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Microbial Dynamics And Biodegradability Of Fuel From Polluted Soil In Artisanal And Conventional Refineries

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ABSTRACT

Microbes play vital roles in the ecosystem during degradation thus reducing the levels of contamination and pollution. This study was basically designed to determine the microbial dynamics and biodegradability of fuel samples in soil from artisanal refineries and conventional refinery in Bayelsa state. Result on baseline biological and physicochemical quality of the soil revealed that the heavy metal was below detection with the exception of iron, meanwhile the soil had petroleum hydrocarbon (2.457 - 4.218 mg/kg), Polycyclic Aromatic Hydrocarbon (1.818 - 2.772 mg/kg), total heterotrophic bacterial ($3.2x10^5$ -$1.01x10^6$) and hydrocarbon utilizing bacterial ($3.6x10^4$ - $3.6x10^4$). Result showed that the soil profile analysis showed that the soils were sandy-clayey and sandy-silt in nature with southern Ijaw sample composed of 69% sand, and 9% clay. Ekeremor soil sample had 61% sand and 28% slit, and NNPC 59% sand and 24% slit respectively. The total heterotrophic count in contaminated soil ranged from 4.6 x 10^5 to $1.8x10^6$cfu/g on day 0. Day 56 being the final day of monitoring had higher counts ranging from $1.39x10^6$ to $6.7x10^6$cfu/g indicating a gradual reduction in the viable bacterial population. However, hydrocarbon utilizing bacteria population were static for 56 days studied as there were no significant differences in counts between Day 0 and 56 at $p \leq 0.005$. *Bacillus sp. Pseudomonas sp., Corynebacterium sp., Micrococcus sp.*, were the most predominant heterotrophic bacterial species isolated during monitoring.

Keywords*:* Petrol, Diesel, Artisanal refinery, Biodegradation, Microbial dynamics

1.0 INTRODUCTION

The toxins arising from the release of hydrocarbons from anthropogenic activities to the air, water and soil, have always placed biodiversity in a vulnerable position (Fowzia & Fakhruddin, 2018). One of the crucial biotic elements that are in charge of the biochemical processes in the soil are soil organisms. The activities of soil organisms are adversely affected by petroleum hydrocarbons (Fowzia & Fakhruddin, 2018). Oil spills have an impact on biodiversity by causing situations where vital nutrients like nitrogen and oxygen required for plant growth are depleted (Shukry *et al*., 2013).

Exploiting microorganisms' capacity to eliminate these organic contaminants from contaminated locations is one promising therapeutic strategy (Finley *et al.*, 2010). This alternative therapy approach is efficient, reasonably safe, affordable, adaptable, and environmentally friendly (Finley *et al.*, 2010). Nevertheless, microbial degradation refers to an ability that microorganisms have to naturally change organic compounds into a different form. This process could result in the complete conversion of organic molecules into straightforward inorganic substances like carbon dioxide, water, and biomass (Abu & Moro, 2004).

Currently, biodegradation and techniques are being positively promoted as viable, eco-friendly and cost-effective hydrocarbon remediation technology (Sathishkumar *et al.,* 2008; Guerra *et al.,* 2018). This method has received a lot of attention and is a superior alternative to the degrading of recalcitrant hydrocarbons (Lea-Smith *et al.,* 2015; Dombrowski *et al.,* 2016; Dvorak *et al.,* 2017). This study assessed the microbial dynamics and biodegradability of polluted soil from artisanal refineries.

2.0 MATERIALS AND METHODS

2.1 Sampling

The soil samples selected for this study were from two artisanal refining sites at Korokorosei, Southern Ijaw LGA (Latitude 4^{o} 44' 49.182" N; Longitude 6^{o} 0' 34.92" E) and Owegbene, Ekeremor LGA (Latitude 5^{o} 1' 15.432" N; Longitude 5^{o} 42' 11.268" E) in Bayelsa State. However, the control Soil samples were sourced from NNPC Petrol station located in Yenagoa, Bayelsa State. Polluted surface soil samples were collected from different spots from a site pooled together and properly mixed to obtain composite soil samples. The soil samples were collected from the surface to a depth of 0-15cm with sterile soil auger into fresh unused black polyethylene bags and transported to the microbiology laboratory for setup and analyses.

2.2 Soil Sample Preparation

The soil was obtained using manual soil auger to a 0-15cm depth into sterile plastic bags and transported to the laboratory. Samples were de-stoned, sieved, and homogenized with a 2mm sieve, homogenize and acclimatized (air dried) for 24 hours and stored in dark polyethylene bags for subsequent usage.

2.3 Determination of soil texture and Physicochemistry

The materials used for soil particle test were Glass cylinder, 1000ml capacity, Thermometer, Hydrometer, Bouyoucos (fisherbrand model #14-331-5c) Electric mixer with dispersing cup, Plunger, Balance sensitive to ±0.01g. The ASTM D 5765 GC-MS method was used to determine the TPH and PAH of the samples studied. Aliphatic hydrocarbon is quantified within ranges C9-C36 while the Aromatic hydrocarbons are quantified within C11-C22. The materials used were GC-MS machine. Heavy metals were assessed using Flame Atomic Adsorption Spectrophotometer.

