

Assessment of the Effect of Auto-Mechanic Activity on Microbial Population and Availability.

Abstract

The long-term presence of the resulting pollutants in the soil increases concerns about soil microorganism poisoning, which can lower soil fertility, which is dependent on both the type and quantity of microorganisms living there as well as the soil's chemical makeup. Contaminated soil samples were collected using a soil auger in a randomized method along transect of automobile mechanic workshop within the study area. Soil pollution from used engine oil has continued to pose threat to the existence of bacteria. Research has proven that these action from man affects negatively the population of bacteria by affecting respiratory processes. Laboratory results indicates that spent oil spill from automechanic activity has a direct effect on microbial population. It can hence be concluded that spent oil spill reduces microbial population in soil which can affect soil fertility.

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Keywords: soil fertility, microorganism, bacteria, climate change

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Introduction

In Nigeria, automechanic activities have significantly deteriorated the environment. According to predictions, Africa will be the first continent to face anthropogenic-induced climate change, particularly in highly polluted countries like Nigeria (Mwambazambi, 2010). Auto-mechanic activities in the vicinity of municipalities provide a portion of this anthropogenic pollution. Urbanization, population growth, and technology advancements are the main causes of this

pollution, which has negative consequences on the environment and has made Nigeria a major environmental concern (Nkwoada et al., 2018).

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Orji et al. (2015) state that self-employed artisan mechanics work at auto-mechanic repair stations to perform auto-mechanic (motor and motorcycle) repairs and maintenance services. The used petroleum products produced by these repair services mostly consist of used diesel, petrol (premium motor spirit, or PMS), and engine lubricating oil, which are utilized to remove grease from machine parts. These wastes are frequently dumped on soil, where they might have an impact on plant growth and the microbial community. According to reports, the information and knowledge gaps caused by low literacy levels in these places have been linked to pollution and environmental degradation (Adelana and Adeosun, 2011).

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According to Orjiakor et al. (2015), the percentage of vital components including microorganisms, soil water, soil air, and mineral compounds that are present in good quality soil defines its quality. Nonetheless, the amount of each of these components will probably vary depending on the kind of activity found in that particular soil. According to Orji et al. (2015), these waste materials made from petroleum and derivatives (from auto-mechanic service stations) are regularly dumped into the soils inside the service stations within a 2-meter radius. According to records, everyday careless disposal into the soil may not appear like much because the majority of auto-mechanic repair facilities are not cemented; nonetheless, the cumulative discharge over time may have an impact on the microbial community in the receiving soils.

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The impact of auto-mechanic operations and disposed-of waste is contingent upon the nature and amount of contaminants, as well as the physical, chemical, and biological characteristics of the contaminated soil, all of which contribute to the persistence of pollutants (Idemudia et al., 2014).

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The long-term presence of the resulting pollutants in the soil increases concerns about soil microorganism poisoning, which can lower soil fertility, which is dependent on both the type and quantity of microorganisms living there as well as the soil's chemical makeup. Most of the biological activity in the soil occurs in the upper layer, which is exposed to more contaminants. Orjiakor et al. (2015) found that auto-mechanic action resulted in a comparatively low microbial count in contaminated soil. It shows that the microbial population in the environment is unavoidably harmed by auto-mechanic activities that are now taking place in some parts of Nigeria.

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Materials and Methods

Study Area

Ozoro is the headquarters of Isoko North Local Government Area of Delta State. It is one of the administrative units of the Isoko regions in Delta State Nigeria situated at Latitude: 5.5383 and Longitude: 6.2161 with approximate population of 13,411 (at 2015) inhabitants and land mass of 1.136km². It is host to spills from petroleum products and subject to frequent flooding which helps in dispersing pollutants over a large area. Ozoro falls within the southern tropical evergreen forest zone and characterized by two climatic seasons. It comprises also of commercial activities and other municipal practices which causes environment pollution.

Collection of Sample/Preparation

Contaminated soil samples were collected using a soil auger in a randomized method along transect of automobile mechanic workshop within the study area. These sampling points have been subject to spent engine oil discharge and were labeled as samples A, B, C, D and E (Triplicate samples were collected). An additional soil sample from a point free from spent oil

discharge was obtained as sample control. At each sampling location, auger-boring instrument was used to bore holes of depths 5 - 25cm. The samples were homogenized in a clean plastic bucket, poured into a polythene bag, labeled adequately and transported to the laboratory immediately for analysis of the effect of auto mechanic activities on microorganisms.

