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Bioremediation of Crude Oil Polluted Soil in the Niger Delta Area of Nigeria Using Enhanced Natural Attenuation

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Abstract: Remediation by Enhanced Natural Attenuation (RENA) is a land farming treatment technology, which relies on natural processes to clean up or attenuate pollution in soil and groundwater. There is the need for remediation because crude oil spills may cause damage to the environment in many ways. Oil spill on land may lead to retardation of vegetation growth and cause soil infertility for a long period of time until natural processes reestablish stability. In the present study, RENA was employed to remediate oil-contaminated site in the Gokana local government area of Rivers State of Nigeria between January and September 2006. Mineral salt medium to which crude oil had been added was used as a sole source of carbon and energy to isolate hydrocarbon utilizers from soil samples collected from different plots of the contaminated site. Two fungi *Articulosprium inflata* and *Zoopage mitospora* as well as five bacterial genera; *Lactobacillus, Arthrobacter, Bacillus, Pseudomonas* and *Micrococcus* were isolated and identified. This study also indicated that the counts of Total Heterotrophic Bacteria (THB) varied over a period of 18 weeks. The microbial and physicochemical properties of the soil samples varied with the different plots and at different periods of remediation.

Key words: Bioremediation, biodegradation, hydrocarbon utilizers, RENA

INTRODUCTION

Petroleum in its natural state is called crude oil (Ukoli, 2003). There are many different varieties of crude oil, ranging from very fluid volatile liquids to viscous, semi-solid materials (Ojo and Adebusuyi, 1996). Crude oil is mainly either black or green but it can also be light yellow. It varies considerably in density and is described as heavy, average or light (API) (Ojo and Adebusuyi, 1996).

The discharge of crude oil into the environment constitutes a serious pollution problem. Pollution is an undesirable change in the physical, chemical and biological characteristics of all the components of an environment (Aboribo, 2001). This can threaten human health and that of beneficial organisms in the environment (Aboribo, 2001). Increasing petroleum exploration, refining and the operation of petroleum companies in the Niger Delta region of Nigeria have led to the wide scale contamination of most of its creeks, swamps, rivers and streams (Okpokwasili, 1996). In 1998 alone, a total of 390 cases of oil spills were reported in the Niger Delta region of Nigeria (Mitchell, 1999).

The presence of oil has significant social and environmental impacts, from accidents and routine activities such as seismic explosions, to drilling and generation of polluting wastes (Atlas, 1995). Oil extraction is costly and sometimes environmentally damaging. Over 70% of the reserves in the world are associated with visible macro seepages and many oil fields are found due to natural leaks (Atlas, 1995). Offshore exploration and extraction of oil disturbs the surrounding marine environment. Extraction may involve dredging, which stresses up the seabed, killing the sea plants that marine creatures need to survive (Gbadegesin, 1997). Crude oil and refined fuel spills from tanker ship accidents have damaged fragile ecosystems in Alaska, the Galapagos Islands, Spain and even the Niger Delta Region in Nigeria.

As a result of the small size of the oil fields in the Niger Delta there is an extensive network of pipelines between the fields, as well as numerous small networks of flow lines - the narrow diameter pipes that carry oil from well heads to flow stations - allowing many opportunities for leaks (Oil Spill Intelligence Reports, 1998). In onshore areas, most pipelines and flow lines are laid above ground and many pipelines and flow lines are old and subject to corrosion: fifteen years is the estimated safe lifespan of a

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pipeline, but in numerous places in the Niger delta, pipelines aged twenty to twenty - five years can be found (Oil Spill Intelligence Report, 1998). The main causes of oil spill in Nigeria, according to Okpokwasili (1996) include criminal damage (sabotage and crude theft, bunkering), equipment failure e.g. wellhead blow out, valve and flanges failure etc, corrosion: either through chemical or biological agent, Human error and Technical failure.

Crude oil spills may cause damage to the environment in many ways. In water, oil film floating on the water surface could prevent natural aeration leading to the death of fresh water or marine life. Fish may ingest spilled oil or other food materials impregnated with oil. Such fish have been observed to be unpalatable (Ukoli, 2003). Oil spill on land may lead to retardation of vegetation growth and cause soil infertility for a long period of time until natural processes re-establish stability ((National Environmental Protection Regulations, 1991; Sada and Odemerho, 2000).

