BIODEGRADATION OF TOTAL PETROLEUM HYDROCARBONS IN SHAMBAT SOIL, SUDAN

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Abstract
Purpose: The aim of this study was to investigate the biodegradation of petroleum hydrocarbons in crude oil when added to Shambat soil in Sudan. Design/methodology/approach: A laboratory experiment was conducted in the Soil Science Unit laboratories at the Department of Environment and Environmental Pollution, ENRRI, National Center for Research. The experiment aimed to study the biodegradability of light crude oil, which was refined by the Petrodar Oil Company. Petri dishes were filled with Shambat soil, amended with three different concentrations of light crude oil and incubated at optimum temperature (37°C). Samples were taken after 2, 14 and 28 days following crude oil addition. The recovery of petroleum hydrocarbons was determined to calculate the biodegradation percentage. Findings: The results indicated highly significant differences among the various concentrations and sampling durations. The highest concentration of crude oil showed a significant decrease in biodegradation of petroleum hydrocarbons. Originality/value: This research was carried out by four researchers from two institutions concerned with the environmental aspects of soil pollution due to the implementation of the oil industry in Sudan. The paper emphasizes the microbial degradation of total petroleum hydrocarbons in soils in order to facilitate management practices in polluted sites.

Keywords: Biodegradation, Crude oil, Total petroleum hydrocarbons, Sudanese soil

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INTRODUCTION

Many challenges occur with respect to environment and environmental management programmes. The uprising of industrial activities in recent years has resulted in many environmental problems that affect both the natural world and human lives. Crude oil is the product of heating ancient organic materials over geological periods. It is formed from pyrolysis of hydrocarbon in a variety of reactions, mostly endothermic at high temperature and/or pressure (Arun et al., 2011).

The problem of petroleum hydrocarbons in the environment is that they have serious health effects on humans and animals. They are known to cause irritation, inflammation, redness, itching and swelling of the skin, mucous membranes, nose, trachea and bronchioles. They also produce anaesthesia and problems in the central nervous system (Luch, 2005).

Mechanical and chemical methods for remediation of hydrocarbon-polluted environments are often expensive, technologically complex and lack public acceptance (Vidali, 2001). Thus, bioremediation remains the method of choice for effective removal of hydrocarbon pollutants in the environment (Okoh and Trejo-Hernandez, 2006). Microorganisms play a major role in saving our environments by degrading xenobiotic compound chemicals wastes, which are toxic either in their native form or modified to be non-toxic. The efficiency of the biomass in the biodegradation of crude oil may be affected by many factors, including initial concentration, temperature, the presence of other nutrients, the presence of other pollutants and the biomass abundance (Marrot et al., 2006; Kira et al., 2000).

The objective of this study is to determine the natural role of soil microorganisms on the biodegradation of Total Petroleum Hydrocarbons (TPHs) in soil to minimize soil contaminants.

MATERIALS AND METHODS

Materials

The soil used in the laboratory studies was collected from the Faculty of Agriculture, University of Khartoum, demonstration farm, Shambat, Khartoum North, Sudan. It classifies as Fine, loam mixed Isohyperthermic,
Typic, Torrifluvent, as reported by Mohammed (2011). The crude oil used in the experiment was delivered by Petrodar Oil Company/Khartoum, Sudan. It was light with a bulk density of 0.81 g cm$^{-3}$. Seven petroleum hydrocarbon components were found in the crude oil: decane, undecane, tridecane, tetradecane, pentadecane, nonadecane and heneicosane. These findings are supported by the results of Gustafson (1997), who found hydrocarbon fractions containing a number of carbons ranging between C8 and C22.

**TOTAL PETROLEUM HYDROCARBONS INCUBATION EXPERIMENT**

Fifty grams of the soil samples were weighed. Twenty-seven soil samples were placed in Petri dishes and treated with 0.16, 0.32 and 1.28 ppm of crude oil (mixed with solvent to ease distribution on the samples) using a 1 ml syringe. 16 ml of distilled water were then poured into the soil to fill the pore volume and to activate the soil microorganisms. The dishes were incubated at 37$^\circ$C in an incubator and sampling was carried out after 2, 14 and 28 days with a 40 g subsample from each treatment.

