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Full Length Research Paper

Microbial population changes in tropical agricultural soil experimentally contaminated with crude petroleum

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Impacts of crude petroleum pollution on the soil environment and microbial population dynamics as well as recovery rates of an abandoned farmland was monitored for seven months spanning the two major seasons in Nigeria with a view to establishing process conditions necessary for development of effective strategies for bioremediation. The physico-chemistry of the control and contaminated soils differed just significantly ($P < 0.05$). Whereas these factors were relatively stable over the period of investigation for the control site, a downward trend was observed for the experimental. The polluted soil showed significant diversity in structure and number of flora .There was an initial drop in microbial population densities at the onset of pollution but, a gradual increase was observed thereafter. Higher counts of microflora were obtained for April, May, June and July samples which coincided with the onset and peak of wet season. A rapid and significant reduction in residual oil concentration was observed during this period. Overall, nearly 100% of the crude oil pollutant was degraded within the 28-week study period. The residual oil concentration gave a high but negative correlation coefficient ($r = -0.84$ to -0.90) with total heterotrophic and hydrocarbon-utilizing populations. On application of data generated to model equations, approximately 60.5 weeks would elapse before the contaminated soil could recover from the impact of the oil. Our results show that a natural population readily able to degrade crude oil is present in the soil chosen for this study. However, it may be necessary to monitor the level of inorganic nutrients and adjust some appropriately to enhance biodegradation of the organic pollutant.

Key words: Biodegradation, crude petroleum, heterotrophic counts, hydrocarbon-utilizers, microflora, pollution, residual oil concentration.

INTRODUCTION

Petroleum production and operations produce serious ecological problems. Pollution of environment due to the accidental oil spillage and seepage and ruptured pipelines is very common and has become a major concern to governments, individuals, environmental activists and the communities in the immediate environment. This problem has been further compounded by sabotage and vandalization of pipelines in restive communities, particularly in the Niger-Delta region of Nigeria. Thus, oil pollution, despite the progress of recent years, will remain a considerable problem.

Microbial degradation is known to be an efficient process in the *in situ* decontamination of oil-polluted environments due to its plasticity and, particularly, environmental compatibility. Two approaches can be envisioned for the application of the method: either the contaminated sites can be inoculated with specific, capable organisms (bioaugmentation), or the activity of indigenous organisms can be enhanced *in situ* by addition of appropriate nutrients and inducers (biostimulation) (Morgan and Watkinson, 1989; Sylvestre and Sondossi, 1994). Owing to the high degree of success on the abilities of micro-organisms (majority of which are bacteria) to completely mineralize crude petroleum and petroleum products under laboratory conditions, many researchers have suggested the augmentation of these

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strains to hasten remediation (Amund and Nwokoye, 1993; Nwachukwu, 2001; Adenipekun and Fasidi, 2005; Ojomu et al., 2005; Ilori et al., 2006). Interestingly, some commercial strains have also been developed with capability to degrade petroleum products. Despite the apparent simplicity of bioaugmentation, there have been many failures (Wagner-Dobler, 2003; Thompson et al., 2005). One limitation of these strains is their survival in the competitive environment with the indigenous populations. Another major problem is that the intermediary products of degradation often become toxic to the organisms (Barriault and Sylvestre, 1993; Unterman, 1996; Vidali, 2001). Since it is well documented that oil-degrading organisms are ubiquitously distributed in both polluted and undisturbed soil, natural attenuation is the simplest and the most cost-effective approach to decontamination of polluted matrices. This is generally the first choice of remediation because quite obviously it requires no intervention, just monitoring of the natural progress of degradation.

Whichever approach is eventually employed in bioremediation, there is no doubt that the success of it will depend largely on many environmental conditions not all of which are fully understood. If bioremediation of contaminated matrices are to be effectively used, in-depth knowledge about the physico-chemical properties of the contaminated site, the amount of target pollutant, microbial flora and the nature and types of the indigenous organisms which mediate the detoxification of the pollutant will help determine the process conditions that should be used in practical treatment systems. Results obtained from such studies could be useful in designing novel strategies that may be necessary in rehabilitating polluted land, particularly under field conditions where application of data generated at the laboratory level are prone to scale-up problems. In other words, field research has an important role to play in evaluating the intrinsic degradation abilities of the indigenous microorganisms in eliminating crude oil pollutant (Solano-Serena et al., 2000) and in providing clues to scale-up problems associated with applications of laboratory experiments to field conditions, which are usually larger, more complex and, therefore, more difficult to manage. In this preliminary study, a pilot project was designed to monitor the natural recovery rates in terms of microbial communities and physico-chemistry of an agricultural land contaminated with crude petroleum. Thus, the main focus was to generate information on a time-programmed basis about the degradation kinetics of crude oil and impacts on the soil. Kinetic data of this nature could be useful in developing models and predict the time required for environments polluted with the target compound to recover naturally.

