Original Research Article

Bacteria Associated with Petroleum Hydrocarbon Contaminated Soil from NNPC depot

ABSTRACT

The microorganisms associated with soil polluted with petroleum hydrocarbon were isolated at NNPC depot Ibadan, in Ido Local Government Areas of Oyo State. Soil samples were taken from three different points along the point of discharge.The spread plate method was used to isolate the microorganisms found in these rivers and were later identified.From the research, seven different species of microorganism were isolated, which are Pseudomonas sp, Bacillus sp., Micrococcus sp. *Staphylococcus aureaus sp., Enterobacter sp., Klebsiella sp. and Escherichia coli.*Pseudomonas species were found to be more prominent in the samples collected in a research work. This suggested that the isolates are resistant to the petroleum hydrocarbon and can be recommended as candidates for the clean-up of petroleum hydrocarbon contaminated soil.

Keywords: Microorganism, Soil, Petroleum, Resistant, Discharge.

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1.0 INTRODUCTION

During petroleum production, storage and transportation, refining and processing, as well as spills and discharges of petroleum hydrocarbons often occur as a result of blowout accidents during oilfield development, leakage from oil pipelines and storage tanks, oil tanker and tanker leakage accidents, oil

well waxing, and during overhauls of refineries and petrochemical production equipment (Chaerun*et al*., 2004; Chen *et al*., 2015; Wang C. *et al*., 2018).Petroleum hydrocarbon released in to the sea, especially during transportation, leading to the pollution of several sites, and can eventually reach the coasts.

Petroleum oil is an important strategic resource for which all countries compete fiercely (Sun, 2009). Indeed, anthropogenic activity is reliant on oil to meet its energy demands, which causes the petrochemical industry to flourish. However, petroleum use results in environmental deterioration (Xue*et al*., 2015). Oil spills ranging from lowlevel discharges to catastrophic accidents threatened coastal environments; large spills commonly are followed by clean-up efforts, but complete containment is rare. Large spills should be recycled or eliminated to as great a degree as possible, but in some cases it is difficult to recover the spilled materials, resulting in its remaining in the affected area, and posing persistent risks to the environment..

Petroleum hydrocarbon contamination is highly dangerous to the environment. It has severe impacts on the plants as well as animal ecosystem including human health. Although oil pollution is difficult to treat, petroleum hydrocarbon-degrading bacteria have evolved as a result of existing in close proximity to naturally occurring petroleum hydrocarbons in the environment. Such organisms are candidates for the treatment of oil pollutants (Margesin*et al*., 2003; Ron and Rosenberg, 2014; Lea-Smith *et al*., 2015).

Accordingly, there is a constant threat of contamination wherever oil is exploited when coupled with an insufficient ability to deal with oil-contaminated environments, especially in extreme or unique environments such as polar regions, deep sea areas, deserts, and wetlands.The continuous development and improvement of microbial remediation technology has also provided a new method for the remediation of petroleum hydrocarbon pollution, which has attracted much attention (Dombrowski*et al*., 2016; Dvořák *et al*., 2017).

The aim of this study is to isolate and identify the bacteria associated with soil contaminated with petroleum from NNPC depot in Apata, Ibadan, Oyo state.

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Justification.

People living in and around this study sites are exposed to the effect of this discharge into the environment because this petroleum contaminates both surface and underground water and agricultural soil during seepages. This study therefore would enable us determine the bacteria available in the petroleum hydrocarbon contaminated soil. It would also serve as a background to make useful suggestions to the Departments and Ministries of Environment on the proper management of petroleum discharge into the environment and also present microorganisms identified as a possible candidate for bioremediation of hydrocarbon contamination because of their ability to tolerate the contamination.

Study Site.

This study was conducted on Nigerian National Petroleum Corporation (NNPC) depot, Apata, Ibadan, Oyo State located between latitude (07° 23° 26.9′) and longitude (03º 49º, 02.3′) in Ibadan Metropolis, Oyo state Nigeria. A petroleum depot is an industrial facility used for storing oil and/ or petrochemical products where these products are transported to end users or for further storage.

