

Bacterial Population and Physicochemical Characteristics of Hydrocarbon Polluted Soil around Generator Houses in a Tertiary Institution in Nigeria

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ABSTRACT

Heavy-duty generators constitute a form of hydrocarbon pollution but enrich microorganisms to having the ability to breakdown hydrocarbons hence can be used for remediation. Bacterial population and physicochemical characteristics of hydrocarbon polluted soil around generator houses in a Tertiary Institution in Nigeria was studied. Soil samples were collected from six different generator houses and control. Analysis for bacterial population and physicochemical characteristics were done using standard methods. Total heterotrophic bacterial (THB) counts ranged from $1.60 \pm 0.37 \times 10^6$ cfu/g to $3.32 \pm 1.36 \times 10^6$ cfu/g. Hydrocarbon utilizing bacteria (HUB) counts ranged from $0.00 \pm 0.00 \times 10^4$ cfu/g to $2.75 \pm 0.92 \times 10^4$ cfu/g. The percentage occurrence of bacterial isolates ranges as follows; *Bacillus* sp. 21%, *Pseudomonas* sp. 18%, *Staphylococcus* sp. 14%, *Enterobacter* sp. 13%, *Escherichia coli* 9%, *Chromobacterium* sp. 8%, *Corynebacterium* sp. 7%, *Micrococcus* sp. 6%, and *Vibrio* sp. 4%. Sequence analysis revealed the presence of *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Bacillus megaterium*, *Chromobacterium violaceum* and *Micrococcus luteus*. Range of physicochemical characteristics of the different soil samples analysed were: pH 6.49 to 6.84, Moisture contents ranged from 6.22 to 14.70 %, Nitrate 69.05 to 2.88mg/kg, Sulphate 1.73 to 3.48mg/kg, Phosphorus 7.03 to 50.54mg/kg, Potassium 0.003 to 357.90mg/kg, Total organic carbon (TOC) 1.05 to 2.89% and Total petroleum hydrocarbon (TPH) 0.00 to 31.445mg/kg. This study revealed presence of reasonable population of indigenous hydrocarbon utilizing bacteria which can be monitored and enhanced to improve their bioremediation abilities. Finally, the study revealed that the waste hydrocarbon oil spilled within the generator houses in the institution are at minimum but also required clean-up process to remove total petroleum hydrocarbon in the soil.

Keyword: Electricity generators, hydrocarbon polluted soil, Bacteria, physicochemical characteristics.

Introduction

Hydrocarbon products continue to be used as the major source of energy, but, despite its vital utilization, petroleum hydrocarbons also operate as a global environmental pollutant, (Benson *et al.*, 2007, Dorn *et al.*, 2005). Since the petroleum hydrocarbons are used widely, oil spills are inevitable even in virtually uninhabited places. These may be through oil well blow outs, tanker accidents, accidental or deliberate rupture of oil pipelines and dumping of used petroleum products.

According to Saravana and Amruta, (2013), about 0.08-0.46 % of the total oil produced is continuously being spilled into the environment and this eventually pollutes land and water.

This is a major concern not only here in Nigeria but worldwide, as the presence of hydrocarbons pollution in the environment poses great hazard to livelihood, human and environmental health, (Margesin *et al.*, 2009; Mnif *et al.*, 2017). As a result, there are worldwide concerns about remediating and restoring the hydrocarbon polluted environment.

The sole of pollution relies on the quantity of oil and the damage done to the ecosystem. In locations where there's heavy oil pollution, there will be an instant harmful influence on the lives of plants and animals. Therefore, there is an urgent need to evolve viable technologies to combat oil or hydrocarbon pollution that may accompany activities of oil industries.

Especially, the frequent spills of crude oil or its products in part of the Niger Delta region demand a refocused attention on the problem of hydrocarbon contaminant in the area (Al-Saleh *et al.*, 2009; Alvarez *et al.*, 2008). Microbial enhanced remediation process has a proven eco-friendly advantage over every other method such as mechanical removal, burying, evaporation, dispersion, incineration, land filling and washing, among other physical and chemical treatments which can be quite expensive, in remediating hydrocarbons contaminated environment (Al-Wasify and Hamed, 2014; Ukaegbu-Obi and Mbakwem-Aniebo, 2014; Zhang *et al.*, 2005). This has made it an attractive and acceptable technology for restoration of hydrocarbon contaminated sites (Das and Chandran, 2011; Juwarkar *et al.*, 2010; Moliterni *et al.*, 2012).

