# EX-SITU BIOREMEDIATION OF OILY SLUDGE CONTAMINATED SOIL USING BIOPILES

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***Abstract***

*A combination of treatments, consisting of the applications of fertilizer, straw, saw dust, oxygen and other organic materials exposure, was evaluated ex-situ during a period of six weeks. Biopile remediation is an environmental clean-up technology that uses naturally occurring microbes such as bacteria and fungi to destroy organic pollutants as a food source, thus detoxifying the pollutants. The remedial treatments were then applied and the soil characteristics analysed after set periods. Soil physicochemical parameters, such as moisture content, PH value electrical conductivity as well as, organic-carbon and total-nitrogen contents showed distinct variations with time. The total heterotrophic bacteria (THB) count in all the treatment cells increased with time. The hydrocarbon losses (60 to 96%) experienced in the first other treatment-cells revealed the effectiveness in degrading the hydrocarbon contaminant. The results of this study indicate that the application of increased concentrations of nutrients (by the application of fertilizer, straw and saw dust) lead to greater rates of biodegradation of oily sludge contaminated soil.*

***Keywords****: Bioremediation, oily sludge, Biopiles soils physicochemical characteristics.*

## Introduction

Large quantities of organic and inorganic compounds are released into the environment every year as a result of human activities causing serious environmental problems. Among those problems are oil contamination of soil and water from industrial sources and other activities which create a great environmental hazard (Bosset and Bartha, 1994). Also, oil spills result in an imbalance in the carbon and hydrogen. This causes a nitrogen deficiency in an oil-soaked soil, which retards the growth of bacteria and the utilization of carbon source(s).

In addition to nitrogen deficiency like phosphorus may be growth-rate limiting (kogbara et al., 2006). Furthermore, large concentrations of biodegradable organics in the top layer of agricultural soils deplete oxygen reserves in the soil and slow down the rates of oxygen diffusion to deeper layers (Ayotamuno, 2006). Crude-oil pollution tends to persist in soils until remediation measures, involving the application of nutrients are resorted to, because oxygen and nitrogen are limiting factors in all types of petroleum degradation (Ayotamuno, 2006).

The effect of oily sludge on soil nutrient status is related to the energy and carbon obtained by soil microbes from decomposition of the oil (Johnsen, 2005). According to Johnsen and Odu (1981), most of the nutrients are tied up in the cell and tissues of microbes that use these nutrients for growth and development. David (2002) reported increases in organic matter content, soil pH and total Nitrogen concentration following oily sludge application. Similarly, organic carbon and matter had been reported to increase with crude oil spillage rate (Isirimah 1989). Soil total organic carbon content has been reported to increase with the addition of oil (Rowell, 1977).

Biopiles refers to the piling of the material to be biotracted by adding nutrients and air into piles or windrows usually to a height of 30-40cm. Biopiles may be amended with a bulking agent, usually with straw, saw dust back or wood chips or some other organic materials.

The application of fertilizer plus implementing certain agro-technical processes like tilling were as effective as the use of bio-augmentation with indigenous hydrocarbon utilizing bacteria (HUB) plus fertilizer application and tilling, in the degradation of the hydrocarbon contaminant. This research is highly relevant to the Niger Delta region of Nigeria, where frequent oil-spills arising from crude-oil exploration and development activities have devastated farm lands and other agricultural settlements. Hence, biopiles with fertilizers was investigated in this remediation study. The aims were:

- To create an optimum environment for microorganisms to degrade the contaminants.
- To determine the effects of biopiles in the degradation of oily sludge contaminated soil.

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## MATERIALS AND METHODS

### Study Area

This investigation was undertaken at the research farm of the Rivers State University, Port Harcourt, Nigeria. Port Harcourt is the capital of Rivers State and economically the most important city in the Niger Delta region of Nigeria. From this region, more than 98% of Nigeria's current economic mainstay namely crude-oil, is derived. Port Harcourt is within the tropical rain-forest zone with an ambient environment having; a mean annual rainfall of 2400mm; a mean monthly relative humidity of 85%; a mean daily minimum temperature of about $23^0$C and a mean daily maximum temperature of $31.5^0$C.

