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Changes in residual concentration of PAHs in planted and bioaugmented crude oil polluted tropical soils

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Abstract

Effects of bioaugmentation of vegetated crude oil polluted soils on the residual concentration of PAHs were examined in microcosms in a green house study. Weathered crude oil polluted soils (2% w/w) in 4 sets of pots were respectively planted and bioaugmented with hydrocarbon utilizing species (*Micrococcus* sp. RZI, *Pseudomonas* sp. RZIII, *Bacillus* sp. RZIII and *Bacillus* sp. GSIII). Polluted vegetated and polluted non-vegetated control soil samples were also set up. At intervals between setup and the tenth week of incubation, soil samples from three replicates of each treatment chosen randomly were analysed for soils' residual PAHs concentrations. The results obtained showed that 10 weeks after planting (WAP) residual PAHs concentrations in polluted soils were 34.3 mg/kg; 31.6 mg/kg; 26.1 mg/kg and 25.9 mg/kg respectively for vegetated control soil, vegetated soil bioaugmented with *Micrococcus* sp.RZI, vegetated soil bioaugmented with *Bacillus* sp. RZIII and vegetated soil bioaugmented with *Pseudomonas* sp. RZIII. Compared to concentrations in polluted non-vegetated samples, there was 78.8%, 76.6%, 80.8% reductions in pyrene, benzo (b) fluoranthene, indeno (1,2,3-C-D) pyrene and 1,2,5,6-dibenzanthracene respectively in polluted vegetated samples bioaugmented with *Pseudomonas* sp. RZIII. These show that bioaugmentation of vegetated polluted soils with hydrocarbon utilizing species can enhance PAHs removal from weathered petroleum polluted soils. This can be used to improve on the gains of phytoremediation particularly with respect to carcinogenic PAHs.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants with two or more benzene rings. PAHs are of great concern owing to their hydrophobicity, toxicity, tendency to adsorb to particulate materials and bioaccumulation. These compounds are mainly released into the environment via incomplete combustion or pyrolysis of organic compounds (Sheng-wang *et al.*, 2008). They are also components of the petroleum mixture. A number of PAHs are known to have mutagenic, carcinogenic, and teratogenic effects and are thus considered as hazardous (Kalf *et al.*, 1997; Nasseri *et al.*, 2010). PAHs in oil-polluted soil thus pose a major environmental and health problem, and its removal has become of particular concern for the protection of the environment (Diab, 2008).

To achieve this removal of PAHs, a process of remediation is required. These include bioremediation which harness the natural activities of microbes and higher organisms to degrade, transform and/or accumulate a wide range of compounds including PAHs, leading ultimately to the removal of pollutants from the environment (Diaz, 2008). It has proved successful in enhancing the cleanup of pollutants in contaminated environments (Adieze *et al.*, 2003; Odokuma and Dickson, 2003; Adieze, 2012).

Bioremediation can be spurred on via biostimulation, bioaugmentation or phytoremediation. Biostimulation involves the addition of fertilizers to increase the population of microbes able to utilize the contaminants within a medium. Bioaugmentation involves the addition of competent contaminant degrading microbial strains to contaminated media to enhance the resident population's ability to break down contaminants, while phytoremediation uses plants and their associated rhizosphere microorganisms to degrade, contain or render harmless, contaminants in the soil or ground water (Cunningham *et al.,* 1996). Phytoremediation is presumed to be based on the stimulation of microbial degradation in the rhizosphere. Microorganisms and plants complement each other here in the cleanup of polluted soil. Plants' growth impairment in phytoremediation may arise from contaminants toxicity in the soil or water. This will limit the benefits of phytoremediation. To overcome this, hydrocarbon utilizing bacterial inoculants have been used to detoxify contaminants in close proximity to planted seeds and plant's roots. This reduces possible stressors, thus enhancing plants' growth (Siciliano and Germida, 1997; Huang *et al.,* 2004). If plants are introduced to contaminated sites, with the addition of hydrocarbon degrading microorganisms, the plants may enhance inoculant's survival and the inoculants may effectively detoxify contaminants in close proximity to the plant's roots, aiding plant's survival. The increased microbial population and in turn increased microbial activity in the rhizosphere as result of bioaugmentation, supported by source of nitrogen and carbon from root exudates and sloughing cells can degrade organic pollutants such as PAHs. Plants also modify the soil structure and increase its aeration and humidity. The can physically transfer organic pollutants into their tissues, where they will mineralize them (Yu *et al.*, 2010). The roots' exudates also contain oxidative enzymes that can contribute to the degradation of PAHs.

