altering soil pH (Hu *et al.,* 2006; Wang *et al.,* 2009; 2010) and other chemical properties, hydrocarbon contamination can also increase soil total organic carbon, as well as other soil parameters (Arocena and Rutherford, 2005; Kisic *et al.,* 2009). The aim of this research work was to determine the effect of crude oil on the microbiological and physiochemical characteristics of soil in Port Harcourt Rivers State, Nigeria.

Materials and Methods

Study Area and Sample Collection

This study was carried out in Port Harcourt, Rivers State University Rivers State, Nigeria. Soil samples were collected from Rivers State University Demonstration Farm with coordinates 4°48’3.59” N, 6°58’46.09”E.

Source of Crude Oil and Contamination of Soil

Bonny light crude oil was obtained from the Shell Petroleum Development Company of Nigeria Limited (SPDC) Port Harcourt Nigeria.

Soil samples were aseptically collected from the study area from between the depths of 0 to 15 cm using a sterile hand-held soil auger. Samples were bulked up into a composite sample in disinfected polythene bags and transported immediately to the Microbiology Laboratory at the Rivers State University for microbiological and physicochemical analyses. About 2000g of each soil sample was weighed into two different sterile containers labeled appropriately. One was evenly contaminated with 300ml crude oil (polluted soil) while the other was not contaminated with crude oil (unpolluted soil). Soil samples were immediately analyzed on the first day (Day 1), and thereafter at 2 weeks (14 days) interval for 8 weeks (56 days).

Microbiological Analysis of the Soil

Enumeration of Total heterotrophic Bacteria and Fungi count

The spread plate count method was used to determine the Total heterotrophic Bacteria (THB), Hydrocarbon and Fungi (F) in the soil was conducted with the following; About 1g of the soil samples were aseptically transferred into a 9.0ml normal saline. The weighed soil samples were subjected to a serial ten-fold dilution with a dilution factor ranging from 10-3 to 10-6 onto nutrient agar, an aliquot (0.1ml) of the appropriate dilutions were spread plated onto suitable agar medium in triplicate. For twenty-four hours, the plates were incubated at 37°C. The total heterotrophic bacterial (THB) counts were estimated from the colonies formed on nutrient agar, which were counted and described morphologically. For fungi aliquot of 0.1ml of the 10^{-3}dilution was inoculated onto Sabouraud Dextrose Agar (SDA) plate in duplicates.

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The inoculated plates were incubated for 48-72hrs at ambient temperatures (26-32°C). After incubation, the number of discrete colonies that formed were counted and used to calculate the total fungi in spore forming units per gram (SFU/g) soil.

Enumeration of Hydrocarbon Utilizing Bacteria and Fungi

For the enumeration of Hydrocarbon utilizing bacterial (HUB) an aliquot of 0.1ml of the 10^{-3} dilution was inoculated onto Bushnell-Haas agar (BHA) supplemented with Ketoconazole in duplicates. Inoculated plates were inverted and filter paper was placed inside the cover and was flooded with 1ml of sterile crude oil as source of carbon and energy. The plates were incubated at 30^{0}C for 5 to 7 days. Discrete colonies that developed were counted and used to calculate the HUB. To obtain pure cultures, the HUB colonies were subcultured onto NA. The ensuring pure cultures were characterized through physicochemical and biochemical tests so as to ascertain their identity.

While for Hydrocarbon utilizing fungi (HUF) an aliquot of 0.1ml of the 10^{-3} dilution was inoculated onto Bushnell-Haas agar (BHA) supplemented with chloramphenicol. The plates were inverted and filter papers were placed inside the cover and were flooded with 1ml of sterile crude oil as source of carbon and energy. The plates were incubated at 28^{0}C for 5 to 7 days. Discrete colonies that developed were counted and used to calculate the HUF. To obtain pure cultures, the HUF colonies were subcultured onto SDA. The ensuring pure cultures were characterized through macroscopic and microscopic examination (Odokuma *et al.,* 2008).

