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CHARACTERIZATION OF BACTERIAL SPECIES FROM OIL CONTAMINATED SOILS IN SOME SELECTED PETROLEUM STATIONS WITHIN BIRNIN KEBBI, KEBBI STATE

[1]Yusuf, A. B., [2]Ismaila, M. B., [3]Keta, J. N., [3]Keta, M. N., [3]John, R. P., [4]Hauwa, M. K & [1]Mubarak, A.

[1]Department of Microbiology, Federal University, Birnin Kebbi
[2]Department of Plant Biology, Federal University of Technology, Minna
[3]Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero Nigeria
[4]Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi

Corresponding author's email address: **Moubaraqameenuaburga30@gmail.com;** Phone no.: +2348163330338.

ABSTRACT
Soil provides home and food to both plants and organisms when it is not altered by any materials that make it unfavourable for the growth of species. Characterization of bacterial species in oil-contaminated soils was conducted. Five soil samples were collected from five (5) selected petroleum mega stations namely, A.A Rano, NNPC, Ap 2, Oando and Yahaya Mekera all within Birnin Kebbi, Kebbi State. The species of bacteria were enumerated and classified using serial dilution, standard plate count and biochemical tests. From the results of the bacterial analysis, six (6) species of bacteria were encountered from the contaminated soil samples collected and these were *Bacillus cereus* which had the highest rate of percentage occurrence with 6 (30%) while *Yersinia enterocolitica, Closteridium tetani* and *Staphylococcus aureus* had the lowest, each with 2(10%) others are *Pseudomonas aeruginosa*3(15) and *Bacillus subtilis*5(25). The total mean of the bacterial counts from soil samples analyzed in this study ranged from $8.75x10^6$cfu/g to $9.45x10^6$cfu/g. Based on petroleum stations, NNPC had the highest bacterial load of $9.45x10^6$cfu/g, while A.A Rano had the lowest bacterial load of $8.75x10^6$cfu/g. There is a need for concern over human health risk that could be related with some of these bacterial isolates that are pathogenic species in our environment. Therefore, public enlightenment, good environmental hygiene and prevention of oil spillages on the soil could provide a solution to soil contamination problems in our petroleum stations.

Keywords: Soil, bacteria, percentage and petroleum stations

INTRODUCTION

Soil is the outer layer cover of the planet surface that serves as a natural medium for the growth of the plant and animal species as well as gave shelter, food or nutrients that are necessities for the survival (Aislabie and Deslippe, 2013).

However, spills of petroleum oil on the soil may lead to damage to environmental life by effecting the species found above or below the soil. The defile of the soil layer due to petroleum compounds often result in major problem that cause infertile of the soil for any agricultural uses (Gojgic-Cvijovic *et al*., 2012).

Kebbi State has different petroleum stations where oils is sold and spills from tankers or pipelines on the soil during the selling/uploaded.

Petroleum oil is known as a source of energy, daily life and income to both developing and developed countries all over the world, but its spillage within surroundings have greatly caused contamination of the environment resulting in producing harmful compounds that lead to the changes of soil nature and entire ecosystem (Walker *et al*., 2005).

Moreover, discharge of petroleum oil have negative influent on the distribution of plants and microbes over a time (Keta *et al*., 2020). The decrease or increase population of microbes on the oil contaminated soil depend on the physical structure of the soil, chemical composition of the soil and oil as well as plant species present. Additionally, soil can favour the distribution of different genera of microbes at different temperatures (Westlake *et al*. 1990).

Rosco *et al*. (1989) also reported an increase in anaerobic microorganisms in crude oil-polluted soil. Oil causes imbalances in the carbon nitrogen (C-N) ratio at the spilled site. For the efficient growth of bacteria, the C-N ratio should be around 60-100:1 (Dibble and Bartha, 2000; Jobson *et al*., 2001). Thus, when this ratio reduce or increase, the development of bacteria will be delayed. Urea is used as a nitrogen source at the spilled site which stimulates the development of the microorganisms and simultaneously accelerates the vanished of the harmful compounds (Garg *et al*., 1994).

The environment is pollutant with petroleum oil due to agitation of petroleum stations/companies and soon (Sharma, 2014). Petroleum stations and industries unavoidably generate massive quantities of oily wastes and oily contaminated soil, which constitute a major challenge for hazardous waste management as well as environmental management. Oil spills on soil, insect and worms are killed due to toxicity, lack of oxygen supply and reduce the pH of the soil and its productivity (Keta *et al*., 2020).

However, in terms of plant growth Oil spills kill wildlife and damage the ecosystem (Mohammed *et al*., 2021). The effect can last up to generations by enforcing changes in the reproduction and compromising the complex food web if not rectified. For these reasons, there is need for research, which would focus on identifying the various bacterial species that can growth on crude oil-contaminated soils. Thus, this study aimed to isolates and characterizes the bacterial species from petroleum contaminated soils in some selected petroleum stations within Birnin Kebbi, Kebbi State North western Nigeria.

