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Biodegradation of Polycyclic Aromatic Hydrocarbons in Agricultural Soil Contaminated with Crude Oil from Nigeria Refinery using *Pleurotus sajor-caju*

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Abstract

Contamination by petroleum hydrocarbons has rendered soil from oil exploring areas in Nigeria unwholesome for agricultural practices. Because of the carcinogenic properties of the contaminant, its removal from soil is therefore an absolute necessity to promote a sustainable development for society and sound human health. Thus, a rapid cost effective method of biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) using *Pleurotus sajor-caju* was employed. Soil samples were collected from agricultural sites in Akpan town near Nigerian refineries and were analysed for sixteen PAHs by Gas Chromatography (GC). The total PAHs concentrations ranged from 56.8 to 112 mg kg^{-1}. Using the ratios of phenanthrene to anthracene (Phe/Ant) and fluoranthene to pyrene (Flu/Pyr) to identify sources of contamination, PAHs concentrations in Akpan soils possibly had pyrogenic and petrogenic sources. These sources included crude oil petroleum combustion and spills. Pleurotus Sajor-Cajor offered significant reduction in PAHs concentrations after 6 weeks of incubation.

Keywords: Crude oil spill; Soil; PAHs; Biodegradation; *Pleurotus sajor-caju*

Introduction

Soil contamination with crude oil derived pollutants is a significant threat to human health as well as to the wider environment [1-3]. Such contaminated soil from where crude oil spill has occurred has devastating effects on plants and animals. Crude oil spill occurs as a result of petroleum manufacturing activities such as exploration and transport of oil through oceans and on land. As a consequence of such oil spill, Polycyclic Aromatic Hydrocarbons (PAHs) cause the most environmental damage [4], PAHs contaminated soil resulting from crude oil spill has characteristic that render it less useful to human beings in most cases. The fertility of soil is reduced, resulting in reduced crop yields and poorer harvests. The land then becomes economically disadvantageous to the landowner. In addition, such soil has the potential to pollute nearby groundwater drinking supplies and become detrimental to humans utilizing this water source [5-7]. These impacts can last decades because of the persistence of PAHs in soil for many years where it is slowly removed by natural processes such as microbial degradation, volatilization and photooxidation [8]. Bioremediation of these molecules is, therefore, regarded as one of the best options to restore petroleum-contaminated soil [9-11]. The bioremediation process is widely achieved by using bacteria to degrade contaminants. However, the enzymatic capabilities of bacteria are limited by environmental conditions such as pH, temperature and metal ions and, consequently recalcitrant compounds or toxic products remain in the soil [12,13]. To overcome this drawback, fungi are found to have capability to degrade molecules such as polyaromatic and chlorinated nitroaromatic previously thought to be recalcitrant. A number of studies have been conducted regarding the effectiveness of white rot fungi (WRF) as an agent for biodegradation [14,15]. Of these white rot fungi, there are reports concerning *Pleurotus ostreatus* [16-18] and *Trametes trogii* [19-21] which have shown potential for bioremediation of recalcitrant molecules as pesticides and PAHs [16,19,22-25]. Decision was made to employ *Pleurotus sajor-caju* in this study because of dearth information on its use for degradation of organic pollutants especially PAHs.

Remediation of soil contaminated with elevated levels of PAHs is of great importance to the inhabitants of Niger-Delta region of Nigeria due to proven pollution from oil spills. Non-remediated contaminated soil from this region does not fulfill the quality requirement as stipulated by Canadian soil quality guidelines and others from other countries for agricultural purpose. Reference is made to these guidelines since there is no national soil quality guideline for land use presently. With respect to this quality standards set by Canadian Council of Ministers of the Environment (CCME) [26], the maximum limit of PAHs in soil for agricultural purpose is 0.1 mg kg^{-1}. So, there has been effort to find technical and economic solutions for this environmental issue. It is expedient, giving this background, to explore biodegradation of PAHs using fungi to remediate oil-spilt contaminated soil from the Niger-Delta region. Oil industries cited in this region have contributed greatly to the economy growth and development of the country. However, crude oil exploration activities have rendered this region severely damaged due to oil spills. Cases of oil spill resulting to gross pollution of soil with organic pollutants such as PAHs abound [27].

