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Spent Automobile Oil Degradation Potential of Bacteria Isolates within Makurdi Town Journal of Environmental Science April 2023

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Abstract:- The discharge of spent automobile oil from automobile, industrial machines, plants and electric power generators are great concern in automobile workshop as they littered and contaminate soil. Spent automobile oil degradation potentials of bacteria isolates from soil in mechanic workshops within Makurdi was carried out. Eighty (80) samples comprising of 40 top soil and 40 sub soil were collected from the four mechanic workhouses in Makurdi; (Apir, Wurukum, North bank and New Garage). The samples were pour plated on mineral salt agar from where total viable counts was done and discrete colonies sub-cultured to obtain pure isolates. Fresh automobile spent oil was inoculated with bacteria isolates and incubated for five days to determine their biodegradation. Optical density of the spent automobile oil was determined in Aucm^{-1} at 600nm for every 24 hours. Bacteria specie found to possess biodegradation potentials were subjected to molecular analysis for full identification. Result shows mean total viable counts to be 61.8±7.76x10^{3}, 41.7± 5.23x10^{3}, 57.2±6.36x10^{3} and 96.0±6.11x10^{3} Cfu/g for North bank, for North bank, Apir, New Garage and Wurukum Mechanic workhouses respectively. There is significant difference (P=0.000) in the total viable count across locations. Staphylococcus, Serratia, Eschrichia coil and pseudomonas species were found to possess bioremediation potential. Further test to evaluate quantitatively the biodegradations shows that pseudomonas specie degraded spent oil from an optical density of 1.762±0.001 to 0.834±0.000 Aucm^{-1}, Staphylococcus specie had an optical density from 1.694±0.000 to 0.992±0.002 Aucm^{-1} and Escherichia Coli had an optical density of dropped from 1.785±0.000 to 0.854±0.000 indicating a significant drop in optical due to biodegradation of the spent oil as these bacteria to be pseudomonas putida strain Mpstv 12.2, Escherichia coli strain Kenece 3 and staphylococcus equorum strain planc 5. Phylogenetic analysis shows relationship between pseudomonas putida and Escherich ia Coli. This study showed that the bacteria strains used in this study could be relevant in the bioremediation of ecosystem that may be contaminated with hydrocarbons. Keyword: Biodegradation, Bioremediatio n, Biostimulation.

I. INTRODUCTION

Environmental pollution with spent automobile oil and other petroleum products has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale (Althalb and Singleton., 2017). The presence of different substrates and metabolites in hydrocarbon contaminated soils has no doubt provided an environment for the development of a quite complicated microbial community (Ichor *et al.*, 2014).

The discharge of spent oil from automobiles, industrial machines, plants and electric power generators are the main sources of oil pollution in Makurdi Benue state, North central Nigeria (Aloysius, 2013). According to (Umanu *et al*. 2013), oil released into the environment affects many plants, animals, microorganisms and humans within the oil impacted environment. Additionally, prolonged exposure to oil and high concentration of oil could cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Abioye *et al*., 2012).

Spent auto oil is a brown-to-black oily liquid removed from a motor vehicle, when the oil is changed (Nwinyi, 2014). Spent motor oil is similar to unused oil, except that it contains additional chemicals that are produced or build up in the oil, when it is used as the engine lubricant at high temperatures and pressures, inside the engine as it runs (Eniola *et al*., 2014). In addition, these added chemical impurities contribute significantly to chronic hazards because of their solubility in soil surface and groundwater (Abdulsalam *et al*., 2012).

Some of these heavy metals in used motor oil can dissolve in water and move through the soil easily; absorbed and distributed into various tissues of human, plants and animals by their movement in the environment, which can result in serious health problem, such as anemia, tremor and consequently, resulting in death (Obi *et al.*, 2022).

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In most countries of the world, oil spills at auto-mechanic workshops have been left uncared for over the years, and its continuous accumulation is of serious environmental concern because of the hazard associated with it (Omoni *et al.*, 2015). The physicochemical treatment technologies currently in use are expensive and not environmentally friendly. In addition, some of these technologies only transfer the contamination from one place to another (Joshi *et al*., 2011).

In recent times, a lot of efforts have been made towards reducing environmental pollution, by using natural processes to treat environmental pollution. These techniques include: bioremediation "use of microorganisms to degrade pollutants" and phytoremediation "use of plants to clean pollutants by bioaccumulation into the plant's tissues" (Khashayar and Mahsa, 2010). Bioremediation for the soil requires the identification of microbes that are present in the soil itself so that in case of a large spill these can be stimulated further in order to clean-up the area. Identification of such strains can ensure better efficiency of remediation as these strains will be well adapted to grow in the soil system (Opasols and Adewaye, 2010).

Bioremediation, using biological processes to ameliorate hydrocarbons including spent automobile oil from the environment, is already a successful technology for cleaning up soil and marine sediments (Balogun *et al.*, 2015). When the oil spill by British Petroleum (BP) in the Gulf of Mexico occurred in April 2010, stakeholders in fishing communities, environmentalist, and tourism agencies doted along Miami Beach and elsewhere got tensed up because the problem had gotten out of hand. It was expected that the spill would reach the shorelines and dwindle the fortunes of the communities doted along the coastline but that never occurred. How was the problem solved, and who were the unsung heroes? Some microbes (bacteria) appeared and consumed the contaminant which had spread over a long distance in nautical miles for several weeks (Adene, 2013). This research was primarily carried to investigate spent automobile oil degradation potential of bacteria isolate from spent automobile oil contaminated soil in Makurdi, Benue State Capital, Nigeria.

