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BIOSTIMULATION POTENTIALS OF SPENT MILLED MAIZE AND COWBLOOD ON A CRUDE OIL POLLUTED SOIL

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ABSTRACT

This study was carried out to investigate the biostimulation effect of the application of spent milled maize and cow blood to crude oil polluted soil. The treatment groups were; Control (0 L crude oil (CO) + 0 kg spent milled maize (SMM)), group 1 (1 L CO + 1 kg SMM + 1 L cow blood), group 2 (2 L CO + 2 kg SMM) and group 3 (5 L CO + 5 kg SMM). The experiment was laid out in a completely randomized design A total of four treatment combinations were applied and replicated 3 times giving a total of 48 plots.The physicochemical properties and bacterial load of the soil were determined before pollution, two weeks after pollution, four weeks and eight weeks after remediation. The results for physicochemical properties of soil indicates a decrease in total organic carbon and nitrogen while there was an increase in the levels of cation exchange capacity, phosphorus and electrical conductivity after crude oil pollution. The mean levels of total petroleum hydrocarbon and polycyclic aromatic hydrocarbon reduced after the pollution. The application of the spent milled maize and cow bloodwere observed to improve the physicochemical properties of soil. There was also an increased bacterial count for the treated groups compared to the control; the values ranged from 1.3 x 10^3cfu/g to 1.24 x 10^8cfu/g. The identified bacteria wereFlavobacterium, Pseudomonas, Bacillus, Micrococcus, Proteus, Clostridium and Nocardia species.Generally, this study has revealed that spent milled maize and cow blood are effective in the restoration of crude oil polluted soil.

Keywords: Biostimulation, cow blood, crude oil, spent milled maize

INTRODUCTION

Crude oil pollution is considered to be a worldwide threat to the environment.Pollution of the environment by crude oil occurs when crude oil or its derivatives are released into the environment at levels that is harmful to the entire ecosystem (Odeyemi, 2014). Oil exploration and exploitation gave birth to crude oil pollution as a natural consequence and this occurs when oil is accidentally discharged due to human error, sabotage and in the course of transportation (Oriapkono et al., 2018). The level of crude oil spill in Nigeria, specifically the Niger Delta Region is significant and this emanate from different sources such as leakage of pipeline, sabotage, transportation and others (Nwilo, 1998). Crude oil pollution has been reported by many authors to exert negative effects on the soil by increasing carbon and reducing soil nitrates and phosphorus, it has also been reported to increase soil total organic carbon, total petroleum hydrocarbons and poly aromatic

hydrocarbons which all have adverse effect on plants and soil organisms (Oriapkono et al., 2018). It is also known to lead to organic pollution of groundwater which negatively affects its use and also causes a reduction in agricultural productivity of the soil (Obiakalaije et al., 2015). The contamination of agricultural soils specifically with polyaromatic hydrocarbon and total petroleum hydrocarbon are of great concern because these substances are toxic, mutagenic and carcinogenic. Since it is generally known that contaminated soil is detrimental to human health, there is need to clean up these sites in response to the risk of adverse health or environmental effects caused by crude oil contamination. Among different methods of clean up and decontamination, bioremediation is an option that offers the possibilities of using natural biological activity to destroy and render the different contaminants harmless (Vidali, 2001). Bioremediation involves three principal approaches; natural attenuation, biostimulation and bioaugumentation. Amidst the three approaches, the one of interest in this study is biostimulation. Biostimulation is a method of biodegradation that is aimed at enhancing the process via addition of materials that supplies the limiting nutrients such as nitrogen and phosphorus. (Ijah and Antai, 2003; Chikere et al., 2012). Poultry droppings and cow dung are among the most common materials used in the process of bioremediation (Obiakalaije et al., 2015; Oriapkono et al., 2018). This study was therefore aimed at evaluating the effect of biostimulation with spent milled maize and cow blood on crude oil polluted soils.

MATERIALS AND METHODS

Study Area: This study was undertaken at the research farm of the University of Port Harcourt, Rivers State, Nigeria.

