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# Biodegradation Potential of Bacterial Isolates from Crude Oil Contaminated Soil Samples from Gelegele River, Edo State

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**Abstract**

*The dependency on fossil fuels as energy source has resulted in serious environmental issues in recent times. Bioremediation is an efficient, economic and environmentally friendly method of treatment. The purpose of this study was to determine the degradative potential of bacterial isolates from soil samples collected from Gelegele river. Eight (8) soil samples were collected from eight sampling locations around the flow station at Gelegele River. The physiochemical studies were carried out according to the methods of Association of Official Analytical Chemist (A.O.A.C) for the determination of the soil physicochemical parameters. Standard microbiological techniques were used for the morphological and biochemical analysis. Molecular characterization of the bacterial isolates was carried out using 16S rRNA, and shake flask technique was used for the bioremediation studies. Gas chromatography with flame ionization detector (GC-FID) method was used for the determination of total petroleum hydrocarbon. The physiochemical parameters, pH, electrical conductivity (EC), carbon showed significance ($p<0.05$) with respect to normal control. The total petroleum hydrocarbon (TPH) was recorded above the 1,000 mg/kg permissible limit by World Health Organization (WHO) and United States Environmental Protection Agency (USEPA) for hydrocarbon contaminated soil. The total heterotrophic bacterial counts ranged from $2.3 \pm 3.5 \times 10^4$ cfu/g - $5.2 \pm 3.5 \times 10^4$ cfu/g, while the total hydrocarbon utilizing bacterial counts ranged from $0.3 \pm 0.01 \times 10^3$ cfu/g - $3.6 \pm 2.60 \times 10^3$ cfu/g. The screening test revealed that out of the seven bacterial isolates screened for hydrocarbon utilization, six had the potentials to utilize hydrocarbon as carbon and energy sources. The bioremediation studies revealed changes in pH, which ranged from 5.0 - 7.3, temperature 31.0 $^0$C - 33.2 $^0$C and optical density 0.1300 - 1.236, of the mineral salt broth. Bacterial isolates showed degradation of aliphatic components of crude oil with respect to normal control. Molecular characterization revealed the bacterial isolates to include Citrobacter freundii Citrobacter cronae, Bacillus cereus, Citrobacter sp., Citrobacter cronae and Providencia vermicola. This study shows that the identified bacterial isolates may constitute potential isolates for biotechnological application in environmental clean-up of petroleum hydrocarbon.*

## 1. Introduction

There is growing public concern as a wide variety of toxic chemicals are being introduced inadvertently or deliberately into the environment. Petroleum hydrocarbons are one common example of these chemicals, which enter the environment frequently and in large volumes through numerous routes. Petroleum hydrocarbons come into the environment through accidents, spills or leak, urban input, industrial releases and commercial or domestic uses [28]. The term hydrocarbon has been used to describe petroleum complex mixtures in which there are hundreds of organic compounds ranging from light, volatile, short-chained, long-chained and branched compounds [35]. Soil polluted with organic compounds such as hydrocarbon products pose an environmental problem challenge world-wide [18]. Studied properties of soils include soil texture, moisture content, density, soil organic matter, pH, electrical conductivity and cation exchange capacity, among others. Oil pollution has deleterious effect on plant growth, soil macronutrients, microorganism and the terrestrial ecosystem in general [27][25]. One of the most important characteristics of hydrocarbon degrading bacteria is the ability to emulsify hydrocarbons in solution by producing surface active agents such as bio-surfactants [10]. Petroleum-contaminated soil can be decontaminated by environmental-friendly and cost-effective natural remediation techniques [7] such as bio-stimulation and bio-augmentation through enhancing the activity of hydrocarbon degrading microbes in contaminated soil [32]. The level of the contaminated soil is a crucial step in the planning for the remediation program for the oil-contaminated soil [22]. As such, analyses of both soil physicochemical and biological parameters are vitally important in the characterization of petroleum-contaminated soil. However, a reduced contaminant concentration may not always indicate a decrease in soil toxicity [8] due to the possibility of producing toxic metabolites in soil during bioremediation thus the microbiological characterization and identification is needed [21]. The objective of this study was to investigate the biodegradation potential of bacterial isolates from crude oil contaminated soil samples from Gelegele River, Edo state.

## 2.0: Materials and Method

### 2.1 Study site

The site used in this study was around the flow station at Gelegele Seaport in Ovia North East Local Government, Edo State. Benin City is the capital and largest city of Edo State in Southern Nigeria. It is the fourth largest city in Nigeria after Lagos, Kano and Ibadan, with a population of 1,782,000 as of 2021. It is situated approximately 40 kilometers (25 ml) north of the Benin river and 320 kilometers (200 ml) by road east of Lagos. Gelegele is a village located in Ovia North East Local Government Area of Edo State, Nigeria. Figure 1 represent the study site map. The control sample (sampling site A was about 300 meters away from sampling site B, while the others sampling sites (B to H) were 50 meters away from each other.

### 2.2 Sample collection

Soil samples of approximately 1kg (0 – 120 cm depth) were collected using a core sediment grab sampler from around the flow station at different locations along the shore line of Gelegele River, Edo State, (Figure 1). Samples were collected to represent a range of hydrocarbon contaminated soil and to represent various sources of pollution. Samples were collected in plastic bags and transported to the laboratory for analysis.