The ultimate objective of this research is to investigate the potential application of WRF Pleurotus for PAHs removal from crude oil contaminated soil. In this study, we report the PAHs concentrations in crude oil contaminated soil from Ekpan town near refinery in Delta State, Nigeria. Ex-situ biodegradation of PAHs in the soil using WRF *Pleurotus sajor-caju*, over incubation time, was also assessed. The choice of this fungus is due to its capacity to colonize a wide spectrum of wastes. Also, it has an advantage of growing over a wide range of temperatures especially in this temperate region.

Materials and Methods

Description of sampling area and soil sample collection

The crude oil contaminated soil samples were collected from Ekpan town, near Nigerian National Petroleum Corporation (NNPC) refinery in Delta State, Nigeria (Figure 1). The town is situated in Niger-Delta region located in the eastern Nigeria coastal zone covering a surface of about 70,000 km2. It lays at the intersection of 5°34′N latitude and 5°43′E longitude. It is densely populated and serves as home to gas industries with most of Nigeria's oil wells. This, therefore, makes the region more vulnerable to environmental pollution from crude oil waste products.

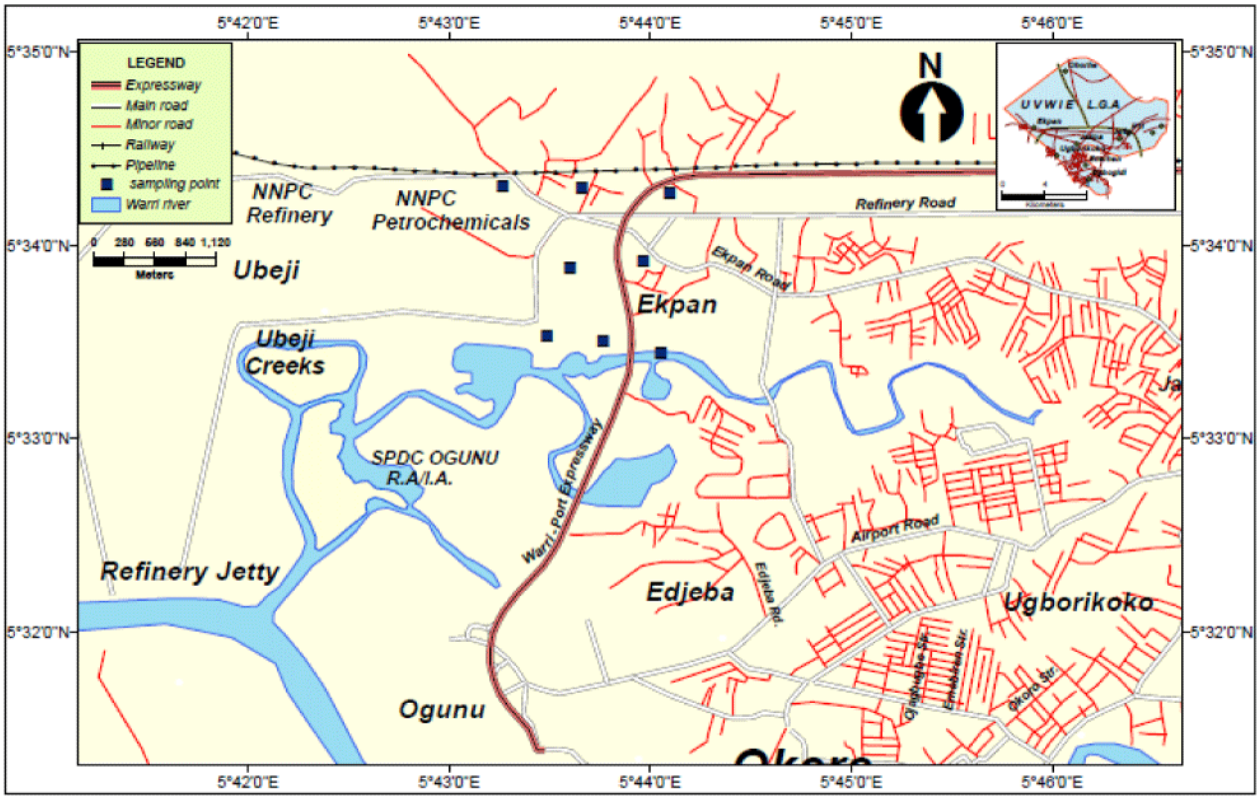

Figure 1: The study area showing sampling locations in Akpan town near refinery and petrochemicals situated in Nigeria.

Five farmlands were located as sampling points within Ekpan town. These farmlands were chosen to reflect PAHs levels of the soil receiving crude oil spills that constantly contaminate the land. Sampling of topsoil (0-15 cm) was done monthly using a stainless hand trowel for a period of three months denoted as A, B and C. Five composite samples were made from soils collected from transects of a farmland to make a total of twenty five composite samples for five farmlands in a month designated as SA. This purposive sampling scheme was repeated consecutively for two other months designated as SB and SC. Soils were also collected as control samples from the Botanical garden in the University of Ibadan. Each composite soil samples was air-dried, sieved and kept in glass bottles wrapped with an aluminium foil for PAHs analysis. A blank sample was incorporated for every five soil extracts analysed.