2.0 Methodology

2.1 Collection of Samples

Samples were aseptically collected in a sterile polythene with icepacksfrom source of effluent discharge and along other point of flow from the depot. The samples were collected from three different points for three months: June, July andAugust 2019. The samples were immediately taken to the laboratory for further examination.

2.2 Preparation of Media

Twenty eight gramof Nutrient Agar was weighed and dissolved in 1000ml of distilled water boil to dissolve and distributed into McCartney bottles before they were autoclaved at 121^{o}C for 15 minutes.

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Eosin methylene blue agar and mannitol salt agar were compounded and were autoclaved as described for nutrient agar. Pre-pour plate method was used to plate out the sample.

1ml of each dilution discharged into the centre of the appropriate petri-dish. The plates were allowed to cool and set. They were incubated inverted at 37^{o}C for 24-48 hours. Only plate which contain between 30-300 colonies were counted with colony counter and the number got were multiplied by dilution factor to obtain the viable counts per ml of the original sample.

The desired dilution factor was plated out different media such as Nutrient Agar. Eosin Methylene Blue Agar, MacConkey Agar and Potato Dextrose Agar for fungi growth.

2.3 Method of Analysis

2.3.1 Isolation and characterization of the isolated organisms

Isolation and characterization of the organism was base on two criteria.

1. Cultural and morphological characterization of the colonies
2. Biochemical characteristics

All the isolate were cultured on the prepared medium in duplicate and incubated aerobically at 37^{o}C they were observed on the agar medium plate while the cell morphology was observed microscopically after staining various biochemical test were carried out on the pure bacteria isolate for possible identification.

One millilitre of bath culture of each isolate was used for all except otherwise stated.

2.3.2 Motility Test

Motility test was carried out on the isolate using the method stated by (Seely and Vam Demark, 1972). A loopful of isolates was placed on the cover slip and inverted on the cavity slide on which immersion oil was observed flagellated organisms are observed in constant motion.

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2.3.3 Catalase Test

Catalase is an enzyme produced by wide varieties of Gram positive and Gram negative organisms. It decomposes hydrogen peroxide to form oxygen and water. A drop hydrogen peroxide was placed on a clean free slide. A colony of the test organism was emulsified in it. Evolution of gas bubbles indicated a positive result.

2.3.4 Citrate Utilization Test

From a young colony suspension in peptone water, a loopful of each of the bacterial isolates was inoculated on the Simon's citrate agar and incubated at 37^{o}C for 24 hours. After change in colour of agar from green to blue indicated citrate utilization is positive result. Uninoculatedbottle which served as control remained green in colour after 24 hours of incubation at 37^{o}C.

2.3.5 Gram Staining Technique

A colony of the organism was emulsified on a clean-free slide with a loop-full of distilled water.

This was dried and heat-fixed by passing over flame thrice. The slide was then flooded with crystal violet for 30 seconds and rinsed with water.

It was then decolorized with acetone for 30 seconds and rinse with water and counter stained with safranin for 30 seconds, rinsed with water and allows drying. The slide was then examined with a drop of immersion oil under x 100 objective.

Two results were obtained from this test and these are the Gram reaction cell shape of each of the bacterium. The organism that retained the purple colorations is Gram positive and those that were able to take up the red colour are the Gram positive.

2.3.6 Indole Production

Inoculate the organism provided into a testtubeofindole peptone water. The control was set up along with the test; incubate the tube along the other tube of sterile indole peptone, in a water bath at 44^{o}C to 25^{o}C for 48 hours.At the end of the incubation period, test for the production of

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indole(from the decomposition of tryptophane constituent of peptone water), as reagent along the wall of the test-tube, into the culture obtained. Do not stake water for the formation of a pink layer which would not dissolve on shaking. Absence of pink colouration shows negative test.

2.3.7 Coagulase Test

This test differentiates the pathogenic*Staphylococcusaureus* from non pathogenic staphylococci. There are two methods for this test, slide and tube method. The slide method involves the use of a clean grease-free slide andloopful of sterile normal saline was placed on it and emulsify a bit for 18-24 hours agar culture in the drop into a homogenous suspension. A drop of human plasma (diluted 1 in 5 sterile).

