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Assessment of Bacterial Loads in Clogged Drainage Systems in Urban City for the Remediation of Petroleum Polluted Soil

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Abstract: *The use of bacterial loads in clogged drainage systems for the bioremediation of petroleum hydrocarbon contaminated soil was investigated. Soil samples were contaminated with diesel, engine oil and petrol. The contaminated soil samples were inoculated with cultured bacteria isolated from clogged drainage systems and monitored for 56 days. Results obtained indicate that effective biodegradation process occurred in a neutral to slightly alkaline condition (pH 7.0 – 8.0) and temperature range of 27 to 29^{o}C. Pseudomonas, Micrococcus, Acinetobacter, Bacillus cereus and Providencia species actively participated in the bioremediation process. The results on day 56 showed that the biodegradation of diesel, engine oil and petrol by the active bacteria were in the following order: Pseudomonas (92.39, 94.67 and 92.43%) > Micrococcus (88.29, 71.45 and 90.4%) > Acinetobacter (88.11, 31.0 and 87.8%) > Bacillus cereus (86.91, 28.37 and 87.73%) > Providencia (29.64, 28.25 and 87.67%). This biodegradation was found to be statistically highly significant at 95% confidence interval (p<0.05). It is concluded that bacterial loads found in clogged drainage systems have the potential to biodegrade petroleum products, especially if the bacterial loads consist ofthe active bacteria (Pseudomonas, Micrococcus, Acinetobacter, Bacillus cereus and Providencia).*

Keywords: *Bacterial loads, Clogged drainage systems, Bioremediation, Petroleum products, Contaminated soils*

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I. Introduction

Drainage systems are provided to remove domestic wastewater and storm water in order to prevent flooding. Drainage systems have been identified as part of an infrastructural development in cities (Marcelo et al., 2012) and play an important environmental role in urban cities. Majority of drainage systems in urban cities of developing countries are poorly designed, constructed and managed leading to complete failure. When drainage systems fail, the areas become subjected to flooding, resulting in possible environmental degradation, poor sanitation services thereby causing health problems.

Drainage systems become clogged due to the dumping of garbage in them as well as gross negligence and poor management by the Citizenry, Local and State Governments. A blocked drain will normally produce odours, and this can pose reasonable health effects (Blom, 2015; Baker, 2013). Prolong exposure to odorous drains can cause stress, anxiety, headaches, poor moods and fatigue (Kolsky, 1998). Offensive odours from stagnant water in clogged drains can irritate or exacerbate existing health problems in people (Blom, 2015; Baker, 2013). Contacting wastewater around blocked drains can also lead to skin problems. Stagnant water in clogged drains can become a breeding ground for harmful bacteria which in turn can lead to diseases like cholera, gastroenteritis and typhoid (Blom, 2015).

However, the bacteria in clogged drains can find useful application in bioremediation of petroleum hydrocarbon polluted soil. Petroleum hydrocarbon spills are common in our society which results from leakages, storage facilities, broken pipeline, accidental discharge, etc., and have negative impacts on soils and farmlands. Several studies have been carried out on bioremediation (Al-Awadhi et al., 1996; Zhongyun, 1998; Kafilzadeh et al., 2011; Tanzadeh and Ghasemi, 2016). These studies concluded that bioremediation is a cost-effective, simple, innovative and attractive approach for the remediation of petroleum contaminated soils. Thus, the aim of this study is to identify the concentration load of bacteria in clogged drainage systems and to determine their potential for the remediation of petroleum polluted soil.

II. Materials And Methods

2.1. Study area

Obio/Akpor Local Government Area in Rivers State of Nigeria was selected for this study (Fig. 1). It is located between longitudes 6°50'E and 8°00'E and latitudes 4°45'N and 4°60'N. It covers 260 square kilometres and has a population of 626,400 according to the 2015 National Population Commission of Nigeria population

projection (NPC, 2015). Obio/Akpor is influenced by urbanization or conurbation whereby smaller communities have merged together to form a large urban city. This is mainly caused by high influx of people due to boom in oil and allied industries as well as commercial activities resulting in rapid growth of the population. Drainage systems are constructed along major streets in many parts of Obio/Akpor Local Government Area to convey storm water and wastewater from residential buildings to nearby streams or canal. However, these drainages are not maintained and thus are often blocked. Most of the drainage systems in the area are littered with plastic bottles and other non-biodegradable materials. Some residents dump domestic waste into the drainages and street sweepers often swept debris and sand into them leading to their blockage.

