Rhizosphere and Hydrocarbon Utilizing Bacteria of Wetland Plants in Oil Polluted Areas of the Niger Delta Region, Nigeria

***Pondei, J. O. and Aladese, M. A.**
Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria

Article Information

Article # 1018
Received date: 15th Jan., 2018
1st Revision 6th March, 2018
2nd Revision: 20th May, 2018
Acceptance: 23rd Oct., 2018
Published: 28th Dec., 2018

Key Words
Heterotrophic bacteria, Hydrocarbon utilizing bacteria, Petroleum, rhizosphere, Wetland plants

Abstract

The study investigated the presence of heterotrophic bacteria and petroleum hydrocarbon utilizing bacteria (using Minimal Salt Medium) in the rhizosphere of wetland plants in six-month old and twelve-month old petroleum-contaminated sites and an uncontaminated site in Oloibiri Oil Field, Ikarama and Otuoke respectively, Bayelsa State, Nigeria. Nineteen species of wetland plants were identified and three unidentified species. Plant species common to all the three sites are *Commelina benghalensis*, *Chromoleana odorata* and *Aspilia Africana. Ipomoea involucrata*, *Kyllinga* species and *Ageratum conyzoides* are peculiar to the petroleum-contaminated sites. Other plant species are site specific. Bacteria isolated belong to the phyla Proteobacteria, Bacteroidetes and Firmicutes with the γ-Proteobacteria and particularly *Pseudomonas* spp. dominating in all the sites, both as heterotrophic and hydrocarbon utilizing bacteria. More heterotrophic bacterial species occurred at the uncontaminated site (38.46%) than the contaminated sites (30.77% each). Hydrocarbon utilizing bacterial species diversity was greater at the twelve-month old contaminated site (44.44%), followed by the uncontaminated site (38.89%) while fewer species (16.67% *Pseudomonas* spp) were at the six-month old site. The total heterotrophic bacterial counts (THB) of the soils were $1.37 \times 10^8 - 2.80 \times 10^8$ cfu/g, the hydrocarbon utilizing bacterial counts (HUB) were $4.00 \times 10^7 - 1.33 \times 10^8$ cfu/g with the petroleum-contaminated sites having higher counts. The rhizosphere bacterial counts were $1.25 \times 10^7 - 1.03 \times 10^8$ cfu/g (THB) and $1.20 \times 10^7 - 3.20 \times 10^7$ cfu/g (HUB), with the uncontaminated site having the most. The findings show high numbers of HUB in the uncontaminated soil indicating soil pollution. These findings may be of immense help in planning bioremediation strategies but further investigation on the role of the individual plants and rhizosphere populations would be required.

*Corresponding Author: Pondei, J. O.; julipondei@gmail.com

INTRODUCTION

Wetlands comprise of marshes, bogs and swamps, which occur through waterways and floodplains. Wetlands are regarded as the most biologically diverse of all ecosystems (Okoro, 2010). The Niger Delta Region of Nigeria, with its abundance of oil, is among the world's ten most significant ecosystems comprising of wetlands with fresh water swamps and coastal marine swamps dominating. (Imogie, 2012; Isikhuemen, 2012; Onyechi *et al.*, 2016).

Oil contamination of the aquatic ecosystem has been going on for decades. Petroleum hydrocarbons enter this environment directly through spills or indirectly has become a recurrent phenomenon since oil was first located at Oloibiri in 1956. Oil spills have posed and caused extensive damage to the aquatic environments; marine and freshwater. Oil spills are occurring frequently in the Niger Delta Region, particularly in Bayelsa State where it occurs on a monthly basis. Reports on the number of spills recorded are conflicting. Statistics shows that about 40 spill cases occur monthly in Bayelsa State which was the case in 2014 (MoE, 2018). An estimated 1103 total number of spills occurred between 1999

and 2012, and 1095 spills occurred between 2013 and March, 2017 with some undocumented cases (MoE, 2018).

Contamination of wetlands with oil is quite deleterious to the habitat. The aquatic sediments consist of biological, organic and inorganic matter which act as sinks for a diverse array of pollutants (Perelo, 2010). Natural microbial communities found in a specific petroleum contaminated site degrade or transform pollutants to less deleterious forms over a time lapse in a process called natural bioattenuation. Wetlands support hydrophytes, or plants emerging from water, soil, or on a substance with irregular oxygen supply due to excessive water content. These wetland plants play important roles as indicators of water pollution, oil bioremediation, wetland restoration and nutrient cycling, (Prasad, 2001, 2005; Goudarzi and Afrous, 2012; Chen *et al*., 2012; Abed *et al.,* 2017).