MATERIALS AND METHODS

Study site

An experimental site (0.7 × 0.7 m) was mapped out on an abandoned farmland behind the Faculty of Science complex, University of Lagos, Nigeria. The site was flooded with 5 liters of Escravos crude oil (specific gravity, 0.84; colour, dark brown; pH, 5.24) and monitored over a period of 8 months spanning the two major seasons in Nigeria (December 2005 – July 2006; dry and wet seasons) for natural rate of removal of the oil pollutant and changes in community structure.

Sample collection

Soil samples were collected randomly from 0 – 15 cm depth at an interval of 4 weeks throughout the study period. Oil penetration into the soil was very slow and hardly exceeded 13.5 cm. Each sample was a composite of collection from 5 different sectors of the site. Sample for physico-chemical analysis were collected in sterile wide-mouthed screw-cap glass bottles. Analysis commenced immediately upon arrival in the laboratory. Similarly, uncontaminated soil samples (control) were also taken routinely from the area outside the experimental site for comparison.

Physico- chemical analysis of soil samples

The pH of the soil samples was determined with a pH meter (Jenway 3051) in 1:1 soil solution in distilled water. The moisture level, organic content, total nitrogen content, potassium content and available phosphorous were determined as described previously (Bray and Kurtz, 1945; Black, 1965; Chopra and Kanwar 1998). The Residual hydrocarbon concentration was extracted from the soil using n-hexane: dichloromethane solvent systems (1:1) and quantified gravimetrically (Venosa et al., 1990; Yveline et al., 1997). The oil extract was placed in an oven at 80^{o}C for 5 – 10 min to evaporate the solvent system after which the residual oil was obtained by difference in mass.

Microbiological analysis

The total heterotrophic bacterial and fungal counts were enumerated by plating aliquots (100 µl) of appropriate diluted soil samples on nutrient agar and acidified potato dextrose agar containing streptomycin (1 mg/100 ml), respectively. Starch casein agar was employed in determining the population density of actinomycetes according to the method of Kuster and Williams (1964). The nitrogen fixing bacterial counts were estimated using the Ashby's mannitol agar. All plates were incubated aerobically at room temperature (30^{o}C) counted after 48, 96, 120, and 168 h, respectively for bacteria, fungi, actinomycetes and nitrogen fixers.

Similarly, the population of hydrocarbon-utilizers was estimated on mineral salts (MS) medium formulated by Kästner et al. (1994). The medium contained (in g/L) Na_2HPO_4, 2.13 g; KH_2PO_4, 1.30 g; NH_4Cl, 0.50 g and $MgSO_4.7H_2O$, 0.20 g. Sterile trace element solution (1.0 ml/L) of Bauchop and Elsden (1960) was aseptically added to the medium after sterilization. The pH of the medium was adjusted to 7.2 and 5.6, respectively for bacterial and fungal estimations. The MS medium was also fortified with nystatin (50 µg/ml) for bacterial and 1 mg/100 ml of streptomycin for fungi. Sterile crude petroleum served as the sole carbon and energy source and made available to the cultures through vapour-phase transfer (Raymond et al., 1976). Plates were counted after incubation at room temperature for 5 – 7 days. The percentage of hydrocarbon-utilizers relative to the heterotrophic population for each time point was subsequently determined.

Bacterial colonies were screened and identified based on the taxonomic schemes of Cowan and Steel (Barrow and Feltham, 1995). The microscopic and macroscopic features of the hyphal mass, morphology of cells and spores, nature of the fruiting bodies were some of the criteria adopted for the classification of fungal isolates.

Statistical analysis

The data obtained for both the polluted and control soils over the experimental period were fitted to linear and non-linear regression, correlation, analysis of variance and t-test statistics using the Prism version 4.03 computer software programme (GraphPad Software, San Diego, CA. USA). Significance limits were set at the 95% probability level.