II. MATERIALS AND METHODS

A. Sample Collection

Spent automobile oil (100 mL) was collected from each mechanic workhouse. Spent automobile oil contaminated soil were also collected from the four (4) mechanic workhouses. Again, Soil, not contaminated with spent automobile oil were collected away from each mechanic workhouse (100 meters respectively) and homogenously mixed to serving as control sample. The collected soil samples were designated as: A (Mechanic Village, North-Bank), B (Mechanic Village, Apir), C (Mechanic Village, New Garage), D (Benue State University Technical Workshop, Wurukum) and E (Control Soil Sample).

For each sample station, soil samples were randomly collected, using a sterile spatula at a tillage depth of 1-2 cm, from different points. All soil samples were collected into clean polythene bags and transported to Biological Sciences Postgraduate Research Laboratory, Joseph Sarwuan Tarka University, Makurdi for analysis. All collected samples were temporally stored in an icebox at 4 °C as described by Zulfa *et al.* (2016).

B. Isolation and Identification of Spent Automobile Oil Degrading Bacteria

Total spent oil degrading bacterial counts (TSDBC) were carried out as described by Balugun *et al*., (2015). Ten–fold serial dilution in the range of 10^{-6} was carried out on 1 g of each soil sample using sterile distilled water. Aliquot (0.1 mL) of the diluents was dispensed into sterile Petri dish aseptically. It was then plated out on plate count agar, using pour plate method. The plates were incubated at 37 ^{o}C for 24 hours, after which the colonies were counted. This was carried out in replicates. Discrete colonies were sub-culture to obtain pure cultures. The pure cultures were subjected to the following biochemical test; catalase, citrate, urease, indole, methyl red, voges-pasteure and sugar fermentation, Bergey's *et al;* (2009) Bacteria types (groups) were serially arranged (1, 2, 3, ……).

C. Degradation Potential of the Identified Soil Bacteria on the Spent Automobile Oil

A weighed filter paper was soaked in spent automobile oil and reweighed. This was then placed on a culture plate of test organisms and incubating at 37 ^{o}C for one week. Oil-weight loss as a result of bacterial degradation of the spent automobile oil was assessed by gravimetric method after extraction with n-hexane; dichloromethane, as reported by Umanu *et al.* (2013).

D. DNA Extraction

DNA was extracted using ZR Fungal/Bacterial DNA MINIPREP, Manufactured by Zymo Research, [name of the country], following the manufacturers' instructions. About 2 mL of bacterial cells were added to a ZR BashingTM lysis tube, then 750 µL lysis solution. To this was secured a bead fitted to the tube holder assembly and process at maximum speed for > 5 min utes. The ZR lysis tube was micro-centrifuged at > 10,000 x g for 1 minute and 400 µL supernatant was transferred to a Zymo-SpinTM IV spin filter (orange top) in a collection tube and centrifuged at 7,000 x g for 1 minute. Then 1,200 µL of bacterial DNA binding buffer was added to the filtrate in the collection tube, 800 µL was transferred to the mixture, then to a Zymo-SpinTM IIC column in a collection tube and centrifuge at 10,000 x g for 1 minute. The flow through the collection tube was discarded. This process was repeated. Then 200 µL DNA Pre-Wash buffer was added to the Zymo-SpinTM IIC column in new collection tube and centrifuged at 10,000 x g for 1 minute, 500 µL bacterial DNA wash buffer was added to the Zymo-SpinTM IIC column and centrifuged at 10,000 x g for 1 minute. This was tranfered from Zymo-SpinTM IIC column to a clean 1.5 mL micro centrifuge tube. To this was added 100 µL NA Elution Buffer directly to the column matrix. Then centrifuged at 10,000 x g for 30 seconds to elute the DNA.

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E. Electrophoresis for DNA and PCR

To 1 g of agarose (for DNA); 2g of agarose for PCR was mixed agarose powder with 100 mL 1xTAE in a microwavable flask and microwave for 1-3 min until the agarose completely dissolved (The solution was not over boiled so that the buffer will not evaporate and alter the final percentage of agarose in the gel). The agarose solution was allowed to cool down to about 50 °C (a convenient temperature to keep one's hand on the flask), for about 5 minutes. To this was added 10 µL EZ vision DNA stain. EZ vision that binds to the DNA was allowed to visualize the DNA under ultraviolet (UV) light. The agarose was then pour into a gel tray with the well comb in place. Then newly poured gel was placed at 4 °C for 1-15 minutes (or at room temperature for 20-30 minutes), until it completely solidified.

F. Loading samples and running an agarose gel

To load buffer of each DNA sample or PCR product once solidified, the agarose gel was placed into the gel box (electrophoresis unit) and filled with 1xTAE (or TBE) to the brim carefully until the gel is covered. Carefully, a molecular weight ladder was loaded into the first lane of the gel, followed by samples into the additional wells of the gel. The gel was run at 80-150 V for about 1-1.5 hours. Afterwards, the power was turned off and the electrodes from the power source were disconnected and then carefully, the gel was removed from the gel box. Then DNA fragments or PCR products were visualized under UV transilluminator.

G. PCR mix components

The PCR mix was made of 12.5 µL of Taq 2 X master mix from New England Biolabs (M0270); 1 µL each of 10 µM forward and reverse primer; 2 µL of DNA template and then made up with 8.5 µL nuclease free water.

