



Bacteriological and Physicochemical Profiles of Soils in Selected Oil-Contaminated Sites in Yorla, Ogoni Land

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Authors' contributions

This work was carried out in collaboration among all authors. Author OSU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GCO and EOA contributed to both the design and supervision of the study. All authors read and approved the final manuscript.

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ABSTRACT

Bacteriological and physicochemical properties of oil-polluted sites in Yorla, Ogoniland was studied. Soil samples were collected from three different points and analyzed for bacteriological and physicochemical characterization using standard methods. The total culturable heterotrophic bacteria (THC) counts from the polluted sites ranged from $7.2 \cdot 10^7$ to $9.5 \cdot 10^7$ cfu/g while the THC of the unpolluted sites ranged from $11.9 \cdot 10^7$ to $12.9 \cdot 10^7$ cfu/g. Hydrocarbon utilizing bacterial (HUB) counts from the polluted sites ranged from $2.8 \cdot 10^6$ to $3.3 \cdot 10^6$ cfu/g while the hydrocarbon utilizing bacterial (HUB) counts of the unpolluted sites ranged from $1.1 \cdot 10^6$ to $1.5 \cdot 10^6$. Bacteria obtained from the various sites were isolated using standard microbiological methods. Identification of isolates was carried out using 16S rRNA (Ribonucleic acid) gene amplification and sequencing. Electropherograms of generated sequences were inspected with Chromas Lite 2.0.1 software. Sequence identification was carried out using GenBank's Basic Local Alignment Search Tool (BLAST) algorithm of National Centre for Biotechnology and Information (NCBI). Sequence

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analysis revealed the presence of *Acinetobacter venetianus*, *Bacillus cereus*, *Bacillus flexus*, *Bacillus thuringiensis*, *Acinetobacter junii*, *Shewanella* sp., *Lysinibacillus xylanilyticus* and *Pseudomonas pseudoalcaligenes*. The physicochemical properties of the soil samples analyzed showed that the pH values of the polluted sites ranged from 7.86 to 9.16 while those of the unpolluted sites were 6.19 and 7.10. Total organic carbon (TOC) contents of the polluted sites ranged from 3.18% to 3.96% while the unpolluted sites TOC ranged from 3.00% -3.06%. Statistical analyses of the results were carried out using analysis of variance (ANOVA) and t-test analysis. This work revealed the presence of reasonable population of indigenous hydrocarbon-utilizing bacteria in oil-polluted sites in Yorla community which can be monitored and enhanced to improve their bioremediation abilities in the oil-polluted sites.

Keywords: Hydrocarbon-pollution; Niger Delta; Biodegradation; Hydrocarbon utilizers.

1. INTRODUCTION

The Niger Delta region is Nigeria's hub of hydrocarbon exploration and exploitation. The over 50 years of crude oil production has left severe consequences on farmlands, aquaculture and fishing settlements due to intermittent hydrocarbon spills [1]. One of the main reasons for extended negative impact of oil spill on the environment could probably be unavailability of adequate and qualitative scientific baseline data which is required to provide informed and quick response to emergent environmental challenges [2]. Hydrocarbon exploration and exploitation activities in Ogoniland, Niger Delta, Nigeria began in the 1950's, but was halted due to conflict between international oil companies and the host communities. Most crude oil fields and installation have been left dormant for several decades due to the restiveness in Ogoniland. Lack of maintenance, decades of un-remediated spills, oil trapping and damage to oil infrastructures have been a common sight in this region and the environment has been without remediation or partially remediated over the years [3].

Hydrocarbon spill in the environment can be traced to several reasons including human error, equipment failure, sabotage and accidents. Accidents from the transportation of petroleum products from production points to different end users is increasingly becoming a major source of hydrocarbon pollution [4]. The deleterious effect of hydrocarbon on the environment (marine and terrestrial) and on humans and other living organisms makes it a contaminant of global concern [5]. Biodegradation of hydrocarbons by indigenous microbial species, which are often ubiquitous is a primary mechanism for eliminating petroleum pollution from the environment [6].

Several bacterial and fungal species have been demonstrated to have the capacity to degrade a wide range of hydrocarbons including complex aromatic fractions [4]. Constant deposit of pollutants of petroleum sources has given rise to development of microbial community with the capacity to withstand and survive toxic environments. Micro-organisms can easily sense alteration in their immediate environment. When the physical or chemical composition of their environment is altered suddenly, there exists a lag period or acclimation period within which the microbial community began to adjust to adapt in the new environment [6,7,8]. This acclimation period helps the micro-organisms to develop metabolic repertoire needed for their adaptation [9,10,11,12]. It has been observed that this process or phenomenon occurs in both marine and terrestrial environments [6,13,14,15].

