Application of seawater microbial inocula for the remediation of hydrocarbon polluted mangrove swamp in the Nigerian oil rich Niger Delta

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Abstract: Seawater microbial inocula was used for the remediation of hydrocarbon polluted mangrove swamp located in the Nigerian oil rich Niger Delta. The indices used to monitor the progress of bioremediation were; the gradual loss in the gravimetric weight of oil, the biomarker index exemplified by the decreasing trend in the ratios of nC17/Pristane and nC18/Phytane, Gas chromatographic analysis of the residual total petroleum hydrocarbon (TPH) and the population dynamics of hydrocarbon degrading bacteria. The polluted section of the plot where sea water microbial inocula, aeration and nutrients were applied (Plot C) showed very rapid and more enhanced biodegradation of the original petroleum hydrocarbon pollutants. About 96% of the original TPH pollutants were degraded within 2 weeks. The second section of the plot (Plot B) where only nutrients and aeration were applied without seawater microbial inocula, recorded relatively slower biodegradation rates with only about 51.5% and 87.5% of the original TPH pollutants degraded after 2 and 3 weeks of nutrient and aeration application respectively. The third section of the plot where nothing was applied (Plot A) served as a control plot and biodegradation rates on this plot was relatively very slow as expected. After the 5 week period the experiment lasted, only 54% of the original TPH pollutants were degraded from the control plot. Statistical analysis using analysis of variance (ANOVA) and Duncan multiple range tests(DMRT) revealed that application of seawater microbial inocula and nutrients significantly influenced bacterial growth and subsequent degradation of petroleum hydrocarbons and this naturally improved both the speed and efficiency of the bioremediation program. Secondly for hydrocarbon pollution in some inaccessible and difficult mangrove environment, sea water microbial inocula can be naturally regarded as safe and also one of the best bioremediation approaches. [Nature and Science 2010;8(8):152-162]. (ISSN: 1545-0740).

Keywords: Seawater microbial inocula, biodegradation, bioremediation, mangrove swamp, hydrocarbon.

1. Introduction:

The Niger Delta region of Southern Nigeria is a region of intense oil exploration and production activities, consequently the mangrove environment which dominate the wetland habitat in the Niger Delta are subject to oil spills resulting from shipping accidents, production related incidents and pipeline ruptures and vandalization.

The term mangrove refers to a non-taxonomic grouping of woody halophytic spermatophytes that occur along low energy coastlines, deltas, estuaries and embayment throughout the tropics and subtropics (Proffitt, 1996). Mangroves root in soft sediments, their stems and proproots are islands of hard substrate where plants and animals such as sponges, algae, molluscs and other invertebrates attach and grow (Rutzler and Feller, 1987). Mangroves also support wide and varied group of mobile organisms ranging from birds that nest on the trees to fishes that feed and live among submerged proproots and also snails that feed on attached oysters (Odum *et al*, 1982). Oil spills into the mangrove environment therefore have the potential to affect not only the mangroves but also on other animal and plant species that attach, nest, feed and shelter in the mangrove environment.

Generally, the limiting factors in the biodegradation of petroleum hydrocarbon in wetland soils and sediment are nutrients and aeration because oxygen and nutrients in wetland soils are generally not sufficient for biological activity (Mendelssohn and Lin 2003, Prince 2005, Hambrick *et al.*, 1990).

Some investigators have documented the effects and remediation measures of petroleum hydrocarbon pollution in the mangrove environment (Scherrer and Mille, 1990, Lee and Levy, 1991, Okoro, 2009) while others have evaluated the impact of oil cleanup procedures in the sensitive mangrove environment (Proffitt, 1996). What is more important is the fact that the mangrove environment is a very sensitive one and any clean up or remediation procedure must not negatively impact the mangrove wetland ecosystem.

One of the promising technologies that that can be used to quickly and effectively remedy the adverse effect of petroleum hydrocarbon pollution in the mangrove swamp is bioremediation. Bioremediation is simply an act of adding materials such as nutrients, microbial products or microorganisms and aeration to contaminated environments such as oil spill sites to cause an acceleration of the natural biodegradation process (US Congress, 1991). It is a promising means

by which oil pollution in the mangrove as well as other wetland types can be removed with minimal impact to the habitat.

Scherrer and Mille (1990) confirmed enhanced degradation of oil in West Indies mangrove swamp after the addition of oleophyllic fertilizer. Lee and Levy (1991) also found enhanced degradation of oil in salt marsh sediments treated with inorganic nutrients. Okoro (2009) have also observed enhanced biodegradation of hydrocarbon in mangrove swamp using oleophyllic fertilizer and crude biosurfactant extract. In order to address the limitation of low oxygen status of the mangrove swamp, Mendelssohn and Lin (2003) have demonstrated that addition of nutrients, soil aeration and microbial seeding using indigenous microorganisms significantly enhanced the rate of biodegradation of petroleum hydrocarbons in the mangrove swamp. Some investigators have argued that commercial microbial products that are not indigenous to the environment have not been found to be effective in enhancing the progress of biodegradation of petroleum hydrocarbons (Mendelssohn and Lin 2003, Means 1991, Pritchard 1992, Tagger *et al.,* 1983) but interestingly the application of microbial seeding using indigenous microorganisms have produced better results (Taberk *et al.,* 1991, Jones and Greenfield, 1991,Okoro and Amund, 2002, Okoro and Amund, 2008, Okoro and Amund, 2010). The reason is that commercial cultures do not compete well in non-indigenous environments but indigenous microorganisms when re-introduced to their natural environments does not have any problem with adaptation and competition.

