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ENHANCED BIOSTIMULATION OF SOIL ARTIFICIALLY POLLUTED WITH CRUDE OIL AFTER AMENDMENT WITH BOVINE FEACES AND *GALLUS GALLUS DOMESTICUS* (CHICKEN) DROPPINGS

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ABSTRACT: *Environmental degradation as a result of oil spillage during extraction, processing, transportation and corrosion of pipeline or damage is one of the many disasters that have been caused by humans throughout history. The study investigates the influence of incorporating different types of organic waste in bioremediation of crude polluted soils. Five treatment levels of crude oil pollution (0, 20, 40, 60, and 80 ml) were used, while amendment treatments were done after two weeks. The different organic waste: chicken dropping (CD), bovine feaces (BF), chicken dropping + bovine feaces (CD + BF), with two sets of control: pollution + no amendment and no pollution + no amendment were used for this investigation. Results shows that the crude oil significantly affected the physicochemical properties of the soil.Two weeks after crude oil pollutiona decline in pH values was observed for all crude oil polluted soil with increased amount of crude oil. Other parameters that had notable reduction in values with increased crude oil volume include Calcium, Potassium and Phosphorus while percentage total organic carbon,total organic matter and total hydrocarbon content (THC) significantly increased. The results also showed that the organic manure amendment treatments significantly decreased crude oil toxicity at different degrees by improving the nutrient content and decreasing the total hydrocarbon content of the soil after 4 weeks of amendment. The results indicated the order of their remediation potential as CD+BF > CD > BF. The microbial composition of the CD and BF which could have contributed in the biodegradation process as identified using 16SrRNA sequencing include: Lysinibacillus sphaericus, Lysinibacillus fusiformis, Enterobacter cloacae, Bacillus amyloliquefaciens, Aspergillus niger and Enterobacter asburiae. Nucleotide sequences of the isolates retrieved from this study have been deposited in the GenBank nucleotide sequence database under accession nos. MT560691, MT500581, MT498090, MT500681 and MT500680 (NCBI GenBank, www.ncbi.nlm.nih.gov). Therefore, this study shows that the reduction of crude oil polluted soil using organic waste should be encourage and locally propagated for economic reasons.*

KEYWORDS: biostimulation, soil, crude oil, bovine feaces, *gallus gallus domesticus* (chicken)

INTRODUCTION

Environmental degradation which result from oil spillage during extraction, processing, transportation and corrosion of pipeline or damage is one of the many disasters that have been caused by humans throughout history. In Nigeria most of the terrestrial ecosystem and shore lines in oil producing community are important agricultural land under cultivation. Any contact with crude oil usually results in damage to the soil, microorganism and plants (Adedokun & Ataga, 2007). Oil spillage constitutes the most momentous source of oil pollution in the Niger Deltal region of Nigeria. Oil spillage also destroys farmlands and has significant effect on plant growth (Agbogidi *et al*. 2005). The high request or demand for petroleum products in form of gas oil, engine lubricating oil, cooking gas, aviationfuel increases its production and then eventually results in oil spills and hydrocarbon pollution of the environments (Resinger, 1995).

Biological (bioremediation) techniques arecost-effectiveand eco-friendly compared to thephysical and chemical methods of remediation[Silva-Castro*et al*. 2015; Dados*et al.* 2015]. Furthermore, biological method of remediation conserves soil characteristics andtexture [Vidali, 2001; Yerushalmi *et al,* 2003].The success of the application of bioremediation depends on nature of the pollutant.for example; degree of pollution, aggregate, and oxidationstate of crude oil and environmental conditions such as temperature,oxygen concentration,pH, moisture content, presence of alternate carbon sourcesand microbes with degradation capability, soil property, andnutrient availability [Agarry and Jimoda, 2013;Gavrilescu, 2006; Bamforthand Singleton, 2005]. In bioremediation of polluted environment, biostimulation is paramount for optimal nutrient concentrationsespecially nitrogen and phosphorus to offset the disparity caused by high carbon content of crude oil during pollution, which may impede the growth and activities of hydrocarbonoclastic bacteria [Silva-Castro*et al*. 2015; Bamforthand Singleton, 2005; Ayotamuno *et al*, 2006].

