

A Screen House Experiment to Evaluate the Biostimulation Potentials of Cow Dung on a Crude Oil Polluted Soil

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Abstract – This screen house study was conducted to investigate the effect of the application of cow dung to crude oil polluted soils. Four rates of cow dung (0, 1, 2 and 3g) and four rates (0, 5, 10 and 15ml) of crude oil per 10kg of soil were used, a total of sixteen (16) treatment combinations were applied. Each treatment was replicated three times, for a total of forty eight (48) pots. The rate used is equivalent to 0, 200, 400 and 600kg/ha and 0, 1000, 2000 and 3000 litres/ha of cow dung and crude oil respectively. The experiment was laid out in a completely randomized designed. Bacteria, fungi and Physico-chemical properties of the soils were determined before pollution, two weeks after pollution and at the end of the experiment. The results for the physico-chemical properties of soil indicate an increased in pH, carbon to nitrogen ratio (C:N), total organic carbon (TOC) and nitrogen (N) while Phosphorus (P) decreased as the level of crude oil increased despite remediation with cow dung. There was an increase in bacterial count for both the control and the treatment groups. The result range from 1.3×10^4 to 28.6×10^4 . The genus of bacteria identified were *Pseudomonas*, *Bacillus*, *Micrococcus*, *Proteus*, *Clostridium* and *Nocardia* species. Four isolates were gram positive while 2 were gram negative. Five were rod-shaped while one was in coccus form, while the fungi isolates are *Cladosporium*, *Pichia*, *Aspergillus*, *Fusarium* species. Generally remediation for the oil contaminated soil at the end of tenth week revealed a positive correlation coefficient in the degree of remediation during the trial periods.

Keywords – Biostimulation, Cow Dung, Crude Oil, Soil Properties, Total Petroleum Hydrocarbon.

I. INTRODUCTION

Oil exploration and exploitation activities in economies that produce and consume oil products bring about crude oil pollution as a natural consequence. This pollution is due mainly to accidental discharge human error, sabotage, transportation and other natural causes. In Nigeria, there is a significant level of spilled oil in the terrestrial and aquatic environments from a number of sources including damaged oil tankers, storage vehicles, leakages of oil pipelines and oil tankers overflow due to increase in crude oil exploration, exploitation, storage, sabotage and transportation. This is because crude oil has for decades been the life wire of the Nigerian economy and accounts for over 90% of the national income (Nwilo, 1998). Significant among the damages done to the environment by crude oil spills is pollution of soil which renders it less useful for agricultural activities and affects soil dependent organisms adversely.

Bioremediation is an option that offers the possibilities to

destroy or renders harmless various contaminants using natural biological activity. The effects of crude oil pollution on the properties of soil have been the subjects of many studies. Okoloet *al.*, (2005) reported that oil pollution increase carbon and reduces soil nitrates and phosphorus. Crude oil pollution prevents oxygen exchange between soil and the atmosphere due to hydrophobic properties of oil. Any contact of soil with crude oil results in damage to the soil micro-organisms and plants.

Cow dung is widely available at almost cost free in the environment. Cow dung also known as cow manure is the undigested plant matter (waste product) of bovine animal species which has passed through the animal guts. The resultant fecal matter usually combined with soil bedding and urine is rich in minerals and often used as agricultural manure, providing food for a wide range of animals and fungus species. The use of cow dung on crude oil contaminated soils will also protect the soil structure, provide utilizable nutrients (Ogboghodoet *al.*, 2005).

The objectives of this study therefore were to evaluate the effect of crude oil on soil properties and to access the biostimulative effect of cattle's dung on crude oil polluted soils.

II. MATERIALS AND METHODS

The pot experiments were conducted at the Faculty of Agriculture, University of Benin at the department of Soil Science and Land Management screen house. The experiment comprising of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) buckets.

Treatments and Experimental Design

48 buckets measuring 12 litres volume capacity were used for this study. The buckets were perforated at the sides and bottom. Each bucket was filled with 10kg of top soil equivalent to 0.10m^2 . Soils were collected at 0 - 15 cm of soil depth from the field experimental site, air dried, carefully cleaned by picking away all litter of leaves and roots that could decompose with time and weighed into plastic buckets. The soils were allowed to settle for two weeks, watered and treated with four rates (0, 5, 10 and 15 ml) of crude oil (bonny light blend). The crude oil was spilled on the surface of the soil in simulating what generally occurs in case of oil spills. Two weeks after crude oil treatment, four rates (0, 1, 2 and 3 gm) of air-dried, ground cow dung manure was applied to polluted soils. The cow dung manure was thoroughly mixed with the soil using

hand trowel to ensure uniform distribution within the soil. Each quantity of crude oil served as a treatment with the 0ml treatment serving as the control. The experiment was a 4*4 factorial laid out in a complete randomized design.