Materials

Petri dishes, Test tubes, Pipette, Syringe/bent glass rod, Measuring cylinder, Agar bottle, Autoclave, Incubator, Oven, Colony counter

Reagents/Chemicals

- I. Growth media (Nutrient Agar for Bacteria and Malt Extract Agar for Fungi)
- II. Growth media (Mineral salt Agar – Bushnell Haas)
- III. Distilled water
- IV. Streptomycin (Antibiotic)
- V. Fungusol (Antifungal)
- VI. Carbon source (crude oil/diesel/petrol)

Methods

Determination of Total Heterotrophic Bacteria (THB)

1g of representative soil samples were weighed and diluted with 9ml sterilized distilled water in sterilized test tubes to make serial dilutions of 10^{-1} to 10^{-9} . 0.1ml aliquot from the 5th serial dilutions (mostly low concentration for bacteria) were collected and pour on already prepared nutrient agar plates. These plates were incubated at 37°C for 24 hours before colonies were enumerated or counted using colony counter.

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Image 1 :Laboratory analyses

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Determination of Hydrocarbon Utilizing Bacteria (HUB)

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1g of representative soil samples were weighed and diluted with 9ml sterilized distilled water in sterilized test tubes to make serial dilutions of 10^{-1} to 10^{-6} . 0.1ml aliquot from the 5th serial dilutions (mostly low concentration for bacteria) were pour on already prepared mineral salt agar (Bushnell Haas Agar) plates with antifungal (fungusol) added to suppress fungi growth. Ascetically, a filter paper saturated with sterile crude oil were placed on the inside of the inverted petri dishes of the cultured plate and then Incubate at 37°C for 4 days. Afterwards, the colonies on the plate were counted using colony counter.

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Determination of Total Heterotrophic Fungi (THF)

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1g of representative soil samples were weighed and diluted with 9ml sterilized distilled water in sterilized test tubes to make serial dilutions of 10^{-1} to 10^{-4} . 0.1ml aliquot from the 9th serial dilutions (mostly high concentration for fungi) were pour on already prepared malt extract agar plates and Incubate at $28\pm 2^{\circ}\text{C}$ for 4-5 days before counting using a colony counter.

Determination of Hydrocarbon Utilizing Fungi (HUF)

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1g of representative soil samples were weighed and diluted with 9ml sterilized distilled water in sterilized test tubes to make serial dilutions of 10^{-1} to 10^{-3} . 0.1ml aliquot from 3rd serial dilutions (mostly high concentration for fungi) were pour on already prepared mineral salt agar (Bushnell Haas Agar) plates with antibiotic (streptomycin) added to suppress bacteria growth. Ascetically, a filter paper saturated with sterile crude oil on the inside of the inverted petri dishes was placed on the cultured plate and Incubate at $28\pm 2^{\circ}\text{C}$ for 7 days before colony count on a colony counter.

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Results and Discussions

Results

Result from laboratory analysis of soil sample indicates considerable influence from automechanic operation. Soil samples were subjected to laboratory analysis with result indicated below;

Table 1: Result of Total Heterotrophic Microbial Count

TOTAL HETEROTROPHIC COUNT						
Microorganism	A	B	C	D	E	Control
Bacteria (cfu/g x10 ⁹)	3.67	3.12	2.77	3.04	2.94	4.89
Isolates	<i>Pseudomonas sp; Bacillus sp</i>					<i>Micrococcus sp; Bacillus sp;</i> <i>Staphylococcus sp;</i> <i>Pseudomonas sp.</i>
Fungi (cfu/g x10 ⁴)	0.33	0.24	0.20	0.21	0.22	0.51
Isolates	<i>Aspergillus sp; Tricophytonsp</i>					<i>Aspergillus sp; Mucor sp;</i> <i>Penicillium sp</i>

Table 2: Result of Hydrocarbon Utilizing Microbial Count

HYDROCARBON UTILIZING MICROORGANISM						
Microorganism	A	B	C	D	E	Control
Bacteria (cfu/g x10 ⁹)	0.64	1.33	0.40	0.97	0.64	1.64
Isolates	<i>Pseudomonas sp; Bacillus sp</i>					<i>Bacillus sp;</i> <i>Staphylococcus sp;</i> <i>Pseudomonas sp.</i>
Fungi (cfu/g x10 ⁴)	0	0	0	0	0	0.11
Isolates						<i>Aspergillus sp;</i>