Spawn preparation and bio-treatment of soil samples

Pure spawn cultures of *Pleurotus sajor-caju* was collected from the Pathology Department of Forestry Research Institute of Nigeria (FRIN), Ibadan. Dried freshly harvested rice straws were cut into 0.1-3.0 cm using a stainless scissor. About 20 g of moistened rice straw was laid on 200 g of crude oil contaminated soil contained in a clean bottle, separated with wire gauze and covered with an aluminium foil. The bottle was autoclaved at 121°C for twenty minutes. The culture bottles were set up in tree replicates. After cooling, each bottle was inoculated at room temperature with 10 g of vigorously grown spawn of *P. sajur-caju*. From composite soil samples, a set of replicate culture bottle was incubated for two weeks, four weeks and six weeks respectively in an incubator. Two control experiments using control soil samples were equally set up in which they were inoculated with the fungus, and incubated for the same periods. The mycelia ramified substrate was carefully separated from the soil layer after the incubation periods. Crude oil contaminated soil, control soil and WRF *Pleurotus sajor-caju* biotreated soil samples were subjected to extraction and prepared for PAHs analysis.

Extraction of oil, clean up and PAH's analysis

The residual oil was extracted using standard analytical protocols described by the Association of Official Analytical Chemists [27]. A five gramme soil sample was extracted with 100 mL of double distilled hexane and dichloromethane (3:1, v/v) in a sonicator. The extracted oil was dried with anhydrous sodium sulphate and concentrated in a stream of nitrogen. The concentrated oil was fractionated into

aliphatic and polyaromatic hydrocarbons fractions by alumina liquid column chromatography. The concentrated oil was poured onto the alumina and eluted down the column with double distilled hexane to remove aliphatic fraction. The aromatic fraction was recovered by eluting the column with a mixture of hexane and dichloromethane (3:1). The most polar polyaromatic hydrocarbons were removed by eluting with dichloromethane into a pre-cleaned borosilicate beaker. This fraction was concentrated to 0.1 mL in a stream of nitrogen before being chromatographed.