H. Primer sequences

27F: AGAGTTTGATCMTGGCTCAG
1525R: AAGGAGGTGWTCCARCCGCA

I. Cycling conditions

Initial denaturation at 94 ˚C for 5 minutes was followed by 36 cycles of denaturation at 94 ˚C for 30 seconds, annealing at 56 ˚C for 30 seconds and elongation at 72 ˚C for 45 seconds, followed by a final elongation step at 72 ˚C for 7 minutes and hold temperature at 10 ˚C forever.

J. Sequencing

The amplified fragments were sequenced using a genetic analyzer 3130 x l sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis.

K. Statistical analysis

Statistics used include: t-test, ANOVA, chi square and Pearson correlation analysis.

III. RESULTS

Table 1 presents the viable count of bacteria at North Bank Mechanic Site. Total viable count in top soil ranged from 32 x 10^3 to 104 x 10^3 cfu/g while the average bacteria count was 61.8±7.76. The control top soil was significantly higher than the North Bank Mechanic top soil (T==6.52, P<0.05) as the mean control value was 135.8±8.28. The sub soil at the mechanic site contained more bacterial viable count than the top soil with values ranging from 124 x 10^3 to 224 x 10^3 cfu/g. The average viable count in the sub soil was 172±11.4 while that of the control site was significantly lower (T==8.70, P<0.05 recording mean value of 59.9±5.93).

The various types of soil bacteria found at North Bank Mechanic are shown in Table 2. Out of Five bacteria isolated from the site, both the top and sub soil contained 40 % of bacteria species with Micrococcus spp. only identified at the top and *Citrobacter* spp only identified at the sub soil.

Table 3 gives the viable count of bacteria at Apir Mechanic Site. The average viable count in top soil was 41.7±5.23 ranging between 27 x 10^3 and 76 x 10^3 cfu/g whereas the control top soil had higher bacterial load with an average value of 135.8±8.28. The difference recorded in viable count in top soil of the Apir Mechanic Site and that of the control site was significant (T==9.61, P<0.05). Bacterial load was higher in sub soil than in the top soil. In the former, the average viable count was 178.4±12.2 with values ranging from 144 x 10^3 to 272 x 10^3 cfu/g. The control sub soil contained lower bacterial load than the sub soil of the mechanic site. In the control sub soil, mean viable count was estimated as 59.9±5.93. Thus, a significant difference was established (T==8.74, P<0.05).

The various types of soil bacteria found at Apir Mechanic are shown in Table 4. The top soil contained four bacteria types (33.33 %) while the sub soil had five bacteria types (41.67 %).

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Table 1: Viable Bacterial Count (10^3 cfu/g) at North Bank Mechanic Site

S/No.	Top Soil (Workshop)	Top Soil(Control)	Sub Soil(Workshop)	Sub Soil(Control)
1	84	156	128	42
2	46	128	204	76
3	32	172	176	88
4	58	116	124	64
5	48	152	216	84
6	36	164	136	68
7	104	108	224	36
8	76	124	152	48
9	88	92	184	41
10	46	146	176	52
Total	618	1358	1720	559
Mean±SE	61.8±7.76	135.8±8.28	172±11.4	59.9±5.93

T (top soil and control) =6.52, p=0.000
T (sub soil and control) =8.70, p=0.000

Table 2: Soil Bacterial Types at North Bank Mechanic Site

S/No.	Bacteria	Top Soil	Sub Soil
1	*Staphylococcus* spp.	✓	✓
2	*Micrococcus* spp.	✓	×
3	*Citrobacter* spp.	×	✓
4	*Bacillus* spp.	✓	✓
5	*Klebsiella* spp.	✓	✓
% Presence		40 %	40 %

Table 3: Viable Bacterial Count (10^3 cfu/g) at Apir Mechanic Site

S/No.	Top Soil (Workshop)	Top Soil(Control)	Sub Soil(Workshop)	SubSoil (Control)
1	34	156	156	42
2	66	128	272	76
3	28	172	152	88
4	76	116	196	64
5	32	152	144	84
6	41	164	183	68
7	38	108	157	36
8	44	124	168	48
9	27	92	152	41
10	31	146	204	52
Total	417	1358	1784	559
Mean±SE	41.7±5.23	135.8±8.28	178.4±12.2	59.9±5.93

T (top soil and control) =9.61, p=0.000
T (sub soil and control) =8.74, p=0.000

Table 4: Soil Bacterial Types at Apir Mechanic Site

S/No.	Bacteria	Top Soil	Sub Soil
1	*Staphylococcus* spp.	✓	✓
2	*Micrococcus* spp.	×	✓
3	*Pseudomonas* spp.	✓	✓
4	*Bacillus* spp.	✓	✓
5	*Klebsiella* spp.	✓	×
6	*Serratia* spp.	×	✓
% Presence		33.33 %	41.67 %

Table 5 gives the viable count of bacteria at New Garage Mechanic site. The average viable count in top soil was 57.2±6.36 and ranged between 32 x 10^3 and 96 x 10^3 cfu/g whereas the control top soil had higher bacterial load with an average value of 135.8±8.28. The difference recorded in viable count in top soil of the New Garage Mechanic site and that of the control site was significant (T=7.53, P<0.05). Bacterial load was higher in sub soil than in the top soil. In the former, the average viable count was 185.6±8.67 with values ranging from 152 x 10^3 to 236 x 10^3 cfu/g. The control sub soil contained lower bacterial load (59.9±5.93) than the sub soil of the mechanic site. Thus, a significant difference was established (T=11.97, P<0.05).

The various types of soil bacteria found at New Garage Mechanic are shown in Table 6. The top soil contained four bacteria types (25 %) while the sub soil had five bacteria types (31.25 %).

