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Environmental Assessment of Petrophilic Bacteria Associated with Bioremediation and Biodegradation of Engine Oil Contaminated Soil in Maiduguri

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ABSTRACT

Soil contamination by petroleum hydrocarbons is a major problem resulting from activities related to petroleum industry, automobile service stations and accidental release of oil in the soil. Bioremediation/biodegradation can be considered as an effective aid to clean up oil spills, aside from conventional methods that are uneconomical and produce residues that harm the surrounding biota. There are an estimated over 250 mechanic shops with more than 5000 technicians in Maiduguri. Mechanics working therein frequently spill oil which is a potent source of immunotoxicants and carcinogenic to humans and animals. The aim of this study was to screen for petrophilic bacterial isolates from engine oil contaminated soil, that can utilize crude oil as sole source of carbon for growth in Maiduguri Metropolis. Ten isolates from engine oil contaminated soils in Bola and Tashan Kano areas of Borno State were obtained. The bacteria were individually cultured in Bushnell Haas Mineral Salt Medium devoid of carbon source except for crude oil for 21 days at 37^0C. Bacterial growth from visible increased turbidity was enumerated by CFU/g on nutrient agar. *Pseudomonas sp.* isolate exhibited relatively higher ability to grow on crude oil with TNTC followed by *Bacillus sp.,* 6.7×10^4, *Arthrobacter sp.,*6.5×10^4, *Flavobacterium sp.,*6.2×10^4, *Pseudomonas sp.,* 6×10^4, *Nocardia sp.,*5.9×10^4, *Acinetobacter sp.,* 5×10^4, *Bacillus sp.,* 5×10^4. *Proteus sp.,* 4.7×10^4 and *Flavobacterium sp.* 4×10^4. It is therefore concluded that all the bacterial genera present in this study are effective hydrocarbon oil degraders.

Keywords: Bioremediation, Biodegradation, Petrophilic Bacteria, Engine Oil, Soil

INTRODUCTION

Engine oil is made from a heavier and thicker petroleum hydrocarbon, used as lubricating oil, that has various substances enhanced with additives, particularly anti-wear additives, detergents and dispersants (Adsodun *et al.,* 2008, Nathalia *et al.,* 2020). It is used for lubricating internal combustion engines (Vitalina *et al.,* 2020). The main functions of engine oil are to reduce friction and wear on moving parts of a vehicle and to clean engine sludge, the functions of dispersants and varnish detergents (Nivedita *et al.,* 2020). Due to the chemical composition of engine oil, it constitutes a serious environmental threat to human and animal health (Ibe *et al*., 2021). Contamination of soil with used engine oil causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutation (Alvarez *et al*., 1991). In the body of humans or animals these chemicals are capable of mimicking the inherent actions of reproductive hormones and, hence, have the ability to disrupt the neuroendocrine system or the function of the gonads directly (Colborn *et al.,* 1993). Naphthalene a chemical component of used engine oil can cause hazardous effects to kidneys, hearts, lungs and the central nervous system (Mandri and Lin 2007., Mishra *et al.,* 2001., Propst *et al.,* 1999., Irwin *et al.,* 1997). The release of hydrocarbons such as used engine oil into the environment whether accidentally or due to human activities is the main cause of water and soil pollution in most regions of the world (Ajona *et al*, 2021).

Additionally, used engine oil reduces soil productivity and fertility by depositing aliphatic and aromatic hydrocarbons such as naphthalene, fluorine and other chemicals of crankase base oil into the soil. (Stegmann *et al*., 2011). It can also change the physical, chemical and microbiological properties of the soil (Okonokhua *et al*., 2007). Indigenous petrophilic bacterial can be isolated, identified, and be used to remediate these polluted soils and the chemicals deposited, from toxic

to nontoxic un-harmful substances (Effendi *et al*., 2018). Studies have however indicated that environmental pollution with petroleum and petrochemical products, complex mixture of hydrocarbons has been recognized as one of the serious environmental problems facing Nigeria (UNEP, 2011). Used engine oil stands responsible for contamination of soils with huge levels of aliphatic and poly aromatic and heavy metals (Nweke and Okpokwasili, 2004).

