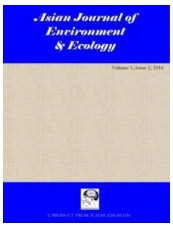

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Microbial Community Profiling of Spent-oil Contaminated Soil in Odukpani, Nigeria

A. A. Unimke[1*], I. U. Bassey[1], A. O. Mmuoegbulam[1] and H. N. Ikat[1]

[1]Department of Microbiology, Faculty of Biological Sciences, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IUB and AOM managed the analyses of the study. Authors AAU and HNI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The evaluation of microbiological and heavy metals concentration of oil contaminated soil samples from three different locations, thus; Okurikang, Inuakpa and Creek Town of Odukpani Local Government Area of Cross River State was investigated. The Total Heterotrophic Bacteria (THB) count ranged from $1.09x10^3$ to $3.6x10^3$cfu/ml, $1.11x10^3$ to $6.4x10^3$ cfu/ml, and $1.26x10^3$ to $4.6X10^3$ cfu/ml for the three locations respectively and the Hydrocarbon Utilizing bacteria (HUB) population obtained ranged from $2.28×10^3$ to $7.24×10^3$cfu/ml, $1.63×10^3$ to $6.96×10^3$cfu/ml and $1.62×10^3$ to $7.57×10^3$cfu/ml respectively foSr the three locations. The percentage occurrence of bacteria isolates for THB includes; *Enterobacter cloacae* 27.27%, *Lactobacillus* sp 45.45%, *Mycobacterium* sp 18.18% and *Enterobacter aerogenese* 9.09%, the percentage occurrence of bacteria isolates for HUB includes; *Acinetobacter calcoaccticus* 18.18%, *Micrococcus* sp 36.36% and *Pseudomonas aeruginosa* 45.45%. The levels of physicochemical properties; pH, Nitrate, Nitrite, Phosphate, Calcium, Magnesium, Potassium, Lead, Cobalt, Nickel, Iron, Ammonia and electrical conductivity obtained varied greatly with each soil sample. These findings provide adequate information on the microbial levels and heavy metals concentration of oil contaminated soil in Odukpani, Nigeria.

**Corresponding author: E-mail: austinraney.unimke@gmail.com;*

Keywords: Microbial community; heavy metals; spent-oil; contaminated soil; profiling.

1. INTRODUCTION

One of the major environmental challenges today is hydrocarbon pollution arising from activities related to petrochemical industry. Accidental and deliberate release of petroleum products is of particular concern in the environment [1-2]. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products.

The amount of natural crude oil seepage is estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [1-2]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [3-5]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [1]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry [6-9]. In addition, bioremediation technology is believed to be non-invasive and relatively cost-effective. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [10-11] and is cheaper than other remediation technologies.

The success of oil spill bioremediation depends on the ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influences the rate of oil biodegradation [3,11]. One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9. The physical and chemical characteristics of the oil and oil surface area are also important determinants of bioremediation success [1,9-14].

The aim of this study was to critically evaluate the microbial community structure and heavy metals concentration of spent-oil polluted soil in Odukpani Local Government Area of Cross River State of Nigeria. The objectives were; the evaluation of the microbial indices of spent-oil contaminated soil, the determination of the relationship between hydrocarbon pollution and heavy metals bioavailability and bioaccumulation, and the determination of the biodegradability of microbes isolated from spent-oil contaminated soil.

2. MATERIALS AND METHODS

The samples were collected from three mechanic workshops in three different towns (Okurikang, Inuakpa and Creek Town) in Odukpani Local Government of Cross River State of Nigeria. Microbial analysis was carried out in microbiology research laboratory university of Calabar while physiochemical and heavy metal analyses where done at the laboratory of cross river state water board, Calabar.

