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Potentials of using *Moringa oleifera* seeds in the bioremediation of soil contaminated by crude oil

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ABSTRACT

The potential of *Moringa oleifera* in increasing the rate of the microbial degradation of crude oil-spilled soil was investigated. The investigation was carried out at the National Research Institute for Chemical Technology, Zaria, Nigeria, within a 28 day period in the month of May, 2012. The proximate analysis of Moringa *oleifera* seed cake was found to contain nitrogen (10.55%), phosphorus (18.33ppm) and organic carbon (1.06%). Likewise hydrocarbon degrading bacteria were isolated using spread plate method. The results revealed that the counts of crude oil degrading bacteria in crude oil polluted soil amended with *Moringa oleifera* increased to 3.6 x 10^6 CFU/g as compared with that of unamended polluted soil, having 1.5 x 10^6 CFU/g. *Moringa oleifera* seed cake was found to enhance the degradation in crude oil polluted soil compared to unamended crude oil polluted soil by 64.3%. pH of crude oil polluted soil sample amended with *Moringa oleifera* ranged between 7.24 and 7.70 while that of unamended crude oil polluted soil ranged between 7.24 and 7.44. This implies that *Moringa oleifera* has a buffering effect which enables micro-organisms to proliferate. Statistical analysis showed no significant difference ($p>0.05$) between rate of biodegradation of crude oil in soil amended with *Moringa Oleifera* seed cake as against that of unamended crude oil polluted soil. The results of this study show that *Moringa oleifera* seed cake is suitable for bioremediation of crude oil polluted soils.

Keywords*: Moringa oleifera seed cake, hydrocarbon degrading bacteria, polluted soil, buffering effect, bioremediation*

1. INTRODUCTION

Oil and gas has become a major source of energy, the world over. Not only does oil and gas impact our lives in various ways but revenue generated thereof is of immense benefits. The vast gains accrued from the oil and gas industry through its exploration and exploitation brings along with it significant negative effect to the ecological and human environments

(Oyegun, 1998), subsequently impacting negatively on the socioeconomic stratum of a nation. Oil spillage is one of the problems that could result from drilling oil wells for exploitation. Oil spillage could also occur during discharge from refineries, accident of ship/tankers, rupture on seabeds, etc. These spills endanger public health, devastate natural resources, contaminate potable water and disrupt the economy. This problem is widespread and estimated number of contaminated sites is substantial and also emerging (John et *al*., 2010).

In Nigeria, oil pollution problems 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 nutrients 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 potential of *Moringa oleifera* seed cake in enhancing crude oil degradation in soil was determined. Some changes in physicochemical properties of soil during bioremediation of oil polluted soil were also determined.

2. MATERIAL AND METHODS

2.1 Collection and Processing of Samples

Nigerian light crude oil was obtained from the Petrochemical Laboratory of the Niger Delta University, Bayelsa State, Nigeria. The *Moringa Oleifera* seeds were collected from the Botanical Garden of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The *Moringa oleifera* seeds were grinded into fine powder with mortar and pestle. The size was monitored continuously by sieving with a 2mm Tyler sieve until homogenous samples were gotten.

The soil sample used was collected from the farm of National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. The soil was transported using marked sterile plastic bags in an ice chest and transported to the laboratory to be analyzed within 24 hours.

2.2 Sample Source, Collection and Processing

Two kilograms of soil samples each were introduced into three 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 (Abioye *et al*., 2009).

2.3 Crude Oil Biodegradation

The quantity of crude oil reduced from 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. The mixture was 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 kept open at room temperature overnight to allow the solvent to evaporate completely (Ijah and Ukpe 1992). The percentage of oil degraded was calculated using the formula below (Abioye *et al*., 2009).

$$\textit{Wt. loss of crude oil (\%)} = \frac{Weight\ of\ crude\ oil\ (initial) - Weight\ of\ crude\ oil\ (after\ treatment)}{Weight\ of\ crude\ oil\ (initial)}\ x\ 100$$

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 Analysis

The total heterophobic bacteria count was performed using nutrient agar (NA). The medium was prepared according to manufacturer's specifications. Aliquots of the serially diluted soil samples were plated out in duplicates on nutrient agar plates using spread plate method. The plate was incubated at room temperature for 48 hours.