The aim of this study was therefore to assess the microbial and physicochemical profiles of soils in selected oil contaminated sites in Yorla, Ogoniland, Niger Delta region in Nigeria in order to determine their role in hydrocarbons degradation. Following the chronic pollution of the study site and an attendant increasing adaptation of the microbial community, there are possibilities for recovery of new strains of organisms capable of accelerated degradation of hydrocarbon, adapted to the *in situ* conditions of the polluted sites. This study will also explore this opportunity.

2. METHODOLOGY

2.1 Sample Collection

Soil samples were collected in sterile polyethylene bags using soil auger. The soil samples were collected using random sampling method at 0-15cm and 15-30cm depths.

Approximately 1 Kg and 50 g of soil was respectively collected for chemical and microbiological analyses. Composite samples collected at these depths were immediately transported to the laboratory for analyses.

2.2 Physico-Chemical Analysis of Soil Samples

2.2.1 pH measurement

pH meter was switched on for ten minutes to stabilize. The electrode was dipped in buffer 4 solution and the meter was calibrated using the appropriate knob. The electrode was transferred into buffer 9 solution and was calibrated to same pH, the electrode was rinsed with distilled water jet from the wash bottle and dipped finally to the soil solution in the beaker after stirring several minutes to homogenized properly. The pH value displayed on the pH meter screen that remained constant for 30 seconds was recorded as the pH value of the solution (APHA 4500H⁺B).

2.2.2 Total organic carbon (TOC)

TOC was determined as described in APHA [16] (BS 1377-3:1990 clause3). Exactly 0.1 g air-dried sieved soil was weighed into a clean 250 ml Pyrex conical flask. Five millilitres potassium dichromate solution was added with 7.5 ml concentrated sulphuric acid respectively. Five millilitres and 7.5 ml of potassium dichromate and sulphuric acid were introduced into another clean conical flask and was labelled blank test. The conical flasks were subjected to heating on an electro-thermal heater for 10-15 minutes as the case may be. When the oxidation/reduction process was accomplished by the appearance of greenish-yellow solution of the digest then, digest was cooled to room temperature and was diluted to 100 ml with distilled water. Twenty-five millilitres of this solution was pipette into a clean conical flask and was titrated with Ferrous Ammonium Sulphate to reddish solution using ferrion as indicator. Blank test was titrated and the respective titres were recorded.

Calculation:

$$\% \text{TOC} = \frac{\text{Blank titre} - \text{Sample titre} \times 0.2 \times 0.3}{\text{Weight of sample}}$$

2.2.3 Nitrate – Nitrogen

Determination of nitrate was also based on APHA [16] method (APHA 4500-N₃⁻). One gram

of soil sample was weighed into a clean conical flask using weighing balance. 100 ml distilled water was added and content was stirred for 5 minutes with magnetic stirrer. This solution was filtered through Whatman filter paper into a 100 ml volumetric flask.

One millilitre of filtrate was pipette into a clean test tube then 0.5 ml brucin reagent was added and 2 mls concentrated sulphuric acid. A blank was prepared using distilled water. The yellowish colour developed by sample filtrate was read at 420nm wavelength in a spectrophotometer using blank test to zero the spectrophotometer. Standard NO₃⁻ solution was prepared and was treated as was described above in the sample test. The colour was read at same wavelength using blank test to zero the spectrophotometer. A calibration graph was plotted using the standard values. The concentration of the NO₃⁻ (Nitrate-Nitrogen) ion in the soil solution was interpolated from the standard graph (EPA 352.1).

2.2.4 Phosphate – Phosphorus

The determination of phosphate was based on standard method (APHA 4500-PD). One gram of soil sample was weighed into 250 ml conical flask. 50 ml solution of glacial acetic acid was added and was stirred for 5 minutes. The mixture was filtered into a clean volumetric flask through Whatman filter paper.

2.2.5 Standard Phosphate Phosphorus

This was prepared, from where lesser concentration ranges were further prepared. Fifty millilitres of test sample; distilled water and standard phosphate phosphorus solution were measured into 100 ml volumetric flask respectively and labelled appropriately. To this flask was added 8.0 mls combined ascorbic acid reagent respectively and were made up to 100 ml with distilled water; allowed to stand for 20 minutes for proper colour development.

The colour developed was read at 880nm wavelength in the spectrophotometer using distilled water as blank. The absorbance of the standard phosphate concentration ranges was recorded and same for the sample. Standard graph of absorbance against concentration was plotted to obtain a straight line graph that passed through the origin. The concentration of the PO₄³⁻P in the sample was interpolated from the graph (APHA 4500-PD).