Spent engine oil contains aromatic hydrocarbons which is a toxic environmental contaminant (Dominguez-Rosado and Pichtel, 2004). When disposed into the soil as commonly practiced by motor mechanics, generator mechanics and engine oil sellers, it could contribute to chronic animal and human health hazards including mutagenicity and Carcinogenicity (Mandri and Lin, 2007). Studies have also indicated that contaminating the soil with engine oil may cause dermatitis and other skin disorders, including skin cancer to human and animal (English *et al.,* 2003). These products tend to also harden and change the colour of the soil, which have untold health hazard on artisans (Udeani *et al*., 2008). During rainfall, runoff of contaminated soil with engine oil from the mechanic workshops might be source of water to both animals and plants. Toxic components of the runoff water contain lead chromium, copper and zinc as components of the spent and spilled engine oil, these can directly kill some plants and animals (Delistraty and Stone, 2007). It can also become concentrated in plant and animal tissues (Moyo and Masika, 2009). Furthermore, concentrations of toxic compounds in plants and animal tissues consumed by humans may also be toxic to them (Moyo and Masika, 2009). Additionally, it can cause lead poisoning in livestock (Delistraty and Stone, 2007). Continuous contact with used motor oil has caused skin cancer in animals and swallowing of which may cause stomach cramps and diarrhoea (Moyo and Masika, 2009).

Microorganisms, mainly bacteria such as *Pseudomanas, Moraxella,Alcaligens, Oligella Acinetobacter***,** *Methylobacterium, Stenotrophomonas,Morganella, Flavobacterium* and *Bacillus sp,* have been isolated from hydrocarbon polluted soil and they play a significant role in bioremediation processes of this contaminated soils (Kaszycki *et al*., 2011). Microbial bioremediation as a process utilizes living organisms, mainly bacteria and fungi, green plants and their enzymes, to remove, degrade, detoxify and transform the environmental pollutants into less toxic form (Azubuike *et al.,* 2016, Kuppusamy *et al.,* 2017).

Maiduguri has an estimated 250 and above mechanic shops amounting to more than 5000 technicians. Mechanics working therein are always exposed to oily sludge which are potent immunotoxicant and carcinogenic (Propst *et al.,* 1999, Perez-Cadahia 2007). The release of used engine oil onto the soil by human activities in Bank of the North area (Bola) and Tashan Kano in Borno State pollutes the environment and constitute a serious environmental threat due to possibilities of contaminating nearby water bodies through runoff. There is paucity of documented information on confirmation of indigenous petrophilic bacteria around the study area. Therefore, this study was designed to assess petrophilic bacteria associated with bioremediation and biodegradation of engine oil contaminated soil in Maiduguri.

MATERIALS AND METHODS

Study Area

The present study was carried out at two locations in the Maiduguri Metropolitan Area, Borno State, Nigeria. These two locations have the greatest number of auto-mechanic workshops in Maiduguri. The areas are Bank of the North (Bola) area, and Tashan Kano areas.

Soil Sample collection

A simple random sampling technique was used to select 5 samples from each of the two locations. The samples were selected to represent each of the locations based on the convenient available contaminated soil. A total of 10 soil samples were collected from the two different automobile service stations.
Soil samples of top soil (0-15cm depth) were collected in a well labelled sterile polythene bag at Bola site (B1-B5) and Tashan Kano site (T1-T5). Samples were then transported to the Veterinary Microbiology Department Laboratory, University of Maiduguri for analysis.

