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Isolation and Characterization of Hydrocarbon-Degrading Bacteria in Top and Subsoil of selected Mechanic Workshops in Benin City Metropolis, Nigeria

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ABSTRACT: The isolation of hydrocarbon-degrading bacteria in topsoil and subsoil samples of selected mechanic workshops located in the Benin metropolis was carried out. The highest and lowest bacterial counts in topsoil samples were found out to be 2.94 x 10^6 CFU/g and 2.39 x 10^6 CFU/g in CENTRAL road and OGBELAKA road automobile workshops respectively. The highest and lowest bacterial counts in subsoil samples were found out to be 1.14 x 10^6 CFU/g and 1.09 x 10^6 CFU/g in OGBELAKA r oad and CENTRAL road automobile workshops respectively. The bacterial species isolated were *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Streptococcus* spp. The isolates were also subjected to hydrocarbon degradation/utilization test where it was observed that *Pseudomonas* spp utilized the hydrocarbon in the medium more efficiently than the other isolates. The isolates from this study are thus recommended as bacterial species for possible use in bioremediation of hydrocarbons. © JASEM
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Accidental spills, illegal dumping and careless handlings of petroleum products in mechanic workshops have been a significant source of environmental pollution, because of the predominantly unstructured practice of automobile vehicle repair services. The environments of soil and groundwater have been contaminated from the continuous spill and disposal of these products, which could lead to health hazards. Petroleum products used in mechanic workshops include petrol, diesel, and lubricants (engine oil). These Petroleum products contain metals and heavy polycyclic aromatic hydrocarbons (PAHs) which could contribute to chronic hazards including mutagenicity and carcinogenicity (Keith and Telliard, 1979; Haramaya *et al*., 2005.). As they are inevitable for the efficient and effective functioning of the automobile engines, soil contamination with these products is becoming one of the major environmental problems (Mandri and Lin, 2006) and this is due to uncontrolled disposal, particularly in developing countries like Nigeria (Mandri and Lin, 2006). In recent years, leakage of gasoline from underground storage tanks primarily at automobile service stations and from pipelines has been experienced at an alarming rate. Marine spills are also now becoming a frequent and major source of water and coastal contamination (Salam, 1996).

A variety of technologies is currently available to treat soil contaminated with hazardous materials, including excavation and containment in secured landfills, vapour extraction, stabilization and solidification, soil flushing, soil washing, solvent extraction, (Russell, 1992). Many of these technologies, however, are either costly or do not result in complete destruction of contamination.

Bioremediation is one of the processes used in the cleanup of such soil contamination and it is defined as the use of biological processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil and water (Vidali, 2001). Bioremediation is a natural process which relies on bacteria, fungi, and plant, to alter contaminants as they carry out their normal life functions (Donlon and Bauder, 2006). Microorganisms are found throughout the environment; in air, soil, and water, on the surface of objects and humans both internally and externally. In the environment, many organisms in soil play important geochemical roles in processing vital elements such as phosphorus and nitrogen making them available for use by plants.

The widespread ability of microorganisms to assimilate these hydrocarbons is of great significance. This process whereby microorganisms break down these pollutants especially as it relates to hydrocarbon spillage is known as bioremediation. A wide range of hydrocarbon utilizing bacteria (HUB) useful for bioremediation are found to be in the soil; they

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include the following species: *Pseudomonas, Rhodococcus, Mycobacterium, Bacillus, Acinetobacter, Providencia, Flavobacter, Corynebacterium, Streptococcus, Serratia* (Bhattacharya *et al*., 2002).

The main goal of a bioremediation design should be the creation of the most favourable conditions for microbial growth and activities which would further enhance the breakdown of contaminants (mostly hydrocarbons) that need to be broken down (Balba *et al*., 1998). Thus, there is urgency in the isolation of microorganisms capable of breaking down and utilizing hydrocarbons to minimize their toxic effect to other biological agents in the environment. This work was aimed at isolating hydrocarbon degrading bacteria from three (3) mechanic workshop soils in Benin City and to ascertain their ability to grow efficiently in hydrocarbon based medium.

MATERIALS AND METHOD

The Study Site: Topsoil and subsoil samples were collected from auto-mechanic workshops at three different locations in Oredo, Benin City, namely; OGBELAKA Street, ADESUWA road, and CENTRAL road.

*Sample Collection***:** Eighteen (18) soil samples were collected using a clean auger at three different points in the auto-mechanic workshops. The topsoil sample was collected at different intervals 0 – 15 cm down the soil horizon, ensuring that the soils clogged to the auger was scraped off after collection at each interval. Soil samples collected were transferred into well labelled, clean polyethylene bags. The procedure for collection of topsoil samples was repeated for subsoil samples except for that sub- soil samples were collected at intervals 15 – 30 cm down the soil horizon. The whole process of topsoil and subsoil sample collection was repeated for two other points at each auto-mechanic workshop. The soil samples were arranged in a box and transported to the laboratory for microbial analysis.

Sterilization of Glass Wares and Other Materials: All the glass wares used were washed, dried and sterilized in a hot-air oven at a temperature of 160 ^{o} C for 1 hour. The area (bench) where the work was done was properly swabbed with cotton wool and methylated spirit. The wire loop was also sterilized by flaming before and after use, using a spirit lamp.

