ISSN 2581 – 4303

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2021; 12(2):73-79

RESEARCH ARTICLE

Isolation and Identification of Bacteria Associated with Lubricant Oil Contaminated Soil

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Received: 21 February 2021; Revised: 25 March 2021; Accepted: 15 April 2021

ABSTRACT

Environmental pollution with petroleum and petroleum products has been recognized as one of the significant causes of environmental pollution, and in concert to reduce these effects, bacterial organisms are known to adapt to these condition which could serve as potential bioremediators. Isolation and identification of bacteria associated with lubricant oil contaminated soil were carried out using two mechanic workshops and gasoline stations in Kebbi state, Nigeria. Ten grams of the sample soil were taken from each workshop and cultured using the conventional bacteriological isolation and identification technique. The bacterial organisms identified WERE *Staphylococcus aureus*, *Pseudomonas* spp., *Micrococcus* spp. *Lactobacillus fermentum*, *Proteus vulgaris,* and *Salmonella* spp. *S. aureus* was the dominant species having highest percentage prevalence 23.80%. *Pseudomonas* spp. had a prevalence of 19.04% while the organisms with the least percentage occurrence were *L. fermentum*, *Salmonella* spp., and *P. vulgaris* with 4.76% prevalence. This study examined the ubiquity of *S. aureus* showing ITS ability to adapt to conditions present in soil contaminated with lubricant oil.

Keywords: Kebbi, Lubricant oil, Public health, Soil, *Staphylococcus aureus*

INTRODUCTION

Bioremediation has become an alternative way to remedy oil polluted sites, where the addition of specific microorganism (bacteria, cyanobacteria, algae, fungi, and protozoa) or enhancement of microorganism already present, can improve biodegradation efficiency.[1] These microorganisms can degrade a wide range of target constituents present in oil sludge.[2] A large number of *Pseudomonas* strains capable of degrading polycyclic aromatic hydrocarbons have been isolated from soil.[3] Other petroleum hydrocarbon degraders include *Yokenella* spp., *Alcaligenes* spp., *Roseomonas* spp., *Stenotrophomonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Cyanobacterium* spp., *Capnocytophaga* spp., *Moraxella* spp., and *Bacillus* spp.[4] Other microorganisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer period of time to grow compared to their bacterial counterparts.[5]

Petroleum products such as engine oil, petrol, diesel, and kerosene are used daily in various forms in mechanic workshops. These products tend to harden and change the color of the soil, which may have untold health hazard on the technicians and artisans.

Used/waste engine oil is defined as used lubricating oils removed from the crankcase of internal

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combustion engines.[6] Before they are used, they consist of hydrocarbons (80–90% by volume) and performance enhancing additives (10–20% by volume). Engine oils are altered during use by vehicles, motor bikes, generators, and other machinery 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. It is recognized that the major components consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene, benz(a)antracene, benzo(a)pyrene, fluoranthene, lead, cadmium, and other potentially toxic metals.[6]

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. High concentration of aliphatic, polycyclic aromatic hydrocarbon, and heavy metals contributes to the inherent toxicity of used oil.[7] 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.[8]

Spent lubricating oil, when present in the soil, creates an unsatisfactory condition for life in the soil, which is due to the poor aeration it causes in the soil, immobilization of soil nutrients, and lowering of soil pH.[9] Various contaminants such as used engine oil and heavy metals have been found to alter soil biochemistry which includes alteration in soil microbes, pH, O_2, and nutrient availability.[8,9]

In spite of the increasing number of automechanic workshops in Birnin Kebbi, with their attendants indiscriminately dumping waste engine oil in the environment, we are not aware of any study that has attempted to isolate and identify bacteria present in used lubricant contaminated soil environment here in Birnin Kebbi. The present study was, therefore, undertaken with a view to isolate and identify bacteria in soil samples contaminated with used lubricant oil.

MATERIALS AND METHODS

Source of sample

The sampling site was two mechanic workshops and two filling stations in Birnin Kebbi Town, Kebbi state, Nigeria. The site includes the AP II filling station Haliru Audu Road, Oando filling station Sarkin Gabas Road, mechanic workshop along Unity Girls College Ibrahim Bashar Road, and Bayan Tasha Mechanic workshop Ahmadu Bello way in Birnin Kebbi, Kebbi state, Nigeria.