Experimental Design: The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times. Each replication was made up of four beds each carrying a treatment. Each bed measured 1.0m x 1.0m. A total land size of 24.75m^2 (5.5m x 4.5m) was marked out for the study. Alleys of 0.5m were left between plots, and 0.75m between replicate to prevent treatment drift to adjacent plots. After the preparation of beds, the soils were left for two weeks and treated with four rates (0, 1, 2 and 5 L) of crude oil (Bonny light blend).

Soil Amendment Agents: The soil amendment agents used were spent milled maize and cow blood. The spent milled maize was collected from Rumuokoro market while the cow blood was collected from cattle slaughterhouse at Choba; both in ObioAkpor Local Government Area of Rivers State. The spent milled maize was air-dried for 21 days and then grounded before its application.

Experimental Groups: The experimental groups were designated as follows;

- Control: Unpolluted soil + No Amendment Agent
- Group 1: Polluted soil + 1 kg Spent milled maize + 1 L Cow blood
- Group 2: Polluted sol + 2 kg Spent milled maize
- Group 3: Polluted sol + 5 kg Spent milled maize

Treatment: 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 pollution, three rates; Group 1 (1 kg spent milled maize and 1 litre of blood), Group 2 (2 kg spent milled maize) and Group 3 (5 kg spent milled maize) were applied to polluted soils. The spent milled maize and blood were thoroughly mixed with the soil using hand trowel to ensure uniform distribution within the soil. Each quantity of soil spiked with crude oil served as treatment while the unpolluted soil without amendment agent served as the control. Treated soils were left for about two months for revegetation to occur before final samples were collected.

Sampling: Soil samples were collected from the plots at four different times. First was before crude oil application to ascertain the physicochemical nature of the unpolluted soil. Second was two weeks after pollution, third was one month (4 weeks) after remediation

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and lastly was two months (8 weeks) after remediation.

Physicochemical Analysis: Samples were collected, properly labeled, and then taken to the laboratory for analysis. In the laboratory, soil samples were air dried, passed through a 2mm plastic sieve and analyzed for the following parameters. The pH of the soil samples was determined in distilled water at a ratio of 1:1 using a glass electrode pH Meter. Organic carbon was determined using wet oxidation method by Walkey and Black (1934). The total nitrogen of the soil was extracted by Kjeldahl′s method. The available phosphorous in the soil was extracted from the soil using the Bray and Kurtz (1945) solution. Phosphorus was determined using calorimetric method.

TPH was analyzed with the GC-FID (Gas Chromatography - Flame Ionization Detector) while the PAH was analyzed with the GC-MS (Gas Chromatography - Mass Spectrometry) Clarus -500 Perkin Elmer according to the method of Ashraf (2014). The GC-FID system consists of a HP5890 SERIES II, Hewlett Packard, Waldbrown, 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 (30 m × 250 µm inner diameter × 0.20 µm film thickness) (USA). Helium gas was used as the carrier gas at a low flow rate of 1 ml/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.

The determination of exchangeable cations (Ca, Mg, K and Na) was by atomic absorption spectrophotometry. Thirty millilitres (30 ml) of 1 N NH_4OAC (i.e. ammonium acetate) solution was added to 5 g of oven dried soil sample and shaken for 15 minutes. CEC was obtained by summing the values of sample exchangeable acidity and exchangeable bases. Soil conductivity was determined using conductivity meter method (HACH, Ectestr microprocessor series model).

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. The bacterial isolates were characterized using the schemes of Treagon and Pullcan (1982) and Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994).

Statistical Analysis

The results were presented as mean ± standard deviation and subjected to analysis by using one-way analysis of variance (ANOVA). A post hoc test was used to determine the significant difference among means of different groups. The SPSS (Statistical Package for Social Sciences) software (version 20) was used for the analysis of data and the level of significance was set at $P \leq 0.05$.