2.4 Preparation of Experimental Setup for Degradability Tests

Soil samples were mixed and sieved thoroughly into clean sterile containers. The spiking method of Durumin-Iya (2021) was adopted with slight modifications. One thousand grams (1000g) of soil sample was weighted into each of the six plastic containers labeled AP, AD, BP, BD, CP and CD and 100mls of the respective petroleum product were added to spike the soils. AP was amended with 10% (v/w) (i.e. 100mls of Petrol with 1000g of soil) of petrol sourced from an artisanal refinery in Southern Ijaw; AD was amended with 10% (v/w) of Diesel from an artisanal refinery in Southern Ijaw BP was amended with 10% (v/w) petrol from an artisanal refinery in Ekeremor; BD with 10% (v/w) of Diesel from an artisanal refinery in Ekeremor; CP was amended with 10% (v/w) of petrol from conventional station (NNPC) in Bayelsa State. While CD with 10% (v/w) Diesel from conventional station (NNPC) in Bayelsa State. Biodegradability setup was monitored for 56days on a 28days interval (0 day to 56day) and Total Petroleum (TPH) and Polycyclic Aromatic Hydrocarbon (PAH) were analyzed on the various days respectively.

2.5 Sterilization of Materials

Glasswares including Petri dishes used were washed, dried and placed in the appropriate cans. All glass ware used were sterilized with the aid of autoclave (Thorn, Model 1604, England) at 121 ^{o}C at 15 min and 15 psi (Pounds per square inch unit of pressure). Plastic containers were sterilized by rinsing the inside of the container with 95 % ethanol. The materials were thereafter rinsed using adequate sterile distilled water to remove traces of ethanol from contaminating the plastic containers and other materials used in conducting the experiment.

2.6 Media preparation

Microorganisms were grown using various prepared culture media. The culture media used in carrying out this study included Bushnell Haas Agar (BHA), and Nutrient Agar (NA). All media used in the study were prepared aseptically following the manufacturer's protocol and Standard microbiological methods.

Bushnell Haas agar used in determining degradation yields of PAHs, was prepared by dissolving 23.27g of Commercially available Bushnell Haas agar powder in 1000 ml of distilled water and the pH adjusted to 7.1 (Okolo & Amadi, 2004; Ezekoye et al.,2015). One millilitre (1ml) of crude oil was then added into the medium before autoclaving. Nutrient agar was prepared by dissolving 28g of commercially available Nutrient agar powder in 1000mls of distilled water. After which they were sterilized for by autoclaving at 121^{o}C for 15minutes at 15psi.

2.6.1 Purification of Isolates

Discrete bacterial colonies were picked and subcultured onto nutrient agar (NA) plates using the streak plate method. Stock cultures were prepared on sterile NA in Bijou bottles, coded for ease of identification and stored in the refrigerator at 4^{o}C until needed for further tests (Abu & Akomah, 2008).

2.6.2 Characterization and Identification of Isolates

The isolates were identified on the basis of their cultural, morphological and biochemical characteristics in accordance with schemes and methods previously described (Cheeseborough, 2005; Chikere & Ekwuabu, 2014; Ibiene *et al*.,2011). Microscopic examination of the various isolates was carried out using oil immersion objectives (×100). Identification of bacterial colonies was based on Gram reaction, biochemical, cultural and colonial morphologies of the bacterial isolates as previously described by Bergey & Holt (1994) & Cheeseborough (2005).

2.7 Molecular Identification

A total of four (4) pure bacterial isolates in triplicates were analyzed for phylogenetic and evolutionary relatedness. The selection was based on the predominant hydrocarbon degrading bacteria. Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tubes were centrifuged at 10,000x*g* for 1 minute.

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 x*g* for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute.The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000x*g*microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

2.8 Statistical Analysis

The version 23 of SPSS was used for the statistical analysis. Data was expressed as Mean±Standard deviation. The mean was separated using analysis of variance (ANOVA), while Duncan was used to test the degree of significance.

3.0 RESULT AND DISCUSSION

3.1: Baseline Properties of Soil used Before Pollution

The biological and chemical parameters of the soil samples collected from the artisanal refining site and a conventional refinery used in the study before the analysis are presented in Table1. Physiochemical parameters of heavy metals contents as well as heterotrophic microbial count of the soil are presented. Most heavy metals except iron were not detected in the three soil samples. Total Petroleum Hydrocarbons concentrations of soil samples were similar. It ranged from 2.457 to 4.218 mg/kg. Polycyclic Aromatic Hydrocarbon concentrations in the three soils were similar. It ranged from 1.818 to 2.772 mg/kg.

Hydrocarbon Utilizing Bacterial populations were high in the three soils indicating previous exposure to hydrocarbon.

