Enhanced Bioremediation of Hydrocarbon Contaminated Mangrove Swamp in the Nigerian Oil Rich Niger Delta using Seawater Microbial Inocula amended with Crude Biosurfactants and Micronutrients

Chuma C. Okoro[1]

[1] Department of Biological Sciences and Biotechnology, Caleb University, Imota-Lagos, Nigeria
e-mail: chuma2k2001@yahoo.com

Abstract: Enhanced bioremediation of hydrocarbon contaminated mangrove swamp in the Nigerian oil rich Niger Delta was carried out using sea water microbial inocula amended with crude biosurfactants and micronutrients in a pilot scale experimental study that was monitored for 5 weeks. The indices used to monitor the progress of bioremediation were; the gradual loss in the gravimetric weight of oil, the biomarker index as exemplified by the decreasing trend in the ratios of nC17/Pristane and nC18/Phytane, the Gas chromatographic analysis of the residual oil and the population dynamics of hydrocarbon degrading bacteria. The bioremediation protocol employed in plot D where sea water microbial inocula was used in combination with crude biosurfactants and micronutrients was very efficient, fast and economical as after 7 days of application, about 97% of the total petroleum hydrocarbons (TPH) originally present in the contaminated mangrove swamp were removed. In plot C where sea water microbial inocula was applied along with micronutrients but without biosurfactants, the original TPH was reduced by 71% after 7 days of application. The other two plots (A and B) where neither sea water microbial inocula nor crude biosurfactants were applied showed no appreciable biodegradation after 14 days of application. Statistical analysis using analysis of variance (ANOVA) and Duncan multiple range test (DMRT) showed that seawater microbial inocula amended with crude biosurfactants and micronutrients significantly influenced bacterial growth and subsequent biodegradation of the petroleum hydrocarbon within a relatively short time. The short duration of the bioremediation program must have prevented the disastrous ecological consequences the prolonged presence of petroleum hydrocarbons would have caused the mangrove swamp environment. [Nature and Science 2010;8(8):195-206]. (ISSN: 1545-0740).

Keywords: Bioremediation, Mangrove swamp, Seawater microbial inocula, Biosurfactants, Micronutrients, Hydrocarbon degrading bacteria, Petroleum hydrocarbons.

1. Introduction

Mangrove wetlands are inertial ecosystems that grow in tropical and subtropical regions along the coastlines constituting important nurseries for fishes, crustaceans, sponges, algae and other small invertebtares (Rutzler and Feller, 1987). Mangroves also support wide and varied group of mobile organisms ranging from birds that nest in the trees to fishes that feed and live among submerged proproots (Odum *et al.*, 1982). In subtropical and tropical regions of the world, tidal salt marshes give way to mangrove swamp (Zhu *et al.*, 2004). The mangrove swamp are the dominant wetland ecosystem in the Nigerian oil rich Niger Delta which is the main zone of oil production and exploration activities in Nigeria. The mangrove habitat therefore are exposed to frequent oil spill that may result from shipping accidents, production related incidents and pipeline ruptures and vandalisation. In the mangrove environment, the sediment behave like a sink, retaining pollutants from contaminated tidal water. In that case, the spilled and stranded oil containing large amount of recalcitrant polycyclic aromatic hydrocarbons (PAHs) will penetrate and accumulate in the mangrove sediment (Bayoumi and Nager, 2009).

Oil spills have been known to cause acute and long term damage to salt marshes and mangrove swamps. These impacts include; reduction in population and growth rate of the mangrove trees, acute and long term damage to salt marshes and mangroves, wide spread animal mortality as a result of the toxic effects of the oil and the disruption of the entire ecosystem (Zhu *et al.*, 2004). Efforts to minimise the damage of oil spills to the mangroves and enhance the recovery of mangroves are very important in order to minimise the complete loss of ecological functions of these mangroves to the coastal zone.

Bioremediation has been advanced as one of the promising technologies that can be used to quickly and effectively remedy the adverse effects of petroleum hydrocarbon pollution in the mangrove swamp. Bioremediation is simply the 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. This technology has been used by many scientist to treat petroleum hydrocarbon pollution in the mangrove swamp. Scherrer and Mille (1990), have confirmed enhanced degradation of oil in the West Indies mangrove swamp after the addition of oleophyllic fertilizer. Lee and Levy (1991), have equally demonstrated enhanced degradation of oil in the salt marsh sediment treated with inorganic nutrients.

One of the limiting factors in petroleum biodegradation by microorganisms is the bioavailability of the many fractions of the oil to microorganisms. There must be a form of emulsification to make various components of petroleum hydrocarbons available to microorganisms for effective degradation. In order to cope with this challenge, some hydrocarbon degrading microorganisms produces biosurfactants of diverse chemical nature and molecular size. These surface active materials increases the surface area of hydrophobic water insoluble substrates and their bioavailability thereby enhancing the growth of microorganiams and rate of bioremediation (Rosenberg *et al.*, 1988; Ron and Rosenberg, 2002).

