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Full Length Research Paper

Bacterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria

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This investigation was carried out to determine the bacterial flora of soils contaminated with used oil (petroleum products) in Keffi town. Pour plate method involving the use of serial dilutions was employed for the isolation of the bacteria. Soil samples from 10 different mechanic workshops in Keffi town were obtained and plated out on plate count agar, eosin methylene blue agar, brilliant green agar, desoxycholate citrate agar and mannitol salt agar to isolate the bacterial species from the soils contaminated with petroleum products. The bacterial species isolated were *Pseudomonas* sp., *Streptococcus* sp., *Escherichia coli, Staphylococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Mycobacterium* sp., *Enterobacter aerogenes*, *Salmonella* sp., and *Micrococcus* sp. The hydrocarbon substrates (petroleum products) used were petrol, kerosene, diesel and engine oil. *Pseudomonas* sp., *Streptococcus* sp., and *Bacillus* sp., were found to utilize all the four petroleum products as their sole source of carbon and energy. *Staphylococcus* sp., and *Micrococcus* sp., utilized petrol, kerosene and diesel, while *Klebsiella* sp., and *Mycobacterium* sp., utilized only petrol and diesel. *Salmonella* sp., *E. aerogenes* and *E. coli* did not utilize any of the test substrates (Petrol, kerosene, diesel and engine oil). The results of this study revealed that *Pseudomonas* sp., *Streptococcus* sp., and *Bacillus* sp., are the most versatile species of bacteria that could utilized petroleum products in the soil environment of Keffi. The investigation demonstrates that *Pseudomonas* sp., *Streptococcus* sp. and *Bacillus* sp. could be harnessed for use in bioremediation of land polluted with petroleum and petroleum products.

Key words: Bacterial species, contaminated soils, used petroleum products, Keffi, Nigeria.

INTRODUCTION

The term petroleum came from two Latin words, Petra, “rock” and oleum “oil”. It is used to describe a broad range of hydrocarbons that are found as gases, liquids, or solids beneath the surface of the earth. The two most common forms are natural gas and crude oil.

Petroleum is a fossil fuel produced from the decomposition of decayed organic matter. Petroleum, like other fossil fuels consists primarily of a complex mixture of molecules called hydrocarbons (hydrogen and carbon). In large concentrations, the hydrocarbon molecules that make up petroleum products are usually highly toxic to many organisms, including human beings (Alexander, 1994).

Ojumu (2004) reported that the dominance of petroleum products in the world economy creates the condition for distributing large amounts of these toxins into areas having high human populations as well as other ecosystems around the globe. The most rational way of decontamination of the environment loaded with petroleum derivatives is by application of methods based on metabolic activities of microorganisms (Leahy and Colwell, 1990).

Microbial degradation is the major mechanism for the elimination of used petroleum products from the environment (Walker et al., 1977; Ibe, 1984; Atlas and Bartha, 1992). Barka and Atlas (1977) also reported that the ability to actively decompose specific fractions of petroleum oil is displayed by many microorganisms. Hydrocarbon degradation has been investigated in both complex mixtures as well as with individual model constituents while considering the material as it generally occurs in nature.

Studies of the degradation of petroleum and crude oil as mixtures are difficult to interpret because of the vast

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array of compounds present (Mitchell, 1992). Generally, the accepted pattern of susceptibility of hydrocarbon components to microbial degradation is n-alkane > branched alkanes > low-molecular weight aromatics > polycyclics. However, system-specific exceptions to this pattern have been found (Mitchell, 1992).

Some factors have been reported to influence biodegradation of hydrocarbon, which include soil, water, oxygen availability, redox potential, pH of the environmental, nutrient status and temperature of the affected areas (Leahy and Colwell, 1990). The strategies used by bacteria to degrade hydrocarbons have been found to involve the insertion of molecular oxygen into these carbon-rich structures catalysed by oxygenase enzymes (Atlas and Bartha, 1992). Aerobic conditions are therefore necessary for microbial oxidation of hydrocarbons in the environment.

Crude oil is a complex mixture that is between 50 and 95% hydrocarbon by weight. The first step in refining crude oil involves separating the oil into different hydrocarbon fractions by distillation (Mitchell, 1992). Since there are a number of factors that influence the boiling point of a hydrocarbon, these petroleum fractions are complex mixtures.