Table 4.1 Baseline Properties of Soil used before Pollution

S/N	PARAMETER	UNIT	SAMPLE ID		
			Southern Ijaw (A)	Ekeremor (B)	NNPC Refinery (C)
1	pH	-	8.37	8.45	7.97
2	Electrical Conductivity, E.C	µS/cm	68.00	25.00	25.00
3	Salinity	PSU	0.03	0.01	0.01
4	Nitrate, NO_3^-	mg/kg	2.66	2.34	2.26
5	Phosphate, PO_4^{3-}	mg/kg	12.85	5.69	7.77
6	Sulphate, SO_4^{2-}	mg/kg	28.33	8.56	10.56
7	Cation Exchange Capacity, CEC	Cmol (+)kg^{-1}	132.93	131.92	133.46
8	Total Organic Carbon, TOC	%	0.49	0.49	0.42
9	Water Activity	%	18.55	7.49	10.12
10	Iron, Fe	mg/kg	0.473	<0.001	0.026
11	Nickel, Ni	mg/kg	0.011	<0.001	<0.001
12	Lead, Pb	mg/kg	0.001	<0.001	<0.001
13	Vanadium, V	mg/kg	<0.001	<0.001	<0.001
14	Total Petroleum Hydrocarbon, (TPH)	mg/kg	3.436	2.457	4.218
15	Polycyclic Aromatic Hydrocarbons, PAHs	mg/kg	2.772	1.818	1.981
16	Total Heterotrophic Bacteria Count	CFU/g	$3.2x10^5$	$5.3 x10^5$	$1.01x10^6$
17	Hydrocarbon Utilizing Bacteria Count	CFU/g	$9.5x10^4$	$3.6x10^4$	$7.6 x10^4$

3.1 Properties of Soil Used for Degradation

Soil profile analysis showed that all samples sourced from the different locations (conventional and artisanal refineries) were sandy-clayey and sandy-silt in nature with southern Ijaw sample composed of 69% sand, and 9% clay. Ekeremor soil sample 61% sand and 28% slit and NNPC 59% sand and 24% slit respectively (Table 2).

Table 2: Properties of Soil Used for biodegradability Studies

Sample	Particle Size			Porosity (%)	Texture
	Sand (%)	Clay (%)	Silt (%)		
Southern Ijaw (A)	69	9	3.58	3.58	Sandy Clayey
Ekeremor (B)	61	11	28	2.46	Sandy Silt
NNPC (C)	59	17	24	2.01	Sandy Silt

3.1 Total Heterotrophic Bacteria Counts during Degradation Monitoring

The total heterotrophic bacteria count for the different samples studied showed varying counts on day 0, ranging from $4.6x10^5$ for sample AD to $1.8x10^6$ CFU/g observed for sample CD. However, Samples BD had a count of $6.0x10^5$, AP $4.8x10^5$, BP $6.0x10^5$ and $1.2x10^6$cfu/g for sample CP. Monitoring on day 28 showed that sample CD had the highest viable count of $2.77x10^6$ while sample BD had the least heterotrophic bacteria count of $3.55x10^5$cfu/g. Counts of $9.1x10^5$, $1.62x10^6$, $5.15x10^5$, and $7.0x10^5$ CFU/g were recorded for samples AD, AP, BP, and CP respectively. Monitoring of heterotrophic bacteria on day 56 showed that sample CD had the highest count of $6.7x10^6$ while sample BP had the least viable bacteria count of $1.39x10^6$ CFU/g as seen in Table 3. Counts of $2.0x10^6$, $2.42x10^6$, $1.64x10^6$ and $6.15x10^6$ CFU/g

were recorded for samples AD, AP, BD, and CP respectively. (Table 4.1). The Total heterotrophic bacteria count showed no significance difference in day0, 28 and day 56.

Table 3: Total Heterotrophic Bacteria Counts for Day 0, 28 and 56

Code	Day 0 CFU/g	Day 28 CFU/g	Day 56 CFU/g
AD	$4.6x10^{5b}$	$9.15x10^{5c}$	$2.0x10^{6c}$
AP	$4.8x10^{5b}$	$1.62x10^{6d}$	$2.42x10^{6d}$
BD	$6.0x10^{5c}$	$3.55x10^{5a}$	$1.64x10^{6b}$
BP	$1.27x10^{6a}$	$5.15x10^{5a}$	$1.39x10^{6a}$
CD	$1.80x10^{6d}$	$2.77x10^{6e}$	$6.7x10^{6e}$
CP	$1.21x10^{6a}$	$7.0x10^{5b}$	$6.15x10^{6e}$

Column means with different alphabet is significant

Table 4: Hydrocarbon Utilizing Bacteria Counts for Day 0, 28 and 56

Code	Day 0 CFU/g	Day 28 CFU/g	Day 56 CFU/g
AD	$1.14x10^{5e}$	$2.44x10^{5e}$	$1.67x10^{5a}$
AP	$1.04x10^{5d}$	$1.11x10^{5c}$	$1.42x10^{5a}$
BD	$5.4x10^{4b}$	$1.69x10^{4a}$	$1.17x10^{5a}$
BP	$4.1x10^{4a}$	$2.64x10^{5f}$	$9.85x10^{4a}$
CD	$1.04x10^{5d}$	$1.99x10^{5d}$	$1.03x10^{5s}$
CP	$8.0x10^{4c}$	$6.05x10^{4b}$	$1.70x10^{5a}$

Column means with different alphabet is significant

3.2: Hydrocarbon Utilizing Bacteria Counts during Degradation Monitoring

Table 4 shows the hydrocarbon utilizing bacteria count for the different samples studied. The results showed varying counts on day 0, ranging from $4.1x10^4$ for samples BP to$1.14x10^5$ CFU/g observed for sample AD. However, Samples BD had a count of $5.4x10^4$, AP $1.04x10^5$, CD $1.04x10^5$ and $8.0x10^4$ CFU/g for sample CP. Monitoring of hydrocarbon utilizing bacteria on day 28 showed that sample AD had the highest count of $2.44x10^6$ while sample CP had the least hydrocarbon utilizing bacteria count of $6.05x10^4$ CFU/g.