RESULTS

The changes in the physico-chemical profiles in both contaminated and undisturbed (control) soils over a period of 28 weeks are summarized in Figure 1. Generally, the trends observed for these variables were much more remarkable in contaminated soil, with almost stable pH values in control soil over the period. The pH of soils at the oil-polluted site ranged between 5.5 and 6.7 while the control ranged from 6.7 – 7.0. There was a gradual and significant reduction in the nitrogen content, available phosphorous and potassium content in the polluted soil. Similar trend was observed for moisture content until week 12 after which it increased consistently and peaked at 12.0% on week 28 (Figure 1). Interestingly, these periods coincided with wet season. In contrast to other physico-chemical parameters, there was a surge in the organic matter level of the contaminated soil. This trend continued until week 12 after which it decreased significantly. Generally, the physico-chemistry of the contaminated soil varied very significantly (t-test) from those obtained for soil at $P < 0.05\%$ level of significance.

The distribution and population densities of different microbial groups present in the soil samples are given in Figure 2. Although the differences were not significant at 5% level, at any week, the microbial communities were found to be higher in contaminated soil than the control. The exception to this observation was the population densities of heterotrophic fungi and nitrogen fixers which were respectively 50 – 100% and 45 – 70% more than what was obtained for polluted soil. In the contaminated soil, population densities of fungi and bacteria increased gradually but slowly between the first 12 weeks of pollution (Figure 2) with a corresponding insignificant decrease in the oil content. However, samples collected between the month of March (week 12) and July (week 28) gave higher counts of total heterotrophic bacteria and fungi, hydrocarbon-utilizing bacteria and actinomycetes, especially in polluted soil than observed in other months. Incidentally, these periods coincided with the beginning and the peak periods of wet season. Similarly, the moisture contents were higher during these months. The percentage hydrocarbon-utilizers obtained for the polluted and control soils relative to the total heterotrophic microorganisms is shown in Figure 3. Interestingly, variance analysis revealed that the values differed just significantly ($P < 0.05$).

The gravimetric data as depicted in Figure 4 for the residual hydrocarbon concentration gave a downward trend over the period of study. Similar to observations in microbial populations, approximately 14.61% degradation of the oil was obtained between the months of December (week 0) and March (week 12) indicating a degradation rate of 53.33 ppm/week. However, in the following month (April; week 16) percent degradation obtained was 89.50 giving a record increase of 74.89%. Overall, the results revealed that the oil content decreased to 206 from 4380 ppm at week 28, giving a total disappearance rate of approximately 150.5 ppm/week (Table 1, Figure 4).

Table 1 gives a summary of the growth characteristics of the microbial flora in both contaminated and control soils. Mean generation times were generally low in polluted soil while corresponding higher values were obtained for control soil. The highest generation time was obtained for fungi (82.52 week). When the data obtained over the period were fitted into population model dynamics as described by Nwachukwu (2000), it was observed that it would still require additional 32.5 weeks for the soil to recover its original conditions in terms of microbial population densities, suggesting that approximately a total of 60.50 weeks would elapse before the ecological balance of the soil is restored (Table 1). Both the heterotrophs and hydrocarbon utilizers correlated highly but negatively with residual oil content ($r = -0.90$ for heterotrophs and -0.84 for the hydrocarbon-utilizers) indicating that the increase in the microbial flora was associated with significant reduction in the concentrations of the oil pollutant over the period.

Table 2 summarizes the kinds and relative abundance of microbial communities in the two soil types. Generally, the microbial population of the contaminated soil was higher both in number and types of species than the control. The organisms were more abundant during the wet season and when the level of the oil pollutant has significantly reduced. An unidentified bacterium P6, which was present in the control soil, was not detected in the contaminated soil, thus, suggesting oil toxicity and zero oil-tolerance of isolate P6. Also *Micrococcus roseus, Micrococcus* sp., *Nocardia* sp., *Hansenula* sp. and *Candida* sp. that were almost absent in the control were abundant in the oil-polluted soil (Table 2).

