Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 4, p. 152-165, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Effect of crude oil pollution on the rhizosphere microbial communities of *Mangifera indica* L and *Elaeis guineensis* Jacq in Rivers State, Nigeria

Chima Uzoma Darlington*, Omokhua Godwin Ejakhe, Njoku Ogechi

Department of Forestry and Wildlife Management, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Article published on April 21, 2015

Key words: Trees, rhizosphere, bacteria, fungi, crude oil pollution.

Abstract

This study investigated the effect of crude oil pollution and remediation on the fungi and bacteria communities of *M. indica* and *E. guineensis* rhizospheres using three sites - Unpolluted Site (UPS), Polluted and Treated Site (PTS), and Polluted and Untreated Site (PUS). Population of fungi in both *M. indica* and *E. guineensis* rhizospheres was highest in UPS while the bacteria population was highest in PUS and UPS in *M. indica* and *E. guineensis* rhizospheres, respectively. The highest similarity in fungi species was observed between UPS/PTS (67%) and PTS/PUS (87%) in *M. indica* and *E. guineensis* rhizospheres, respectively. Similarity in bacteria species was highest between UPS/PTS (50%) in *M. indica* rhizosphere while it was highest between UPS/PUS (60%) and PTS/PUS (60%) in *E. guineensis* rhizosphere. The diversity of fungi was highest at UPS in both *M. indica* (H = 1.04; Simpson 1-D = 0.63) and *E. guineensis* (H = 1.17; Simpson 1-D = 0.67) rhizospheres. Bacteria diversity in *M. indica* rhizosphere was highest in PUS (H = 0.70) when Shannon-Wiener index was used but highest in PTS (Simpson 1-D = 0.42) when Simpson index was used; and highest in PTS (H = 039; Simpson 1-D = 0.20) for *E. guineensis* rhizosphere. Most of the evaluated attributes compared better in UPS; however, bacteria population and diversity in *M. indica* rhizosphere was highest in PUS and PTS, respectively.

***Corresponding Author:** Chima Uzoma Darlington ✉ uzoma.chima@uniport.edu.ng

Introduction

Crude oil pollution constitutes a big threat to the environment, and effective remediation of oil-polluted ecosystems remains a daunting challenge for environmental research. Oil spillage is a widespread phenomenon, though; it is comparatively more frequent in the developing countries than in the technologically developed nations. In Nigeria, a large amount of crude oil is spilled annually into the environment. Incidences of environmental pollution due to high rate of petroleum related activities in the Niger Delta area of Nigeria and other oil producing areas of the world have been associated with frequent oil spills, especially through blowing out of oil wells, tanker accidents, bunkering, rupture of pipelines and sabotage.

Crude oil is a known source of energy and income in the world, but its introduction into the environment poses a lot of pollution problems as it distorts the soil originality, thus leading to loss of agricultural land and deforestation (Walker *et al.* 2005). Oil spillages have been known to exhibit various deleterious effects on both plants and microorganisms. The presence of petroleum in the soil has been known to affect plant diversity, canopy and productivity (Strickland, 1990). It has been reported that in tropical conditions, crude oil disappears rapidly in freely well –drained soil but degradation is slowed down by poor aeration (Odu, 1981). Roscoeet *et al.* (1989) also reported increase in anaerobic microorganisms in crude oil polluted soil.

Rhizosphere microorganisms are found in and around the roots of plants. Some penetrate into the cells of plant root while others grow between the roots of woody plants. These microorganisms help plants absorb minerals and water from ground by increasing the surface area in contact with the soil (Hackl *et al.*, 2004). Their cell membranes possess a biochemistry different from that of the roots, which aids in the uptake of phosphate ions and other nutrients like nitrogen (Cairns *et al.,* 1993). Sabate *et al.* (2004) reveal that oil spills result in an imbalance in the carbon-nitrogen ratio at the spill site because crude oil is essentially a mixture of carbon and hydrogen.

Several studies have been conducted previously in oil spill sites to determine the oil and grease contents of soil (Amajor, 1985), ecological post impact assessment (IPS, 1990), effect of spilled oil on soil properties and microflora (Amadi *et al.*, 1996), and effect on plant growth and soil productivity (Onweremadu and Duruigbo 2007, Smith *et al*, 1989). However, no study has been carried out to ascertain the impact of crude oil pollution on the microbial populations of the rhizosphere, which plays a vital role in the survival of plants under adverse chemical conditions (Izaguirre-Mayoral *et al.*, 2002).

The main objective of this study was to develop a comprehensive understanding on the effect of crude oil pollution on the fungal and bacterial communities of the rhizospheres of two economically important tree species – *M. indica* and *E. guineensis*, in Kagbere-Dere oil-producing Community located in Gokana Local Government Area of Rivers State.

Materials and methods

Description of the study area

Kagbere Dere Community is located in Gokana Local Government Area of Rivers State. The town is basically linear. This pattern is as a result of the direction of expansion of the town northwards and southwards. Kagbere Dere lies in the humid tropical zone with annual rainfall that ranges from 2000-2470mm and with an annual temperature ranging from 23^{o}C minimum to 32^{o}C maximum (NDES, 2001).