Table 5: Morphology, Gram's Reaction and Biochemical Characteristics of Total Heterotrophic Bacteria Isolated From Soil Samples on Day 0

													TSIA											
	Catalase	**Citrate**	**Oxidase**	**Indole**	**Lactose**	**Glucose**	**Sucrose**	**MR**	**VP**	**Motility**	**Starch Hydrolysis**		**Butt**	**Slant**	**H_2S**	**Gas**	**Elevation**	**Edge**	**Shape**	**Surfaces**	**Pigmentation**	**Cell shape**	**Gram's**	**Isolated Organism**
1AD1	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Curled	Iregular	Smooth	Cream	Rod	+	*Brevibacillus sp.*
1AD2	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Flat	Entire	Round	Smooth	Cream	Rod	+	*Bacillus sp.*
1AD3	+	+	-	-	-	A	A	+	+	-	+	-	A	B	-	+	Raised	Entire	Round	Smooth	White	Rod	+	*Viridissp*
1AD4	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Rough	Cream	Rod	+	*Bacillus sp.*
1AD5	-	+	+	-	+	+	-	-	-	+	-	-	A	B	-	+	Raised	Entire	Regular	Smooth	White	Cocci	+	*Micrococcus sp*
1AD6	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*
1AP1	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Curled	Iregular	Smooth	Cream	Rod	+	*Brevibacillus sp.*
1AP2	+	-	+	-	-	A	-	+	-	+	-	-	A	B	-	+	Raised	Entire	Irregula	Rough	Cream	Rod	-	*Derxialacustris*
1AP3	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Flat	Entire	Round	Smooth	White	Cocci	+	*Staphylococcus sp*
1BD1	+	+	-	-	-	A	A	+	+	-	+	-	A	B	-	+	Raised	Entire	Round	Smooth	White	Rod	+	*Viridissp*
1BD2	-	+	+	-	+	+	-	-	-	+	-	-	A	B	-	+	Raised	Entire	Regular	Smooth	White	Cocci	+	*Micrococcus sp*
1BD3	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Flat	Entire	Round	Smooth	White	Cocci	+	*Staphylococcus sp*
1BD4	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*
1BP1	+	+	-	-	-	A	A/G	+	+	-	+	-	A	B	+	-	Raised	Entire	Round	Smooth	Brown	Rod	-	*Acinetobacter sp.*
1BP2	+	-	-	-	-	+	-	+	-	-	-	-	A	A	+	-	Raised	Oval	Round	Smooth	Cream	Rod	+	*Corynebacterium sp*
1BP3	+	-	+	-	-	A	-	+	-	+	-	-	A	B	-	+	Raised	Entire	Irregula	Rough	Cream	Rod	-	*Derxialacustris*
1BP4	-	+	+	-	+	+	-	-	-	+	-	-	A	B	-	+	Raised	Entire	Regular	Smooth	White	Cocci	+	*Micrococcus sp*

Morphology, Gram's Reaction and Biochemical Characteristics of Total Heterotrophic Bacteria Isolated from Soil Samples on Day 0 (Contd.)

													TSIA											
Samples	**Catalase**	**Citrate**	**Oxidase**	**Indole**	**Lactose**	**Glucose**	**Sucrose**	**MR**	**VP**	**Motility**	**Starch Hydrolysis**		**Butt**	**Slant**	**H_2S**	**Gas**	**Elevation**	**Edge**	**Shape**	**Surfaces**	**Pigmentation**	**Cell shape**	**Gram's Reaction**	**Isolated Organism**
1CP1	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	Green	Rod	-	*Pseudomonas sp*
1CP2	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Flat	Entire	Round	Smooth	Cream	Rod	+	*Bacillus sp.*
1CP3	+	+	-	-	-	A	A	+	+	-	+	-	A	B	-	+	Raised	Entire	Round	Smooth	White	Rod	+	*Viridissp*
1CD1	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Rough	Cream	Rod	+	*Bacillus sp.*
1CD2	-	+	+	-	+	+	-	-	-	+	-	-	A	B	-	+	Raised	Entire	Regular	Smooth	White	Cocci	+	*Micrococcus sp*
1CD3	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*

Table 6: Morphology, Gram's Reaction and Biochemical Characteristics of Hydrocarbon Utilizing Bacteria Isolated From Soil Samples on Day 0

