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Gravimetric Profile of Hydrocarbon Degrading Bacterial and Fungal Isolates from Contaminated Soil Samples in Ado-Ekiti, Nigeria

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Abstract
One of the biological compounds limiting soil water retention capacity is oil when present due to its hydro-nature. However, some microorganisms exhibit the capacity to degrade oil as a source of carbon, whereby the soil quality is retained and enhanced. Hence, the gravimetric profile of hydrocarbon degrading bacteria and fungi isolated from oil contaminated soil samples was investigated. Soil samples were collected from surface and 10m depth from six different mechanic workshops and generator sites. The pour plate technique was used to isolate the microorganisms. All pure isolates were sub-cultured using Bushnell Haas agar and the isolated bacteria were identified by their morphological and biochemical characteristics. The soil samples pH range was 4.3 - 6.4. Bacteria isolated included Pseudomonas spp., Staphylococcus spp., Micrococcus spp., Acinetobacter spp., and Bacillus spp. The fungi isolated included Aspergillus spp., Rhizopus spp., Candida spp., Trichoderma spp. and Penicillium spp. Degradation of kerosene, diesel, crude oil, engine oil, and spent engine oil was allowed using Acinetobacter baumanni, P. aeruginosa, B. subtilis, and S. aureus. Gravimetric analyses were used to determine the percentage of petroleum hydrocarbon degraded by bacterial isolates. The highest percentage of degradation was between P. aeruginosa and B. subtilis. Pseudomonas aeruginosa degraded 97.4% diesel, 88.2% kerosene, 71.3% crude oil, 80.7% engine oil and 78.2% spent engine oil; while Bacillus subtilis degraded 71% diesel, 97% kerosene, 89.6% crude oil, 87% engine oil and 72.6% spent engine oil. This study revealed that bacterial and fungal isolates from oil contaminated soils exhibited the potentials to degrade oil and bioremediation using these microorganisms was possible.
Keywords: *Hydrocarbon, Contaminated soils, Bacteria, Fungi, Degradation, Gravimetric analyses*

INTRODUCTION

Environmental pollution caused by petroleum products is an unavoidable outcome of the production, transportation, and distribution of oil. The likelihood of land contamination is brought about by a large volume of petroleum products processed on land yearly in addition to large volumes of crude and refined petroleum products being transported all over the world from production sites to various countries for their usage (Ohanmu *et al.*, 2019). The widespread contamination of arable lands, swamps, creeks, and natural sources of water with petroleum and petrochemical products particularly in the region of Southern Nigeria, is largely because of increasing petroleum exploration, refining, and other associated industrial activities (Okpokwasili and Odokuma, 1996; Okpokwasili and Odokuma, 1990). An increase in the world's population has led to an increase in petroleum and petroleum products' demand; this has become an avenue for pollution to the environment (Akoachere *et al.*, 2008).

Soil contamination by oil spills which most times require cleaning up of such contaminated sites poses an environmental problem in Nigeria (Bundy *et al.*, 2002). The release of petroleum products into lakes, streams, rivers, beaches, oceans, seas, and lands is called oil spillage. This is poisonous resulting in water and land becoming fouled and threatening the rich coastal habitat. The main constituents of petroleum oil spills and refined product molecules will be gradually diminished from the environment as they are biodegradable with microorganisms utilizing them for their metabolic activities (Prince, 2002). Due to these habitats being contaminated, it poses major risks to public health and socio-economy leading to a joint attempt in surveying the practicability of removing oil contaminants by making use of organic and inorganic wastes (Akoachere *et al.*, 2008; Adenipekun and Isikhuemhen, 2008). Under certain conditions, crude oil