Characterization and Identification of Bacterial and Fungal Isolates

Each bacterial and fungal isolates obtained after isolation was characterized based on colonial, microscopic and macroscopy. Bacterial colonies were identified based on their color, morphology, elevation, size, and margin etc. Fungal growth on plate culture was observed, surface, spore, and underside color. Stained (lactophenol cotton blue) slide was examined using a microscope (x40) for structure of hyphae and details of sporulation structure (Devi, 2011). Discrete bacterial and fungal colonies were purified through sub-culturing.

The isolates were identified according to descriptions in the Bergeys Manual of Systematic (Holt, 2000) and recorded accordingly.

Purification and Stocking of Isolates

Following colony observation, the specific colonies of interest were selected from a mixed population and transferred using sterile straight wire loops onto the appropriate agar plates, also known as isolation plates. Using a sterile wire loop, the inocula were processed by streaking them across the surface of their respective agar plates. The plates were then incubated for 24 hours at 37°C for bacteria and for 48 hours at room temperature for fungi. Finally, purified colonies were kept in bijou bottles with 5% glycerol at 20°C (freezer) for storage.

Measurement of Physicochemical Parameters

Determination of pH

About 20g of each air-dried soil was weighed into 50ml beaker and 20ml of distilled water was added. It was stirred with a glass rod and allowed to stand for 30 minutes. Calibrated HANNA PH meter (Model Jenway 3510) was inserted into the liquid and pH recorded.

Determination of Moisture content

About 1g of sieved soil sample was weight into dry crucible. The crucible was then placed in an air circulated oven at 105oC and dried to constant weight (for 6 hours). The sample was cooled in a desiccator and re-weighed. The percentage air dried moisture from the loss weight was then determined as fellows (Nrior and Onwuka, 2017): % moisture content = Loss in weight x 100 initial weight.

Determination of Total Organic Carbon (TOC)

The method of Nrior and Onwuka (2017) was used in measuring the total organic carbon (TOC). One gram (1g) of the sample was transferred into a clean Pyrex conical flask. 5 ml of potassium chromate solution and 7.5 ml of concentrated sulphuric acid were added. The mixture was heated on an electro thermal heater for 15 minute to reflux. The sample was allowed to cool at room temperature and was diluted to 100mls with distilled water. 25ml of the sample solution was titrated with 0.2 molar ferrous ammonium sulphate using Ferrion as an indicator.

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A blank containing oxidant (potassium chromate) and sulphuric acid was titrated as in the sample. The titre value was recorded (EPA, 2012). The percentage of TOC was calculated as follows; / TOC = (Titre value of the blank – sample tire × 0.003×100/ sample weight).

Determination of Nitrogen

Total Nitrogen was determined by semi-micro Kjeldahl method using the method described by (Nrior and Onwuka, 2017).

Determination of Potassium

Digested samples from the Kjeldah analysis were made up to 50ml with distilled water. A standard potassium ion concentration was aspirated into the spectrometer's burner chamber to calibrate the equipment and to plot a graph of standard ion concentration. Wavelength used was 760nm. Prior to aspirating the sample, the aspirators tubing system in the spectrometer was flushed with water. The concentration of the potassium ion in the sample was automatically displayed on the screen of the spectrophotometer.

Determination Phosphorus

Digested samples from the kjeldah analysis were made up to 50ml with distilled water. A standard potassium ion concentration was aspirated into the spectrometer's burner chamber to calibrate the equipment and to plot a graph of standard ion concentration. Wavelength used was 690nm. Prior to aspirating the sample, the aspirators tubing system in the spectrometer was flushed with water. The concentration of the potassium ion in the sample was automatically displayed on the screen of the spectrophotometer.

Statistical Analysis

In order to compare the treatments and test whether the various nutrients given to the polluted soil were statistically significant at a level of 95% or P>0.05, data obtained from the bioremediation setup were subjected to statistical analysis using computer-based programs, SPSS version 25 for Analysis of Variance (ANOVA) and Microsoft Excel® on microbiological and physicochemical parameters.

Results

The results of the microbial counts of uncontaminated and contaminated soil is shown in Table 1. The uncontaminated and contaminated soil showed average counts of $4.77\pm0.47\times10^6$ and $5.2\pm2.80\times10^8$, CFU/g for Total heterotrophic bacteria, $4.3\pm0.96\times10^4$ and $5.1\pm1.11\times10^3$ SFU/g for Fungi.