Sampling

Soil samples were collected from the pots at three different times. The first was before crude oil application to ascertain the physico-chemical nature of the unpolluted soil. Second was 4 weeks after pollution and amendment and third after 10 weeks.

Determination of Physiochemical Parameters

Soil Samples were collected, properly labeled, and then taken to the laboratory for analysis.

The pH of the soil samples was determined in distilled water at a ratio of 1:1 using a glass electrode pH Meter. Total Organic carbon was determined using titrimetric method by Walkey and Black (1934). The total Nitrogen, CEC and available phosphorous in the soil was determined by spectrophotometry method (Ogboghodo *et al.*, 2005). Soil conductivity was determined using a conductivity meter.

Determination of total Petroleum Hydrocarbon and Pah in Crude Oil Samples

Procedure

The samples were cold-extracted in a conical flask for two hours in each case using 100% dichloromethane according to the method of (Shahunthala *et al.*, 2004). The solvent from the resultant solution was removed by means of a rotary evaporator under vacuum (pressure not greater than 200mbar) and finally by a flow nitrogen at not more than 30°C to yield the extracted organic matter (EOM).

The extracted organic matter (EOM) was analysed by capillary gas chromatography. TPH was analysed with the GC-FID (Gas Chromatography–Flame Ionization Detector) while the PAH was analysed with the GC-MS (Gas Chromatography - Mass Spectrometry) Clarus -500 Perkin Elmer according to the method of (Ashraf, 2014). The GC-FID system consist of a HP5890 SERIES II, Hewlett-Packard, Wald brown, Germany GC equipped with flame ionization detector and ATLAS software data processor (USA). The gas chromatographic column used was Ultra-1932530, a non - polar, fused-silica capillary column (30m × 250µm inner diameter × 0.20µm film thickness) (USA). Helium gas was used as the carrier gas at a low flow rate of 1ml/min at a pressure of 75kpa. The injector temperature was set at 250°C, and detector temperature at 310°C. The temperature program used was; 2 minutes hold time at 250, a ramp to 13°C at 3°C/min followed by 3 min hold time, a ramp to 240 °C at 7 °C /min and a final ramp to 285 °C at 12 °C with an 8 minute hold time.

Table 1. Concentration of PAH's in crude oil

PAH (ml/l)	Nigerian crude oil
Acenaphthene	1.072
Acenaphthylene	1.046
Anthracene	0.522
Benzo (a) pyrene	0.076
Benzo (b) flouranzthene	0.023
1, 12-Benzoperylene	0.007
1, 2, 5, 6 Dibenzanthracene	0.002
Fluoranthene	0.450

PAH (ml/l)	Nigerian crude oil
Fluorene	0.284
Indeno (1, 2, 3) pyrene	0.002
Naphthalene	0.163
Phenanthrene	0.143
Pyrene	0.621
Benzo (k) fluorathene	BDL

Enumeration of total Heterotrophic Bacteria (THB)

The viable bacteria were enumerated on nutrient agar plates by spread plate method using 0.1 ml of dilutions 10^{-1} to 10^{-7} of the bacterial suspensions. All inoculated plates were incubated for 24-48 hours at 37 °C. The bacterial colonies on the plates were counted then randomly picked and purified by sub-culturing unto fresh agar plates using the streak plate technique. Isolated colonies that appeared on plates were then transferred into nutrient agar slants, properly labelled and stored as stock cultures. The bacterial isolates were identified based on their morphology, Gram reaction and biochemical characterization.

III. RESULT

Effects of Remediation Amendments on Soil Physico-Chemical Properties Ph

The mean pH ranges from (4.41 – 4.69) pre exposed soil, (4.14 - 5.74) 4 weeks after pollution and amendment and (4.31 - 4.85) after 10 weeks. The highest mean was (5.74 ± 0.02) gotten from pH (4 weeks after pollution and amendment) in treatment (15ml of crude oil, 3g of cow dung application). Similarly, the lowest mean was recorded (4.14) from (4 weeks after pollution and amendment) in treatment (10ml of crude oil, 1g of cow dung and 15ml of crude oil, NA of cow dung application).

