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INFLUENCE OF SALINITY AND FUNGAL PREVALENCE ON BIOREMEDIATION OF CRUDE OIL POLLUTED SOIL

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ABSTRACT

The effect of NaCl salt on bioremediation of crude oil polluted soil was studied. Salt· treatments included NaCl amendments to adjust the soil solution electrical conductivities to 50, 130, 210 dsm-^{1}. Oil biodegradation was estimated from quantities of CO_2 evolved. Salt concentration at 210 dsm-^{1} in oil polluted soil resulted in a significant decrease ($p < 0.05$) in oil biodegradation. A salt concentration of 50 dsm-^{1} reduced bioremediation by about 12%. The physico-chemical properties of the soil samples examined showed that the total hydrocarbon (THC) content increased with the oil pollution but significantly decreased ($p < 0.05$) with NaCl addition. The prevalence of fungal species in the soil samples during each sampling intervals showed that the oil contaminated soil and the uncontaminated soil supported fungal growth while addition of NaCl reduced the fungal population in the soil

KEY WORDS: Salinity Effect, Bioremediation, Fungal Prevalence, Crude Oil Polluted Soil.

INTRODUCTION

Petroleum hydrocarbon spills in oil processing and production in the Niger Delta environments of Nigeria amounts to 1.7 to 10.8 million metric tons each year (national Academy of Science, 1985) in marine and land environment. Oil contaminated soils have posed severe difficulties for Agricultural crop production in oil producing and processing areas in Nigeria (Odoemena, 2002).

Bioremediation of oil contaminated soils is a cost effective biotechnological mechanism for the removal of hydrocarbon pollutants from the environment. This is because they drastically affect the physiocochemical and microbiological properties of such soils (Eja *et al., 2003).*

A factor complicating bioremediation of crude oil spills is salinity. Oil field brine has an electrical conductivity of about 200 dsm-^{1} which is nearly four times that of sea water (Kinghorn, 1983). Oil contamination of soil results in higher salinity of the soil and microbial degradation of hydrocarbons in hypersaline soil and water (50 to 440 dsm-^{1}) is relatively slow (Ward and Brock, petroleum hydrocarbons in soil has not been adequately studied. The objective of this study is to investigate the effect of NaCl on biodegradation of oil in the sandy loam soil and the prevalence of biodegrading fungi load in the soil.

Materials and Methods

Three experimental soil sampling sites designated as S_1, S_2 and S_3 of 1 metre square each and 5 metre apart from each other were selected within the Department of Botany and Ecological Studies Research garden of the University of Uyo, Uyo, Akwa Ibom State. Site one (S_1) was neither treated with crude oil nor NaCl salt which served as the control. Site two (S_2) was contaminated with 4 litres of crude petroleum oil by pouring it evenly on the site. Site three (S_3) was contaminated with 4 litres of crude oil and 500g of NaCl salt was evenly spread on the site. The three sites were given basal fertilizer treatment of Nitrogen as NH_4N0_3 at 0.80g kg-^{1} and phosphorus as K_2HPO_4 at 0.20g Kg-^{1} soil. This amendment is believed to enhance the

microbial agents (Atlas & Bartha, 197:3; Pritchard and Costa, 1991).

The sites were thoroughly irrigated two hours after pollution and after other treatments. On the second day after pollution, 10g each of the sites soil samples were collected respectively using hand driven auger at a depth of 4cm. The soil samples were taken to the laboratory in labeled polythene bags stored in ice-cooled boxes at 5±1^{o}C. The soils were analysed for their physiochemical characteristics after collection. There after the evaluation of the fungal load and CO_2 evolution at 7 days interval for 28 days were carried out.

Physioco-chemical analysis of soil samples.

