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Biostimulation Potentials of Cow Dung on a Crude Oil Polluted Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Author Okunwaye Iris designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Ogboghodo Ikponmwosa and ES managed the analyses of the study. Author OO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This field study was conducted to investigate the biostimulation effect of the application of cow dung to crude oil polluted soils. Four rates of crude oil (0, 100, 200 and 300 mL) and four rates of cow dung (0, 20, 40 and 60 g) were used respectively. It comprised of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) plots with each plot measuring 1m x 1m. The experiment was laid out in a randomized complete block design. 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 physicochemical properties of soil indicate an increase 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.

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There was an increase in the bacterial count for both the control and the treatment groups. The result range from 1.3 $X10^4$ to 77.2 $X10^4$. 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 coccus in form, while the fungal isolates are *Cladosporium, Pichia, Aspergillus, Fusarium* species. Soil analysis during the experiment revealed a general negative correlation coefficient implying enhanced remediation during the trial periods.

Keywords: Biostimulation; cow dung; crude oil; soil properties; total petroleum hydrocarbon.

1. INTRODUCTION

Crude oil is a naturally occurring liquid found in the earth and it is a complex mixture of hydrocarbons and hydrocarbon-like chemicals [1]. Crude oil also contains some inorganic elements like sulphur, nitrogen, phosphorus; trace elements such as vanadium, nickel, iron, aluminum, copper, and some heavy metals like lead and cadmium [2].

Nigeria is an oil producing and exporting nation producing medium and light crude oil, such as Bonny Light [3]. Since commercial exploration of oil started in Nigeria in 1958 [4], it has become the mainstay of the Nigerian economy, as the annual budgets are based on oil revenue. However, the exploration of petroleum has led to the pollution of both terrestrial and aquatic environments in Nigeria. The agricultural lands have become less productive while the creeks and the fishing waters have become more or less dead [5]. Spill incidences are now common either by accident or deliberate actions via pipeline vandalism. Between 1958 and 2012, several spill incidences were recorded and large quantities of crude oil were discharged into the environment in each case there by polluting both terrestrial and aquatic ecosystem. Several civil unrests due to environmental degradation caused by oil pollution have also been witnessed in the Niger Delta region of Nigeria. Crude oil can enter into the environment through leakage from storage containers, refueling of vehicles, wrecks of oil tankers and through improper disposal by mechanics when cleaning Crude oil tankers. In onshore areas, most pipelines and flow lines are laid above ground passing through farmlands. Plants can take in some of the spilled oil either through foliar penetration or absorption by roots and can cause injury as well as alterations in both physiological and biochemical processes in the plant.

Changes in soil properties due to contamination with petroleum-derived substances can lead to water and oxygen deficits as well as a shortage of available forms of nitrogen and phosphorus [6]. Crude oil is a major source of pollution to the environment [7].

Crude oil spills on agricultural land reduce plant growth and soil microflora population [7].

Soil contamination with crude oil causes organic pollution of groundwater which limits its use, as well as economic loss, environmental problems, and decreases in the agricultural productivity of the soil. Hydrocarbon contamination of soil and freshwater especially polyaromatic hydrocarbon (PAHs) attract public attention because PAHs are toxic, mutagenic and carcinogenic. Since it is widely recognized that contaminated land poses threat to human health, there is need to remediate many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

In recent years Bioremediation is an option that offers the possibilities to destroy or render harmless various contaminants using natural biological activity. Bioremediation involves three principal approaches namely, natural attenuation (reliance on natural biodegradation activities and rates), which is sometimes called intrinsic bioremediation; biostimulation (stimulation of natural activities by environmental modifications such as fertilizer addition to increase rates of biodegradation); and bioaugmentation (addition of exogenous microorganisms to the hydrocarbon-impacted ecosystem to supplement the existing microbial population). The effect of crude oil pollution on the properties of soil increase carbon and reduces soil nitrates and phosphorus.

Organic manures such as cattle dung as well as plants have over time been used to improve soil fertility. Cow dung is widely available at almost cost free in the environment. The use of cow dung on crude oil contaminated soils will also

protect the soil structure, provide utilizable nutrients [8].

The objectives of this study therefore were to access the effect of biostimulation with cattle's dung on crude oil polluted soils.

2. MATERIALS AND METHODS

The field experiments were conducted at the Faculty of Agriculture Teaching and Research Farm; University of Benin. The experiment comprised of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) plots.