Total Organic Carbon (%)

The total organic carbon range from (0.04-0.85) pre exposed soil, (0.68-1.32) 4 weeks after pollution and amendment and (0.12-0.96) after 10 weeks. The mean for the control groups are (0.85 ± 0.02, 0.85 ± 0.02 and 0.90 ± 0.03) and the highest mean for the treatment group was (1.32 ± 0.04) recorded at treatment (15ml of crude oil, 3g of cow dung), while the lowest mean was (0.04 ± 0.86) recorded at treatment (0ml of crude oil, 1g of cow dung).

Phosphorus (mg/kg)

The result for phosphorus range from (18.14 - 18.55) for normal soil (17.33 - 18.06) 4 weeks after pollution and amendment and (12.11 - 12.62) after 10 weeks. The mean for the control groups are (18.32 ± 0.03, 17.35 ± 0.25 and 12.13 ± 0.13) respectively.

Total Nitrogen (%)

Total nitrogen (TN) content is presented in (Table 3), the highest mean for the treatment group was (0.67 ± 0.04) recorded at treatment (10ml of crude oil, 2g of cow dung), while the lowest mean was (0.22 ± 0.01) recorded at treatment (10ml of crude oil, NA of cow dung).

Electrical Conductivity

Electric conductivity was low in treatment (10ml of crude oil, NA of cow dung) after 10 weeks, but high in treatment (15ml of crude oil, 1g of cow dung).

Cation Exchange Capacity (meq/100g)

The cation exchange Capacity (CEC) value range from (1.31-1.38) pre-exposed soil, (1.35-1.38) 4 weeks after pollution and amendment and (1.23-1.28) after 10 weeks. The mean for the control groups are (1.35 ± 0.01, 1.35 ± 0.02 and 1.28 ± 0.01).

Carbon/Nitrogen Ratio

The C: N ratio content, the highest mean for the treatment group was (29.0 ± 3) recorded at treatment (5ml of crude oil, 1g of cow dung), and the lowest mean was (11.0 ± 1) recorded at treatments (0ml of crude oil, 3g of cow dung, 10ml of crude oil, 2g of cow dung), details of carbon to nitrogen ratio result are shown in (Table 5).

Effect on Bacteria and Fungi Population

The result for bacteria count range from (1.3 X10⁴ to 1.3 X10⁴) pre-exposed soil, (1.3 X10⁴ to 28.6 X10⁴) 4 weeks after pollution and amendment and (1.4 X10⁴ to 12.3 X10⁴) after 10 weeks. The mean for the bacterial count of the control group for pre-exposed soil, 4 weeks after pollution and amendment and after 10 weeks are (1.3 X10⁴, 1.3 X10⁴, 1.4 X10⁴) respectively and the highest bacterial count for the treatment group is (28.6 X 10⁴) recorded at treatment (15ml of crude oil, 3g of cow dung application), While the lowest mean value was (1.3 X10⁴).

The results of biochemical test and colony characteristics for identification of the bacterial isolates are found in (Table 5). The bacteria are identified as *Pseudomonas*, *Bacillus*, *Micrococcus*, *Proteus*, *Clostridium* and *Nocardia* species. Four isolates were gram positive while 2 were gram negative. Five were rod-shaped while one was in coccus form

Pseudomonas, *Bacillus*, *Clostridium* and *Nocardia* species were found in the pre exposed soil. *Pseudomonas*, *Bacillus*, *Micrococcus*, *Proteus*, *Clostridium* and *Nocardia* species were found in soil samples polluted and amended with cow dung 4 weeks after pollution and amendment while *Pseudomonas*, *Bacillus*, *Clostridium* and *Nocardia* species were found in after 10 weeks. *Proteus sp* was introduced base on cow dung amendment only. It was not found in the pre-exposed soil and at the expiration of the experiment.

The fungal isolates are *Cladosporium*, *Pichia*, *Aspergillus*, *Fusarium* species. *Aspergillus* and *Cladosporium* were the only soil samples found in the pre exposed soil samples, *Cladosporium*, *Pichia*, *Aspergillus*, *Fusarium* were found in soil samples polluted and amended with cow dung before planting while *Aspergillus* and *Cladosporium* were found in after 10 weeks.