The soil samples were air dried and passed through a 2mm sieve. Soil organic carbon was determined according to the method of Fawole and Oso (1988) by igniting the dried sieved soil (2.5g) in a pre-weighed crucible and calculating the loss in weight by difference, and thus the percentage of organic carbon in the soil was calculated. The pH of the soil was determined in 1:2 soil/water ratio. Available phosphorus was determined by the methods of Bray and Kurtz (1945). Total Nitrogen was obtained by micro-kjeldahl digestion method (Black *et al.,* 1965). The particle size measured was determined by the Bououcos hydrometer method (Day, 1965) as modified by Gee and Bauder (1986). This involved weighing about 100g of the air-dried soil samples into a container and adding 5.0ml of sodium hexametaphosphate solution followed by stirring for 30 minutes. The mixture was left overnight in a 250ml measuring cyclinder followed by shaking and inverting the cyclinder several times. After 40 seconds, the first reading which gave the percentages of clay ·and silt was recorded (Gee and Bauder, 1986) while second reading was taken after two hours as the percentage of sand (Gee and Bauder, 1986).

Total hydrocarbon (THC) was determined following extraction with redistilled n-hexane solvent before measuring the total hydrocarbon content colorimetrically at 430nm using a DR/3000 HACH spectrophotometer (England)

range of 10-1 to 10-6 were each plated on malt extract Agar (oxoid) acidified to a pH of 4.8 to suppress the growth of bacteria and incubated at room temperature (27±1^{o}C) for three to four days. Total fungal counts were made on any plate showing discrete colonies. The number of visible colonies was multiplied by the reciprocal of the dilution factor and recorded as colony forming units (cfu) per gram of soil (Fawole and Oso 1988, APHA, 1998).

Estimation of CO_2 Evolution During Bioremediation

The rate of crude oil degradation in the soil samples with and without NaCl was estimated by CO_2 evolution technique (Caxufield, 1961). Twenty grams each of the crude oil polluted soil (S_2) and crude oil and salt contaminated soil (S_3) were respectively transferred into two different screw capped bottles.

Different concentrations of NaCl solution were prepared by dissolving 0, 0.20, 0.50 and 0.80g to 50g of the oil polluted soil corresponding to salt-water electrical conductivities of 0, 50, 130 and 210 ds m-^{1}. One milliliter of the different NaCl conductivity levels was added into each of the screw-capped bottles containing 20g of the oil contaminated soil. The bottles were then filled to full capacity with distilled water by adding a mixture of 1.0g of barium peroxide and 10ml of distilled water contained in a vial into each of the screw capped bottles and tightly closed. The bottles were later incubated at room temperature (27± 1^{o}C). The vials were withdrawn and replaced with fresh sets at 7 days intervals for 28 days.

RESULTS

The mean levels of some physico-chemical characteristics of the S_1, S_2 and S_3 soil samples are presented in Table 1. The contaminated soil samples with crude petroleum oil showed very high levels of total hydrocarbon (THC) compared with the control (S_1). The oil polluted soil samples (S_3) amended with NaCl indicated lower THC content than that polluted with petroleum oil alone (S_2). THC levels ranged from 10.5mg/kg in the unpolluted soil (control) to 3, 245.2mg/kg in S_2 and 1,252.6mg/kg in NaCl amended polluted soil (S_3). The oil pollution

Table 1: Some Physicochemical Properties of the Experimental Soils Polluted with Crude Oil and Amended with Sodium Chloride Salt

Soil Properties	***Garden Soil Unpolluted*** S_1 ***(Control)***	***Garden Soil Polluted with Crude Oil*** S_2	**Garden Soil Polluted with Crude Oil and amended with NaCl** S_3
Sand	75.34±0.01	75.32±0.02	75.24±0.03
Silt %	58.73±0.30	20.25±0.25	17.56±0.15
Clay %	16.12±0.12	16.23±0.13	16.20±0.20
pH	6.55±0.03	5.34±0.12	7.40±0.10
THC (mg/kg)	10.5±0.15	3,245.2±0.80	1,252.6±0.21
Organic Carbon (%)	1.23±0.02	4.26±0.01	4.25±0.03
Total Nitrogen (%)	0.16±0.20	0.12±0.30	0.14±0.10
Phosphorus (%)	14.21±0.03	7.14±0.25	7.25±0.30
C/N Ration	7.68±0.05	35.50±1.15	30.36±2.03

Each value IS the mean ± standard deviation of triplicate soil samples collected from S_1-S_3.

Pollution of the soil sample with crude petroleum oil stimulated the CO_2 production in the soil but the addition of NaCl reduced CO_2 production (Table 2). Carbon dioxide evolution from the soil amended with 210 dsm-^{1} was decreased by 56.25% when compared with that of the control. The result also showed that increased concentration of NaCl decreased CO_2 evolution. Also the production of CO_2 increased significantly ($P < 0.05$) with time with a peak at 21 days which gave a significant fall ($p < 0.05$) at the 28th day of the experiment.