Pelczer (2004) reported that about 87% of the crude oil refined in 1980 went into the production of fuels as gasoline and kerosene, while the remainder went for non fuel uses, such as petroleum solvents, industrial greases and waxes or as starting materials for the synthesis of petrochemicals. Petroleum products are used to produce synthetic fibre such as nylon, Orlon, dacron and other polymers such as polystyrene, polyethylene and synthetic rubbers. They also serve as raw materials in the production of refrigerants, aerosols, antifreeze, detergents, dyes, adhesives, alcohols explosives, weed killers insecticides and insect repellents (Mitchell, 1992).

Hans (1993) reported that hydrocarbons are rapidly and completely degraded in well-aerated active soils. It is only when the contamination of the soil is severe or under anaerobiosis, or if the oil has penetrated to great depths, that there is any danger of its (soil) conservation and contamination of drinking water (Joanne et al*.,* 2008).

Petroleum spillage at sea presents an immediate danger to marine fauna and flora, but it is also subject to degradation by bacteria. However, residues of long-chain alkanes, polyaromatic hydrocarbons and asphalt-like mixtures may occur which can resist biological attack for considerable periods (Hans, 1993; Evans, 1947).

The physical state of petroleum hydrocarbons has a marked effect on their biodegradation. At very low concentrations hydrocarbons are soluble in water, but most oil spill incidence release petroleum hydrocarbons in concentrations far in excess of solubility limits (Atlas and Bartha, 1972). The degree of spreading determines in part the surface area of oil available for microbial colonization by hydrocarbon-degrading microorganisms in aquatic ecosystems where the oil normally spreads, forming a thin slick (Cohen, 2002). The degree of spreading is reduced at low temperatures because of the viscosity of the oil. In soils, petroleum hydrocarbons are absorbed by plant materials and soil particles, limiting its spread (Cohen, 2002).

Used motor oil is a common environmental contaminant. This is defined by the United State environmental protection agency as any oil that has been refined from crude oil or any synthetic oil that has been used, and as a result of such use is contaminated by physical or chemical impurities (U.S., EPA, 2001).

Used motor oil can cause great damage to sensitive environments and soil microorganisms. Hydrocarbons are rapidly and completely degraded in well-aerated active soil (Hans, 1993). Used oil may contain components such as Lead, Cadmium, Barium and other potentially toxic metals. Most of the microbial groups found in soil contaminated with used oil are bacteria and fungi (Joanne et al., 2008). Substantial volumes of soil have been contaminated by used oil in many countries of the world, especially industrialized nations. High concentration of aliphatics, polycyclic aromatic hydrocarbon and heavy metals contribute to the inherent toxicity of used oil (Vazguez–Duhalt and Bartha, 1989).

Bacteria and fungi are the primary agent for degradation of organic contaminants in soil (Alexander, 1994). Increasing diversity of microbial populations and common structure can accelerate the degradation of the contaminants (Cole and Liu., 1994).

Hydrocarbon soil degrading microorganisms require an environmental habitat that has a sufficient and preferably substainable source of nutrients, water, air, mild ambient temperature, and a moderate pH (Stegmann et al., 1991). At optimum levels these environmental factors provide the energy and metabolic resources that create a widely diverse group of beneficial microorganisms that will suddenly reproduce on a very rapid scale (Rahman et al., 2002). While these degrader microorganisms increase their populations, they also work rapidly and effectively to degrade petroleum hydrocarbons for food (from carbon) to substain their growth pattern (Stevenson, 1994; US, EPA, 2001).

Hydrocarbon utilizing microorganisms are important in combating the problem of oil pollution in our environment (Atlas and Bartha, 1992). The fate of hydrocarbons in the soil and aquatic environments depends on the distribution of hydrocarbon-utilizing microorganisms in the affected areas. Although, hydrocarbon-utilizing microorganisms are ubiquitous, their proportion within the microbial community is thought to be a sensitive index of environmental exposure to hydrocarbons (Leahy and Colwell, 1990). Hydrocarbon-utilizing microorganisms are widely distributed and most of them can be found in arable, pasture and forest soils. The ability to utilizing mineral oil as an energy source is not restricted to a few species of microorganisms but it is rather found in numerous species of bacteria. Buckley et al. (1976)

reported that liquid aromatic hydrocarbons were utilized by bacteria at the water-hydrocarbon interface, but solid aromatic hydrocarbons were not metabolized. Wodzinasky et al (1977) reported that at 30°C diphenyl ethane is liquid and could be degraded at 20°C, while the solid form of diphenyl methane could not be utilized by *Pseudomonas* species. Atlas et al. (1976) also found that naphthalene could not be utilized in the solid form but could be utilized if dissolved in a liquid hydrocarbon. Alexander (1994) reported that there are certain bacteria which utilize petroleum hydrocarbons. Some of these microorganisms had been isolated from soils environment and these include *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp. and *Streptococcus* sp.