Isolation of oil Degrading Bacteria

Bacteria capable of degrading oil were isolated from oil contaminated soils on mineral salt medium (Bushnell Haas enriched medium). The composition of Bushnell and Haas medium used for the isolation of oil metabolizing bacteria was prepared by adding (g/L): KH_2PO_4, 1; K_2HPO_4, 1; NH_4NO_3, 1; $MgSO_47H_2O$, 0.2; $FeCL_3$, 0.05; $CaCL_2.2H_20$, 0.02; Final pH-7.00.2 and Bushnell Haas agar with same composition but with the addition of agar 20 grams. The medium was autoclaved at 121^0C for 15 minutes in a sealed Erlenmeyer Flasks. Sterile crude oil was used as the sole carbon source at 1%. Contaminated soil samples of 2g was added to 99ml of Bushnell Hass medium containing 1% crude oil. Enrichment was carried out with rotary shaking at 120rpm for 7 days at 37^0C (Gunasinghe *et al.,* 2021)

At 7 days post incubation, 1ml of sample from primary enrichment was transferred to a fresh medium containing 1 % crude oil as the primary culture and continued to incubate. After several transfers, hydrocarbon oil degraders were isolated and enumerated by streaking the inoculum from final flask on Bushnell Haas (BH) agar with crude oil as the sole carbon source and incubated at 37^oC for 48hours. After incubation several colonies appeared and these were further purified by sub culturing onto Nutrient agar plates through streaking to get pure discrete colonies (Gunasinghe *et al.,* 2021)

Identification of Bacterial Isolates

The bacteria screened for hydrocarbon oil degrading abilities were identified based on Colonial morphology, Microscopic observations and Biochemical reactions

Gram Staining technique

Smear was prepared using each isolate then heat fixed. Crystal violet was applied on the bacterial smear for 30

seconds and then washed off by distilled water for 10 seconds. Lugol's iodine (mordant) was then applied on the smear and then kept for 30 seconds before washing off with distilled water. Smear was decolorized with 95% alcohol and then safranin (counter stain) was applied on the smear for 30 seconds before it was washed off with distilled water (Colaninno, 2021)

Microscopic Observation

Gram positive bacteria stained purple and gram-negative bacteria-stained pink from the counter stain upon observation.

Biochemical Tests

Biochemical tests were carried out to identify the bacteria with the aid of a microbiology laboratory manual. The bacteria tested were grown on nutrient agar plates, mannitol salt agar plates and McConkey agar plate (Jabir and Lahmood, 2021).

Motility, Indole Production Test

A motility indole (MI) semisolid medium was used to determine motility, indole production of bacteria. Fresh bacteria from a pure culture were used to inoculate with a sterile inoculating wire loop. The inoculating loop was stabbed 2/3rd way of the medium in the test tube approximately at the center. The medium was kept at 37^{o}C in an incubator for 24 hours. The growth and result were interpreted as follows (Aina *et al.,* 2021).

Motility: the bacteria was considered positive for motility if there was turbid growth spreading from the stab line in the media.

Indole Production: bacterial colony was cultured in peptone broth for 37^{o}C for 24 hours. Kovac's reagent was added at the end of incubation. Appearance of cherry red reagent layer indicated positive reaction for indole production while negative reaction was considered if the layer remains yellow or brown.

Catalase Test

Hydrogen Peroxide (catalase reagent) was dropped on a sterile clean dry glass slide. A bacterial colony was placed on the clean dry glass slide with a sterile wooden stick, and mixed. Production of bubbles (oxygen) within 5-10 seconds indicate positive for catalase activity.

Oxidase Test

Whatman filter paper (1mm) was soaked with the oxidase reagents (N, N, N'N'-tetramethyl-p-phenylenediamine. A pure culture of bacterial isolate was streaked on it. Within 1-30 seconds the appearance of purple colour shows a positive result.

Citrate Utilization Test

Simmon citrate agar medium was used to test for organisms that can utilize citrate. The bacteria from a pure culture were streaked on the slant of agar in a test tube. The change in colour was observed after an incubation period of 24hours at 37^{o}C. In positive reaction the colour of the medium is turned blue. No change in colour indicated negative result for citrate utilization.