While the uncontaminated and contaminated soil showed average counts $3.4\pm0.10\times10^4$ and $3.9\pm0.59\times10^3$CFU/g for Hydrocarbon utilizing bacteria HUB, $2.8\pm0.59\times10^4$ and $3.0\pm0.53\times10^3$ SFU/g for Hydrocarbon utilizing fungi, respectively.

Table 1: Microbial Counts of Uncontaminated and Contaminated Soil

Sampling	Microbial counts (CFU/g)							
	Total heterotrophic bacteria (×10^8CFU/g)		Total fungi (×10 SFU/g)		Hydrocarbon utilizing bacteria (×10 CFU/g)		Hydrocarbon utilizing fungi (×10^3SFU/g)	
	Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted
Day 1	5.2 ± 2.80^b	4.7 ± 0.47^a	5.1 ± 1.11^a	4.3 ± 0.96^a	3.9 ± 0.59^b	3.4 ± 0.10^a	3.0 ± 0.53^b	2.8 ± 0.59^a
Day 14	4.97 ± 0.51^{ab}	3.60 ± 0.40^c	5.23 ± 0.38^a	4.03 ± 0.40^{ab}	2.59 ± 0.37^a	2.17 ± 0.55^a	4.30 ± 3.98^a	3.23 ± 0.96^a
Day 28	3.67 ± 2.93^a	3.43 ± 2.61^a	2.87 ± 0.40^a	2.33 ± 0.35^a	2.35 ± 0.32^a	2.22 ± 0.50^b	1.60 ± 0.20^a	1.00 ± 3.02^a
Day 42	4.37 ± 1.01^a	4.13 ± 1.80^a	4.63 ± 0.38^a	4.43 ± 0.42^a	3.45 ± 0.34^a	3.01 ± 3.10^a	2.63 ± 1.06^b	1.53 ± 0.40^a
Day 56	3.13 ± 0.50^b	3.00 ± 2.10^a	5.11 ± 0.55^{ab}	5.01 ± 0.98^b	2.05 ± 0.77^a	1.27 ± 0.19^a	4.11 ± 3.25^a	3.03 ± 3.28^a
P value	0.446	0.341	0.322	0.356	0.371	0.012	0.035	0.026

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The physicochemical parameters of uncontaminated and contaminated soil are shown in Table 2. The pH is acidic with value of 5.63±0.03 for uncontaminated soil and 5.83±0.02 for contaminated soil. The temperature was 27.47±0.35^{O}C for uncontaminated while the contaminated was 27.80±0.10^{O}C. The moisture content was 12.17±0.11% and 45.37±0.25% respectively, electrical conductivity was 78.33±0.25µS/cm for uncontaminated soil and 19.43±0.21µS/cm for contaminated soil. Nitrogen was 9.15±0.03mg/kg for uncontaminated and 3.63±0.04 mg/kg for contaminated soil. The phosphorus was 6.29±0.04mg/kg and 35.37±0.25 mg/kg for uncontaminated and contaminated. The potassium was 12.17±0.05 mg/kg for uncontaminated and 22.47±0.15 mg/kg for contaminated. TOC was 0.65±0.03% and 0.37±0.02% for contaminated soil, SOM was 1.15±0.03% and 0.69±0.01% for contaminated and uncontaminated soil respectively and THC for uncontaminated soil was 353.51±2.03 mg/kg and for contaminated soil was of 14576.56±0.073 mg/kg.

Table 2: Physicochemical Parameters of Uncontaminated and Contaminated Soil

Parameters	Unpolluted soil	Crude oil polluted soil
pH	5.63±0.03	5.83±0.02
Temperature (^{o}C)	27.47±0.35	27.80±0.10
Moisture content (%)	12.17±0.11	45.37±0.25
Electrical conductivity (µS/cm)	78.33±0.2	19.43±0.21
Nitrogen (mg/kg)	9.15±0.03	3.63±0.04
Phosphorus (mg/kg)	6.29±0.04	35.37±0.25
Potassium (mg/kg)	12.17±0.05	22.47±0.15
Total Organic Carbon (%)	0.65±0.03	0.37±0.02
Soil Organic Matter (%)	1.15±0.03	0.69±0.01