Sample collection

Using a sterile spoon, the samples was collected aseptically by scrapping the top most soil and the soil sample was then collected at about 4 cm down the ground. The sample was transferred into a sterile container and was fully labeled then taken to microbiology laboratory of the Federal University Birnin Kebbi, Kebbi state, Nigeria. All the samples were collected using the same procedure.[10]

Sample processing

After sample collection, the samples was homogeneously mixed and carefully sorted to remove unwanted and larger soil debris. Ten grams of the sample were measured using weighing balance and mix with 90 ml of sterilize distilled water in 500 ml sterilized beaker. The mixture was stirred to ensure proper mixing. The mixture was allowed to settle. One milliliter was drawn from mixture and serially diluted in five test tubes; each tube was containing 9 ml of sterile distilled water.[10]

Media preparation

Twenty-eight grams of nutrient agar solute were suspended in 1000 ml of distilled water,
in a conical flask which was then mixed very well and heated on hot plate to dissolve completely. The medium was autoclaved at 121°C for 15 min, allowed to cool at 45°C then 20 ml of the media was dispensed in each sterile Petri dish.[11]

Inoculation

From 10^{-2} and10^{-4} dilution factors of each of the samples, 0.1 ml was drawn and inoculated to two separate media plate by spread plate method and incubated at 37°C for the growth of aerobic bacteria, the same procedure was repeated but incubated in the anaerobic jar for 24 h for culturing anaerobic bacteria.[10]

Enumeration of bacteria

The total bacteria counts were determined by standard plate count method and the colonies were counted using colony counter.[12]

Isolation of bacteria

Distinctive colonies from different culture plate after 24 h were selected and further subcultured on nutrient agar using streak plate method. Cells from some selected colonies were picked up using sterile wire loop to one area by rubbing the wire loop across the surface of the agar, creating visible streaks. After streaking area one, the loop was sterilized and then rubbed across the edge of area 1 to make area 2. This procedure is repeated for area 3 and 4 and incubated at 37°C for 24 h while for anaerobic bacteria was incubated in the anaerobic jar for 24 h.[13]

Identification of isolates

The isolates were identified by Gram staining and biochemical tests which include Coagulase test, catalase test, indole test, motility test, and carbohydrate fermentation test.[11,12]

Gram stain

A thin smear of bacterial isolates was prepared on a glass slide, air dried, and then heat fixed by passing over a burner flame 3 times. The smear was covered with crystal violet stain for 60 s and flooded with water, it was then covered with Lugols' iodine and flooded with water after 60 s, and it was then decolorized with 95% acetone and flooded with water immediately for 10 s. The smear was covered with safranin and flooded with water after 60 s. The back of each of the slides was wiped out with cotton wool and allowed to air dry. The dried smear was then examined microscopically with oil immersion using objective lens of ×100.[11]

Catalase test

A drop of 3% or 6% hydrogen peroxides was placed on a glass slide. Then, a bit of growth of an isolate was picked from a solid medium with a sterilize wire loop and mixed with the drop of hydrogen peroxide (H_2O_2) on a glass slide and emulsified. The bubbles formed within seconds, indicates a positive test. This test is used to determine the ability of an organism to liberate (O_2) from H_2O_2.[11]

Coagulase test

Two drops of normal saline were placed on a clear glass slide. A colony was picked from an isolate and emulsified with the drops of the saline. Then, a loop full of plasma was added to the bacterial suspension on the slide and mixed. Clumping of the cells suspension mixed with plasma indicates a positive coagulation test, and for negative result, there was no coagulation.[11]

Indole test

This test was used to determine the ability of an organism to split indole from tryptophan (amino acid) which is present in peptone water. It is used as an aid in differentiation of Gram-negative bacilli. Pure cultures of isolate were inoculated into test tubes and incubated at 37°C for 48 h. After the incubation period, 2–4 drops of Kovac's reagent was added and shaken gently and allowed to stand for about 5 min to permit the reagent layer indicated indole production while no change indicated indole negative.[11]

Carbohydrate fermentation test

This test was carried out to determine the ability of an organism to utilize different carbohydrates. The test organism was inoculated into a purple broth or a phenol red tube with a sterile transfer loop. The tube was incubated at 35°C for 48 h before examination. The broth was observed for color

change and gas production. Bacteria that ferment carbohydrate produce acid or acid and gas as end products. If bacteria do not ferment a carbohydrate, the medium remains red.[11]

Motility test

The test organism was inoculated into the tube containing motility test medium with a transfer needle. The needle was inserted and withdrawn in a straight line in the center of the medium. The tube was incubated at 35°C for 48 h before examining the growth along the line. Bacteria cell with flagella are spread away from the line of inoculation. When the tube is held up to the light, growth is seen macroscopically as turbidity extending through the medium. Growth away from the line of inoculation indicates that organism is motile. Bacterial cells without flagella do not spread away from the line of inoculation, and this indicates that the organism is non-motile.[11]