The total fungi count was performed using potato dextrose agar (PDA). Aliquots of the serially duplicated soil samples were plated out in duplicates on dextrose agar plates using spread plate method. The plate was incubated at room temperature for 72 hours.

The crude oil utilizing bacteria were enumerated on oil agar OA (1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 1.2g KH_2PO_4, 0.01g $FeSO_4.7H_2O$, 0.1g NaCl, 20g agar, 1% crude oil in 1000ml distilled water, pH 7.4). The oil agar was incubated at 30^oC for seven days. Sterile Whatman No. 1 filter paper saturated with crude oil was placed inside the cover of each petri dish. The petri dishes were incubated with the agar side up at 30^oC for 7 days.

The colonies which developed were expressed as colony forming units per gram ($CFUg^{-1}$) of sample. Isolated bacterial colonies were identified based on their gram stain reaction and biochemical tests. The organisms were identified to the genus level using the scheme of Cowan (1974) and Abioye *et al*. (2009). The fungi isolates were characterized based on macroscopic and microscopic examinations (Ijah *et al.* 2003a and Abioye *et al*. 2009) and were identified using the scheme of Alexopoulos and Mims (1979).

2.6 Determination of Crude Oil Utilization by Bacterial Isolates

The method of Kokub *et al.* (1989) was used whereby 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^oC, 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 for 21 days (Abioye *et al*., 2009). A control universal bottle containing 9.9ml of sterile mineral salt broth with 0.1ml of crude oil was not inoculated. Crude oil utilizing bacteria turned the broth into a cloudy suspension. 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.

2.7 Statistical Analysis

A paired t-test was run on the weight loss (%) of crude oil for samples B and C, using SPSS (version 17) software. This was to determine whether there was a statistically significant mean difference between the weight loss (%) of crude oil for sample B and that of sample C.

3. RESULTS AND DISCUSSION

Table 1: Proximate Composition of *Moringa oleifera* Seeds used for Bioremediation Studies

Physiochemical Properties of *Moringa oleifera* seeds	
pH	7.06
Moisture (%)	1.95
Nitrogen (%)	10.55
Phosphorus (ppm)	18.33
Organic carbon (%)	1.06

Table 1 shows the proximate composition of *Moringa oleifera* seeds used for bioremediation studies. The results from Table 1 revealed that the *Moringa oleifera* seed cake had appreciable quantities of nitrogen and phosphorus; 10.55 % and 18.33 ppm, respectively, which are major nutrients required by bacteria to degrade organic substances from the environment (Dibble and Bartha, 1979).

Table 2: Crude Oil Utilization by Bacterial Isolates

Bacterial Isolates	Growth in crude oil medium
Pseudomonas sp.	+++
Micrococcus sp.	+++
Bacillus sp.	++
Acinebacter sp.	++

Key: +++ = Heavy growth, ++= Moderate growth, + = Minimal growth

Table 2 shows the ability of crude oil degrading bacteria (CUB) isolates to utilize crude oil revealed that *Pseudomonas* sp. and *Micrococcus* sp. utilizes crude oil at higher rate than other isolates. This is in confirmation with the work carried out by Abioye et *al.* (2009). This implies that these bacteria can use crude oil are a source of carbon and energy.

Figure 1 show that the counts of aerobic heterotrophic bacteria in unpolluted soil ranged from 2.0 x 10^6 CFU/g to 3.5 x 10^6 CFU /g of soil while that of polluted but unamended soil ranged from 1.0 x 10^6 CFU/g to 1.5 x 10^6 CFU /g of soil (Lürling and Beekman, 2010). The counts in oil- polluted soil amended with *Moringa oleifera* seed cake ranged from 1.2 x 10^6 CFU/g to 3.6 x 10^6 CFU/g of soil. The results indicate an increase in the counts of total aerobic heterotrophic bacteria in soil amended with *Moringa oleifera*. This may be as a result of high nitrogen and phosphorus contents present in the amended soil.