MATERIALS AND METHODS

Study Area

Birnin Kebbi is situated in Kebbi State Northern-western part of Nigeria. The city as of 2007 had estimated population of 125,594 (NPC, 2007). It is located along Sokoto River and connected by Argungu road (45 km northeast), Jega (35 km southeast) and Bunza (45 km southwest). Birnin Kebbi has a tropical continental-type climate with a wet season that lasts from April to October in the South and from May to September in the north, the dry season lasts for the remaining period of the year. Agriculture is the main occupation of the people especially in rural areas, including farming, fishing and livestock rearing. The annual temperature varies from 21^{o}C to 38^{o}C.

Sample Collection

A total of five soil samples were collected from different spots field (soil) contaminated with petroleum oil in five selected petroleum stations namely, A.A Rano, NNPC, Ap 2, Oando and Yahaya Mekera are all within Birnin Kebbi, Kebbi State. The samples were collected in February, 2020using a simple sampling technique. This was collected at a depth of 10.0 cm, from the soil surfaces. The samples of 10.0 g were collected at each mega station, put into sterile polythene bags well labelled according to each mega station for easy identification. All the samples were taken to the Department of Microbiology Laboratory of Kebbi State University of Science and Technology Aliero within 1 hour for further analysis.

Sterilization of Glassware

All glassware used were soaked overnight in 70% ethanol, washed with detergent, rinsed with distilled water and air-dried. Petri dishes, glass slides and bottles used were sterilized by heating them in an oven at 160^{o}C for one hour.

Medium Preparation
Nutrient Agar (NA) was prepared and used according to manufacturer's instruction.

Enumeration of Bacteria
These samples were subjected to serial dilution. One gram (1.0g) of each soil sample was weighed and suspended in 9ml sterile water contained in a test tube (10^{-1}) and shaken. An aliquot of 1ml from test tube 10^{-1} was conveyed into the second test tube containing 9ml of distilled water (10^{-2}). Another 1ml was transferred from the second test tube to the third test tube containing 9ml of distilled water (10^{-3}).

These were repeated until 10^{-6} (Cheesebrough, 2000). After the serial dilution, 0.1ml of the dilution was transferred from10^{-5} and 10^{-6} dilution factors of each sample since it had the least bacteria, which were then aseptically inoculated on the solidified plates of media agar respectively. The plates were incubated at 30^{o}C for 48hrs, before colonies of bacteria were counted and reported as colony-forming unit per gram (CFU/g).

Isolation of Bacteria
Due to the appearance of the different colonies, an individual colony was picked and sub-cultured into a new prepared plate of nutrient agar that was enriched with 2.5ml of crude oil by streaked plate method using sterile were loop and incubated at 30^{o}C for 48hrs as described by Manga (2004).

Characterization and Identification of Bacteria
Identification of the bacteria was based on their biochemical reactions, which included triple sugar fermentation tests, indole production, citrate utilization test, H_2S production, motility test, urease enzyme production, MR-VP test, spore tests and gas production test, catalase tests, coagulate tests and mannitol tests as described by Manga (2004).

Statistical Analysis
Determination of the dominant Bacteria species in soil was determine using:
Percentage Frequency of Occurrence

$$= \frac{\text{Occurrence of the isolate}}{\text{Total number of the isolate}} \times \frac{100}{1}$$

RESULTS
The total mean of the bacterial counts from soil samples obtained from A.A Rano, NNPC, Ap 2, Oando and Yahaya Mekera analyzed in this study ranged from $8.75x10^6$cfu/g – $9.45x10^6$cfu/g. However, NNPC soil had the highest bacterial load of $9.45x10^6$cfu/g, while A.A Rano had the lowest bacterial load of $8.75x10^6$cfu/g presented in (Table 1). A total of 20 bacterial isolates were characterized and identified from the oil-contaminated soil samples. This isolate belongs to six (6) bacterial genera; *Bacillus cereus*, had the highest rate of occurrence with 6 (30%) while *Yersinia enterocolitica*, *Closteridium tetani* and *Staphylococcus aureus* which had the least, each with 2 (10%) (Table 2). However, the biochemical tests revealed various morphological features of the encountered species as shown in (Table 3) respectively.

