



## Original Research Article

# APPLICATION OF BIOSTIMULANTS AND BIOPARTICULATES FOR THE BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL

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### ABSTRACT

*The bioremediation of crude oil contaminated soil using organic biostimulant (poultry droppings) and bioparticulate (sawdust) was investigated in this study. Soil samples were contaminated with crude oil in four different compartments. These were Cell A (soil + oil) which served as the control. Other cells were labelled as: Cell B (soil + oil + sawdust), Cell C (soil + oil + poultry Droppings) and Cell D (soil + oil + poultry droppings+ sawdust). Bioremediation indicating parameters of the polluted soil (pH, residual hydrocarbon content (RHC) and total microbial count (TMC)) were monitored in the course of bioremediation which lasted for a total of seven weeks. The results obtained showed that the pH of the control did not vary significantly throughout the treatment period. There was however a slight decrease in the pH of the sample amended with poultry droppings and sawdust thus creating a slightly acidic condition which enhanced biodegradation of the oil. There was a general increase in the total microbial count of the amended samples. The highest microbial count was observed in the cell amended with poultry droppings and sawdust showing that these amendments stimulated the growth of the indigenous microbes. The control sample showed a decrease in residual hydrocarbon content in the course of bioremediation although this was not very significant (47%). In comparison, the amended samples showed a significant reduction in RHC and the highest reduction (99.6%) was recorded for the sample amended with both poultry droppings and sawdust. The results obtained suggests that amendment of crude oil contaminated soil with poultry droppings and sawdust enhanced the bioremediation process.*

## 1. INTRODUCTION

Crude oil is the major pollutant in marine environments (Hidayat and Tachibana 2012). It is a complex biodegradable substance containing a large variety of hydrocarbons. Incidences of crude oil spillage is commonplace and this typically occurs through tanker accidents, natural oil seepage, washing of oil tankers, well blow out, sabotage and accidental rupture of pipelines, resulting in the release of crude oil into terrestrial and aquatic environments (Hasanuzzaman et al., 2007). A statistical analysis by Ifeadi and Nwankwo (1989) revealed that the most common cases of oil spills occur in the mangrove swamp and near offshore areas of the Niger Delta region of Nigeria. In large concentrations, the hydrocarbon molecules that make up crude oil are highly toxic to many organisms including plant and humans thus necessitating the need to decontaminate polluted sites (Obahiagbon et al., 2009).

The complex hydrocarbon composition of crude oil makes it a recalcitrant substance to degrade. Adsorption, volatilisation, abiotic transformation (chemical or photochemical) and biotransformation are some of the processes that can influence the fate of hydrocarbons in soil (Santos et al., 2011). However, the limitations of these methods in terms of cost and safety has necessitated the need for more environmentally friendly and less expensive options.

Bioremediation has been identified as the most rational choice for the decontamination of crude oil contaminated environment. It involves treating the petroleum pollutants with hydrocarbon degrading microorganisms possessing the kind of enzymes required for such a process. Many microorganisms such as species of *Pseudomonas*, *Escherichia*, *Clostridium*, *Candida*, *Aspergillus*, *Yeasts*, *Penicillium* etc have been established to utilize petroleum and its derivatives for metabolic activities (Adekunle and Adebambo, 2007). Bioremediation has been shown to have advantages including viability, relatively low cost of operation, low technology requirement and widespread use. Furthermore, the hydrocarbons are broken down in a relatively short time (Erdogan and Karaca 2011; Otokunefor and Obiukwu, 2010). The pollutants are broken down into simpler substances such as carbon dioxide and water through biostimulation and bioaugmentation (Agbor et al., 2012). Biostimulation involves the use of nutrients and Bioparticulates to stimulate the growth of indigenous microorganisms in regions of surface and subsurface contamination while bioaugmentation involves the introduction of exogenous microorganisms to the remediation medium to boost the microbial population (Amenaghawon and Obahiagbon, 2014).

