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Full Length Research Paper

Effect of inorganic fertilizer on microbial utilization of hydrocarbons on oil contaminated soil

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The effect of inorganic fertilizer (NPK agricultural fertilizer) on biodegradation of soil (5 kg) contaminated with crude oil (50 g) was investigated for seven weeks. Four different test options were prepared namely; (i) 100 g of contaminated soil + 30 g of NPK agricultural fertilizer; (ii) 100 g of contaminated soil + 60 g of NPK agricultural fertilizer; (iii) 100 g of contaminated soil + 90 g of NPK agricultural fertilizer; and (iv) 100 g of contaminated soil only (control). The microbial degradation was monitored by the measurement of total heterotrophic count (THC), hydrocarbon utilizing bacterial count (HUB) and gravimetric loss of the crude oil with time. At the end of the seven weeks of incubation, the THC of $6.9x10^7$, $9.0x10^7$, $1.03x10^8$ and $3.1x10^7$cfu/g were recorded for test options (i), (ii), (iii) and (iv), respectively. The hydrocarbon utilizing bacterial counts (HUB) were $1.68x10^5$, $1.63x10^5$, $1.9x10^5$ and $4.8x10^4$ cfu/g for tests options (i), (ii), (iii) and (iv), respectively. The corresponding gravimetric hydrocarbon losses were 40.0, 50.52, 36.84 and 29.47% for test conditions (i), (ii), (iii), and (iv), respectively. The results of the study suggest that addition of inorganic fertilizer (especially 60 g NPK agricultural fertilizer) will further enhance microbial utilization of hydrocarbons.

Key words: Biodegradation, crude, NPK agricultural fertilizer.

INTRODUCTION

Microbial degradation of petroleum hydrocarbon is a very important factor in the treatment of oil pollution both in aquatic and terrestrial environment (Colwell and Walker, 1977; Ibe and Ibe, 1984). The bacteria and fungi genera associated with degradation of petroleum have been documented (Atlas, 1981; Bossert and Bartha, 1984; Okpokwasili and Amanchukwu, 1988). The use of lipophilic fertilizer to enhance microbial utilization of crude oil has been suggested (Olivieri, et al., 1978; Abu and Ogiji, 1996). Ijah and Okang (1993) reported that the growth and proliferation of oil utilizing microorganisms in polluted soil is greatly influenced by the availability of nutrients and their hydrocarbonoclastic property.

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This study reports the effects of inorganic fertilizer (NPK agricultural fertilizer) on the microbial utilization of petroleum hydrocarbon on polluted soil. Several concentrations of NPK fertilizer were added to different quantities of polluted soil samples in order to determine the nutrient ratio that gives the best performance for remediation purposes.

MATERIALS AND METHODS

Sample collection and preparation

Garden topsoil (0-15 cm) with no previous history of crude oil contamination was collected from the Federal University of Technology, Owerri. The crude oil (Bonny light) was obtained from Nigeria Agip Oil Company, Port Harcourt. The fertilizer (NPK 15:15:15) used for this study was bought from the open market. 5 kg of the soil samples contained in plastic bags were contaminated with crude oil at the ratio of 10 g/kg. The experimental samples

Table 1. A summary of test conditions and sample treatment employed in the microbial utilization of petroleum hydrocarbons in contaminated soil.

Treatment	Condition	Description of condition
(i)	Nutrient added	100 g of contaminated soil + 30 g NPK fertilizer
(ii)	Nutrient added	100 g of contaminated soil + 30 g NPK fertilizer
(iii)	Nutrient added	100 g of contaminated soil + 90 g NPK fertilizer
(iv)	No nutrient added	100 g of contaminated soil only

The NPK fertilizer contained nitrogen, phosphorus and potassium at the ratio of 15:15:15.

