

Biostimulation potentials of saw dust and cow blood on a spent engine oil polluted soil

Obemeata E. Oriakpono^{1,*}, Oluwatumise Adetayo¹ and Iris O. Oriakpono²

¹Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria.

²Department of Crop and Soil Science, Faculty of Agriculture, Rivers State University, P. M. B. 5080, Nkpolu-Oroworukwo, Rivers State, Nigeria.

ARTICLE INFO

Article history:

Received: 27 July 2021

Received in revised form:
17 September 2021

Accepted: 4 October 2021

Published: 25 February
2022

Edited by KC Chinsembu

Keywords:

Biostimulation potential

Cow blood

Saw dust

Spent engine oil

ABSTRACT

The study was carried out to examine the biostimulatory effect of saw dust and cow blood on remediation of soil polluted with spent engine oil. A completely randomized design with four treatments and three replicates each were used; three rates of saw dust and 1 litre of blood was applied to crude oil polluted soils. The results for soil physicochemical parameters showed significant variations ($P < 0.05$) as the levels of total organic carbon, total petroleum hydrocarbon, polycyclic aromatic hydrocarbon and nitrogen increased 2 weeks after pollution but decreased 4 weeks after remediation. The bacteria count at two weeks after pollution was (1.4×10^3 to 21.07×10^3) but increased at 4 weeks after remediation to (1.67×10^3 to 80.13×10^3). This study showed improved soil physicochemical properties after the application of saw dust and cow blood as biostimulatory agents on the soil polluted with spent engine oil.

© 2022 ISTJN. Published by ISTJN. All rights reserved.

1 Introduction

Crude oil and its accompanying refined petroleum products such as gasoline, diesel, petrol, lubricating oils and other products comprises of complex mixtures of organic compounds which have been reported to be toxic to plants (Adedokun and Ataga, 2007). These petroleum derivatives are observed to pollute the soils in municipal areas, around industrial plants and in regions where these petroleum and natural gas are obtained (Adam *et al.*, 2002; Clark, 2003; Ogbuehi *et al.*, 2011).

In addition, the processing, distribution and subsequent utilization of the petroleum product also contribute significantly to the pollution and contamination of the soil (Ayotamuno *et al.*, 2006; Ogbuehi *et al.*, 2011). One of the products of crude oil refinement that is generally used is engine oil which usually contains chemical additives such as amines, zinc, phenols, benzene, calcium, phosphorus, sulphur, barium, magnesium, and lead. These oils are mainly used for the purpose of providing a film between the different moving parts of engines and auto machines. This helps in the reduction of wear and tear and prevents the loss of power. In addition, it can also help in the prevention of corrosion of the different auto machine parts.

However, the problem arises due to improper disposal of the engine oil after use. These spent engine oil or lubricant are usually disposed into gutters, drainage, open vacant areas and farms. This indiscriminate disposal is usually done by mechanics and vehicle repairers after changing the oil of cars, trucks, and other power generating machines. Due to the increase in the number of vehicles on the roads and owners of power generating machines,

*Corresponding author: E-mail address: obemeata.oriakpono@uniport.edu.ng (O.E. Oriakpono)

these indiscriminate disposal is widespread across the country and is not localized like crude oil spill (Kalichevsky and Peters, 1960; Obidike, 1985; Atuanya, 1987; Odjegba and Sadig, 2002; Nwoko *et al.*, 2007). Soils that are contaminated with these oil is of important environmental concern as they are rendered unsuitable for agricultural purposes as it adversely affects the soil microorganisms, plants, and increases the risk of surface and ground water pollution due to the presence of highly toxic polycyclic aromatic hydrocarbons that are contained in the oils (Nwoko *et al.*, 2007; Adenipekun *et al.*, 2008; Ogbuehi *et al.*, 2011).

The presence of oil in the soil due to pollution hinders the normal exchange of oxygen between the soil and atmosphere, which is caused by the hydrophobic properties of oil (Atlas, 1977; Adedokun and Ataga, 2007). It has been reported that with a concentration of more than 3% of oil in an environment, the soil microorganisms and the growth of plants becomes severely affected. The germination of seeds is also negatively affected (Onuoha *et al.*, 2003; Odjegba and Sadig, 2002; Hazel, 2005; Adedokun and Ataga, 2007). Soil provides the vital macronutrients needed for the growth and development of plants, and these minerals are in ionized or solubilized form that is available for uptake by plants (Epstein, 1972; Nwoko *et al.*, 2007).

