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Remediation Potentials of Spent Milled Maize and Cowblood on Agricultural Soil Polluted with Crude Oil

[1] Oriakpono Iris O, [2] Egena Innocent, [3] Oriakpono Obemeata E

[1, 2] Department of Soil Science, Rivers State University Port Harcourt, P.M.B. 5080 Port Harcourt, Nigeria
[3] Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Choba, Rivers State, Nigeria

Corresponding Author: **Oriakpono Obemeata E**

Abstract

In Nigeria especially in Niger Delta region, soil pollution and degradation such as crude oil spillage is of a paramount concern. As such, the only strategy to remediate such soil condition in Niger Delta region is through the application of organic nutrient amendment material such as Cowblood and spent milled maize. Cow blood and spent Milled Maize is easy to find and has a great potential as raw materials for Boiremediation as shown in this study carried out. Three rather of crude oil (0,10 and 15ml) and three rates of Spent Milled Maize (0, 2 and 3g) were used respectively. It comprises of the three-treatment replicated Nine Times, for a total of Twenty-Seven pots with each pot containing 5kg of soil. Microbial population and physico chemical properties of soil were determined after pollution and at the end of the experiment the result for physico chemical properties of the soil indicates and increase across most of the soil Parameter analysed such as Nitrogen, Potassium, Available Phosphorus etc. Also, there was a notable reduction (decrease) in the level of total hydrocarbon found in the soil. There was also an increase in the microbial population (bacteria Count) from 2.3X10'3 in the control to 3.5X10'6 in souls polluted with 15ml of crude oil,3G of Spent Milled Maize and 3ml of Cowblood. The genus of bacteria identified in the Course of this study *Pseudomonas, bacullus, Norcardia* and *Proteus* species were found in the soil samples.

Keywords: Spent Milled Maize, Cow Blood, Soil, Pollution, Crude Oil

1. Introduction

Commercial exploration of petroleum started in Nigeria in the year 1958 many Nigerian oil fields have become unproductive many of which have resulted in major land degradation thus rendering the land non useful for agricultural purposes (Okoh, 2003) [7].

These smaller unproductive oil fields account for about 62.6% of the nation's oil production. Due to the distance between the smaller oil fields and for product transportation purposes, there is need to connect these smaller and unproductive areas hence, the reason for laying an extensive pipeline network in and around the communities. In course of the oil exploration in these oil bearing communities, large amount of compounds containing organic and inorganic materials are introduced or released into the environment as a result of anthropogenic activities. This act of introduction or release could either be a deliberate act or well regulated (e.g. industrial emission) or accidental discharge (e.g. oil spill resulting from colliding tanker or pipeline leakage etc. Different constituent of petroleum and crude oil such as polycyclic aromatic hydrocarbon (PAHs) which are mutagenic, carcinogenic and toxic in nature has been found in water ways as a result of petrochemical products and effluent from industrial pollutions (Beckles *et al.,* 1998) [2]. In Niger Delta area of Nigeria for example, the high rate of environmental pollution recorded due to high rate of petroleum related activities has been attributed to tanker accident, pipeline leakages, oil well blow out, use of faulty materials or equipment, transportation and It can also be from anthropogenic sources (Hemsbergen *et al.*, 2004) [6]. Like any other technical appliance, pipelines are not exceptional as they are subject to wear and tear hence, as a result of corrosion can fail with time (Beller, *et al.,* 1996) [3]. The spill oil degrades both the aquatic and terrestrial ecosystem by polluting the water and soil making the soil less viable for agricultural activities e.g. farming with soil dependent microbes being adversely affected (Alexander, 2009) [1].

Looking at the socio economic health risk and enormous agronomic problems caused by hydrocarbon, there is need to device eco-friendly measures or techniques for an effective remediation of our polluted environment. Environmental bioremediation is seen as an idea remedy which offers the possibilities to eider render harmless or destroy the various contaminants through the activities of living things. It involves three principles which are; Biostimulation: Which is the stimulation of the remedial activities of microorganism by environmental modifications such as fertilizer addition or application to increase the rate of biodegradation and remediation of the polluted site. Bioaugumentation: Which has to do with the addition of oxogenous microorganism to the hydrocarbon impacted ecosystem to supplement the existing microbial population in the biodegradation of the soil? And finally Natural attenuation or natural biodegradation and rate which is sometimes called intrisic bioremediation which involves allowing nature to take it tor on the recovery process without the usual activities of man though man can control it.