In the present study, the most efficient bioremediation approach that can speedily eliminate petroleum hydrocarbons in the mangrove swamp within a relatively short time was sort for and seawater microbial inocula was found to be very effective and promising. Seawater microbial inocula is a formulation of mixed hydrocarbon degrading bacterial culture that are indigenous to the polluted environment using seawater and molasses as the sole carbon and energy sources with the aim of increasing the population density of the bacterial culture up to 10 folds before reintroducing the bacterial culture to the polluted environment. This application is also in combination with nutrients and aeration with the sole aim of significantly enhancing the progress of bioremediation of the polluted petroleum hydrocarbon in the mangrove swamp within a relatively short time.

2. Materials and Methods:

Study area:

The study area is located within Escravos mangrove swamp (Longitude E, 4^0, 32',21' and Latitude 6^0, 22',10.) in Ugborodo town, Delta State, Nigeria. The environment is near shore with moderate salinity (780mg/L).

Experimental Design

A 30 square meter portion of mangrove swamp was clearly demarcated with an oil resistant polythene lined in a wooden platform of about 40 inches deep from the surface into 3 equal segments otherwise referred to as plots A, B and C in this study and the design was in such a way that fluid movement from one plot to another was totally restricted. The entire plot was polluted with 30 liters of crude oil (Escravos light, obtained from Chevron Nigeria Limited) at the rate of 10 liters per plot which was evenly distributed in all the plots and the set up was allowed to stand for 48hrs undisturbed. This was followed by the application of 2.5 liters of oleophylic fertilizer (obtained from IITA Ibadan) enclosed in a semi-permeable membrane to each of the plots (B and C). Plot C was later flooded with 6 liters of seawater microbial inocula followed by further addition of fertilizer (2.5 liters). There was intermittent mixing of surface sediments by tilling to increase aeration in plots B and C. Plot A served as a control plot with only crude oil application without any form of treatment.

Microbiological and Physicochemical Analysis of the study area.

Enumeration of Total Heterotrophic Bacteria.

Total heterotrophic bacterial counts were enumerated by adopting the standard plate count technique using spread plate method. Appropriate dilutions of samples were plated out on nutrient agar plates in triplicates and incubated aerobically at 29^0C for 24hrs. as described in Eaton *et al.,* (1995).

Enumeration of Hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacterial counts were obtained by plating out at low dilutions $10^{-1} - 10^{-3}$ of samples on mineral salt medium of *Mills et al*., (1978) The composition of the medium in (g/L) is as follows NaCl (10), $MgSO_4.7H_20$ (0.42), KCl (0.29), KH_2PO_4 (0.83), Na_2HPO_4 (1.25), $NaNO_3$ (0.42), Agar bacteriological (15), distilled water (1000 ml), and pH (7.2). The medium was autoclaved at 1.1 kg/cm^2 for 15 mins. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light) and held in the lid of the petri dishes. The dishes were wrapped round with a masking tape so as to increase the vapor pressure within the Petri dishes while the plates were incubated at 29^0C for 6 days after which the growth of hydrocarbon degrading bacteria were estimated.

pH, Temperature and Salinity determination.

The pH of the mangrove swamp was measured with a portable water proof pH meter (Jenway, 3150, USA),Temperature was measured using portable thermometer (Hanana , H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described in Eaton *et al.,* (1995).

Estimation of background nutrient concentration in the mangrove swamp sediment.

Interstitial water samples were withdrawn with a simple apparatus as described by McKee *et al*,(1998). The collected interstitial water was filtered and inorganic nutrients such as Phosphorus and Potassium were analysed with ICP (Inductively coupled argon plasma emission spectrometer) as described by Eaton *et al,* (1995). Amonium-Nitrogen was analysed with auto analyser as described by Eaton *et al*.,(1995).

Determination of mangrove Swamp redox potential and dissolved oxygen (DO):

Mangrove swamp redox potential at 2 and 5cm depths were determined with bright platinum electrodes and a calomel reference electrode. Readings were taken with a portable pH/mV digital meter. The potential of a calomel reference electrode (+244mV) was added to each value to calculate the Eh as described by Patrick *et al.*,(1996.) Dissolved oxygen was measured with a DO meter as described by Eaton *et al*.,(1995).