2.1 Treatments and Experimental Design

The experiments were laid out in 4 x 4 factorial arrangement fitted into a Randomized Complete Block Design (RCBD) and replicated three times. Each replication was made up of sixteen beds each carrying a treatment, after the preparation of beds the soil was left for two weeks and treated with four rates (0, 100, 200 and 300 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, 20, 40 and 60 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.

2.2 Sampling

Soil samples were collected from the plots at three different times. The first was before crude oil application to ascertain the physico-chemical nature of the unpolluted soil. The second was 4 weeks after pollution and amendment and third was at the expiration of the experiment at 10 weeks. 50g of soil were collected at 0-15 cm depth using a soil auger.

3. DETERMINATION OF PHYSIOCHEMI-CAL PARAMETERS

Soil Samples were collected, labeled, and then taken to the laboratory for analysis. The pH of the soil samples was determined by meter method using distilled water at a ratio of 1:1 with a glass electrode pH Meter (Hanna, HI 8314 model). Total Organic carbon was determined using titrimetric method by [9]. The total Nitrogen, CEC and available phosphorous in the soil was determined by spectrophotometry method [8]. Soil conductivity was determined using a conductivity meter.

4. DETERMINATION OF TOTAL PETROLEUM HYDROCARBON AND PAH IN CRUDE OIL SAMPLES

4.1 Procedure

The samples were cold-extracted in a conical flask for two hours in each case using 100% dichloromethane according to the method of [10]. 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^{o}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 [11]. The GC-FID system consist of a HP5890 SERIES II, Hewlett-Packard, Waldbrown, Germany GC equipped with flame ionization detector and ATLAS soft ware data processor (USA). The gas chromatographic column used was Ultra-1932530, a non- polar, fused-silica capillary column (30m × 250 µm 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 75 kpa. The injector temperature was set at 250°C, and detector temperature at 310^{o}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.

4.2 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 labeled and stored as stock cultures. The bacterial isolates were identified based on their morphology, Gram reaction and biochemical characterization.

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
1,2,5,6Dibenzanthracene	0.002
Fluoranthene	0.450
Fluorene	0.284
Indeno(1,2,3)pyrene	0.002
Naphthalene	0.163
Phenanthrene	0.143
Pyrene	0.621
Benzo(k)fluorathene	BDL

5. RESULTS

5.1 Effects of Remediation Amendments on Soil Physico-Chemical Properties

5.1.1 Total petroleum hydrocarbon content (TPH)

Total Petroleum Hydrocarbon inall samples pre-exposed to crude oil were below detecting limits (BDL).

5.1.2 pH

The mean pH ranged from (4.26 – 4.73) pre exposed soil, (4.56 -5.93) 4 weeks after pollution and amendment and (4.63- 4.93) after 10 weeks. The highest mean pH was (5.93±0.02) 4 weeks after pollution and amendment in treatment (200ml of crude oil, 60g of cow dung application). Similarly, the lowest mean pH recorded (4.26±0.03) from pre-exposed soil in treatment (0ml of crude oil, 60g of cow dung application).

Statistical analysis of pH indicate there were no statistically significant differences ($P>0.05$) in pH between the pre exposed soil and soil samples collected after 10 weeks but there were statistically significant differences ($P<0.05$) in pH of soil collected and analyzed 4 weeks after pollution and amendment (Table 2).

5.2 Total Organic Carbon (%)

Total organic carbon (TOC) range from (0.83-0.86) pre exposed soil, (0.89-2.37) 4 weeks after pollution and amendment and (1.12-2.30) after 10 weeks. The mean of the control group for pre exposed soil, 4 weeks after pollution and amendment and after 10 weeks was (0.85±0.02, 0.89±0.07 and 1.12±0.09) respectively and the highest mean for the treatment group was (2.37±0.04) recorded at treatment (300ml 0f crude oil, NA of cow dung application), While the lowest mean value was (0.83) in majority of the treatments (Table 2).

Statistically there were significant differences ($P<0.05$) in total organic carbon between the pre exposed soil sample and soil 4 weeks after pollution and amendment and after 10 weeks.

5.3 Phosphorus (mg/kg)

The result for phosphorus had a mean value range of (18.3-18.8) pre-exposed soil, (16.4-17.6) 4 weeks after pollution and amendment and (13.3-13.6) after 10 weeks. The mean of the control group of pre-exposed, 4 weeks after pollution and amendment and after 10 weeks was (18.3±0.03, 16.6±0.45 and 13.6±0.14) respectively.

