Bio-stimulatory Potential of Intestinal Waste of Cow on a Crude Oil Polluted Soil

Oriakpono, Iris O[1]*, Wemejiri Anwuri [2], Ukaulor, Victor C[1] and Oriakpono, Obemeata E[2]

[1]Department of Soil Science, Rivers State University Port Harcourt, P.M.B.5080 Port Harcourt

[2]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Choba, Rivers State, Nigeria.

*(Corresponding Author Email ID: obemeata.oriakpono@uniport.edu.ng)

DOI: 10.56201/ijaes.vol.11.no4.2025.pg29.41

Abstract

The study was carried out to examine the biostimulatory effect of intestinal wastes of cows on the bioremediation on a crude oil contaminated soil. A completely randomized design was applied with four rates (0, 1, 2 and 7Kg) of intestinal waste of cow and (0, 1, 2 and 5L) of crude oil were applied to contaminate the soil per plot. The soil physico-chemical parameters analysed includes pH, Total Organic Content (TOC), Nitrogen (N), Carbon to Nitrogen ratio (C:N), Phosphorus (P), cation exchange capacity (CEC), total petroleum hydrocarbon (TPH) and poly aromatic hydrocarbon (PAH). The result for soil physio-chemical parameters revealed that the mean value of soil nitrogen, C:N, TOC, TPH and PAH increased in the 2 weeks after pollution while the level reduced significantly (p< 0.05) at 4 weeks after bioremediation. The mean value of CEC, soil conductivity and soil phosphorus decreased in the 2 weeks after pollution when compared to the pre-exposed soil while the level increased significantly (p< 0.05) at 4 weeks after bioremediation. This research has proven that using intestinal waste of cow which is an animal waste that is generated in abattoirs has a strong bio-stimulatory properties and is effective in bio-remediation, therefore should be adopted in the restoration of crude oil polluted soil.

Key words: *Biostimulation, Intestinal Waste, Cow waste, Crude oil and Polluted soil*

INTRODUCTION

Crude oil comprises of a complex mixture of different hydrocarbons such as alkanes, aromatics, alicyclics, branched hydrocarbons, and other non-hydrocarbon compounds that encompasses polar fractions which contains hetero-atoms of nitrogen, sulfur and oxygen (NSO fraction), and asphaltens,(Dutta and Harayama, 2001; Reisinger 2005; Ekpo and Udofia 2008). Due to the different uses of petroleum products and the corresponding increase in the demand of the products, there have been a need for an increase in the production and consequently, an increase in all crude oil related activities ranging from drilling to transportation to installation of need pipelines. In the course of all these, there have been an increase in the rate of oil spills and hydrocarbon contamination of the environment particularly via, tanker accidents, vandalization or accidental rupture of pipelines and routine clean-up operations and oil well blow out. These usually result in the discharge of oil into the environment, and whenever they occur a large region is affects as seen in most developed and developing countries such as Nigeria (Gerhart *et al.*, 1981; Marshal 1988; Okpokwasili and Amanchukwu, 1988; Mentzer and Ebere, 1996; SVMS, 2001; Ugochukwu *et al.*, 2016). In an event of a crude oil spill, the soil ecosystem is adversely affected as the crude oil components gets adsorbed into the soil particles, and there is an excessive increase in carbon which indirectly affects the level of soil nitrogen and phosphorus, also different constituent of petroleum and crude oil like polycyclic aromatic hydrocarbon (PAHs) which have been reported to be mutagenic, carcinogenic and toxic in nature has been found in water ways as a result (Atlas, 1981; Beckles, *et al.*, 1998; Ugochukwu *et al.*, 2016), the effects are very enormous on the ecosystem and results in the loss of microbial communities in the soil, and when the spill occurs in the aquatic environment, it leads to the loss of habitat of economically important fish species and other different aquatic animals, oil spills also damages the wet lands in the coastal regions as well as areas of vegetation that are meant to be used for agricultural purposes etc. (Nwachukwu and Ugorji, 1995; SVMS, 2001; Nnadi and Osakwe 2017).