may flow without control onto the earth's solid surface thereby causing significant risk to the environment. These cases are mostly attributed to oil spills and by-products during oil exploration, processing, and transportation. Microorganisms occurring naturally in crude oil are often used to remove this type of pollution because of their ability to break down (biodegrade) crude oil and by-products (Wolicka and Borkowski, 2011; Wolicka *et al.*, 2009; Nazina *et al.*, 2007; Mokhatab, 2006). The decomposition potential by microorganisms (biodegradation) of petroleum compounds can, however, be said to be weak when compared to the biological carbon cycle in most organic molecules (Bertrand *et al.*, 1990). Hundreds of various hydrocarbon molecules form the major compositions of crude oil, of which alkanes from C1 to C40 straight chain are major; cyclohexanes, aromatics, C6–C8 branched-chain, and sulphur, oxygen and nitrogen containing compounds (Stafford *et al.*, 1982). The isolation of a high number of particular microorganisms with oil-degrading abilities from environments polluted with oil usually serves as proof that these microorganisms are the main decomposers (bio-degraders) of the environmental pollutants (Adegbola *et al.,* 2014).

Pollution caused by crude oil and by-products from the soil, groundwater, and seawater found close to exploration sites, leaking pipelines, and in various locations including petrol stations or roadsides are removed by microorganisms activities. Biodegradation of oil-derived products in the soil is influenced by basic factors that include chemical structure, concentration and toxicity of hydrocarbons to the microflora, microbiological soil potential (biomass concentration, population variability, enzyme activity), physical-chemical environmental parameters (such as reaction, temperature, organic matter content, humidity), and availability of hydrocarbons for microorganisms cells. Various groups of microorganisms, especially the bacteria indigenous to the soil help in degrading sites contaminated with hydrocarbons. Many strains of *Pseudomonas* isolates from aquifers and soil can degrade Polyaromatic Hydrocarbons (PAHS) (Wong et al., 2001). Other petroleum hydrocarbons degrading bacteria include *Alcaligens* spp., *Bacillus* spp., *Corynebacterium* spp., *Flavobacterium* spp., *Micrococcus* spp. and *Streptococcus* spp. (Saadoun, 2002). Also, fungi are capable of degrading hydrocarbon to a certain extent.

This study was aimed at investigating the gravimetric profile of hydrocarbon degrading bacterial and fungal isolates from contaminated soil samples collected from mechanic workshops and generator sites in Ado-Ekiti.

MATERIALS AND METHODS

Sample collection, isolation, and identification of microorganisms

Soil samples were collected from three different mechanic workshops and three generator sites located within Ado-Ekiti between January to April 2019. The surface and 10 cm depth soil were collected from each site with separate clean polyethylene bags. Samples of uncontaminated soil adjacent to the mechanic workshops and generator sites were collected as control and transported to the Microbiology Laboratory, Department of Biological Sciences, Afe Babalola University, where they were analyzed. The culture media used included nutrient agar, nutrient broth, potato dextrose agar, and Bushnell Haas agar. The media preparation was according to the instructions by the manufacturer and was sterilized at 121°C for 15 minutes. Ten (10) grams of the contaminated and uncontaminated soil (control) samples each were suspended in 90 ml of sterile distilled water as diluents and tenfold serial dilutions of the soil samples from 1:10 to 1:100000 were carried out; 0.1ml of the 10^{-3}, 10^{-4}, 10^{-5} for each sample was plated in triplicate on nutrient agar and potato dextrose agar (PDA) plates with amoxicillin to inhibit bacterial growth using pour plate methods. The nutrient agar plates were incubated at 37°C for 24-48 hours while the potato dextrose agar plates were incubated in a cupboard at room temperature for 72 hours. The number of viable microorganisms in the plates was calculated from the number of colonies formed and the dilution factor; expressed as colony forming unit per ml (cfu/ml). Standard microbiological protocols were used in the identification of bacterial and fungal isolates.

