African Journal of Biotechnology Vol. 6 (16), pp. 1939-1943, 20 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Studies on the effect of petroleum hydrocarbon on the microbial and physico-chemicals characteristics of soil

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Accepted 20 June, 2007

The microorganisms capable of degrading crude oil are present in any conceivable environment. This study was aimed at ascertaining the bacteria and fungi that are able to survive in soils contaminated with 3 different petroleum hydrocarbon fractions (premium motor spirit 'PMS', domestic purpose kerosene 'DPK' and automotive gas oil 'AGO'). Soil surface samples (0.5 cm) were collected randomly from different locations in Ile-Ife in Osun State of Nigeria. Soil samples were contaminated with the hydrocarbon fractions in a ratio of 1:1 and estimated for total bacterial counts (TBC), total fungal counts (TFC), and total hydrocarbon utilizing bacterial counts (HYCUB). The following anions and cations were also determined in the soil samples; Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, NO_3^{+}, and NO_2^{+}. The results showed that TBC ranged from 2.1 x 10^3 to 3.6 x 10^6 cfu/g, while TFC was in the range of 0 to 2.0 x 10^3 cfu/g and HYCUB was in the range of 4.8 x 10^1 to 4.5 x 10^3 cfu/g. Apart from average HYCUB counts that was highest in DKP samples, other counts were highest in the PMS samples. Similar bacteria of diverse species were isolated from the contaminated soil samples, although their months of occurrences and numbers differed, Mg^{2+}, K^{+} and Na^{+} all increased in all the contaminated soils, at the end of the study. In this study, the HYCUB counts were negatively correlated with Ca^{2+} ($r = -0.667$, $p<0.05$). This study revealed a significant correlation between total fungal counts and hydrocarbon-utilizing bacterial counts ($r = 0.700$, $p< 0.05$) and a negative correlation between total bacterial counts and Na^{+} ($r = -0.677$, $p< 0.05$). The results of the study has revealed that refined petroleum can have an increasing or decreasing effect on soil physico-chemical characteristics, and that some of these physico-chemical characteristics may have effect on microbial counts.

Key words: Hydrocarbon, microbial counts, physico-chemical characteristics.

INTRODUCTION

Petroleum is a complex mixture of liquid and solid hydrocarbons, whose composition also varies with the source. The components are paraffin hydrocarbon, saturated alicyclic hydrocarbon and aromatic hydrocarbon (Atlas and Bartha, 1998). Petroleum can be accidentally or deliberately released into the environment leading to serious pollution problems (Thouand et al. 1999; Okoh et al., 2002; Okoh 2006). Still small releases of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in excess of regulatory limits (Spence et al., 2005). These pollution problems often result in huge disorder of both the biotic and abiotic com ponents of the ecosystems (Mueller et al. 1992), more so that some hydrocarbon components have been known to belong to a family of carcinogenic and neurotoxic organ pollutants (Hallier-Soulier et al., 1999). The processes leading to the eventual removal of hydrocarbon pollutants from the environment has been extensively documented and involves the trio of physical, chemical and biological alternatives (Okoh, 2006).

Current practices of oil wastes management include the use of reserve pit for drilling wastes and land spreading of reserve pit content for disposal of aqueous effluents through Class II underground injection well (EPA, 2002; Trejo-Hernández et al., 2007). Trejo-Hernández et al. (2007) reported the biological alternative in the treatment of pollution associated with petroleum hydrocarbon. Petroleum oil biodegradation by bacteria can occur under

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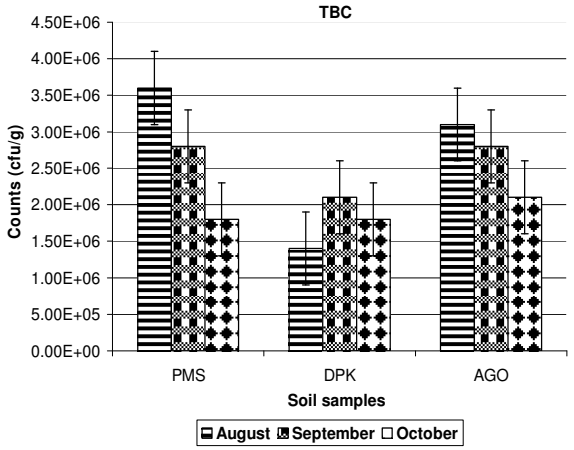

Figure 1. Fluctuations in total bacterial counts (TBC) of the soil samples during the sampling period (PMS = premium motor spirit, DPK = domestic purpose kerosene, and AGO = automotive gas oil contaminated soils).

both oxic and anoxic conditions (Zengler et al., 1999), albeit by the action of different consortia of organisms (Okoh et al., 2002). In the subsurface, oil biodegradation occurs primarily under anoxic conditions, mediated by sulfate reducing bacteria (Holba et al., 1996) or other anaerobes using a variety of other electron acceptors as the oxidant. The fate of petroleum hydrocarbons in the environment is largely controlled by abiotic factors which influence rates of microbial growth and enzymatic activities that determine the rates of petroleum hydrocarbon utilization (Atlas 1995; Ojo, 2005).

