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Seasonal Influence on Microbial Load in Waste Engine Oil Polluted Soil of Automechanic Workshop in Port Harcourt Metropolis

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Abstract: *Automechanic workshops are engine servicing centres and are source of automobile waste oil, the significant environmental impact associated with automechanic activities is the seepage of waste engine oil into the soil. This study was aimed at identifying the microbial species that are associated with waste engine oil polluted soil in automechanic workshops at Rumuoke and Elechi all in Port Harcourt City using molecular characterization. The soil samples were collected with the aid of a hand augar for microbiological analysis. The data obtained from this study were analyzed using analysis of variance (ANOVA) and Duncan Multiple using (DMRT). Based on Duncan multiple range test. The bacterial counts obtained during dry season ranged from 1.0x 10^4cfu/g to 9.1x10^5cfu/g while the rainy season were within the range of 1.0x10^4cfu/g to 3.2x10^6cfu/g. The fungal counts obtained during dry season ranged from 2.0x102cfu/g to 5.1x103cfu/g while the rainy season were within the range of 2.0x10^3cfu/g to 6.3x10^3cfu/g, The bacterial counts obtained from depth 0-15cm were within the range of 1.1 x 10^4cfu/g to 1.22 x 10^6cfu/g while depth of 16-30cm ranged from 1.0 x 10^4 cfu/g to 1.64 x 10^6cfu/g. The fungal counts obtained in depth of 0-15cm ranged from 1.2 x 10^3 cfu/g to 9.0 x 10^3 cfu/g while depth of 16-30cm ranged from 1.1 x 10^2 cfu/g to 7.0 x 10^3 cfu/g. The bacterial species isolated were Klebsiella pneumoniae, Plesiomonas shigelloide, Acinetobacter species, Bacillus licheniformis, and Pseudomonas aeroginosa while the fungal species included Aspergillus alabamensis, Rhodotorula mucilaginosa, Aspergellus terreus, Aspergillus flavus, Sarocladium hominis, Trichoderma capillare, and Aspergillus aculeatus.*

Keywords*: Automechanic Workshop, Waste Engine Oil Polluted Soil*

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I. Introduction

Industrial production of chemicals as well as their inappropriate use, improper disposal and accidental leakage has resulted in contamination of many areas. Engine oils are altered during use by vehicles, motor-bikes, generators and other machineries because of the breakdown of additives, contamination with the products of combustion and the addition of metals from the wear and tear of the engine. 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 around Port Harcourt metropolis (Ugoh and Moneke, 2011). Oil pollution is a predominant feature in Nigeria, being a major oil producing country and whose economy solely depends on crude oil. Pollution of this nature is either directly from crude oil or from waste engine oil (WEO). This is even becoming very widespread and more devastating than crude oil pollution because it is a major source of pollution within communities, where it constitutes a major risk to both plants and animals, as well as soil organisms. The fact that motor mechanic workshops as well as workshops of other artisans that use and dispose WEO, are situated in urban and rural areas, bushes and open plots, gardens and farms in such areas are constantly under threat of pollution by WEO through run offs which eventually delivers water soluble fractions of the contaminants to other places (Ikhajiagbe *et al*., 2013).

The precipitous discharge of large amount of hydrocarbons poses stress to the environment, thereby disrupting the economic life of the populace. Oil spillage as it is referred to have deleterious impact on flora, fauna and microbiota of the ecosystem. The most commonly found environmental contaminants are petroleum hydrocarbon, though they are not usually classified as hazardous wastes (Akpe *et al.*, 2015).

On the terrestrial environment, oil spills cause extensive damage ranging from the destruction of terrestrial flora and fauna to biomagnification of the toxic components of the petroleum conversion of arable land to barren soils and the destruction of the aesthetic quality of the environment. Other environmental

consequences of oil pollution include the adverse effects on the soil microflora and ground water contamination (Obire and Ayanwu, 2009).

