PHYTOREMEDIATION OF CRUDE-OIL CONTAMINATED MANGROVE SOIL BY
Rhizophora racemosa

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ABSTRACT

This study examined the phytoremediative potentials of *Rhizophora racemosa* in remediation of crude-oil contaminated mangrove soil ecosystem. Results revealed that hydrocarbon utilizing bacterial load increased substantially over the ninety days growth period. The highest increase (77.5%) was recorded in acute crude-oil contaminated soil with three *R. racemosa* seedlings and poultry manure and lowest increase was recorded in acute oil treatment without poultry manure. The total petroleum hydrocarbon (TPH) and heavy metals levels decreased substantially at the end of period of growth. The highest percentage reduction in TPH of 96.16% was recorded in acute crude-oil contaminated soil containing three *R racemosa* seedlings and poultry manure. The lowest percentage reduction of 38.3% was recorded in acute crude-oil contaminated soil without poultry manure. Generally, mangrove seedlings with acute and chronic treatments showed potentials for phytoremediation of crude-oil contaminated soil, the former demonstrated greater phytoremediation than the later. Seedlings treated with poultry manure showed more remarkable potentials and promptness for phytoremediation of crude-oil contaminated soil than untreated ones. Equally, there was gradual reduction in pH during the ninety-day growth period. Results from this study also indicated that acute contamination of crude-oil has more adverse effect on the mangrove ecosystem than chronic contaminations. The stem height, stem girth (diameter), number of leaves and leaf area (length and width) of the mangrove seedlings, *Rhizophora racemosa* increased comparably fortnightly in the ninety days growth period. This study has shown that mangrove plants together with microbial actions are efficient and fast in phytoremediation of crude-oil in a mangrove ecosystem.

Keyword: Phytoremediation, oil polluted mangrove soil, *Rhizophora racemosa* .

INTRODUCTION

Petroleum oil is a major source of energy that sustains society as the principal feedstock of the petroleum industry. It is also a serious environmental pollutant. The increase in activities of petroleum exploration and production has brought increase in the discharge of waste materials into the environment. The impact of these wastes on the ecosystem is an obvious problem of environmental concern particularly with regard to their persistence and ecotoxicity. Degradation of toxic substances such as petroleum hydrocarbon can occur naturally by microbial degradation (Okpokwasili and Okorie, 1988; Amanchukwu *et al*., 1989).

The presence of hydrocarbon and surfactant in the environment will select for the existence of hydrocarbon utilizing microorganism within the total heterotrophic population (Nnubia and Okpokwasili, 1992). Oil utilizing microbes have been reported to include both Gram positive and Gram negative organisms (Fought and Westlake, 1987). Diverse group of bacteria and fungi called hydrocarbonoclastic microbes occur in nature and are capable of degrading petroleum hydrocarbon in aquatic, terrestrial and mangrove environment.

Plant roots were defined from phytoremediation point of view as "exploratory, liquid phase extractors that can find, alter and/or translocate elements and compound against large chemical gradients" (Fought and Westlake, 1987). Since the roots of majority of higher plant live naturally in symbiosis with different types of mycorrhizal fungi, this association should be regarded as an organic components of phytoremediation systems.

By various mechanisms mycorrhizal fungi are able to take direct or indirect part in different processes of phytoremediation of

contaminated soils including phytostabilisa-tion, phytoextraction or phytodegradation. Indirectly, mycorrhiza can increase plant ability to withstand soil phytotoxity due to improved nutrition, particularly in the soil with relatively immobile phosphorus, protect plants against root pathogens and drought stress and enhance soil aggregation and consequently increase retention of xenobiotics (Donnelly and Fletcher, 1994).

Mangrove forests are found at the edge of tropical oceans where regular flooding occurs. They are highly productive areas and in many places an underdeveloped resource. Mangroves are highly susceptible to oil exposure; oiling may kill them within a few weeks to several months. Oil-impacted mangroves may suffer yellowed leaves, defoliation, and tree death (Quilici *et al.,* 1995). Mangroves have proven to be an important source of food and materials for many coastal people. Crabs, clams, oysters, fish and other foods are often collected from mangrove forests. Even mangrove fruit as sometimes eaten and the trees are also useful. If properly managed, mangroves can provide firewood, timber for construction, charcoal for energy, food for livestock, shellfish for local consumption and so on. (Adeqehin, 1993).