However, due to the presence of spent engine oil in the soil, the soil chemistry is negatively affected and this adversely influences the process of nutrient release and uptake. Studies carried out by different authors have indicated that there is a significant decrease in the growth of plants (Rowell, 1977; Kinghorn, 1983; De Song, 1980; Odjegba and Sadig, 2002; Nwoko *et al.*, 2007). Amongst the ways oil pollution affects the growth of plants is also by affecting soil aeration, as oil displaces air from pore spaces in addition to the increased demand for oxygen by the activities of oil-decomposing microorganisms (Gudin and Syrratt, 1975; Odjegba and Sadig, 2002). Baker (1970) also revealed that oil causes damage to the cell membranes and cause leakage of cellular content by penetrating and accumulating in plants. The presence of spent engine oil in the soil have also been reported to cause dehydration in plants and a decrease in the moisture content of cowpea seeds, reduction in protein and crude fibre contents of cassava and maize (Udo and Fayemi, 1975; Agbogidi *et al.*, 2007; Adenipekun *et al.*, 2008; Ogbuehi *et al.*, 2010, 2011).

Generally, soil is an essential component of our natural ecosystem as environmental sustainability is largely dependent on a sustainable soil ecosystem (Adriano *et al.*, 1998; Adedokun and Ataga, 2007). Whenever the soil gets polluted, the ecosystem will be altered and the different agricultural activities will be negatively affected, in addition, the protective functions of the soil ecosystem can be limited, disrupt the metabolic activity of the soil, reduce soil fertility and adversely influence plant production (Gong *et al.*, 1996; Wyszowski *et al.*, 2004; Ogbuehi *et al.*, 2011).

Nonetheless, efforts are being made to mitigate these effects and one of the widely used approach to the mitigation is bioremediation. For bioremediation to be efficient, the soil amendment or additives such as sawdust, peat, waste cotton, manure, fertilizers etc. are introduced to the soil in a bid to increase microbial activities. A soil amendment is any material that upon its introduction to the soil can enhance the physical properties of the soil, for instance water infiltration, drainage, water retention, permeability, aeration and structure of the soil (Davis and Wilson, 2005; Adedokun and Ataga, 2007).

This study evaluates the biostimulatory potentials of saw dust and cow blood in the bioremediation of soil polluted with spent engine oil. The physicochemical parameters of soil analysed were; pH, total organic content, nitrogen, carbon to nitrogen ratio, phosphorus, cation exchange capacity, electrical conductivity, total petroleum hydrocarbon and poly aromatic hydrocarbon while bacteria cells were also isolated.

2 Materials and methods

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

2.1 Treatments and experimental design

The experiment was carried out using a Randomized Complete Block Design (RCBD) with three replicates. Each replicate was made up of four beds each carrying a treatment. Each bed measured $1.0\text{ m} \times 1.0\text{ m}$. A total land size of 24.75 m^2 ($5.5\text{ m} \times 4.5\text{ m}$) was marked out for the study. Alleys of 0.5 m were left between plots, and 0.75 m between replicate to prevent treatment drift to adjacent plots.

After the preparation of beds, the soil was left for two weeks and treated with four rates (0, 1, 2 and 5 L) of spent engine oil. The spent engine oil was spilled on the surface of the soil in simulating what generally occurs in case of oil spills. Two weeks after spent engine oil pollution, 1 litre of cow blood was applied to the polluted soils alongside the application of four rates (0, 2, 5 and 7 kg) of saw dust to the polluted soils with the control having 0kg, while group 1, group 2 and group 3 were 2, 5 and 7 kg respectively. The saw dusts were thoroughly mixed with the soil using hand trowel to ensure uniform distribution within the soil. Each quantity of spent engine oil served as a treatment with the 0 mL treatment serving as the control.