Microorganisms play important role in the biogeochemical cycling and mineralization. Substances whose presence in the environment affect these microbial activities also adversely affect plant growth as well as detoxification of organic pollutants (Obire and Ayanwu, 2009).

Hydrocarbon degrading microbes play a paramount role in bioremediation and they include bacteria, fungi, yeasts and some algae. These organisms have been isolated from heavily oil-polluted deposits or in a variety of soils. Microbial transformation of organic contaminants normally occurs because the organisms can use the contaminants for their own energy needs, growth and reproduction (Gerard *et al.*, 2008).

The presence of petroleum may also affect the microbial community through selection of species. Variety of oil degrading microorganisms have been isolated from oil polluted soils the species of bacteria that have been frequently isolated include *Pseudomonas, Acetobacter*, *Chromobacterium* and *Corynebacterium*, which occured as dominant species. Among the fungi are *Candida* species which is the yeast most commonly found while *Penicillium* and *Cladosporium* are among other fungal species that are found. The major genera of bacteria active in oil polluted soils were *Pseudomonas, Bacillus, Seratia* and *Acinetobacter*, while the fungal genera were *Aspergillus*, *Penicillium* and *Mucor* (Gerard *et al.,* 2008).

The disposal of waste engine oil (WEO) into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics. This oil, also called spent lubricant or waste engine oil, is usually obtained after servicing of automobiles and generator engines (Okonokhua *et al.,* 2007).

Service stations in some parts of Nigeria dispose WEO indiscriminately into plots, land, sewage, and drainage ditches, thus increasing pollution in the environment (Kayode *et al.,* 2009).

This study was carried out to identify the microorganisms associated with waste engine oil polluted soil from the different automobile workshops in Port Harcourt metropolis.

II. Materials And Method

Study Area

The soil samples were collected from three different automechanic workshops in Port Harcourt, Rivers State, Southern part of Nigeria

Sampling locations	**GPS coordinates**
Okilton	N4.84285^{o}, E6.97589^{o}
Rumuoke	N4.84421^{o}, E6.96554^{o}
Elechi	N4.79958^{o},E6.98511^{o}
Control	N4.84230^{o},E6.96537^{o}

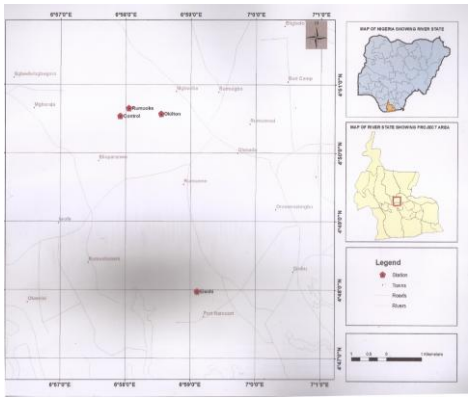

Figure 1: Map Showing Study Location in Port Harcourt, Rivers State

Sample Collection

A sampling device (hand auger) was used to collect the soil samples. The samples were collected from the waste engine oil polluted areas between 0-30cm depth, with respect to 0-15cm and 16-30cm depths and taken as polluted soils. Soil samples were also collected from sites far away from the mechanic workshops and taken as control sites. This procedure was carried out for the three different workshops two times for each season (dry and wet seasons). The soil samples were transported to Rivers State University microbiology laboratory for analysis.

Microbiological Analysis of Soil Samples

Enumeration of Bacteria and Fungi

One gram (1g) of the soil sample was mixed with 9ml of sterile distilled water in test tubes lined up in test tube racks. One millimeter (1ml) was taken from test tube one to the second test tube; this one tenth serial dilution continued upto 10^{-3} dilution. From the dilutions 10^{-1}, 10^{-2}, 10^{-3} aliquot (0.1m) was spread plated onto sterilized media plates of nutrient agar, maconkey agar, sabouraud dextrose agar and oil agar which were prepared according to Manufacturer's prescription (Panda *et al*., 2013).