Hydrocarbon utilization by bacterial isolates

Bushnell Haas agar, a selective medium for hydrocarbon degrading bacteria was used which consisted of 1.0 g K_2HPO_4, I.0 g KH_2PO_4, 1.0 g NH_4N0_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $FeCl_2$, 0.02 g $CaCl_2 \cdot 2H_2O$, and 15 g agar. The components were added to distilled water and brought to a volume of 990ml, mixed thoroughly, and heated in a water bath to boiling before sterilizing at 121 °C for 15 minutes. The agar was dispensed into Petri dishes and allowed to set. Twenty-four hours old culture of bacterial isolates was streaked on the plates and incubated at 37°C for 5 days. Selective colonies were taken for culture in nutrient agar. To further test for hydrocarbon utilization, 100 ml each of the different hydrocarbons including diesel, crude oil, spent engine oil, engine oil, and kerosene were introduced into 300 ml of sterilized nutrient agar respectively in conical flasks, mixed thoroughly, poured on plates, and incubated at 37°C for 4 days.

Hydrocarbon utilization by fungal isolates

Using sterilized cock borer, pure fungal colonies were placed on the Bushnell Haas medium and kept at room temperature for 7 days. Fungi were also introduced to the potato dextrose agar plates containing different hydrocarbons to check for growth.

Biodegradation tests

The ability of microorganisms isolated from hydrocarbon contaminated soil to degrade hydrocarbon was tested by introducing spent oil, engine oil, crude oil, kerosene, and diesel to 100 mL of sterilized Nutrient Broth medium in 250 mL flasks. The flasks were inoculated with microorganisms and incubated at 37°C under aerobic condition at 200 rpm in a shaking incubator for 14 days (Jyothi *et al.,* 2012).

Gravimetric analysis

The oil degradation profile of the isolates was studied using the gravimetric method. The flasks were taken out following the desired time interval, and 1% 1N-HCL was added to stop bacterial activities. Oil extraction was carried out using the mixture of 50 mL culture broth, 20 mL petroleum ether, and acetone in a ratio of 1:1 in a separating funnel was shaken vigorously to get a single emulsified layer. Acetone was subsequently added and mixed gently to break the emulsification, which resulted in three layers (Bharti and Irfan, 2011). The top layer, a mixture of petroleum ether, oil, and acetone; clumping cells make a middle layer and the bottom aqueous layer contains acetone, water, and biosurfactant in soluble form. The two lower layers were spread out while the top layer containing petroleum ether mixed with oil and acetone was taken in a pre-weighed beaker. The extracted oil was passed through anhydrous sodium sulphate to remove moisture. The petroleum ether and acetone were evaporated on a water bath. The gravimetric analysis of residual oil left after biodegradation was obtained by weighing the quantity of oil in a tarred beaker. The percentage of degradation was calculated as follows:

Weight of Residual oil = Weight of beaker containing extracted oil - Weight of empty beaker.

Amount of crude oil degraded = Weight of oil added - Weight of residual oil.

$$\% \text{ degradation} = \frac{\text{Amount of oil degraded media}}{\text{Oil added in media}} \times 100$$

(Guru *et al.,* 2013)

Statistical analysis

All the data obtained were subjected to Statistical analyses using analysis of variance (ANOVA) using a computer-aided SPSS (V.23) statistical program. All the means and standard deviations were separated and compared using the Duncan Multiple Range Test at 5% level of significance.

RESULTS

The bacterial and fungal isolates from six sample sites which included three mechanic workshops (A, B, and C) and three generator sites (D, E, and F) with dilution factors 10^{-3}, 10^{-4}, and 10^{-5} were identified using standard microbiological methods. Table 1 showed the frequency of occurrence of bacterial isolates from hydrocarbon contaminated soil samples. A sum of 36 identified bacterial isolates were obtained from the hydrocarbon contaminated soil samples. The most frequently occurred bacterial isolates were Acinetobacter baumannii 7 (24%) followed by Pseudomonas aeruginosa 6 (21%) while the least occurring bacterial isolates were Aeromonas veronii, Jannibacter terrae, Acinetobacter ursingii and Bacillus spp. with 1 (3%) respectively.

