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Microbiological Assessment of Soil Contaminated with Refined Petroleum: A Case Study of Eluama in Isuikwuato LGA, Abia State, Nigeria

Ezeigbo, O. R. [a]*, Agomoh, N. G.[b], Okike-Osissiogu, F. U.[c], Ibebuike, C. [d], Ike-Amadi C. A.[e]

[a,b,c,d]*Department of Biology/Microbiology, Abia State Polytechnic, Aba, Nigeria*

[e]*Department of Chemistry/Biochemistry, Abia State Polytechnic, Aba, Nigeria*

[a]*Email: obyezeigbotxt1@yahoo.com*

Abstract

A microbiological assessment of soil polluted by refined petroleum was carried out in Eluama community, Isuikwuato LGA, Abia State, Nigeria between March and October, 2012. The aim of the study is to examine the long-term kinetics of refined petroleum oil contaminated soil in this area from pipeline vandalization in the year 2000 and to assess the extent of biodegradation with respect to length of time of the spill. The microbiological examination of the soil samples were conducted by serial diluting and then inoculating the soil samples on different growth media. Several microbiological and biochemical methods were applied in order to isolate and identify the microorganisms accustomed to the soil sample. An unpolluted farmland served as control. Results showed a decrease in microbial load of soil as distance approaches seepage area. The control has a total heterotrophic bacteria count of 22.3 x 10^6 CFU/ml which decreases towards the seepage area (4.1 x 10^6 CFU/ml). The total fungi count also decreases from the control (5.9 x 10^6 CFU/ml) towards the seepage area (1.2 x 10^6 CFU/ml). *Micococcus sp* and *Pseudomonas aeroginosa* were predominant in the seepage area up to 30m away from the seepage area. Other bacteria identified after this distance include *Bacillus sp, Klebsiella pneumonia, Streptomyces sp, Streptococcus sp* and *Staphylococcus aureus* while the predominant fungi were *Aspergillus niger* and *Mucor sp.* The marked decrease of heterotrophic bacteria and fungi in the petroleum polluted area compared with the control (the unpolluted farmland) shows the unsuitability of the soil for agricultural purpose, as full remediation has not taken place.

Keywords: microbiological assessment; refined petroleum oil; soil contamination; pipeline vandalization

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* Corresponding author.
E-mail address: obyezeigbotxt1@yahoo.com

1. Introduction

Petroleum continues to be used as a principal source of energy in Nigeria; however, despite its usage, petroleum hydrocarbons also act as a global environmental pollutant. There is growing public concern as a wide variety of toxic chemicals are being introduced inadvertently or deliberately into the environment. Petroleum hydrocarbons are now common example of these chemicals, which enter the environment frequently and in large volumes through numerous routes.

A significant amount of petroleum oil is refined, stored and transported through mangrove ecosystem in Nigeria and as a result, the risk of an oil spill. Petroleum oil pollution results from human activities such as drilling, manufacturing, storing, transporting, waste management of oil and vandalization of oil pipe lines [1]. The massive and extensive pollution of the environments by petroleum industries constitute socioeconomic and public health hazards [2]. Petroleum oil pollution exerts adverse effects on plants indirectly by making toxic minerals in the soil more available to plants [3]. Han [4] reported that hydrocarbons especially petroleum oil are rapidly and completely degraded in well aerated soils. It is only when the contamination of the soil is severe or under anaerobiosis, or if the oil has penetrated to great depths that there is any danger of soil conservation and contamination of drinking water [5]. Bacteria and fungi are the primary agent for degradation of organic contaminants in soil [6] Increasing diversity of microbial populations and common structures can accelerate the degradation of the contaminants [7]. Although hydrocarbon utilizing microorganisms are ubiquitous, their population within the microbial community is thought to be a sensitive index of environmental exposure to hydrocarbons [8]. It is now generally accepted by scientific community that no one species of microorganisms will completely degrade any particular oil [9]. The degradation of both crude and refined oils involves a consortium of microorganisms, including eukaryotic and prokaryotic forms. The common genera known to be responsible for oil degradation comprises mainly of *Narcadia, Pseudomonas, Acinetobacter, Corynebacterium, Achromobacter, Rhodococcus, Alcaligenes, Mycobacterium, Bacillus, Aspergillus, Mucor, Fusarium, Penicillium, Rhodotorula, Candida* and *Sporobolomyces* [10, 11, 12, 13]. While bioremediation has many advantages, it is a site-specific process and successful biological treatment of contaminated soil presents a challenge to environmental scientists and engineers. Such challenges include the heterogeneity of the contaminants, extreme concentration of the hydrocarbons which can be toxic or inhibit microorganisms while extremely low concentration may not be adequate to support microbial activities. The variable site environmental conditions such as soil type, soil depth and soil microorganisms as well as physical conditions such as pH, temperature, oxygen availability, redox potential, moisture content and substrate bioavailability can substantially affect the microbial growth and biodegradation of organic contaminants. This study therefore is aimed at assessing the level of microorganisms associated with biodegradation of oil polluted soil in Eluama community with a view of ascertaining the level of remediation of the polluted soil for agriculture after 12 years of oil spill.

