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BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOILS USING ORGANIC AND INORGANIC BIOSTIMULANTS ENHANCED WITH NUTRIENT AGAR: EFFECT ON PHYSICO-CHEMICAL PROPERTIES

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ABSTRACT

Environmental pollution caused by the spillage of crude oil and its primary constituents on land and water has become a serious problem in Nigeria. This study investigated the performance of organic and inorganic biostimulants enhanced with nutrient agar for the bioremediation of crude oil contaminated soil. Uncontaminated soil samples were collected from Ologbo in Edo State and subjected to detailed laboratory characterization to determine their physico-chemical characteristics. Crude oil contaminated soil was prepared by adding 100ml of crude oil to 1kg of the uncontaminated soil. The contaminated soil samples were placed in plastic buckets and left open throughout the period of experimentation to allow for the influence of atmospheric oxidation. The remediation process involved the use of organic (cow dung) and inorganic biostimulant (NPK fertilizer) enhanced with nutrient agar. Remediation was carried out for a period of about ten (10) weeks, during which soil samples were taken out from the plastic buckets and analysed to determine their physico-chemical properties. Results obtained show that crude oil contamination resulted in a drastic alteration of the physico-chemical properties of the soil. A progressive increase in the total heterotrophic bacterial count for all the biostimulant used was observed with increasing remediation time. In addition, there was a gradual decrease in the total nitrogen, total phosphorous and total hydrocarbon content (THC). The performance evaluation based on the efficiency of crude oil removal, showed that cow dung enhanced with nutrient agar was best for the treatment of the crude oil contaminated soil.

1. INTRODUCTION

The exploration, production and distribution of crude oil have given rise to environmental degradation - the most prevalent of which is crude oil spillage. The spills may occur as a result of blowouts due to overpressure,

equipment failure, operator's errors, corrosion, sabotage (vandalisation of pipelines), flow line replacement, flow station upgrades, and tank rehabilitation (Abioye, 2010). The problem of oil spill is most prevalent in the Niger Delta region of Nigeria which constitutes the centre of most of the country's oil exploration and exploitation activities. The region is endowed with high amount of hydrocarbon deposits and accounts for over 80% of the Nation's foreign exchange earnings (Agarry *et al.*, 2013)

Environmental pollution caused by the spillage of crude petroleum hydrocarbons and its primary constituents on land and water has become a serious problem in Nigeria and have developed into a widely studied area (Alexander, 2000). Crude oil pollution renders soils unproductive for years after spillage, reducing the growth, performance and productivity of most plants (Baker and Moore, 2000). The Niger Delta region is not left out of this menace as most plants have suffered serious depletion of vital nutrients needed to support growth and productivity owing to incessant spillage.

A number of technologies have been evaluated to remediate crude oil polluted sites. These include: solvent extraction, bioremediation, phytoremediation, chemical oxidation, electrokinetic remediation, thermal technologies, ultrasonication, flotation, etc. (Lim *et al.*, 2016). Each of these techniques has advantages and disadvantages, and most times, it is usually better to use a combination of them (Lim *et al.,* 2016; Yoo *et al.*, 2017). According to Chen *et al.* (2015), biological treatment methods should be utilised more for soil remediation than chemical and physical methods, as the later methods are either incompetent or too costly.

Biological treatment methods have been found to be a less sophisticated natural method of clean-up of hydrocarbon polluted sites. The low solubility and adsorption of high molecular weight hydrocarbons limits their availability to microorganisms and thus tend to slow down the method of biological treatment (Jorgensen *et al.*, 2000; Grossi *et al.*, 2002). In addition, the specificity of the biological degradation process is highly related to the genetic potential of the microorganism to introduce molecular oxygen into hydrocarbon and to generate the intermediates that subsequently enter the general energy-yielding metabolic pathway of the cell (Khan *et al.*, 2004). More also, the driving force for petroleum hydrocarbon biodegradation depends on the ability of the microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs. One of the basic limitations to this practice of using isolated living cells microorganism is the problem of disposal. If not properly disposed, microorganism can cause outbreak of disease (Burland and Edwards, 1999).