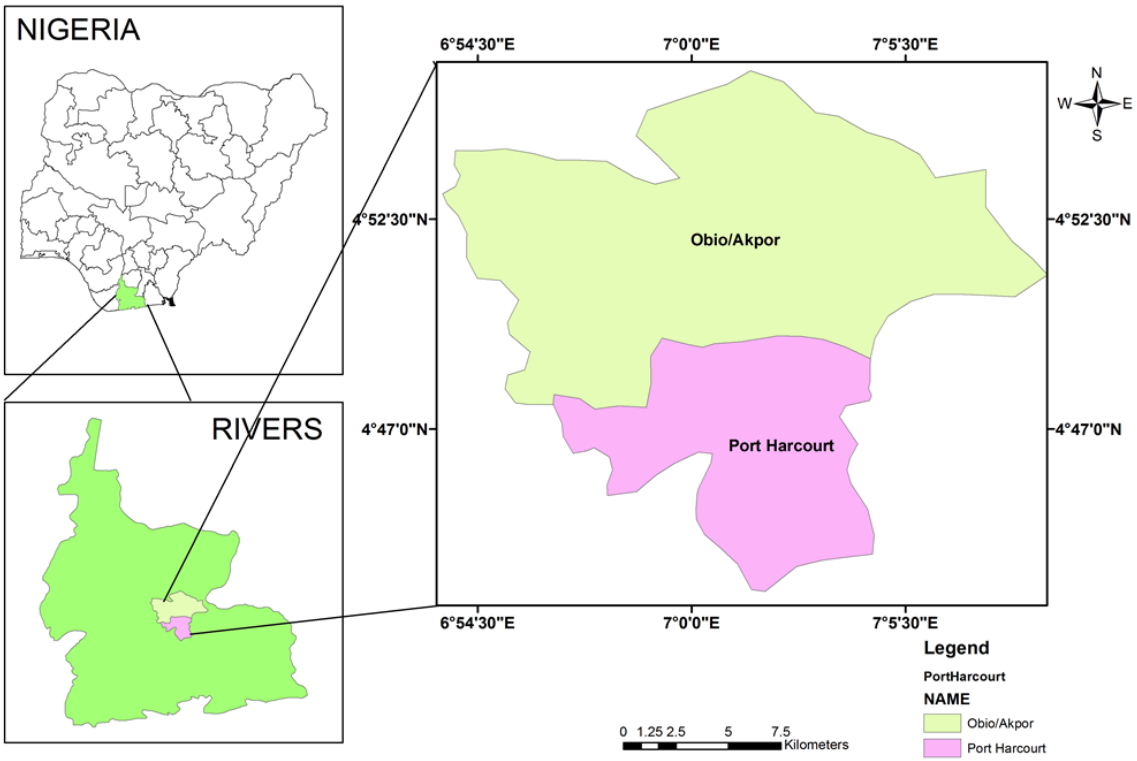

Fig. 1: Study area

2.2. Petroleum contaminants

Three petroleum products were used in this study. They are: (i) Premium Motor Spirit (PMS) popularly called petrol, (ii) Automotive Gas Oil (AGO) popularly called diesel and (iii) Used Lube Oil (ULO) popularly called waste engine oil. Petrol and diesel were bought from Londa fuel station at Bori, while waste engine oil was obtained from a motor mechanic workshop adjacent to the fuel station. The petroleum products were first analysed in the laboratory to determine their total petroleum hydrocarbon (TPH) contents.

2.3. Collection of clogged drainage sediment samples

Grab-samplers were used to collect sediment samples with water from clogged drainage systems at selected locations in the study area. The sampling containers were sterilized to maintain good microbial quality in the samples. Collected samples were labelled and stored in a cooler with ice packs prior to transportation to laboratory for analysis.

2.4. Identification of bacteria in clogged drainage

All collected samples from clogged drainage systems were analysed to identify bacterial types as discussed below.

2.4.1. Bacterial Culture

The spread plate method, using glass containers, was adopted (Sanders, 2012). All the glass containers were sterilized by putting them into a hot air oven at a temperature of 160^{o}C for 1hour. Three agars (Mueller Hinton ager, MacConkey agar and plate count agar) were used. The plates were inoculated at the temperature of 37^{o}C for 24 hours.

2.4.2. Isolation of Bacterial Colonies

The bacteria were first sub-cultured to have a pure culture after enumeration by counting the numbers of each bacterium. Thereafter, biochemical tests were carried out on each of the bacteria to identify them. The bacterial species were counted using a method of Total Viable Plate Count (TVPC) (Kafilzadeh et al., 2011). The bacteria were cultured and incubated for a period of 10 days to allow for growth and multiplication.

2.5. Collection of soil samples

Loamy soil commonly found in most farmland in the study area was used for the experiment. Samples of loamy soil were collected from a depth of 0 to 60cm using a standard auger. Collected soil samples were stored in a cooler with ice packs and taken to laboratory. The soil samples were analysed for indigenous bacteria, hydrocarbon content, pH and temperature to establish baseline condition before use in the experiment. Soil samples were heated at 1200^{o}C to destroy the indigenous bacteria before use in the experiment.