Many scientists have employed the use of biosurfactants to facilitate the process of bioremediation (Bannat *et al.*, 1995; Cameotra and Bollang, 2003) and biosurfactant enhanced bioremediation approach has been frequently used to treat hydrocarbon pollution in some sensitive environments like the mangrove swamp where accessibility and human intrusion is difficult (Teas *et al.*, 1993). Bayoumi and Nagar (2009), while monitoring a bioremediation program on the Egyptian red sea mangrove discovered that surface mangrove sediments harboured diverse petroleum hydrocarbon degrading bacteria which produces biosurfactants that accelerates the rate of degradation of petroleum hydrocarbons. Okoro (2009) have also observed enhanced biodegradation of hydrocarbons in the mangrove swamp using oleophyllic fertilizer and crude biosurfactant extracts. Banat (1995) and Banat *et al.*, (1995) have demonstrated the use of biosurfactants in pollution control, oil tank clean up and enhanced oil recovery. Enhanced bioremediation of n-Alkane in a sludge using bacterial consortium amended with rhamnolipid biosurfactant and macronutrients have been demonstrated by Rahman *et al.*, (2003). Millioli *et al*,(2009) have also observed that addition of rhamnolipid biosurfactant to hydrocarbon contaminated soil significantly increased the biodegradation of the hydrocarbon by the indigenous hydrocarbon degrading microbial community.

The indices used to monitor the progress of bioremediation in this study were the population dynamics of hydrocarbon degrading bacteria, the loss in the gravimetric weight of oil, the gas chromatographic analysis of the residual oil and the use of biomarker index such as the decreasing trend in the ratios of nC17/Pristane and nC18/Phytane. 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 according to Pritchard and Coaster (1991).

The purpose of the present study therefore was to establish a fast and efficient bioremediation protocol for the bioremediation of petroleum hydrocarbon pollution of the mangrove swamp. Enhancement of biodegradation was achieved through a combination of bioaugmentation and biostimulation approaches by employing the use of seawater microbial inocula amended with crude biosurfactants, micronutrients and aeration to achieve significant biodegradation within a relatively short time.

2. Materials and Methods:

Study area:

The study area is located within Escravos mangrove swamp (Longitude E, 4^0, 33',24' and Latitude 6^0, 28',12.) in Ugborodo town, Delta State, Nigeria. The environment is near shore with moderate salinity (798mg/L). A comprehensive physicochemical and microbiological analysis was carried out in the study area 48 hrs before the commencement of the experiment.

Experimental Design.

A 40 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 4 equal segments otherwise referred to as plots A, B, C and D 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 40 liters of crude oil (Escravos

light, obtained from Chevron Nigeria Limited) at the rate of 10 liters per plot which was evenly distributed to 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) which was enclosed in a semi-permeable membrane to plots B, C and D. Plots C and D was later flooded with 6 liters of sea water microbial inocula followed by further addition of fertilizer (2.5 liters). This was followed by the additional 5 liters of mixed crude biosurfactants extracts to plot D. There was intermittent mixing of surface sediments by tilling to increase aeration in plots B, C and D. Plot A served as a control plot with only crude oil application without any form of treatment. 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.

Microbiological and Physicochemical Analysis of the study area.

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^{0}C 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), while temperature was measured using portable thermometer (Hanana , H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described by E*aton 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 at,* (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 2cm 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) of the mangrove surface water.

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 in the mangrove sediment:

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 of the mangrove sediment:

The moisture content of the mangrove sediment was measured by simple gravimetric analysis. 10g of the soil sample containing water 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 in *(Eaton et al,1995)*

Solvent extraction of Residual Oil

One gram (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 Sea Water Microbial Inocula.

Sea water microbial inocula was formulated from four hydrocarbon utilizing bacterial cultures that were previously isolated from the experimental site. The bacterial isolates 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.), $MgSO_4.7H_20$ (1.26g), KCl (1g), KH_2PO_4 (2.50), 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 into 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 was regarded as the ideal bacterial population density for field application.

Growth and Biosurfactant Production

The two bacterial isolates that were used for biosurfactant production, *Pseudomonas. mallei* and *Alkaligenes sp.* were grown on the minimal salt medium of *Rosenberg et al*,(1988) with diesel oil as the sole carbon source. The composition of the medium in g/L, is as follows, NaCl (5), $NaHPO_4$ (13.7), KH_2PO_4 (7.26), $(NH4)_2S0_4$(3), $MgS0_4$ (0.4) and diesel (10ml). The culture medium was contained in a 5 liter Wheaton glass bottle which was covered with a non absorbent cotton wool and placed in a slanted position to allow air passage through the pores of the cotton wool. The bottles were shaken at regular intervals to allow adequate homogeneity of contents and the incubation was at room temperature. At the end of the 6 days incubation, the supernatant which consists of the microbial cells and the crude biosurfactant were aseptically collected and the microbial cells were separated from the biosurfactant by centrifugation.