The use of plant materials (phytoremediation) have also been investigated and found to be an effective and less expensive natural method of petroleum hydrocarbon degradation (Moubasher *et al.*, 2015). Root exudates can aid in degradation of toxic organic chemicals and act as biostimulants for soil microorganisms. Although phytoremediation can be highly effective, it may not be a good option for sites with high concentrations of petroleum hydrocarbons due to the phytotoxic effects of hydrocarbons on the plants (Frick *et al.*, 1999; Izinyon and Seghomise, 2013). Recently, bioaugmentation technology, which involves the introduction of exogenous microorganisms into the contaminated environment usually as a result of insufficient population of the indigenous microorganisms; and biostimulation technology, which involve the use of organic and inorganic biostimulants, is gradually becoming the centre of attraction owing to their low cost, ease of operation and availability (Isitekhale *et al.*, 2013).

While various studies have investigated the performance of some of these biomaterials in relation to treatment of crude oil contaminated soils, very few studies have considered the effect of adding enhancers (e.g. nutrient agar) to these biostimulants as a way of influencing their performances as bioremediation materials. In this study, nutrient agar was used as an enhancer in combination with an organic biostimulant (cow dung) and an inorganic biostimulant (NPK fertilizer), for the treatment of crude oil contaminated soils.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Soil sampling and contamination

Soil samples were collected from the test location, which is located at Ologbo in Edo State, Nigeria (Figure 1). The collected samples were divided into two parts. Crude oil obtained from local sources was added to one part of the uncontaminated soil samples at a mix proportion of 100 ml of crude oil to 1 kg of uncontaminated soil. These were mixed together, homogenised and kept for a period of four (4) days before commencing treatment. The contaminated soil sample (CS) was passed through 2 mm sieve mechanically, and thereafter stored in black polyethylene bags prior to use. The other part of the soil sample (uncontaminated soil or UCS) was also stored in polythene bags and were used mainly for comparative analysis, to determine the effect/efficiency of the various bioremediation methods used in the study.

Figure 1: Google earth imagery of sampling location

2.1.2. Bioremediation materials

Four treatment materials were used for this study:

- Cow dung (CD)
- NPK fertilizer (NPK)
- Cow dung enhanced with nutrient agar (CD+NA)
- NPK fertilizer enhanced with nutrient agar (NPK+NA)

The cow dung was collected from the University of Benin livestock farm, which is located at the University of Benin, Benin City, Nigeria. After collection, it was air dried for one month, pulverized and stored in black polyethylene bags before use. The NPK fertilizer and nutrient agar were purchased from local chemical vendors.

2.2. Soil Treatment Procedure

The contaminated soil was placed in five different buckets perforated at the bottom and treated using different procedures as shown in Table 1. After adding the treatment material, the soil sample was mixed homogeneously under air. Samples were extracted from each of the buckets on a weekly basis, for a period of ten weeks. These were tested to determine the physicochemical properties of the soil - Total Heterotrophic Bacteria (THB), Total hydrocarbon content (THC), available phosphorus and total nitrogen. The moisture content of the samples in each bucket was maintained at 45 – 50% throughout the period of the treatment.

Table 1: Details of soil treatment procedure

Treatment procedure	Contaminated soil (g)	CD (g)	NPK (g)	NA (ml)
No treatment (Control)	500	-	-	-
Treated with CD	500	250	-	-
Treated with NPK	500	-	250	-
Treated with CD+NA	500	250	-	200
Treated with NPK+NA	500	-	250	200

2.3. Analytical Methods

2.3.1. Total heterotrophic bacteria (THB)

THB was determined using procedures similar to that used by Obire *et al.* (2008). About 1 g of the soil sample was mixed with 9 ml of phosphate buffered saline solution and was diluted with 0.85% NaCl to give 10^{-1} dilution. Then, 0.1 ml of each dilution was plated on nutrient agar and Petri plates and incubated at 37°C for 48 hrs. After incubation, the colonies were counted. The resultant colony forming unit (cfu/g) was calculated for both the untreated and treated soil samples.

2.3.2. Total hydrocarbon content (THC)

The total amount of THC present in the soil sample before and after treatment was determined using a method similar to that used by Izinyon and Seghomise (2013). The contaminated soil sample was dried properly and ground to particles passing through 75 μm sieve. About 5 g of the dried sieved sample was weighed and placed in 100 ml conical flask. To this was added 25 ml of n-hexane and the entire mixture was shaken properly. The mixture was then covered and allowed to stand for about 1 hr. Thereafter, the mixture was filtered and the filtrate was analysed with the aid of a visible/UV spectrophotometer at 420 nm wavelength. The total hydrocarbon content was calculated using the mass balance equation given as:

$$THC\ (in\ ppm) = \frac{Visible\ spec.\ reading \times slope\ reciprocal \times 25}{Weight\ of\ sample} \quad (1)$$