Table 3: Microbial Isolates from Soil Samples

Samples	Morphological Test		Biochemical Test					Species Name	
	Grain Stain	Shape	Motility	Catalase	Coagulase	Oxidase	Spore		Indole
A, B, C, D, E and Control	-	Rod	-	+	-	+	-	-	<i>Pseudomonas sp.</i>
A, B, C, D, E and Control	+	Rod	+	+	-	-	+	-	<i>Bacillus sp.</i>
Control	+	Coccus	-	+	-	-	-	-	<i>Staphylococcus sp.</i>
Control	+	Coccus	-	+	-	+	-	-	<i>Micrococcus sp.</i>

Table 4: Geographical Coordinates of Sample Point

Samples	Coordinates
A	N5°32'53.94", E6°14'8.808"
B	N5°32'55.07", E6°13'40.182"
C	N5°52'50.20", E6°13'40.386"
D	N5°32'52.07", E6°13'40.7"
E	N5°52'55.07", E6°13'41.182"
Control	N5°32'47.42", E6°13'31.14"

Discussion

Soil pollution from used engine oil has continued to pose threat to the existence of bacteria. Research has proven that these action from man affects negatively the population of bacteria by affecting respiratory processes.

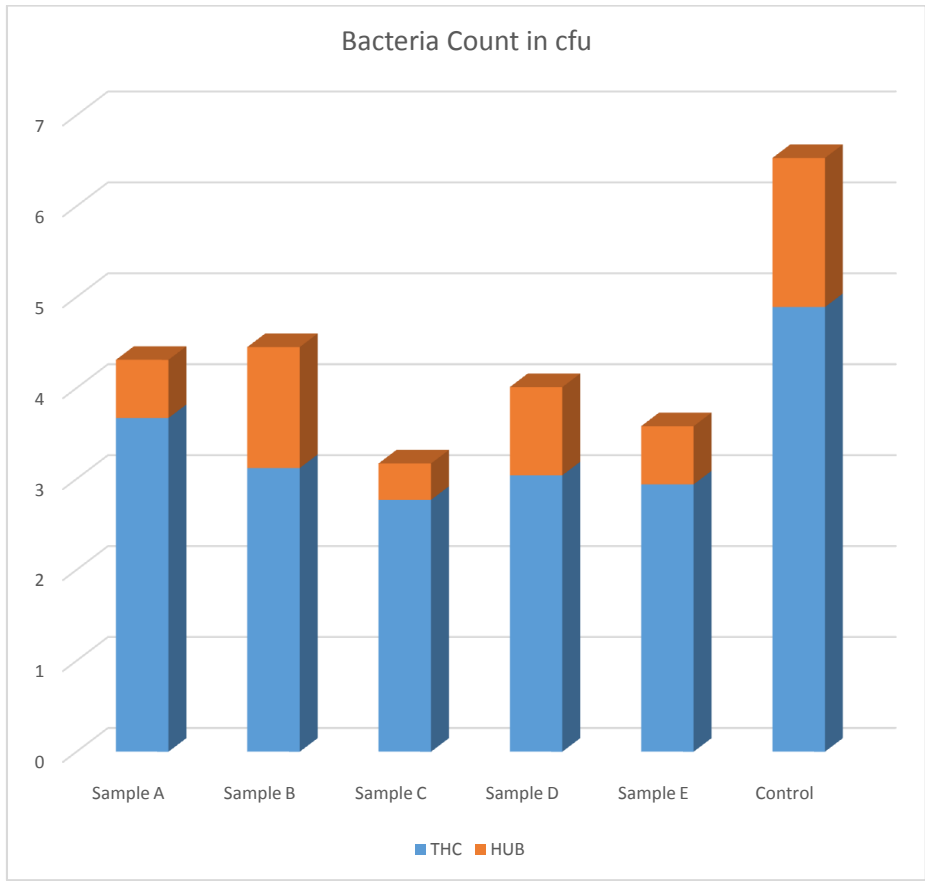


Fig. 1: Heterotrophic and Hydrocarbon Utilizing Bacteria Count

Result obtained from laboratory analysis indicates considerable reduction in bacteria population as a result of petroleum pollution around automechanic workshops in the study area. Orji *et al.*, (2015) reported similar results with decrease in number of heterotrophic bacteria of years of existence of workshops and reduction through counts on the numbers of hydrocarbon utilizing bacteria. Their study shows that there were more bacteria populations and isolated from soils in mechanic workshops abandoned for over a year than soils from the functional workshops showed that petroleum hydrocarbon discharged by mechanic into the soils have reducing

