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The status of the soil microbial flora and oil content of Imore, a coastal community in Lagos state, Nigeria after petroleum spill and fire outbreak

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Abstract

Petroleum spill on soil is perilous, however, natural occurring hydrocarbon degrading microorganisms have the capacity to utilize the hydrocarbon as source of carbon and energy, thereby, enhancing their transformation and mineralization. Therefore, to ascertain the extent of damage and natural restoration of an oil spill site, this study compared its microbial flora and hydrocarbon content with an adjacent unpolluted zone, after 40 weeks. The results revealed that the number of heterotrophic bacteria and hydrocarbon utilizers remained at least one order of magnitude higher in the polluted zones (2.5×10^7 to 3.2×10^4) than in the unpolluted zone (2.3×10^3 to 2.8×10^4) throughout the study period. Conversely, the fungi population, which was three orders of magnitude higher at 0 d, drastically decreased later (120 - 150 d) with all zones having approximately 1.3×10^4 $cfug^{-1}$. At the end of the study, the extent of oil reduction was 1.6 % at the control zone while the polluted sites showed between 33 to 35%. The rate of hydrocarbon reduction was between 9.2×10^{-5} to 9.9×10^{-5} $mgd^{-1}$by 90 d, but declined to 3.3×10^{-5} to 4.1×10^{-5} $mgd^{-1}$by 150 d. Consequently, though the soil microbial flora seems to have adapted to the excess oil, the extent and rate of oil removal is not satisfactory, hence, additional remediation treatment is required for complete restoration.

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Introduction

The importance of crude oil production, refining, transportation and marketing to Nigeria external revenue cannot be over emphasized. This is basically because her economy is heavily dependent on the oil sector, whose revenue accounts for nearly 80% of the Government's foreign exchange earnings, over the past four decades (hwr.org, 2004; Anoliefo *et al.,* 2006). The refined product distribution network has a total of 5,001km of oil pipelines, consisting of 4,315km of multiproduct pipelines and 666km of crude oil pipelines (Osuno, 1989). These oil pipelines criss-cross the country and inter link the twenty-two petroleum storage depots strategically dispersed across the country and linked to the various refineries.

The communities that have oil facilities as well as oil producing communities are therefore, exposed to the hazards of oil pollution as a result of oil spills. In addition to oil spills that occur at every activity stage of the oil industry – oil prospecting, production, refining, transportation and marketing, these oil pipelines are frequently vandalised by miscreants to siphon their products to sell illegally. These activities are known to have ended disastrously on many occasions when they occurred, with incidents resulting in explosions followed by fire that consumes hundreds of people, vegetation and animal life (Nigeria oil directory, 1989, George-Okafor, 2009).

Oil spills result in the release of excess hydrocarbons into the environment leading to the pollution of land masses and water bodies. Due to the toxicity of oil, these spills adversely affect plant and animal species, including soil and water resources (Kinako 1997). The fate of hydrocarbon pollutants in the environment is determined primarily by its nature, amount and also by the relationship between the chemical, geochemical and biological characteristics of the environment (Bossert and Bartha, 1984; Bordenave *et al.*, 2007). Soil microorganisms are remarkable biological force due to their versatile metabolic activities that are essential for plant growth and soil fertility. Furthermore, Leahy and Colwell (1990) had stated that hydrocarbons in the environment are primarily degraded by the bacteria and fungi. And the ability of soil microbes to degrade organic pollutants in both laboratory culture and natural environment is well documented (Roelof van der Meer, 2006). The known hydrocarbon degrading bacterial genera include *Pseudomonas, Achromobacter, Flavobacterium, Nocardia, Arthrobacter and other coryne-forms, Vibrio, Bacillus, Micrococcus, Acinetobacter Actinomyces, Aeromonas, Alcaligenes, Coryne-bacterium*. Among the fungi genera are *Candida, Rhodotorula, Penicillium, Aspergillus, Cladosporium, Fusarium, Gonytrichum, Hansenula, Helminthosporium, Mucor, Oidio-dendrum, Paecilomyces, Phialophora, Rhodosporidium, Saccharomyces, Saccharomycopsis, Scopulariopsis, Torulopsis, Trichoderma* (Bossert and Bartha, 1984; Atlas and Cerniglia, 1995; Balba *et al.,* 1998).