Cow blood and Akamu is used as an amendment to enhance and remediate the polluted soil by increasing the soil fertility and at the same time, this judicious use of this amendments in bioremediation solves the problem of waste management thereby making the environment friendly and free from contaminants which may have resulted from it.

The objectives of this study were to determine the effect of bacteria population on cow blood and spent milled maize on crude oil polluted soils and the effect on soil properties.

2. Materials and Methods

Description of Experimental Site

The experiment was a pot experiment and was conducted in the department of Crop/Soil science screen house at the Rivers State University, Port Harcourt for 6 weeks.

Treatments and experimental design

The pot experiment was carried out at the Screen House of the Department of Crop/Soil Science Rivers State University. 27 buckets of 12 litres capacity were used for this study. The buckets were perforated at the sides and bottom. Each bucket was filled with 10 kg top soil. Soils will be collected randomly with a metal soil auger at the surface soil between the depths of 0 to 15 cm from faculty of agriculture farm, air dried, carefully cleaned by picking away all litter of leaves and roots that could decompose with time, the soil samples were bulked together, homogenized and 10 kg was weigh into plastic buckets. A total of 27 pots filled with experimental soil was used for the experiment. The soils were allowed to settle for two weeks, watered and treated with three rates (0, 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 oil spills. Two weeks after crude oil treatment, three rates (0, 1 and 3 gm) of air‐dried, spent milled maize and cow blood (0, 5 and 10 ml) were applied to the polluted soils. The spent milled maize and cow blood were 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. Soil samples were collected from the pots one month after remediation.

Determination of chemical properties

Soil pH, was carried out using the glass electrode pH meter. Organic Carbon, was done using the Walkley and Black wet oxidation method. Total Nitrogen was carried out using the Kjeldahl method. Available Phosphorus was carried out using the Bray and Kurtz no.1 method.

Isolation of bacteria and fungi

One gram of soil sample was extracted from the various test media under aseptic conditions and diluted serially up to x 10^{-9} in physiological saline as suspending medium. After a preliminary investigation, 0.1ml aliquots of the serial dilutions were inoculated onto nutrient agar, mineral salt agar and potatoes dextrose agar plates for the cultivation of total heterotrophic bacteria, and hydrocarbon-utilizing bacteria. The inoculation technique applied was the spread plate method, using a sterile bent glass rod. The nutrient agar plates for cultivation of total aerobic heterotrophic bacteria were be incubated at 28± 2°C overnight, while the hydrocarbon-utilizing bacteria culture plates were incubated at 30°C for 5 days. All inoculations were made in triplicates and incubated along with corresponding un-inoculated plates which served as controls. At the end of each incubation period, colonies which developed were enumerated as total viable organisms. The relative abundance of hydrocarbon-utilizing bacteria, expressed as a percentage of the total viable heterotrophic bacteria were calculated. Discrete colonies were sub-cultured to obtain pure bacterial isolates for subsequent investigations.

3. Result and Discusion

Bacteria population on crude oil polluted soils

The bacteria count range between ($2.3x10^3$) in the control to ($3.5\ x10^6$) in soils polluted with 15 ml of crude oil, 3 g of Spent Milled Maize and 3 ml of cow blood.

The identified bacteria in the cause of the study are as follows; *Pseudomonas*, *Bacillus*, *Nocardia* and *Proteus species*. *Pseudomonas* sp was found in the control only while *Bacillus*, *Nocardia* and *Proteus species* were found in soil samples polluted and remediated with spent milled maize and cow blood. The *Proteus sp* observed is believed to be as a result of the spent milled maize which was used as amendment (Table 1).

Most bacteria are seen to be bio-indicators of soil properties as determine by their numbers and the type present in the polluted environment. The result from this study indicate the bacteria count increased after remediation. It is in agreement with similar studies (Oriakpono *et al.*, 2018) [9]. The presence of increased number of bacteria in the polluted soil are indications that they have the ability to accumulate and stay in polluted areas and as well replicate and increase in number. The high number of bacteria in the soil after remediation shows their high tolerance rate and rapid colonization of soils polluted by crude oil. The result of the isolated bacteria is in agreement with the work of (Okpokwasili and James, 1995) [8].