Table 1. Chemical Composition of Ground Spent Milled Maize

S/NO	PARAMETERS	VALUE
1	Organic carbon (%)	30.2
2	Total nitrogen (%)	2.11
3	Sodium (ppm)	0.20
4	Potassium (ppm)	0.52
5	Calcium (ppm)	2.40
6	Magnesium (ppm)	0.63
7	Available phosphorus (ppm)	10.5
8	pH	6.40
9	Hydrogen ion (H^+)	0.10
10	Microbial count (cfu/g)	$12.50*10^1$

Table 2. Concentration of TPH in Crude Oil

TPHs (mg/kg)	Nigerian crude oil
C_{10}	0.2002
C_{11}	0.0432
C_{12}	0.0421
C_{13}	0.0523
C_{14}	0.4934
C_{15}	0.0060
C_{16}	BDL
C_{17}	0.1480
Pritane	0.1218
C_{18}	0.3200
Phytane	BDL
C_{19}	1.7480
C_{20}	1.6860
C_{21}	1.7100
C_{22}	1.5910
C_{23}	1.3930
C_{24}	1.1830
C_{25}	0.0330
C_{26}	0.8110
C_{27}	0.7070
C_{28}	1.120
C_{29}	0.7450
C_{30}	0.2260
C_{31}	0.7570
C_{32}	0.4300

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Table 3. Concentration of PAH in Crude Oil

PAH (ml/l)	Nigerian crude oils
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	0.45
Fluorene	0.284
Indeno (1,2,3) pyrene	0.002
Naphthalene	0.163
Phenanthrene	0.143
Pyrene	0.621
Benzo(k)fluorathene	BDL

RESULTS

Effects of Remediation Amendments on Soil pH

The mean value for pH ranged from 8.30 ± 0.19 to 8.66 ± 0.15, 6.71 ± 0.08 to 8.30 ± 0.17, 4.95 ± 0.84to 8.31 ± 0.26 and 6.86 ± 0.35to 8.31 ± 0.26 for pre-exposed soil, 2 weeks after pollution, 4 weeks after remediation and 8 weeks after remediation respectively (Table 4).

Total Organic Carbon (%)

The highest and lowest mean value for total organic carbon were 2.51 %and 2.31 %, 6.20 % and 2.44 %, 6.19 % and 2.38 %, 6.19 % and 2.83 % for the control, groups 1, 2 and 3 respectively (Table 5).

Total Nitrogen (%)

The data obtained for total nitrogen (TN) content are presented in Table 6. The highest mean TN content obtained for the treatment group was 8.41 % in soil sample collected from treatment Group 3 (2 weeks after pollution), while the lowest mean TN content was 0.58 % obtained in soil from treatment group 1 (pre-exposed soil).

Carbon/Nitrogen Ratio

The carbon to nitrogen ratio was 6:01 in group 1 (2 weeks after pollution) and group 3 (pre-exposed soil) whereas it remained at 5:01 in all other groups (Table 7).

Phosphorus (mg/kg)

The mean values for phosphorus in the treated groups ranged from 3.82 mg/kg in group 2 (4 weeks after remediation) to 9.34 mg/kg in group 1 (8 weeks after remediation) (Table 8). The values for the control were 19.05 mg/kg (pre-exposed soil), 19.05 mg/kg (2 weeks after pollution), 19.39 mg/kg (4 weeks after remediation) and 16.09 mg/kg (8 weeks after remediation).

Cation Exchange Capacity (meq/100g)

The mean value for cation exchange capacity ranged from 4.41 meq/100g to 4.48 meq/100g (pre-exposed soil), 0.84 meq/100g to 4.42 meq/100g (2 weeks after pollution), 2.31 meq/100g to 4.24 meq/100g (4 weeks after remediation) and 3.34 meq/100g to 4.44 meq/100g (8 weeks after remediation). The highest value in the treated group was 3.79 meq/100g in group 3 (8 weeks after

remediation) while the lowest value was 0.84 meq/100g in group 2 and 3 (2 weeks after pollution).

Total Petroleum Hydrocarbon (TPH)

The Total Petroleum Hydrocarbon (TPH) was below detectable limits (BDL) in the pre-exposed soil for all the treatment and in the control. The levels of TPH ranged from 327.86mg/kg to 1780.68mg/kg, 442.92mg/kg to 2464.55mg/kg and 507.54mg/kg to 2730.59mg/kg in group 1, 2 and 3 respectively.