The soil is normally moisture laden due to the high annual rainfall which results in surface run-offs, rivulets and streams conveying substance like crude oil to contaminate nearby land and rivers (Adenipekun and Fasidi 2005).

### Experimental Design

The soil was divided into six (6) treatment cells that were made into beds, each with dimension (1m x 1m) and tilled to a depth of (0.3m). This was in line with studies by Vance (2002). The beds were constructed so that the depth and exposed surface area of the soil, and in turn its temperature, nutrient concentration, moisture content and oxygen availability could be controlled (Mattewson & Grubbs, 1988). Furthermore, the beds were necessary in order to prevent excessive run-off of the oily sludge contaminant, which was inhibited since the remediation study took place from August to October 2010, in the open air and so exposed to the rains.

Cell O was the control volume, i.e. did not receive any treatment, whereas Cell A: receive 1kg of oily sludge, 50g of 20-10 NPK fertilizer and 0.7 litres of water ($H_2O$). Cell B, receive 1kg of sludge, 75g of 20-10-10 NPK Fertilizer and 0.75 litres of water ($H_2O$). Cell C, receive 1kg of sludge, 100g of 20-10-10 NPK Fertilizer and 1.5 litres of water ($H_2O$). Cell D, receive 1kg of sludge, 150g of 20-10-10 NPK Fertilizer and 1.5 litres of water ($H_2O$). Cell E, 1kg of sludge, 200g of 20-10-10 NPK Fertilizer and 1.7 litres of water ($H_2O$) respectively.

### Soil Treatment

Prior to the Fertilizer application, oily sludge was added to each treatment cells (including the control cell). The cells were left undisturbed (i.e in the open air) for three days. Then the treatments i.e. different amounts of Fertilizer were applied but equal rates of tilling were used. The various treatment cells were tilled twice a week with cutlasses and shovels to provide the necessary aeration and mixing of nutrients and microbes with the contaminated soil.

The aforementioned quantities of fertilizer were applied, to the relevant cells and well worked to at least 0.3m depth in each cell.

## Soil Sample

These were obtained using a 22-cm hand dug soil auger and put in labelled polyethylene bags. The samples for the Total Hydrocarbon Content (THC) measurements were placed in one litre glass bottles and sealed with aluminium foil. This procedure was undertaken three times to form three replicates. The bags and glass bottles were immediately transferred to the laboratory for analysis.

## Analysis of Soil Characteristics

Measures were made of some of the soils physicochemical parameters, such as particle size distribution; THC; concentrations of organic carbon, nitrogen and moisture in the soil, the soils PH value; electrical conductivity and bacteria counts. Particle size analysis was obtained by the Bouyoucous hydrometer method as modified by Day (2002). The THC was measured using the procedure described by Odu et al. (1985), while the organic-carbon content was determined by the wet combustion method of Walkey and Black. The total nitrogen and moisture contents as well as the soil's PH value and electrical conductivity were determined using method adapted from Odu et al., Smith and Smith (1998).

The microbial analysis and bacterial counts were carried out following the procedure described by Harrigan and Mc cane (1990).

## Least Significant Difference (LSD)

Least Significant Difference, Analysis of Variance (ANOVA) and Correlation Coefficient Methods were employed to analyse the measured data. These were used to determine the relationship between time and the soil characteristics during the remediation process.

## Results

The soil characteristic that were used as indicator of the levels of pollution and remediation, before and after the oily sludge contamination, as well as during remediation process, are presented in Tables 1 to 5. The particle-size analyses of the 30cm thick, top layers of the soil before treatment showed that the soils texture is silty clay (see Table 1).

The soils moisture-content prior to contamination averaged 14%, it dropped to about 9% after contamination, prior to remediation, and increased later in all the cells during remediation. There was a correlation ($r = + 0.073$) between soil moisture content and the remediation period, which was not significant either at the 1% or 5% probability levels (Table 6).