Table 1: Microbial Count and Microorganisms Identified

Rate	Treatment	Microbial count (cfu/g)	Microorganisms Identified
0(NA)	0(NA)	2.3×10^3	Pseudomonas sp, Bacillus sp,
	0(1/1)	2.5×10^3	Nocardia sp, Bacillus sp
	0(3/3)	2.7×10^3	Nocardia sp, Bacillus sp *Proteus* sp
10ml	10(NA)	2.3×10^6	Nocardia sp, Bacillus sp, *Proteus* sp
	10(1/1)	2.6×10^6	Nocardia sp, Bacillus sp, *Proteus* sp
	10(3/3)	2.8×10^6	Nocardia sp, Bacillus sp, Fusarium sp
15ml	15(NA)	2.4×10^3	Nocardia sp, Bacillus sp
	15(1/1)	3.3×10^6	Nocardia sp, Bacillus sp, *Proteus* sp
	15(3/3)	3.5×10^6	Nocardia sp, Bacillus sp, *Proteus* sp

KEY: 0(NA) = 0 ml of crude oil, No amendment (NA)
0(1/1) = 0 ml of crude oil, 1 g of Spent Milled Maize / 1 ml of cow blood
0(3/3) = 0 ml of crude oil, 3 g of Spent Milled Maize / 3 ml of cow blood
10(NA) = 10 ml of crude oil, No amendment (NA)
10(1/1) = 10 ml of crude oil, 1 g of Spent Milled Maize / 1 ml of cow blood
10(3/3) = 10 ml of crude oil, 3 g of Spent Milled Maize / 3 ml of cow blood
15(NA) = 15 ml of crude oil, No amendment (NA)
15(1/1) = 15 ml of crude oil, 1 g of Spent Milled Maize / 1 ml of cow blood
15(3/3) = 15 ml of crude oil, 3 g of Spent Milled Maize / 3 ml of cow blood.

Effect of Bioremediation of the crude oil polluted soil on soil physicochemical parameters

pH: The pH ranged between 5.42 – 5.87 (0 ml), 5.39- 6.19 (10 ml) and (5.27- 6.41) in 15 ml. The soil pH is essential in regulating the conditions of soil flora and fauna (Petersen *et al*., 2014). The result for pH of the soil tends acidic as the soil were exposed to pollution and more acidic when they were remediated. There was slight significant difference across the treatment.

Organic Carbon (%): The range was between (1.73 - 3.49) in the 0 ml control, (2.56 - 5.93) in the 10 ml treated group and (4.67 - 6.49) for the 15 ml treated group. The highest mean (6.49) was found in soils polluted with 15ml of crude oil and remediated with 3 g of Spent Milled Maize, 3 ml of cow blood.

Total Nitrogen (%): Total Nitrogen range was between (1.58- 4.53) in the 0 ml control, (1.89 - 5.48) in the 10 ml treated group and (1.93 - 5.95) in the 15 ml treated group. The highest mean (5.95) was found in soils polluted with 15ml of crude oil and remediated with 3 g of Spent Milled Maize, 3 ml of cow blood. The result shows significant difference $P < 0.05$ between the various treatments and the control. Total nitrogen shows a significant difference across all the treatment. This is because the material used in the remediation process (spent milled Maize) is a product of corn which is seen to have high nitrogen content, therefore enhancing the multiplication of bacteria through nutrient enrichment and availability after remediation. Nitrogen is a compound that can be added to the soil either through natural processes or fixation by microorganisms, that increases the nitrogen content of the soil. The result for the soil nitrogen in this study is in accordance with similar research carried out by other authors (David *et al.*, 2009). The phosphorus content in the study shows slight difference in all the treatments. It could be seen that they are needed by bacteria for their high proliferation since a high bacteria count is recorded.