2.6. Experimental setup for bioremediation of hydrocarbon contaminated soil

A 10g soil sample was mixed with 1ml of each contaminant (diesel, engine oil and petrol) to provide known quantity of the soil sample and volume of the contaminants. The contaminated soil samples were thoroughly mixed to ensure that the contaminants were evenly distributed in the soil samples. The experiment was set up in glass beaker. For each contaminated soil sample, 11 experiments were setup, one for each bacterium, given a total of 33 experimental setups. Three (3) controls were also setup, one for each contaminant, given an overall total of 36 setups for the study. Isolated bacteria (9ml) from the clogged drainage systems were inoculated into the 33 experimental setups while no inoculation was done to the 3 controls. 0.5g of nitrogen and 0.5g of phosphorus were mixed in 100ml of distil water and 1ml was transferred into each sample to provide nutrients for the bacteria. The 36 setups were monitored for a period of eight (8) weeks and samples were taken from each on a weekly basis and analysed for total hydrocarbon petroleum (TPH) contents. Variations in pH and temperature of the setups were also monitored weekly.

2.7. Data analysis

Analysis of variance (ANOVA) was performed to determine the significant level of biodegradation by each of the bacterium isolates.

III. Results And Discussion

3.1. Isolation of colonies

The result of the biochemical test to identify bacterial species in clogged drainage system from Obio/Akpor Local Government Area is shown in Table 1. A total of eleven (11) bacterial species were identified. Ogbonna and NiaBari (2017) obtained similar results in a study carried out on the characteristics of bacteria from clogged drainage system within Rivers State University premises which is a part of Obio/Akpor Local Government Area. The result is also in agreement with the findings of Sonali (2011). All the identified bacteria have rod shape except Micrococcus and Staphylococcus which have spherical (cocci) shape. Gram-positive bacteria are spherical cells ranging from about 0.5 to 3 micrometres in diameter and typically appear in tetrads (Kocur et al., 2006; Ogbonna and NiaBari, 2017). They are catalase positive and oxidase positive. Gram-negative bacteria are facultative anaerobic, rod-shaped, non-spore-forming bacteria of the family of Enterobacteriaceae.

Table 1: Identified bacteria isolates from clogged drainage system

Bacteria type	Indole test	Catalase test	Citrate test	Methyl red test	Oxidase test
Salmonella	-	-	-	+	-
Micrococcus	-	-	+	-	-
Staphylococcus	-	+	-	+	-
Vibrio cholerae	-	-	+	-	+
Shigella	+	+	-	+	-
Bacillus cereus	-	+	+	+	+
Pseudomonas	-	-	-	-	+
Acinetobacter	-	-	+	-	-
Escherichia	+	-	-	-	-
Proteus	-	-	+	-	-
Providencia	+	-	+	-	-

+ = Gram-positive; - = Gram-negative

3.2. Bacterial culture

The growth phase of the bacteria during culture and incubation period is shown in Fig. 2. The bacteria went through four (4) main growth phases, namely lag phase, log or exponential growth phase, stationary phase and decline or death phase. Similar observation was reported by Davis and Cornwell (2004). There was a rapid increase in the cell biomass of the bacteria within first two weeks of culturing and then a decline as some bacteria stopped growing and began to die (Davis and Cornwell, 2004). The observed decline or death phase at week eight (8) inspired the choice of eight weeks as the experimental period.