*Preparation of Culture Media***:** Nutrient Broth (NB) was used for serial dilution and Nutrient Agar (NA) was used for culturing. In preparing the media, 13 g of nutrient broth was dissolved in 1000 ml of distilled water and 28 g of nutrient agar was dissolved in 1000 ml of distilled water. The media were autoclaved at 121 ^{o} C for 15 minutes.

Microbiological Analysis: Under an aseptic condition, topsoil samples collected at different levels in the soil from a particular auto-mechanic workshop were mixed together and grounded to break soil clogs using a sterile mortar and pestle. This was repeated for the subsoil samples. A ten-fold serial dilution was made for each soil sample, after which 1 ml of 10^{-5} dilutions was plated using pour plate method and incubated at 37 ^{o}C for 48 hours.

*Microbial Count and Pure Culture Isolation***:** Total viable counts of bacteria were determined by enumerating the colony forming units (CFU) after incubation for 48 hours. Pure cultures of bacterial isolates were obtained by sub-culturing on the nutrient agar plates and pure cultures were then transferred to agar slants for further biochemical tests.

Characterization and Identification of the Isolates: Bacterial isolates were characterized by morphological and biochemical characteristics. The biochemical tests carried out on each bacterial isolates were: Catalase, Oxidase, Citrate utilization, Urease, Methyl red, Indole, Sugar fermentation (glucose, maltose, and lactose) (Cheesbrough, 2000; Harrigan and MacCane, 1976; Aneja, 2003)

Hydrocarbon Degradation Test: 100 ml of Bushnell Hass broth was prepared into four conical flasks, supplemented with 1 ml of spent engine oil. The media was inoculated with a loop full of the bacterial isolates and conical flasks were placed in a shaker. The absorbance of the culture was also measured using a spectrophotometer. Serial dilution and pour plate was done on the cultures in the conical flask every two days to enumerate the number of colonies that thrived and/or can survive the Bushnell Hass broth-engine oil mixture.

RESULTS AND DISCUSSION

Table 1 shows the total heterotrophic count of soil samples (topsoil and subsoil) collected from three auto-mechanic repair workshops in Oredo, Benin City. The topsoil samples collected from OGBELAKA street has the highest mean bacterial count of 2.94×10^{6} CFU/g while the topsoil sample collected from CENTRAL road has the lowest mean bacterial count of 2.39×10^{6} CFU/g. The subsoil samples collected from CENTRAL road has the highest bacterial count of 1.0×10^{6} while the subsoil samples collected from OGBELAKA street has the lowest mean bacterial count of 1.09×10^{6} CFU/g. The top soil samples contain more microorganisms (bacteria) than the subsoil samples. Bacteria isolated were *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp.

Table 1: Total Heterotrophic Bacteria Count of Soil Samples

Location	MEAN (CFU/g)	
	SUBSOIL	TOPSOIL
Adesuwa Street	1.13×10^6	2.67×10^6
Central Road	1.14×10^6	2.39×10^6
Ogbelaka Street	1.09×10^6	2.94×10^6

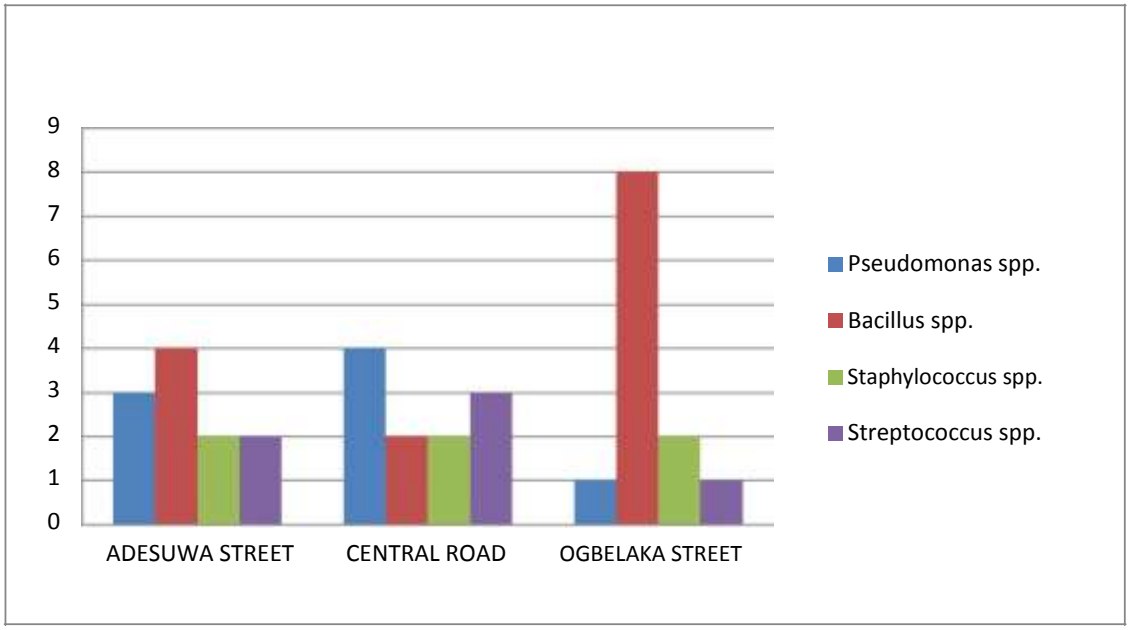

Fig 1: The frequency of occurrence of bacterial isolates in the soil samples from the three locations