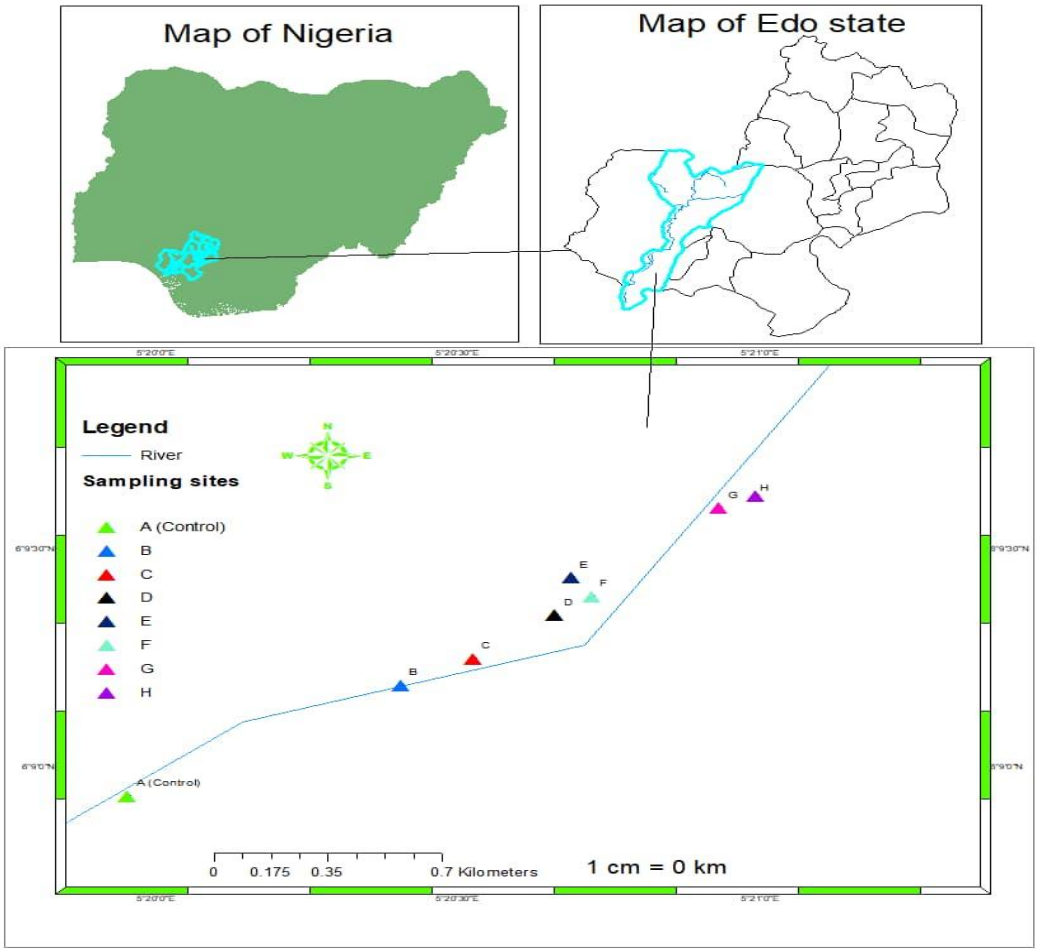


Figure 1: Map showing GPS coordinates of soil samples collected around the flow station of Gelegele River. (Key: A, B, C, D, E, F, G and H are the sampling sites)

**2.3 Physiochemical properties of the soil samples**

The physiochemical properties of the soils studied were determined by standard methods generally used in chemical soil laboratories [9].

**2.3.1: Determination of total petroleum hydrocarbon (TPH)**

Five grams (5 g) of crude oil contaminated soil from the field was suspended in 25 ml of hexane and shaken for 20 min using a shaker. The mixture was filtered using a Whatman No 1 filter paper and the filtrate diluted by transferring 1 ml of the extract into 50 ml of hexane. The absorbance of this mixture was read at 460 nm with HACH DR/2010 Spectrophotometer using n-hexane as blank [3].

The actual TPH concentration (mg/kg) was deduced as follows;

$$TPH = \frac{\text{Instrument reading (Conc. obtained from calibration)} \times \text{Volume of extract (ml)} \times DF}{\text{Weight of sample (kg)}} \quad (1)$$

Where TPH = Total petroleum hydrocarbon, DF = Dilution factor, Conc. = Concentration

**2.4: Bacterial enumeration**

One gram (1 g) of the contaminated soil was weighed using analytical balance (Model no AX423/E, USA Ohaus Cooperation) into test tubes containing 9 ml distilled water and 10 fold dilution was carried out up to $10^{-3}$ dilution. One milliliter (1 ml) of each dilution was inoculated into nutrient agar for the isolation of bacteria using the pour plate method. The plates were incubated at 30 $^0$C for 24 h. Hydrocarbon utilizing bacteria counts (HUB) were enumerated by inoculating 1 ml of aliquot of $10^{-1}$, $10^{-2}$, $10^{-3}$, sediments suspension into mineral salt agar plates prepared according to [11]. The plates were incubated at 30 $^0$C for 7 days. After the seven days incubation, emerging discrete colonies of the isolates were enumerated.