Statistical analysis indicate 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 (Table 3).

5.4 Total Nitrogen (%)

Total nitrogen (TN) content has a higher value of (1.45±0.03) 4 weeks after pollution and amendment is done in Treatment (300 ml of crude oil, 60 g 0f cow dung application) compared with those of the other treatments and a lower value of (0.33±0.02). TN contents fluctuated significantly in pre-exposed samples.

Statistical analysis indicate 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.

5.5 Electrical Conductivity

Conductivity was low in Treatment (100 ml of crude oil, 20 g of cow dung) after 10 weeks, but high in treatment (0 ml of crude oil, 60 g of cow dung) 4 weeks after pollution and amendment.

Statistical analysis for electrical conductivity indicate there were statistically significant differences ($P<0.05$') in electrical conductivity between the pre exposed soil, soil 4 weeks after pollution and amendment and after 10 weeks (Table 4).

5.6 Cation Exchange Capacity

The cation exchange Capacity (CEC) ranged from (1.32-1.39) pre-exposed soil, (1.33-1.49) 4 weeks after pollution and amendment and (1.28-1.35) after 10 weeks. The mean of the control group for pre-exposed soil, 4 weeks after pollution and amendment and after 10 weeks are (1.35±0.01, 1.35±0.02 and 1.32±0.03) respectively and the highest mean for the treatment group is (1.49±0.01) recorded at treatment (300ml 0f crude oil, 20 g of cow dung application), While the lowest mean value was (1.28±0.01) recorded at treatment (300 ml of crude oil, NA of cow dung application).

Statistical analysis indicate 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.

5.7 Carbon/Nitrogen Ratio

C:N ratio content has a higher value of (26.67±2) pre-exposed samples at treatment (300 ml of crude oil, 60g 0f cow dung application) as compared with those of the other treatments and a lower value of (11.33±1.53) at treatment (100ml of crude oil, NA of cow dung application).

Statistical analysis for C:N ratio indicate no statistically significant difference ($P>0.05$) in C:N ratio between the pre exposed soil and soil samples collected after 10 weeks but there were statistically significant differences ($P<0.05$) in C:N ratio of soil collected and analyzed 4 weeks after pollution and amendment.

5.8 Effect on Bacteria and Fungi Population

Results from the field experiment revealed an increase in bacterial count of both the control and the treatment groups. The bacterial count for soil samples collected after 10 weeks were higher than that of 4 weeks after pollution and amendment while that of the pre exposed soil had the lowest bacterial count for both control and the treatment groups. The bacterial count range from ($1.3 X10^4$ to $1.4 X10^4$) pre exposed soil, ($1.3 X10^4$ to$18.5 X10^6$) 4 weeks after pollution and amendment ($1.6 X10^4$ to $77.2 X10^4$) and 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 ($1.3 X10^4$, $1.3 X10^{4,}$ &$1.6 X10^4$) respectively and the highest bacteria count for the treatment group is ($77.2 X10^4$) recorded at treatment (300 ml 0f crude oil, 60g of cow dung application), While the lowest mean value was ($1.3 X10^4$).

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 dung4 weeks after pollution and amendment while *Pseudomonas, Bacillus, Clostridium and Nocardia* species were found 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 dung4 weeks after pollution and amendment while *Aspergillus* and *Cladosporium* were found.