influence on the microbial population. As obtained in this study, reduction (in bacteria count of heterotrophic and hydrocarbon utilizing bacteria) can be attributed to pollution concentration as also revealed by Ekpo and Ebeagwu (2009). This is supported by report from Ayandele (2018) on the effect of spent oil discharge on soil microbial population.

Isolates from laboratory analysis were similar to those published by Orji *et al.*, (2015) and Emoghene and Eyong (2008) in which bacteria such as *Micrococcus sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Pseudomonas sp.* were identified in soil samples. However, laboratory analysis indicates that only *Pseudomonas sp.* and *Bacillus sp.* were identified in polluted soil samples and this is due to petroleum effects which has affected the existence of other species as compared to the control sample.

Petroleum pollution has continued to have its effect on population of fungi in soil especially in places where spills are persistent.

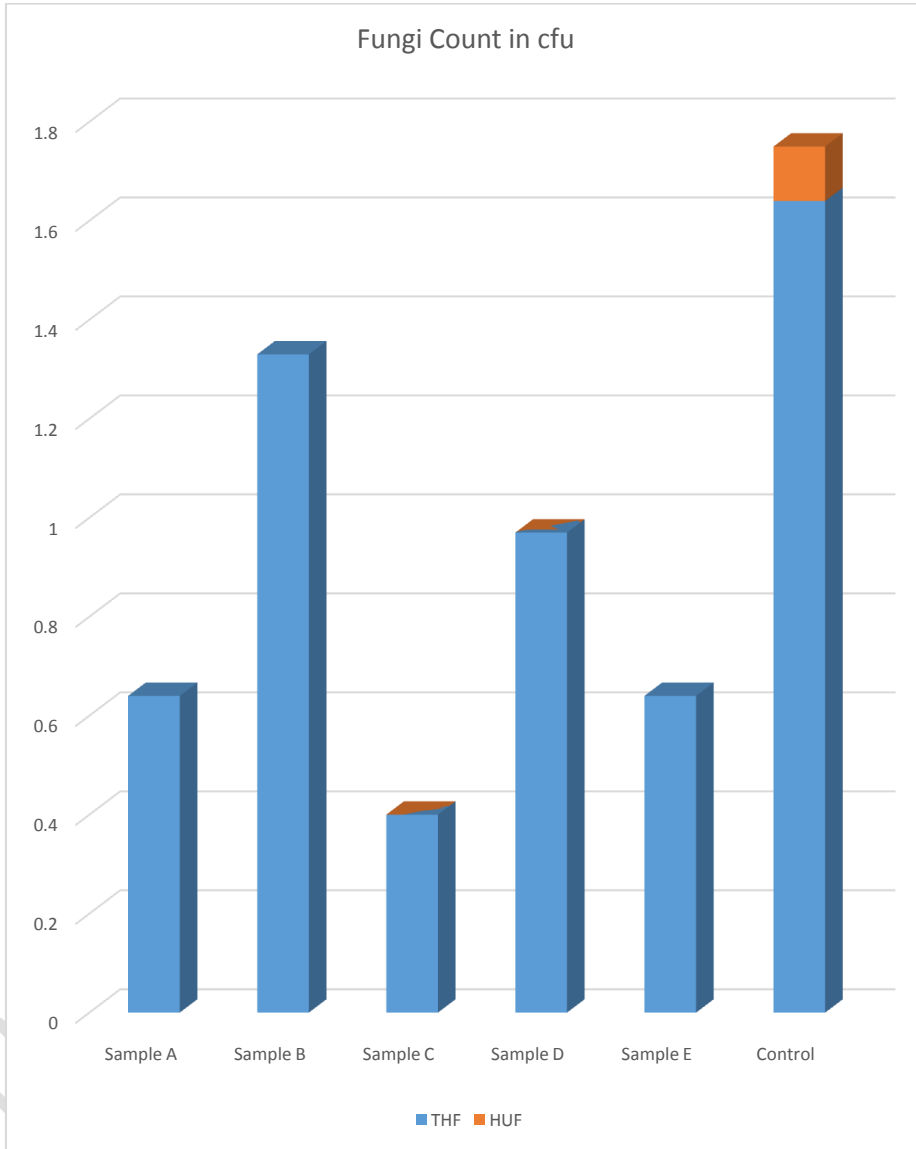


Fig. 2: Heterotrophic and Hydrocarbon Utilizing Fungi Count

Result on fungi pollution reveals that automechanic activities and its spill actions reduces fungi population. This is seen through reduction in fungi count for polluted and pollution free soil

(control). Similar report was made by Emoghene and Eyong (2008) in which *Aspergillus* sp. recorded decrease in count over concentration increase. Adeleye *et al.*, (2018) noted that fungi such as *Aspergillus* sp. are potential microorganisms responsible for crude oil hydrocarbon remediation. However, the degree of pollution was recorded to have had a major effect on fungi pollution as no fungi was recorded for hydrocarbon utilizing fungi count.

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Conclusion and Recommendations

Conclusion

Laboratory results indicates that spent oil spill from automechanic activity has a direct effect on microbial population. It can hence be concluded that spent oil spill reduces microbial population in soil which can affect soil fertility. Statistical test revealed that significant difference ($p < 0.05$) in fungi population exist between the observed and expected population count at 5% level of significance on pollution activities on the soil which kills both bacteria and fungi.

Recommendations

From the above conclusion, it is recommended that;