2.2.6 Total Hydrocarbon Content

One gram of sieved sample was extracted with 10mls chloroform (CCl₃), in a glass test tube. The extraction was partitioned, between distilled water in a separatory flask. The ccl₃ layer (lower phase) was taken with a clear test tube and was dehydrated by adding a spoonful of anhydrous sodium sulphate. The clear extracted solution was absorbed at 420nm wavelength with Thermospectromic Spectrophotometer. The concentration of THC in the sample was extrapolated from a standard bonny light, bonny medium crude oil graph plotted (ASTM D3921).

2.3 Microbiological Analysis of Samples

2.3.1 Enumeration of Total Culturable Heterotrophic Bacteria

One gram of soil sample was weighed into 9ml sterile diluent (0.85% NaCl) under aseptic condition. It was then shaken vigorously to homogenize and serially diluted. Determination of total culturable heterotrophic bacteria was based in the method described previously by Chikere and Ekwuabu [17]. Briefly, 0.1ml aliquot of the inoculums was collected from dilution 10⁻⁵ using a sterile pipette and inoculated on Nutrient Agar (NA) surface. The inoculum was spread evenly with a sterile hockey stick. Plates were incubated at 37^oC for 24 hours. Thereafter, colonies were counted to obtain colony forming units (cfu) per ml of the soil sample. Distinct colonies were picked and streaked on freshly prepared nutrient agar medium to obtain pure culture after 24 hours incubation at 37^oC. The pure culture was Gram stained for microscopic examination. It was also used to carry out biochemical tests for characterization and identification of the isolates.

2.3.2 Enumeration of Hydrocarbon-Utilizing Culturable Bacteria (HUB)

One gram of soil sample was weighed into 9ml sterile diluents (0.85% NaCl) under aseptic condition. It was then shaken vigorously and serial diluted. An aliquot (0.1 ml) of inoculums was inoculated from dilution 10⁻³ on mineral salt agar (MSA) using the spread plate technique as described by Okpokwasili [5]. Sterile filter paper was soaked with crude oil and placed in the lid of Petri dish. Plates were incubated in inverted position at ambient temperature for 3-5 days. Thereafter, distinct colonies were purified by sub

culturing on a freshly prepared medium and incubated for 24 hours, from which microscopic examination and biochemical tests were done for characterization and identification.

2.3.3 Sub-culturing and Purification of Isolates

A loopful of each distinct colony was picked out with sterile wire loop and transferred to the edge of a freshly prepared nutrient agar plate to make a smear. The smear was then streaked out over the surface of the medium in one of several patterns. The streaking was done in three different segments, heating the wire loop at interval. Streaked plates were incubated at 37^oC for 24 h. Thereafter, distinct colonies that developed from streaked plates were transferred on agar slants and incubated at 37^oC for 24 h to obtain stock culture.

2.4 Molecular Identification

2.4.1 DNA extraction

DNA extraction was done according to manufacturer's instruction using Zymo Research Fungal/Bacterial DNA extraction KitTM. The extracted genomic DNA was quantified using the Nano-drop 1000 spectrophotometer.

2.4.2 16S rRNA amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were given as: 95^oC Initial denaturation which lasted for 5 minutes, 95^oC denaturation which lasted for 30 seconds; 52^oC annealing which lasted for 30 seconds; 72^oC extension which lasted for 30 seconds for 35 cycles and 72^oC final extension which lasted for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator. The BigDye Terminator kits were used for sequencing on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

2.4.3 Phylogenetic analysis

The bioinformatics algorithm Trace edit was used to edit the sequences obtained; similar sequences were sourced from the National Center for Biotechnology Information (NCBI) data base using BLASTN. The ClustalX was used to align the sequences. Inference of the evolutionary history was done using Neighbor-Joining method in MEGA 6.0 [18]. The bootstrap consensus tree inferred from 500 replicates [19] is taken to represent the evolutionary history of the taxa analyzed. Jukes-Cantor method was used in computation of the evolutionary distances [20].

3. RESULTS

3.1 Physicochemical Characteristics of Study Sample

The results of the physicochemical properties of the soil samples are represented in Table 1. The phosphorus content of the samples ranged from 2.302 to 22.768 mg/kg, pH 7.10 to 9.16, total organic carbon (TOC) 3.00 to 3.96%, THC 135.00 to 645.00 mg/kg, and nitrate from 1.99 to 12.957 mg/kg.

3.2 Total Hydrocarbon Content (THC) in the Soil Samples

The polluted soil samples had higher values of total hydrocarbon as compared to the unpolluted sample. The values of total hydrocarbon content for the unpolluted soil sample from 15 and 30 cm depth were 150.00 and 135.00 mg/kg respectively. However, the values of hydrocarbon content for the polluted soil samples ranged from 270.00 to 645.00 mg/kg.