Table 2. Effect of the remediation amendments on soil pH & 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)	4.65^{aA}	4.56^{cA}	4.63^{aA}	0(NA)	0.85^{aB}	0.89^{cB}	1.12^{bA}
0(20)	4.44^{aB}	5.32^{cA}	4.84^{aAB}	0(20)	0.83^{aB}	1.18^{cA}	1.15^{bA}
0(40)	4.56^{aC}	5.52^{cA}	4.85^{aB}	0(40)	0.83^{aC}	1.14^{cB}	1.19^{bA}
0(60)	4.26^{aC}	5.25^{cA}	4.82^{aB}	0(60)	0.84^{aC}	1.14^{cB}	1.24^{bA}
100(NA)	4.49^{aB}	5.04^{bA}	4.91^{aA}	100(NA)	0.85^{aC}	1.61^{bA}	1.20^{abB}
100(20)	4.51^{aB}	5.77^{bA}	4.79^{aB}	100(20)	0.83^{aC}	1.57^{bA}	1.15^{abB}
100(40)	4.57^{aB}	5.58^{bA}	4.82^{aA}	100(40)	0.85^{aB}	1.67^{bA}	1.21^{abAB}
100(60)	4.43^{aC}	5.79^{bA}	4.81^{aB}	100(60)	0.86^{aC}	1.95^{bA}	1.22^{abB}
200(NA)	4.51^{aB}	5.62^{aA}	4.80^{aAB}	200(NA)	0.83^{aC}	2.11^{aA}	1.14^{abB}
200(20)	4.48^{aB}	5.82^{aA}	4.88^{aB}	200(20)	0.85^{aC}	2.20^{aA}	1.18^{abB}
200(40)	4.33^{aC}	5.89^{aA}	4.93^{aB}	200(40)	0.85^{aC}	2.35^{aA}	1.24^{abB}
200(60)	4.49^{aC}	5.93^{aA}	4.91^{aB}	200(60)	0.83^{aC}	2.35^{aA}	1.23^{abB}
300(NA)	4.54^{aC}	5.03^{cA}	4.81^{aB}	300(NA)	0.84^{aC}	2.37^{aA}	1.67^{aB}
300(20)	4.55^{aC}	5.05^{cA}	4.88^{aB}	300(20)	0.84^{aC}	2.35^{aA}	1.20^{aB}
300(40)	4.42^{aB}	5.03^{cA}	4.87^{aA}	300(40)	0.85^{aC}	2.35^{aA}	1.24^{aB}
300(60)	4.73^{aB}	5.13^{cA}	4.85^{aAB}	300(60)	0.86^{aC}	2.32^{aA}	2.30^{aB}

$^{a-c}$ 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)**	**Treatments**	**N (Pre-exposed Soil)**	**N (4 weeks AP/A)**	**N (After 10 Weeks)**
0(NA)	18.3^{bA}	16.6^{aB}	13.6^{aC}	0(NA)	0.57^{aA}	0.45^{cB}	0.57^{aA}
0(20)	18.5^{bA}	16.4^{aB}	13.3^{aC}	0(20)	0.53^{aB}	0.88^{cA}	0.55^{aB}
0(40)	18.4^{bA}	16.9^{aB}	13.6^{aC}	0(40)	0.56^{aB}	0.93^{cA}	0.60^{aB}
0(60)	18.4^{bA}	17.4^{aB}	13.4^{aC}	0(60)	0.53^{aB}	0.95^{cA}	0.64^{aAB}
100(NA)	18.6^{bA}	16.9^{aA}	13.5^{aB}	100(NA)	0.56^{aA}	0.51^{cA}	0.51^{aA}
100(20)	18.5^{bA}	16.9^{aB}	13.3^{aC}	100(20)	0.56^{aB}	0.85^{cA}	0.34^{aB}
100(40)	18.5^{bA}	17.2^{aA}	13.5^{aB}	100(40)	0.54^{aB}	0.88^{cA}	0.62^{aB}
100(60)	18.5^{bA}	17.5^{aB}	13.4^{aC}	100(60)	0.56^{aB}	0.95^{cA}	0.70^{aB}

	P				N		
Treatments	**P (Pre-exposed Soil)**	**P (4 weeks AP/A)**	**P (After 10 Weeks)**	**Treatments**	**N (Pre-exposed Soil)**	**N (4 weeks AP/A)**	**N (After 10 Weeks)**
200(NA)	18.5^{aA}	17.5^{aB}	13.3^{aC}	200(NA)	0.54^{aA}	0.55^{bA}	0.58^{aA}
200(20)	18.5^{aA}	17.3^{aB}	13.5^{aC}	200(20)	0.54^{aB}	1.07^{bA}	0.47^{aB}
200(40)	18.7^{aA}	17.2^{aB}	13.6^{aC}	200(40)	0.54^{aC}	1.23^{bA}	0.71^{aB}
200(60)	18.8^{aA}	16.9^{aB}	13.3^{aC}	200(60)	0.56^{aB}	1.25^{bA}	0.55^{aB}
300(NA)	18.8^{aA}	17.0^{aB}	13.4^{aC}	300(NA)	0.49^{aA}	0.53^{aA}	0.54^{aA}
300(20)	18.8^{aA}	17.1^{aB}	13.5^{aC}	300(20)	0.33^{aB}	1.45^{aA}	0.59^{aB}
300(40)	18.5^{aA}	17.5^{aB}	13.6^{aC}	300(40)	0.55^{aB}	1.33^{aA}	0.67^{aB}
300(60)	18.6^{aA}	17.6^{aB}	13.4^{aC}	300(60)	0.56^{aB}	1.45^{aA}	0.66^{aB}