This study aimed at assessing in a green house study, the effect of bioaugmentation of the rhizosphere of *P. maximum* on the residual concentration of PAHs in crude oil polluted soil. The study reported here was a part of a larger investigation, which was aimed at evaluating the efficiency of bioaugumentation of crude oil polluted rhizosphere and non-rhizosphere soil of *P. maximum* in reducing the concentration of crude oil contaminant in soil. The results of the first part of the study have been reported in details in a previous papers (Adieze, 2012; Orji *et al.*, 2015) which studied the effect of bioaugumentation on soil microbial populations and residual crude oil concentration, and the effect of bioaugmentation on the growth of *P. maximum* in crude oil polluted tropical soils. Although the use of plants to increase

the rates of PAH degradation in the rhizosphere has been reported by other studies (Ferro *et al.*, 1999; Reilley *et al.,* 1996; Liste and Alexander, 2000), none of the studies considered PAHs degradation in a weathered petroleum mixture. None of the studies also took place in the tropics.

Materials and methods

Collection of samples

Soil samples

The soil samples are uncontaminated surface soil (0 – 20 cm) from a fallow patch of land within the National Root Crops Research Institute Complex, Umudike. They samples were collected and taken to the laboratory following procedures described in Adieze (2012).

Plants

Seedlings of *Panicum maximum*, were obtained from those growing in uncontaminated soil in the wild within the National Root Crops Research Institute Complex, Umudike. They were transplanted into uncontaminated soil (150 g) in thin membrane polyethylene bags, watered and grown under about 90% shading for 24 hours before transplanting into experimental pots containing 2% (w/w) crude oil polluted soil samples.

Microbial species

Five hydrocarbon utilizing bacterial species (*Micrococcus* sp. RZ1, *Pseudomonas* sp. RZIII, *Pseudomonas* sp. GSIII, *Bacillus* sp. RZIII, and *Bacillus* sp. GSIII) isolated in an earlier study and stored were obtained and used for this study. The bacterial species were selected for adaptation in the rhizosphere of *P. maximum* and hydrocarbon utilization in submerged culture.

Pollution of soil

To artificially pollute the uncontaminated soil sample, crude oil was mixed with acetone, and then mixed with 10% of the total soil (Adieze *et al.*, 2012). The crude oil laddered soil was then added to the bulk of the soil and then mixed to obtain the final concentration of 2% w/w crude oil in soil. The crude oil-polluted soil was then stirred several times for 2 days to remove the acetone (Banks *et al.,* 2000), watered intermittently, and allowed to undergo weathering for six weeks.

Preparation and standardisation of microbial inoculums

A loopful of each of the stored bacterial isolates was sub cultured into 50 ml nutrient broth contained in 250 ml conical flasks and incubated in a shaker (150 rpm) at 30 ^{o}C for 24h. The cells were harvested by centrifugation at 10,000 g for 20 mins. The cell pellets collected were washed twice in 20 ml of sterile tap water. Washed cells were resuspended in 10 ml sterile normal saline (0.85% NaCl) in 20 ml sterile test tubes.

To standardize the bacterial inocula in normal saline, the optical density (OD) readings of different concentrations of given species of bacteria were determined using a colorimeter set at 660 nm. The numbers of viable organisms per ml of the different concentrations of the bacterial species were determined by plating out 0.1 ml aliquots of the cell suspensions on nutrient agar (duplicate plates for each dilution). A standard curve was then drawn matching specific OD readings to specific numbers of viable organisms. Cells were standardised by adjusting cell suspensions to specific OD readings on the colorimeter at 660 nm.

Screening bacterial species for survival in the rhizosphere of P. maximum.

Soil preparation and planting of P. maximum

Seven black cylindrical plastic planting pots of approximately 8 cm diameter and 20 cm height were filled with 800 g, 2% (w/w) crude oil contaminated soil. The soil samples were then moistened with tap water to bring the soil's moisture level to about 80% of its water holding capacity and allowed to undergo weathering for six weeks.

