DYNAMIC RESPONSE OF MICROBIAL SYSTEM OF SELECTED COMMUNITIES IN THE RIVERS STATE TO CRUDE OIL (HYDROCARBONS) POLLUTION

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ABSTRACT
The activity of indigenous microbial system is a required tool to remediate an environment that has been contaminated by hydrocarbons. This work deals with the dynamic response to crude oil contaminants of undefined microbial systems in three selected communities of the Rivers State of Nigeria. The activities of indigenous (undefined) consortia were studied and compared using Respiratory technique. The soil samples were incubated with 2 % ($^{v}/_{v}$)crude oil in mineral salt medium at 37 °C in three phases of two weeks in a shake flask at 150 rpm. At the end of the last phase, components of the crude oil degraded by the undefined consortia in the soils were identified with the gas chromatographic techniques. The results obtained showed that the consortia from the different soil samples exhibited different degrees of capacities to degrade the crude oil. On the whole, 50.00 to 85.70 % of hydrocarbon components of the crude oil were degraded thus making the areas potentially suitable for in-situ bioremediation. The study has shown that the obtained microbiological characteristics and activities of the soils are adequate for bioremediation technologies.

KEY: Microbial activities, Undefined Consortium, Crude oil, Bioremediation, Soil, Respiratory Technique.

INTRODUCTION

Rivers State is one of the highly ranked oil-producing states of Niger-Delta of Nigeria,and a lot of petroleum production and activities have directly exposed this area to large or repeated spills or leaks frequently. The presence of hydrocarbon pollution in the environment poses a great hazard for aerial, terrestrial and aquatic life, andthe impact of these pollutants is of great public concern due to the severe ecosystem imbalances caused by them (Whyte *et al*., 1997; Bicca *et al.*, 1999; Margesin *et al.*, 2003). Regardless of the level of pollution, they are potentially dangerous to the microfauna and microflora, and have detrimental effects on the ecosystem (Amund *et al.*, 1987; Akpofure *et al.*, 2001). As a result there are worldwide concerns about remediating and restoring environments of hydrocarbon pollution (Bicca *et al.*, 1999).

Microbial influenced remediation and restoration have been accepted as a promising tool to cleaning up hydrocarbon contaminated environments (soil, water, and estuaries). It has gained a wide application in combating the menace of hydrocarbon pollution to the environment which is usually encountered in the oil and gas industries. Apart from being environmentally friendly, because it does not introduce additional chemicals to the environment, bioremediation usually competes well on a cost basis, especially in biodegrading petroleum products and most solvents (Admassu and korus, 1996). Going by the success story of application of bioremediation in so many hydrocarbon contaminated places for instance in Prince William Sound in Alaska after the Exxon Oil spill in 1989 (Boopathy, 2000), the method can also be employed in cleaning the contaminated environments in Niger Delta area of Nigeria.

Bioremediation systems or technologies are used in cleaning up environment such as soil, water and air contaminated with hydrocarbons. It is basically an application of biodegradation, which is a microbial driven process to clean up the environments contaminated with organic compounds (hydrocarbons). The effectiveness of these systems thrives on the ability to enrich and maintain microbial populations and activities within the target environment. This is achieved with two major inputs like operational conditions and the organic contaminants as illustrated in Fig. 1. The operational conditions include the physical and chemical properties of the environment, which are the environmental variables that govern the soil (environment) microbial activities, populations and communities. Other factors include toxicity, bioavailability and degradation rate of the contaminants. Finally, the strategic methods of optimizing the system are also inputs in the bioremediation system. From the foregoing, it is important that the evaluation of the activity of the microbiota of the soil is

vital for well-designed and implemented bioremediation efforts. Soil biological investigations, such as measurement of soil respiration, enzyme activities and microbial counts (depending on the desired information) can give information about the presence of viable microorganisms and on the impact of the effects of environmental stresses, such as hydrocarbon contamination on the metabolic activity and biogeochemical cycles taking place in soils (Margesin *et al*., 2000).

Fig. 1: Bioremediation System.

The engineering of bioremediation processes relies on information about the site and about the candidate microorganisms. This work aims at obtaining this basic information such as response of the microbial system which can enhance the bioremediation technologies involved in the Niger Delta, particularly Rivers State of Nigeria. The activities of pristine and contaminated sites will also be compared with a view to improving the developed bioremediation technologies.