To determine the Total Petroleum Hydrocarbons, 40 g fresh soil (sieved < 2 mm) was extracted for 30 min in 100 ml 1:1 acetone: dichloromethane in a shaker (200 rev/min). The extract was filtered with (Whatman No. 42) into a 100 ml volumetric flask and the volume was made up to 100 ml with 1:1 acetone: dichloromethane. This extract was analyzed by GC-FID using the conditions described below and the total petroleum hydrocarbon (TPH) value was calculated. The method for total petroleum hydrocarbons analysis by capillary GC-FID was modified from the US EPA method 8100 for the analysis of polynuclear aromatic hydrocarbons (US EPA, 1986).

**STATISTICAL ANALYSES**

Data collected from the experiment were analyzed as a factorial complete randomized design. Analysis of variance (ANOVA) was performed according to the method described by Gomez and Gomez (1984). Means were separated by using Duncan's Multiple Range Test (DMRT). Ten assigned peaks were used for verification of acceptable replicate analysis. The average total peak area from the three replicates was calculated and used to work out the total TPH content of that section.
RESULTS AND DISCUSSION

Decane and undecane hydrocarbon fractions were not detected under all concentrations in all sampling dates within the period of incubation (28 days) as shown in Figures 1 and 2. This may be due to the complete degradation of these fractions by soil microorganisms during the first two days, since this fraction consists of simple, straight chain petroleum hydrocarbons and is most readily degraded by soil microorganisms. Jain et al. (2005) found that reduction by biodegradation for crude oil was achieved within one month of incubation.

Figure 1. Biodegradation of decane at different sampling dates and different concentrations of crude oil

Figure 2. Biodegradation of undecane at different sampling dates and different concentrations of crude oil
Biodegradation of tridecane, tetradecane, pentadecane, nonadecane and heneicosane hydrocarbon fractions showed negative values as indicated in Figures 3, 4, 5, 6 and 7 respectively. Salam et al. (2011) and Obayori et al. (2009) reported that more than 90% of the degradation of light crude oil was performed by each of the *Pseudomonas* isolates over a period of 21 days incubation. Obayori et al. (2009) reported that as indicated in the GC fingerprints of the two isolates on crude oil for 21 days, the C\textsubscript{10}-C\textsubscript{14} fractions of the crude oil were almost completely utilized by the *Pseudomonas* species with a drastic reduction in the major peaks C\textsubscript{15}, C\textsubscript{17}, C\textsubscript{19} and C\textsubscript{20}. Johnsen et al. (2005) and Wang...
et al. (2001) reported the same results and they attributed them to the fact that petroleum hydrocarbons, like many other hydrophobic organic contaminants, have very low solubility. Furthermore, they are rapidly sorbed onto soil clay and organic matter particles. The strong association of petroleum hydrocarbons with sediment particles affects their biodegradation, and as a consequence, they become

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**Figure 5.** Biodegradation of pentadecane at different sampling dates and different concentrations of crude oil

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**Figure 6.** Biodegradation of nonadecane at different sampling dates and different concentrations of crude oil
potentially unavailable for microbial degradation since bacteria are known to degrade chemicals only when they are dissolved in water. Another explanation as reported by Zhihuan et al. (2008) is that any odd values in hydrocarbon biodegradation assessment may be due to analytical problems encountered for the compounds with the highest boiling temperatures during the detection process, and they reported that dodecane is volatile at 216°C. This may be due to the condensation of the vapours in batch bottles and syringes used for injection in GC-FID.

Hohener et al. (2003) demonstrated that during longer exposure to volatile organic compound (VOC) vapours, the microbial numbers rose more slowly and the assumption of constant biomass for exposure times of a few weeks may be justified.

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