Rhizosphere, the area surrounding the root, is colonized by a variety of bacteria beneficial to the plant, called the growth promoting rhizobacteria (PGPR). These micro-organisms are essential in plant degradative processes while their nutrients are provided by plant exudates (Amora-Lazcano *et al*., 2010). The rhizosphere soil determines microbial activity around the root from discharged root exudates (Jussila, 2006). Rhizoremediation, a distinct type of phytoremediation requires plants and microorganisms of the rhizosphere (Divya and Kumar, 2011) living and benefitting from each other. Degradation of contaminants with rhizosphere microbial communities differ with plant species and varieties. Petroleum hydrocarbon degraders are increased in the rhizosphere of plants exposed to hydrocarbon contamination (Banks *et al*., 2003; Stephen and Ijah, 2011, Odokuma and Ubogu, 2014; Iffis *et al.,* 2017). The positive outcome of phytoremediation of hydrocarbon polluted soil is attributed to the plant's ability to boost microbial rhizosphere functioning and the capacity of the rhizosphere microbes themselves to survive, proliferate and colonize this zone (Kuiper *et al.,* 2004; Diab and Sandouka, 2010; Bisht *et al*., 2015).

Successful bioremediation using rhizosphere bacterial populations on petroleum hydrocarbon are documented (Banks *et al*., 2003; Diab and Sandouka, 2010; Gkorezis *et al.,* 2016). The hydrocarbon utilizing rhizosphere bacteria include *Acinetobacter, Micrococcus, Pseudomonas, Enterobacter, Klebsiella, Flavobacterium, Edwardsiella, Escherichia, Proteus, Citrobacter, Nocardia, Bacillus, Lactobacillus and Staphylococcus* (Eze and Okpokwasili, 2010; Mmom and Deekor, 2010; Chikere and Okpokwasili, 2015; Alegbeleye *et al.,* 2017).

With the few reports on the use of wetland or aquatic plants in petroleum bioremediation, the research was conducted to ascertain the diversity and number of petroleum hydrocarbon utilizing bacteria in soils and rhizosphere of some wetland plants common in this oil polluted region.

MATERIALS AND METHODS

Sampling Location and Collection

Wetland plants were picked from two aged petroleum-contaminated sites and a non-contaminated site as control. The first is a six-month old petroleum-contaminated soil at Oloibiri Oil field, Ogbia LGA, Latitude 4.695^{o}N Longitude 6.35043^{o}E (Latitude 4^{o}41′30.12″N Longitude 6^{o}21′33.3″E.). Crude Oil spill occurred in November, 2013 while sampling was in May, 2014. The second is a twelve-month old petroleum-contaminated soil at Ikarama, Okordia Clan, Yenagoa LGA; Latitude 5.14931^{o}N Longitude 6.45287^{o}E. Oil spill occurred in November, 2013. Sampling took place in November, 2014. The uncontaminated control is situated at the Federal University Otuoke, Ogbia LGA, Latitude 4.8019^{o}N Longitude 6.3189^{o}E. Sampling was done in June, 2015.

Triplicate samples of ten aquatic plant species were selected randomly per site, put in clean press lock plastic bags and transported to the laboratory. Plants were identified at the Plant Taxonomy Unit, University of Port Harcourt. Thirty gram aliquots of soil samples were collected at a depth of 30 cm in six random locations per site using a clean spade and put into clean plastic bags. These were transported to the laboratory on ice packs and stored at 4^{o} C in the refrigerator before use. Soil samples were also taken from uncontaminated areas, 50 – 100 meters away from the petroleum contaminated sites.

Sample Processing for Rhizosphere and Total Heterotrophic Bacteria

Rhizosphere soil of all the plants from each site was collected carefully by detaching soil adhering to the plant roots and aseptically introduced into sterile containers. All rhizosphere soil samples per site were mixed to form one composite sample. To isolate rhizosphere bacteria, a tenfold dilution of one gram of sample in sterile normal saline (0.85 % NaCl) was done, and 100 µl of dilutions 10^{-5} and 10^{-6} inoculated onto nutrient agar plates in triplicates using the spread plate method. Plates were incubated at room temperature 25^{o} C ± 2^{o} C for 18 – 24 hours after which colonies were picked, subcultured two to three

times onto fresh nutrient agar plates until pure colonies were obtained and identified.

The total heterotrophic bacterial (THB) count of the rhizosphere and soil bacteria were determined using a slightly modified version of the drop plate method of Miles and Mishra (1938) in which 10µl of 10^{-5} dilution was inoculated onto the plates in triplicates and left for 20 – 30 minutes to diffuse into the medium. Incubation of plates were at 25^{o} C ± 2^{o} C for 18 – 24 hours and the resulting colonies were counted using a colony counter.

Number of colonies / ml = Average number of colonies / Dilution factor X 100.