The results of the morphological and biochemical characterization of the bacteria isolates are presented in Table 3 and 4. Based on the results, the bacterial isolates are suspected as follows: *Serratia* spp. *Micrococcus* spp., *Aeromonas* spp. *Bacillus mycoides, Chryseobacterium* spp. *Bacillus thuringiensis, Pseudomonas* spp., *Staphylococcus* sp. and *Bacillus subtillis*. Results of the macroscopic and microscopic characterization of the fungal isolates were; *Trichoderma* spp., *Aspergillus niger, Penicillium sp, Aspergillus* spp. *Fusarium* sp. *Colletotrichum* spp. *Rhizopus* spp. *Alternaria* spp., *Aspergillus* spp. *Trichophyton* spp. and *Aspergillus* spp.

Table 3: Morphological and Biochemical Characteristics and Probable Identity of Bacteria Isolated US and CS

Isolate Code	Morphology	Gram's Reaction	Catalase	Oxidase	Citrate	Motility	Indole	Methyl Red	Voges Proskauer	Glucose	Lactose	Sucrose	Mannitol	Probable Organism
US-POG1	Rods tiny, pale, raised and smooth	-ve rods	+	+	-	+	+	-	+	A	A	A	A	*Serratia* spp
US-POG2	Creamy circular with entire and fimbrate edges	-ve rod	+	+	+	+	+	-	+	A G	-	-	-	*Aeromonas* spp.
CS-POG3	Creamy, opaque rhizoid shape	+ve rods	+	-	+	-	-	+	+	A	-	A	-	*Bacillus mycoides*
CS-POG4	Small grayish-white, slightly mucoid	+ve cocci	+	-	-	-	-	-	+	A	A	A	-	*Staphylococcus* spp
US-POG5	Small clustered cocci	+ve rod	+	+	-	-	+	+	+	A	+	A	A	*Micrococcus* spp
CS-POG6	Small rough colony, wrinkled with radiant crest	-ve rod	+	+	+	-	-	-	-	-	-	-	A	*Pseudomonas* spp.
CS-POG7	Small circular dark yellow, smooth	-ve rod	+	+	-	-	+	+	+	-	A	A	A	*Chryseobacterium* spp
US-POG8	Milky cream round dull surface	+ve rods	+	+	+	+	-	-	+	A	A	A	A	*Bacillus subtilis*
CS-POG9	Milky serrated flat	+ve rods	+	-	+	+	-	+	+	A	A	A	A	*Bacillus thuringiensis*

Key: AG= acid and gas, A= acid, += positive, -= negative, US- Uncontaminated soil, CS- Contaminated soil

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Table 4: Macroscopic and Microscopic Features of Fungi Isolated from Uncontaminated Soil and Crude Oil Contaminated Soil

Isolate code	Colony Morphology	Microscopic Characteristics	Identity of Fungi
US-GOF1	Green dusty growth with white patches and brown reverse	Paired branches like pyramid, with dense conidia branced conidiophores	*Trichoderma spp*
CS-GOF2	Black cottony growth, brown reverse	Septate hyphae bearing globose conidiophores with scattered conidia	*Aspergillus* spp
CS-GOF3	Milk colour cottony growth with radial surface and yellow reverse	Columnar bunched philiades branching conidia, with septate hyphae	*Aspergillus* spp
CS-GOF4	Radially furrowed blue-green velvety growth with white periphery and yellow reverse	Septate hyphae, with branched conidiophores bearing phialides. Conidia, arranged in chains on the phialides.	*Penicillum* spp
CS-GOF5	Gray colour fluffy growth with black spores and brown reverse	Non-septate hyphae with round conidia head	*Rhizopus* spp
CS-GOF6	Greyish to blackish suede-like and black reverse	Septate hyphae branched and oblong septate conidia head	*Alternaria* spp
CS-GOF7	Creamed coloured, smooth round glistening colonies	Oval shaped cells	*Candida* spp
US-GOF8	White fluffy growth with brown reverse	Septate hyphae branched round shaped spores	*Colletotrichum* spp
US-GOF9	Lemon dark green, radial symmetry white periphery	Radiate conidial heads, septate long hyphae	*Aspergillus* spp
CS-GOF10	White cottony lawn like growth, with reverse yellow colour	Septate hyphae, with presence of banana shaped septate conidia	*Fusarium* spp.
CS-GOF11	White lawny growth with radial surface and red reverse side	Septate branching hyphae, with microconidia formed along hyphae	*Trichophyton* spp
CS-GOF12	Dark green and dark brown color on the revers	Hyphae are septate and hyaline, columnar conidial heads.	*Aspergillus* spp

