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Microbiological and physiochemical analyses of oil contaminated soil from major auto mechanic workshops in Ado-Ekiti metropolis

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Abstract

The ability of microorganisms to utilize used oil in contaminated soil from some selected major mechanic workshops in Ado Ekiti metropolis as a sole source of carbon and energy was studied. Soil samples collected from the two major mechanic workshops at Adebayo, and Irona Quarters in Ado-Ekiti were analyzed for the microbiological and physicochemical qualities using the basic microbiological methods. The physicochemical properties of the soil samples shows that the value of pH range from 5.45 to pH 5.58, percentage of carbon range from 3.98 % to 4.20%, nitrogen content varies from 2.20 to 2.40. The total heterotrophic bacterial counts from the mechanic workshops ranges from 7.52 x 106 to 8.25 x 106cfu/g while the total heterotrophic fungal counts ranges from 5.54 x 106 to 6.25 x 106 cfu/g. The bacterial recovered from the oil contaminated soils include *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *E. coli* and *Acinobacter* spp. The frequency of occurrence of the microbial isolates revealed that *Bacillus cereus* 5 (31.25%) occurred most, followed by *Pseudomonas aeruginosa* (18.75%), *Acinetobacter* spp. (18.75%) and *E. coli* (18.75%) while *Citrobacter freundii* (12.50%) had the lowest frequency of occurrence. The results of this study have showed that some of the isolated organism can be used for bioremediation of the contaminated soil.

Keywords: Oil contaminated soil; Bacteria; Fungi; Frequency; Mechanical workshop

1. Introduction

Oil released in to the environment is a well-recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans [1]. Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment large amount of them are liberated into the environment when the motor oil is changed and disposed into the soil which is a common practice by motor mechanics and generator mechanics including small scale engine oil sellers along the road [2]. The oil is also released into the environment from the exhaust system during engine use and due to engine leaks.

The release of oil into the environment causes environmental concern and attracts public attention. Used motor oil contains aromatic hydrocarbons (PAHs) that could contribute to chronic hazards including mutagenicity and Carcinogenicity [3]. These products tend to harden and change the colour of the soil, which have untold health hazard on technicians and artisans [4]. Bioremediation makes use of indigenous oil-consuming microorganisms, called petrophiles by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source. Microbial remediation of a hydrocarbon contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge [5] thereby, can be used in cleaning up contaminated sites [3]. The main objective of this study is to isolate bacteria from engine oil polluted soil around mechanics workshop in Ado Ekiti.

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2. Methodology

2.1. Sample Collection

Soil samples contaminated with spent engine oil were collected from a mechanic workshop Adebayo and Irona in Ado Ekiti, Ekiti State. The samples were collected from the same spot but different depths (5cm, 10cm, and 15cm). The age of oil dumping at this site was 3years.

2.2. Media used for isolation

Media used are Nutrient Agar, Peptone water, Nutrient broth and Modified Minimal salt medium and sterilized for 15minutes at 121 ^{O}C.

2.3. Serial dilution of samples

About 1 mL of each of the sample was aseptically transferred into test tube containing 9 mL of distilled water to give 10^{-1} (Ten-fold serial dilution). Further ten-fold dilution was carried out to factor 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilution factors.

2.4. Pour plate

After the serial dilution of the water samples, pour plate method was used for the culturing procedure, this involves taking 1 mL of the serially diluted samples from the 10^{-5} and 10^{-6} tubes and transferring it into sterile petri dishes labeled equally according to each samples and appropriate sterilized agar cooled at 45^{0}C was added. The plates were allowed to cool, solidify, inverted and incubated at 37^{0}C for 18-24 hours of incubation, the plates were examined for growth of viable bacteria. The well-developed colonies were counted using the colony counter and recorded in colony forming unit per milliliter (CFU/mL).

2.5. Bacterial load enumeration

Samples were enumerated by making tenfold dilutions of the soil samples from 1:10 to 1:100000 from broth culture. They were covered or corked and incubated for one week at 30^{o}C in an incubator. From the diluted sample, using a dropper pipette, 0.025 mL of each dilution was dropped on the solid nutrient agar surface. Each inoculum of microorganism developed into a discreet colony. The number of viable micro-organisms in the sample was calculated from the number of colonies formed, the volume of inoculum used by dropper pipette and the dilution factor expressed in colony forming unit.

2.6. Isolation of microorganisms

Microorganisms were isolated using Nutrient Agar (NA) for total bacterial counts. About1g of the soil sample was taken from each of the soil samples contaminated with the different hydrocarbons and suspended in 10ml of sterile water. The suspension was serially diluted and 0.1ml of appropriate diluents was evenly spread on the surface of the already prepared Nutrient Agar. Duplicate plates were incubated for 24 hours at 37 ^{o}C and morphologically distinct colonies were sub-cultured onto fresh plates. Pure colonies of each isolated bacteria strains were stored on Nutrient agar slants at 4 ^{o}C for further study [6].