Table 2. Effect of the Remediation Amendments on Ph of Soil total Organic Carbon (Toc)

pH				TOTAL ORGANIC CARBON			
TREATMENTS	pH (Pre-exposed Soil)	pH(4 weeks AP/A)	pH (After 10 weeks)	TREATMENTS	TOC (Pre-exposed Soil)	TOC (4 weeks AP/A)	TOC (After 10 weeks)
0(NA)	4.57aA	4.71bA	4.83aA	0(NA)	0.85aA	0.85cA	0.90cA
0(1)	4.57aA	4.20bB	4.31aAB	0(1)	0.04aA	0.89cA	0.95cA
0(2)	4.56aA	4.41bA	4.61aA	0(2)	0.85aB	0.68cA	0.94cAB
0(3)	4.54aA	4.16bB	4.63aA	0(3)	0.85aB	1.14cA	0.89cB
5(NA)	4.65aA	4.24bcB	4.82aA	5(NA)	0.84aB	1.19bA	0.94cB
5(1)	4.58aA	4.51bcA	4.32aA	5(1)	0.83aC	1.17bA	0.94cB
5(2)	4.67aA	4.17bcB	4.71aA	5(2)	0.84aC	1.15bA	0.95cB
5(3)	4.47aAB	4.19bcB	4.85aA	5(3)	0.84aC	1.18bA	0.94cB
10(NA)	4.45aA	4.26cA	4.39aA	10(NA)	0.84aC	1.13bA	0.94bB
10(1)	4.56aA	4.14cB	4.64aA	10(1)	0.83aC	1.16bA	0.94bB
10(2)	4.54aA	4.17cB	4.64aA	10(2)	0.83aC	1.17bA	0.96bB
10(3)	4.55aA	4.15cB	4.33aAB	10(3)	0.84aC	1.17bA	0.12bB
15(NA)	4.51aA	4.14aB	4.33aAB	15(NA)	0.85aB	1.27aA	0.16aA
15(1)	4.67aB	5.13aA	4.65aB	15(1)	0.83aC	1.19aA	0.14aB
15(2)	4.69aB	5.15aA	4.59aB	15(2)	0.84aC	1.22aA	0.12aB
15(3)	4.41acB	5.74a A	4.71aB	15(3)	0.85aC	1.32aA	0.15aB

^{a-c} Different letters in the same column indicate significant difference (P<0.05)

^{A-C} Different letters in the same row indicate significant difference (P<0.05)

Table 3. Effect of the Remediation Amendments on Soil Phosphorus (P) and Total Nitrogen

P				N			
TREATMENTS	P (Pre-exposed Soil)	P (4 weeks AP/A)	P (After 10 weeks)	TREATMEN TS	N (Pre-exposed Soil)	N (4 weeks AP/A)	N (After 10 weeks)
0(NA)	18.32aA	17.35aB	12.13aC	0(NA)	0.47aA	0.25dB	0.23aB
0(1)	18.31aA	17.61aA	12.58aB	0(1)	0.47aA	0.39dA	0.35aA
0(2)	18.35aA	17.93aB	12.28aC	0(2)	0.45aA	0.33dB	0.30aB
0(3)	18.35aA	17.67aA	12.39aB	0(3)	0.39aA	0.37dA	0.23aB
5(NA)	18.34aA	17.60aB	12.40aC	5(NA)	0.44aA	0.23bB	0.28aB
5(1)	18.55aA	17.46aA	12.62aB	5(1)	0.43aA	0.52bA	0.27aB
5(2)	18.46aA	17.71aA	12.51aB	5(2)	0.47aA	0.54bA	0.29aB
5(3)	18.24aA	17.90aA	12.47aB	5(3)	0.45aAB	0.57bA	0.29aB
10(NA)	18.14aA	17.33aB	12.11aC	10(NA)	0.52aA	0.22aB	0.24aB
10(1)	18.54aA	17.34aB	12.34aC	10(1)	0.44aA	0.60aA	0.39aA
10(2)	18.38aA	18.06aA	12.38aB	10(2)	0.49aA	0.67aA	0.45aA
10(3)	18.31aA	17.73aA	12.42aB	10(3)	0.45aAB	0.61aA	0.42aB
15(NA)	18.28aA	17.99aA	12.43aB	15(NA)	0.46aA	0.23cB	0.25aB
15(1)	18.40aA	17.44aB	12.30aC	15(1)	0.45aA	0.43cA	0.38aA
15(2)	18.55aA	17.33aB	12.34aC	15(2)	0.55aA	0.45cB	0.55aA
15(3)	18.46aA	17.42aB	12.36aC	15(3)	0.50aA	0.44cAB	0.27aB