Discussion

Results of the microbial population of the crude oil uncontaminated and contaminated soil showed the total heterotrophic bacterial count (THBC) ranged from 4.7×10^8 to 2.24×10^9 CFU/g. The increased bacterial count which was observed in the sample uncontaminated with crude oil was due to the soil not being contaminated with crude oil, which in disagrees with the findings of Okerentugba and Ezeronye where their findings showed higher bacterial count for the contaminated soil as a result of increased hydrocarbon content. Fungal count ranged from 4.3×10^4 to 5.1×10^4 for uncontaminated and contaminated soil.

The hydrocarbon utilizing bacteria (HUB) ranged from 3.4 to 3.9 $\times10^4$ and the hydrocarbon utilizing fungi (HUF) had the least population ranging from 2.8 to 3.0 $\times10^4$. There was a significant difference ($p\leq0.05$) across the soil microbial counts. The increases in the hydrocarbon-utilizing bacterial and fungal counts should have resulted in the removal of hydrocarbons resulting in the decrease in the value. But because the oil is discharged continuously into the surrounding environment the level of the hydrocarbons was not affected hence the difference in the value between the contaminated soil and the control (Vieira, 2020).

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The uncontaminated soil had the highest population of total heterotrophic bacteria at all counts as also revealed in the study of Williams and Hakam (2016). It is known that crude oil is toxic and seriously affect soil productivity and cause different degrees of harm to microorganisms (Mambwe *et al.,* 2021), only those that could decompose it and survived contamination lived after the decline. A study by Akpoveta *et al.* (2011) found a positive correlation between this observation and a decrease in the colony forming unit of microorganisms following crude oil contamination.

When natural environments are contaminated with pollutants the indigenous microbial communalities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating waste. Degradation of macromolecules in waste to smaller molecules is enhanced by soil microorganisms which produce a tremendous range of potentially useful enzymes that help in breaking down or decomposition of these macromolecules. Most Studies have shown that oil degrading microbes are abundant and are not limited to oil contaminated areas (Okoh, 2006).

Soils have been a favorable habitat for the proliferation of microorganisms, but the addition of refractory humic substances slows down the activities of these microorganisms, thus giving room to diverse bacterial and fungal species that have evolved the metabolic capacity to degrade hydrocarbons (Wu *et al.,* 2019). In this study, similar bacteria were recovered from both the control and contaminated samples apart from few species. The bacterial isolates obtained were characterized based on their biochemical, morphological (colour, elevation, edge, surface, and optic characteristics) properties and they belong to the genera: Serratia spp. Micrococcus spp., Aeromonas spp. Bacillus mycoides, Chryseobacterium spp. *Bacillus thuringiensis, Pseudomonas* spp., *Staphylococcus* sp. and *Bacillua subtillis*. The fungal isolates from obtained were characterized based on their microscopic and macroscopic properties and they belong to the genera: *Trichoderma spp., Aspergillus niger, Penicillium sp, Aspergillus* spp. *Fusarium sp. Colletotrichum* spp. *Rhizopus* spp. *Alternaria* spp., *Aspergillus* spp. *Trichophyton* spp. and *Aspergillus* spp. This is consistent with other studies that found comparable bacterial and fungal isolates from soils contaminated by crude oil (Williams and Ambrose, 2023; Aleruchi and Dike, 2022; Chikere *et al.,* 2009; Talat *et al.,* 2015).