Although the majority of the oil spill literature documents the adverse effects of oil on mangroves, some workers have documented an apparent "stimulating" effect (Page *et al.,* 1985; Thomas, 1987). Thomas 1987 recorded an apparent stimulation in 28 experimental treatments versus inhibitory responses in 75 experimental treatments. It was also observed at the TROPICS site in Panama that mangroves regenerating or growing faster than similar-sized *Rhizophora mangle* at a nearby control station (Dodge *et al.,* 1995). The mangrove forests of the Niger Delta principally comprise only three tree families and size species; *Rhizophoraceae (Rhizophora racemosa, Rharrosonii and R mangle), Avicenniaceae (Avicennia Africana)* and *Combretaceae (Laguncularia raremosa* and *Conocarpus erectus). Rhizophora racemosa (*red mangrove), which forms a dense growth throughout Niger Delta region, is the most common species, estimated to cover 90 percent of the mangrove area (Linden and

Jernelov, 1980; Powell, 1993). This study examined the phytoremediative potentials of *Rhizophora racemosa* in remediation of crude-oil contaminated mangrove soil ecosystem.

MATERIALS AND METHODS
Samples Collection, Experimental Set-up, Soil/oil Treatments

Matured healthy mangrove seedlings of *Rhizophora racemosa* in good condition were carefully up rooted with shovel from mangrove soil in Ugbokodo community of Uvwie Local Government area of Delta State. They were transplanted into transparent plastic containers containing 5 kilograms of the mangrove soil each and were left to acclimatize for 40days. Surface soil samples 0-15cm from the study area were collected during tidal recession. The seedling were watered through out the period of acclimatization. Acute and chronic crude-oil treatments were applied to the loamy-sand mangrove soil containing the seedlings together with other remedial treatments such as application as kilned poultry manure.

Treatments commenced at the end of 40-day acclimatization period. Bonny light crude-oil was used for the treatment. Bonny-light has been reported to consist of saturates (56%), aromatics (31%) pollars (11%) and asphaltenes (2%). It also has 35.3^0 API gravity and contains 0.1% sulphur (Qui *et al.* 2004). The acute treatment consists of a one-time application of 150ml of crude oil added on the surface of the soil. The chronic treatment consists of weekly application of smaller amount (20ml) of same crude for a period of 90days (Managhan and Koons, 1975; Proffit *et al.,* 1995).

In addition to the treatments (acute and chronic) four extra containers were set-up. The first of the four containers contained soil samples collected from the study area and 20ml of the same crude-oil applied. The second contained soil samples in which 20ml of crude-oil was applied in addition with little amount (20g) of kilned poultry manure. The third contained soil samples having three seedlings of *Rhizophora racemosa* in which 150ml of crude-oil was applied in addition with little amount of kilned poultry manure. The fourth contained soil samples having three seedlings

of mangrove plant and 150ml of crude-oil applied without poultry manure. Particle-size analysis of the soil was equally determined using hydrometer method to ascertain the soil type.

Isolation and Enumeration of Total Heterotrophic Bacteria and Total hydrocarbon utilizing Microbial Counts

The preliminary isolation was done by adding one gram of sieved unimpacted soil sample in 9ml of sterile water. A tenfold serial dilution of soil samples were prepared and dilutions in tube 10^4 and 10^5 were pour-plated in 0.1ml volumes unto duplicate plates of nutrient agar (incorporated with fulcin antifungi agent). The plates were incubated at 35^0c for 24-48 hours. Colonies numbering between 30 to 300 colonies forming units (cfu) on plates were recorded. Isolation and enumeration of total fungi count in the soil samples were performed using aliquots of 10^{-1} and 10^4 dilutions which were inoculated in duplicates onto potato-dextrose agar (incorporated with antibacterial agent) using pour plate method. Plates were incubated at 28 ± 2^0c for 5-7days after which plates containing 10-100 colonies were counted.