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Table 7 presents the viable count of bacteria at Wurukum Mechanic Site. Total viable count in top soil ranged from 64 x 10^3 to 124 x 10^3 cfu/g while the average bacteria count was 96.0±6.11. The control top soil was significantly higher than the Wurukum Mechanic top soil (T=3.87, P<0.05) as the mean control value was 135.8±8.28. The sub soil at the mechanic site contained more bacterial viable count than the top soil with values ranging from 196 x 10^3 to 284 x 10^3 cfu/g. The average viable count in the sub soil was 238.8±9.41while that of the control site (59.9±5.93) was significantly lower (T=16.08, P<0.05).

The various types of soil bacteria found at Wurukum Mechanic are shown in Table 8. The top soil contained four bacteria types (33.33 %) while the sub soil had five bacteria types (41.67 %).

Table 5: Viable Bacterial Count (10^3 cfu/g) at New Garage Mechanic Site

S/No.	Top Soil (Workshop)	Top Soil(Control)	Sub Soil(Workshop)	SubSoil (Control)
1	52	156	216	42
2	76	128	192	76
3	38	172	168	88
4	64	116	156	64
5	32	152	152	84
6	36	164	164	68
7	72	108	204	36
8	54	124	192	48
9	52	92	176	41
10	96	146	236	52
Total	572	1358	1856	559
Mean±SE	57.2±6.36	135.8±8.28	185.6±8.67	59.9±5.93

T (top soil and control) =7.53, p=0.000
T (sub soil and control) =11.97, p=0.000

Table 6: Soil Bacterial Types at New Garage Mechanic Site

S/No.	Bacteria	Top Soil	Sub Soil
1	*Staphylococcus* spp.	×	✓
2	*Pseudomonas* spp.	✓	×
3	*Citrobacter* spp.	×	✓
4	*Enterobacter* spp.	✓	×
5	*Escherichia coli*	✓	×
6	*Bacillus* spp.	✓	✓
7	*Klebsiella* spp.	×	✓
8	*Serratia* spp.	×	✓
% Presence		25 %	31.25 %

Table 7: Viable Bacterial Count (10^3 cfu/g) at Wurukum Mechanic Site

S/No.	**Top Soil (Workshop)**	**Top Soil(Control)**	**Sub Soil(Workshop)**	**SubSoil (Control)**
1	112	156	236	42
2	76	128	216	76
3	92	172	252	88
4	104	116	268	64
5	76	152	204	84
6	108	164	284	68
7	112	108	252	36
8	124	124	264	48
9	92	92	196	41
10	64	146	216	52
Total	960	1358	2388	559
Mean±SE	96.0±6.11	135.8±8.28	238.8±9.41	59.9±5.93

T (top soil and control) =3.87, p=0.001
T (sub soil and control) =16.08, p=0.000

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Table 8: Soil Bacteria Types at Wurukum Mechanic Site

S/No.	Bacteria	Top Soil	Sub Soil
1	***Micrococcus* spp.**	✓	✓
2	*Pseudomonas* spp.	×	✓
3	*Citrobacter* spp.	×	✓
4	*Enterobacter* spp.	✓	×
5	*Bacillus* spp.	✓	✓
6	*Klebsiella* spp.	✓	✓
% Presence		33.33 %	41.67 %

Top soil bacterial viable counts among sample locations is presented on Figure 1. The Control Soil had the highest viable count (1358 x 10^3 cfu /g) and Apir Mechanic Site recorded the lowest (417 x 10^3 cfu/ g) count. Bacterial load in top soil was significantly associated with sample locations (χ^2 =723.103, p<0.05).

Sub soil bacterial viable counts among sample locations is presented on Figure 2. The Control Soil had the lowest viable count (559 x 10^3 cfu/g) and Wurukum Mechanic recorded the highest (2388 x 10^3 cfu/g) count. Bacterial load in sub soil was significantly associated with sample locations (χ^2 =1083.16, p<0.05).

Variation in absorbance of bacterial treated spent automobile oil at 600 nm based on time and sample location is presented on Table 9. Spent oil degradation in five days (Time) revealed the highest absorbance on day 3 at site E (Control Site; 1.808±0.001) and the lowest absorbance on day 5 at site A (North Bank Mechanic Village). Generally, bacterial treated spent automobile oil absorption at 600 nm decreased from day one to five and increased from site A (North Bank Mechanic Village) to E (Control Site) with significant differences (15.32, p=0.000 and p=6.95, p=0.002 respectively).

Degradation ability of bacterial treated spent automobile oil with time (days) is demonstrated on Figure 3 with a negative correlation (r = -0.714) while degradation ability of bacterial treated spent automobile oil according to sample location is demonstrated on Figure 4 with a positive correlation (r = 0.64).

Site related degradation ability of spent automobile oil based on bacterial counts is presented on Figure 5. Sample site D (Benue State University Technical Workshop, Wurukum) recorded the highest bacterial count of 167.4 x 10^3 cfu/g and lowest spent automobile oil concentration of 1.350 mg/kg. Similarly, Sample site E (Control Site) recorded the lowest bacterial count of