The physical and chemical characteristics of the contaminated soil sample revealed the presence of crude oil hydrocarbon components in the soil. The observed outcome exhibits a positive correlation with the research conducted by Ogbonna and Amajuoyi (2009), who examined the physical and chemical characteristics of a site, contaminated with crude oil and concluded that the contamination caused the values of the parameter to exceed the designated restrictions (BIS, 2012). The physicochemical properties of the soil before and after crude oil contamination showed that there was an increase in the pH and temperature after contamination. A reduction in pH for the soil implied slight acidity, this concurs with the study of Williams and Amaechi (2017), which is a problem for agricultural soils (Otterson, 2015; Abate *et al.,* 2017). The type of microorganisms that participate in hydrocarbon degradation is determined by the pH of the soil (Chen *et al.,* 2015). Bacteria have limited tolerance for acid conditions and fungi are more tolerant (Aguilera, 2013). Since the pH in this study was at low pH, it could be assumed that fungi were more involved in the degradation of the oil.

The observed reduction in conductivity correlated positively with the findings of Osuji and Nwonye (2007). Reduced conductivity could be due to the non-polar nature of the crude oil bringing about reduced ionic movement in the soil. Moisture content increased from 12.7 to 45.37%. Nutrient parameters such as nitrogen decreased from 9.15 to 3.63mg/kg, potassium and phosphorus increased after crude oil contamination, correlating with the study of Ogbonna *et al.* (2019). Total Organic Carbon (TOC) and Soil Organic Matter (SOM) both reduced.

In conclusion, this study showed that, bacteria had the highest microbial counts when compared to that of fungi. This shows the abundance of bacterial in soil. Nine (9) bacterial were isolated and identified from both uncontaminated and contaminated soil which include: *Serratia spp, Aeromonas spp, Micrococcus* spp. *Bacillus subtilis* while *Bacillus mycoides,* C*hryseobacterium spp, Bacillus thuringiensis, Pseudomonas* spp. and *Staphylococcus* sp. from the contaminated soil. While twelve (12) fungi species were isolated and identified which include *Aspergillus* spp.*, Trichoderma* spp.*, Colletotrichum* spp, from uncontaminated soil *and Aspergillus spp, Aspergillus* spp. *Penicillum* spp, *Fusarium* spp. *Aspergillus spp, Candida* spp. *Trichophyton* spp. *Alternaria* spp. and

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Rhizopus spp from contaminated soil. The study revealed that fungal species were isolated more than the bacterial species. This reveals fungi are more tolerant soil pH that is acidic.

The alterations in the physicochemical characteristics were a direct result of the crude oil's toxicity. These unfavorable circumstances have had a detrimental effect on the soils and may have an impact on nutrient cycles, which may then have an impact on the surrounding vegetation. It can be inferred that the soil contaminated with crude oil contains a large number of microorganisms that break down oil which large-scale campaigns to clean up oil spills can employ.

References

Abate, E., Hussein, S., Laing, M., & Mengistu, F. (2017). Soil acidity under multiple land-uses: Assessment of perceived causes and indicators, and nutrient dynamics in small-holders' mixed-farming system of northwest Ethiopia. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science, 67*(2), 134–147.

Aguilera, A. (2013) Eukaryotic organisms in extreme acidic environments, the Río Tinto Case. *Life, 3,* 363–374.

Aislabie, J. M., Balks, M. R., Foght, J.M. & Waterhouse, E. J. (2004). Hydrocarbon spills on Antarctic soils: Effects and management. *Environmental Science & Technology*, 38(5), 1265–1274.

Akpoveta, O. V. Okoh, B. & Osakwe, S. A. (2011). Quality Assessment of Borehole Water used in the Vicinities of Benin, Edo State and Agbor, Delta State of Nigeria. *Environmental Science, Chemistry*, 34, 45-55.

Aleruchi, O. & Dike, C.L. (2022). Fungal Population in Farming Soils in a Community in Rivers State. *Journal of Biology and Genetic Research (JBGR), 8(1),* 28-42.

Arocena, J.M. & Rutherford, P.M. (2005). Properties of hydrocarbon-and salt-contaminated flare pit soils in northeastern British Columbia (Canada). *Chemosphere, 60(4),* 567-75

Bureau of Indian Standards (BIS) (2012). *Specification of drinking water. Indian Standard Institute,* New Dehli, India.