^{a-d} Different letters in the same column indicate significant difference (P<0.05)

^{A-C} Different letters in the same row indicate significant difference (P<0.05)

Table 4. Effect of the Remediation Amendments on Soil Electrical Conductivity and Cation Exchange Capacity

CONDUCTIVITY				CATION EXCHANGE CAPACITY			
TREATMENT S	COND (Pre-exposed Soil)	COND (4 weeks AP/A)	COND(After 10 weeks)	TREATME NTS	CEC (Pre-exposed Soil)	CEC (4 weeks AP/A)	CEC (After 10 weeks)
0(NA)	222.39aA	222.44aA	196.03aB	0(NA)	1.35aA	1.35aA	1.28aB
0(1)	222.42aA	222.17aA	211.05aA	0(1)	1.34aB	1.38aA	1.23aC
0(2)	222.39aA	223.28aA	205.37aB	0(2)	1.33aA	1.36aA	1.27aB
0(3)	222.36aA	223.18aA	208.24aB	0(3)	1.33aA	1.37aA	1.24aB
5(NA)	222.35aA	221.34bB	193.79aC	5(NA)	1.35aA	1.37aA	1.24aB
5(1)	222.28aA	222.33bA	205.08aB	5(1)	1.33aA	1.36aA	1.23aB
5(2)	222.27aA	222.35bA	204.59aB	5(2)	1.31aB	1.37aA	1.26aC
5(3)	222.37aA	222.27bA	207.63aB	5(3)	1.34aB	1.38aA	1.24aC
10(NA)	222.56aA	204.54aB	192.26aC	10(NA)	1.35aA	1.37aA	1.23aB
10(1)	222.46aA	224.14aA	204.60aB	10(1)	1.34aA	1.37aA	1.25aB
10(2)	222.32aA	223.15aA	206.06aB	10(2)	1.34aB	1.38aA	1.28aC
10(3)	222.25aB	223.05aA	208.79aC	10(3)	1.34aA	1.37aA	1.25aB
15(NA)	222.13aA	201.71aB	197.70aC	15(NA)	1.38aA	1.36aA	1.23aB
15(1)	222.36aA	225.97aA	205.72aB	15(1)	1.35aA	1.35aA	1.25aB
15(2)	222.49aA	225.03aA	204.00aB	15(2)	1.34aA	1.37aA	1.26aB
15(3)	222.47aB	225.26aA	208.20aC	15(3)	1.33aB	1.38aA	1.26aC

^{a-c} Different letters in the same column indicate significant difference (P<0.05)

^{A-C} Different letters in the same row indicate significant difference (P<0.05)

Table 5. Effect of the Remediation Amendments on Soil Carbon/Nitrogen Ratio and Effect of Bacteria Population on Crude Oil Polluted Soil