This investigation is aimed at determining the bacterial species found in the soils contaminated with used oil in Keffi town in Nasarawa State of Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in Keffi town, Nasarawa State, Nigeria. Keffi is about 58km from Abuja (the Federal Capital of Nigeria), and is situated on latitude 8°5'N and longitude 7°50'E. The town (Keffi) is located on the altitude of 850 m above sea level and it is in the North-west of Lafia, the capital of Nasarawa State (Akwa et al., 2007).

Sample collection

The soil samples were collected from 10 different mechanic sites situated at different location of the town. These locations were Dadin Kowa, Tudun Amama, Sabon Layi, Makwalla, Angwan NEPA, Main Garage, Kofar Hausa, Angwan Mada, Kofar Kokona, and Angwan Waje. 200 g of soil samples were aseptically collected with spatula into sterile sample collection bottle from each site and taken to the laboratory for analyses.

Isolation and identification of soil bacteria

The soil bacteria were isolated by both the direct soil inoculation and the soil dilution techniques using the pour plate method. The media used were nutrient agar for determination of total aerobic bacterial count, brilliant green agar for the isolation of *Pseudomonas* and *Salmonella*, desoxycholate citrate agar for the isolation of *E. coli*, mannitol salt agar for the isolation of *Staphylococcus*, eosin methylene blue agar for the isolation of *E. coli* and *Enterobacter aerogene,* and blood agar for the isolation of *Bacillus Streptococcus, Mycobacterium, Klebsiella* and *Micrococcus*. The plates were prepared and inoculated in duplicates. They were incubated at 35°C for 24 h. The plates were observed and organisms were identified by their cultural, morphological and biochemical characteristics as described by Bergey's manual of determinative bacteriology as revised by Buchannan and Gibbons (1974).

Utilization of petroleum products by isolates

A loopful of isolates on agar plates were picked and inoculated on agar-agar. A quantity of 0.2 ml of each test substrate (kerosene, petrol, diesel and engine oil) was measured and spread aseptically on the surface of the agar-agar. The plates were incubated at 35°C for 24 - 48 h and the number of colony forming units (cfu) was determined (Mitchell, 1992).

Table 1. Bacterial counts of soil samples from different sites in Keffi town.

Sites	Total bacterial count (cfu/ml)
A	$4.0 \times 10^4 \pm 0.6$
B	$8.0 \times 10^4 \pm 3.4$
C	$5.0 \times 10^4 \pm 0.4$
D	$4.0 \times 10^4 \pm 0.6$
E	$7.0 \times 10^4 \pm 2.4$
F	$3.0 \times 10^4 \pm 1.6$
G	$5.0 \times 10^4 \pm 0.4$
G	$3.0 \times 10^4 \pm 1.6$
I	$4.0 \times 10^4 \pm 0.6$
J	$3.0 \times 10^4 \pm 1.6$

A = Angwan-Waje mechanic site; B = Sabon-Layin mechanic site; C = Makwalla mechanic site; D = Main-Garage mechanic site ; E = Kofar-Kokona mechanic site; F = Tudun-Amama mechanic site; G = Angwan-Mada mechanic site; H = Kofar-Hausa mechanic site; I = Dadin-Kowa mechanic site; J = Angwan-NEPA mechanic site.

RESULTS AND DISCUSSION

Table 1 shows bacterial counts of soil samples, obtained from the ten different sites, while Table 2 shows the bacterial isolates found in the soil of the different sites. Table 3 shows the results of utilization of petroleum products (kerosene, petrol, diesel and engine oil) by the bacterial isolates.

The results of the bacterial count show that Sabon Layi mechanic site had the highest count of 8.0×10^4 cfu/ml, followed by Kokona mechanic site which had 7.0×10^4 cfu/ml. Main Garage, Angwan Waje and Angwan Mada mechanic sites had 5.0×10^4cfu/ml, respectively, while the mechanic sites at Tudun Wada, Kofar Hausa and Angwan NEPA had the lowest counts of 3.0×10^4cfu/ml. The bacterial isolates from the soil contaminated with petroleum products from the different sites showed that *Pseudomonas* sp. and *Bacillus* sp. had the highest percentage occurrence frequency of 60%, followed by *Streptococcus* sp and *Micrococcus* sp. which had 50% each. *Staphylococcus* sp. had 40%, *Mycobacterium* sp. had 30%, while *E. coli, Klebsiella* sp and *Enterobacter aerogenes* had the lowest percentage occurrence frequency of 10%, respectively. The result of utilization of petroleum products by isolates showed that most of the bacterial isolates have the ability to utilize the test substrate which showed that they are capable of utilizing petroleum products found in the soil environment. *Pseudomonas, Streptococcus* and *Bacillus* species

Table 2. Bacterial species isolated from the soil contaminated with petroleum products in different soil sites in Keffi town.