**2.5: Characterization and identification of bacterial isolates**

The bacterial isolates were characterized and identified based on the preliminary cultural, and biochemical characteristics. Identification of the isolates was performed according to [11]. The pure cultures of the test organisms were maintained in slants in McCartney bottles containing tryptic soy broth-gylcerol (TSB-glycerol). They were then stored as stock cultures in 4 $^0$C until required for analyses.

**2.6: Screening test for biodegradation potential of crude oil contaminated soil**

The isolates were screened for the ability to utilize crude oil using mineral salt medium. Nine milliliters (9 ml) of mineral salt medium was dispensed into seven test tubes. In each of the test tubes was added one milliliter of crude oil to serve as the main source of carbon and then sterilized. Thereafter, all the test tubes were inoculated with 0.5 Macfarland ($10^8$ cfu/ml) of an isolate previously grown in saline water. All the tubes were incubated at temperature of 30 $^0$C for 7 days, after which the turbidity of the solution was checked to determine the potentials of the isolates Those with high degradation potentials were utilized, while does with poor degradative potentials were eliminated from the experiment [20] [30].

**2.7: Determination of the ability of the bacterial isolates to utilize crude oil contaminated soil using shake flask method**

A known volume of 98 ml of the mineral salt medium was transferred into 250 ml conical flask followed by 1 ml crude oil before sterilization. Thereafter, all the conical flasks except that of the control sample were inoculated with 0.5 Macfarland ($10^8$ cfu/ml) of a twenty-four (24 h) culture previously grown in normal saline. The utilization of crude oil was monitored at three days interval for fifteen (15) days by monitoring the optical density readings at 600 nm using M501 UV-Vis Camspec Spectrophotometer, changes in ionic concentration with pH meter and temperature using thermometer [33].

**2.8: Total petroleum hydrocarbon (TPH) depletion analysis**

The method in extracting the residue crude oil in the conical flask was conducted by transferring twenty milliliters (20 ml) of the residual sample into a separating funnel, transfer 10 ml of n-Hexane was transferred into the flask and shaken vigorously for 10 to 15 mins and left to settle. 3 g of $NaSO_4$ was added and shaken to dry up water from the solvent. The solvent was transferred into a round bottom flask of a rotary evaporator, concentrate and filtered through the silica gel clean up. Cotton wool was placed at the bottom of the glass syringe cartridge and n-Hexane was allowed to move through the column until the head of the liquid in the column was above the

column frit, and the n-Hexane was collected. The extract was loaded into the column and the elutant was immediately collected in a 25 ml volumetric flask labelled Aromatics. Prior to exposure of the column frit to air, additional 5 ml of n-Hexane was transferred into the column. The contents (extracts) were transferred into 25 ml volumetric flask of a round bottom flask. The extract was concentrated to a final volume of 1 ml. The one milliliter (I ml) was transfer into labelled 2 ml flask auto sampler vials with Teflon-Lined rubber caps and was read using the HP 6890 Gas Chromatograph [23].

**2.9: Molecular characterization of the bacterial isolates**

The bacterial isolates characterized using biochemical methods were subjected to strain level identification applying molecular methods. The DNA of the bacterial were extracted using standard protocols and then amplification of the DNA using PCR, sequencing the gene of interest using next generation sequencing (NGS) [15].

**2.10: DNA extraction and PCR amplification of 16S rRNA gene in the bacterial isolates**

The DNA of six bacterial isolates were extracted using genomic DNA purification kit according to the manufacturer instructions (Thermo Fisher Scientific). The quality of extracted genomic DNA was evaluated by UV spectrophotometry at 260 and 280 nm. The ratio of 260/280 gives an idea of the quality of the solution of the DNA. The concentration of the DNA was measured by Pico-Green method using Quant-iT$^{TM}$ Pico-Green$^{TM}$ DNA assay kit (Thermo Fisher Scientific). The DNA extracted was stored at -20 $^{0}$C. Bacteria 16S rRNA gene was amplified using the forward and reverse primer: Forward primer 27F (5' – AGAGTTTGATCMTGGCTCAG-3') and Reverse primer 1492R (5' –TACGGYACCTTGTTACGACTT-3'). Each PCR reaction mixture contain (10 µl) 1 µl DNA template, 0.1 µl Q5 High –Fidelity DNA Polymerase (New England Bio-labs LTd), 0.2 mMdNTP, 1X buffer 5X Q5 (New England Bio-Lab Ltd) and 0.2 µM of each primer. Conditions for PCR amplification were as follows: Initial denaturation for 30 sec at 98 $^{0}$C for 10 sec, 62 $^{0}$C for 15 sec and 72 $^{0}$C for 2 min. Products from the PCR were visualized on 1 % agarose gel electrophoresis [15].