3.3 Total Heterotrophic Bacterial (THB) Count

The total heterotrophic bacteria count for each site sampled were obtained and presented in Fig. 1a. This was carried out in order to ascertain the presence of microbial activity in the sites investigated. The total heterotrophic bacterial (THB) count for the soil samples ranged from 7.2 to 12.9 x 10⁷cfu/g. The unpolluted soil sample was observed to have a higher number of total heterotrophic bacterial counts than the polluted soil samples.

3.4 Hydrocarbon Utilizing Bacterial (HUB) Count

Presented in Fig 1b. is the hydrocarbon utilizing bacteria counts in both the polluted and unpolluted soil samples. The results revealed that the unpolluted soil sample had a lower count of hydrocarbon utilizers when compared to the polluted soil samples. The hydrocarbon utilizing bacteria (HUB) count for the unpolluted soil sample ranged from 1.1 to 1.5 x 10⁶, while the counts for the polluted soil samples ranged from 1.5 to 3.3 x 10⁶(Fig . 1c).

3.5 Comparison of the Composition/Frequency of Occurrence of Organisms Recovered from the Polluted and Unpolluted Soils

The comparison of the frequency of occurrence of bacterial species recovered from the polluted and unpolluted soil samples are presented in Fig 1d. The frequency of the organisms in polluted and unpolluted soil samples are; *Bacillus thuringiensis* (3 and 5), *Bacillus cereus* (3 and 6), *Shewenella* sp. (2 and 4), *Lysinbacillus xylanilyticus* (1 and 3), *Acinetobacter junii* (3 and 0), *Bacillus flexus* (3 and 6), *Pseudomonas pseudoalcaligenes* (4 and 0), and *Acinetobacter venetianus* (1 and 2).

3.6 Molecular Characterization of Bacterial Isolates

PCR amplification of the 16S rRNA gene fragment was obtained for all the isolates indicating that DNA was successfully extracted and inhibition of PCR did not occur. The extracted DNA all yielded PCR products of 1500bp. The products were visualized in 5% tris acetate EDTA (TAE) agarose gel with ethidium bromide.

The obtained 16S rRNA gene sequence of the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database (Fig. 2). The 16S rRNA of the isolate S1 showed a percentage similarity to other species at 100%. The evolutionary distances obtained using Jukes-Cantor method were in line with the phylogenetic placement of the 16S

Table 1. Physicochemical Characteristics of Study Sample

S/No	Sample code	Sample Identity	Phosphorus (mg/kg)	pH	TOC (%)	THC (mg/kg)	Nitrate-Nitrogen (mg/kg)
1.	PFP 1	0-15cm	16.629	9.16	3.66	645.00	1.99
2.	PTP 1	15-30cm	21.745	8.86	3.96	375.00	7.973
3.	PFP 2	0-15cm	22.768	9.16	3.60	435.00	6.977
4.	PTP 2	15-30cm	11.768	7.86	3.54	270.00	7.973
5.	PFP 3	0-15cm	15.861	9.04	3.54	585.00	8.970
6.	PTP 3	15-30cm	18.419	8.87	3.18	330.00	3.987
7.	NPF	P 0-15cm	2.558	6.91	3.06	150.00	12.957
8.	NP	P 15-30cm	2.302	7.10	3.00	135.00	7.973

KEY: PFP1 = Polluted soil at 15cm depth (composite soil at point 1)

PTP1 = Polluted soil at 30cm depth (composite soil at point 1)

PFP 2 = Polluted soil at 15cm depth(composite soil at point 2)

PTP 2 = Polluted soil at 30cm depth (composite soil at point 2)

PFP 3 = Polluted soil at 15cm depth (composite soil at point 3)

PTP 3 = Polluted soil at 30cm depth (composite soil at point 3)

NPF = Non polluted soil at 15cm depth (composite)

NPT = Non polluted soil at 30cm depth (composite)