Enumeration and Identification of Hydrocarbon Utilizing Bacteria (HUB)

Hydrocarbon utilizing bacteria (HUB) of the rhizosphere soil were isolated and counted using the vapour phase transfer method in modified minerals salts medium of Eziuzor and Okpokwasili (2009); pH adjusted to 7.0 and the medium sterilized at 121^{o} C, 15 psi for 15 minutes. One hundred microliters (100 µl) of diluted (10^{-5}) soil was inoculated onto triplicate plates of sterile mineral salts agar plates, spread evenly on the surface and left to diffuse into medium for 20 – 30 minutes. Bonny light crude oil was fully soaked in filter paper (Whatman No. 1) before placing aseptically onto lids of petridishes containing the medium. Hydrocarbon supply to the inoculum was by vapour phase transfer. All petridishes were inverted and incubated at 25^{o} C ± 2^{o} C for 5 – 7 days, colonies counted and further subcultured on nutrient agar, purified and identified.

Identification of Isolates

Colonial morphology, cultural characteristics and biochemical tests in the identification of isolates were carried out using standard procedures. Characterization of isolates were done depending on their morphology on gram staining, motility, biochemical tests; oxidase, catalase, citrate utilization, coagulase, urease, nitrate reduction, and sugar fermentation reactions (Abbott *et al.*, 2003; Grimont and Grimont, 2006; Manos and Belas, 2006). Gram negatives were further identified using API APIWEB (Biomereux, France). The test strip contained tests for beta-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, hydrogen sulphide production, urea hydrolysis, deaminase, indole and acetoin production (Voges-Proskauer), gelatinase and sugar fermentation tests. Isolates were grouped into various genera using Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) and Bergey's Manual of Systematic Bacteriology (Madigan *et al.*, 2012)

RESULTS

Identification of Aquatic Plant Species

Aquatic plant species identified from the petroleum-contaminated sites (Site A and Site B) and the uncontaminated control site (Site C), and their occurrences are presented in Table 1. Wetland plant species common to all the three sites are C. *benghalensis*, *C. odorata* and *A. africana*. In addition, plant species common to both petroleum-contaminated sites include *Ipomoea involucrata*, *Kyllinga* species and *Ageratum conyzoides*. Other plant species were site specific. Three aquatic plant species from the petroleum-contaminated Site A were unidentified.

Rhizosphere and Hydrocarbon Utilising Bacteria (HUB)

Rhizosphere and Hydrocarbon Utilising Bacteria (HUB) isolated from the petroleum-contaminated sites and the control site, their frequencies of occurrence are shown in Table 2 and Table 3 respectively. *Pseudomonas* species were common isolates of the rhizosphere of plants of the three sites while *Aeromonas* species were common to the petroleum-contaminated sites. Bacterial species *C. violaceum*, *P. pnuemotropica* and *C. indologenes* were common to both Sites A and C, and *B. cepacia* for Sites B and C. *Alcaligenes*, *Providencia rettgeri* and *Serratia* were specific isolates for Site B and *Bacillus* for Site A. *Myroides* sp, *Ochrobactrum anthropi* and *Staphylococcus* sp. were isolated from the rhizosphere of plants from Site C. For the Hydrocarbon Utilizing Bacteria (HUB), *Pseudomonas* species were common to all the sites while *Morganella morganii* and *Alcaligenes* were common for Sites B and C. *Klebsiella*, *Vibrio* and *Acinetobacter* were isolated from Site B while *Ochrobactrum anthropi* was isolated from Site C only.

A comparison of the bacterial counts; total heterotrophic (THB) and hydrocarbon utilizing (HUB), of the rhizosphere sediments and soil is given in Table 4. Generally, more bacterial counts were recorded in the soils than the rhizosphere and the HUB were just a little lower than the THB for all locations. Comparing the rhizosphere counts only, the twelve-month old site had higher numbers of both THB and HUB. For the soils, THB was higher in the uncontaminated site but HUB more in the twelve-month old site. Less THB counts occurred in the six-month old contaminated site but the highest THB was recorded in the soil of this site. The HUB counts were

very close to the THB counts in both the soil and the rhizophere.

DISCUSSION

Plants in association with microorganisms are used to clean up environmental pollutants through many phytoremediation mechanisms such as phytoextraction, rhizofiltration, phytostabilization, phytotransformation and phytodegradation or rhizoremediation. A variety of species of wetland plants with possible phytoremediation abilities were identified. Wetland and terrestrial bioremediation studies have exposed an array of microorganisms with pollutant degradative abilities in the rhizosphere. In this research work, the microorganisms of the individual plants were not analyzed but rhizosphere soil bacteria of all plants per site were compared. Rhizosphere bacteria present were of the phyla; Proteobacteria, Bacteroidetes and Firmicutes. The Proteobacteria were mostly isolated. Of the α-Proteobacteria, only *Ochrobactrum anthropi* (Family Brucellaceae) was isolated. β-Proteobacteria members were *Alcaligenes* (Alcaligenaceae), *Chromobacterium* (Neisseraceae) and *Burkholderia* (Burkholderiaceae). The γ-Proteobacteria are *Pseudomonas* (Pseudomonadaceae), *Aeromonas* (Aeromonadaceae) and *Pasteurella* (Pasteurellaceae) with members of the Enterobacteriaceae; *Providencia* and *Serratia*. *Bacillus* (Bacillaceae) and *Staphylococcus* (Staphylococcaceae) were the Firmicutes isolated while *Chryseobacteria indologenes* and *Myroides* (Flavobacteriaceae) were of the phylum Bacteroidetes.

