Research article

Achife, C. E., et al., Microbial Population of Soil and Water around Petroleum Depot Suleja, Nigeria, and their Hydrocarbon Utilization. International Journal of Life Sciences and Biotechnology, 2021. 4(1): p. 90-113. DOI: 10.38001/ijlsb.791853

Microbial Population of Soil and Water around Petroleum Depot Suleja, Nigeria, and their Hydrocarbon Utilization

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ABSTRACT

Contamination by petroleum products is a common occurrence in various depots in Nigeria. Suleja depot in recent times has recorded several petroleum spillages and fire incidence attributed to petroleum products storage and distribution. This study was aimed at determining the microbiological quality of soil and water sources in communities around petroleum products depot in Suleja, Nigeria**.** Soil and water samples were collected from petroleum depot and the five communities around the petroleum products depot and a control site. Microorganisms in the soil and water samples were enumerated by spread inoculation on general purpose media and selective media. Bacterial and fungal isolates were tested for their potential to utilize petroleum products in a Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) as a source of carbon and energy. The utilization rate was determined by spectrophotometry. The capacities of selected bacterial and fungal isolates to mineralize crude oil were further tested in minimal salt medium. The bacteria isolated were *Staphylococcus aureus, Streptococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli*. The microbial isolates were not evenly distributed in the six experimental and control plots. Soil samples had higher aerobic heterotrophic bacterial counts than the water samples. Crude oil was most utilized by the microbial isolates. Bacterial isolates from genera *Pseudomonas* and *Bacillus* had the highest capacity in utilizing the petroleum products. Among the fungal species, *Aspergillus niger* and *Penicillium notatum* exhibited greater capacity to utilize the petroleum products. Present study revealed isolates capable of utilizing the various petroleum products which can be useful in oil spill bioremediation in the tropical environments.

ARTICLE HISTORY
Received
10 September 2020
Accepted
20 October 2020

KEYWORDS
Bacteria,
fungi,
soil,
water,
hydrocarbons

Introduction

Crude oil pollution is currently considered to be a great threat to the health of living things in the environment including humans. Nigeria records an average of 300 oil spills in the oil producing States annually, making the Niger Delta regions the most polluted part of Nigeria, affecting the air, soil and water bodies [1]. Apart from the Niger Delta regions, other places that serve as depots for petroleum products such as diesel, premium motor

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spirit, and kerosene among others have in one way or the other encountered oil spills during transportation or storage either accidentally or due to human error contributing to soil and water pollution [2,1].

Crude oil also known as petroleum is composed of saturated alkanes and cycloalkanes, unsaturated alkynes and alkenes, as well as polycyclic aromatic hydrocarbons (PAHs) [3]. PAHs are difficult to degrade from the environment and their presence in the environment has been proven to have debilitating effects on living things [3]. Nwakanma *et al*. [2] reported that Nigerian crude oil could be hepatotoxic and hemotoxic which can cause cancer and infertility. Furthermore, crude oil has been reported to contain heavy metals and their accumulation in plant or animal tissues can cause mutation or even death depending on the dose and period of exposure [1, 4]. Oil spills have adverse effects on the environment, productivity of farmlands, water bodies and birds of the sky as well as microbial community and their distribution in the environment compared to a pristine environment [5].

Nigeria has 22 Nigerian National Petroleum Corporation/Pipeline and Products marketing Company (NNPC/PPMC) depots, saddled with the responsibility of transportation, storage and marketing of petroleum products such as; Premium Motor Spirit (PMS), Automotive Gas Oil (AGO) and Dual Purpose kerosene (DPK) in the domestic and efficient evacuation of refined petroleum products from the local refineries. The operation of the Nigerian Pipelines and Storage Company Ltd (NPSC) started in 1979 [6]. There are total of twelve storage tanks in Suleja depot in Niger State. Among these storage thanks, 4 tanks are for PMS, 4 storage tanks for AGO, and 4 storage tanks for DPK. Each storage tank of PMS, AGO, and DPK has a storage capacity of 12.6million, 7.6million and 7.6million liters respectively. These storage tanks are prone to leakages and washing which may lead to contamination of the soil and water in the surrounding communities.

Microorganisms are ubiquitous, and when faced with extreme conditions, some still find a way of surviving in the environment through various means of adaption such as alteration in membrane permeability, change of metabolic pathways, spore formation, mutation among others [5, 7]. Once faced with extreme conditions as is the case of crude oil pollutants, some microorganisms survive and eventually thrive in the environment utilizing

the hydrocarbons in the petroleum products as a source of carbon and energy. Others may assume a dormant form whereas those microorganisms that cannot withstand this extreme exposure die leaving the promising ones, which have been sought for in carrying out bioremediation process[2].

From the exploration, distribution and storage of crude oil and its products, several incidences of oil spillage have occurred, which caused huge adverse effects on health safety and the environment [8,9]. Several attempts have been tried to clean up oil spillage from the environment, but bioremediation is preferred [10]. Bioremediation is a process that involves the use of living microorganisms or their enzymes in detoxifying and degrading environmental pollutants, thus, restoring a polluted environment. The microbial population and activities in the affected environment such as microbial counts, respiration, biomass diversity, and enzyme activities can be used to evaluate the extent of bioremediation [1, 11]. Microbial biodegradation of petroleum contaminants is cheap and environmentally friendly and can be enhanced for better cleanup operations. Petroleum utilizing microorganisms are more in number in oil polluted environment than pristine environment. However, microorganisms that are indigenous to a petroleum contaminated site have been reported to best remediate the environment from oil spills and other pollutants than the non-indigenous microorganisms to that site [2, 10].