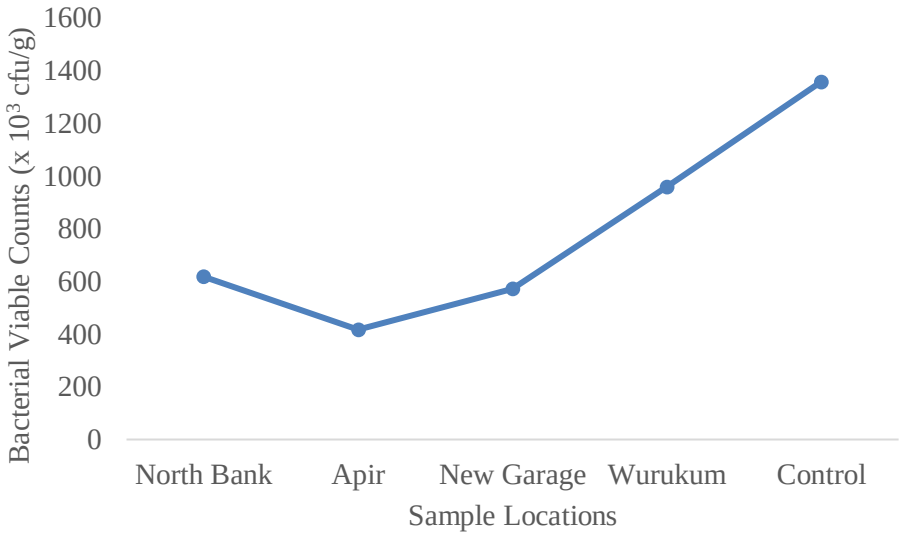

Fig. 1: Top Soil Bacterial Viable Counts (x 10^3 cfu/g) among Sample Locations

χ^2 (4DF) =723.103, p=0.000

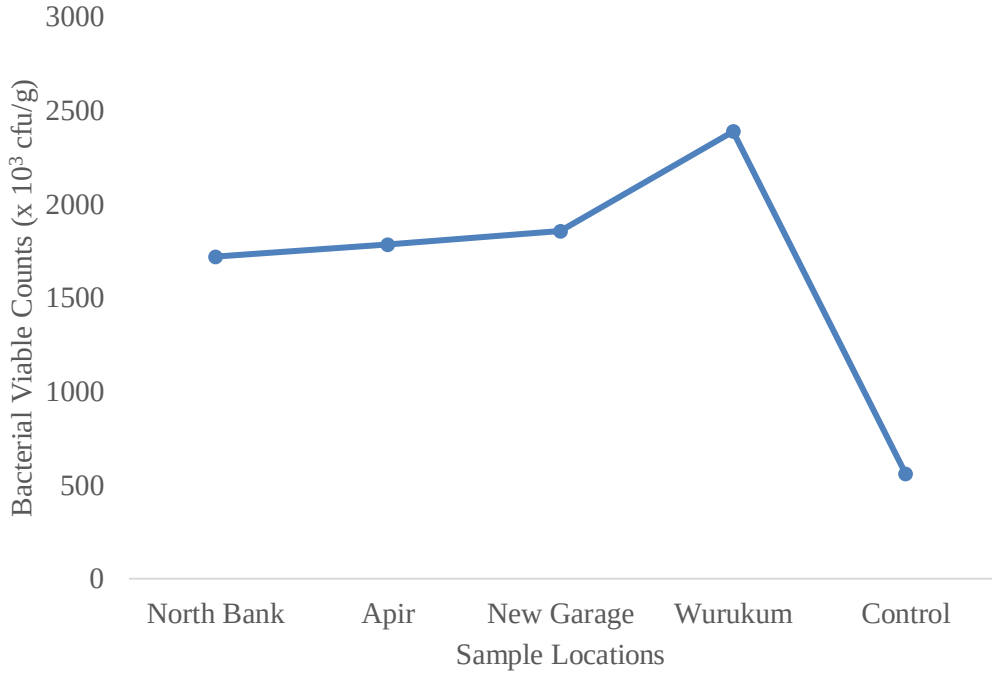

Fig. 2: Sub Soil Bacterial Viable Counts (x 10^3 cfu/g) among Sample Locations

χ^2 (4DF) =1083.16, p=0.000

Table 9: Variation in Absorbance of Bacteria Treated Spent Automobile Oil (600nm) based on Time and Sample Location

Time (Days)	**Sample Locations**					**Mean Separation**
	A	**B**	**C**	**D**	**E**	
Day 1	1.762±0.001	1.694±0.000	1.806±0.004	1.785±0.000	1.803±0.000	1.770^{a}
Day 2	1.691±0.001	1.633±0.019	1.723±0.002	1.632±0.000	1.806±0.000	1.697^{a}
Day 3	1.468±0.055	1.453±0.045	1.560±0.040	1.366±0.067	1.808±0.001	1.531^{ab}
Day 4	1.103±0.002	1.231±0.000	1.302±0.002	1.111±0.001	1.801±0.002	1.309^{bc}
Day 5	0.834±0.000	0.992±0.002	1.063±0.011	0.854±0.004	1.783±0.004	1.105^{c}
Mean Separation	1.372^{b}	1.400^{ab}	1.491^{ab}	1.350^{b}	1.800^{a}	

Means that share different letters along the column/row are significantly different at P≤0.05
F (Absorbance/Treated Spent Oil) = 6.95, p=0.002
F (Absorbance/Time) = 15.32, p=0.000