2.2 Sampling

Soil samples were collected from the plots at three different times. The first was before spent engine oil application to ascertain the physicochemical nature of the unpolluted soil. Second was two weeks after pollution and third was one month (4 weeks) after remediation.

2.3 Determination of physicochemical parameters

Samples were collected, properly labelled, and then taken to the laboratory for analysis. In the laboratory, soil samples were air dried, passed through a 2 mm plastic sieve and analyzed. 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 of 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 1.

The Total Hydrocarbon (THC) and concentration of PAH in spent engine oil at each sampling period was determined gravimetrically by toluene extraction method described by (Adesodun and Mbagwu, 2008). Determination of exchangeable cations (Ca, Mg, K and Na) was by atomic absorption spectrophotometry. 30 mL of $\text{NH}_4\text{CH}_3\text{CO}_2$ (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, Ecstestr microprocessor series model).

2.4 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, 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 to nutrient agar slants, properly labelled and stored as stock cultures. The bacterial were then characterized using the schemes of Bergy's Manual of Determinative Bacteriology (Bergey and Holt, 1994).

2.5 Statistical analysis

Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) and Tukey test using Assistat en (2017). The data were expressed as mean \pm standard deviation, results were considered significant when p -value was less than 0.05 ($P < 0.05$).

3 Results

3.1 Total organic carbon (TOC) (%)

The values for TOC ranged from (2.23 - 3.24) in pre-exposed soil, (2.23 - 6.15) two weeks after pollution, and (2.56 - 5.24) in the 4 weeks after remediation (Table 1). The highest mean was (6.18 ± 0.02) recorded in group 1 in 2 weeks after pollution and the lowest mean was (2.23 ± 0.25) recorded in control pre-exposed soil and 2 weeks after pollution.

3.2 Total petroleum hydrocarbon (TPH)

The results of TPH ranged from (975.80 - 3213.65), with the highest been (3213.65 ± 5.37) at two weeks after pollution at group 3, and the lowest (975.80 ± 84.58) found in 4 weeks after remediation in group (Table 1). There was a significant difference ($P < 0.05$) when comparing the different results obtained in 2 weeks after pollution with that of 4 weeks after bioremediation.

3.3 Poly aromatic hydrocarbon (PAH)

The results for PAH as presented in Table 1 ranges between (495.74 - 1848.60), with the highest as (1848.60) found in group 3 at two weeks after pollution and the lowest is (495.74) in group 1 at four weeks after remediation. There was a significant difference ($p < 0.05$) when comparing the different results obtained in 2 weeks after pollution with that of 4 weeks after bioremediation.

3.4 Total nitrogen (TN) (%)

The TN content is presented in Table 2; it ranged from (0.54 - 0.57) in pre-exposed soil, (0.56 - 8.41) two weeks after pollution, and (0.58 - 6.13) at 4 weeks after remediation. The highest mean was (8.41 ± 0.02) recorded in group 1 at 2 weeks after pollution and the lowest mean was (0.54 ± 0.02) obtained in control in pre-exposed soil.

3.5 Carbon/nitrogen (C:N) ratio

C:N ratio content was with a higher value of (6.1) in group 2 and group 3 at 2 weeks after pollution (Table 2) as compared with those of the other treatments and a lower value of (3.1) at group 1 at 4 weeks after remediation. Statistical analysis for C:N ratio showed that there was a significant difference ($P < 0.05$) in C:N ratio between the pre-exposed soil and soil samples collected after 2 weeks after pollution and 4 weeks after remediation.

3.6 Soil phosphorus (mg/kg)

The values for phosphorus had a range of (2.16 - 20.51), with highest mean (20.51) seen in four weeks after remediation in group 3 (Table 2). The lowest was (2.16) in four weeks after remediation in group 3. The result indicated a significant difference ($P < 0.05$) when comparing pre-exposed soil to that of the 4 weeks after remediation.

3.7 pH

The mean pH ranges from (8.43 - 8.56) in pre-exposed soil, (8.54 - 6.67) two weeks after pollution, and (4.67 - 8.27) 4 weeks after remediation (Table 3). The highest mean was (8.56 ± 0.22) recorded in group 2 at pre-exposed soil and the lowest mean was (4.67 ± 0.23) recorded in group 1 at 4 weeks after remediation.