Kagbere Dere consists of tropical rainforest; however, towards the coast the typical Niger Delta environment features many mangrove swamps. Generally, the vegetation of the area is made up of an intricate mixture of plants which belongs to different plant families, genera and species. However, the plants in the polluted sites are in patches and are sparsely distributed on the soil (Chima and Vure, 2014).

A Port Harcourt Appeal Court Report (1994) noted that the soils of the polluted site are resistant to penetration by plant roots, have high bulk densities,

low hydraulic conductivities and infiltration capacities, and consequently very poor plant growth. The report equally observed that soil samples studied were very acidic, poor in total nitrogen, available phosphorous, organic carbon and generally low levels of exchangeable cations and micronutrients; and that the level of manganese in the soil was found to be toxic to plant life.

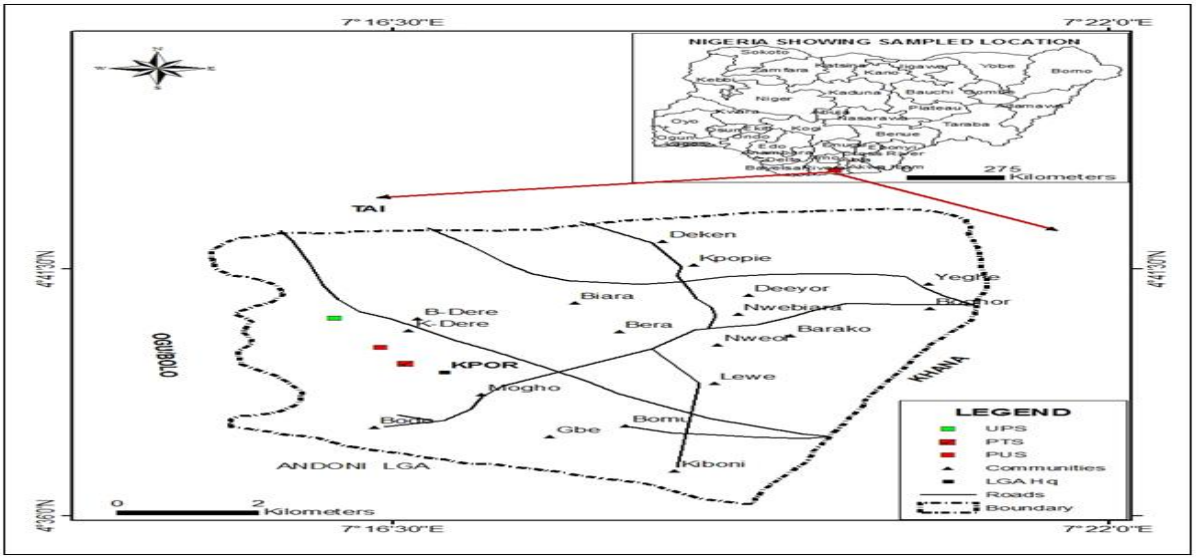

Fig. 1. Map of Ghokana Local Government Area showing the study sites.

Selection of the study sites

Three sites were purposively selected for the study. The Unpolluted Site (UPS), which served as the reference ecosystem, was selected from the section of the study area without any history of crude oil pollution. The Polluted and Untreated Site (PUS) was selected from a crude oil polluted section of the study area. There have been series of crude oil pollution in the area of which the recent ones occurred in 2001 and 2009. The Polluted and Treated Site (PTS) was selected from the crude oil polluted section but where remediation had been carried out at different times including in 2003 and 2012. The type of remediation done is called remediation by enhanced natural attenuation, and this involved tillage and excavation of the polluted soil up to a depth of 1.2m, replacement with soil from an unpolluted area, and addition of fertilizers. These sites were considered to ascertain the effect of crude oil pollution and remediation on the rhizophere microbial communities of *M. indica* and *E. guineensis*. Figure 1 is the map of Ghokana Local Government Area showing the study sites, and an inset map of Nigeria showing the location of the study area.

Methods of data collection

Selection of tree species

M. indica and *E. guineensis* were purposively chosen for the study based on their economic importance, and availability in the three sites considered for the study. Three individual trees belonging to each of the selected tree species were randomly selected in each site for soil sampling.

Collection of soil samples

Soil samples were collected up to a depth of 1m and distance of 10cm from the root collar, on four sides of each of the selected trees. Soil samples collected were bulked separately for each tree/site, divided into three equal parts, and one randomly selected for microbial analysis. This gave rise to three samples for each of the selected tree species in each site. The samples were enclosed in polythene bags, and taken to University of Port Harcourt soil microbiology laboratory for isolation, enumeration and identification of bacteria and fungi using standard microbiological methods.