Key
A =
B =
C =
D =
E =

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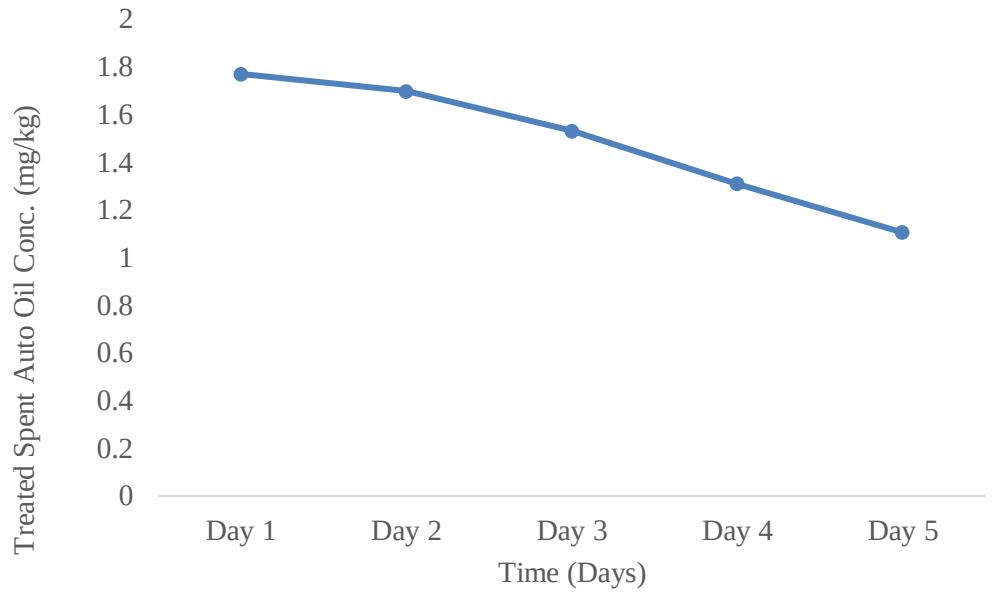

Fig. 3: Degradation Ability of Bacterial Treated Spent Automobile Oil with Time (days)

r = -0.714

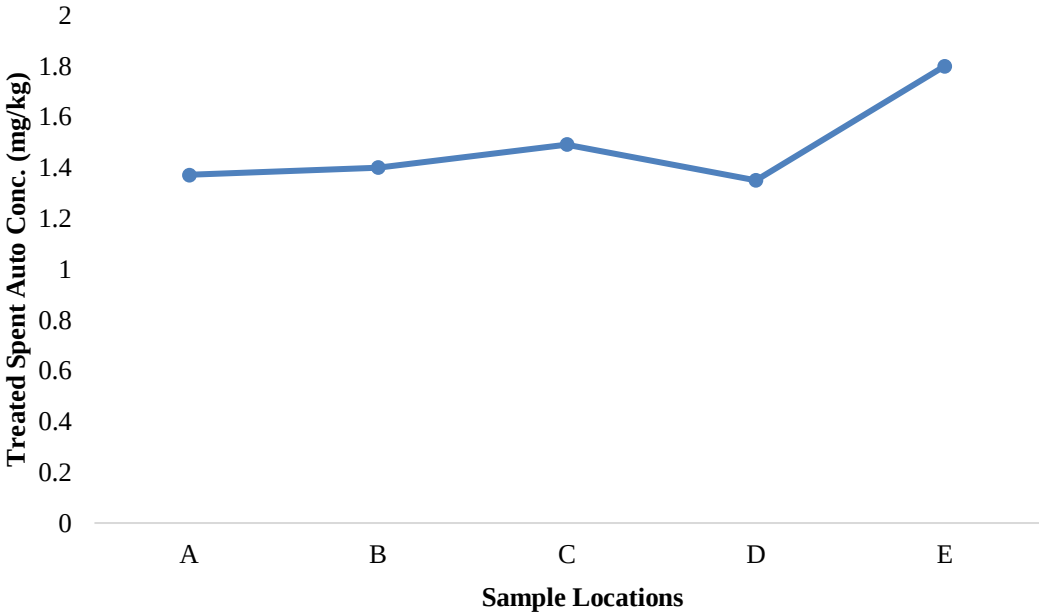

Fig. 4: Degradation Ability of Bacterial Treated Spent Automobile Oil according to Sample Location

r = 0.64

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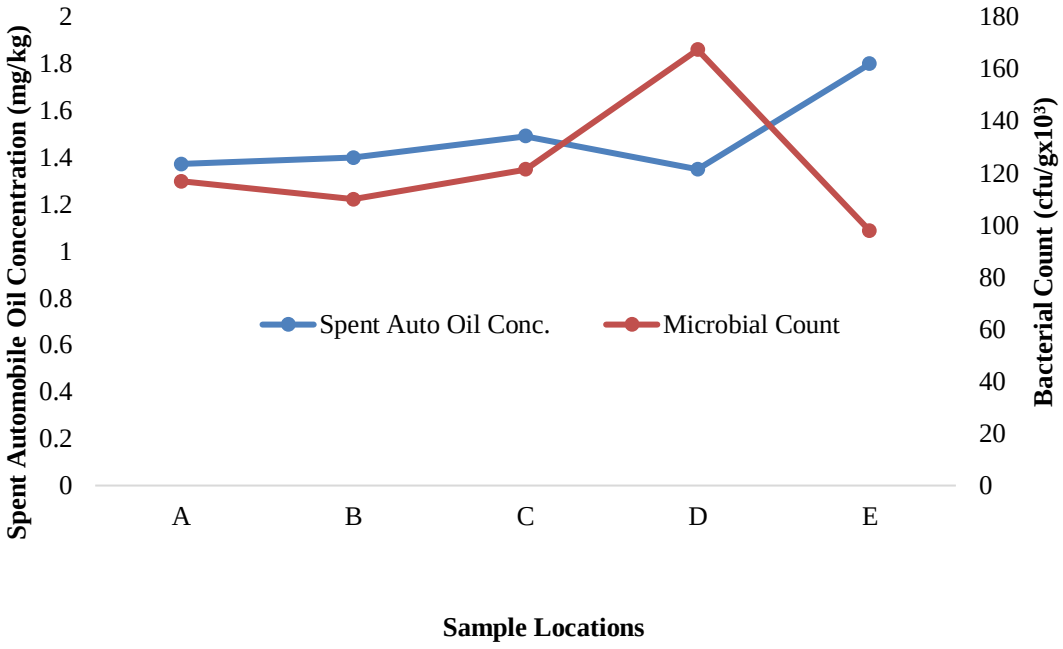

Fig. 5: Site Related Degradation Ability of Spent Automobile Oil based on Bacterial Counts

r = 0.64

97.85 x 10^3 cfu/g and highest spent automobile oil concentration of 1.80 mg/kg. Variables of microbial degradation of spent automobile oil here demonstrate inverse asymmetrical proportionality.

