Global Journal of Applied, Management and Social Sciences (GOJAMSS); Vol.24, April 2022; P.183 – 187 (ISSN: 2276 – 9013)

BACTERIOLOGICAL EXAMINATION OF OIL CONTAMINATED SOIL FROM AUTOMOBILE WORKSHOPS AT OBA, ANAMBRA STATE, NIGERIA

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Abstract
Automobile repair is a lucrative economic activity found across different cities across Nigeria. One of the major challenges that come with this business is unregulated allocation of these workshops and consequent oil pollution of the soil and surrounding environment. This study aimed at evaluating bacterial communities found in soil samples from automobile shops located at Oba, Anambra State. A 10 g portion of each soil samples (n=5) were examined using 1 in ten-fold serial dilution, culturing on nutrient agar, and characterizing the isolates using Gram stain and other biochemical procedures. Microbial counts were noted as well as percentage occurrence of isolates in each soil sample. Hydrocarbon degraders were also isolated using the vapour-phase transfer method. Staphylococcus, Bacillus, Pseudomonas, Streptococcus and Enterobacter species were identified. Soil samples from automobile sites B and E had the highest bacterial counts of 7.3 x 10^3 cfu/g, while Bacillus and Staphylococcus species were the most occurring isolates with occurrence of 100%. The vapour phase transfer method showed the presence of Rhodococcus, Campylobacter, Bacillus, Alcaligenes and Pseudomonas species as the hydrocarbon degraders present in the examined soil samples. This study thus showed that oil polluted soils from these mechanic workshops contain bacterial communities that can possibly biodegrade them through natural attenuation, if these sites are left to fallow for a given period of time.

Introduction

Oil released in the environment is a well recognized environmental problem in recent times. Oil spills affect many species of plants and animals in the environment, as well as humans. Spent engine oil is a common and toxic environmental pollutant in mostly automobile workshops. It is usually released during automobile oil changes and repairs at the workshops and also released by oil vendors and other engine repairers like power generator technicians into their surrounding environment.

Used motor oil contains aromatic hydrocarbons (such as phenol, naphthalene, pyrene, fluoranthene) that contribute to chronic environmental hazards as mutagens and carcinogens (Adeleye et al., 2018). Petroleum

products such as engine oil, premium motor spirit, diesel and dual purpose kerosene are daily used in automobile workshops for various repair functions, which end up contaminating the surrounding environment, changing soil colour, texture and possibly soil profile.

Bioremediation has been adopted as an alternative way to remedy oil polluted soils whether by remediation by enhanced natural attenuation (RENA) or by controlled bioremediation using microorganisms and other amendments. Bioremediation is defined as the use of microorganisms to remove or detoxify toxic pollutants from any polluted substrate or environment (Agu *et al*., 2015; Otobong and Ediene, 2016; Hambali *et al*., 2021; Achife *et al*., 2021). Adeleye (2018), reported that there exist more than 200 species of bacteria sometimes indigenous to the contaminated soils, that form a consortium of microorganisms that act on hydrocarbons and degrade them *in situ*. Some genera he reported are *Arthrobacter, Brevibacterium, Flavobacterium, Sporobolomyces, Achromobacter, Thiobacillus, Staphylococcus* amongst others. These microbes are believed to contain several abilities which include enzyme complexes production, surfactant production and genetic ability to hydrolyze hydrocarbons from spilled crude oil which is why they are used for bioremediation studies. This study aims to evaluate bacterial communities present in oil contaminated soils from automobile workshops located at Oba, Anambra state, Nigeria.

Methods

Sample Collection

A 10 g portion of soil samples from top surface soils, were collected from five different automobile workshops situated at Oba, Anambra State, Nigeria into plastic bags already sterilized with 70% ethanol, and sent to the laboratory for microbial analysis using modified method described by Eziuzor and Okpokwasili.(2009).

