

# Bioremediation of Crude Oil Polluted Soil Using a Blend of NPK Fertilizer and Periwinkle Shell Ash

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## Abstract:

Analysis on the bioremediation of crude oil polluted soil using a blend of NPK (15-15-15) fertilizer and Periwinkle Shell Ash (PSA) is presented. The analysis involves using 7-batch bioreactors under aerobic conditions with each bioreactor containing 2 kg of soil sample and spiked with 200 ml of crude oil except for the control experiment, and were properly mixed and periodically tilled during the experimentation period of 90 days. The substrate concentration was analysed as Total Petroleum Hydrocarbon (TPH) while the microbial concentrations Total Heterotrophic Bacteria (THB) and Hydrogen Utilising Bacteria (HUB) was also analysed, including the soil pH, during the period. Soil parameters (nitrogen, organic compound, phosphorus and potassium) were also measured at the beginning and at the end of the experiments. The results showed that THB increased with time from  $6.0 \times 10^5$  cfu/g to  $3.02 \times 10^8$  cfu/g for the control experiment, to  $3.3 \times 10^8$  cfu/g for sample with 100 g NPK, to  $3.95 \times 10^8$  cfu/g for sample with 100 g NPK and 200 g PSA during the period. HUB also increased from  $2.74 \times 10^4$  cfu/g to  $4.45 \times 10^7$  cfu/g for the sample during the same period. TPH values reduced from 58,200 mg/kg to 34,591 mg/kg for the control and to 18,045 mg/kg been the lowest for sample with 100 g NPK and 500 g PSA during the same period. pH values varied from 5.7 – 8.8 for all sample during the experimentation. Also nitrogen increased from 0.42% to 0.91%, organic carbon from 1.65% to 1.78%, organic matter from 2.58% to 3.20% phosphorus from 26.8% - 31.57%, and potassium from 0.35% - 0.46%.

**Keywords** — Bioreactors, Biodegradation, Monod growth kinetics, substrate concentration, Total Heterotrophic Bacteria

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## I. INTRODUCTION

Crude oil pollution, as a result of exploration, exploitation, production and processing operations is an environmental challenge globally and particularly in Nigeria's Niger Delta area (Odukoya, Lambert and Sakrabani, 2019). For the past forty years of petroleum exploration and exploitation in Nigeria, over 6000 cases of crude oil spills have been recorded with an average of about 150 crude oil spills per year (Department of Petroleum Resources, 1997). Crude oil spills into the environment may be from technical errors, deliberate human sabotage (vandalism), transportation problems and storage faults (Khamchhiyan, Hossein and Tajik, 2007; Ajagbe, Omokehinde, Alade and Agbede, 2012;

Ivshina, Kuyukina, Krivoruchko, Evkin, Makaraov, Cummngham, Peshkur, Allas, and Philip, 2015; Ndimele, Saba, Ojo, Ndimele, Anetekhai, and Erundu, 2018).

## II. RELATED LITERATURES

Schmidt-Etkin 2011, observed that although large spills may sometimes occur with severe environmental and socio-economic damage, small spills are often more common. The impacts and damages as a result of crude oil spills depends generally depend on the spill location, the type of crude oil, the volume of the spill, the closeness to sensitive or critical resources and the season it occurred, for example in Nigeria whether rainy or dry season, among other factors (Schmidt-

Etkin,2011). Odukoya *et al.*, 2019 explained in their work, that a significant part of the petroleum hydrocarbon contamination in the world is as a result of large-scale accidents of crude oil spills. They further explained that, there are more cases of crude oil spills on land, than those recorded in aquatic environment in which plant life especially in farmland becomes exposed to petroleum hydrocarbon with subsequent effects on agricultural produce.

In Nigeria for example, there is significant spillage of crude oil into the environment from various sources including leakages of crude oil pipelines, human sabotage, damaged crude oil tankers and storage vehicles, oil tankers overflow, equipment failures, technical errors (Nas, 1975; Nicolotti and Egli, 1998). Bioremediation is defined as the use of micro-organisms for the degradation or removal of environmental pollutants in an established and feasible technique and is now being used for the removal of crude oil contaminants from soil (Yelebe, Samuel, Yelebe, 2015). Bacteria that metabolize naturally occurring hydrocarbons such as petroleum hydrocarbon are wide spread in the environment (Yelebe *et al.*, 2015).

