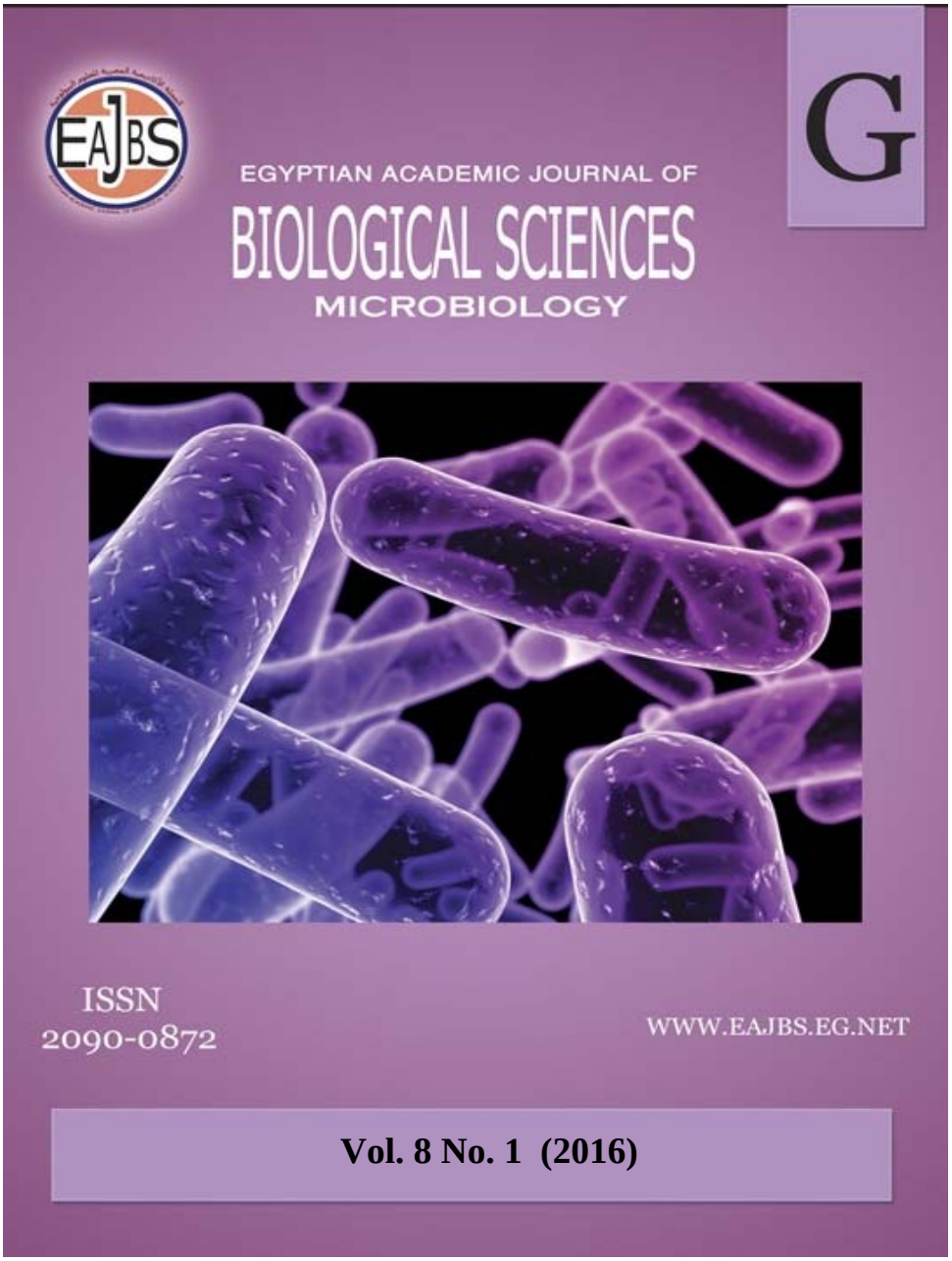

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Microflora of the Petroleum Farm Tank Soil at Mosimi Depot Sagamu Ogun State Nigeria