3.8 Cation exchange capacity (CEC) (meq/100g)

The CEC value ranged from (3.87 - 4.02) in pre-exposed soil, (0.84 - 3.88) two weeks after pollution, and (2.17 - 4.19) one month after remediation (Table 3). The highest mean was (4.19 ± 0.62) recorded in control at 4 weeks after remediation and the lowest mean was (0.84 ± 0.30) in group 2 at 2 weeks after pollution.

3.9 Electrical conductivity (EC)

The EC ranged from (19.41 - 20.04) in pre-exposed soil, (11.89 - 19.41) two weeks after pollution, (12.61 - 20.27) 4 weeks after remediation (Table 3). The highest mean was (20.51 ± 0.53) recorded in group 3 at pre-exposed soil and the lowest mean was (11.89 ± 0.95) recorded in group 2 at weeks after pollution. There was a significant difference ($P < 0.05$) when comparing the results in 2 weeks after pollution with 4 weeks after remediation.

3.10 Effect on Bacteria population (cfu/g)

The result for bacteria count ranged from (1.4×10^3 to 1.4×10^3) in pre-exposed soil, (1.4×10^3 to 21.07×10^3) two weeks after pollution, and (1.67×10^3 to 80.13×10^3) 4 weeks after remediation (Table 3). The highest bacteria count was (80.13×10^3) recorded in group 3 at 4 weeks after remediation and the lowest bacteria count was (1.4×10^3) recorded in the different groups and control at pre-exposed soil. The bacteria were identified as *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Clostridium* and *Nocardia* species. Four isolates were gram-negative while four isolates were gram-positive. Six were rod-shaped, one was in coccus form and one was in a spherical form.

4 Discussion

In this study, the results of TOC, TPH and PAH increased significantly ($P < 0.05$) upon the application of spent engine oil in the soil when compared to the pre-exposed soil. In TPH and PAH, the values were below detectable limits in the pre-exposed soil and the control. The increase in the levels of TOC, TPH and PAH can be attributed to the presence of hydrocarbon in the spent engine oil as engine oil is a derivative of crude oil and is in agreement with the study carried out by Ogboghodo *et al.* (2005); Njoku *et al.* (2009) and Iris *et al.* (2018).

The results obtained 4 weeks after the application of the amendment material which are saw dust and cow blood showed that the level of TOC, TPH and PAH were all reduced significantly ($P < 0.05$) when compared to the results of week 2 after pollution and this indicates that the process of biodegradation of the hydrocarbons is taking place and given sufficient time, the levels will be within the permissive concentrations. At appropriate concentrations, TOC, which is essential in the soil can be utilised by plants; TPH can also be biodegraded and the product can also be utilised by plants unlike PAH (Basumatary *et al.*, 2012).

The level of soil nitrogen increased in the 2nd week after pollution when compared to the pre-exposed soil while after 4 weeks of post remediation, the levels decreased significantly ($P < 0.05$) and the result obtained is similar with the reports of Iris *et al.* (2018) as they also recorded an increase in soil nitrogen in soils contaminated with crude oil. Iris *et al.* (2018) proposed that the increase in nitrogen level may be as a result of the fixation of atmospheric nitrogen by the microbes which assimilate the hydrocarbons. The level of carbon to nitrogen ratio was observed to increase in the 2nd week after pollution and this ratio might have been influenced by the presence of hydrocarbon in the soil.

The soil phosphorus was recorded to be below detectable levels in week 2 after pollution and this indicates that the pollutant has a strong negative effect on the availability of the soil phosphorus as observed in the 4th

week after bioremediation where the level of phosphorus was recorded albeit significantly lower than the control ($P < 0.05$). According to Wyszokowska and Kucharski (2000), the changes in soil properties caused by the presence of pollutants that are petroleum-derived can lead to deficit in the level of soil water and oxygen and also a shortage to available forms of phosphorus.

The level of soil phosphorus recorded in the 4th week after remediation was still less than the permissive concentration, as the proposed suitable concentration of phosphorus for crop production is > 10 mg/kg (FAO, 1976). The value of soil phosphorus recorded in the pre-exposed soil and the control were within the recommended levels and did not exceed 20 mg/kg which is the highest tolerable concentration of phosphorus. (Holland *et al.*, 1989; Iris *et al.*, 2018).