Poly Aromatic Hydrocarbon (PAH)

The Poly Aromatic Hydrocarbon (PAH) was below detectable limits (BDL) in the pre-exposed soil for all the treatment and in the control group. The levels of PAH were 1001.63mg/kg (2 weeks after pollution), 428.51mg/kg (4 weeks after pollution) and 291.46mg/kg (8 weeks after pollution) for group 1; 1371.40 mg/kg (2 weeks after pollution), 588.08mg/kg (4 weeks after pollution) and 368.44mg/kg (8 weeks after pollution) for group 2; and 1687.50mg/kg (2 weeks after pollution), 671.68mg/kg (4 weeks after pollution) and 504.43mg/kg (8 weeks after pollution) for group 3.

Effect on Bacteria Population

The result for bacteria count range from (1.37 $x10^3$cfu/g to 1.4 x 10^3cfu/g) pre-exposed soil, (1.4 x 10^4cfu/g to 1.24 x 10^8cfu/g) 2 weeks after pollution, (1.6 x 10^3cfu/g to 7.86 $x10^7$cfu/g) after 4 weeks remediation, and (1.90 x 10^3cfu/g to 8.49 x 10^7cfu/g) after 8 weeks after remediation. The highest bacterial count for the treatment group is (1.24 x 10^8cfu/g) recorded on Group 3 and 2 weeks after pollution, while the lowest mean value was (1.4 $x10^3$cfu/g). The identified bacteria were*Flavobacterium, Pseudomonas, Bacillus, Micrococcus, Proteus, Clostridium and Nocardia* species.

Electrical Conductivity

The mean values of electrical conductivity ranged from 19.26µS/cm to 19.32 µS/cm in the pre-exposed soil, 11.85 µS/cm to 19.26µS/cm in the 2nd week after pollution, 13.67µS/cm to 20.36µS/cm in the 4th week after remediation and 14.97 µS/cm to 15.74 µS/cm in the 8th week after remediation.

Table 4. Effect of the Remediation Amendments on the pH of soil of crude oil polluted soil

	pH (Pre-exposed Soil)	pH (2 weeks after pollution)	pH (4 weeks after remediation)	pH (8 weeks after remediation)
Control	8.30 ± 0.19^{aA}	8.30 ± 0.17^{aA}	8.31 ± 0.26^{aA}	8.31 ± 0.26^{aA}
Group 1	8.63 ± 0.27^{aA}	6.71 ± 0.025^{cB}	5.68 ± 0.081^{dB}	7.86 ± 0.065^{aB}
Group 2	8.66 ± 0.15^{aA}	6.71 ± 0.061^{bB}	4.95 ± 0.84^{bC}	6.86 ± 0.35^{cB}
Group 3	8.55 ± 0.33^{aA}	6.73 ± 0.078^{bB}	5.12 ± 0.45^{bC}	7.23 ± 0.61^{bB}

$^{a-d}$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 5. Effect of the Remediation Amendments on the Total Organic Carbon (TOC) of crude oil polluted soil

	TOC (Pre-exposed Soil) (%)	TOC (2 weeks after pollution) (%)	TOC (4 weeks after remediation) (%)	TOC (8 weeks after remediation) (%)
Control	2.33 ± 0.14^{bA}	2.31 ± 0.13^{bA}	2.51 ± 0.46^{bA}	2.39 ± 0.10^{bA}
Group 1	2.44 ± 0.19^{abD}	6.20 ± 0.02^{Aa}	5.13 ± 0.19^{aB}	3.31 ± 0.37^{aC}
Group 2	2.38 ± 0.16^{bC}	6.19 ± 0.01^{aA}	5.17 ± 0.38^{aB}	3.11 ± 0.44^{abC}
Group 3	2.83 ± 0.12^{aD}	6.19 ± 0.01^{aA}	5.38 ± 0.08^{aB}	3.30 ± 0.02^{aC}

$^{a-d}$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)

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Table 6. Effect of the Remediation Amendments on the Soil Nitrogen of crude oil polluted soil