The bacterial genera that have been isolated from varying crude oil contaminated sites include *Nocardia*, *Pseudomonas*, *Gordonia*, *Micrococcus*, *Rhodococcus*, *Arthrobacter*, *Mycobacterium*, *Flavobacterium*, *Corynebacterium*, *Klebsiella*, *Alcaligenes* and *Bacillus* while the fungal genera include *Penicillium, Aspergillus, Trichoderma,* and *Fusarium* [12-15]. The aim of this study was to assess microbial population and their hydrocarbon utilizing potentials from soil and water sources around petroleum products depot in Suleja, Nigeria.

Materials and Methods

Experimental areas

[The sampling areas were five surrounding communities and depot premises (Table 1)] designated Plots 1-6. These communities include Dikko, Maje, Tunga Shanu, Dagweru,

and Gwatupe. Plots 1 and 5 are farthest to the petroleum products depot and are about 1500 meters away from the depot. They are Tunga Shanu and Tunga Koro communities respectively. Plot 4 and 6 are about 1000 meters each away from the depot while Plot 2 is 500 meters away and is closest to the petroleum products depot. They are Dagwe, Maje and Dikko communities respectively. Plot 3 hosts the petroleum product depot. [The control is Federal University of Technology (FUT) Bosso Campus, Minna, Niger State of Nigeria, which has no previous history of oil pollution]. The choice of control was selected to help obtain isolates in an unpolluted and uncontaminated habitat with similar soil characteristics as the plots.

Table 1 Distances of various plots to petroleum products depot and their coordinates

Plot	Distance (meters)	Community	Coordinates
1	1500	Tunga shanu	9.250958° N, 7.160060° E
2	500	Dikko	9.253754° N, 7.164931° E
3	(1500 to farthest communities)	Petroleum products depot	9.254262° N, 7.1668195° E
4	1000	Dagwe	9.2512229° N, 7.1687292° E
5	1500	Tunga koro	9.2559345° N, 7.1786521° E
6	1000	Maje	9.255818° N, 7.1788452° E
Control	93,000	FUT Minna (Bosso campus)	9.655311° N, 6.5267310° E

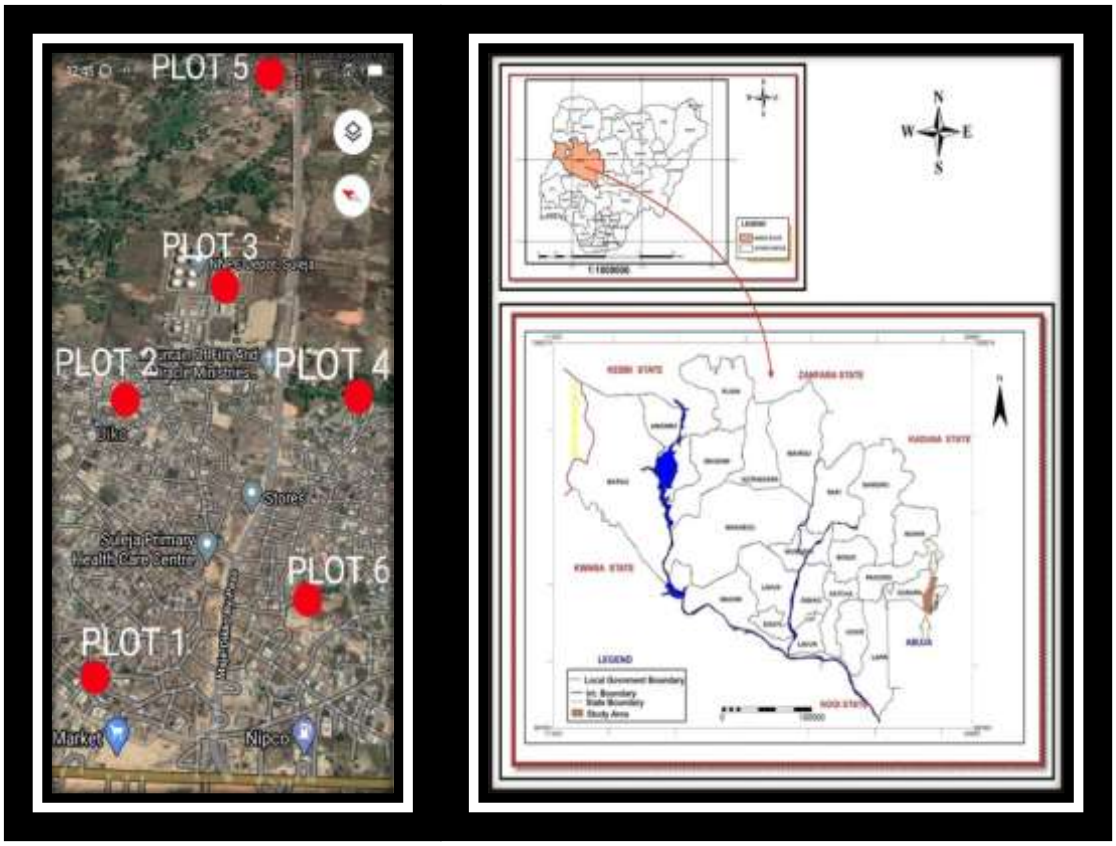

Fig 1 Map of study site showing various Communities (Plots)
Legend: Plot 1- Tunga shanu; Plot 2- Dikko; Plot 3- Petroleum products depot; Plot 4- Dagwe, Plot 5- Tunga Koro, Plot 6 Maje.