The cleaned fractions were analyzed for PAHs by a gas chromatography (GC). The GC model is HP 6890 equipped with flame ionization detector (FID) and powered with Chemstation Rev. A09.01 (1206) software, using a glass column (30 m length x 0.25 µm i.d) and column film (0.25 µm). Injector and detector temperatures were 250°C and 350°C respectively with nitrogen as mobile phase or carrier gas flowing at 30 psi. Hydrogen and compressed air pressures were 28 psi and 32 psi respectively. Detection limit (µg/g) was 1.0 for all PAHs. Recovery study was carried out by spiking an appropriate volume of known concentration of naphthalene standard in contaminated soil whose PAHs concentrations had been previously determined. The spiked soil was thoroughly homogenized and subjected to extraction procedure for PAHs determination. Experiment on recovery was replicated with five different soil samples and the average recovery was 91.7 ± 0.4% naphthalene.

Results and Discussions

Table 1 shows the occurrence of PAHs with their concentrations in crude oil contaminated soil samples from Ekpan town. The PAHs are composed of two to six fused rings compounds, which include those PAHs considered as carcinogens by the US environmental protection agency. The occurrence of these PAHs is attributed to transport of crude oil and its spill around NNPC refinery and NNPC petrochemical industry located in the town. The most abundant of PAHs were napththane with concentration ranging from 9.74 mg/kg to 17.2 mg/kg. Benzo(b)fluoranthene, indeno (1,2,3,cd) pyrene and dibenzo(a,h)anthracene had the lowest concentrations of 0.44 mg/kg, 0.13 mg/kg and 0.08 mg/kg for SA, SB and SC respectively. The relative occurrences were 15.4-17.1% (two rings PAHs), 33.3-38.8% (three rings PAHs), 37.2-40.2% (four rings PAHs), 6.9-7.1% (five rings PAHs) and 0.6-1.6% (six rings PAHs) of the total PAHs. This implies that the concentrations of PAHs with 5-6 rings were relatively low in all soil samples.

The total PAHs concentrations were 112 mg/kg, 67.3 mg/kg and 56.8 mg/kg for SA, SB and SC respectively (Table 1). Surprisingly, PAHs concentrations in soils from this town were much higher in magnitude than what was reported (0.395 ± 0.190 mg/kg) as PAH concentration in a conventional farming site in Orgeval catchment of early industrialized region in France [28]. PAHs concentrations in soils from Ekpan town were compared with some environmental quality criteria for PAHs in soil released by the Netherlands Ministry, Canadian Councils of Ministers of Environment, British Columbia Ministry of Environments, for assessing risk of contaminated sites. The necessity for employing these standards stems from the fact that such environmental standards for PAHs in soil are not established yet in Nigeria.

The significance difference between PAHs concentrations measured and 0.1 mg/kg of PAHs set as the Canadian soil quality criterion for agricultural use [29] may possibly be due to pollution generated by crude oil exploration in Ekpan town. This implies that the soil is polluted with PAHs compounds and may pose risk to human health if cultivated for agricultural purpose. The inhabitants of Ekpan town may be predisposed to high risk of cancer due to long-term exposure to such compounds through bioaccumulation in the food chains. PAHs have very high lipid solubility and hence may quickly be absorbed into the gastrointestinal tract of humans [30,31]. More hydrocarbons were found in Ekpan soil contaminated possibly by spills when compared with soils from the control site (Botanical garden) as far as in Ibadan, which is usually uncontaminated.

PAHs	Ring group	Molar mass	SA	SB	SC	Environmental standards for PAHs	
						CCME (Agric)	BCME (Agric)
Naphthalene	2 rings	128	17.2^{c}	12.4^{b}	9.74^{a}	0.1	0.1
			(16.8-18.3)	(11.5-13.2)	(7.45-10.2)		
Acenaphthaene	3 rings	166	7.10^{b}	3.30^{a}	5.53^{b}	-	-
			(6.23-8.45)	(2.10-4.22)	(3.23-6.38)		
Acenaphthylene	3 rings	166	6.32^{b}	1.25^{a}	1.40^{a}	-	-
			(6.01-6.52)	(0.21-3.04)	(1.12-1.78)		
Fluorene	3 rings	166	7.25^{b}	2.98^{a}	3.19^{a}	-	-
			5.12-8.12)	(2.11-3.12)	(2.34-4.7)		
Phenanthrene	3 rings	178	14.0^{c}	7.99^{b}	5.64^{a}	0.1	0.1
			(12.5-14.9)	(6.27-8.12)	(4.23-6.23)		
Anthracene	3 rings	178	8.78^{b}	6.86^{a}	5.09^{a}	-	-
			(8.10-9.14)	(5.78-6.57)	(3.21-5.89)		

