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Evaluation of the physico-chemical state of the soil contaminant isolate indigenous bacterial species

Nwankwo CC [1], John Godson N [2,*] and Daodu Bamidele T [3]

[1] *Department of Microbiology Technology, School of Science Laboratory Technology, University of Port Harcourt.*
[2] *Department of Biochemistry/Chemistry Technology, School of Science Laboratory Technology, University of Port Harcourt.*
[3] *Department of Science Laboratory Technology, Delta State University of Science and Technology, University of Port Harcourt.*

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Abstract

The aim of the study is to isolate indigenous bacterial species which have the ability to degrade crude oil. Samples were collected from an oil polluted site in Ejama-Ebubu Eleme Rivers state. The samples were taken from five spot (A-E), of depth 0-15 (A1– E1) and 15-30 (A2– E2) in sterile polyethylene bags, using appropriate equipment, then taken to the laboratory for analysis, Physicochemical parameters such as pH, nitrate, sulphate, phosphate, total petroleum hydrocarbon (TPH), poly aromatic hydrocarbon (PAH), salinity, temperature, conductivity and heavy metals (iron, zinc, nikel, lead, chromium) were determined, The THB count was determined using the spread plate method on nutrient agar. Soil, sediment and water physicochemical parameters determined indicated that the samples had been exposed to hydrocarbon contamination. The Gram negative bacteria belonging to the genus pseudomonas is the most frequent. Other genera isolated were *Nocardia, Micrococcus,Chromobacterium, Burkholdia, Corynebacterium.* The study revealed the presence of petroleum hydrocarbons in the Ejama_Ebubu site as well as known genera of hydrocarbon utilizing bacteria. The biases associated with culture-dependent microbial enumeration techniques may limit the full description of the bacterial diversity in Ejama-Ebubu site. From the study, it is concluded that microorganisms that can degrade hydrocarbons are found in oil contaminated soil and can easily be isolated from these contaminated sites, although it is very difficult to work with aromatic hydrocarbons due to their volatility and toxic effects.

Keywords: Physiochemical; Microorganisms; Biodegradation; Bacterial

1. Introduction

The release of hydrocarbon into soil and water promotes the growth and proliferation of hydrocarbon utilizing microorganisms (HUM), which includes both bacteria and fungi. Incidentally, these hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) are also the organisms that are responsible for the biodegradation and eventual cleanup of oil spills in our environment Bioremediation, which is accomplished by adding exogenous microbial populations or stimulating indigenous ones, attempts to raise the rates of degradation found naturally to significantly higher rates (Watanabe,2001).

The biodegradation is not a new concept when dealing with oil pollutants, it has been intensively studied in controlled conditions (Chaillan *et al*., 2004) and in open field experiments (Chaıneau *et al*., 2003; Gogoi *et al*., 2003), but it has acquired a new significance as an increasingly effective and potentially inexpensive cleanup technology. Its potential contribution as a countermeasure biotechnology for decontamination of oil polluted systems could be enormous.

*Corresponding author: John Godson N
Department of Biochemistry/Chemistry Technology, School of Science Laboratory Technology, University of Port Harcourt.

Bioremediation using microorganisms of hydrocarbon contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil (Sebiomo *et al*., 2010). Bioremediation is emerging as one of the most promising technologies for the removal of petroleum hydrocarbons from the environment. For this, the screening of potential crude oil degrading organisms is one of the key steps (Varjani *et al* 2013).

Accidental releases of petroleum products are of particular apprehension in the environment. Conventional disposal methods of incineration or burial insecure landfills currently can become prohibitively costly when dealing with large amounts of contaminants. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms (Nalineekumari *et al* 2013).

The release of crude oil into the environment can be accidental or deliberately leading to serious pollution problems. Even small releases of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in excess of regulatory limits (Spence *etal*., 2005). Removal of poly aromatic hydrocarbons (PAHs) from the environment is very difficult due to their high hydrophobicity which increases with increasing molecular weight and this results in higher toxicity and longer persistence in the environment (Chauhan *etal* 2008). But there are a variety of microorganisms (bacteria and fungi) that are capable of degrading certain PAHs therefore there is a significant interest in studying microorganisms in contaminated sites as a mean for bioremediation (Daane*etal* 2001).

Hydrocarbon (HC) are organic compounds which are composed of two main elements-carbon and hydrogen. They also contain small quantities of molecules containing sulphur, nitrogen, metals, oxygen, etc., (Vieira *et al.,* 2007). Petroleum, in Latin means rock oil, which occurs as a dark, sticky, viscous liquid. Petroleum products such as gasoline, kerosene, diesel / fuel oil, crude oil are complex mixture of organic compounds basically of paraffinic, oleifinic and aromatic hydrocarbons (Mittal and Singh, 2009; Singh and Lin, 2008; Vieira *et al.,* 2007).

HCs in crude petroleum can be classified as alkanes, cycloalkanes, aromatics, polycyclic aromatics, asphaltines, and resins. Among the petroleum HCs, n-alkanes are the most amenable to biodegradation. Normally alkanes in the range of C5 to C10 are inhibitory to majority of the HC degraders at higher concentration as they disrupt lipid membrane when present as solvent. Alkanes in range of C20 to C40, also referred to as waxes, are less biodegradable as they being hydrophobic solids have low solubility in water. During degradation the alkanes are converted to alcohol by the action of oxygenase enzymes that attack the terminal methyl group. The alcohol is further oxidized to aldehyde and then to fatty acids. Further utilization of fatty acid occurs by β-oxidation of aliphatic chain. Higher the methyl branching lower is the extent of β-oxidation.

