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Isolation and identification of bacteria from engine oil polluted soil from mechanic workshops in Okija Town

Abstract

This research was conducted for the isolation and identification of bacteria from engine oil polluted soil from mechanic workshops in Okija village. Two different mechanic workshops within Okija were selected and soil samples were collected differently based on top soil and depth soil from each site. Then 0.1ml was taken from the dilution 10-3 to 10-6 diluents and inoculated into a Bushnell Haas agar (containing: (g/l) 0.2, MgSO4; 0.02, CaCl2; 1.0, KH2PO4; 1.0, K2HPO4; 1.0, NH4NO3; 0.05, FeCl3; 20.0, Agar and 5ml of fresh engine oil as sole carbon source) and was incubated at 370C for 5 -7 days. Spread plate method involving the use of serial dilutions was employed for the isolation of bacteria. The culture was examined for bacterial growth and subsequently cultured the specific bacteria growth on nutrient agar plates with 2ml of fresh engine oil for 24 hours to determine engine oil degradability by the isolated bacteria. The numbers of viable bacterial count were 20, 26, 18 and 28 as expressed in colony forming units per milliliter (cfu/ml). The bacterial count was furthermore expressed in percentage per the dilution factor 10-3; S1Top, S1Depth, S2Top and S2Depth were 21.74%, 28.26%, 19.56% and 30.44% respectively. The bacteria species isolated were Serratia, Micrococcus, Pseudomonas and Bacillus species. Bacillus was the most dominant. There was presence and activity of bacteria species in the depth soil than top soil. The bacterial species isolated were able to degrade fresh engine oil especially Bacillus species. From, the data obtained it was found that depth soil has more presence and activity of bacterial species because there is less disturbance in the depth soil than top soil. Bacillus species and Pseudomonas species are most adapted to conditions present in soils polluted with used engine oil and hence could be exploited in bioremediation activities in cases of accidental oil spillage.

Keywords: engine oil, polluted, isolate, degrade, bushell haas

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Osuji MI,[1] Ogbulie JN,[1] Nweke CO,[1] Nwanyanwu CE,[1] Ndiukwu PC,[2] Udeogu CV[3]

[1]Federal University of Tehnology Owerri Imo State, Nigeria
[2]Legacy University Okija Anambra Sate, Nigeria
[3]Nnamdi Azikiwe University Awka Anambra State, Nigeria

Correspondence: Osuji MI, Federal University of Tehnology Owerri Imo State, Nigeria, Email malachyosuji760@gmail.com

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Introduction

The spillage of oil is a constant threat to the global environment.[1] The production, transportation and consumption of petroleum together enable a myriad of avenues for which oil enters into the environment.[2] Oil spillage also impacts the industrial, commercial and residential sectors posing issues of operational productivity and environmental impact for refineries, wastewater plants and motor vehicles use, respectively.[3] Much like the bed of any ocean floor, terrestrial- soil microbial organisms capable of preferentially metabolizing oil exist and flourish and have so for millions of years.[1] Soil environments richly composed of diverse population of microorganisms which can fully metabolize entire hydrocarbon chains of different types of petroleum oil. As a class, microorganisms specialized in degrading a wide range of petroleum oils from gasoline to jet fuel to skin moisturizers.[1]

Many bacterial genres like that of *Pseudomonas*, Acinetobacter, *Micrococcus*, *Bacillus*, *Serratia*, and Clostridium specifically metabolize petroleum oil. Although motor, diesel and kerosene petroleum oils are highly volatile, they are largely and readily biodegraded by most soil bacteria and or fungi.[3] As microorganisms in soil are present in lower concentrations prior to the spillage of oil, the microorganisms augment their numbers during a lag phase in adjustment to oil volume and concentrations once oil is introduced.[3] While some strains of bacteria prefer certain grades of oil, not all strains are selective. As oil volumes released into the environment are brought under control, so should the potency of bacteria population with the depletion of primary carbon source.[1] Oil is best metabolized by microorganisms when other microbes are non-competitive and oil is light and viscous, structurally branched or occurring in liner chains and warm climates of soils rich in oxygen, and nitrogen and phosphorus.[1]

Engine oil could simply be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine.[4] Used engine oil represent oil that has undergone destructive changes in the property when subjected to oxygen, combustion gases, and high temperature. The said oil also undergoes viscosity changes as well as additive depletion and oxidation (Mark *et al*., 2018). The disposal of spent engine oil (SEO) into gutters, water drains, open plots and farms is a common practice in Nigeria especially by motor mechanics.