The effects that crude oil pollution exerts on the physico-chemical properties of soils have been emphasized and carefully studied alongside the socio-economic impact and health challenges that accompanies the pollution (Adeniyi *et al.*, 1983; Isirimaru , 1989; Ugochukwu *et al.*, 2016). Except the situation is effectively managed, the hydrocarbon polluted environments can remain impacted for an extended period of time. Nonetheless, with the implementation of active biodegradation process and remediation programme a significant higher rates of oil removal from the affected environment could be achieved (Adams and Jackson, 1996). The current technologies that are being implemented for the purpose of cleaning up of a hydrocarbon contaminated soil include thermal treatment, composting, soil washing, solvent extraction, chemical oxidation (Fenton's reagent, permanganate, ozone etc) and bioremediation (bioaugmentation, biostimulation and phytoremediation) (Romantschuk *et al.*, 2000; Nwankwegu *et al.*, 2016; Ugochukwu *et al.*, 2016)

Bioremediation can be defined as the use of naturally occurring microorganisms in the environment or microorganisms that have been genetically engineered by man to detoxify man-made pollutants. (Jobson *et al.*, 1974; Ugochukwu *et al.*, 2016). The most generally used bioremediation procedure is the biostimulation of the indigenous microorganisms via the introduction of nutrients, as input of large amounts of carbon sources tends to lead to a rapid depletion of the available store of major inorganic nutrients that are essential to plants, such as nitrogen and phosphorus. Biostimulation can be defined as the addition of appropriate nutritional amendments by addition of different forms of limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses), which are otherwise available in low quantities in other to increase the microbial metabolism and to encourage bioremediation, and this process may be carried out in-situ or ex-situ (Morgan and Watkinson, 1989; Elektorowicz, 1994; Piehler *et al.*, 1999; Rhykerd *et al.*, 1999; Ugochukwu *et al.*, 2016). The main goal of this process is to adequately stimulate the indigenous microbial flora of the waste to bring about its degradation. In other to achieve this objective, different material both organic and inorganic have been adopted. However, the use of organic materials such as animal waste is widely adopted, as animal waste has over time been used for the purpose of increasing the fertility of the soil (Odgen and Adams, 1989; Perfumo *et al.*, 2007; Ugochukwu *et al.*, 2016). Studies have been carried out on the usage of organic materials to improve microorganism degradation of polluted soils (Nduka *et al.*, 2012; Adams *et al.*, 2015; Chikere *et al.*, 2016; Oriakpono *et al.*, 2018). Animal waste includes livestock and poultry manure, bedding and litter, it also includes by-products after processing a livestock for meat such as intestinal waste, etc. Such waste can effectively enhance the organic matter which improves the water holding capacity and improves other properties of the soil. Animal waste can also provide an economical source of nitrogen, phosphorus and potassium in addition to other nutrients for plant growth (Odu *et al.*, 1989; Keeney and Nelson, 1994; Ugochukwu *et al.*, 2016). In line with this, the objectives of this study is to determine the bio-stimulatory potential of cattle intestinal waste which is a common animal waste in abattoirs on a crude oil polluted soil.

MATERIALS AND METHODS

The experiment comprising of four (4) treatment combinations was replicated thrice giving rise to a total of twelve (12) plots

Treatments and 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. The land was prepared manually using cutlass and hoe. It was cleared of existing vegetation, marked out into blocks and plots using ranging poles and pegs, after which beds were constructed using hoes and shades. 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 5L) of crude oil (bonny light blend) for control, group 1, group 2 and group 3 respectively. 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, four rates (0, 2, 5 and 7kg) of air- dried intestinal waste were applied to polluted soils. The intestinal wastes 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. Treated soils were left for about two months for revegetation to occur before final samples were collected.