Thus, the present work was undertaken to explore the use of locally sourced biostimulant (poultry droppings) and bioparticulate (sawdust) for the enhancement of the bioremediation of crude oil contaminated soil. The main advantage of this option is that these materials are waste products and they are abundantly and freely available locally. Moreover, utilisation of these materials will also solve the problem of environmental pollution caused by their disposal.

## 2. MATERIALS AND METHODS

### 2.1. Materials Collection and Preparation

The soil type used for this study was loamy soil and it was obtained locally from around the University of Benin, Benin City, Edo State, Nigeria. The soil samples were randomly collected with a Dutch auger and trowel at a depth of 0-15 cm. Large particles including stones and other unwanted materials were removed and the soil was then stored in perforated polythene bags. Sawdust was obtained from a wood mill at Isihor, Benin City, Edo State. Large wood particles were removed from the sawdust prior to use. Fresh poultry droppings were collected from the University of Benin Model Farm and it was dried for 3 days until constant weight was achieved. Crude oil with a specific gravity of 0.921 was kindly provided by an oil producing company located in the Niger Delta region of Southern Nigeria.

### 2.2. Bioremediation Studies

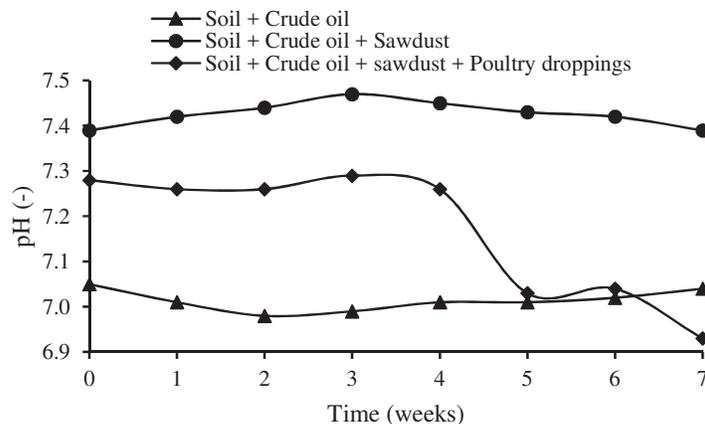
Four kilograms (4 kg) each of soil was transferred into four cells of dimension 1 m × 1 m × 1 m. Crude oil polluted soil was simulated by contaminating the soil in the cells with 800 g of crude oil. The cells were labelled as Cell A (soil + oil), Cell B (soil + oil + sawdust), Cell C (soil + oil + poultry droppings) and Cell D (soil + oil + poultry droppings + sawdust). The oil was thoroughly mixed with the soil in the cells and the cells were allowed to stand for one week for acclimatization between the soil, the oil and the indigenous microorganisms. Poultry droppings (400 g) served as a biostimulant while sawdust (1 kg) served to improve soil quality and these were applied a week after contamination. The soil sample in Cell A without amendment served as control for this study. The moisture content of the samples was adjusted by adding 250 ml of water on a weekly basis (Agbor et al., 2012).

### 2.3. Analytical Methods

Bioremediation indicating parameters of the polluted soil were monitored in the course of the remediation process. The following parameters; pH, residual hydrocarbon content (RHC) and total microbial count (TMC) were monitored in the course of bioremediation. Sampling was done on day zero (before amendments) and subsequently at intervals of seven days (one week) for a total of 49 days (seven weeks). These parameters were measured according to the methods reported in Obahiagbon et al. (2014a).