Samples	Catalase	Citrate	Oxidase	Indole	Lactose	Glucose	Sucrose	MR	VP	Motility	Starch Hydrolysis	Urease	TSIA				Elevation	Edge	Shape	Surfaces	Pigmentation	Cell shape	Gram's Reaction	Isolated Organism
													Butt	Slant	H_2S	Gas								
HUB BD3	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Moist	Cream	Rod	+	*Brevibacillus sp.*
HUB BD1	+	-	-	+	-	A	-	+	-	-	-	+	B	B	-	-	Raised	Entire	Round	Moist	Cream	Rod	+	*Bacillus sp.*
HUB BD2	+	+	-	-	-	A	A	+	+	-	+	-	A	B	-	+	Flat	Entire	Round	Moist	Yellow	Rod	+	*Viridissp*
HUB CP1	+	-	-	+	+	A	A	+	-	-	-	+	B	B	-	-	Raised	Entire	Round	Moist	Brown	Rod	+	*Paenibacillus sp.*
HUB CP2	+	+	-	-	-	-	-	-	+	+	-	-	B	B	-	-	Flat	Entire	Oval	Smooth	Cream	Cocci	+	*Micrococcus sp.*
HUB CP3	+	+	+	-	-	A	A	+	-	+	-	-	A	B	-	-	Raised	Serate	Rhizoid	Moist	White	Rod	+	*Bacillus sp*
HUB CD1	+	-	-	+	-	A	-	+	-	+	-	+	A	B	-	-	Raised	Entire	Round	Moist	Brown	Rod	+	*Paenibacillus sp.*
HUB CD2	+	+	+	-	-	A	A	+	+	-	+	-	A	B	+	+	Flat	Entire	Round	Dry	Milky	Rod	+	*Bacillus Atrophaues*
HUB AP1	+	-	+	-	-	+	+	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Smooth	White	Rod	+	*Bacillus sp.*

HUB AP2	+	+	-	-	-	-	-	+	+	+	-	-	A	B	-	-	Flat	Wavy	undulate	Smooth	Brown	Rod	+	*Lynsinibacillussp*
HUB AP3	+	-	-	-	-	+	-	+	-	-	-	-	A	A	+	-	Flat	Entire	Round	Smooth	White	Rod	+	*Corynebacterium sp*
HUB BP1	+	+	-	-	-	-	-	+	+	+	-	-	A	B	-	-	Flat	Wavy	undulate	Smooth	White	Rod	+	*Lynsinibacillussp*
HUB BP2	+	+	-	-	-	-	-	-	+	+	-	-	B	B	-	-	Flat	Entire	Oval	Smooth	Cream	Cocci	+	*Micrococcus sp.*
HUB BP3	+	-	-	-	-	+	-	+	-	-	-	-	A	A	+	-	Flat	Entire	Round	Smooth	White	Rod	+	*Corynebacterium sp*
HUA D1	+	-	-	-	-	+	-	+	-	-	-	-	A	A	+	-	Flat	Wavy	undulate	Dry	White	Rod	+	*Corynebacterium sp*
HUA D2	+	-	-	+	-	A	-	+	-	-	-	+	B	B	-	-	Raised	Entire	Round	Moist	Cream	Rod	+	*Bacillus sp.*
HUA D3	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Moist	Cream	Rod	+	*Brevibacillus sp.*
HUB BP4	+	+	-	-	-	-	-	-	+	+	-	-	B	B	-	-	Flat	Entire	Oval	Smooth	Cream	Cocci	+	*Micrococcus sp.*

Table 7: Morphology, Gram's Reaction and Biochemical Characteristics of Total Heterotrophic Bacteria Isolated From Soil Samples on Day 28

Samples	Catalase	Citrate	Oxidase	Indole	Lactose	Glucose	Sucrose	MR	VP	Motility	Starch Hydrolysis	Urease	TSIA				Elevation	Edge	Shape	Surfaces	Pigmentation	Cell shape	Gram's Reaction	Isolated Organism
													Butt	Slant	H_2S	Gas								
28AD1	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Flat	Entire	Round	Smooth	White	Rod	+	*Bacillus sp.*
28AD2	-	+	+	-	+	+	-	-	-	+	-	-	A	B	-	+	Raised	Entire	Regular	Smooth	White	Cocci	+	*Micrococcus sp*
28CP1	+	-	+	-	-	A	-	+	-	+	-	-	A	B	-	+	Raised	Entire	Irregula	Rough	Cream	Rod	-	*Derxialacustris*
28CP2	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Entire	Round	Smooth	White	Rod	+	*Brevibacillus sp.*
28BP1	+	+	-	-	-	-	-	+	+	+	-	-	A	B	-	-	Raised	Entire	Round	Moist	yellow	Rod	+	*Lynsinibacillus sp.*
28BP2	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Entire	Round	Smooth	White	Rod	+	*Brevibacillus sp.*
28BD1	+	-	+	-	-	+	+	+	-	+	+	-	B	B	-	-	Flat	Curled	Irregula	Smooth	White	Rod	+	*Bacillus sp.*
28BD2	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Entire	Roundr	Smooth	White	Rod	+	*Brevibacillus sp.*
28CD1	+	-	+	-	-	-	-	-	+	+	-	-	B	B	-	-	Raised	Entire	Round	Moist	yellow	Rod	+	*Paenibacillus sp.*
28CD2	-	-	-	-	+	+	+	-	+	-	+	-	B	B	-	-	Raised	Entire	Round	Smooth	White	Rod	+	*Bacillus sp*
28AP1	+	-	-	-	-	+	-	+	+	-	-	-	A	A	+	-	Flat	Lobate	Rhizoid	Smooth	Cream	Rod	+	*Corynebaceriu msp*
28AP2	+	+	-	-	-	-	-	+	+	+	-	-	A	B	-	-	Raised	Entire	Round	Moist	yellow	Rod	+	*Lynsinibacillus sp.*

Table 8: Morphology, Gram's Reaction and Biochemical Characteristics of Hydrocarbon Utilizing Bacteria Isolated From Soil Samples on Day 28