Materials and Sampling

Soil samples were collected from the plots at three different times. First was before crude oil application to ascertain the physio-chemical nature of the unpolluted soil. Second was two weeks after pollution and third was one month after remediation. Materials Used includes: Crude oil Bonny Light (BL) that was obtained from Shell Petroleum Development Company (SPDC) of Nigeria and cattle intestinal waste.

Determination of Physiochemical Parameters

Soil Samples were collected, labelled, 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 (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

The samples were cold-extracted in a conical flask for two hours in each case using 100 % dichloromethane according to the method of (Shahunthala, 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. The GC-FID system consist 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 Ultra1932530, 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 1 ml/min at a pressure of 75kpa. The injector temperature was set at 250°C, and detector temperature at 310°C. The temerature 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

Statistical Analyses

The Assistat 7.6 beta statistical analysis software program for windows was used in the statistical analysis of data, the data was subjected to analysis of variance (ANOVA). Mean differences among treatments were evaluated with the Tukey Least Significant Difference t-test (LSD) test. Results were regarded significantly different at a significance level below ($P \leq 0.05$).

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

Table 2. Chemical Composition of Ground Intestinal Waste

Parameters	Value
Organic carbon (%)	49.5
Total Nitrogen (%)	1.82
Sodium (%)	0.40
Potassium (%)	5.75
Calcium (%)	2.83
Magnesium (%)	2.28
Available Phosphorus (%)	0.91
pH	6.43
Microbial count (cfu/g)	5.4×10^2

RESULTS

The effects of bioremediation using intestinal waste of cow on the physio-chemical properties of a crude oil polluted soil.

The results are present in tables 3 to 5, the pH mean ranged between 8.19 – 8.76 (pre- exposed), two weeks (5.93- 8.74) and four weeks (5.88- 7.41). The highest mean (8.76 ± 0.19) found in first week of pre-exposed soil in control. Also, the lowest mean of pH was recorded as (5.88± 0.17) and from one month after remediation in treatment of group 1. The Cation Exchange Capacity (CEC) ranges between (3.48-4.50 meq/100g) found in pre-exposed soil, (0.79-3.62 meq/100g) two weeks after pollution and (1.66-3.62 meq/100g) found in four weeks after pollution, the highest mean value is (4.50 meq/100g) found in group 3 of pre-exposed soil, and lowest (0.79 meq/100g) found two weeks after pollution and group 1. The Electrical Conductivity had a mean range between (19.33-20.53) for pre-exposed soil, (11.25-19.13) for two weeks after pollution, and (12.52-19.59) in four weeks after pollution. The highest range was (20.53±0.48) found in group 3. The lowest (11.25±0.38) was in group 1. For soil nitrogen levels, the values ranges between (0.53-0.57) in the pre-exposed soil, the highest mean was (0.57±0.02) found group 2 at two weeks after pollution and the lowest (0.52±0.03) found in control 2 weeks after pollution. The result shows significant different ($P<0.05$) across rows and column. The C:N had the lowest value of (5.10) per-exposed soil, and the highest mean (20.76) in group 3 of two weeks pre-exposed soil. The values for Phosphorus had a range of (4.34-20.96), with highest mean (20.96) recorded in group 3 in pre-exposed soil. The lowest mean was (4.34) in group 1 at four weeks after remediation, while group 1, 2 and 3 had phosphorus vales that were below detectable limits at 2 weeks after pollution. For the Total Organic Carbon, the values ranges between (2.33-6.40), the highest mean is (6.40) found in group 3 at two weeks after pollution, the lowest (2.33) in control at pre-exposed and two weeks after pollution.