2.1 Collection of Samples

Soil samples were collected in a sterile polyethylene bag using a sterile spatula from the edge of a motor-oil stained patch at different locations of each workshop by scooping to about 5 cm. They were transported immediately to the laboratory for analysis [3,11-15]. Samples were homogenized and sub-samples taken to obtain a good representation of the hydrocarbon degrading microorganisms required for the study. Physiochemical properties of the soil such as conductivity, pH, temperature and heavy metals were examined.

2.2 Sample Preparation

Before proceeding to the analysis, an aliquot (portion) was taken from the bulk sample and in order to be sure that what was analysed was a representative of the collected samples, the samples were homogenized for the extraction. Soil samples from the various sampling locations

were mixed and the composite samples from all the stations were analysed properly for various physiochemical parameters and heavy metals using standard procedures [3,11].

2.3 Microbiological Analysis

2.3.1 Sample processing

Soil samples for microbial analysis were collected aseptically labelled and transported to the laboratory immediately where analysis was done. Prior to analysis, the soil samples were homogenized. One (1)g of each sample was weighed out, added to 90ml of sterile water and vigorously shaken for one minute. Treated samples were allowed to settle in order for withdrawal of supernatant for serial dilution. Tenfold serial dilution of the soil sample was carried out for enumeration of densities of microbial isolates [3,11-15].

2.3.2 Culture media

The analytical media used for this research included Nutrient Agar (NA), mineral salt medium (MSM), the media were prepared according to recommendations by the manufacturers (Difco; Biotech). The mixture was thoroughly shaken and allowed to dissolve. It was sterilized by autoclaving at 121℃ for 15 minutes. After the sterilization, it was allowed to cool at 45℃ (before dispensing into sterile Petri-dishes).

2.3.3 Estimation of heterotrophic micro-organisms

Ten (10) grams of the oil contaminated soil was suspended in 90ml of distilled water and tenfold serial dilutions of the samples from 1.10 to 1.100000 were carried out, 0.1ml of the 10^{-5} dilution for each soil sample was plated in triplicates on Nutrient Agar amended with Nystatin (50 µg/ml) to suppress the growth of fungal contaminants. The counts of THB were determined using pour plate techniques [3,11-16]. The Nutrient Agar plates were incubated at 37℃ for 24 h. The number of viable microorganisms in the samples was calculated from the number of colonies formed.

2.3.4 Isolation, purification and maintenance of pure culture

Distinct representative colonies from the culture plates were selected for characterization. Bacterial colonies were repeated transferred to freshly prepared nutrient agar plates by streak-plate method and allowed to grow for 24 h before stocking [3,11].

2.3.5 Enumeration of hydrocarbon utilizing microorganisms

The counts of hydrocarbon utilizing bacteria were enumerated by pour plate techniques [11-17] using vapour phase transfer on mineral salt medium (MSM), which basically contains mineral salts and vital nutrients. The medium was supplemented with 30 gml^{-1} fungizol miconazole to inhibit the growth of fungal contaminants. The crude oil used was sterilized by Millipore filtration (0.45 µm pore size) and stored in sterile bottles. The oil agar plates were incubated at room temperature for 5 days before enumeration.

2.3.6 Physiochemical analysis

Physicochemical analyses were used to evaluate the physical and chemical properties of the soil including [3,11-17]. The parameters analyzed included:

2.3.7 pH value determination

The 50 ml beaker was half-filled with the samples. Water was added to sufficient depth to allow immersion of the electrode. Mixing was carried out using a gentle shaker and stirred frequently for a few minutes then allowed to stand for another 15 minutes. The electrode of the meter was immersed into the starry and waited for middle drift to cease. The pH was recorded for each sample [3,11].

2.3.8 Electrical conductivity (EC) determination

A saturated paste of the crushed/soil samples were made using distilled water. The electrical conductivity of the samples was determined electrometrically with a calibrated electrical conductivity meter [3,11].