Chrysene	4 rings	228	11.8 ^{c}	8.53^{b}	6.28^{a}	-	-
			(10.2-12.4)	(7.14-9.45)	(4.37-7.10)		
Pyrene	4 rings	202	11.8^{b}	6.86^{a}	5.69^{a}	0.1	0.1
			(9.81-12.9)	(6.10-7.11)	(3.54-6.10)		
Benzo(a)anthracene	4 rings	228	8.78^{b}	6.86^{a}	5.69^{a}	0.1	0.1
			(8.10-8.98)	(5.32-7.23)	(4.25-6.78)		
Fluoranthene	4 rings	271	9.23^{b}	4.82^{a}	4.16^{a}	-	-
			(8.95-10.3)	(3.68-5.21)	(3.23-5.67)		
Benzo(k)fluoranthene	5 rings	232	6.07^{b}	3.44^{a}	3.0^{a}	-	-
			(5.78-6.89)	(2.34-4.23)	(2.34-3.89)		
Benzo(b)fluoranthene	5 rings	252	0.44^{b}	0.26^{a}	0.24^{a}	0.1	0.1
			(0.27-0.61)	(0.17-0.37)	(0.15-0.38)		
Benzo(a)pyrene	5 rings	252	0.72^{a}	0.64^{a}	0.71^{a}	0.1	0.1
			(0.53-0.97)	(0.56-0.71)	(0.48-0.97)		
Dibenzo(a,h)anthracene	5 rings	273	0.67^{c}	0.30^{b}	0.08^{a}	0.1	0.1
			(0.56-0.72)	(0.26-0.43)	(0.05-0.14)		
Benzo(g,h,i)perylene	6 rings	273	0.96^{b}	0.70^{b}	0.11^{a}	-	-
			(0.86-1.03)	(0.41-1.54)	(0.07-0.21)		
Indeno(1,2,3,c,d)pyrene	6 rings	278	0.78^{b}	0.13^{a}	0.20^{a}	0.1	0.1
			(0.69-0.8)	(0.11-0.15)	(0.11-0.38)		
Total PAHs			112	67.3	56.8		
			3.28	4.6	5.8		
Organic carbon (%)			(3.01-3.91)	(2.45-5.67)	(3.45-6.2)		

Table 1: Initial concentrations (mg/kg) of PAHs and organic carbon content (%) in crude oil contaminated soil samples. SA, SB and SC=soil sample from location A, B and C respectively. These concentrations in each column are averages of nine results (n=27). CCME=Canadian Council of Ministers of the Environment (Agriculture); BCME=British Columbia Ministry of the Environment (Agriculture) (30); Phe/Ant ratio (SA=1.59, SB=1.16, SC=1.11); Flu/Pyr ratio (SA=0.78, SB=0.70, SC=0.73). Values in the brackets are ranges. Mean values in the same row with different superscripts (a,b,c) are significantly different ($p \leq 0.05$).

Figures 2-5 give overview of studies on incubating contaminated soil with *P. sajor-caju* for 2-, 4- and 6 weeks respectively. PAHs were potentially biodegradable with the fungus. Significant reductions in all the PAHs concentration were observed with increase in incubation periods. The ranges of the extent of degradation in PAHs concentrations, between the incubation periods of two weeks to six weeks were indicated in the figures.

As a way to evaluate possible sources, the ratios of some PAHs compositions regarded as molecular indices were used to distinguish the natural and anthropogenic PAHs in soils [32,33]. The use of these molecular indices depends on the fact that PAHs distribution is dependent on temperature [33,34]. The PAHs distribution is governed by thermodynamic properties during low temperature processes such as catagenesis of organic matter leading to the formation of petroleum. On the other hand, their distributions are governed by kinetic properties during high temperature processes such as pyrolysis of organic matter [32].