Any substance whose presence in the environment impacts these microbial activities, would also adversely affect plant growth, as well as detoxification of organic pollutions. Microbes play a very essential role in forecasting the impact of a particular stress on the environment by their ability to adapt to these unfavourable conditions through a change in their numbers (Obire *et al.*, 2007) and the eradication of some species or types (Adeyemo *et al.*, 2013). The aim of the current study was to evaluate the bacterial population and physicochemical parameters of hydrocarbon products in polluted soil.

Materials and Methods

Study Area

The study area was Port Harcourt Metropolis and sampling locations of five generator houses were chosen in Rivers State University, Port Harcourt with longitude and latitude 4048'4"N6'58'57.5"E.

Rivers State University has several generator houses which are located within the main campus, namely Estate and works phase one generator house, Estate and works phase two generator house, Rivers State University Central Library generator house, Amphitheatre generator house and Law faculty generator house. An unpolluted soil sample from the Rivers State University School farm, served as a control.

Sample Collection, Transportation and Processing

A total of fifty four (54) composite soil samples were collected for three months using random sampling method. 9 Estate and works phase one generator house soils, 9 Estate and works phase two generator house Soils, 9 Central Library generator house Soils, 9 Amphitheatre generator house soil, 9 Law faculty generator house soils and 9 Agricultural school farm soil (Control) were randomly collected from 6 different locations with the Rivers State University, Port Harcourt. The soil samples were collected at 0 to 15cm depth using a sterile soil auger. The samples were aseptically collected into sterile zip-lock bags with the aid of sterile hand trowel, labeled properly and immediately sent to Postgraduate laboratory of Microbiology Department, Rivers state university Port Harcourt for analysis.

Bacteriological Analysis of Samples

The weighed soil samples were subjected to a serial ten-fold dilution with a dilution factor ranging from 10^{-3} to 10^{-6} onto nutrient agar, an aliquot (0.1ml) of the appropriate dilutions were spread plated onto suitable agar medium in triplicate. For twenty-four hours, the plates were incubated at 37°C. The total heterotrophic bacterial (THB) counts were estimated from the colonies formed on nutrient agar, which were counted and described morphologically. To obtain pure cultures, representative discrete colonies were purified by sub-culturing on newly prepared sterile nutrient agar plates and then incubated at 37 °C for 24 hours.

Enumeration of Hydrocarbon Utilizing Bacteria

Hydrocarbon utilizing bacterial (HUB) were enumerated and isolated using mineral salt agar. The mineral salt medium composition (g/L) is as follows: 0.5 K₂HPO₄, 0.3 NaCl₂, 0.02 FeSO₄.6H₂O, 0.3 ZnCl₂, 0.3 MgSO₄.7H₂O, 0.03 NaNO₃, 0.2 MnSO₄.H₂O and pH adjusted to 7–7.2. The medium was treated with Ketoconazole to limit the growth of fungal contamination. Sterile filter paper was moistened with crude oil and inserted into the lid of Petri dish. The Mineral salt agar (MSA) plates were inoculated in duplicate with 0.1ml aliquots of 10^{-1} dilution of each soil samples and incubated at 35°C for 3-7 days. After incubation, colonies on the agar plates were counted, yielding the total number of hydrocarbon-degrading bacteria for each soil samples (Odokuma *et al.*, 2008).

Calculation of colony forming units per gram of soil samples

The incubated plates were placed on counter machine and the average was taken and expressed as colony forming units per gram (cfu/g) of sample using the formula as adopted by Prescott, (2005).

$$\text{cfu/g} = \frac{N}{D \times V}$$

Where:

N = Number of Colonies

D = Dilution

V = Volume plated

Sub-culturing and Purification of Isolates

A loopful of each discrete 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°C for 24 hours to obtain pure culture.

Measurement of Physicochemical Parameters

Determination of pH

About 20g of each air-dried soil was weighed into 50ml beaker and 20ml of distilled water was added. It was stirred with a glass rod and allowed to stand for 30 minutes. Calibrated HANNA pH meter (Model Jenway 3510) was inserted into the liquid and pH recorded.