Partial Biochemical Characterisation of Crude Bioemulsifuers

The crude biosurfactants extracts from the two microbial isolates were analysed for protein using the method of Bradford (1976), Lipid contents of the biosurfactants were determined using the method of Kates (1972) while carbohydrates were determined as described by Spiro (1966). *Pseudomonas mallei* whose biosurfactants had both carbohydrate and protein components was tentatively classified as glycoproteins while *Alcaligenes* sp. with Carbohydrate and lipid component biosurfactants was classified as glycolipid.

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

To ensure that the project area is pristine and have not received any significant hydrocarbon pollution in the past, microbiological and physicochemical analysis was carried out before the commencement of the experiment. The low population density of hydrocarbon degrading bacteria counts relative to the high heterotrophic bacterial counts and low concentration of total petroleum hydrocarbon is enough indication that the environment is pristine and have not received any significant hydrocarbon pollution in the past. The background nutrient concentration was also not high enough to sustain high microbial activity. The detailed results of the microbiological and physicochemical properties of the study area before the commencement of the experiment are shown in Table 1.

Application of Seawater Microbial Inocula, Micronutrients, Aeration and Crude Bioemulsifier for the Bioremediation of Hydrocarbon Contaminated Mangrove Swamp

Plot A is the control plot where no treatment was applied and it is the plot that represents a typical natural biodegradation scenario. The initial TPH level of 5360 ppm was slowly reduced to 2360ppm after 5 weeks of natural biodegradation without any form of enhancement as shown in Table 2.

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)	2.80 X 10^5 cfu/ml	3.20 x 10^6 cfu/g	2.20 x 10^4 cfu/g
Hydrocarbon utilizing bacterial counts (Cfu/ml or g)	0.02 X 10^5 cfu/ml	0.16 x 10^6 cfu/g	0.048 x 10^4 cfu/g
pH	7.20	6.80	6.70
Temperature	28^0C	24^0C	22^0C
Salinity (mg/l or g)	6456mg/l	6230	5910 mg/g
Phosphorus(mg/l or g)	23mg/l	121mg/g	108 mg/g
Potassium (mg/l or g)	11mg/l	93 mg/g	66mg/g
Amonia-Nitrogen (mg/l or g)	1.70mg/l	3.10 mg/g	2.30 mg/g
Dissolved Oxygen (mg/l)	10.40mg/l	ND	ND
Redox Potential (Eh) mV	342mV	261 mV	88
BOD_5(mg/l)	60mg/l	ND	ND
Heavy metals detected (ppm)	Fe (0.0065), Cr(0.16)	Cd(0.05), Pb(0.018), Zn.(0.03)	Cr.(0.16), Pb(0.42)
Total Petroleum Hydrocarbon (TPH) ppm	26.50	18.60	6.70

ND: NOT DETERMINED

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 ± 28.2^A**	**5160 ± 7.07^D**	**4780 ± 7.07^D**	**3640 ± 7.07^D**	**2760 ± 42.42^C**	**2360 ±5.56^C**
B	**5430 ± 7.07^A**	**4150 ± 7.07^C**	**2280 ± 8.48^C**	**1620 ± 5.65^C**	**360 ± 8.48^B**	**36 ± 5.65^B**
C	**5450 ± 7.07^A**	**1580 ± 2.82^B**	**310 ± 2.82^B**	**160 ± 11.31^B**	**42 ± 2.82^A**	**12 ± 2.82^A**
D	**5410 ± 2.82^A**	**140 ± 11.31^A**	**86 ± 2.82^A**	**28 ± 2.82^A**	**5.20 ± 0.14^A**	**0.60 ± 0.28^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 AERATION APPLICATION ALONE. PLOT C = SEA WATER MICROBIAL INOCULA NUTRIENTS AND AERATION. PLOT D= SEA WATER MICROBIAL INOCULA, NUTRIENTS, AERATION AND CRUDE BIOSURFACTANT

The population dynamics of hydrocarbon degrading bacteria and the ratios of nC17/Pristane and nC18/Phytane are shown in Tables 3 and 4 respectively while the GC chromatograms of the residual TPH concentrations in plot A are shown in Figure 1.

In plot B where nutrients and aeration were applied, the initial TPH level of 5430 ppm was reduced to 36 ppm after 5 weeks of application as shown in Table 2. Plot B showed a more enhanced biodegradation when compared with plot A. The

population dynamics of hydrocarbon degrading bacteria and the ratios of nC17/Pristane and nC18/Phytane in plot B are shown in Tables 3 and 4 respectively while the GC chromatograms of the residual TPH concentrations are shown in Figure 2.