The relative kinetic and thermodynamic stabilities of different PAHs molecules during their formations in petroleum source rocks under different heating or cooling conditions and by their comparable environmental fate were related to their distribution in substrates. So, the molecular indices were derived by evaluating the ratios of phenanthrene/anthracene (Phe/Ant) within the 3-ring PAHs group and fluoranthene/pyrene (Flu/Pyr) within the 4-ring PAHs [35]. Phenanthrene is much more stable than anthracene so that at low temperature molar fraction of phenanthrene produced is higher than that of anthracene. This might possibly explain higher levels of phenanthrene than that of anthracene as reflected in Figure 3. Consequent upon this, biodegradation of phenethrane by *P. sajor-caju*

in terms of reduction in concentration (21.5-35.7%) was low compared to that obtained for the biodegradation of anthracene (35.9-47.9%).

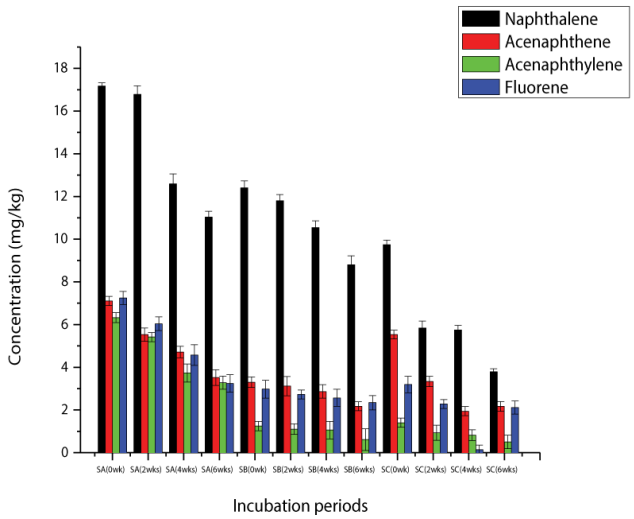

Figure 2: Biodegradation pattern of some PAHs in crude oil contaminated soil incubated with *P. sajor-caju* during weekly incubation periods (% reduction in concentration for naphthalene=29.0-61.1%; acenaphthene=34.2-60.7%; acenaphthylene=48.2-64.1% and fluorene=21.4-55.3%).

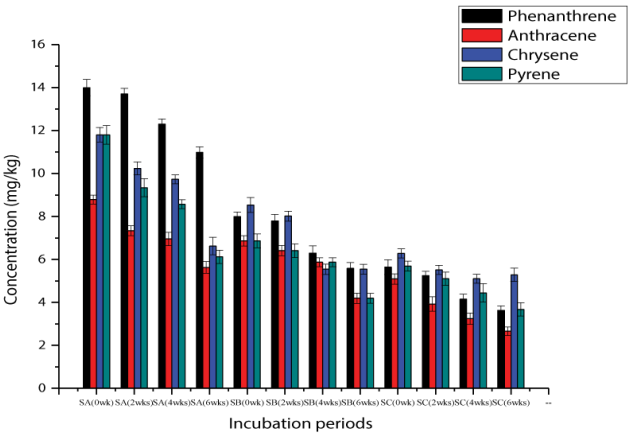

Figure 3: Biodegradation pattern of some PAHs in crude oil contaminated soil incubated with P. sajor-caju during weekly incubation periods (% reduction in concentration for phenanthrene=21.5-35.7%; anthracene=35.9-47.9%; chrysene=15.7-43.8% and pyrene=35.4-48.1%).