METHOD

Sample Sites and Collection: The methods used in the works of Margesin *et al*. (2003), Uzoamaka *et al*. (2009) and Obire and Anyanwu (2009) were integrated and adopted in this work. This involvedsoil samples, both contaminated with crude oil and pristine (uncontaminated), serving as control were collected from surface soil (0-15 cm depth) from three communities (as sites) in River states. These communities are Pete, Ebubu and Kpor in the Local Government Area of Tai, Eleme and Gokana respectively, and they are abbreviated as RTP, REE, and RGK respectively in this study (Fig. 2).The soil sample collections were made from 3-4 random points per sample sites and mixed to form a composite soil sample with a sterile scoop and transferred aseptically into sterile polyethene bags.

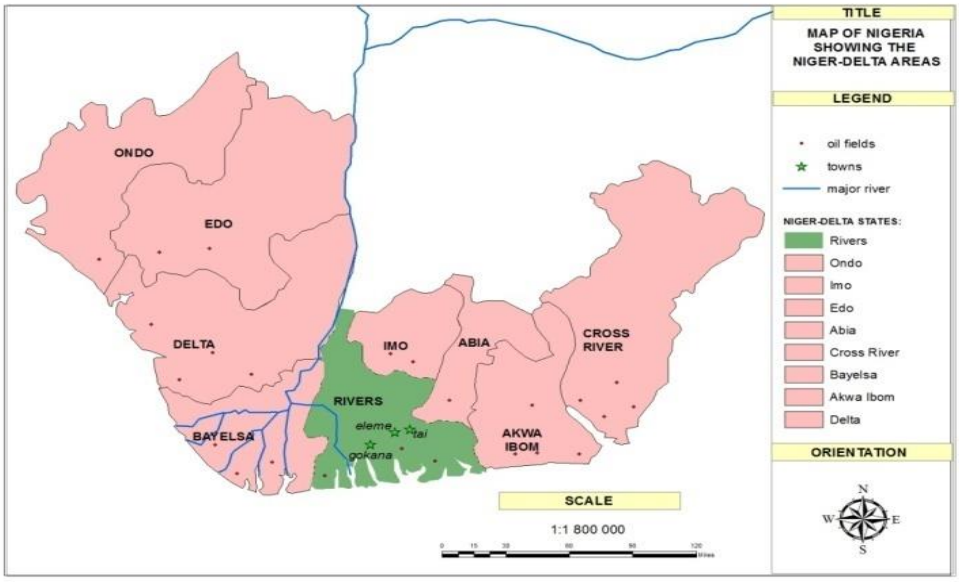

Fig. 2: Map of Nigeria (Niger Delta area) Showing Sample sites in River State

Microbial Activties Analysis

Respirometric technique was used, since it allows an in depth analysis of the microbial population dynamics (Margesin *et al*., 2003; Eze and Eze, 2010). The soil respiration was measured from incubation of 100g soil samples kept in hermetically sealed 250 ml glass flask for 21days at room temperature (Fig. 3). To achieve this, 100 ml conical flask containing 50 ml of 0.50 $moldm^{-1}$ of NaOH solution was used to capture the CO_2 produced from the respiration.

Fig. 3: Set up for the respiratory study.

The conductance of the NaOH solution was measured using Jensy Model 712 Conductance Instrument after each incubation time (1, 3, 5, 7, 13, 17 and 21 days). The microbial activity was monitored in triplicate for the released CO_2 (Critter *et al*., 2004). The produced CO_2 was calculated from the following equation:

$$m = 22\left[\frac{\lambda 1-\lambda x}{\lambda 1-\lambda 2}\right]VC \qquad 1.0$$

Where λ_x is the conductivity value of the sample, λ_2 is the conductivity value of Na_2CO_3, λ_1 is the conductivity value of NaOH, V is the volume of the standard NaOH solution, C is its concentration in mol dm^{-3} and m is the estimated mass of absorbed CO_2 in mg.