Determination of Moisture content

About 1g of sieved soil sample was weight into dry crucible. The crucible was then placed in an air circulated oven at 105°C and dried to constant weight (for 6 hours). The sample was cooled in a desiccator and re-weighed. The percentage air dried moisture from the loss weight was then determined as fellows (Nrior and Onwuka, 2017): % moisture content = Loss in weight x 100 initial weight.

Determination of Total Organic Carbon (TOC)

The method of (Nrior and Onwuka, 2017) was used in measuring the total organic carbon (TOC).

One gram (1g) of the sample was transferred into a clean Pyrex conical flask. 5 ml of potassium chromate solution and 7.5 ml of concentrated sulphuric acid were added. The mixture was heated on an electro thermal heater for 15 minute to reflux.

The sample was allowed to cool at room temperature and was diluted to 100mls with distilled water. 25ml of the sample solution was titrated with 0.2 molar ferrous ammonium sulphate using Ferrion as an indicator. A blank containing oxidant (potassium chromate) and sulphuric acid was titrated as in the sample. The titre value was recorded (EPA, 2012). The percentage of TOC was calculated as follows; / TOC = (Titre value of the blank – sample titre × 0.003×100/ sample weight).

Determination of Nitrate – Nitrogen

Total Nitrogen was determined by semi-micro Kjeldahl method. Using the method described by (Nrior and Onwuka, 2017).

Determination of Potassium

Digested samples from the kjeldah analysis were made up to 50ml with distilled water. A standard potassium ion concentration was aspirated into the spectrometer's burner chamber to calibrate the equipment and to plot a graph of standard ion concentration. Wavelength used was 760nm. Prior to aspirating the sample, the aspirators tubing system in the spectrometer was flushed with water. The concentration of the potassium ion in the sample was automatically displayed on the screen of the spectrophotometer.

Phosphate – Phosphorus

Digested samples from the kjeldah analysis were made up to 50ml with distilled water. A standard potassium ion concentration was aspirated into the spectrometer's burner chamber to calibrate the equipment and to plot a graph of standard ion concentration. Wavelength used was 690nm.

Prior to aspirating the sample, the aspirators tubing system in the spectrometer was flushed with water. The concentration of the potassium ion in the sample was automatically displayed on the screen of the spectrophotometer.

Sodium – Sulphate

Digested samples from the kjeldah analysis were made up to 50ml with distilled water. A standard potassium ion concentration was aspirated into the spectrometer's burner chamber to calibrate the equipment and to plot a graph of standard ion concentration. Wavelength used was 589.0nm. Prior to aspirating the sample, the aspirators tubing system in the spectrometer was flushed with water. The concentration of the potassium ion in the sample was automatically displayed on the screen of the spectrophotometer.

Total Petroleum Hydrocarbon (TPH) Content

Total petroleum hydrocarbon (TPH) content in soil samples was being determined using the spectrophotometric method described in Peekate, (2018).

Data Processing and Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to statistically analyse the data obtained from counts and the measurement of the zones of inhibition. Descriptive statistics was used to summarize all data obtained. Analysis of variance (ANOVA) was carried out to test for significant difference ($p \leq 0.05$) in the bacterial counts from the various locations. Duncan multiple range test was used to separate the means where difference existed.

Results

The results obtained during the study are presented in Tables, Figures and Plate below.

Table 1: Mean values of Total Heterotrophic Bacterial counts from the different soil samples

| S/N | Soil/Generator Location | R1 (CFU/g) | R2 (CFU/g) | MN \pm SD (CFU/g) | NDI |
|---------|--------------------------|--------------------|--------------------|-----------------------------|-----|
| 1 | Estate & Works phase one | 1.85×10^6 | 1.33×10^6 | $1.60 \pm 0.37 \times 10^6$ | 3 |
| 2 | Estate & Works phase two | 1.23×10^6 | 1.54×10^6 | $1.39 \pm 0.22 \times 10^6$ | 2 |
| 3 | Amphitheatre | 4.48×10^6 | 3.04×10^6 | $3.80 \pm 1.01 \times 10^6$ | 3 |
| 4 | Central Library | 1.55×10^6 | 1.42×10^6 | $1.49 \pm 0.92 \times 10^6$ | 3 |
| 5 | Law Faculty | 2.92×10^6 | 3.36×10^6 | $3.14 \pm 0.31 \times 10^6$ | 3 |
| 6 | School Farm (Control) | 1.28×10^6 | 8.4×10^6 | $4.84 \pm 0.32 \times 10^6$ | 3 |
| P-value | | | | 0.006 | |