The primary aims of any remediation according to Atlas (1995) are reduction of actual or potential environmental threat and reduction of potential risks so that unacceptable risks are reduced to acceptable levels. Consequently, the need for remediation will depend on the degree of actual or potential environmental threat or the level of risk. Aspects of risk will in turn depend partly on the expected end use of the site following remediation, as different at risk targets can be associated with different end-uses (Ukoli, 2003). Remediation of a contaminated site is achieved by one or more of the following objectives: removal or destruction of the contaminants, modification of the contaminants to a less toxic form and isolation of the contaminant from the target by interrupting the pathway of exposure.

RENA is a land farming treatment technology adopted by Shell Petroleum Drilling Company (SPDC) for intervention in petroleum hydrocarbon impacted soils in the Niger delta (Ukoli, 2003). It relies on natural processes to clean up or attenuate pollution in soil and groundwater. RENA is a full-scale bioremediation technology in which contaminated soils, sediments and sludges are periodically turned over or tilled into the soil to aerate the waste (Ukoli, 2003). Soil conditions are often controlled to optimize the rate of contaminant degradation as the right conditions must exist underground to clean sites properly, if not, cleanup will not be quick enough or complete enough. Conditions normally controlled include moisture content (usually by irrigation or spraying), oxygen level (by mixing the soil using tilling or aerating), nutrients primarily nitrogen and phosphorus (by fertilizing), pH (increased in some instances by adding lime) and soil bulking (by adding soil amendments and by mixing using tilling etc).

The ubiquitous nature of microorganisms and their ease of isolation from oil-contaminated environment confirm the fact that they play an important role in the degradation of oil spills. Atlas (1995) noted that microbacteria grew well on mineral agar exposed to hydrocarbon vapour. Duffy *et al.* (1997) also observed that liquid culture media designed to isolate hydrocarbon utilizing bacteria yielded large numbers of *Pseudomonas* species.

In the present investigation, RENA was employed to remediate oil-contaminated site in the Gokana local government area of Rivers State of Nigeria

MATERIALS AND METHODS

Collection of samples: Soil samples used in this research were obtained from an oil spilled area in the Gokana Local Government Area of Rivers State, Nigeria, between January and June 2006. Controls were obtained from both the right and left sides of the right of way.

The samples were aseptically collected using soil sampler at a depth of 20cm, stored in sterile aluminum foils and transported to the laboratory within 48 h of collection. The spill site was cleared and divided into 3 portions which were labeled A, B and C, respectively. Samples were collected from different parts of each portion and bulked for homogenecity. Thereafter, windrows were constructed and leveled at intervals of two weeks. This is in accordance with the methods employed by RENA.

Enumeration of total heterotrophic bacteria and fungi in the soil samples: Total heterotrophic bacterial and fungal counts in the soil samples were enumerated by diluting 1g of each sample serially (10^{-1} to 10^{-7}). One mililiter from dilutions of 10^{-2} and 10^{-3} were plated in duplicate on sterile saboraud dextrose agar plates while dilutions of 10^{-5}, 10^{-6} and 10^{-7} were plated in duplicate on sterile nutrient agar plates, using the pour plate method. Incubation was carried out at 28+2°C for 7days for the saboraud dextrose agar plates and 37°C for 24h for the nutrient agar plates. Colonies on the plates were afterwards enumerated in accordance with the American Public Health Association (1995).

Enumeration of hydrocarbon utilizing bacteria and fungi: One gram of each sample was diluted serially (10^{-1} to 10^{-7}). One mililitre from dilutions of 10^{-2}, 10^{-3}, 10^{-5}, 10^{-6} and 10^{-7} were plated in duplicate on pre dried mineral salt agar using the spread plate technique and a hockey stick. For the 10^{-2} and 10^{-3} dilutions, 0.5 mL of streptomycin was added to the mineral salt agar to suppress bacterial growth. A filter paper saturated with sterile crude oil was

aseptically placed on the inside of the inverted petridishes and the culture plates were incubated for 7days at 28+2°C for fungi and 4 days at 37°C for bacteria. Plates yielding 30 to 300 colonies were afterwards enumerated for bacterial isolates. Plates with fungal colonies were also enumerated.

