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Isolation Of Bacteria From Soil Contaminated With Used Engine Oil In Federal Capital Territory, Nigeria

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***Abstract*—The oil degrading bacteria were identified and isolated from soil samples contaminated with used engine oil in this study. The results of the four soil samples collected from four different mechanic workshops showed a prevalence yield of bacteria degrading agents with the highest growth in Colony Forming Units (CFU) recorded in between day 3 and 4 while the results obtained from the biochemical analysis of bacteria isolates from the various soil contaminated with used engine oil in mechanic workshops across FCT revealed the presence of these bacteria; Pseudomonas aeruginosa, Bacillus Stearothermophillus, Streptococcus spp and Listeria spp which have the ability to utilize the engine oil as source of carbon. This study showed that the bacteria strains used in this study could be relevant in the bioremediation of soil contaminated with hydrocarbons. Thus, this study recommends that Government should stop indiscriminate sitting of mechanic workshops as it affect land use for agricultural production and human health among others.**

Keywords—Bacteria, used engine oil, contaminated soil, Colony Forming Units.

INTRODUCTION

Used oil is oil which has been contaminated by chemical impurities which contributes to chronic hazards including mutagenicity and carcinogenicity as well as environmental hazard with global ramifications (Blodgette, 2001). Used motor oil is the brown-to-black oily liquid removed from a motor vehicle, when the oil is changed. Used 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 an engine lubricant at high temperatures and pressures, inside an engine as it runs (Deziel et al., 1996). Large amounts of used engine oil are liberated into the environment when the oil from motor cars, motor-bikes, generators etc is changed and disposed into gutters, water drains, open vacant plots and farmlands a common practice by motor and generator mechanics (Odjegba and Sadiq, 2002). High concentration of aliphatic polycyclic aromatic hydrocarbon and heavy metals contribute to the inherent toxicity of used oil (Barathi and Vasudevan, 2001). Used motor oil can cause great damage to sensitive environments and soil microorganisms. Substantial volumes of soil have been contaminated by used oil in many countries of the world, especially industrialized nations. Various contaminants such as used engine oil and heavy metals have been found to alter soil biochemistry, which includes alteration in soil microbial properties such as pH, O_2 and nutrient availability (Atuanya, 1987; Odjegba and Sadiq, 2002).

Bioremediation has become an alternative way to remedy oil polluted sites, where the addition of specific microorganism (bacteria, cyanobacteria, algae, fungi, protozoa) or enhancement of microorganism already present can improve biodegradation efficiency (Hagwell et al., 1992). These microorganisms can degrade a wide range of target constituents present in oil sludge (Barathi and Vanudevan, 2001). A large number of pseudomonas strains capable of degrading polycyclic aromatic hydrocarbons have been isolated from soil (Johnson et al., 1996). Other petroleum hydrocarbon degraders include *Yokenella* spp., *Alcaligenes* spp., *Roseomanas* spp., *Sreanotrophomanas* spp., *Acinetobacter* spp., *Flavobacter* spp., *cyanobacterium* spp., *capnocytophage* spp., *Moraxella* spp. and *Bacillus* spp. (Antai, 1990). Other microorganism such as fungi is also capable of degrading the hydrocarbons in engine oil to a certain extent.

Engine oil, petrol, diesel and kerosene are used daily in various forms in mechanic workshops across FCT. These used oil products tend to harden and change the colour of the soil, which may have untold health hazard on the technicians and artisans. This present study, however, was aimed to assay and investigate the bacteria present in used engine oil-contaminated soil in FCT Nigeria.

Materials and Methods

Study Area

The federal Capital Territory is located in the geographical centre of Nigeria for easy accessibility to all parts of the country. It lies between the latitude of

8°25` N and 9° 20` W and longitude 6° 45` S and 7° 39` E. It is bounded to the North by Kaduna and Niger States, to the South by Kogi State, to the East by Nasarawa State and to the West by Niger State.