2.3.8 Oxidase Test

A few drops of P-aminodemethyl were dropped onto piece of (ND1)Whatman filter paper in a Petri dish with a glass rod, some bacteria growth was smeared on the moistened filter paper with the aid of the edge of clean grease-free glass slide.

A purple colouration was introduced with 5 seconds of oxidase-positive cultures. A delayed reaction was recorded as negative.

2.0 Results and Discussion

Table 1. List of various microorganism isolated from different point

Microorganism Isolated	Month	Point A	Point B	Point C
Pseudomonas sp.	June	+	+	+
	July	+	+	+
	August	+	+	+
Bacillus sp.	June	_	_	+
	July	_	_	+
	August	+	_	_
Micrococcus sp.	June	+	_	+

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	July	+	+	+
	August	+	_	+
Staphylococcus sp.	June		+	+
	July	+	+	+
	August	+	+	_
Enterobacter sp.	June	+	+	+
	July	+	+	+
	August	+	+	_
Klebsiella sp.	June	+	+	+
	July	+	+	_
	August	_	_	+
Escherichia coli	June	_	_	+
	July	_	_	+
	August	_	_	+

Different organisms were isolated in percentage at different points along the course of the rivers used for the study site which is from Temidire (point A), Charity(point B) to Peku Area(Point C) all in Ido local government, Ibadan, Oyo State. The study indicated that the organisms were not equally dispensed.

From all the tables showing the results from the colony counts, it shows that more organisms were isolated from the sample taken from Temidire and this can be taken as a result of so many reasons like in the month of July, the rains were just coming in, so there were not much dilution in the river at that particular time. Also *Enterobacter* is present in the Temidire because sometimes the river enter some animals like chicken, goat and rodents at the bank of the river, in that process of trapping this animals, the animals pass out their waste in the river and this might bring about the growth of *E.coli* and *Micrococcus* which is sometimes referred to as an opportunist microorganism.

At the Temidire point, there are no domestic activities, so is only when there is a downpour that the organisms are wash down to the other rivers.

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Temidire Point does not flow as much as Charity and Peku which are fast flowing. Petroleum Hydrocarbon is heavier at the Temidire point, making the level of the contamination very concentrated.

Human activity is very low in this river because of the bitumen concentration, which makes the river not very accessible.

Pseudomonas sp. was also isolated in Temidirepoint in very high percentage and this will be due to reasons like, it uses a wide range of organic materials for food, and with this the organism will receive more nutrients from the partially stagnant or not too flowing river with high level bitumen. It is also referred to as an opportunistic human and animal pathogen.

At Charity (Point 2), the water flows steadily and this brings the organism down the river very fast, not much substrate is found here and since the water is fast flowing fewer numbers of organisms were isolated in Charity.

Peku is the point 3, where there are much of human activities, because the dilution here is very high, traces of bitumen was also on the low side during the rainy season. In Peku,*Klebsiella sp*,*E. coli* were isolated. *Klebsiella* is ubiquitous in nature.

An ability to isolate high number of certain oil-degrading bacteria from an environment is commonly taken as evidence that those bacteria are responsible for the biodegradation of oil hydrocarbons.

Klebsiella sp. was also isolated, its gram negative, oxidase negative and rod shaped.

Klebsiella sp. can lead to wide range of diseases notably urinary tract infections, septicaemia and soft tissue infections. Also isolated was *Staphylococcus aureus*which is facultative anaerobes, gram positive, cocci in shape, it is a nosocomial and community acquired pathogen, it is the leading cause of skin and soft tissue infections. Micrococcus sp,Bacillus sp, Enterobacter sp and *Escherichia coli*were also isolated and they are all known to be pathogenic in nature. Seven Bacteria were isolated and identified in this study with the prominent to be the pseudomonas.

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4.0**Conclusion and Recommendation**

The bacteria isolated from the petroleum contaminated soils are more of pathogenic and can contaminate both surface and underground water they also showed the ability to survive the harsh condition in the soil and they are been regarded as being tolerant to petroleum contamination and suggests them as good candidates for clean-up of petroleum contaminated soil.

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