Table 2: Cumulative carbon dioxide evolution (cm^3/wk) crude oil polluted soil treated with NaCl (soil S_3)

Sodium Chloride Concentration in Polluted Soil				
Time in Days	**Site 1 Control**	**Site 3**		
0	**0**	**50**	**130**	**210(dsm-^{1})**
7	63.8±0.75	52.5±1.63	38.4±0.81	28.6±0.50
14	123.2±1.32	103.4±1.32	85.2±0.74	33.0±0.34
21	165.3±1.21	134.1 ±1.54	115.0±1.20	92.5±1.50
28	183.4±2.16	162.4±2.15	143.6±1.85	115.9±1.63
28	156.2±1.65	138.5±1.67	110.7±1.24	106.2±1.45

Each value is the mean ± standard deviation of triplicate determinations of CO_2 evolution during bioremediation process.

The effect of soil pollution with crude oil on fungal counts are shown in Table 3. There was no significant difference ($p < 0.01$) in fungal population between the days of sampling. However, high significant differences ($p < 0.01$) in fungal counts were observed between the uncontaminated (S_1) soil sample and crude oil polluted soil (S_2). However, the NaCl amended polluted soil showed least amount of fungal load (Table 3).

Table 3: Effect of Crude Oil Polluted Soil Sample on the Total Fungal Counts (x 10^4 cfu/g)

Sampling Intervals (days)	**S_1 Unpolluted Soil Control**	**S_2 Crude Oil Polluted Soil**	**S_3 Polluted Soil Amended with NaCl**
0	283.81±0.3	208.31 ±0.5	154.87±0.3
7	178.53±0.5	162.25±1.6	140.46±1.2
14	156.42±0.2	144.40±2.1	120.45±0.4
21	138.26±0.4	135.52±0.1	106.63±1.5
28	122.18±0.5	115.23±0.3	102.32±2.3

Each value is the mean ± standard deviation of fungal counts of triplicate soil samples collected from each site on the marked days.

The prevalence of fungal species in the soil samples during each sampling intervals that of uncontaminated (S_1) supported the growth of the fungal species while the NaCl addition

DISCUSSION

The addition of NaCl salt on crude oil polluted soil reduced the total hydrocarbon (THC) level of the soil sample (S_3) when compared with that of oil polluted (S_2). The mean THC level in the unpolluted soil (S_1) serving as control was as low as 10.5mg/kg indicating that hydrocarbons could be present in unpolluted soils and sediments which is in line with the report of Geiger and Blumer (1974). Addition of NaCl increased the pH level of the soil while oil contamination reduced the soil pH from 6.55 to 5.34. The pH shift towards neutrality in S_2 may have enhanced fungal growth and activity (Dibble and Barth 1979).

The increased stimulation of CO_2 production due to petroleum oil pollution in the soil as observed in this study had earlier been reported by Eja *et al.,* (2006) and the subsequent decrease of CO_2 production on NaCl amendment to the oil polluted soil also corroborates with the findings of Rhykerd *et al.,* (1995) (Table 2). The reduction in CO_2 production with increased concentration of NaCl soil solution electrical conductivity (210 dsm^{-1}) may be attributed to the salinity effect of the soil on the fungi or other microorganisms in the soil (Walker and Calwell 1975). Twenty to forty percent reduction in CO_2 production in soils containing higher concentration of salt (200 dsm^{-1}) have been reported by Rhykerd *et al.,* (1995).

The unpolluted site (S_1) showed the highest fungal population counts (283.8±0.3 cfu/g) indicating that the petroleum products in the polluted site could have initially adversely affected the growth of the organisms. The low incidence of total fungi in NaCl amended polluted soil in the days of sampling (Table 2) was as a result of a lag period caused by hypersalinic effect of the salt coupled with crude oil toxic effect (Clark *et al.,* 1997) on fungi which were less evident in soils polluted with crude oil alone.

In summary therefore, this study has been able to deduce the long delay in bioremedial activity of oil pollution in hypersaline environments in Nigeria coastal regions.

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