**2.11: Sequencing of 16S rRNA**

The genes of interest were amplified, cut and freeze dried at International Institute of Tropical Agriculture. The freeze dried samples of DNA were sent to Macrogen U.S.A laboratory for sequencing. The genomic sequence data was analyzed using bioinformatics tools, sequence identification was performed using National Centre for Biotechnology Information. Basic Local Alignment Search Tool (BLAST) algorithm and similar sequences were down-loaded. The sequences were aligned using Clustal W. The evolutionary history was inferred using the Neighnot-Joining method [31].

**3.0: Results and Discussion**

Soil is a reservoir for many microbes and natural habitat for many bacteria. Bacteria are known to be ubiquitous therefore can easily be isolated from the soil. The hydrocarbon degrading organisms convert hydrocarbons present in the pollutant such as crude oil into harmless products like carbon (iv) oxide, water and cell biomass.

Figure 1 is the map showing the location using GPS where the soil samples were collected.

Table 1 revealed the physicochemical properties of crude oil contaminated soil samples. The result showed that across all the sampling sites (A to H), pH ranged from (6.01 ± 0.02 – 6.49 ± 0.07),

Electrical Conductivity (EC) (416.00 ± 10.70 – 656.45 ± 11.65) mS/m, Carbon (2.13 ± 0.03 – 3.70 ± 0.01[a]) %, Nitrogen (0.04 ± 0.01 – 1.67 ± 0.02[ab]) %, Phosphorus (1.33 ± 0.06 – 2.91 ± 0.04[ab])%, Water holding capacity (WHC) (82.48 ± 0.82 – 92.48 ± 0.82) %, Bulk density (0.70 ± 0.02[ab] – 2.79 ± 0.05) %, Moisture (11.53 ± 9.75 – 26.06 ± 0.38) %. Table 1 revealed the physicochemical properties of the crude oil contaminated soil samples. These results showed that across all the sampling sites (A to H) there were significance in the results with respect to normal control. This study and other studies carried out have shown that the physiochemical properties of soil can be affected by hydrocarbon contamination [29] [1]. These physiochemical properties are suspected to aid the proliferation of the bacteria in the soil [24] [2].

Figure 2 was the result of the total petroleum hydrocarbon (TPH) which showed that the range of TPH contamination was between 216.16 ± 71.21 mg/kg – 4928.80 ± 61.35[ab] mg/kg.

The result of TPH of this study was above 1,000 mg/kg concentration of TPH in polluted soil which is the acceptable value by regulatory standards such as DPR, WHO, USEPA [14]. In this research the highest TPH value gotten was 4928.80 ± 61.35[ab] which was higher than the guideline given by [14]. Overtime, due to oil deposition in the environment due to continuous oil exploration, it will cause high accumulation and deposition of crude oil contaminating the environment, which can pose as great risk to the overall health status of the environment. This is in agreement with the research carried out by [5], who reported TPH values ranging from 1,242 mg/kg – 5,200 mg/kg of hydrocarbon contaminated soils in communities in Niger Delta region. Just like this present study where different sampling sites had different crude oil concentration. The research of [5] reported the highest value of TPH of 3,307 mg/kg in a research where polluted soil samples were collected from different locations in some communities in River State.

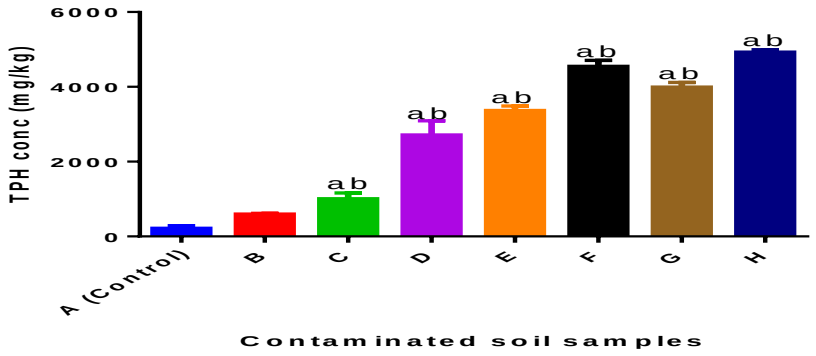


Figure 2: Total petroleum hydrocarbon (TPH) of contaminated soil sample. [ab]P < 0.0001 compare to normal control. The samples were represented as Mean ± SEM of triple replicates.

Key: A = Sampling site A (control sample) (N 06.14888 E 005.33254), B (sampling site B) (N 06.15311 E 005.34011), C (sampling site C) (N 06.15414 E 005.34210), D (sampling site D) (N 06.15581 E 005.34436), E (sampling site E) (N 06.115725 E 005.34481), F (sampling site F) (N 06.15653 E 005.34535), G (sampling site G) (N 06.15991 E 005.334887), H (sampling site H) (N 06.16039 E 005.34989)