The soil pH level was observed to decrease in the 2nd week after pollution with spent engine oil when compared to the pre-exposed soil and the control; the decrement was also recorded at week 4 after remediation. The alteration in the mean pH value can be due to the different metabolic activities that are on-going in the soil during the process of biodegradation of the hydrocarbons in the soil. (Frank *et al.*, 2013).

The recommended pH for soils is within a range of 6-8.5 (Tales and Ingole, 2015). This range is important because pH plays a vital role in the availability of plants nutrients and is essential in regulating the conditions of soil flora and fauna (Agarry *et al.*, 2013; Ekperusi and Aigbodion, 2015). Brady and Weil (2002) reported that when the level of soil pH is low, the solubility of micronutrients in the soil will increase while the reverse will be the case when the pH is high. Extremely high or low pH will cause an ionic imbalance which will negatively affect the crops (Kumar *et al.*, 2011).

The CEC and the soil electrical conductivity were both negatively influenced by the presence of spent engine oil in the soil as indicated by the result in the 2nd week after pollution while the levels significantly increased ($P < 0.05$) at 4 weeks after remediation, revealing that the process of biodegradation of the hydrocarbons are responsible for these adverse effects. The rate of reduction in soil pH increases when the CEC is low, in addition, lower value of CEC increases the risk of developing deficiencies in potassium (K^+), magnesium (Mg^{2+}) and other essential cations (CUCE, 2007). CEC greatly influences the soil structure and stability, it also influences the availability of nutrients, and the soil's reaction to ameliorants (Hazelton and Murphy, 2007).

Soil EC, which is a measure of the amount of salts in the soil, is a good indicator of the soil health status (NRCS, 2013) and is a generally applied method for analysing the salinity of the soil due to its high sensitivity and ease of measurement (Zhu *et al.*, 2001). The value of soil EC is known to increase as the concentration of the ion in the soil increases, and conversely decrease as the concentration of the ion in the soil decreases (Tales and Ingole, 2015). The bacteria count in the treated groups 2 weeks after pollution was higher compared to the control while the population increased further in the 4th week after remediation. This increment can be attributed to the process of biodegradation of the hydrocarbons in the soil.

The result for bacteria count which increased after remediation is in agreement with the works of Iris *et al.* (2018). The overall improvement in the soil physicochemical properties indicates that saw dust and cow blood is a good biostimulant and is in agreement with the studies carried out by Starbuck (1994); Davis and Wilson (2005) and Adedokun and Ataga (2007).

5 Conclusion

The results of this study reveal that the use of saw dust and cow blood as amendment materials for the bioremediation of a soil polluted with spent engine oil is effective in the recovery of the soil.