DISCUSSION

The release of crude oil and petroleum products into the environment has resulted in many problems that are of much global concern (Wang et al., 1994). The long persistence of crude petroleum pollutant in environments was attributed to low numbers of hydrocarbon-utilizers and to the toxicity of crude oil on natural flora (Amund and Igiri, 1990; Atlas, 1991; Vidal, 2001; Hamamura et al., 2006). As a result, bioaugmentation or biostimulation is often suggested to circumvent this problem. However, the lack of appropriate data on the contaminated site such as the microbial flora, environmental conditions and other necessary factors to optimize the degradation of

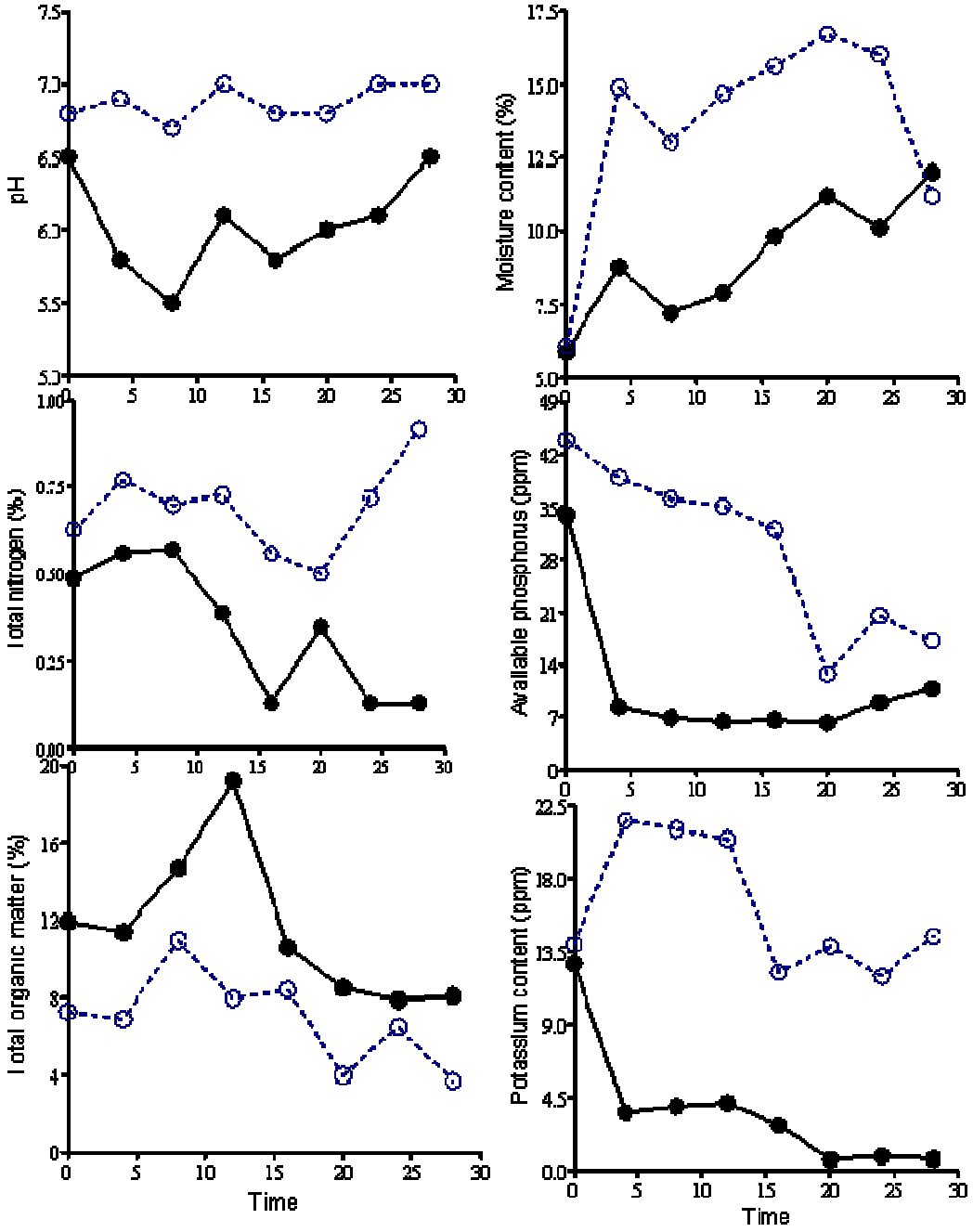

Figure 1. Physico-chemical dynamics of agricultural soil polluted with crude petroleum (●) over a period of 28 weeks spanning both wet and dry seasons. Values obtained for control soil are represented with open symbols. Data presented are averages of three replicate determinations. Error bars were eliminated for clarity. Soil samples were analyzed immediately after collection

crude oil in soils, usually result in failure of such remediation programme. Therefore, in this study, natural attenuation of crude oil contaminated farmland was monitored with a view to gaining an insight into process conditions involved in crude oil degradation. Such information would be useful in predicting approximate timeframe required for recovery and ultimately in developing effective bioremediation strategy for contaminated soils. The results of our investigation show that crude oil exhibited a great impact on the ecology of the soil by causing drastic changes in the microbial community structure, and physical and chemical properties of the soil. The initial drop in both biotic and abiotic components of the contaminated site confirms the toxic impacts of

