

Bioremediation of Soil Contaminated with Crude Oil and Amended with *Moringa Oleifera*

ABSTRACT

The potential of *Moringa Oleifera* in increasing the rate of the microbial degradation of crude oil-spilled soil was observed. The *Moringa oleifera* seed cake was found to contain nitrogen (10.55%), phosphorus (18.33ppm) and hydrocarbon degrading bacteria. The results revealed that the counts of crude oil degrading bacteria in crude oil polluted soil amended with *Moringa oleifera* came up to 3.6×10^6 CFU/g on the 21st day while that in unamended soil could only get to 1.5×10^6 CFU/g. *Moringa oleifera* seed cake was found to enhance the degradation in crude oil polluted soil by up to 64.3% compared to the crude oil polluted and unamended soil. pH of crude oil polluted soil sample amended with *Moringa oleifera* ranged between 7.24 and 7.70 while that of crude oil polluted soil ranged between 7.24 and 7.44. This implies that *Moringa oleifera* has a buffering effect which provides an enabling environment for the micro-organisms to proliferate. The results of this study show that *Moringa oleifera* seed cake is efficient and can be used in reclaiming crude oil polluted soil.

Keywords: Microbial degradation, spilled oil, degrading bacteria, polluted soil, buffering effect, *Moringa oleifera*.

1. Introduction

Oil and gas has become a major source of energy, the world over. This is deduced from application of its various products in our lives and how much revenue is being generated from it. With the immense benefits generated from the oil and gas industry notwithstanding, its exploration and exploitation also brings along with it significant negative impact to the ecological and human environments (Oyegun, 1998), subsequently impacting on the socio-economy of the nation. Oil spillage is one of the problems that could result from oil drilling.

In Nigeria, oil pollution problem have been prevalent since the commencement of oil exploration and development of the petroleum industry (Okoh *et al.*, 2001). However, different methods have been used in restoring petroleum polluted soil, some of these include the use of oil degrading microorganisms, inorganic fertilizers, chicken droppings, periwinkle shell, liming and tilling (Leahy and Colwell 1990; Ijah *et al.* 2003a; and Ijah *et al.* 2003b).

Bio-stimulation using inorganic fertilizer has been extensively employed worldwide in reclaiming oil polluted soil (Jobson *et al.* 1974; Dibble and Bartha 1979; and Song *et al.* 1990 and USEPA 1990). However *Moringa Oleifera* due to its high nitrogen content can be used to enhance biodegradation of crude oil in soil and at the same time solving the problem of wastes from the environment. Treatment of oil contaminated soil is necessary to protect

water supplies, human health and environmental quality (Chang *et al.* 1996), owing to the fact that plants derive nutrient for their living from soil, animals derive theirs from plants and human derive theirs from plants and animals living on soil.

Bioremediation is a method whereby the natural abilities of micro-organisms are employed for the reduction of concentration or /and toxicity of various chemical substances, such as, petroleum derivatives, aliphatic and aromatic hydrocarbons, industrial solvents, pesticides and metals (Korda *et al.*, 1997).

The speed and efficiency of bioremediation of a soil contaminated with petroleum and petroleum products depends on the number of hydrocarbon-degrading microorganisms in the soil. The most important factors for population growth are temperature, oxygen, pH, content of nitrogen and phosphorus, hydrocarbon class and their effective concentration. A single microorganism can degrade only certain types of petroleum compounds, but a mixed population – microbial community enables a higher level of degradation. The crude oil degrading bacteria and fungi identified in soil amended with *Moringa oleifera* seeds and pods are species of *Bacillus*, *Acinetobacter*, *Micrococcus*, *Pseudomonas*, *Rhizopus* and *Aspergillus* species respectively.

The objectives of this work were to determine the potential of *Moringa oleifera* seed in enhancing crude oil degradation in soil and to determine some changes in physicochemical properties of soil during bioremediation of oil polluted soil.

2. Material and Methods

2.1 Collection and Processing of Samples

Nigerian light crude oil was collected from Niger Delta University, Bayelsa State, Nigeria. The *Moringa Oleifera* seeds were collected from Zaria town, Kaduna State, Nigeria. The *Moringa Oleifera* seeds were grinded into fine powder to pass through a Tyler sieve of 2mm mesh-size. The soil sample used was collected from the farm of National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. The soil was sieved with 2mm mesh size before use.

2.2 Bioremediation Studies

Two kilograms of soil sample each was introduced into four different plastic buckets (PB) labelled A to C. The PB B and C were treated with 200ml of crude oil each, while A had no crude oil and served as control. PB C was treated with 400g of grinded seeds of *Moringa oleifera*, 250ml of distilled water was introduced into each of the PB and the contents were thoroughly mixed and incubated at room temperature. Periodic sampling from each PB was carried out at seven days intervals for 28 days. The samples were analyzed for changes in pH, moisture, biodegradation of crude oil as well as microbial counts.