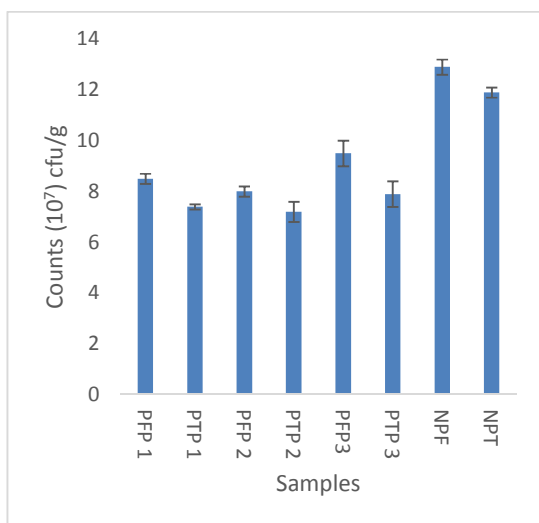


Fig. 1a. Total heterotrophic bacterial count

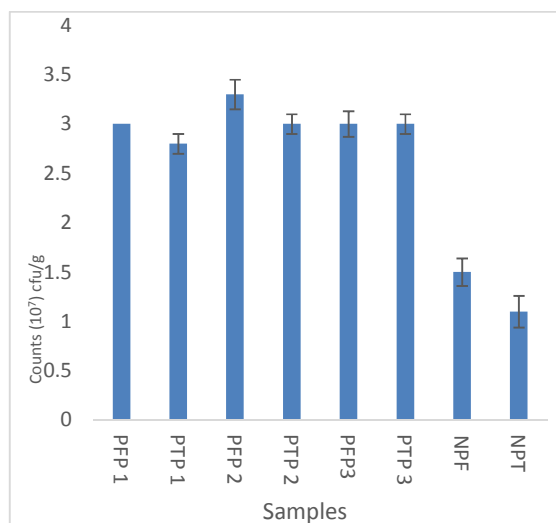


Fig. 1b. Total hydrocarbon utilizing bacteria counts

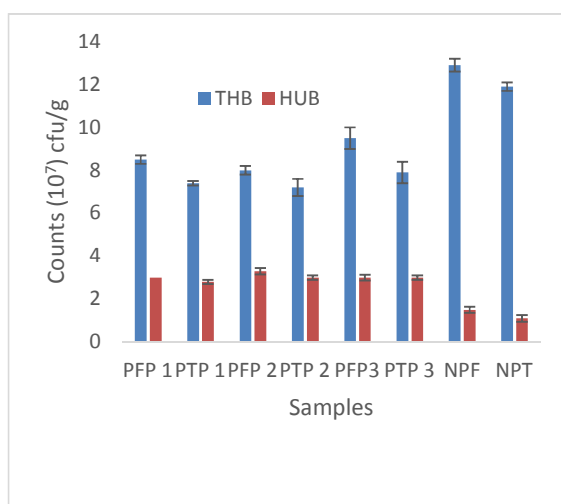


Fig. 1c. HUB and THB proportion in the different samples

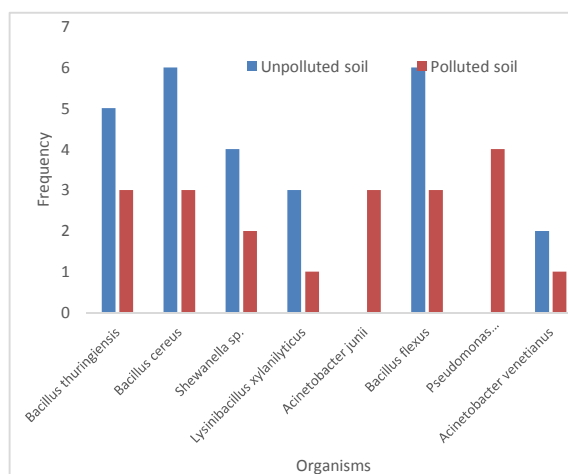


Fig. 1d. Comparison of the composition/frequency of occurrence of organisms recovered from the polluted and unpolluted soils

PFP1 = Polluted soil at 15 cm depth (composite soil at point 1) PTP1 = Polluted soil at 30cm depth (composite soil at point 1), PFP 2 = Polluted soil at 15cm depth (composite soil at point 2) PTP 2 = Polluted soil at 30cm depth (composite soil at point 2), PFP 3 = Polluted soil at 15cm depth (composite soil at point 3) PTP 3 = Polluted soil at 30cm depth (composite soil at point 3), NPF = Non polluted soil at 15cm depth (composite) NPT = Non polluted soil at 30cm depth (composite)

rRNA of the isolates within the *Acinetobacter* sp and revealed a closely relatedness to *Acinetobacter venetianus* (MG571557) than other *Acinetobacter* sp. The 16S rRNA of the isolate S2 showed a percentage similarity to other species at 100%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the

isolates within the *Bacillus* sp and revealed a closely relatedness to *Bacillus cereus* (MG571558) than other *Bacillus* sp. The 16S rRNA of the isolate S4 showed a percentage similarity to other species at 100%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Bacillus* sp and revealed

a closely relatedness to *Bacillus flexus* (MG571559) than other *Bacillus* sp. The 16S rRNA of the isolate S5 indicated a percentage similarity to other species at 100%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Bacillus* sp and revealed a closely relatedness to *Bacillus thuringiensis* (MG571560) than other *Bacillus* sp. The 16S rRNA of the isolate S6 indicated a percentage similarity to other species at 99%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Acinetobacter* sp and revealed a closely relatedness to *Acinetobacter junii* (MG571561) than other *Acinetobacter* sp. The 16S rRNA of the isolate S7 indicated a percentage similarity to other species at 100%. The evolutionary distances were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Shewanella* sp (MG571562). The 16S rRNA of the isolate S8 indicated a percentage similarity to other species at 100%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Lysinibacillus* sp and revealed a closely relatedness to *Lysinibacillus xylanilyticus* (MG571563) than