Microbiological analysis of soil samples

Isolation of bacteria

Soil samples obtained from the sampling sites were analyzed for total heterotrophic and bacterial counts using the spread plate method (Cheesbrough, 2000). Ten grams of the soil sample was weighed using an electronic weighing balance, and dissolved in 90 mls of sterile distilled water; this was homogenized. Serial dilution was then carried on the soil suspension up to 10^6, 100 μL of soil suspension was transferred from the tubes to sterile nutrient agar (Oxoid) plates and spread using a glass spreader. The plates were incubated at 37°C for 24 hrs, colonies were enumerated and colonial morphology (shape, size, consistency, edge elevation and opacity) of the various bacteria isolates was noted. Single colonies on the agar plates were sub cultured into sterile nutrient agar plates and incubated at 37°C for 24 hours. Codes were used to identify each isolate on a plate. Pure isolates were stored in nutrient agar slants at 4°C for identification and characterization using standard microbiological procedure. The bacteria isolates were identified using Gram staining to differentiate them into Gram + ve and Gram - ve bacteria. Different biochemical tests such as oxidase test, catalase test, MR- VP test, indole test, and citrate test were also carried out on the isolates for further identification.

Isolation of fungi

Heterotrophic fungi were isolated from soil using the method of Dubey and Maheshwari, (2007) and Efiuvwevwere (2000) with slight modifications. One gram of soil sample was weighed and added to 99 ml of sterile distilled water. From the soil suspension 1.0 ml was transferred into the first tube containing 9 mls of sterile water. Further serial dilution was also carried out. One hundred micro liters was transferred from each tube to sterile sabaroud dextrose agar plates and incubated at room temperature for five to seven days. Colonies on each plate were counted and predominant colonial morphology was observed. Fungi were identified by staining with lacto phenol cotton blue stain on a slide. The slides were observed under the microscope, and fungi identified following the mycological literature.

Methods of data analysis

Measurement of the diversity of fungi and bacteria at different sites

Both the diversity of rhizosphere fungi and bacteria for each tree species/site was measured using Simpson Diversity Index (Simpson, 1949) and Shannon-Wiener Diversity Index (Odum, 1971). Simpson diversity index is expressed as:

$$D = \frac{\sum_{i=1}^{q} ni(ni-1)}{N(N-1)} \quad \text{Eqn. 1}$$

Where: N = total number of individuals encountered.

ni = number of individuals of ith species enumerated for i=1.....q

q = number of different species enumerated.

Since Simpson diversity index as computed with the formula above shows an inverse relationship with diversity, the final result was presented as Simpson (1 – D), to allow for a direct relationship with diversity.

Shannon-Wiener diversity index is expressed as:

$$H = -\sum_{i-1}^{s} pi \log pi \quad \text{Eqn. 2}$$

Where: pi = the proportion of individuals in the ith species

s = the total number of species

Measurement of similarity in fungi and bacteria species between sites

Sorensen's similarity index (SI) was used to ascertain the level of similarity of rhizosphere bacteria and fungi species between sites for each tree species. Sorensen's similarity index was computed after Margurran (2004) with the formula below.

$$SI = 2a / (2a + b + c) \quad \text{Eqn. 3}$$

Where: a = number of species common to both Sites

b = number of species present in Site 1 but absent in Site 2.

c = number of species present in Site 2 but absent in Site 1.

Results

Fungi species composition of M. indica and E. guineensis rhizosphere

The species of fungi found at rhizosphere of *M. indica* and *E. guineensis* are shown in Tables 1 and 2 respectively. Three species of fungi were found at the rhziophere of *M. indica* in each of UPS and PTS while only one species was found in PUS (Table 1). At the rhziophere of *E. guineensis*, four species of fungi were found in UPS, three in PTS, and two in PUS (Table 2). The population of rhizophere fungi for both *M. indica* and *E. guineensis* was highest in UPS followed by PTS while the lowest number was observed for PUS (Figure 2).

Table 1. Fungi species present in the rhizosphere of *M. indica* at various sites.

		Population		
S/No.	Species	UPS	PTS	PUS
1	*Aspergillus flavus*	0	1.2×10^4	0
2	*Aspergillus niger*	1.4×10^4	2.2×10^4	0
3	*Fusarium proliferatum*	1.0×10^4	6.0×10^3	0
4	*Penicillium camemberti*	2.3×10^4	0	3.2×10^4

Values are means of three samples.

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Bacteria species composition of M. indica and E. guineensis rhizosphere

The species of bacteria found at rhizosphere of *M. indica* and *E. guineensis* are shown in tables 3 and 4 respectively. Four species of bacteria were found at the rhizosphere of *M. indica* in each of UPS, PTS, and PUS (Table 3). At the rhizosphere of *E. guineensis* six species of bacteria were found in PUS, and four species in each of UPS and PTS (Table 4). The population of rhizosphere bacteria for *M. indica* was highest in PUS, followed by UPS and PTS respectively, while for *E. guineensis*, it was highest in UPS, followed by PUS and PTS, respectively (Figure 3).

Table 2. Fungi species present in the rhizosphere of *E. guineensis* at various sites.