2.7. Characterization of selected isolates

Isolates were characterized and identified on the basis of their morphological (microscopic, macroscopic) and biochemical properties such as catalase, glucose, sucrose, gram stain, H_2S, maltose, urease, etc, were all carried out

2.8. Morphological Characterization

- i Macroscopic: They were observed based on shape, colour, elevation, size, edge and surface on respective agar plate.
- ii Microscopic: This was carried out using the simple staining technique described by Olutiola *et al.* [6]. A thin smear of the isolate was made on a clean glass slide and heat-fixed. Two drops of Crystal violet were applied onto the smear for 60 seconds. It was washed with water and stained with Grams iodine solution for 1 minute. The stain was decolourized by flooding the slides with ethanol until no more violet colouration was observed. Two drops of counter stain Safranin reagent was added for a minute, rinsed with water and blotted dry using filter paper. Microscopic observation was carried out under the oil immersion objective (Fisher Scientific, USA).

Gram positive organisms were characterized by purple colouration after counter staining while Gram negative cells were pink in colour. Their shapes were also observed [6].

3. Results

3.1. Physiochemical properties of oil polluted soil

Table 1 shows the physiochemical properties of oil polluted soil in Ado Ekiti. The results revealed that the contaminated soil samples were found to be acidic in nature. The pH content in oil polluted soil from Adebayo workshop was pH 5.58 while the pH from Irona oil polluted site was pH 5.45. The lowest content of organic carbon was found in oil polluted soil sample collected from Adebayo (3.98%) and the highest from Irona (4.20%). The moisture content (%) of the contaminated soils also ranged from 1.52-1.90 %. Soil nutrients such as nitrogen (N) ranged from 5.30- 8.20 %.

3.2. Total hydrocarbon-utilizing bacteria

The result of total hydrocarbon-utilizing bacteria of soil samples from oil contaminated sites in Ado-Ekiti is shown in Table 2. The mean total hydrocarbon utilizing bacteria count ranges from 7.54 $x10^{-6}$ in oil polluted soil samples from Irona to 8.25 x 10^{-6} in polluted site in Adebayo when compared with uncontaminated soil samples (3.20$x10^{-6}$).

Table 1 Physiochemical properties of oil polluted soil in Ado Ekiti

Parameters	Uncontaminated site	Contaminated site	
		Adebayo Site	Irona Site
Colour	Brown	Light black	Light black
pH	6.78	5.58	5.45
Organic carbon	1.25	3.98	4.20
Moisture content	0.87	1.90	1.52
Nitrogen	8.70	2.20	2.40

Table 2 Bacteria count from oil contaminated soil in Ado-Ekiti

Sample	Total Heterotrophic bacteria count (Log_{10}^{6}CFU/ml)
Uncontaminated samples (Control)	3.20
Adebayo Site	8.25
Irona Site	7.54

3.3. Biochemical characterization of bacteria from oil polluted soil

Table 3 reveals the biochemical characterization of bacterial isolated from oil polluted soil. A total of 16 indigenous bacteria species belonging to five (5) genera were identified. The isolated bacteria were *Staphylococcus* spp., *Pseudomonas aeruginosa, Bacillus* spp., *E. coli* and *Acinobacter* spp.

3.4. Percentage Distribution of bacterial from oil polluted soil

The prevalence and percentage distribution of bacterial isolates from oil polluted soil samples is shown in Table 4. *Bacillus cereus* (31.25%) had highest percentage of occurrence followed by *Pseudomonas aeruginosa* (18.75%), *Acinetobacter* spp. (18.75%) and *E. coli* (18.75%) while *Citrobacter freundii* (12.50%) had the least occurrence.

Table 3 Isolation and identification of bacteria from oil polluted soil in Ado Ekiti

Isolate	Gram Rxn	Catalase	Motility	Indo	Citrate	Oxidase	MR	VP	Gas	H_2S	Urease	Organism
1	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	*Acinetobacter* spp.
2	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	*Staphylococcus* spp.
3	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Bacillus cereus*
4	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	*Pseudomonas aeruginosa*
5	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	*Acinetobacter* spp.
6	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Bacillus cereus*
7	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	*Pseudomonas aeruginosa*
8	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	*Acinetobacter* spp.
9	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	*Pseudomonas aeruginosa*
10	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	*E. coli*
11	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Bacillus cereus*
12	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Bacillus cereus*
13	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	*E. coli*
14	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Bacillus cereus*
15	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	*E. coli*
16	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	*Staphylococcus* spp.

