CRUDE OIL DEGRADATION BY *Bacillus* AND *Micrococcus* SPECIES ISOLATED FROM SOIL COMPOST IN KANO, NIGERIA

*Kawo, A.H. and Bacha, H.Y.
[1]Department of Microbiology, Faculty of Science, Bayero University, PMB 3011, Kano, Nigeria
(Correspondence author: ahkawo.mcb@buk.edu.ng, ahkawo@yahoo.com; +234-(0)-802-315-3895: *Currently on Sabbatical Leave with the Department of Biological Sciences, Faculty of Science, Northwest University, PMB 3220, Kano, NIGERIA)

ABSTRACT
Microorganisms capable of degrading crude oil were isolated from soil compost in Kano, north-western Nigeria. The work was carried out with the aim of determining crude-oil biodegradation potentials of Bacillus and Micrococcus species isolated from the soil compost as well as the assessment of the applicability of compost in bioremediation of hydrocarbon-polluted soils. The physico-chemical and microbiological parameters of the uncontaminated soil compost samples were determined using standard procedures. Similarly, the microbiological properties of the crude oil-contaminated soil compost samples were determined using liquid Mineral Salt medium. The microorganisms isolated from the contaminated soil samples showed an appreciable degree of degradation of the crude oil used, among which the crude oil-utilizing potentials of Micrococcus species and Bacillus species isolated from the soil composts were further analyzed using biodegradation experiment. Here, utilization of the crude oil as a growth substrate by the two bacterial isolates resulted in increased population densities with simultaneous increase in emulsification index (EI$_{24}$) value and decrease in residual crude oil concentration. The Fourier Transform Infrared Spectrometry (FTIR) analyses of the residual hydrocarbons in the liquid minimal salt (MS) medium at the end of 15 days incubation showed that Micrococcus species had the highest degradation rate of 93.7% while Bacillus species had biodegradation rate of 87.5%. Thus, the two bacterial species isolated in this study showed appreciable degrees of degradation of the crude oil used. It could therefore be concluded that bioremediation of crude oil-polluted fields could be achieved using indigenous hydrocarbon utilizers of the soil compost and the process could be enhanced by supplementing the polluted environment with compost.
Keywords: Soil compost, Crude oil-degrading microorganisms, Bioremediation, Kano.

INTRODUCTION

The dramatic increase in production, refining and distribution of crude oil and its products have brought with it an ever increasing problem of environmental pollution. The Nigerian environment is characterized by nonchalant, indiscriminate and highly unregulated disposal of petroleum products including diesel and engine oil (Odjegba and Sadiq, 2000; Adebusoye *et al.*, 2007). Petroleum is a major source of energy globally; wide-scale production, transport, use and disposal of petroleum globally have made it a lead contaminant in both water and soil environments (Onifade *et al.*, 2007). Thus, the release of these compounds poses a threat to water and soil ecosystems. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in plant and animal tissues may cause death or mutation. Consequently, many techniques are being developed to clean up petroleum-polluted environments. Biodegradation of hydrocarbon-contaminated soil, which exploits the ability of microorganisms to degrade and/or detoxify the organic compounds, has been established as an efficient, economical, versatile and environmentally

sound treatment. The biological treatments are more efficient and cheaper than chemical or physical ones (Stroud *et al.*, 2007). In relation to biological treatments, the bioremediation technology is being employed for the degradation of crude oil in soil matrix through microorganisms able to transform petroleum hydrocarbons into less toxic compounds (Omotayo *et al.*, 2012). However, the low solubility and adsorption are two major properties of high molecular weight hydrocarbons that limit their availability to microorganisms (Kastner *et al.*, 1995). On the other hand, compost contributes organic matter to the soil that may serve as a source of nutrients among other functions for the various microbes that inhabit the soil. Compost is a very rich source of nitrogen that maintains and enhances the fertility and productivity of agricultural soils (Chitra *et al.*, 2014). Studies carried out both in microcosms and field experiments showed that organic amendments not only act by improving soil structure and serving as a source of nutrients, but can also strongly enhance the activities of the microflora (Ortego-Calvo *et al.*, 1998).

According to Ralph and Ji-Dong (2010), the success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in contaminated environment. Numerous scientific researchers have reported various factors that influence the rate of oil biodegradation (Barathi and Vasudevan, 2001; Obayori *et al.*, 2008; Haritash and Kaushik, 2009; Ibrahim *et al.*, 2009; Omotayo *et al.*, 2012). One important requirement is the presence of microorganisms with the appropriate metabolic capabilities (Kastner *et al.*, 1995; Omotayo *et al.*, 2012). If these microorganisms are present, then optimal rates of growth can be sustained by ensuring that adequate concentration of nutrients and oxygen are present and that the pH is between 6 and 9 (Rousk *et al.*, 2009). The physical and chemical characteristics of oil and oil surface area are also important determinants of bioremediation success (Barathi and Vasudevan, 2001; He *et al.*, 2000; Ibrahim *et al.*, 2008). In this study, microorganisms with the capacity to utilize crude oil as carbon and energy source were isolated from soil composts and their biodegradation potential of crude oil as well as the applicability of composts in bioremediation of hydrocarbon- polluted soils was assessed.

MATERIALS AND METHODS

Sample Collection

Soil (soil compost) samples were collected randomly using quadrant sampling technique and homogenized from five different spots at Ecological Study Area of Bayero University, Kano, Nigeria (11.9818°N, 8.4801°E) and a dump site at the National Orthopaedic Hospital, Dala, Kano, Nigeria (11.9944°N, 8.5138°E). The soil samples were collected at a depth of 0-10 cm below the soil surface using soil auger and put into sterile polyethene bags and transported to the laboratory (Luyafor *et al.*, 1990). Physico-chemical and microbiological analyses commenced immediately upon arrival in the laboratory at the Department of Microbiology, Faculty of Science, Bayero University, Kano, Nigeria. The sampling was done between October 2015 and January, 2016.