The cycloalkanes or alicyclic HCs are less degradable than alkanes. Here the biodegradability decreases with increase in number of ring structures. Alkyl substituted cycloalkanes can be degraded more easily as compared to non-substituted HCs. Cycloalkanes are degraded to cyclic alcohol by the action of oxidases which further is dehydrogenated to ketone. The primary products of metabolism of cycloalkanes are cycloketones and cycloalkane-carboxylic acids.

Mmom and Igbuku, (2015) reported that Biodegradable Contaminants such as crude oil soaked soil were treated by in-situ and ex-situ (Engineered Bio cell) bioremediation techniques. Bioremediation, involved systematic tilling and application of microbial nutrient amendments– Contaminants at depths of 6m to 10m below ground surface were removed by deep excavation and treated by ex-situ process, Non-Biodegradable waste stream (burnt carbonized residue): These were handled through fixation and stabilization into cement blocks. About 40,000 blocks were produced, Oily-bituminous sludge: These were handled by Thermal desorption process.

Impacted Shallow Groundwater. Free phase oil on shallow groundwater trenches was removed by skimming of oil and application of bio-degrader to remove the oil.

Deep seated contaminated soil is being tackled by use of heavy equipment to excavate and remediate by either ex-situ bioremediation in an Engineered Bio cell or on-site ex-situ. Deep seated impacted soils with relatively light contaminants are excavated and remediated on-site in-situ using bioremediation process. Deep seated impacted soil with heavy contaminants is excavated and remediated using bioremediation process in an Engineered Bio cell.

2. Material and methods

2.1. Sample collection

Samples were collected from an oil polluted site in Ejama-Ebubu Eleme Rivers state. The samples were taken from five spot (A-E), of depth 0-15 (A_1– E_1) and 15-30 (A_2– E_2) in sterile polyethylene bags, using appropriate equipment, then taken to the laboratory for analysis.

2.2. Determination of physicochemical parameters of samples

Physicochemical parameters such as pH, nitrate, sulphate, phosphate, total petroleum hydrocarbon (TPH), poly aromatic hydrocarbon (PAH), salinity, temperature, conductivity and heavy metals (iron, zinc, nikel, lead, chromium) were determined.

2.3. PH

This test is done to determine the acidity or alkalinity of the sample site using Glass-electrode pH apparatus.

20g of soil sample was weighed out and sieved using a 2mm sieve into a 50 mL beaker, 20 mL of distilled water was added and allowed to stand for 30 minutes and stirred occasionally with a glass rod.The pH probe was inserted into the partly settled suspension to measure the pH.

2.4. Nitrate

Apparatus: B and L spectronic-20, electrophotometer.

Reagents: brucine (2.5g of brucine dissolved in 100m L of glacial acetic acid), conc. H_2SO_4, standard NO_3-N solution, 50 ppm- 0.1805g of KNO_3 was dissolved in 500mL of the extracting solution, 0.5 mL of chloroform as a preservative, dilute nitrate standard solution.

The Extraction solution-100g of sodium acetate was dissolved in 500 mL of distilled water and 30 mL of 99.58 acetic acid was added, then diluted to 1L. 5.0g of soil was transferred into a shaking bottle and ¼ teaspoon of activated carbon and 20 m L of extracting solutionwas added and shook for 1 minute then filtered. 1 mL aliquot of the soil extract was transferred to a vial and mixed, 0.5 m L of brucine reagent was added and then 2 m L of sulphuric acid the sample was mixed for 30 seconds and allowed to stand for 5 minutes. The sample was mixed again and 2 mL of distilled water added and mixing continued for about 30 seconds. The tubes were allowed to set in cold water for 15 minutes and transmittance was measured at 470µ.

2.5. Sulphate

Reagent: KH_2PO_4 containing 500ppm P (extracting solution) 5g of air-dried soil sample was weighed out and passed through a 2mm sieve into a flask and 25m L 0f extracting solution was added. The mixture was shake for 30 minutes on a mechanical shaker and centrifuged. The SO_4 content was determined by turbidity.

2.6. Phosphorous

Apparatus: centrifuge, mechanical shaker, 20 mL test tube, B and L spectronic –electrophotometer.

Extracting solution- 15 mL of 1.0 N NH_4F and 25 m L of 0.5 N HCl was added to 460 m L distilled water, Stannous chloride ($SnCl_2.2H_2O$) stock solution- 10g of $SnCl_2.2H_2O$ was dissolved in 25 mL of conc. HCl, Ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$- 15g of ammonium molybdate is dissolved in 350 mL of 10 N HCl in a litre volumetric flask and cooled to room temperature then made up to 1 L with distilled water.

1 m L of stock solution of stannous chloride was mixed with 333 m L of distilled water. 1 g of air dried soil sample was weighed and sieved with a2mm sieve into a 15 mL centrifuge tube and 7 mL of extracting solution was added. The mixture was shaked using a mechanical shaker and centrifuged at 2000 rpm for 15 minutes. 2 mL of the clear supermatant was pipetted into a test tube. 5 mL of distilled water was added to $(NH_4)_6Mo_7O_{24}.4H_2O$ solution and mixed. 1 m L of $SnCl_2.2H_2O$ dilute solution was added and mixed. After 5 minute, the transmittance was measured on the electrophotometer at 660 mµ wave length.

2.7. Determination of heavy metals

2.7.1. Soil pre-treatment

5g of air dried soil sample was weighed out and sieved into a 100mL flask using a 2mm sieve, 2ml of $HNO_{3(aq)}$ and 6ml of $HCl_{(aq)}$ was added in the ratio of 1:3 to the already weighed sample. The mixture was digested by heating on a heating mantle until the sample attained near dryness to enable proper leaching of the sample. The sample was then diluted with distilled water and filtered using the watchman No. 42, 150mmin diameter filter paper into a 50mL volumemetric flask. The filtrate was then made up to 50mL mark. The atomic adsorption spectrometer (AAS) was calibrated using a standard solution for each of the metal of interest (Zn- 1 and 2 ppm, Cr- 2 and 5 ppm, Pb- 0.5 and 1.0 ppm) The digested sample is then introduced into the AAS and the concentration of the metals were displayed.