The soil's pH increased in all the cells after contamination with oily sludge. It later decreased during the remediation treatment; after the six weeks of total remediation, it increased in all the options. Tisdale and Nelso (1975) made a similar observation and reported that the decrease in PH during remediation treatment may have resulted from the production of acid radicals through the process of nitrification of the applied fertilizer. There was no significant change in the effect of remediation treatment on the soil PH value at 5% probability levels.

The sharp increase in the soils organic carbon (C) content resulted from the oily sludge contamination. However, the organic carbon content dropped to near background conditions during the remediation treatment. In this study, the relationship between organic carbon content and remediation period showed a correlation (r = -0.187). This suggests that the amount of carbon reduced with time.

There was a marked decrease in the percentage of the Total Hydrocarbon Content (THC) in all the cells except for that of the control cell, for which the THC increased. After six weeks of remediation, the percentage THC reductions for the treatment cells were 96%, 94%, 97%, 93% and 89% for cells A,B, C,D and E, respectively (see Fig. 1). The results indicate that the applied Fertilizer increased the degradation of the hydrocarbons since the THC of the control cell that received no Fertilizer treatment was on the increase. Results of the total heterophic bacteria (THB) count showed that there was a general increase in the THB in all the cells (see Table 7).

**Table 3.1 Soils Physiochemical Characteristics before Oily Sludge Contamination**

**(Result represents mean and standard deviation of treatment cells)**

| Percentage (%) | | | | $P^H$ 1:2.5 | EC µS/CM | THC MG/KG | Percent | | C/N Ratio |
|---|---|---|---|---|---|---|---|---|---|
| Sand | Silt | Clay | Moisture | | | | Organic C | Total N | |
| 13.7 ± 0.5 | 41.0 ± 0.2 | 45.4 ± 0.5 | 14 ± 1 | 4.65 ± 0.1 | 29 ± 2 | 66 ± 8 | 0.18 ± 0.02 | 0.62 ± 0.3 | 0.4 ± 0.01 |

**Table 3.2: Initial assessment of Sludge before application**

| Percentage (%) | | | | PH 1:2.5 | EC µS/CM | THC MG/KG | Percent | | C/N Ratio | A.Phos Mg/kg | Potass Cm/kg | Textural Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sand | Silt | Clay | Moisture | | | | Organic C | Total N | | | | |
| 6.0 | 10.0 | 11.0 | 20.48 | 5.8 | 4.71 | 69,371,72 | 0.49 | 0.13 | 4 | 1.02 | 1.29 | Loam sand |

**Table 3.3: Soil physiochemical characteristics 3 days after the application of sludge.**

**(Result represents mean and standard deviation of three replicates)**

| Cell | % moisture | $P^H$ 1:2.5 | EC µs/cm | THC mg/kg | Percent | | C/N Ratio |
|---|---|---|---|---|---|---|---|
| | | | | | Organic C | Total N | |
| O | 20.9±1 | 5.8±0.11 | 471±16 | 64,37.72±650 | 0.49±0.04 | 0.13±0.07 | 4±0.6 |
| A | 1.9±1 | 5.87±0.25 | 401±116 | 63,101.23±595 | 0.49±0.04 | 0.13±0.07 | 4±0.5 |
| B | 17±1 | 5.88±0.10 | 467±10 | 63,275.84±640 | 0.47±0.03 | 0.12±0.06 | 4±0.5 |
| C | 16±1 | 5.89±0.10 | 470±9 | 63.345.62±590 | 0.48±0.03 | 0.13±0.7 | 03±0.4 |
| D | 18±1 | 5.93±0.20 | 4.68±7 | 64,11.21±620 | 0.51±0.05 | 0.13±0.07 | 4±0.5 |
| E | 20.1±1 | 5.94±0.25 | 4.67±11 | 63,171.22±567 | 0.42±0.03 | 0.12±0.06 | 30±0.4 |