In plot C where sea water microbial inocula, micronutrients and aeration were applied, a more enhanced biodegradation was observed when compared with that of plot B. The residual TPH concentration was reduced from 5450ppm at week 0 to 42 ppm at week 4 as shown in Table 2. The population density of hydrocarbon degrading bacteria also rose from 0.56 x 10^4 cfu/g at week 0 and reached a peak level of 110.30 x 10^4 cfu/g at week 2 and gradually declined thereafter as shown in Table 3. The ratios of nC17/Pristane and nC18/Phytane also indicated considerable biodegradation as shown in Table 4. The GC Chromatograms of the residual TPH concentrations in plot C are shown in Figure 3

Plot D represents a more comprehensive combination of treatment methods comprising of nutrients, aeration, seawater microbial inocula and crude biosurfactants. The reductions in TPH concentrations was more drastic in plot D than the other plots as shown in Table 2. The residual TPH concentrations of 5410 ppm at week 0 was reduced to 28ppm at week 3. The peak population density of hydrocarbon degrading bacteria was achieved within a relatively short period as shown in Table 3 and the reductions in the ratios of nC17/Pristane and nC18/Phytane as shown in Table 4 also indicated a much more enhanced biodegradation when compared with the other plots. The GC Chromatograms of residual TPH concentrations in plot D are shown in Figure 4.

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	0.48 ± 2.82^B	0.25 ± 7.01^A	3.40 ± 0.28^A	6.50 ± 0.56^A	11.30 ± 0.42^B	14.50 ± 0.70^D
B	0.30 ± 7.07^A	3.50 ± 0.56^B	11.20 ± 0.28^B	36.50 ± 0.71^B	56.50 ± 0.56^C	7.60 ± 0.70^C
C	0.56 ± 6.36^B	36.20 ± 0.28^C	110.30 ± 2.82^C	86.70 ± 0.28^C	11.30 ± 0.42^B	1.20 ± 0.14^B
D	0.32 ± 2.82^A	43.20 ± 0.28^D	230.50 ± 0.56^D	106.50 ± 0.56^D	6.50 ± 0.42^A	0.08 ± 0.11^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 ALONE. PLOT C = SEA WATER MICROBIAL INOCULA NUTRIENTS AND AERATION. PLOT D= SEA WATER MICROBIAL INOCULA, NUTRIENTS, AERATION AND CRUDE BIOSURFACTANT

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	1.47	2.91	1.46	2.78	1.43	2.76	1.30	2.80	1.28	2.71	1.29	2.70
B	1.45	2.86	1.43	2.84	1.30	2.77	1.10	2.40	0.86	0.89	0.10	0.16
C	1.44	2.73	1.21	2.65	0.74	0.88	0.21	0.36	0.08	0.06	TDP	TDP
D	1.46	2.83	0.65	0.72	0.21	0.18	TDP	TDP	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 ALONE. PLOT C = SEA WATER MICROBIAL INOCULA NUTRIENTS AND AERATION. PLOT D= SEA WATER MICROBIAL INOCULA, NUTRIENTS, AERATION AND CRUDE BIOSURFACTANT