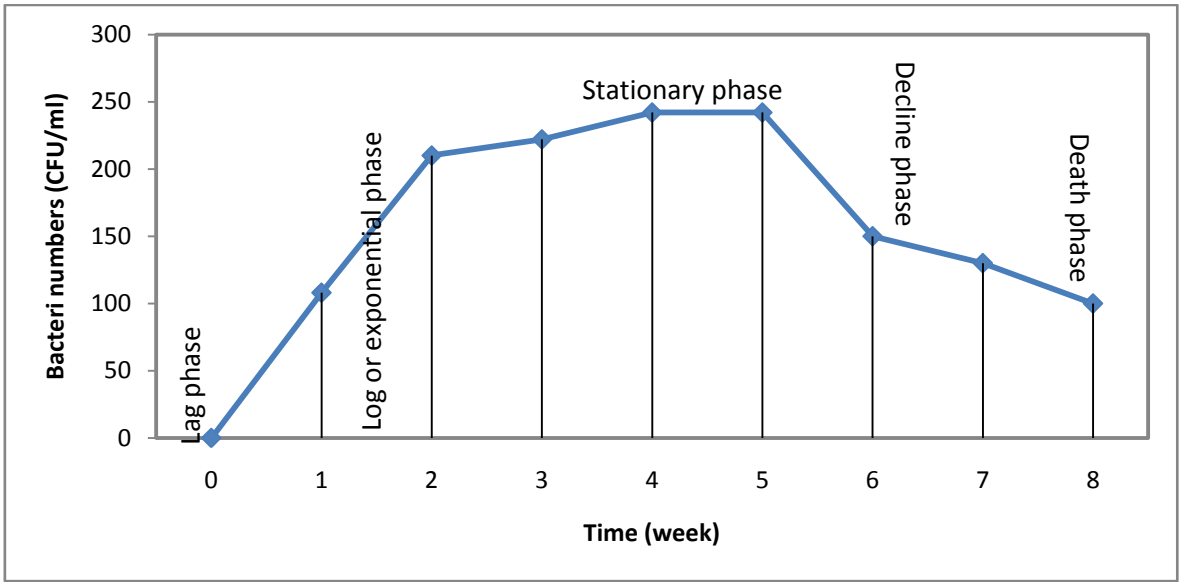

Fig. 2: Bacterial growth process during culture

3.3. Biodegradation of petroleum products

The results of the biodegradation of petroleum products by the 11 bacteria isolates from clogged drainage systems during the eight weeks (56 days) period are presented in Fig. 3, 4 and 5, while the percentage of biodegradation of the petroleum products by each bacterium isolate is shown in Fig. 6. The control samples with no bacteria addition had negligible petroleum hydrocarbon removal. Among the 11 bacteria used, five (5) bacteria species (pseudomonas, micrococcus, Acinetobacter, Bacillus cereus and Providencia) actively participated in the bioremediation process. These bacteria utilized part of the petroleum hydrocarbons in the soil as carbon and energy sources (Zhongyun, 1998; Balba et al., 1998; Kensa et al., 2011; Yudono et al., 2011; Darsa et al., 2014). TPH concentration in the contaminated soils decreased with time during the bioremediation process but became relatively fast after day 14.