*Key: R: Replicate, MN: Mean, SD: Standard deviation, NDI: Number of distinct isolates based on colonial morphology, *T-test shows there was a significant ($p \leq 0.05$) difference in the total heterotrophic bacteria count in the various sampled locations

Table 2: Mean values of Hydrocarbon Utilizing Bacterial (HUB) counts from the soil samples

| S/N | Soil/Generator Location | R1 (CFU/g) | R2 (CFU/g) | MN \pm SD (CFU/g) | NDI |
|---------|--------------------------|-------------------|-------------------|-----------------------------|-----|
| 1 | Estate & Works phase one | 7.4×10^4 | 5.3×10^4 | $6.4 \pm 0.15 \times 10^4$ | 3 |
| 2 | Estate & Works phase two | 5.1×10^4 | 3.4×10^4 | $4.30 \pm 0.12 \times 10^4$ | 2 |
| 3 | Amphitheatre | 6.8×10^4 | 5.2×10^4 | $6.0 \pm 0.11 \times 10^4$ | 2 |
| 4 | Central Library | 3.2×10^4 | 2.5×10^4 | $2.90 \pm 0.50 \times 10^4$ | 2 |
| 5 | Law Faculty | 4.0×10^4 | 2.8×10^4 | $3.40 \pm 0.90 \times 10^4$ | 3 |
| 6 | School Farm (Control) | 0.0×10^4 | 0.0×10^4 | $0.00 \pm 0.00 \times 10^4$ | 0 |
| P-value | | | | 0.003 | |

*Key: R: Replicate, MN: Mean, SD: Standard deviation, NDI: Number of distinct isolates based on colonial morphology, *T-test shows there was a significant ($p \leq 0.05$) difference in the hydrocarbon utilizing bacteria count in the various sampled locations

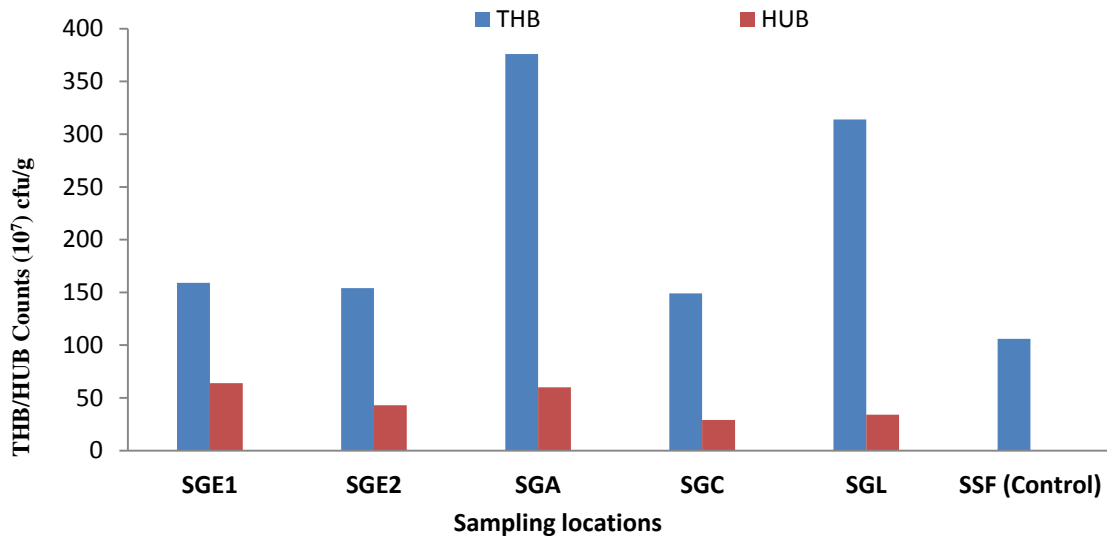


Figure 1: Total heterotrophic bacterial and hydrocarbon utilizing bacterial counts in the various soil samples