Collection of samples

Five soil samples were collected from each of the six plots into clean polyethylene bags for microbial analysis. Soil samples were collected with a hand auger after first stripping away litter to expose the first mineral based horizon (A-horizon). The samples were collected at depth of 0-15 cm, as described by Minnesota Pollution Control Agency, MCPA [16]. Soil samples were also collected from the control site. Samples were placed in WhirlPak bags and transported to the Microbiology Laboratory of the Federal University of Technology (FUT), Minna, Nigeria, for analysis.

Water samples were collected from wells, boreholes and stream of the study area and control. A total of 30 water samples were collected from the plots in sterile containers [16]. Water sample bottles were first rinsed with the water sample to be collected before

collection. Water samples were collected during rainy and dry seasons in icebox and transported to Microbiology Laboratory, FUT, Minna for analysis.

Collection of hydrocarbons

Bonny light crude oil and petroleum products (Diesel, Kerosene, and Engine oil) were collected from Kaduna Refinery and Petrochemical Company (KRPC), Kaduna, Nigeria, in clean bottles. The bottles were first rinsed with the product to be sampled before collection. The products were transported to the laboratory for biodegradation studies.

Enumeration and isolation of microorganisms in soil and water samples

Microorganisms in the soil and water samples were enumerated by spread inoculation. Ten grams (10 g) of soil samples were suspended in 90 mL of sterile distilled water and were serially diluted. One milliliter of the serially diluted soil was dispensed on the surface of Nutrient agar (NA) and Sabouraud dextrose agar (SDA) in petri dishes for the enumeration of total aerobic heterotrophic bacteria (TAHB) and fungi respectively. The NA plates were incubated for 24-48h at 30°C while the SDA plates were incubated for 3-5d at room temperature (28 ±2°C) [17]. Aliquot of the serially diluted water samples were also inoculated on MacConckey agar (MCA), Eosine methylene blue (EMB) agar, *Pseudomonas* base agar (PBA), thiosulfate-citrate-bile salt (TCBS) agar, *Salmonella*/*Shigella* agar (SSA) and spread evenly for the isolation of total coliform bacteria (TCB), *Pseudomonas*, *Vibrio*, *Salmonella* and *Shigella* respectively. Developed colonies on the surfaces of the nutrient medium were enumerated and recorded as colony forming units per gram of soil (cfu/g) and colony forming units per millilitre (cfu/mL) of water. The inoculated plates were incubated for 24 - 48h at 37°C after which colonies were counted. The isolates were sub-cultured repeatedly on fresh media to obtain pure cultures, and were maintained on agar slants for further characterization and identification.

Enumeration of total hydrocarbon utilizing bacteria and fungi

Total hydrocarbon utilizing bacteria (THUB) were enumerated using the method described by Hamamura *et al*. [18]. The method basically involves the dilution and plating out of 0.1 mL of sample suspensions on Bushnell Haas agar (Sigma-Aldrich, USA). Supply of hydrocarbon was carried out through the vapor phase to hydrocarbon utilizers by placing sterile Whatman filter papers (No.1) saturated with 3mL of hydrocarbons (crude oil, diesel,

kerosene, engine oil) on the lids of the inverted plates. The plates were incubated for 14 - 21d at 30°C. Colonies were counted and discreet colonies of different THUB were sub cultured on freshly prepared nutrient agar plates and incubated for 24-48h at 37°C while THUF were sub-cultured on freshly prepared SDA plates and incubated for 3- 5days at 28°C to obtain pure isolates.

Characterization and identification of microbial isolates

Bacterial isolates were characterized and identified on the basis of Gram's reaction and biochemical tests as described by Cheesbrough [19]. Some of the biochemical tests carried were: indole, sugar fermentation, catalase, coagulase, citrate utilisation, motility, spore formation, nitrate reduction, starch hydrolysis, urease and oxidase tests. The identities of the bacterial isolates were confirmed using Bergey's Manual of Determinative Bacteriology [20].

The fungal isolates were identified macroscopically and microscopically according to; aerial and substrate hyphae, type and shape of hyphae, spore formation, presence of specialized structures (foot cell, sporangiophores, conidiophores) and pigmentation. A small portion from each fungal isolate was placed in a drop of lactophenol cotton blue on a clean glass slide and then covered before viewing with 10X and 40X objective lenses of light microscope to confirm hyphae type. Fungal isolates were identified using Atlas of Clinical Fungi [21] and schemes of Domsch and Gams [22].

Screening for the bacterial utilization of petroleum products by bacterial isolates

Bacterial and fungal isolates were tested for their potential to utilize petroleum products under aerobic conditions by inoculating a calibrated loopful of 18-24h old culture of each isolate into 5 mL of Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) which had been sterilized by autoclaving for 15 min at 121°C. Hydrocarbon utilization potential of the isolates was screened by determining the turbidity, total viable counts and emulsification of oil in the medium after 14 days' incubation at 30°C [23]. The optical density of the isolates was determined at a wavelength of 600 nm using PG T70 UV/VIS spectrophotometer.

Utilization efficiencies of petroleum products by selected bacterial and fungal isolates

The capacities of selected bacterial and fungal isolates to utilize crude oil were further tested in minimal salt medium (MSM). One hundred millilitres of MSM (10g NaCl, 0.29g KCl, 0.42g $MgSO_4$, 0.83g KH2PO_4, 0.42g $NaNO_3$, 1.25g $NaHPO_4$, 100ml distilled water, pH 7.2) was introduced into 250 ml conical flasks and 1.0% (v/v) of crude oil was introduced and sterilized using the method described by Okpokwasili and Okorie [24]. A 24 hours old broth culture of each selected organism was seeded into each flask and incubated in a rotatory shaker incubator (New Brunswick Scientific Incubator Shaker) at 150 rev/min and 30°C. The efficiency of utilization of other hydrocarbons was monitored at three days interval for 21d by monitoring bacterial and fungal growth measured by viable counts in the MSM. The optical density was also determined at a wavelength of 600 nm using PG T70 UV/VIS spectrophotometer.