Source of Crude Oil

The Escravos crude oil (dark-brown in color) used in this study was obtained from Kaduna Refinery and Petrochemical Company (KRPC), a subsidiary of the Nigeria National Petroleum Corporation (NNPC) located at Kilometer 16, Kachia Road, Kaduna State, Nigeria.

Physico-chemical Analysis of the Soil Compost Samples

Determination of Hydrogen-ion Concentration (pH)

Twenty (20) grams of soil sample was air dried after sieving through 2-mm mesh size and transferred into a 50ml-beaker to which 20ml of distilled water was added. The soil suspension was stirred several times for 30 minutes using a glass rod. The suspension was allowed to stand for 30 minutes. The electrode of the pH meter (Jenway 3051 model) was then inserted into the partly settled suspension and measured the pH (Omotayo *et al.*, 2012).

Determination of Total Organic Carbon (TOC)

The soil sample was grinned and passed through 0.5mm sieve and 1.00g soil was placed in a 250ml conical flask containing 10ml of 1N K_2CrO_7 solution. The flask was swirled gently to dispense the soil. A quantity (20ml) of concentrated H_2SO_4 was added immediately and swirled gently until the soil and reagents were mixed. The contents were then swirled more vigorously for a minute. The flask was rotated again and allowed to stand on a sheet of asbestos for 30 minutes after which 100ml of distilled water was added and allowed to cool. The suspension was filtered and followed by the addition of 5ml of O-phosphoric acid after which 3 drops of o-phenanthroline indicator were added and the contents titrated against 0.5N ferrous sulphate on a white background. As the end point approached, the solution turned to a greenish cast and then changed to dark green. At this point, the ferrous sulphate was added in drops until the color changed sharply from blue to red (maroon color) in reflected light against the white background. Blank determination in the same way was made but without soil to standardize the dichromate (Mclean, 1965). The result was calculated according to the following formula:

% Organic carbon (air-dried soil)

$$= \frac{(\text{Blank titer} - \text{Actual titer}) \times 0.3 \times M \times F}{\text{Weight (g) of air-dried soil}}$$

Where: M = Concentration of $FeSO_4$ and F= Correction factor = 1.33

Determination of Moisture Content

Five (5) grams of soil sample were transferred into pre-weighed can box with tight-fitting lid. The can box containing the moist soil sample was weighed immediately and placed with its lid off in the drying oven at a temperature of 105°C (to dry the soil to constant weight) for 24hrs after which the set up was removed from the oven and immediately covered with its lids, cooled in dessicator and weighed again (Luyafor *et al.*, 1990). The moisture content of the soil sample was determined using the following formula:

$$\text{Moisture content} = \left[\frac{W_2 - W_{3\,(g)}}{W_3 - W_{1\,(g)}} \times 100 \right]$$

Where: W_1 = Weight of empty soil can box (g).

W_2 = Weight of can box + moist soil (g).

W_3 = Weight of can box + oven dry soil (g).

Air-dried soil was crushed and passed through 2mm sieve to which a round filter paper was placed and fixed to the internal perforated floor of a dish. The weight of the dish and the filter paper was noted. The dish was then filled with soil by tapping the dish briskly and making plane the top soil and weighed. A set of perforated dishes was placed in enamel tray and poured water in the enamel tray to the half of the height of each dish. Water rose in the dishes through the perforated bottom and moistened the soil to its capacity. The soil was kept in for 6 hours in water and the dish was then taken off and placed on filter paper sheet so that the excess water drained away from the pores within half an hour and the dish containing the moist soil was weighed (Luyafor *et al.*, 1990).

Determination of Water Holding Capacity **Thus:**

Water Holding Capacity (%) = $\dfrac{\text{Water retained (ml)}}{\text{100ml of sample}}$

Determination of Available Phosphorous

One (1) gram of air-dried soil was weighed and transferred into a 15ml centrifuge tube and 7ml of extraction solution was added. The suspension was mixed for one minute on a mechanical shaker and centrifuged at 2000 rpm for 15 minutes and then decanted into an acid-washed volumetric flask. The phosphorous content was determined calorimetrically using the ascorbic acid molybdate blue titration technique (Mclean, 1965).

Determination of Total Nitrogen

Two (2) grams of soil was placed into a digestion tube and 20ml of distilled water was added and the flask swirled for a few minutes and then allowed to stand for 30 minutes. Then, 3g of a catalyst (mixture of 0.5g sodium sulphate, 50g anhydrous copper sulphate and 0.5g of selenium powder) and 20ml of conc. H_2SO_4 were added and heated continuously on a low heat. When the water has been removed and frothing has ceased, the heat was increased until the digest cleared. The content was then boiled for 5 hours. The heating was regulated during this boiling so that the H_2SO_4 condensed about half of the neck of the flask. The flask was allowed to cool down slowly with shaking and adding some distilled water. The digest

was carefully transferred to another clean flask and all sand particles in the original digestion flask were retained because sand could cause severe bumping during distillation. The sand residues were washed with 50ml distilled water four times and transferred each portion into the same flask. Then, 10ml H_3BO_3 solution was added into a 500ml Erlenmeyer flask, which was then placed under the condenser of the distillation apparatus (the end of the condenser was at about 4cm above the surface of the H_3BO_3 solution) and 10ml of 40% NaOH was added slowly into 10ml of the digest flask, which has been attached to the distillation apparatus. The temperature was raised until it boiled. The condenser was cooled (below 30°C) by allowing sufficient water to flow through and the heat regulated to minimize frothing and prevent suck back. A quantity (50ml) of the distillate was collected and then the distillation was stopped. Ammonia (NH_3) was titrated against standard H_2SO_4 using three drops of methyl red-bromocresol green indicator. The color changed at the end point from green to pink. The percent nitrogen content in the soil was calculated by running a blank solution similarly but without soil sample (Luyafor *et al.*, 1990). Thus:

Percentage Nitrogen = $\dfrac{0.014 \times VD \times N \times 100 \times TV}{\text{Weight of soil} \times AD}$

Where:

VD	=	Volume of the digest.
N	=	Normality of the acid.
TV	=	Titer value.
AD	=	Aliquot of the digest.