Isolation of hydrocarbon utilizing bacteria and fungi: Colonies of different hydrocarbon utilizing bacteria and fungi were picked randomly using a sterile inoculating wire loop and subcultured to purify, by streaking on nutrient agar plates and sabouraud dextrose agar plates respectively. The plates were and incubated at 30°C for 24 h and at room temperature for 3 days, respectively to obtain pure colonies. This process was repeated to ensure that the isolates are pure.

Identification and characterization of isolates: Representative colonies of hydrocarbon-utilizing bacterial isolates were purified on nutrient agar plates. Isolated pure cultures were afterwards transferred onto bottles of nutrient agar slant, incubated for 24 h and stored at 8+2°C until required for biochemical test. While representative colonies of hydrocarbon utilizing fungi were purified on sabouraud dextrose agar plates to which 0.5 mL of streptomycin had been added. Isolated pure cultures were afterwards transferred onto bottles of sabouraud dextrose agar slant, incubated for 3 days at room temperature and stored at 8+2°C. The methods used for the identification and characterization of the isolates were adopted from Gerhardt *et al.* (1981), Cruickshank *et al.* (1980) and Carpenter (1977).

Analyses of physicochemical properties of the soil samples

Determination of Total Petroleum Hydrocarbon (TPH): This was determined in accordance with American Society for Test and Materials-ASTM D3921` and D5369. Fourier Transform Infrared Spectrophotometer (FTIR, Genesis Series) was used for the test. Prior to analysis the soil samples were extracted with carbon tetrachloride and treated with 2% deactivated silica gel. The equipment was calibrated with isooctane/cetane in carbon tetrachloride. TPH concentrations in the samples were determined by using the stored calibration graph in the software of the equipment as a reference.

Moisture content determination: This was determined in accordance with ASTM D2216-92. A weighed amount of the soil sample was placed in a weighed crucible and dried at 105°C in the oven until a constant weight was reached. From the difference in weight, the percentage moisture content was calculated.

Phosphate content determination: The phosphate content of the soil samples was determined in accordance with Stannous Chloride Reduction Method, based on the method described in American Public Health Association-APHA 424E which was applied using Unicam UV/visible spectrophotometer. Soil samples were extracted with 25% acetic acid and the extract run on the UV at a wavelength of 700 nm. A spike sample was analyzed in every batch of analysis. A standard was analyzed after every batch of samples and the first value of the standard was used to plot the means control chart.

Nitrate content determination: Nitrate content of the samples was determined colorimetrically in accordance with the Water Operational Guide 1987, using Unicam UV/visible spectrophotometer. Soil samples were extracted with sodium acetate in the presence of sulphuric acid and measured at a wavelength of 470nm.

pH determination: This was determined based on the method described in American Public Health Association 424E (1995). The pH electrodes of a multimeter were dipped into a mixture of soil sample and deionized water. The pH values of the samples were subsequently read on the multimeter.

Total organic matter content determination: Five hundrad miligram of soil sample was weighed into a 250 mL conical flask and 25 mL chromic acid mixture was added. This was boiled for 1 h, allowed to cool and diluted with 100 mL of water. This was followed by the addition of 5 mL of indicator solution and titration of unused dichromate with ferrous ammonium sulphate solution. 2.5 mL of dichromate mixture was further added when the first colour change occurred and the titration was completed dropwise. A blank determination was carried out and subtracted as shown below. For example: If T mL of ferrous Ammonium sulphate is used in the titration, then:

$$\% C = \frac{(2.7 - T)\text{mL} \quad 0.12}{\text{Sample weight or sample volume}}$$

Statistical analysis of data: All the data obtained were subjected to statistical Analysis of Variance (ANOVA) using computer aided SPSS statistical program. All the means were separated and compared using Duncan Multiple Range Test at 5% level of significance.