Statistical analysis

Data obtained were subjected to statistical package for social science (SPSS 23) using one-way analysis of variance (ANOVA) and Duncan multiple range tests for the determination of the significant difference between values and comparison of various means respectively. P values less than 0.05 was considered significant while P values greater than 0.05 was considered as non-significant.

Result

Total aerobic heterotrophic bacteria (TAHB) in soil and water samples

The mean counts of total aerobic heterotrophic bacterial (AHB) in soil samples are presented in Table 2. The total mean counts of AHB observed in control were higher than the values obtained in the Plots. In the rainy season, observed mean counts of AHB were lower than the mean counts obtained in the control. The highest mean count (49 x 10^6 cfu/g) for the dry season was obtained in Plot 4 while the lowest count (12.0x10^6 cfu/g) was obtained in Plot 3. There were significant differences ($P<0.05$) among the counts in the Plots.

The results for water samples also revealed that AHB counts for both rainy and dry seasons were higher in the control samples when compared with the results from the Plots. The

mean counts in rainy season were higher than the counts in dry season (Table 2). The highest AHB mean counts in water samples (9.8 x 10^4 cfu/mL) were recorded in Plot 3 while the lowest counts (3.2x10^4 cfu/mL) were recorded in Plot 6. The bacterial counts in the various Plots were significantly differently (P<0.05).

Table 2 Mean counts of total aerobic heterotrophic bacteria (AHB) in soil and water samples

Plots	Soil samples (× 10^6 cfu/g)		Water samples (× 10^4 cfu/mL)	
	Rainy Season	Dry Season	Rainy Season	Dry
1	52.0 ^{d}	26.0 ^{b}	6.7 ^{f}	4.1 ^{f}
2	16.1 ^{f}	12.1 ^{f}	7.6 ^{d}	4.4 ^{d}
3	62.0 ^{b}	12 .0 ^{g}	9.8 ^{b}	6.2 ^{c}
4	26.0 ^{e}	49.0 ^{a}	8.0 ^{c}	6.9 ^{b}
5	11.0 ^{g}	25.1 ^{c}	7.0 ^{e}	4.2 ^{e}
6	54.0 ^{c}	13.0 ^{e}	6.1 ^{g}	3.2 ^{g}
Ctr	84.0 ^{a}	18.0 ^{d}	11.5 ^{a}	7.9 ^{a}

Means with dissimilar superscript across the column differ significantly (p<0.05). Means with same superscript do not differ significantly (P>0.05). cfu/g: colony forming unit per gram, cfu/ml: colony forming units per milliliters, Ctr: control.

Mean counts of total fungi (TF) in soil and water samples

The values obtained for total fungi in soil were higher in the control than the plots in both rainy and dry seasons with the exception of Plot 2. The mean counts of total fungi in soil ranged from 2.20 × 10^3 cfu/g to 14.10 × 10^3 cfu/g for the rainy season and 1.00 × 10^3 cfu/g to 6.20 × 10^3 cfu/g for dry season. The control recorded mean counts of 12.00 × 10^3 and 6.10 × 10^3 cfu/g (Table 3) for rainy and dry seasons respectively.

The values obtained for total fungi in water were higher in the control than the plots in both rainy and dry seasons with the exception of Plot 2. The mean counts of total fungi in water samples ranged from 0.0 × 10^3 cfu/mL to 4.30 ×10^3 cfu/mL in the rainy season and 0.0 × 10^3 cfu/mL to 3.20 × 10^3 cfu/mL in dry season (Table 3).

Table 3 Mean counts of total fungi (TF) in soil and water samples

Plots	**Soil samples (× 10^3 cfu/g)**		**Water samples (× 10^3 cfu/mL)**	
	Wet season	**Dry season**	**Wet season**	**Dry season**
1	5.20 [d]	4.10 [b]	0.00 [d]	1.10 [a]
2	14.10 [a]	6.20 [a]	4.30 [a]	3.20 [a]
3	10.10 [c]	4.50 [b]	2.00 [b]	2.20 [b]
4	3.50 [e]	3.30 [c]	1.20 [c]	0.00 [c]
5	2.20 [f]	1.20 [d]	0.00 [d]	1.12 [c]
6	3.10 [e]	1.00 [d]	2.20 [b]	1.10 [c]
Ctr	12.00 [b]	6.10 [a]	4.10 [a]	2.00 [b]

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). cfu/g: colony forming unit per gram, Ctr: control

Mean counts of total coliforms and other bacteria (TCB) in water samples

The mean counts of total coliform bacteria ranged from 3.10 × 10^3cfu/g to 10.00 ×10^3cfu/mL and 2·03×10^3 cfu/mL to 10.01×10^3cfu/mL in rainy and dry seasons respectively. No count was found in the control water sample (Table 4).

Table 4 Mean counts of total coliforms bacteria (TCB) in water

Plots	Water samples ($\times 10^3$ cfu/mL)	
	Rainy Season	Dry Season
1	15.00 [a]	7.10 [b]
2	8.41 [c]	6.00 [c]
3	10.00 [b]	10.01 [a]
4	4.01 [d]	5.31 [d]
5	3.10 [e]	4.03 [e]
6	4.23 [d]	2.03 [f]
Control	0.00 [f]	0.00 [g]

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). cfu/mL: colony forming units per milliliter.