Table 2 showed the growth and utilization of hydrocarbon by bacterial isolates from mechanic workshops and generator sites. The bacterial isolates that had the ability to utilize hydrocarbon as a carbon source included *Acinetobacter baumannii, Acinetobacter ursingii, Bacillus subtilis, Bacillus megaterium, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Staphylococcus aureus*. However *Aeromonas veronii* and *Jannibacter terrae* were unable to grow on oil agar hence unable to utilize hydrocarbon as a carbon source. The growth and utilization of hydrocarbon by fungal isolates from mechanic workshops and generator sites are showed in Table 3. The fungal isolates from mechanic workshops and generator sites included *Trichoderma* spp., *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Rhizopus* spp., *Rhizopus oryzae, Fusarium* spp., *Mucor* spp., *Candida* spp., *Geotrichium* spp., *Penicillium* spp. *and Trichophyton* spp. All the fungal isolates had the ability to utilize hydrocarbon as a carbon source except *Fusarium* spp., *Mucor* spp., *Geotrichium* spp., *and Trichophyton* spp.

The heavy metal composition of hydrocarbon samples are revealed in Table 4. Statistical analysis at $p \leq 0.05$ showed that for calcium, crude oil had the highest value of 386.53 ± 0.4256 while kerosene had the lowest value of 2.08 ± 0.0928. Magnesium present in petroleum products, crude oil had the highest value of 14.36 ± 0.0232 while kerosene had the lowest (0.17 ± 0.0049). Crude oil had the highest value of Iron with 58.62 ± 0.0489 while engine oil had the lowest value

of 0.14 ± 0.0057. Manganese present in used engine oil was more compared to other samples having a value of 974.24 ± 0.0803 while kerosene had the lowest value of 4.39 ± 0.3586. The highest value of Lead was found in crude oil with 1.3 ± 0.0041 while the lowest was engine oil with 0.26 ± 0.0058. Nickel in the products was more in used engine oil having 7.46 ± 0.0525 but lesser in engine oil with a value of 0.13 ± 0.0054. Table 5 showed the physicochemical characteristics of the hydrocarbon samples. Statistical analysis at $p \leq 0.05$ showed that kerosene had a pH of 6.10 ± 0.0577 and a temperature of 26.25 ± 0.1443, Crude oil had a pH of 8.50 ± 0.0289 and a temperature of 27.20 ± 0.0577, diesel had a pH of 5.17 ± 0.0058 and a temperature of 26.80 ± 0.0577, used engine oil had a temperature of 28.00 ± 0.0577 while engine oil had a temperature of 27.40 ± 0.0577.

The percentage of degradation of different petroleum hydrocarbon by bacterial isolates are showed in Table 6. The highest percentage of degradation was between *Pseudomonas aeruginosa* and *Bacillus subtilis. Pseudomonas aeruginosa* degraded 97.4% of diesel 78.2% of spent engine oil, 88.2% of kerosene, 80.7% of engine oil, and 71.3% of crude oil. *Bacillus subtilis* degraded 89.6% of crude oil, 87% of engine oil, 71% of diesel, 72.6% of spent engine oil, and 97% of kerosene. Table 7 showed the physicochemical composition of contaminated and uncontaminated soil samples. Statistical analysis at $p \leq 0.05$ showed that the pH value in the control (uncontaminated soil sample) was 6.92 ± 0.18 significantly different from the contaminated samples. The product with the lowest pH value was crude oil with 4.87 ± 0.01, while the highest pH value was oil and grease with 6.12 ± 0.00. For total Nitrogen statistical analysis at $p \leq 0.05$ showed a significant difference from the control (uncontaminated soil sample) which was 0.26 ± 0.00. Crude oil and Diesel had the lowest value of Nitrogen as 0.10 ± 0.00, while the highest value was in the oil and grease having 0.15 ± 0.01. For total organic Carbon, statistical analysis at $p \leq 0.05$ also showed a significant difference from the control (uncontaminated soil sample) which was 4.01 ± 0.12. Oil and grease had the lowest value of organic carbon (1.93 ± 0.03), while the highest value was in crude oil having 3.46 ± 0.01. For oil and grease, they were significantly different from the control (uncontaminated soil sample) which was 14.93 ± 0.357. The lowest value of oil and grease was found in Diesel with 394.86 ± 3.10, while the highest value was found in crude oil having 851.43 ± 3.12.