Over the years, synthetic fertilizers have been appliedas biostimulants for enhanced bioremediation of petroleumhydrocarbon polluted sites. However, it's excessive applicationhas been implicated in negative consequences such aseutrophication, blue baby syndrome, and atmospheric pollution[Geddes *et al*, 2015].Moreover, synthetic fertilizers are very expensive in developing countries like Nigeria due to their high demand as an essential agricultural input [Danjuma*et al*, 2012]. These challenges among others are quest for environmental sustainability that motivated researchers to search for organic substrates, which would serve as alternatives to synthetics fertilizers to enhance bio-remediation. Therefore this research has been considered because*Gallus gallus domesticus* (chicken) dropping and bovine feaces (cow manure) is readily available, transportable and affordable.

MATERIALS AND METHODS

Sample Collection:

Soil samples for the experiment were collected randomly with a hand held boring metal soil auger at the surface soil (loamy clay) between the depths of 0 to 15 cm from an agricultural garden in Michael Okpara University of Agriculture Umudike (MOUAU), Abia State. The soil samples were

bulked together, homogenized and put into perforated labeled bags (Onuh *et al*., 2008a). This perforation allows for proper drainage (avoid water logging) and better aeration of the experimental soil.The chicken droppings were collected from poultry farm at Michael Okpara University of agriculture umudike (MOUAU), Abia State. Bovine feaces were collected from ubakala abattoir, Umuahia South LGA, Abia State. The chicken droppings and bovine feaces were compost and crushed before use. The crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Eleme, Port-Harcourt, Rivers State and was applied as pollutant.

Pollution treatment

Crude oil was added to the soil in the bags at various levels (0, 20, 40, 60, and 80ml) and thoroughly mixed with the soil. The polluted and unpolluted soils were allowed to stand under natural environment for 14 days before application of organic manure amendments. During this period, the soil samples were watered at intervals of two days. A total of 140 bags with soil were polluted with crude oil and 28 bags without crude oil pollution.

Amendment treatments

After 14 days of pollution treatments, organic manures which included chicken drooping (CD), bovine feaces (BF) and a combination of chicken drooping + bovine feaces (CD+ BF) were carefully weighed into the bags containing the crude oil treated and un-treated soils.

Sampling

Soil samples were collected from the bag at three different times. First was before crude oil application to ascertain the physico-chemical nature of the unpolluted soil. Second was at 14 days after crude oil pollution and third was at 28 days after amendments of crude oil polluted soil.

Determination of physiochemical parameters

The pH were determined by the method outlined by Bates (1954) using an electronically pH meter at ratio of 1:2.5 soil/water. The rapid titrimetric method as outlined by Osuji and Adesiyan (2005) was used to determine the organic carbon and organic matter. The ascorbic acid method as outlined by AOAC (1999) was used for phosphate determination. Total nitrogen was determined by Kjaldahl method as outlined by AOAC (1999). Total hydrocarbon contents was determined according to the method as outlined in Osuji and Uduetok (2008) while the determination of calcium, potassium and magnesium were done by mixed acid digestion method as outlined by AOAC (1999).

Table 1: The Experimental Design

Control	Treatments with organic manures		
Bag 1A + 5.0 kg soil+ 0 ml crude oil	Bag 1B + 5.0kg soil+ 0ml crude oil + 1.67kg CD	Bag 1C + 5.0kg soil+ 0ml crude oil + 1.67kg BF	Bag 1D + 5.0 kg soil+ 0 ml crude oil + 0.83 kg CD + 0.83kg BF
Bag 2A + 5.0 kg soil+ 20 ml crude oil	Bag 2B + 5.0kg soil+ 20ml crude oil + 1.67kg CD	Bag 2C + 5.0kg soil+ 20ml crude oil + 1.67kg BF	Bag 2D + 5.0 kg soil+ 20 ml crude oil + 0.83 kg CD + 0.83 kg BF
Bag 3A + 5.0 kg soil+ 40 ml crude oil	Bag 3B + 5.0kg soil+ 40ml crude oil + 1.67kg CD	Bag 3C + 5.0kg soil+ 40ml crude oil + 1.67kg BF	Bag 3D + 5.0 kg soil+ 40 ml crude oil + 0.83 kg CD+ 0.83 kg BF
Bag 4A + 5.0 kg soil+ 60 ml crude oil	Bag 4B + 5.0kg soil+ 60ml crude oil + 1.67kg CD	Bag 4C + 5.0kg soil+ 60ml crude oil + 1.67kg BF	Bag 4D + 5.0 kg soil + 60 ml crude oil + 0.83 kg CD + 0.83 kg BF
Bag 5A + 5.0 kg soil+ 80 ml crude oil	Bag 5B + 5.0kg soil+ 80ml crude oil + 1.67kg CD	Bag 5C + 5.0kg soil+ 80ml crude oil + 1.67kg BF	Bag 5D+ 5.0 kg soil + 80 ml crude oil + 0.83 kg CD + 0.83 kg SBF