The isolation of hydrocarbon utilizing bacteria and fungi from soil samples were performed in triplicates by plating-out 0.1ml of serially diluted crude-oil impacted soil samples on modified mineral salt medium of Mills *et al*. (1987) to which 1% of crude oil was added using spread plate technique. The mineral salt medium had in one litre: $MgSO_4$ $7H_2O$, O.4g; KCl,0.28gi KH_2PO_4, 8.0g; K_2H PO_4, 1.25g; $NaNO_3$, 0.42g; pH-7.2; agar, 15g. Two sets of mineral salt media were prepared. The first set contained fulcin while the second set contained chloramphenicol to inhibit the growth of fungi and bacteria respectively. Enumeration was done after incubation of plates at room temperature $28^0 \pm 2^0C$ for 7-14 days.

Determination of Total Petroleum Hydrocarbon, Some Heavy Metals and *Rhizophora racemosa* Plant Growth Measurements

Three grams of air-dried sieved soil were weighed into clean dried separation flask, 16ml of hexane was added for the ex-

traction of crude-oil. The separation flask was shaken for 30minutes in a mechanical shaker of 500rpm and the supernatant decanted was used for initial extraction 5ml was used for the second and then 4ml for the third extraction to be sure of possible total extraction of residual hydrocarbon from the soil. The three different extracts were mixed together and read on a Ciriffin spectrophotometer at a wavelength of 460nm. The standards (blank) obtained from non-oil-impacted soil and distilled water were read before the samples were read.

Determination of heavy metals were achieved by weighing-out 2.0grams of air-dried, sieved soil samples into a dry conical flask. Two milliliters (2ml) of nitric acid and sulphuric acid were added respectively. The resultant solution was heated on a hot-plate until brown fumes ceased to appear, indicating that digestion was complete. The concentrations in mg/kg of the following heavy metals (Pb, Cd, Cr and Zn) in the collected samples were determined (after nitric acid digestion) by means of an atomic absorption spectrophotometers. Specific metal standards in the linear range of the metal were used to calibrate the equipment. The concentrated and digested samples were then aspirated and their actual concentrations were obtained by referring to the calibration graph and necessary calculations.

The electronic method using Mettler Toledo model MP126. pH meter was adapted for pH determination. Five grams (5.0g) of soil samples was dissolved in 5.0ml of distilled water and stirred for 5minutes. After calibration, the electrode was immersed into the sample and the pH read immediately the digits stabilized.

The fate and growth of *Rhizophora racemosa* seedlings were monitored fortnightly and at various intervals for 3months. Any yellowing of leaves and seedlings survival were recorded. Stem height, stem diameter (girth), number of leaves and leaf area (length and width) were measured individually to the nearest centimeter using the criteria of Back et al. (2004). All the generated data from this study were subjected to statistical analysis of ANOVA (Analysis of Variance) and Least Significant Difference (LSD) at 5% probability level.

RESULTS

The hydrocarbon utilizing microbial load increase substantially over the ninety days growth period. The highest increase (77.5%) was recorded in contaminated soil with three *R. racemosa* seedlings and poultry manure and the lowest increase of microbial load was recorded in acute oil-treatment without poultry manure as shown on table 1 and fig.1. The total petroleum hydrocarbon (TPH) and heavy metals levels decrease substantially at the end of the period growth. The highest percentage reduction in TPH of 96.16% was shown in contaminated soil containing the seedlings *R. racemosa* and poultry manure. The lowest percentage reduction of 38.34% was shown in contaminated soil without poultry manure (Fig II and Table 2). The result equally showed gradual reduction in pH (Fig II).