2.3 Biodegradation of Crude Oil

The amount of crude oil degraded in each soil sample was determined by the weight loss method of Bossert and Bartha (1984). This was done by suspending 3g of soil in 10ml of diethyl ether in a universal bottle and shaken vigorously to extract the oil. The solvent-oil mixture was transferred slowly into a beaker of known weight. This was repeated until all the oil was extracted from the soil. The solvent-oil mixture was exposed at room temperature overnight to allow the solvent to evaporate completely. The new weight of the beaker containing the residual oil was taken and the percentage of oil degraded was calculated (Ijah and Ukpe 1992).

2.4 pH Determination

pH of the sample was determined using pH-meter (Crison pH 210 micro-processor) on 1:2.5 (w/v) soil/water mixture.

2.5 Microbial Counts and Type

Bacteria and fungi were enumerated by spread plate technique by inoculating 0.1ml serially diluted sample unto nutrient agar (NA) plates and Potato dextrose agar (PDA) plates respectively. The NA plates were incubated at 30°C for 48 hours while PDA plates were incubated at room temperature for 72 hours. The crude oil utilizing bacteria were enumerated on oil agar OA (1.8g K₂HPO₄, 4.0g NH₄Cl, 0.2g MgSO₄.7H₂O, 1.2g KH₂PO₄, 0.01g FeSO₄.7H₂O, 0.1g NaCl, 20g agar, 1% crude oil in 1000ml distilled water, pH 7.4). The oil agar was incubated at 30°C for seven days. The colonies which developed were counted and expressed as colony forming units per gram (CFUg⁻¹) of sample. Isolated bacterial colonies were characterized based on their gram stain reaction and biochemical tests. The organisms were identified to the genus level using the scheme of Cowan (1974). The fungi isolates were characterized based on macroscopic and microscopic examinations (Ijah *et al.* 2003a) and were identified using the scheme of Alexopoulos and Mims (1979).

2.6 Determination of Utilization of Crude Oil by Bacterial Isolates

The method of Kokub *et al.* (1989) was used, 5ml of mineral salts medium of Zajic and Supplisson (1972) was dispensed into each universal bottles containing 0.05ml of crude oil and sterilized for 15 minutes at 121°C, and the bottles were allowed to cool and inoculated with 0.1ml of nutrient broth-grown culture of crude oil degrading bacterial isolates. The bottles were incubated at room temperature (28±2°C) for 21 days. The turbidity produced as a result of bacterial growth was monitored visually at the end of incubation period and assigned (+) to (+++) depending on the intensity of the growth.

3. Results and Discussion

Table 1: Biological and Physiochemical Properties of *Moringa Oleifera* Seeds used for Bioremediation Studies

Biological/Physiochemical Properties	<i>Moringa Oleifera</i> seeds
pH	7.06
Moisture (%)	1.95
Nitrogen (%)	10.55
Phosphorus (ppm)	18.33
Organic carbon (%)	1.06
Total aerobic bacteria	6.0x 10 ⁶ CFU/g
Crude oil utilizing bacteria	3.0x 10 ² CFU/g
Total fungi	6.0x 10 ¹ CFU/g

Table 1 show the biological and physiochemical properties of *Moringa Oleifera* seed cake used for bioremediation studies. The results from Table 1 above revealed that the *Moringa oleifera* seed cake used had appreciable quantities of nitrogen and phosphorus 10.55 % and 18.33 ppm, which are major nutrients required by bacteria to degrade organic substances from the environment (Dibble and Bartha 1979). The results of bioremediation studies with crude oil polluted soil amended with *Moringa oleifera* shown in Figure 1, shows that the counts of aerobic heterotrophic bacteria in unpolluted soil ranged from 2.0 x 10⁶ CFU/g to 3.5 x 10⁶ CFU /g of soil that of polluted but unamended soil ranged from 1.0 x 10⁶ CFU/g to 1.5 x 10⁶ CFU /g of soil (Lüring and Beekman, 2010). The counts in oil- polluted soil amended with *Moringa oleifera* seed cake ranged from 1.2 x 10⁶ CFU /g to 3.6 x 10⁶ CFU/g

of soil. The results indicate an increase in the counts of total aerobic heterotrophic bacteria in soil amended with *Moringa oleifera*.