The persistence of petroleum pollution depends on the quantity and quality of hydrocarbon mixture and on the properties of the affected ecosystem (Ojo, 2005). In one environment, petroleum hydrocarbon persists indefinitely whereas under another set of conditions the same hydrocarbons may be completely biodegraded within a few hours or days (Atlas and Bartha, 1992). Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats (Okoh, 2006).

The ability to isolate high numbers of certain oil-degrading microorganisms from oil-polluted environment is commonly taken as evidence that these microorganisms are the active degraders of that environment (Okerentugba and Ezeronye, 2003). Although, hydrocarbon degraders may be expected to be readily isolated from an oil-associated environment, the same degree of expectation may be anticipated for microorganisms isolated from a total related environment such as soil.

This study is aimed at assessing the effect of refined petroleum hydrocarbon on the physicochemical and microbial characteristics of soil.

MATERIALS AND METHODS

Surface soil samples (0.5 cm depth) were collected from Ile-Ife; a town in Osun State of Nigeria. Soil samples were collected at random, air-dried and passed through 0.2 mm sieve and contaminated with 3 different petroleum hydrocarbon fractions {Premium motor spirit (PMS), domestic purpose kerosene (DPK), automotive gas oil (AGO)}. The hydrocarbon fractions were obtained from and stored in sterile containers. Sampling was done in the months of August, September and October 2005.

Each petroleum hydrocarbon fraction (PMS, DPK and AGO) was applied at 10% levels to the soil samples. The contamination involved 4 treatments in completely randomized design replica.

Microbial counts were estimated using the dilute plate method, as described by Seeley and Vendermark (1981). At the expiration of incubation, plates were counted, and representative bacteria colonies were counted, isolated, purified and identified. The bacteria isolates were identified as described by Bergey (1989). The following anions and cations were estimated for: Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, $N0_3^{+}$ and $N0^{-}_2$ using atomic absorption spectroscopy (AAS).

Values were subjected to statistical analysis, using the SPSS 11.0 computer software.

RESULTS AND DISCUSSION

The total bacterial counts (TBC) of the soil samples ranged from 1.1 x 10^6 to 2.4 x 10^6, 1.4 x 10^6 to 3.1 x 10^6 to 2.1 x 10^6 to 3.1 x 10^6 cfu/g, premium motor spirit (PMS), domestic purpose kerosene (DPK) and automotive gas oil (AGO) contaminated soils, respectively (Figure 1). Apart from the DPK contaminated soil, a highest bacterial count was observed in the month of August in the remaining two soil samples (Figure 1). The TBC of the various soil samples were different, and these differences were observed to be significant ($p< 0.05$).

The microbial counts reported in this study fell within the limits that have been reported by earlier workers (Obire and Nwaubeta, 2002). There was no correlation between TBC and either TFC or HYCUB counts. There was, however, a significant negative correlation between total bacterial counts and Na^{+} ($r = -0.677$, $p < 0.05$). No correlation was however observed between bacterial counts and the other cations and anions that were investigated.

Also, the total fungal counts (TFC) of the soil samples were in the ranges of 1.5 x 10^2 –1.1 x 10^3, 0 – 1.6 x 10^2, 0 – 2. 0 x 10^3 cfu/g for the soil samples contaminated with PMS, DPK and AGO, respectively (Figure 2), there was no observed growth in the months of August for the DPK and AGO contaminated soils and September for the AGO contaminated soil and October for the PMS contaminated soil. Highest TFC was observed in the month of August for the PMS contaminated soil. As was found in the bacterial counts, the differences in TFC of the soil samples were observed to be significant ($p < 0.05$). This study revealed a significant correlation between total fungal counts and hydrocarbon-utilizing bacterial counts ($r = 0.700$, $p < 0.05$). However, there was no correlation between TFC and the other parameters investigated.