Microbiological Analysis of the Soil Compost Samples

Enumeration of Heterotrophic Bacterial, Fungal and Actinomycetes Counts

One (1) gram of soil from each sample was separately suspended in 10ml of prepared Kringer's solution in a flask and placed on a shaker at 100 rpm at room temperature (37°C) for 30 minutes. At the end of the shaking, the soil samples were serially diluted up to 5 dilutions using the Kringer's solution as a diluent. The total heterotrophic bacterial and fungal counts were enumerated by plating aliquots (1.0ml) of appropriate diluted soil samples on nutrient agar and potato dextrose agar respectively. Starch casein agar was employed in determining the population density of the actinomycetes. All inoculated plates were incubated aerobically at room temperature (30°C) and colony counts were made after 48, 96, 120 and 168 hours (Omotayo *et al.*, 2012).

Bacteriological Analysis of the Crude Oil-Contaminated Soil Compost Samples

Enumeration of Crude Oil-Utilizing Bacterial Counts

The population of hydrocarbon-utilizers was estimated on mineral salt (MS) medium as formulated by Obayori *et al.* (2008). The medium consisted of the following composition (gl^{-1}): Na$_2$HPO (3.6), (NH$_4$)$_2$ SO$_4$ (1.0), KH$_2$PO$_4$ (1.6), MgSO$_4$ (1.0), Fe (NH$_4$) citrate

(0.01), CaCl$_2$.2H$_2$O (0.10) and 10ml of trace elements solution per liter. The trace elements solution used was of the following composition (mg l^{-1}): ZnSO$_4$.7H$_2$O (10), MnCl$_2$.4H$_2$O (3.0), CoCl$_2$.6H$_2$O (1.0), NiCl$_2$.6H$_2$O (2.0), NO$_2$MoO$_4$.2H$_2$O (3.0), H$_3$BO$_3$ (3.0) and CuCl$_2$.2H$_2$O (1.0). The crude oil, which served as the sole carbon and energy source was added into the medium. The plates were incubation at room temperature for 5 days after which the colony counts were recorded and overall mean results expressed in cfu/g of the soil sample (Obayori *et al.*, 2008).

Identification of the Crude Oil-Utilizing Bacterial Isolates

Gram's Staining

After the smear was prepared, the slide was flooded with crystal violet and allowed to stand for one minute. It was washed off with tap water and then flooded with Gram's iodine (a mordant) and left for one minute after which the smear was washed off with tap water again, decolorized with alcohol (95%) until no more color washed off. This was the most critical step; care was taken so as not to over decolorize the smear as many Gram-positive organisms may lose the violet stain easily and thus appear to be Gram-negative after they are counterstained.

The smear was wash off with tap water and safranin (counter stain) was applied and allowed to stand for one minute. The smear was then washed off with tap water, drained and blotted gently with bibulous paper. The slide was air dried thoroughly before examined under the microscope. Gram-positive organisms retained the primary stain while Gram-negative ones took up the secondary (counter) stain (Todar *et al.*, 2005).

Catalase Test
This was carried out by introducing 2.0ml of hydrogen peroxide on a clean, grease-free glass tube. With the edge of a sterile glass rod, a colony of organism was picked and introduced onto the hydrogen peroxide on the slide. The presence of bubbles indicated a positive reaction while absence of bubble indicated a negative reaction (Bhattacharya *et al.*, 2002).

Urease Test
Urea agar medium was inoculated with culture and incubated at 37ºC temperature for 48 hours. After incubation, change of colour to pink showed a positive result (Kummerer, 2004).

Gelatin Hydrolysis
The actively grown cultures were inoculated in nutrient gelatin medium and incubated at 37ºC for 48 hours. Degradation of gelatin indicated a positive reaction (Aneja, 2003).

Citrate Utilization Test
This was carried out by inoculating the test organism in a test tube containing Simon's citrate medium, which was incubated for 24 hours. The development of deep-blue colour after the incubation period indicated a positive result (Udeani *et al.*, 2009).

MR-VP Test
A quantity (5.0 ml) of MR-VP broth was inoculated with the test organism an incubated for 72 hours at 37°C after which 1.0 ml of the broth was transferred into a small test tube. Three drops of methyl-red solution were added. A red colour development on the addition of the indicator signified a positive methyl red test while yellow colour signified a negative test. To the rest of the broth in the original tube, five drops of 4% potassium hydroxide (KOH) were added followed by fifteen (15) drops of 5% -naphtol in ethanol. No colour change indicated a VP negative test (Dubey, 2002).

Indole Test
One percent tryptophan broth in a test tube was inoculated with a bacterial colony and incubated at 37°C for 48 hours. Then, 1.0 ml of chloroform was added to the broth. The test tube was shaken gently and then 2 ml of Kovac's reagent were added and shaken gently and allowed to stand for twenty (20) minutes. The formation of red colouration at the top layer indicated a positive test while yellow colouration indicated a negative test (Udeani *et al.*, 2009).

Starch Hydrolysis
Starch agar medium was inoculated with a bacterial colony and incubated at 37ºC for 24 hours after which iodine solution was added into the medium to determine the capability of bacterial isolate to use starch (Bhattacharya *et al.*, 2002).

