BIODEGRADATION OF TOTAL PETROLEUM HYDROCARBONS IN SHAMBAT SOIL, SUDAN

[1]Nuhad M. Ali Eltayeb, Sarra A. M. Saad and Rayan M. A. Madani
National Center for Research, Sudan

[2]Elamin A. Elamin
University of Khartoum, Sudan

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 (37oC). 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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[1] Soil Science Unit, ENRRI, National Center for Research, Khartoum, SUDAN
[2] Soil and Environment Science, Faculty of Agriculture, University of Khartoum, Khartoum, SUDAN

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°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.

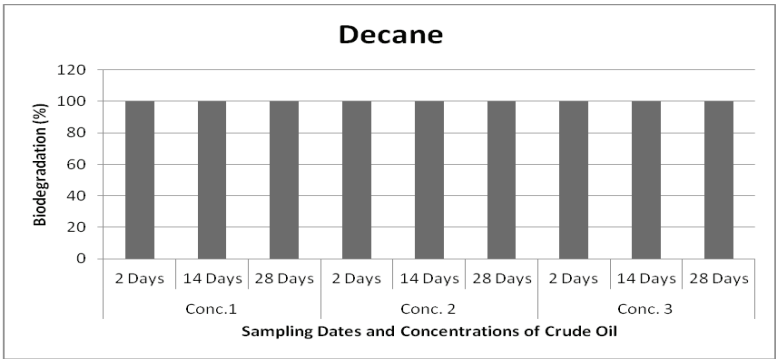

Figure 1. Biodegradation of decane at different sampling dates and different concentrations of crude oil

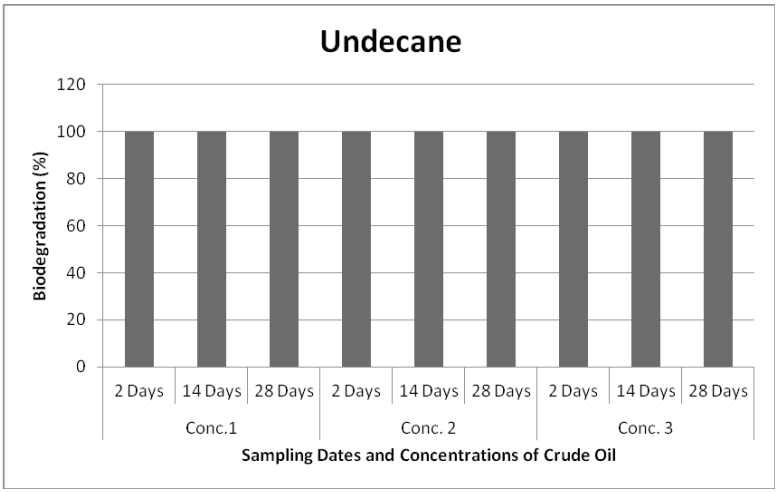

Figure 2. Biodegradation of undecane at different sampling dates and different concentrations of crude oil

Biodegradation of tridecane, tetradecane, pentadecane, nonadcane 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_{10}-C_{14} fractions of the crude oil were almost completely utilized by the *Pseudomonas* species with a drastic reduction in the major peaks C_{15}, C_{17}, C_{19} and C_{20}. Johnsen *et al*. (2005) and Wang

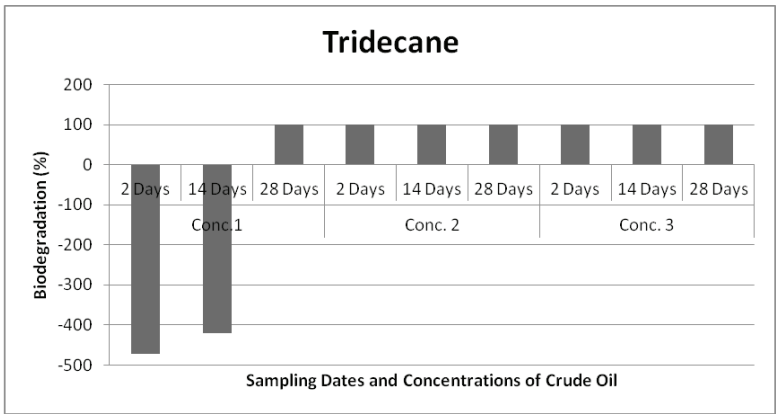

Figure 3. Biodegradation of tridecane at different sampling dates and different concentrations of crude oil