Table 1: Frequency of occurrence of bacterial isolates from hydrocarbon contaminated soil samples

S/N	Bacterial isolates	Frequency	Percentage Occurrence (%)
1	*Staphylococcus epidermidis*	2	7
2	*Aeromonas veronii*	1	3
3	*Bacillus megaterium*	3	10
4	*Escherichia coli*	2	7
5	*Acinetobacter baumannii*	7	24
6	*Jannibacter terrae*	1	3
7	*Pseudomonas aeruginosa*	6	21
8	*Bacillus subtilis*	5	17
9	*Klebsiella pneumonia*	2	7
10	*Micrococcus luteus*	3	10
11	*Acinetobacter ursingii*	1	3
12	*Bacillus* spp.	1	3
13	*Staphylococcus aureus*	2	7

Table 2: Growth of bacterial isolates from mechanic workshops and generator sites on oil agar

Sample code	Bacterial isolates from mechanic workshops	Growth on oil agar	Sample code	Bacterial isolates from generator sites	Growth on oil agar
As-3	*Pseudomonas aeruginosa*	+	Ds-3	*Bacillus subtilis*	+
As-4	*Acinetobacter ursingii*	+	Ds-4	*Acinetobacter baumannii*	+
As-5	*Micrococcus luteus*	+	Ds-5	*Micrococcus luteus*	+
Ad-3	*Acinetobacter baumannii*	+	Dd-3	*Klebsiella pneumoniae*	+
Ad-4	*Acinetobacter baumannii*	+	Dd-4	*Bacillus megaterium*	+
Ad-5	*Staphylococcus aureus*	+	Dd-5	*Micrococcus luteus*	+
Bs-3	*Staphylococcus epidermidis*	+	Es-3	*Staphylococcus epidermidis*	+
Bs-4	*Pseudomonas aeruginosa*	+	Es-4	*Aeromonas veronii*	-
Bs-5	*Bacillus megaterium*	+	Es-5	*Bacillus megaterium*	+
Bd-3	*Bacillus subtilis*	+	Ed-3	*Escherichia coli*	+
Bd-4	*Pseudomonas aeruginosa*	+	Ed-4	*Acinetobacter baumannii*	+
Bd-5	*Bacillus* spp.	+	Ed-5	*Jannibacter terrae*	-
Cs-3	*Acinetobacter baumannii*	+	Fs-3	*Escherichia coli*	+
Cs-4	*Pseudomonas aeruginosa*	+	Fs-4	*Pseudomonas aeruginosa*	+
Cs-5	*Staphylococcus aureus*	+	Fs-5	*Bacillus subtilis*	+
Cd-3	*Acinetobacter baumannii*	+	Fd-3	*Klebsiella pneumoniae*	+
Cd-4	*Bacillus subtilis*	+	Fd-4	*Bacillus subtilis*	+
Cd-5	*Pseudomonas aeruginosa*	+	Fd-5	*Escherichia coli*	+

Keys:
+ = Growth; - = No growth; s - Surface soil sample; d - Depth (10m) soil sample
As-3 - Ad-5: Bacterial isolates from mechanic workshop 1
Bs-3 - Bd-5: Bacterial isolates from mechanic workshop 2
Cs-3 - Cd-5: Bacterial isolates from mechanic workshop 3
Ds-3 - Dd-5: Bacterial isolates from generator site 1
Es-3 - Ed-5: Bacterial isolates from generator site 2
Fs-3 - Fd-5: Bacterial isolates from generator site 3

Table 3: Growth of fungal isolates from mechanic workshops and generator sites on oil agar