Baumard et al. [36] proposed Phe/Ant ratio<10 and Flu/Pyr ratio>1 as quantitative indication of PAHs coming from pyrogenic source (such as petroleum combustion through a flare stack) and Phe/Ant ratio>15 and Flu/Pyr ratio<1 as indication of PAHs originating from petrogenic source (such as oil spill contamination). The Phe/Ant ratios in this study for all the samples ranged from 1.11 to 1.59 and Flu/Pyr ratios ranged from 0.70 to 0.78, indicating that there were both pyrogenic and petrogenic influences or input of PAHs on Ekpan soil samples. This implies that there is a natural leakage of oil related hydrocarbon in this study area. The basic concept of soil sorption of organic molecules suggests that the sorption of hydrophobic organic molecules is related to the organic matter derivable from organic carbon content of the soil [37].

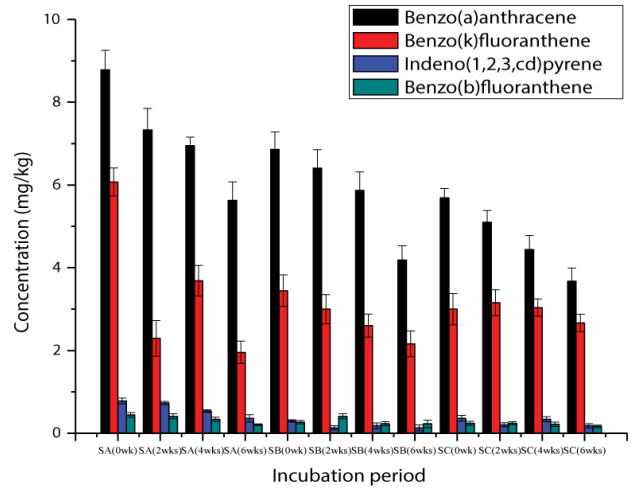

Figure 4: Biodegradation pattern of some PAHs in crude oil contaminated soil incubated with *P. sajor-caju* during weekly incubation periods (% reduction in concentration for benzo(a)anthracene=35.4-40.0%;benzo(k)fluoranthene=11.2-67.8% ; indeno(1,2,3,cd)pyrene=50.0-56.0% and benzo(b)fluoranthene=13.6-54.1%).

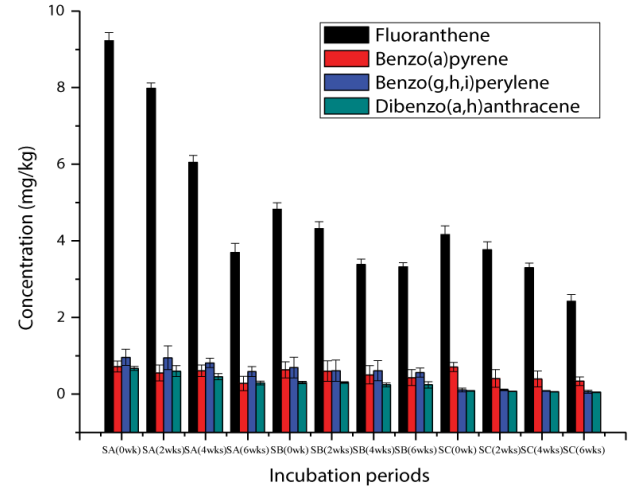

Figure 5: Biodegradation pattern of some PAHs in crude oil contaminated soil incubated with *P. sajor-caju* during weekly incubation periods (% reduction in concentration for fluoranthene=31.1–59.9%; benzo(a)pyrene=32.1-61.7%; benzo(g,h,i)perylene=19.0-44.4%; dibenzo(a,h)anthracene=20.0-57.1%).

Soil organic content varied from 3.28% to 5.80%. The results of regression analysis conducted to determine the relationship between

the total PAHs concentrations and the soil organic contents showed positive exponential relationship with r^2 of 0.8560. The relationship implies that high amounts of PAHs mainly occur in soil with high organic content.

Conclusion

This study revealed that Ekpan soils from agricultural land were contaminated with PAHs of 2- to 6-rings. Based on the molecular indices, PAHs in the soil samples possibly had pyrogenic and petrogenic origins. The retention of PAHs in soils was evident with strong relationship between the total PAHs concentrations and organic carbon contents.

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