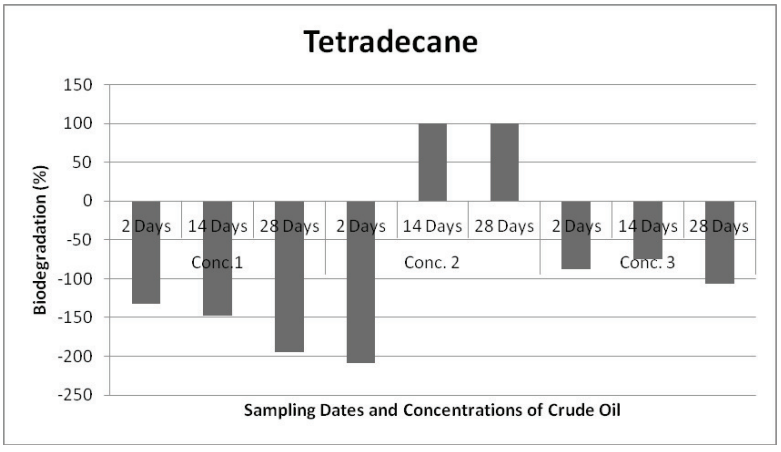

Figure 4. Biodegradation of tetradecane at different sampling dates and different concentrations of crude oil

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

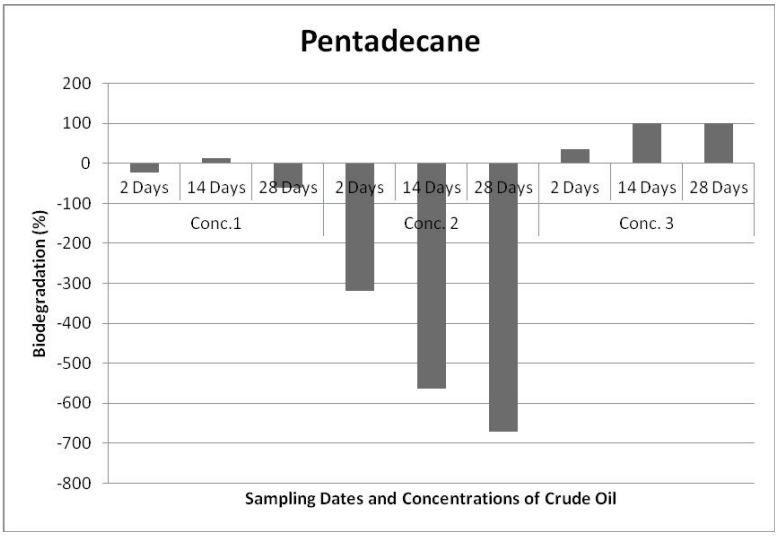

Figure 5. Biodegradation of pentadecane at different sampling dates and different concentrations of crude oil

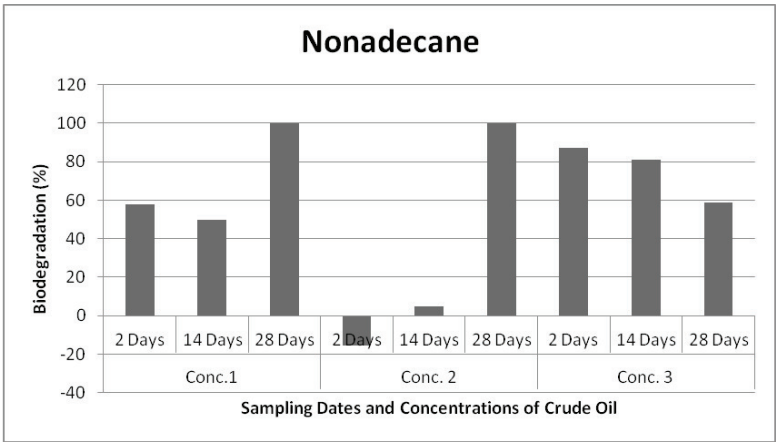

Figure 6. Biodegradation of nonadecane at different sampling dates and different concentrations of crude oil