CARBON/NITROGEN RATIO				BACTERIA POPULATION			
TREATMENTS	C:N (Pre-exposed Soil)	C:N (4 weeks AP/A)	C:N (After 10 weeks)	TREATMENTS	Bacteria count (Pre-exposed Soil)	Bacteria count (4 weeks AP/A)	Bacteria count (After 10 weeks)
0(NA)	23.0bA	12.3bB	20.7aA	0(NA)	1.3 X10 ⁴	1.3 X10 ⁴	1.4 X10 ⁴
0(1)	23.0bA	11.3bB	22.0aA	0(1)	1.3 X10 ⁴	5.3 X10 ⁴	1.4 X10 ⁴
0(2)	21.7bA	12.3bB	20.7aA	0(2)	1.3 X10 ⁴	5.2 X10 ⁴	1.5 X10 ⁴
0(3)	23.0bA	11.0bB	22.7aA	0(3)	1.3 X10 ⁴	5.4 X10 ⁴	1.5 X10 ⁴
5(NA)	22.3bA	11.3aB	21.7aA	5(NA)	1.3 X10 ⁴	9.2 X10 ⁴	4.4 X10 ⁴
5(1)	21.3bA	13.0aB	29.0aA	5(1)	1.3 X10 ⁴	12.2 X10 ⁴	9.3 X10 ⁴
5(2)	21.3bA	15.0aB	22.7aA	5(2)	1.3 X10 ⁴	12.2 X10 ⁴	9.4 X10 ⁴
5(3)	23.0bA	14.3aB	22.3aA	5(3)	1.3 X10 ⁴	12.5 X10 ⁴	7.8 X10 ⁴
10(NA)	22.3aA	12.0bB	25.7aA	10(NA)	1.3 X10 ⁴	11.2 X10 ⁴	4.3 X10 ⁴
10(1)	26.3aA	11.7bC	21.0aB	10(1)	1.3 X10 ⁴	24.2 X10 ⁵	10.7 X10 ⁴
10(2)	25.0aA	11.0bC	19.3aB	10(2)	1.3 X10 ⁴	23.2 X10 ⁵	11.4 X10 ⁴
10(3)	23.0aA	12.3bB	23.3aA	10(3)	1.3 X10 ⁴	22.9 X10 ⁵	11.3 X10 ⁴
15(NA)	24.0abA	17.7abB	22.0aA	15(NA)	1.3 X10 ⁴	11.2 X10 ⁴	5.6 X10 ⁴
15(1)	22.0abA	13.3abB	22.7aA	15(1)	1.3 X10 ⁴	27.3 X10 ⁴	11.3 X10 ⁴
15(2)	26.3abA	11.7abB	20.7aA	15(2)	1.3 X10 ⁴	25.1 X10 ⁴	11.9 X10 ⁴
15(3)	24.3abA	13.7abB	21.0aA	15(3)	1.3 X10 ⁴	28.6 X10 ⁴	12.3 X10 ⁴

^{a-b} Different letters in the same column indicate significant difference (P<0.05)

^{A-C} Different letters in the same row indicate significant difference (P<0.05)

Table 6: Results of TPH in Pre & Crude Oil Exposed Polluted Soil

TRT	TRT	TPH (Pre-exposed Soil)	TPH (4 weeks AP/A)	TPH (After 10 Weeks)
0(NA)	BDL	BDL	BDL	0(NA)
0(1)	BDL	BDL	BDL	0(1)
0(2)	BDL	BDL	BDL	0(2)
0(3)	BDL	BDL	BDL	0(3)
5(NA)	BDL	17.6 ± 084	6.4 ± 0.27	5(NA)
5(1)	BDL	11.3 ± 0.25	BDL	5(1)
5(2)	BDL	10.4 ± 0.26	BDL	5(2)
5(3)	BDL	10.5 ± 0.36	BDL	5(3)
10(NA)	BDL	32.9 ± 0.36	13.24 ± 0.03	10(NA)
10(1)	BDL	26.4 ± 4.58	BDL	10(1)
10(2)	BDL	20.2 ± 5.45	BDL	10(2)
10(3)	BDL	43.8 ± 0.34	BDL	10(3)
15(NA)	BDL	30.4 ± 5.72	14.22 ± 0.05	15(NA)
15(1)	BDL	28.9 ± 5.72	BDL	15(1)
15(2)	BDL	28.9 ± 0.64	BDL	15(2)
15(3)	BDL	28.1 ± 0.09	BDL	15(3)

KEY:

4 weeks AP/A = 4 weeks after pollution & amendment.

NA = NO AMENDMENT.

1 = 1g of cattle dung.

2 = 2g of cattle dung.

3 = 3g of cattle dung.

0 = No crude oil.

5 = 5ml crude oil.

10 = 10ml crude.

15 = 15ml crude oil.

IV. DISCUSSION

Effects of Remediation Amendments on Soil Physico-Chemical Properties

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Marschner, 1995). pH increased significantly after crude oil exposure though there was a decrease in pH after 10 weeks. The positive interactions between the pH of the soils and the amount of crude oil added to the soil may imply that crude oil pollution leads to increase in soil pH. This is similar to the findings of Andrade *et al.*, (2004) who observed increase in the pH of soils polluted with crude oil.