Hydrocarbon utilizing bacteria and fungi in soil and water samples

The mean counts of hydrocarbon utilizing bacteria (HUB) and fungi (HUF) are presented in Table 5. Generally, the results revealed that samples from the six plots have higher HUB counts than the control plot. The mean counts of HUB ranged from 1.28×10^7 cfu/g to 6.32×10^7 cfu/g in the various plots. The mean counts of HUB were highest in Plots 3 and 6 which recorded 6.32×10^7 - 5.05×10^7 cfu/g and 6.11×10^7 - 5.81×10^7 cfu/g in rainy and dry seasons respectively. Plot 5 recorded lowest mean counts of HUB of 1.21×10^7 cfu/g and 1.20×10^7 cfu/g in the rainy and dry seasons respectively.

The mean counts of hydrocarbon utilizing fungi (HUF) were highest in Plot 3 with 3.60×10^4 cfu/g and 3.05×10^4 cfu/g in rainy and dry seasons respectively. The lowest mean counts of HUF were obtained in Plot 5 (Table 5). There were significant differences ($P<0.05$) among mean HUB and HUF counts in the rainy and dry seasons, as well as the control.

The mean counts of HUB and HUF in water sample were presented in Table 6. Hydrocarbon utilizing bacteria recorded highest mean counts in Plot 6 of 2.58×10^7 cfu/mL and 2.18×10^7 cfu/mL) in rainy and dry seasons respectively. The mean counts of HUB were lowest in Plot 5 (Table 6) The mean counts of HUF were highest in Plot 3 with counts of 2.0×10^4 and 1.53×10^4 cfu/mL in rainy and dry seasons respectively. The results obtained showed that the mean counts of HUB and HUF were much lower in the control samples than in the six plots.

Table 5 Mean counts of hydrocarbon utilizing bacteria and fungi in soil samples

Plots	HUB ($\times 10^7$ cfu/g)		HUF ($\times 10^4$ cfu/g)	
	Rainy Season	Dry Season	Rainy Season	Dry Season
1	1.45 [d]	2.30 [e]	1.56 [e]	1.8 [g]
2	4.20 [c]	4.00 [c]	2.65 [c]	2.11 [c]
3	6.32 [a]	5.50 [b]	3.60 [b]	3.05 [b]
4	1.42 [d]	3.40 [d]	2.00 [d]	1.93 [d]
5	1.28 [e]	2.14 [f]	1.21 [b]	1.20 [f]
6	6.11 [c]	5.81 [a]	4.5 [a]	3.71 [a]
Control	1.23 [e]	1.24 [g]	0.10 [f]	0.30 [i]

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). HUB/F= Hydrocarbon utilizing bacteria/fungi, cfu/g = colony forming units per gram.

Table 6 Mean counts of hydrocarbon utilizing bacteria and fungi in water samples

Plots	THUB ($\times 10^7$ cfu/mL)		THUB ($\times 10^4$ cfu/mL)	
	Rainy Season	Dry Season	Rainy Season	Dry Season
1	1.00 [c]	0.50 [f]	0.95 [d]	0.60 [c]
2	1.32 [b]	0.80 [c]	1.74 [b]	1.04 [b]
3	1.50 [b]	1.00 [b]	2.00 [a]	1.53 [a]
4	0.95 [c]	0.80 [b]	0.42 [e]	0.19 [d]
5	0.28 [e]	0.14 [d]	0.13 [f]	0.15 [d]
6	2.58 [a]	2.18 [a]	1.5 [c]	0.13 [d]
Control	0.40 [d]	0.1 [e]	0.05 [g]	0.0 [e]

Means with dissimilar superscript across the column differ significantly ($p<0.05$). Means with same superscript do not differ significantly ($P>0.05$). HUB/F= Hydrocarbon utilizing bacteria/fungi, cfu/mL = colony forming unit per milliliter.

Frequency of occurrences of microbial isolates in the various plots

The bacterial isolates were not evenly distributed in the six experimental Plots and control Plot. The control had the least frequency of occurrences (7.19 %) when compared to the plots. Plot 6 had the highest frequency of occurrence (20.92 %) of isolated bacteria (Table 7). Plots 1,2,3,4 and 5 had frequency of occurrence of 16.26 %, 19.57 %, 16.44 %, 10.02 %, and 9.55 % respectively.

The results showed that *Bacillus* had the highest frequency of occurrence (32.25%) while *Salmonella* had the lowest frequency of occurrence (2.2 %). Staphylococcus, *Pseudomonas* and *Streptococcus* had 18.15%, 13.57% and 13.16% respectively, frequency of occurrences (Table 7).

There were higher frequencies of occurrence of bacterial isolates in rainy seasons than in in dry season. The genera *Micrococcus*, *Enterobacter*, *Salmonella* and *Escherichia* were only observed in one season in some Plots. The results also showed that only the genus *Bacillus* was observed in both rainy and dry seasons in all the Plots (Table 7).

The frequency of occurrence of fungal isolates was presented in Table 8. The results revealed that fungal isolates were observed in all the plots but were not evenly spread. The control plots had the least frequency of occurrence of 5.27% with two fungal genera; *Penicillium* and *Aspergillus*. Plot 6 had the highest frequency of occurrence (28.57%) and had four fungal genera (Table 8). Plots 1 and 2 recorded equal number of frequencies of occurrence (15.80%).

The result showed that *Aspergillus* was the dominant fungal isolate with a total frequency of occurrence of 48.88%, while the *Rhizopus* had the lowest frequency of occurrence Table 8. There were more occurrences of fungal isolates in the rainy season than in the dry season.