Sample code	Fungal isolates from mechanic workshops	Growth on oil agar	Sample code	Fungal isolates from generator sites	Growth on oil agar
As-3	*Trichoderma* spp	+	Ds-3	*Mucor* spp	-
As-4	*Aspergillus flavus*	+	Ds-4	*Candida* spp	+
As-5	*Aspergillus niger*	+	Ds-5	*Geotrichum* spp	-
Ad-3	*Trichoderma* spp	+	Dd-3	*Candida* spp	+
Ad-4	*Aspergillus parasiticus*	+	Dd-4	*Aspergillus flavus*	+
Ad-5	*Aspergillus flavus*	+	Dd-5	*Aspergillus flavus*	+
Bs-3	*Aspergillus niger*	+	Es-3	*Aspergillus niger*	+
Bs-4	*Rhizopus* spp	+	Es-4	*Penicillium* spp	+
Bs-5	*Aspergillus parasiticus*	+	Es-5	*Trichoderma* spp	+
Bd-3	*Aspergillus parasiticus*	+	Ed-3	*Aspergillus niger*	+
Bd-4	*Rhizopus* spp	+	Ed-4	*Penicillium* spp	+
Bd-5	*Aspergillus flavus*	+	Ed-5	*Penicillium* spp	+
Cs-3	*Rhizopus oryzae*	+	Fs-3	*Penicillium* spp	+
Cs-4	*Fusarium* spp	+	Fs-4	*Trichophyton* spp	-
Cs-5	*Trichoderma* spp	+	Fs-5	*Geotrichum* spp	-
Cd-3	*Rhizopus* spp	+	Fd-3	*Rhizopus oryzae*	+
Cd-4	*Fusarium* spp	-	Fd-4	*Penicillium* spp	+
Cd-5	*Aspergillus flavus*	+	Fd-5	*Candida* spp	+

Keys:
+ = Growth; - = No growth; s - Surface soil sample; d - Depth (10m) soil sample
As-3 - Ad-5: Fungal isolates from mechanic workshop 1
Bs-3 - Bd-5: Fungal isolates from mechanic workshop 2
Cs-3 - Cd-5: Fungal isolates from mechanic workshop 3
Ds-3 - Dd-5 = Fungal isolates from generator site1
Es-3 - Ed-5 = Fungal isolates from generator site 2
Fs-3 - Fd-5 = Fungal isolates from generator site 3

Table 4: Heavy metal analysis in hydrocarbon samples

Hydrocarbon samples	Calcium	Magnesium	Iron	Manganese	Lead	Nickel
Kerosene	2.08 ± 0.0928	0.17 ± 0.0049	1.35 ± 0.0513	4.39 ± 0.3586	0.27 ± 0.0055	2.06 ± 0.0476
Crude Oil	386.53 ± 0.4256	14.36 ± 0.0232	58.62 ± 0.0489	18.44 ± 0.0529	1.3 ± 0.0041	5.16 ± 0.0617
Diesel	64.00 ± 0.3215	4.03 ± 0.0546	6.46 ± 0.0365	13.51 ± 0.0564	0.81 ± 0.0023	1.61 ± 0.0041
Used/Spent Engine Oil	7.33 ± 0.0882	3.31 ± 0.0568	35.17 ± 0.0328	974.24 ± 0.0803	1.09 ± 0.0234	7.46 ± 0.0525
Engine Oil	3.60 ± 0.1157	1.12 ± 0.0436	0.14 ± 0.0057	121.42 ± 0.3990	0.26 ± 0.0058	0.13 ± 0.0054

Table 5: Physicochemical characteristics of hydrocarbon samples

Hydrocarbon samples	pH	Temperature (^{o}C)	Colour
Kerosene	6.10 ± 0.0577	26.25 ± 0.1443	Colourless
Crude oil	8.50 ± 0.0289	27.20 ± 0.0577	Darkish Brown
Diesel	5.17 ± 0.0058	26.80 ± 0.0577	Amber
Used/Spent Engine Oil	-	28.00 ± 0.0577	Black
Engine Oil	-	27.40 ± 0.0577	Amber

Table 6: Gravimetric analysis of petroleum hydrocarbon treated with bacterial isolates