Materials and methods

Sample collection

The study sites are two different mechanic workshops situated at different locations in Okija town. The locations include the mechanic workshops along Nkwo Market and the other at Umuofor Street in Okija town of Ihiala LGA in Anambra State of Nigeria. Two soil samples were obtained from each workshop using a spatula to get top soil surface (S.Top) and the other by digging up the soil at 30cm deep (S.Depth). The physicochemical characteristics of the soil samples were analyzed. The various characteristics like texture, pH and temperature were taken for each of the samples. The samples; S_1Top, S_1Depth, and S_2Top, S_2Depth along Nkwo Market and at Umuofor Street respectively were collected into a sterile petri dish and conveyed

to microbiology laboratory of Legacy University Okija within 24 hours of collection. Sealed engine oil (Sea Horse) was bought along the road for the practical as a carbon source. Physicochemical properties of the soil such as temperature and pH were measured using a thermometer and pH meter by Hanna respectively.

Apparatus and reagents used for the research

The apparatuses and reagents used for this research work include: test-tube, test-tube rack, conical flask, sterile pipette, bunsen burner, wire loop, petri dish, autoclave, mechanical shaker, micropipette, micropipette tip, spread rod, wire loop, microscopic slide, cover slip, microscope, colony counter. Bushnell haas agar, nutrient agar, sealed engine oil, distilled water, methylene blue, gram's iodine, ethanol, safranin, peptone water, simmon citrate agar, methyl red broth, vogues-proskauer broth, hydrogen peroxide, kovac's reagent, methyl red indicator, α-naphthol reagent, oxidase reagent, glucose, sucrose and lactose.

Media preparation

Bushnell Haas agar was formulated according to (Bushnell and Haas)[5] for the examination of bacterial contamination and for studying bacterial hydrocarbon deterioration.

Composition of bushnell haas agar	g/l
Magnesium Sulphate	0.200
Calcium chloride	0.020
Monopotassium phosphate	1.000
Dipotassium phosphate	1.000
Ammonium nitrate	1.000
Ferric chloride	0.050
Agar	20.000
Final pH (at 25^{0}C)	7.0 ± 0.2

Addition of sterile engine oil as carbon source. Suspend 23.27g of the media in 1000ml distilled water and was sterilized by autoclaving at 15lbs pressure (121^{0}C) for 15 minutes.

Isolation of bacterial cultures

The isolation of bacteria from engine oil polluted soils was carried out using Bushnell Haas Agar with an addition of 5ml of sealed engine oil (Sea Horse) as sole carbon source, which is an enriched medium for the isolation of engine oil degrading bacteria. One gram (1g) of each sample was measured into a test tube containing 9ml of distilled water to form stock. This was placed in a laboratory shaker (S20) for 3mins to homogenize the solution and this served as the stock solution. A ten-fold serial dilution was done up to ten diluents. Then 0.1ml from dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} were inoculated in duplicates on sterile Bushnell Haas Agar plates. Incubation was carried out at 37°C for 5–7 days. Colonies on the plates were counted using Colony Counter and purify by sub-culturing on nutrient agar containing 2ml of engine oil with sterile 50 mg/ml of fluconazole tablet (Lesanto Laboratories) incorporated into 100ml distilled water for the inhibition of fungi in the medium. The discrete colonies were stored in nutrient agar slant.

Biochemical screening of isolates

Biochemical tests were done on the four isolates and the results are shown in the table.

Physicochemical properties of polluted soil

The various properties like texture, pH and temperature were measured for each sample and the result shown in the below (Table 1).

Table 1 Physicochemical properties of polluted soils

S/No	Properties	S1Top	S1Depth	S2Top	S2Depth
1	Structure	Coarse	Fine	Coarse	Fine
2	pH	6.8	7.6	6.9	7.0
3	Temperature	20.9^{0}C	30.2^{0}C	30.3^{0}C	30.2^{0}C

Measurement of bacterial growth for top and depth of soil

Presence of bacteria growth on culture media (Bushnell Haas agar)[5] for top and depth soil in dilution; 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} were shown in the table below. (Tables 2, 3, 4, Figure 1, 2, & Table 5)

Table 2 Bacterial growth on bushnell haas agar for top and depth soil

S/No	Samples	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1	S1Top	+	-	+	-
2	S1Depth	+	+	+	-
3	S2Top	+	-	-	+
4	S2Depth	+	+	+	+

Table 3 Colony count of bacterial growth from top and depth of polluted soil on bushnell haas agar