The stem height, stem girth (Diameter), number of leaves and leaf area (length and width) of the mangrove seedling *R. racemosa* increased comparably fortnightly in the ninety days growth period (Table 3 and Figure III). Equally crude-oil contaminated mangrove soils with or without mangrove seedling were remediated between 75-100% in ninety days of growth period for all the experimental set-up (Table 3). Mangrove seedling with acute and chronic treatments showed potentials for phytoremediation of crude-oil contaminated soil, the former demonstrating greater phytoremediation than the later. Equally, seedlings with poultry manure showed more remarkable potentials and promptness for phytoremediation of crude-oil contaminated soil than untreated ones (See Table 2 and 3). The result also indicates that acute contamination of crude oil has more adverse effect on the mangrove ecosystem than chronic contamination.

Table 1: Microbiological status of the soil at (Day 90) of treatment after acclimatization

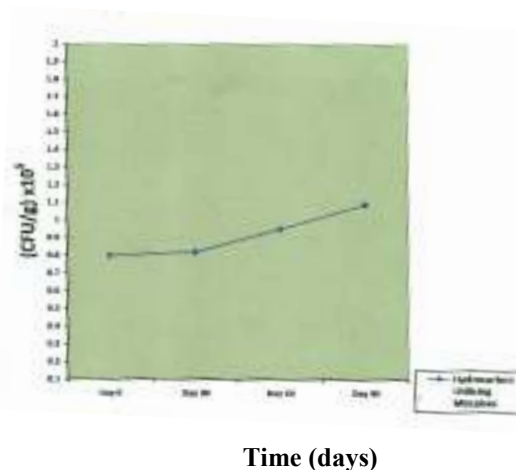|	THB (CFU/g x10^5)	HUB (CFU/g x10^5)	HUF (CFU/g x10^5)	%increase based on HUB
Chronic treatment (Soil + Seedling)	0.66	0.30	0.40	20
Soil + Seedling (without manure)	1.54	0.56	0.45	43.59
Acute Treatment (Soil + Seedling)	0.60	0.72	0.25	0
Soil + Seedling (with manure)	2.05	1.10	0.71	77.5
Contaminated soil without manure	0.96	0.80	0.35	18.33
Soil with manure	1.34	0.71	0.53	44.74

Time (days)

Fig I: A graph showing status of microbial load of the soil over 90 days of growth after acclimatization

Table 2: Physico-chemical status at (Day 90) of treat acclimatization

|	Pb (ppm)	Cd (ppm)	Cr (ppm)	Zn (ppm)	TPH (ppm)	% reduction in TPH
Chronic treatment (Soil + Seedling)	0.138	9.715	4.390	4.390	412.33	95.70
Soil + Seedling (without manure)	0.495	8.750	7.919	7.919	438.32	95.40
Acute Treatment (Soil + Seedling)	0.209	9.017	7.006	28.419	539.01	93/96
Soil + Seedling (with manure)	1.123	8.637	7.827	8.709	396.45	96.16
Contaminated soil without manure	0.405	9.827	5.026	28.378	649.11	38.34
Contaminated Soil with manure	0.315	9.356	4.667	29.670	358.06	95.72
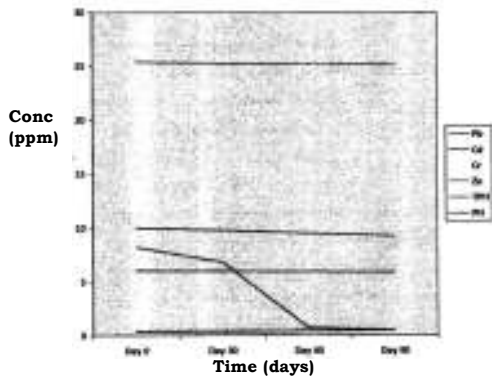
Fig. II: A graph showing Physico-chemical status and pH of the soil over 90 days of growth after acclimatization