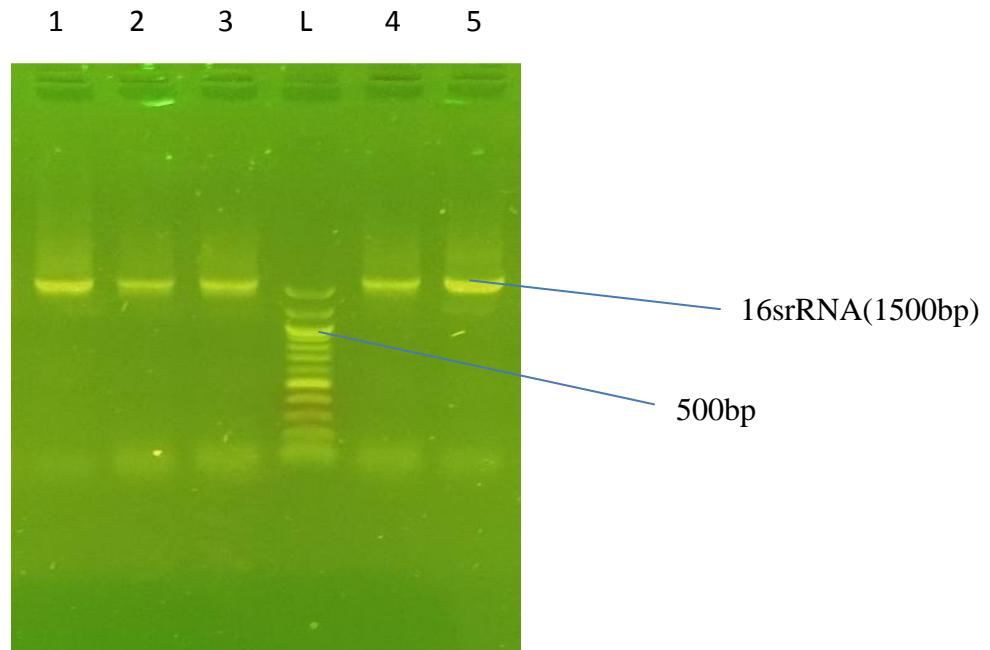


Plate 1: Agarose Gel Electrophoresis showing the Amplified 16S rRNA Gene of the bacteria Isolates at 1500bp while L represents the 500bp Molecular Ladder

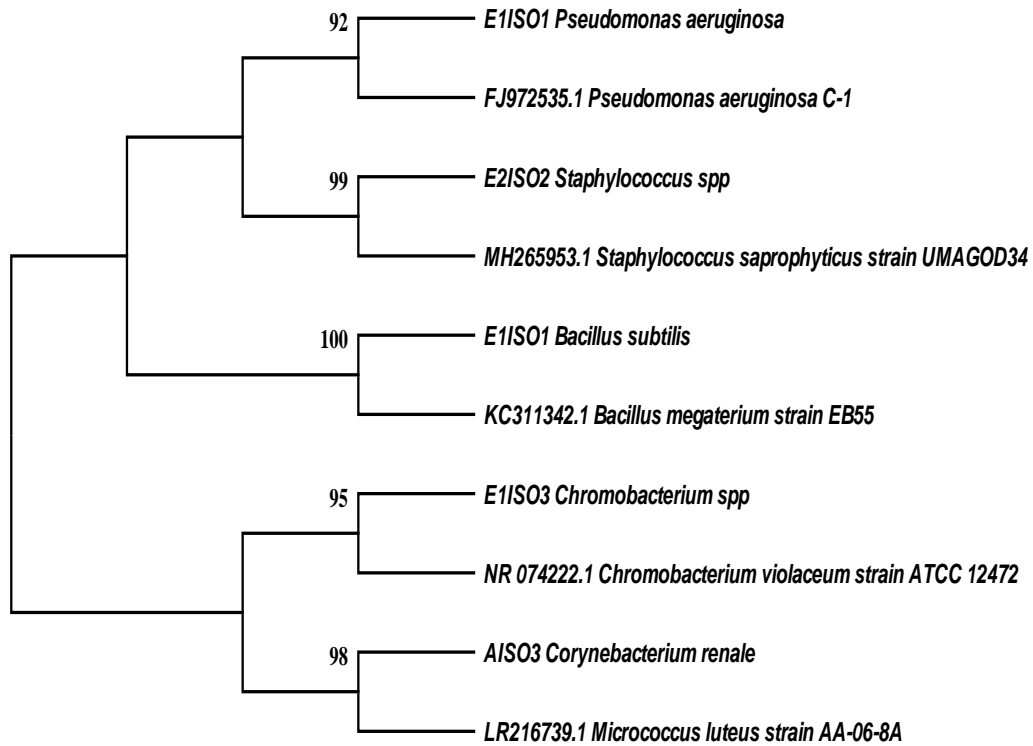


Figure 2: Phylogenetic Tree showing evolutionary distance between bacterial Isolates