Three species of *Pseudomonas*; *Pseudomonas aeruginosa*, *Pseudomonas flourescens* and *Pseudomonas putida* were present in plant rhizospheres of both petroleum-contaminated and uncontaminated sites showing its dominance over the variety of bacterial species present. *Pseudomonas* is often present in the soil, rhizosphere and rhizoplane of plants in petroleum affected sites, and even noted as being dominant irrespective of plant (Mukasheva *et al*. 2014), although individual plants were not analysed here but combined for each site. Gram negative rods, particularly *Pseudomonas*, *Flavobacterium* and *Alcaligenes* are regarded as specific occupants of the rhizosphere (Abdel Ghany *et al*., 2015). *Pseudomonas aeruginosa* was isolated frequently from the six month-old site (unreported data) and could be essential for plant colonization. *Aeromonas* species were common rhizosphere bacteria of the polluted sites but not cultured from the control site. *Aeromonas hydrophila, Aeromonas salmonicida* ssp. *salmonicida* and other *Aeromonas* species were isolates of the six month-old site while *Aeromonas salmonicida* ssp. *salmonicida* was at the twelve month-old petroleum contaminated site. Environmental changes or differences due to microbial activities, or other physical factors may have affected the species variability. The genus *Aeromonas* is a regular environmental organism and frequent isolates of aquatic wastewaters (Alegbeleye *et al*., 2017). *Chromobacterium violaceum* is an inhabitant of soil and water, and a producer of biosurfactants (Antunes *et al*., 2006) and was found as rhizobacteria in polluted mangrove sediments from the Iko River of the Niger Delta (Udotong *et al*., 2008). Non-pigmented *Chromobacterium violaceum* were isolates of this study which is in agreement with other reports (Antunes *et al*., 2006; Dall'Agnol *et al*., 2008). Production of the purple pigment, violacein may be an insignificant factor in the survival of the microorganism (Dall"Agnol *et al*., 2008).

Alcaligenes, Serratia and *Providencia* were noted in the twelve month-old polluted area but not in others. These microorganisms are regular isolates of petroleum polluted sites but their absence in the six month-old site could be because of cultural lapses, environmental factors or stage of the hydrocarbon degradation. *Chromobacterium violaceum, Pasteurella pneumotropica* and *Chryseobacterium indologenes* were all present in the six month-old and control site. These are also documented as rhizosphere and soil bacteria of polluted sites, except for *Pastuerella pneumotropica*. *Pasteurella pneumotropica*, a common carrier of rat and mice could be a regular soil microbe of this region but its prevalence in the wild is unknown (Scharmann and Heller, 2001). *Ochrobactrum anthropi* was an isolate of only the control site implying it as an uncommon petroleum degrading rhizosphere microorganism, since to the best of my knowledge, no history of its occurrence in this region is on record but in other countries (Berg *et al*., 2005; Oliveira *et al*., 2014). *Bacillus* spp. were present as isolates of the six month-old contaminated site while *Staphylococcus* spp. were at the control site. *Bacillus* and *Staphylococcus* are common soil bacteria and rhizobacteria of petroleum polluted sites (Fatima *et al*., 2015).

Pseudomonas spp. were present as Hydrocarbon Utilising Bacteria (HUB) of all three sites. *Acinetobacter baumanii, Vibrio* sp. and, *Klebsiella pneumoniae* ssp. *rhinoscleromatis, Morganella morgani* (all γ-proteobacteria) were present though not isolated as heterotrophic rhizosphere microorganisms of Site B, probably because of experimental flaws or their being dominated by other species. This further shows their preference for

hydrocarbon utilization. *Alcaligenes and Morganella morganii* were in Site B while *Ochrobactrum anthropi* still remained specific to Site C (uncontaminated soil). HUB isolated from soil samples in the Niger Delta include *Acinetobacter, al*., 2008; Eze and Okpokwasili, 2010; Mmom and Deekor, 2010; Chikere and Okpokwasili, 2015). Athough the control site was uncontaminated, petroleum hydrocarbon utilizing bacteria occurred. Otuoke soil, as part of Ogbia LGA where oil was first found in Nigeria, can be said to harbour petroleum degraders. Other reports of hydrocarbon utilizing bacteria in uncontaminated soils of the Niger Delta exist (Odokuma and Ubogu, 2014a). Thus, a vast species of HUB occurs in this oil rich region.