**Table 1: Physiochemical properties of hydrocarbon contaminated soil samples from different sampling sites in Gelegele river**

| Parameters | Sample A | Sample B | Sample C | Sample D | Sample E | Sample F | Sample G | Sample H |
|---|---|---|---|---|---|---|---|---|
| pH | 6.19 ± 0.14 | 6.01 ± 0.02 | 7.01 ± 0.09[a] | 6.48 ± 0.09 | 6.26 ± 0.11 | 6.49 ± 0.07 | 6.47 ± 0.32 | 6.40 ± 0.03 |
| EC (mS/m) | 794.45 ± 31.65 | 719.30 ± 191.40 | 456.0 ± 10.70 | 493.90 ± 14.70 | 417.50 ± 19.60 | 567.15 ± 37.75 | 616.05 ± 10.85 | 592.30 ± 27.10 |
| Carbon (%) | 3.34 ± 0.04 | 2.13 ± 0.03[ab] | 2.44 ± 0.15[ab] | 3.10 ± 0.02 | 3.65 ± 0.13 | 3.66 ± 0.03 | 3.90 ± 0.04[a] | 3.70 ± 0.01[a] |
| Nitrogen (%) | 0.04 ± 0.01 | 1.44 ± 0.12[ab] | 0.84 ± 0.07[a] | 0.53 ± 0.04[a] | 0.98 ± 0.07[ab] | 0.81 ± 0.18[a] | 1.02 ± 0.11[ab] | 1.67 ± 0.22[ab] |
| Phosphorus (%) | 1.73 ± 0.02 | 1.33 ± 0.06 | 1.38 ± 0.19 | 1.55 ± 0.02 | 1.66 ± 0.03 | 1.76 ± 0.03 | 2.50 ± 0.07[ab] | 2.91 ± 0.04 [ab] |
| WHC (%) | 90.80 ± 0.80 | 88.30 ± 5.00 | 82.48 ± 0.82 | 86.65 ± 1.65 | 86.65 ± 3.35 | 89.15 ± 0.85 | 89.15 ± 0.85 | 92.48 ± 0.82 |
| Bulk density ($g/cm^3$) | 2.67 ± 0.02 | 0.70 ± 0.02 [ab] | 0.90 ± 0.03 [ab] | 2.79 ± 0.05 | 2.46 ± 0.16 | 2.82 ± 0.02 | 2.85 ± 0.08 | 2.71 ± 0.01 |

The values were the means±SEM for the physiochemical properties analyzed of the soil samples. Significance at $p< 0.05$ with respect to normal control.

Key: A = Sampling site A (control sample) (N 06.14888 E 005.33254), B (sampling site B) (N 06.15311 E 005.34011), C (sampling site C) (N 06.15414 E 005.34210), D (sampling site D) (N 06.15581 E 005.34436), E (sampling site E) (N 06.115725 E 005.34481), F (sampling site F) (N 06.15653 E 005.34535), G (sampling site G) (N 06.15991 E 005.334887), H (sampling site H) (N 06.16039 E 005.34989), a= Significant, ab= Highly significant

The result in Table 2 revealed that the total heterotrophic bacteria count (THBC) in this study ranging from $2.3 \pm 3.5 \times 10^4$ to $5.2 \pm 3.5 \times 10^4$ cfu/g, while that of Table 3 for the total hydrocarbon utilizing bacteria counts (THUBC) ranged from $0.3 \pm 0.01 \times 10^3$ to $3.6 \pm 2.60 \times 10^3$ cfu/g. The presence of bacterial growth from the contaminated soil samples is an evidence of the ability of these bacteria to adapt to such environment, just like it's been reported by other researchers such as [12] reported THBC of $6.66 \times 10^7$ cfu/g and THUBC of $8.22 \times 10^2$ cfu/g of soil samples collected from Nembe waterside at Port Harcourt. [17] reported THBC of $6.25 \times 10^5$ cfu/g and THUBC of $5.38 \times 10^4$ cfu/g from soil samples collected from hydrocarbon contaminated environment. These researches are in alignment with the result of this study.

**Table 2: Total heterotrophic bacterial counts (cfu/g) for different sampling sites in Gelegele river**

| Soil samples | Mean counts ($x10^4$) cfu/g |
|---|---|
| A | 5.2 ± 3.5 |
| B | 4.9 ± 2.2 |
| C | 3.1 ± 2.0 |
| D | 3.3 ± 2.0 |
| E | 2.3 ± 1.1 |
| F | 4.0 ± 2.6 |
| G | 3.3 ± 1.9 |
| H | 4.0 ± 2.6 |

Key: A = Sampling site A (control sample) (N 06.14888 E 005.33254), B (sampling site B) (N 06.15311 E 005.34011), C (sampling site C) (N 06.15414 E 005.34210), D (sampling site D) (N 06.15581 E 005.34436), E (sampling site E) (N 06.115725 E 005.34481), F (sampling site F) (N 06.15653 E 005.34535), G (sampling site G) (N 06.15991 E 005.334887), H (sampling site H) (N 06.16039 E 005.34989), The values were the means±SEM of triple replicates for the total heterotrophic bacteria count.