P. maximum's seedlings obtained as previously

described were propagated in uncontaminated soil (200 g) in thin membrane polyethylene bags. They were then transplanted into the planting pots by embedding in the top 8 cm of the seven planting pots. After transplanting, the seedlings were grown under about 90% shading for one week, followed by 55% shading for another two weeks before transferring to the green house. The seedlings were moistened every other day to keep the soil water content near field capacity.

Preparation of bacterial inocula and soil inoculation

Suspensions of the test hydrocarbon utilizing bacterial species were inoculated into 10 ml sterile normal saline contained in 20 ml sterile test tubes. The suspension was shaken for five minutes to evenly distribute the organisms and then transferred into a sterile 500 ml conical flask containing 190 ml sterile mineral salt broth containing 1% Bonny light crude oil. This mixture was incubated on a rotary shaker (150 rpm) at 28±2°C for five days (Odokuma and Dickson, 2003). The pH of the cultures were monitored and maintained at between 7 – 7.2 by adjusting the culture with standard phosphate buffer (APHA, 1985).

To obtain the inocula, aliquots of the final cultures were centrifuged at 10,000 x g for 20 minutes. The supernatants were discarded and the cell pellets collected and washed twice in 20 ml sterile tap water. After washing, the cell pellets were resuspended in 100 ml sterile water and adjusted to a final OD giving a population between 10^7 and 10^8 CFU per ml at 660 nm, using sterile distilled water. The suspensions were then used for the inoculation of the planted soil samples by spray irrigation after holes have been made into the soil using sterile glass rods.

Survival of inoculated bacterial species in the rhizosphere of P. maximum

The test bacterial isolates were assayed for survival in the rhizosphere of *P. maximum*. The population of hydrocarbon utilizers were determined on inoculation and re-determined after the 14th day of incubation by comparing the hydrocarbon utilizing population of the plant rhizosphere in inoculated pots with that of the plant's rhizosphere of an uninoculated control. Rhizosphere soils that maintained inoculant's population greater than or equal to 40% of their initial population were selected and stored for further studies (Adieze, 2012).

Setting up and inoculation of planted soil microcosm

Eight hundred grams (800 g) each of weathered crude oil polluted soil (2% w/w) were placed in plastic planting pots. These constituted the polluted pots. A total of 60 polluted pots were set up. The pots were then planted with *P. maximum* following the procedure described in Adieze *et al.* (2012). Of the 60 planted polluted pots, 15 pots each were inoculated with the different selected bacterial inoculants following procedures described in Adieze (2012). Briefly, standardized inocula in 100 ml sterile distilled water was introduced into holes made on the surface of the planted crude oil polluted soils.

Pots of planted polluted soil samples (15) were also set up and maintained as control. Likewise, 100 ml of sterile distilled water alone was introduced into holes made on the surface of the polluted controls soils.

Five sets of 15 plastic planting pots each were thus set up. These are:

Set 1: Pots of vegetated polluted soil.

Set 2: Pots of vegetated polluted soil + *Micrococcus sp.* RZ1

Set 3: Pots of vegetated polluted soil + *Pseudomonas* sp. RZIII

Set 4: Pots of vegetated polluted soil + *Bacillus* sp. RZIII

Set 5: Pots of vegetated polluted soil + *Bacillus* sp. GSIII

After bioaugmentation, the pots were transferred to the green house with approximately 12h day light and incubated for ten weeks following procedures described in Adieze, *et al.* (2012).

Sample analysis

At intervals from the second week to the tenth week of incubation, three replicate pots of each of the various treatments chosen randomly were destructively sampled. The soil samples obtained were analysed for residual PAHs concentration.