- I. Protection of soil microorganisms should be adopted through prevention of Spent oil spill around soil at automechanic workshop by setting up appropriate collection drums and containers.
- II. Analysis of effect on microbial population in soil along these stations should be done at greater depths to ascertain the leaching powers of petroleum products.
- III. Education of automechanic technicians on the adverse effects of unsafe/detrimental actions around the workstations should be conducted.

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References

- Adeleke, A. O., Nkereuwem, M. E., Omokhudu, G. I., Amoo, A. O., Shiaka, G. P. and Yerima, M. B. (2018).** Effect of Microorganisms in the Bioremediation of Spent Engine Oil and Petroleum Related Environmental Pollution. *J. Appl. Sci. Environ. Manage.* 22(2), 157 – 167. <https://dx.doi.org/10.4314/jasem.v22i2.1>
- Adelana, S., and Adeosun, T. (2011).** Environmental pollution and remediation: challenges and management of oil Spillage in the Nigerian coastal areas. *American Journal of Scientific and Industrial Research*, 2(6), 834–845. doi:10.5251/ajsir.2011.2.6.834.845
- Ekpo, M. A. and EbeAgwu, C. J. (2009).** The effect of crude oil on microorganisms and dry matter of fluted pumpkin (*Telfairia occidentalis*). *Scientific Research and Essay*, 4(8), 733-739
- Idemudia, M. I., Osula, A. N. and Odigie, O. (2014).** Comparative Assessment of Degradation Potentials of Bacteria and Actinomycetes in Soil Contaminated with Motorcycle Spent Oil. *Asian Journal of Science and Technology*. 5(8), 482-487
- Mwambazambi, K. (2010).** Environmental Problems in Africa: A Theological Response. *Ethiopian Journal of Environmental Studies and Management*, 3(2), 23-34.
- Nkwoada, A. U., Alisa, C. O. and Amakom, C. M. (2018).** Pollution In Nigerian Auto-Mechanic Villages: A Review. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 12(7), 43-54. DOI: 10.9790/2402-1207014354
- Orji, M. U., Ikechukwu, O., Nwaogo, M. C. and Nwagu, T. N. T. (2015).** Dynamics of Microbial Population in Petroleum-Impacted Soils in Automobile Mechanic Workshops in Awka, Nigeria. *International Journal of Research and Innovations in Earth Science*, 2(4), 122-126

Orjiakor, P. I., Atuanya, E. I., Mbata, T. I., Umar, A. F., Eze, C. N., Ajuzie, C. U., Ugboaja, M. J. and Ogu, G. I. (2015).Effects of Automobile Battery Wastes on Microbial Qualities of the Soil. *NISEB Journal*, 15(3), 188-122

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