The results for Poly Aromatic Hydrocarbon (PAH) ranges between (348.39-2439.30) with the highest been (2439.30) found in group 3 at two weeks after pollution and the lowest is (348.39) in group 1 at four weeks after remediation. In pre-exposed soil and control the values were below detectable limits. There was significant difference $p< (0.05)$ when comparing 2 weeks after pollution and 4 weeks after bioremediation. While the results of Total Petroleum Hydrocarbon (TPH) was (553.96-3280.81), with the highest been (3280.81) in group 1 at two weeks after pollution, and the lowest range (553.96) found in group 2 at 4 weeks after remediation, similarly, there was significant difference $p< (0.05)$ when comparing 2 weeks after pollution and 4 weeks after bioremediation.

Table 3. Effect of the Remediation Amendments on the Soil pH, CEC and Conductivity.

	pH			**CEC (meq/100g)**			**Conductivity**		
	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R
Control	8.76 ± 0.19^{bB}	8.74±0.22^{cB}	7.41±0.28^{bA}	3.48 ±0.19^{aA}	3.62±0.23^{bB}	3.87±0.87^{bA}	19.33±0.38^{aA}	19.13±0.69^{bA}	19.59±0.48^{bA}
Group 1	8.19± 0.11^{aC}	6.55±0.04^{aB}	5.88±0.17^{aA}	4.07±0.63^{aB}	0.79±0.03^{aA}	1.66±0.67^{aB}	19.73±0.47^{aC}	11.25±0.38^{aA}	12.52±1.09^{aB}
Group 2	8.64 ± 0.28^{aC}	5.93±0.46^{aA}	6.08±0.59^{aB}	4.31±0.41^{aB}	0.84±0.03^{aA}	1.96±0.39^{aB}	19.77±0.47^{aC}	11.82±0.11^{aA}	14.01± 061^{aB}
Group 3	8.51± 0.27^{aB}	6.76±0.04^{bA}	6.45±0.03^{aA}	4.50±0.26^{aB}	0.92±0.04^{aA}	2.24 ±0.29^{aB}	20.53±0.48^{bC}	11.88±0.12^{aA}	14.29±0.76^{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)
Key: 4wks A/R= 4 weeks after Remediation
2wks A/P= 2 weeks after Pollution

Table 4. Effect of the Remediation Amendments on the Soil Nitrogen, C:N and Soil Phosphorus.

	Soil Nitrogen%			**C:N**			**Soil Phosphorus (mg/kg)**		
	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R
Control	0.53 ± 0.03^{aA}	0.52 ± 0.03^{aA}	0.54 ± 0.03^{aA}	5.10	5.10	5.76	19.41 ± 0.42^{aA}	19.41 ± 0.44^{bA}	19.47 ± 0.33^{cA}
Group 1	0.56 ± 0.03^{aA}	8.46 ± 0.05^{bC}	6.29 ± 0.11^{cB}	5.10	18.10	15.10	20.08 ± 0.76^{aB}	BDL	4.34 ± 0.57^{aA}
Group 2	0.57 ± 0.02^{aA}	8.53 ± 0.04^{bC}	5.51 ± 0.49^{bB}	5.10	18.10	16.10	19.13 ± 0.23^{aB}	BDL	6.32 ± 0.73^{bA}
Group 3	0.55 ± 0.06^{aA}	8.55 ± 0.04^{bC}	5.05 ± 0.33^{bB}	5.10	20.10	15.76	20.96 ± 0.57^{bB}	BDL	7.05 ± 0.89^{bA}

$^{a-d}$ 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)
Key: 4wks A/R= 4 weeks after Remediation
2wks A/P= 2 weeks after Pollution

Table 5. Effect of the Remediation Amendments on the TOC, TPH and PAH.