S/No	Samples	Colony count (10^{-3})	Total Count (cfu/ml)	Percentage (%)
1	S1Top	20	2×10^{-1}	21.74
2	S1Depth	26	2.6×10^{-1}	28.26
3	S2Top	18	1.8×10^{-1}	19.56
4	S2Depth	28	2.8×10^{-1}	30.44
Total		92		100

Table 4 Morphological characteristics and microscopic observation of bacterial isolates

Bacterial Isolates	Morphology Characteristics	Gram reaction	Endospore reaction
Bacillus	Milky white colour, round, smooth, flat, entire, 0.5 -2µm	Gram-Positive rod	Positive
Pseudomonas	Greenish pigment, raised, smooth surface, convex, opaque, moist, 1.5 – 3.0µm	Gram-Negative rod	Negative
Micrococcus	Creamy-yellow, circular, convex, smooth surface, opaque 0.5 – 2.0µm	Gram-Positive cocci	Negative
Serratia	Bright red colour, smooth, convex, entire, round colonies, umbonate 0.5 – 0.8µm	Gram-Positive rod	Negative

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Table 5 Morphology & microscopic observation and biochemical identification of bacterial isolates

S/ No.	Catalase test	Indole test	Oxidase test	Motility test	Glucose	Lactose	Sucrose	Citrate Utilization test	Methyl Red	Vogues Proskauer	Bacterial Isolates
1	+	-	-	+	+	-	+	-	-	+	*Bacillus spp.*
2	+	-	+	+	+	-	-	+	-	-	*Pseudomonas spp.*
3	+	-	+	+	-	-	-	+	-	+	*Micrococcus spp.*
4	+	-	-	+	+	-	+	+	-	+	*Serratia spp.*

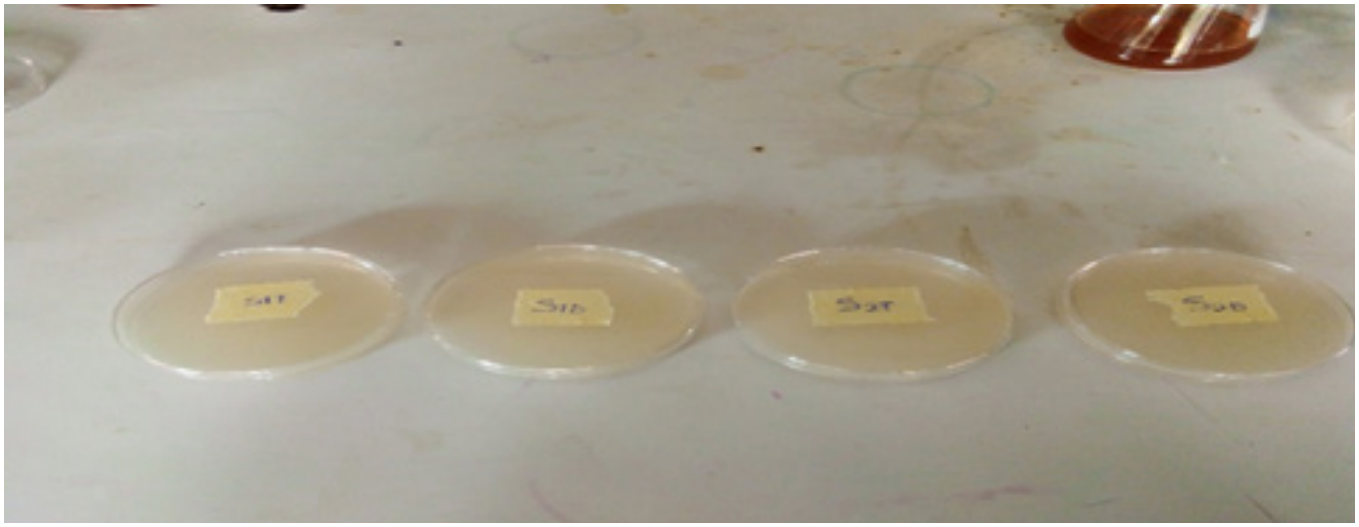

Figure 1 Inoculated plated before incubation.