MR-VP Test

Potassium phosphate broth (MR-VP broth) containing dextrose, peptone and potassium phosphate was inoculated by a loopful of bacteria isolate and incubated at 37^{o}C for 24 hours. The broth (10ml) was divided equally among two tubes to perform MR (methyl red) test and VP (Voges-Proskauer) test.

MR Test Reaction

Bacteria may have produced acid through the incubation period to suppress the phosphate buffer and make the broth acidic. Methyl red was added and the test was considered as positive if red colour is formed.

VP Test Reaction

Barritt's reagent A was added to an already incubated potassium phosphate broth and shaken slightly. Barritt's reagent B was added in equal amounts to reagent A. The test tube was kept still for 15 minutes. Appearance of a red colour on the reagent layer signified positive reaction.

Fermentation Test: Lactose, sucrose and glucose

Labelled test tubes were filled with triple sugar iron agar (TSI) containing glucose, lactose, sucrose and also iron, before slanting. Each tube was aseptically inoculated with pure bacteria culture from nutrient agar plates. The tubes were inoculated for 24 hours at 37^{o}C**.** The result was interpreted by observing change of colours accordingly. Alkaline slant/no change in butt (K/NC) i.e. Red/Red=glucose, lactose and sucrose non-fermenter. Alkaline slant/Alkaline butt (K/K) i.e. Red/Red=glucose, lactose and sucrose non-fermenter. Alkaline slant/acidic butt (K/A); Red/Yellow=glucose fermentation only, gas (+or-) H_2S (+or-). Acidic slant/acidic butt (A/A); Yellow/Yellow=glucose, lactose and/or sucrose fermenter gas (+or-), H_2S (+or-).

Hemolysis Test

Blood agar was streaked with bacterial isolate and incubated for 24hours. Alpha-haemolysis was indicated by presence of brown-green discoloration under the bacterial growth. Beta-haemolysis was observed from the clearing of red colour from the agar around the bacteria due to breakdown of RBC's in the agar. Gamma-haemolysis, here the lack of discoloration or clearing of the medium indicated gamma-haemolytic bacteria (negative for haemolysis)

Growth Analysis

The ability of these organism to utilize crude oil as sole carbon source was determined by observing growth in mineral salt medium (BH) with only crude oil as carbon source. The growth was visibly qualified by increased turbidity and quantitatively studied by measuring colony forming units on nutrient agar plates. CFU/g was measured using the formula

$$CFU/g = \frac{\text{number of colonies} \times \text{dilution factor}}{volume\ of\ culture\ plate}$$

RESULTS

The colony counts of different isolates on nutrient agar after its culture in Bushnell Haas mineral salt broth containing only crude oil as carbon source are presented in Table 1 below. Isolate B4 has the highest colony counts of 135 followed by T1 and B5 with 130 and 125 colony counts respectively. Isolates B1, B2, T3 and T5 have 100,100,119 and 120 colony counts respectively.

The Gram's staining results of ten isolates are presented in Table 2 below. Six out of the ten isolates were tested negative and they are B1, B3,B5,T2,T4 and T5. While the remaining isolates (B2,B4,T3 and T1) all tested positive for Gram's stain.

Table 1: Colony counts of different isolates on nutrient agar after its culture in Bushnell Haas mineral salt broth containing only crude oil as carbon source.

Isolates	Dilution	Colonies counted	CFU/g
B1	10^{-3}	100	5×10^4
B2	10^{-3}	100	5×10^4
B3	10^{-3}	95	4×10^4
B4	10^{-3}	135	6.7×10^4
B5	10^{-3}	125	6.2×10^4
T1	10^{-3}	130	6.5×10^4
T2	10^{-3}	94	4.7×10^4
T3	10^{-3}	119	5.9×10^4
T4	10^{-3}	TNTC	TNTC
T5	10^{-3}	120	6×10^4