The bacterial numbers of the soils were higher than the rhizosphere counts for all the areas studied depicting that specific bacteria attach to plant roots. The uncontaminated control soil had more bacterial numbers, allowing various microorganisms to thrive in. The bacterial counts were more in the twelve month-old contaminated site than the six month-old site because of the possibility of reduced toxicity of hydrocarbon brought about by microbial degradation, and soil restoration. The higher number of HUB in soil of the six-month old contaminated soil shows a greater number of the petroleum degraders in this site. Generally, the hydrocarbon utilizing bacterial counts (HUB) were just a bit lower than the heterotrophic bacterial counts (THB) and far lesser HUB occurred in the control.

A THB of 10^8 occurred in an uncontaminated mangrove soil of the Niger Delta (Odokuma and Dickson, 2003) while individual plant rhizosphere heterotrophic and hydrocarbon utilizing populations where of 10^6. The THB determines the density of HUB and rhizobacteria in plant sediments as a strong correlation has been shown to exist between them (Udotong *et al*., 2008; John *et al*., 2011). The toxic effect of the hydrocarbon could have reduced the microbial population of the polluted soils. Such findings agree with those of Ilori *et al*. (2015). The differences in bacterial counts may be because of the differences in soil type and the environmental condition at the study time.

The importance of the composition and density of rhizosphere microorganisms, the plants, soil type and characteristics in crude oil remediation have been highlighted (Bisht *et al*., 2015; Ubogu *et al.*, 2018). The abundance and composition of micro-organisms residing in the rhizosphere was also noted to be species specific (Yousaf *et al*., 2010; Pizarro-Tobías *et al*., 2015). Microbial activity and proliferation in soil is reliant on soil characteristics (such as pH, soil type or particle size, moisture and nutrient content, among others) as well as vegetation, which are all necessary for contaminant removal. The appearance of vegetation enhances pollutant removal due to the combined activities of soil microorganisms, rhizosphere microorganisms and plants as compared with a non-vegetated soil (Jussila *et al*., 2006; Fatima *et al*., 2015).

Micrococcus, Pseudomonas, Enterobacter, Klebsiella, Flavobacterium, Edwardsiella, Escherichia, Proteus, Citrobacter, Nocardia, Bacillus, Lactobacillus and Staphylococcus (Ijah *et*

CONCLUSION

Environmental problems initiated by widespread petroleum discharge are continuous process that needs to be curtailed. This occurrence has necessitated the search for varying avenues to give lasting solutions to this menace, of which plant-bacterial partnerships are among. Wetland plants present in oil polluted wetland ecosystem may play important roles in bioremediation and wetland restoration. The plant root zone harbor bacteria called rhizosphere bacteria which occur in synergistic relationship vital for successful plant growth and functioning. A variety of wetland plants and their accompanying rhizosphere and hydrocarbon utilizing bacteria with possible petroleum degradative abilities are introduced in this study.

ACKNOWLEDGEMENT

My sincere appreciation goes to laboratory staff of Biological Sciences Department, Federal University Otuoke, Nigeria. To the staff of the Department of Medical Microbiology, Niger Delta University, Amassoma Nigeria for all their support and assistance rendered during the course of this work.

Table 1 List of occurrence of plant species inherent in the study sites

Plant species	Site A (Oloibiri)	Site B (Ikarama)	Site C (Otuoke)
Commelina benghalensis	+	+	+
Chromoleana odorata	+	+	+
Aspilia Africana	+	+	+
Ipomoea involcurata	+	+	-
Ageratum conyzoides	+	+	-
Kyllinga species	+	+	-
Melastomastrum capitatum	+	-	-
Fimbristylis littoralis	-	+	-
Sacciolepsis Africana	-	+	-
Cyperus difformis	-	+	-
Solenostemon monostachyus	-	+	-
Echinochloa obtusiflora	-	+	-
Diplazium sammatii	-	-	+
Dissotis rotundifolia	-	-	+
Anielema sp.	-	-	+
Panicum laxum	-	-	+
Scleria verrucosa	-	-	+
Cyathula prostate	-	-	+
Costus sp.	-	-	+
Unidentified 1	+	-	-
Unidentified 2	+	-	-
Unidentified 3	+	-	-

Key; Site A, six-month old petroleum-contaminated; Site B, twelve-month old petroleum-contaminated; Site C, uncontaminated control

Table 2. Occurrence of culturable rhizosphere bacteria obtained from plant samples of the three sites