Nevertheless, oil contamination of the soil environment may affect the population and activities of soil microflora at different functional levels, and reduce the growth and the yield of plants (Maliszewska-Kordybach and Smreczak, 2003). Meanwhile, hydrocarbon spill generally results in selective enrichment of culturable heterotrophic bacteria and hydrocarbon degrading microorganisms, thus reducing microbial diversity. This increase in hydrocarbon degraders consequently, enhances the breakdown or complete mineralization of the pollutant with time (Nyman, 1999; Saul *et al*., 2005, Roelof van der Meer, 2006). Therefore, the structure and dynamics of the indigenous soil microbial flora are imperative to persistence or biodegradation of the pollutants (Bordenave *et al*., 2007).

One of the numerous activities of petroleum pipeline vandals, led to an oil spill ensued by a fire incident at the Imore fishing community, a Lagos coastal village. This resulted in the loss of over a hundred lives and a

complete destruction of inter-tidal organisms. The fire outbreak that followed would most likely increase the concentration of highly toxic and recalcitrant polycyclic aromatic hydrocarbons (PAH). Thus, after about 40 weeks of the oil spill, this study attempted to establish the difference in the microbial flora, alongside the total petroleum hydrocarbon (TPH) content of the oil polluted site and an adjacent unpolluted site. Soil samples were collected every monthly (30 days) for a period of six months. This work intended to determine the extent of damage that occurred and also to verify the rate of natural restoration, since no remediation treatment was applied.

Materials and methods

Sampling site

The location of Imore community is as marked in the map (Fig 1). The impacted area was divided into approximately equal imaginary Zones; A, B, C and D, away from the epicenter of the spill, in an attempt to demonstrate the presence of a pollution gradient, if any existed. A fifth Zone; E (Ibasa village), served as the control, this was about 1kilometer away from the impacted site, but with similar vegetation.

Soil sample collection

Samples were collected every thirty (30) days for a period of one hundred and fifty (150). This spanned between July and December (wet season to dry season). In each of the impacted zones, A, B, C, D and the control un-impacted zone E, two sampling stations were randomly chosen per zone as replicates, Within each sampling station of the impacted and un-impacted control zone, sampling was done by throwing 3 replicate quadrants (4 x 4111), within which 20 g of soil samples were collected and placed in sterile polythene bags. The samples were transported immediately to the laboratory for analysis.

Enumeration of Total Heterotrophic bacteria and fungi

Heterotrophic bacteria and fungi were enumerated using the spread plate technique. Aliquots of serially diluted polluted soil samples were inoculated on appropriate media. Nutrient agar was used for bacteria, while for fungi; potato dextrose agar containing streptomycin (10 mg/l) was used. Incubation was carried out at room temperature (29 ± 2.0 °C). The total viable count was recorded after 18 to 24 h for bacteria and 48 to 72 h for fungi.

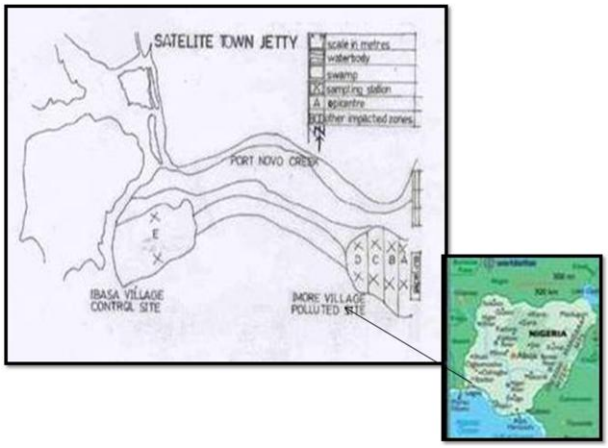

Fig 1. Map showing the study site.