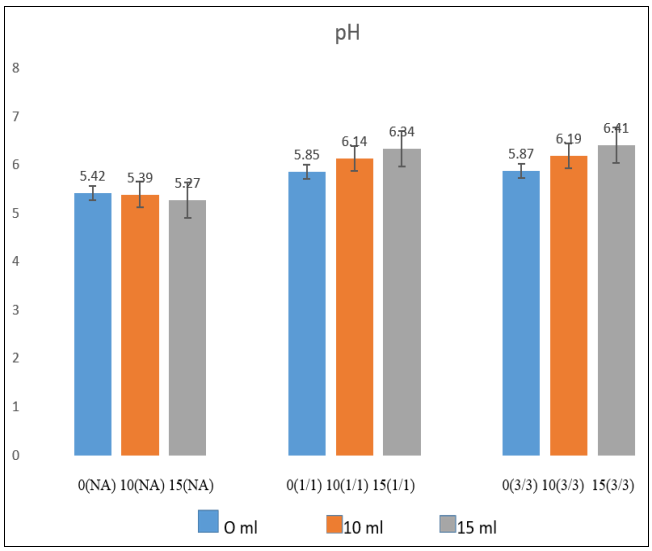

Fig 1: pH in Control and bioremediated Soils

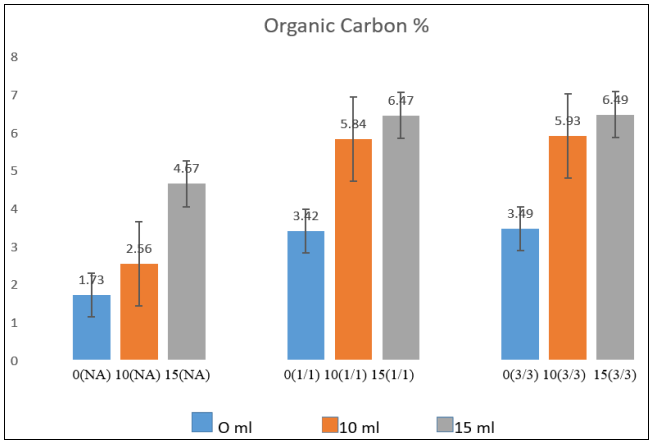

Fig 2: Organic Carbon in Control and Bioremediated Soils

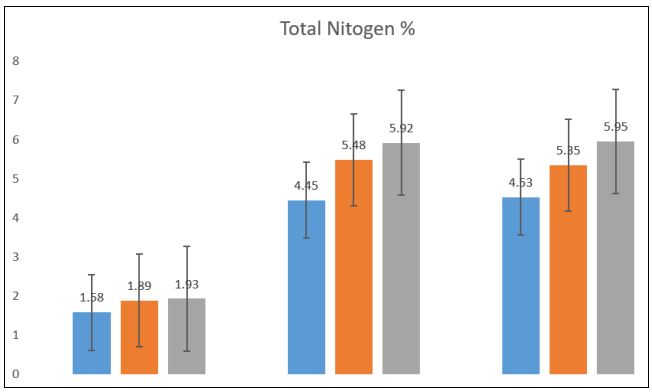

Fig 3: Total Nitrogen concentration in Control and Bioremediated Soils

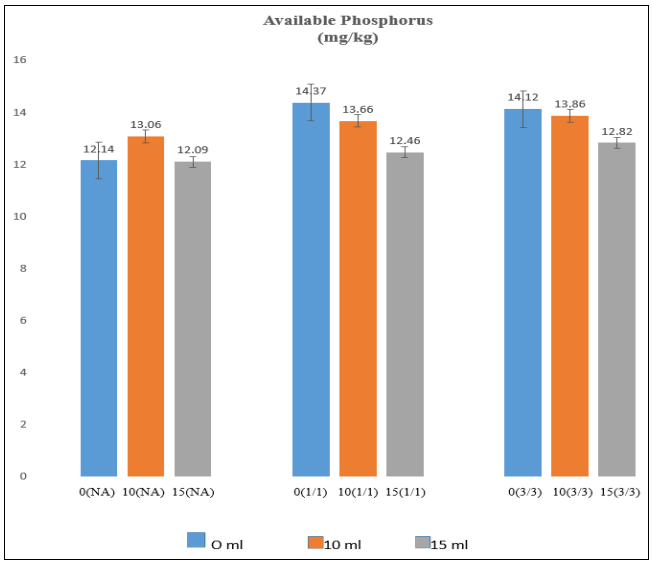

Fig 4: Available Phosphorus in Control and Bioremediated Soils

Total Hydrocarbon (THC): THC was found to below detectable limit in the 0 ml of crude oil but was high in 10 ml and 15 ml of crude oil treated groups. The lowest THC was found in soils polluted with 15 ml of crude oil and amended with 3 g of Spent Milled Maize and 3 ml of cow blood (326.19). The THC limit of the study shows a below detectable limit across the soils that was not exposed to crude oil and the control throughout the study, the high value for THC were recorded in soils that were not remediated, but moves towards minimal as remediation measures were put into place, the reduction in the level of hydrocarbon after remediation shows the potency in spent milled maize as an amendment remediation of polluted soil with spent milled maize seen to be more useful.