References

- Adam, G., Gamoh, K., Morris, D.G., Duncan, H., 2002. Effect and alcohol addiction on the movement and petroleum hydrocarbon fuels in soils. *Science of the Total Environment* 286(113), 15–25.
- Adedokun, O.M., Ataga, A.E., 2007. Effects of amendments and bioaugmentation of soil polluted with crude oil, automotive gasoline oil, and spent engine oil on the growth of cowpea (*Vigna unguiculata* L. Walp). *Scientific Research and Essay* 2(5), 147–149.
- Adenipekun, O., Oyetungi, O.S., Kassim, C.Q., 2008. Effect of spent engine oil on the growth parameters and chlorophyll content of *Corchorus olitonus*. *Environmentalist* 28, 446–450.
- Adesodun, J.K. Mbagwu, J.S.C., 2008. Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal dropping. *Bioresource Technology* 99(13), 5659–5665.
- Adriano, D.C., Chlopecka, A., Kaplan, K.I., 1998. Role of soil chemistry in soil remediation and ecosystem conservation. *Soil Science Society of America Journal Special Publication*, Madison, WI., pp. 361–386.
- Agarry, S.E., Aremu, M.O., 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, 137–147.
- Agbogidi, O.M, Erutor, P.G, Appanbi, S.O., Nagi, G.U., 2007. Evaluation of crude oil contaminated soil on the mineral nutrient element of maize (*Zea mays*). *Journal of Agronomy* 6(1), 188–193.
- Atlas, R.M., 1977. Simulated Petroleum Biodegradation. *Critical Review in Microbiology* 5, 371–386.
- Atuanya, E.I., 1987. Effect of oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated Delta soil in Bendel State. *Nigerian Journal of Applied Science* 5, 155–76.
- Ayotamuno, S.M, Kogbara, R.B., Taleat, M.O., 2006. Bioremediation of Petroleum hydrocarbon polluted agricultural soil at different levels of water application in Port-Harcourt, Nigeria. *Journal of Food and Agriculture* 4(3), 214–217.
- Baker, J.M., 1970. The effect of oil on plants. *Environ. Pollut.* 1, 27–44.
- Basumatary, B., Saikia, R., Bordoloi, S., 2012. Phytoremediation of crude oil contaminated soil using nut grass, *Cyperus rotundus*. *Journal of Environmental Biology* 33(5), 891–896.
- Bergey, D.H., Holt, J.G., 1994. *Bergey's Manual of Determinative Bacteriology*. ninth ed. Williams and Wecket, Baltimore, Washington DC.
- Brady, C.N., Weil, R.R., 2002. *Nature and properties of soils*, thirteenth ed. Prentice Hall.
- Bray, R.H., Kurtz, L.T., 1945. Determination of total organic and available form of phosphorus in soils. *Soil Science* 59(1), 39–46.
- Clark, C.S., 2003. Field detector evaluation of organic clay soils contaminated with diesel fuel. *Florescence* 4(3), 167–173.
- 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.
- Davis, J.G., Wilson, C.R., 2005. Choosing a Soil Amendment. *Colorado State University Cooperative Extension Horticulture* 7, 235.
- De Song, E., 1980. The effect of a crude oil spill on cereals. *Environmental Pollution* 22, 187–96.
- Ekperusi, O.A., Aigbodion, I.F. 2015. Bioremediation of heavy metals and petroleum hydrocarbons in diesel contaminated soil with the earthworm: *Eudrilus eugenia*. *Springer Plus* 4, 540.
- Epstein, E., 1972. *Mineral Nutrition of plants: Principles and perspective*. John Wiley and Sons Inc., New York, pp. 412.