Hydrocarbon samples	***Pseudomonas aeruginosa*** **(% Degraded)**	***Bacillus subtilis*** **(% Degraded)**	***Staphylococcus aureus*** **(% Degraded)**	***Acinetobacter baumannii*** **(% Degraded)**
Diesel	97.4	71	56.1	63.6
Kerosene	88.2	97	51.4	58.9
Crude oil	71.3	89.6	44.2	52
Engine oil	80.7	87	47.4	47.3
Used/Spent engine oil	78.2	72.6	52.8	36

Table 7: Physicochemical analysis of contaminated and uncontaminated soil samples

Hydrocarbon samples	Total Nitrogen	Total Organic Carbon	Oil and Grease	pH
Control (Uncontaminated soil sample)	0.26 ± 0.00	4.01 ± 0.12	14.93 ± 0.357	6.92 ± 0.18
Kerosene	0.13 ± 0.00	2.35 ± 0.09	512.97 ± 0.21	5.63 ± 0.01
Crude oil	0.10 ± 0.00	2.49 ± 0.16	851.43 ± 3.12	4.87 ± 0.01
Diesel	0.10 ± 0.00	3.46 ± 0.01	394.86 ± 3.10	6.10 ± 0.03
Used/spent engine oil	0.14 ± 0.00	3.26 ± 0.03	427.32 ± 0.28	5.29 ± 0.02
Engine oil	0.11 ± 0.00	2.72 ± 0.02	767.45 ± 0.73	4.93 ± 0.01
Oil and grease	0.15 ± 0.01	1.93 ± 0.03	529.37 ± 3.17	6.12 ± 0.00

DISCUSSION

Previous studies have shown that oil degrading bacterial isolates and other microorganisms are best isolated from oil contaminated soil (Vigneshpriya *et al.,* 2017). Microbiological and biochemical tests carried out on the samples determined the population and types of heterogeneous microorganisms found in the contaminated soil samples. The fungal and bacterial isolates were identified (Larone and Ryan, 2003; Bergey *et al.*, 1984). The ability of bacterial isolates to utilize hydrocarbon as a carbon source was studied in *Aeromonas veronii, Acinetobacter baumannii*, *Acinetobacter ursingii*, *Bacillus cereus*, *Bacillus subtilis, Bacillus megaterium, Escherichia coli, Micrococcus luteus, Jannibacter terrae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus*, etc. of which *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the most frequent.

The ability of fungal isolates to utilize hydrocarbon as a carbon source was studied in *Aspergillus flavus,* Aspergillus niger, *Aspergillus parasiticus, Candida* spp., *Penicillium* spp., *Rhizopus* spp., *Rhizopus oryzae*, and *Trichoderma* spp. which exhibited hydrocarbon degrading abilities by their growth on the oil agar. Out of all the fungal isolates studied in a similar study, *Penicillium citrinum* was observed to have the highest ability to efficiently biodegrade crude oil (Barnes et al., 2018). However, *Fusarium* spp., *Mucor* spp., *Trichophyton* spp., and *Geotrichum* spp. did not grow on the oil agar which indicated their inability to degrade or utilize hydrocarbon as a carbon source. Most of the identified bacterial and fungal isolates were able to utilize oil as a carbon source and degrade hydrocarbon. The nutrient status of the contaminated soil produced a higher count of bacterial than fungal isolates and also, the presence of toxic components does not favor fungal growth (Ijah and Antai, 2003).

