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**Bioremediation of Soils Contaminated with Hydro Carbon  
(Oil Spillage) in Nigeria**

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**Abstract**

The aim of the study was to determine the rate at which bioremediation of hydrocarbon occurs in a polluted soil medium and also to examine the effects of organic substrate such as cow dung on the bioremediation processes. A pot experiment (CRD) was conducted for four weeks on two group bases, group 1 contained the polluted soil and group 2 contained polluted soil amended with organic substrate (cow dung). In the group 1 experiment, the nitrate concentration of 8.4 mg/g at zero day of the experiment to 6.24mg/g at week 4, phosphate concentration decreased from 5.2mg/g to 4.4mg/g, TOC reduced from 3.9% to 2.28%, pH reduced from 7.8 to 7.5 and the total petroleum hydrocarbon reduced from 0% to 18% to 22% to 29% and 29% at week zero, week 1, week 2, week 3 and week 4 respectively. Group 2 experiment, the pH increased from 6.8 to a neutral range of 7.1 at week 4, the nitrate concentration, phosphate concentration and TOC reduced drastically from week zero to week four at 14.72mg/g to 4.2mg/g, 10.81mg/g to 2.42mg/g, and 6.44% to 1.28% respectively. The TPH in the group 2 experiment shows a loss in TPH of 32%, 68%, 89% and 98% at week 1, week 2, week 3 and week 4 respectively. The study showed that a natural bioremediation process occurs in the soil and that the bioremediation process could be further enhanced by amending the polluted soil with organic substrates such as cow dung.

Key-Words: Bioremediation, Hydrocarbon, Organic substrate

**Introduction**

The current trend of environmental pollution has raised a great deal of concern over the decades; the safe environment has so far suffered much decadence from the use of hazardous chemicals which are deleterious to lives of both plants and animals. The deleterious effect of pollutants on the environment has led to increased awareness and vigilance against contamination of the Niger Delta Environment. In Nigeria, in relatively recent time, there has been remarkable increase in population, urbanization and industrial activities, (Eze and Okpokwasili, 2010). Pollution caused by petroleum and its derivative is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving world-wide attention (Millioli *et al.* 2009). Therefore in the pursuit of solving the menace, a microbial approach has been developed towards the remediation of soils contaminated to reclaim and stabilize the soil, this method is known as bioremediation. Nigeria's economy is heavily dependent on the oil sector, which accounts for 90- 95% of export revenue. Estimates of Nigeria's estimated proven oil reserve range from 24 billion (Oil and Gas journal) to 31.5 billion barrels (OPEC).

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Nigeria has more than 182 tcf of recoverable gas reserves, which are expected to last for 450 years. The quantity of gas reserves in the country is twice as much as Nigeria's considerable crude oil reserves. However, only 23 tcf, or 12 % of the gas reserves, is so far being exploited. The nation has an estimated and additional undiscovered gas potential of about 100 tcf, making Nigeria one of the world's leading gas producers (EIA, 2003). And as a result of the high crude oil potential, a conscious effort should be made to remedy the end product of our resource and save the succeeding generation from the environmental adverse effects of our explorations and this is best achieved by the process known as bioremediation and its accessories. According to the department of petroleum resource (DPR), between 1979 and 1996 a total of 4647 incidence resulted in the oil spill of approximately 2,369,470 barrels of oil into the environment. Of this quantity, an estimated 1,820,410.5 barrels (77%) were lost to the environment. Available records for the periods 1976 to 1996 indicated that approximately 6%, 25% and 69% respectively, of total oil spilled were in land, swamps and offshore environment. The heaviest recorded oil spill so far in Nigeria occurred in 1979 and 1980 with a net volume of 694,117.13 barrels and 600,511.02 barrels, respectively. This statistics shows the need to reclaim the lost environment and the only

environmentally friendly and cost effective method is bioremediation. This study was designed to achieve the following objectives: To evaluate the fastest means of bioremediation process, to evaluate the use of organic substrates in the bioremediation process of hydrocarbons and to evaluate the most environmentally friendly technique of bioremediation of hydrocarbons (Oil Spillage).

### Material and Methods

**Study Area Description:** The hydrocarbon polluted soil was obtained at the automobile mechanic's shop in Anyigba Kogi state. The project was carried out at the Department of Soil and Environmental management laboratory in the Faculty of Agriculture, Anyigba Kogi State. (Lat.7.06N and Long. 6.43E) located within the South Eastern Guinea savannah Agro-ecology of Nigeria (Kowal and Knabe, 1972).

The site lies within the warm humid climate of the middle belt zone of Nigeria with clear, distinctively dry and wet seasons. The mean annual rainfall is 1260mm with peaks in the month of July and September. Temperature shows some variation throughout the year, with average monthly temperature varying between 17°C and 36.20°C (Amhakhian *et al.*, 2010).