2.3.9 Heavy metals determination

1 g of soil sample was transferred into a dry 100 ml conical flask using a spatula. 30 ml of concentrated nitric acid was measured using a measuring cylinder and added into the conical flask containing 1 g of soil sample. 10 ml of hydrochloric acid was added into the conical flask using a 10 ml measuring cylinder. The two acids – HCl and HNO_3 were added in a ratio of 3:1 respectively, it was then covered with

aluminium foil and heat in the oven at 100℃.Heavy metals content of the soil digest was then determined using atomic absorption spectrophotometer (Model UNICAM 939) [3,11-13].

3. RESULTS

3.1 Total Heterotrophic Bacteria (THB)

In this study, the result represented in Table 1 show the total heterotrophic bacteria count in the samples from various locations. It is observed that the mechanic workshop situated at Inuakpa of Odukpani Area of Cross River State, produced a significantly higher total heterotrophic bacteria count ranging from $1.11x10^3$ to $6.4x10^3$ cfu/ml, Creek Town had counts ranging from $1.260x10^3$ to $4.63x10^3$ cfu/ml, while Okurikang had the least number of heterotrophic counts ranging from $1.09X10^3$ to $3.6\ x10^3$ cfu/ml.

3.2 Hydrocarbon Utilizing Bacteria (HUB)

Table 2 shows the result obtained for hydrocarbon utilizing bacteria. The mechanic work shop at Creek Town had the most significant counts ranging from 1.62×10^3 to 7.57×10^3 cfu/ml, Okurikang had counts ranging from 2.28×10^3 to 7.24×10^3 CFU/ml, while the Inuakpa had the least count ranging from 1.63×10^3 to 6.96×10^3 cfu/ml.

Tables 1 and 2 shows the result in cfu/gof the total heterotrophic bacteria (THB) count and hydrocarbon utilizing bacteria (HUB) in triplicates of the different spent-oil contaminated soil.

3.3 Physiochemical Characteristics of the Contaminated the Soil samples

The physiochemical characteristics of composite soil samples collected from different mechanic workshops were analysed and the result obtained is presented in Table 3. Various characteristics like electrical conductivity and PH were taken into consideration for each of the samples named sample A, B and C representing the three sampling locations (Okurikang, Inuakpa and Creek Town) respectively.

4. DISCUSSION

Evaluating the effects of different hydrocarbons on soil microbial activity and determining the suitability of analytical techniques for

Table 1. Total heterotrophic bacteria count in cfu/ml

Sample location	Sampling point	Sample 1 (cfu/g)	Sample 2 (cfu/g)	Sample 3 (cfu/g)	Mean (cfu/g)
Okurikang	SP 1	$2.8x10^3$	$3.6x10^3$	$2.46x10^3$	2.95×10^3
	SP 2	$1.09x10^3$	$1.48x10^3$	1.53×10^3	$1.37x10^3$
	SP 3	$2.01x10^3$	$1.46x10^3$	$1.57x10^3$	$1.68\ x10^3$
Inuakpa	SP 1	1.26×10^3	$1.42x10^3$	$1.46x10^3$	$1.38x10^3$
	SP 2	$2.66x10^3$	$6.4x10^3$	$1.11x10^3$	$3.39x10^3$
	SP 3	$1.28x10^3$	$3.67x10^3$	$3.33x10^3$	$2.76x10^3$
Creek Town	SP 1	$1.8x10^3$	$1.43x10^3$	$4.63x10^3$	$2.62x10^3$
	SP 2	$1.52x10^3$	$1.46x10^3$	$3.57x10^3$	$2.18x10^3$
	SP 3	$1.45x10^3$	$1.260x10^3$	$2.53x10^3$	$1.75x10^3$