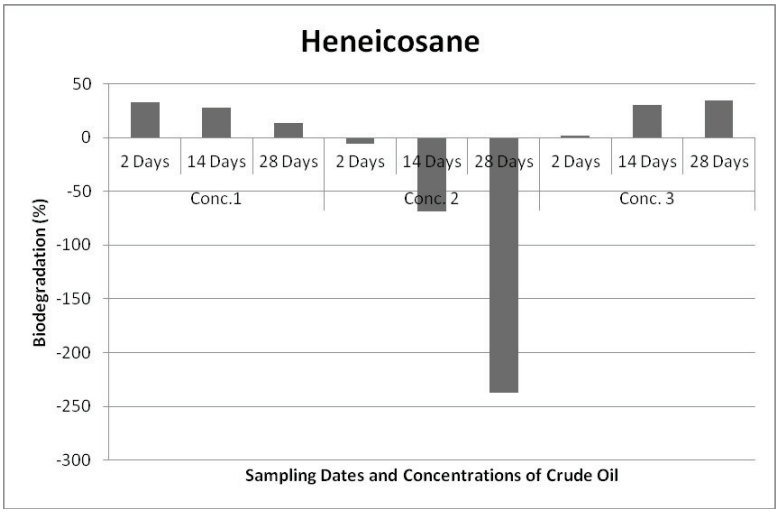

Figure 7. Biodegradation of heneicosane at different sampling dates and different concentrations of crude oil

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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ABOUT THE AUTHORS

Professor Elamin Abdelmagid Elamin is a professor of soil chemistry/reclamation and fertility currently working as Dean of the Faculty of Agriculture, University of Khartoum. He was awarded a PhD in soil science by the University of California, and achieved his MSc (agriculture) and BSc (soil science) at the University of Khartoum, Sudan. He has attended many training courses, symposia, workshops and seminars and has supervised many postgraduate students. He also leads research projects dealing with different aspects of soil problems in Sudan besides acting as a consultant for private and governamental sectors related to soil surveys and land evaluation inside and outside Sudan. He is a member of many scientific societies and has published numerous scientific papers in peer-reviewed journals. He was awarded the Phi Beta Kappa Scholarship by the University of California, Riverside, USA in 1991 and is an Honorary Resident of the City of Riverside, California, USA.

Dr Sarra Ahmed Mohamed Saad was born in Khartoum and is currently Assistant Research Professor of soil science in the Department of

Environment & Environmental Pollution, ENRRI, and the National Center of Research in Khartoum, Sudan. She attained her PhD at the Institute of Soil Science, University of Goettingen, Germany and both her MSc and BSc degrees from the University of Khartoum majoring in soil science. She is an external examiner for postgraduate programmes at many universities in Khartoum, and also acts as a consultant for many private and governmental sectors in producing organic fertilizers with regard to production procedures and quality control. She is also a member of many scientific societies inside and outside Sudan and has published scientific papers in Sudan and abroad, besides contributing to scientific conferences. She is currently finalizing two books: Methods of Soil & Compost Analysis and Soils of Sudan. Dr Saad was awarded a prize for the best supervisor for postgraduate research projects at the Sudan Academy of Science in 2008, and patent No 1900 for producing organic fertilizer from animal wastes.

Ms Nuhad Mergani Ali Eltayeb is a researcher at the Environment and Natural Resources Research Institute (ENRRI) Sudan. She holds a BSc degree in agriculture (soil and environment sciences) and a MSc degree in agriculture from the University of Khartoum. She also holds a certificate of postgraduate diploma in environmental management from Technische Universität Dresden, Germany, and a certificate in Applied Techniques for Water and Waste Water Quality Control, Khartoum, Sudan. Her research interests are soil pollution, soil chemistry, land degradation and conservation.

Ms Rayan Mohammed Ahmed Madani is a research assistant at ENRRI. She holds a BSc Honours degree in biotechnology science from Omdurman Islamic University. She has attended many training courses in Sudan in molecular biology, micro-RNA and DNA CHIP technology and computational chemistry. Her research interest is biodegradation of pollutants in soils and composted organic wastes.