- Food and Agriculture Organization (FAO), 1976. A Framework for land evaluation, FAO Bulletin 32, FAO/UNESCO, France.
- Frank, A.O., Abiye, A.I., Philip, O.O., 2013. Bioremediation of petroleum hydrocarbon polluted mangrove swamps using nutrient formula produced from water Hyacinth (*Eicchornia crassipes*). American Journal of Environmental Sciences 9(4), 348–366.
- Gong, P., Sun, T.H., Beudert, G., Hahn, H.H., 1996. Ecological effect of combined organic and inorganic pollution on soil microbial activities. Water, Air and Soil Pollution 96, 133–143.
- Gudin, C., Syrratt, W.J., 1975. Biological aspects of land rehabilitation following hydrocarbon contamination. Environmental Pollution 8, 107–112.
- Hazel, W., 2005. Suck it up: Phytoremediation. Organic Ade (online). Available at <http://ourgardengang.tripod.com/organicade.htm> (Accessed 15 June, 2006).
- Hazelton, P.A., Murphy, B.W., 2007. Interpreting soil test results: What do all the numbers mean? CSIRO Publishing, Melbourne.
- Holland, M.D., Allen, R.K.G., Barten, D., Murphy, S.T., 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 Nigeria and the Cross River State Government, 1189.
- Iris, O., Ogboghodo, I., Ewansiha, S., Oriakpono, O., 2018. Biostimulation potentials of cow dung on a crude oil polluted soil. International Journal of Plant and Soil Science 27(1), 1–11.
- Kalichevsky, V.A., Peters, E.H., 1960. Petroleum, our man of all work. The raw material, crude petroleum and natural gas, in: Guthrie, V.B. (Ed.), Petroleum Products Handbook, McGraw-Hill Book Company, New York, pp. 1–22.
- Kinghorn, R.R.F., 1983. An introduction to the physics and chemistry of petroleum. John Wiley and sons NY, 34.
- Kumar, S., Iyer, A., Agarwal, S., 2011. Cotton yield in relation to physicochemical properties of cultivated soil of Rajkot Region. International Journal of Advanced Engineering and Technology 2(3), 52–55.
- Njoku, K.L., Akinola, M.O., Oboh, B.O., 2009. Phytoremediation of crude oil contaminated soil: The effect of growth of glycine max on the physicochemistry and crude oil content of soil. Nature and Science 7(10), 1–2.
- NRCS., 2013. Soil electrical conductivity. Soil quality kit guide for educators. Natural resources conservation service. United States department of agriculture p7 google scholar.
- Nwoko, C.O., Okeke, P.N., Agwu, O.O., Akpan, I.E., 2007. Performance of Phaseolus vulgaris L. in a soil contaminated with spent engine oil. African Journal of Biotechnology 6 (16), 1922–1925.
- Obidike, D.I., 1985. Oil spill contingency planning Proc. Int. Seminar Pet. Ind. and the Nigerian Environment, 11–14 Nov. 1985, pp.145–56.
- Odjegba, V.J., Sadiq, A.O., 2002. Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. Environmentalist 22, 23–28.
- Ogboghodo, I.A., Azenabor, U.F., Osemwota, I.O., 2005. Amelioration of crude oil polluted soil with poultry manure and the effect on growth of maize and some soil properties. Journal of Plant Nutrition 28(1), 21–32.
- Ogbuehi, H.C., Onuh, M.O., Ezeibekwe, I.O., 2011. Effects of spent engine oil pollution on the nutrient composition and accumulation of heavy metal in cowpea [*Vigna unguiculata* (L.) Walp]. Australian Journal of Agricultural Engineering 2(4), 110–113.
- Ogbuehi, H.C., Ezeibekwe, I.O., Agbakwuru, U., 2010. Assessment of crude oil pollution the proximate composition and macro element of cassava crop in Owerri, Imo State. International Journal Science and Resource (2), 62–65.

- Onuoha, C.I., Arinze, A.E., Ataga, A.E., 2003. Evaluation of growth of some fungi in crude oil polluted environment. *Global Journal Agricultural Science* 2, 1596–2903.
- Rowell, M.J., 1977. The effect of crude oil spills on soils: In the reclamation of agricultural soils after oil spills, in: Good, J.A (Ed.). Edmonton: Department of Soil Science, University of Alberta, pp. 1–33.
- Starbuck, C. 1994. Using sawdust as a soil amendment. *Applying Research in Forestry*. p. 6.
- Tales, K., Ingole, S., 2015. A review on role of physicochemical properties in soil quality. *Chemical Science Review and Letters* 4(13), 57–66.
- Udo, E.J., Fayemi, A.A., 1975. The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. *Journal of Environmental Quality* 4, 537–440.
- Walkey, A., 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.
- Wyszkowski, M., Wyszokowska, S., Ziochowska, A., 2004. Effect of soil contamination with diesel oil on yellow lupine, field and macroelements content. *Plant Soil Environment* 50(5), 218–226.
- Wyszokowska, S., Kucharski, S., 2000. Biochemical properties of soil contaminated by petrol. *Polish Journal of Environmental Studies* 9(6), 476–485.
- Zhu, X., Venosa, A.D., Suidan, M.T., Lee, K., 2001. *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*; U.S. Environmental Protection Agency, Cincinnati, OH.

Table 1: 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	2.23 ± 0.25 ^{aA}	2.23 ± 0.25 ^{aA}	2.56 ± 0.26 ^{aA}	BDL	BDL	BDL	BDL	BDL	BDL
Group 1	2.36 ± 0.64 ^{aA}	6.18 ± 0.02 ^{bC}	5.31 ± 0.51 ^{bB}	BDL	2707.5 ± 1503 ^{bB}	975.80 ± 84.58 ^{bA}	BDL	1067.1 ± 103.8 ^{bB}	495.74 ± 59.9 ^{cA}
Group 2	3.02 ± 0.36 ^{aA}	6.15 ± 0.00 ^{bC}	5.24 ± 0.26 ^{bB}	BDL	3167.5 ± 80.68 ^{cB}	1403.5 ± 134.24 ^{cA}	BDL	1832.1 ± 174.1 ^{cB}	759.85 ± 100.5 ^{cA}
Group 3	3.24 ± 0.61 ^{bA}	6.15 ± 0.003 ^{bB}	5.60 ± 0.19 ^{bC}	BDL	3213.6 ± 5.37 ^{cB}	1561.9 ± 147.91 ^{cA}	BDL	1848.6 ± 441.0 ^{cB}	863.09 ± 254.6 ^{cA}