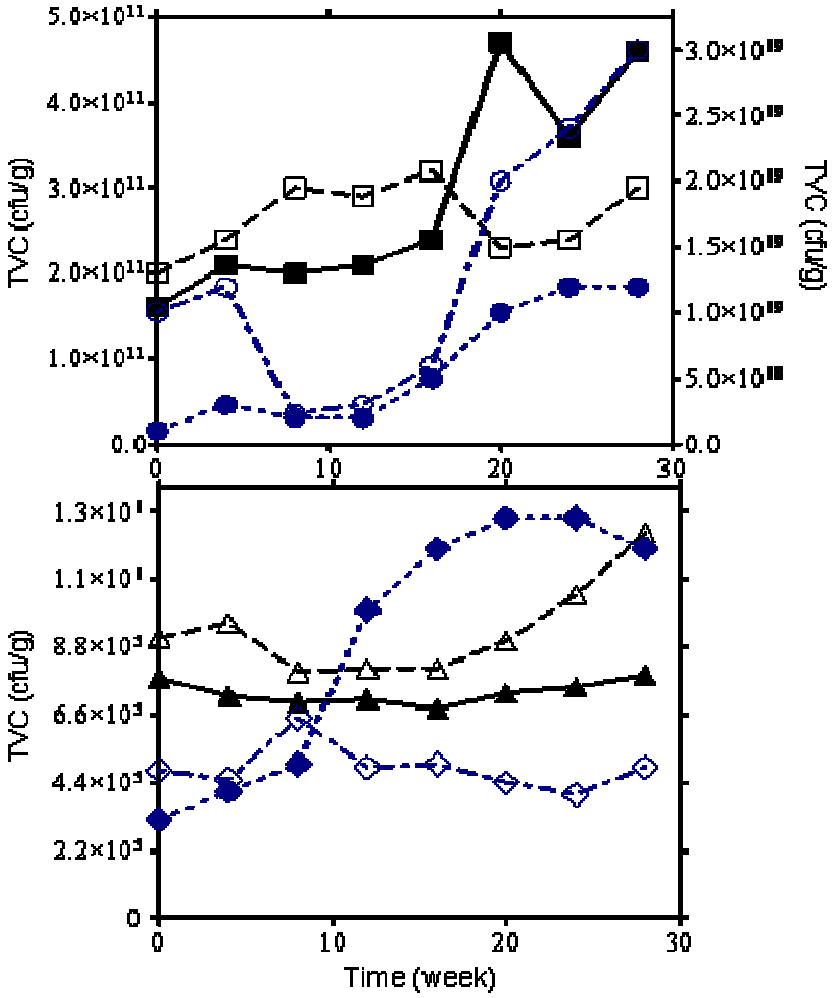

Figure 2. Population dynamics of microbial communities in contaminated and control soils. Compartment A: total heterotrophic bacteria (■), total heterotrophic fungi (●); compartment B: total actinomycetes (♦), total nitrogen fixing bacteria (▲). Respective values obtained from control soil are represented with open symbols. All values are averages of three replicate determinations. Error bars were eliminated for clarity.

crude oil (Ward et al., 1980; Ladousse and Tramirr, 1991).

Microorganisms generally require mineral (inorganic) nutrients sources for growth (Andrew and Jackson, 1996). If any of the required nutrients is lacking or becomes limiting, particularly the macromineral elements, pollutant degradation reaction may be slow even though carbon and energy sources required for growth are available (Giordani et al., 1998; Lehtola et al., 1998; Vidali, 2001). This may partially explain the persistence of pollutants in soil in spite of capable microbial population. For instance, the consistent decrease in phosphorus and nitrogen may be due to their high demand by micro-organisms for sugar phosphorylation, nucleic acid synthesis and other cellular processes (Andrew and Jackson, 1996). Also, it has been reported that crude oil pollutant could destroy inorganic nutrient sources by reacting with them along with other substances present in soil, for example, sulphonation, nitration and chlorination etc., with SO_4^{2-}, NO_3^-, and pesticides or chloride sources, respectively (Stevens and Udall, 1981; Teal et al., 1992; Andrew and Jackson, 1996). Therefore, the observed decreases for these mineral nutrients may be the reason for the initial drop in the population of micro-organisms when, in fact, the total organic matter showed increasing trend. This inference is further corroborated by the fact that higher values of N, P, K were encountered in the control soil (Figure 1). Therefore, in soils with low level of available nutrients, such as those from which this study was carried out, it may be necessary to adjust N and P concentrations to enhance biodegradation of the organic pollutant.

