

Microbial Population Dynamics and Composition in Crude Oil Contaminated Soils Treated with Microorganisms and Guinea Grass (*Panicum maximum*)

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Abstract

Crude oil pollution can cause alterations in the soil physico-chemical properties and microbial activities, with deleterious effects on soil productivity indices. Studies were conducted on a sandy loam soil contaminated with levels of crude oil, bioremediated with bacteria, fungi and guinea grass. The experiment was a 3 x 4 factorial in completely randomized design (CRD) in the screen house in the University of Port Harcourt. Results showed that all the treatments had significant ($p < 0.05$) effects on microbial population, diversity and total petroleum hydrocarbons (TPHs). Bacteria significantly ($p < 0.05$) reduced TPH within 6 to 8 days. *Bacillus Streptococcus*, *Staphylococcus*, *Aeromonas* and *Pseudomonas* spp. were the dominant bacteria found in the soil whereas, *Aspergillus*, *Fusarium*, *Rhizopus*, *Blastomyces* and *Saccharomyces* species were the dominant fungi isolates. Guinea grass was not effective in bioremediation when the crude oil was above 5% w/w level of contamination.

Keywords: Crude oil, bioremediation, bacteria, fungi, soil fertility, petroleum hydrocarbon.

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Introduction

Soil contamination with petroleum and petroleum based products is a major concern to humans and the ecosystem indicators including farmlands, crop plants and forest trees species (Ogari, 2001; Agbogidi, 2003). Most often, the problems of crude oil pollution occur as a result of accidental discharge, seepage, loading and offloading and improper disposal (Njoku *et al.*, 2009). The amount of contamination due to natural seepage is estimated at 600,000 metric tons per year, with arrangement of uncertain components of about 200,000 metric tons (Kvenolden and Cooper, 2003). High content of hydrocarbons and certain hazardous compounds in relatively high concentrations in crude oil has also been reported to affect soil physical, chemical and biological properties (Franco *et al.*, 2004). On the other hand, diverse microorganisms such as bacteria, fungi, algae, viruses, and protozoa form biological phase (Tanee and Albert, 2011) and they play vital roles as ecosystem indicators, including nutrient cycling (Zucchi *et al.*, 2003). They are responsible for decomposition of organic matter (Wolicka *et al.*, 2009), which invariably promote plant health and growth as bio fertilization (Liu *et al.*, 2008), and aid in bio remediation processes.

Degradation of petroleum hydrocarbon by natural population of microorganisms is the main process acting in the cleanup of hydrocarbons. In situ degradation involves greater degradation of oil pollutants on site by a consortium of microorganisms (Ojumu *et al.*, 2004) and more than 200 species of bacteria, fungi and algae can biodegrade hydrocarbons. Various genera of these organisms are hydrocarbon degrading species such as *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Staphylococcus*, *Aeromonas*, *Flarobacterium*, *Aspergillus*, *Fasarium* and have been isolated in large numbers from many polluted waters and soils (Okoh, 2003).

Although, there are several studies to show that microorganism can remediate crude oil polluted soils, there is still need to study the effect of crude oil pollutions at different concentrations on the growth, distributions and diversity of soil microorganisms. The aim of this study therefore is to determine the microbial population dynamics and composition with varying levels of crude oil pollution in a sandy loam soil in Ibaa community, Emohua local government of Rivers State.

Materials and Methods

Study Area and Sample Collection: Surface soil samples (0-20 cm) was collected from Ibaa community in Emohua Local Government Area of River State, Nigeria. The sampled site is located at Latitude 04°57'N, and Longitude 6°48'E. the soil was taken to the screen house at the Faculty of Agriculture, Department of Crop and Soil Science, University of Port Harcourt, River State, Nigeria for the simulation study. The mean annual rainfall of the area ranged between 2000 mm and 45000 mm with peak periods in July and October (Inyang, 1978). The annual temperature ranged from a minimum of 22°C to a maximum of 31°C.

Experimental procedure and Soil Treatment: Crude oil was obtained from the Shell Petroleum Company Nigeria in Rivers State and used to contaminate the soil at 0, 2, 5, and 10% w/w levels of contamination. Six kilogram (6 kg) of the soils was filled in experimental pots and further treated with Fungi, Bacteria and guinea grass. The experiment was a 4x3 factorial, arranged in completely randomized design (CRD) in 3 replications.

Treatment combinations are shown in Table 1. Initial properties of composite soil samples used for the experiment are shown in Table 2. In treating the soils, the methods of Njoku *et al.*, (2009) were used by thoroughly mixing the crude oil with soils. The experiment commenced in late August to October, 2018 (8 weeks).

Isolation of Total Heterotrophic Bacteria THB): Total heterotrophic bacterial counts were determined using the serial dilutions and spread plate technique on nutrient agar as described by Fawole *et al.* (2001). The colony forming units (cfus) were counted after incubating the samples at 37°C for 24 h. transferred onto agar slant for storage. Identification of isolations was one by biochemical test as described in Bergey's manual to determine bacteriology.