Materials Used

Polluted soil, Sterile bottles, Conical flask , Paper soil, Spatula, Weighing boat, Cotton wool, Rubber band, Syringes, Petri dishes, Sprayer, Glass beaker, Masking tape ,Test tubes, and measuring cylinder, sterile bottle, Autoclave,Incubator, Laminar air flow, Refrigerator, Microscope. **Reagents used;** Bushhell Hass Media (BHM), Disodium phosphate, Potassium phosphate, Magnesium tetra-oxosulphate(vi)SO_4 ($MgSO_4$),Calcium chloride, Ethanol(70%), Engine oil(as carbon source), Distilled water, Simmon's Citrate Agar, MacClonky Agar, Methyllvogesproskauer, Nutrient Agar media.

Collection of Soil Samples

The experimental soil samples were collected from Federal Capital Tartary Abuja. Four different soil samples were collected in sterile containers at the depth of 5cm because organic matter accumulates on the topsoil. The collection sites include motor mechanic sites at Apo, Zuba, Gwagwalada and Sheda generator site. Soil samples were collected from specific locations at each workshop. The soil samples were collected aseptically with sterile bottles to avoid external contamination.

Enrichment cultures and isolation.

The culture media used were Bushnell – Haas medium according to Nnamchi *et al* (2006) which is an enrichment medium for isolation of bacterial degrading organism. Inoculation was done using the enrichment medium of 20 ml in MacCartney bottles into which 10 grams of the contaminated soil was added and incubated at 30°C for one week. After a week, 10mls of the enrichment media was transferred into a fresh sterile Bushnell Hass Media (BHM) and incubated at the same conditions as stated above. This was repeated for the third time after which serial dilutions (1/10) of the 3rd enrichment process was plated out on to Bushnell Hass (BH) agar plates and were then incubated for 48hours at 30^{o}C to observe the morphological characteristics of the isolates. The ability of isolates to utilize the oil was indicated by an increase in turbidity of the medium and the colony forming unit (cfu/ml) of the isolates on plate count agar taken every 48 hours for 2 weeks. After 24hours the Colony Forming Units (CFU) of isolates were counted and this formed the standard for the microbial screening.

Identification of Isolates

The morphological characteristics of the Isolates were identified by Gram stain and biochemical reactions (Nnamchi *et al* 2006). The biochemical reactions include glucose fermentation, Catalase production reaction, coagulase reaction, Methyl red-voges-proskauer broth reaction, Simmons Citrate Agar reaction.

RESULTS

The results of the four soil samples collected from four different mechanic workshops showed a prevalence yield of bacteria degrading agents. Figure 3.1. Shows the total Colony Forming Units (CFU) of organisms cultured for 7 days. The highest growth in Colony Forming Units (CFU) was recorded in between day 3 and 4 because the cells were at cellular stage called log phase while all organism showed significant drop in CFU after 7 days of exposure due to retardation of cells called death phase.

Table 3.1: Microbial count in the different sampling sites

	Microbial count in CFUs in the four sampling sites at dilution factor of 10^6			
DAYS	A (GWAGWALADA)	B (ZUBA)	C (APO)	F (SHEDA)
DAY1	35	51	20	24
DAY2	68	70	75	63
DAY3	138	141	150	95
DAY4	130	129	132	143
DAY5	110	105	110	122
DAY6	80	80	80	101
DAY7	60	70	60	71

Source: Fieldwork

The graph below trends the table above showing the highest microbial counts at Days 3 and 4 after which the microbial counts began to decline.

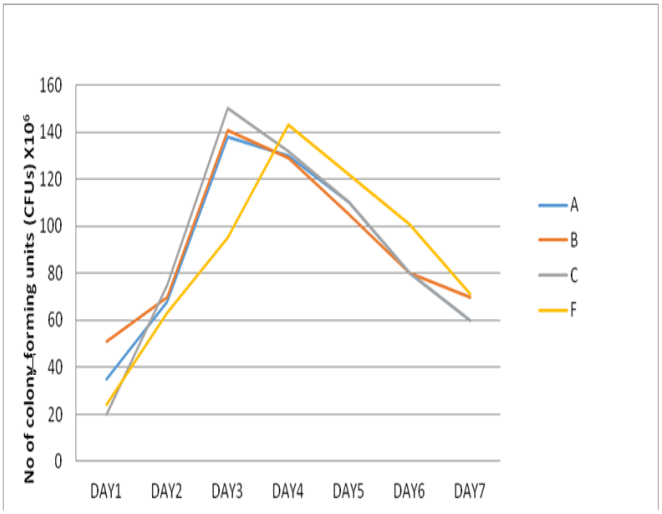

Figure 3.1: Ability of organisms to utilize motor oil as sole carbon source after 7 days of exposure