Determination of biological oxygen demand (BOD)

BOD bottles were filled with appropriate dilutions of the samples (50ml) and the initial dissolved oxygen was measured. The BOD bottles with samples were sealed to exclude air followed by incubation at 20^{O}C for 5 days after which the BOD was computed from the difference between the initial and the final dissolved oxygen *(Eaton et al, 1995)*

Detection of heavy metals:

Heavy metals were detected using the Atomic absorption Spectrophotometer (Perlkin Elmer 5100PC, England) after sample preparation and digestion as previously described (*Eaton et al.,1995).*

Moisture content:

The moisture content of the mangrove soil was measured by simple gravimetric analysis. 10g of the soil sample was dried in the oven at a temperature of 200^{o}C after which, the sample was measured again and the difference in weight is the moisture content as previously described *(Eaton et al.,1995)*

Solvent extraction of residual oil

1g of the sample was introduced into a separating funnel containing 50mls of Methylene chloride, this was followed by vigorous shaking for 10mins and filtration using Watman no.1 filter paper as previously described *(Eaton et al., 1995)* and the filtrate was collected in a clean conical flask.

Gas Chromatography of Oils

Degraded oil were analyzed by Gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1micron film thickness were used. A temperature program of 50-305^{0}C increasing at 3.5^{0}C per minute for 27.15min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was 325^{0}C while the injection port temperature was 305^{0}C. 1 ml of the residual oil extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of 0.2 µl was injected into the GC.

Formulation of Seawater microbial inocula

Sea water microbial inocula was formulated from four hydrocarbon utilizing bacterial cultures that were previously isolated from the experimental site. They include *Pseudomonas mallei* (SWMI-1), *Alcaligenes* sp.(SWMI-2), *Acinetobacter* sp.(SWMI-3), and *Flavobacterium* sp.(SWMI-3). The bacterial cultures were grown in a 5 liter capacity Weaton glass bottle containing the sea water nutrient broth. The composition of the broth is as follows; Sea water (3liters), Liquid molasses (250mls.), MgSO4.7H20 (1.26g), KCl (1g), KH_2PO_4 (2.50g), Na_2HPO_4 (3.75g), and $NaNO_3$ (1.29g). Starter cultures of the mixed bacterial culture were prepared in a 250ml capacity Wheaton glass bottle before transferring to the 4 liter capacity broth. The mixed bacterial cultures were incubated for 48hrs. to achieve maximum population density and ensure near complete utilization of the molasses. Culture samples were taken for colony counts after the 48hr. incubation and bacterial counts of up to 10^{9} cfu/ml or more were regarded as the ideal bacterial population density for field application.

The experimental set up was allowed to stand for 5 weeks and at each weekly interval, samples were collected and analyzed for residual total petroleum hydrocarbon and hydrocarbon utilizing bacteria.

Monitoring level of oil penetration and sample collection for analysis:

The level of oil penetration in the mangrove swamp was monitored during the 5 week period the experiment lasted and it was noted that the oil did not penetrate beyond the 2cm mark. It was also observed that during mixing and tilling of the oil with the mangrove swamp, the little water that was originally present on the surface got entrapped in the mangrove sediment. All samples were collected within the 2cm mark in form of wet sediment and measured in grams.

Statistical Analysis:

One way analysis of variance (Anova) and Duncan tests were used to test for significant differences (5%level) in the mean concentrations of Total Petroleum Hydrocarbons (TPH) and Population densities of indigenous hydrocarbon degraders in the various plots and the analysis was performed with a computer statistical package SPSS 10 for windows.

3. Results

Microbiological and Physicochemical properties of the study area before the commencement of the experiment

Microbiological and physicochemical properties of the study area was carried out to ensure that the environment is pristine and has not received any significant petroleum hydrocarbon pollution in the past. The low population density of hydrocarbon degrading bacterial counts relative to very high heterotrophic bacterial counts and low concentration of total petroleum hydrocarbon is enough indication that the environment is pristine. The original background nutrient concentration of the study area showed some levels of concentrations of Nitrogen, Potassium and Phosphorus which though may be enough to support indigenous microbial activity insitu but may not be enough to sustain higher microbial activity in case of pollution. The detailed results of the microbiological and physicochemical properties of the study area are shown in Table 1.

Application of Seawater microbial inocula, nutrients and aeration on the experimental plots

Experimental plot A is the control plot where nothing was applied throughout the duration of the experiment. The mean concentrations of residual total petroleum hydrocarbon(TPH) in plot A are shown in Table 2. The initial TPH concentration of 5360ppm at week 0 was slowly reduced to 2360ppm at week 5. This is a typical scenario of natural biodegradation process which is usually slow. The population density of hydrocarbon degrading bacteria also rose slowly from 0.26 x 10^4 cfu/g at week 0 to 16.50 x 10^4 cfu/g at week 5 as shown in Table 3. The decreasing trend of the biomarker indices (nC17/Pristane and nC18/Phytane) was equally slow as can be seen in Table 4. The GC Chromatograms of the residual TPH concentrations in plot A are shown in Fig. 1 and there was no evidence of enhanced biodegradation after the 5 week period the experiment lasted.