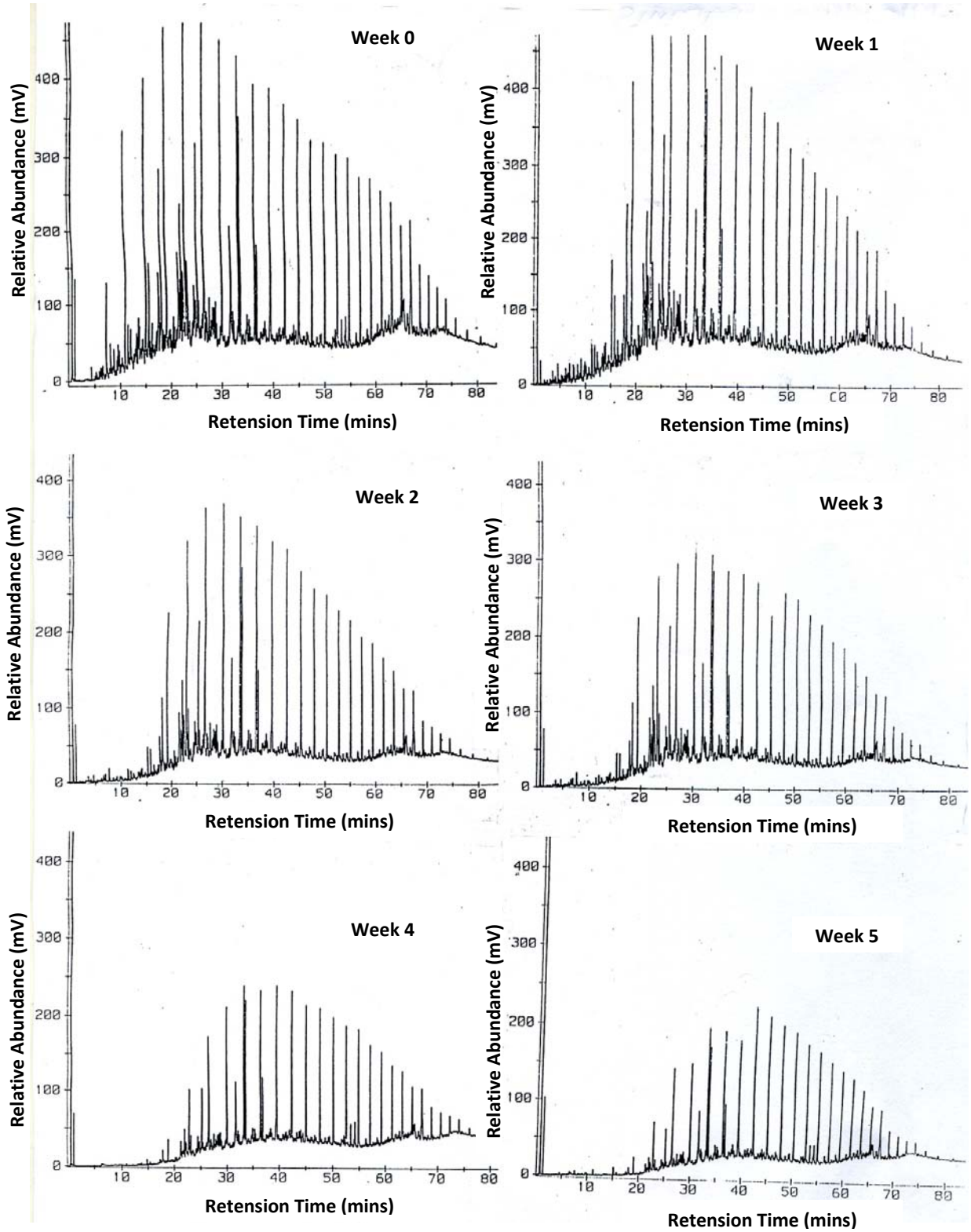

Fig. 1: GC Chromatograms of residual TPH Concentrations in Plot A

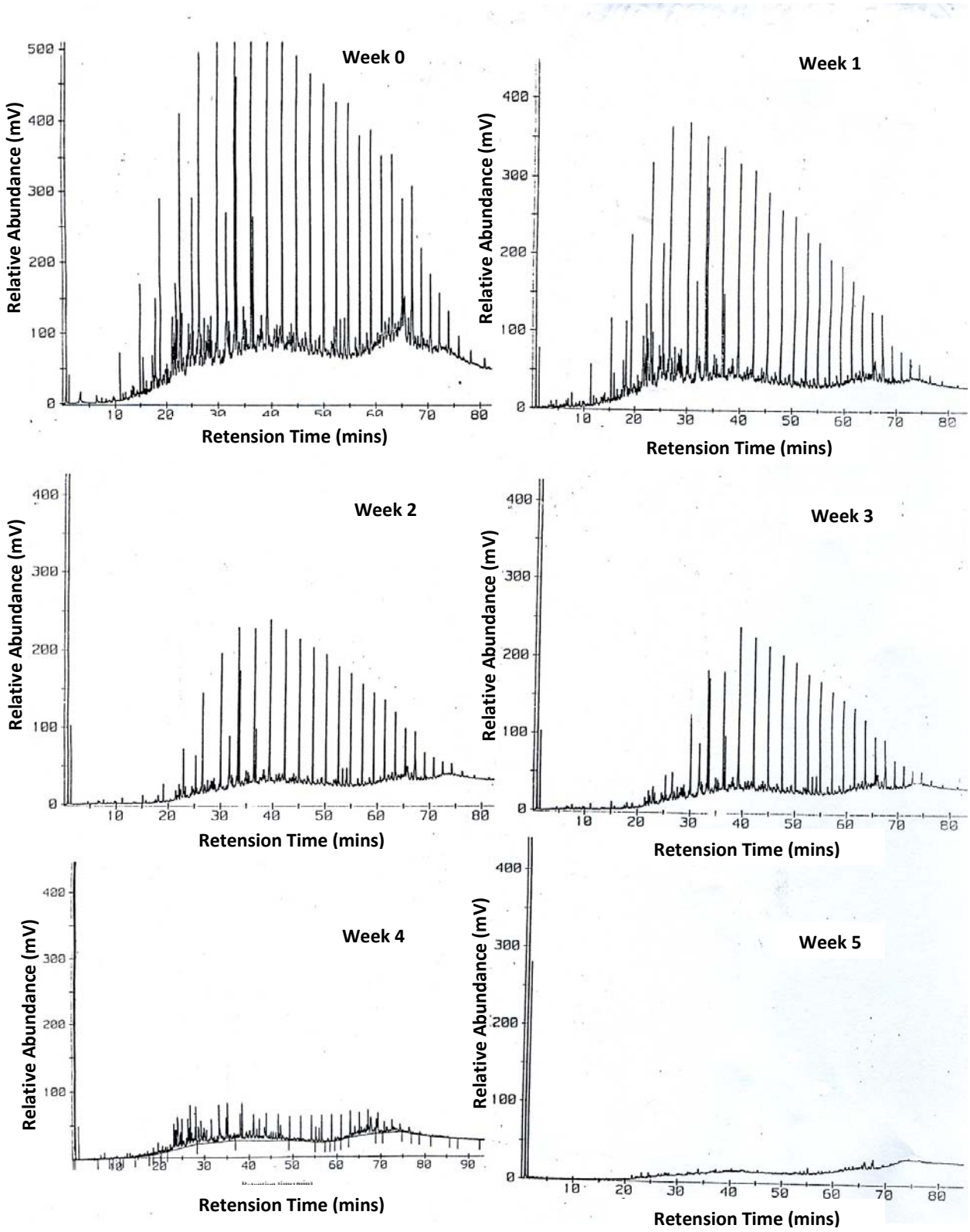

Fig. 2: GC Chromatograms of residual TPH Concentrations in Plot B

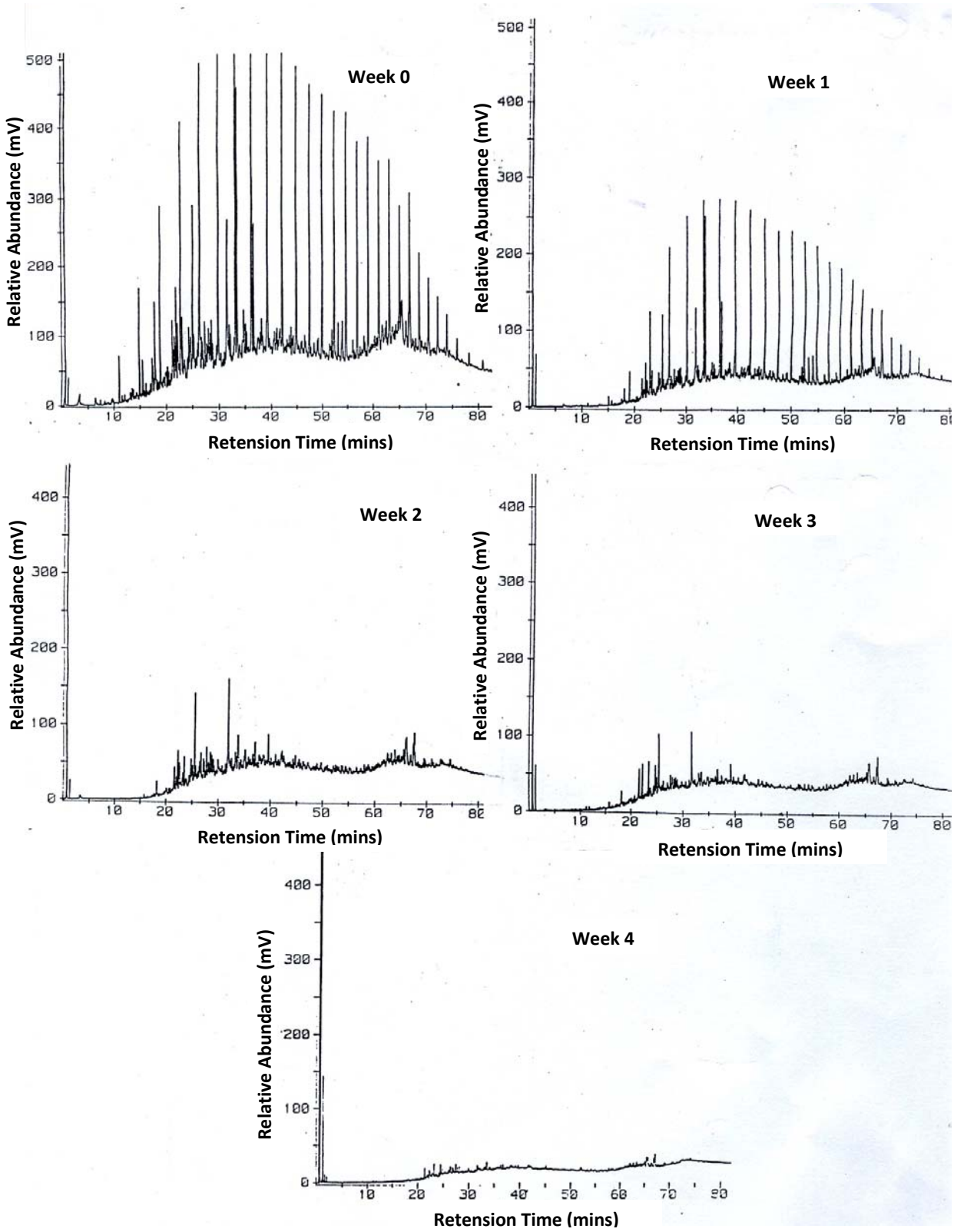

Fig. 3: GC Chromatograms of residual TPH Concentrations in Plot C

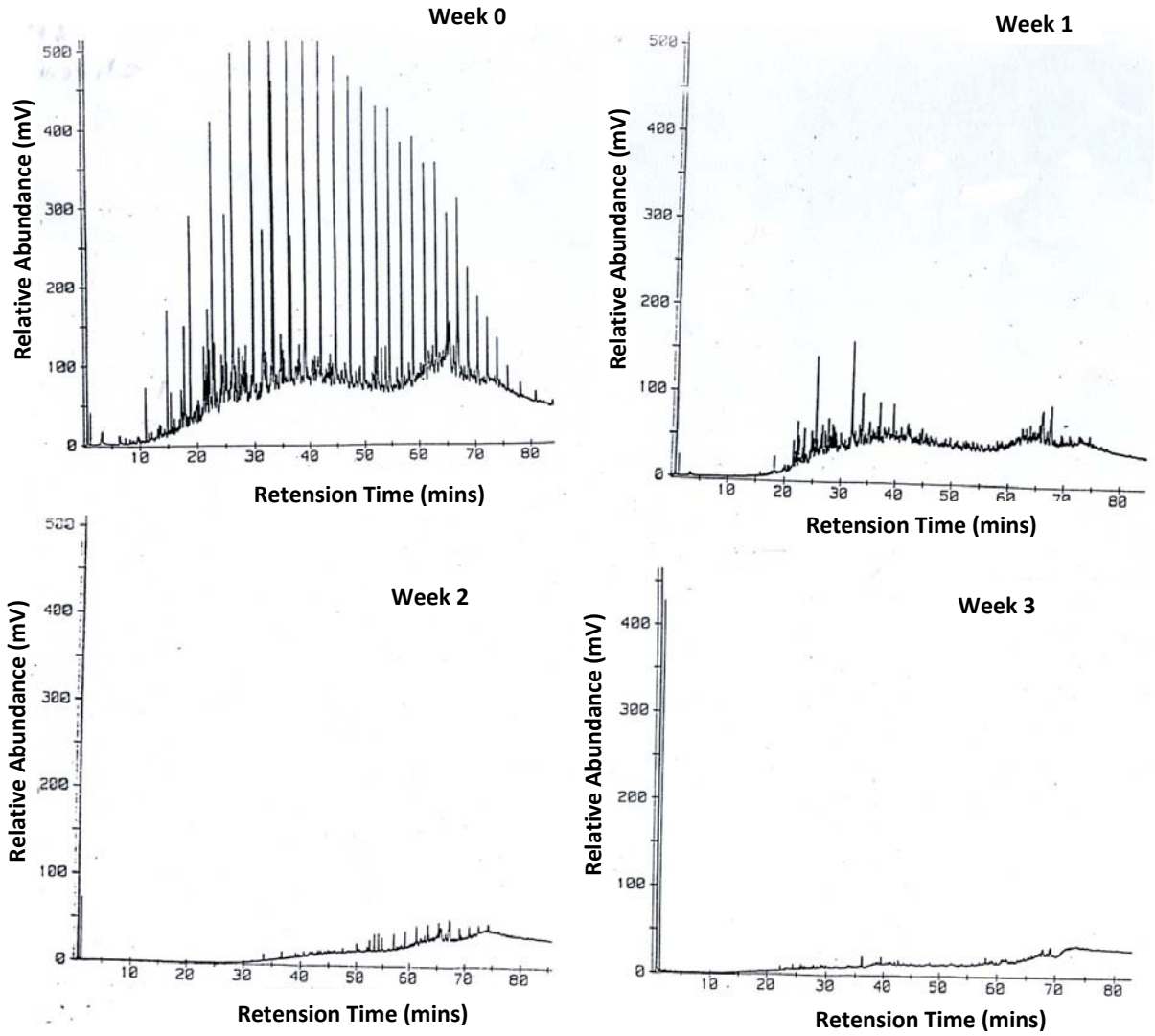

Fig. 4: GC Chromatograms of residual TPH Concentrations in Plot D

4. Discussions:

In the present study, biosurfactants produced by two bacterial isolates from a mangrove swamp were used to enhance the process of biodegradation in the same hydrocarbon polluted mangrove swamp. It has been reported in several literatures that most marine organisms produces biosurfactants. Bayoumi and Nagar (2009) while monitoring a bioremediation program in the Egyptian red sea mangrove forest discovered that the surface mangrove sediments harboured diverse petroleum crude oil degrading bacteria which produces biosurfactants, such organisms include; *Bacillus polymyxa, Pseudomonas pseudomallei, Bacillus subtilis and Bacillus lichiniformis*. Some of the properties produced by marine microorganisms which make them perfect biodegradation enhancers according to Kosaric (2001) include; lowering of surface and interfacial tensions, wetting and penetrating actions, hydrophylicity and hydrophobicity actions and microbial growth enhancements. For these reasons many authors have concluded that addition of biosurfactants to a petroleum hydrocarbon contaminated environment enhances solubility and removal of hydrocarbon contaminants and this also improves and quickens oil biodegradation rates (Millioli *et al.*, 2009).