Crude Oil Utilization by the Bacterial Isolates
The ability of the bacterial isolates (*Bacillus* and *Micrococcus* species) to utilize crude oil was confirmed by inoculating each isolate in separate, cotton-plugged 50ml test tube containing sterile liquid Minimal Salt (MS) medium. The medium according to the American Type Culture Collection (ATCC) was composed of the following: Na_2HPO_4 (12.8g), KH_2PO_4 (3.0g), NaCl (0.5g), NH_4Cl (1.0g) and distilled water (478ml). The contents were autoclaved at 121°C for 15 minutes and then cooled to 50°C after which the following filtered sterilized solutions were aseptically added: 20% glucose solution (20ml), 1M $MgSO_4$ solution (2ml), 1M $CaCl_2$ solution (0.1ml) and thiamine. The MS medium and the crude oil were autoclaved separately at 121°C for 15 minutes. The sterile crude oil, which served as the sole source of carbon and energy, was added (1ml) to make up a final volume of 100ml MS medium. Each isolate was subsequently inoculated in separate tube of the medium. Control tube containing the liquid MS medium and 1ml of crude oil but without the organism was also prepared. The tubes were monitored and agitated daily for a period of ten (10) days. Isolates with the highest turbidity were selected for further study. Here, an aliquot of each isolate (with the highest turbidity) was transferred into a fresh medium containing the crude oil to re-confirm its ability to utilize the crude oil (Omotayo *et al.*, 2012).

Biodegradation Studies
The biodegradation study was performed using liquid MS medium as described by Omotayo *et al* (2012). The growth medium used was prepared as described above. In this study, *Micrococcus* and *Bacillus* species were selected based on their ability to grow well in the crude oil used as the only carbon and energy source. The isolates were inoculated into each tube and incubated in the dark at ambient temperature with constant shaking (using shaker) for 15 days. Emulsification index was monitored at 3 days intervals. The residual hydrocarbon content after biodegradation was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) technique.

Determination of Emulsification Index
In determining the emulsification index, 2ml of the medium was centrifuged for 30 minutes to separate the cells. The supernatant was collected in a test tube while the cells were discarded. The emulsification stability of the isolate was determined by adding 2ml of sterile crude oil to the test tube containing the spent medium. The tubes were properly vortexed for 5 minutes and allowed to stand for 24 hours. The emulsification index was calculated as the height of the emulsion divided by the total height of the supernatant with added crude oil multiplied by 100 (Abbasi and Amiri, 2008).

Thus:

Emulsification index (EI$_{24}$) = $\dfrac{\text{Height of emulsion x 100}}{\text{Height of supernatant}}$

Determination of Residual Functional Groups
Fourier Transform Infrared Spectroscopy is the technique, which was used to obtain an infrared spectrum of absorption or emission of a liquid or gas. The FTIR analysis for this research was conducted in accordance with the method of Griffiths and Hasseth (2007) at the Instrumental Laboratory of the Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria.

RESULTS AND DISCUSSION
Physico-Chemical Properties of the Soil Compost Samples
The soil compost samples obtained from the National Orthopedic Hospital, Dala dump site were found to be

more acidic (pH 5.8) compared to the soil compost samples from BUK Ecological Study Area (pH 6.6). The moisture content of both the two soil samples was found to be the same (14.4%). The total organic carbon, percentage nitrogen, water holding capacity and available phosphorous of soil samples from Dala (2.5%, 0.4%, 0.11% and 12.9 ppm respectively) were higher than those of BUK (1.8%, 0.3%, 0.08% and 10.0 ppm respectively). However, the potassium content of BUK sample (1.8 ppm) was higher than that of Dala (0.5 ppm) (Table1).

Table 1: Physico-chemical properties of the soil compost samples

	Sample Sites	
Parameters	BUK	DALA
Hydrogen-ion Concentration (pH)	6.6	5.8
Organic carbon (%)	1.8	2.5
Water Holding Capacity (%)	0.08	0.11
Moisture Content (%)	14.4	14.4
Available Phosphorus (ppm)	10.0	12.9
Total Nitrogen (%)	0.3	0.4

Microbiological Properties of the Soil Compost Samples
In this study, the heterotrophic bacterial counts of the soil compost samples obtained from Dala after 48, 96 and 120hrs of incubation were 1.93×10^5 cfu/g, 2.40×10^5 cfu/g and 2.75×10^5 cfu/g respectively. These values were comparatively higher than those of soil samples obtained from BUK (1.50×10^5 cfu/g, 1.80×10^5 cfu/g and 2.00×10^5 cfu/g (Table 2). This is probably because the soil from Dala contained more nutrients compared to that of BUK sample. This could also explain the reason why Dala soil samples harbored high actinomycetes counts (7.10×10^4 cfu/g, 8.60×10^4 cfu/g, 9.80×10^4 cfu/g and $1.08 \times$

10^5 cfu/g) than BUK samples (6.80×10^4 cfu/g, 7.20×10^4 cfu/g, 8.60×10^4 cfu/g and 9.80×10^4 cfu/g) after 48, 96, 120 and 168 hours respectively (Table 4). On the contrary, the heterotrophic fungal counts of soil samples obtained from BUK were 3.00×10^4 cfy/g, 3.40×10^4 cfu/g, 3.45×10^5 cfu/g and 7.50×10^5 cfu/g after 48, 96, 120 and 168 hrs respectively. These values were higher than those of Dala with 1.00×10^4 cfu/g, 1.10×10^4 cfu/g, 1.25×10^4 cfu/g and 1.60×10^5 cfu/g respectively (Table 3). This could be due to fact that even though the soil sample from BUK was less acidic (Table 1), however, it could have retained high moisture content due to the availability of trees within the sample site.

Table 2: Heterotrophic bacterial counts of the soil compost samples

	*Heterotrophic bacterial counts (cfu/g)	
Duration of incubation (hours)	Sample A (BUK)	Sample B (DALA)
48	1.50×10^5	1.93×10^5
96	1.80×10^5	2.40×10^5
120	2.00×10^5	2.75×10^5
168	2.18×10^5	Too numerous to count

*Values are means of triplicate determinations.