Isolation of Microorganisms from the organic manure samples

The serial dilution technique was employed in the inoculation of the samples. Each of the samples was diluted in the 10-fold serial dilution technique described by Gurung *et al.* (2009). One (1) g of each soil sample was diluted in 9 ml of sterile water and diluted serially upto the sixth tube in the row. An inoculum of 0.1ml diluents from tubes No. 3 and 4 were inoculated onto Bushnell-Hass Agar Plates. The diluents were spread plated on the agar plates using an alcohol flame sterilized glass rod. The inoculated plates were incubated at 30^0C for 7days. Upon establishment of growth, colonies were sub cultured onto freshly prepared nutrient agar plates and incubated overnight. The resulting pure colonies were transferred aseptically and stored in nutrient agar slants for further studies.

Genomic DNA Preparation

The molecular analysis including DNA extraction Gel electrophoresis were conducted using the facilities of the Centre for Molecular Biosciences and Biotechnology, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria.

DNA Extraction

The bacterial cells grown in nutrient broth were mixed by vortexing. An aliquot of 1.5ml of each isolate suspension was centrifuged at 10,000rpm for 2minutes, the supernatant was decanted and

blotted on a paper towel. One ml of sterile distilled water was added to the pellet in the eppendorf tube, vortexed and centrifuged at 10,000rpm for 5 minutes. The supernatant was again discarded and the tube blotted on a paper towel. The cells were washed again at 10,000rpm for 5 minutes and supernatant discarded. Two hundred (200µl) of sterile distilled water was added and vortexed to homogenize the pellets. The resuspended cells were transferred into a ZR Bashing Bead™ Lysis Tube. This was followed by the addition of 750µl Lysis solution into the tube. The bead containing the solution was secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 2minutes. The ZR Bashing Bead™ Lysis Tube was centrifuged in a micro-centrifuge at 10,000 x g for 1 minute. Four hundred (400µl) of the supernatant was pippeted into a Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 x g for 1 minute. This was followed by the addition of 1,200µl of Fungal/Bacterial DNA binding buffer into the filtrate in the collection tube. After this 800µl of the mixture was transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute. The flow through was discarded from the collection Tube and the process was repeated to obtain the remaining products. Two hundred (200µl) DNA pre-wash buffer was added into the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10,000 x g for 1 minute. This was followed bythe addition of 500µl Fungal/Bacterial DNA Wash Buffer into the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IIC column was transferred into a clean 1.5 ml micro-centrifuge tube and 100 µl of DNA Elution Buffer was then added directly to the column matrix. This was centrifuged at 10,000 x g for 30 seconds to elute the DNA. The Ultra-pure resulting filtrate (DNA) obtained was used as a template for Polymerase Chain Reaction (PCR).

TAE (tris-acetate-EDTA)-Agarose Gel Electrophoresis

Extracted DNA was resolved through Tris Acetic acid EDTA (TAE) agarose gel electrophoresis prepared in a concentration of 0.8% of molecular biology grade agarose (Bioline, UK) in 1×TAE buffer. The gel was mixed with 10µl Ethidium bromide. Ten microlitre (10µl) of DNA template were mixed with 2µl of loading dyeand visualized under UV light using UV transilluminator (Edvotek, USA) and were photographed using a camera. Molecular weight standard marker was used to estimate the size of extracted DNA.

DNA Sequencing

DNA sequencing was performed by Sanger (dideoxy) sequencing Technique to determine the nucleotide sequence of the specific microorganism isolated using automated PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems (Russell, 2002; Metzenberg 2003). This result was obtained as nucleotides sequence analysis from resultant nucleotides base pairs was performed by BLAST analysis by direct blasting on American data base (http://blast.ncbi.nlm.nih.gov). For every set of isolate, a read was BLASTED and the resultant top hits with minimum E-score for every BLAST result showing species name was used to name the specific organism.