Table 3: The status of the mangrove seedlings at (Day 90) of treatment after acclimatization

|	Stem Length	Leaf Area	Stem Diameter	Fate and Growth seedlings sand soils
Chronic treatment (Soil + Seedling)	93cm	60cm²	6cm	Addition to six leaves. Small amount of crude oil observed
Contaminated Soil + 3 Seedling (without manure)	a) 66cm b) 52cm c) 47cm	a) 47.25cm² b) 33cm² c) 33.25m²	a) 7cm b) 4.5cm c) 5cm	Addition of four leaves each to (a,b,c). no discolorations. No trace of crude oil observed on the surface of the soil. Growth of other plants on the soil.
Acute Treatment (Soil + Seedling)	89cm	57.85cm²	6cm	No discoloration. Addition of six leaves. little or no trace of crude oil observed.
Contaminated Soil +3 Seedling (with manure)	a) 75cm b) 74cm c) -	a) 49.5cm² b) 40.5cm2 All dried off	a) 6cm b) 5cm c) -	Addition of five leaves each to (a & b). No discoloration. No trace of crude oil seen, growth of other plants on the soil.
Contaminated soil without manure	-	-	-	Trace of crude oil observed
Contaminated soil with manure	-	-	-	No trace of crude oil observed. Growth of spirogyra observed
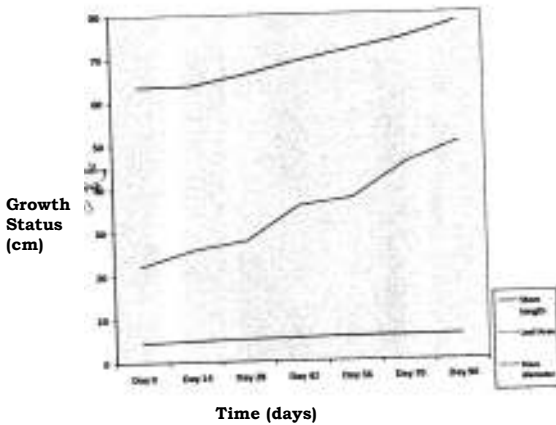
Fig. III: A graph showing Growth Status (cm) over 90 days of growth after acclimatization

DISCUSSION

Findings from these experiments on mangrove seedlings *Rhizophora racemosa* exposed to different crude-oil treatment demonstrated considerable growth with respect to the shoot growth, stem height, and leaves development. Which includes leaf length, with and yellowing of leaves that may be as a result of the effect of crude-oil. There is little statistical significant difference observed between various treatments and controls. This situation can be attributed to the stringent polycyclic aromatic components associated with crude-oil as earlier reported by April and Sims (1990).

Microbial load increased considerably over the ninety days of growth. The highest increase (77.5%) was recorded in contaminated soil with three R. *racemosa* seedlings and poultry manure and the lowest increase of microbial load recorded in soil have acute crude oil treatment. Thus, from this investigation it was observed that the contaminated soil containing poultry manure and mangrove seedling had the fastest rate of total petroleum hydrocarbon (TPH) reduction during the ninety day growth period. This is attributed to the effect of mycorrhizal technology.

Mycorrhizal technology involves the use of mycorrhizal fungi as bioremediation agents with special attention focused on both fungus and host plant. The host plant gives the fungus a selective advantage for surviving at a contaminated site (Donnelly and Fletcher, 1994). Our analysis of the rhizosphere microflora of *R. racemosa* seedlings showed that the population of hydrocarbon degrading microorganisms increased remarkably in the rhizosphere of the seedlings with growth time. The increase in the population of the hydrocarbon utilizing microorganisms in the rhizosphere of the seedlings might have contributed to the observed remediation of the crude-oil and the tolerance of the seedlings to hydrocarbon pollution. Leigh *et al*. (2002) observed that the rhizosphere of most plant promotes a wealth of microbes that can contribute significantly to degradation of hydrocarbon during phytoremediation. Thus, a plant may not directly act on those contaminates but can influence the microbial community within its root zone to carry out phytoremediation (Leigh *et al*., 2002).