Table 3: Physicochemical Constituents of the Soils from the Different Locations

| Elements | SGE1 | SGE2 | SGA | SGC | SGL | SSF Control | FEPA Limit |
|--------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-------------------------|------------|
| Moisture (%) | 14.70±0.14 ^d | 13.16±0.01 ^d | 10.55±0.01 ^d | 9.96±0.03 ^d | 12.52±0.02 ^d | 6.22±0.01 ^c | 55.00 |
| Nitrate (mg/kg) | 7.42±0.01 ^e | 2.89±0.01 ^b | 4.53±0.01 ^f | 14.08±0.01 ^f | 35.82±0.01 ^f | 69.05±0.01 ^f | 45.00 |
| pH | 6.68±0.01 ^c | 6.72±0.01 ^c | 6.63±0.01 ^c | 6.69±0.01 ^c | 6.86±0.02 ^c | 6.49±0.01 ^d | 6.50-8.50 |
| Phosphorus (mg/kg) | 19.65±0.01 ^e | 23.52±0.01 ^e | 16.15±0.01 ^e | 7.03±0.01 ^e | 20.54±0.01 ^e | 39.31±0.01 ^f | 50.00 |
| Sulphate (mg/kg) | 3.48±0.01 ^a | 2.30±0.14 ^a | 2.37±0.01 ^a | 1.89±0.01 ^a | 1.73±0.01 ^a | 2.13±0.01 ^b | 5.00 |
| Potassium (mg/kg) | 31.90±0.01 ^f | 0.0030.01 ^a | 25.100.01 ^e | 44.000.01 ^e | 357.900.01 ^f | 100.300.01 ^f | Not fixed |
| TOC (%) | 1.06±0.01 ^a | 2.97±0.01 ^b | 2.66±0.02 ^b | 2.88±0.02 ^b | 2.19±0.01 ^b | 4.14±0.01 ^b | 5.00 |
| TPH | 31.445±11.141 ^f | 16.046±1.749 ^e | 13.004±2.662 ^d | 13.992±0.532 ^d | 11.521±0.266 ^d | 0.00±0.00 | 100 |
| Texture | Sandy loam | Sandy loam | Sandy loam | Sandy loam | Sandy loam | Loamy | |
| P-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 | |

*Key: TOC: Total organic carbon, FEPA: Federal Environmental Protection Agency. TPH: Total Petroleum Hydrocarbon

*Means with different alphabet along the rows shows a significant difference (p<0.05).

Discussion

Hydrocarbon polluted soil from generator houses in a tertiary institution, Rivers State University and unpolluted soil from the school agricultural farm (Control) were investigated using microbiological standard procedures. The identities of the bacterial isolates from the polluted and unpolluted soil were confirmed using conventional and molecular techniques. The same method was used by Chikere and Ekwuabu (2014).

The presence of microbial activity was ascertained by the enumerations of total heterotrophic bacteria and hydrocarbon utilizing bacteria. The result obtained from Table 1 and 2 shows the enumeration of these organisms however, indicates that indigenous microbial communities survived in the existence of hydrocarbon contamination, although the total heterotrophic bacterial counts ranges ($1.39 \pm 0.22 \times 10^6$ cfu/g to $4.84 \pm 0.32 \times 10^6$ CFU/g.) in the soil around generator houses in Rivers State University (RSU) were higher when compared to the hydrocarbon utilizing bacterial ranges ($0.00 \pm 0.00 \times 10^4$ CFU/g to $6.4 \pm 0.15 \times 10^4$ CFU/g). Similar observations were made by Ibiene *et al.*, (2011), Chikere and Ekwuabu (2014) and Eze and Okpokwasili (2010).

The current study showed that the level of hydrocarbon utilizing bacteria were significantly low in the control soil than the polluted soil. This could be attributed to the availability of nutrients, and high organic matters as well as other biological factors that encourage the growth and survival of the microorganisms whose roles are important in degradation and nutrient recycling. Chikere and Ekwuabu (2014) findings reported that continuous input of petroleum based products/pollutants in the soil normally leads to enriched microbial community that can survive toxic contaminants.