The environmental consequences of crude oil pollution are enormous. Crude oil spills, especially in the Niger Delta have destroyed agricultural lands, traditional fishing grounds, fishing ponds etc. Studies have shown that, there is a reduction in soil fertility leading to low productivity and hence, the need for a solution (Odjuvwuederhie, Douglason and Adun, 2006; Osuagwu, Okigbo, Ekpo, Chukwurah, and Agbor, 2013). Insufficient aeration of the soil as a result of the oil pollution has been observed to be the major contributing factor to the retarded growth identified in plants and the chlorosis of the leaves (Udo and Fayemi, 1975).

Considering the overall adverse effects of crude oil pollution and consequently, its implications on food security and environment in the oil rich Niger Delta region, it has become increasingly necessary to develop new technology or improve on the existing technologies on the treatment of the crude oil polluted soils to mitigate the food security crises.

### III. MATERIALS AND METHODOLOGY

Materials used for this work include; crude oil, Periwinkle shell ash, NPK fertilizer, Soil sample, GC/MS.

#### A. Methods

The method in this research involves the collection and preparation of the following samples:

1. Crude Oil Sample: Crude oil (Bonny light) was obtained from Agip flow station in Brass, Bayelsa State, Nigeria.
2. Periwinkle Shell Sample: Periwinkle shell (*Tympanotomus Fuscatus* Specie) samples used for this experiment were collected from Amassoma waste dump in Southern Ijaw Local Government Area of Bayelsa State.
3. Preparation of Periwinkle Shell Ash: Fresh periwinkle shells were weighed, then heated in an oven to a temperature of 1000°C, and then weighed again. Fine particles were obtained using a 2mm mesh after the periwinkle shells have been crushed.
4. NPK Fertilizer: N.P.K fertilizer used for this work was obtained from Rivers State Ministry of Agriculture, Port-Harcourt.

#### B. Chemical Analysis of Periwinkle Shell Ash

Results of chemical analysis of the Periwinkle shell ash was obtained from literature and presented in Table 1

TABLE I  
CHEMICAL ANALYSIS OF PERIWINKLE SHELL (SOURCE: UKPAKA AND OKOCHI, 2018)

Element	Content	Element	Content	Element	Content
Mg	0.0000	Cr	0.0000	Ca	60.5899
Al	0.3685	Mn	0.0079	Ti	0.0000
Si	0.3033	Co	0.0007	V	0.0041
P	0.2704	Fe	0.1604	Zn	0.0436
S	0.4609	Ni	0.0261	As	0.0000
K	0.0000	Cu	0.0230	Pb	0.0000

### C. Collection and Preparation of Soil Sample

Random collection of soil samples from fallow land that is not polluted with crude oil within the Niger-Delta University was done using a shovel and hand auger digging up to 20 cm. The collected soils were homogenized and air dried for a period of one week in a clean well-ventilated laboratory and sieved by passing through a 2 mm mesh sieve to remove stones, sticks and any unwanted particles. 2 kg of soil was each measured into clean dry experimental planting containers and moistened with distilled water to ensure proper mixing with the crude oil.

### D. Characterization of Soil Samples

Standard procedures using Boyoucos hydrometer method to carry out the physical and chemical analysis of the soil sample was followed (Sheldrick & Wang, 1993).

### E. Total Petroleum Hydrocarbons (TPH) Determination

The method of Ahmadi *et. al.* (2006), which is described in ASTM D3291 was used. This is the standard method for TPH extraction.

### F. Preparation of Broth Culture

The broth culture was prepared by seeding three different types of bacteria obtained from soil into medium containing vital broth nutrient at a pH of 7.4. Before been used for the study, the inoculated broth was incubated for 24 hours at 37°C. These experiments were carried out at the Microbiology Laboratory of the Department of Biological Science, Niger Delta University, Bayelsa State.

### G. Experimental Procedure

2 kg of soil sample was put into each container, and 200 ml of crude oil was measured and poured into the container also. Proper mixing was done to achieve 10% pollution as reported by Osuji and Adesiyon (2005). In variation, 100 g, 200 g, and 300 g and 500 g of the periwinkle shell ash were added to the containers and 100 g of N.P.K fertilizer respectively and thoroughly mixed to obtain homogeneity, for one week with constant watering to allow proper decomposition.