other *Lysinibacillus* sp. The 16S rRNA of the isolate S9 indicated a percentage similarity to other species at 99%. The evolutionary distances were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Pseudomonas* sp and revealed a closely relatedness to *Pseudomonas pseudoalcaligenes* (MG571564) than other *Pseudomonas* sp. The 16S rRNA of the isolate S10 indicated a percentage similarity to other species at 100%. The evolutionary distances calculated were in line with the phylogenetic placement of the 16s rRNA of the isolates within the *Bacillus* sp and revealed a closely relatedness to *Bacillus flexus* (MG571565) than other *Bacillus* sp. The results are all presented in Table 3.

4. DISCUSSION

Selected crude oil contaminated sites in Yorla community of Ogoni Land were investigated using molecular techniques. The identities of the bacterial isolate from the polluted and unpolluted sites were confirmed using PCR and 16s rRNA gene sequencing. The same method has been previously employed by Chikere and Ekwuabu [3].

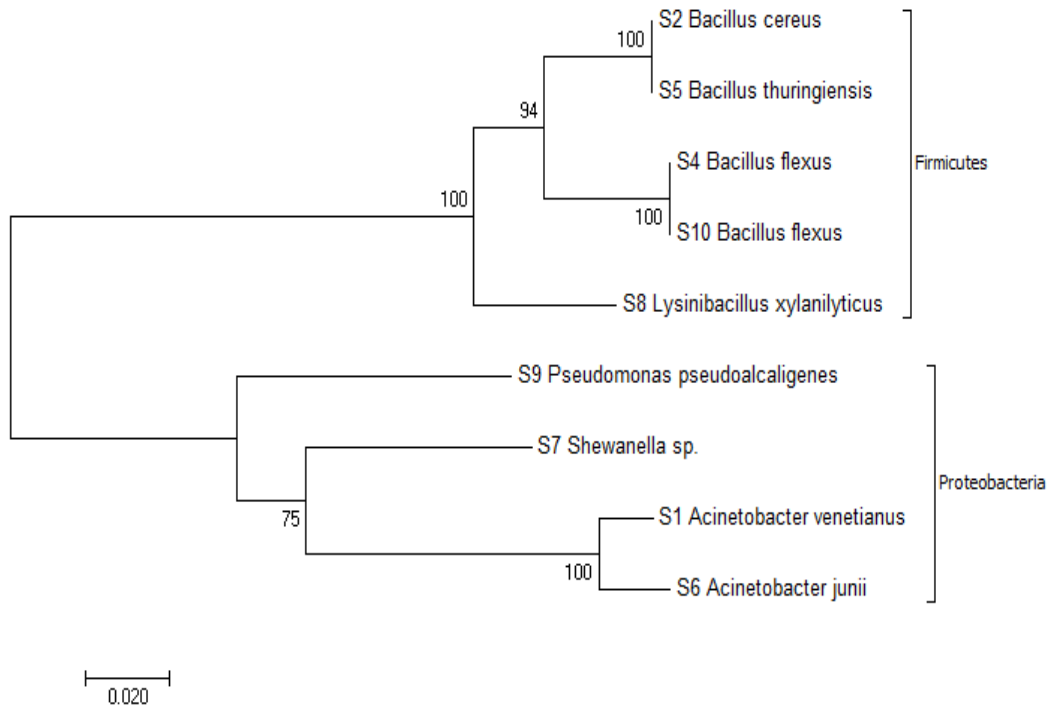


Fig. 2. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

Table 2. Occurrence of bacteria recovered from different soil depths of the oil-polluted and unpolluted soil