Statistical analysis

The results were expressed as mean ± standard deviation of three replicates. Analyses of variance (ANOVA) were carried out using SPSS version15.0 and mean values were separated using the Ducans multiple range test (DMRT) at $P \leq 0.05$.

RESULT AND DISCUSSION

Effect of crude oil level on the physicochemical properties of the soil

The result of the physicochemical properties of the soil before and two weeks after different level of crude oil pollution is shown in Table 2. The pH ranges (5.10 to 5.35) of the unpolluted and the crude oil-polluted soils indicated acidity. The results also showed significant increases in organic carbon and organic matter as the level of crude oil pollution increased. Table 2also showed that the total nitrogen and phosphorus significantly decreased correspondingly as pollution levels increased. The results (Table 2) also showed a significant decrease ($P \leq 0.05$) in the calcium, potassium and magnesium content as the levels of crude oil pollution increased but a corresponding significant increase ($P \leq 0.05$) in total hydrocarbon (THC) as the crude oil pollution levels increased.

Table 2:Mean and Standard Deviation of Physico-chemical properties of the soil after 2 weeks of crude oil treatment

Parameters	0ml (control)	20ml	40ml	60ml	80ml
pH	5.35±0.03	5.20±0.01	5.18±0.10	5.15±0.02	5.10±0.01
OC(%)	2.43±0.01	3.48±0.10	4.98±0.02	5.10±0.01	5.62±0.03
OM(%)	4.18±0.02	5.02±0.01	7.68±0.11	9.20±0.02	10.02±0.04
N(%)	0.15±0.07	0.13±0.03	0.12±0.01	0.11±0.02	0.09±0.01
Ca(Mg/Kg)	1.42±0.02	1.32±0.01	1.30±0.01	1.26±0.01	1.23±0.02
K(Mg/Kg)	1.01±0.01	1.87±0.03	0.82±0.02	0.77±0.01	0.75±0.01
Mg(Mg/Kg)	0.58±0.02	0.55±0.01	0.53±0.05	0.50±0.01	0.51±0.01
P(Mg/Kg)	1.37±0.03	1.12±0.01	1.05±0.01	0.86±0.03	0.62±0.01
THC(Mg/Kg)	-	3500.20±1.61	5692.5±7.02	6612.19±5.10	7003.27±10.05

The results showed a decrease in the pH as the levels of crude oil pollution increased, agreeing with the reports of Amadi *et al*. (2005) who observed increased soil acidity following increased crude oil pollution. The observed pH, 4.98 to 5.30 in the crude oil polluted samples was acidic and compared favorably with pH values, 4.7 to 5.4 reported by Osuji and Adesiyan (2005). The decrease in pH as the levels of crude oil pollution increased as observed in this study contradicts the reports of Onuh *et al*. (2008a) who observed an increase in pH as the levels of crude oil pollution increased. The decrease in pH as the levels of crude oil pollution increased as observed in this study is in line with the reports of Obasi *et al*. (2013) who observed an increase in pH as the levels of crude oil pollution increased. These observed pH values however, do not fall completely within the acceptable standards of 5.5 to 6.5 (DPR, 2002). The pH (the degree of acidity and alkalinity) affects not only the physico-chemical properties but also the flora and fauna of soil. Thus, it determines the availability of many nutrients for plant growth and maintenance.

Strong acidic soils (pH 4 to 5) have been reported to have high concentration of soluble aluminum and manganese salts, which are toxic to plants. Consequently, the lowered pH values observed in the polluted soils can be raised by liming through appropriate application of calcium and

magnesium compounds. Also, it is known that carbon mineralization and organic matter breakdown are rapid in neutral-to-slightly alkaline soils (Hunt, 1996).