Potassium (K): It ranged between (9 - 13) in the 0 ml control, (7 - 11) in the 10 ml treated group and (7 - 11) in the 15 ml treated group. The highest mean (13) was found in the control.

Sodium (Na): sodium ranged from (13 – 15) in all the treatment groups.

Calcium (Ca): It ranged between (0.7 – 0.9) in the 0 ml control, (0.5 – 1.3) in the 10 ml treated group and (0.6 – 1.4) in the 15 ml treated group. The highest mean (1.4) was found in soils polluted with 15ml of crude oil and

remediated with 3 g of Spent Milled Maize, 3 ml of cow blood.

Magnesium (Mg): Magnesium ranged between (0.3 – 0.4). The highest mean (0.4) was found in soils polluted with 15ml of crude oil and remediated with 3 g of Spent Milled Maize, 3 ml of cow blood.

Available Phosphorus (mg/kg): Phosphorus was within the range of (12.14 - 14.37) in the 0 ml control, (13.06 - 13.86) in the 10 ml treated group and (12.09 - 12.82) in the 15 ml treated group. The highest mean (14.37) was found in the control soils remediated with 1 g of Spent Milled Maize, 1 ml of cow blood.

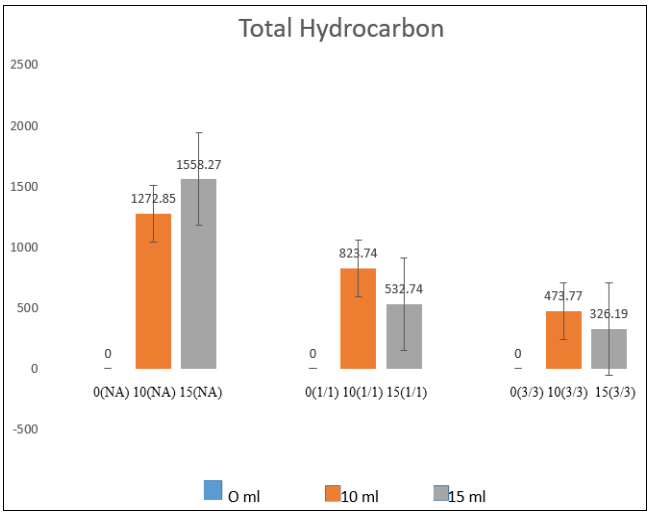

Fig 5: Total Hydrocarbon concentration in Control and Bioremediated Soils

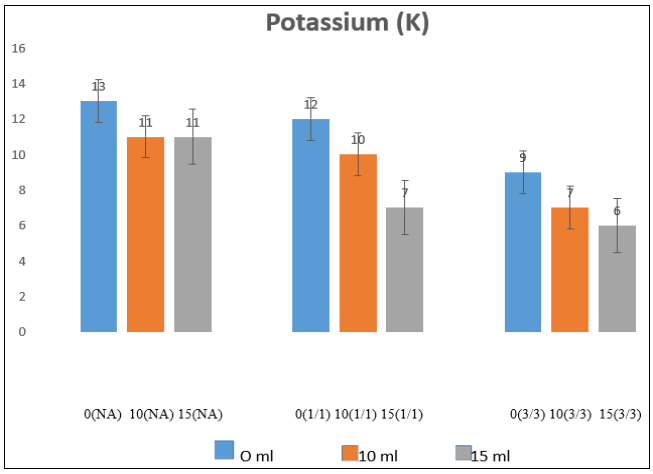

Fig 6: Potassium concentration in Control and Bioremediated Soils

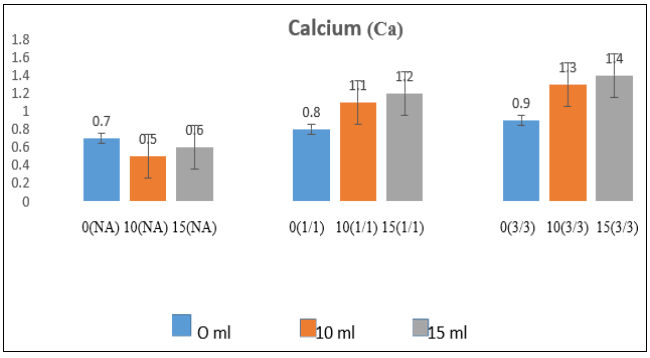

Fig 7: Calcium concentration in Control and Bioremediated Soils

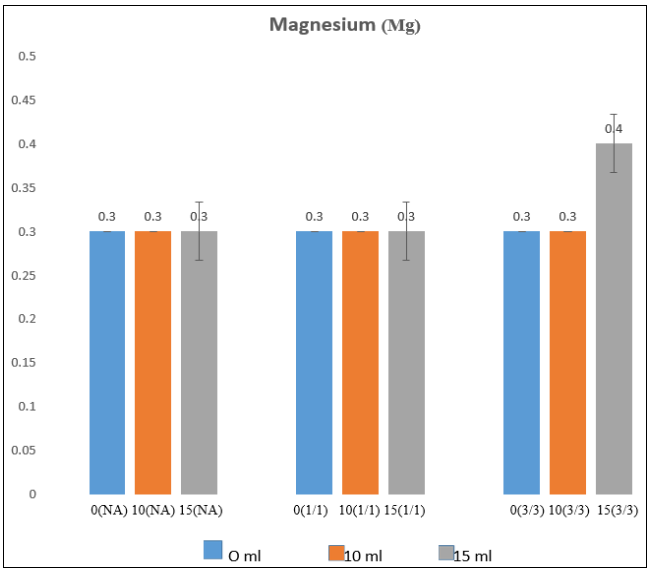

Fig 8: Magnesium concentration in Control and Bioremediated Soils

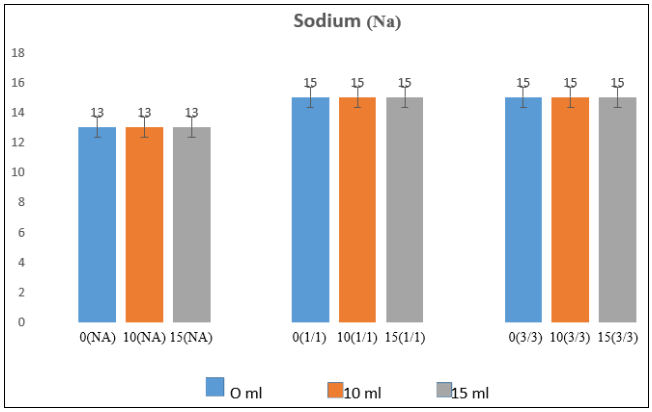