Crude Oil Utilization-ability of the Un-defined Consortium in the Environment

The abilities of the un-defined consortium in the sampled soils were confirmed by inoculating 10 g of each sample in separate cotton plugged 250 ml Erlenmeyer flasks containing sterile liquid minimal salt medium (whose composition was (g/l): NaCl, 10.00; $MgSO_4.7H_2O$, 0.42; KCl, 0.29; KH_2PO_4, 0.83; $Na_2HPO_4.H_2O$, 1.25 and $NaNO_3$, 0.42) (Nwachukwu, 2000). The liquid medium and crude oil were autoclaved separately at 121 ^oC for 15 min. sterile crude oil which served as source of carbon and energy was added at 1-2 %(v/v) to make up a final volume of 90 ml sterile liquid minimal salt medium (i.e. to give 10^{-1} dilution). Control flask containing the liquid medium and the 1-2 % crude oil but without soil sample was also prepared. The flasks were monitored and agitated in water bath shaker for a period of 14 days at 15 rpm. This is the first enrichment. For second enrichment phase, 10 ml of aliquot of each soil sample was transferred to fresh 90 ml medium containing 1-2 % (v/v) crude oil and also monitored for 14 days in water bath shaker at 15 rpm. The same procedure was repeated for the third enrichment phase. These were to re- confirm the biodegradation abilities of the undefined consortium. At the end of each phase, microbial loads were determined by plating aliquots (0.1 ml) from $10^{-3}$and 10^{-4} dilutions of samples on PDA plates and 10^{-5}, 10^{-7} and 10^{-8} dilutions on NA plates for estimation of the total fungi and bacteria respectively. The PDA plates were incubated aerobically at room temperature for 3-5 days and NA plates also aerobically at 37 ^oC for 1-2 days. At the end of the incubation periods, the population densities of fungi and bacteria were estimated according to previous methods (Nwachukwu, 2000; Omotayo *et al*., 2012). The residual hydrocarbons present in the media after the last phase of enrichment were determined by GC/MS analysis (Omotayo *et al.*, 2012; Malatova, 2005).

RESULT AND DISCUSSION

The respirometry technique has allowed an in-depth analysis of the microbial population dynamics, by comparing the activities of indigenous microbial population in both pristine (uncontaminated) and contaminated soils. The plots of accumulated carbon (IV) oxide production against time of incubation are shown in Fig. 3- 5. The cumulative values obtained reflect the distinct behavior for the contaminated and uncontaminated soil samples.

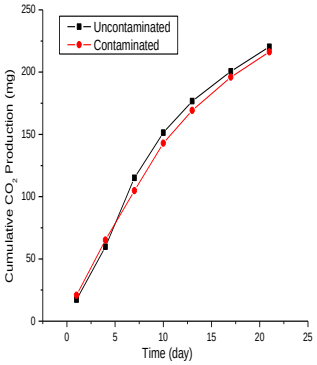

Fig.3: Accumulated CO_2Production during 21 days incubation of soil sample from Eleme, Rivers State, REE.

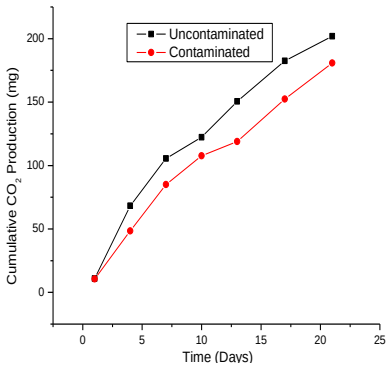

Fig.4: Accumulated CO_2Production during 21 days incubation of soil sample from Gokana, Rivers State, RGK.

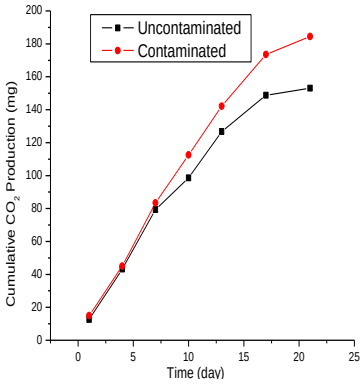

Fig.5: Accumulated CO_2Production during 21 days incubation of soil sample from Tai, Rivers State, RTP.

The results obtained as reflected in Fig. 3 and 4 (representing Eleme and Gokana, communities in Rivers State respectively) showed that the activities of microbes in uncontaminated soils (actual) are higher than that of the contaminated (potential). This was similar to the observation reported by other researchers (Margesin *et al*., 2003). The results indicated that there was a decrease in the total heterotrophs in the contaminated soils apparently due to the toxic effects of the crude oil.Moreover, the indigenous microbial systems lack adequate abilities to mineralize the crude oil hydrocarbons. This is an indication that such environments would require enhancement (biostimulation or bioaugmentation) to be remediated or restored.

The results plotted in Fig. 5, depicting the microbial activities in Pete, Rivers State showed that the potential activity of the indigenous microbes was higher than that of the actual. Thus there is a strong positive response from the indigenous microbial population, indicating their biodegradative contributions. This suggests that the microbial activities of this sitewere not so impacted and interfered with by the crude oil pollution or the recovery of the site had improved by the time this study started.