However, the enumeration of hydrocarbon utilizing bacteria counts in the control sample was very low ranging from $0.00 \pm 0.00 \times 10^4$ CFU/g to $6.4 \pm 0.15 \times 10^4$ CFU/g compared to the polluted soils around the generator houses. This implies that organisms which are capable of utilizing the hydrocarbons present in the polluted soil samples around the generator house 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) was observed to be normal, suggesting that most of the bacteria present in the soil samples were hydrocarbon utilizers able to survive in the presence of hydrocarbon contamination. A similar observation was reported by Ros *et al.*, (2010).

The outcome of the diversity of the organisms isolated from the various soils around the different generator houses and control soil samples in the present study revealed that *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Enterobacter* sp, *Escharichia coli*, *Staphylococcus* sp., *Corynebacterium* sp., and *Chromobacterium* sp. were in existence in the soil samples. This result was also in line with the study performed by Chikere and Ekwuabu (2014), where *Bacillus* sp. and *Pseudomonas* sp. was isolated from soil contaminated with crude oil in Bodo community in Rivers State (Eze and Okpokwasili, 2010; Ibiene *et al.*, 2011). This result therefore, demonstrates the presence of a diversity of microbes in the contaminated soil that could potentially degrade hydrocarbons. This also indicated that hydrocarbon contamination shifts the dynamics of microbial population towards crude oil degrading microbes. Furthermore, Das and Chandran, (2010) revealed that a mixed population with broad enzymatic abilities are required to degrade complex mixtures of hydrocarbon such as crude oil in the soil and marine sediments.

According to Brito *et al.* (2005), Alonso-Guiterz *et al.* (2009) and Chikere and Ekwuabu (2014), the organisms were characterized using 16srRNA. This technique has been used by other authors in the past and has proven to be more reliable and sensitive than culture dependent techniques and the results obtained from this current study is consistent with past research on hydrocarbon polluted environments (Sarma and Sarma, 2010; Kostka *et al.*, 2011; Kadali *et al.*, 2012). 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 (Okerentugba and Ezeronye, 2008). The finding in this study is also in agreement with the report of Atlas and Cerniglia, (2006) that carried out a research on microorganisms capable of surviving in hydrocarbon polluted environments.

It has been established that the physiochemical parameters of soil is greatly affected when there is oil spill in the environment, however, this is determined by the type of soil, crude oil and extent of pollution (Polyak *et al.*, 2018). The results of the physiochemical parameters of soil in Table 3 showed that moisture content (%), nitrogen-nitrate (mg/kg), phosphorus (mg/kg), sodium-sulphate (mg/kg) potassium (mg/kg) and total organic carbon (TOC %), were at moderate level except in the control soil sample.

The pH of the samples was between near neutral and alkaline. This study has however, revealed that the optimal pH range for biodegradation is between 6.0-7.0 and it is in line with the report of Oboh *et al.* (2016) and Aparna *et al.*, (2010). Result in this study revealed that total petroleum hydrocarbon in the soil was low in the different selected generator houses this concurred with Edori *et al.* (2017) that worked on total petroleum hydrocarbon categories concentration in soils within the vicinity housing heavy-duty diesel generators in three universities and this was also in line with the study by Okpo *et al.* (2012) that observed that total petroleum hydrocarbon concentration decreased with increase in depth.

In conclusion, this study has revealed that the activities from the different generator house caused an increase in the total petroleum hydrocarbon (TPH) in comparison to the control soil. The effects of uncontrolled disposal of the hydrocarbon products will results in high concentration of polycyclic aromatic hydrocarbon which can be hazardous for human in the long run and it projects the demaging effects of the petroleum waste practice around the generator houses in Rivers State University (RSU).

The population of indigenous hydrocarbon utilizing bacteria in the hydrocarbon polluted soil around the generator houses can be monitored, stimulated and enhanced to improve their bioremediation abilities in the oil polluted soils. The pollution of soil around the generators houses triggered the proliferation of most organisms known to be potential hydrocarbon degraders. Three organisms such as *Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Bacillus megaterium* were not recovered from the control soil sample; however, they may be playing a major role in the hydrocarbon degradation.

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