Table 7 Frequency of occurrence of bacterial genera in the plots

Bacterial Genera	Plot 1 R/D	Plot 2 R/D	Plot 3 R/D	Plot 4 R/D	Plot 5 R/D	Plot 6 R/D	Ctr R/D	Total (%)
Bacillus	16/12 (4.38)	32/15 (7.35)	28/22 (6.26)	12/10 (3.45)	11/8 (2.98)	26/13 (6.10)	7/4 (1.72)	206 (32.25)
Staphylococcus	13/0 (2.03)	10/6 (2.50)	13/10 (3.60)	6/3 (1.41)	8/4 (1.88)	23/10 (5.17)	5/5 (1.56)	116 (18.15)
Streptococcus	13/6 (2.98)	14/0 (2.19)	12/8 (3.13)	6/0 (0.94)	5/3 (1.25)	5/4 (1.41)	10/0 (1.56)	86 (13.46)
Proteus	3/2 (0.78)	3/0 (0.47)	5/0 (0.78)	0/0 (0.00)	2/0 (0.32)	0/3 (0.47)	0/0 (0.00)	18 (2.82)
Pseudomonas	10/8 (2.82)	5/3 (1.26)	8/4 (1.88)	12/7 (2.97)	3/2 (0.78)	12/7 (2.92)	0/6 (0.94)	87 (13.57)
Escherichia	5/2 (1.09)	12/2 (2.19)	0/0 (0)	2/1 (0.47)	4/3 (1.09)	11/6 (2.66)	0/3 (0.47)	51 (7.97)
Klebsiella	0/6 (0.94)	5/2 (1.09)	1/0 (0.16)	3/1 (0.62)	3/0 (0.47)	4/0 (0.63)	0/0 (0.00)	25 (3.91)
Salmonella	3/0 (0.47)	4/2 (0.94)	0/0 0 (0)	0/0 (0.00)	1/0 (0.16)	3/0 (0.47)	1/0 (0.16)	14 (2.20)
Enterobacter	0/1 (0.16)	2/0 (0.31)	1/0 (0.16)	0/0 (0.00)	4/0 (0.62)	2/2 (0.62)	5/0 (0.78)	17 (2.65)
Micrococcus	3/1 (0.62)	0/8 (1.25)	3/0 (0.47)	1/0 (0.16)	0/0 (0.00)	3/0 (0.47)	0/0 (0.00)	19 (2.97)
Total	104 (16.26)	125 (19.57)	105 (16.44)	64 (10.02)	61 (9.55)	134 (20.92)	46 (7.19)	639 (100)

Ctr: Control, Percentage (%) in parenthesis, R/D: Rainy/Dry Seasons

Table 8 Frequency of occurrences of fungal isolates in the various plots

Fungal genera	Plot 1 R/D	Plot 2 R/D	Plot 3 R/D	Plot 4 R/D	Plot 5 R/D	Plot 6 R/D	Ctr	Total (%)
Aspergillus	6/3 (6.77)	6/2 (6.02)	8/3 (8.27)	3/2 (3.76)	6/5 (8.27)	11/6 (12.78)	2/2 (3.01)	65(48.88)
Penicillium	5/3 (6.02)	5/2 (5.26)	2/1 (2.25)	4/2 (4.51)	2/1 (2.26)	8/4 (9.02)	2/1 (2.26)	42(31.58)
Mucor	3/1 (3.01)	0/2 (1.50)	2/0 (1.50)	2/0 (1.50)	0/0 (0.0)	4/1 (3.76)	0/0 (0.00)	15(11.27)
Rhizopus	0/0 (0)	3/1 (3.01)	0/1 (0.75)	2/0 (1.50)	0/0 (0.0)	3/1 (3.01)	0/0 (0.00)	11(8.27)
Total (%)	21 (15.80)	21 (15.80)	17 (12.77)	15 (11.27)	14 (10.53)	38 (28.57)	7 (5.27)	133 (100)

Ctr: Control, (%): Percentage in parenthesis, R/D: Rainy/Dry Seasons

Utilization of petroleum products by microbial isolates

The ability of selected bacterial isolates from the various plots with potential to degrade hydrocarbons was tested on some petroleum products (Crude oil, kerosene, Diesel and engine oil), which served as sole source of carbon and energy in modified minimal salt nutrient broth. The bacterial isolates were; *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus thuringensis*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidemidis*, *Bacillus licheniformis* and *Pseudomonas putida***.** The bacterial isolates utilized the petroleum products to varying capacities after 21d of incubation (Table 9). Bacterial isolates from the genera *Pseudomonas* and *Bacillus* had higher absorbance of 0.663±0.55 and 0.601±0.14 respectively on crude oil than other isolates. The lowest absorbance on crude oil (0.081±0.41) was from *Proteus mirabilis* (Table 9). *Pseudomonas aeruginosa* recorded highest absorbance in kerosene (0.43± 0.002) while *B. thuringiensis* recorded highest absorbance in diesel (0.311±0.46) and engine oil (0.284±0.24) (Table 9), meaning that these organisms had efficient abilities in utilizing the hydrocarbons. The isolated fungi utilized the petroleum products to varying capacities within 21d of incubation. The organisms had highest percentage utilization in crude oil (0.211-0.713) and

lowest in engine oil (0.024-0.213). Kerosene and diesel recorded moderate rates of 0.124-0.453 and 0.053 – 0.325 respectively) by the fungal isolates (Table 10). Among the fungal species, *Aspergillus niger* and *Penicillium notatum* exhibited greater capacity to utilize the petroleum products when compared with other fungal isolates (Table 10).