Chen, M., Xu, P., Zeng, G., Yang C., Huang D. & Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnology Advances, 33,* 745–755.

Chikere, C. B., Tekere, M. & Adeleke, R. (2019). Enhanced microbial hydrocarbon biodegradation as stimulated during field-scale landfarming of crude oil-impacted soil. *Sustainable Chemistry and Pharmacy,* 14, 1–11.

Chikere, C.B., Okpokwasili, G.C. & Chikere, B.O. (2009). Bacterial diversity in a tropical crude oil polluted soil undergoing bioremediation. *African Journal of Biotechnology, 8(11),* 2535-2540.

Cho, C., Choi, S. Y., Luo, Z. W. & Lee, S. Y. (2015). Recent advances in microbial production of fuels and chemicals using tools and strategies of systems metabolic engineering. *Biotechnology Advances,* 33, 1455–1466.

Devi, M. A. (2011). *Slide culture technique for fungi. www.vlab.amrita.edu.* Accessed 13/07/2023.

Doro, K. O., Kolapkar, A. M. & Becker, A. M. (2021). Using shallow subsurface geophysical models to guide restoration of old agricultural fields in northwestern Ohio. *In: SEG/AAPG/SEPM first international meeting for applied geoscience & energy, Texas,US.*

EGASPIN (2002). *Environmental guidelines and standards for the petroleum industry in Nigeria (EGASPIN) from Department of Petroleum Resources (DPR).* Revised Edition. *pp.* 277-288.

Fan, M. Y., Xie, R. J. & Qin, G. (2014). Bioremediation of petroleum-contaminated soil by a combined system of biostimulation-bioaugmentation with yeast. *Environmental Technology, 35,* 391–399.

Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology. 9th edn. Williams and Wilkins: A waverly company,* Baltimore Maryland, USA. *pp*. 73-589.

Hu, Y., Wang, S. & Yan, S. (2006). Research advances on the factors influencing the activity and community structure of soil microorganism. *Chinese Journal of Soil Science*, 37(1), 170–176.

Ijah U. J. J., Safiyanu, H. & Abioye, O. P (2008). Comparative study of biodegradation of crude oil in soil amended with chicken droppings and NPK fertilizer. *Science World Journal, 3(2),* 2.

Ijah, U. J. J. & Antai, S. P. (2003). The potential use of chicken-drop microorganisms for oil spill remediation. *Environmentalist, 23,* 89–95.

Kisic, I., Ferdo Basic, Brkic, V., Mesic, M., Durn, G., Zgorelec, Z. & Bertovic, L. (2009). The effect of drilling fluids and crude oil on some chemical characteristics of soil and crops. *Geoderma, 149(149),* 209-216.

Labud, V., Garcia, C. & Hernandez, T. (2007). Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere, 66(10),* 1863–1871.

Lăzăroaie, M. M. (2010). Multiple responses of gram-positive and gram-negative bacteria to mixture of hydrocarbons. *Brazilian Journal of Microbiology, 41,* 649–667.

Lin, Q. & Mendelssohn, I. (2012). Impacts and recovery of the Deepwater Horizon oil spill on vegetation structure and function of coastal salt marshes in the northern Gulf of Mexico. *Environmental Science and Technology,* 46(7), 3737-43.

Mambwe, M., Kalebaila, K.K. & Johnson, T. (2021). Remediation technologies for oil contaminated soil. *Global Journal of Environmental Science Management, 1,* 1–20.

Nrior, R. R. & Onwuka N. F. (2017). Bioremediation of Crude oil Contaminated Marshland Muddy Soil by Bioaugmentation Approach using *Candida tropicalis* and *Penicillium chrysogenum. Journal of Environmental Science, Toxicology and Food Technology, 11 (10),* 2319-2399.

Nwogu, T. P., Azubuike, C. C. & Ogugbue, C. J. (2015). Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons after Amendment with *Capra aegagrus hircus* (Goat) Manure. *Biotechnology Research International, 1,* 1-7.

Odokuma, L. O. & Ibor, M. N. (2008). Nitrogen fixing bacteria enhanced bioremediation of a crude oil polluted soil. *Global Journal of Pure and Applied Sciences*, 86, 455-468.