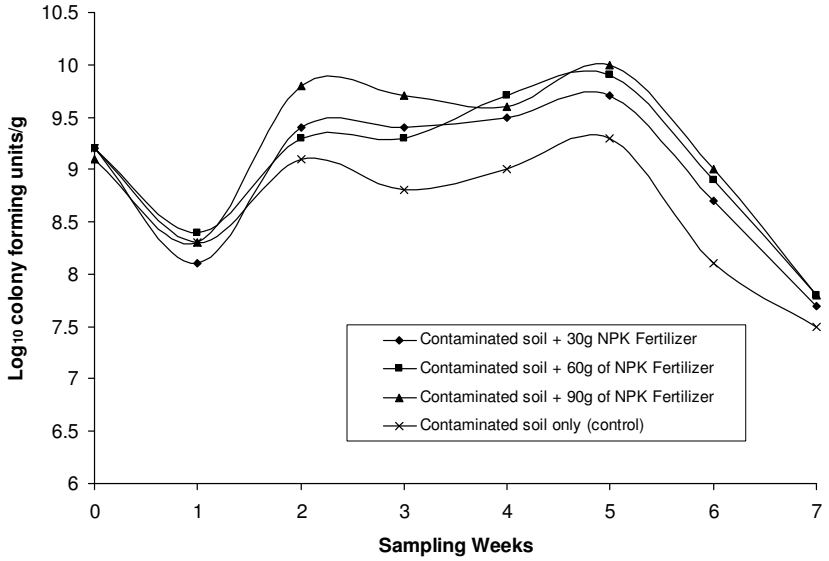

Figure 1. Total heterotrophic count of microbial population in different treatment options.

were set up as shown in Table 1 and monitored for a period of seven weeks.

Heterotrophic bacterial count

The mean total aerobic bacteria present in the samples at the beginning of the experiment (day zero) and at weekly intervals for each of the treatment options were estimated using spread plate method with nutrient agar as medium. A ten-fold dilution using physiological saline was prepared and 0.1 ml of appropriate dilution was plated in duplicates and incubated for 18-24 h at room temperature after which the colonies were counted.

Enumeration of hydrocarbon utilizing bacteria

Aliquots (0.1 ml) of appropriate dilutions of soil samples were plated on to modified mineral salts medium of Mills et al. (1978) containing the following in g/l: NaCl, 10.0; $MgSO_4.7H_2O$, 0.42; KCl, 0.29; KH_2PO_4, 0.53; NH_4NO_4, 0.42; agar, 15.0 and distilled water. The vapour phase transfer technique of Okpokwasili (1988) was adopted, which employs the use of sterile filter paper soaked in crude oil, which served as the carbon and energy source. The soaked sterile filter papers were then aseptically placed onto covers of the inoculated inverted plates and incubated for 5 to 7 days at room temperature. Average mean counts of colonies from duplicate plates were recorded and used for the calculation of colony forming units per gram (cfu/g) of soil.

Measurement of crude oil utilization using gravimetric method

Residual crude oil was extracted from the soil samples using a modified method of Abu and Ogiji (1996). Quantitative determination of the crude oil extracts was carried out as described by Udeme and Antai (1988). A standard curve of absorbance (A520 nm) against varying concentrations of Bonny light crude oil (1 to 5%) in chloroform was drawn after taking readings from a PYE UNICAM SP6-550 UV/VS spectrophotometer. The hydrocarbon concentrations were calculated from the standard curve after multiplying by the appropriate dilution factor.

RESULTS AND DISCUSSION

The total heterotrophic microbial population is presented in Figure 1. The total aerobic heterotrophic count (THC) decreased from 1.8×10^8 cfu/g in week zero to 1.4×10^8

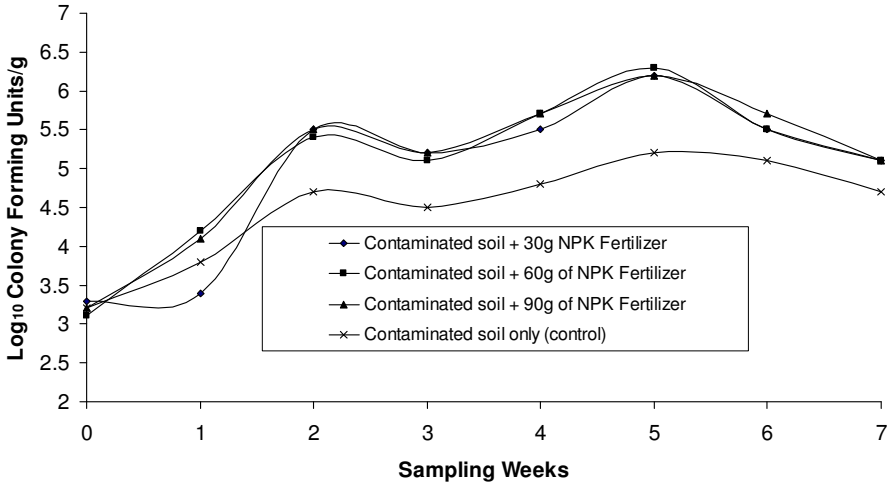

Figure 2. Total count of hydrocarbon utilizing bacteria in different treatment options.