Table 9 Utilization efficiencies of bacterial isolates in petroleum products after 21d of incubation

Bacterial Isolates	**Absorbance at 600 nm**			
	Crude oil	**Kerosene**	**Diesel**	**Engine oil**
Staphylococcus aureus	0·302±0.21^{e}	0·291±0.31^{f}	0·250±0.13^{d}	0·180±0.15^{e}
Bacillus subtilis	0·580±0.13^{c}	0·391±0.13^{d}	0·291±0.25^{b}	0·264±0.26^{b}
Bacillus thuringensis	0·601±0.15^{b}	0·413±0.42^{b}	0·311±0.46^{a}	0·284±0.24^{a}
Streptococcus faecalis	0·131±0.34^{g}	0·049±0.38^{i}	0·030±0.59^{i}	0·062±0.33^{g}
Pseudomonas aeruginosa	0·663±0.55^{a}	0·431±0.02^{a}	0·281±0.23^{c}	0·250±0.16^{c}
Proteus mirabilis	0·081±0.41^{h}	0·103±0.35^{h}	0·125±0.31^{h}	0·031±0.11^{i}
Staphylococcus epidemidis	0·180±0.30^{h}	0·116±0.41^{g}	0·135±0.21^{g}	0·051±0.42^{h}
Bacillus licheniformis	0·282±0.40^{f}	0·381±0.16^{e}	0·190±0.29^{f}	0·152±0.13^{f}
Pseudomonas putida	0·501±0.25^{d}	0·410±0.44^{c}	0·193±0.18^{e}	0·183±0.43^{d}

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). Values are replicate mean determination ± SD

Table 10 Utilization efficiencies by fungal isolates of petroleum products after 21 days of incubation

Fungal Isolates	Absorbance at 600nm			
	Crude oil	Kerosene	Diesel	Engine oil
Aspergillus niger	0·713±0·41[a]	0.453±0·31[a]	0.325±0·43[a]	0.213±0·14[a]
Penicillium notatum	0.654±0·10[c]	0.412±0·25[ab]	0.250±0·15[a]	0.213±0·23[a]
Mucor plumeus	0.301±0·2[e]	0.125±0·11[d]	0.181±0·22[b]	0.051±0·15[d]
Rhizopus stolonifera	0.211±0·41[f]	0.124±0·23[d]	0.053±0·33[c]	0.024±0·43[e]
Aspergillus fumigatus	0.648±0·30[b]	0.386±0·17[b]	0.260±0·32[a]	0.200±0·41[b]
Aspergillus flavus	0.524±0·15[d]	0.314±0·28[c]	0.254±0·25[a]	0.180±0·18[c]

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). Values are replicate mean determination ± SD

Counts of selected hydrocarbon utilizing bacteria and fungi in MSM amended with crude oil

There were variations in the viable cell counts of selected HUB and HUF in minimal salt broth amended with crude oil during 21d of incubation. The crude oil gradually emulsified as the degradation progressed. There were increases in the viable cell counts up to day 15 and 18 for *Bacillus* and *Pseudomonas* species respectively. *Pseudomonas aeruginosa* recorded the highest viable cell counts when compared with other isolates (Table 11). The viable cell counts increased from 4.32×10^6 to 12.15×10^6 cfu/mL and declined to 12.00×10^6 cfu/mL on day 21. *Penicillium notatum* and *Aspergillus niger* recorded a consistent increase in viable counts to 5.80×10^4 and 7.60×10^4 up to day 15 and 18 respectively, and decreased slightly after 21st day (Table 11). The results showed that there were significant differences ($p<0.05$) in the viable cell counts of microbial isolates within the period of incubation.

Table 11 Mean viable cell counts of selected hydrocarbon utilizing bacteria and fungi in MSM amended with crude oil

Time(days)	*Bacillus thuringiensis* ($\times 10^6$ cfu/mL)	*Pseudomonas aeruginosa* ($\times 10^6$ cfu/mL)	*Penicillium notatum* ($\times 10^4$ cfu/mL)	*Aspergillus niger* ($\times 10^4$ cfu/mL)
0	3.40 ^{h}	4.32 ^{h}	2.60 ^{h}	3.30 ^{g}
3	3.80 ^{g}	4.71 ^{g}	3.70 ^{g}	4.11 ^{f}
6	4.61 ^{e}	5.70 ^{f}	4.50 ^{f}	5.23 ^{e}
9	4.91 ^{d}	6.15 ^{e}	4.90 ^{d}	6.30 ^{c}
12	5.82 ^{c}	8.45 ^{d}	5.60 ^{b}	5.51 ^{d}
15	7.21 ^{a}	10.71 ^{c}	5.80 ^{a}	7.40 ^{a}
18	6.50 ^{b}	12.15 ^{a}	5.00 ^{c}	7.00 ^{b}
21	4.51 ^{f}	12.00 ^{b}	4.81 ^{e}	5.20 ^{e}

Means with dissimilar superscripts across the column differ significantly ($p<0.05$). Means with same superscripts do not differ significantly ($P>0.05$). cfu/mL: colony forming unit per milliliter

Discussion

The total mean counts of aerobic heterotrophic bacteria (AHB) in soil were greater than the counts obtained in water sample. This may be due to the fact that the soil is not mobile on its own and eventually leads to the deposition of organic substances that help in providing nutrients for microbial proliferation. The highest aerobic heterotrophic bacterial counts obtained during the rainy season is in concordance with the report of Olukunle [26] and Ikuesan [27] that moisture is one of the factors that influence microbial proliferation in soil. A dry soil can cause desiccation of microbial cells thereby reducing the overall microbial activities and causes some cells to be dormant. The soil can also hold substances in stationary mode allowing microorganisms to act on them conveniently without any disturbance unlike the liquid medium [28]. The low counts of AHB in water samples may be due to the fact that oxygen concentration in water was lower compared to soil sample, which may be due to presence of pore spaces that allowed diffusion of atmospheric oxygen into them, thus enhancing bacterial proliferation in the soil [27].