Samples	Catalase	Citrate	Oxidase	Indole	Lactose	Glucose	Sucrose	MR	VP	Motility	Starch Hydrolysis	Urease	TSIA				Elevation	Edge	Shape	Surfaces	Pigmentation	Cell shape	Gram's Reaction	Isolated Organism
													Butt	Slant	H_2S	Gas								
28AP1	-	+	-	-	-	A	-	+	-	-	+	-	A	B	-	-	Raised	Entire	Round	Smooth	Brown	Rod	+	*Bacillus sp.*
28AP2	+	+	-	-	-	-	-	-	+	+	-	-	B	B	-	-	Flat	Entire	Oval	Smooth	Cream	Cocci	+	*Micrococcus sp.*
28AP3	+	+	-	-	-	A	A/G	+	+	-	+	-	A	B	+	-	Raised	Entire	Round	Smooth	Brown	Rod	-	*Acinetobacter sp.*
28AP4	-	+	+	-	-	A	-	+	-	+	-	-	A	B	+	+	Flat	Filamt	Irregula	Smooth	White	Rod	+	*Brevibacillus sp.*
28BD1	-	+	-	-	-	-	-	+	-	+	-	-	B	B	-	-	Flat	Serate	Round	Smooth	White	Cocci	+	*Enterococcus sp.*
28BD2	+	+	-	+	-	A	A	-	+	-	+	+	A	B	-	-	Flat	Entire	Oval	Smooth	Brown	Rod	+	*Bacillus sp.*
28BD3	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*
28BD4	+	+	-	-	-	A/G	-	-	+	-	-	+	A	B	+	+	Raised	Filamt	Round	Dry	Brown	Rod	+	*Bacillus mycoides*
28AD1	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*
28AD2	+	+	-	-	-	-	-	-	+	+	-	-	B	B	-	-	Flat	Entire	Oval	Smooth	Cream	Cocci	+	*Micrococcus sp.*
28BD4	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Raised	Entire	Round	Smooth	White	Rod	+	*Brevibacillus sp.*
28AD3	+	+	-	+	-	A	A	-	+	-	+	+	A	B	-	-	Flat	Entire	Oval	Smooth	Brown	Rod	+	*Bacillus sp.*

Table 9: Morphology, Gram's Reaction and Biochemical Characteristics of Total Heterotrophic Bacteria Isolated From Soil Samples on Day 56

													TSIA											
Samples	**Catalase**	**Citrate**	**Oxidase**	**Indole**	**Lactose**	**Glucose**	**Sucrose**	**MR**	**VP**	**Motility**	**Starch Hydrolysis**	**Urease**	**Butt**	**Slant**	**H_2S**	**Gas**	**Elevation**	**Edge**	**Shape**	**Surfaces**	**Pigmentation**	**Cell shape**	**Gram's Reaction**	**Isolated Organism**
56CP1	+	+	+	-	-	-	-	-	-	+	+	-	A	B	-	-	Flat	Entire	Round	Dry	White	Rod	+	*Bacillus sp.*
56CP2	+	+	-	-	-	-	A	+	-	-	-	-	A	B	-	-	Raised	Curled	Round	Dry	White	Rod	+	*Bacillus endophytis.*
56CP3	+	+	+	-	-	A/G	A/G	+	+	+	-	+	A	A	+	-	Flat	Lobate	Rhizoid	Dry	White	Rod	-	*Proteus Sp*
56CP4	+	-	+	-	-	-	-	-	+	+	-	-	B	B	-	-	Raised	Entire	Round	Moist	yellow	Rod	+	*Paenibacillus sp.*
56BD1	+	+	-	-	-	A	-	-	-	-	+	-	B	B	-	-	Raised	Entire	Round	Moist	Cream	Rod	-	*Providential sp.*
56BD2	+	+	-	-	-	A	A	+	-	-	+	-	A	B	-	-	Flat	Entire	Iregular	Dry	White	Cocci	+	*Micrococcus sp.*
56BD3	+	+	-	-	-	-	-	-	-	-	-	-	B	B	-	-	Flat	Entire	Iregular	Dry	White	Rod	+	*Lynsibacillus sp.*
56BP1	+	+	+	-	-	-	+	-	+	-	-	-	A	B	-	-	Raised	Oval	Rhizoid	Dry	White	Rod	+	*Unknown*
56BP2	+	+	-	-	-	-	-	+	-	-	-	+	A	B	-	-	Flat	Entire	Round	Dry	White	Cocci	+	*Micrococcus sp.*
56CD1	+	-	-	-	-	-	-	+	-	-	+	-	A	B	-	-	Raised	Entire	Iregular	Moist	yellow	Rod	+	*Bacillus sp.*
56CD2	+	+	-	-	-	-	-	-	-	-	+	+	A	B	-	-	Flat	Entire	Round	Moist	yellow	Rod	+	*Unknown*
56CD3	+	+	-	-	-	-	-	+	+	+	-	-	A	B	-	-	Raised	Entire	Round	Moist	yellow	Rod	+	*Lynsibacillus sp.*
56AP1	+	-	-	-	-	-	-	-	+	-	-	-	A	B	-	-	Flat	Entire	Round	Dry	White	Rod	+	*Unknown*
56AP2	+	-	+	-	-	-	-	+	-	+	-	+	B	B	-	-	Flat	Entire	Oval	Moist	Cream	Rod	+	*Bacillus sp.*
56AD1	+	-	+	-	-	A	A	+	-	+	-	+	A	A	+	+	Raised	Oval	Round	Moist	Cream	Rod	+	*Salimcrobium sp.*