**Table 3: Total hydrocarbon utilizing bacterial counts (cfu/g) for different sampling sites in Gelegele river**

| Soil samples | Mean counts ($x10^3$) (cfu/g) |
|---|---|
| A | 0.3 ± 0.09 |
| B | 1.3 ± 2.40 |
| C | 1.3 ± 0.70 |
| D | 1.6 ± 0.90 |
| E | 1.8 ± 1.00 |
| F | 2.6 ± 1.70 |
| G | 2.9 ± 2.00 |
| H | 3.6 ±2.60 |

Key: A = Sampling site A (control sample) (N 06.14888 E 005.33254), B (sampling site B) (N 06.15311 E 005.34011), C (sampling site C) (N 06.15414 E 005.34210), D (sampling site D) (N 06.15581 E 005.34436), E (sampling site E) (N 06.115725 E 005.34481), F (sampling site F) (N 06.15653 E 005.34535), G (sampling site G) (N 06.15991 E 005.334887), H (sampling site H) (N 06.16039 E 005.34989), The values were the means±SEM of triple replicates for the total hydrocarbon utilizing bacteria count.

Table 4 revealed the result of screening test which showed that *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Enterobacter* sp., *Citrobacter* sp. and *Pseudomonas* sp. had better hydrocarbon

utilization that *Klebsiella* sp. Out of the seven bacteria isolates, *Klebsiella* sp. had the least degradative ability and was not used for further studies. This result supports the study of [15] [4] [13] [26]. where bacteria were screened to ascertain their hydrocarbon degrading potential.

**Table 4: Screening test result for biodegradation analysis**

| S/N | Isolates | Activity | Reference |
|---|---|---|---|
| 1 | *Acinetobacter* sp. | 0.200 | +++ |
| 2 | *Alcaligenes* sp. | 0.189 | ++ |
| 3 | *Bacillus* sp. | 0.207 | +++ |
| 4 | *Enterobacter* sp. | 0.203 | +++ |
| 5 | *Klebsiella* sp. | 0.080 | + |
| 6 | *Citrobacter* sp. | 0.210 | +++ |
| 7 | *Pseudomonas* sp. | 0.214 | +++ |

Key: + = Little Growth, ++ = Moderate Growth, +++ = Heavy Growth

Figure 3 showed the PCR product of 1500 bp of the six bacterial isolates analyzed using 1 % agarose gel electrophoresis. The use of molecular techniques using 16S rRNA have been used in identification of bacterial isolates just like this study (Obafemi *et al*., 2018; Dilmi *et al.*, 2017. This research is in agreement with this present study.

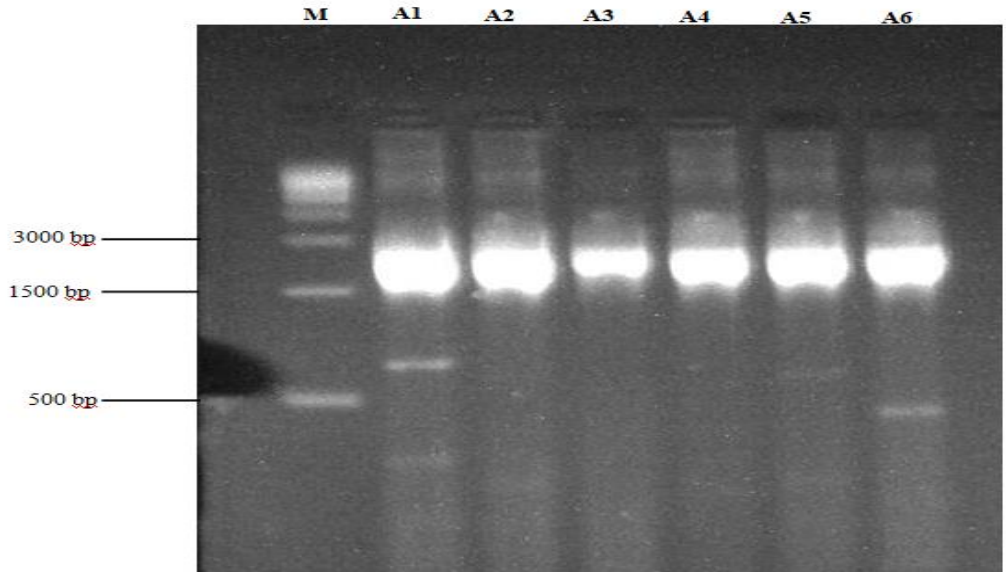


Figure 3: Agarose gel electrophoresis of PCR amplification product of 16 S rRNA gene band of the isolates.

Key: M, 100 bp DNA size marker, A1 (*Citrobacter freundii* strain BCB380 KT260592.1), A2 (*Citrobacter cronae* MN548424.1), A3 (*Bacillus cereus* strain JS10 MT102922.1), A4 (*Citrobacter* sp. strain FDAARGCS CP014030.2), A5 (*Citrobacter cronae* MN548424.1), A6 (*Providencia vermicola* strain Bu15–38).

Table 5 revealed the results of the gene bank profile of 16S rRNA sequence of bacterial isolates showing A1 (*Citrobacter freundii* strain BCB380 KT260592.1), A2 (*Citrobacter cronae* MN548424.1), A3 (*Bacillus cereus* strain JS10 MT102922.1), A4 (*Citrobacter* sp. strain FDAARGCS CP014030.2), A5 (*Citrobacter cronae* MN548424.1), A6 (*Providencia vermicola* strain Bu15–38). The results revealed that the bacterial isolates were identified to their species level. The use of cultural, morphological and biochemical test was used to identify the bacterial isolates

used in this study at first before proceeding to using 16S rRNA techniques, which revealed a change in almost all the isolates. [34] [13]. The research of this study agrees with the research of [15] where 16S rRNA gene sequencing was used for the identification of four bacterial isolates used for bioremediation studies to their species level.