RESULTS

The count of total Heterotrophic Bacteria (THB) in crude oil polluted soil ranged from 4.10×10^4 cfu g^{-1} to 2.73×10^7 cfu g^{-1}, while in the control (crude oil free soil),

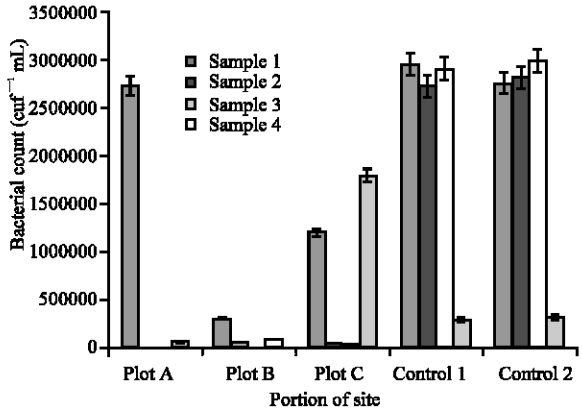

Fig. 1: Trend of change in the total heterotrophic bacterial population

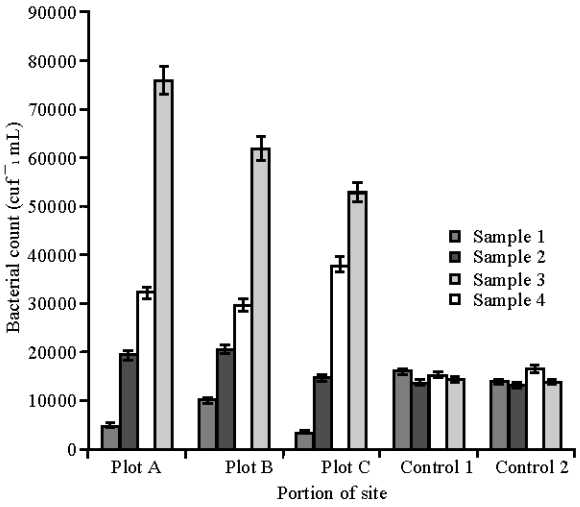

Fig. 2: Trend of change in the hydrocarbon utilizing bacterial population

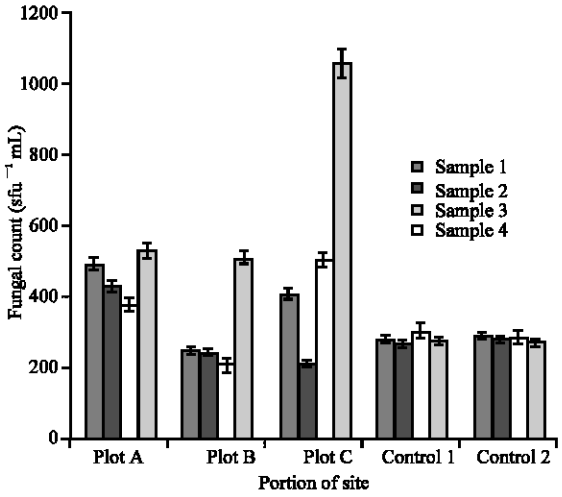

Fig. 3: Trend of change in the total heterotrophic fungal population

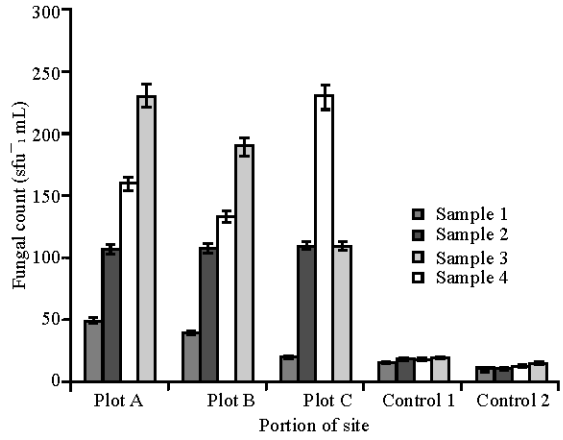

Fig. 4: Trend of change in the hydrocarbon utilizing fungal population

the counts ranged from 3.00×10^{6} cfu g^{-1} to 2.99×10^{7} cfu g^{-1} of soil (Fig. 1 and 2). Generally, the THB counts were higher in crude oil free soil than in crude oil polluted soil. However, statistical analysis revealed that the difference in counts between the two sites was not significant. There were higher counts of Hydrocarbon Utilizing Bacteria (HUB) in crude oil polluted soils (3.60×10^{3} cfu g^{-1} to 7.60×10^{4} cfu g^{-1} of soil) than crude oil free soil (1.33×10^{4} cfu g^{-1} to 1.66×10^{4} cfu g^{-1} of soil). However, there was no significant difference (p=0.05) between the 2 sites.