Table1: Mean bacterial species counts of crude oil contaminated soils samples in petroleum stations

Soil Sample Site	Bacterial count forming per unit (cfu/g)
AA Rano Petroleum Station	$8.75x10^6$
Ap 2Petroleum Station	$9.1x10^6$
Oando Petroleum Station	$9.8x10^6$
NNPC Petroleum Station	$9.45x10^6$
Yahaya Mekara Petroleum Station	$9.17x10^6$

Table 2: Frequency of occurrence of the bacteria isolated from the petroleum oil contaminated soil samples

Bacterial Species	Occurrence of Isolates (%)
Bacillus subtilis	5 (25)
Pseudomonas aeruginosa	3 (15)
Bacillus cereus	6 (30)
Clostridium tatani	2 (10)
Yersinaentrocolitica	2 (10)
Staphylococcus aureus	2 (10)
Total	**20 (100)**

Table 3: Morphological and biochemical features of bacteria isolates from petroleum contaminated soils

Gram React	Shape	Lac	Glu	Suc	Man	Cat	Coa	Cit	Mot	Ind	Ura	H2S	Oxi	Gas	Mr	Vp	Spo	Isolates
+	Rod	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+	+	*Bacillus cereus*
_	Rod	-	+	-	+	-	-	+	+	-	-	+	+	-	+	-	-	*Pseudomonas auruginosa*
+	Rod	-	+	+	+	+	-	+	+	-	-	+	-	-	-	+	+	*Bacillus subtills*
_	Rod	-	+	+	+	-	-	-	+	-	+	-		-	+	-	-	*Yersinia entrocolitica*
+	Cocci	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	*Staphylococcus aureus*
+	Rod	-	-	-	-	-	-		+	-	-		-	+	+	-	+	*Clostrdium tatani*

Keys:
Lac = Lactose Test, Glu = Glucose Test, Suc = Sucrose Test, Man =Mannitol Test, Cat = Catalase Test, Coag = Coagulase Test, Cit = Citrate Test, Mot = Motility Test, Ind= Indole Test, Ura = Urase Test, H_2S = Hydrogen-sulphate Test, Oxi = Oxidase Test, Gas = Gas Production test, MR = Methyl Red Test, Vp = Voges Proskauer Test, Spor = Spore Test

DISCUSSION

This study had shown higher bacterial counts in filling station soil sample of NNPC and lower bacteria counts in soil sample AA Rano filling station, the mean of the total bacterial counts ranged from $8.75x10^6$cfu/g to$9.45x10^6$cfu/g (Table1). These mean counts were slightly higher than those reported by (Mujahid *et al*., 2015; Agu *et al.,* 2015). The difference in counts might be due to pH, temperature and substances of petroleum-impurities soil which possible aid the proliferation of microorganisms.

A total of 20 bacterial spp were isolated from the soil samples, the isolates belongs to six (6), bacterial genera. This result is supported by Atlas (1981), who found out that isolates representing 6 genera of bacteria were the predominant microbial genera responsible for hydrocarbon oxidation in soil samples. Moreover, Ameh and Kawo (2017), observed and reported similar bacterial species in their findings. The presence of these isolates in soil samples indicated that, the isolates were able to exits in the oil-contaminated environment, while those that could not survive in this environment being eliminated by the unfavorable conditions caused by the oil.

Furthermore, Rosco *et al*. (1989) also reported an increase of anaerobic microorganisms in crude oil-polluted soil.

The bacterial characterized and identified from petroleum oil soil samples in this study belonged to six (6), genera of bacteria and were: *Bacillus subtilis*, *Pseudomona aeruginosas*, *Bacillus cereus*, *Closteridium tetani*, *Yersinia enterocolitica*, and *Staphlococcus aureus* (Table 2).*Bacillus cereus* was frequently isolated, and the least were *Closteridium tetani*, *Yersinia enterocolitica* and *staphylococcus aureus*. The frequently isolation might be due to its diverse metabolic activity the result agree with Panda *et al* (2013), who reported that *Bacillu s*sp was isolated based on the hydrocarbon degradation efficiency. Also, the findings of Agu *et al.* (2015), supported the results of our finding, based on the diversity of the isolated bacterial spp.

Amadi *et al.* (1996) shows that release or discharge of petroleum have negative impact on the soil quality and distribution of other organisms. In addition to its effects on visible plants and animals, petroleum contamination impacts microbial populations. This probably explains why the population of bacteria was higher or lower in the crude contaminated soils. The soil environment is very complex and provides diverse microbial habitats. Soils vary greatly depending on climate, organisms, land form, and parent material. According to Westlake *et al*. (1990), stated that the influence of oil on microbes varied as different factors (abiotic) and nutrient. Furthermore, some oils contain volatile bacteriostatic compounds that must degrade before microbial populations can grow.

CONCLUSION

Bacteria species are distributed in contaminated soil in oil stations as indicated in this research which could be a problem in our environment. Therefore, this means that they are available in significant numbers, and this may lead to huge economic loss more especially agricultural in addition to the health conditions that could results from some pathogenic species causing health dangers. The results of our finding conclusively showed that, there is a need to come out with a good method that would stop the spits of petroleum or its products as this would reduce the concentration of these bacterial species in the soils causing health problem.

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