Table 10: Morphology, Gram's Reaction and Biochemical Characteristics of Hydrocarbon Utilizing Bacteria Isolated From Soil Samples on Day 56

													TSIA											
Samples	**Catalase**	**Citrate**	**Oxidase**	**Indole**	**Lactose**	**Glucose**	**Sucrose**	**MR**	**VP**	**Motility**	**Starch Hydrolysis**	**Urease**	**Butt**	**Slant**	**H_2S**	**Gas**	**Elevation**	**Edge**	**Shape**	**Surfaces**	**Pigmentation**	**Cell shape**	**Gram's Reaction**	**Isolated Organism**
56CD1	+	+	+	-	-	A	-	+	-	+	+	-	B	B	-	-	Flat	Entire	Round	Moist	yellow	Rod	+	*Unknown*
56CD2	+	-	-	+	+	+	+	+	-	-	-	+	B	B	-	-	Raised	Entire	Round	Moist	Brown	Rod	+	*Paenibacillus sp.*
56CD3	+	-	+	-	-	A	A	+	-	-	+	-	A	B	+	-	Flat	Entire	Round	Moist	Cream	Rod	+	*Bacillus sp.*
56CD4	+	+	+	-	-	A	-	-	-	-	+	-	A	B	-	-	Raised	Lobate	Iregular	Moist	yellow	Rod	+	*Bacillus sp.*
56AD1	+	+	-	-	-	A/G	-	-	+	-	-	+	A	B	+	+	Raised	Filamt	Round	Dry	Brown	Rod	+	*Bacillus mycoides*
56AD2	+	+	+	-	-	A	-	+	-	+	+	+	A	B	-	-	Flat	Entire	Round	Moist	Cream	Rod	+	*Bacillus sp.*
56AD3	+	+	+	+	A/G	A	-	+	-	+	-	+	A	B	+	-	Flat	Entire	Round	Moist	Cream	Rod	-	*Proteus sp.*
56AD4	-	-	+	-	-	A	-	+	-	+	+	+	A	B	-	-	Raised	Entire	Round	Moist	Cream	Rod	+	*Paenibacillussp*
56CP1	+	+	+	-	-	A	-	-	-	+	-	+	B	B	-	-	Flat	Entire	Round	Moist	White	Rod	-	*Pseudomonas sp*
56BD1	+	+	+	-	-	A	A	+	-	+	-	-	A	B	-	-	Raised	Serate	Rhizoid	Moist	White	Rod	+	*Bacillus sp*

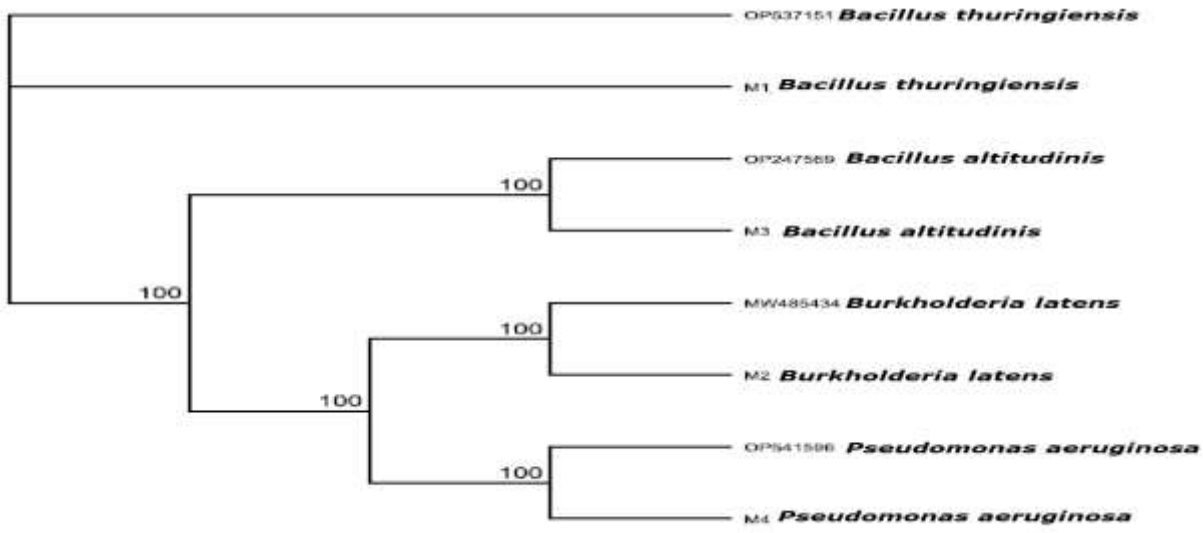

Figure 2: Phylogenetic Tree showing the Evolutionary Distance between the Bacterial Isolates

The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Bacillus, Burkholderia,* and *Pseudomonas sp* and revealed a closely relatedness to *Bacillus thuringiensis*, *Bacillus altitudinis. Burkholderia latens*, and *Pseudomonas aeruginosa* (Figure 2). Agarose gel electrophoresis was used to check for the different base pairs and their lanes. Lanes 1 – 4 represent 16SrRNA gene bands (1500bp). Lane M represents the 100bp Molecular ladder indicated at 100b.