Table 4 Percentage distribution of bacterial isolated from oil polluted soil in Ado Ekiti

Isolated organism	Occurrence	Percentage %
Acinetobacter spp.	3	18.75
Bacillus cereus	5	31.25
Pseudomonas aeruginosa	3	18.75
E. coli	3	18.75
Staphylococcus spp.	2	12.5
Total	16	100.00

4. Discussion

The quality of soils is generally assessed based on physicochemical indices, microbiological and eco-toxicological parameters [7]. The total concentration of heavy metal in soils gives little information on their mobility and toxicity. In this study, the presence of oil spillage had some significant adverse effects on the chemical properties of the contaminated soil in two different sites in Ado Ekiti as shown in Table 1. There is an increase in moisture content of oil polluted soil when compare with unpolluted soil. The high moisture content of oil polluted soils could be due to the presence of hydrocarbons and polycyclic aromatic hydrocarbons, which can cause an increase in soil hydrophobicity, leading to an increase in the moisture holding capacity of soil [7]. A review of existing data on the Niger Delta by Osuji [8] showed that extremely high hydrocarbon levels in soil affect both above and belowground flora and fauna, which are essential factors in the biogeochemical cycle, as they affect availability of plant nutrients. Among soil fertility indices, the concentrations of macronutrients in Nitrogen (%) of contaminated soils were low when compared with uncontaminated soil as compared to acceptable ranges of 15 000, 2 000 and 10 000 mg kg−1 for N, P, and K respectively, as recommended for agricultural soils [9]. The lower concentration of Nitrogen (%) content in contaminated soil could

be due to utilization of the nutrients by resident microflora. Osuji and Nwoye [10] suggested that it is unlikely that the oil release is directly responsible for the loss of macronutrients from soil. However, higher concentration of nitrogen (8.70%) in unpolluted soil in comparison to polluted soil (2.20-2.40) is in agreement with the findings of Ujowundu *et al*. [11], who studied the biochemical and physical properties of diesel-contaminated soil in southeastern Nigeria.

Similarly, Lehtomake and Niemela [12] reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contamination In addition, the high amount of organic carbon in the contaminated soil samples when compared with uncontaminated soil could be due to gasoline fuel, which is composed of hydrocarbon and polycyclic aromatic hydrocarbons [13]. The contamination resulted in the soil pH (5.45-5.58) as compared to pH 6.78 in the uncontaminated soil. The low pH may have affected fungal growth in the contaminated soil, which was observed to be low. It has been shown that optimal activity for microbial degradation occurs at pH 7.4 while considerable inhibition can be seen both at pH 4.5 and 8.5 [14]. The resulting slightly acidic pH in contaminated soil could be due to the fact that hydrocarbons contain many free cations causing them to have properties of a weak acid. A reduction in pH implies increased acidity which is a problem for agricultural soils because many metal cations are more soluble and available in the soil solution at very low pH including Cd, Cu, Hg, Ni. Pb and Zn [10].

Five bacterial isolates, namely *Acinetobacter* spp., *E. coli, Bacillus cereus, Pseudomonas* spp., and *Staphylococcus* spp. were recovered from oil polluted soil in mechanical workshop in Ado Ekiti. The result of this study is in correlation with the work reported by Lin and Madri [3]. Khan and Rizvi [15] also reported the isolation of *Streptococcus* spp, *E. coli, Pseudomonas* spp from oil contaminated site. Abioye *et al*. [16] also reported an isolation of *Pseudomonas*, *Bacillus, Micrococcus* and other bacterial strains from engine oil contaminated soil. *Pseudomonas*, *Bacillus,* and *Rhodococcus* were isolated from engine oil contaminated soil as reported by Ogunbayo *et al*. [17]. Chikere and Okpokwasili [18] also made similar findings on petroleum effluents. To date, generals belonging to *Pseudomonas, Micrococcus, Bacillus* and *Mycobacterium* have been characterized and reported in the literature as hydrocarbon degrading strains [19]**.** The presence of Bacillus species could be attributed to their ability to produce spores which enable them to survive in a different environment including hydrocarbon polluted soils [20].

4.1. Bacteria count

The results obtained in this study indicated that bacteria utilized oil in contaminated soil from mechanic workshop as their sole sources of carbon and energy. There was variation in the total hydrocarbon utilizing bacterial counts in the oil contaminated soil samples from the different sampling locations. Butier and Mason [21] indicated that there is an increase in heterotrophic bacteria population in the presence of dispersant agent. Also, Antai [22] reported two major response to crude oil in which there is an increase in microbial population. Although, this disagrees with the work of Ekhaise and Nkwelle [23], who observed that petroleum hydrocarbon has little or no effect on the total bacterial heterotrophic environment.

5. Conclusion and Recommendation

Soil samples contaminated with engine oil examined from different mechanic repair workshop in Adebayo and Irona harbour bacteria. About five bacterial isolates, namely *Acinetobacter* spp., *E. coli, Bacillus cereus, Pseudomonas* spp. and *Staphylococcus* spp. were recovered. Thus, further studies, need to be done to identify and characterize these organisms down to the species level and more effective strains could also be developed to aid the bioremediation of oil-contaminated soil. This could help reduce the scourge of oil spill pollution caused by drilling operations of oil exploration companies in the Niger-Delta region of Nigeria.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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