The first container having 2 kg of crude oil polluted soil served as control 1, while the second container, containing 200 ml of crude oil stimulated soil plus 100 g of NPK served as control 2, another container, containing 200 ml of crude oil stimulated soil and 500 g periwinkle shell ash, control 3, and the fourth, fifth, sixth and seventh containers, containing 200 ml of crude simulated soil with variations of 100 g, 200 g, 300 g and 500 g of periwinkle shell ash plus 100 g of NPK fertilizer were set up. Standard procedure as described by Ofunne (1999) was used in the enumeration and isolation of THB.

## IV. RESULTS AND DISCUSSION

### A. Microbial Population Evaluation

Bioremediation of Petroleum hydrocarbon polluted soil was investigated using a combination of NPK fertilizer and Periwinkle Shell Ash (PSA) to stimulate the population of the indigenous micro-organisms: The baseline bacterial count for the total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) obtained were  $6.0 \times 10^5$  cfu/g and  $2.74 \times 10^4$  cfu/g respectively. THB and HUB increased progressively during the period of study.

Figure 1 is a comparative plot of changes in THB against time for crude oil polluted soil for three different cases (i.e., (1) control (2) only NPK, and (3) a blend of NPK and PSA). THB increased progressively from an initial value of  $6.0 \times 10^5$  cfu/g at the beginning of the experiment to  $2.64 \times 10^8$  cfu/g after 30 days and then to  $3.02 \times 10^8$  cfu/g after 60 days of experimentation for the control experiment. The increase showed that indigenous micro-organism in the solid have the ability to use petroleum hydrocarbon as a carbon source and energy. This has been reported by other researchers (Rosenberg and Ron, 1996).

The THB counts for crude oil polluted soil amended with 100 g of NPK fertilizer was higher compared to the counts for the control experiment. From the same initial value of  $6.0 \times 10^5$  cfu/g at the commencement of the experimental process, the THB increased to  $2.88 \times 10^8$  cfu/g after 30 days and then to  $3.3 \times 10^8$  cfu/g after 60 days of experiments,

figures higher than that of the control process. This could be ascribed to be the effect of the NPK fertilizer as these nutrients are necessary for bacterial growth and degradation activities (Gadhvi, 2019; Ekenwosu, 2019).

TABLE III  
RESULTS OF MICROBIAL POPULATION DURING THE EXPERIMENTATION PROCESS

		Time		
		0 day	30 days	60 days
THB values in cfu/g	Control	$6.0 \times 10^5$	$2.64 \times 10^8$	$3.02 \times 10^8$
	100 g NPK	$6.0 \times 10^5$	$2.88 \times 10^8$	$3.3 \times 10^8$
	100 g NPK + 200 g PSA	$6.0 \times 10^5$	$2.95 \times 10^8$	$3.95 \times 10^8$
HUB values in cfu/g	Control	$2.74 \times 10^4$	$2.31 \times 10^5$	$2.59 \times 10^6$
	100 g NPK	$2.74 \times 10^4$	$2.6 \times 10^7$	$3.33 \times 10^7$
	100 g NPK + 200 g PSA	$2.74 \times 10^4$	$4.2 \times 10^7$	$4.45 \times 10^7$

given values for THB higher than the control experiment and that when only NPK fertilizer was used. This trend is supported by studies carried out by Obiakalaje et. al., 2015; Oyedele and Amoo, 2014; Adesodu and Mbagwu, 2008; Ijah and Antai, 2003, and Nakasaki et. al., 1992.

The changes in the hydrocarbon utilizing bacteria (HUB) count also followed the same pattern in all three cases. For Figure 2, HUB increased from  $2.74 \times 10^4$  cfu/g at the commencement of the experiment to  $2.3 \times 10^5$  cfu/g after 30 days and to  $2.59 \times 10^6$  cfu/g after 60 days of the experimentation process for the control experiment.

For the sample amended with 100 g of NPK fertilizer, HUB grew from  $2.74 \times 10^4$  cfu/g to  $2.6 \times 10^7$  cfu/g after 30 days and further increased to  $3.33 \times 10^7$  cfu/g after 60 days.