Key: SP_x = Sampling Point, cfu/g = colony forming unit per gram

Table 2. Hydrocarbon utilizing bacteria counts in cfu/ml

Sample location	Sampling point	Sample 1 (cfu/g)	Sample 2 (cfu/g)	Sample 3 (cfu/g)	Mean (cfu/g)
Okurikang	SP 1	3.8×10^3	5.9×10^3	5.46×10^3	5.05×10^3
	SP 2	2.28×10^3	6.7×10^3	3.34×10^3	4.11×10^3
	SP 3	7.24×10^3	3.32×10^3	3.14×10^3	4.57×10^3
Inuakpa	SP 1	1.75×10^3	3.41×10^3	1.63×10^3	2.26×10^3
	SP 2	5.23×10^3	6.96×10^3	2.46×10^3	4.88×10^3
	SP 3	4.78×10^3	3.94×10^3	5.92×10^3	4.88×10^3
Creek Town	SP 1	7.57×10^3	6.41×10^3	4.36×10^3	6.11×10^3
	SP 2	2.57×10^3	3.67×10^3	3.74×10^3	3.33×10^3
	SP 3	1.62×10^3	2.1×10^3	1.73×10^3	1.82×10^3

Key: SP_x = Sampling Point, cfu/g = colony forming unit per gram

Table 3. Physicochemical characteristics of the soil sample from the three sampling locations

Parameters	Sample A (Okurikang)	Sample B (Inuakpa)	Sample C (Creek Town)
pH	7.07	7.04	7.16
Conductivity (μScm^{-1})	39.5	35.7	105.4
Nitrate (%)	394	249.5	374.5
Nitrite (%)	0.325	0.395	0.435
Phosphate (ppm)	11	27.5	12.5
Calcium (mg/g)	0.35	BDL	BDL
Magnesium (mg/g)	BDL	0.1	0.05
Potassium (mg/g)	20	11.5	22
Ammonia	0.85	0.7	0.5

Key: BDL = Below Detection Level

Table 4. Concentration and distribution of heavy metals in the three sampling locations

Parameters ($mgkg^{-1}$)	Sample A (Okurikang)	Sample B (Inuakpa)	Sample C (Creek Town)
Lead (Pb)	825	255	240
Cobalt (Co)	0.31	0.74	0.91
Nickel (Ni)	0.95	1.07	0.96
Iron (Fe)	43	48.5	34.7
Manganese (Mn)	BDL	BDL	BDL

Key: BDL = Below Detection Level

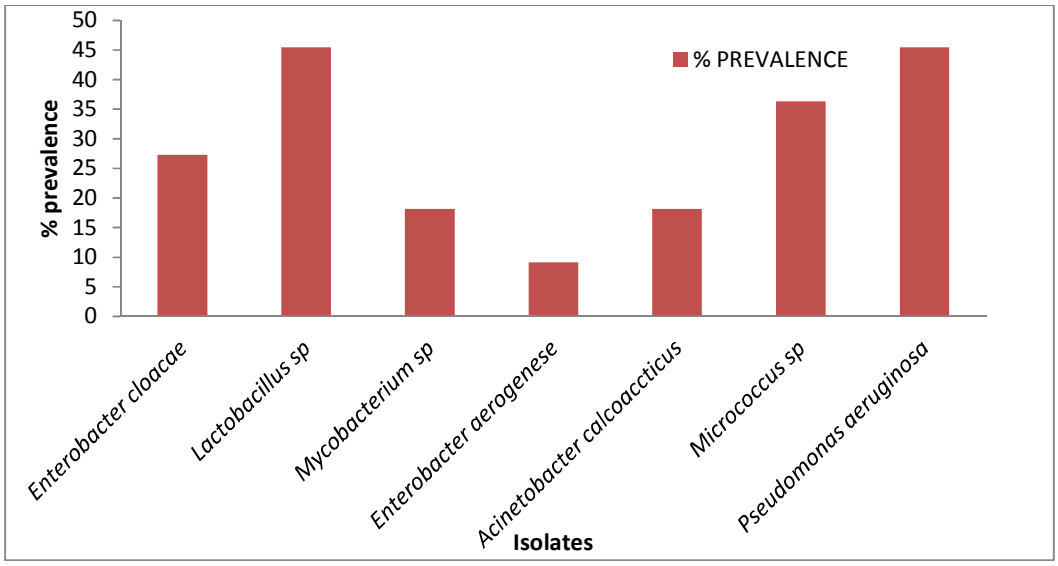

Fig. 1. Percentage prevalence of the bacterial isolates from the different spent-oil contaminated soil samples