At two weeks after pollution, percentage organic carbon and organic matter content of the soil samples increased with increase in the concentration of crude oil pollution (Table 2). The increase in the percentage organic carbon and organic matter observed in this study had been observed earlier (Onuh *et al.,* 2008a, Amadi *et al*.,2005;Ogboghodo *et al*., 2005;Obasi *et al*., 2013) and may be attributed to the microbial degradation of the crude oil.Available percentage nitrogen and phosphorus of the soil decreased with increase in the levels of crude oil pollution. Onuh *et al.* 2008a; Obasi *et al*., 2013 had also observed a decrease in nitrogen availability with increased levels of crude oil pollution. Similarly, a decrease in phosphorus availability with increased levels of crude oil pollution had been reported (Okolo *et al*., 2005; Ogboghodo *et al*., 2005; Isirimah *et al.,* 1989). The decrease in the available nitrogen and phosphorus with increased levels of crude oil pollution may be attributed to the limitation induced by the introduction of excess carbon to the soil since crude oil is a rich source of hydrocarbon (Atlas, 1981).

Exchangeable bases (calcium, potassium and magnesium) were observed to have decreased with increased levels of crude oil pollution. This may be attributed to the use of these exchangeable bases by the microbes present in the experimental soil samples.

The result in table 2 above shows a significant increase in the THC with increased levels of crude oil pollution. The total hydrocarbon levels of the polluted soils significantly exceeded the compliance limit of 50 ppm set for the petroleum industry in Nigeria for oil and grease contamination (DPR, 2002). A number of studies have shown that high concentration of THC in soils is detrimental to the growth and productivity of plants and animals (Okolo *et al*., 2005; Osuji *et al*., 2004; Salanitro *et al*., 1997). Thus, the presence of high hydrocarbons of the range obtained in this study creates a clear condition that demands rehabilitation process for a meaningful existence of flora and fauna in crude oil polluted soils.

Effect of organic manures on the physico-chemical properties of the unpolluted and crude oil polluted soil

The results of the physicochemical properties of the unpolluted and crude oil polluted soil four weeks after amendments with two different organic manures are shown in Tables 3 to 11 below.The results showed that addition of organic manures to the crude oil polluted soils slightly raised the soil pH. The pH of the oil polluted soil amended with CD followed by CD + BF raised the pH higher than those amended with BF which was only slightly raised. The results obtained are in harmony with those obtained by Ijah *et al*. (2008). The results also confirm earlier findings (Ijah and Antai, 2003) that organic manures (for example, chicken droppings) have buffering effect on crude oil polluted soil. This rise in the pH of the amended soils may favor oil degradation by microorganisms as observed in similar studies that higher pH range (6 to 9) provides better conditions for degradation of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH conditions close to neutrality (Tanee and Kinako, 2008; Manuel *et al.,* 1993; Atlas and Bartha, 1992).

The results in table 4 and 5 below shows that the organic carbon and organic matters contents of the unpolluted and crude oil polluted soil increased significantly ($P \leq 0.05$) on application of the different types of organic manures. Bags treated with chicken droppings gave the highest organic carbon and organic matter contents followed by bags treated with CD+ BF and then followed by BF only. Organic carbon and organic matter affect soilproperties such as their water holding capacity, bulk density and mobilizes nutrients for plants (Atlas and Barth, 1973).McGill (1976) also reported that organic carbon and organic matter when present in sufficient quantity have beneficial effect on soil chemical and physical properties. Thus, the significant increase in the organic carbon and organic matter content of the amended soil observed in this study may have beneficial effect on the soil chemical and physical properties. This is in line with earlier reports (Mbah *et al*., 2006, 2009;Shimp and Pfender,1984) which stated that organic carbon and organic matter from wastes can influence the ability of microorganisms to degrade pollutants.

Table 3: Mean and Standard Deviation of pH values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	5.38±0.01	5.26±0.02	5.22±0.01	5.19±0.02	5.12±0.02
Chicken dropping (CD)	6.01±0.03	5.52±0.02	5.42±0.01	5.38±0.02	5.23±0.02
Bovine feaces (BF)	5.49±0.01	5.43±0.01	5.34±0.00	5.29±0.01	5.17±0.01
CD + BF	5.59±0.02	5.50±0.00	5.36±0.04	5.31±0.03	5.18±0.03

Table 4: Mean and Standard Deviation of Organic Carbon (OC) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	2.95±0.01	3.61±0.01	5.14±0.02	5.18±0.05	5.75±0.01
CD	3.39±0.02	3.84±0.02	5.33±0.01	5.37±0.06	5.87±0.03
BF	3.22±0.01	3.68±0.05	5.23±0.10	5.29±0.02	5.81±0.02
CD+ BF	3.37±0.02	3.86±0.01	5.30±0.02	5.31±0.01	5.84±0.01