Unpolluted Soil			Polluted Soil		
Sample code	Organism	CM	Sample code	Organism	CM
PFP 2	<i>Bacillus thuringiensis</i>	15	NPF 1	<i>Bacillus thuringiensis</i>	15
PFP 1	<i>Bacillus thuringiensis</i>	15		<i>Bacillus thuringiensis</i>	15
PFP 2	<i>Bacillus thuringiensis</i>	15		<i>Bacillus thuringiensis</i>	15
PTP 1	<i>Bacillus thuringiensis</i>	30			
PFP 2	<i>Bacillus thuringiensis</i>	15			
PFP 2	<i>Bacillus cereus</i>	15	NPF 1	<i>Bacillus cereus</i>	15
PT 2	<i>Shewanella</i> sp.	30			
PTP 3	<i>Shewanella</i> sp.	30	NPT 1	<i>Shewanella</i> sp.	30
PFP 1	<i>Shewanella</i> sp.	15	NPT 2	<i>Shewanella</i> sp.	30
PFP 2	<i>Shewanella</i> sp.	15			
PF 3	<i>Lysinibacillus xylanilyticus</i>	15	NPT	<i>Lysinibacillus xylanilyticus</i>	30
PTP 1	<i>Lysinibacillus xylanilyticus</i>	30			
			PFP 1	<i>Acinetobacter junii</i>	15
			PF 3	<i>Acinetobacter junii</i>	15
			PFP 2	<i>Acinetobacter junii</i>	15
PT 3	<i>Bacillus cereus</i>	30	NPF 1	<i>Bacillus cereus</i>	15
PTP 2	<i>Bacillus cereus</i>	30	NPF 3	<i>Bacillus cereus</i>	15
PFP 2	<i>Bacillus cereus</i>	15			
PF 3	<i>Bacillus cereus</i>	15			
PFP 1	<i>Bacillus flexus</i>	15	NPT 2	<i>Bacillus flexus</i>	30
PTP 2	<i>Bacillus flexus</i>	30			
PTP 1	<i>Bacillus flexus</i>	30			
PFP 1	<i>Bacillus flexus</i>	15			
			PF 3	<i>Pseudomonas pseudoalcaligenes</i>	15
			PFP 2	<i>Pseudomonas pseudoalcaligenes</i>	15
			PTP 2	<i>Pseudomonas pseudoalcaligenes</i>	30

Unpolluted Soil			Polluted Soil		
Sample code	Organism	CM	Sample code	Organism	CM
			PT 3	<i>Pseudomonas pseudoalcaligenes</i>	30
PTP 2	<i>Acinetobacter venetianus</i>	30	NPF 2	<i>Acinetobacter venetianus</i>	15
PF 3	<i>Acinetobacter venetianus</i>	15			
PTP 1	<i>Bacillus flxus</i>	30	NPT 3	<i>Bacillus flxus</i>	30
PTP 1	<i>Bacillus flxus</i>	30			
PTP 1	<i>Bacillus flxus</i>	30			

KEY: PFP1 = Polluted soil at 15cm depth (composite soil at point 1) PTP1 = Polluted soil at 30 cm depth (composite soil at point 1)
 PFP 2 = Polluted soil at 15cm depth (composite soil at point 2) PTP 2 = Polluted soil at 30 cm depth (composite soil at point 2)
 PFP 3 = Polluted soil at 15cm depth (composite soil at point 3) PTP 3 = Polluted soil at 30 cm depth (composite soil at point 3)
 NPF = Non polluted soil at 15 cm depth (composite)
 NPT = Non polluted soil at 30 cm depth (composite)

Table 3. Characterization of bacterial isolates using Genbank blast

Code	Organism	Accession number	GenBank relative	Accession number of relative	Similarity (%)
S1	<i>Acinetobacter venetianus</i>	MG571557	<i>Acinetobacter venetianus</i>	LC057712.1	100
S2	<i>Bacillus cereus</i>	MG571558	<i>Bacillus cereus</i>	MF977355.1	100
S4	<i>Bacillus flexus</i>	MG571559	<i>Bacillus flexus</i>	MF470198.1	100
S5	<i>Bacillus thuringiensis</i>	MG571560	<i>Bacillus thuringiensis</i>	KX664088.1	100
S6	<i>Acinetobacter junii</i>	MG571561	<i>Acinetobacter junii</i>	MF426261.1	99
S7	<i>Shewanella</i> sp.	MG571562	<i>Shewanella</i> sp.	MF373562.1	100
S8	<i>Lysinibacillus xylanilyticus</i>	MG571563	<i>Lysinibacillus xylanilyticus</i>	KX254351.1	100
S9	<i>Pseudomonas pseudoalcaligenes</i>	MG571564	<i>Pseudomonas pseudoalcaligenes</i>	GU447236.1	99
S10	<i>Bacillus flexus</i>	MG571565	<i>Bacillus flexus</i>	MF470198.1	100

The presence of microbial activity was ascertained by the enumeration of total heterotrophic bacteria and hydrocarbon utilizing bacteria. The result obtained from enumeration of these organisms however indicated that indigenous microbial communities survived in the existence of hydrocarbon contamination, although the total heterotrophic bacteria counts in the unpolluted soil samples were higher compared to the contaminated soil samples. Similar observations were made by Chikere and Ekwuabu [3], Ibiene et al. [21], and Eze and Okpokwasili [22]. The high counts however could be ascribe to the availability of nutrients, and high organic matter, and other biological factors that encourage the growth and survival of the microorganisms whose role are important in degradation and nutrient cycling. Chikere and Ekwuabu [3] reported that continuous input of petroleum-based pollutants normally leads to enriched microbial community that can survive toxic contaminants.