The contaminated soil from the mechanic workshops was brown, clay-like and moist, brown, sandy, and had large particle size while that of the generator sites were black, sandy, and dry while others were gray, sandy, and moist. Soil contamination due to hydrocarbon reduces its bulk density thereby accelerates the porosity of such soil (Atlas and Cerniglia, 1995). *Bacillus* spp. was predominant in this study and is similar to the report of the study by Ijah and Antai (2003) where *Bacillus* spp. was reported as the most frequent isolate of all the crude oil utilizing bacteria, characterized by highly polluted soil samples. Also, a direct relationship between *Bacillus cereus* cell growth and crude oil biodegradation was reported in a previous study (Abdulla *et al.*, 2019). Similarly, Latha and Kalaivani (2011) reported that Gram-positive *Bacillus* species were effective engine oil-degrading bacteria. The presence of the peptidoglycan cell wall helps Bacillus spp. tolerate a high concentration of engine oil. *Bacillus* (rod) species are known as the most effective petroleum hydrocarbon-degrading bacteria (Latha and Kalaivani, 2012; Hamza *et al.*, 2010). Rahman *et al*. (2002) postulated that Bacillus spp. were tolerant to high levels of hydrocarbons in soil due to their resistant endospores. Among the Gram-negative organisms isolated were *Pseudomonas* spp. and *Aeromonas* spp. The cell membrane of Gram-negative rods contains an additional efflux pump that ensures the removal of metabolites from the cell to prevent saturation of waste products by ensuring the excretion and removal of metabolites from the cell to prevent feedback inhibition; this accounts for *Pseudomonas* spp. being adjudged as the best petroleum hydrocarbon degraders. Most bacterial isolates were able to utilize the different hydrocarbons introduced in varying amounts due to the differences in their competence; that is, the capacity to degrade hydrocarbons. The bacterial isolates that did not grow on the Bushnell Haas agar (which is a selective medium for growing hydrocarbon degrading organisms) included *Aeromonas veronii* and *Jannibacter terrae* while other bacterial species grew on the selective medium proving their hydrocarbon degrading abilities.

The number of heterogeneous bacteria was observed to be higher than oil degrading bacteria in the media. The increase in microbial count after initial decrease could indicate the adaptation of the microorganisms to the environment that is bacteria degrading activities of contaminated soil samples could have stimulated the growth of the adapted organisms; a similar case was reported by Okoh (2006). The presence of *Pseudomonas* spp. in soil was in line with the work of Panda *et al.* (2013) who reported that Pseudomonas is a naturally occurring potent oil degrading bacteria. Heavy metal analysis in petroleum samples revealed the presence of calcium, magnesium, iron, manganese, lead, and nickel in varying quantities. It was observed that the presence of a contaminant like petroleum hydrocarbon accumulated more heavy metals and also has a drastic negative effect on the texture of the soil and environmental hazards associated with heavy metals (Szulc *et al*., 2014).

The level of oil *degradation* by *Pseudomonas* spp., *Staphylococcus* spp., *Micrococcus* spp., *Acinetobacter* spp., and *Bacillus* spp. was determined using gravimetric analysis. *Pseudomonas aeruginosa* and *Bacillus subtilis* were observed from the result to be more efficient oil degraders. Dayamrita *et al*. (2020) had previously reported a biosurfactant producing *Bacillus* spp. as an efficient degrader of crude oil. *Pseudomonas*

aeruginosa and *Bacillus subtilis* are known to possess a more competent and active hydrocarbon degrading enzyme system than *Staphylococcus* spp., *Micrococcus* spp. and *Acinetobacter* spp. They are fast-growing and can degrade a wide variety of organic compounds (Ijah and Okang, 1993). *Staphylococcus* spp., *Micrococcus* spp., and *Acinetobacter* spp. have been observed to have the property and considerable efficiencies to be used as an oil degrader, but it requires more time and in larger amount than *Pseudomonas* spp. and *Bacillus* spp. The use of such organisms is useful in biological processes such as bioremediation which consists of bioaugmentation strategies; this usually lowers the cost as when compared with chemical treatment processes for various contamination sites (Nrior and Jirigwa, 2017; Cattaneo *et al*., 1997).

Conclusion

This study showed that bacterial and fungal isolates from soils contaminated with oil exhibited the potentials to degrade oil and the bioremediation process using microorganisms was made possible. The bioremediation process using microorganisms is effective and also cost-efficient compared to chemical methods; although it takes a longer period and requires the microorganisms in large quantities. Also, changes in soil parameters such as structure, texture, total Nitrogen present, and increase in the concentration of heavy metals were found in the oil contaminated soil which has been reported to be toxic to life forms.

Conflict of interests

The authors' declare that there is no conflict of interest of any kind about this research.

Authors' contribution

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