^{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 2: 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	0.54 ± 0.02 ^{aA}	0.56 ± 0.05 ^{aB}	0.58 ± 0.19 ^{aA}	5.1 ^{aA}	5.1 ^{aA}	5.1 ^{aA}	19.31 ± 0.21 ^{aA}	19.31 ^{bA}	20.34 ± 0.10 ^{bB}
Group 1	0.55 ± 0.04 ^{aA}	8.41 ± 0.02 ^{bC}	6.13 ± 0.02 ^{bB}	5.1 ^{aA}	5.1 ^{bC}	3.1 ^{bB}	20.07 ± 1.13 ^{aB}	BDL	2.78 ± 0.64 ^{aA}
Group 2	0.55 ± 0.02 ^{aA}	8.37 ± 0.04 ^{bC}	6.08 ± 0.69 ^{bB}	5.1 ^{aA}	6.1 ^{bC}	4.1 ^{bB}	20.37 ± 1.05 ^{aB}	BDL	2.36 ± 0.83 ^{aA}
Group 3	0.57 ± 0.03 ^{aA}	8.40 ± 0.03 ^{bC}	6.08 ± 0.84 ^{bB}	5.1 ^{aA}	6.1 ^{bC}	4.1 ^{bB}	20.51 ± 0.68 ^{aB}	BDL	2.16 ± 0.48 ^{aA}

^{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 3: Effect of the Remediation Amendments on the Soil pH, CEC, Conductivity and Bacteria Count.

	pH			CEC (meq/100g)			Conductivity			Bacteria Count (cfu/g)		
	Pre-exposed Soil	2 weeks after pollution	4 weeks after remediation	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	8.54 ± 0.008 ^{aA}	8.54 ± 0.008 ^{bA}	8.27 ± 0.60 ^{bA}	3.87 ± 0.48 ^{aA}	3.88 ± 0.51 ^{bA}	4.19 ± 0.62 ^{bB}	19.41 ± 0.18 ^{bA}	19.41 ± 0.18 ^{bA}	20.27 ± 0.19 ^{bB}	1.4 × 10 ³	1.4 × 10 ³	1.67 × 10 ³
Group 1	8.43 ± 0.06 ^{aC}	6.67 ± 0.07 ^{aB}	4.67 ± 0.23 ^{aA}	4.02 ± 0.33 ^{aC}	0.85 ± 0.25 ^{aA}	2.55 ± 0.37 ^{aB}	20.04 ± 0.69 ^{aC}	11.96 ± 0.50 ^{aA}	13.62 ± 0.54 ^{aB}	1.4 × 10 ³	21.07 × 10 ³	49.73 × 10 ³
Group 2	8.56 ± 0.22 ^{aC}	6.69 ± 0.96 ^{aB}	4.68 ± 0.18 ^{aA}	4.34 ± 0.26 ^{aC}	0.84 ± 0.30 ^{aA}	2.55 ± 0.11 ^{aB}	20.11 ± 0.69 ^{aC}	11.89 ± 0.95 ^{aA}	13.13 ± 0.69 ^{aB}	1.4 × 10 ³	18.20 × 10 ³	69.40 × 10 ³
Group 3	8.43 ± 0.13 ^{bC}	6.68 ± 0.13 ^{aB}	4.84 ± 0.52 ^{aA}	4.51 ± 0.12 ^{aC}	0.86 ± 0.30 ^{aA}	2.17 ± 0.76 ^{aB}	20.51 ± 0.53 ^{aC}	11.99 ± 0.31 ^{aA}	12.61 ± 0.31 ^{aB}	1.4 × 10 ³	15.83 × 10 ³	80.13 × 10 ³

^{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