Urease test

A speck of each isolate was inoculated into urea agar and incubated at 37° C for 72 h. Th e formation of red color indicates urease positive test while initial yellow color indicates negative.[11]

Table 1: Bacterial load from soil sample contaminated with lubricant oil isolated under aerobic condition

Sample number	Total plate count (cfu/ml)
1	1.06×10^7
2	1.33×10^7
3	4.78×10^6
4	5.10×10^4

Key
Sample 1 – Bayan Tasha Mechanic Workshop Amadu Bello Way
Sample 2 – Along Govt. Girls Unity Mechanic Workshop Ibrahim Bashar Road
Sample 3 – AP II Filling Station Haliru Audu Road
Sample 4 – Oando Filling Station Shehu Sarkin Gabas Road

Table 2: Bacterial load from soil sample contaminated with lubricant oil isolated under anaerobic condition

Sample number	Total plate count (cfu/ml)
Sample number 1	1.38×10^7
Sample number 2	1.09×10^7
Sample number 3	5.96×10^6
Sample number 4	4.96×10^6

Key
Sample 1 – Bayan Tasha Mechanic Workshop Amadu Bello Way
Sample 2 – Along Govt. Girls Unity Mechanic Workshop Ibrahim Bashar Road
Sample 3 – AP II Filling Station Haliru Audu Road
Sample 4 – Oando Filling Station. Shehu Sarkin Gabas Road

RESULTS

Table 1 shows the bacterial load from soil sample contaminated with lubricant oil isolated under aerobic condition. Table 2 also portrays the bacterial load from soil sample contaminated with lubricant oil isolated under anaerobic condition while Table 3 shows the Gram stain and biochemical identification of the bacteria isolated under aerobic condition. Table 4 also shows the Gram stain and biochemical identification of the bacteria isolated under anaerobic condition. Table 5 depicts the frequency and percentage of occurrence of the bacteria isolated under aerobic and anaerobic conditions per sample. Table 6 shows the overall frequency and percentage of occurrence of the bacteria isolated under aerobic and anaerobic conditions.

DISCUSSION

Bacteria associated with lubricant oil contaminated soil were isolated and identified using standard procedure. The colony count of bacteria isolated from lubricant oil contaminated soil under aerobic condition ranges between 5.10×10^4 and 1.06×10^7, as shown in Table 1, the sample number four (Oando Filling Station) has least colony count of 5.10×10^4 and the sample number two (Bayan Tasha Mechanic Workshop) has the highest colony count of 1.06×10^7. This result is relatively higher in comparison to the colony count reported by[14] Ugoh and Moneke, 2011, whose colony count ranges between 1.5×10^4 and 7.6×10^4, it is also identified by[15] Jane-Francis *et al*., 2008, work which shows the colony count of 6×10^4–49×10^4, while for anaerobic growth, the colony count ranges between 4.96×10^6 and 1.38×10^7, as shown in Table 2; the sample number four (Oando Filling station) has the least colony count of 4.96×10^6 and sample number one (Bayan Tasha Mechanic Workshop) has the highest colony count of 1.38×10^7.

In general, from this result, mechanic workshops tend to has higher colony count than filling stations, this may be due to excessive use of lubricant and their spillage on the soil in the garages.

Nine different bacteria species were biochemically identified from lubricant oil

Table 3: Gram stain and biochemical identification of the bacteria isolated under aerobic condition

Sample	Gram	Glu.	Suc.	Lact	Mot.	Indole	Urease	H_2S	Gas	Cat	Coa	Isolate
Sample 1	+rod	+	+	+	-	-	+	-	+	-	+	*Lactobacillus fermentum*
	+rod	+	+	+	-	-	+	-	-	+	+	*Staphylococcus aureus*
	- rod	+	+	-	+	-	+	+	+	+	+	*Proteus vulgaris*
Sample 2	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus* spp.
	+rod	+	+	-	-	-	-	+	+	+	+	*Micrococcus* spp.
	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus aureus*
Sample 3	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus aureus*
	+rod	+	-	-	-	-	-	+	+	+	+	*Micrococcus* spp.
Sample 4	+rod	+	+	+	-	-	+	-	+	-	+	*Lactobacillus* spp.
	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus aureus*
	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus aureus*

Key
Sample 1 – Bayan Tasha Mechanic Workshop
Sample 2 – Along Govt. Girls Unity Mechanic Workshop
Sample 3 – AP II Filling Station
Sample 4 – Oando Filling Station
Glu: Glucose test, Suc: Sucrose test, Lact: Lactose test, Mot: Motility test, Cat: Catalase test, Coa: Coagulase test