Table 1. Growth kinetics of total heterotrophic and hydrocarbon-utilizing organisms in crude petroleum contaminated and control soils.

Soil site	Bacteria				Fungi				Actinomycete		%Dg[1]	Dg rate (ppm/w)[2]	Recovery time (w)
	Heterotroph		HC-U[3]		Heterotroph		HC-U		Tg (w)	μ (w^{-1})			
	Tg (W)[4]	μ (w^{-1})[5]	Tg (w)	μ (w^{-1})	Tg (w)	μ (w^{-1})	Tg (w)	μ (w^{-1})					
Polluted	17.18	0.04	21.69	0.03	9.2	0.02	82.52	0.01	18.34	0.04	96.21	150.5	32.50 (from week 28) or 60.50 (from week 0)
Control	125.6	0.006	$6.93x10^6$	$1 x 10^{-7}$	8.75	0.079	$6.93x10^6$	$1x10^{-7}$	$6.93x10^{-7}$	$1x10^{-7}$	N/A[6]	N/A	N/A

[1]Percent degradation; [2]overall degradation rate; [3]hydrocarbon-utilizers; [4]mean generation time; [5]specific growth rate; [6]not applicable.

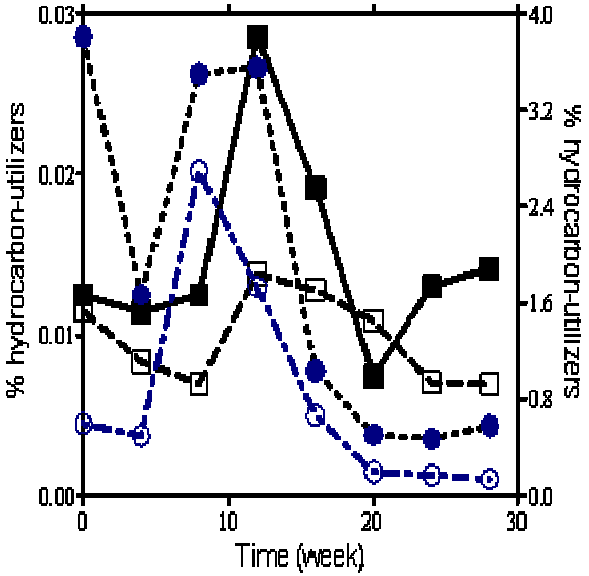

Figure 3. Percent population dynamics of hydrocarbon degrading bacteria (■) and fungi (●) relative to total heterotrophs obtained for contaminated soil. Respective values obtained from control soil are represented with open symbols. All values are averages of three replicate determinations. Error bars were eliminated for clarity.

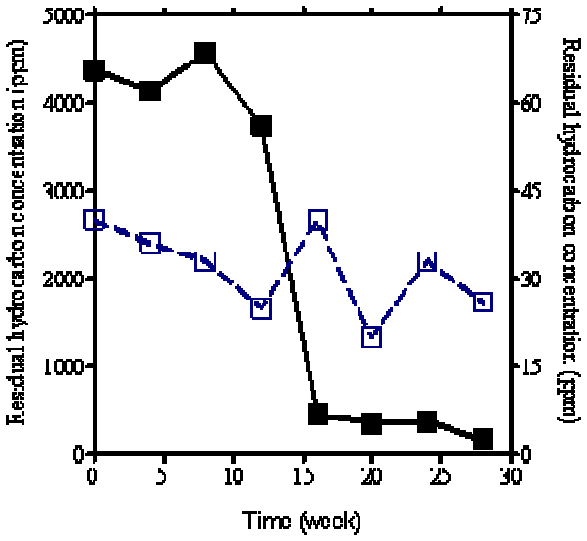

Figure 4. Dynamics of hydrocarbon concentration of crude petroleum contaminated (■) and control (□) soils. All values are averages of three replicate determinations. Error bars were eliminated for clarity.