Enumeration of hydrocarbon utilizers

Hydrocarbon utilizers were enumerated on mineral salts agar medium as described by Kastner *et al.* (1994) using crude oil as sole carbon source. The medium contained per litre 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. The pH of the medium was adjusted to 7 - 7.2. Trace elements solution (1 ml/l) described by Bauchop and Elsden (1960) was sterilized separately and added aseptically to the media. The inoculated plates were then inverted over sterile filter papers moistened with crude oil and held over the lid of the Petri dishes (Raymond *et al.*, 1976). The dishes were taped round with masking tape to increase the vapour pressure within them. Incubation was carried out at 27 °C for 48 to 72 h.

Identification of some bacteria and fungi isolates

A number of the bacteria and fungi isolates were randomly picked from the culture plates, and further identified up to the generic level. The pure cultures of the bacteria isolates were obtained, and identified based on their colonial morphology, cellular morphology and biochemical characteristics following the method of Cowan and Steel (Barrow and Feltham, 1995). Pure cultures of fungi were identified by viewing wet mounts on slides in lacto-phenol, observing and recording characteristics which were subsequently compared with the established identification key of Barnett and Hunter (1972).

Determination of the oil content

The residual oil in the soil was extracted twice with an n-hexane:dichloromethane solvent system (1:1) and quantified gravimetrically as described elsewhere (Nwachukwu, 2001; Adebusoye *et al*., 2010).

Statistical analysis

The analysis of variance (ANOVA) and t-test were determined using Prism version 5.03 computer software programmes (GraphPad Software, San Diego, CA. USA). Tests were carried out at 5% significance level.

Results

Total heterotrophic bacteria

The total viable count of the heterotrophic bacteria in all the plots during the experimental period is presented in Fig. 2.The total viable count in the control plot was 2.2 x 10^4 cfug^{-1} during all the sampling days, except at 120 d when a count of 2.1 x 10^4 cfug^{-1}was observed. Meanwhile the highest total viable count of 3.2 x 10^9 cfug^{-1} was noted in Zone A, the epicenter and B on 60 d, this was approximately five order of magnitude higher than the population in the control plots. These two Zones (A and B) also showed higher bacterial population than the Control throughout the experimental period. A population range of between 3.0 x 10^6 and 3.6 x 10^8 cfug^{-1} was observed in Zones C and D, from 0 d to 90 d. However, the population dropped to between 2.4 x 10^5 and 2.4 x 10^4 cfug^{-1} towards the end of the experimental period. This population range was close to the value (2.2 x 10^4 cfug^{-1}) noted in the Control Zone at that time. Nevertheless, there was a significant difference ($p< 0.0001$) between populations of heterotrophic bacteria when each of the polluted Zones was compared with the Control Zone using the student's t-test. However, 2way ANOVA showed no significant difference among all the plots during the period of the investigation.

Enumeration of the heterotrophic fungi

The population of the heterotrophic fungi in the Control Zone was 1.0 x 10^4 cfug^{-1} throughout the sampling period (Fig 3). However, at the polluted Zones (A,B,C and D), between 0 d and 90 d, a population (1.0 x 10^7 to 2.1 x 10^8 cfug^{-1}) which was three to four order of magnitude higher than that of the Control Zone was observed. Interestingly, towards the end of the sampling period (120 to 150 d), both the polluted and the unpolluted Zones had equivalent (between 1.0 x 10^4 and 1.3 x 10^4 cfug^{-1}) fungal population.

Table 1. Total oil content of soil samples (mg g-1).

Time(day)	A	B	C	D	Control
0	3.26	3.2	3.17	3.12	1.22
90	2.37	2.35	2.3	2.29	1.31
150	2.17	2.13	2.07	2.04	1.2

Values are averages of three replicate determinations.

Table 2. Percent and rate of hydrocarbon reduction at 90 and 150 d in all Zones.