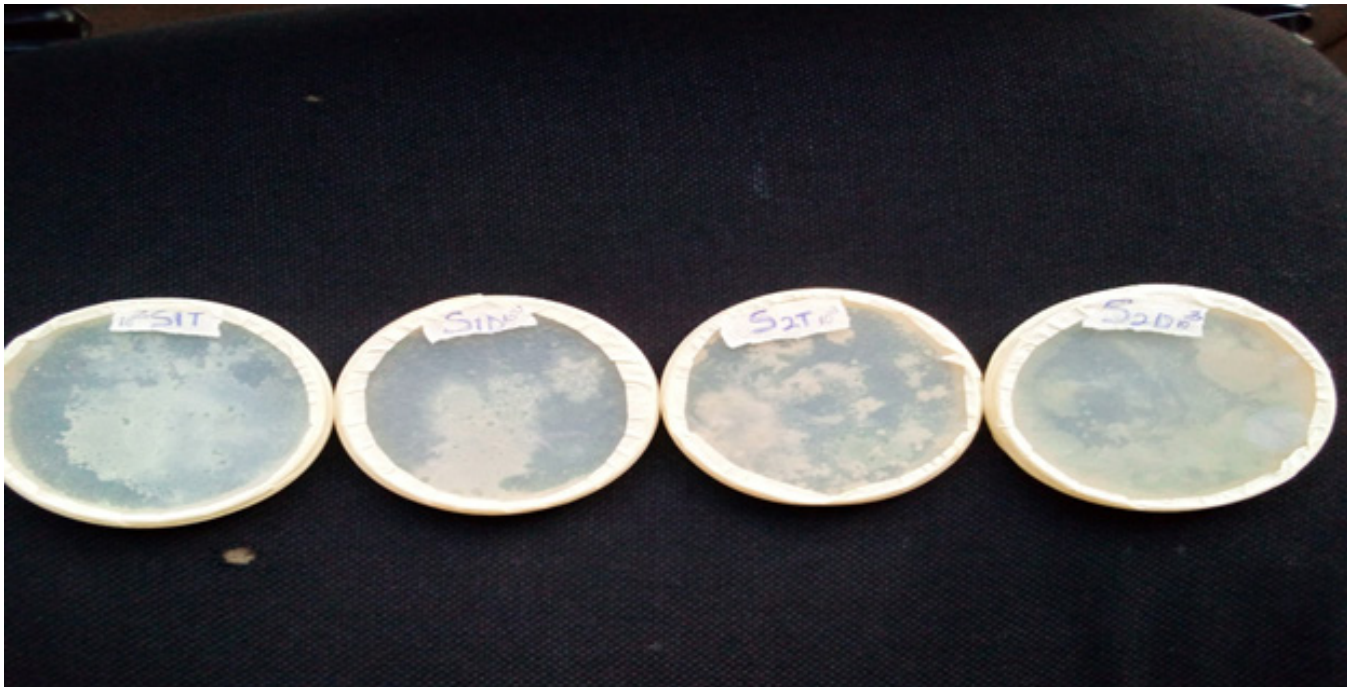

Figure 2 Bacterial growth on plates after incubation.

Engine oil degradability

From the morphological and microscopic characteristics, four oil degrading bacteria isolates; *Bacillus species, Pseudomonas species, Micrococcus species and Serratia species* were taken for culture in nutrient agar containing 1ml of engine oil by streaking method for degradation studies and were allowed to undergo degradation of engine oil for 3 days. The degradation of oil was examined according to the bacteria growth from day 1-3. The results were shown in the table below (Table 6).

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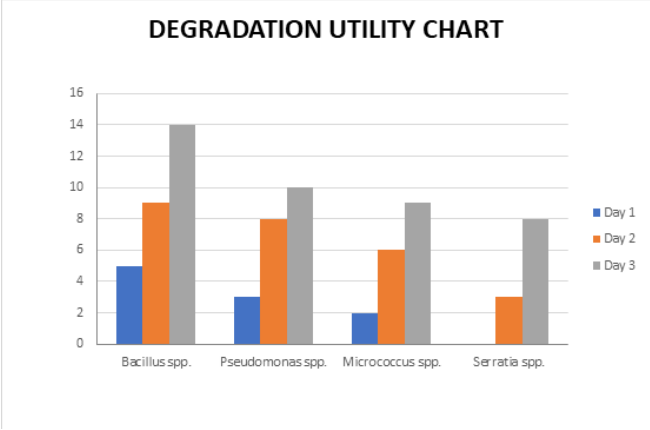
Table 6 Degradation of fresh engine oil by bacterial isolates

Bacterial Isolates	Day 1	Day 2	Day 3
Bacillus spp.	5	9	14
Pseudomonas spp.	3	8	10
Micrococcus spp.	2	6	9
Serratia spp.	0	3	8