Residual PAHs Concentration

Ten grams of the air-dried soil samples were mixed with 10 g of anhydrous sodium sulphate to remove moisture. The hydrocarbons were soxhlet extracted with chloroform for 8h. The extract was evaporated in a preweighed dish. The extracted residual oil was suspended in n-hexane and filtered through tared filter paper to remove the insoluble fraction (asphaltene). The hexane - soluble fraction was fractioned by liquid - solid chromatography into saturates, aromatics and resins. Gas chromatography (GC) analysis was used the aromatic fraction resolution of PAH compounds. To identify PAH compounds of priority importance in the aromatic fraction, Varian 3900 gas chromatography equipped with a FID was used, using helium as a carrier gas, with a flow rate of 1ml/min. A column (25 m long x 0.32 mm diameter x 0.2 mm thickness) for the stationary phase 1 was used. Temperature programming of initial holding at 40° C (2 min) and then heating with a rate of 10° C/min to 295° C (holding 2 minutes) was applied. The total time of analysis was 45 min. Injector and detector temperatures were 250° C and 280° C respectively injection volume was 1 µL or 2 µL for some samples. The quantification of PAHs was based on the application of reference standard of the PAHs (100 ppm for each). Samples were run in duplicates and the mean values were taken.

Results

Hydrocarbon utilization by bacterial isolates

The result of the hydrocarbon utilization by isolates in submerged culture is shown in Table 1.

Table 1. Hydrocarbon utilization by isolates.

Bacterial Isolates	Petrol	Xylene	Diesel oil	Kerosene	Crude oil
Micrococcus sp. RZI	++	+	+++	+++	+++
Pseudomonas sp. RZIII	++	++	+++	+++	+++
Pseudomonas sp. GSIII	+	+	+++	+++	+++
Bacillus sp. RZIII	+	++	+++	+++	+++
Bacillus sp. GSIII	++	+	+++	+++	+++

Table 1. Legend: + - Low turbidity, ++ - Moderate turbidity, +++ - High turbidity.

The result showed that five bacterial species (*Micrococcus* sp. RZ1, *Pseudomonas* sp. RZIII, *Pseudomonas* sp. GSIII, *Bacillus* sp. RZIII, and *Bacillus* sp. GSIII) tested, were able to utilize the various hydrocarbon sources with intense cloudiness (+++) in at least three of the five carbon sources tested. Diesel, Kerosene and crude oil were the carbon sources most utilized by the isolates.

Survival of Inoculated bacterial species in the rhizosphere of P. maximum

The result of the quantities of bacterial species introduced into and recovered from the rhizosphere of *P. maximum* in 2% w/w oil polluted soil samples two weeks after inoculation is presented in Table 2.

Table 2. Population of bacterial species (CFU/g soil) introduced and recovered from the rhizosphere of *P. maximum* after two weeks.

Bacterial Species	Number introduced	Number recovered	% recovered
Micrococcus sp. RZ1	7.0×10^7	3.3×10^7	47.1
Pseudomonas sp. RZIII	1.3×10^8	6.7×10^7	51.5
Pseudomonas sp. GSIII	8.8×10^7	27.9×10^6	31.7
Bacillus sp. RZIII	8.0×10^7	4.3×10^7	53.8
Bacillus sp. GSIII	4.7×10^7	2.2×10^7	46.8
Consortium of the five isolates	5.3×10^7	10.2×10^6	19.2

Of the five bacteria species, and a consortium of the five inoculated into the rhizosphere of *P. maximum* in polluted soil, four (*Micrococcus* sp. RZ1, *Pseudomonas* sp. RZIII, *Bacillus* sp. RZIII, and *Bacillus* sp. GSIII) were able to maintain a population 40% and above (of the initial hydrocarbon utilizing bacterial population) 2 weeks after inoculation (Table 2). The consortium of the five isolates produced the poorest population, 19.2% of their initial population recovered. It was followed by the *Pseudomonas* sp. GSIII, which had 31.7% of its initial inoculums population recovered. However, *Bacillus* sp. RZIII had the best percentage recovery (53.8%).

PAHs removal studies

The result of the concentrations of residual PAHs in the polluted soil samples and polluted soil samples with various treatments are shown in Table 3.

Table 3. Concentrations of residual PAHs (mg/kg) and percentage removed 10 weeks after planting and bioaugmentation.