Table 3: Heterotrophic fungal counts of the soil compost samples

	*Heterotrophic fungi counts (cfu/g)	
Duration of Incubation (hours)	Sample A (BUK)	Sample B (DALA)
48	3.00×10^4	1.00×10^4
96	3.40×10^4	1.10×10^4
120	3.45×10^5	1.25×10^5
168	7.50×10^5	1.60×10^5

*Values are means of triplicate determinations.

Table 4: Heterotrophic actinomycetes counts of the soil compost samples

Duration of incubation (hours)	*Heterotrophic actinomycetes counts (cfu/g)	
	Sample A (BUK)	Sample B (DALA)
48	6.80×10^4	7.10×10^4
96	7.20×10^4	8.60×10^4
120	8.60×10^4	9.80×10^4
168	9.80×10^4	1.08×10^5

*Values are means of triplicate determinations.

The cultural, morphological and biochemical properties of the crude-oil utilizing bacteria (*Bacillus* and *Micrococcus* species) isolated from the soil compost are presented in Table 5 while Table 6 presents the overall crude oil-utilizing bacterial counts of the contaminated compost soil samples. It has been reported that population level of hydrocarbon utilizers and their composition within microbial community appear to be a sensitive index of environmental exposure to hydrocarbons (Rahman *et al.,* 2002). In unpolluted ecosystems, hydrocarbon utilizers generally constitute about 0.1% of the microbial community and in oil-polluted ecosystems, they can constitute up to 100% of viable microorganisms (Rahman *et al.,* 2002). Thus, the microbial population quantitatively reflects the degree or extent of exposure of an ecosystem to hydrocarbon contamination (Atlas, 1992; 1995). In this study, a lag phase of 5 days was experienced for the growth of hydrocarbon-utilizing bacteria (Table 6). After 5 days incubation of the hydrocarbon-utilizing bacteria, it was found that the soil samples from Dala had highest number of hydrocarbon-utilizers (2.00×10^4 cfu/g) than those from BUK (Table 6). The low proportion of crude oil utilizers after 5 days (Table 6) compared to the total heterotrophic populations (Tables 2-4) indicates that the soil ecosystem from which the samples were obtained probably had not been previously exposed to heavy and consistent crude oil pollution. Several hydrocarbon-utilizing organisms have been isolated from diverse environments; soil and aquatic sources, which are the two major environments affected by hydrocarbon pollution (Mittal and Singh, 2009). The soil compost samples used in this study harboured crude oil utilizers, which mainly included the species of *Micrococcus* and *Bacillus* (Table 5). The crude oil utilizers obtained in this study showed varying degrees of the hydrocarbon degradation. However, *Micrococcus* species appeared to be the fastest growing species in the crude oil compared to the *Bacillus* species (Figures 4-5).

Table 5: Cultural, morphological and biochemical properties of the crude oil-utilizing bacterial isolates

Biochemical Characteristics	Isolate A	Isolate B
Gram's Reaction	+	+
Shape	Bacilli	Cocci
Catalase Test	+	+
Citrate Utilization	-	+
Starch Hydrolysis	-	+
Indole Test	-	-
Methyl Red Test	+	-
VP Test	+	-
Urease Test	-	+
Gelatin Hydrolysis	+	+
Oxidase Test	-	+
Probable Identity	*Bacillus* species	*Micrococcus* species

Table 6: Overall crude oil-utilizing bacterial counts of the contaminated soil compost samples

Duration of incubation (days)	Crude oil-utilizing bacterial counts (cfu/g)	
	Sample A (BUK)	Sample B (DALA)
Five (5)	1.20×10^4	2.00×10^4

Emulsification indices of the crude oil-utilizing bacterial isolates
Figure 1 shows the results of the emulsification index of the bacterial isolates used in the biodegradation studies. The population density of Isolate A (*Bacillus* species) and Isolate B (*Micrococcus* species) showed that there was a considerable period of lag phase of five (5) days before the isolates entered an exponential phase of growth. The population density of *Bacillus* species increased steady higher than that of the *Micrococcus* species before the stationary phase after 15 days. The emulsification index of the *Bacillus* species rose steadily from 30 to 40 EI_{24} (Fig. 1) while its growth rate constant (μ) was 0.01. On the other hand, utilization of substrates by the bacterial isolates resulted in increased population densities with simultaneous increase in the emulsification index value and decrease in residual crude oil concentration. It is clear that *Micrococcus* species had the highest emulsification index, which signified high viable count as the number of cells increased after 3 days (Fig. 1).