Table 4: Gram stain and biochemical identification of the bacteria isolated under anaerobic condition

Sample	Gram	Glu.	Suc.	Lact	Mot.	Indole	Urease	H_2S	Gas	Cat	Coa	Isolate
Sample 1	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus* spp.
	-rod	+	-	-	+	-	-	+	-	+	+	*Salmonella* spp.
	-rod	-	-	-	+	-	-	-	+	+	-	*Pseudomonas* spp.
Sample 2	-rod	-	-	-	+	-	-	-	+	+	-	*Pseudomonas* spp.
	-rod	-	-	-	+	-	-	-	+	+	-	*Pseudomonas* spp.
	+Cocci	+	+	+	-	-	+	-	-	+	+	*Staphylococcus* spp.
Sample 3	+rod	+	+	+	-	-	+	-	+	-	+	*Lactobacillus* spp.
	-rod	-	-	-	+	-	-	-	+	+	-	*Pseudomonas* spp.
Sample 4	-rod	+	+	-	-	-	-	-	+	+	-	*Klebsiella* spp.
	-rod	+	+	-	-	-	-	-	+	+	-	*Klebsiella* spp.

Key
Sample 1 – Bayan Tasha Mechanic Workshop
Sample 2 – Along Govt. Girls Unity Mechanic Workshop
Sample 3 – AP II Filling Station
Sample 4 – Oando Filling Station
Glu: Glucose test, Suc: Sucrose test, Lact: Lactose test, Mot: Motility test, Cat: Catalase test, Coa: Coagulase test

contaminated soil from different sites in Birnin Kebbi, namely, *Staphylococcus aureus*, *Micrococcus* spp., *Lactobacillus* spp., *Proteus vulgaris*, *Lactobacillus fermentum*, Klebsiella spp., *Salmonella* spp., *Staphylococcus* spp., and *Pseudomonas* spp., as shown in Tables 3 and 4. Some of these isolates are similar to those identified by[14] Ugoh and Moneke, 2011, which identified four different bacterial species, namely, *Micrococcus* spp., *Pseudomonas* spp., Serratia, and *Bacillus* sp. Ekanem and Ogunjobi,[16] 2017, also identified eight different species, namely; *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Micrococcus* spp., *Proteus mirabilis*, *Bacillus* spp., *Nocardia* spp., and *Acinetobacter* spp.

Six different species were isolated under aerobic growth condition which includes *Lactobacillus fermentum*, *S. aureus*, *P. vulgaris*, *Staphylococcus* spp., *Micrococcus* spp., and *Lactobacillus* spp.

Five different bacteria species were isolated under anaerobic growth condition, which include *Staphylococcus* spp., *Pseudomonas* spp., *Salmonella* spp., *Lactobacillus* spp., and *Klebsiella* spp. Out of nine different bacteria isolated two isolates were found under both

Table 5: Frequency and percentage of occurrence of bacteria isolated under aerobic and anaerobic conditions per sample

Isolate	Occurrence	Percentage
Sample 1		
Lactobacillus fermentum	1	16.66
Staphylococcus aureus	1	16.66
Proteus vulgaris	1	16.66
Staphylococcus spp.	1	16.66
Salmonella spp.	1	16.66
Pseudomonas spp.	1	16.66
Total	6	99.96
Sample 2		
Staphylococcus spp.	2	33.33
Micrococcus spp.	1	16.66
Staphylococcus aureus	1	16.66
Pseudomonas spp.	2	33.33
Total	6	99.98
Sample 3		
Staphylococcus aureus	1	25
Micrococcus spp.	1	25
Lactobacillus spp.	1	25
Pseudomonas spp.	1	25
Total	4	100
Sample 4		
Lactobacillus spp.	1	20
Staphylococcus aureus	2	40
Klebsiella spp.	2	40
Total	5	=100

Table 6: Overall frequency and percentage of occurrence of bacteria isolated under aerobic and anaerobic conditions

Isolate	Occurrence	Percentage
Lactobacillus fermentum	1	4.76
Staphylococcus aureus	5	23.80
Proteus vulgaris	1	4.76
Staphylococcus spp.	3	14.28
Salmonella spp.	1	4.76
Pseudomonas spp.	4	19.04
Micrococcus spp.	2	9.52
Lactobacillus spp.	2	9.52
Klebsiella spp.	2	9.52
	=21	=99.96

aerobic and anaerobic growth conditions, these isolates are *Staphylococcus* spp. and *Lactobacillus* spp. which are facultative anaerobes they can live and survive under both aerobic and anaerobic conditions.[17]

The frequency of occurrence of bacteria isolated from the soil contaminated with lubricant oil for both aerobic and anaerobic conditions shows that *S. aureus* has the highest percentage of occurrence of 23.80%, and it was found in all samples, followed by *Pseudomonas* spp. which has 19.04%, while *Salmonella* spp. has the least percentage of occurrence of 4.76%. These findings correspond with the ones[18] Makut and Ishaya, 2010, isolated, who also found *Pseudomonas* spp. with 19.0% and *Salmonella* spp. with least percentage of occurrences of 4.72%.