Treatments	Residual PAHs (mg/kg)	% PAHs removed 10 WAP
Vegetated + bioaugmentation with *Micrococcus* sp.RZI	31.6	87.3
Vegetated + bioaugmentation with *Pseudomonas* sp.RZIII	25.9	89.6
Vegetated + bioaugmentation with *Bacillus* sp. RZIII	26.1	89.5
Vegetated + bioaugmentation with *Bacillus* sp. GSIII	33.7	86.5
Vegetated Control	34.3	86.3
Non-vegetated Control	45.7	81.7
Weathered polluted soil	249.7	-

The concentration of poly aromatic hydrocarbons (PAHs) at the end of the study was greatly reduced in all the samples from 249.7 mg/kg in the weathered polluted soil sample. There was 45.7 mg/kg residual PAHs (81.7% reduction) in the non-vegetated control soil sample, while the vegetated polluted control sample had 86.3% reduction (34.3 mg/kg residual PAHs). The vegetated bioaugmented samples had percentage PAHs reduction ranging from 86.5% to 89.6% (25.9 mg/kg to 33.7 mg/kg residual PAHs) Table 3.

The results of the gas chromatographic analyses of residual PAHs in the polluted soil samples with various treatments are shown in Fig. 1, Table 3 and Fig. 2. The results showed that five weeks after incubation, bioaugmented samples had residual PAHs concentrations ranging between 44.3 mg/kg and 94 mg/kg as against 112.4 mg/kg and 164.4 mg/kg in vegetated polluted control and polluted control soil samples respectively (Fig. 1). Also, 81.7% to 89.6% PAHs were removed from the treated soil samples within the 10 weeks period of incubation (Fig. 1 and Table 3).

The results of the residual PAHs in soil samples revealed that the concentrations of individual PAHs in the polluted soils with various treatments were in the following order: vegetated + bioaugmentation < vegetated control < non-vegetated control < polluted soil (Fig. 2).

For the probable human carcinogenic PAHs, pyrene, benzo(b)fluoranthene, indeno(1,2,3-C-D)pyrene and 1,2,5,6-dibenzanthracene their removal from treated samples was greatest for indeno(1,2,3-C-D)pyrene followed by 1,2,5,6-dibenzanthracene, pyrene and benzo(b)fluoranthene, respectively. For the PAHs, there were reductions with vegetation of polluted soil samples and a further reduction with the bioaugmentation of the vegetated control samples. Compared to concentrations of PAHs in polluted non-vegetated samples, there was 32.2% and 78.8% reduction in pyrene respectively in polluted vegetated soil samples and in polluted vegetated samples bioaugmented with *Pseudomonas* sp.RZIII. There

was also 62.6% and 76.6% reduction in benzo(b)fluoranthene respectively in polluted vegetated soil samples and in polluted vegetated samples bioaugmented with *Pseudomonas* sp.RZIII. In 1,2,5,6-dibenzanthracene, there was also 34.5% and 80.8% reduction respectively in polluted vegetated soil samples and in polluted vegetated samples bioaugmented with *Pseudomonas* sp.RZIII (Fig. 2).

Discussion

Due to the toxicity of petroleum hydrocarbons in soils, and their potential to cause far reaching environmental and health consequences, there are needs for quick removal of these pollutants from the environment. In this study, bacterial inoculation of the rhizosphere of *Panicum maximum* was assessed for efficacy in the removal of PAHs from aged petroleum contaminated soil.