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ABSTRACT

Isolation and characterisation of microorganisms from petroleum contaminated soil was carried out on the soil samples collected from seven different spots at the NNPC Depot in Mosimi, Ogun State, Nigeria. The highest bacterial count of $2.6X10^6$cfu/g was recorded at Tank 1 which was at the North-Eastern part of the Tank Farm while the least bacterial count of $5.0X10^5$ cfu/g was recorded at Tank 3, about 30 metres away from Tank1. The fungal count ranged from 1.3 X 10^5 cfu/g to 1.7 X 10^5 cfu/g. The bacterial count ranged from 6.4 X 10^5 to 2.6 X 10^6. *Bacillus subtilis* had the highest occurrence (76.2%) among the bacterial isolate while *Aspergillus niger* had the highest occurrence among the fungal isolates.Using biochemical and morphorlogical characteristics that are based on established standards, the bacterial isolates were identified as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Proteus vulgaris.* The fungi isolated were *Aspergillus flavus, Aspergillus niger, Rhizopus oryzae*and *Penicillium chrysogenum. Aspergillus niger* showed highest count and fastest growth pattern among all the isolated fungi. Using biochemical and morphorlogical characteristics that are based on established standards, the bacterial isolates were identified as*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Proteus vulgaris.* The fungi isolated were *Aspergillus flavus, Aspergillus niger, Rhizopus oryzae*and *Penicillium chrysogenum. Aspergillus niger*s howed highest count and fastest growth pattern among all the isolated fungi.This investigation provides information that could be useful in designing bioremediation protocols for environments polluted with petroleum products.

INTRODUCTION

Petroleum is a natural product which is a mixture of aliphatic, aromatic and various heterocyclic compounds. It includes oxygen, nitrogen and sulphur containing compounds. Petroleum continues to be used as the principal source of energy. However, despite its important usage, petroleum products also pose as a globally environmental pollutant(Plohl *et al.*, 2002).

Petroleum hydrocarbon accumulations are found in porous sedimentary rock strata, such as limestone or in other fractured rock such as fissured shale. Hydrocarbon molecules that make up petroleum products are seldom toxic to many organisms, including human beings(Alexander, 1994).

The composition of crude oil depends upon the type of oil formation, the location and the underground conditions where it is found. The majority of crude oil contains high amounts of hydrocarbons compared to the non-hydrocarbon fraction. Typically, crude oil also contains a wide variety of trace metals like nickel, iron, aluminium, vanadium, and copper. Heavy metals commonly found in land-treated refinery wastes in concentrations greater than 10 parts per million (ppm) include chromium, copper, lead, nickel, and zinc (Atlas, 1975).

Tank farms are used as transit depots for the storage of crude oil and natural gas. Prior to its export, crude oil is temporarily housed in storage tanks. Facilities at Tank Farms require scheduled maintenance. Maintenance and improvement projects at these terminals generate huge (in terms of pollution potential) volumes of oily sludge during the cleaning of the storage tanks and heaters (Ayotamuno *et al.,* 2007). The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration, environmental factors and the biological state of the organisms at the time of contamination. In heavily polluted areas, there are immediate detrimental effects on plant and animal(Baker, 1970). Nevertheless, different species and different life stages of organisms have different susceptibilities to pollution (Nelson-Smith, 1973). In addition to its effects on plants and animals, petroleum contamination impacts microbial populations(Ahearn and Meyers, 1976). The effect of oil on microbial populations depends on the chemical composition of the oil and the species of microorganisms present. Populations of some microbes increase; typically, such microbes use the petroleum hydrocarbons as nutrients. The same crude oil can favour different genera at different temperatures. However, some crude oils contain volatile bacteriostatic compounds (Atlas, 1975; Larkia *et al.,* 2005).

At present, various microbial genera such as *Bacillus* species and *Aspergillus* species have been detected in petroleum-contaminated soil or water (Farinazleen *et al.,* 2004). Fungi are of interest because of their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin degradation. They have also been implicated in the degradation of high molecular weight complexes and recalcitrant toxic compounds(Colombo *et al.,* 1996). Response of microorganisms to organic contaminants has been studied for many years. It has been found that individual microorganisms can mineralize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with a broad enzymatic capacities are required to increase the rate and extent of petroleum biodegradation (Farinazleen *et al.,* 2004; Chikere and Chijioke-Osuji, 2006). Although, hydrocarbon degraders may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for microorganisms isolated from other environments (Adenipekun and Fasida, 2005).

However, it has been reported that adapted communities previously exposed to hydrocarbons exhibit higher biodegradation rates than communities with no history of hydrocarbon contamination(Leahy and Colwell, 1990). The microorganisms capable of surviving in such a polluted environment are those that develop specific enzymatic and physiological responses that allow them to use the hydrocarbon compounds as substrates (Farinazleen *et al.,* 2004).