Table 1: Kinetic Parameters of Growth of the Undefined Consortium as function of CO_2 generation at the sites.

Location	Description of Soils used	Lag Time, (hr)	Specific Growth Rate(mg/l/hr)	Mean Generation Time(hr)
REE	A33.601.060.65			
	B28.800.611.13			
RGK	A14.401.140.61			
	B36.000.960.72			
RTP	A24.000.441.56			
	B33.600.461.50			

Key: A-uncontaminated soil, B- contaminated soil, RTP-Tai (Pete), REE-Ebubu (Eleme) and RGK-Kpor (Gokana)

These observations are further strengthened by the kinetic parameters generated for the sites as a function of carbon (iv) oxide production (Table 1). In the locations (REE and RGK), where the actual activities were greater than the potential activities, the specific growth rates were also higher while the mean generation times were lower. This is an indication that increase in activities was as result of increase in the growth rate. In other words, when activity was higher, less time would be required by the microbial population to double itself.

Furthermore, this may imply that the biodegradability potential of hydrocarbons in soils of these sites was high, and that the soil could be successfully remediated by natural attenuation. In other words, not only were viable hydrocarbons utilizing microbes present but they were efficient in mineralizing the hydrocarbons.

Biodegradability in the undefined Environments (Soils)

The biodegradability of the total petroleum hydrocarbon, (TPH) of Nigerian crude Oil within the un-defined consortium of microbial population in the Niger Delta soils was qualitatively evaluated after the last phase of two-week enrichment technique using Gas Chromatography, GC. Table 2 shows the microbial load of the soil samples while Fig. 6-11 show the chromatograms of the residuum hydrocarbons after the enrichment period.

It was observed that the biodegradation or utilization of the hydrocarbons in the crude oil by the un-defined consortium resulted in the growth of the microbial populations, as there were increases in the microbial load of both bacteria and fungi during the period of enrichment as shown in Table 2.

The results showed that all the soil samples harbored some population (consortium) of hydrocarbon degraders whose activities resulted in the biodegradation of hydrocarbons in the crude oil.Consequently, this could be the reason forthe disappearance of majority of the components of petroleum hydrocarbons. It is a strong implication that the indigenous consortium in the Niger Delta of Nigeria, though non-defined, could effectively biodegrade crude oil. This is in agreement with the result of previous work, which reported the degradation ability of indigenous microorganisms on component of petroleum hydrocarbons (Margesin *et al*., 2003). Consequently, there is need to encourage the use of bioremediation in the region.

Table 2: Indigenous Microbial Populations in Enrichment Medium

Site Location	Site Description	Microbial Type	Phase I (cfu/g)	PhaseII (cfu/g)	Phase III (cfu/g)
REE	A	Bacteria	2.00×10^8	1.60×10^9	1.50×10^{10}
		Fungi	ND	0.43×10^6	6.25×10^6
	B	Bacteria	7.2×10^8	3.18×10^9	6.00×10^{10}
		Fungi	7.50×10^6	0.93×10^6	1.05×10^7
RGK	A	Bacteria	2.35×10^8	2.40×10^9	5.00×10^{10}
		Fungi	1.35×10^6	3.30×10^6	5.75×10^6
	B	Bacteria	7.18×10^8	7.00×10^9	2.00×10^{10}
		Fungi	7.40×10^6	3.37×10^6	5.50×10^6
RTP	A	Bacteria	3.45×10^8	8.25×10^9	5.00×10^{10}
		Fungi	3.65×10^6	2.70×10^6	1.25×10^7
	B	Bacteria	5.93×10^8	1.20×10^9	5.50×10^{10}
		Fungi	3.35×10^6	2.98×10^6	$1,75 \times 10^7$

Key: A-uncontaminated soil, B- contaminated soil, RTP-Tai (Pete), REE-Ebubu (Eleme) and RGK-Kpor (Gokana).

The analysis of crude oil hydrocarbons by GC-MS is a tool to evaluate the biodegradation process and can play an important role in validating the CO_2 evolution data as a tool for evaluating hydrocarbons degradability.

In comparing the chromatographic properties of the sample from different locations, control flasks (not containing soil either crude oil contaminated or not) and the experimental flasks (containing soil either crude oil contaminated, A or not, B) shown in Fig. 6-11, some disappearances ofthe hydrocarbons could be related to abiotic factors such as weathering, dissolution, evaporation and others.A similar result was observed in some previouswork (Malik and Ahmed 2012) and these factors were known to have contributed to the disappearance and decrease of significant quantities of the aliphatic and aromatic hydrocarbons particularly the fractions with low molecular weights.