**Table 3.4: Soil physiochemical characteristics two weeks after remediation.**

**(Result represents mean and standard deviation of three replicates)**

| Cell | % moisture | $P^H$ 1:2.5 | EC µs/cm | THC mg/kg | Percent | | C/N Ratio |
|---|---|---|---|---|---|---|---|
| | | | | | Organic C | Total N | |
| O | 14±1 | 5.81±0.25 | 442±16 | 64,217.12±650 | 0.49±0.04 | 0.13±0.07 | 4±0.6 |
| A | 12±1 | 5.58±0.15 | 3.52±11 | 46,212.42±430 | 0.31±0.03 | 0.11±0.06 | 5±0.70 |
| B | 16±1 | 5.47±0.20 | 345±12 | 48,657.17±457 | 0.42±0.03 | 0.90±0.05 | 4±0.62 |
| C | 14±1 | 5.42±0.05 | 364±14 | 42,897.82±385 | 0.41±0.03 | 0.12±0.06 | 3±0.5 |
| D | 12±1 | 5.61±0.25 | 358±14 | 40,178.92±381 | 0.39±0.03 | 0.80±0.04 | 3±0.5 |
| E | 13±1 | 5.32±0.21 | 367±13 | 42,765.65±411 | 0.41±0.043 | 0.87±0.04 | 4±0.60 |

**Table 3.5: Soil physiochemical characteristics six (6) weeks after remediation.**

**(Result represents mean and standard deviation of three replicates)**

| Cell | % moisture | $P^H$ 1:2.5 | EC µs/cm | THC mg/kg | Percent | | C/N Ratio |
|---|---|---|---|---|---|---|---|
| | | | | | Organic C | Total N | |
| O | 18±0.5 | 5.91±0.30 | 412±16 | 42,896.82±385 | 0.44±0.4 | 0.11±0.06 | 4±0.60 |
| A | 20±0.5 | 5.95±0.15 | 456±18 | 22,145.65±115 | 0.268±0.01 | 0.90±0.05 | 3±0.5 |
| B | 16±1 | 5.85±0.25 | 364±14 | 19,414±90 | 0.315±0.01 | 0.87±0.04 | 4±0.60 |
| C | 19±0.5 | 5.94±0.30 | 442±16 | 21,650±105 | 0.305±0.06 | 0.80±0.04 | 5±0.70 |
| D | 16±1 | 6.08±0.30 | 345±12 | 14,678.72±40 | 0.068±0.06 | 0.75±0.32 | 3±0.5 |
| E | 14±1.5 | 6.05±0.30 | 358±14 | 13,789.62±30 | 0.28±0.02 | 0.70±0.30 | 4±0.60 |

**Table 3.6: The relationship between time elapsed after remediation to measured soil characteristics during remediation as expressed by correlation coefficients and regression: the g level is not significant at the 1-5% probability levels.**

| Correlation regression factor | Correlation coefficient, r | Significance equation |
|---|---|---|
| time vs. moisture | +0.073 | y = 17.1 + 0.3x |
| Time vs. pH | +0.037 | y = 5.54 + 0.05x |
| time vs. THC | -0.260 | y = 3170 – 255x |
| time vs. organic C | -0.187 | y = 0.196 – 0.01x |
| time vs. total N | -0.341 | y = 0.037 – 0.0x |
| time vs. C/N ratio | +0.182 | y = 4.0 + 0.2x |
| time vs. EC | +0.106 | y = 98.6 + 2.6x |

**Table 3.7: Total Heterotrophic Bacterial Count.**

| Cell | Sampling Period Week | | |
|---|---|---|---|
| | 0 | 2 | 6 |
| | Heterotrophic bacteria count ($10^5$ Cfu/ml) | | |
| O | 8.9 | 12.8 | 15.3 |
| A | 3.5 | 18.6 | 26.8 |
| B | 6.2 | 16.2 | 26.4 |
| C | 5.9 | 17.0 | 28.4 |
| D | 10.1 | 19.4 | 28.4 |
| E | 4.8 | 19.6 | 21 |