Isolation of Total Heterotrophic Fungi (THF): Total heterotrophic fungi count was determined using serial dilution and spread plate technique on potato dextrose agar methods as described by Monica (2001). The soil samples were prepared by 10 fold serial dilutions of 10³ dilutions and incubated at room temperature of 25°C for 5 days. Pure fungal isolates were identified using lacto phenol blue and direct microscopy (Monica, 2001).

Table 1. Treatment Combinations

Treatments	Designation
C	Control
B	Bacteria alone
F	Fungi alone
G	Guinea grass alone
BCu ₂	Bacteria and 2% crude oil
FCu ₂	Fungi and 2% crude oil
GCu ₂	Guinea grass and 2% crude oil
BCu ₅	Bacteria and 5% crude oil
FCu ₅	Fungi and 5% crude oil
GCu ₅	Guinea grass and 5% crude oil
BCu ₁₀	Bacteria and 10% crude oil
FCu ₁₀	Fungi and 10% crude oil
GCu ₁₀	Guinea grass and 10% crude oil

Isolation of Hydro Carbon Utilizing Bacteria and Fungi and Total Petroleum Hydrocarbon One gram of each soil sample was diluted serially 10⁻¹ to 10⁻⁷ in triplicates and inoculated in minerals salt agar, using the spread plate technique. Colonies of hydrocarbon utilizing bacteria and fungi were randomly obtained using potatoe dextrose. Identification was done using biochemical test described in Bergey's Manual. Total petroleum hydrocarbon content was measured using the method of cold extraction method as described by Odu *et al.* 1995. The total petroleum hydrocarbon (TPH) concentrations in the samples were determined using the stored calibration curve.

Determinations of Particle-size Distribution, pH and Organic Matter: Particle size distribution was determined by the method of Gee and Bauder (1986) after soil dispersion with sodium hexametaphosphate. Soil pH was measured with a glass electrode in a 1:2.5 soil water solution (McLean, 1982). Total organic carbon was determined by Walkley and Black wet dichromate oxidation method (Nelson and Sommers, 1996).

Data Analysis: A two-way analysis of variance was carried out using the SAS software (SAS, 2001), and the least significant difference (LSD) of the treatment means was determined at 5% probability.

Results and Discussion

The soil is sandy loam with sand, silt and clay content are 75.2, 2.3, and 21.5% respectively (Table 1), and slightly acidic (pH 6.2). Total nitrogen and organic carbon were low, indicating low fertility status of the soil. Total petroleum hydrocarbon was near threshold value of Odu *et al.* (1995) for sandy loam soils of the Niger Delta. Total heterotrophic bacteria and fungi were low (270 and 12 x 10³ cfus g⁻¹, respectively).

Table2: Initial soil properties before contamination

Soil Properties	Mean value
pH	6.2
Total petroleum hydrocarbon (mg kg ⁻¹)	1578.95
Sand (%)	75.2
Salt (%)	2.3
Clay (%)	21.5
Texture	SL
TOC (%)	0.91
Total N (%)	0.046
Available P (mg kg ⁻¹)	8.50
Total heterotrophic bacteria (x10 ³ cfus g ⁻¹)	270
Total heterotrophic fungi (x10 ³ cfus g ⁻¹)	12

TOC- total organic carbon, SL- sandy loam

Effect of Treatments on Total Petroleum Hydrocarbons and Soil pH: Changes in total petroleum hydrocarbon (TPH) and pH are shown in Table 3. Guinea grass was not effective in reducing TPH compared to fungi treated soils. Total petroleum hydrocarbon (TPH) ranged from 11457.2 in fungi treated soils to 1676.8 for guinea grass soils. When heavy load of crude oil (5% and 10%), fungi consistently showed positive ($p < 0.05$) effect in degrading TPH. On the other hand, fungi tend to increased soil acidity. For example, pH was 5.3, 5.8 and 5.9 for F, FCu₅ and FCu₁₀, respectively, compared with bacteria which increased soil pH significantly ($p < 0.05$). This implied that bacteria modified soil acidity to a minimum. This is consistent with previous works by Nester *et al.* (2001) and Okoh (2006) that bacteria modified the soil temperature and consequently, soil pH to favour biodegradation. The use of fungi in bioremediation process led to significant increase in soil pH, whereas, guinea grass did not show significant short-term effect in removal of TPH.

Effects of Treatments on Hydrocarbon Utilizing Bacteria and Fungi: Hydrocarbon utilizing bacteria didn't show any significant difference with the treatments applied until four weeks after planting (4 WAP), where the bacteria population was highest (540.50 Cfus × 10³g) at 0% and significantly reduced to the lowest (372.00 Cfus × 10³g) level at 10% load of crude oil at $P \leq 0.05$ (Table 4). The treatments had significant influence on the hydrocarbon utilizing fungi across the period of assessment with the fungi population increasing with increase in weeks after application. At 2 WAP, a sharp fall was noticed in the population of fungi at 2% treatment level before the population began to increase with increase in the levels of treatment, while at 4 WAP, the hydrocarbon utilizing fungi significantly increased with a rise in the treatment levels at $P < 0.05$. This is consistent with the report of Agbobidi *et al.* (2005), that hydrocarbon utilizing micro-organisms usually increased during the early period of introduction of petroleum hydrocarbon and reduced to minimum after a long period as observed at 8 weeks after application (8 WAA).