2. Materials and Methods

Study Area: Eluama is a community in Isuikwuato LGA of Abia State, Nigeria. It is located along the Nigeria National Petroleum Corporation (NNPC) high pressure oil pipe lines used for the distribution of petroleum products from refinery in Port Harcourt, River State, Nigeria, to other parts of the country. Being a rural community, the people survive on subsistence farming. However, due to vandalization of the pressure pipe lines by members of the community in year 2000, the area was gutted by fire from oil spill thus damaging the vegetation and soil.

Collection of Soil Samples: One hundred meters (100m) were mapped out from the sampling area. The area was divided into 5 with 20m distance spacing. The seepage area was identified and labeled while an unpolluted farm

land in the community served as control. The soil samples were collected at different depth of 0-15cm using an auger and kept in sterile plastic bag. The samples were transported in refrigerated coolers to arrest microbial growth to Federal College of Land Resources and Technology, Owerri, Imo State, Nigeria, for analysis.

Preparation of Soil Samples for Analysis: Ten –fold serial dilutions of the soil samples were made as described by [14]. 1g of soil sample was homogenized in 9ml distilled water. 1ml of homogenized soil was transferred into a second tube containing 9ml distilled water. This was continuously repeated until the tenth tube (10^{-10}).

Inoculation and Enumeration: Four different nutrient media (Nutrient agar, MacConkey agar, Eosin methylene blue agar and Potato dextrose) were used. All nutrient media were prepared according to manufacturer's procedures.

0.1ml of appropriate ten-fold serial dilutions (10^{-6}) of the soil sample was inoculated into each of the nutrient media in triplicates using pour plate method [14]. Inoculated plates were incubated at 37^0C for 24 hours for the enumeration of total heterotrophic bacteria. Inoculated plates of potato dextrose agar (PDA) were kept at room temperature for 5-7 days. Visible discrete colonies in inoculated plates were counted and expressed as colony forming units per gram (CFU/ml) of soil samples.

Characterization and Identification of Microbial Isolates: Discrete colonies were purified by sub-culturing into appropriate agar media. Pure cultures of microbial isolates were identified based on cultural parameters. Grams staining as well as biochemical tests were carried out in agar slants as described by [15] and [16].

Statistical Analysis: The values obtained were expressed in Bar Charts for quick appreciation of the data.

3. Result

The microbial counts of microorganisms isolated from the soils are shown in Table 1. Total heterotrophic bacteria counts (THBC) from the polluted soil ranged from 4.1 x 10^6 to 20.0 x10^6 with that of unpolluted soil which served as control being 22.3 x 10^6. The total fungal counts (TFC) ranged from 1.2 x 10^6 to 5.9 x10^6 with that of the unpolluted soil which served as control being 5.9 x10^6. The study revealed an increase in microbial counts of isolates as distance increases from the seepage site. The following bacteria genera were isolated: *Bacillus, Pseudomonas, Micrococcus, Staphylococcus, Streptococcus* and *Klebsiella* (Table 2). This result was expressed in a Bar chart for a quick appreciation of the data (Figure 1). The fungi isolated belong to the genera *Aspergillus* and *Mucor* (Table 3).