Fig 9: Sodium concentration in Control and Bioremediated Soils

Table 2: Result of Chemical Properties

Rate	Treatment	pH	TN (%)	Org. C (%)	THC	Av. P (mg/kg)	Exchangeable ions (meq/100g of soil)					
							K	Ca	Mg	Na	H^+	Al^+
0 ml	0(NA)	5.42	1.58	1.73	<0.001	12.14	13	0.7	0.3	13	2.35	0.4
	0(1/1)	5.85	4.45	3.42	<0.001	14.37	12	0.8	0.3	15	3.05	0.2
	0(3/3)	5.87	4.53	3.49	<0.001	14.12	9	0.9	0.3	15	3.13	0.2
10 ml	10(NA)	5.39	1.89	2.56	1272.85	13.06	11	0.5	0.3	13	2.51	0.5
	10(1/1)	6.14	5.48	5.84	823.74	13.66	10	1.1	0.3	15	3.17	0.3
	10(3/3)	6.19	5.35	5.93	473.77	13.86	7	1.3	0.3	15	3.35	0.3
15 ml	15(NA)	5.27	1.93	4.67	1558.27	12.09	11	0.6	0.3	13	2.59	0.5
	15(1/1)	6.34	5.92	6.47	532.74	12.46	7	1.2	0.3	15	4.27	0.3
	15(3/3)	6.41	5.95	6.49	326.19	12.82	6	1.4	0.4	15	4.52	0.2

KEY: 0(NA) = 0 ml of crude oil, No amendment (NA)
0(1/1) = 0 ml of crude oil, 1 g of Spent Milled Maize / 1 ml of cow blood
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15(3/3) = 15 ml of crude oil, 3 g of Spent Milled Maize / 3 ml of cow blood

4. Conclusion

Soil pollution due to crude oil spill has been a great problem of concern as they have the ability and potentials of devastating soil properties since they alter some parameters needed for biogeochemical processes to take place. Bioremediation with spent milled maize have been proven by this research to be effective in remediation, hence should be employed as a natural amendment to restore polluted soil that have been affected by crude oil pollutions.

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