Percent Occurrence of Heterotrophic and Hydrocarbon Utilizing Bacteria and Fungi: *Bacillus* and *Aspergillus* were found to be more populated when compared to other bacteria and fungi isolates in both the treated and non-treated soils (Table 5). *Bacillus* and *Aspergillus* species were consistently found in all the samples showing adaptability of the two species. On the other hand, the *pseudomonas* were inhibited in the contaminated soils, indicating that *pseudomonas* do not grow in crude oil contaminated environment. The result is consistence with the previous assertions of Santhoshkumar *et al.* (2015) who identified *Bacillus subtilis*, and insignificant numbers of *Pseudomonas putida*, in *Chlorpyrifos* polluted soil in India. *Bacillus* and *Aspergillus* species are well known species in the breakdown of petroleum hydrocarbons in soil. This result further agreed with the findings of Tesar *et al.* (2002) who reported a broad phylogenetic range of bacteria and fungi including species/strains of *Bacillus*, *Pseudomonas*, *Areomonas spp*, *Staphylococcus spp*, *Streptococcus spp* in contaminated soils

Conclusion: The following revelations were made from this study: *Bacillus* and *Aspergillus* species of microorganisms were commonly found in treated soils than in untreated soils. They both have the potential to tolerate and grow in crude oil contaminated soils and can be used in remediation of contaminated soils. Fungi alone were not very effective in bioremediation technology. Bacteria were more effective in cleanup of the crude oil contamination soils above 5% w/w, whereas, Guinea grass is recommended in low levels of crude oil contamination. Both the total heterotrophic and hydrocarbon utilizing microorganisms increased significantly during the early 4 to 6 weeks of contamination and reduced significantly at 8 weeks after application (WAA).

Table 3. Effects of treatment on total hydrocarbon and pH at 8 weeks after application

Treatments	Total hydrocarbon (mg kg ⁻¹)	pH(H ₂ O)
C	1672.5	6.2
B	1554.8	6.8
F	1457.2	5.1
G	1676.8	5.3
BCu ₂	1577.9	6.4
FCu ₂	1581.7	6.1
GCu ₂	1512.6	5.8
BCu ₅	1591.2	6.1
FCu ₅	1588.4	6.3
GCu ₅	1591.1	6.1
BCu ₁₀	1622.5	6.6
FCu ₁₀	1597.5	5.9
GCu ₁₀	1623.9	5.2
LSD (0,05)	102.18	1.3

Table 4. Effects of Treatments on Hydrocarbon Utilizing Bacteria and Fungi

Treatment	Hydrocarbon Utilizing Bacteria (cfus x 10 ³)			Hydrocarbon Utilizing Fungi (cfus x 10 ³)		
	2 WAA	4 WAA	8 WAA	2 WAA	4 WAA	8 WAA
C	272.8	540.5	569.8	2.30	4.28	4.36
BCu ₀	252.7	412.4	569.08	2.11	5.61	6.33
FCu ₀	230.2	422.5	468.19	2.46	4.88	5.19
GCu ₀	231.5	432.8	332.18	2.39	4.22	5.125
BCu ₂	236.5	436.5	411.32	2.14	4.50	6.25
FCu ₂	248.0	421.6	472.16	2.04	4.36	7.11
GCu ₂	241.5	511.7	406.52	3.71	4.11	6.0
BCu ₅	257.9	484.3	426.12	3.63	6.91	6.38
FCu ₅	141.3	461.3	431.16	3.33	6.21	6.33
GCu ₅	221.9	433.7	418.22	3.49	6.66	6.48
BCu ₁₀	195.0	372.0	303.05	3.71	7.55	7.34
FCu ₁₀	183.1	318.5	310.44	3.11	7.23	7.21
GCu ₁₀	241.6	402.1	384.55	3.59	6.49	7.09

LSD(0.05): Bacteria =NS, Bacteria x weeks = 31.87, Fungi = 0.508, fungi x weeks = 0.3584

Table 5. Occurrence of heterotrophic and hydrocarbon utilizing bacteria and fungi at 2 weeks after crude oil application

Bacteria Isolated	Occurrence (%)		Fungi Isolated	Occurrence (%)	
	THB	HUB		THF	HUF
<i>Bacillus spp</i>	40	45	<i>Aspergillus spp</i>	50	40
<i>Streptococcus spp</i>	15	10	<i>Fusarium spp</i>	2	3
<i>Staphylococcus</i>	30	30	<i>Rhizopus</i>	35	50
<i>Aeromonas spp</i>	5	10	<i>Blastomyces</i>	10	5
<i>Pseudomonas</i>	5	5	<i>Saccharomyces</i>	2	2

THB- Total heterotrophic bacteria, HUB- Hydrocarbon utilizing bacteria,

THF- Total heterotrophic fungi, HUF- hydrocarbon utilizing fungi

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