Isolation of Bacteria

Method described by Nwankwegu and Onwosi (2017) was used. Nutrient agar medium was used for the isolation of bacteria from oil polluted soil samples. A 1g portion of each soil sample was diluted using one-in-ten-fold serial dilution in sterile water. A 1 ml aliquot of 10^{-2} dilution factor was pour plated on sterile nutrient agar and incubated for 24 h at room temperature. Colonies developed on the plates were counted, and sub-cultured on fresh nutrient agar plates to obtain pure cultures which were stored on Nutrient Agar slants.

Biochemical Characterization of Bacteria Isolates

Bacterial isolates were characterized using Gram stain, motility test, coagulase, catalase, citrate, methyl red, voges prouskauer, oxidase and sugar fermentation tests.

Isolation of Bacterial Hydrocarbon Degraders/Users

The enumeration of bacterial hydrocarbon degraders (BHD) was done by using the vapour phase method described by Bento *et al*. (2005) and Orji *et al*. (2012). A 10^5 dilution of each soil samples was inoculated into modified mineral salt medium. The medium components in gl^{-1} are: $MgS0_4 \cdot 7H_2O$, 0.42; KCl, 0.297; KH_2PO_4, 0.85; $NaNO_3$, 0.42; K_2HPO_4,1.27; NaCl, 0.1; agar powder (Oxoid, United Kingdom), 20 g. These components were weighed out and were dissolved in 1000 ml distilled water in Erlenmeyer flask. The medium was sterilized by autoclaving at 121°C, 15psi for 15mins before pouring into sterile petri dishes to solidify. The solidified mineral salt agar (MSA) was inoculated separately with 10^{-1} and 10^{-5} dilutions of polluted soil samples. Whatman No. 1 filter papers were saturated with crude oil and the crude oil impregnated filter papers were aseptically placed onto the covers of the petri dishes while inverted. The hydrocarbon saturated filter papers supplied hydrocarbons by vapour phase transfer to the inoculum. The plates were incubated at room temperature (28 ± 2°C) for 5 – 7 days and colonies were counted and recorded in colony forming unit per gram (cfu/g). Each colony that developed on plates inoculated with 10^{-1} dilution was sub-cultured and pure cultures were identified.

Results

Isolation and Characterization of Bacteria from Soil Samples

Examination of soil samples showed the presence of *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus* and *Enterobacter* species as shown in Table 1. Soil samples from automobile sites B and E had the highest bacterial counts of 7.3 x 10^3 cfu/g, as shown in Table 2. *Bacillus* and *Staphylococcus* species were the most occurring isolates with occurrence frequency of 100% (n=5) as shown in Table 3.

Determination of Hydrocarbon Degraders

The vapour phase transfer method revealed the presence of *Rhodococcus*, *Campylobacter*, *Bacillus*, *Alcaligenes* and *Pseudomonas* species as the hydrocarbon degraders present in the examined soil samples as shown in Table 4.

Table 1: Identification of Bacteria Present in Examined Soil Samples

Cell shaape	Cocci	Cocci	Rod	Rod	Rod
Cell arrangement	Cluster	Colonies	Colonies	Colonies	Colonies
Motility	-	+	+	-	-
Gram	+	-	-	-	-
Catalase	+	+	+	+	+
Oxidase	+	-	-	+	-
H2S	-	-	-	-	-
Methyl red	-	+	-	-	-
Voges proskauer	-	-	+	-	-
Coagulase	+	-	-	-	-
Citrate	-	-	+	+	-
Glucose	G	G	G	G	G
Lactose	G	AG	AG	G	G
Presumptive organism	*Staphylococcus* species	*Bacillus* species	*Pseudomonas* species	*Streptococcus* species	*Enterobacter* species

+ = positive; - = negative; AG = acid and gas; G = gas production.