2.7.2. Determination of total petroleum hydrocarbon (TPH) and poly aromatic hydrocarbon (PAH)

Residual total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were extracted from the samples and quantified using gas chromatograph-flame ionization detector (GC-FID).

2.7.3. Extraction

2g of sample was weighed into a clean extraction amber container. 10 m L of extraction solvent (pentane) was added into the sample and mixed thoroughly and allowed to settle. The mixture was carefully filtered into clean solvent rinsed extraction bottles using filter paper fitted into Buchner funnels. The extracts were concentrated to 2 mL and then transferred for separation.

2.7.4. Separation

1 cm of moderately packed wool was placed at the bottom of 10mm ID X 250 mm long chromatographic column. Slury of 2g activated silica in 10 mL methylene chloride was prepared and placed into the chromatographic column. To the top of the column was added 0.5 cm of sodium sulphate. The column was rinsed with additional 10 mL o methylene chloride. The column was pre eluted with 20 mL of pentane, this was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulphate layer, immediately, 1 mL of the extracted sample was transferred into the column. The extraction was rinsed with 1 mL of pentane and added to the column, the eluent was collected with graduated cylinder. Prior to exposure of the sulphate layer to air, pentane was added to the column in 1-2 mL increments accurately measured volume of 8-10 mL of the eluent was collected and was labelled aliphatics.

2.7.5. Gas chromatographic analysis

The concentrated aliphatic fraction were transferred into labelled glass vials with Teflon or rubber crimp caps for GC analysis, 1 µL of the concentrated aliphatic fraction was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituent partition of between the gas and liquid phases. The sample was automatically detected as it emerges from the FID whose response is dependent upon the composition of the vapour. The result was recorded.

2.8. Bacterial enumeration

2.8.1. Enumeration of total heterotrophic bacteria (THB)

The THB count was determined using the spread plate method on nutrient agar. The nutrient agar was prepared by dissolving 28g of nutrient agar powder in 1000mL of distilled water according to manufacturer's standard Soil suspensions were prepared by 10-fold serial from each sample 1g was homogenized in 9 ml of 0.85% normal saline dilutions and then 10^{-4} and 10^{-5} dilution was spread on the plates in duplicates. The colony forming unit (CFU) of total heterotrophs was counted after incubation at room temperature for 24hrs.

2.8.2. Enumeration of hydrocarbon utilizing bacteria (HUB)

The HUBs were enumerated using mineral salts medium (composition: 0.063g of NH_4NO_3, 0.19g of K_2HPO_4, 1.5g OF NaCl, 0.063g of $MgSO_4$, 0.044g OF KCl, 2.28g of agar and 150 mL 0f distilled water) with crude oil as the sole source of carbon source. Hydrocarbon utilizing bacteria (HUB) were enumerated by a method adopted from (Hamamura*et al* 2006) which involved the dilutions of appropriate sample suspensions and plating out on BushnellHaas agar from dilutions of 10^{-1} – 10^{-3} in duplicates. Hydrocarbons were supplied through the vapour phase by placing sterile Whatman No.1 filter papers impregnated with crude oil on the lids of the inverted plates and incubated for 5days at room

temperature. Isolated colonies were further purified by sub-culturing and identified using biochemical tests and microscopy. Enumeration of THB and HUB was done every fort night.

2.8.3. Purification and identification of hydrocarbon utilizing bacteria

Discreet colonies of different HUB were randomly picked using a sterile inoculating wire loop and sub cultured by streaking on nutrient agar plates and incubated at 38°C for 24 h. The purity of each colony was confirmed by Gram staining, the micro and macro morphological characteristics were recorded. Individual colonies were identified using the following biochemical tests: catalase and oxidase production, gas/acid production from fermentation of lactose, indole production, citrate utilization, triple sugar iron fermentation, and methyl red-VogesProskaeur.

2.8.4. Gram's staining technique

This test is differentiate between Gram positive and Gram negative bacteria species.

On clean grease free slides, one loopful of distilled water was placed and aseptically, a loopful of the subcultured organisms were smeared on different slides and allowed to air dry.The slides were heat fixed by passing through flame 5 times. The slides were stained with safranin for 1 minute and rinsed. The mordant Gram's iodine was added to the slide for 30 seconds and rinsed off, 75 % ethanol was added and rinsed immediately. The secondary dye safranin was then added for 1 minute and rinsed. The slides were allowed to dry and viewed under the microscope.

2.8.5. Catalase test

Most bacterial have flavoprotein oxidases which produces H_2O_2, no H_2O_2 will be produced anaerobically since oxygen is absent to accept electrons. Peroxide is inhibitory to bacteria. Aerobes which are exposed to peroxide, normally possess catalase which decomposes peroxide to give water and oxygen gas, as in the eqation below:

$2H_2O_2 \rightarrow 2H_2O + O_2$. Bubble formation is indicative of a positive result.

On clean slides, hydrogen peroxide was dropped, each organism was asceptically smeared on hydrogen peroxide and observed for bubble formation, the results were recorded.

2.8.6. Oxidase test

Sterile filter paper was impregnated with oxidase reagent. Sterile wire loop was used to streak each organism on the filter paper. The streakings were observed for purple colour formation and the results were recorded.

2.8.7. Citrate utilization test

The citrate test uses a medium in which sodium citrate is the only carbon and energy source. In Simon's citrate agar, the p H indicator is bromthymol blue, which is green at neutral pH and becomes blue when the medium becomes alkaline. If an organism can utilize citrate as the sole source of carbon and energy source, it will then need to use the ammonium salts for nitrogen source. This results in the release of ammonia, causing the pH to change to alkaline causing a colour change from green to royal blue.