The main purpose of the present study therefore is to investigate possible methods of enhancing biodegradation rates of oil pollution in the mangrove environment with the aim of reducing significantly the holding time of the entire bioremediation program while sustaining the efficiency of the process.

Enhancement of biodegradation in the present study was achieved through the combination of biostimulation and bioaugmentation approaches. A combination of glycoprotein biosurfactant produced by *Pseudomonas mallei* and a glycolipid type produced by *Alkaligenes* sp. were used in combination with seawater microbial inocula, micronutrients and aeration to facilitate the degradation of petroleum hydrocarbons in the mangrove swamp within a relatively short time. It is expected that the combination of two different biosurfactants will provide a synergistic effects that can lead to faster degradation of petroleum hydrocarbons in the mangrove swamp. Some investigators have shared the same opinion, for instance Cameotra and Makker (2008), have suggested mixture of surfactants rather than individual surfactants to facilitate the bioremediation process of petroleum hydrocarbons. They argued that in most cases when different types of biosurfactants are purposely mixed, synergism is observed and better results are obtained. Cameotra and Makker (2008) have used mixture of anionic and non-anionic biosurfactants which exhibited synergistic solubulisation of hydrophobic contaminants that enhanced the efficiency of biodegradation. They have also applied mixed biosurfactants in oil tank recovery, oil spill management, microbial enhanced oil recovery and heavy oil dispersants and de-emulsification. Lai *et al.*,(2009) have also identified the capability of two biosurfactants to remove TPH from soil compared with single synthetic surfactants. Their results indicate that the combined biosurfactants exhibited much higher TPH removal efficiency when compared with single synthetic one. On the contrary, Maneerat (2005) identified a single glycolipid biosurfactant from *Alkaligenes* species which was effectively used to enhance the biodegradation of petroleum hydrocarbons. Millioli *et al.,* (2009) have also observed that addition of rhamnolipid biosurfactant to hydrocarbon contaminated soil significantly increased the biodegradation efficiency of the indigenous hydrocarbon degrading microbial community. Enhanced biodegradation of n-alkane sludge using bacterial consortium amended with rhamnolipid and micronutrients have also been demonstrated by Rhahman *et al.,* (2003). From the foregoing, it very obvious that both single and mixed biosurfactants enhances the process of biodegradation of petroleum hydrocarbons but it is expected that the efficiency of biodegradation with mixed biosurfactants will be better than that of single biosurfactant.