Table 1. Microbiological and Physicochemical Properties of the Study Area before the Commencement of the Experiment

	Mangrove Surface water (Mean Value)	Mangrove Sediment (Mean value)	
		2cm depth.	5cm depth.
Total Heterotrophic Bacterial Counts (Cfu/ml or g)	3.50 X 10^5 cfu/ml	4.40 x 10^6 cfu/g	4.20 x 10^4 cfu/g
Hydrocarbon utilizing bacterial counts (Cfu/ml or g)	0.06 X 10^5 cfu/ml	0.46 x 10^6 cfu/g	0.045 x 10^4 cfu/g
pH	7.30	6.80	6.70
Temperature	28^0C	24^0C	22^0C
Salinity (mg/l or g)	6355mg/l	6210	5810 mg/g
Phosphorus(mg/l or g)	28mg/l	125mg/g	110 mg/g
Potassium (mg/l or g)	12mg/l	96 mg/g	68mg/g
Amonia-Nitrogen (mg/l or g)	1.50mg/l	3.40 mg/g	2.60 mg/g
Dissolved Oxygen (mg/l)	10.20mg/l	ND	ND
Redox Potential (Eh) mV	340mV	260 mV	80
BOD_5(mg/l)	55mg/l	ND	ND
Heavy metals detected (ppm)	Pb(0.02), Cr(0.16)	Cd(0.05), Pb(0.018), Cr.(0.03)	Cr.(0.16), Pb(0.32)
Total Petroleum Hydrocarbon (TPH) ppm	16.50	8.60	5.40

ND; NOT DETERMINED

In plot B, nutrients and aeration were applied to speed up the rate of biodegradation of petroleum hydrocarbons by the indigenous bacterial flora but there was no additional microbial seeding with seawater microbial inocula. The mean concentrations of residual TPH as seen in Table 2 showed a more enhanced biodegradation of petroleum hydrocarbons when compared with plot A. The residual TPH was reduced from 5430 ppm at week 0 to 36 ppm at week 5. The mean population densities of indigenous hydrocarbon degrading bacteria in plot B as shown in Table 3 indicated the peak population density of 56.20 x 10^4 cfu/g after nutrient application at week 4 and a gradual decline thereafter. Ratios of the biomarker indices (nC17/Pristane and nC18/Phytane) also showed significant gradual decline from week 0-5 as shown in Table 4. The GC Chromatograms of the residual TPH concentrations in plot B as shown in Fig. 2 also indicated enhanced biodegradation of the petroleum hydrocarbons after 5 weeks of treatment with nutrient and aeration.

Table 2. Mean Concentrations of Residual Total Petroleum Hydrocarbons (TPH) in the Experimental Plots

PLOTS	MEAN TPH CONC.(PPM) WEEK O±SD	MEAN TPH CONC.(PPM) WEEK 1±SD	MEAN TPH CONC.(PPM) WEEK 2±SD	MEAN TPH CONC.(PPM) WEEK 3±SD	MEAN TPH CONC.(PPM) WEEK 4±SD	MEAN TPH CONC.(PPM) WEEK 5±SD
A	5360 ± 28.3^A	5160 ± 5.65^C	4780 ± 11.3^C	3640 ± 2.82^C	2760 ± 7.07^C	2360 ±2.82^C
B	5430 ± 7.07^A	4150 ± 11.3^B	2280 ± 2.82^B	1620 ± 5.7^B	360 ± 21.2^B	36 ± 2.82^B
C	5460 ± 7.07^A	1580± 8.48^A	310 ± 4.24^A	52 ± 5.48^A	5.80 ± 1.41^A	2.60 ± 5.65^A

***Means with the same superscript letter(s) in a column are not significantly different in the Duncan tests (at P=0.05) , ± SD = Standard deviation at n=2. * ANOVA TEST : P<0.05 (Significant), P>0.05 (Not significant),**
PLOT A = CONTROL PLOT(NO TREATMENT).
PLOT B = NUTRIENT AND AERATTION APPLICATION ALONE
PLOT C = APPLICATION OF SEA WATER MICROBIAL INOCULA, NUTRIENTS & AERATION

Table 3. Mean Population Densities of Hydrocarbon Degrading Bacteria in the Experimental Plots

PLOTS	MBPD(Cfu/g x 10^4) WEEK O ±SD	MBPD(Cfu/g x 10^4) WEEK 1 ±SD	MBPD(Cfu/g x 10^4) WEEK 2 ±SD	MBPD(Cfu/g x 10^4) WEEK 3 ±SD	MBPD(Cfu/g x 10^4) WEEK 4 ±SD	MBPD(Cfu/g x 10^4) WEEK 5 ±SD
A	O.26 ± 8.48^A	0.03 ± 2.82^A	2.83 ± .47^A	8.50 ± .14^A	14.20 ±.14^B	16.50 ±.28^C
B	0.18 ± 2.82^A	2.65 ± .21^B	12.40 ± .28^B	48.50 ± .35^C	56.20 ± .14^C	14.80 ±.42^B
C	0.21 ± 1.41^A	68.50 ± .56^C	120.4 ± .28^C	44.60 ± .14^B	6.60 ± .42^A	0.86 ± 2.82^A