		Population		
S/No.	Species	UPS	PTS	PUS
1	*Aspergillus flavus*	2.1×10^4	0	0
2	*Aspergillus niger*	1.4×10^4	2.2×10^4	0
3	*Fusarium proliferatum*	1.3×10^3	8.0×10^3	6.0×10^3
4	*Geomyces traen*	0	4.0×10^3	4.0×10^3
5	*Penicillium chrysogenum*	1.5×10^4	0	0

Values are means of three samples.

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Similarity in rhizosphere fungi species composition of M. indica and E. guineensis at various sites

The similarity in rhizosphere fungi species of *M. indica* at various sites is shown in Table 5. The highest similarity (67%) was observed for UPS and PTS, followed by UPS and PUS (50%), while similarity between PTS and PUS was (0%). Table 6 shows the similarity in rhziophere fungi species of *E. guineensis*. Similarity was highest between PTS and PUS (87%), followed by PUS and PTS (57%), while in UPS and PUS the similarity was (33%).

Similarity in rhizosphere bacteria species composition of M. indica and E. guineensis at various sites

The similarity in rhizosphere bacteria species of *M. indica* was highest in UPS and PTS (50%), followed by UPS and PUS (25%), and PTS and PUS (25%) (Table7). At the rhizosphere of *E. guineensis,* the bacteria species similarity was 60% between each of UPS and PUS and PTS and PUS, while it was 25% for UPS and PTS (Table 8).

Table 3. Bacteria species present in the rhizosphere of *M. indica* at various sites.

		Population		
S/No.	Species	UPS	PTS	PUS
1	*Bacillus subtilis*	2.3×10^4	6.4×10^4	3.9×10^6
2	*Chromobacterium violaceum*	0	0	6.4×10^5
3	*Citrobacter freundii*	0	0	4.4×10^5
4	*Micrococcus luteus*	0	2.6×10^4	0
5	*Micrococcus lylae*	4.8×10^4	0	0
6	*Proteus vulgaris*	3.2×10^3	3.2×10^2	0
7	*Pseudomonas putida*	0	0	3.2×10^4
8	*Staphylococcus epidermis*	3.2×10^5	0	0
9	*Staphylococcus saprophyticus*	0	3.9×10^2	0

Values are means of three samples.

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Table 4. Bacteria species present in the rhizosphere of *E. guineensis* at various sites.

		Population		
S/No.	Species	UPS	PTS	PUS
1	*Bacillus subtilis*	2.5×10^6	4.9×10^5	7.2×10^5
2	*Bacillus cereus*	0	5.6×10^4	0
3	*Burkholderia cepacia*	2.4×10^4	0	6.2×10^2
4	*Chromobacterium violaceum*	0	2.3×10^3	3.3×10^3
5	*Micrococcus lylae*	0	3.2×10^3	5.9×10^3
6	*Serratia marcescens*	0	0	3.9×10^3
7	*Staphylococcus epidermis*	3.6×10^4	0	3.2×10^4
8	*Staphylococcus saprophyticus*	5.6×10^4	0	0

Values are means of three samples.

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Diversity of rhizosphere fungi for M. indica and E. guineensis at various sites

The highest diversity of fungi at the rhizosphere of *M. indica* was found in UPS, followed by PTS, while it was zero in PUS (Table 9). At the rhizosphere of *E. guineensis,* fungi diversity was also highest in UPS, followed by PTS, and PUS respectively (Table 10).

Diversity of rhizosphere bacteria for M. indica and E. guineensis at various sites

The diversity of rhizosphere bacteria for *M. indica* at various sites is shown in Table 11. Using the diversity indices (Shannon H), the highest diversity was observed in PUS, followed by PTS and then UPS, while with Simpson (1– D) the diversity was highest in PTS, followed by PUS, and then lowest in UPS. The

diversity of bacteria at the rhizosphere of *E. guineensis* was highest in PTS, followed by PUS and lowest in UPS (Table 12).

Discussion

The species richness and population of the rhziosphere fungi were higher in the unpolluted sites and the polluted and treated sites than in the polluted and untreated sites for both *M. indica* and *E. guineensis*. In fact, population of the rhziosphere fungi showed a declining trend from unpolluted sites through polluted and treated sites to polluted and untreated sites. This can be attributed to the effect of crude oil pollution on species of fungi. Amadi *et al.* (1996) show that crude oil affects soil properties and microflora. In addition to its effects on visible plants and animals, petroleum contamination impacts microbial populations (Aheam and Meyers, 1976). This probably explains why population of rhziosphere fungi was lowest in the polluted and untreated site for both tree species.

Table 5. Sorensen's similarity indices for *M. indica* rhizosphere fungi in different sites.

	UPS	PTS	PUS
UPS	*	0.67	0.50
PTS		*	0.00
PUS			*

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Table 6. Sorensen's similarity indices for *E. guineensis* rhizosphere fungi in different sites.