Similarly, the total hydrocarbon utilizing bacterial counts (HYCUB) ranged from 8.2 x 10^2 to 6.8 x 10^3, 5.2 x 10^2 to 4.5 x 10^3 and 4.8 x 10^1 to 5.3 x 10^2 cfu/g, for soils conta-

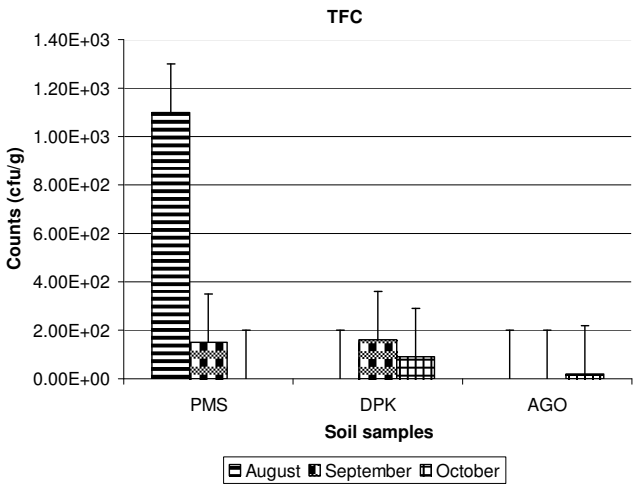

Figure 2. Fluctuations in total fungal counts (TFC) of the soil samples during the sampling period (PMS = premium motor spirit, DPK = domestic purpose kerosene, and AGO = automotive gas oil contaminated soils).

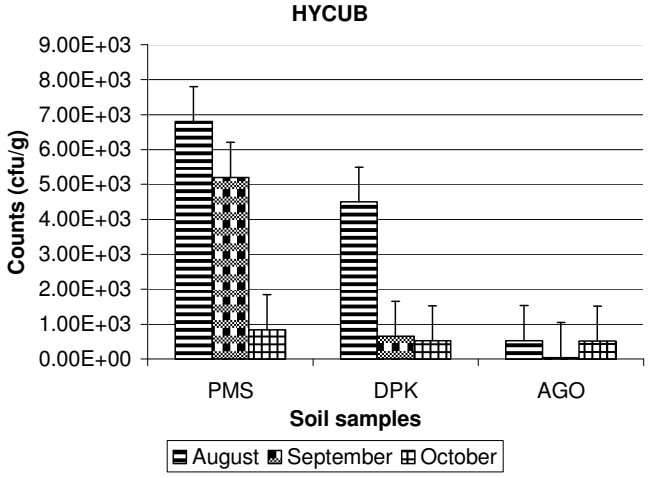

Figure 3. Fluctuations in total hydrocarbon utilizing bacterial counts (HYCUB) of the soil samples during the sampling period (PMS = premium motor spirit, DPK = domestic purpose kerosene, and AGO = automotive gas oil contami-nated soils).

minated with PMS, DPK and AGO, respectively, (Figure 3). Highest HYCUB counts were observed in the month of August in all the samples. Significant difference was observed between the HYCUB counts of the samples ($p<0.05$). In this study, the HYCUB counts were negatively correlated with Ca^{2+} ($r = -0.667$, $p < 0.05$). Also, in all the counts monitored (TBC, TFC and HYCUB), there were significant variations between the months of sampling, ($p < 0.05$). In this study, similar bacteria were recovered from both the control and contaminated samples, apart from a few species. Studies have shown that oil-degrading microorganisms are abundant and not limited to oil-producing areas (Ajisebutu, 1987; Okoh et al., 2002).

Soil had been known to be a favourable habitat for the proliferation of microorganisms, with microcolonies developing around soil particles, but the addition of refractory humic substances slow down the activities of these organisms, thus giving room to organisms that have the ability of metabolising such products and limiting the growth of non-metabolizers of the products (Atlas and Bartha, 1998). Diverse microorganism, including many species of bacteria and fungi has evolved the metabolic capacity to degrade hydrocarbons (Atlas and Cerniglia, 1995). Similar bacteria of diverse species were isolated from the contaminated soil samples, although their months of occurrences and numbers differed. Other workers, (Atlas and Cerniglia, 1995), Obire and Nwaubeta (2002) had reported this trend.

In this study, the most predominant bacteria species was *Pseudomonas.* Other prevalent bacteria that were isolated include *Arthrobacter, Corynebactericum* and *Klebsiella*. Atlas and Cerniglia (1995) reported that the most prevalent bacteria hydrocarbon degrades belong, in decreasing order, to the genera *Pseudomonas, Achromobacter Flavobacterium, Nocardia*, *Arthrobacter, Bacillus, Micrococcus* and *Acintobacter*.