Microorganism	Site A (Oloibiri)	Site B (Ikarama)	Site C (Otuoke)	Frequency
Pseudomonas aeruginosa	+	+	+	3
Pseudomonas flourescens	-	+	+	2
Pseudomonas putida	-	+	+	2
Chromobacterium violaceum	+	-	+	2
Aeromonas hydrophila	+	-	-	1
Aeromonas sp.	+	-	-	1
Aeromonas salmonicida ssp. *salmonicida*	+	+	-	2
Pasteurella pneumotropica	+	-	+	2
Alcaligenes sp.	-	+	-	1
Providencia rettgeri	-	+	-	1
Serratia sp.	-	+	-	1
Burkholderia cepacia	-	+	+	2
Myroides sp.	-	-	+	1
Chryseobacterium indologenes	+	-	+	2
Ochrobactrum anthropi	-	-	+	1
Bacillus sp.	+	-	-	1
Staphylococcus sp.	-	-	+	1
Total %	8 (30.77)	8 (30.77)	10 (38.46)	26 (100)

Key; Site A, six-month old petroleum-contaminated; Site B, twelve-month old petroleum-contaminated; Site C, uncontaminated control

Table 3 Occurrence of Hydrocarbon Utilizing Bacteria (HUB)

Microorganism	Site A (Oloibiri)	Site B (Ikarama)	Site C (Otuoke)	Frequency
Pseudomonas spp.	+	+	+	3
Pseudomonas aeruginosa	+	+	+	3
Pseudomonas flourescens	-	-	+	1
Pseudomonas putida	+	+	+	3
Klebsiella pneumoniae ssp. *Rhinoscleromatis*	-	+	-	1
Morganella morganii	-	+	+	2
Alcaligenes spp.	-	+	+	2
Vibrio sp.	-	+	-	1
Ochrobactrum anthropi	-	-	+	1
Acinetobacter baumanii	-	+	-	1
Total %	3 (16.67)	8 (44.44)	7 (38.89)	18 (100)

Key; Site A, six-month old petroleum-contaminated; Site B, twelve-month old petroleum-contaminated; Site C, uncontaminated control

Table 4. Comparison of Bacterial Counts of the Rhizosphere Sediments and Soils in the Study Locations

Counts	Bacterial counts (cfu / g)			
Location	Rhizosphere		Soil	
	THB	HUB	THB	HUB
Site A	1.25×10^7	1.20×10^7	1.37×10^8	1.33×10^8
Site B	1.82×10^7	1.78×10^7	2.67×10^8	8.67×10^7
Site C	1.03×10^8	3.20×10^7	2.80×10^8	4.00×10^7

Key; Site A, six-month old petroleum-contaminated; Site B, twelve-month old petroleum-contaminated; Site C, uncontaminated control; THB, total heterotrophic bacteria; HUB, hydrocarbon utilizing bacteria

REFERENCES

Abbott, S. L., Cheung, W. K. W. and Janda, J. M. (2003). The Genus *Aeromonas*: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes. *Journal of Clinical Microbiology*, 41(6): 2348 – 2357.

Abed, R. M. M., Al-Kharusi, S., Gkorezis, P., Prigent, S. and Headley, T. (2017). Bacterial communities in the rhizosphere of Phragmatis australis from an oil-polluted wetland. *Archives of Agronomy and Soil Science* https:// doi.org/ 10.1080/ 03650340. 2017.1352087

Alegbeleye, O. O., Opeolu, B. O. and Jackson, V. (2017). Bioremediation of ploycyclic aromatic hydrocarbon (PAH) compounds: (acenaphthene and flourene) in water using indigenous bacterial species

isolated from the Diep and Plakenburg. Brazilian Journal of Microbiology, 48(2): 314 – 325.

Amora-Lazcano, E., Guerrero-Zúñiga, L. A., Rodriguez-Tovar, A., Rodriguez- Dorantes, A. and Vasquez-Murrieta, M. S. (2010). Rhizospheric plant-microbe interactions that enhance the remediation of contaminated soils. *Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. A. Mendez-Vilas ed. pp 251 – 256.

Antunes, A. A., Silva, M. L. R. B., da Silva, C. A. A. and de Campos-Takaki, G. M. (2006). Characterization of *Chromobacterium violaceum* isolated from Paca River, Pernambuco, Brazil *Revista de Biologia e Ciências da Terra Suplemento Especial*, (1 – 2): 48 – 55.

Banks, M. K., Kulakow, P. A., Schwab, A. P., Chen, Z. and Rathbone, K. (2003). Degradation of Crude Oil in the Rhizosphere of *Sorghum bicolor*. *International Journal of Phytoremediation*, 5(3): 225 – 234.

Berg, G., Eberl, L. and Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology*, 7: 1673 –1685.

Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V. and Sharma, K. D. (2015). Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. *Brazilian Journal of Microbiology*, 46(1): 7 – 21.

Chen, W-M., Tang, Y-Q., Mori, K. M. and Wu, X-L. (2012). Distribution of culturable endophytic bacteria in aquatic plants and their potential for bioremediation in polluted waters. *Aquatic Biology*, 15: 99 – 110.