**Table 5: Closest relatives of identified bacteria based on 16S rRNA gene sequences**

| S/N | Isolates | Closest hit | Phylum | Accession number | % Similarity |
|---|---|---|---|---|---|
| 1 | *Acinetobacter* sp. | *Citrobacter freundii* | Proteobacteria | KT260592.1 | 99.41 |
| 2 | *Alcaligenes* sp. | *Citrobacter cronea* | Proteobacteria | MN548424.1 | 99.57 |
| 3 | *Bacillus* sp. | *Bacillus cereus* | Firmicutes | MT102922.1 | 99.43 |
| 4 | *Enterobacter* sp. | *Citrobacter* sp. | Proteobacteria | CP014030.2 | 99.44 |
| 5 | *Citrobacter* sp. | *Citrobacter cronea* | Proteobacteria | MN548424.1 | 99.63 |
| 6 | *Pseudomonas* sp. | *Providencia vermicola* | Proteobacteria | KY671146.1 | 100.00 |

The research of [15] is in conformity with the results of this study in Figure 3, 4 and 5, which revealed the results of pH, temperature and optical density (OD) of the bioremediation experiment. These parameters were used to explain the metabolic activities of bacterial isolates during the process of biodegradation. These parameters helped to give a clear indication if the bacterial subjected to bioremediation process in the medium were able to adapt, proliferate and utilize the hydrocarbon in the medium as the source of carbon. The results in Figure 3, 4 and 5 clearly revealed that the six bacterial isolates used in this study were able to utilize, proliferate and degrade the crude oil present in the mineral salt medium. Like reported in this study and other researchers, there was a gradual increase in optical density and turbidity of the mineral salt medium of the bioremediation setup which lasted for fifteen days, indicating adaptation, survival and proliferation of the bacterial isolates, thereby the bacteria utilizing carbon as a sole source of energy [16] [19].

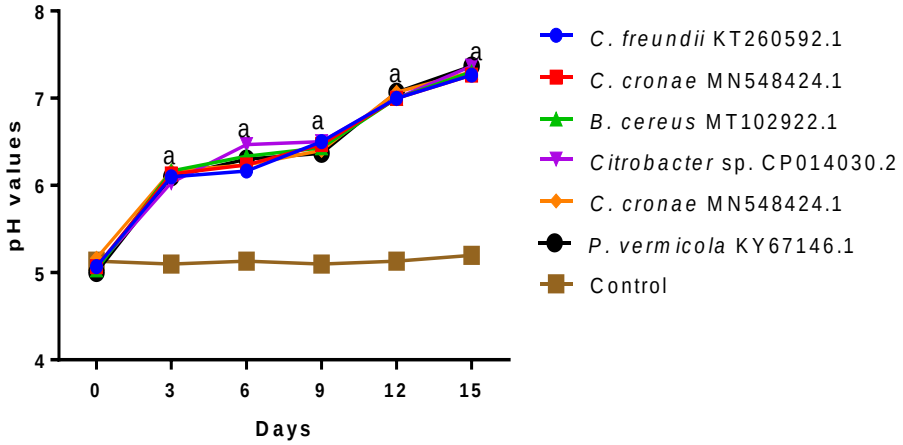


Figure 3: Growth curve of pH values in mineral salt medium (MSM) broth with 1 % crude oil for 15 days incubation. $^{a}P < 0.05$ compared to control, values were represented as Mean ± SEM, n = 3

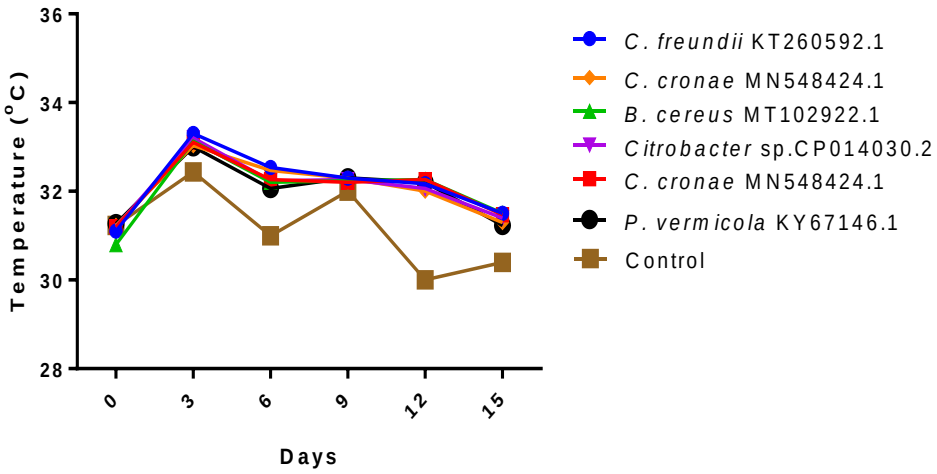


Figure 4: Growth curve of temperature values in mineral salt medium (MSM) broth with 1 % crude oil for 15 days incubation. [a]P < 0.05 compared to control, values were represented as Mean ± SEM, n = 3