Similar to the earlier report of Amund et al. (1993), we observed higher moisture level in the control site (Figure 1). The observed reduction in moisture of the contaminated soil could be attributed to the factor that hydrocarbons could render some surface hydrophobic properties to the soil thereby reducing the water holding capacity of the soil (Dibble and Bartha, 1979). Hydrocarbon pollution also reduces the bulk density of soil and increases its porosity. It is noteworthy that the moisture level was highest for both sites during the months of March (week 12) to July (week 28). Interestingly, this coincided with the period when the changes in microbial populations increased exponentially and also the beginning and peak of wet seasons with significant reduction in the oil content of the soil, indicating that the increase in microbial population was the key factor responsible for oil depletion.

As depicted in Figure 2, the population densities of heterotrophic bacteria, fungi, actinomycetes, and nitrogen-fixers were higher during the first 16 weeks of study in the control soil. This trend readily suggests toxicity of crude oil to some of the microbial communities in the contaminated soil. Although microbial counts were highest for both sites during the months of March and July which incidentally are wet season periods as earlier indicated, probably, run-offs after rainfalls carrying materials including microorganisms and nutrients contributed to the higher levels of microorganisms. There was an over 100% increase in the density of actinomycetes in the contaminated site during week 12. Interestingly, this was the period of active removal of crude oil pollutant.

Table 2. Relative abundance and types of micro-organism in control and contaminated.

Microorganisms	Control								Contaminated							
Bacteria	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**
Micrococcus roseus	-	-	-	-	-	-	-	-	-	-	†	†	†	†	†	†
Micrococcus luteus	†	†	†	†	†	†	†	†	-	-	-	-	-	†	†	†
Micrococus sp.	-	-	-	-	-	-	-	-	-	†	-	†	†	†	†	†
Acinetobacter sp.	†	†	†	†	†	†	†	†	†	†	†	†	††	††	††	††
Bacillus subtills	†	†	†	††	††	††	††	††	†	†	†	†	††	†	†	†
Bacillus sp.	†	†	†	†	†	†	†	†	-	-	-	-	-	-	-	-
Pseudomona aeruginosa	†	-	-	†	†	†	†	†	†	-	†	††	††	††	††	††
Pseudomonas sp.	-	-	†	†	†	†	†	†	†	-	†	††	††	††	††	††
Nocardia sp.	-	-	-	-	†	-	-	-	-	†	-	†	††	†	††	†
Corynebacterium sp.	†	-	-	†	†	†	†	†	†	†	†	††	†	†	††	††
Streptomyces sp.	†	†	†	†	††	††	-	-	†	†	†	††	††	†	†	††
Unidentified isolate p6	†	†	†	-	-	-	-	†	-	-	-	-	-	-	-	-
Fungi																
Aspergillus niger	†	†	†	†	†	-	†	†	†	††	††	††	††	††	††	††
Aspergillus flavus	-	-	-	-	-	†	†	†	-	-	-	-	†	†	†	†
Trichoderma sp.	†	†	†	†	†	†	†	†	†	†	†	††	††	††	††	††
Penicillium sp.	†	-	-	-	-	†	†	†	-	-	-	†	†	†	†	†
Hansenula sp.	-	-	†	-	-	†	-	-	-	-	†	†	†	†	††	††
Thodotorula glutini	-	-	-	-	†	†	-	-	††	††	†	†	†	†	-	†
Saccharomyces sp.	††	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†
Candida sp.	†	-	-	-	-	-	-	-	†	†	†	†	†	†	†	†
Unidentified isolate F5	†	-	-	†	†	†	-	-	†	†	†	†	†	†	†	†

†, Present; ††, abundant; -, absent. D, J, F, M, A, M, A, M, J, J, respectively represent December, January, February, March, April, May, June and July. Sites were sampled every 4 weeks spanning the two major seasons in Nigeria.

Actinobacteria have been shown to have hydrocarbon degrading abilities even though they do not seem to compete successfully in contaminated soil. However, their slower growth may infer a more dominant role in later stages of hydrocarbon degradation (Jensen, 1975), as shown by a significant surge in population between weeks 20 and 28. In contrast to population dynamics of bacteria, there was a consistent increase in total fungal population in the control site more than the oil affected site throughout the study period (Figure 2). This suggests to us that fungi are perhaps, less oil tolerant than bacteria and more importantly, it reinforces the fact that bacteria are the major microbial group that actively partake in the degradation of organic pollutants in the environment.

