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Microbiological And Physicochemical Analyses Of Oil Contaminated Soil From Major Motor Mechanic Workshops In Benin City Metropolis,Edo State, Nigeria

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ABSTRACT: The ability of microorganisms to utilize used oil in contaminated soil from some selected major mechanic workshops in Benin City metropolis as sole source of carbon and energy was studied. Soil samples collected from the three major mechanic workshops at Igun, Evbareke and Uwelu Quarters in Benin City were analyzed for the microbiological and physicochemical qualities using the basic microbiological methods. The total heterotrophic bacterial counts from the three mechanic workshops ranges from 1.98 X 10^7 to 2.95 X 10^7cfu/g while the total heterotrophic fungal counts ranges from 6.7 X 10^6 to 9.2 X 10^6 cfu/g. The hydrocarbon utilizing microbial isolates were isolated using the Bushnell Haas enrichment techniques. The hydrocarbon utilizing microbial populations were recorded to range from 3.1 X 10^6 to 9.7 X 10^6cfu/g and 3.4 X 10^6 to 6.0 X 10^6 cfu/g for the bacterial and fungal counts respectively. The bacterial isolates include the genera *Bacillus, Pseudomonas, Micrococcus, Flavobacterium, Klebsiella,* while the fungal isolates include the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Yeast.* The biodegradation potentials of the hydrocarbon degrading microbial isolates monitored by gas chromatography (GC) showed *Pseudomonas* sp. and *Aspergillus versicolor* to have recorded the highest biodegradation potentials among the microbial isolates. The frequency of occurrence of the microbial isolates revealed the microbial isolates *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Aspergillus versicolor, Trichoderma* sp *and Penicillium* sp. as the most frequently occurring isolates. The physicochemical properties of the soil samples analysed shows the pH ranges from 5.63 to 6.01, percentage of carbon 2.345 to 4.800, total organic matter 1.59 to 7.81%, total petroleum hydrocarbon 86.10 to 286.50 mg/kg and sulphate 1.49 to 6.10ppm. The correlation analysis showed that there was significant difference in the mean values for both total heterotrophic bacterial and fungal population among the locations. It was also showed that there exist significant difference in the optical density and pH among the microbial isolates. @JASEM

Keywords: Oil contaminated soil, microbial isolates, mechanical workshops and physicochemical parameters

Pollution of the environment by petroleum products is an inevitable consequence of oil production, transportation and distribution activities. Large amounts of petroleum products handled on land every year create the possibility for land contamination. In addition, large volumes of crude oil and/or refined petroleum products are transported across the world's oceans from producing areas to consumer countries (Atlas, 1981). Consequently, a substantial fraction of this oil is released into the sea either by accident or during normal tanker operations. It has been estimated that approximately 0.1 % of transported crude oil (about 35 million metric tons) enters the sea annually from tankers alone *(*Energy Information Administration, 1992). Other pollution sources include ballast water discharges, natural seepage, blow out of wells, leakages from pipelines and storage tanks, industrial wastes and runoffs and sometimes sabotage (Atlas, 1981).

The toxicity of crude oil or petroleum products varies widely, depending on their composition, concentration, environmental factors and on the biological state of the organisms at the time of the contamination. The discharge of used oil from vehicles or motorcycles is a major source of oil pollution in mechanic workshop and its environs. Biodegradation of hydrocarbons by natural population of microorganisms represents one of the primary mechanisms of eliminating petroleum pollution from the environment (Leahy and Colwell, 1990). The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide range of bacteria and fungi (Atlas, 1981).

The ability to isolate high numbers of certain oil-degrading microorganisms from oil-polluted environment is commonly taken as evidence that these microorganisms are the active degraders of the pollutants in the environment (Okerentugba and Ezeronye, 2003).

Microbial degradation of hydrocarbon-contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. A large number of *Pseudomonas* strains capable of degrading Poly aromatic hydrocarbons have been isolated from soil and aquifers (Kiyohara *et al.* 1992; Johnson *et al*. 1996). Other petroleum hydrocarbon degrading bacteria include *Bacillus, Micrococcus*, *Alcaligenes* spp., *Flavobacterium*, *Corynebacterium* spp. and *Streptococcus* spp. (Antai, 1990; Bhattacharya *et al*. 2002). Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent.

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Petroleum products such as engine oil, petrol, diesel and kerosene are used daily in various forms in mechanic workshops. These products tend to harden or change the texture of the soil, which may have effects on the microbiological and physicochemical properties of the contaminated soil. Therefore, due to the increasing number of mechanic workshops and their indiscriminate disposal of used oil into the environment, there is need to consider options for their removal from the environment due to the environmental hazards associated with petroleum products. The objective of this study was to evaluate the potential of microorganisms isolated from oil-contaminated soil in mechanic workshop to utilize petroleum hydrocarbon.