	Percent reduction (%)		Rate of reduction (mgd^{-1})	
Zone	90 d	150 d	90 d	150 d
A	27.3	33.4	9.9 x 10^{-5}	3.3 x 10^{-5}
B	26.6	33.4	9.4 x 10^{-5}	3.7 x 10^{-5}
C	27.4	34.7	9.6 x 10^{-5}	3.8 x 10^{-5}
D	26.6	34.6	9.2 x 10^{-5}	4.1 x 10^{-5}
Control	-	1.6	-	1.8 x 10^{-5}

Enumeration of hydrocarbon utilizers

The population of hydrocarbon utilizers in each of the polluted Zones was significantly higher than what was noted in the Control Zone (Fig 4). At 0 d, the spill epicenter (A) and the Zone (B) next to it had higher number (3.4 x 10^6 and 3.2 x 10^6 cfug^{-1}) of viable hydrocarbon utilizers. This was two orders of magnitude higher than the population range of 2.4 x 10^4 to 3.2 x 10^4 cfug^{-1} observed at Zones C, D and E. However, at 30 d, Zone C had the highest population (4.1 x 10^7 cfug^{-1}) but a decline in population occurred in all the polluted Zones during the course of sampling. And at 150 d, a population of between 3.2 x 10^4 and 3.8 x 10^4 cfug^{-1} was observed while the Control Zone had 2.8 x 10^3 cfug^{-1}. The highest population noted in the Control Zone for the duration of the experiment was 2.4 x 10^4 cfug^{-1}.

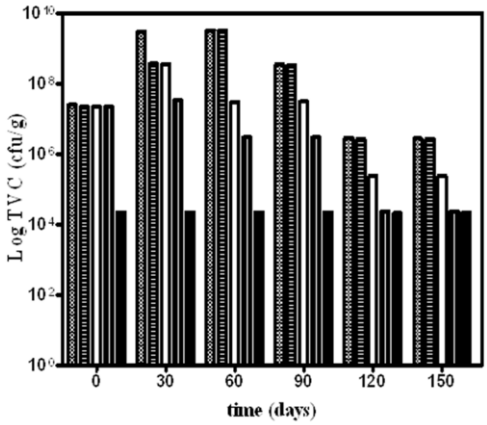

Fig. 2. Total viable count of heterotrophic bacteria at the different zones with time. A, B, C, D, Control. Data represents average of three replicate determinations.

Total oil content, extent and rate of reduction

The total oil content of the polluted and unpolluted zones is presented in Table 1. Expectedly, the spill epicenter (A) had the highest (3.26) value at the beginning of the sampling period. The oil content gradually decreased from Zone B to D, with the Control Zone showing the least value of 1.22 g. A consistent reduction in the TPH content was recorded for all the polluted sites from 0 to 150 d. Nonetheless, the Control Zone displayed an inconsistent increase to 1.31 at 90 d but this was still lower than the contents in the polluted sites. The extent and rate of reduction of the hydrocarbon content of the Zones were determined at 90 and 150 d. All the polluted Zones had approximately 27% hydrocarbon reduction at 90 d (Table 2). However, the highest rate of reduction (9.9 x 10^{-5} mgd^{-1}) occurred in Zone A, while Zones B, C and D showed 9.4 x 10^{-5}, 9.6 x 10^{-5} and 9.2 x 10^{-5} mgd^{-1} respectively. Conversely, at 150 d, Zone D showed a rate of degradation of 4.1 x 10^{-5} mgd^{-1}which was slightly higher than the range (3.3 x 10^{-5} to 3.8 x 10^{-5} mgd^{-1}) noted on the other polluted Zones. Similarly, the extent of hydrocarbon reduction by 150 d was higher (35%) in Zones C and D while Zones A and B had 33.4%. The Control Zone exhibited the lowest values (≈2% and 1.8 x 10^{-5} mgd^{-1}) of percent and rate of hydrocarbon reduction respectively.