4.2 DISCUSSION

The Niger Delta region is regarded as oil and gas hub of Nigeria and has several networks of pipelines. This has created a social problem as youths of the region engage in illegal refining of crude to earn living. Most products used around the region are products refined from artisanal refineries and pose so much environmental and health risks. This study was set to investigate the degradability of artisanal refinery products and survivability of macro and microorganism in impacted soil in Bayelsa state, Nigeria.

The initial total heterotrophic bacteria count on the various samples studied showed that soils contaminated with petrol product had counts of $4.8x10^5$ cfu for AP (Petrol product from Southern Ijaw), $1.27x10^6$ for BP (Petrol product from Ekeremor) and $1.21x10^6$ for CP (petrol product from NNPC). However, soil contaminated with diesel products from the same locations had counts of $4.6x10^5$ (AD), $6.0x10^5$ (BD) and $1.8x10^6$cfu/g (CD). The petroleum products AP, BP and CP had counts of $1.6x10^6$, $5.15x10^6$, and $7.0x10^5$cfu/g respectively on Day 28. Diesel products however showed counts of $9.15x10^5$, $3.55x10^6$ and $2.77x10^5$cfu/g on same day of monitoring. The total heterotrophic Bacteria count on day 56 revealed that sample AP, BP and CP had counts of $2.42x10^6$, $1.36x10^6$ and $6.15x10^6$ respectively. Diesel samples however had counts of $2.0x10^6$, $1.64x10^6$ and $6.15x10^6$cfu/g for the respective product sites. Comparing these results showed that diesel sample from Southern Ijaw (AD) recorded the highest value of THB count of $9{,}15x10^5$ on day28 with significant difference and diesel product from NNPC (CP) on day 0, recorded the least count of THB $1.2x10^5$ showing no significant difference.

The entire samples generally show day 56 having the highest counts across the samples studied, implying that the more the samples are allowed to undergo natural biodegradation, the more the microbial community has potential to proliferate, Likewise the hydrocarbon utilizing bacteria counts varied on the different days monitored with other samples increment in counts as those of the total heterotrophic bacteria. Samples AP, BP and CP had initial counts of $1.04x10^5$,$7.1x10^4$ and $8.0x10^4$cfu on day 0 of the analysis. Same sample had counts of $1.11x10^5$, $2.64x10^5$ and$6.05x10^5$cfu/g respectively on day 28 and day

56; The samples AP, BP and CP showed counts of $1.42x10^5$, $9.5x10^4$ and $1.70x10^5$ respectively. Ekeremor sample (BD) on day 56 had the highest HUB count ($9.85x10^4$) with no significant difference with other samples but significantly different with CD. NNPC (CD) recorded the least HUB with $1.03x10^5$CFU/g.

The cultural and morphological properties were employed in the identification of various isolates, the colonial properties examined and showed varying characteristics include color, shapes, elevations, margins etc. Morphological Gram's stain and biochemical analysis in the bacterial isolate revealed the prevalence of ten bacterial genera including *Bravibacillus* sp., *Bacillus* sp., *Vindis* sp., *Micrococcus* sp., *Pseudomonas* sp., *Acetobacter* sp., *Corynbacterium* sp., *Derxia* sp., *Staphylococcus* sp *and bacillus sp.* Predominant hydrocarbon utilizers isolate were members of the Bacillus, Pseudomonas, Micrococcus, bacillus and Paenicibacillus.

The release of hydrocarbon pollutants into the environment whether accidentally or due to human error is the main cause of water and soil pollution. Fortunately, microorganisms are highly efficient and versatile in their ability to degrade hydrocarbons; (Odokuma & Dickson 2003; Head et al., 2006; Odokuma & Smith 2007; Adebusoye *et al*., 2007).

The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method agreed with the phylogenetic placement of the 16S rRNA of the isolates within the *Bacillus, Burkholderia,* and *Pseudomonas sp* and revealed a closely relatedness to *Bacillus thuringiensis*, *Bacillus altitudinis. Burkholderia latens,* and *Pseudomonas aeruginosa*

Different factors influencing hydrocarbon degradation have been reported by Cooney et al. (1985). One of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment (Rahman *et al*. 2003; Brooijmans *et al*., 2009). Similar report of Crude petroleum oil from petroleum contaminated soil from North East India was reported by Das & Mukherjee, (2007). Similar organisms where reportedly found in petroleum degradation; commonly found bacterial genera, include: Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia, and Mycobacterium isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation (Chaillan *et al*., 2004).

CONCLUSION

This study was basically designed to determine the microbiological dynamics and degradability of soil samples contaminated by six different diesel and petrol samples in soil from two artisanal refineries, and a conventional refinery in Bayelsa state. The assay was carried out in compliance with standard analytical protocol. The findings from the study strongly suggest that most of the petroleum products are readily biodegradable having biodegradability. Although the process is relatively slow. Microorganisms responsible for degradation included *Bacillus sp. Pseudomonas sp. Corynebacterium sp. Bukhoderia sp*. Artisanal refining should be prohibited and more effective methods of degradation such as bioremediation should be encouraged. Also, modular refineries can be set up and adequately monitored to reduce the high levels of contamination in the Niger Delta.

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