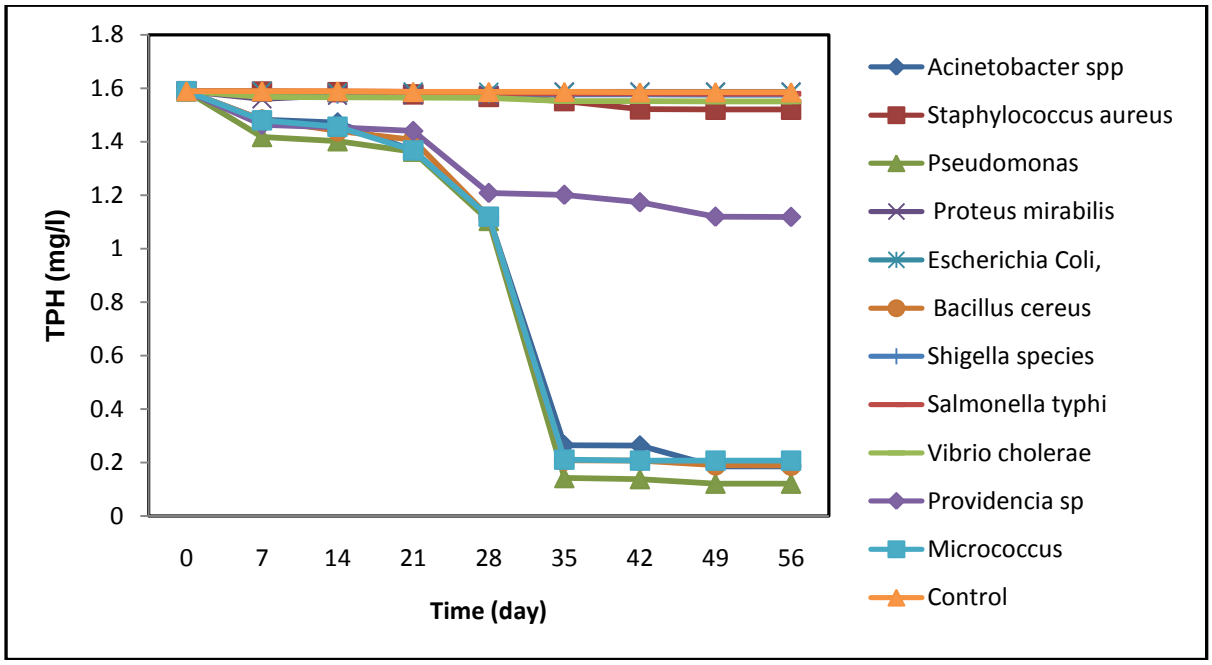

Fig. 3: Biodegradation of diesel by clogged drainage bacteria isolates

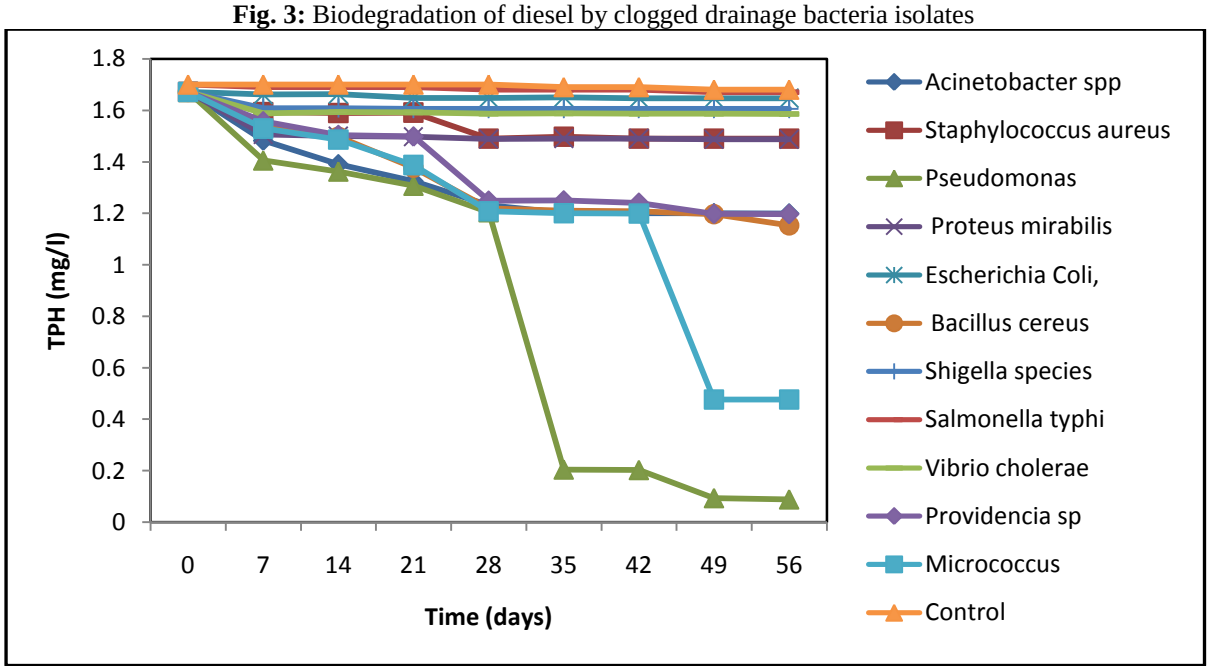

Fig. 4: Biodegradation of engine oil by clogged drainage bacteria isolates

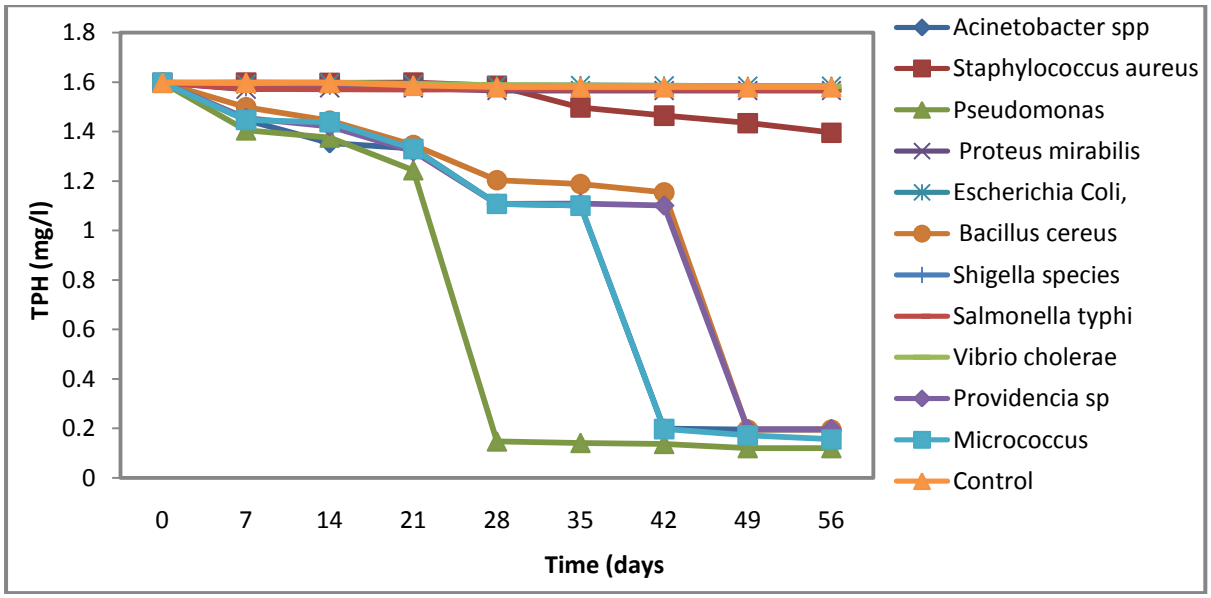

Fig. 5: Biodegradation of petrol by clogged drainage bacteria isolates

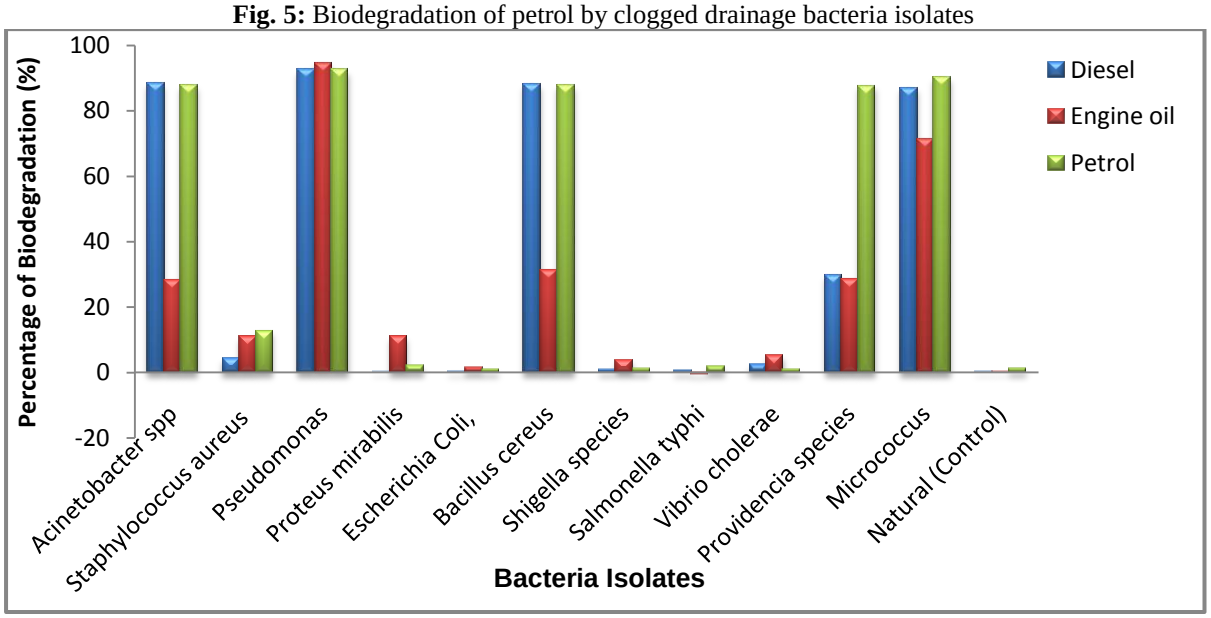

Fig. 6: Percentage of biodegradation of the petroleum products by each bacterium

The initial TPH concentration of the diesel contaminated soil was 1.589mg/l. At the end of the bioremediation period (day 56), the concentration of diesel in the soil was reduced to 0.121mg/l by pseudomonas, 0.186mg/l by Acinetobacter, 0.189mg/l by Bacillus cereus, 0.208mg/l by micrococcus, and 1.118mg/l by Providentia, while TPH concentration in the control sample was insignificantly reduced to 1.585mg/l. The percentage of biodegradation of TPH in soil contaminated with diesel by each of the five active bacteria ranged from 92.39% (Pseudomonas) to 29.64% (Providencia) at the end of the bioremediation period. This result compared favourably well with results obtained by Yudono et al. (2011) and Darsa et al. (2014).