TNTC = too numerous to count

Table 2: Gram's staining results of ten isolates

Isolate Designation	Gram's staining	
	Gram's	Morphology
B1	-ve	Rods
B2	+ve	Rods
B3	-ve	Rods
B4	+ve	Rods
B5	-ve	Rods
T1	+ve	Rods
T2	-ve	Rods
T3	+ve	Rods
T4	-ve	Rods
T5	-ve	Rods

KEY: +ve = Positive, -ve = Negative

The biochemical test results of ten isolates and their identification are presented in tables 3 and 4. The catalase test indicated a positive result for all the isolates. Oxidase test only indicated negative result for isolates B4 and T2. The motility test indicated a negative result for isolate B1 and T3 only. Indole production test indicated a negative test for isolates B1, B4,T4 and T5. The citrate utilization test indicated positive results for B4, T4 and T5. The hemolysis blood agar test indicated an α hemolysis result for T5, B4,B2 and B1. The Methyl-red test was only positive for isolates B4 and B5. The Presumptive organisms for the ten isolates (B1, B2, B3, B4, B5, T1, T2, T3, T4 and T5) were identified as *Acinetobacter sp., Bacillus sp., Flavobacterium sp., Bacillus sp., Flavobacterium sp., Arthrobacter sp., Proteus sp., Nocardia sp., Pseudomonas sp., and Pseudomonas sp.,* respectively.

DISCUSSION

In the current study, hydrocarbon degrading bacteria were isolated from engine oil contaminated soil based on their ability to utilize crude oil as sole carbon and energy source. The isolate that showed greatest growth in the crude oil and Bushnell Haas medium belong to *Pseudomonas sp*., with TNTC. Report has shown that the environmental exposure to hydrocarbon has a profound role in bioremediation/biodegradation abilities of microorganisms (Rahman *et al.,* 2002). Prince *et al.*1993 reported in his research findings about *Pseudomonas sp.,* as a common bacterium capable of degrading hydrocarbon. *Pseudomonas sp.,* was reported to be found in consortium of bacteria from soil that can degrade hydrocarbon in light fuel oil Prenafeta *et. al*. (2001). *Pseudomonas sp.,* have been associated with biodegradation of hydrocarbon and specifically petroleum products in many studies (Rahman *et al.,* 2002, Onwurah, 2003).

Table 3: Biochemical test results of ten isolates and their identification

Isolate Designation	Catalase	Oxidase	Motility	Indole Production	Citrate utilization	Haemolysis blood Agar
B1	+ve	+ve	-ve	-ve	-ve	α hemolysis
B2	+ve	+ve	+ve	+ve	-ve	α hemolysis
B3	+ve	+ve	+ve	+ve	-ve	γ hemolysis
B4	+ve	-ve	+ve	-ve	+ve	α hemolysis
B5	+ve	+ve	+ve	+ve	-ve	γ hemolysis
T1	+ve	+ve	+ve	+ve	-ve	γ hemolysis
T2	+ve	-ve	+ve	+ve	-ve	γ hemolysis
T3	+ve	+ve	-ve	+ve	-ve	γ hemolysis
T4	+ve	+ve	+ve	-ve	+ve	γ hemolysis
T5	+ve	+ve	+ve	-ve	+ve	α hemolysis

KEY: +ve = Positive, -ve = Negative, α = Alpha hemolysis, γ = Gamma hemolysis

Table 4: Biochemical test results of ten isolates and their identification

Isolate Designation	Methyl-red test (MR)	Voges-Proskauer test (VP)	Triple Sugar Iron (TSI)	Presumptive organisms
B1	-ve	-ve	A/A	*Acinetobacter sp.*
B2	-ve	-ve	K/A	*Bacillus sp.*
B3	-ve	-ve	K/A	*Flavobacterium sp.*
B4	+ve	-ve	K/A	*Bacillus sp.*
B5	+ve	-ve	A/A	*Flavobacterium sp.*
T1	-ve	-ve	K/K	*Arthrobacter sp.*
T2	-ve	+ve	K/A	*Proteus sp.*
T3	-ve	-ve	A/A	*Nocardia sp.*
T4	-ve	-ve	K/K	*Pseudomonas sp.*
T5	-ve	-ve	K/K	*Pseudomonas sp.*

KEY: +ve = Positive, -ve = Negative, A/A = Glucose, Lactose and/or Sucrose fermenter gas (+or-), H_2S (+or-). K/A = Glucose fermentation only, gas (+or-) H_2S (+or-). K/K = Glucose, Lactose and Sucrose non fermenter.