***Means with the same superscript letter(s) in a column are not significantly different in the Duncan tests (at P=0.05) , ± SD = Standard deviation at n=2. * ANOVA TEST : P<0.05 (Significant), P>0.05 (Not significant),**
MBPD = Mean Bacterial Population Density.
PLOT A = CONTROL PLOT(NO TREATMENT).
PLOT B = NUTRIENT AND AERATION APPLICATION
PLOT C = APPLICATION OF SEA WATER MICROBIAL INOCULA, NUTRIENTS & AERATION.

Table 4. Ratios of nC17/Pristane and nC18/Phytane taken as biodegradation progressed in the experimental plots.

PLOTS	WEEK O		WEEK 1		WEEK 2		WEEK 3		WEEK 4		WEEK 5	
	nC17/ Pr.	nC18/ Ph.	nC17/ Pr.	nC18/ Ph.	nC17/ Pr.	nC18/ Ph.	nC17/ Pr.	nC18/ Ph.	nC17/ Pr.	nC18/ Ph.	nC17/ Pr.	nC18/ Ph.
A	1.46	2.82	1.43	2.40	1.43	2.44	1.20	2.54	1.21	2.56	1.16	2.55
B	1.47	2.85	1.41	2.66	1.09	1.29	0.68	0.82	0.34	0.42	0.21	0.32
C	**1.42**	**2.76**	**1.25**	**2.11**	**0.35**	**0.42**	**0.15**	**0.22**	**TDP**	**TDP**	**TDP**	**TDP**

TDP= Total depletion of nC17/Pristane and nC18/Phytane
PLOT A = CONTROL PLOT(NO TREATMENT).
PLOT B = NUTRIENT AND AERATION APPLICATION
PLOT C = APPLICATION OF SEA WATER MICROBIAL INOCULA ,NUTRIENTS & AERATION