Time related degradation ability of spent automobile oil based on bacterial types is presented on Figure 6. Day 1 recorded the highest spent automobile oil concentration (1.770 mg/kg). Thereafter, spent automobile oil concentration decreased steadily to day 5 (1.105 mg/kg). Groups of bacteria involved in the degradation were lowest on day 1 (5 groups) and highest on day 4 (8 groups). Statistics however revealed negative correlation (r = -0.714) between spent automobile oil and bacterial types.

Gel image showing the quality of DNA extracted from three bacteria isolates is shown in Plate 1. Amplification of 16SrRNA gene of the isolates is given in Plate 2. The amplicons confirmed that the isolates possessed the gene of 1500 base pair known for degradation of hydrocarbon.

Based on gene sequencing data from the amplicons, isolate 1 was identified as *Staphylococcus equorum* strain Planc 5 using 98 % pairwise identity of the NCBI database. Isolate 2 was identified as *Pseudomonas putida* strain M.pstv.12.2 using 95.8 % pairwise identity of the NCBI database. Isolate 3 was identified as *Escherichia coli* strain KENECE3 using 95.5 % pairwise identity of the NCBI database. The base sequence results of the three isolates are shown in the appendix section Phylogenetic evolutionary tree of the three hydrocarbonoclastic bacteria (Figure 7). *Pseudomonas putida* strain M.pstv.12.2 and *Escherichia coli* strain KENECE 3 were closely related while *Staphylococcus equorum* strain Planc 5 was divergent.

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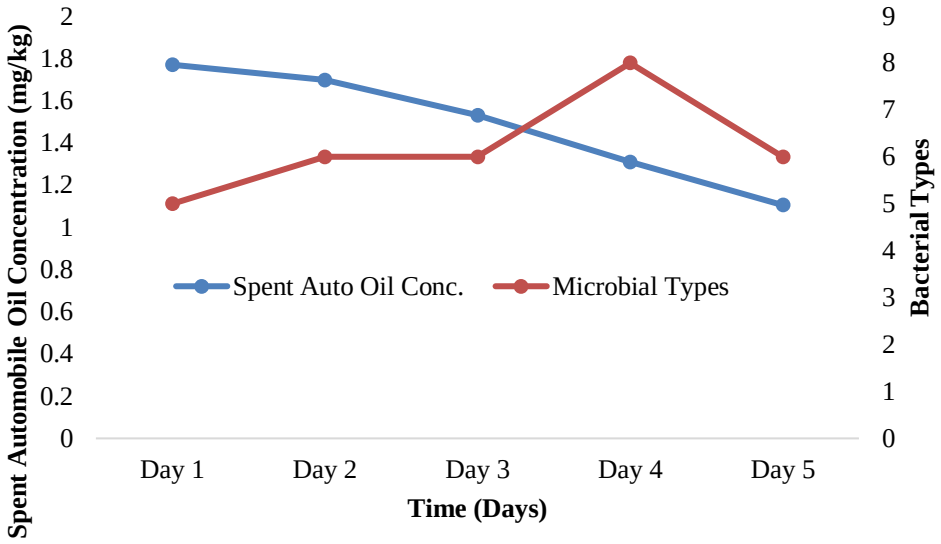

Figure 6: Time Related Degradation Ability of Spent Automobile Oil based on Bacterial Types