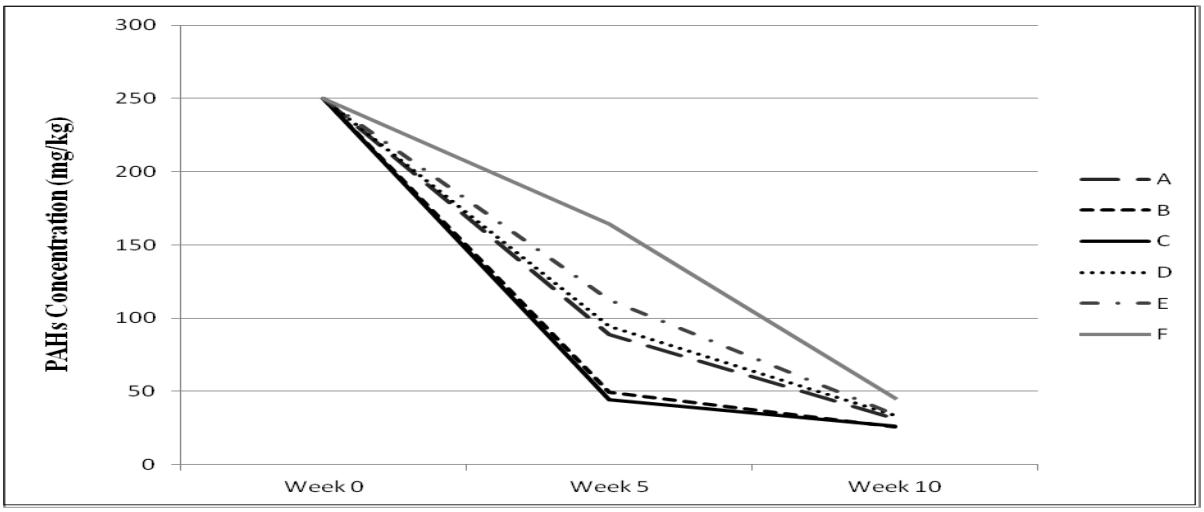


Fig. 1. Reduction in concentrations of PAHs in polluted, polluted vegetated, and bioaugmented polluted vegetated soil samples.

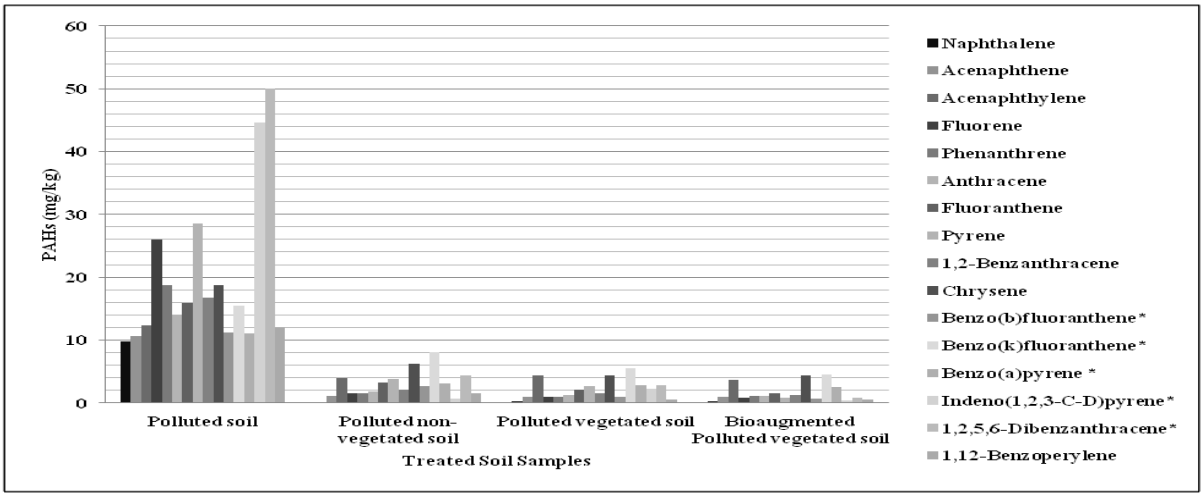
Fig. 1. Legend. A- Vegetated polluted soil bioaugmented with *Micrococcus* sp.RZI, B- Vegetated polluted soil bioaugmented with *Pseudomonas* sp.RZIII, C- Vegetated polluted soil bioaugmented with *Bacillus* sp. RZIII, D- Vegetated polluted soil bioaugmented with *Bacillus* sp.GSIII, E- Vegetated polluted control, F- Polluted control soil.

All the bacteria species in the present study were isolated from petroleum contaminated soil samples, they survived and adapted in oil contaminated liquid environment. The testing of the bacterial inoculants for survival in the plants' rhizosphere revealed poor percentage recovery in the inoculants composed of a consortium of five isolates.

The observation above could be as a result of antagonism between the various isolates resulting from competition for nutrient and space. It may also be due to the production of antimicrobial agents by some isolates. Nweke *et al.* (2006) also observed that a pure culture of *Bacillus* sp. K9 utilized kerosene better than a consortium of *Bacillus* sp. C4, *Bacillus* sp. K9 and *Flavobacterium* sp.C11. This highlights the need to assay the ability of a consortium to produce a required synergy before its use in field. Microorganisms used for soil inoculation need to survive in the environment and be tolerant of the soil conditions at a contaminated site. Once the microorganisms are in a soil system, they need to persist over long periods of time to allow for sufficient levels of degradation. The survival of the plant and inoculants is thus a deciding factor in the rate of degradation of hydrocarbons (Mishra *et al.,* 2001).