2.3.3. Available phosphorus

Acetic acid extraction method (Koralage *et al.,* 2015) was employed for the determination of available phosphorous present in the contaminated soil samples. About 5 g of soil sample was added to a clean and dry plastic bottle. To this was added 50 ml of 2.5% acetic acid solution, followed by extraction for 2 mins. The mixture was thoroughly mixed and kept for about 3 hrs until the supernatant solution was separated. The supernatant solution was then analysed for phosphorus using the Murphy and Riley (1962) method.

2.3.4. Total nitrogen content

The method used for the determination of the total nitrogen content in the contaminated soil was adopted from ASTM D2973-10 (ASTM, 2010). About 0.2 g of finely ground contaminated soil was weighed and placed in 30 ml Kjeldahl digestion flask. One tablet of catalyst plus 4 ml of concentrated sulphuric acid (H_2SO_4) was then added to the sample in the digestion flask. The mixture was well agitated to ensure complete mixing of the contaminated soil and catalyst mixture. The digestion flask and its content was placed on a hot plate and heated to boiling point. Thereafter, digestion was allowed to occur for about 45 mins. On completion of the digestion, the mixture was removed from the hot plate and allowed to cool to room temperature. About 10 ml of distilled water was added to the mixture and the resulting mixture was filtered through a filter paper (Whatman No. 42) into a 100 ml volumetric flask, from which 5 ml of the filtrate was taken and placed in a 25 ml flask. To this was added 2.5 ml of alkaline phenol and the resulting solution well agitated followed by the addition of 1 ml sodium potassium and 2.5 ml of sodium hypochlorite. The solution was again agitated and made up to 100 ml, before it was read at 630 nm wavelength using a visible/UV spectrophotometer. The percentage of nitrogen was computed using the mass balance equation given as:

$$\%\ N = \frac{UV\ Reading \times Reciprocal\ of\ slope \times Colour\ vol. \times Digest\ vol \times 10^{-6} \times 100 \times Cf}{Weight\ of\ sample \times Aliquot\ taken} \quad (2)$$

where C_f is the correction factor

3. RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of the contaminated and uncontaminated soil samples. From the Table, it can be seen that the impact of the contamination resulted in a significant increase in the total hydrocarbon content of the soil from 0.00 mg/kg to 12.45 mg/kg. The total nitrogen and total phosphorus were all decreased by about 66% while the total heterotrophic bacteria (THB) were decreased by about 90%. Similar findings were also reported in the study by Wang *et al.* (2013) on the effects of crude oil contamination on soil physical and chemical properties in Momoge Wetland of China and can be attributed to the anaerobic environment created by the crude oil particles, which would lead to smothering of the soil particles and blocking of air diffusion in the soil pores. It is well known that nitrogen and phosphorus are essential nutrients for all living organisms, especially growing crops. Thus, it can be seen that the effect of the crude oil contamination is very detrimental to the soil and will greatly affect its use for agricultural purposes.

Table 2: Impact of crude oil contamination on the physico-chemical properties of the experimental soil

Parameter	Uncontaminated soil	Contaminated soil
Total Nitrogen (g/kg)	9.21	3.12
Total Phosphorous (g/kg)	6.49	2.17
Total Hydrocarbon Content (mg/kg)	0.00	12.45
Total Heterotrophic Bacterial (cfu/g)	8.6×10^5	0.98×10^5

3.1. Effect of Treatment on the Total Heterotrophic Bacterial Count (THB)

Figure 2 shows the effect of treatment on the total heterotrophic bacteria (THB) count. From the Figure, it can be seen that there was a significant increase in the total heterotrophic bacteria (THB) count as the bioremediation process commenced. After two weeks of treatment, the THB count of the contaminated soil sample treated with cow dung enhanced with nutrient agar (CD+NA) had grown from 1.0×10^5 cfu/g to about 4×10^5 cfu/g, while

that of cow dung only had grown to about 3 x 10^5 cfu/g. Similar results were also obtained by Obire *et al.* (2008) in their study on saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents, and they attributed it to the propagation of petroleum-utilizing fungi, which was enhanced by the addition of the organic biostimulant. Comparing the four bioremediation methods, the method which involved the use of organic biostimulant enhanced with nutrient agar (CD+NA) was seen to produce the highest amount of THB. This was followed by CD (organic biostimulant without nutrient agar). The treatment method which involved the use of inorganic biostimulant (NPK) was seen to produce the lowest amount of THB.