r = -0.714

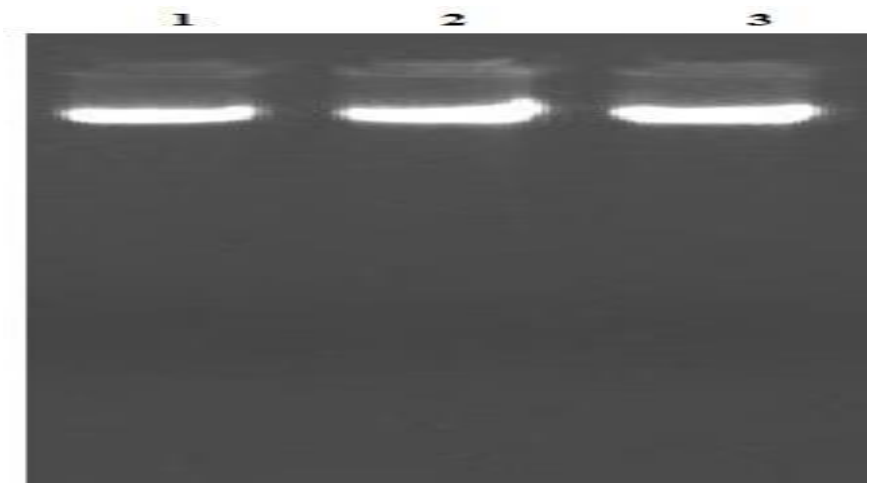

Plate 1: Quality of Genomic DNA Extracted from Bacterial Isolates

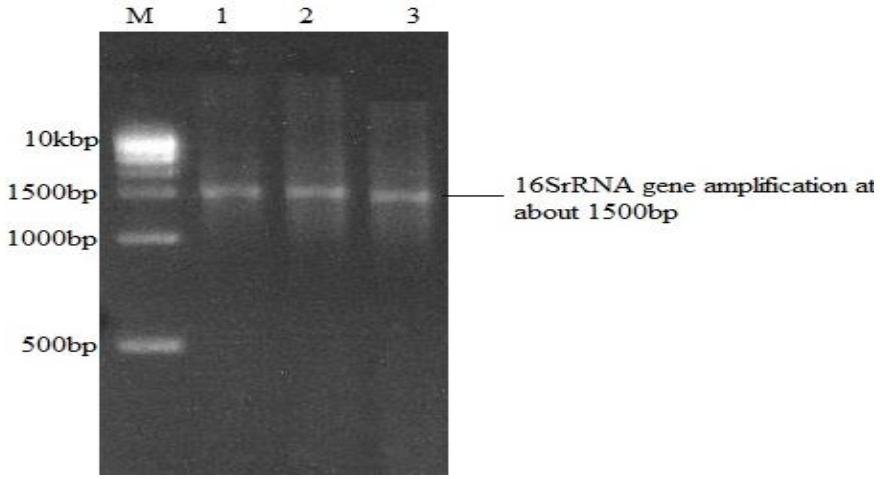

Plate 2: Gel Image Showing Amplification of 16SrRNA Gene at 1500bp
Lane M= 1kb ladder

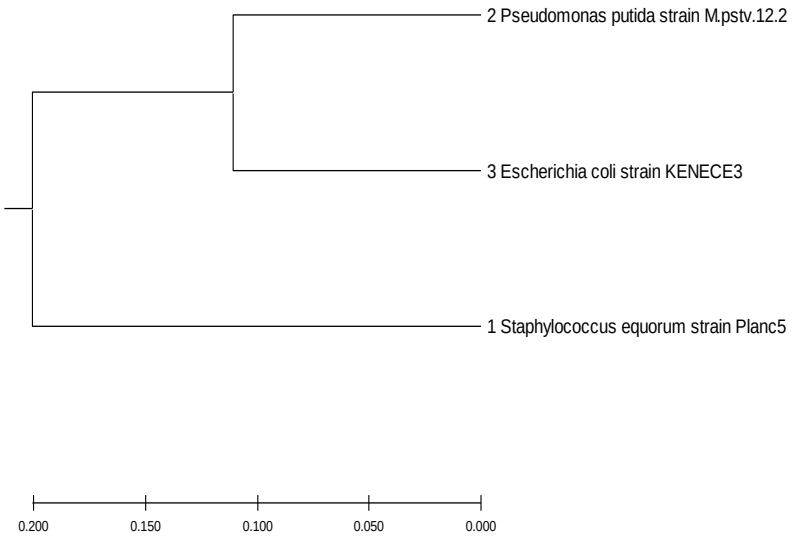

Fig. 7: Phylogenetic Tree of Three Identified Hydrocarbonoclastic Bacteria

IV. DISCUSSION

A total of nine (9) species of bacteria namely, *Staphylococcus* spp, *Micrococuss* spp, *Pseudomonas* spp, *Citrobacter* spp, *Enterobacter* spp, *Escherichia* Coli, *Bacillus* spp, *Klebsiella* spp and *Serratia* spp, were isolated from top and sub soil of the studied mechanic workshops spilled with spent automobile oil. The isolated soil contaminated spent automobile oil bacteria varied in classification and concentrations at different sample locations.

The topsoil had a larger number of microorganisms due to the presence of humus (decayed organic matter), the presence of oxygen e.t.c but the reverse is the case for the subsoil due to a reduction in the oxygen and humus levels. A small number of microorganisms especially the anaerobes were found in the subsoil from this study.

Micrococcus spp, Bacillus spp. ,Staphylococcus spp and Klebsiella spp, Were isolated from indigenous organisms to the waste Auto engine oil – contaminated soil samples collected from the Four sites. These organisms have been reported to utilize hydrocarbon, particularly Pseudomonas spp. and Bacillus spp. (Odoligie. I. 2017) Pseudomonas and Acinetobacter species are the most common bacterial hydrocarbon–degraders reported in literatures. (Mandri *et al,;* 2007) The isolation of these organisms from these environments also shows that these organisms have evolved strategies of adapting to the environment and/or utilizing these substances as energy sources. The presence of Bacillus species could be attributed to their ability to produce spores which enable them to survive in a different environment including hydrocarbon polluted soils (Ghazali et al., 2004).Pseudomonas spp. showed a rapid and prolific growth during the hydrocarbon degradability test, due to its ability to utilize these hydrocarbons as their energy source. A similar report by Christopher.,et al 2016.

The absorbance level of turbidity as a measure of degradation of hydrocarbon by four species bacteria at different periods in days was. In day 1, higher absorbance as evidence in (Table 9)

In other species absorbance was low, the reduce optical density of the spent oil by the test bacteria indicate the ability to utilize spent oil hence the reduction in optical density. At this point it is obvious that sample A (Staphylococcus species), B (Escherichia coli), and D (Pseudomonas species) possess greater bioremediation potential than Serratia. This do not agree with Wu et al., 2012 who identified Sarretia as a good bioremediator of hydrocarbon. This discrepancies may be as a result of differences in the strain of the organism used by Wu et al., 2012.

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