The counts of THF ranged from 2.10×10^{2} sfu g^{-1} to 1.06×10^{3} sfu g^{-1} of soil in crude oil polluted soil and from 2.67×10^{2} sfu g^{-1} to 3.02×10^{2} s fu g^{-1} of soil in crude oil free soil. While the counts of Hydrocarbon Utilizing Fungi (HUF) ranged from 2.0×10^{1} sfu g^{-1} to 2.31×10^{2} sfu g^{-1} of soil in crude oil polluted soil and 1.1×10^{1} sfu g^{-1} to 2.0×10^{-1} sfu g^{-1} of soil in crude oil free soil (Fig. 3 and 4). Statistical analysis revealed that the difference in counts between the 2 sites for THF was not significant (p=0.05). The difference in counts between the 2 sites for HUF was however significant (p = 0.05)

The hydrocarbon utilizing microbial isolates were identified as species of *Bacillus, Micrococcus, Pseudomonas, Arthrobacter, Lactobacter, Zoopage* and *Articulosporium. Bacillus* and *Zoopage* were more frequently isolated among the bacteria and fungi, respectively. Trend of change in Total Petroleum Hydrocarbon (TPH), Total Organic matter Content (TOC), Nitrate content (NO_3), Phosphate content (PO_4),

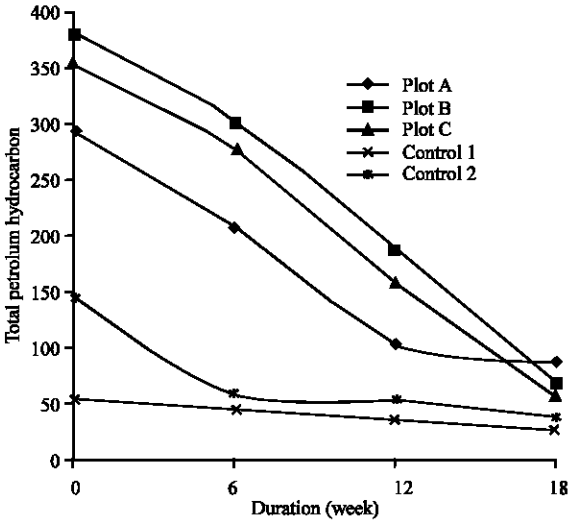

Fig. 5: Trend of change in total petroleum hydrocarbon content of the soil samples during the investigation period

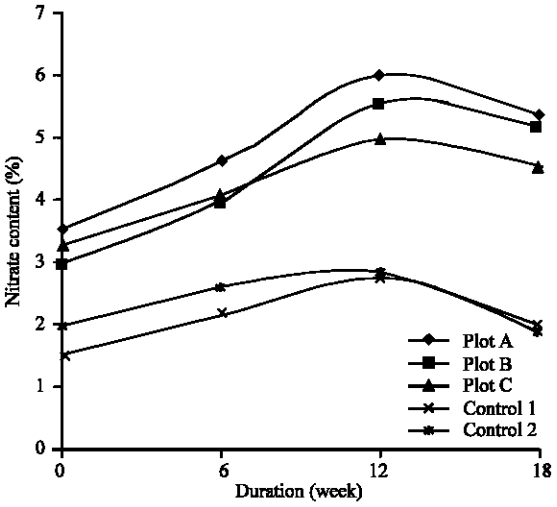

Fig. 7: Trend of change in nitrate content of the soil samples during the investigation period

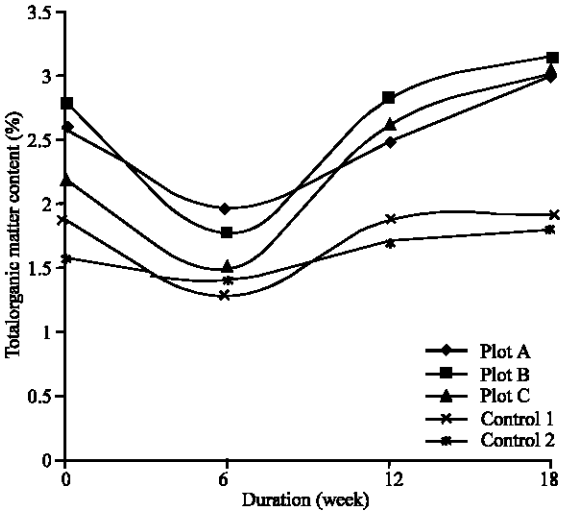

Fig. 6: Trend of change in total organic matter content of the soil samples during the investigation period