The initial TPH concentration in the engine oil contaminated soil was 1.671mg/l. At the end of the bioremediation period, the TPH concentration in the engine oil contaminated soil was reduced to 0.089mg/l by Pseudomonas, 0.4776mg/l by Micrococcus, 1.153mg/l by Bacillus cereus, 0.197mg/l by Providentia and 1.199mg/l by Acinetobacter, while the TPH concentration in the control sample was inconsequentially reduced to 1.667mg/l. The percentage of biodegradation of TPH in soil contaminated with engine oil by each of the five active bacteriaranged from 94.67% (Pseudomonas) to 28.25% (Acinetobacter) at the end of the bioremediation period. Surajudeen (2012) achieved 75% removal of spent motor oil within 70 days of treatment with bacteria.

The initial TPH concentration in the petrol contaminated soil was 1.598mg/l. At the end of the bioremediation period, the TPH concentration in the petrol contaminated soil was reduced to 0.121mg/l by Pseudomonas, 0.156mg/l by Micrococcus, 0.195mg/l by Acinetobacter, 0.196mg/l by Bacillus cereus, and 0.197mg/l by Providentia, while the TPH concentration in the control sample was trivially reduced to 1.58mg/l. The

percentage of biodegradation of TPH in the soil contaminated with petrol by each of the five active bacteria ranged from 92.43% (Pseudomonas) to 87.67% (Providencia) at the end of the bioremediation period.