The FTIR analyses of the residual hydrocarbons in the liquid minimal salt (MS) medium at the end of 15 days incubation showed that *Micrococcus* species had the highest degradation rate of 93.7% while the *Bacillus* species had biodegradation rate of 87.5%. Thus, the FTIR of the biodegradation experiment after 15 days showed a reduction in the concentration of residual hydrocarbons present in the medium. The FTIR results showed that the positive control, which contained only the crude oil without the organism (Fig. 2), contained sixteen (16) different hydrocarbon functional groups out of which only one (1) was not utilized by the *Micrococcus* species (Fig. 4). This gave a degradation rate of 93.7% for the *Micrococcus* species. Figure 3 presents the FTIR results of the negative control. On the other hand, *Bacillus* species was able to degrade all except two (2) of the functional groups (Fig. 5), thus giving a hydrocarbon degradation rate of 87.5%. However, both the *Micrococcus* and *Bacillus* species were found to utilize certain functional groups contained in the crude oil, although the *Micrococcus* species had a higher biodegradation capacity than the *Bacillus* species (Figures 4-5).
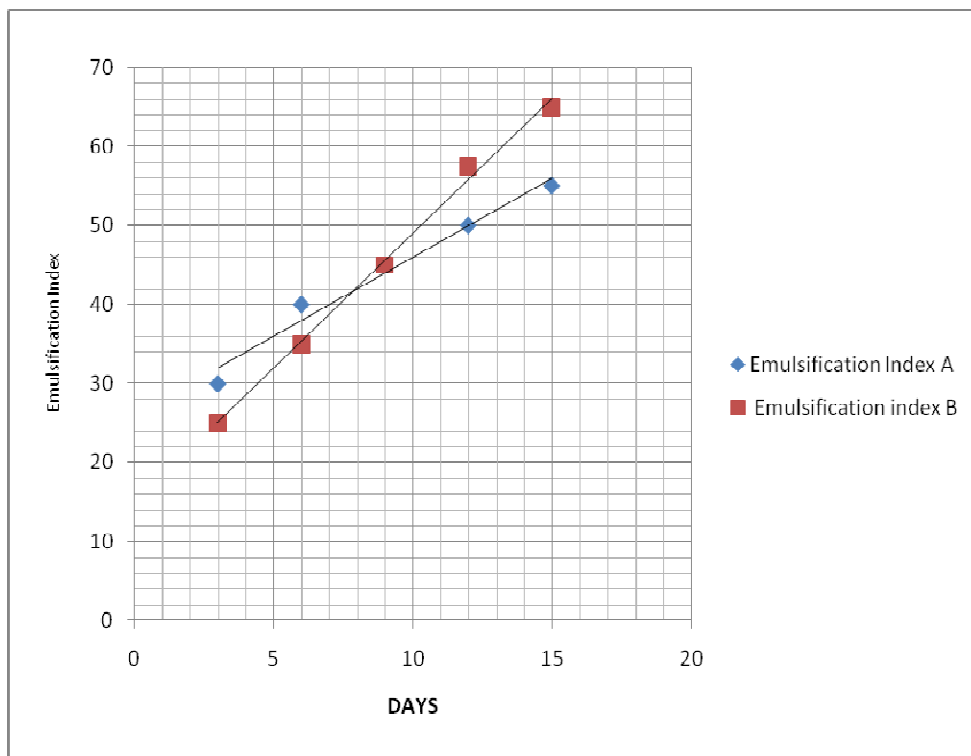
Figure 1: Emulsification indices of the crude oil-utilizing bacterial isolates
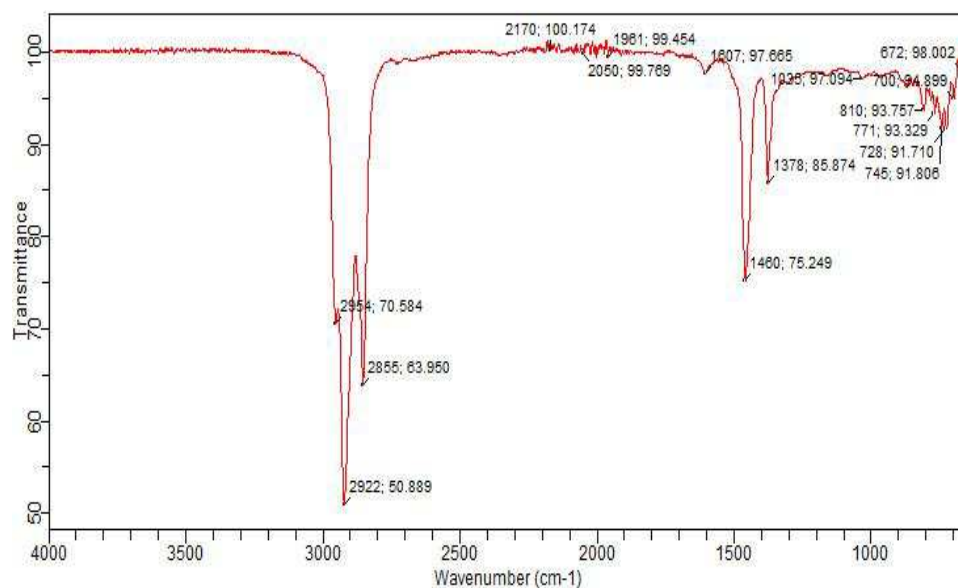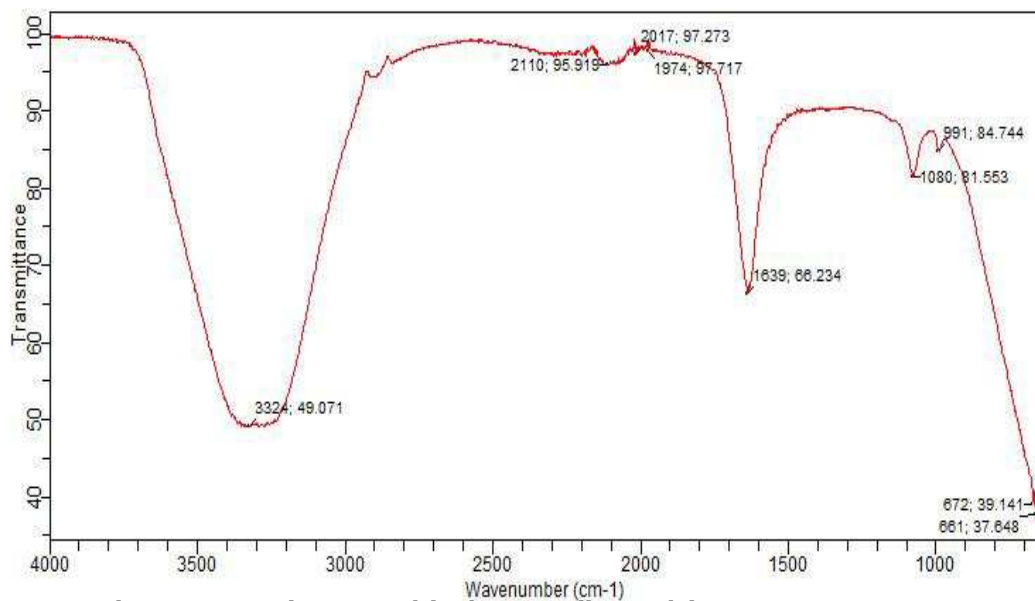