Table 2. Percentage hydrocarbon utilizing bacterial population in crude oil contaminated soil with increasing addition of NPK agricultural fertilizer.

*Treatment	Weeks							
	0	1	2	3	4	5	6	7
(i)	0.0001	0.0052	0.0100	0.0065	0.0127	0.0290	0.0581	0.2326
(ii)	0.0001	0.0054	0.0086	0.0073	0.0094	0.0276	0.0413	0.1843
(iii)	0.0001	0.0064	0.0046	0.0037	0.0050	0.0154	0.0533	0.2093
(iv)	0.0001	0.0035	0.0035	0.0058	0.0067	0.0084	0.1158	0.1548

*See table 1 for details of the treatment i,ii,iii and iv.

cfu/g in week one in response to oil contamination and this sequence followed in all the treatment options. The THC increased again from week 2 up to week 5 before reducing again in weeks 6 and 7. At the end of week 7 the cumulative THC were 5.2 x 10^7, 6.4 x 10^7, 6.7 x 10^7 and 3.1 x 10^7 cfu/g for treatment options (i) (see Table 1), (ii), (iii) and (iv), respectively. The drop in the total heterotrophic counts in the contaminated soil in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic components of petroleum and also as a result of reduced aeration and upsets in carbon/Inorganic nutrient balance for the indigenous population caused by the presence of petroleum (Atlas, 1984).

Figure 2 shows the hydrocarbon utilizing bacterial counts (HUB). The HUB counts increased over the period of the study. At the end of week 7 the HUB counts were 1.2 x 10^5, 1.18 x 10^5, 1.34 x 10^5 and 4.6 x 10^4 cfu/g for treatment options (i), (ii), (iii) and (iv), respectively. This shows that the soil supplemented with 90 g NPK fertilizer gave the highest number of hydrocarbon utilizing bacteria. The increase in both THC and HUB in response to the addition of inorganic nutrient has been reported (Henry et al, 1991; Mark and Jeffrey, 1991; Abu and Ojigi, 1996). The fluctuations in the 3rd and subsequently in the 6th and 7th week of both THC and HUB can be attributed to low moisture level since this study was done within October and December. The 3rd week marks the onset of dry season which starts from November to March and the 6th and 7th week marked the onset of harmattan when the North East Monsoon Wind known for its drying up effects sweeps over the whole region. The drying up effect is presumed to be harsh on the growth and activation of microorganisms. According to Dibble and Bartha (1979) and Alexander (1994), decreasing the moisture content of the soil diminishes rates of degradation, as a result of inadequate supply of water to sustain proliferation, metabolism or both.

The percentage hydrocarbon utilizing bacterial population is shown in Table 2. The result shows that the sample treated with 30 g NPK fertilizer gave the highest percentage of 0.23% while the least percentage (0.15%) was recorded with the un-supplemented soil sample. The difference in percentage HUB population between the different treatments was not significant ($p<0.01$).

Table 3. Loss of crude oil from the crude oil contaminated soil samples for each treatment option after seven weeks of treatment with increasing addition of NPK agricultural fertilizer.

*Treatment	Treatment	Conc. of residual crude oil (mg/ml)	Total percentage loss in crude oil[a]	Net per. Loss due to treatment
(i)	30 g fertilizer	0.0057	40.00	10.53
(ii)	60 g fertilizer	0.0047	50.52	21.05
(iii)	90 g fertilizer	0.0060	36.84	7.37
(iv)	Control	0.0067	29.47	N/A
0^c	N/A	0.0095	N/A	N/A

*See table 1 for details of the treatment i,ii,iii and iv.
N/A = Not applicable.

$$^{a}\text{Total percentage loss in crude oil} = \frac{(\text{conc. of crude oil } (0^c) - \text{conc. of crude oil (treatment)})}{\text{conc. of crude oil } (0^c)} \times 100$$

The bioremediation potential of the treatment options was showed by the percentage reduction of soil in the samples (Table 3). The application of 60 g NPK fertilizer proved the best treatment option with the removal of 50.52% of crude oil from the sample, followed by 30 g NPK fertilizer (40.0%) and 90 g NPK fertilizer (36.84%). There was also 29.47% removal of crude oil in the control experiment (no nutrient addition).

This study supports the fact that nutrient supplementation enhances biodegradation and favours the use of 60 g NPK agricultural fertilizer for soil quality similar to the one used for this study.

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