3.4. Statistical analysis of biodegradation of petroleum products

The results of test statistics indicating the levels of significance of bioremediation of petroleum products contaminated soil are presented in Tables 2 - 4. The results reveal that the rate of degradation by the five active bacteria isolates is statistically highly significant at 95% confidence interval ($p<0.05$).

Table 2: Percentage biodegradation of TPH in soil contaminated with diesel

Bacteria type	% biodegradation	P-value	Level of significance
Pseudomonas	92.39	0.003	Significant
Acinetobacter	88.29	0.004	Significant
Bacillus cereus	88.11	0.004	Significant
Micrococcus	86.91	0.004	Significant
Providencia	29.64	0.002	Significant

Table 3: Percentage biodegradation of TPH in soil contaminated with engine oil

Bacterial type	% biodegradation	P-value	Level of significance
Pseudomonas	94.67	0.002	Significant
Micrococcus	71.45	0.007	Significant
Bacillus cereus	31.0	$9.3x10^{-5}$	Significant
Providencia	28.37	0.0001	Significant
Acinetobacter	28.25	$1.3x10^{-6}$	Significant

Table 4: Percentage biodegradation of TPH in soil contaminated with petrol

Bacteria type	% biodegradation	P-value	Level of significance
Pseudomonas	92.43	0.0005	Significant
Micrococcus	90.24	0.004	Significant
Acinetobacter	87.8	0.003	Significant
Bacillus cereus	87.73	0.009	Significant
Providencia	87.67	0.005	Significant

3.5. Changes in pH and temperature during the bioremediation process

The variation of pH and temperature during the 8 weeks bioremediation process is shown in Fig. 7 while the variation of TPH degradation with pH and temperature are presented in Fig. 8 and 9, respectively.