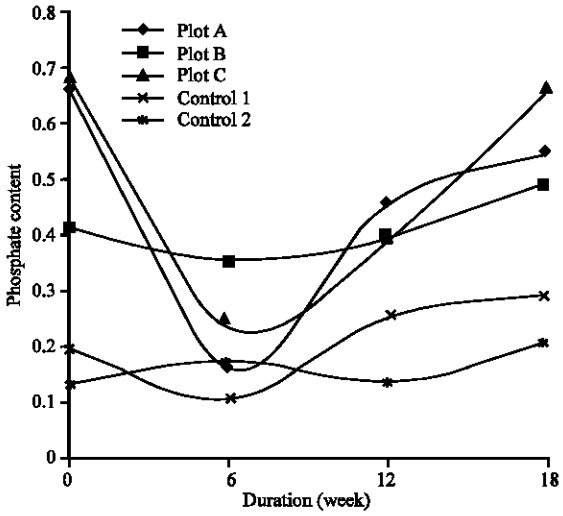

Fig. 8: Trend of change in phosphate content of the soil samples during the investigation period

acidity/alkalinity level (pH) and Moisture Content (MC) of the soil samples are shown in Fig. 5-10, respectively. The pH of crude oil polluted soil ranged from 4.00 - 4.54 while that of crude oil free soil ranged from 4.52 - 4.85. The results revealed that the pH values of crude oil polluted soil were lower compared to those of crude oil free soil. Statistical analysis showed a significant difference between the 2 sites. The TOC of crude oil polluted soil (1.49-3.13%) was higher than that of crude oil free soil (1.26-1.90%). Statistical analysis showed a significant difference between the 2 sites. The nitrogen level of the crude oil polluted soil ranged from (3.00-6.00%), while that of crude oil free soil ranged from (1.51-2.86%), indicating the availability of more nitrogen in the polluted soil than the free soil. The values were significantly different, (p =0.05).

The available phosphorus level of crude oil polluted soil (0.16-0.69) was igher than that of crude oil free soil (0.10-0.29) and the differences were also significant, (p = 0.05). However, the MC of crude oil polluted soil (5.60-8.20%) was lower than that of crude oil free soil (10.44-13.71 %) and statistical analysis showed a

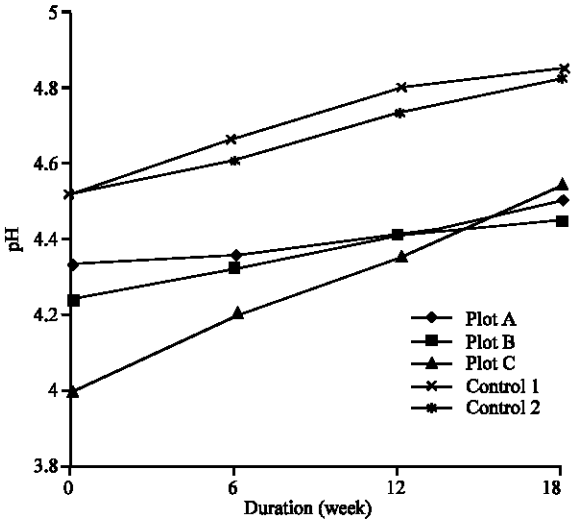

Fig. 9: Trend of change in pH of the soil samples during the investigation period