The values for Ca^{2+}, Mg^{2+}, K^+ and Na^+ ranged from 4.10 – 8.50, 0.70-3.42, 0.02 – 2.00, 0.25 - 0.79 and 0.25 – 0.79 meq/100g soil, respectively (Table 2). Highest values for Ca^{2+} and k^+ were recorded in the AGO contaminated soil and were recorded in the months of August and October, respectively. Also, highest values for Mg^{2+} and Na^+ were in the month of October and were recorded in the PMS sample for Mg^{2+} and DPK sample for Na^+, (Table 1). $N0_3^-$ and NO_2^- were in the ranges of 12 to 18 and 2 to 4 Meq/100g soil, respectively. In these, highest values were mostly in the month of August (Table 1).

Nitrate was significantly higher in soil sample contaminated with AGO than the rest two. Also, Na^+ significantly different between soil sample contaminated with DPK and AGO. There were also observed significant Differences between the Ca^{2+} and Mg^{2+} concentrations of the 3 soil samples

In this study, similar bacteria cultural and distinct types were isolated and were observed in all the contaminated samples (PMS, DPK and AGO), although their months of occurrences differed (Table 2). Some representative bacteria isolates recovered were *Pseudomonas, Bacillus, Flavabacterium, Corynebacterium, Pseudomonas, aeruginosa, Alcaligenes faecalis, Arthrobacter, Proteus, Micrococcus, Klebsiclla and Serratia marcescens*.

Available Ca^{2+} showed no visible trend in the contaminated soils; Ca^{2+} value was lower for PMS, than in the other soil. This is in agreement with the findings of Obire and Nwanbeta (2002). Mg^{2+}, K^+ and Na^+ all increased in all the contaminated soils at the end of the study. A similar finding has been reported by Amund and Akangbou (1993) but different from the trend reported by

Table 1. Some physicochemical characteristics of the soil samples

Months	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	NO_3^{-}	$NO2^{-}$
August	a. 4.10 b. 7.60 c. 8.50	a. 3.35 b. 0.80 c. 2.00	a. 0.02 b. 0.54 c. 0.48	a. 0.53 b. 0.76 c. 0.48	a. 18 b. 28 c. 14	a. 2 b. 4 c. 3
September	a. 4.30 b. 7.70 c. 8.40	a. 3.40 b. 0.90 c. 2.00	a. 0.04 b. 0.53 c. 0.21	a. 0.65 b. 0.77 c. 0.45	a. 18 b. 28 c. 14	a. 2 b. 4 c. 2
October	a. 4.32 b. 7.60 c. 8.48	a. 3.42 b. 0.95 c. 2.00	a. 0.04 b. 0.55 c. 2.00	a. 0.60 b. 0.79 c. 0.53	a. 18 b. 28 c. 12	a. 2 b. 4 c. 2

Values are means of duplicate samples, ND-not determined (a) - Preminun Motor Spirit (PMS), (b)- Domestic Purpose Kerosene (DPK), (c)- Automotive Gas Oil (AGO).

Table 2. Bacteria species distribution from the soil samples.

Month	PMS	DPK	AGO
August	*Bacillus. Pseudomonas aeruginose Flavobacterium.* sp *Pseudomonas.* sp *Corynebacterium*	*Bacillus.* sp *Klebsiella* sp *Pseudomonas.* sp *Proteus.* Sp	*Pseudomonas.* sp *Micrococcus.* sp *Websiella* .sp *Pseudomonas aerugionsa*
September	*Pseudomonas.* Sp *Arthrobacter* sp *Serratia marcescens* *Proteus.* sp *Pseudomonas* aeruginosa	*Bacillus.* sp *Micrococcus.* sp *Klebsiella* sp Pseudomonas. *sp*	*Pseudomonas.* sp *Bacillus.* sp *Arthrobacter.* sp
October	*Corynebacterim* sp *Bacillus* sp, *Serratia Marcescens,* *Arthrobacter* sp	*Bacillus* sp *Pseudomonas* sp *Arthrobacter* sp *Proteus* sp	*Flavobacterium* sp *Bacillus* sp *Pseudomonas* sp *Proteus* sp

PMS = Premium Motor Spirit, DPK = Domestic Purpose Kerosene, and AGO = Automotive Gas Oil.

Obire and Nwanbeta (2002).

Conclusion

The results of the study has revealed that refined petroleum can have an increasing or decreasing effect on soil physico-chemical characteristics, and that some of these physico-chemical characteristics may have effect on microbial counts. Although the study cannot be regarded as total and exhaustive, it has given on overview on the effect of hydrocarbon products on soil microbial and physico-chemical characteristics, over a period of time.

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