1.6g of Simon citrate agar was dissolved in 70 mL of distilled water and heated to homogenize**.** the medium is then poured into Biju bottles and sterilized by autoclaving at. 121^{o}C, at 15 psi, for 15 minutes, the medium was then slanted to solidify. Aseptically the different isolates were inoculated into the medium by streaking on the slant and stabbing the butt. Then incubated at 38^{o}C for 48 hrs and observed for colour change.

2.8.8. lactose fermentation (acid and gas production)

1g of lactose broth was dissolved in 100m L of distilled water.Bromocresol purple indicator was added to the broth.Durham's tubes were placed in test tubes and the lactose broth were added to the tubes amount enough to cover the Durhaam's tube and the tubes were sterilized by autoclaving at 121^{o}C, at 15 psi, for 15 minutes.Aseptically each isolate was inoculated in different tubes and incubated at 38^{o}C for 48 hrs, each test tubes were observed for colour change and gas collection in the Durham's tube and the results recorded.

2.8.9. Triple sugar iron agar (TSIA) test

3 g of TSIA was dissolved in 50 mL of distilled water and heated to homogenize. The medium was poured into test tubes and sterilized by autoclaving 121^{o}C, at 15 psi, for 15 minutes. The media was kept slanted and allowed to solidify. The

test tubes were inoculated with the different isolates and incubated at 38°C for 48 hrs. The test tubes were then observed for changes and results were recorded.

3. Results and discussion

3.1. Physiochemical parameter and heavy metals

Table 1 Physiochemical parameter and heavy metals obtained from samples

S/N	Parameters(s)	A_1	A_2
1	Ph	5.77	5.68
2	Conductivity (µS/cm)	98	35
3	Nitrate (mg/kg)	7.8	2.5
4	Phosphate (mg/kg)	0.11	0.08
5	Sulphate (mg/kg)	9	4
6	Iron, Fe (mg/kg)	5715.3	5,923.5
7	Zink, Zn (mg/kg)	3.31	3.7
8	Nikel, Ni (mg/kg)	1.06	2.22
9	Lead, Pb (mg/kg)	4.12	2.86
10	Chromium, Cr (mg/kg)	5.17	4.3
11	Vanadium (mg/kg)	0.5	0.1
12	TPH (mg/kg)	51.14	25.56
13	PAH (mg/kg)	0.136	0.071

