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INFLUENCE OF ELECTRICAL CONDUCTIVITY ON MICROORGANISMS AND RATE OF CRUDE OIL MINERALIZATION IN NIGER DELTA ULTISOL

J. P. ESSIEN, A. Y. ITAH and S. I. EDUOK

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ABSTRACT

The effect of salinity on the population density of hydrocarbonoclastic microorganisms and oil degradation in a tropical ultisol was determined. Soils were simulated with 50gkg $^{-1}$ of Qua Iboe Light (QIL) crude oil. Salt treatments included NaCl amendments to adjust the soil solution electrical conductivities (EC) to 40. 120 and 200 dSm^{-1}. Treated soils were incubated at 28^{0}C. Oil degradation was estimated from the gravimetric determinations of remaining oil. The results showed that amending the ultisol with crude oil stimulated the growth of oil degrading microorganisms, while salt concentration inducing an EC of 200 dSm^{-1} in oil amended ultisol resulted in a decrease in the amount oil mineralized. A significant positive correlation was found between the number of hydrocarbonoclastic microorganisims and levels of oil mineralization at EC 40 dSm^{-1} (r = 0.980, P =0.05) and EC 120 dSm^{-1} (r = 0.976, P = 0.05) except at EC 200 dSm^{-1} (r = 0.855, P = 0.05). This is an indication that desalting of soils contaminated with oil and salts is required for a speedy bioremediation of an ultisol.

Key words: Electrical conductivity, crude oil, hydrocarbonoclastic microorganismineralization.

INTRODUCTION

Oil spills near production sites result in contamination of soil with oil and salts (Rhykered *et al.*, 1995). The persistence of petroleum pollutants in soil depend on the quality and quantity of the hydrocarbon mixture and on the environmental factors of the affected ecosystem (Atlas, 1981, Rhykered *et al.*, 1995). Successful degradation of hydrocarbon in soil requires optimum environmental parameters which ensures that bioremediation processes can occur at maximum rates.

Hydrocarbonoclastic microbial activities are probably one of the most important factors that determine the fertility of petroleum contaminated soils. Like other groups of microorganisms they play a major role in the mineralization of nutrients in soil (Odu 1972, Balloni and Favilli, 1987) and also influence soil structure and aggregate stability (Gupta and Germida 1988; Torstensson 1993). Microbial decomposition of hydrocarbon in high salinity environment (50 to 440 dSm^{-1}) has been reported to be relatively slow (Ward and Brock 1978, Rhykered *et al*, 1995).

To assess soil fertility, indicators of the three controlling components, the physical, chemical and biological, are needed. However, while optimal and threshold values for many physical and chemical parameters have been identified, the biological components is far less utilized. This is inspite of the frequently stressed importance of biological processes for soil functioning (Beck 1984, Kennedy and Papendick 1995, Bo-Sternberg *et al.*, 1998). In this study, the influence of electrical conductivity on the population of hydrocarbonoclastic microorganisms and rate of crude oil mineralization in the Niger-Delta ultisol was assessed .

MATERIALS AND METHODS

Collection and Treatment of Soil samples.

The soil type used was ultisol, an acidic sandy loam soil (D'Hoore, 1964), obtained from Uyo in the Niger Delta region of Nigeria. Samples were collected in the dry season from the depth of 10cm and within an area of 0.02m^2 at sites presumed to be free from contamination with petroleum hydrocarbons. Approximately 1kg of soil was taken in clean perforated plastic container, which in turn was seated in a plastic bowl with a single perforation at the bottom.

J. P. ESSIEN, Department of Botany and Microbiology, University of Uyo, Uyo, Nigeria
A. Y. ITAH, Department of Botany and Microbiology, University of Uyo, Uyo, Nigeria
S. I. EDUOK, Department of Botany and Microbiology, University of Uyo, Uyo, Nigeria

Distilled water was used to keep the soil moistened.