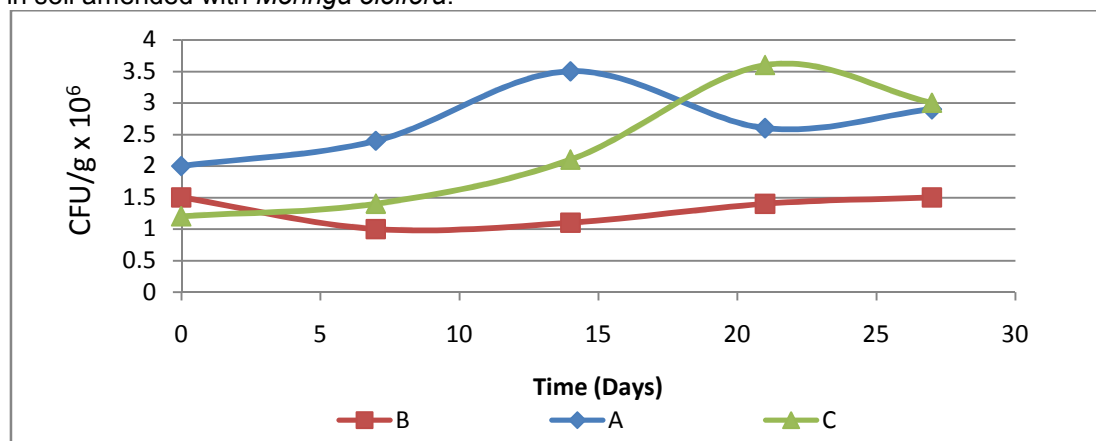


Fig. 1. Microbial Growth Pattern in the Soil Samples

KEY:

Sample A = Unpolluted control soil;

Sample B = Untreated polluted soil;

Sample C = Polluted soil treated with *Moringa oleifera* seed cake;

The crude oil utilizing bacteria isolates were identified as species of *Bacillus*, *Micrococcus*, *Pseudomonas* and *Acinetobacter* with *Bacillus* sp.

The microbial growth pattern in the untreated polluted soil sample (B) begins at the initial point when the crude oil was first introduced into the soil some of microorganisms that were in the oil were unable to survive, while some microorganisms were trying to gather enough enzymes, adapting to the new environment and was able to feed on the substrate (crude oil) to some extent and started dying due to high concentration of the substrate and insufficient microorganisms in the soil.

In samples C, as shown in Figure 1, the lag phase is very much reduced compared to the growth phase. The growth phase is elongated which indicate that there is adequate proliferation of the microbes in sample C and subsequently higher rate of bio-degradation of crude oil in the samples, compared to sample B. Sample A is regarded as the control in this case. The reason for the proliferation of the microbes in the samples can be attributed to the variation of pH in the samples, as shown in Figure 3.

The microbial growth pattern begins with the lag phase, as shown in Figure 2, at which phase the microorganism keeps adapting to the new environment and trying to gather enough enzyme to survive. Beyond that lag phase begins the experimental growth phase meaning that the microorganisms are now used to the environment and can effectively make use of the substrate (feeding on crude oil). At the end of the experiment, growth sets in the stationary phase where there is possible depletion of the substrate, there is possible inhibition, there is possible contamination and change in temperature. This is the period of dynamic equilibrium where the death phase is equal to the growth phase. In the death phase, the activities of the enzymes reduce because the substrate and nutrients are used up and the microorganisms begin to die gradually.

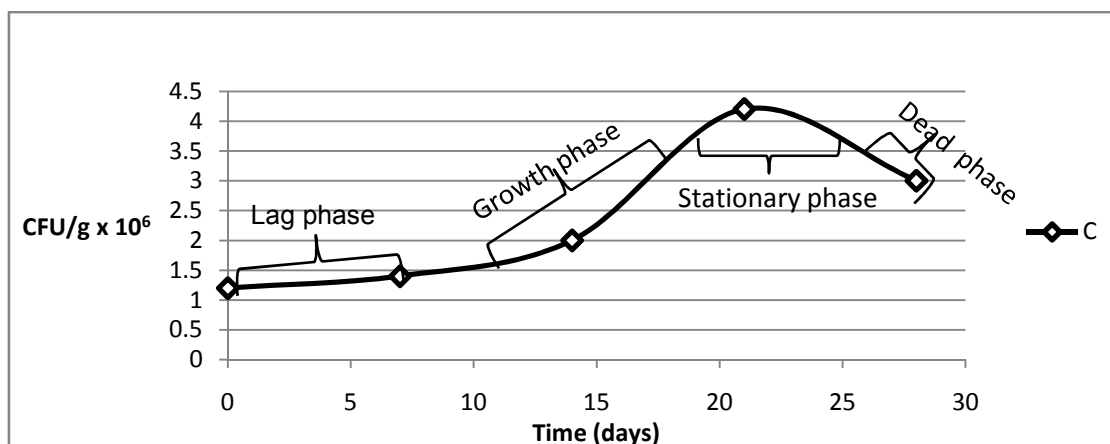


Fig. 2. Analysis of the Growth Pattern in the Soil Sample (C) Treated with *Moringa Oleifera* Seed Cake