discriminating between soil treatments are essential for investigating petroleum polluted soils. Microbiological and heavy metal concentration of oil contaminated soil in (Okurikang, Inuakpa and Creek Town) in Odukpani, Nigeria was carried out. The total heterotrophic bacteria count was significantly high ranging from $1.09x10^3$ to $3.6X10^3$, $1.11x10^3$ to $6.4x10^3$ and $1.26x10^3$ to $4.63x10^3$cfu/g and the hydrocarbon utilizing bacteria (HUB) population obtained ranged from $2.28×10^3$ to $7.24×10^3$, $1.63×10^3$ to $6.96×10^3$ and $1.62×10^3$ to $7.57×10^3$ cfu/g respectively from the three study locations. From the results obtained, the THB counts were slightly higher than the (HUB).

The slight increase is in agreement with the report of Unimke, et al. [25-26], who reported that such increase could be attributed to the high level of nutrients available in the soil which favours the growth of heterotrophic microorganisms. The slightly high levels of HUB obtained may be due prior exposure enabling the organisms to have high survivability rate. The morphology and biochemical characterization of bacteria isolates from contaminated soil samples showed the following suspected organisms *Enterobacter cloacae, Lactobacillus* spp*, Mycobacterium sp, Enterobacter aerogenese, Acinetobacter calcoacoticus, Micrococcus aeruginosa, Pseudomonas aeruginosa, etc.* The percentage occurrence of bacteria isolates for THB includes; *Enterobactercloacae* 27.27%, *Lactobacillus* sp 45.45%, *Mycobacterium* sp 18.18% and *Enterobacter aerogenese* 9.09%. The percentage occurrence of bacteria isolates for HUB includes; *Acinetobacter calcoaccticus* 18.18%, *Micrococcus sp* 36.36% and *Pseudomonas aeruginosa* 45.45%.

The contamination of the soil environment with heavy metals has been on the increase due to high level of industrialization [1-3, 11]. The mean concentrations of heavy metals and physiochemical parameters shows variation within the following ranges pH 7.07, 7.04 and 7.16 , Nitrate 394, 249.5 and 374.5, Nitrite 0.325, 0.395 and 0.435, Phosphate 11, 27.5, and 12.5, Calcium 0.35, 0 and 0, Magnesium 0, 0.1 and 0.05, Potassium 20, 11.5 and 22, Lead 825, 255 and 240, Cobalt 0.31, 0.74 and 0.91, Nickel 0.95, 1.07 and 0.96, Iron 43, 48.5 and 37.7, Ammonia 0.85, 0.7 and 0.5, and electrical conductivity 39.5, 35.7 and 105.4 for each soil sample respectively. This report is in agreement with the report of Cavalho et al. [5] that heavy metals are some of the most toxic, persistent and widespread contaminants in aquatic and terrestrial ecosystems.

The high levels of heavy metals and physicochemical characteristics recorded can be ascribed to the petrochemical pollution and run-off [3-5,11]. The soil samples contain different kinds of heavy metals at different volumes; this might be due to the level of contamination and period of pollution.

5. CONCLUSION

From the study it can be concluded that hydrocarbon degrading bacteria are abundant in soils contaminated with spent oil. The understanding of the influence of different perturbation and the fate of the release of oil in the soil environment is useful in the assessment of the environmental impact of the oil pollutant and its remedial investigation. The variation in the physiochemical parameters were as a consequence of the build-up on the soil of the product of oil degradation. The result of this study revealed that indigenous microbial populations in soils of oil contaminated areas are capable of mineralizing these pollutants in their environment to a save and acceptable level. It also proves that the soils contain different kinds of heavy metals at different volumes; this might be due to the level of contamination and period of pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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