Pritchard *et al.* (1992), reported the organism *Bacillus sp.,* as a predominant isolate of crude oil utilizing bacteria characterized from highly contaminated soil sample. It was hypothesized that the organism *Bacillus sp.,* was more tolerant to high levels of hydrocarbons in the soil due to their resistant endospores (Roling *et al.,* 2002). Onwurah (2003), also reported the genera *Bacillus sp.,* having capabilities to utilize toxic components of crude oil for growth. Although biodegradation activity of the genera *Acinetobacter sp., Flavobacterium sp., Arthrobacter sp., Proteus sp., and Nocardia sp.,* on crude oil have been documented, in this study. *Acinetobacter sp.,* was reported to assimilate saturated and aromatic hydrocarbon in contaminated soil and inorganic compounds such as phosphate (Speight, 1991; Sepahi 2008).

Sarma and Sarma (2010), reported the organism *Acinetobacter sp.,* from crude oil infested fields as potential soils microbial strain that could be effective in the bioremediation of crude oil and its different compounds. *Acinetobacter sp.,* was also reported among the best hydrocarbon degrading bacteria in a study carried out by Onwa *et al.* (2018). In the study of Kumar *et al.* (2007), *Bacillus sp.,* were isolated from oil contaminated soil, and it was capable of utilizing hydrocarbon as sole carbon and energy source across a wide range of temperatures.

Vilayutham *et al.* (2012) isolated two hydrocarbon degrading isolates and identified them as *Pseudomonas sp.,* and *Bacillus sp*. In a study reported by Chikere and Ekwuabu (2014) *Bacillus sp.,* and *Pseudomonas sp.,* were extracted from soil contaminated with crude oil in Bodo community of Rivers State.

Bacillus and *Pseudomonas sp.* have been previously isolated from oil contaminated soil samples (Obire and Nwanbeta 2002; Eze and Okpokwasili, 2010; Ibiene *et al.,* 2011). Ibiene *et al.* (2011) identified *Pseudomonas* genus as the most proficient among hydrocarbon degrading microorganisms. This genus was reported to produce rhamnolipids which increases the surface area of hydrocarbons, thereby increasing bioavailability. The isolation of *Bacillus sp.,* in our current study is in accordance with the study of Okpokwasili and Okorie (1988), who reported the organism *Bacillus species* are predominant Gram-positive organisms found in soil contaminated with polycyclic aromatic hydrocarbons.

The total heterotrophic microbial populations that consist of various genera in this study have been detected in petroleum contaminated soil in other studies as well Adebusoye *et al.* (2008). The hydrocarbon utilizing bacteria genera isolated from (Tables 4.2 and 4.4) were *Pseudomonas sp, Bacillus sp, Acinetobacter sp, Arthrobacter sp, Flavobacterium sp,*

Proteus sp, and *Nocardia sp.* Okpokwasili and Okorie (1988), isolated similar hydrocarbon utilizing bacteria from the Niger Delta aquatic systems.

Chikere and Okpokwasili (2012), also reported similar findings in a study on petroleum effluents. The hydrocarbon degradation ability of *Acinetobacter sp.,* and *Flavobacterium sp.,* was recounted in a study by Onwa *et al.* (2018). The hydrocabonoclastic bacteria isolated in this study are six Gram negative bacteria and four Gram positive bacteria. Numerous researchers have correspondingly shown in different studies that mixed populations of bacteria with broad enzymatic abilities are required to degrade complex mixture of hydrocarbon such as crude oil in soil, marine, and sediments (Das and Chandran, 2011).