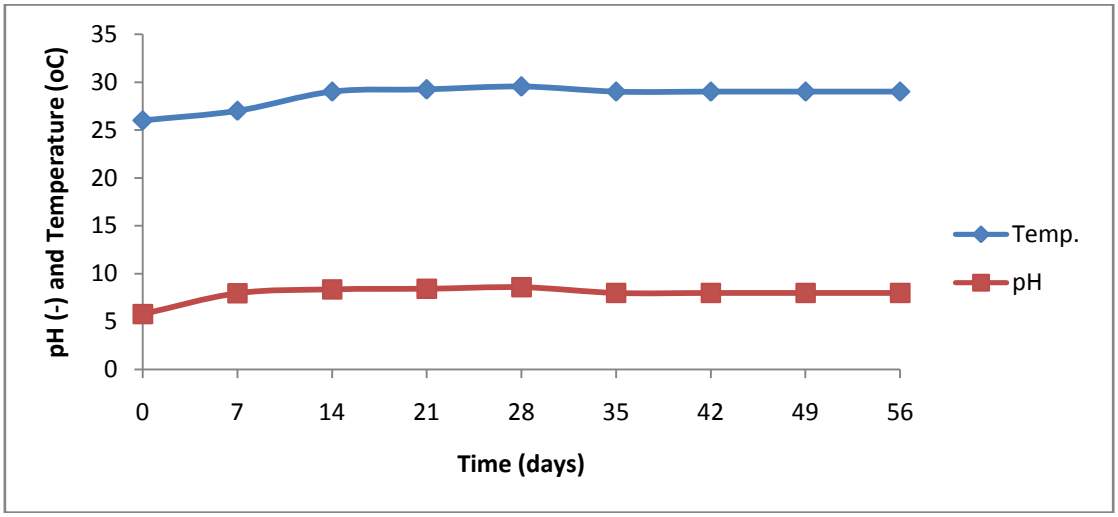

Fig. 7: pH and temperature variations during experimental period

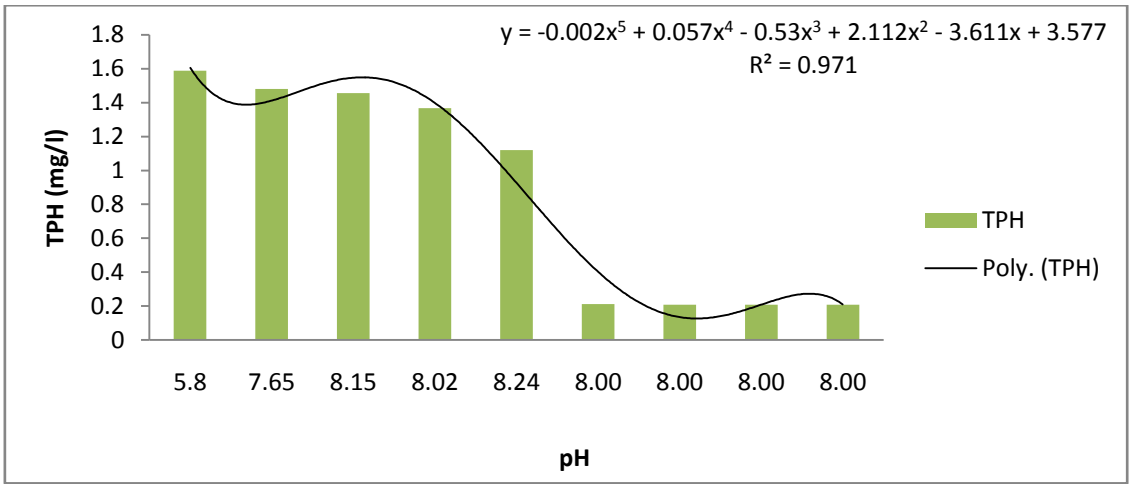

Fig. 8: TPH variation with pH during biodegradation

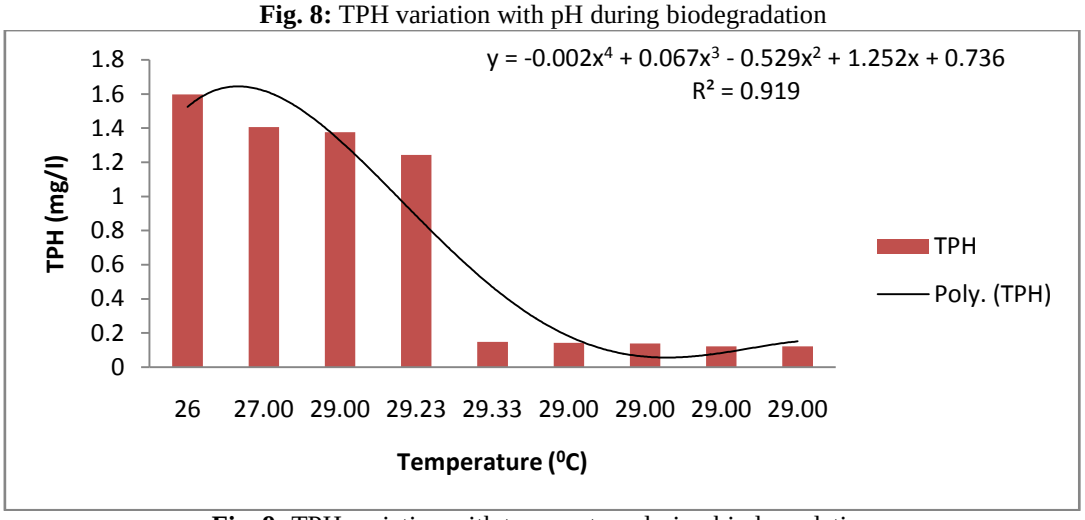

Fig. 9: TPH variation with temperature during biodegradation

In natural condition, the loamy soil had an initial pH value of 5.8 which is acidic. During the experiment, after nutrients and bacterial species were injected, the pH increased from 5.8 on day 0 and peaked at 8.59 on day 28 and then decreased to stabilize at 8.0 on day 35. Experimental results indicated that effective biodegradation process occurred in a neutral to slightly alkaline condition (pH 7.0 – 8.0). It was observed that maximum TPH removal occurred at pH 8.0. A polynomial regression model of the fifth order provided the best description of the TPH / pH relationship (R^2 = 0.971). Previous studies have shown that bacterial degradation of petroleum hydrocarbons is best in pH range of 6 – 8 (Mentzer and Ebere, 1996; Zhongyun, 1998; Darsa et al., 2014). Similarly, the initial temperature of the loamy soil was 26^{o}C. During the experiment, the temperature increased from 26^{o}Con day 0 and peaked at 29.53^{o}C on day 28 and then decreased to stabilize at 29^{o}C on day 35. Experimental results indicated that effective biodegradation process occurred within the temperature range of 27^{o}C and 29^{o}C. It was observed that maximum petroleum hydrocarbon degradation occurred at a temperature of 29^{o}C. A polynomial regression model of the fourth order provided the best description of the TPH / Temperature relationship (R^2 = 0.919). These results indicate that pH and temperature are important factors in the bioremediation of petroleum products contaminated soil.

IV. Conclusion

The potential for bioremediation of petroleum contaminated soil using bacteria from clogged drainage systems was evaluated. Based on the results obtained the following conclusions can be drawn:

a. Degradation of total petroleum hydrocarbon using bacteria from clogged drainage is possible.
b. Five bacterial species (Pseudomonas, Micrococcus, Acinetobacter, Bacillus cereus and Providencia) out of the eleven isolated actively participated in the degradation.
c. The biodegradation rate was significantly greater with Pseudomonas compared to other bacterial species.
d. Petroleum hydrocarbon removal efficiency can reach 90% over a period of 56 days within the experimental conditions investigated in this study.
e. Optimum rate of degradation of petroleum hydrocarbon was achieved at a temperature of 29°C.

f. Optimum rate of degradation of TPH was obtained at a neutral pH of 8.0.

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