Bacterial Isolates	Sites										
	A	B	C	D	E	F	G	H	I	J	% Occurrence
Pseudomonas sp.	+	+	-	+	-	+	+	-	+	-	60
Streptococcus sp.	+	+	-	-	+	-	+	+	-	-	50
E. coli	-	+	-	-	-	-	-	-		-	10
Staphylococcus sp.	+	-	+	-	-	-	-	+	-	+	40
Klebsiella sp.	-	+	-	-	-	-	-	-	-	-	10
Bacillus sp.	-	+	+	+	-	+	-	+	-	+	60
Mycobacterium sp.	-	-	+	-	+	-	+	-	-	-	30
Enterobacter aerogenes	-	+	-	-	-	-	-	-	-	-	10
Micrococcus sp.	+	-	-	+	+	-	+	+	-	-	50
Salmonella sp.	-	-	-	-	-	+	-	-	-	-	10

+ = Present; - = absent; A = Angwan-Waje mechanic site; B = Sabon-Layi mechanic site; C = Makwalla mechanic site; D = Main Garage mechanic site; E = Kokona mechanic site; F = Tudun Amama mechanic site; G = Angwan Mada mechanic site; H = Kofar Hausa mechanic site; I = Dadin Kowa mechanic site; J = Angwan NEPA mechanic site.

Table 3. Results of Utilization of petroleum products by various isolates.

Bacteria isolates	Test substrates			
	Kerosene	Petrol	Diesel	Engine oil
Pseudomonas sp.	+	+	+	+
Streptococcus sp.	+	+	+	+
E. coli	-	-	-	-
Staphylococcus sp.	+	+	+	-
Klebsiella sp.	-	+	+	-
Bacillus sp.	+	+	+	+
Mycobacterium sp.	-	+	+	-
Enterobacter aerogenes	-	-	-	-
Micrococcus sp.	+	+	+	-
Salmonella sp.	-	-	-	-

- = No growth; + = growth.

utilized all the test substrate (petrol, kerosene, diesel and engine oil). Staphylococcus sp. and Micrococcus sp. utilized petrol, kerosene and diesel but did not utilized engine oil. *Klebsiella* sp. and *Mycobacterium* sp. utilized petrol and diesel but did not utilized kerosene and engine oil. Unlike *E. coli, Salmonella* and *Enterobacter aerogenes* did not utilized any of the products. However, *Pseudomonas* sp., *Streptococcus* sp. and *Bacillus* sp. utilized all the petroleum products. These results confirm the report of Alexander (1994) that certain bacteria do utilize petroleum hydrocarbons. In this case there was growth on all the fractions, but more growth was witnessed on petrol, diesel, kerosene than the other fraction. This agrees with the report of Atlas (1992) that hydrocarbon utilizing microorganisms are important in combating the problem of oil pollution.

Conclusion

The investigation revealed that *Pseudomonas, Streptococcus, E. coli, Staphylococcus, Klebsiella, Bacillus, Mycobacterium, Enterobacter aerogenes, Micrococcus and Salmonella* species were isolated from soils contaminated with used petroleum products in Keffi Metropolis. However, *E. coli, Enterobacter aerogenes* and *Salmonella* species were not able to utilize any of the petroleum products used as substrates.

Pseudomonas, *Streptococcus* and *Bacillus* species were found to utilize all the different substrates (kerosene, petrol, diesel and engine oil) tested. *Staphylococcus* species and *Micrococcus* species did not utilized engine oil, but utilized petrol, kerosene and diesel. *Klebsiella* species and *Mycobacterium* species utilized petrol and

diesel, but did not utilize kerosene and engine oil.

This investigation provides information that would lead to selection of bacterial species/strains that could be employed for bioremediation in environments polluted with petroleum and petroleum products. However, further studies need to be carried out to develop strains that would be more efficient in the utilization of the different fractions of petroleum hydrocarbons.

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