In the present study, application of seawater microbial inocula and micronutrients as demonstrated in plot C caused the removal of about 71% of the residual TPH after 1 week of application but when a combination of biosurfactants were added to facilitate the enhancement, about 97% of the residual TPH were removed from the mangrove swamp within the same period and the result was better when compared with a similar study conducted by Okoro (2009) where only one type of biosurfactant was used for enhancement of biodegradation.

Conclusively, it has been advanced that eliminating the hazardous hydrocarbon pollutants in the mangrove swamp as fast as possible is one of the primary goals of an efficient bioremediation program because the longer the contaminants stay in the mangrove environment the more the damage. The present study has clearly demonstrated that a mixture of glucoprotein and glycolipid biosurfactants can be used to significantly enhance the rate of biodegradation of petroleum hydrocarbon in the mangrove swamp when used in combination with seawater microbial inocula, micronutrients and aeration. A significant biodegradation which was achieved after 7 days of application is an indication that the bioremediation program used in the present study was economical, effective and efficient.

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Correspondence to:
Dr. Chuma C. Okoro
Department of Biological Sciences and Biotechnology
Caleb University, Lagos
Tel: 08033072754, 01-7430285
e-mail: chuma2k2001@yahoo.com
P.O. Box 146, University of Lagos Post Office, Lagos, Nigeria

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