Figure 2: Positive Control (Crude oil only)
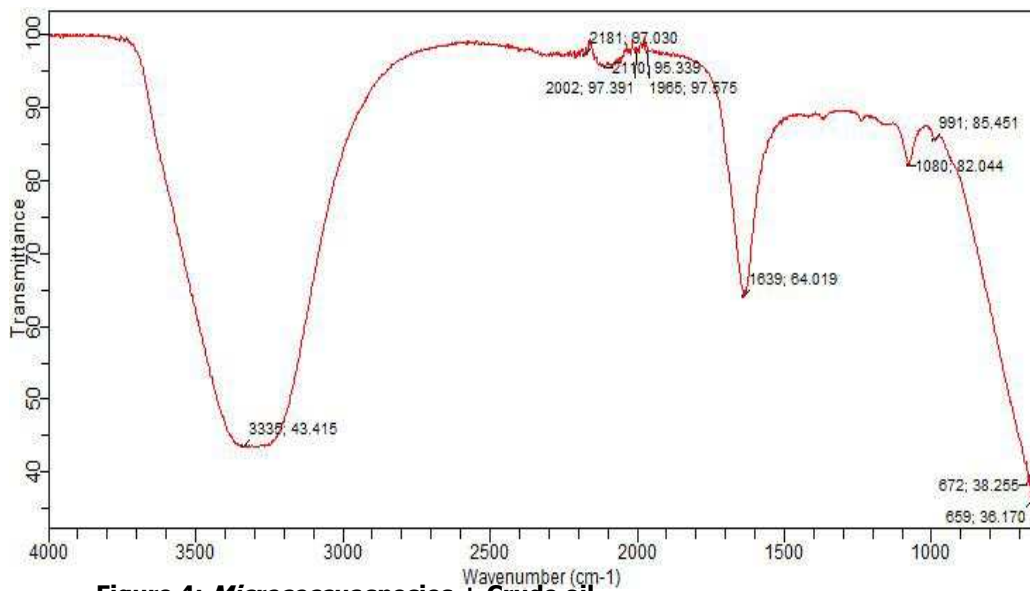
Figure 3: Negative Control (Culture medium only)

Figure 4: *Micrococcus* species + Crude oil
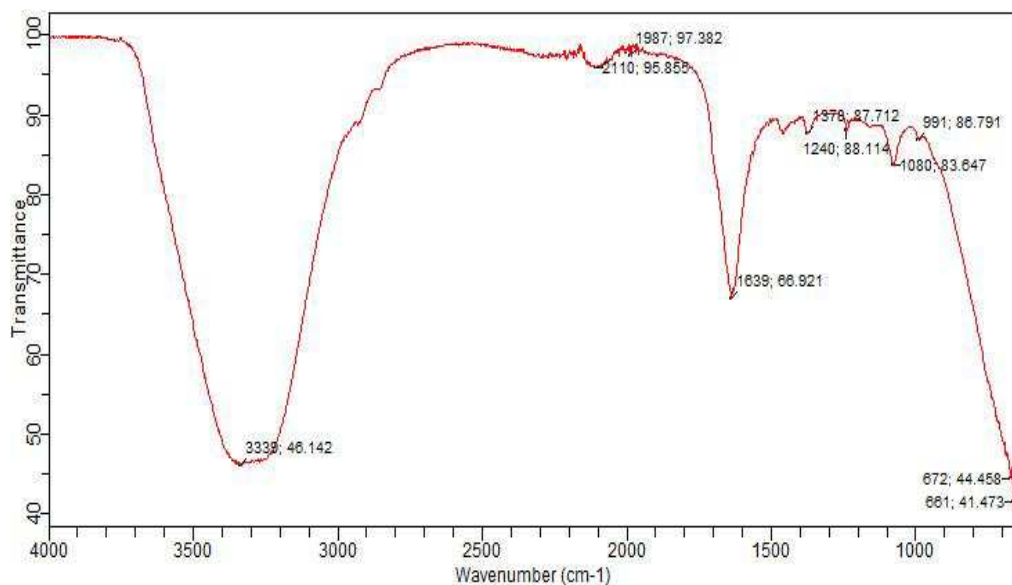

Figure 5: *Bacillus* species + Crude oil   115

CONCLUSION AND RECOMMENDATIONS

The crude-oil degradation potentials of *Bacillus* and *Micrococcus* species isolated from compost soil in Kano, northwestern Nigeria were determined. The natural microbial community of the compost soil included a variety of microorganisms that could degrade the crude oil components with *Micrococcus* species having the highest potential. Thus, bioremediation of crude oil-polluted fields could be achieved using indigenous hydrocarbon utilizers of the soil compost and the process could be enhanced by supplementing the polluted environment with compost.

REFERENCES

Abbasi, A. and Amiri, S. (2008): Emulsifying behavior of an exopolysacharide produced by *Enterobacter cloacae*. *African Journal of Biotechnology* 7:1574-1576.

Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O.D. and Olatope, S.O. (2007): Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal of Microbiology and Biotechnology* 23:1149-1159.

Aneja, K.R. (2003): *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th edition, New Age International Publishers, New Delhi, India.

Atlas, R.M. (1992): *Petroleum Microbiology. In*: J. Lederberg (Ed.). *Encyclopedia of Microbiology*. Academic Press, Baltimore. Pp363-369.

Atlas, R.M. (1995): Petroleum biodegradation and oil spill bioremediation. *Marine Pollution Bulletin* 31:178-182.

Barathi, S. and Vasudevan, N. (2001): Utilization of petroleum hydrocarbons by *Pseudomonas fluorescence* isolated from a petroleum-contaminated soil. *Environment International* 26: 413-416.