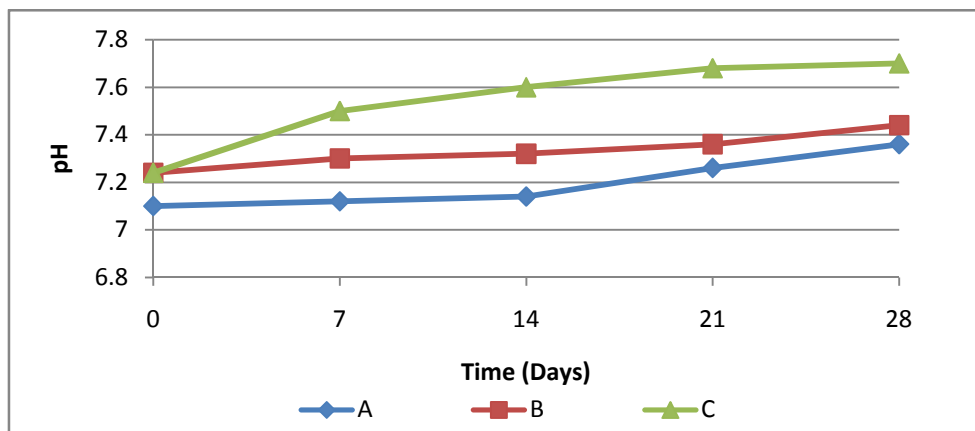


Fig.3: pH of the Soil Samples

KEY:

Sample A = Unpolluted control soil;

Sample B = Untreated polluted soil;

Sample C = Polluted soil treated with *Moringa oleifera* seed cake;

The pH of the polluted soil amended with *Moringa oleifera* seed cake was raised from 7.24 to 7.70, as shown in Figure 3. This is one of the conditions that increased the rate biodegradation of crude oil in amended soil since crude oil degrading bacteria grow and utilize hydrocarbons better at slightly alkaline pH (Dibble and Bartha 1979).

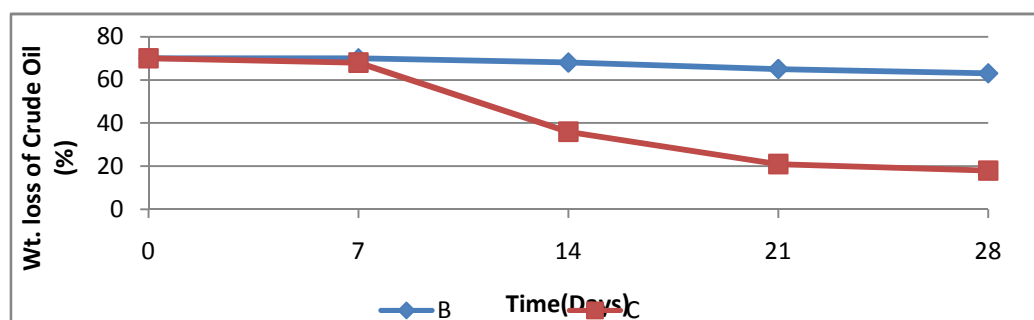


Fig.4: Rate of Biodegradation of Crude Oil in Soil Amended with *Moringa Oleifera* Seed Cake.

KEY:

Sample B = Untreated polluted soil;

Sample C = Polluted soil treated with *Moringa Oleifera* seed cake;

The results of bioremediation study also show that the rates of oil breakdown in oil-polluted soil with time. In untreated polluted soil, sample B, 10% of the oil was biodegraded after 28 days whereas in polluted soil treated with *Moringa oleifera* seed cake, sample C, 74.3% of oil was degraded within the same period of time, as shown in Figure 4. This is most likely due to the high nitrogen and phosphorous contents in the *Moringa oleifera* seed cake which favour the growth of crude oil utilizing bacteria in the treated soil.

4. CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that *Moringa Oleifera* seed cake possesses the ability to enhance the biodegradation of crude oil in soil by up to 64.3%.

Moringa Oleifera seed cake can serve as good materials for reclaiming/rehabilitating crude oil polluted soil. Their use in reclaiming oil polluted soil could also solve the problem of solid waste disposal in the environment.

There should be collaboration between Federal Environmental Petroleum Agency [FEPA] and various Research bodies on the use of bioremediation in soil reclamation.

There should be an oil pollution Act on the development of a National contingency plan on the use of bioremediation as an oil spill response tool.

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