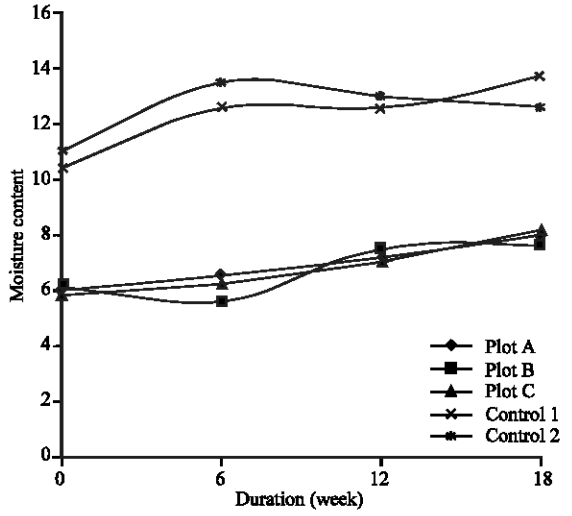

Fig. 10: Trend of change in moisture content of the soil samples during the investigation period

significant difference between them. In the crude oil polluted soil, the TPH decreased sharply through the investigation period while the decrease in the crude oil free soil was gradual and statistically, the difference in the TPH level between both soils was significant ($p = 0.05$).

DISCUSSION

The variation in counts of Total Heterotrophic Bacteria (THB) over the period of 18 weeks may be due to changes in the physicochemical properties of the soil. However, the difference in counts of THB and THF between the crude oil polluted soil and the crude oil free soil was not significant, probably due to rapid biodegradation of the crude oil in the soil. The counts of Hydrocarbon Utilizing Bacteria (HUB) in crude oil polluted soil were higher than those of the crude oil free soil. Statistical analysis revealed that the difference was not significant ($p = 0.05$). The reason for higher counts in crude oil polluted soil may be due to the presence of residual crude oil in the polluted soil which boosts the carbon supply in the soil, hence favor the growth of the hydrocarbon utilizing bacteria as compared to crude oil free soil (Ijah and Abioye, 2003; Ijah and Antai, 2003).

The bacterial counts in both polluted and free soil where higher than the fungal counts in both soils and the HUF count in the polluted soil was higher than that of the crude oil free soil. This agrees with the results of Ijah *et al.* (2003). However, it is a sharp contrast to the results of Ijah and Abioye (2003). The higher counts of bacteria compared to fungi may be as a result of the nutrient status of the soil (Jobson *et al.*, 1974) and the presence of some toxic components which do not favor fungal growth (Colwell and Walker, 1977).

The pH values of crude oil polluted soil were lower as compared to those of crude oil free soil, a finding, which is in line with the report of Ijah and Abioye (2003). The decrease in pH value may be due to increased degradation of crude oil by microorganisms in the soil, resulting in accumulation of acidic metabolites. However, the moisture content of crude oil polluted soil was lower than that of crude oil free soil.

This is in contrast to the work of Ijah and Abioye (2003) and may be due to the fact that crude oil can coat the soil and consequently prevent the penetration of water compared to kerosene that was used in their study. Both nitrogen and phosphorus levels were higher in crude oil polluted soil than crud oil free soil. This agrees with the finding of Odu (1972) who reported increase in nitrogen and phosphorus contents of a crude oil polluted soil. The reason could be due to higher organic matter content of the polluted soil.

The rate of crude oil biodegradation in the soil seems to be rapid. This may be due to the fact that the microorganisms in the soil have efficient ability in utilizing the residual crude oil as a source of carbon and energy (Ijah and Antai, 2003). Crude oil contains hydrocarbon and does not resist attack by microorganisms (Atlas, 1995). The hydrocarbon utilizing microorganisms isolated from the soil were species of *Bacillus*, *Lactobacter*, *Arthrobacter*, *Pseudomonas*, *Micrococcus*, *Zoopage* and *Articulosporium*. *Bacillus* species predominated, especially in the crude oil polluted soil. This may be due to the ability of the organisms to produce spores, which may shield them from the toxic effects of the hydrocarbons.

CONCLUSION

The study shows that there is residual crude oil in the soil after 18 weeks of investigation. The active crude oil utilizing microorganisms identified were species of *Bacillus*, *Pseudomonas Micrococcus*, *Arthrobacter*, *Lactobacter*, *Zoopage* and *Articulosporium*. The study also shows that the physiochemical properties of the soil were significantly affected, ($p = 0.05$). Finally, the study has shown that Remediation by Enhanced Natural Attenuation is effective in the clean up of polluted sites in the Niger Delta.

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