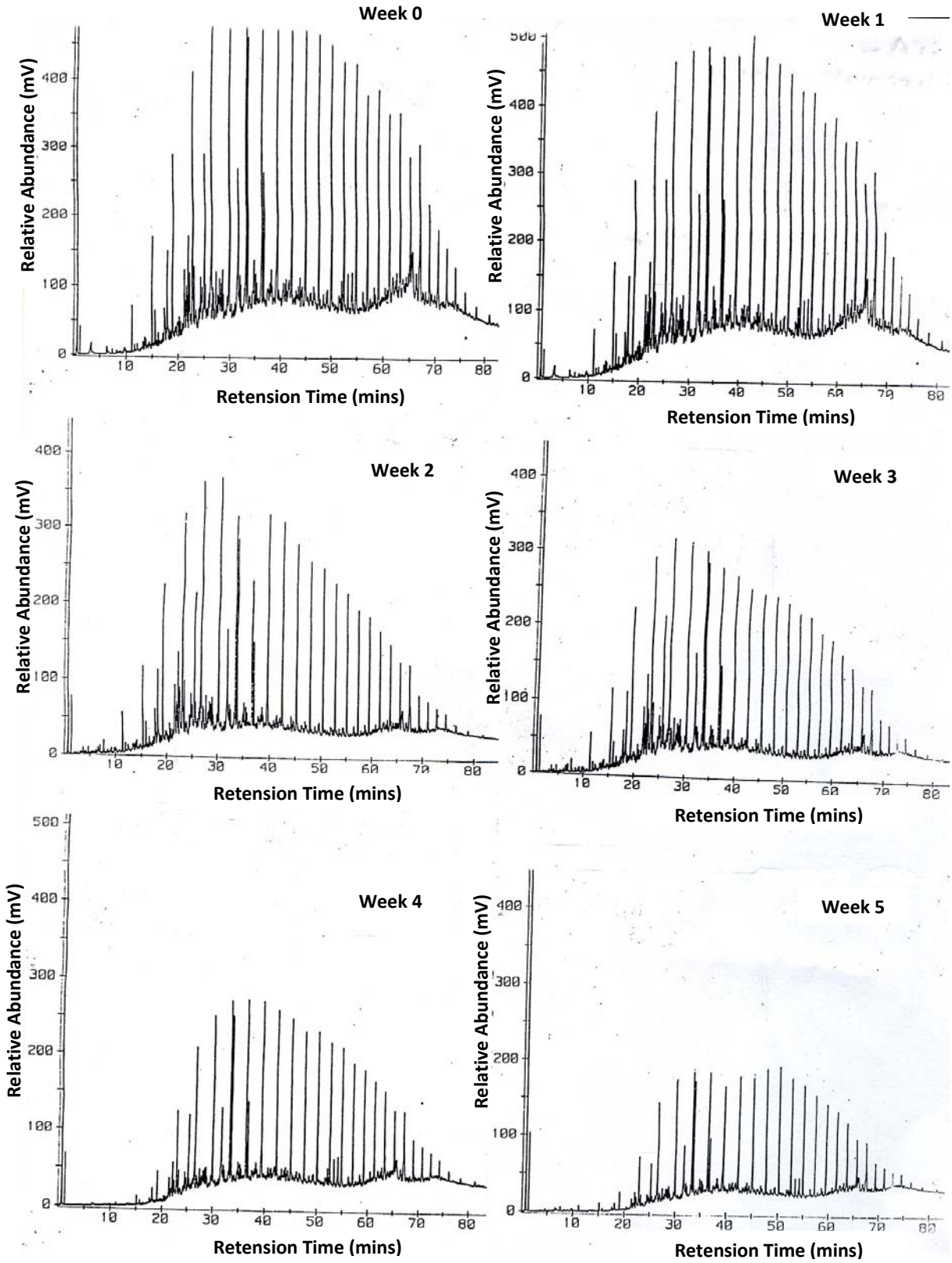

Fig. 1: GC Chromatograms of residual TPH Concentrations in Plot A

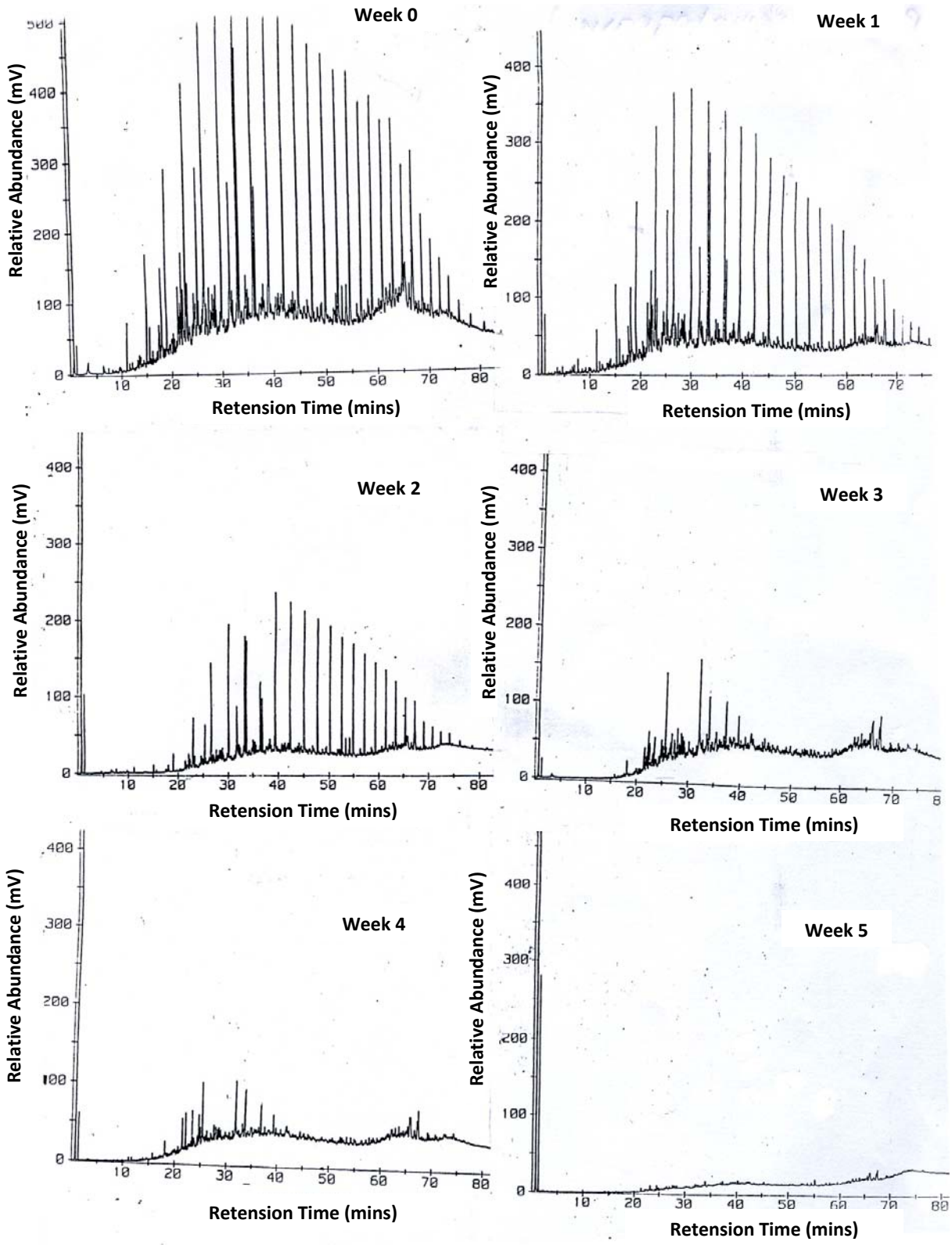

Fig. 2: GC Chromatograms of residual TPH Concentrations in Plot B

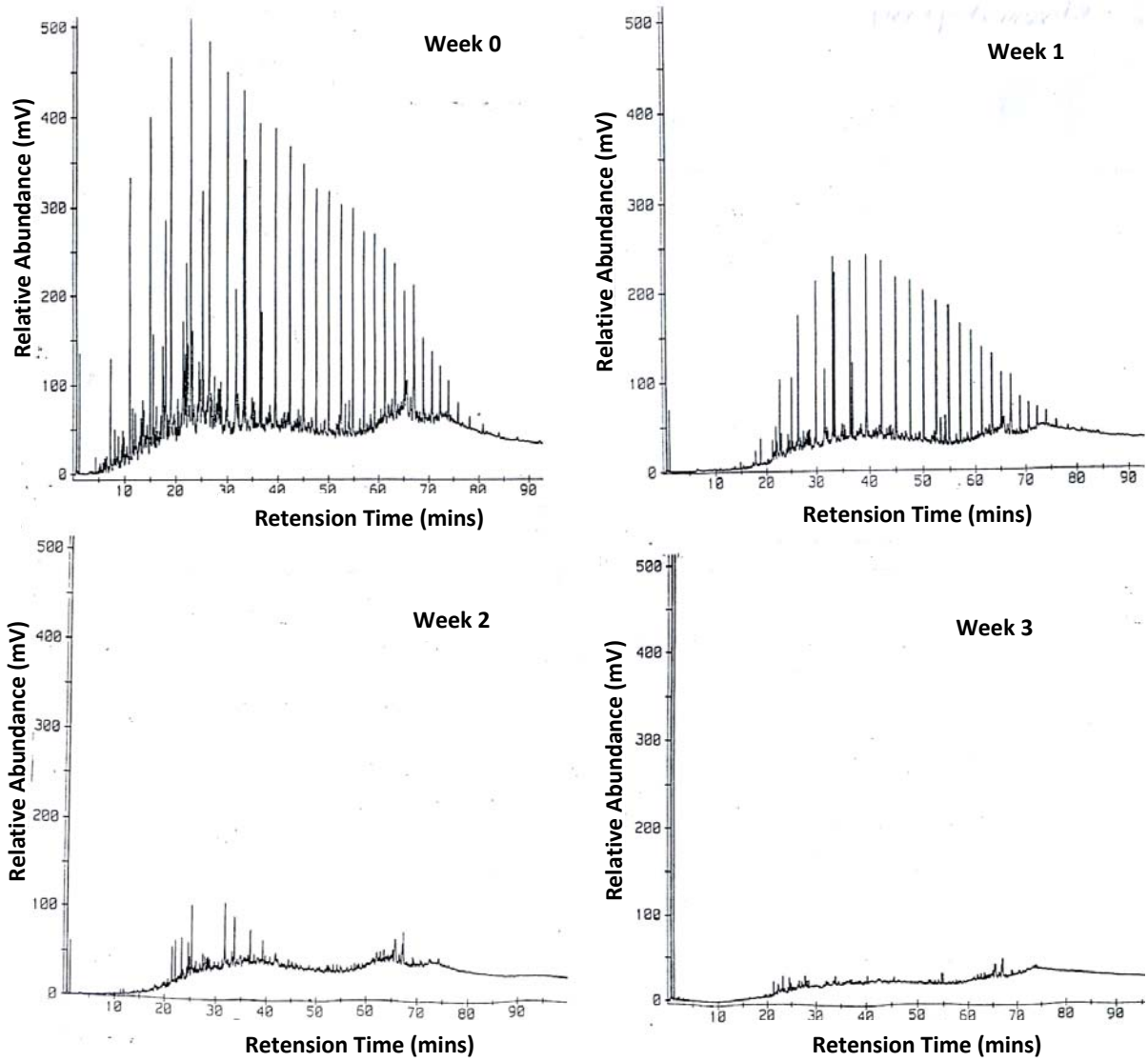

Fig. 3: GC Chromatograms of residual TPH Concentrations in Plot C

The seeding of plot C with seawater microbial inocula, nutrients and aeration provided us with the fastest and most enhanced biodegradation process in the present study. The mean concentration of residual TPH as shown in Table 2 showed very drastic reduction of residual TPH from 5460ppm at week 0 to 52ppm at week 3. The mean population density of hydrocarbon degrading bacteria as stated in Table 3 showed very high population counts of hydrocarbon degraders which rose from 0.21 x 10^4 cfu/g at week 0 to 68.5 x 10^4 cfu/g at week 1 and reached a peak of 120.4 x 10^4 cfu/g at week 2 and thereafter begins to decline after significantly degrading the residual TPH present in the plot. The decreasing trend of the biomarker indices (nC17/Pristane and nC18/Phytane) was also drastic as can be seen in Table 4. The GC chromatograms of residual TPH concentration in plot C as shown in Fig. 3 also indicated very fast and more enhanced biodegradation process after 3 weeks of treatment.