Figure 1 is a bar chart showing the occurrence of bacterial isolates in the soil samples from these three locations: ADESUWA Street, CENTRAL road, and OGBELAKA Street. *Bacillus species* are found to be dominant in soil samples collected from ADESUWA and OGBELAKA Street while *Pseudomonas* spp dominate soil samples collected from CENTRAL road.

Table 2: Total Count during hydrocarbon biodegradation test

ISOLATES	MEAN (cfu/ml)		
	DAY 2	DAY 4	DAY 6
Pseudomonas spp.	1.12×10^5	1.32×10^6	1.25×10^8
Bacillus spp.	0.23×10^5	2.64×10^6	7.05×10^7
Staphylococcus spp.	0.08×10^5	5.20×10^5	5.95×10^7
Streptococcus spp.	1.05×10^5	1.20×10^6	1.62×10^8

Table 2 shows the plate count after two, four and six days of the degradation culture serving as part of the biodegradation tests. All the organisms were found to thrive successfully after six days in the Bushnell Hass-spent oil-organism, with *Streptococcus* spp. having the highest mean plate count of 1.62×10^8 CFU/ml and the *Staphylococcus* spp. having the least mean plate count of 5.95×10^7 cfu/ml after six days. *Streptococcus* spp. showed tremendous growth with 1.62×10^8 CFU/ml at the end of the sixth day. This was followed by *Pseudomonas* spp. at 1.25×10^8 CFU/ml, *Bacillus* spp. with 7.05×10^7 CFU/ml and *Staphylococcus* spp. with 5.95×10^7 CFU/ml at the end of the sixth day.

Table 3 shows the absorbance of the different culture media at a wavelength of 540nm where the *Bacillus* spp. has the highest absorbance of 0.889 at the end of the sixth day, followed by *Pseudomonas* spp. with

0.725, *Streptococcus* spp. with 0.652 and *Staphylococcus* spp. with 0.548.

Table 3: Absorbance (at wavelength 540 nm) of the hydrocarbon biodegradation culture

Isolates	Absorbance (Wavelength 540 nm)		
	DAY 2	DAY 4	DAY 6
Pseudomonas spp.	0.337	0.420	0.725
Bacillus spp.	0.490	0.675	0.889
Staphylococcus spp.	0.228	0.507	0.548
Streptococcus spp.	0.410	0.578	0.652

Hydrocarbon-degrading microorganisms are widely distributed in marine, freshwater and soil ecosystems. The ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as evidence that those organisms are the active degraders of the constituents of that environment. Similar organisms which include;

Pseudomonas, *Bacillus*, *Streptococcus* and *Staphylococcus* species were isolated by Okerentugba and Ezeronye, (2003). 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, *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp. were isolated from indigenous organisms to the waste engine oil – contaminated soil samples collected from the three sites. These organisms have been reported to utilize hydrocarbon, particularly *Pseudomonas* spp. and *Bacillus* spp. (Amund and Adebiyi, (1991), Nardell *et al*., (1997), Nwachukwu *et al*. (2009) and Arotupin and Ogunmalu, (2012)). *Pseudomonas* and *Acinetobacter* species are the most common bacterial hydrocarbon–degraders reported in literatures (Rusansky *et al*., 1987; Kiyohara *et al.*, 1992; Johnson *et al*., 1996; Barathi and Vasudevan, 2001; Bhattacharya *et al*., 2002; Pokethitiyook *et al.*, 2003; Van Hamme *et al*., 2003).

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 and Christopher (2004) and Mandri and Lin (2007) showed that *Flavobacterium* and *Pseudomonas* spp. were the most prevalent species in hydrocarbon polluted soils. All isolates showed an increasing plate count and absorbance because of they were all capable of utilizing the spent engine oil introduced into the growth medium.

Conclusion: Soil samples contaminated with hydrocarbons examined from different auto-mechanic repair workshop harboured bacteria of possible biodegradation importance. Per this research, the following bacterial species: *Pseudomonas* spp., *Bacillus spp*., *Staphylococcus* spp. and *Streptococcus* spp. were isolated and were found to be able to utilize and/or degrade spent engine oil as energy source. It is, however, evident from the outcome of this study that hydrocarbon utilizing and/or degrading microorganisms could readily be isolated from oil-contaminated soil samples in an auto-mechanic repair workshop and possibly used for bioremediation of hydrocarbon polluted soil. Further studies, however, need to be done to identify and characterize these organisms down to the species level and more effective strains could also be developed to aid the bioremediation of oil-contaminated soil. This could help reduce the scourge of oil spill pollution caused by drilling operations of oil exploration companies in the Niger-Delta region of Nigeria.

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