Key:A =Gwagwalada; B = Zuba; C = Apo; F = Sheda

The Above areas are locations within the F.C.T , Abuja Nigeria

The results of some biochemical tests including Catalase, Coagulase, Simmon's Citrate Agar, MacCorky Agar, MR-VP Broth, Motility tests from the

various soil contaminated with used engine oil in mechanic workshops across FCT were described in Table 3.2 below. It showed some of the probable microorganisms present as *Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp* . The occurrence of these microorganisms may be as a result of the nature of soil, or the nutrients in the contaminated soil.

Table 3.2: Different tests done to identify possible microbes present in the samples

Reagents	Grand Reaction	Colour Change	Probable Organisms
Catalase	Positive	-	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*
Coagulase	Negative	-	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*
Simmon's Citrate Agar	Positive	Green to Blue	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*
MacCorky Agar	Positive	Blood to Golden Yellow	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*
MR-VP Broth	Positive	Golden to Brown	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*
Motility	Negative	No spread of growth	*Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp*

Source: Fieldwork

Discussion

The use of microorganisms for removing oil pollution from contaminated sites than any order method is recommended because of the economic values in conserving the soil. This study identified bacteria that can degrade used motor oil contaminated soil in the Federal Capital Territory, Nigeria. In this study, *Pseudomonas*, *Bacillus*, *Streptococcus* and *Listeria* were isolated from oil contaminated soil samples. A large number of *Pseudomonas spp* capable of degrading polycyclic aromatic hydrocarbons have been reported in many studies (Zhang et al, 2004). However, *Bacillus spp* capable of degrading hydrocarbon have also been reported by (Shafiee *et al*., 2006; Atlas, 1981). *Pseudomonas* spp was reported to have specificity for a range of hydrocarbon compounds including biphenyl PAHs and petroleum products commonly used in the Nigerian environment (Paul, 1997) Therefore, its presence in all the soil samples analyzed in this study is not a surprise. The study identified the biodegradation potential of *B. subilis* and *P. aeruginosa* strains which was similar to the results recorded by (Amund et al., 1994) in *B. subtilis* and *P.aeruginosa* strains isolated from crude oil-polluted soil from Nigeria.

Conclusion and Recommendations

This study showed that *Pseudomonas aeruginosa, Bacillus stearothermophillus, Streptococcus spp and Listeria spp* are present in the used oil contaminated soil. Therefore the bacteria isolated from this study could be relevant in bioremediation of soil contaminated with used engine oil especially microorganism that are indigenous to the contaminated sites. Additionally, application of appropriate concentrations of nutrient sources such as nitrate, phosphate and sulphate could accelerate biodegradation of engine oil polluted soil. Also the bacteria isolates obtained from this study could be exploited for oil spill clean-up in similar environments. However, environmental consciousness could be instilled into automobile mechanics to avoid indiscriminate disposal of engine oil.

Based on the findings of this study, it is recommended that:

The FCT administration should adopt bioremediation as a system of cleaning up used oil contaminated sites instead of fertilizer that has effects on the streams and ground water bodies as well as food crops such as vegetables, beans, legumes among others. This system is environmentally friendly.

Government should stop indiscriminate sitting of mechanic workshops as it affect land use for agricultural production and human health.

It is recommended that mechanics who are the drivers of the spill should have designated sites outside their workshop for the final disposal of their used products such as engine oil cans, scrap metals where they can easily be recycled to other by-products.

Government should create a regulatory body that check the activities of mechanics both within and outside their workshops as this could help in reducing unnecessary spill or discard of harmful used motor oil and other materials that are injurious to human health and the environment in general.

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