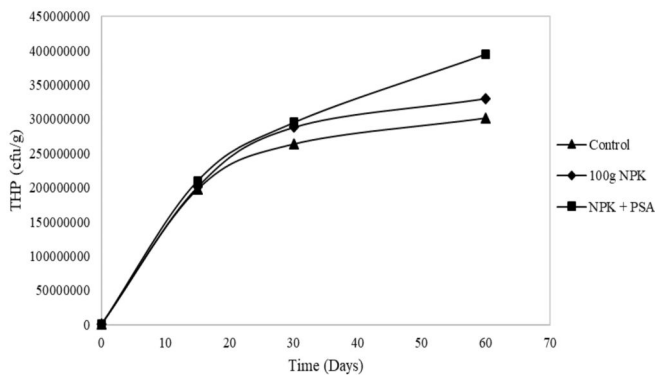


Figure 1: Plot of comparative changes in THB with time

For crude oil polluted soil amended with a blend of 100 g NPK fertilizer and 200 g PSA. As clearly seen, THB count increased from  $6.0 \times 10^5$  cfu/g to  $2.95 \times 10^8$  cfu/g after 30 days and increased further to  $3.95 \times 10^8$  cfu/g after 60 days of experimentation,

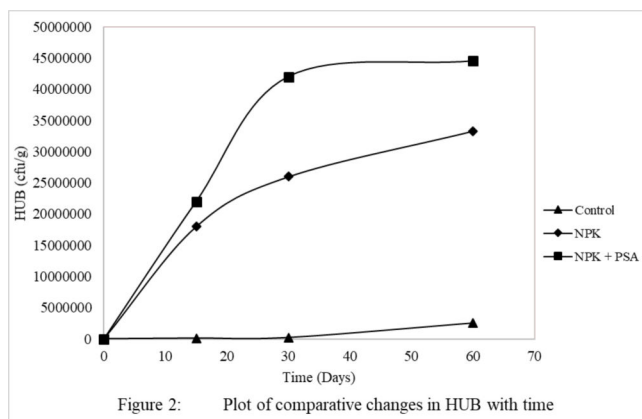


Figure 2: Plot of comparative changes in HUB with time

### B. Total Petroleum Hydrocarbon (TPH) Count Evaluation

The degree of biodegradation of petroleum hydrocarbon polluted soil was investigated using a blend of NPK fertilizer and Periwinkle Shell Ash as stimulants to stimulate the indigenous microorganisms population. The Total Petroleum Hydrocarbon (TPH) content removal effect was used as the indicator parameter for the bioremediation and it was seen to reduce with time during the period of study. The result also shows that the effect of time was highly significant.

Figure 3 is a plot of comparatives changes in TPH of the polluted soil samples with Time. The results

shows a decrease in TPH concentration with Time during the period of the experiment for all cases. The TPH decreased from an initial value of 58,200 mg/kg at the commencement of the experiment to 48,007.55 mg/kg to 34,891 mg/kg on the 90th day of experimentation for the control experiment. This depletion of TPH in the soil shows that indigenous micro-organisms in the soil have the natural ability to degrade the petroleum hydrocarbon and use it as a carbon source and energy. This is in line with the findings of Rosenberg and Ron (1996). Rosenberg and Ron (1996) in their bioremediation study carried out shortly after the Exxon Valdez oil spill during the summer of 1989 in which they used Oleophilic fertilizer to remediate the crude oil polluted shorelines. A similar study carried out by Obiakalije et. al., (2015), in which they used various animal wastes as amendment to treat crude oil polluted soil, also observed a reduction in TPH with time in the control sample. However, in this study it is shown that the stimulation of the crude oil polluted soil with NPK fertilizer, and/or Periwinkle Shell Ash enhanced the rate of TPH degradation.