The development, through genetic manipulation, microbial strains able to degrade a variety of different types of hydrocarbons has been of interest to many

researchers. The use of genetically engineered inoculum during seeding would preclude the problems associated with competition between strains in a mixed culture. However, there has been considerable controversy surrounding the release of such genetically engineered microorganisms into the environment. Issues of safety, containment and ecological damage should be considered before field testing of genetically engineered microbes. This study examined the microflora of the petroleum farm tank soil at Mosimi Depot, Sagamu,Ogun State, Nigeria. Emphasis was laid on isolation, characterisation, identification and enumeration of associated microbes.

MATERIALS AND METHODS

Study Site

The Nigeria National Petroleum Corporation (NNPC) Mosimi (6.849320, 3.639859) can be found in Sagamu, Sagamu Local Government Area of Ogun State, a South-Western State in Nigeria. Most soil around the Farm Tank are contaminated with hydrocarbon, obviously because of oil spills and leakages of pipelines both on or under the ground.

Collection of Samples

Soil samples used were obtained from NNPC[Nigerian National Petroleum Corporation] Mosimi Depot, Sagamu, Ogun State. The samples were aseptically collected from a depth of 10-20 cm. The samples were collected in sterile sample bags from 7 different points which were selected around the Farm Tanks. The samples were collected in triplicate at each point.

Sterilization of Glassware

Glassware such as conical flask, measuring cylinder, McCartney bottles andbeakers were washed and drained to dry. They were all sterilized in hot air oven at 160^{o}C for one hour. After sterilization, they were off-loaded and placed on a swabbed bench to prevent contamination. They were then wrapped in foil paper and kept under aseptic condition (Fawole and Oso, 2001).

Preparation of Media

The Nutrient Agar and Potatoe Dextrose Agar used were prepared according to the manufacturers' instructions.

Isolation of Microorganisms

The pour plate technique was used.One gram of each soil sample was diluted serially to a dilution factor of 10^{-5}. One milliliter of the 10^{-4} dilution was dispensed into sterile cell-culture dishes labelled for fungal isolation. Potatoe dextrose agar was then poured on the inoculum at a temperature of about 40^{o}C. One milliliter of the10^{-4} dilution was dispensed into sterile Petri dishes labelled for the isolation of bacteria. Nutrient agar was poured on the inoculum at a temperature of about 40^{o}C. After pouring, the plates were well swirled to achieve a uniform spread of the inoculate. The media were then allowed to set. Plates containing Nnutrient Agar were incubated at 37^{o}C for 24hours, while the Potato Dextrose Agar plates were incubated at room temperature for 72 hours. All the plates were duplicated (Fawole and Oso, 2001).

Purification of the Isolates

Distinct colonies with different morphological characteristics were picked with sterile inoculating loop from the mixed culture and the colonies were subculture on separate plates using streaking method. The plates containing bacteria were incubated at 37^{o}C for 24hours, while the platescontaining fungi were incubated at room temperature for 72 hours (Fawole and Oso, 2001).

Characterisation and Identification of the Bacterial Isolates

The colonial morphology and biochemical characteristics of the bacterial isolates were used for characterisation and identification. Biochemical tests used were catalase test, oxidase test, citrate test, indole test, urease test, methyl red test, motility test, Voges-Proskauer test (VP), oxidase test, oxygen test, coagulase test and sugar fermentation test (Fawole and Oso, 2001; Robert *et al.,* 2005; Joanne *et al.,* 2009).

Characterisation and Identification of Fungal Isolates

A drop of cotton blue-in-lactophenol was put on a clean glass slide and using an innoculating loop mycelia were transferred on the slide and coverd with cover slip. The slide was then examined using an imaging microscope and observations were recorded. Colonial morphology and cellular characteristics were observed and recorded for the purpose of identification (Robert *et al.*, 1988; Olutiola *et al.*, 2000; Fawole and Oso, 2001; Joanne *et al.*,2009).