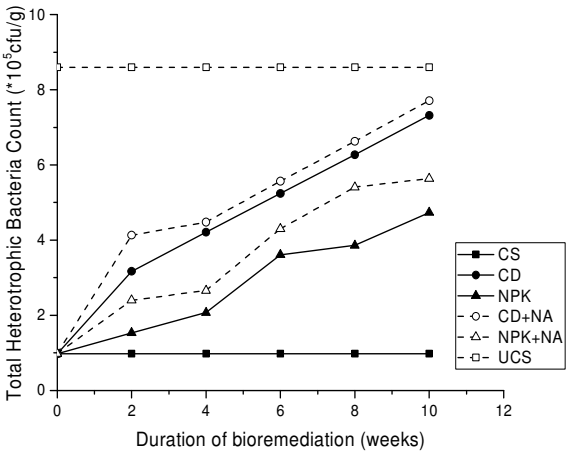

Figure 2: Effect of bioremediation on total heterotrophic bacteria

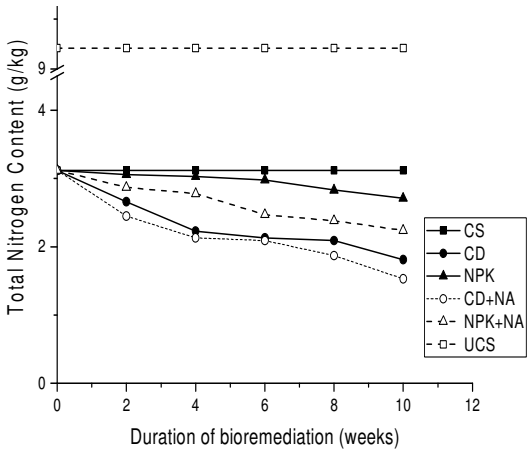

Figure 3: Effect of bioremediation on total nitrogen content

3.2. Effect of Treatment on the Total Nitrogen and Total Phosphorus Content

The effect of bioremediation on the total nitrogen and total phosphorus contents of the soil is shown in Figures 3 and 4 respectively. Similar trends were observed in both figures, in that the total nitrogen and total phosphorus contents of the contaminated soil (CS) were further reduced by the bioremediation process. The soils treated with CD+NA had the lowest total nitrogen and phosphorus content, followed by those treated with CD and NPK+NA.

Nitrogen and phosphorus are essential nutrients in the soil that are needed by plants and microorganisms for growth, so one would expect that the treatment process should result in an increase in these nutrients and not a decrease, as seen in the study by Eneje *et al.* (2012). However, here we see a decrease in the nitrogen and phosphorus content. This may be due to the increase in the population of THB count occasioned by nutrient utilization. Heterotrophic bacterial usually utilize the soil's available nutrient in the form of total nitrogen, total organic carbon and total phosphorous, for their growth and cell development. This consequently leads to a drastic reduction in the nutrient level as can be seen in both figures. Therefore, as the remediation time increases, there is a corresponding increase in the population of THB present in the contaminated soil. This explains why the soils treated with CD+NA had the lowest total nitrogen and phosphorus contents, as these soils also contained the highest number of total heterotrophic bacteria (as seen in Figure 2).

3.3. Effect of Treatment on the Total Hydrocarbon Content (THC)

Figure 5 shows the variation of the total hydrocarbon content with bioremediation time for all the treatment materials. From the Figure, it can be seen that cow dung enhanced with nutrient agar (CD+NA) is the best biostimulant for the clean-up of crude oil polluted soil, followed by cow dung without nutrient enhancement (CD). After a period of 10 weeks, it was observed that CD+NA had reduced the concentration of the crude oil hydrocarbon from the initial 12.45 mg/l to 3.47 mg/l, which is equivalent to about 72% reduction in the THC of the contaminated soil. For CD, NPK+NA and NA, the percentage of reduction in THC after 10 weeks of bioremediation was 62%, 56% and 53% respectively. The high rate of hydrocarbon removal can be attributed to the stability and adaptation of the microorganism to the hydrocarbon polluted environment. As the microorganism stabilizes and becomes well adapted to the polluted environment, they tend to grow and develop based on the available nutrient, while also eating up the hydrocarbon. This process results in possible clean-up.