Table 1: Total Heterotrophic Bacteria Count and Total Fungi Count in Different Soil Samples

Samples (m)	THBC (CFU/ml) x 10^6	TCF (CFU/ml) x 10^6
Seepage Area	4.1	1.2
10	7.2	1.6
30	8.8	2.4
50	15.5	3.0
70	18.4	3.8
100	20.0	5.0
Control	22.3	5.9

THBC=Total Heterotrophic Bacteria Count; TFC= Total Fungi Count

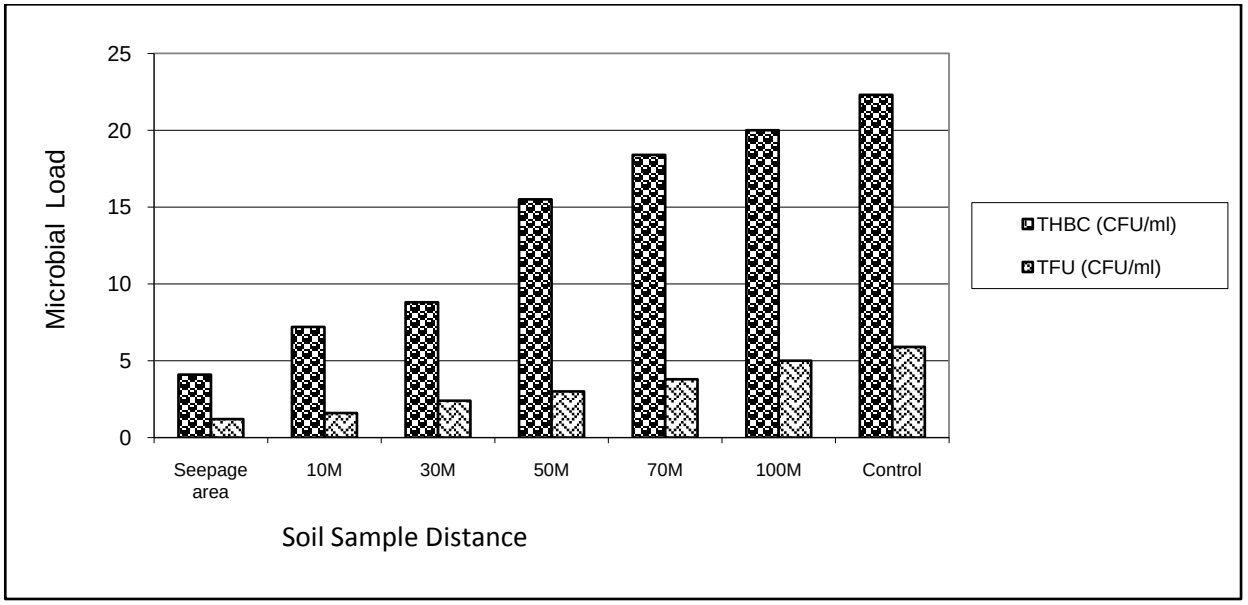

Figure 1: Bar Chart Representing Microbial Load of Different Soil Samples

Table 2: Cultural Characteristics, Gram Reaction and Biochemical Properties of the Various Isolates