	N (Pre-exposed Soil) (%)	N (2 weeks after pollution) (%)	N (4 weeks after remediation) (%)	N (8 weeks after remediation) (%)
Control	0.53 ± 0.025^{aB}	0.53 ± 0.025^{bB}	0.61 ± 0.02^{bA}	0.57 ± 0.01^{bAB}
Group 1	0.58 ± 0.04^{aD}	8.39 ± 0.015^{aA}	6.04 ± 0.56^{aB}	4.28 ± 0.27^{aC}
Group 2	0.64 ± 0.06^{aD}	8.39 ± 0.03^{aA}	5.9 ± 50.56^{aB}	4.11 ± 0.27^{aC}
Group 3	0.66 ± 0.09^{aD}	8.41 ± 0.02^{aA}	5.91 ± 0.40^{aB}	4.07 ± 0.17^{aC}

[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 7. Effect of Remediation Amendments on the Carbon to Nitrogen Ratio of crude oil polluted soil

	C:N (Pre-exposed Soil)	C:N (2 weeks after pollution)	C:N (one months after remediation)	C:N (8 weeks after remediation)
Control	5:01	5:01	5:01	5:01
Group 1	5:01	6:01	5:01	5:01
Group 2	5:01	5:01	5:01	5:01
Group 3	6:01	5:01	5:01	5:01

Table 8. Effect of the Remediation Amendments on the Soil Phosphorus of crude oil polluted soil

	P (Pre-exposed Soil) (mg/kg)	P (2 weeks after pollution) (mg/kg)	P (4 weeks after remediation) (mg/kg)	P (8 weeks after remediation) (mg/kg)
Control	19.05 ± 0.02^{aA}	19.05 ± 0.02^{A}	19.39 ± 0.12^{aA}	16.09 ± 5.17^{aA}
Group 1	19.08 ± 0.04^{aA}	BDL	5.67 ± 0.23^{bC}	9.34 ± 0.14^{abB}
Group 2	19.10 ± 0.03^{aA}	BDL	3.82 ± 0.39^{cC}	8.05 ± 0.39^{bB}
Group 3	19.09 ± 0.035^{aA}	BDL	4.19 ± 0.65^{cC}	8.28 ± 0.44^{bB}

[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 9. Effect of the Remediation Amendments on the Soil Cation Exchange Capacity (CEC) of crude oil polluted soil

	CEC (Pre-exposed Soil) (meq/100g)	CEC (2 weeks after pollution) (meq/100g)	CEC(4 weeks after remediation) (meq/100g)	CEC(8 weeks after remediation) (meq/100g)
Control	4.41 ± 0.027^{aA}	4.42 ± 0.015^{aA}	4.24 ± 0.59^{aA}	4.44 ± 0.04^{aA}
Group 1	4.46 ± 0.04^{aA}	0.84 ± 0.004^{bD}	2.31 ± 0.12^{bC}	3.34 ± 0.17^{bB}
Group 2	4.48 ± 0.02^{aA}	0.84 ± 0.04^{bD}	2.58 ± 0.23^{bC}	3.44 ± 0.29^{bB}
Group 3	4.45 ± 0.02^{aA}	0.88 ± 0.03^{bD}	2.80 ± 0.31^{bC}	3.79 ± 0.27^{bB}

[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 10. Effect of the Remediation Amendments on the Total Petroleum Hydrocarbon (TPH) contents of crude oil polluted soil

	TPH (Pre-exposed Soil) (mg/kg)	TPH (2 weeks after pollution) (mg/kg)	TPH (4 weeks after remediation) (mg/kg)	TPH (8 weeks after remediation) (mg/kg)
Control	BDL	BDL	BDL	BDL
Group 1	BDL	1780.68 ± 179.14^{bA}	425.78 ± 57.61^{bB}	327.86 ± 50.22^{bC}
Group 2	BDL	2464.55 ± 376.89^{abA}	625.68 ± 80.90^{aB}	442.92 ± 41.47^{abC}
Group 3	BDL	2730.59 ± 234.53^{aA}	609.85 ± 113.32^{aB}	507.54 ± 71.04^{aB}

[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 11. Effect of the Remediation Amendments on the Poly Aromatic Hydrocarbon (PAH) contents of crude oil polluted soil