April and Sims (1990) reported that microbial degradation in the rhisosphere might be the most significant mechanism for removal of crude-oil range of organics in mangrove contaminated soils because crude-oil range of organics are considered to be highly hydrophobic and their sorption to soils de-

creases their bioavailability for plant uptake and phytoremediation. Based on data obtained from development of stem (height and girth) and leaf (length and width) in relation to *R racemosa* exposure to chronic and acute crude -oil treatment, the relative growth rate suggests that the acute exposure of seedlings had more damaging effects on seedlings than the chronic exposure, (Table 3). Similar observation was made on mangrove seedlings by Proffitt *et al*. (1995) at different exposure levels.

The present investigation showed that the levels of total petroleum hydrocarbon (TPH) not only reduced during ninety days growth of mangrove seedling than the positive control (ie mangrove soil + crude oil), but equally increased the biodegradation rate. With the highest rate occurring between sixty and ninety days of growth. The later is probably associated with the observed increase of population of hydrocarbon degradation microorganisms in the rhizosphere. The earlier observation indicates that the growth of mangrove seedlings is associated with active phytoremedication of crude-oil. Also, this investigation showed that the levels of TPH on the contaminated soils with poultry manure reduced more and faster than the contaminated soils without poultry manure (Table 2). This indicates that the rate of bioremedation is equally associated with the activities of microorganism from poultry manure.

Results shown on figure II showed a gradual reduction of pH of the test soils samples at the end of the ninety days growth period. The reduction in pH could be attributed to biodegradation of hydrocarbon in the soil. The pH of test soil samples became acidic probably due to the production of acidic metabolites. Ayotamuno *et al.* (2006) reported similar findings. Leathy and Colwell (1990) reported that pH is a predominant factor in determining biodegradation of petroleum hydrocarbon in soils.

As shown in figure II and table 2, the content of the following heavy metals Cd, Cr and Zn were observed to have reduced gradually in concentration over the ninety day period of growth except in Pb. This may be due to the fact that the metals might have been used for plant and microbial growth develop-

ment and there was no such use of lead(pb). The composition of the microbial population in the rhzosphere varies with plant species, the composition of root exudates, root type, plant age, soil type and history, environmental factors and the pollutants (Anderson *et al.,* 1993; Kuiper *et al.,* 2004; Chaudhry *et al.,* 2005).

From the results of physical observation of the mangrove seedlings, it appears that the application of crude-oil were initially inhibitory to development of mangrove seedlings and that about three to four weeks were needed for the plants to be able to tolerate and grow better. In conclusion results from this study suggests that the mangrove seedlings of *Rhizosphora racemosa* responded differently to various crude-oil exposures which has implications for restoration activities. The study has equally demonstrated that mangrove seedlings of *R. racemosa* were negatively impacted by both acute and chronic exposures, but more so with seedlings under acute exposure.

REFERENCES

Adegbehun, J. O. (1993). Mangrove in Nigeria. Conservation and Sustainable utilization of mangrove forest in Latin America and Africa Regions Par II-Africa. International Society for Mangrove Ecosystem and Coastal Marine project of UNESCO. Mangrove Ecosystem Technical Reports. In ED. Diop, Vol. 3 edition

Anderson, T. A., Guthrie, E.A. and Walton, B.T. (1993). Bioremediation in the Rhizosphere. *Environmental Science & Technology* **27:** 2630-2636.

Amanchukwu, S. C., Obafemi, T.A. and Okpokwasili, G. C. (1989). Hydrocarbon degradation and utilization by palmwine Yeast isolates *FEMS Microbial Letters* **57:**151-154.

April, W. and Sims, R.C. (1990). Evaluation of the use of Priairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere* **20**: 253-265.

Ayotamuno, M.J., Kogbara, R. B, Ogaji, S.O. T. and Probert, S.D. (2006). Bioremediation of crude-oil polluted agricultural soil in Port-Harcourt, Nigeria. *Applied Energy*. **83 (11):** 1249-1257.

Back, K., Kim, H., Oh, H., Yoon, B., Kim, J. and Lee, I. (2004). Effect of crude-oil, oil componenets and bioremediation of plant growth. *Journal of Environmental Science & Health. Aza* **9**: 2465-2472.