Table 5: Mean and Standard Deviation of Organic Matter (OM) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	5.07±0.02	5.40±0.02	7.91±0.05	9.33±0.02	10.24±0.03
CD	5.83±0.01	5.63±0.01	8.23±0.31	9.66±0.02	10.45±0.02
BF	5.54±0.02	5.52±0.01	8.10±0.11	9.53±0.05	10.35±0.01
CD + BF	5.80±0.00	5.55±0.05	8.15±0.16	9.58±0.03	10.41±0.03

Table 6: Mean and Standard Deviation of Nitrogen (N) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	0.18±0.02	0.14±0.01	0.14±0.04	0.13±0.01	0.10±0.01
CD	0.27±0.04	0.20±0.01	0.20±0.10	0.21±0.09	0.17±0.03
BF	0.22±0.02	0.17±0.02	0.16±0.12	0.18±0.11	0.13±0.10
CD+ BF	0.25±0.02	0.19±0.05	0.18±0.01	0.19±0.01	0.15±0.05

Table 7: Mean and Standard Deviation of Phosphorus (P) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	1.38±0.03	1.17±0.10	1.12±0.05	1.06±0.03	0.71±0.01
CD	1.42±0.04	1.26±0.01	1.21±0.01	1.18±0.01	0.80±0.02
BF	1.40±0.13	1.21±0.14	1.61±0.11	1.12±0.11	0.75±0.01
CD + BF	1.41±0.11	1.22±0.01	1.17±0.02	1.16±0.02	0.78±0.02

Table 8: Mean and Standard Deviation of Calcium (Ca) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	1.43±0.03	1.39±0.01	1.34±0.05	1.29±0.05	1.25±0.04
CD	1.51±0.04	1.51±0.02	1.49±0.02	1.38±0.02	1.40±0.02
BF	1.48±0.11	1.43±0.01	1.42±0.01	1.33±0.01	1.27±0.01
CD + BF	1.49±0.03	1.48±0.01	1.44±0.05	1.34±0.05	1.28±0.03

Table 9: Mean and Standard Deviation of Potassium (K) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	1.02±0.12	1.02±0.01	0.85±0.23	0.83±0.25	0.92±0.02
CD	1.09±0.21	1.10±0.05	0.91±0.05	0.90±0.04	0.99±0.09
BF	1.06±0.03	1.06±0.02	0.88±0.01	0.87±0.01	0.95±0.07
CD+ BF	1.07±0.02	1.08±0.01	0.89±0.01	0.89±0.01	0.97±0.03

Table 10: Mean and Standard Deviation of Magnesium (Mg) values obtained after 4 weeks of Treatments with different manures

Treatments	0ml (Control)	20ml	40ml	60ml	80ml
Un-amended	0.03±0.02	0.77±0.03	0.54±0.02	0.51±0.01	0.55±0.03
CD	0.82±0.01	0.72±0.02	0.60±0.00	0.55±0.02	0.61±0.10
BF	0.80±0.02	0.65±0.05	0.57±0.02	0.53±0.01	0.58±0.04
CD + BF	0.81±0.02	0.66±0.01	0.58±0.10	0.54±0.02	0.59±0.02

Table 11: Mean and Standard Deviation of Total Hydrocarbon Content (THC) values obtained after 4 weeks of Treatments with different manures

Treatments	20ml	40ml	60ml	80ml
Un-amended	3028.10±1.17	5092.23±7.12	6026.24±6.14	6403.12±2.08
CD	1716.53±1.49	2179.76±11.4	3079.65±10.5	3164.62±1.32
BF	2641.23±1.21	2952.34±1.25	3632.03±1.24	4200.42±1.86
CD + BF	1011.00±1.25	1710.21±1.98	2668.7±2.10	2760.09±2.20

The result in table 6 and 7 shows that Total nitrogen and phosphorus content of the amended unpolluted and crude oil polluted soils were significantly higher than those of the un-amended soils, respectively.Bags amended with CD gave the highest value of nitrogen and phosphorus followed by bags amended with CD+ BF and BF only had the lowest increased values of nitrogen and phosphorus. However, the increment was all significant ($P \leq 0.05$) using Duncan's multiple range test (DMRT). This increase in the percentage nitrogen and phosphorus may be as a result of anthropogenic inputs of these nutrients from the organic manures because organic manures have been reported as being capable of increasing soil nutrients by supplementing the limiting nutrients (Mbah *et al*, 2009:2006; Tanee and Kinako, 2008). Reports have shown that addition of nitrogen and phosphorus enhances biodegradation of polluted soil presumably by removing the nitrogen and phosphorus limitation resulting from low natural level (Odokuma and Ibor, 2002; Lee *et al.*, 1995). Thus, the increase in the percentage nitrogen and phosphorus content of the amended soils induced by the various organic manures may enhance the biodegradation of the crude oil polluted soil and as such enhance its fertility.