## 3. RESULTS AND DISCUSSION

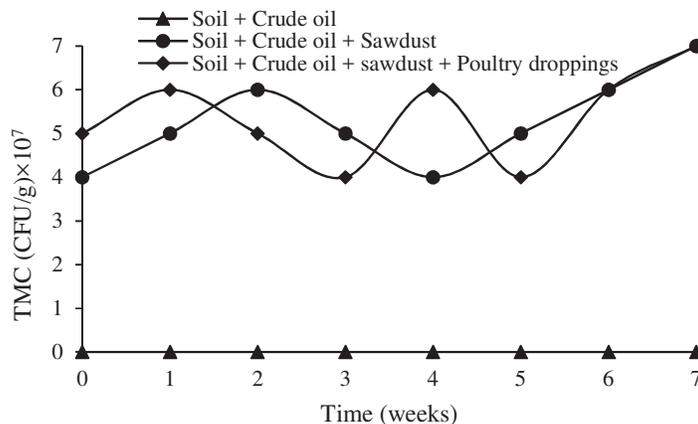
Figure 1 shows the variation of pH in the course of the remediation process. The results showed that the pH of the amended samples as well as the control did not vary significantly throughout the treatment period. There was however a slight decrease in the pH of the sample amended with poultry droppings and sawdust thus creating a slightly acidic condition of the soil which encourages the growth of crude oil degrading bacteria, and thereby promoting biodegradation of the oil (Ebere et al., 2011).



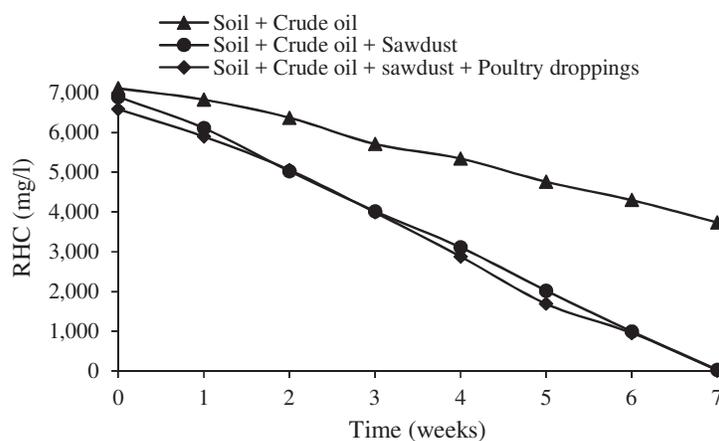
**Figure 1:** Variation of pH during bioremediation

The total microbial count of the control sample did not show any significant increase as shown in Figure 2. This is an indication of lack of growth which could have resulted from inadequate acclimatisation or lack of required growth nutrients. However, there was a general increase in the total microbial count of the amended samples. This trend could be attributed to the growth of the indigenous microorganisms which was reflected in the increase in the population of the microorganism (Obahiagbon et al., 2014b). The highest microbial count was observed in the cell amended with poultry droppings and sawdust showing that these amendments stimulated the growth of the indigenous microbes.

The control sample showed a decrease in residual hydrocarbon content in the course of bioremediation although this was not very significant (47%). In comparison, the amended samples showed a significant reduction in RHC and the highest reduction (99.6%) was recorded for the sample amended with both poultry droppings and sawdust as shown in Figure 3. The reduction in the RHC values is indicative of a reduction in the crude oil content of the soil samples as a result of the mineralisation of the hydrocarbons by the microorganisms to less toxic substances such as  $\text{CO}_2$  and  $\text{H}_2\text{O}$  (Okoh, 2006; Otokunefor and Obiukwu, 2010). Similar reductions in RHC have been reported by previous researchers (Alwan et al., 2013; Mukred et al., 2008; Okoh, 2006). These researchers all attributed the reductions observed to the biodegrading activity of the microorganisms indigenous to the soil.



**Figure 2:** Variation of TMC during bioremediation



**Figure 3:** Variation of THC during bioremediation

#### 4. CONCLUSION

From the results obtained in this study, it can be concluded that the amendment of the contaminated soil with poultry droppings and sawdust enhanced the biodegradation process. The poultry droppings helped in stimulating the growth of the indigenous microbial population while the sawdust helped in improving the properties of the soil.

#### 5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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