**Table 3.8: Percentage THC Reduction**

| Cell | Sampling Period Week | |
|---|---|---|
| | 2 | 6 |
| A | 56% | 94% |
| B | 54% | 92% |
| C | 57% | 67% |
| D | 50% | 71% |
| E | 45% | 88% |

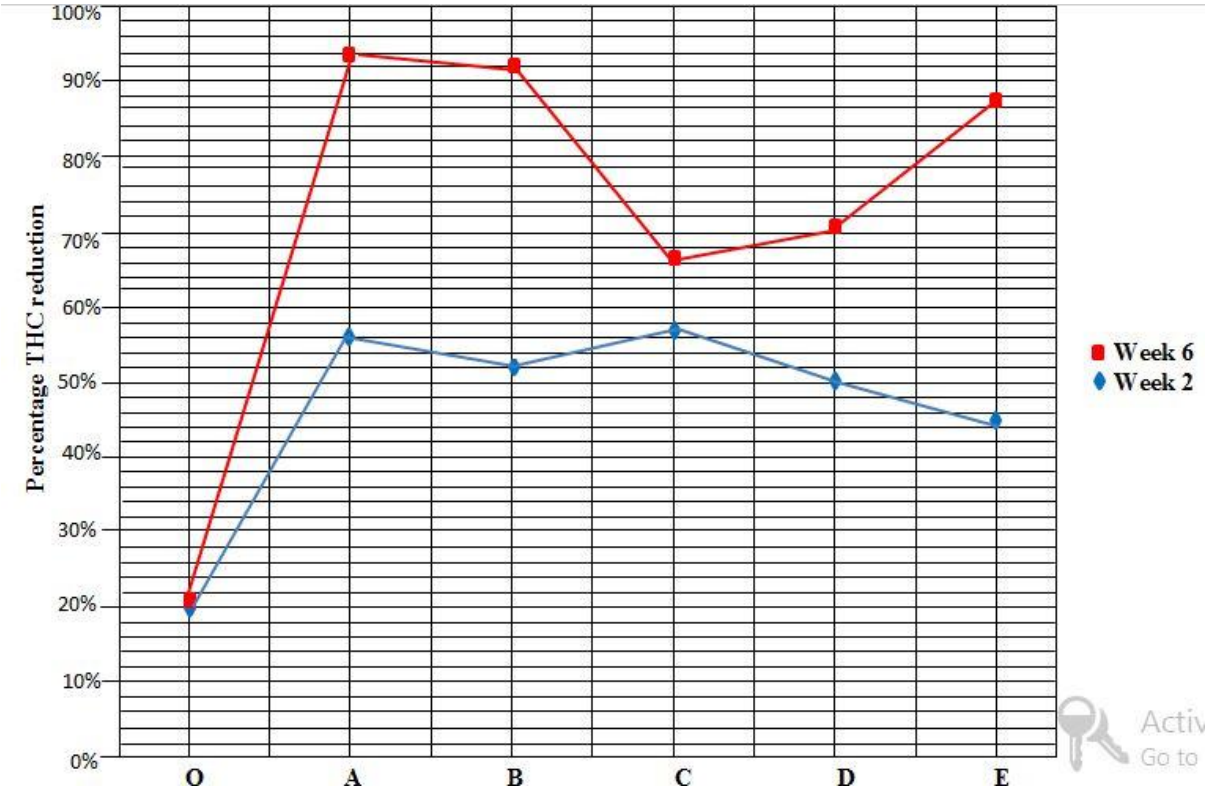


**Fig 4.1: Rate of Hydrocarbon Loss**

## Conclusion

Nutrient-enhanced bioremediation can achieve the degradation of petroleum hydrocarbons in agricultural soils. What is necessary is the supply of the right type(s), quantities or application rates of Fertilizer and also the provision of other suitable environmental conditions for the accelerated development of soil microbes.

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