	UPS	PTS	PUS
UPS	*	0.57	0.33
PTS		*	0.80
PUS			*

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

The fungi - *Penicillium chrysogenum,* was found only in the unpolluted site at the rhizosphere of *E. guineensis*. This species which is naturally found in soil provides plenty quantity of carbon and nitrogen for mycorrhizal growth (Barkai-Golan, 1974). It plays a significant role in the medical community as an antibiotic because it can create penicillin which inhibits the biosynthesis of bacterial cell walls affecting lyses of the cell (Fleming, 1929). It can also play a role as either a pathogen (Adrin *et al.*, 2005; Galland *et al.*, 2004), an allergen (Shen *et al.*, 2003), and may aid in protecting crops from certain pathogenic attacks (Thuerig *et al.*, 2006). Mycorrhizal fungi have been reported to reduce plant responses to other stresses such as high salt levels and noxious compounds associated with mine pollution, landfills, heavy metal and micro-element toxicity (Linderman, 1988). Therefore, their absence as a result of crude oil spillage will have adverse effect on tree or plant growth and productivity.

Table 7. Sorensen's similarity indices between sites for *M. indica* rhizosphere bacteria.

	UPS	PTS	PUS
UPS	*	0.50	0.25
PTS		*	0.25
PUS			*

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

The population of bacteria in the rhziosphere of *M. indica* was highest in the polluted and untreated site followed by the unpolluted site and polluted and treated site. This trend could be as a result of high abundance of *Bacillus subtilis* (a species capable of degrading crude oil) at the polluted and untreated site. This trend agrees with that of Sextone and Atlas (1977). Sabate *et al.* (2004) show that bioremediation occurs through high metabolic activity of indigenous microbial populations in degrading total petroleum hydrocarbon (TPH). Ghazali *et al.* (2004) also report on the usage of consortia of bacteria that include species in Bacillus and Pseudomonas genera to degrade linear chain hydrocarbon. The populations of such microbes that use the petroleum hydrocarbons as nutrients are bound to increase as a result of crude oil spillage. Westlake *et al.* (1974) observe that the same crude oil can favour different genera at different temperatures.

Table 8. Sorensen's similarity indices between sites for *E. guineensis* rhizosphere bacteria.

	UPS	PTS	PUS
UPS	*	0.25	0.60
PTS		*	0.60
PUS			*

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

At the rhziosphere of *E. guineensis* however, the population of bacteria was highest in the unpolluted site, followed by the polluted and untreated site, and the polluted and treated site, respectively. This could be attributed to favourable growth conditions for the species of bacteria found at the rhizosphere of *E. guineensis* at the unpolluted sites. Despite the fact that the bacteria species found at the rhizosphere of *E. guineensis* were only 50% similar to those found at the rhizosphere of *M. indica*, the bacteria populations at the rhizosphere of *E. guineensis* were comparatively higher. The root exudates can be used to increase the availability of nutrients and they provide food sources for microorganisms. Plants exude readily degradable substances into the soil that augment microbial activity in the rhziosphere (Joner *et al.*, 2002).

Table 9. Diversity indices for *M. indica* rhizosphere fungi at the various sites.

	UPS	PTS	PUS
No. of species	3	3	1
Shannon (H)	1.04	0.97	0
Simpson (1 – D)	0.63	0.56	0

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

However, bacteria species richness was equal for all the sites at the rhizosphere of *M. indica*, and highest in the polluted and untreated site at the rhizosphere of *E. guineensis*. Higher bacteria species richness in the polluted and untreated site at the rhizosphere of *E. guineensis* is not out of place because some of the bacteria species - *Bacillus subtilis*, *Burkholderia cepacia* and *Micrococcus lylae,* found in this site are capable of degrading crude oil. Tesar *et al.* (2002) observe that a broad phylogenetically range of bacteria, including the genera *Bacillus, Pseudomonas, and Micrococcus,* are involved in the breakdown of hydrocarbons.

Table 10. Diversity indices for *E. guineensis* rhizosphere fungi at the various sites.

	UPS	PTS	PUS
No. of species	4	3	2
Shannon (H)	1.17	0.87	0.67
Simpson (1 – D)	0.67	0.51	0.48

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Table 11. Diversity indices for *M. indica* rhizosphere bacteria at the various sites.

	UPS	PTS	PUS
No. of species	4	5	4
Shannon (H)	0.63	0.67	0.70
Simpson (1 – D)	0.32	0.42	0.37

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

Table 12. Diversity indices for *E. guineensis* rhizosphere bacteria at the various sites.

	UPS	PTS	PUS
No. of species	4	4	6
Shannon (H)	0.23	0.39	0.28
Simpson (1 – D)	0.09	0.20	0.11

UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

The level of similarity and/or variation in fungi species composition showed different trends for both tree species at the various sites. For instance, the highest similarity in fungi species was observed between the unpolluted site and the polluted and treated site at the rhizosphere of *M. indica*, while the highest similarity was observed between the polluted and treated site and the polluted and untreated site at the rhizosphere of *E. guineensis*. Also the similarity in fungi species at the rhizosphere of *M. indica* was zero between polluted and treated site and polluted and untreated site, while it was 87% between the two sites at the rhizosphere of *E. guineensis*. Considering the similarity in bacteria species, different trends were also observed at the various sites for the two tree species. For instance, the level of similarity in bacteria species was 50% between the unpolluted site and the polluted and treated site at the rhizosphere of *M. indica*, while it was 25% for both sites at the rhizosphere of *E. guineensis*. Also similarity in bacteria species was 25% between polluted and treated site and polluted and untreated site at the rhizosphere of *M. indica*, while it was 60% between the two sites at the rhizosphere of *E. guineensis*. Although, the exact reason for these variations is not known, factors like spatial variations in the effectiveness of the remediation carried out, differences in rhizosphere characteristics of the two tree species and varying degrees of resistance and resilience to the impacts of crude oil pollution, may be contributing factors. Westlake *et al.* (1974) note that the effect of crude oil on microorganisms is dependent on different factors; some organisms utilize petroleum hydrocarbon as nutrients, and crude oil also favours different genera of microorganisms at different temperatures. Furthermore, some crude oils contain volatile bacteriostatic compounds that must degrade before microbial populations can grow (Atlas and Bartha, 1972).

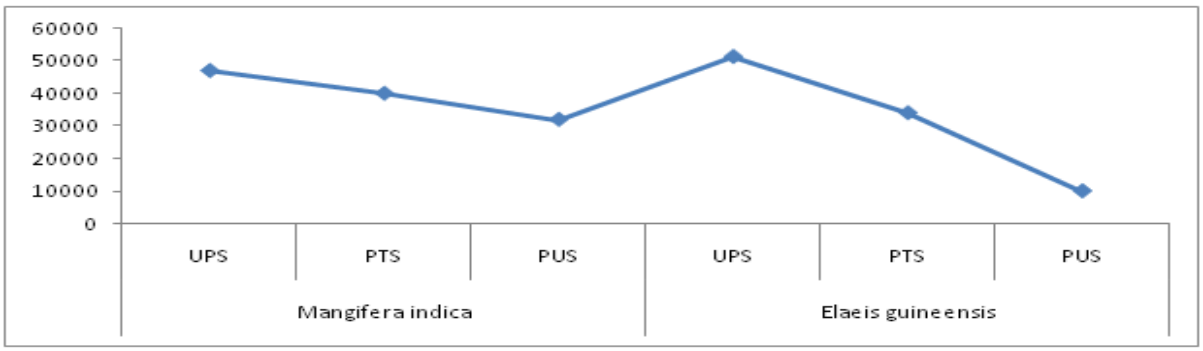

Fig. 2. Population of fungi found within the rhizosphere of *M. indica* and *E. guineensis* at the various sites
UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

The diversity of fungi showed a similar trend for both *M. indica* and *E. guineensis* rhizospheres, decreasing from the unpolluted site through the polluted and treated site, and the polluted and untreated sites respectively. However, a different and opposing trend was observed for the two tree species when the diversity of bacteria was considered, with the unpolluted site having the lowest diversity. Since diversity considers both species richness and evenness in the distribution of individuals among the species encountered, it then follows that the fungi populations are more evenly distributed among species in the unpolluted site than in the other sites while the bacteria populations are more evenly distributed at the polluted and treated site than the other two sites. This may be attributed to better growth conditions for the microorganism communities at the respective sites. It should be noted that some of the bacteria species - *Bacillus subtilis*, *Burkholderia cepacia* and *Micrococcus lylae,* encountered in the study are capable of degrading crude oil. Although, much research has not been carried out to ascertain the impact of the diversity of microorganisms in crude oil polluted soils, there is an assumption that higher microbial diversity could lead to effective removal of pollutant from a substrate (Dejonghe *et al* ., 2001).

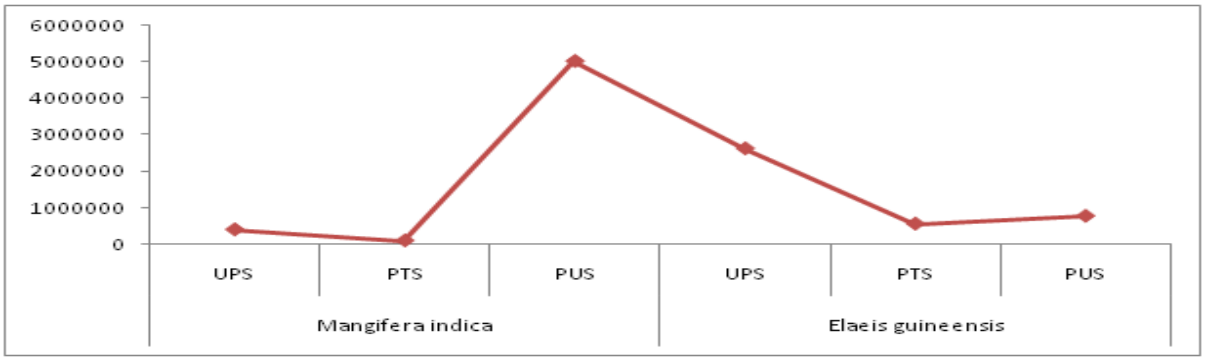

Fig. 3. Population of bacteria found within the rhziophere of *M. indica* and *E. guineensis* at the various sites
UPS = Unpolluted Site; PTS = Polluted and Treated Site; PUS = Polluted and Untreated Site.