The inoculated plates were incubated. Nutrient agar plates incubated at 37^{o}C for 24 hours for colony formation, sabouraud dextrose agar plates were incubated at room temperature for 72 hours while oil agar plates were incubated for 3-7 days. After incubation, the plates with thirty to three hundred colonies were selected, counted and recorded.

Bacterial Identification

Different morphological characteristics of colonies were subcultured to get pure culture.
Microbes were identified using the morphological characteristics of isolates, biochemical test macroscopic and molecular identification

III. Results And Discussion

Results for total heterotrophic bacteria and hydrocarbon utilizing bacteria for both wet and dry seasons and depths are presented in Fig 2 and 3. Total petroleum hydrocarbon ranged from 0.12 to 76.36. The total heterotrophic fungi and hydrocarbon utilizing fungi for both wet and dry seasons and depths are presented in Fig 4 and 5 respectively.

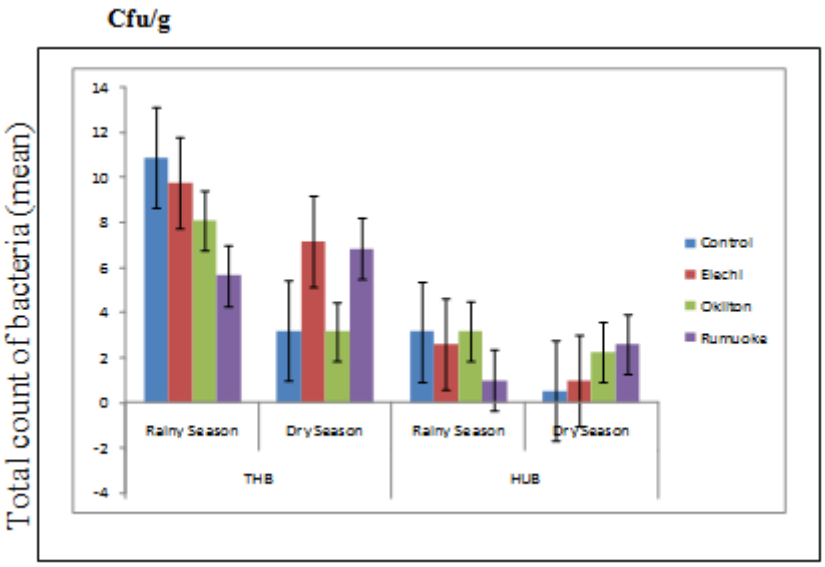

Fig. 2 Total Count of Bacteria for Rainy and dry Seasons at 0-15cm depth

The total heterotrophic bacteria enumerated from the contaminated sites during the rainy season showed that the control recorded the highest colonies of bacteria followed by Elechi, Okilton, and Rumuoke respectively. There is a significant difference between the control sample and the contaminated sites at depth 0-15cm; while the result from the dry season showed that the control and the sample from Okilton had equal microbial load, while the samples from Elechi and Rumuoke showed significant difference when compared with the control.

Hydrocarbon utilizing bacteria isolated during the wet season from all the contaminated area showed little or no significant difference with the control, Elechi and Okilton, while there is a significant difference between the other samples with that of Rumuoke at depth 0-15cm.

The HUB for the dry season showed a significant increase in all the contaminated samples when compared with the control, Rumuoke recorded the highest count of microorganisms followed by Okilton, Elechi and then the control having the lowest count of HUB at depth 0-15cm.

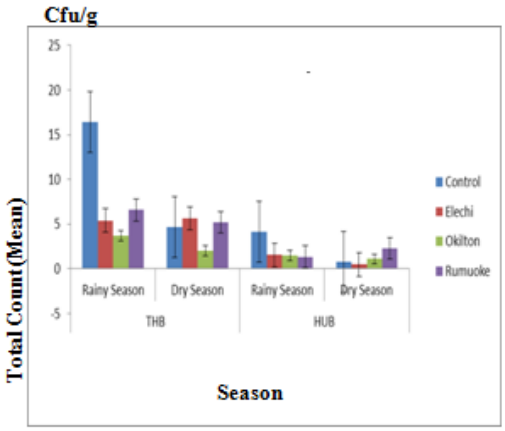

Fig. 3: Total Count of Bacteria for rainy and dry Seasons at 16-30cm depth

The THB isolated during the rainy season at the depth of 16-30cm in all contamined samples showed a significant difference with a decrease when compared with the control, while that of the dry season showed no significant difference with the control.