Table 2: Heterotrophic Bacterial Counts from Examined Soil Samples

Samples	Total Bacteria Count ($\times 10^3$cfu/g)
A	3.9
B	7.3
C	5.6
D	3.3
E	7.3

Table 3: Occurrence of Bacterial Isolates

Samples	*Bacillus* spp	*Pseudomonas* spp	*Staphylococcus* spp	*Streptococcus* spp	*Enterobacter* spp
A	+	+	+	+	-
B	+	-	+	+	+
C	+	+	+	-	-
D	+	+	+	-	-
E	+	-	+	+	-
Percentage	100%	60%	100%	60%	20%

+ = positive; - = negative

Table 4: Hydrocarbon Degraders Present in Examined Soil Samples

Isolates	Gram Stain	Motility	Citrate	Indole	Catalase	Coagulase	Oxidase	Methyl red	Vouges prouskeur	Sucrose	Maltose	Glucose	Lactose	Rhamnose	organism
A	+ve cocci	-	+	-	+	+	-	+	-	A/G	-	-	-	-	*Rhodococcus* sp.
B	-ve curved rod	-	+	-	+	-	-	+	-	A/G	A/G	A/G	A/G	A/G	*Campylobacter* sp.
C	+ve rod	+	+	+	+	-	+	+	-	A/G	A/G	A/G	-	A/G	*Bacillus* sp.
D	+ve rod	+	+	-	+	+	+	+	+	A/G	A/G	A/G	-	A/G	*Bacillus subtilis*
E	-ve short rod	+	+	-	+	+	-	-	+	A/G	A/G	A/G	A/G	A/G	*Alcaligenes* sp.
F	-ve rod in chains	+	+	-	+	-	+	-	-	-	-	A/G	-	A/G	*Pseudomonas aeruginosa*

Discussion

The present study showed that presence of *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus* and *Enterobacter* species in the oil polluted soil samples. This finding corresponds with that of Achife *et al*. (2021) who also identified *Bacillus*, *Streptococcus* and *Pseudomonas* from oil contaminated soil from Suleja, Minna Nigeria. It also corresponds with the reports of Agu *et al*. (2015), on their bacterial examination of oil polluted soil in Kwata, Awka, Anambra State. Heterotrophic bacteria counts showed that two workshop sites had the highest counts of 7.3 x 10^3 cfu/g which partly correspond with the reports of Hambali *et al*. (2021).

Highest occurrence of *Bacillus* and *Staphylococcus* partly corresponds with the review of Adeleye *et al*. (2018) on the effects of bacterial organisms on bioremediation of engine oil polluted soil.

Hydrocarbon degraders isolated from this study were *Rhodococcus*, *Campylobacter*, *Bacillus*, *Alcaligenes* and *Pseudomonas* species, this partly corresponds with the report of Hambali *et al*. (2021) who identified *Pseudomonas* and *Bacillus* as part of hydrocarbon degraders present in engine oil contaminated soil samples in Maiduguri, Nigeria. It also corresponds with that of Achife *et al*. (2021) who also reported *Bacillus* and *Pseudomonas* as hydrocarbon degraders.

Spent engine oil contamination of soil has been reported to affect soils adversely by increasing their organic matter content, altering soil pH to acidic and increasing heavy metal content of the polluted soil (Otobong and Ediene, 2016); (Adeleye *et al*., 2018). These implications adversely affect arable soils after a long period of time, thus rendering them infertile. It is also good to know that these contaminated soil samples contain in them some indigenous hydrocarbon degraders that can remediate the soil back to agricultural usefulness. However, it is advised that town planning officers be made to properly allocate selected areas for automobile workshops to be situated, so as to discourage scattered workshop allocation patterns in the town being witnessed at the this current time. This will help to control areas affected by oil spills.

Conclusion

This study has been able to show the microbial communities present in engine oil contaminated soil samples from mechanic workshops at Oba, Anambra State. It has been shown that these contaminated soil samples contain in them bacterial organisms that can perform bioremediation of the oil pollution, if the right conditions be provided.

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