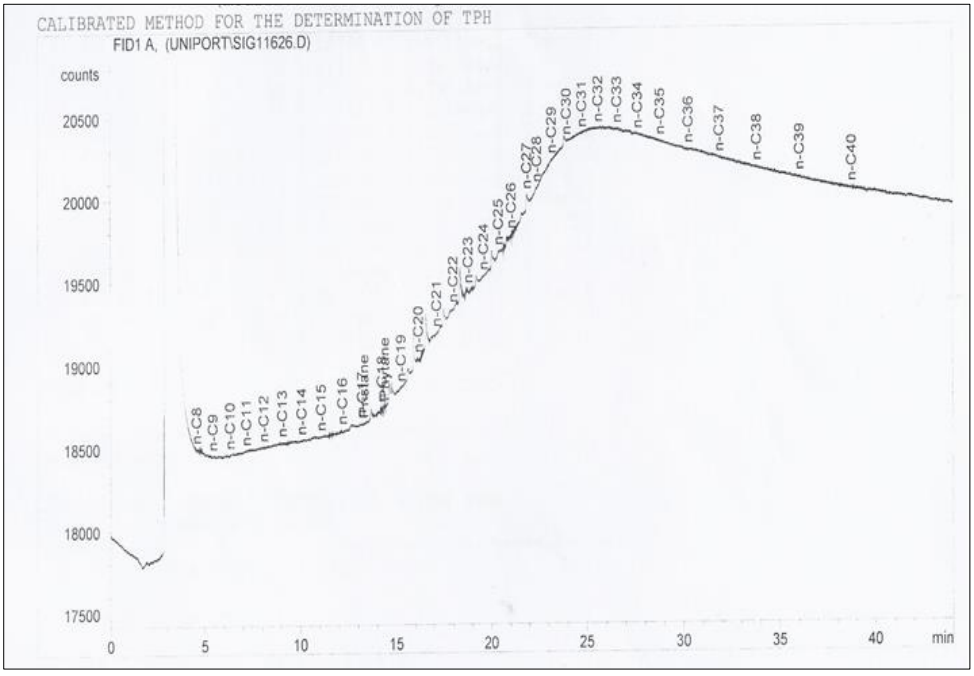

Figure 1 Total petroleum hydrocarbon for sample A_1

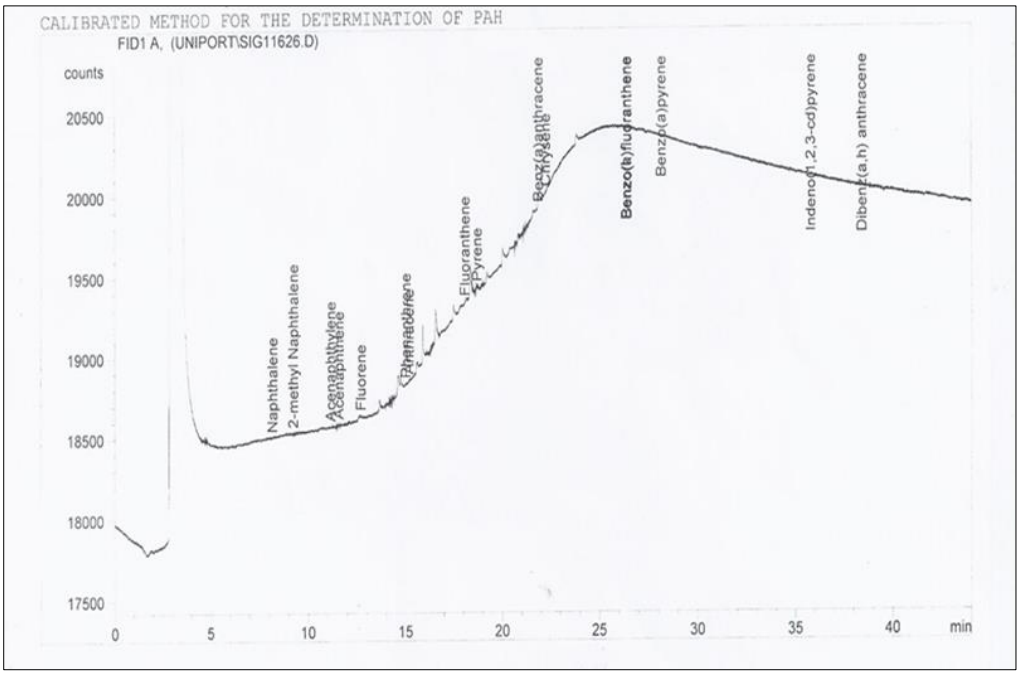

Figure 2 Poly aromatic hydrocarbon (PAH) for sample A_1

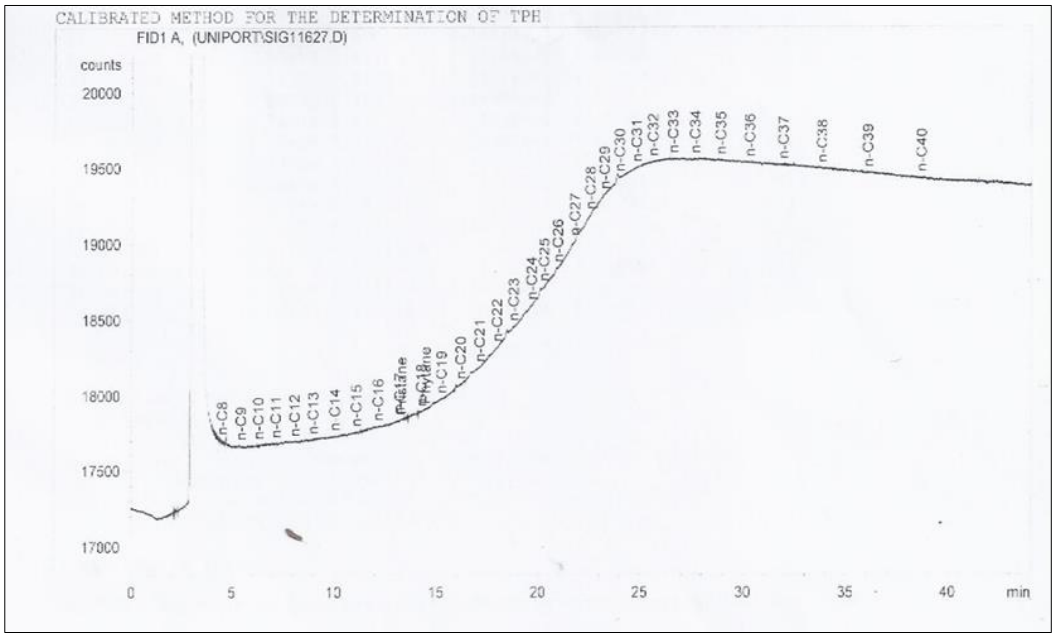

Figure 3 Total petroleum hydrocarbon for sample A_2

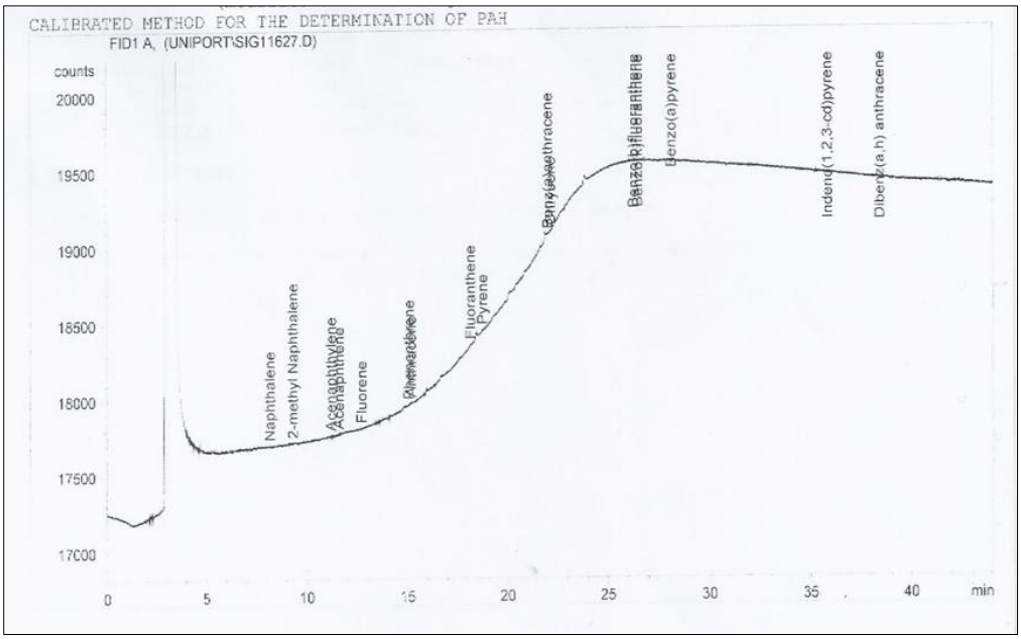

Figure 4 Poly aromatic hydrocarbon (PAH) for sample A_2

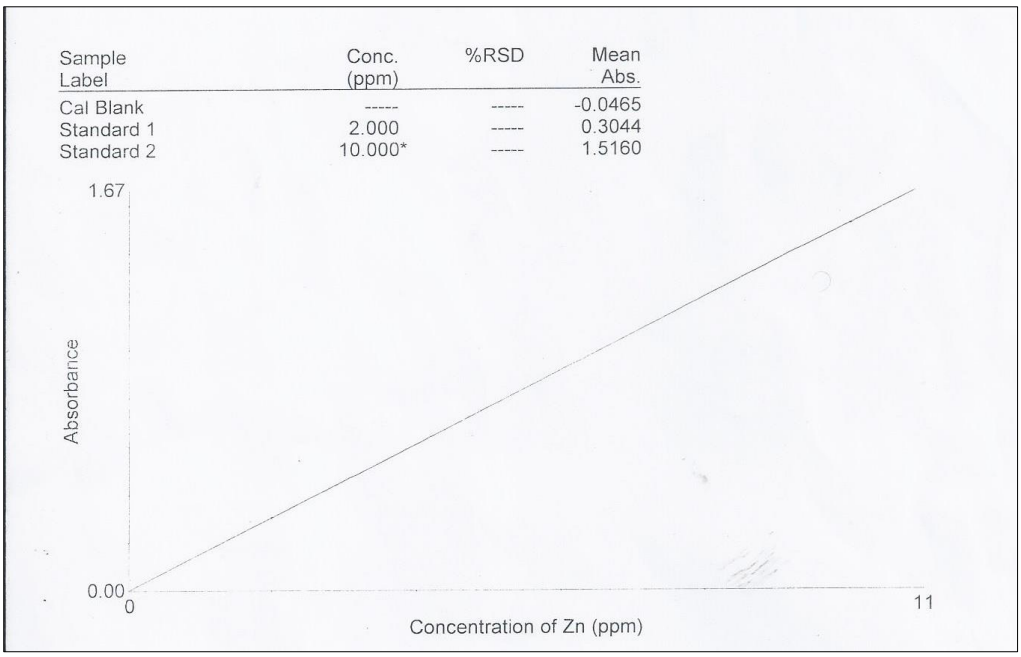

Figure 5 Concentration of Zn for sample A_1 and A_2

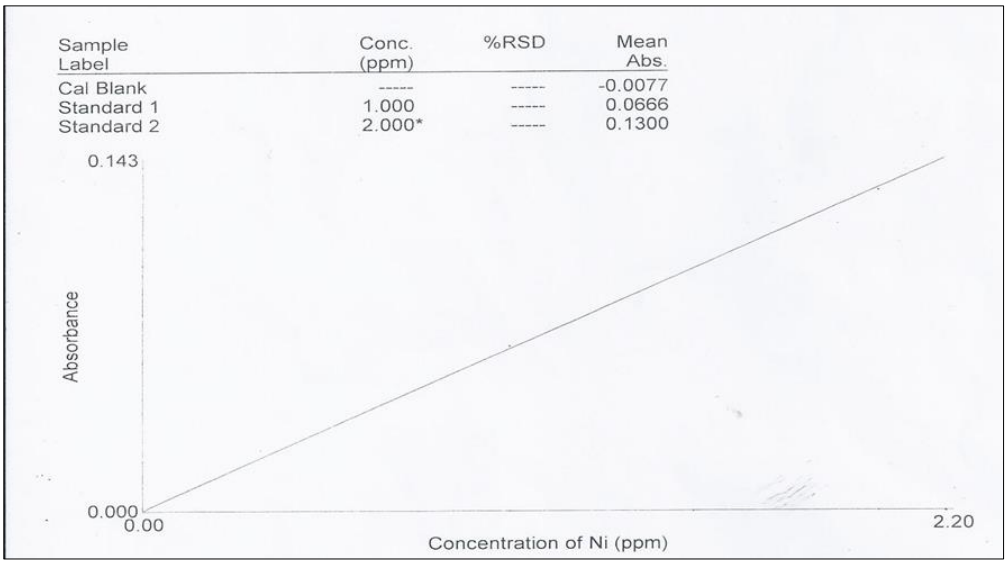

Figure 6 concentration of nitrogen for sample A_1 and A_2

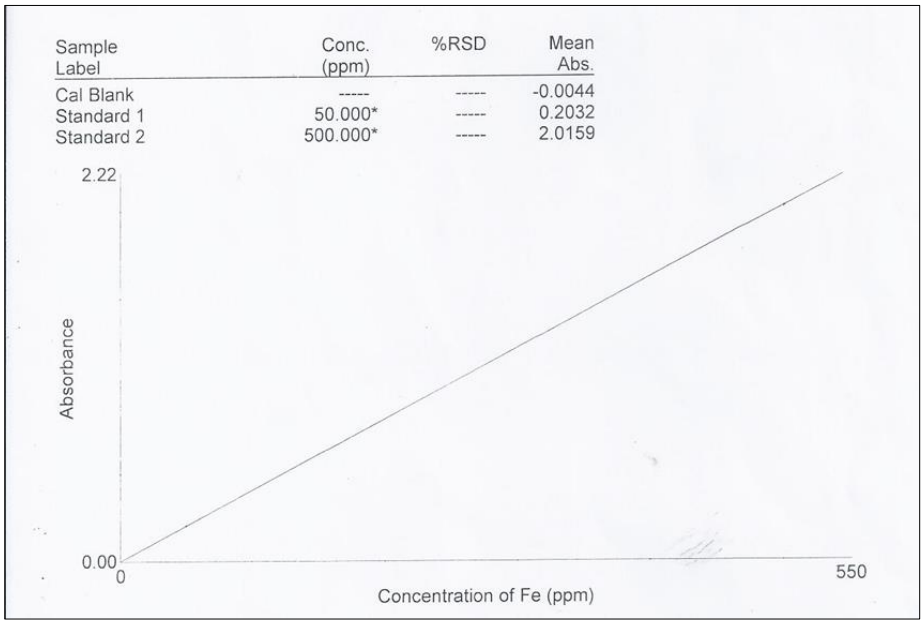

Figure 7 Concentration of iron for sample A_1 and A_2