The results of this study shows that planting of polluted soil samples with *P. maximum*, resulted in

86.3% reduction in PAHs (4.6% increased reduction of PAHs over that in the unplanted control sample). With bioaugmentation, the percentage reduction increased by 4.8 - 7.9%. The reduction of PAHs in polluted soil samples have been reported in other studies. Results of an investigation by Reilley *et al.* (1996) indicated that planting grasses and legumes significantly enhance the removal of PAHs (pyrene and anthracene) from contaminated soils than the unplanted soils, with 30 to 40% more degradation in the planted soils. The greater degradation observed by Reilley *et al.* (1996) could be as a result of the fewer number of rings (3 and 4 respectively for anthracene and pyrene) in his PAHs sample compared to those of petroleum used in our studies. Also, the fate of a single PAH in the rhizosphere may differ from those of the same compound in a mixture of PAHs (Binet *et al.*, 2000). Our study was also on weathered polluted soil.



*Probable human carcinogens

Fig. 2. Concentrations of individual PAHs in crude oil polluted soil, polluted non-vegetated soil, polluted vegetated and polluted vegetated + *Pseudomonas* sp.RZIII bioaugmented soil.

The observed reduction of PAHs in our study could be as a result of the more intense microbial activity in the rhizosphere of *P. maximum*. Epuri and Sorensen (1997) also reported marginally higher mineralization of [^{14}C]benzo[*a*]pyrene, as well as higher microbial numbers, in soil planted to tall fescue compared to unplanted soil. Frick *et al.* (1999) also reported that enhanced biodegradation in the rhizosphere soil appeared to be the primary mechanism of petroleum hydrocarbon dissipation in a polluted planted system, while leaching, plant uptake, abiotic degradation, mineralization, and irreversible sorption have been shown to be insignificant.

Bioaugmentation of the polluted planted soils also effected increased reduction of persistent PAHs. This could be as a result of a synergy between the plant and the microbial species. It has been reported that plants and microbes can evolve a mutually beneficial strategy for dealing with phytotoxicity where microorganisms benefit from plants roots exudates while plants benefit from the ability of microorganisms to breakdown toxic chemicals. PAHs removal from the bioaugumented samples may also have been as a result of cometabolism. Organic molecules as plant exudates may have provided substrates and energy to support populations of microbes to co-metabolise petroleum hydrocarbons. Petroleum hydrocarbons as oil and grease can also serve as co-substrate for degradation of PAHs with four or more rings. Kanaly *et al.* (1997) reported 95% degradation of a five ring PAH Benzo(a)pyrene by soil microbes in the presence of suitable co-substrate from a crude oil mixture. This shows the importance

of cometabolism in the degradation of such recalcitrant hydrocarbons. There is however the need for further research to optimize bioaugmented polluted-planted systems for enhanced PAHs reduction. The reduction in PAHs in petroleum polluted soils as a result of the combination of plant species and hydrocarbon utilizing species as observed here is of great importance to the bioremediation petroleum polluted soils in the tropical.

References

Adieze IE. 2012. Effect of bioaugumentation on soil microbial populations and residual crude oil concentration of a polluted tropical soil. Journal of Nigerian Environmental Society. **7**.

Adieze IE, Nwabueze RN, Onyeze GOC. 2003. Effect of poultry manure on the microbial utilization of hydrocarbons in oil–polluted soil. Nigerian Journal of Microbiology **17,** 12 – 16.

Adieze IE, Orji JC, Nwabueze RN, Onyeze GOC. 2012. Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms, International Journal of Environmental Studies. **69,** 490-500. http://dx.doi.org/10.1080/00207233.2012.665785.

American Public Health Association. 1985. Standard methods for water and waste water analyses, 13th ed., Washington D.C.

Banks MK, Govindaraju RS, Schwab AP, Kulakow P. 2000. Part I; Field demonstration. In: Fiorenza S, Oubre CL, Ward CH. (eds.) Phytoremediation of hydrocarbon-contaminated soil. Lewis Publishers, Boca Raton, Fl. 3-88.