$^{a-c}$ 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 4. Effect of the remediation amendments on soil electrical conductivity and cation exchange capacity

	Conductivity				Cation Exchange Capacity		
Treatments	**Cond (pre-exposed soil)**	**Cond (4 weeks ap/a)**	**Cond (after 10 weeks)**	**Treatments**	**CEC (pre-exposed soil)**	**CEC (4 weeks ap/a)**	**CEC (after 10 weeks)**
0(NA)	222.15^{aA}	222.83^{aA}	214.70^{bB}	0(NA)	1.35^{aA}	1.35^{aA}	1.32^{aA}
0(20)	222.14^{aAB}	226.87^{aA}	217.59^{bB}	0(20)	1.33^{aAB}	1.34^{aA}	1.29^{aB}
0(40)	222.49^{aB}	228.88^{aA}	216.54^{bC}	0(40)	1.33^{aAB}	1.35^{aA}	1.31^{aB}
0(60)	223.47^{aB}	255.74^{aA}	216.33^{bB}	0(60)	1.34^{aA}	1.33^{aA}	1.31^{aA}
100(NA)	222.61^{aA}	173.02^{cB}	217.44^{bA}	100(NA)	1.35^{aA}	1.35^{aA}	1.32^{aA}
100(20)	222.38^{aB}	232.70^{cA}	214.34^{bC}	100(20)	1.35^{aB}	1.42^{aA}	1.30^{aB}
100(40)	222.62^{aB}	236.36^{cA}	215.05^{bC}	100(40)	1.34^{aB}	1.4^{aA}	1.31^{aB}
100(60)	222.14^{aB}	247.83^{cA}	216.67^{bB}	100(60)	1.33^{aAB}	1.39^{aA}	1.31^{aB}
200(NA)	222.16^{aA}	184.89^{bC}	217.30^{bB}	200(NA)	1.36^{aB}	1.46^{aA}	1.30^{aB}
200(20)	222.48^{aB}	235.99^{bA}	215.73^{bC}	200(20)	1.33^{aB}	1.43^{aA}	1.32^{aB}
200(40)	222.36^{aB}	235.23^{bA}	216.87^{bC}	200(40)	1.33^{aB}	1.44^{aA}	1.31^{aB}
200(60)	222.55^{aB}	253.91^{bA}	214.93^{bC}	200(60)	1.32^{aB}	1.46^{aA}	1.31^{aB}
300(NA)	255.70^{aA}	186.87^{bB}	220.55^{aA}	300(NA)	1.39^{aB}	1.47^{aA}	1.28^{aC}
300(20)	222.14^{aB}	238.39^{bA}	222.16^{aB}	300(20)	1.33^{aB}	1.49^{aA}	1.31^{aB}
300(40)	222.16^{aC}	237.37^{bA}	227.15^{aB}	300(40)	1.35^{aB}	1.48^{aA}	1.31^{aC}
300(60)	226.13^{aB}	254.51^{bA}	222.79^{aB}	300(60)	1.34^{aB}	1.48^{aA}	1.35^{aB}