Chaudhry, Q., Blom, Z.M., Gupta, S and Joner, E.J. (2005). Utilising the synergy between plants and rhizosphere microorganism to enhance breakdown of organic pollutants in the environment. *Environmental Science & Pollution Research* **12:** 34-48.

Dodge, R.E, Baca., B.D, Knap, A. H, Snedaker, S.C. and Sleetes, T.D. (1995). The effects of crude-oil and dispersed oil in tropical ecosystem. 10years of monitoring experimental sites (Marino Spill Response Corporation Technical Report series 95-x) Marine Spill Response Corporation Washigton. D.C in Review.

Donnelly, P.K and Fletcher, J. S. (1994). Growth of PCB-degrading bacteria on compounds from photosynthetic plants *Chemosphere* **28:** 981-988.

Fought, J. M and Westlake, D.W.S. (1987). *Biodegradation of hydrocarbons in Fresh waters chemistry.* Pergamon Press, New York.

Kuiper, I, Krachenko, L, Bloemberg., G. V and Lugtenbergy, B.J.J (2004) Pseudo Putila strain PCL 1444, Selected for efficient root colonization and naphthalene degradation, effectively utilizes root exudates components. ***Molecular Plant-Microbe Interactions*** **15**: 734-741.

Leahy, J.C and Colwell, R. R. (1990). Microbial degradation of hydrocarbon in the environment. *Microbiology Reviews* **54:** 305-315.

Leigh, M.B., Fletcher, J.S., Fu, X.O and Schmitz, F.J (2002). Polychlorinated Bipheny (PCB) degrading bacteria associated with trees in a PCB contaminated site. *Environmental Science & Technology* **36:** 1579-1581.

Linden, O. and Jernelov, A. (1980). The mangrove Swamp: an ecosystem in danger. *Ambio* **9(2):** 81-88.

Mills, A. L, Breuil, C. and Colwell, R.R. (1978). Enumeration of petroleum-degrading marine and estuarin microorganisms by the most-probably number method. *Canadian Journal of Microbiology* **24**: 552-557.

Monaghan, P.H and Koons, C.B (1975). Research needed to determine the chronic effect of oil on the marine environment. *Marine Pollution Bulletin* **6(10):** 157-159.

Nnutia, C. and Okpokwasili, G.C. (1992). The microbiology of drill mud cutting from a new offshore oil-field in Nigeria. *Environmental Pollution* **82:** 153-156.

Okpokwasili, G, C, and Okorie, B.B (1998). Biodegradation potentials of microorganism isolated from car engine lubricating oil. *Tribiology International* **21(4):** 215-220.

Page, D.S., Giliffan, E.S, Foster, J.C., Hotham, J.R. and Gonzalez, L. (1985). Mangrove leaf tissue sodium and potassium on concentration as sublettal indicators of oil stress in mangrove trees. Proceedings of 1985 oil spill conference. American Petroleum Institute, Washington, D.C, Pg391-393.

Powell, C.B. (1993). Sites and species of conservation interesting the central axis of the Niger Delta. A report of recommendation of the Natural Resources Conservation Council. (NARESCON) Abuja.

Profit, C.E, Devlin, D.J. and Lindsay, M. (1995). Effects of oil on mangrove seedlings grown under different environmental conditions. *Marine Pollution Bulletin* **30(12):** 788-793.

Qui, X, Sorensen, C.I. Sims, E.W, Engelke, D.L and Grays, M.C. (2004). Enhanced bioremediation for clay soils contaminated with polynuceararomatic hydrocarbons, In: *Bioremediation through rhizosphere technology.* (Anderson, T.A and Coats, J.R eds). American Chemical Society. Washington, D.C.

Quilici, A, Infants, C., Rodriguez-Grau, J, La Schiazza, J.A., Briceno, H. and Pereira, N. (2005). Mitigation strategies at an estuarine mangrove area affected by an oil spoll, in Proceedings of the 1995 International oil spill conference pp 249-433.

Thomas, C. and Ong, J.E. (1981). Effect of heavy metal zinc and lead on Rhosophora mucronata lam wna Avicennia alba Bl seedlings. In: Proceedings of the Asian