The HUB isolated during the wet season showed a significant difference with a slight decrease in all contaminated samples when compared with the control, while the HUB for dry season showed an increase at Rumuoke, while the control, Elechi and Okilton showed no difference at depth 16-30cm.

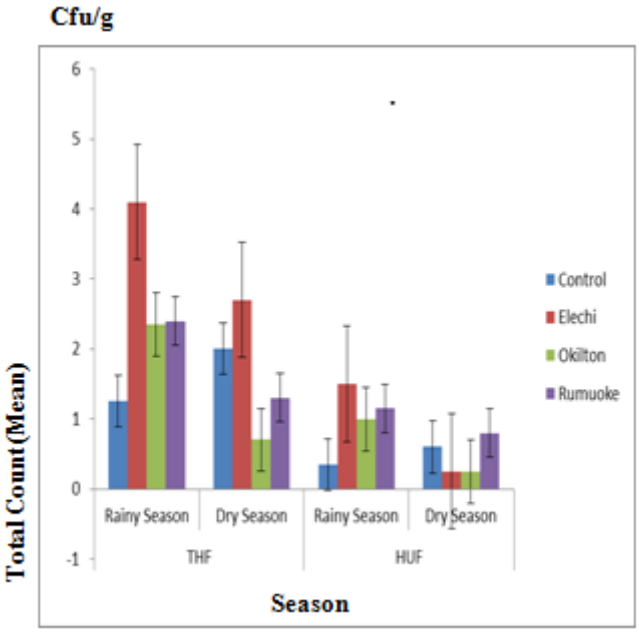

Fig. 4 Total Count of Fungi for rainy and dry Seasons at 0-15 depth

The Total Heterotrophic Fungi (THF) isolated in all the contaminated sampled area during the rainy season showed a significant difference with increase, Elechi recorded the highest fungal isolates, followed by Rumuoke and Okilton when compared with the control which showed the lowest counts at depth 0-15cm.

The THF for the dry season showed that the highest counts were recorded at Elechi, followed by the control and Rumuoke while Okilton recorded the lowest fungal counts at 0-15cm depth.

Hydrocarbon Utilizing Fungi (HUF) isolated during the wet season showed a significant difference with increase in all the contaminated sampled sites when compared with the control which showed lower populations at depth 0-15cm.

The HUF for dry season showed that the highest counts were recorded at Elechi, followed by the control, while the isolates from Okilton and Rumuoke showed equal fungi counts at depth 0-15cm.

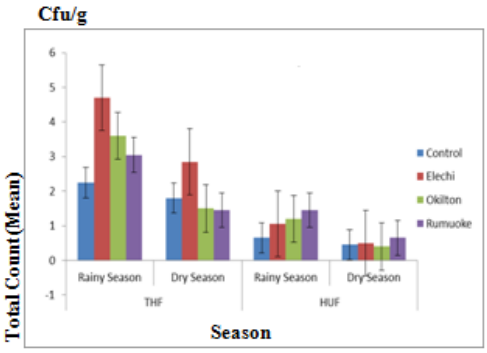

Fig. 5 Total Count of Fungi for rainy and dry Seasons at 16-30cm depth

The total heterotrophic fungi (THF) counts in all the contaminated sites at depth 16-30cm for rainy season showed a significant difference with increase compared with the control which showed a lower percentage of fungal isolates.

The THF for the contaminated sites during the dry season showed that Elechi had the highest counts of fungal flora, followed by the control, Okilton and finally Rumuoke respectively.