The isolation of more number of indigenous microbes proficient of utilizing hydrocarbon from a precise oil contaminated environment is habitually seen as an indication that those organisms are the efficient hydrocarbon degraders in that particular environment Okerentugba *et al.,* (2003), The microorganisms proficient of persisting in such environments are those that have developed physiological and enzymatic reaction which allow them to utilize the hydrocarbon constituents as substrates (Atlas *et al.,* 1995).

Some microorganisms are more abundant in areas of high concentration of hydrocarbons. These petrophilic micro floras are constantly utilizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. Individual organisms can utilize only a limited array of hydrocarbon substrates, therefore assemblages of the mixed populations of microorganisms with broad enzymatic capacities would be required to achieve effective biodegradation of petroleum hydrocarbons as is obtained in a natural environment. Some physiochemical parameters like $_P$H, total organic carbon TOC, and total hydrocarbon content THC, phosphorus, nitrate and nitrogen also makes bioremediation more effective. Studies revealed that the optimal $_P$H range for biodegradation is between 6-7 (Sarma *et al.,* 2010, Oko *et al.,* 2016, Aparna *et al.,* 2010). Microorganisms, which degrade petroleum hydrocarbons, are extremely efficient at certain temperatures, which control production of enzymes used by microbes during degradation pathway, therefore three types of enzymes are produced within the optimal temperature ranges identified as thermophiles above 50 ^{0}C, mesophiles 15^{0}C-45^{0}C, and psychrophiles below 20^{0}C.

Majority of microorganisms have an efficiency for petroleum hydrocarbon degradation at optimum temperature ranging between 20^{0}C and 35^{0}C (mesotherm), with which they give maximum degradation values. Moreover, the degradation process for specific compound needs specific temperature Das and Chandran (2011), Generally, temperature range determines the types of organisms, to a certain extent suitable to the process of biodegradation. Primarily, biodegradation is slow within cold environment and it highly influence microbial physiological properties. As a result of low temperature, the crude oil viscosity rises, the simple hydrocarbon volatility reduces and the solubility of these hydrocarbons rises. This makes the crude oil highly toxic, as well as resistant to be degraded by microorganisms.

Das and Chandran (2011), reported that hydrocarbon biodegradation of microbial activities increases with temperature between 30 and 40^{0}C within soil environments, range between 20 and 30^{0}C in some fresh water environments, and in the range 15-20^{0}C in marine environments. Therefore, the proficiency of microbial biodegradation is directly influenced by temperature ranges. Especially within the study area where the temperature range is above 40^{0}C.

Conclusion

The present study is a screening of microorganisms capable of utilizing crude oil as a carbon source. The study showed that *Acinetobacter sp., Bacillus sp., Flavobacterium sp., Bacillus sp., Flavobacterium sp., Arthrobacter sp., Proteus sp., Nocardia sp., Pseudomonas sp., and Pseudomonas sp.,* were isolated from soil samples contaminated with used engine oil in an auto mobile service station at Maiduguri metropolis, of Borno State Nigeria where the temperature range is above 40^{0}C. The outcome of this study indicates that indigenously it is possible to isolate bacteria micro flora capable of degrading multifaceted hydrocarbon compounds. This study provides information that would lead to selection of bacteria that could be employed for bioremediation in environment polluted with hydrocarbons. It can therefore be concluded that hydrocarbon oil degrading microbes are abundant in soils found within mechanic shops in Maiduguri metropolis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors Contribution

This study was conceived and designed by IUH and AJO. The study was carried out by IUH, AJO and IAA. Data was analysed by AJO, IS, FUY, AMH, FAA, NBA, AOT, OMN and FFAJ. The study was overseen by IUH. The final manuscript was read and approved by all authors.

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