The results in table 8, 9 and 10 shows that there were slight increases in the levels of calcium, potassium and magnesium in the amended unpolluted and crude oil polluted soils relative to the un-amended soils.Calcium, potassium and magnesium were all influenced by the addition of the various organic manures to the unpolluted and crude oil polluted soils. There were significant increases in these metals on addition of these manures with CD producing the highest values in each case. Addition of Bovine feaces (BF)gave the lowestincrement in the value of these metals.This showed that chicken droppings is a rich source of these exchangeable bases than bovine manure. The addition of these exchangeable bases to soils improves soil fertility. Thus, these amendment options will definitely improve the soil fertility thereby amending the crude oil pollution. The increment in the values of these metals were however significant ($P \leq 0.05$). Mbah *et al*. (2006, 2009) observed similar results.

The result in table 11 shows that there was significant decrease in the total hydrocarbon content of amended crude oil polluted soils relative to the un-amended crude oil polluted soils. Highest loss of total hydrocarbon was evident in the CD + BF followed by CD treatments. This reduction in THC of the organic manures amended soils is in line with the reports of Tanee and Kinako (2008) who observed significant loss in the THC of poultry manure and NPK amended crude oil polluted soil. The high hydrocarbon loss in the organic manures amended soils is in line with Lee et al. (1995) who reported that organic manures have effect in stimulating crude oil degradation by increasing the total heterotrophic microbial growth and activity.