However, the enumeration of hydrocarbon utilizing bacteria count in the unpolluted soil samples was lower compared to polluted samples. This implies that organisms which are capable of utilizing the hydrocarbon present in polluted samples were somehow stimulated by the presence of the hydrocarbon present in the polluted soil sample. Although the difference between the total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) were observed to be minimal ($p > 0.05$), suggesting that most of the microorganisms present in the various sample sites were hydrocarbon utilizers able to survive in the presence of crude oil contamination. Similar findings were reported by Chikere et al. [23], and Okpokwasili [24].

The proportion of the organisms obtained from two depths (15 and 30 cm) from each collection site shows that 60% of the organisms enumerated were from 15 cm depth, while 40% were enumerated from 30 cm depth. Previous studies by Odokuma and Ibor [25] revealed that most organisms that have to do with biodegradation of hydrocarbon contaminants are aerobic organisms, and mostly inhabit depths between 0 to 15 cm (top soil). However, higher numbers of organisms were enumerated in the unpolluted soil when compared to the polluted soil samples.

The outcomes of the diversity of organisms extracted from the polluted and unpolluted soil samples revealed that *Bacillus thuringiensis*,

Bacillus cereus, *Shewenella* sp., *Lysinibacillus xylanlyticus*, *Acinetobacter junli*, *Bacillus flexus*, *Pseudomonas pseudoalcaligenes*, and *Acinetobacter venetianus* were in existence in the samples.

In a related study by Chikere and Ekwuabu [3], *Bacillus* sp. and *Pseudomonas* sp were extracted from soil contaminated with crude oil in Bodo community of Rivers state. Ibiene et al. [21]; Obire and Nwanbeta [26], and Eze and Okpokwasii [22] also isolated *Bacillus* and *Pseudomonas* sp. from contaminated soil samples. Many scientists have also revealed that mixed population with broad enzymatic abilities are required to degrade complex mixtures of hydrocarbon such as crude oil in soil, marine and sediments [27].

The dominant genera observed from all samples were *Bacillus* sp., *Pseudomonas* sp and *Acinetobacter*. These genera have been reported to be dominant in hydrocarbon polluted environment by many researchers [28,21,22, 23,3].

The organisms were characterized by 16s rRNA. This method has been used by authors in the past and is proven to be more reliable and sensitive than culture dependent techniques [3,29,30] and the results obtained from these studies are consistent with past research on hydrocarbon polluted environments [31,32,33]. Previous results from Ibiene et al., [21] have identified *Pseudomonas* genus as the most efficient among hydrocarbon degrading microorganisms. This genus produces rhamnolipids which increases the surface area of hydrocarbon, thereby increasing bioavailability. Sarma and Sarma, [31] identified *Acinetobacter* from crude oil contaminated field as potential soils microbial strain that could be effective in the bioremediation of crude oil and its compounds.

The extraction of greater number of microorganisms capable of utilizing hydrocarbon from a particular environment is usually seen as an indication that those organisms are the functional hydrocarbon degraders in that particular environment [34]. The microorganisms capable of surviving in such environments are those that have developed physiological and enzymatic response which allow them to utilize the hydrocarbon compounds as substrates [35].

Some physicochemical parameters investigated were pH, total organic carbon (TOC), total hydrocarbon content (THC), phosphorous and

nitrate-nitrogen. The pH of the samples was between near neutral and alkaline. Studies have however revealed that the optimal pH range for biodegradation is between 6–7 [31,36,37]. Nutrients are essential elements for successful hydrocarbon biodegradation of polluted samples, especially nitrogen and phosphorous [3]. The nutrients investigated were in considerably good amounts in the samples to encourage the growth of the organisms in the sample. Koren et al. [38]; and Odokuma and Ibor [25] demonstrated that essential nutrients such as nitrogen/phosphorous play key role in the biodegradation of soil samples impacted with crude oil. The soil samples however had high values of total hydrocarbon content (THC) indicating prior contamination by hydrocarbons.

5. CONCLUSION

This work revealed the presence of reasonable population of indigenous hydrocarbon utilizing bacteria in oil polluted sites in Yorla community which can be monitored and enhanced to improve their bioremediation abilities in the oil polluted sites. The pollution of soil with hydrocarbon contaminants did not affect microbial richness, but only triggered a proliferation of established hydrocarbon degraders. Two organisms *Acinetobacter junni* and *Pseudomonas pseudoalcaligenes* were not recovered from the unpolluted soil, suggesting they are playing important roles in the oil-polluted soil. Statistically, significant differences were observed in microbial counts when samples recovered from 15 cm depth were compared to those recovered from 30 cm depth indicating that diversity reduced with depth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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