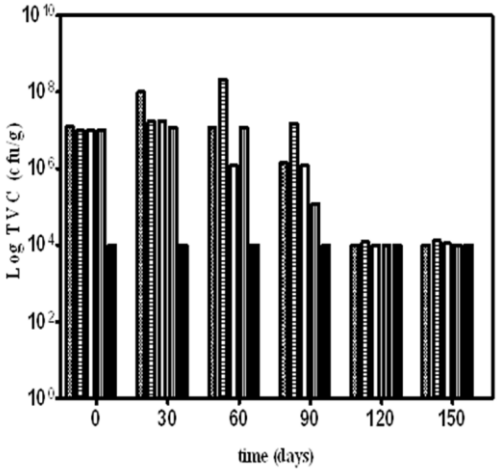

Fig. 3. Total viable count of heterotrophic fungi at the different zones with time. A, B, C, D, Control. Data represents average of three replicate determinations.

Identification of bacteria and fungi isolates

According to the cultural, morphological and biochemical characteristics, majority of bacterial

isolates were probably identified as *Corynebacterium* spp, *Pseudomonas* spp, while the fungal isolates belongs to *Aspergillus* spp, *Candida* spp and *Rhodotorula* spp.

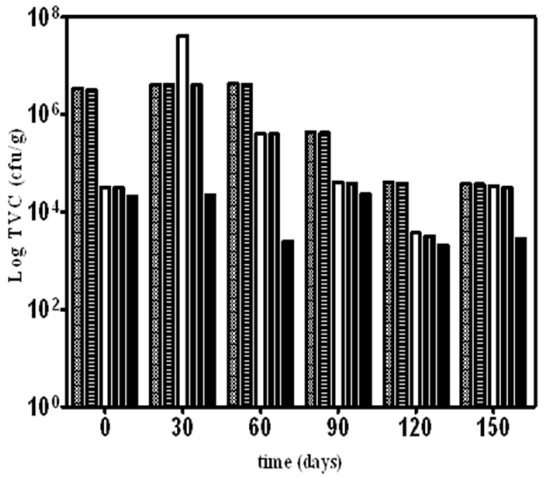

Fig. 4. Total viable count of hydrocarbon utilizers at the different zones with time. A, B, C, D, Control. Data represents average of three replicate determinations.

Discussion

Petroleum contamination of the soil ecosystem is always regarded as an environmental hazard. It usually leads to the inadequacy of the soil to serve as a habitat for plants, microorganisms and soil- living animals (Song *et al*., 1990). All the same, hydrocarbons are prone to degradation, transformation and mineralization by natural agents including microorganisms. Hence, this work attempted to assess the soil micro flora and petroleum (oil) content of an oil spill site.

Apparently, the number of heterotrophic bacteria, fungi and hydrocarbon utilizers were higher in the polluted zones than in the control zone particularly at the early stage of the study (Fig 2, 3 and 4). This observation can be attributed to the fact that hydrocarbon spill raises the concentration of soil organic carbon, which serve as substrates for microbial growth. Similarly, Saul et al. (2005) observed that a higher portion (29–100%) of the culturable aerobic heterotrophic colony-forming units (CFU) was cultured in a hydrocarbon contaminated soil, while only 0.41–2.8% represented the total direct counts for the unpolluted (control) soil. In any case, the population of microorganism usually increases after an oil spill because, they easily adapt by mutating and expression of the genes required for hydrocarbon degradation. Consequently, they multiply their population size at the expense of the easily utilizable carbon fractions of oil. It is also remarkable that the 40 weeks period that elapsed after the spill before this work commenced, must have given the indigenous heterotrophic microbes and the hydrocarbon utilizers, sufficient time to adapt and develop the requisite enzymes for degradation of the abundant hydrocarbons. Still, oil concentration beyond 3% may prove toxic to microbial growth, activity and also decrease diversity (Nyman, 1999, Saul *et al*., 2005, Osuji et al., 2006).