**Sample Collection:** The samples were collected with a spade into a plastic bucket which was cleaned with cotton soaked in 70% alcohol (Eziuzor and Okpokwasili, 2009). Four sampling points were mixed together after the excavations. The excavated soil samples were transported to the Environmental laboratory of the Department of Soil and Environmental Management, Faculty of Agriculture, Kogi State University Anyigba.

**Experimental Design:** This is a laboratory scale experiment carried out in plastic pots; the experiment has three (3) replicates and five (5) treatments. The experimental design is complete randomized design (CRD) and it has two (2) experimental groups

Group A= 500 g of polluted soil + 50 g of dried cow dung; Group B= 500 g of polluted soil + no nutrient

**Preparation of Cow Dung:** Cow dung of about 3 kg was obtained from the Animal Research farm at the cattle section and was transported to the Environmental laboratory of the Soil and Environmental Department. The cow dung was sun dried for five days until was completely dried. The cow dung was stored for usage.

**Physico-Chemical Analysis:** The pH of samples at week 0, week 1, week 2, week 3, week 4 of the study were determined using a digital pH meter (Jenway 3015, united kingdom). At each point, three values were obtained and the mean values were used.

**Determination of the nitrate(NO<sub>3</sub><sup>-</sup>) level of polluted soil** was determined using microKjeldahl method

(Bremner and Mulvaney, 1982) **Determination of the phosphate content of polluted soil** was colorimetric method as described in United Nations Environmental Programme (UNEP, 2004). Total organic carbon is an alternative method for measuring petroleum hydrocarbon using the wet oxidation techniques as previously reported by Nelson and Sommers (1975). Calculation was as follows:

$$\% \text{TOC} = \frac{\text{titre value of blank} - \text{sample titre}}{\text{sample weight}} \times 0.003 \times 100$$

**Total petroleum hydrocarbon (TPH)** was done with Dichloromethane (DCM) using cold extraction method with ASTM-D-3694 heavy machine for 1 hour (Saari *et al.*, 2007). **2.5.6 Calculation of percentage loss in TPH.** This was calculated using this formula;

$$\% \text{ loss in TPH} = \frac{\text{concentration at a point} - \text{concentration at week zero}}{\text{concentration at week zero}} \times 100$$

Data collected were subjected to Analysis of Variance (ANOVA) using SPSS software (version 17.0) to test for significant differences. Where significant differences were observed, Duncan Multiple Range Test (DMRT) was used to rank the means at 95% confidence level.

### Results and Discussion

#### Physico-Chemical Analysis of The Polluted Soil

**Without Amendment:** The pH of the soil was found to be 7.8 at week zero and towards the 4<sup>th</sup> week, there was a slight decline in the pH content to 7.5. This decrease was due to the production of metabolites in the process of bioremediation as also reported by (Zhu *et al.*, 2001). Abu and Akomah (2008) reported that the decrease in pH was as a result of uptake of exchangeable ions by microorganisms. The nitrate content of the soil also decreased from 8.4 mg/g at week zero to 6.24 mg/g at the fourth week of the experiment. The phosphorus content of the study sample was also measured to be 5.2 mg/g at week zero, there after the 4<sup>th</sup> week, it reduced to 4.4 mg/g. The decrease in phosphorus and nitrate content was due to the use of both nitrate and phosphorus as source of nutrients as reported by Abu and Ogiji (1996). (Zhu *et al.*, 2001). The total organic carbon of the study sample was 3.90 mg/g and after the fourth week of the experiment, it reduced to 2.48 mg/g. This reduction was due to the use of carbon as source of energy as reported by (Ibiene *et al.*, 2011).

**Table 1: The physico-chemical analysis of the polluted soil without amendment**

Properties	Week 0	Week 4
pH	7.8	7.5
Nitrate(mg/g)	8.4	6.24
Phosphate(mg/g)	5.2	4.4
Total organic carbon (%)	3.9	2.48

The total petroleum hydrocarbon content of the soil was measured to be 3332 ppm, 2699.33 ppm, 2574 ppm, 2340 ppm and 2351.67 ppm at week zero, week 1 week 2 week 3 and week 4 respectively.

Table 2 shows the mean concentrations of petroleum hydrocarbons in the sample and the statistical analyses showing the significant differences at different intervals of time (treatments).

From the below table obtained from the study, it shows that the concentration of the petroleum hydrocarbon had reduced to a concentration of 2340 ppm at week 3. It also shows the progressive reduction in the TPH, the result of the ANOVA test show that the experiment is highly significant at 5% level of significance. The experiment shows a significant reduction in total petroleum hydrocarbon from 3332 ppm at 0 week to 2340 ppm at week 3. But there was a slight fluctuation in the process at week 4 of the experiment and this makes week 3 and week 4 non-significant.