The significance of this study cannot be overemphasized. Some of the species encountered in the study are among those known to metabolize hydrocarbons and which thrive in crude oil contaminated sites as reported by (Bartha and Altlas 1977; Llanos and kjoller, 1976; Obire *et al*., 2008). Nyns *et al*. (1969) reported that the genera of *Aspergillus* and *Penicillium* are most common in hydrocarbon assimilation, and that although the initiation of degrading synthetic petroleum mixture was done by bacteria, it is twice much degraded when both bacteria and fungi are present. *Aspergillus niger* is used in waste management and biotransformation (Schuster *et al*., 2002). *Penicillium* species are commonly found naturally in moist soil with plentiful quantities of carbon and nitrogen for mycorrhizal growth (Barkai-Golan, 1974). *Burkholderia cepacia* complex species are soil-dwelling bacteria commonly found on plant roots. They are of significant environmental interest as they are capable of degrading a large variety of toxic compounds. This makes them extremely useful in bioremediation.

However, *Penicillium camemberti* was not found in the polluted and treated site at *M. indica* rhizospere. Also *Aspergillus flavus* was not found in both polluted and treated site and polluted and untreated site, while *Aspergillus niger* was not found in the polluted and untreated site at *E. guineensis* rhizosphere. Considering bacteria, *Micrococcus lylae* was not found at the rhizosphere of *M. indica* in both the polluted and treated site and the polluted and untreated site, while *Burkholderia capacia* was not found at the rhizosphere of *E. guineensis*. Efforts at remediating the impact of crude oil in the study area should endeavour to introduce and create favourable

growth conditions for these species.

Conclusion and recommendation

The impact of crude oil showed different trends for different attributes of the rhizosphere fungi and bacteria in *M. indica* and *E. guineensis*. Although, the exact reason for these variations is not known, factors like spatial variations in the effectiveness of the remediation carried out, differences in rhizosphere characteristics of the two tree species, and varying degrees of resistance and resilience of the rhizosphere microbial species to the impacts of crude oil pollution, may be contributing factors. The remediation carried out seems to have favoured the fungi more than the bacteria. However, bacteria diversity still compared better in PTS than the other sites.

Concerted effort should be made to effectively remediate the crude oil polluted sites to enhance the recovery of the microbial populations. Such efforts should include the introduction of species of microorganisms capable of degrading hydrocarbons including the ones identified in this study. *M. indica and E. guineensis* should be planted in the polluted sites where initial attempts had been made to improve the soil conditions, since activities in their root-region (rhizosphere) probably promote and favor the growth of some microorganisms capable of degrading hydrocarbons.

References

Adrian BL, Burdette SD, Herchline TE. 2005. Intestinal invasion and disseminated disease associated with *Penicillium chrysogenum*. Ann Clin Microbiol Antimicrob **4,** 21.

Aheam DG, Meyers PS. 1976. Fungal degradation of oil in the marine environment, p 127-130. In: Gareth J. (ed). Recent Advances in Aquatic Mycology.

Amadi A, Abbey SD, Nma A. 1996. Chronic effects of oil spill on soil. properties and microflora of a rainforest ecosystem in Nigeria. Water, Air and Soil Pollution **86,** 1-11.

Amajor LC. 1985. The Ejamah – Ebubu oil spill of 1970: A case history of a 14 year-old spill, Crit. Rev. Microbiol. **5,** 423 – 445.

Atlas RM, Bartha R. 1972. Degradation and Mineralization of petroleum by two bacteria isolated from coastal water. Biotechnol. Bio Eng. **14,** 297- 308

Barkai-Golan R. 1974. Species of Penicillium causing decay of stored fruit in Isreal. Mycopathologia **54,** 141-145.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. 3rd edition, Burgess Publishing Co., 273 p.

Bartha R, Atlas RM. 1977. The microbiology of aquatic oil spills. Adv. Appl. Microbiol. **22,** 225-266.

Cairns J, McCormic PV, Belanger SE. 1993. Prospects for the continued development of environmentally-realistic toxicity tests using microorganisms. Journal of Environmental Science **5,** 253-268.

Cheesbrough M. 2000. District Laboratory practice in Tropical Contries. United Kingdom: Cambridge University press.

Chima UD, Vure G. 2014. Implications of crude oil pollution on natural regeneration of plant species in an oil producing community in the Niger Delta Region of Nigeria. Journal of Forestry Research **25(4),** 915-921.
http://dx.doi.org/10.1007/s11676-014-0538-y

Dejonghe W, Boon N, Seghers D, Top EM, Verstraete W. 2001. Bioaugmentation of soils by increasing microbial richness, missing links. Environmental Microbiology **3,** 649- 657.

Efiuvwevwere BJO. 2000. Microbial spoilage agent of tropical and assorted fruits and vegetables. 1st ed. Port Harcourt: Paragraphics Publishers.

Fleming A. 1929. On the antibacterial action of cultures of Penicillium, with special reference to their use in the isolation of *B. Influenzae*. British Journal of Experimental Pathology **10,** 226-236.