	TOC%			**TPH**			**PAH**		
	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R	Pre-exposed Soil	2wks A/P	4wks A/R
Control	2.33± 0.03 aA	2.33±0.03aA	3.63±0.06 aB	BDL	BDL	BDL	BDL	BDL	BDL
Group 1	2.35 ± 0.05aA	6.34±0.09bC	5.34±0.17bB	BDL	3280.81±376.63bB	666.24±83.76bA	BDL	1660.1±167.17bB	348.39±142.79bA
Group 2	2.37 ± 0.08aA	6.33±0.01bC	5.49±0.18bB	BDL	3133.29±76.956 bB	553.96±98.20bA	BDL	2145.9±182.26cB	621.93± 26.19cA
Group 3	3.04± 0.54aA	6.40±0.07bC	5.66±0.21 bB	BDL	3181.22±75.25.25 bB	625.80±106.99bA	BDL	2439.3±234.74cB	658.62±34.23cA

[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)
Key: 4wks A/R= 4 weeks after Remediation
2wks A/P= 2 weeks after Pollution

DISCUSSION

The study reveals that crude oil pollution negatively affects the different soil physiochemical components. The pH of the soil after pollution indicates a reduction in the mean value 2 weeks after pollution which decreased further in group 1 and group 3 while in group 2, the value increased 4 weeks after bioremediation. The changes in the mean value can be as a result of the different metabolic activities that are taking in the soil as the introduction of crude oil and the subsequent application of intestinal waste of cow stimulated the different soil microorganisms. (Frank *et al.*, 2013). A soil pH that is lower than 6 is said to be acidic, while a soil pH that is higher than 8.5 is said to be alkaline. Normal pH level of soil is at a range of 6-8.5 (Tales and Ingole, 2015). pH plays a crucial role in the availability of plants nutrients and is vital in the regulation of the conditions of soil flora and fauna (Ekperusi and Aigbodion, 2015), it have been reported the level of soil pH can also determine the availability of certain nutrients (Agarry *et al.*, 2013). When the pH is low, the solubility of micronutrients in the soil is high whereas when the pH is high, micronutrient solubility and availability to plant will decrease (Brady and Weil 2002), and when the pH is extremely high or low crops that are planted on the soil will be negatively affected due to ionic strength imbalance (Kumar et al., 2011). The results of cation exchange capacity reveals that the pollutant decreased the value significantly, low value of CEC increases the rate of reduction in soil pH and this is in agreement with the low pH that was recorded in the soil. The low value of CEC increases the likelihood of the development of deficiencies in potassium (K^+), magnesium (Mg^2+) and other essential cations (CUCE, 2007). The results obtained at 4 weeks after bioremediation indicates that the CEC was recovering and it is a good indicator that bioremediation is taking place in the polluted soil. The soil electrical conductivity (EC) is the measure of the amount of salts in the soil. It is a good indicator of the health status of the soil (NRCS, 2013) and is commonly adopted method for salinity analysis due to its high sensitivity and ease of measurement (Zhu *et al.*, 2001). The soil electrical conductivity also recorded a decrement in the 2 weeks after pollution when compared to the control and afterwards at 4 weeks after bioremediation, the values was observed to be on the increase. The value of soil electrical conductivity increases as the concentration of the ion in the soil increases (Tales and Ingole, 2015). Hence, the decrease recorded indicates that the concentration of the soil ions was low.

The level of soil nitrogen increases significantly 2 weeks after pollution when compared to the pre-exposed soil, although the mean values decreased at 4 weeks after bioremediation. When carefully observed, one can note the rate of reduction in the soil nitrogen levels increased significantly when comparing group 1 to group 2 and group 3, this shows that an increase in the concentration and quantity of the treatment material increases the rate at which the process of bioremediation is taking place. The increase the was observed at 2 weeks after pollution have been reported by (Okunwaye *et al.*, 2018) to be as a result of an increment in the rate at which nitrogen fixation is taking place in the soil due to the actions of different microorganisms that are present in the polluted soil. The records of soil phosphorus among the different groups in 2 weeks after pollution was below detectable limits while in the 4 weeks after bioremediation, the values increased with significant different $p < (0.05)$ when comparing group 1, 2 and 3. The results reveals that group 3 which has the highest quantity of intestinal waste had the highest rate of recovery. However, the level is still lower than the permissive, as the phosphorus values is suggested to be more than 10 mg/kg for it to be considered suitable for crop production (FAO, 1976). But generally, the value of soil phosphorus in the pre-exposed soil and the control were within the permissive levels and not exceeding 20 mg/kg which is the highest tolerable limit of P for soils as stipulated by (Holland *et al.*, 1989; Okunwaye *et al.*, 2018).