4. Discussion:

Microbiological and physicochemical analysis of the study area prior to the commencement of the experiment showed that the area was pristine with no evidence of past hydrocarbon pollution before the commencement of the experiment. The nutrient level in form of indigenous Nitrogen, Phosphorus and Potassium though moderate but may not be high enough to sustain a hydrocarbon pollution incident.

In the present study, a combination of treatment methods comprising of nutrients, aeration and seawater microbial inocula were applied to facilitate the progress of biodegradation in the hydrocarbon polluted mangrove swamp. The results obtained revealed that plot C where seawater microbial inocula, nutrients and aeration were applied had a more enhanced biodegradation when compared with plot B where only nutrients and aeration were applied without seawater microbial inocula. Mendelssohn and Lin (2003) in a similar study concluded that microbial seeding without nutrient addition did not significantly increase the rate of biodegradation of the petroleum hydrocarbon but addition of oxidants and nutrients encouraged the indigenous hydrocarbon degraders present in the environment to degrade petroleum hydrocarbon. Tagger *et al*. (1983) used microbial seeding to facilitate crude oil pollution in seawater but discovered that the exogenous strains of bacteria used disappeared from the dominant hydrocarbon degrading bacterial flora after 4 days of application and there was no significant increase in the rate of biodegradation of petroleum hydrocarbon.

We decided to use indigenous microbial culture in the present study because the use of commercial bacterial strains that are not indigenous to the environment for bioremediation has not produced positive results (Pritchard 1992, Tagger *et al*, 1983, Means 1991, and Atlas, 1981). Addition of hydrocarbon degrading microorganisms that are indigenous to the environment (Bioaugmentation) have been proposed as one of the most effective bioremediation strategy (Zhu *et al*, 2004). This is mainly due to the fact that with recently contaminated site, indigenous microorganisms capable of degrading the contaminants are probably non functional at the onset due to the toxic effect of the freshly spilled oil, therefore massive application of microbial culture possessing metabolic capabilities for the degradation of the spilled contaminant will be necessary to initiate appreciable degradation and that was the idea behind the application of seawater microbial inocula.

Taberk *et al. (*1991) also formulated seawater source microbial inocula which was used in the enhancement of biodegradation of the Alaskan weathered crude oil components. In a similar study, Jones and Greenfield (1991) have also confirmed that addition of indigenous bacterial populations and nutrients provided the highest % reduction (85%) in total petroleum hydrocarbons within 3 weeks. An attempt was also made by Okoro (2009) to use crude biosurfactant to improve the biodegradation potential of the indigenous hydrocarbon degrading bacterial flora in the mangrove swamp.

Careful analysis of the results obtained from the present study showed that nutrient application and aeration without microbial seeding achieved 58% reduction in residual TPH concentrations after 2 weeks of treatment but with microbial seeding 94.4% of residual TPH reduction was achieved within the same period and the microorganisms that constitute the seawater microbial inocula remained the dorminant bacterial flora throughout the duration of the experiment.

The biomarker indices used in the present study (ratios of nC17/Pristane and nC18/Phytane) also confirmed a more enhanced biodegradation using sea water microbial inocula. Nutrient application and aeration without microbial seeding in plot B gave respective nC17/Pristane and nC18/Phytane ratios of 1.09 and 1.29 but with microbial seeding in plot C, the respective ratios of nC17/Pristane and nC18/Phytane were 0.35 and 0.42. Pritchard and Coaster (1991) used the same index to monitor the progress of biodegradation during the EPA Alaska oil spill biodegradation project. The application of this concept is based on the principle that during biodegradation, decreases of total oil residues could occur because of other non biological processes, thus changes in hydrocarbon composition that are indicative of biodegradation must be measured accurately. This is done historically by examining the weight ratios between hydrocarbons known to be readily biodegradable such as the C17 and C18 alkanes and those that biodegrade slowly such as the branched alkanes (Pristane and Phytane) but with very close chromatographic behaviour. A weight ratio less than 1 signifies considerable biodegradation Pritchard and Coaster (1991). Ward *et al*. (1984) have also used the nC17/Pristane and nC18/Phytane biomarker index to monitor biodegradation of the Amoco oil spill and decreasing ratios of the biomarker index were observed to be nC17/Pristane(1.7) and nC18/Phytane(1.1) after 9 days of spill. The ratios decreased to nC17/Pristane(0.3) and nC18/Phytane(0.2) after 20 days, signifying significant biodegradation.

Conclusively, the present study has clearly demonstrated that in hydrocarbon polluted mangrove swamp, seeding the polluted environment with sea water microbial inocula coupled with aeration and nutrient application significantly enhanced the progress of biodegradation of the petroleum hydrocarbon pollutants within a relatively short time. Seawater microbial inocula application therefore can be regarded as a very safe, efficient and economic technology that can be effectively used to tackle the frequent cases of hydrocarbon pollution in the Nigerian oil rich Niger-Delta mangrove swamp.

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