Galland F, Le Goff L, Conrath J. 2004. *Penicillium chrysogenum* endophthalmitis: a case report. Journal Français d'Ophtalmologie **27,** 264 – 266.

Ghazali MF, Zaliha NR, Salleh B, Basri M. 2004. Biodegradation of hydrocarbon in soil by microbial consortium. Inter Biodeter Biodegrad **54,** 61-67.

Hackl E, Zechmeister-Boltenstern S, Bodrossy L, Sessitsch A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl. Environ. Microbiol. **70,** 5057–5065.

Holt JG, Krieg RN, Sneath HA, Staley JT, Williams ST. 1994. Bergey's Manual of Determinative Bacteriology (Ninth Edition). Baltimore: Willams and Wilkins.

Institute of Pollution Studies (IPS). 1990. *Ecological Impact Assessment of Ebubu Ochani, SPDC,* Institute of Pollution Studies Rivers State University of Science and Technology, Port Harcourt, 236 p.

Izaguirre-Mayoral ML, Flores S, Carballo O. 2002. Determination of acid phosphatase and dehydrogenase activities in the rhizosphere of nodulated legume species native to two contrasting savanna sites in Venezuela. Biology and Fertility of Soils **35**, 470-472.

Joner EJ, Corgié SC, Amellal N, Leyval C. 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biology and Biochemistry **34,** 859-864.

Linderman RG. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology **78,** 366-371

Llanos C, Kjøller A. 1976. Changes in the flora of soil fungi following oil waste application. Oikos **27,** 377-382.

Margurran AE. 2004. Measuring Biological Diversity. Oxford UK: Blackwell Publishing.

NDES. 2001. Biological Environmental Research Report. RSUST, Port Harcourt. Volume 46. 251 p.

Nyns EJ, Auquiere JP, Wiaux AL. 1969. Adaptive or constitutive nature of the enzymes involved in the oxidation of n-hexadecane into palmitic acid by *Candida lypolytica*. Z.Allg. Mikrobiol. **9**, 373-380.

Obire O, Anyanwu EC, Okigbo RN. 2008. Saprophytic and crude oil-degrading fungi from cow dung and poultry droppings as bioremediating agents. International Journal of Agricultural Technology **4(2),** 81-89.

Odu CTI. 1981. Degradation and weathering of crude oil under tropical conditions. In:proceeding of an international seminar on the petroleum industry and the Nigerian Environment, November 1981, Petroleum Training Institute, Warri, Nigeria.

Odum EP. 1971. Fundamentals of Ecology. Philadelphia: W.B Saunders Co.

Onweremadu EU, Duruigbo CI. 2007. Assessment of cadmium concentration of crude oil polluted arable soils. Int J. Environ Sci. Tech. **4,** 409-414.

Port Harcourt Appeal Court. 1994. Report of Case between SPDC Nigeria Limited (Appellant/Defendant) and the Kegbara Dere People (Respondents/Plaintiffs).

Roscoe YL, Mcgill WE, Nbbiry MP, Toogood JA. 1989. Method of accelerating oil degradation in soil. pp. 462-470. In proceeding of workshop on reclamation of disturbed Northern Forest, Research Center, Alberta.

Sabate J, Vinas M, Solanas AM. 2004. Laboratory Scale bioremediation experiments on hydrocarbon contaminated soils. Intl. J. Biodeter. Biodegrad. **54**, 19-25.

Schuster E, Dunn-Coleman N, Frisvad J, Van Dijck P. 2002. On the safety of *Aspergillus niger* – a review". Applied Microbiology and Biotechnology **59**, 426-435.

Shen HD, Chou H, Tam MF. 2003. Molecular and immunological characterization of Pen ch 18, the vacuolar serine protease major allergen of *Penicillium chrysogenum*. Allergy **58(10),** 993-1002.

Simpson EH. 1949. Measurement of diversity. Nature **163,** 688

Smith B, Stachuwisk M, Volkenburgh E. 1989. Cellular processes limiting leaf growth in plants under hypoxic root stress. Journal of Experimental Botany **40,** 89-94.

Strickland RM. 1990. The pacific Northwest coast: Fossil fuel frontier. Environment Journal **6(4),** 25-77

Tesar M, Reichenauer TG, Sessitsch A. 2002. Bacterial rhizosphere communities of Black poplar and herbal plants to be used for phytoremediation of diesel fuel. Soil Biology and Biochemistry **34,** 1883-1892.

Thuerig B, Binder A, Boller T. 2006. An aqueous extract of the dry mycelium of *Penicillium chrysogenum* induces resistance in several crops under controlled and field conditions. European Journal of Plant Pathology **114,** 185-197.

Walker JD, Cooney JJ, Colwell RR. 2005. Ecological aspect of microbial degradation of petroleum in the marine environment. Critical Review in Microbiology **4,** 423- 445.

Westlake DW, Jobson A, Phillippe R, Cook FD. 1974. Biodegradability and crude oil composition. Can Journal of Microbiology **20(7),** 915-928.