Chikere, C. B. and Okpokwasili, G. C. (2015). The dominance of the genera *Myroides* and *Proteus* within hydrocarbon utilizing populations in some crude oil-impacted sites. *Nigerian Journal of Microbiology*, 29: 3089 – 3095.

Dall'Agnol, L.T., Martins, R. N., Vallinoto, A. C. R. and Ribeiro, K. T. S. (2008). Diversity of *Chromobacterium violaceum* isolates from aquatic environments of state of Pará, Brazilian Amazon. *Memorias do Instituto Oswaldo Cruz*, 103 (7): 678 – 682.

Diab, A. and Sandouka, M. (2010). Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) in the Rhizosphere Soil of *Cyperus conglomeratus*, an Egyptian Wild Desert Plant. *Nature and Science*, 8(12): 144 – 153.

Divya, B. and Kumar, M. D. (2011). Review: Plant - Microbe Interaction with Enhanced Bioremediation. *Research Journal of Biotechnology*, 6 (4):72 – 79.

Eze V.C. and Okpokwasili, G. C. (2010). Microbial and other related changes in a Niger Delta river sediment receiving industrial effluents. *Continental Journal of Microbiology*, 4: 15 – 24.

Eziuzor, S. C. and Okpokwasili, G. C. (2009). Bioremediation of Hydrocarbon Contaminated Mangrove Soil in a Bioreactor. *Nigerian Journal of Microbiology*, 23(1): 1777 – 1791.

Fatima, K., Afzal, M., Imran, A. and Khan, Q. M. (2015). Bacterial Rhizosphere and Endosphere Populations Associated with Grasses and Trees to be Used for Phytoremediation of Crude Oil Contaminated Soil. *Bulletin of Environmental Contamination and Toxicology*, 94(3):314 - 320 doi 10.1007/s00128-015-1489-5

Gkorezis, P., Daghio, M., Franzetti, A., Van Hamme, J. D., Sillen, W. and Vangronsveld, J. (2016). The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. *Frontiers in Microbiology*, 7: 1836. doi: 10.3389/fmicb.2016.01

Goudarzi, S. and Afrous, A.(2012). Phytoremediation of the Sludge Contaminated with Chromium by Aquatic Plants in Dezful. *Bulletin of Environment, Pharmacology and Life Sciences*, 1(9): 58 – 60.

Grimont, F. and Grimont, P. A. D. (2006). The Genus *Serratia*. *Prokaryotes*, 6: 219 – 244.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. and Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology. 9th. ed. Baltimore, Williams, Wilkins.

Iffis, B., St-Arnaud, M. and Hijiri, M. (2017). Petroleum Contamination and Plant Identity Influence Soil and Root Microbial Communities while AMF Spores Retrieved from the same Plants Possess Markedly Different Communities. Frontiers in Plant Science
https://doi.org/ 10.3389/fpls.2017.01381

Ijah U. J. J., Safiyanu, H. and Abioye, O. P. (2008). Comparative Study of Biodegradation of Crude Oil in soil amended with chicken droppings and NPK fertilizer. *Science World Journal*, 3(2): 63 – 67.

Ilori, M. O, Amund, O. O., Obayori, O. S. and Omotayo, A. E. (2015). Microbial Population and Physico-Chemical Dynamics of a Soil Ecosystem upon Petroleum Contamination. *Journal of Scence. Research and Development*, 15: 25 – 33.

Imogie, A. E., Udosen, C.V., Airede, C. I. and Enonuya, D. O. (2012). Utilization of Niger Delta Basin Coastal Fresh Water Swamps for Meaning Agricultural Production. *Greener Journal of Agricultural Sciences*, 2(5): 180 – 187.

Isikhuemen, E. M. (2012). Status, threats and priority for conservation of freshwater swamp forest in protected areas in Edo State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, 8(1): 38 – 46.

John, R. C., Itah, A.Y., Essien, J. P. and Ikpe, D. I. (2011). Fate of nitrogen-fixing bacteria in crude oil contaminated wetland ultisol. *Bulletin of Environmental Contamination and Toxicology*, 87: 343 – 353.

Jussila, M. M. (2006). Molecular biomonitoring during rhizoremediation of oil - contaminated soil. PhD Dissertation, University of Helsinki, Finland. Pp79

Kuiper, I., Lagendijk, E. L., Bloemberg, G. V. and Lugtenberg, B. J. (2004). Rhizoremediation: A beneficial plant-microbe interaction. *Molecular Plant Microbe Interaction*, 17: 6 – 15.

Kumar, A., Bisht, B., Joshi, V. D. and Dhewa, T. (2011). Review on Bioremediation of Polluted Environment: A Management Tool. International

Journal of Environmental Sciences, 1(6): 1079 – 1093.

Madigan, M. T., Martinko, J. M., Stahl, D. A. and Clark, D. P. (2012). Bergey's Manual of Systematic Bacteriology (2nd ed.). *In*: Brock's Biology of Microorganisms. Pearson Education, Inc. Benjamin Cummings. 1043pp.