RESULTS

The highest bacterial count of $2.6X10^6$cfu/g was recorded at Tank 1 which was at the North-Eastern part of the Tank Farm while the least bacterial count of $5.0X10^5$ cfu/g was recorded at Tank 3, about 30metres away from Tank1 this is shown in Table 1. The fungal count ranged from 1.3 X 10^5 cfu/g to 1.7 X 10^5 cfu/g as shown in Table 1. The bacterial count ranged from 6.4 X 10^5 to 2.6 X 10^6 as shown in Table 1.*Bacillus subtilis* had the highest occurrence (76.2%) among the bacterial isolate while *Aspergillus niger* had the highest occurrence among the fungal isolates as shown in Table 2 and Table 3 respectively.Using biochemical and morphorlogical characteristics that are based on established standards, the bacterial isolates were identified as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Proteusvulgaris.* The fungi isolated were *Aspergillus flavus, Aspergillus niger, Rhizopus oryzae* and *Penicillium chrysogenum. Aspergillus niger* showed highest count and fastest growth pattern among all the isolated fungi.

Table 1: Description of Sampling Points and Microbial Count

S/N	Tank ID	Location of the Tank	Description	Bacterial Count (cfu/g)	Fungal Count (cfu/g)
1.	Tank 1	N/E after crossingthe bridge leading to tank labelled 61	Dry muddy area	$2.6X10^6$	1.4 X 10^5
2.	Tank 2	N/Eof the pipelinescoming directly from the tank	Marshy area (soil+water)	$6.7X10^5$	1.7 X 10^5
3.	Tank 3	About 30m from the tank	Dry muddy area	$5.0X10^5$	1.5 X 10^5
4.	Tank 4	N/W anterior of the pipelines	Dry sandy area	$1.3X10^6$	1.3 X 10^5
5.	Tank 5	S/W of the rear end of the pipelines entering the ground	Solidified sandy area	$8.0X10^5$	1.5 X 10^5
6.	Tank 6	N/E after the bridge linking the tank	Muddy marshy area	$1.2X10^6$	1.6 X 10^5
7.	Tank 7	Adjacent the base of pipelines	Moist sandy area	$6.4X10^5$	1.3 X 10^5

Table 2: Frequency of Occurrence (Bacterial Isolates)

S/N	Microbe	No of Occurrence (Per 21 Samples)	Percentage Occurrence (%)
1	*Bacillus subtilis*	16	76.2
2	*Proteus vulgaris*	6	28.6
3	*Klebsiella pnuemoniae*	4	19.0
4	*Escherichia coli*	5	23.8
5	*Pseudomonas aeroginosa*	9	42.9

Table 3: Frequency of Occurrence (Fungal Isolates)

S/N	Microbe	No of Occurrence (Per 21 Samples)	Percentage Occurrence (%)
1	*Aspergillus flavus*	13	61.9
2	*Aspergillus niger*	15	71.4
3	*Rhizopus oryzae*	6	28.6
4	*Penicillium chrysogenum*	8	38.1

DISCUSSION

Both bacteria and fungi were isolated from the research site. Research reports have shown the isolation of bacteria such as *Achromobacter,Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia, Pseudomonas* species in hydrocarbon contaminated soil. Leahy and Colwell (1990) mentioned that, the participation of bacteria, yeasts, and filamentous fungi in biodegradation is a function of the ecosystem and local environmental conditions. Studies on the isolation of filamentous fungi in environments containing oil or its subproducts found similar diversity of genera to those found in this study, such fungi included *Aspergillus* and *Penicillium* species (Cerniglia, 1997; Bento, Camargo, Okeke, & Frankenber-ger, 2005).Results in this workis also supported by (Akpoveta,*et al.* (2011)who isolated *Penicillium* and *Aspergillus* species along with *Fusarium and Rhizopus* species during their studies.

CONCLUSION

The biodegradation of petroleum and other hydrocarbons in the environment is a complex process which quantitative and qualitative aspects depend on the nature and amount of the oil or hydrocarbons, the microbial consortium and the ambient and seasonal environmental conditions. Microbial degradation of oil has been shown to occur by attack on aliphatic or light aromatic fractions of the oil. High-molecular-weight aromatics and resins are some of the pollutants considered to be recalcitrant or exhibiting only very low rates of biodegradation. This investigation provides information that could be useful in designing bioremediation protocols for environments polluted with petroleum products.

RECOMMENDATIONS

Considering the spate of environmental degradation incidents, occasioned by increased crude-oil and natural gas activities in some parts of the globe, urgent efforts should be geared towards implementing remedial activities aimed at mitigating or remediating environmental pollution. Bioremediation is fast becoming one of the most economic and environmentally-friendly technologies for hydrocarbon-contaminated site restoration and it use is therefore recommended.

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