Discussion, conclusion and recommendation

Discussion

The four (4) bacteria isolated from the soil samples are gram-positive *Bacillus*, *Micrococcus*, *Serratia* and gram-negative *Pseudomonas species*. The result indicated a higher activity of bacterial species from the depth soil than those from the top. The study also revealed that the colony count from soil samples S1Top, S1Depth, S2Top and S2Depth were (20, 26, 18, and 28) cfu/ml and expressed in percentage as 21.74%, 28.26%, 19.56% and 30.44% respectively. The bacterial species were examined for utility of fresh engine oil and the result revealed that Okija soil harbours hydrocarbon degraders which may have occurred as a result of indiscriminate exposure to used engine oil collected from motor vehicles, motor bikes and other machinery in Okija village. An increase in oil degradation was corresponding to an increase in cell number during the degradation processes demonstrating their ability to utilize engine oil as an energy source. The result is in correlation with the work reported by Mandri and Lin,[6] Khan and Rizvi, Ugoh, & Moneke[7] and Abioye *et al*.,[8] who isolated *Pseudomonas*, *Bacillus*, *Micrococcus* and other bacterial strains from engine oil contaminated soil. *Pseudomonas*, *Bacillus*, and Rhodococcus were isolated from engine oil contaminated soil as reported by Ogunbayo *et al*.,[9]

Conclusion

This research work has shown the presence of *Bacillus species*, *Pseudomonas species*, *Micrococcus species*, and *Serratia species* from engine oil polluted soil in Okija village. The result of this work indicates that indigenous bacteria isolates capable of degrading hazardous compounds found in polluted soil can be isolated in this area.[10–15] It also provides information based on the selection of top or depth soil for the isolation of indigenous bacteria that could be employed for biodegradation in environments polluted with engine oil. Furthermore, it revealed information on the physicochemical requirements of the soil for optimum degradation by these microorganisms. Finally, the bacterial utilization of fresh engine oil as carbon source resolved that oil-degrading bacteria are abundant in soils in Okija village and this can be exploited for large oil-spill clean-up campaigns and contracts.

Recommendations

The following are being proposed for consideration. Further research could be conducted to compare the performance or efficiency of oil degrading microbes in other locations. Similar research or study could be carried out on fresh engine oil in comparison with used engine oil as carbon source and the results compared for oil degradability of microbes, more research on oil degrading bacterial strain. Finally, further research could be carried out to determine the amount of engine oil utilizable by microbes in each time.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Okoh A. Biodegradation alternative in the cleanup of petroleum hydrogen pollutants. *Journal of Biotechnology and Molecular Biology*. 2006;1(2):38−50.
2. Banks MK, Shultz KE. Comparison of plants for germination toxicity tests in petroleum-contaminated soils. *Water, Air and Soil*. 2005;167(1-4):211−219.
3. Bossert I, Bartha R. The fate of petroleum in soil ecosystems. *Petroleum Microbiology: New York, NY.* 1984;3(2):6−8.
4. Shahida AA, Sambo S, Salau IA. Biodegradation of used engine oil by fungi isolated from mechanic workshop soils in sokoto metropolis, Nigeria. *Sky Journal of Soil Science and Environmental Management.* 2015;4(6):54−69.
5. Bushnell, Haas. The utilization of certain hydrocarbons by microorganisms. *J Bacteriol.* 1941;41(5):653−673.
6. Mandri T, Lin J. Isolation and characterization of engine oil degrading indigenous microorganism in Kwazulu, South Africa. *Journal of Bioremediation*. 2017;6(1):23−27.
7. Ugoh SC, Moneke LU. Isolation of bacteria from engine oil contaminated soils in auto-mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. *Academia Arena*. 2011;3(5):28−33.
8. Abioye OP, Agamuthu P, Abdulaziz AR. Biodegradation of used motor oil in soil using organic waste. *Biotechnol Res Int*. 2012;2012:587041.
9. Ogunbayo AO, Bello RA, Nwagbara U. Bioremediaton of engine oil contaminated site. *Journal of Emerging Trends in Engineering and Applied* Sciences. 2012;3(3):483−489.
10. Jyothi K, Surendara B, Nancy KC, et al. Identification and characterization of hydrocarbon degrading by molecular characterization. *Helix*. 2012;2:105−111.
11. Ravi A, Praveen RP. Isolation, biochemical, characterization and identification of oil degrading bacteria occurring in oil contaminated sites of mechanical workshops. *Int J Pure App Biosci*. 2016;4(6):102–106.
12. Sutton NB, Maphosa F, Morillo JA. Impact of long-term diesel contamination on soil microbial community structure. *Appl Environ Microbiol*. 2013;79(2):619–630.