Microbial Diversity of the samples

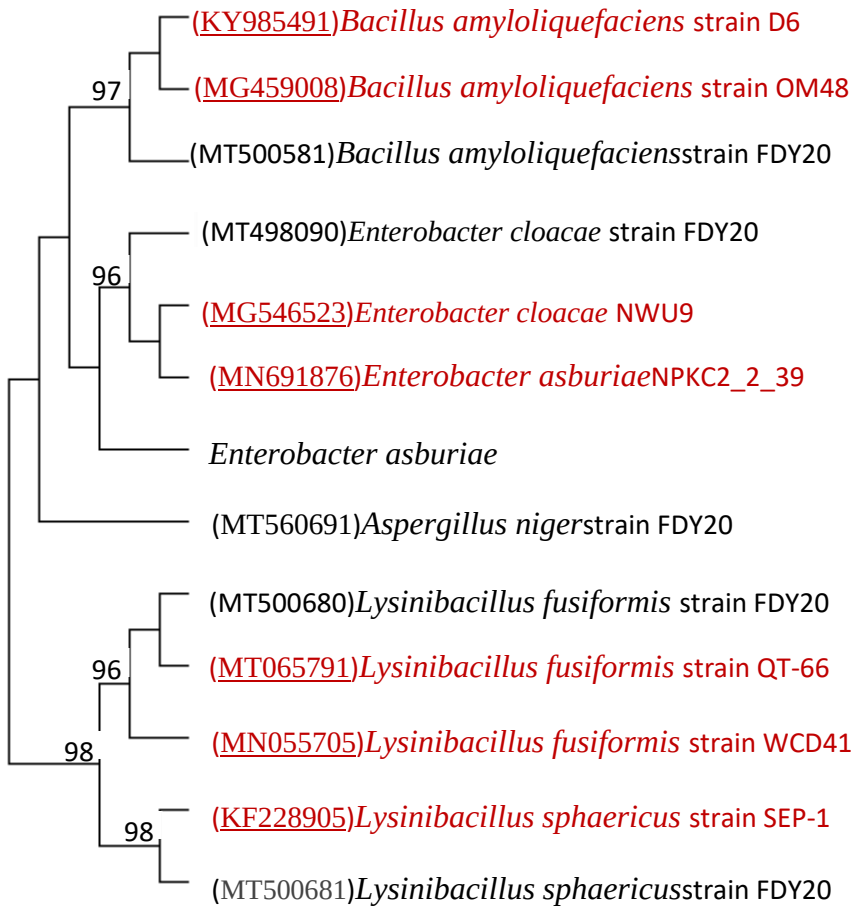

Fig1: Phylogenetic Tree showing similarities between the isolates

The present study elucidated microbial ecology of two animal enriched wastes providing better insights into their microbial community structure. The microbial isolates were identified using analysis of 16S rRNA gene. A number of studies suggest that 16S rRNA gene sequencing provides genus identification in most cases (Janda and Abbott, 2007; Joao *et al.*, 2014). The DNA sequence and subsequent BLAST analysis indicated a high similarity of the obtained sequence

corresponding to the respective isolates as shown in the phylogenetic tree (Figure1). The microbial composition of the CD and BF which could have contributed in the biodegradation process as identified using 16SrRNA sequencing included *Lysinibacillus sphaericus, Lysinibacillus fusiformis, Enterobacter cloacae, Bacillus amyloliquefaciens, Aspergillus niger* and *Enterobacter asburiae*. Nucleotide sequences of the isolates retrieved from this study have been deposited in the GenBank nucleotide sequence database under accession nos. MT560691, MT500581, MT498090, MT500681 and MT500680 (NCBI GenBank, www.ncbi.nlm.nih.gov).

The isolates obtained following the molecular assay were members of various genera such as *Bacillus, Lysinibacillus, Enterobacter* and *Aspergillus*. The dominant genera belonged to *Enterobacter* and *Lysinibacillus* species. All the above genera have been reported in previous biodegradation studies by Alonso-Gutiérrez *et al*. (2008). The poultry dropping contaminated soil was dominated by sequences belonging to the bacterial groups Alphaproteobacteria, Gammaproteobacteria and Firmicutes, and these groups have been previously encountered in earlier studies including those of (Militon *et al.,* 2010; dos Santos *et al.,* 2011; Sutton *et al.,* 2013). Several members of these bacterial groups are known for their ability to degrade aliphatic and aromatic hydrocarbons (Alonso-Gutierrez *et al.,* 2008; Vila *et al.,* 2010; Kostka *et al.,* 2011).However, bovine feaces (cowdung) contaminated soil had sequences dominated by genera including *Bacillus, Lysinibacillus* and *Aspergillus*.

Earlier studies by Hamamura *et al*., (2006); Chikere *et al*., (2009); Obayori and Salam, (2010) reported the most important hydrocarbon degraders of bacterial origin as *Achromobacter,Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Corynebacterium, Dietzia, Flavobacterium, Pseudomonas, Rhodococcus,* and other bacterial clones that cannot be cultured. Contrary to the reports of these authors, most of their isolates were not recovered in this study because of direct metagenomic studies carried out in the studies.The findings (decreased genera of microorganisms) of this study is in conformity with earlier assertion by Simon and Daniel (2010) that the exploration of bacterial diversity in polluted soil undergoing bioremediation requires molecular approaches that can comprehensively extract DNA from the soil microbial community sufficient enough to capture majority of the species that are dominant (Simon and Daniel, 2010)

CONCLUSION

The results indicated that crude oil adversely affect soil physicochemical properties. Results from this study also showed improved soil physicochemical properties on crude oil contaminated soils amended with organic manures. Poultry manure in particular performed significantly better ($P \leq 0.05$) for the improvement of all soil physicochemical parameters in relative to cow manure. In light of the above, the results provided ample evidence that showed that organic manure supplements modify the physical, chemical and biological properties of crude oil polluted soils and improve their nutritional status for enhanced agronomic performances.The result also indicated that the microbial composition of the CD and BF which could have contributed in the biodegradation process as identified using 16SrRNA sequencing include: *Lysinibacillus sphaericus, Lysinibacillus fusiformis, Enterobacter cloacae, Bacillus amyloliquefaciens, Aspergillus niger* and *Enterobacter asburiae*. Nucleotide sequences of the isolates retrieved from

this study have been deposited in the GenBank nucleotide sequence database under accession nos. MT560691, MT500581, MT498090, MT500681 and MT500680 (NCBI GenBank, www.ncbi.nlm.nih.gov).

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