Qua Iboe Light (QIL) crude oil was added to the soils at the rate of 50gkg^{-1} of soil (5%) which is the range considered optimal for bioremediation (Dibble and Bartha 1976). The salinity of the oil soil samples was adjusted by the addition of 4.00g, 11.75g and 18.50g of NaCl to 1 kg of soil, resulting in soil water electrical conductivities of 40, 120 and 2000 dSm^{-1}. Three replications of each treatment were utilized. Two sets of control experiments were also prepared. Control experiment (E_1) consisted of uncontaminated soil samples (free of oil and NaCl) while the other control experiment (E_2) consisted of oil contaminated soil without NaCl. The treated soils were incubated at 28^{0}C.

Analysis of Soil

Prior to treatment representative soil samples from the study site were dried in a draft oven at 35^{0}C and homogenized to pass through a 2mm sieve. Using the pipette method (Gee and Bouder 1980) the soil samples were sorted into three size classes, namely clay (<0.002mm) silt (0.002 – 0.2mm) and sand (0.2 – 2mm). The pH of soil was determined in a 1:2 soil/water volume ratio. Total carbon (C) and nitrogen (N) were determined after combustion at 1400^0 C (CNS 2000 analyser). Total C was corrected to carbonate to give organic carbon. Available P, K. Na Ca and Mg were extracted with the acetate ammonium lactate method (AL) (Vekemans *et al* 1989). The salinity of the soil samples were determined as the electrical conductivity in soil-water samples by means of an electronic conductivity meter (D. C Jenway Model).

Microbiological Analysis

Microbial analysis of freshly collected soil samples was carried out. Subsequent analysis was done within two days interval after treatment. The population of oil degrading (hydrocarbonoclastic) microorganisms was enumerated using the surface spreading technique. Serial dilutions ranging from 10^{-1} to 10^{-10} of the soil samples were prepared. One ml quantities of each dilution were planted on Zajic and Supplisson (1972) agar (ZS) to serve as control. Three plates from each dilution (10^{-1} to 10^{-5}) were prepared and incubated at 28^{0}C for 7 days. Counts of oil degrading microorganisms were estimated by subtracting the microbial count on ZS agar plates from the counts on oil agar plates. The difference in counts represented the total count of hydrocarbonoclastic microorganism. This method reported by Zajic and Supplison (1972) has been previously used by Antai and Mgbomo (1989). It is based on the fact that non oil degrading microorganism are not expected to grow on oil agar.

Estimation of the Rate of Crude Oil Mineralization in Soil.

This was done by gravimetric method described by Rhykered *et al* (1995). Briefly 25g of soil from each treatment container was dried by mixing it with anhydrous sodium sulphate. The oil was extracted by adding 50ml of dichloromethane and shaking the mixture overnight. The extrac was filtered through a Whatman No 2 filter tc