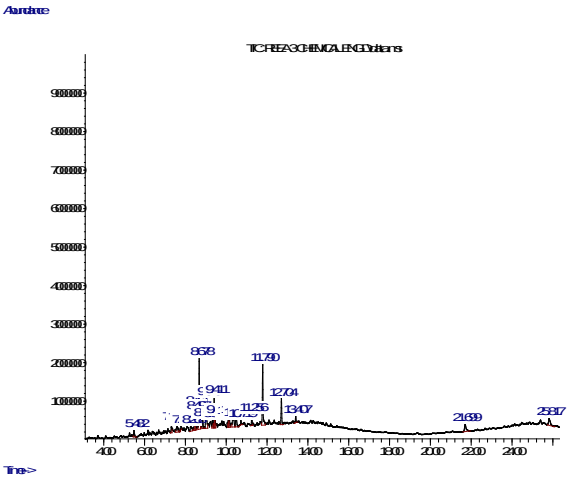

Fig. 6: chromatograms of crude oil in mineral salt medium with soil sample of REEA after the last phase enrichment.

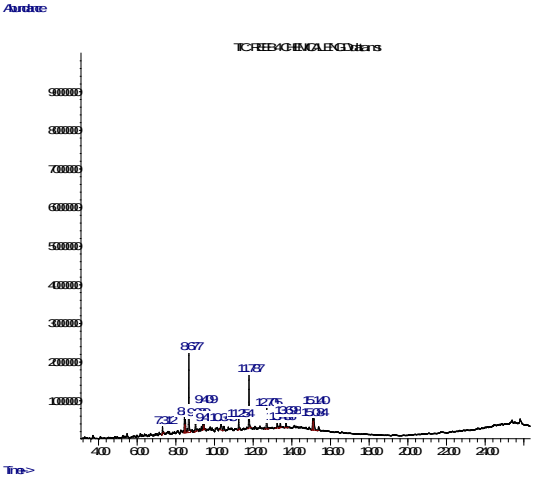

Fig. 7: GC chromatograms of crude oil in mineral salt medium with soil sampleof REEB after the last phase enrichment.

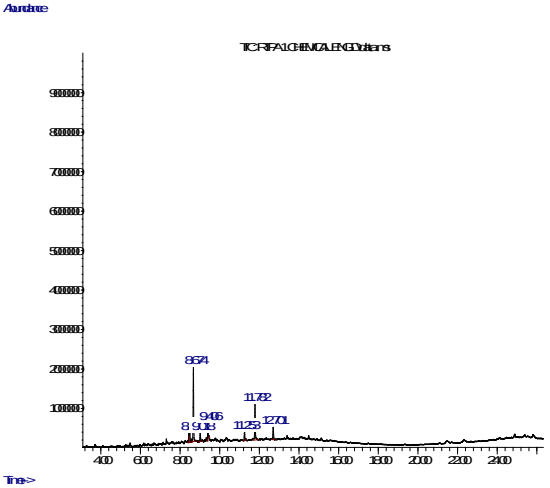

Fig. 8: GC chromatograms of crude oil in mineral salt medium with soil sample of RTPA after the last phase enrichment.

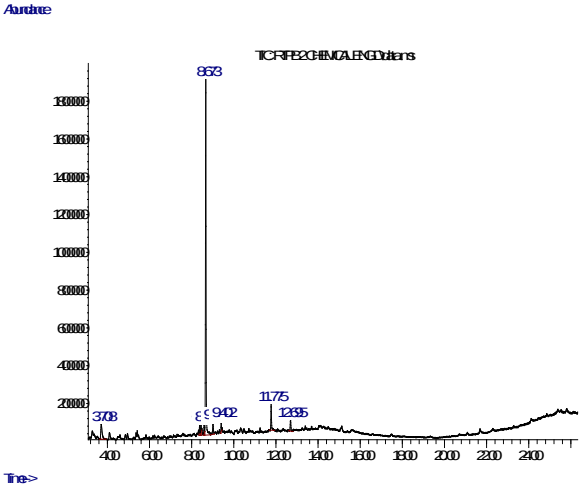

Fig. 9: GC chromatograms of crude oil in mineral salt medium with soil sample of RTPB after the last phase enrichment.