CONCLUSION

This investigation revealed that *Pseudomonas* spp., *Lactobacillus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Micrococcus* spp., *Lactobacillus fermentum*, *Salmonella* spp., *P. vulgaris,* and *Klebsiella* spp. were isolated from soil contaminated with lubricant oil obtained from two mechanic workshops and two filling stations in Birnin Kebbi, Kebbi state, Nigeria. *S. aureus* has the highest frequency and percentage of occurrence of 23.80% and *Salmonella* spp. has the least percentage of occurrences of 19.04%. The result of this study indicates that it is possible to isolate and identify bacteria associated with lubricant oil contaminated soil under aerobic and anaerobic growth conditions.

RECOMMENDATION

Further research needs to be conducted on the microorganisms associated with lubricant oil contaminated soil, so as to ascertain the pathogenic potential of the isolated bacteria. People visiting the mechanic workshops should employ safe hygienic measures. The possibility of using these organisms as potential biodegrading agents of lubricant or crude oil polluted environment.

REFERENCES

1. Hagwell IS, Delfino LM, Ras JJ. Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. Afr J Biotechnol 1992;8:6301-3.
2. Udeani KC, Obroh AA, Okwuosa C, Achukwu PU, Azubike N. Isolation of bacteria from mechanic workshops' soil environment contaminated with used

engine oil. Afr J Biotechnol 2009;8:6301-3.
3. Johnson HA, Pelletier DA, Spormann AM. Anaerobic degradation of monoaromatic hydrocarbons. Appl Microbiol Biotechnol 1996;64:437-46.
4. Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Mandal SC, Bhattacharya SK. Antimicrobial and anti-inflammatory activity of folklore: Mallotuspeltatus leaf extract. J Ethnopharmacol 2002;82:229-37.
5. Boldu P, Kuhn A, Luykx AM, Anke H, van Groenestijn JW, de Bont JA. Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycol Res 2001;105:477-84.
6. Jain PK, Gupta VK, Lowry M, Karolina DP, Chauhan UK. Bioremediation of spent oil contaminated soils using poultry litter. Res J Eng Appl Sci 2009;3:124-30.
7. Vasquez-Duhalt R, Bartha R. Isolation of bacteria from engine oil contaminated soils in auto mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. Acad Arena 1989;3:28-33.
8. Odjegba VJ, Sadiq AO. Isolation of bacteria from engine oil contaminated soils in auto mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. Acad Arena 2002;3:28-33.
9. Atuanya E. Effect of oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated delta soil in Bendel State, Nigeria. J Appl Sci 1987;55:155-76.
10. Samson RA, Frisvad JC. New taxonomic approaches for identification of food-borne fungi, International biodeterioration and biodegradation. Int Bio1993;32:99-116.
11. Cheesbrough N. District Laboratory Practice in Tropical Countries. Cambridge, United Kingdom: Cambridge University; 2006.
12. Fawole OM, Oso AB. Bacterial Profile of Indoor Air of Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. ATBU J Sci Technol Educ 1998;6:27-33.
13. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG. The sequence of the human genome. Science 2001;291:1304-51.
14. Ugoh SC, Moneke LU. Isolation of Bacteria from engine oil contaminated soils in auto mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. Acad Arena 2011;3:28-33.
15. Jane-Francis TK, Akenji TN, Yongabi FN, Nkwelang G, Ndip RN. Lubricating oil-degrading bacteria in soils from filling station and auto-mechanic workshops in Buea, Cameron: Occurrence and characteristics of isolates. Afr J Biotechnol 2008;7:1700-6.
16. Ekanem JO, Ogunjobi AA. Hydrocarbon degradation potentials of bacterial isolated from spent lubricating oil contaminated soils. J Appl Sci Environ Manag 2017;21:973-9.
17. Stieglmeier M, Wirth R, Moissl-Eichinger C. Cultivation of anaerobic and facultatively anaerobic bacteria from space-craft associated clean room. Appl Environ Microbiol 2009;75:3484-91.
18. Makut MD, Ishaya P. Bcaterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria. Afr J Microbiol Res 2010;4:1608-702.