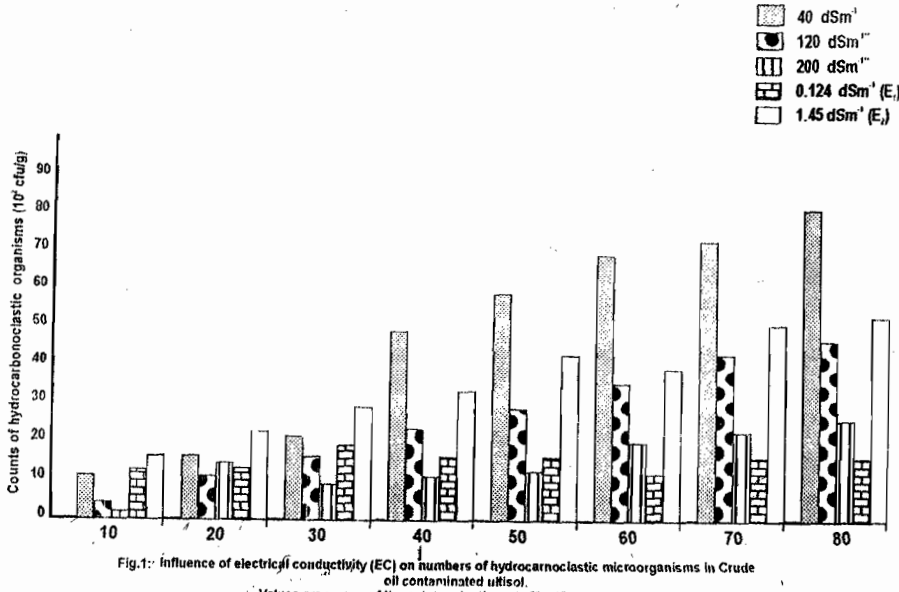

Fig.1: Influence of electrical conductivity (EC) on numbers of hydrocarnoclastic microorganisms in Crude oil contaminated ultisol.
Values are means of three determinations. ** Significant at P=0.05

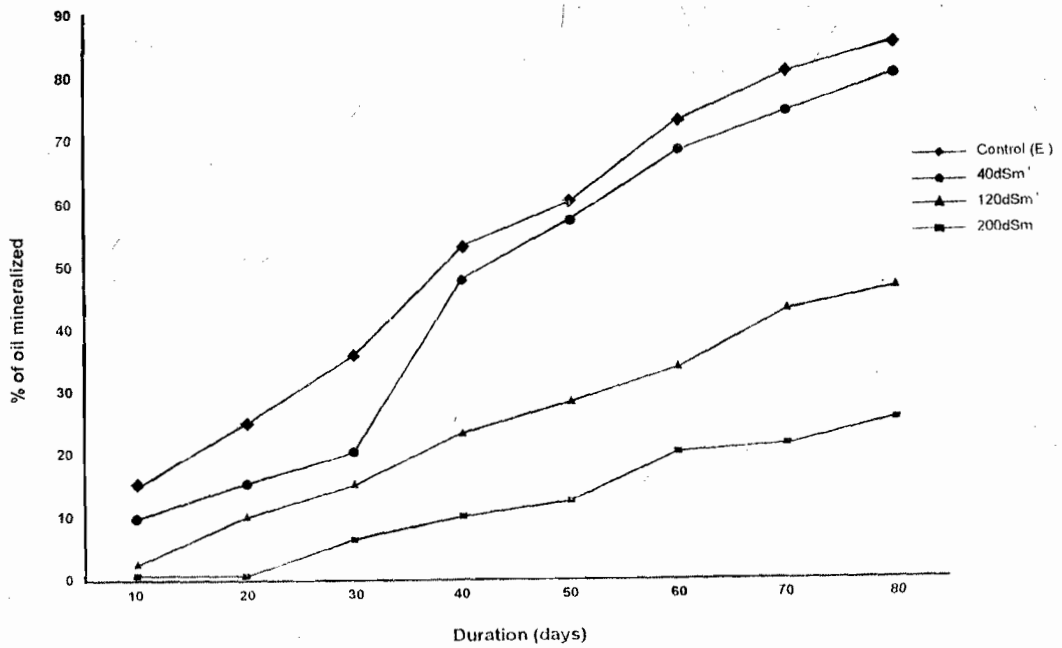

Fig. 2 Influence of electrical conductivity on mineralization of crude oil in ultisol

remove soil particles. The extraction was repeated with 25ml of dichloromethane followed by filtration. The extracts from each sample were combined and evaporated in a rotoevaporator. Soil samples from the control experiments (E_1 and E_2) were also extracted to determine background concentrations of oil to be subtracted from values recorded for the oil contaminated soil. The relationship between the number of hydrocarbonoclastic microorganisms and rate of oil mineralization in the ultisol was determined by simple correlation analysis using the SAS CORRELATION model (SAS Inst. Inc. 1985).