Sample	Gram React.	Mot.	Shape	Ind.	Cata.	Coag.	Oxid.	Spore Form.	Met. red	Voges pros	Glucose	Citrate	Urease	Probable organism
Seepage	+	-	Cocci	-	+	-	+	-	-	+	A	+	-	*Micrococcus sp*
Seepage area	-	+	Rod	-	+	-	+		-	+	A	-	-	*P. aerugonisa*
10m/ A_1	+	-	Cocci	-	+	-	+	-	-	+	A	+	-	*Micrococcus sp*
10m/B_1	-	+	Rod	-	+	-	+		-	+	A	-	-	*P. aerugonisa*
30m/A_1	+	-	Cocci	-	+	-	+		-	+	A	+	-	*Micrococcus sp*
30m/B_1	-	+	Rod	-	+	-	+		-	+	A	-	-	*P. aerugonisa*
50m/A_1	+	+	Rod	-	+	-	+	+	-	-	A		-	*Bacillus sp*
50m/A_1	-	+	Rod	-	+	-	+		-	-	A	-	-	*P. aerugonisa*
50m/A_2	-	-	Rod	-	+	-	-	+	-	+	A/G	+	+	*K. pneumonia*
70m/A_2	-	+	Rod	-	+	-	+		-	-	A	-	-	*P. aerugonisa*
70m/B	+	+	Rod	-	+	-	+	+	-	-	A		-	*Bacillus sp*
70m/B														*Streptococcus sp*
100m/A_1	+	+	Rod	-	+	-	+	+	-	-	A		-	*Bacillus sp*
100m/A_2	-	+	Rod	-	+	-	+		-	-	A	-	-	*P. aerugonisa*
100mB_2														*Streptococcus sp*
100m/A_2														*Streptomyces sp*
Control	+	-	Rod				-	+						*Streptomyces sp*
Control	-	+	Rod	-	+	-	_		-	-	A	-	-	*P. aerugonisa*
Control	+	+	Rod	-	+	-	+	+	-	-	A	-	-	*Bacillus sp*
Control	+	-	Cocci	-	+	+	-	-	+	-	A	-	-	*S. aureus*
Control	-	-	Rod	-	+	-	-	+	-	-	A/G	+	+	*K. pneumonia*
Control	-	+	Rod	+	+	-	-	-	+	-	A/G	-	-	*E. coli*

Gram react. =gram reaction; Mot. = Motility; Ind= Indole ; Cata.= Catalase; Coag. = Coagulase, Oxid.= Oxidase; Spore form= spore forming; Met. Red= methyl red; Voges pros= voges proskauer

Table 3: Cultural and Morphological Characteristics of the Different Fungi Isolates from the Samples

Samples	Culture	Morphology	Probable Fungi
Seepage Area	1.Bluish green colony	Possess stalled –like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
10m	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
30m	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
50m	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
70m	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
100m	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*
	2.White colony	Smooth coinoidiophores that are non-septate.	*Mucor spp*
Control	1.Bluish green colony	Possess stalled-like coinoidiophores with large vesicles.	*Aspergillus niger*

4. Discussion

This work on the microbiological studies of soil with refined petroleum contaminated area in Eluama, Isuikwuato LGA, was designed to assess the current suitability of the soil for land utilization. The presence of heterotrophic bacteria is attributed to the tolerance of these microbes to wide variations of soil properties. This is linked with their ability to thrive well on soils range of 37^0C to 42^0C. This agrees with the report of [17] that consortium of degraders like *Pseudomonas* sp and *Micrococcus* sp are the most predominant bacteria capable of degrading constituents of oil. The result also showed a decrease in microbial and fungal counts with increase in petroleum contamination as revealed in distances 30m, 10m and oil seepage area, with microbial isolates, *P. aeruginosa* and *Micrococcus spp* inhabiting such areas, whereas Bacillus spp and Streptococcus spp were found in distance 50m to 70m. Similar species of microbial isolates have been reported with petroleum contaminated soil [10, 11, 18, 19]. The presence of *Pseudomonas aeruginosa* and *Micrococcus* spp at the high petroleum contaminated soils is indicative of the petroleum degrading ability of these microorganisms while Bacillus and Klebsiella spp present in low petroleum

contaminated soils are low-petroleum degraders. However, P. aeruginosa was found in all soil samples, thereby displaying the characteristics of being a normal flora of the soil with or without petroleum. The study revealed that the microbial activities in the petroleum polluted soil is not suitable to support plant growth, therefore much time is needed for proper restoration of the soil for agricultural purposes.

5. Conclusion

The microbiological study of petroleum polluted soil in Eluama community was carried out to assess the suitability of the soil for land utilization after 14 years of petroleum oil pollution. The result revealed a decrease in microbial load of the soil samples as distance approaches the seepage area. The presence of *Pseudomonas aeruginosa* and *Micrococcus species* at the high petroleum contaminated sites is indicative of the petroleum degrading ability of the organisms. The data provided from this work showed the inability of the polluted soil to support plant growth. It is therefore recommended that more time should be allowed for full remediation to take place.

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