^{a} Different letters in the same column indicate significant difference (P<0.05)
$^{a-c}$ 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						
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)	21.0^{abA}	13.7^{aB}	22.0^{aA}	0(NA)	$1.3 X10^4$	$1.3 X10^4$	$1.6 X10^4$
0(20)	21.0^{abAB}	17.7^{aB}	24.7^{aA}	0(20)	$1.3 X10^4$	$5.4 X10^4$	$1.7 X10^4$
0(40)	21.3^{abA}	20.0^{aA}	22.7^{aA}	0(40)	$1.3 X10^4$	$8.6 X10^4$	$1.7 X10^4$
0(60)	23.0^{abA}	21.3^{aA}	25.7^{aA}	0(60)	$1.3 X10^4$	$8.6 X10^4$	$1.7 X10^4$
100(NA)	12.0^{bB}	11.3^{bB}	23.0^{aA}	100(NA)	$1.3 X10^4$	$10.5 X10^5$	$19.8 X10^4$
100(20)	22.3^{bA}	13.7^{bB}	25.7^{aA}	100(20)	$1.4 X10^4$	$18.3 X10^6$	$47.2 X10^4$
100(40)	23.7^{bAB}	17.3^{bB}	26.3^{aA}	100(40)	$1.3 X10^4$	$18.2 X10^6$	$45.1 X10^4$
100(60)	23.0^{bA}	14.3^{bB}	26.0^{aA}	100(60)	$1.4 X10^4$	$18.5 X10^6$	$46.0 X10^4$
200(NA)	22.7^{abA}	15.7^{bB}	24.7^{aA}	200(NA)	$1.3 X10^4$	$13.3 X10^5$	$21.2 X10^4$
200(20)	22.3^{abA}	14.3^{bB}	23.0^{aA}	200(20)	$1.4 X10^4$	$11.2 X10^7$	$58.7 X10^4$
200(40)	24.7^{abA}	14.7^{bB}	24.3^{aA}	200(40)	$1.4 X10^4$	$18.0 X10^6$	$60.2 X10^4$
200(60)	22.0^{abA}	12.0^{bB}	25.0^{aA}	200(60)	$1.3 X10^4$	$9.5 X10^7$	$61.6 X10^4$
300(NA)	25.3^{aA}	15.0^{bB}	25.3^{aA}	300(NA)	$1.4 X10^4$	$10.5 X10^6$	$24.5 X10^4$
300(20)	20.7^{aAB}	13.3^{bB}	26.0^{aA}	300(20)	$1.4 X10^4$	$15.5 X10^6$	$74.4 X10^4$
300(40)	25.3^{aA}	14.7^{bB}	25.7^{aA}	300(40)	$1.4 X10^4$	$8.6 X10^7$	$76.3 X10^4$
300(60)	26.7^{aA}	14.0^{bB}	25.0^{aA}	300(60)	$1.3 X10^4$	$6.3 X10^7$	$77.2 X10^4$

$^{a-b}$ 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	20	BDL	BDL	BDL
0	40	BDL	BDL	BDL
0	60	BDL	BDL	BDL
100	NA	BDL	163±2.82	112.31±1.53
100	20	BDL	124.07±3.14	30.31±1.24
100	40	BDL	116.46±3.16	33.61±0.92
100	60	BDL	104.83±2.48	37.83±1.27
200	NA	BDL	245.15±3.31	185.43±2.30
200	20	BDL	192.23±7.83	96.97±3.13
200	40	BDL	179.41±4.49	103.89±2.08
200	60	BDL	181.21±14.4	107.95±15.25
300	NA	BDL	389.9±2.53	200.41±1.93
300	20	BDL	322.9±4.18	117.59±2.58
300	40	BDL	312.46±6.93	124.28±2.53
300	60	BDL	305.72±3.28	136.93±3.02

Key: 4 weeks AP/A= 4 weeks after pollution & amendment; NA = No Amendment; 20= 20g of cattle dung; 40=40g of cattle dung; 60=60g of cattle dung; 0= No crude oil; 100= 100ml crude oil in a 1sqm or 1x1m; 200= 200ml crude oil in a 1sqm or 1x1m; 300=300ml crude oil in a 1sqm or 1x1m

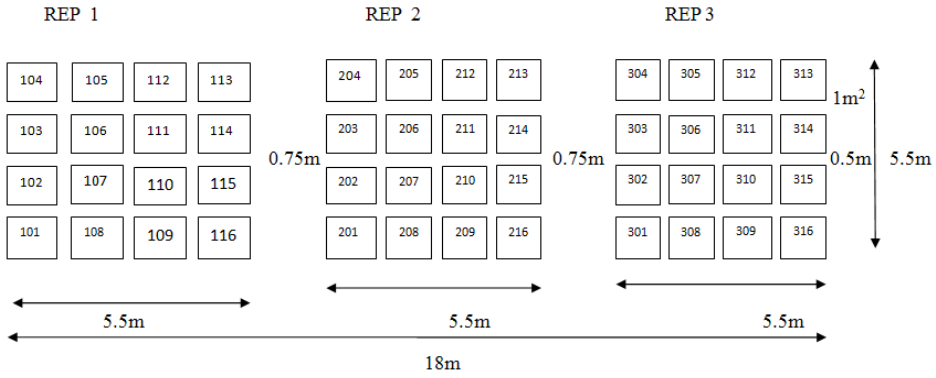

Fig. 1. Field experimental layout in a randomized complete block design (RCBD)