The concentration of soil TOC, TPH and PAH were observed to be higher in the treated groups 2 weeks after pollution with subsequent significant reduction ($p<0.05$) in 4 weeks after remediation. This proves that the intestinal waste of cow has bio-stimulatory effects and is very effective in the reduction of the level of soil TOC, TPH and PAH after crude oil pollution. The results are in agreement with the works of different authors (Ogboghodo *et al.,* 2005; and Njoku *et al.,* 2009; Iris et al, 2018), however, it is important to note that the level of organic carbon in the soil also determines the soil properties such as water retention capacity and an increase in the level of organic carbon enhances the binding process of the soil. One of the constituents of crude oil that can be biodegraded and utilised by plants is TPH (Basumatary *et al.,* 2012).

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 using intestinal waste of cow which is an animal waste that is generated in abattoirs have been proven by this research to be effective in remediation, therefore should be adopted in the restoration of crude oil polluted soil.

REFERENCES

Adams, G.O., Fufeyin, P.T., Okoro, S.E. and Ehinomen, I. (2015). Bioremediation, Biostimulation and Bioaugumentation: *A review / international Journal of Environmental Bioremediation and Biodegradation ,3(1): 28-39.*

Adams, P.Z., Jackson, P. P. (1996). Bioremediation of oil spill. Theory and Practice. In Nigeria National Petroleum Corporation Publication. Department of Petroleum Resources. 3-10.

Adeniyi, E. O., Oluwole, B. A., Amyaye, G. K. (1983). Environmental and social economic impacts of oil spillage in the petroleum producing service area of Nigeria. Environmental Impact Statement and Assessment.8, 233-237.

Agarry, S.E., Aremu, M.O. and Aworanti, O.A. (2013). Kinetic modelling and half- life study on enhanced soil bioremediation of bonny- light crude oil amended with crop and animal derived organic waste. *Petroleum and Environmental Biotechnology.4:137-14.*

Atlas, R. M. (1981). Biodegradation of Petroleum Hydrocarbon: An Environmental Perspective Microbiological Review 45,180-209.

Basumatary B, Saikia R, Bordoloi S. (2012). Phytoremediation of crude oil contaminated soil using nut grass, cyperusrotundus. Journal of Environmental Biololgy.;33(5):891-896

Beckles, M. D., Ward, C. H., Hughes, J. E., (1998). Effect of mixtures of polycyclic aromatic hydrocarbons and sediments of fluoromethane biodegradation pattern Environ. Toxicol. Chem., 17, 1246-1257.

Brady C N, and Weil RR (2002). Nature and properties of soils, 2002,13th Ed. Prentice Hall

Chikere, C.B., Okoye, A.U. and Okpokwasili, G.C. (2016). Microbial community profiling of active oleophilic bacteria involved in bioreactor based crude oil polluted sediment treatment. *Journal of Applied and Environmental microbiology. 4(1): 1-20.*

Cornell University Cooperative Extension (CUCE) (2007) Cation Exchange Capacity (CEC). Agronomy Fact Sheet Series # 22. Department of Crop and Soil Sciences, College of Agriculture and Life Sciences, Cornell University

Dutta, T. K. and Harayama, S. (2001). Analysis of long side chain alkyl aromatics in crude oil for evaluation of their fate in the environment.Environ.Sci. Technol. 35, 102-107.

Ekperusi, O. A. and Aigbodion, I. F. (2015). Bioremediation of heavy metals and petroleum hydrocarbons in diesel contaminated soil with the earthworm: *Eudrilus eugeniae,* Springer Plus, 4: 540.