	PAH (Pre-exposed Soil) (mg/kg)	PAH (2 weeks after pollution) (mg/kg)	PAH (4 weeks after remediation) (mg/kg)	PAH (8 weeks after remediation) (mg/kg)
Control	BDL	BDL	BDL	BDL
Group 1	BDL	1001.63 ± 23.32^{cA}	428.51 ± 105.50^{bB}	291.46 ± 50.64^{cB}
Group 2	BDL	1371.42 ± 111.98^{bA}	588.08 ± 81.41^{abB}	368.44 ± 43.23^{bC}
Group 3	BDL	1687.50 ± 125.12^{aA}	671.68 ± 36.49^{aB}	504.43 ± 80.82^{aB}

$^{a-d}$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 12. Effect of the Remediation Amendments on the Bacterial Population of Crude Oil Polluted Soil

	Bacteria count (Pre-exposed Soil) (cfu/g)	Bacteria count (2 weeks after pollution) (cfu/g)	Bacteria count (one months after remediation) (cfu/g)	Bacteria count (8 weeks after remediation) (cfu/g)
Control	$1.37\ X10^3$	$1.4\ X10^3$	$1.6\ X10^3$	$1.9\ X10^3$
Group 1	$1.4\ X10^3$	$1.4\ X10^6$	$7.86\ X10^7$	$8.41\ X10^7$
Group 2	$1.4\ X10^3$	$1.47\ X10^7$	$7.23\ X10^7$	$8.49\ X10^7$
Group 3	$1.4\ X10^3$	$1.24\ X10^8$	$5.62\ X10^7$	$6.98\ X10^7$

Table 13. Effect of the Remediation Amendments on the Soil Electrical Conductivity of crude oil polluted soil

	Conductivity (Pre-exposed Soil) (µS/cm)	Conductivity (2 weeks after pollution) (µS/cm)	Conductivity (4 weeks after remediation) (µS/cm)	Conductivity (8 weeks after remediation) (µS/cm)
Control	19.26 ± 0.05^{aB}	19.26 ± 0.05^{aB}	20.36 ± 0.06^{aA}	19.43 ± 0.13^{aB}
Group 1	19.28 ± 0.03^{aA}	11.85 ± 0.05^{cD}	13.67 ± 0.25^{cC}	14.97 ± 0.12^{cB}
Group 2	19.30 ± 0.03^{aA}	11.85 ± 0.04^{cD}	13.96 ± 0.19^{cC}	15.15 ± 0.33^{bcB}
Group 3	19.32 ± 0.06^{aA}	12.00 ± 0.02^{bD}	14.32 ± 0.04^{bC}	15.74 ± 0.27^{bB}

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

DISCUSSION

The soil pH varied in the treated groups from the 2 weeks after pollution and remediation to the 8 weeks after remediation, but was generally below the level recorded in the control and in the pre-exposed soil before the pollution was carried out. This shows that the crude oil pollution affected the soil pH negatively by making it slightly acidic. The fluctuations in the soil pH can also be due to the metabolites produced by the microorganisms during the period of remediation (Obiakalaije et al, 2015). This might explain why the values were different in the different weeks after remediation. It was observed that the pH in the treated groups was higher in the second weeks after pollution and remediation and then reduced in the fourth week after remediation and later increased in the eighth weeks after remediation. Group 1 on the 8 weeks after remediation had the pH that was closest to the control showing that the addition of cow blood in the group had a positive effect on the remediation process compared to the other groups. Soil pH is known to be one of the major factors that influence the availability of elements in the soil for plant uptake (Marschner, 1995; Oriakpono et al, 2018)

The TOC level was significantly ($P< 0.05$) higher in the treated groups compared to the control, the level of TOC was also

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significantly different (P<0.05) in the treated groups when comparing the level in the pre-exposed soil to the different weeks after remediation. The increase in TOC levels due to crude oil pollution have been reported by many authors and TOC is known to improve the binding process and water retention ability of soils (Njoku et al, 2009; Obiakalaije et al, 2015; Oriakpono et al, 2018). TOC levels reduced across the weeks in all the treated groups indicating that the remediation process is going on progressively and when left over a considerable period of time, the level of TOC will normalize.

There was reduction in the level of nitrogen after crude oil pollution in the 4th and 8th week after remediation which is an indication of high depletion in the nutrient level during the remediation process. This observation revealed that microorganisms need nitrogen formetabolism and in bio-oxidation of the crude oil polluted soil.