Total petroleum hydrocarbon (TPH) was found to be below detectable limit (BDL) in soils before pollution. Analysis of TPH content after exposure of soil to crude oil revealed statistically significant decrease ($P < 0.05$) in concentration of TPH in the treatment groups. The TPH content of crude oil contaminated soil showed clearly that there was a reduction in the concentration of petroleum hydrocarbon in soil at the end of the experiment. This is in agreement with the work of Efe and Elenwo, (2014).

Electrical conductivity of soil reported in this research was within the range reported for research conducted within the same area (Ogboghodo *et al.*, 2005). The highest value for electrical conductivity was recorded in soils exposed to the highest concentration of crude oil and amendment. Anions, metallic ions and carbonic acids contribute to electrical conductivity of crude oil polluted soils and have been found to increase with increasing concentration of remediating agent (Xiao-yuet *et al.*, 2009).

Carbon to Nitrogen (C: N) ratio was observed to increase with increased crude oil concentration and increased amendment. The observed result of C: N ratio may be attributable to the increase in microbial activity of the carbon utilizing agent since microbes are known to be heavy carbon utilizers (April and Simms, 1990). The amendment used in this study had significant effect ($P < 0.05$) on the soil C: N ratio.

CEC is an indication of the relative ability of K, Na, Ca and Mg to displace other cation. There were also statistically significant differences ($P < 0.05$) between the treatment groups on the same column as found in the pre-exposed soil, 4 weeks after pollution and amendment and soil collected after 10 weeks indicating no effect of amendment. The CEC reported in this study is in agreement with other researchers who worked within the same area (Ogeh and Osiomwan, 2012).

The TOC content had a positive relationship between soil and contaminant concentrations as organic carbon concentrations increased with increase in crude oil concentrations. This observation is in support with Njoku *et al.*, (2009) who concluded that organic carbon contents improved the binding processes and water retention ability of soils, as well as a good dependable source of energy necessary for microbial growth and development.

There were statistically significant differences ($P < 0.05$) between the treatment groups on the same column as found in the pre exposed soil, 4 weeks after pollution and amendment and after 10 weeks indicating significant effect of amendments. Nitrogen (N) reported in this study was found to be within the range reported by other research carried out within the same area (Ogeh and Osiomwan, 2012). The increase of total nitrogen may be due to the fixation of atmospheric nitrogen by the microorganisms which assimilate the hydrocarbons (Schwendinger, 1963).

Phosphorus (P) concentration reported in this study is in agreement with report of other research carried out within the same area (Ogeh and Osiomwan, 2012). There were no statistically significant differences ($P > 0.05$) in P between the soil collected 4 weeks after pollution and amendment and soil samples collected after 10 weeks but there were statistically significant differences ($P < 0.05$) in P of pre exposed soil samples. The available phosphorus obtained in soils from this experiment could be regarded as agricultural limitations since the values were below 20 mg/kg which is the maximum tolerable limit of P for soils as stipulated by (Holland *et al.*, 1989).

The high bacterial and fungal counts in the treatment groups and the control observed in this experiment could be attributed to the presence of diverse species of microorganisms. The demand for crude oil caught up best with the supply of it for the oil degraders in the soil. This is in agreement with the statement made by American society for microbiology (ASM, 2013) that when there is a pollution of crude oil, the bacteria capable of degrading hydrocarbons proliferate quickly. The bacteria species isolated in the course of this experiment are in agreement with the works of (Okpokwasili and James, 1995). A wide range of microorganisms can utilize hydrocarbon from petroleum source as their sole carbon and energy source. *Pseudomonas* and *Bacillus* species are effectively use to remediate crude oil polluted soils.

V. CONCLUSION

The study shows that crude oil pollution encouraged rapid development of some hydrocarbon degrading microbes which utilize the crude oil as source of food thereby breaking down the hydrocarbon chain. Such microbes are useful in the process of bioremediation of crude oil polluted sites. Some microbes are however affected by the crude oil pollution thereby inhibiting their growth. The process involving the use of organic manure has been considered for the potential for biodegradation and biotransformation of petroleum products, which indicates that bioremediation methods are more efficient and cheaper than chemical processes. Thus, bioremediation technique

employed for this remediation of crude oil soil by promoting soil microbe's ability to bio transform petroleum hydrocarbons into less toxic compounds. Although, this laboratory scale research study can also be applied on a large scale study because the manure used are environmentally friendly and have been observed to promote the bioremediation of hydrocarbons.

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