Ekpo, M. A., and Udofia, U. S. (2008). Rate of Biodegradation of Crude Oil by Microorganisms isolated from Oil Sludge Environment. Afr. J. Biotechnol. 7, 4495-4499.

Elektorowicz M (1994) Bioremediation of petroleum-contaminated clayey soil with pretreatment. Environ Technol15: 373-380.

FAO, (1976). A Framwork for Land Evaluation, FAO Bulletin 32, FAO/UNESCO, France

Frank, A.O., Abiye, A.I. and Philip, O.O. (2013). Bioremediation of petroleum hydrocarbon polluted mangrove swamps using nutrient formular produced from water Hyacint (*Eicchornia crassipes). American Jour. of Environmental sciences. 9(4):348-366*

Gerhart, P. Murray., R. R. E., Kneg, N. R., Phillips, C. B. (1981). Manual of Methods of General Bacteriology.American Society for Microbiology, Washington D.C. W. A.

Holland MD, Allen RKG, Barten D, and Murphy ST. (1989). Land Evaluation and Agricultural for Cross River National Park, Oban Division. Prepared by the Overseas Development National Resources Institute in Collaboration with WWF for the Federal Republic of and the Cross River State Government.;1189

Isirimaru, N. O., Zoufa, K.,Loganathan, P. (1989). Effect of Crude oil on maize performance and soil chemical properties in humid forest zone of Nigeria". Discovery and Innovation. 1, 95-98.

Jobson, A. M., Mclaughlin, M., Cook, F. D.,Westlake, D.W.S. (1974). Effects of amendment on microbial utilization of oil applied to soil, Appl. Microbiol. 27, 166-171

Keeney, D. R., and Nelson, D. W. (1994). Determination of numeral (inorganic) Nitrogen. In: Miller, A. L, and Keeney, D. R. (Ed.). Methods of Soil Analysis. Animal Science Publishers. Madison. 643

Kumar S, Iyer A, and Agarwal S, (2011). Cotton Yield In Relation To Physicochemical Properties Of Cultivated Soil Of Rajkot Region.Int.J.of Adv.Engi.Tech.,2(3),52-55.

Marshal. T. R. (1988). Biodegradation of Petroleum wastes in soil. The Microbial Ecosystem and the Optimization of a Treatment Process.DoctoralThesis.University of Southern California, California, USA.

Mentzer, E. and Ebere D. (1996). Remediation of hydrocarbon of hydrocarbon contaminated sites. Proceeding industry and the Nizena Enviornment, Nigerian National Petroleum (Corporation NNPC) 1:5-59

Nduka, J.K., Umeh, L., Okerulu, I.O., Umedum, L.N. and Okoye, H.N. (2012). Utilization of different microbes in bioremediation of hydrocarbon contaminated soils stimulated with organic and inorganic fertilizers. *Journal of petroleum Environmental Biotechnology. 3:116-125.*

Njoku KL, Akinola MO, Oboh BO. (2009). Phytoremediation of crude oil contaminated soil: The effect of growth of Glycine max on the physico-chemistry and crude oil content of soil. Nature & Science.;7(10):1-2

Nnadi P. C and Osakwe J. A. (2017). Bioremediation of Crude Oil Contamination Soil with Livestock Waste. International Journal of Agriculture and Earth Science Vol. 3 No. 7. 32-42.

NRCS (2013). Soil electrical conductivity. Soil quality kit guide for educators. Natural resources conservation service. *United States department of agriculture* p7 google scholar

Nwachukwu S.C.U and Ugorji, E.O (1995) Impacts of crude petroleum spills on microbial communities of tropical soils. International journal of ecology and environmental sciences 21:169-176

Nwankwegu, A.S., Onwosi, C.O., Orji, M.U., Anaukwu, C.G., Okafor, U.C., Azi, F., and Marins, P.E. (2016). Reclamation of DPK hydrocarbon polluted agricultural soil using a selected bulking agent. J.Environ. Manag. 172, 136-42.