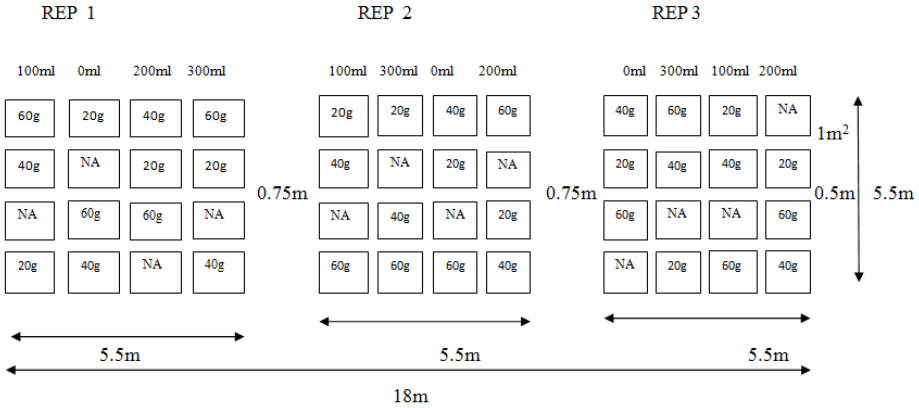

Fig. 2. Field experimental layout in a randomized complete block design (RCBD) Showing the different concentration of crude oil and cow dung

6. DISCUSSION

In this study, pH increased significantly (P< 0.05) after crude oil exposure though there was a decrease in pH after 10 weeks. The pH range observed in this study implies that crude oil pollution makes the soil to be acidic thereby increasing the toxicity of the soil. Soil pH is an important factor that controls various physicochemical reactions. It regulates the solubility, mobility, and the availability of the ionized forms of contaminants. The growth and activity of soil microorganisms are very much dependent on the soil pH. An increase in soil toxicity results in less availability of materials like nutrients [12].

There was a positive interaction between the pH of the soils and the amount of crude oil added to the soil. This may imply that crude oil pollution leads to increase in soil pH. This is similar to the findings of [13] 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.TPH is one of the constituents of crude oil that is easily biodegradable or utilized by plant during an oil spill [14].

Electrical conductivity (EC) is a measure of ionic concentration in the soils which is related to dissolved solutes. As salt content increases, so does Electrical conductivity. The highly significant conductivity values obtained from conductivity 4 weeks after pollution and amendment and conductivity of pre exposed soil samples could be as a result of the high concentration of charged ions (cations and anions) in the crude oil polluted soils.

The increase in TOC in this study is in agreement with [8]. Who attributed the increase to the microbial mineralization of the crude oil and the cow dung.The TOC content had a positive relationship with concentrations of crude oil in soil (P> 0.05) as organic carbon concentrations increased with increase in crude oil concentrations. This observation is in agreement with the work of [15] who concluded that organic carbon contents improved the binding processes and water retention ability of soils, as well as serve as good dependable sources of energy necessary for microbial growth and development.

The increase of total nitrogen may be due to the fixation of atmospheric nitrogen by the microorganisms which assimilate the hydrocarbons. 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 after 10 weeks indicating significant effect of amendment with cow dung. Nitrogen tends to increase in soil contaminated with crude oil [16]

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 [17]. It was noted that more than 80% of the available phosphorus becomes immobile and unavailable for plant uptake as a result of adsorption, precipitation and conversion to the organic form.

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 [18]. The amendment used in this study had significant effect (P <0.05) on the soil C:N ratio. This may be attributed to the crude oil and the concentration of cow dung used as amendment [19].

7. CONCLUSION

Bioremediation has been recognized as a suitable tool to restore contaminated site. Amendment of soil with cow dung manure have shown the effectiveness of cow dung manure at enhancing the degradation of crude oil in polluted soils and also its beneficial role in creating optimized conditions for plants to grow. Remediation of crude oil polluted soil with cow dung manure has been established to be highly effective towards the improvement of the minerals, nutrients and physico-chemical properties of the amended soil, thereby making bioremediation a success. The bacterial species identified in this study when produced in large quantity, can be used for bioaugmentation in hydrocarbon biodegradation processes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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