RESULTS AND DISCUSSION

A summary of the values for some of the properties of the ultisol is given in Table 1. Its low hydrocarbon and salt contents justifies the choice for this investigation. The addition of crude oil stimulated an increase in the numbers of hydrocarbonoclastic microorganisms in the ultisol (control E_1) higher than its average count of 10.2 x 10^2cfu/g (Fig. 1). The population density of oil degrading microorganism in oil contaimated soil (E_2) increased exponentially and significantly more than the initial population of degraders in the control experiment (E_1). The proliferation of oil degraders appeared to have occurred in much slower rate in oil free soil (E_1) due to the absence of crude oil. The population density of oil degrading microorganisms in the oil amended soil given salt treatments varied with the concentration of salt applied. On day 80 there were more hydrocarbon degrading microorganisms in the oil soil compared to oil soils treated with salt except for the treatment with an electrical conductivity of 40 dSm^{-1} where counts were similar to that of the oil soil control (E_2). Other levels of salt treatment (120 dSm^{-1} and 200 dSm^{-1}) significantly ($p>0.05$) decreased the number of hydrocarbonoclastic microorganisms in the ultisol.

Hydrocarbon degrading microbial population normally increase following the addition of oil to soil. (Atlas *et al.*, 1980, Rhykered *et al.*, 1995), and their decrease in number after salt treatment, may be attributed to an osmotic effect of salt on the organisms that reduced their activities, but not their viability. Salinity reduces the rate of mineralistion in many species of microorganisms (Rhykered *et al.*, 1995, Ward and Brock 1978, (Rhykered *et al.*, 1978). The low rate of microbial multiplication in soils with high salt concentrations may be attributed to the influence of salt on microbial metabolism, which may certainly affect their hydrocarbonoclastic potential.

The results presented in Figure 2 show that addition of NaCl to the ultisol has a decreasing effect on the amount of oil mineralized by the hydrocarbonoclastic microorganisms. Expectedly the rate of oil minerlization decreased with increase in the concentration of salt in soil. The

amount of oil recovered from the soil was approximately 16% and 20% (representing about 84% and 80% of oil mineralized) of the volume added to the control (E_2) and soil induced to EC of 40 dSm^{-1} respectively .while the amount of oil mineralize within 80 days in soil induced to EC of 120 dSm^{-1} was comparatively low (46%), and much lower (25%) in soil with EC of 200 dSm^{-1}

Correlation between hydrocarbonoclastic microbial counts and oil mineralization at different salt concentrations in soil are listed in Table 2. A positive significant correlation was observed between counts of oil degraders and rate of oil mineralization in control E_2 and soils with EC 40 dSm^{-1} and 120 dSm^{-1}. Both parameters were however insignificantly correlated in soil treatment with EC 200 dSm^{-1}. The significant correlation among the two parameters in soil suggests that the rate of oil degradation in hydrocarbon polluted soil is dependent upon the ability of oil degrading organisms to multiply in the microcosm.The results also suggest that high salt concentration may also be a factor which influences oil degradation in soil. Coefficients of variation (C.V.) as high as 96% and 95% were derived in soils with EC 40 dSm^{-1} and 120 dSm^{-1} respectively. The lower C.V. observed in soil with EC 200 dSm^{-1} is an indication of the negative influence of high salt levels on the number and oil degradability of microorganism in soil.

The gravimetric method of analysis used in this study for the evaluation of the amount of oil mineralized in soil has been reported to be more reliable because oil incorporated into microbial biomass is also accounted for (Rhykered *et al.*, 1995). Therefore, it is a useful index of assessing the effect of environmental parameters on the biodegradtion of oil in soils. The present study indicates that oil degrading organisms can tolerate salinity as much as 120dSm^{-1} in tropical ultisol. It also shows that oil mineralization in an ultisol is greatly determined by the population density of hydrocarbonoclastic microorganisms which appears to be osmotolerant although their oil degrading capabilities may be depressed by extremely high salt content in soils. Removal of salts from oil and salt contaminated soils before undertaking bioremediation may reduce the time required for the bioremediation of polluted ultisols.

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