The results for hydrocarbon utilizing fungi for all the contaminated area during the wet season showed a significant difference in an ascending order, while that of the dry season showed no significant difference in all samples.

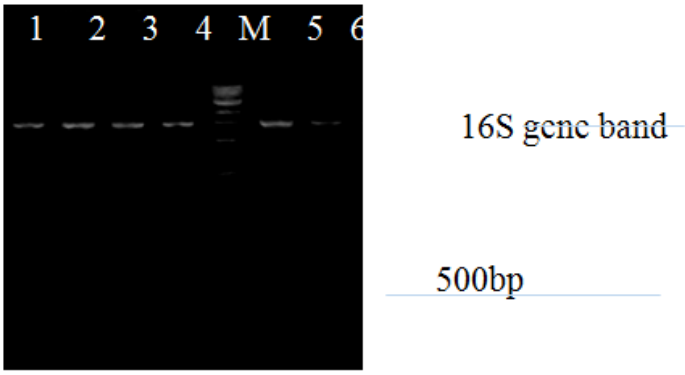

Fig 6: Agarose gel electrophoresis picture showing the 16S gene band (1500bp) of fungi isolates. Lane 1-4 and 5 and 6 represent the isolates while lane M represents Quick- Load 1kb molecular ladder.

Fig 47: Gel electrophoresis showing the *IMB* gene of bacterial isolate with no positive bands. Lane 1-10 represents the samples and lane M represents the 1000bp Quick-Load DNA molecular ladder

Molecular identification of bacterial and fungal isolates characterized the organisms which made possible to identify them to species level. The bacterial species isolated were *Plesiomonas shigelloides*, *Klebsiella pneunaniae*, *Pseudomonas aeroginoa*. *Bacillus licheniformis*, *Acinebacter specie* while the fungi included *Aspegillus alabamensis*; *Aspegillus terreus*, *Aspegillus flavus*, *Aspegillus aculeatus*, *Sarochadium hominis*, *Trichoderma capillare* and *Rodotorulla mucilaginosa*

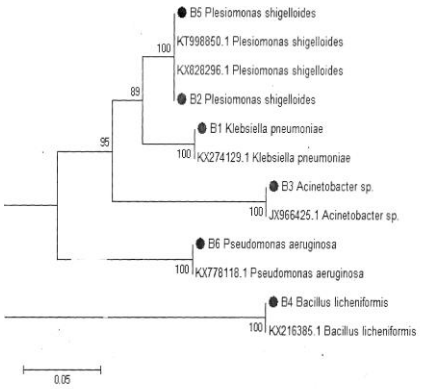

Fig 8: Phylogenetic tree showing relationship between the bacterial isolates

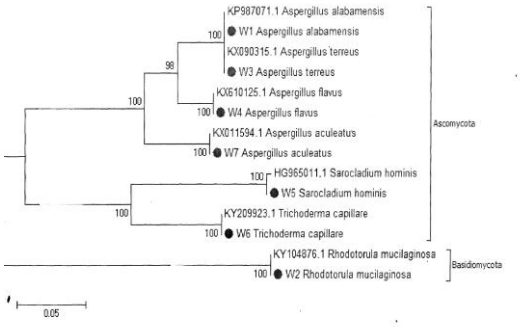

Fig 9: Phylogenetic analysis showing the evolutionary relationship between the fungal isolates

IV. Conclusion and Recommendations

Waste engine oil had negative effect on microbiological characteristics of the sampled soils and survival. Farmers should consider the environment before cultivation. The presence of this waste engine oil in the soil influenced microbial load negatively, its degradation is slow and there is limited microbes that utilize it as source of energy waste engine oil in the soil cause elimination of decomposers which liberate nutrients from dead organic matters. The spread of used engine oil in residential area and agricultural farmlands should be discouraged because of it has mutagenic and carcinogenic potentials and long lasting effects which may lead to unproductivity.

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