Bhattacharya, D., Sarma, P.M., Krishnan, S., Mishra, S. and Lal, B. (2002): Evaluation of genetic diversity among *Pseudomonas citronellosis* strains isolated from oily sludge-contaminated sites. *Applied Environmental Microbiology* 69(3):14351-441.

Chitra, B., Harsha, P. Gupta, S. and Roy, S. (2014): Isolation and characteriazation of bacterial isolates from agricultural soil at Durg district. *Indian Journal of Science Research* 4(1):221-226.

Dubey, R.C. (2002): *Practical Microbiology*. 1st edition, S. Chand Publications, New Delhi, India.

Griffiths, P. and Hasseth, J.A. (2007): *Fourier Transform Infrared Spectrometry.* Second edition. John Wiley publishers, UK.

Haritash, A.K, and Kaushik, C.P. (2009): Biodegradation aspects of polycyclic aromatic hydrocarbons: A Review. *Journal of Hazardous Materials* 169(1-3):1–15.

He, Z.L., Alva, A.K., Calvert, D.V. and Li, Y.C. (2000): Nutrient availability and changes in microbial biomass of organic amendments during field incubation compost. *Science and Utilization* 8:293-302.

Ibrahim, M.L., Ijah, U.J.J, Manga,S.B. and Rabah, A.B. (2008): Occurrence of hydrocarbon-utilizing bacteria in the rhizosphere of *Eucalyptus camaldulensis*, *Lablab purpureus* and M*oringa oleifera*. *International Journal of Pure and Applied Sciences* 2(3):21-26.

Ibrahim, M.L., Manga, S.B., Rabah, A.B. and Ijah, U.J.J. (2009): Biodegradation of Escravos light crude oil by bacteria isolated from the rhizosphere of *Eucalyptus camaldulensis*, *Lablab purpureus* and *Moringa oleifera*. *Proceedings of International Conference, Workshop and Exhibition on Biotechnology for Improved Production of Oil and Gas in the Gulf of Guinea Held in Abuja, Nigeria Between 1st and 3rd April, 2009*.

Kafhilzadeh, F., Schatnezhad, N., Kadivar, E. and Rezacian, A.A. (2012): Bioremediation of chrysene by isolated bacteria from petroleum-polluted soils in Iran. *International Journal of Biology, Pharmacy and Allied Sciences* 1(8):1047-1056.

Kastner, M., Breuar-Jammali, M. and Mahro, B. (1995): Enumeration and characterization of soil microflora from hydrocarbon-contaminated soil site able to mineralize polycyclic aromatic hydrocarbon. *Applied Microbial Biotechnology* 41:267-273.

Kummerer, K. (2004): Resistance in the environment. *Journal of Antimicrobial Chemotherapy* 45:311–320.

Luyafor, V.O., Esu, S.E., Odeare, V. and Abalarin, O.M. (1990): *Selected Methods for Soil and Plant Analyses.* Department of Soil Science, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

Mclean, E.O. (1965): *Aluminum. In*: C.A. Black (ed.). *Methods of Soil Analyses* 2:986-994.

Mittal, A. and Singh, P. (2009): Isolation of hydrocarbon-degrading bacteria from soils contaminated with crude oil spill. *Indian Journal of Experimental Biology* 47:760-765.

Obayori, O.S., Illori, M.O., Adebusoye, S.A., Amund, O.O. and Oyetibo, G.O. (2008): Microbial population changes in tropical agricultural soil experimentally contaminated with crude petroleum. *African Journal of Biotechnology* 7:4512-4520.

Omotayo, A.E., Ojo, O.Y. and Amund, O.O. (2012): *Crude oil degradation by microorganisms in soil composts*. Department of Microbiology, University of Lagos, Nigeria.

Odjegba, V.J. and Sadiq, A. (2000): Effects of spent engine oil on the growth parameters, chlorophyll and protein level of *Amaranthus hybridus* (L.). *Nigerian Journal of Applied Sciences* 7:1-46.

Onifade, A.K., Abubakar, F.A. and Ekundayo, F.O. (2007): Bioremediation of oil-polluted soil in the Niger Delta area of Nigeria using enhanced natural attenuation. *Research Journal of Animal Science* 2:498-504.

Oluwafemi, S.O., Lateef, B.S. and Ibiyemi, M.O. (2012): Degradation of weathered crude oil (Escravos Light) by bacterial strains from hydrocarbons-polluted site. *African Journal of Microbiology Research* 6(26):5426-5432.

Ortego-Calvo, J. J. and Saiz-Jimenz, C. (1998): Effect of humic fractions and clayon biodegradation of phenanthrene by *Pseudomonas fluorescens* strain isolated from soil. *Applied Environmental Microbiology* 64: 3123-3126.

Rahman, K.S.M., Rahman, T., Lakshmanaperumalsamy, P. and Banat, I.M. (2002): Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. *Journal of Basic Microbiology* 42:284-291.

Ralph, M. and Ji-Dong, G. (2010): *Environmental Microbiology*. 2nd edition, John Wiley and Sons Inc. Publications, USA. pp177-206.

Rousk, J., Brookes, P.C. and Baath, E. (2009): Contrasting soil pH effects on fungal and bacterial growth suggest fundamental redundancy in carbon mineralization. *Applied Environmental Microbiology* 75:1589-1596.

Stroud, J.L., Paton, G.I. and Semple, K.T. (2007): Microbe-aliphatic hydrocarbon interactions in soil implication for biodegradation and bioremediation. *Journal of Applied Microbiology* 102: 1239-1253.

Todar K., Ubukata M. and Hamada, M. (2005): *Microbiology of Human Perspective*. McGraw-Hill Publishers, London.

Udeani, C.K.T., Obroh, A.A., Okwuosa, N.C., Achukwu, U.P. and Azubike, N. (2009): Isolation and characterization of bacterial isolates. *African Journal of Biotechnology* 8(22):6301-6303.