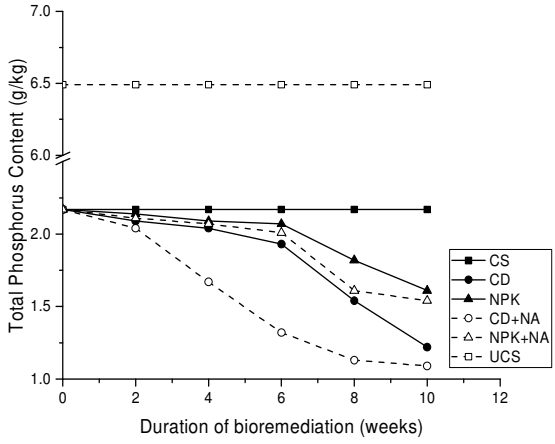

Figure 4: Effect of bioremediation on total phosphorus content

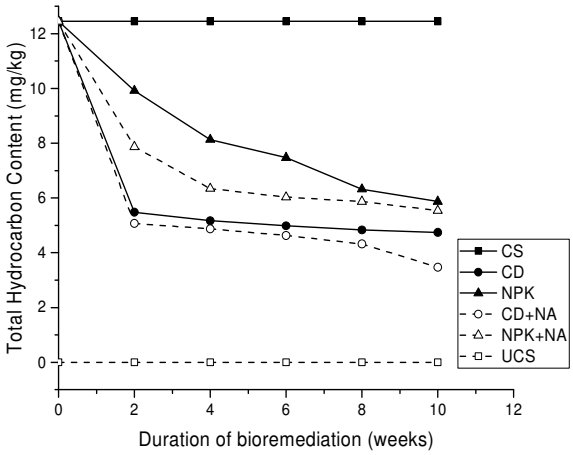

Figure 5: Effect of bioremediation on total hydrocarbon content

3.4. Efficiency of Crude Oil Degradation

The efficiency of crude oil removal was calculated using the mass balance equation proposed by Eba *et al.* (2010):

$$E\ (\%) = \frac{100}{C_0}(C_0 - C_e) \tag{3}$$

where C_o = total hydrocarbon content in contaminated soil sample before treatment in mg/l; C_e = total hydrocarbon content in contaminated soil sample after treatment in mg/l.

The computed efficiencies for the four bioremediation materials used in the study are presented in Table 3. From the Table, it can be seen that cow dung enhanced with nutrient agar (CD+NA) and NPK fertilizer (NPK) had the best and worst performance as bioremediation materials respectively.

Table 3: Efficiency of crude oil degradation (%)

Time (weeks)	CD	NPK	CD + NA	NPK + NA
2	55.98	20.32	59.28	36.79
4	58.47	34.70	60.88	49.08
6	60.00	40.00	62.81	51.57
8	61.20	49.24	65.30	52.85
10	61.93	52.85	72.13	55.50

After 2 weeks of treatment, cow dung (CD) was able to remove about 56% of the crude oil from the contaminated soil, while NPK fertilizer could only remove about 20% of the crude oil. At the end of the treatment period, which was 10 weeks, CD had effectively removed about 62% of the crude oil while NPK had removed about 53%. This falls within the range of removal efficiencies obtained by Isitekhale *et al.* (2013). It can be seen that the addition of nutrient agar as an enhancer further increased the effectiveness of the organic and inorganic biostimulants. This increase was more evident on the organic biostimulant (CD), with the efficiency being increased by about 10% as compared to the inorganic biostimulant (NPK), whose efficiency was only increased by about 3%.

4. CONCLUSION

This study investigated the performance of organic and inorganic biostimulants as bioremediation materials for the removal of crude oil from crude oil contaminated soils. The influence of adding an enhancer to these biostimulants was also investigated. From the results obtained, the following conclusions were drawn:

- Crude oil contamination has a significant negative effect on the physico-chemical properties of soils.
- Treatment of the crude oil contaminated soil resulted in a significant increase in the THB count of the soil and a decrease in the THC, total nitrogen and total phosphorus content of the contaminated soil.

In terms of efficiency of crude oil removal, the organic biostimulant (cow dung) performed better than the inorganic biostimulant (NPK fertilizer). Addition of an enhancer (nutrient agar) to the biostimulants resulted in increase in the efficiency of crude oil removal, with the effect being more pronounced on the organic biostimulant.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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