Ogbonna, D. N., Nrior, R. R. & Ezinwo, F. (2019). Bioremediation Efficiency of *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* with the Nutrient Amendment on Crude Oil Polluted the Soil. *Microbiology Research Journal International, 29(5),* 1-13,

Ogbonna, J.F. & Amajuoyi, C.A. (2009). Physicochemical Characteristics and Microbial Quality of an Oil Polluted Site in Gokana, Rivers State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 13(3), 99-103.

Okerentugba, P. O., & Ezeronye, O. U. (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *African Journal of Biotechnology,* 2(9), 288-292.

Okoh, A.I. (2006). Biodegradation alternatives in the cleanup of petroleum hydrocarbon pollutants, *Biotechnology and Molecular Biology Review, 1 (20),* 38-50.

Okoye, C.O. & Okunrobo, L. A. (2014). Impact of oil spills on land and water and its health implications in Odu-Gboro community, Sagamu, Ogun State, Nigeria. *World Journal of Environmental Sciences and Engineering*, 1, 1-12.

Osuji, L.C., Egbuson E.J.G. & Ojinnaka C.M. (2005). Chemical reclamation of crude-oil- inundated soils from Niger Delta, Nigeria. *Chemical Ecology, 21(1),* 1-10.

Otterson, D. W. (2015). Tech Talk: (11) pH Measurement and Control Basics. *Measurement and Control, 48 (10),* 309–312.

Quyum, A., Achari, G. & Goodman R.H. (2002). Effect of wetting and drying and dilution on moisture migration through oil contaminated hydrophobic soils. *The Science of the Total Environment, 296(1-3),*77-87.

Sutton, N. B., Maphosa, F., Morillo, J. A., Abu Al-Soud, W., Langenhoff, A.A., Grotenhuis, T., Rijnaarts, H.H. & Smidt, H. (2013). Impact of long-term diesel contamination on soil microbial community structure. *Applied and Environmental Microbiology*, 79(2), 619–630.

Talat, Y.M., Abdul, W., Safia, H.P., Syed, A.S., Muneera, N. & Zaid, A.P. (2015). Isolation and characterization of hydrocarbon degrading bacteria from petrol contaminated soil. *Journal of Basic and Applied Sciences, 11,* 223-231.

Townsend, G. T., Prince, R. C. & Suflita, J. M. (2003). Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. *Environmental Science and Technology,* 37(22), 5213–5218.

Vidali, M. (2001). Bioremediation: An overview. *Pure and Applied Chemistry, 73,* 1163–1172.

Vieira, A. (2020). Bacterial colonization of minerals in grassland soils is selective and highly dynamic. *Environmental Microbiology, 22 (3),* 917–933.

Wang, X. Y., Feng, J. & Zhao, J. M. (2010). Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. *Environmental Monitoring and Assessment, 161(1),* 271–280.

Wang, X., Feng, J. & Wang, J. (2009). Petroleum hydrocarbon contamination and impact on soil characteristics from oilfield in Momoge wetland. *Environmental Science, 30(8),* 2394–2401.

Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang, X., & Wang, G. (2013). Effects of crude oil contamination on soil physical and chemical properties in Momoge wetland of China. *Chinese Geographical Science, 23(6),* 708–715.

Williams, J. O. & Amaechi, V. C. (2017). Bioremediation of hydrocarbon contaminated soil using organic wastes as amendment. *Current Studies in Comparative Education, Science and Technology*, 4(2), 89-99.

Williams, J. O. & Ambrose, F. C. (2023). Pigeon droppings as compositing amendment for enhancement of the biostimulation of soil artificially polluted with diesel oil. *Journal of Advances in Microbiology Research, 4(2),* 112-121

Williams, J. O. & Hakam, K. (2016). Microorganisms associated with dump sites in Port Harcourt metropolis. *Journal of ecology and Natural Environment, 8(2),* 9-12.

Wu, M., Wu, J., Zhang, X. & Ye X. (2019). Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere, 237,* 124456.

Zahir, Z., Seed, K. D. & Dennis, J. J. (2006). Isolation and characterization of novel organic solvent-tolerant bacteria. *Extremophiles, 10,* 129–138.

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