**Table 2: Showing the chemical analysis of soil containing spent engine oil**

Treatments	Mean Value (ppm)
Week 0	3332 <sup>a</sup>
Week 1	2699.33 <sup>b</sup>
Week 2	2574 <sup>c</sup>
Week 3	2340 <sup>d</sup>
Week 4	2351.67 <sup>d</sup>
Significance	**
LSD (0.05)	144.19
C.V	3.0%

From the table above, week Zero to week 3 means were significant, week zero to week one shows the highest significant to bioremediation with total petroleum hydrocarbon loss of 18% and thereafter 22%, 29% and 29% for the week 2 week 3 and week 4 respectively.

**Physico-Chemical Analysis of Polluted Soil Amended With Cowdung**

The pH of the soil was found to be at 6.8 at week zero of the study and thereafter 7.1 after week 4 of the study. This is in alignment with the neutral range of PH which optimum for agronomic use. The nitrate level of the study sample is found to be 14.72 mg/g at week zero and thereafter week 4 of the study it reduces to 4.2 mg/g. The decrease in nitrate level is due to high degradation activity of microbes. The phosphate level of the study sample is found to be 10.81 mg/g at week zero after the incorporation of cow dung and thereafter at week 4 it reduced drastically to 2.42 mg/g.

The decrease in nitrate and phosphate level is attributed to the fact that they were been used in the metabolism of organism in building biomass. There is a positive

correlation in the utilization of both nitrate and phosphate and this indicate their importance in cell metabolism. It was establish that the availability of nitrogen and phosphorus limit the microbial degradation of hydrocarbon (Abu and Ogiji, 1996; Zhu *et al.*, 2001)

Total organic carbon rose to 6.44% at amendment with cow dung at first measurement at week zero and it reduced to 1.28%. The reduction is as a result of the degradation by microorganism that degrade hydrocarbon and use carbon as source of energy (Ibiene *et al.*, 2011). The reduction in total organic carbon is also an analytical method of determination percentage loss of Total petroleum hydrocarbon as reported by Nelson and Sommers (1975).

**Table 3: The physico-chemical properties of polluted soil amended with cow dung**

Descriptions	At week zero	At week4
pH	6.8	7.1
Nitrate (mg/g)	14.72	4.2
Phosphate (mg/g)	10.81	2.42
Total organic carbon (%)	6.44	1.28

**Total Petroleum Hydrocarbon**

From the experimental study, it was observed that the mean concentration of total petroleum hydrocarbon reduced from 33320.67 ppm to 2240.67 ppm to 1050 ppm to 360.3 ppm to 35.3 ppm at week zero, week 1, week 2, week 3 and week 4, respectively, as shown in the Table 4 below

**Table 4: The petroleum hydrocarbon content (ppm) of soil under a period of 4 weeks**

Treatment	Mean value (ppm)
Week Zero	3320.67 <sup>a</sup>
Week One	2240.67 <sup>b</sup>
Week Two	1050 <sup>c</sup>
Week Three	360.3 <sup>d</sup>
Week Four	35.3 <sup>e</sup>
Significance	**
LSD (0.05)	37.40
C.V	1.47%

From the above table, there were sharp declines in the oil content of the study sample. The second columns shows the progressive decline in the petroleum content of the test sample, week 4 shows a high level of degradation with a reduction of 3324 ppm at week zero to 36 ppm at week 4. This is in alignment with (Chikere *et al.*, 2009) which had a reduction of TPH 3666.0 mg/g of soil to 135.01 mg/g of soil. The above table shows the significance of the experiment. The experiment is highly significant at 5% level of significant

Table 4 above shows the Duncan ranking for all the treatments of the study.

The Duncan ranking shows that all the treatments were significant due to the progressive reduction in petroleum content from week zero to week four without much fluctuations as seen in group A above. There was a high decline in the level of the hydrocarbon at week 1 of the experiment with a total petroleum hydrocarbon loss of 32%, week 2 to be 68%, week 3 to be 89% and week 4 to be 98%. After the week four of the experiment, the petroleum hydrocarbon content of the soil had reduced to a minimal level and therefore leaves the soil in a state favourable for Agronomic uses

### Conclusion

The result of this research has proven that there exist a natural bioremediation process in the soil but this bioremediation is subject to variations and fluctuation due to certain environmental factors that affects microbial activities of the soil. Also the research shows that the addition of cow dung or organic matter can stabilise the microbial activity and place the soil under a stable hydrocarbon degradation leading to the bioremediation of the polluted soil resulting in 98% (percentage) loss of total petroleum hydrocarbon in the soil. The use of cow dung for bioremediation is in line with international convention on waste utilization (Awodun, 2008). The use of cow dung in the reclamation by bioremediation has proven not just cost effective and efficient. It provides the soil microbes with the needed nutrient for the degradation of hydrocarbon, it's also seen to be very environmentally friendly as it does not leave the soil too acidic or Alkaline for agronomic use and the nutrient left behind in the remediated soil supports the growth of arable crops. Therefore I recommend that cattle should be allowed to graze, pass out dung and mix with the polluted land using their feet as they graze along the polluted land.

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