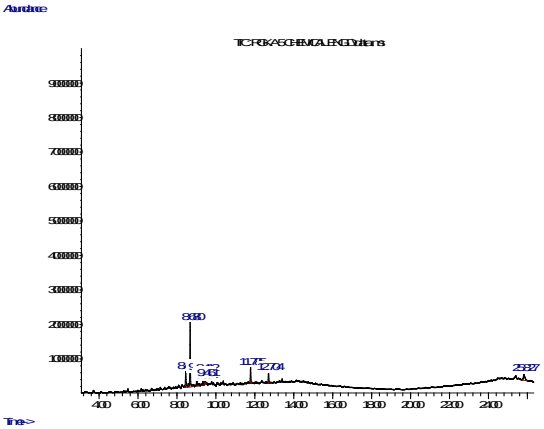

Fig. 10: GC chromatograms of crude oil in mineral salt medium with soil sample of RGKA after the last phase enrichment.

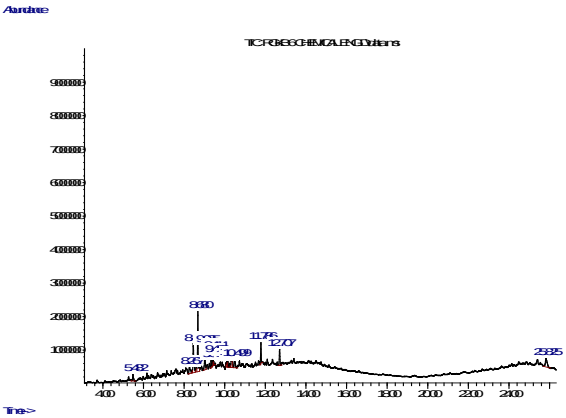

Fig. 11: GC chromatograms of crude oil in mineral salt medium with soil sample of RGKB after the last phase enrichment.

Chromatographic characteristic of various soil samples showed removal or disappearance of different hydrocarbons. The degree of disappearance was found to be more in the location RGKA and RTPB, where 2, 6, 11- trimethyldodecane and 2, 6- dimethyl heptadecane respectively were the only identified hydrocarbon peaks in chromatograms (Table 2). The 2, 6- dimethyl heptadecane was identified in every other location except in RGKA while 2, 6, 11- trimethyldodecane was found only in locations such as RGKA and RTPA. This could be explained that consortium of hydrocarbon utilizing microbes present in different locations have different capabilities. Hence, there is need of bioaugumentation with selected strains to degrade the recalcitrant molecules.

The location REEB was found to have least biodegradation capability of 50% when comparing its chromatographic properties with other locations. This could be attributed to depressed microbial activities and lower percentages of hydrocarbon utilisers observed for this location during the respiratory study and microbiological properties carried out on the site (Fig. 3). Therefore, activities of the consortia as with the respiratory study validated the major observation in the disappearances of the hydrocarbons. It could be deduced that the disappearances of the hydrocarbons in the crude oil were actually proportional to the microbial activities.

Table 3: Interpretation of the Gas Chromatograph Analysis Showing Residual Hydrocarbons (After the last two weeks of degradation in enrichment)

CRUDE OILCONTROL	**REEA**	**REEB**	**RGKA**	**RGKB**	**RTPA**	**RTPB**
2-methyl Naphthalenexxx						
2, 6, 10- trimethylDodecane						
2, 7- dimethyl Naphthalenexxx						
1,7- dimethyl Naphthalenexxx						
2, 3, 6- trimethyl Naphthalenexxx	xxx	xxx		xxx		
Tridecane						
Heptadecane						
2, 6- dimethyl heptadecanexxx	xxx	xxx		xxx	xxx	xxx
Hexadecanexxx						
2, 6 ,11-trimethylDodecanexxx			xxx		xxx	
Nonadecane						
Heptacosane						
Heneicosane						
Dodecane		xxx				
Tricosane						
Hexadecanexxxxxx		xxx				
Octadecane						
5- butyl docosane						
Hexacosane						
Octacosane						
% Biodegradation	62.50	50.00	87.50	75.00	75.00	87.50

Key: xxx- indicates Presence, A-uncontaminated soil, B- contaminated soil, RTP-Tai (Pete), REE-Ebubu(Eleme) and RGK-Kpor (Gokana).

CONCLUSIONS

From the findings of this work, the following conclusions are made:

(i) The environment under study has a natural consortium of microorganisms that have biodegradation abilities.

(ii) It has been established that with slight exception, application of bioremediation in this region is possible.

(iii) At locations such as REE and RGK where biodegradation of the crude oil hydrocarbon components were relatively lower in comparison to RTP, there would be need to bioaugument and biostimulate the process of remediation in order to restore the environment.

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