Nonetheless, the increase in microbial number in an oil polluted site is usually temporal because, after a while, the need for various growth limiting factors such as nutrients (nitrogen and phosphorus), water and other physicochemical parameters may tend to limit microbial growth (Roelof van der Meer, 2006). This could be attributed to the 1 to 3 order of magnitude decrease in the population of the various groups of microbes assayed towards the end of the study (Fig 2, 3 and 4). As a matter of fact, it is noteworthy that the sampling period commenced during the wet season (July) and proceeded into the dry season (December). Hence, the absence of rain water, an essential factor for microbial growth and metabolism would have affected cell viability and multiplication. Even though, the more consistent population observed concurrently in the control zone suggests that the hydrocarbon contamination had a huge impact on the micro flora. However, further studies establishing links between

other environmental parameters will provide more explanations.

Although, hydrocarbons in the environment are biodegraded primarily by the bacteria and fungi, other physical factors such as evaporation of volatile fractions, photo-degradation, vertical penetration and horizontal movement also contributes to its disappearance (Bossert and Bartha, 1984). In other words, the decrease in the oil content with time (Table 1) may be ascribed to both microbial degradation and the other factors. Nevertheless, hydrocarbon degrading microorganisms are still regarded as the main players in mineralization of hydrocarbons in the terrestrial ecosystem, thus their presence in any oil polluted site is consequential. They act mainly at the oil-water interface, and are usually observed growing over the entire surface of an oil droplet while no growth occurs within the oil droplets in the absence of entrained water (Leahy and Colwell, 1990). Interestingly, Atlas and Cerniglia (1995) averred that the number of hydrocarbon utilizers in most natural ecosystems will initially limit the rate of hydrocarbon degradation but may not be the principal rate-limiting factor after a period of exposure. Accordingly, the lower number of hydrocarbon utilizers experienced at the later part of this study (dry season), would have also influenced the rate of hydrocarbon reduction which was obviously higher between 0 and 90 d (9.2×10^{-5} to 9.9×10^{-5} mgd^{-1}), than the range (3.3×10^{-5} to 4.1×10^{-5} mgd^{-1}) noted between 90 and 150 d (Table 2).

Importantly, it was evident at the end of this study that only >35 % of the total oil content was removed in all the polluted Zones (Table 2). This implies that a substantial amount (65%) was still present in the environment. This obviously could be due to the difference in the susceptibility of the various complex petroleum hydrocarbons to microbial attack. A generalized sequence in order of decreasing biodegradation is usually represented as follows: n-alkanes > branched-chain alkanes > low molecular weight aromatics > poly nuclear aromatics > asphaltenes. This implies that rates of biodegradation is highest for saturates, followed by light aromatic with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation. In addition, the incomplete combustion of petrol leads to production of polycyclic aromatic hydrocarbons (PAH) (Leahy and Colwell, 1990; Van Hamme *et al.,* 2003). In other words, since this particular oil spill was accompanied by fire outbreak, an incidence that will increase the concentration of PAHs, there is no doubt that the bulk of the hydrocarbons remaining would be made of this recalcitrant group.

The various bacterial and fungal genera (*Corynebacterium*, *Pseudomonas*, *Aspergillus*, *Candida* and *Rhodotorula*) identified among the heterotrophic microorganisms was consistent to the groups well known to be excellent hydrocarbon degraders (Atlas and Cerniglia, 1995; Bossert and Bartha, 1984). Similarly, Bordenave *et al.* (2007) had reported the dominance of *Gammaproteobacteria* genera, which are also recognized for their hydrocarbon biodegradation potential after hydrocarbon contamination of a site.

Conclusion

The results of this study strongly suggest that natural restoration will take a long time to completely remove all hydrocarbons present. Therefore, for complete oil removal, interventions such as enhanced bioremediation (bioaugmentation or biostimulation), physical or chemical remediation treatments should be applied. Besides, high concentrations of PAH is expected in this site, and a number of these PAHs are carcinogenic. For this reason, apart from the harm to the soil ecosystem, this spill will also be detrimental to humans living around this area if adequate pollution management measures are not put in place.

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