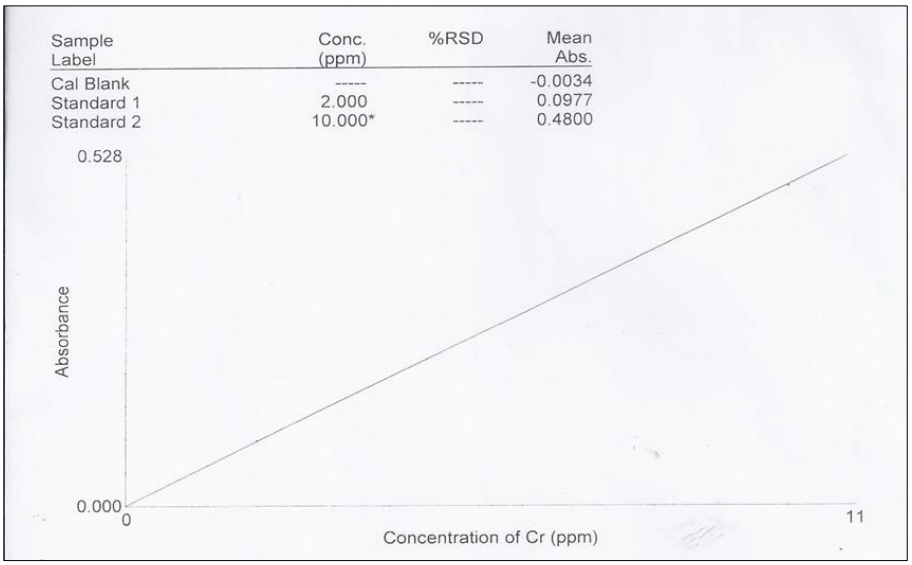

Figure 8 Concentration of chromium Zn for sample A_1 and A_2

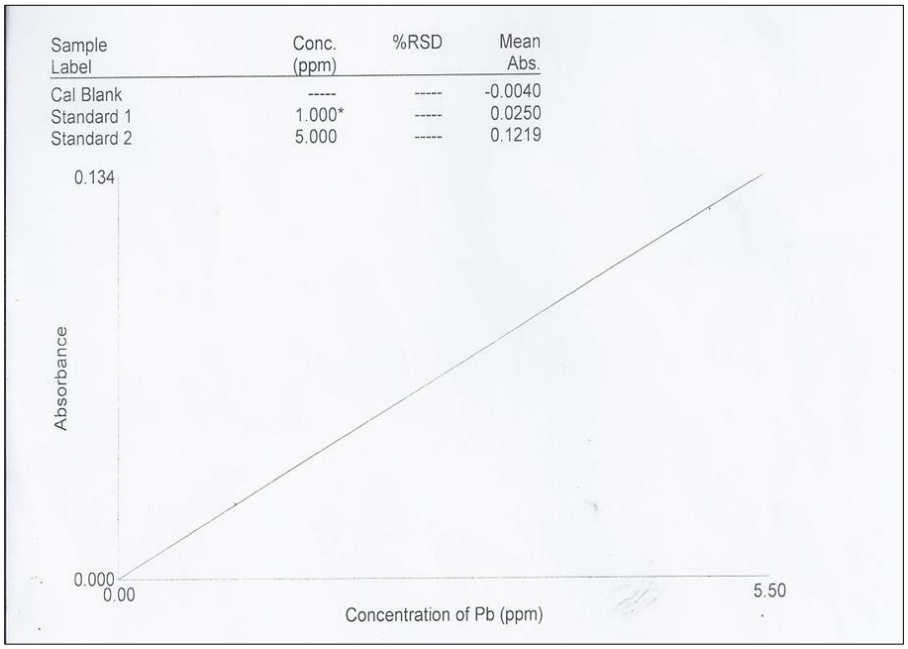

Figure 9 Concentration of lead for sample A_1 and A_2

3.2. Bacterial enumeration

After 24 hours of incubation for day 0- day 28 of isolation, the following results were obtained for THB as shown in table 2 below.

Soil, sediment and water physicochemical parameters are shown in Table 1. The parameters determined indicated that the samples had been exposed to hydrocarbon contamination.

These pollutants cause damages to humans and the ecosystem if not effectively remediated. The contamination may have resulted in the low pH of 5.77 depth 0-15cm and 5.68 at depth 15-30cm observed in soil. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and in some cases iron. Addition of nutrients is necessary to enhance biodegradation of crude oil pollutants (Chikere*etal*, 2014).

Table 2 Total heterotrophic bacteria count obtained for all samples

Nutrient agar (NA)	Sample	Dilution	Average	CFU/mL
Day0	A_1	10^{-4}	191	$1.91 X 10^7$
		10^{-5}	100	$1 X 10^8$
	A_2	10^{-4}	76	$7.6 X 10^6$
		10^{-5}	40	$4 X 10^7$
Day 14	A_1	10^{-4}	116	$1.16 X 10^7$
		10^{-5}	64	$6.4 X 10^7$
	A2	10^{-4}	54	$5.4 X 10^6$
		10^{-5}	43	$4.3 X 10^7$
Day 28	A1	10^{-4}	103	$1.03 10^8$
		10^{-5}	61.5	$6.15 X 10^7$
	A2	10^{-4}	34	$3.4 X 10^6$
		10^{-5}	18	$1.8 X 10^7$