Among the fungi isolated in this study, *Penicillium notatum* was prominent across all plots sampled. Elemuo *et al.* [25] have reported the versatility of *P. notatum* in the environment

with ability to produce various enzymes, which helped the microorganisms degrade crude oil in contaminated environment.

The mean total fungi (TF) counts in the soil and water sample in this study was highest in Plot 2, which is closer to the petroleum products depot both in wet and dry seasons. This may be due to the ability of fungi to tolerate adverse environmental conditions, especially with the formation of resistant spores.

The low mean total coliform bacterial (TCB) counts generally observed in this study may be due to the inability of coliform bacteria to produce spores, which have been noted in the past to help microorganisms survive in environment that are not favorable for their existence and metabolisms. Moreover, there is paucity of information concerning the low proliferation of coliform bacteria on environment polluted by crude oil.

The total mean counts of hydrocarbon utilizing bacteria (HUB) and fungi (HUF) were lowest in the control soil both in wet and dry seasons. This indicated that the microbial population found in communities surrounding petroleum products depot had the ability to utilize hydrocarbon due to periodic exposure to petroleum products. Though, there was no established pattern of growth observed in the microbial community in this study, their counts were quite higher, than in the control. The total mean counts of HUB and HUF were generally low in the water sample in rainy and dry seasons compared to the soil sample. This is because, nutrient and oxygen availability in water is quite low compared to that of the soil sample. Different microbial genera use different pathways in the degradation of petroleum products, some of which take little time to complete a degradation process whereas others take longer time. Also, the types of enzymes utilized by microorganisms as well as the constituent of the petroleum products plays a great role in influencing biodegradation processes [29].

Microbial population and diversity are usually high in a pristine environment unlike an environment experiencing influx of contaminants from crude oil spills. The reduction and changes in the microbial communities occur as a result of the inability of the indigenous microorganisms to withstand such extreme conditions and those that are able to withstand and even strive often have machineries and pathways they utilize to achieve that [30, 29,

31]. The indigenous bacterial diversity obtained in this study was similar to the one obtained by Nkiru *et al.* [1]

This study reported some microorganisms that were peculiar to crude oil polluted environment, in which the genera *Bacillus* had the highest number of species, which include *B. subtilis, B. thurengiensis and B. licheniformis.* These bacteria in addition to *Pseudomonas aeruginosa* were all isolated from all the plots sampled. The dominant presence of *Bacillus* and *Pseudomonas* in this study supports the fact established by Osarumwense *et al.* [32] that these bacterial genera efficiently degrade hydrocarbons in the environment through the production of surfactants and enzymes such as lipase. Agu *et al.* [33] have reported that spore forming ability of *Bacillus* helped the organisms in resisting toxic effects exerted by the petroleum compounds.

All the microorganisms (bacteria and fungi) tested for biodegradation of petroleum products in this study were able to utilize hydrocarbons although at different rates. This observation was also made by Ikuesan (2017) where the test isolates utilized hydrocarbons at varying rates. Microbial genera such as *Bacillus* and *Pseudomonas*. *Penicillium* and *Aspergillus* had high degradation capabilities in this study. Ikuesan (2017) has suggested that these differences in degradation rates were associated with the natural ability of the different microorganisms. It may also be due to the presence of enzymes and biosurfactants, and petroleum products constituents [31, 34, 35].

The bacterial genus; *Bacillus* and *Pseudomonas* had high frequency of occurance and utilising efficiencies when compared with other bacterial genus. This may be connected with their high ability to breakdown complex organic and recalcitrant compounds due to the production of specialized enzymes [17].

The mean viable cell count (VCC) of bacteria and fungi in MSM amended with crude oil generally showed a progressive growth of microbial cells untill day 15 across all bacterial and fungal isolates. However, the microbial cells of *Bacillus thuringiensis*, *Penicillium notatum* and *Aspergillus niger* began to decrease after day 15, while the cells of *Pseudomonas aeruginosa* increased till day 18 before it declined. The general progressive increase of microbial cells observed at the initial and later stages as well as gradual decline of microbial cells depicts a typical microbial growth curve. The reduction in the number of

cells occurred as a result of cell aging as well as the exhaustion of nutrients in the mineral salt medium used. As microbial cells grow, they metabolize and release their products into the environment in which they live. Since the medium used in this study is typical of a batch culture, there was no renewal of nutrients and toxic metabolites, which eventually deterred the growth of the microorganisms, thus, a decline in the number of cells was observed in this study and is in concordance with the report of Nwakanma *et al.* [2].

Conclusion

The microorganisms isolated from soil and water close to petroleum products depot in this study were capable of utilizing crude oil, kerosene, diesel and engine oil in varying amount. *Pseudomonas aeruginosa* had the highest ability to utilize kerosene while *B. thuringiensis* was most efficient in utilizing diesel and engine oil. *Aspergillus niger* recorded a consistent increase in viable counts and also exhibited the highest capacity to utilize the petroleum products compared with other fungal isolates. The findings in this study therefore revealed that bacterial genera *Pseudomonas* and *Bacillus* as well fungal genera *Aspergillus* and *Penicillium* were effective in the bioremediation of petroleum products contaminated sites. It also showed that water sources from the plots were contaminated with coliform bacterial and poses great challenge to public health.

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