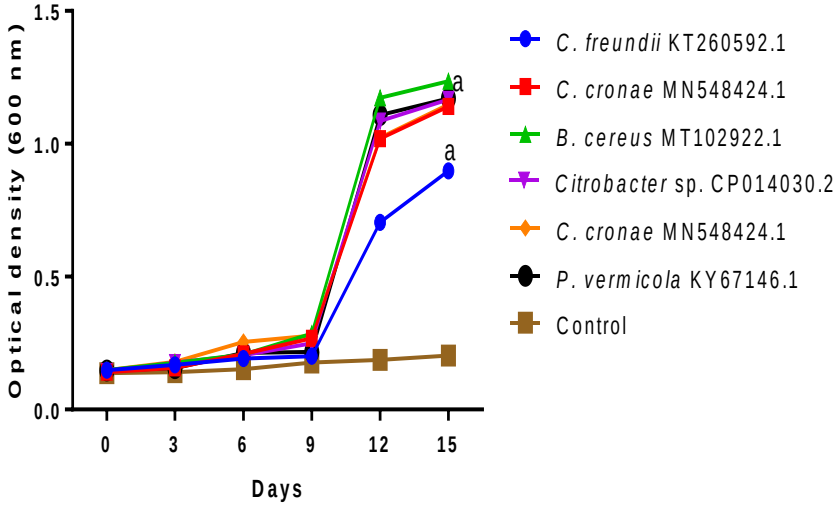


Figure 5: Growth curve of optical density values in mineral salt medium (MSM) broth with 1 % crude oil for 15 days incubation. [a]P < 0.05 compared to control, values are represented as Mean ± SEM, n = 3

The results in Table 6 revealed the aliphatic components degradation during shake flask experiments at day 15. This result revealed reductions in the quantity of aliphatic components present in the medium. This reduction signified utilization of the carbon and mineralization of the hydrocarbons by the bacterial isolates. The components of crude ranging from $C_{11}$ to $C_{20}$ were reduced by the activity of the bacterial isolates. All the bacterial isolates were active in degrading the aliphatic components of the crude oil at different intensity. The research of [23] revealed that almost all the component of crude oil ranging from $C_9$ to $C_{30}$ were drastically reduced by the action of hydrocarbon utilizing bacteria in a bioremediation experiment. This research is in agreement with this present study.

Table 6: Aliphatic components of crude oil degradation

| Component mg/kg | Control | A1 | A2 | A3 | A4 | A5 | A6 |
|---|---|---|---|---|---|---|---|
| n-undecane | 20.142 | 0.132 | 0.389 | 0.593 | 0.469 | 0.178 | 0.422 |
| n-dodecane | 23.802 | 0.216 | 0.788 | 1.273 | 1.056 | 0.399 | 0.872 |
| n-tridecane | 28.494 | 0.213 | 1.219 | 1.444 | 1.257 | 0.458 | 0.965 |
| n-tetradecane | 30.809 | 0.353 | 0.000 | 2.441 | 2.237 | 0.778 | 1.516 |
| n-pentadecane | 33.182 | 1.180 | 8.094 | 1.372 | 2.250 | 0.842 | 1.713 |
| n-hexadecane | 32.245 | 0.384 | 3.346 | 1.401 | 0.683 | 0.551 | 1.025 |
| n-heptadecane | 29.545 | 0.252 | 1.156 | 1.605 | 0.325 | 0.596 | 1.087 |
| n-octadecane | 2.683 | 0.311 | 1.103 | 1.311 | 0.716 | 0.688 | 1.087 |
| n-nonadecane | 4.019 | 0.407 | 1.263 | 0.213 | 0.162 | 1.081 | 1.198 |
| n-eicosane | 2.170 | 1.000 | 0.170 | 0.150 | 0.173 | 0.000 | 0.066 |

Key: A1 (*Citrobacter freundii* strain BCB380 KT260592.1), A2 (*Citrobacter cronae* MN548424.1), A3 (*Bacillus cereus* strain JS10 MT102922.1), A4 (*Citrobacter* sp. strain FDAARGCS CP014030.2), A5 (*Citrobacter cronae* MN548424.1), A6 (*Providencia vermicola* strain Bu15–38).

## 4.0: Conclusion

This study was able to reveal that the soil samples collected from the flow station were high in total petroleum hydrocarbon beyond the acceptable limit by regulatory organizations connoting hydrocarbon pollution of the environment. The physiochemical parameters analyzed revealed that the hydrocarbon contamination in the soil affected the parameters used to evaluate the structural integrity of a soil. The bacterial isolates from the hydrocarbon contaminated soil samples showed high potentials in carrying out bioremediation. This research showed that the bacterial isolated had the potential to carry out mineralization of pollutants and can be used to remediate polluted environments. Conclusively, the characterized bacterial isolates may constitute potential isolates for biotechnological application in environmental cleanup of petroleum contamination.

## Conflict of Interest

There is no conflict of interest associated with this work.

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