MATERIALS AND METHODS

Study site: The soil samples were collected from mechanic workshops in Evbareke, Igun and Uwelu quarters, Benin City, Edo state, Nigeria.

Sample collection: Soil samples were collected in a sterile polyethylene bag using a sterile spatula from the edge of a motor oil-stained patch in the workshops by scooping to about 5cm. They were immediately transported to the laboratory for analyses.

Enumeration of microorganisms: Ten (10) grams of the oil-contaminated soil was suspended in 90 ml of distilled water and ten fold serial dilutions of the soil samples from 1:10 to 1:100000 were carried out. 0.1 ml of the 10^{-5} dilution for each soil samples were plated in triplicate on Nutrient agar amended with nystatin to suppress the growth of fungi and potato dextrose agar amended with streptomycin to inhibit the growth of bacteria using pour plate methods. The nutrient agar plates were incubated at 37^0C for 24 - 48hours while the potato dextrose agar plates were incubated at 28 ± 2^0C for 72 hours. The number of viable micro-organisms in the sample was calculated from the number of colonies formed and the volume of inoculums and the dilution factor expressed in colony forming unit.

Isolation of hydrocarbon utilizing microorganisms: One gram of the oil-stained soil from a motor-mechanic workshop was inoculated in Bushnell Haas medium in test tubes. The test tubes were incubated at room temperature (28 ± 2^Oc) for 5 to 7 days. After incubation, the content of each test was serially diluted using distilled water. One milliliter (1ml) aliquot of the 10^{-5} dilution was plated in Bushnell Haas medium amended with nystatin for hydrocarbon utilizing bacteria while in the case of the hydrocarbon utilizing fungi, the medium was amended with streptomycin to prevent the growth of bacteria. The hydrocarbon utilizing microorganisms were enumerated after incubation at room temperature for 5 to 7 days.

Biodegradation tests: The ability of the microorganisms isolated from the oil contaminated soil to degrade crude oil was tested by introducing pure diesel oil to 100ml of sterilized Bushnell Haas medium in conical flasks. The flasks were inoculated with microbial isolates and incubated, under aerobic condition at 200 rev/min at room temperature (28±2°C). The microbial growth was monitored by measurement of optical density and pH using spectrophotometer and standardized pH meter respectively. The residual oil was measured and the qualitative changes in the hydrocarbon profile of the oil were monitored by gas-liquid chromatography as previously described (Amund, 1984).

Physicochemical analysis of soil samples: The pH of the soil samples was determined using a pH meter (Jenway 3051) in 1:1 soil solution in distilled water in accordance with the manufacturer's directions and a thermometer of a range 0 – 100^oC (Cowan and Steel, 2004). The carbon organic content, total nitrogen content, potassium content and available phosphorous were determined according to the methods of Chopra and Kanwar (1998). Conductivity was determined using a conductivity meter (PW 9504 Philips, USA) with a cell constant of 1.2, while the heavy metal content of the soil was determined using atomic absorption spectrophotometer (Alpha 4, AAS) following digestion with nitric acid and extraction of the soil samples. The total hydrocarbon content of the soil was extracted using n-hexane: dichloromethane solvent systems (1:1).

RESULTS

The total heterotrophic bacterial (THB) counts ranged from $2.00 \times 10^7 - 2.80 \times 10^7$ while the total heterotrophic fungal (THF) counts ranged from $7.2 \times 10^6 - 9.2 \times 10^6$. The hydrocarbon utilizing bacterial (HUB) counts ranged from $3.1 \times 10^6 - 5.6 \times 10^6$ while the hydrocarbon utilizing fungal (HUF) counts ranged from $3.7 \times 10^6 - 6.0 \times 10^7$.

Table1: Total Mean Microbial Counts of oil contaminated soil from Igun

Locations	THB	THF	HUB	HUF
S1	2.80×10^7	9.2×10^6	4.1×10^6	6.0×10^6
S2	2.00×10^7	7.2×10^6	5.6×10^6	5.5×10^6
S3	2.19×10^7	7.5×10^6	5.0×10^6	3.7×10^6
S4	2.15×10^7	7.7×10^6	3.1×10^6	4.8×10^7
S5	2.11×10^7	7.7×10^6	4.3×10^6	4.7×10^6

Table2: Total Mean Microbial Counts of oil contaminated soil from Uwelu

Locations	THB	THF	HUB	HUF
S1	2.40×10^7	8.1×10^6	8.6×10^6	4.7×10^6
S2	2.26×10^7	8.9×10^6	5.3×10^6	5.4×10^6
S3	2.11×10^7	6.7×10^6	4.4×10^6	3.4×10^6
S4	2.16×10^7	8.6×10^6	8.9×10^6	4.6×10^6
S5	2.01×10^7	7.0×10^6	8.6×10^6	4.5×10^6