Binet P, Portal JM, Leyval C. 2000. Dissipation of 3±6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biology and Biochemistry **32,** 2011 -2017.

Cunningham SD, Anderson TA, Schwab AP,

Hsu FC. 1996. Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy **56,** 55 – 114.

Diab EA. 2008. Phytoremediation of Polycyclic Aromatic Hydrocarbons (PAHs) in a Polluted Desert Soil, with Special Reference to the Biodegradation of the Carcinogenic PAHs, Australian Journal of Basic and Applied Sciences **2,** 757-762.

Epuri V, Sorensen DL. 1997. Benzo(a)pyrene and hexachlorobiphenyl contaminated soil: phytoremediation potential. In: Kruger EL, Anderson TA, Coats JR, Ed. Phytoremediation of Soil and Water Contaminants. American Chemical Society: Washington, D.C. ACS Symposium Series **664,** pg. 200 - 222.

Ferro AM, Rock SA, Kennedy J, Herrick JJ, Turner DL. 1999. Phytoremediation of soils contaminated with wood preserva- tives: Greenhouse and field evaluations. International Journal of Phytoremediation **1,** 289–306.

Frick CM, Farrel RE, Germida JJ. 1999. Assessment of Phytoremediation as an In-situ Technique for Cleaning Oil-Contaminated Sites, Petroleum Technology Alliance of Canada, Calgary.

Huang XD, El-Alawi Y, Penrose DM, Glick BR. Greenberg BM. 2004. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution **130,** 465-476.

Kalf DF, Commentuijn T, Vande Plassche EJ. 1997. Environmental quality objectives for 10 polycyclic aromatic hydrocarbons (PAHs), Ecotoxicology and Environmental Saftey **36,** 89-97.

Kanaly R, Bartha R, Fogel S, Findlay M. 1997.Biodegradation of [14C]benzo[a]pyrene added in crude oil to uncontaminated soil. Applied and Environmental Microbiology **63,** 4511-4515.

Liste HH, Alexander M. 2000. Plant-promoted pyrene degradation in soil. Chemosphere **40,** 7-10.

Mishra S, Jyot J, Kuhad RC, Lal B. 2001. In situ bioremediation potential of an oily sludge-degrading bacterial consortium. Current Microbiology **43,** 328-335.

Nasseri S, Kalantary R, Nourieh N, Naddafi K, Mahvi A, Baradaran N. 2010. Influence of bioaugmentation in biodegradation of PAHs-contaminated soil in bio-slurry phase reactor. Iranian Journal of Environmental Health Science and Engineering **7,** 199–208.

Nweke CO, Mgbachi LC, Nwanganga C, Nwanyanwu CE. 1996. Heavy metal tolerance among hydrocarbon utilizing bacteria isolated from oil-contaminated soil. Nigerian Journal of Microbiology **20,** 1057 – 1065.

Odokuma LO, Dickson AA. 2003. Bioremediation of a crude oil polluted Tropical rain forest soil. Global Journal of Environmtal Sciences **2,** 29-40.

Orji JC, Adieze IE, Nwabueze RN, Onyeze GOC. 2015. Effect of bioaugmentation of crude oil polluted tropical soils on the growth of Panicum maximum. Journal of Biodiversity and Environmental Sciences **6,** 227-235. http://www.innspub.net

Reilley KA, Banks MK, Schwab AP. 1996. Organic chemicals in the environment: dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality **25,** 212-219.

Sheng-wang PAN, Shi-qiang WEI, Xin Y, Sheng-xian CAO. 2008. The removal and remediation of phenanthrene and pyrene in soil by mixed cropping of alfalfa and rape. Agricultural Sciences in China **7,** 1355–1364. http://dx.doi.org/10.1016/S1671-2927(08)60185-6.

Siciliano SD, Germida JJ. 1997. Bacterial inoculants of forage grasses that enhance degradation of 2-chlorobenzoic acid in soil. Environmental Toxicology and Chemistry **16,** 1098-1104.

Yu XZ, Wu SC, Wu FY, Wong MH. 2010. Enhanced dissipation of PAHs from using mycorrhizal ryegrass and PAH-degrading bacteria. Journal of Hazardous Materials **186,** 1206-1217.