A comparison of the populations of hydrocarbon utilizers in the two ecosystems shows some remarkable differences (Figure 3). The greater number of hydrocarbonutilizers of the contaminated soil indicates that the presence of the crude oil in the site attracted and stimulated faster multiplication of hydrocarbon utilizers than obtained in the control soil. According to Calomiris et al. (1976) and Nwachukwu (2000), there is always an increase in the population density of hydrocarbon-utilizers in the ecosystems exposed to crude petroleum and petroleum products. The proportions of hydrocarbon utilizers relative to the total heterotrophs observed in this study were generally less than 0.01%, thus indicating that only a small fraction of the population actually participated in the decontamination of the organic pollutant (Figure 3).

Appreciable levels of hydrocarbons were observed in the control soil, although the concentration reduced significantly during the sampling period. It is, however, difficult to trace the hydrocarbon content of the control soil to any human activity since this land has yet to experience any oil discharge. Therefore, the hydrocarbon content was predominantly biogenic in origin. The pattern of degradation of the crude oil pollutant in the contaminated soil presents an interesting observation. The amount of oil degraded during the first 12 weeks was insignificant (14.61%). It is noteworthy that this coincided with periods of low microbial activity when most of the microorganisms were probably recovering from the toxic effects of crude oil (see Figure 2). Significant drop in the oil content (96.21%), concomitant with increase in microbial populations, were observed thereafter (between weeks 16 and 28), indicating a rapid recovery of the soil from the oil impact; hence, these weeks are highly critical in this study regarding recovery of the contaminated soil.

The presence of hydrocarbon contamination was associated with reduced phylotype diversity usually at the

onset of crude oil pollution (Stapleton et al., 2000; Van Hamme et al., 2003; Hamamura et al., 2006). Our results are comparable with these findings. This study demonstrates that crude oil contamination results in the selection of diverse microbial populations. The bacterial and fungal community structures of the contaminated soil demonstrated a marked rise in the proportion of species than the control site, especially during the wet season. Many of the organisms encountered in this study have been known to effectively degrade hydrocarbon pollutants (Atlas, 1992; Hamamura et al., 2006).

Kinetics of data emanating from this study indicate that it would take approximately 32.5 weeks more before the soil regains its natural original conditions. In other words, a total about of 60.5 weeks would elapse before the crude oil is completely eliminated from the environment. Practically, however, at week 20, plants were observed sprouting in the soil, indicating recovery of the land from oil pollution. The estimated recovery time was possible because of the sensitive and robust nature of the indigenous microbial flora of the soil in effectively utilizing the pollutant. As the oil decreased, trends in the succeeding species, particularly the hydrocarbon-utilizers started adjusting to less oiled environment. Eventually, the heterotrophic counts and hydrocarbon-utilizers would probably decrease to the initial population density after depleting oil when the soil would have regained its ecological balance as reflected by hydrocarbon-utilizers. This was not until after 32.5 weeks from week 28. Thus, the recovery time-frame represents the total time it would take the contaminated soil to regain its ecological balance and not when the pollutant would completely be degraded. Of course, we expect complete disappearance of the oil pollutant to occur much earlier and going by the overall degradation rate of 150.5 ppm/week, an additional 1.03 week may be required. However, Nwachukwu (2000) contended that with the long-term persistence of crude oil in the environment in addition to sampling problems, it may be impossible to monitor residual oil on a routine basis over a long period of time whenever there is oil spill in order to determine when it would be completely degraded. Therefore, this method of oil impact assessment using model equations can be adopted using the initial reproducible data generated to make a reliable prediction concerning the impact of the oil and when the contaminated soil would regain its ecological balance. Additionally, it can be adopted as a reliable biological index for reference purposes, policies and action plans particularly in deciding compensations to be paid to communities after crude oil pollution of their land.

The data in this investigation point out the need to give proper weight to the interrelated variables that influence microbial community structure in complex habitats such as soil. This study represents a successful attempt to investigate natural attenuation of crude petroleum in an abandoned farmland. The main conclusion of this study is that a natural population readily able to degrade crude oil is present in the soil chosen for this study. This is quite remarkable since this soil has never experienced oil spill major or minor. Therefore, decommissioning of such contaminated matrix by bioaugmentation may not be an option to consider. It is expected that environments such as Niger Delta region of Nigeria would have higher proportions of crude oil-degraders due to continuous exposure to crude oil contaminants. What may be limiting factor for biodegradation to occur *in situ* therefore could be nutrients such as P and N. The proper baseline studies therefore are needed to understand the site physico-chemistry before appropriate nutrient amendment could enhance the number of indigenous bacteria able to promote *in situ* biodegradation.

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