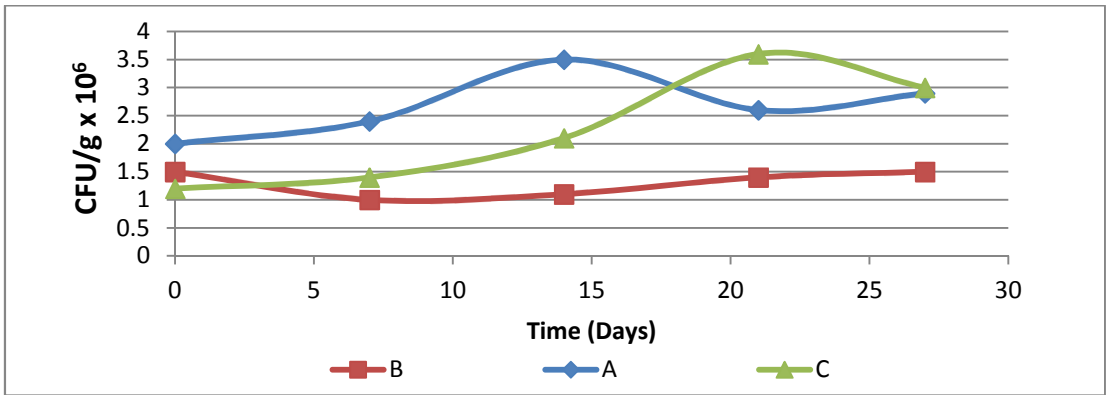

Fig. 1: Microbial Growth Pattern in the Soil Samples

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 sp.,* being frequently isolated than other species. This is in agreement with previous works (Ijah 1998, Bossert and Bartha 1984, Abioye *et al*., 2009).

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

In samples C (Figure 1) the lag phase is very much reduced compared to the growth phase. The growth phase was elongate indicating that there was 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 was 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 began with the lag phase, as shown in Figure 2, at which phase the microorganism kept adapting to the new environment and tried to survive. Beyond the lag phase began the experimental growth phase, implying that the microorganisms got used to the environment and could effectively make use of the substrate (feeding on crude oil) to proliferate. At the end of the experiment, growth set in the stationary phase where there was possible depletion of the substrate, there was possible inhibition, notwithstanding, and there could be possible contamination and change in temperature. This was the period of dynamic equilibrium where the death phase was equal to the growth phase. In the death phase, the activities of the enzymes reduced because the substrate and nutrients were used up and the microorganisms began to die gradually.

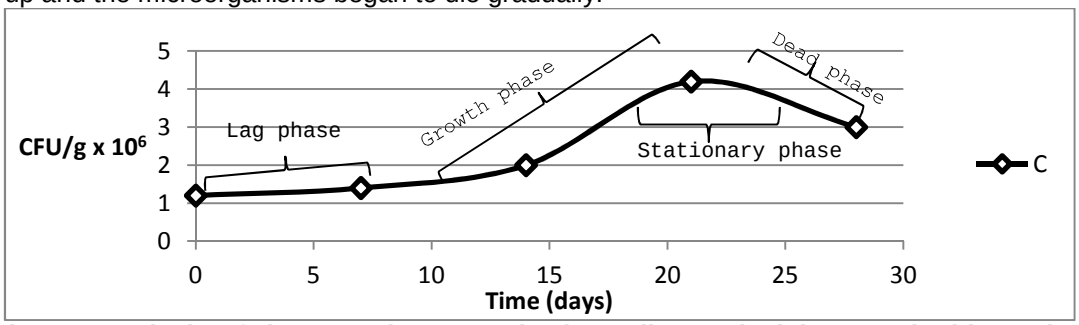

Fig. 2: Analysis of the Growth Pattern in the Soil Sample (C) Treated with *Moringa oleifera* Seeds