The colonial morphology of each colony in pure culture is presented in colonial morphology of the isolates.Table 3 & 4

Table 3 Biochemical test result for the different isolates hydrocarbon utilizing bacteria

[1]		[2]	**Size(mm)**	[3]	**Transparency**	[4]	**Colour**	[5]	**Form**	[6]	**Elevation**
[7]	Isolate 1	[8]	2.0	[9]	Opaque	[10]	Cream	[11]	Entire	[12]	Umbonate
[13]	Isolate 2	[14]	0.5	[15]	Opaque	[16]	White	[17]	Serrated	[18]	Flat
[19]	Isolate 3	[20]		[21]	Opaque	[22]	White	[23]	Entire	[24]	Umbonate
[25]	Isolate 4	[26]	1	[27]	Transparent	[28]	Cream	[29]	Serrated	[30]	Flat
[31]	Isolate 5	[32]	3	[33]	Transparent	[34]	Cream	[35]	Entire	[36]	Unbonate
[37]	Isolate 6	[38]	2	[39]	Opaque	[40]	Cream	[41]	Entire	[42]	Unbonate
[43]	Isolate 7	[44]	1	[45]	Translucent	[46]	Green	[47]	Entire	[48]	Flat
[49]	Isolate 8	[50]	2	[51]	Opaque	[52]	Cream	[53]	Entire	[54]	Flat
[55]	Isolate 9	[56]	1	[57]	Transparent	[58]		[59]	Entire	[60]	Flat
[61]	Isolate 10	[62]	1	[63]	Transparent	[64]	Green	[65]	Entire	[66]	Flat

The presence of microbial activity was determined by the enumeration of culturable total heterotrophic bacteria and hydrocarbon utilizing bacteria as presented in Table 1 and 2 respectively. It was observed that the Ejama-Ebubu sample contained THB count ranging from 1.8×10^7 -1.19×10^7 CFU mL^{-1} and a mean HUB count of 9×10^3 – 1.3×10^4 CFU mL^{-1}. This site is continuously exposed to petroleum hydro-carbons owing to navigational activities and this may have enriched the sediment with hydrocarbon utilizing bacteria. However, the fewness in number of the HUB counts may be attributed to the inadequacy of nutrients at that depth especially nitrogen and phosphorus which deplete with input of hydrocarbons. The Gram negative bacteria belonging to the genus *pseudomonas* is the most frequent. Other genera isolated were*Nocardia*, *Micrococcus*,*Chromobacterium, Burkholdia, Corynebacterium*. After every forth night, the bacterial count reduces, this may be due to depletion of available nutrient in the soil sample.

Table 4.5 also indicates that the crude oil aerobic degradative ability of the individual bacterial isolates was significant as evidenced by turbidity and emulsification of 1 ml of crude oil in 100 ml of Bushnell-Haas broth after 14days incubation when compared with the test isolates on day zero incubation. Bacteria that are capable of utilizing hydrocarbons as energy and carbon sources in broth culture have been shown to produce bioemulsifiers or bio-surfactants that assist in the transport of hydrocarbons into the cell via efficient uptake systems (Atlas*et al* 2005).

Table 4 Biochemical test result for the different isolates hydrocarbon utilizing bacteria

	Gram's reaction	***Oxidase***	***Catalase***	***MR***	***VP***	***Citrate utilization***	***Lactose fermentation***		***Indole***	***TSIA***				
							A	**Gas**		**Slant**	**butt**	**H_2S**	**Gas**	**Probable Organism**
Isolate 1	Gram negative rod	-	+	+	-	-	+	-	-	B	A	-	+	*Escherichia coli*
Isolate 2	Gram positive rod	+	+	-	+	-	-	-	+	A	B	-	-	*Norcardia sp.*
Isolate 3	Gram negative rod	+	+	+	-	-	+	+	-	B	B	-	-	*Burkholderia sp.*
Isolate 4	Gram positive rod	-	+	-	+	-	+	-	-	B	B	-	-	*Corynebacterium sp.*
Isolate 5	Gram positive cocci	-	+	-	+	+	+	-	-	B	B	-	-	*Micrococcus*
Isolate 6	Gram negative rod	-	-	-	+	+	-	-	-	B	A	-	+	*Chromobacterium sp.*
Isolate 7	Gram negative rod	+	-	-	-	+	-	-	-	B	B	-	-	*Pseudomonas sp.*
Isolate 8	Gram positive cocci	-	+	-	+	+	+	-	-	B	B	-	-	*Micrococcus coli*
Isolate 9	Gram negative rod	-	+	+	-	-	+	-	-	B	A	-	+	*Pseudomonas sp.*
Isolate 10	Gram negative rod	+	-	-	-	+	-	-	-	B	B	-	-	*Pseudomonas sp.*

Key (+) positive, (-) negative, (B) base, (A) acid.

4. Conclusion

This show the presence of petroleum hydrocarbons in the Ejama_Ebubu site as well as known genera of hydrocarbon utilizing bacteria. The biases associated with culture-dependent microbial enumeration techniques may limit the full description of the bacterial diversity in Ejama-Ebubu site. From the study, it is concluded that microorganisms that can degrade hydrocarbons are found in oil contaminated soil and can easily be isolated from these contaminated sites, although it is very difficult to work with aromatic hydrocarbons due to their volatility and toxic effects.

Pseudomonas sp. Isolate 7, 9, *Burkholderia* sp. Isolate 2, showed appreciable aerobic degradative ability of hydrocarbons, while isolates 1, 5, and 6 showed little emulsication of the crude oil, this could imply that this isolates are unable to degrade hydrocarbon under aerobic condition. Bacterial strains capable of degrading complex hydrocarbons present in the environment have a potential to be used as an effective tool for removing ecotoxic compounds.

Compliance with ethical standards

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Disclosure of conflict of interest

No Conflict of interest.

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