The carbon to nitrogen ratio was high in the treated group and 8 weeks after remediation, the ratio was same with the control group. The increase in carbon to nitrogen ratio might be as a result of increase in the microbial activities of the carbon utilizing agents since microbes are known to be heavy carbon utilizers (April and Simms, 1990; Oriakpono et al, 2018).

The soil phosphorus was below detectable limits in 2 weeks after pollution indicating that the crude oil pollution had adverse effect on the level of soil phosphorus. The resurgence of phosphorus in subsequent weeks might be as a result of the application of amendment materials (spent milled maize and cow blood). The following weeks recorded a gradual increase in the level of soil phosphorus indicating that the remediation process that is going on is indeed improving the soil phosphorus level. There was no statistically significant difference (P>0.05) in the level of soil phosphorus across the weeks and also across the treated groups but there was a significant difference (P<0.05) in the level of soil phosphorus when compared to the control. The level of soil phosphorus obtained from this study on the treated groups after the 8 weeks after bioremediation was still below 20mg/kg which is the maximum tolerable limits of phosphorus for soils (Holland et al 1989; Oriakpono et al 2018).

The cation exchange capacity is known to be an indicator of the relative ability of elements like K, Na, Ca and Mg to displace other cations (Oriakpono et al, 2018). There was a reduction in the level of soil CEC 2 weeks after pollution in the groups that received treatment showing that crude oil exerts a negative effect on the soil CEC. The later weeks had a gradual increase in the level of CEC. This indicates that the remediation process using the spent milled maize andcow blood improved the soil cation exchange capacity. There was a significant difference (P<0.05) in the level of CEC across the weeks and across the groups.

The level of soil TPH and PAH was very high 2 weeks after pollution in the treated groups but below detectable limits in pre-exposed soil and also in the control. The reason for high concentrations of TPH and PAH was as a result of spiking the soil with crude oil in such a quantity as to simulate pollution. There was a significant decrease (P<0.05) in the level of TPH and PAH across the weeks; from 2 weeks to 8 weeks after remediation. There was a significant reduction in the concentration of TPHand PAHat the end of the experiment. This might be attributed to the additionalbiodegradative activities performed by the microbial diversity from the amendment materials. This reduction could also be due to the ability of microorganisms to make use of spent milled maize and cow blood as both carbon and nitrogen sources to degrade hydrocarbon compounds. This reduction revealed that spent milled maize and cow blood enhanced the biodegradation of crude oil polluted soil by supplying nutrients to the microbial community; thereby increasing the microbial count with increasing degradation over time.This result is in agreement the work of Oriapkono et al (2018). Authors such as Agarry et al (2010) and Obiakalaije et al, (2015) also recorded lower levels of TPHafter remediation and their result is similar to the

one gotten using spent milled maize and cow blood as amendment materials for crude oil polluted soil.

The bacterial counts were observed to be generally higher in the treated groups than in the control.This showed that there were active indigenous organisms that could bring about biodegradation when enhanced with spent milled maize and cow blood.When there is crude oil pollution, the microorganisms capable of degrading hydrocarbons proliferate quickly making use of nutrients supplied by the amendment materials (ASM, 2013; Oriakpono et al, 2018). The microorganisms isolated from this study are in agreement with the study carried out by Oriakpono et al (2018), Okpokwasili and James (1995) and Obiakalaije et al, (2015).

The soil electrical conductivity was negatively affected by the crude oil pollution as the treated groups recorded a reduction in the level of conductivity. The levels increased in the 4^{th} and 8^{th} week after remediation significantly ($P<0.05$). The increased levels of electrical conductivity might be as a result of the spent milled maize and cow blood which helped in the release of dissolved solutes.

CONCLUSION

The toxic substances in crude oil polluted soils can be removed during bioremediation making use of amendment materials as revealed in this study. The amendment of crude oil polluted soil with spent milled maize and cow blood have revealed the effectiveness of these agents at enhancing the degradation of toxic constituents in polluted soils. The data obtained have shown that spent milled maize and cow blood are effective towards the betterment of the physicochemical properties of the amended soil; hence, making bioremediation a success.

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