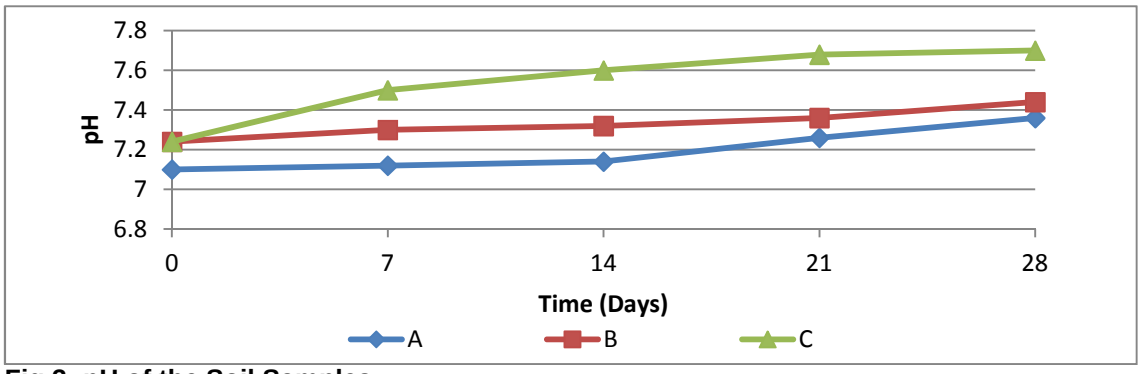

Fig.3: pH of the Soil Samples

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 was one of the conditions that increased the rate of 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).

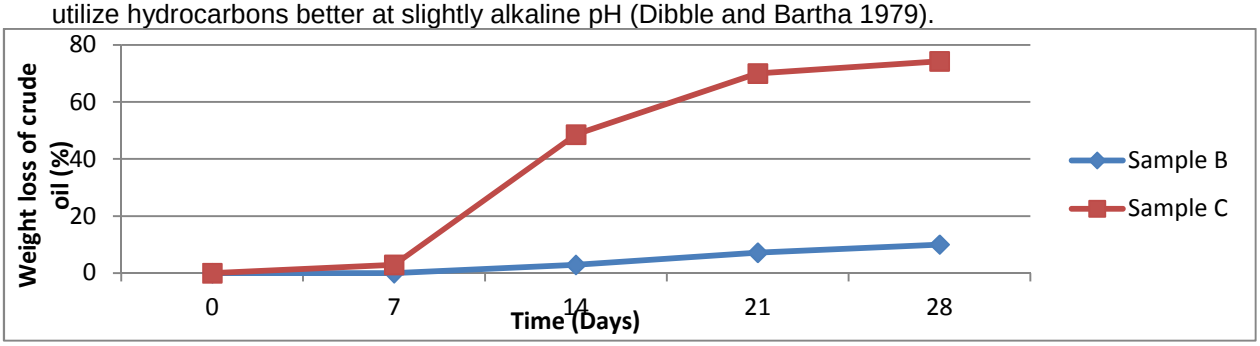

Fig.4: Rate of Biodegradation of Crude Oil in Soil Amended with *Moringa oleifera* Seed Cake.

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 favoured the growth of crude oil utilizing bacteria in the treated soil.

Table 3: Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	% wt loss of crude oil Sample B	4.0000	5	4.44857	1.98946
	% wt loss of crude oil Sample C	39.1420	5	35.79198	16.00666

Table 4: Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	% wt loss of crude oil Sample B & % wt loss of crude oil Sample C	5	.940	.017

Table 5: Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	% wt loss of crude oil Sample B - % wt loss of crude oil Sample C	-35.14200	31.64680	14.15288	-74.43670	4.15270	-2.483	4	.068

From Table 3, biodegradation of crude oil in soil was more in sample C (39.142±35.792) as opposed to that of sample B (4.00±2.00). The *P*-value is 0.068 (*P* >.05), (as shown in Table 5), we therefore rejected the alternative hypothesis and concluded that the rate of biodegradation of crude oil in soil amended with *Moringa oleifera* seed cake as compared with that of crude oil in soil unamended with *Moringa oleifera* seed cake was not effective at 5% significant level.

4. CONCLUSION

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%. the link between the isolation of crude oil degrading bacteria and amendment with Moringa Oleifera seed cake will be researched upon.

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.

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COMPETING INTERESTS

There are no conflicting interests.

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