Table3: Total Mean Microbial Counts of oil contaminated soil from Evbareke

Locations	THB	THF	HUB	HUF
S1	2.39×10^7	7.0×10^6	9.7×10^6	3.7×10^6
S2	2.19×10^7	7.2×10^6	8.3×10^6	3.9×10^6
S3	2.32×10^7	6.9×10^6	7.8×10^6	3.7×10^6
S4	1.98×10^7	7.0×10^6	5.1×10^6	3.5×10^6
S5	2.98×10^7	7.2×10^6	9.1×10^6	3.4×10^6

Table4: **Physicochemical properties of the soil samples from the mechanic workshops**

Properties	S1	S2	S3
PH	6.01	5.69	5.63
EC (µs/cm)	71.1	69.2	70.1
C (%)	4.800	2.345	4.672
N (%)	0.342	0.228	0.328
OM (%)	8.299	5.760	8.078
TOC (%)	7.81	1.59	4.03
TPH (mg/kg)	286.50	86.10	187.46
P (ppm)	47.946	19.180	47.866
$SO4^{2-}$ (ppm)	6.10	1.49	1.91
Ca (meg/100g)	3.68	2.56	3.98
Mg (meg/100g)	0.48	0.33	0.40
K (meg/100g)	0.709	0.100	0.714
Na (meg/100g)	0.698	0.050	0.751
Al (meg/100g)	0.0	0.0	0.0
ECEC	5.567	3.568	5.845
Clay (%)	30.6	7.5	70.3
Silt (%)	6.0	1.4	6.0
Sand (%)	63.4	91.1	23.74
Fe (ppm)	244.36	197.50	228.13
Zn (ppm)	45.504	19.00	44.458
Mn (ppm)	1.083	0.098	1.042
Cu (ppm)	6.826	1.50	6.501

DISCUSSION

The results obtained in this study indicate that microorganisms which included bacteria and fungi 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 and fungal counts in the oil contaminated soil samples from the different sampling locations. Butier and Mason (1997) indicated that there is an increase in heterotrophic bacteria population in the presence of dispersant agent. Also, Antai (1990) reported two major response to crude oil in which there is an increase in microbial population. Although, this disagrees with the work of Lizarraga-Partida *et al*. (1982), who observed that petroleum hydrocarbon has little or no effect on the total bacterial heterotrophic environment.

Correlation analysis, the total viable counts increased significantly with optical density as the days of incubation progressed until the 15^{th} day ($P<0.05$). There was significant decrease in pH ($P<0.05$) as microbial isolates utilized the oil contaminated soil. The highest optical density value of 0.593 was recorded with *Pseudomonas* after 6^{th} day of incubation among all the bacterial isolates in the oil contaminated soils. The lowest pH value of 4.53 was also recorded with *Pseudomonas* at the end of 15^{th}days of incubation among bacterial isolates. *Klebsiella* recorded the lowest optical density value of 0.028 of all bacterial isolates at the end of the incubation period*; Klebsiella* sp. had the highest pH value of 5.20. Among all the fungal isolates isolated from the contaminated soils, *Aspergillus* spp. recorded the highest optical density and pH values of 0.432 and 5.31 respectively at the end of 15^{th} day of incubation while *Rhizopus* sp. had the lowest optical density and pH values of 0.109 and 5.39 respectively at the end of the 15^{th} day of incubation.

The hydrocarbon-utilizing bacterial genera isolated from the oil contaminated soil were *Pseudomonas*, *Bacillus, Micrococcus, Flavobacterium* and *Klebsiella*. Okpokwasili and Okorie (1990) isolated similar hydrocarbon utilizing bacteria from the Niger Delta aquatic systems. Chikere and Okpokwasili (2004) also made similar findings on petroleum effluents. It has also been observed that some microorganisms are more abundant in areas of high concentration of hydrocarbons. These microflora are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. The hydrocarbon-utilizing fungal isolated from the oil-contaminated soil were *Aspergillus niger*, *Aspergillus versicolor, Penicillum* , *Trichoderm* and *Rhizopus*. These genera of fungi have been reported by other authors in the degradation of hydrocarbon (Bossert and Bartha, 1984; Okpokwasili and Amanchukwu, 1988; Eze and

Okpokwasili, 2010). The result of this study revealed that, the indigenous microbial populations in soils of oil contaminated mechanic workshops are capable of mineralizing these pollutants in the environment to safe and acceptable level.

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