Odgen, R., and Adams, D. A., (1989). Recombinant DNA Technology: Applications In: Carolina Tops, Vol. 52, Carolina Biological Supply company, Burlington, North Carolina. 18-19

Odu, C. T., Ogunbi, A. A., Fagade, O. F. (1989). Microbiology of Soil Contaminated with Petroleum Hydrocarbon in Natural Rehabilitation and Reclamation Petroleum Technology Publication, Warri Nigerian. 11-48

Ogboghodo, I. A., Azenabor, U. F. and Osemwota, I. O. (2005). Amelioration of Crude oil polluted soil with poultry manure and the effect on growth of maize and some soil properties. J. Plant Nutr.;28(1):21-32

Okpokwasili G. C. and Amanchukwu, S.C. (1988). Petroleum hydrocarbon degradation by Candida spp. Environmental interaction 14:243-247.

Okunwaye, I., Ogboghodo, I., Ewansiha, S. and Oriakpono, O. (2018). Biostimulation Potentials of Cow Dung on a Crude Oil Polluted Soil. International Journal of Plant & Soil Science 27(1): 1-11.

Oriakpono,O.E., Okunwaye, I. and Imafidor, O.H. (2018).A screen house experiment to evaluate the biostimulation potentials of cow dung on a crude oil polluted soil .*International Journal of Agriculture innovation and research vol.* 6(5): 2319-1473.

Perfumo, A., Banat, I. M., Marchant, R. and Vezzulli, L., (2007). Thermally enhanced approaches for bioremediation of hydrocarbon-contaminated soils. Chemosphere66(1): 179-184

Piehler, M.F., Swistak, J.G., Pinckney, J.L. and Paerl, H.W. (1999) Stimulation of Diesel Fuel Biodegradation by Indigenous Nitrogen Fixing Bacterial Consortia. Microb Ecol38: 69-78

Reisinger, H. J. (1995). Hydrocarbon bioremediation an overview. In: Hinchee. R. E., Kittel, J. A. and Reisinger, H. J.(Eds.). Applied Bioremediation of Petroleum Hydrocarbons. Battelle Press, Columbus. 7.

Rhykerd RL, Crews B, McInnes KJ, Weaver RW (1999) Impact of bulking agents, forced aeration and tillage on remediation of oil-contaminated soil.Bioresour Technol 67: 279-285.

Romantschuk, M., Sarand, I., Petanen, T., Peltola, R., Jonssonvihanne, M., and Koivula, T. (2000). Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. Environ. Pollut. 107, 179-185

Shahunthala D. Ramachandran, P. V. Hodson, C. W. and Khan, K. L. (2004). Oil dispersant increases PAH uptake by fish exposed to crude oil Ecotoxicology and Environmental Safety.;59:300-308.

SVMS (2001). SPDC people and environment annual report. Shell virtual media services (SVMS) of the shell petroleum development of Nigeria of Nigeria, pp.36-37

Tales, K. and Ingole, S. (2015). A Review on Role of Physico-Chemical Properties in Soil Quality. Chemical Science Review and Letters, 4(13), 57 – 66

Ugochukwu C. O., Michael U. O., Kingsley C.A., Nsikak S. A., Benjamin C. O., Onyedika I. O., and Nnenna C.N. O, (2016) “Bioremediation of Crude Oil-polluted Soil Using BroilerChicken Droppings.” Journal of Applied & Environmental Microbiology, vol. 4, no. 4: 75-84. doi: 10.12691/jaem-4-4-2.

Walkey A, and Black, C. A. (1934). An examination of the micro-kjeldahl method of determining soil organic matter and a proposed modification of chromic acid titration method. Soil Science.;37:29-38

Zhu, X.; Venosa, A.D.; Suidan, M.T. and Lee, K. (2001) Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands; U.S. Environmental Protection Agency, Cincinnati, OH.