Manos, J. and Belas, R. (2006). The Genera *Proteus*, *Providencia*, and *Morganella*. *Prokaryotes*, 6: 245 – 269.

Miles, A. A., Misra, S. S. and Irwin, J. O. (1938). The estimation of the bactericidal power of blood. *Journal of Hygiene*, 38(6): 732 – 749.

Mmom, P. C. and Deekor, T. (2010). Assessing the Effectiveness of Land farming in the Remediation of Hydrocarbon Polluted Soils in the Niger Delta, Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, 2(7): 654 – 660.

MoE, Ministry of Environment, (2018). *Oil Spill Logbook.* Ministry of Environment, Bayelsa state, Nigeria.

Mukasheva, T. D., Omirbekova, A. A., Sydykbekova, R. S., Berzhanova, R. Zh. and Ignatova, L. V. (2014). Biodiversity of Plants Rhizosphere and Rhizoplane Bacteria in the Presence of Petroleum Hydrocarbons. *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering*, 8(7): 538-542.

Odokuma L. O. and Dickson, A. A. (2003). Bioremediation of a crude oil – polluted tropical rain forest soil. *Global Journal of Environmental Sci*ence, 2: 29 – 40.

Odokuma, L. O. and Ubogu, M. (2014). Quantitative Assessment of Hydrocarbon Utilizing Microflora of the Rhizosphere of Some Plants in the Rainforest and Mangrove Swamp in Niger Delta. *Australian Journal of Biology and Environment Research*, 31 – 42.

Oliveira, V., Gomes, N. C. M., Almeida, A., Silva, A. M. S., Simões, M. M. Q., Smalla, K. and Cunha, A. (2014). Hydrocarbon contamination and plant species determine the phylogenetic and functional diversity of endophytic degrading bacteria. *Molecular Ecology*, 23: 1392 – 1404. doi: 10.1111/mec.12559

Onyechi, K. C. N., Eseadi, C., Ugwuozor, F. O., Omeje, J. C. and Ngwoke, D. U. (2016). Probable Psychological Impacts of Environmental Pollution (Oil Spills) in the Coastal Areas of the Niger Delta of Nigeria: a Philosophical Discourse. *American-Eurasian Journal of Agriculture and Environmental Science,* 16 (2): 374 – 379.

Perelo, L. W. (2010). Review: In situ and bioremediation of organic pollutants in aquatic sediments. *Journal of Hazardous Materials,* 177: 81 – 89.

Pizarro-Tobías, P., Niqui, J. L., Roca, A., Solano, J., Fernández, M., Bastida, F., García, C. and Ramos, J. L. (2015). Field trial on removal of petroleum-hydrocarbon pollutants using a microbial consortium for bioremediation and rhizoremediation. *Environmental Microbiology Reports,* 7(1): 85 – 94.

Prasad, M.N.V., Greger, M. and Aravid, P. (2005). Biogeochemical cycling of trace elements by aquatic and wetland plants: relevance to phytoremediation. *In* Prasad, M. N. V., Sajwan, K. S. and Naidu, R (eds.). *Trace Elements in the Environment: Biogeochemistry, Biotechnology and Bioremediation.* CRC Press, Florida USA, New Taylor and Francis. Chapter 24 pp 443 – 474.

Prasad, M. N. V., Greger, M. and Smith, B. N. (2001) Aquatic macrophytes. In: *Metals in the Environment: Analysis by biodiversity*. Prasad, M. N. V. (ed.). Marcel Dekker Inc., New York, 259pp.

Scharmann, W. and Heller, A. (2001). Survival and transmissibility of *Pastuerella pnuemotropica. Laboratory Animals,* 35: 163 – 166.

Stephen, E.1. and Ijah, U. J. J. (2011). Comparison of *Glycine max* and *Sida acuta* in the Phytoremediation of Waste Lubricating Oil Polluted Soil. *Nature and Science*, 9(8): 190 – 193.

Ubogu, M., Akponah, E., Vinking, G. E. and Loho, N. G. (2018). Assessment of the Hydrocarbon Utilizing Mycoflora of the Root Zones of *Saccharum officinarum. SciFed Journal of Mycology*, 1:1

Udotong, I. R., John, O. V. M., and Udotong, J. I. R. (2008). Microbiological and physicochemical studies of wetland soils in Eket, Nigeria. *International Journal of Biology, Veterinary, Agriculture and Food Engineering*, 2(8): 30 – 35.

Yousaf, S., Ripka, K., Reichenauer, T. G., Andria, V., Afzal, M. and Sessitsch, A. (2010). Hydrocarbon degradation and plant colonization by selected bacterial strains isolated from Italian ryegrass and birdsfoot trefoil. *Journal of Applied Microbiology,* 109(4): 1389 – 1401.