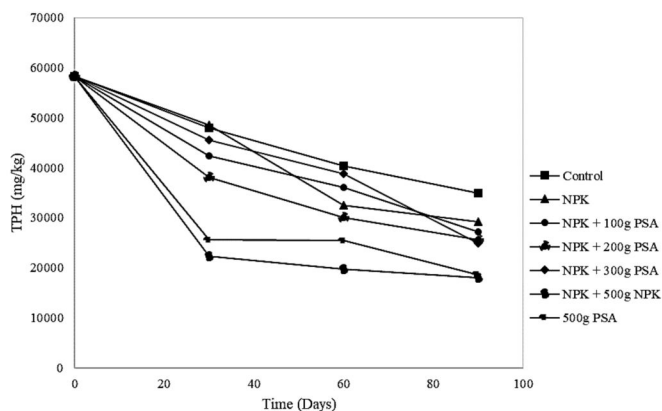


Figure 3: Plot of comparative changes in TPH with time

For TPH concentration against time for crude oil polluted soil with 100g of NPK fertilizer as stimulant. The result shows a significant reduction in TPH from the initial value of 58,200 mg/kg at the commencement of the experiment to 29,183 mg/kg after 90 days of the experiment.

Table 3: Results of TPH concentration during the Experimentation Process

Sample \ Time		Time			
		0 day	30 days	60 days	90 days
TPH values in mg/kg	Control	58,200	48,007.53	40,383.20	34,891
	100 g NPK	58,200	48,561.53	32,527.80	29,183
	100 g NPK + 100 g PSA	58,200	42,381.00	36,006.60	27,103
	100 g NPK + 200 g PSA	58,200	38,103.8	30,086.60	25,636
	100 g NPK + 300 g PSA	58,200	45,529.25	38,728.40	24,857
	100 g NPK + 500 g PSA	58,200	22,254.20	19,729.30	18,045
	500 g PSA	58,200	25,522	25,407.31	18,640
ln(TPH)	Control	10.972	10.78	10.61	10.46
	100 g NPK	10.972	10.79	10.39	10.28
	100 g NPK + 100 g PSA	10.972	10.65	10.49	10.21
	100 g NPK + 200 g PSA	10.972	10.55	10.31	10.15
	100 g NPK + 300 g PSA	10.972	10.73	10.56	10.12
	100 g NPK + 500 g PSA	10.972	10.01	9.89	9.8
	500 g PSA	10.972	10.15	10.14	9.83

This clearly shows the effect of the stimulation on the remediation process when compared to the control experiment. Gadhvi (2019) explained that nitrogen deficiency in crude oil polluted soil as a result of increase in organic carbon retards bacteria growth and hence a limiting factor. This position is also corroborated by Asuquo, Ibanga, and Idunafa (2001), where they observed that increase in organic carbon in petroleum hydrocarbon polluted soil creates an initial scarcity of nitrogen. The supply of nitrogen through the NPK fertilizer has now stimulated the bacteria growth and enhanced the ability of the nitrogen fixing bacteria and hence enhanced the biodegradation activity leading to the reduction in TPH, since nitrogen is a source of nutrient for micro-organisms (Ekenwosu, 2019).

For the case of TPH concentration against time for crude oil polluted soil with a blend of 100 g of NPK



fertilizer and 100 g of Periwinkle Shell Ash. From the results obtained, there was a further reduction of TPH from 58,000 mg/kg to 27,103 mg/kg during the same 90 days experimentation period, a value lower than the 29,183 mg/kg obtained when only 100 g of NPK fertilizer was used. This difference was believed to be the effect of the addition of the periwinkle shell ash used in combination with the NPK fertilizer. The additional nutrients supplement being supplied by the PSA like calcium, zinc, iron and phosphorus have microbially mineralized the stimulated soil and have contributed to the remediation. This is in agreement with the findings of researchers like Oyedele and Amoo (2014), and Gadhvi (2019).

When, only 500 g of PSA was used with no addition of NPK fertilizer, and the result shows a significant reduction of TPH to 18,640 mg/kg during the same period of study. This clearly agrees with the findings of Ofoegbu et. al., (2015).

## V. CONCLUSION

A simple, very effective, cheap, readily available and environmentally friendly technology for bioremediation of crude oil polluted soil was employed in this research work (Periwinkle Shell Ash (PSA) and N.P.K fertilizer). The experiment was carried out under aerobic conditions using seven batch bioreactors in the laboratory. Indigenous micro-organisms were identified, cultured and used for the process. The result showed that for crude oil polluted soil amended with NPK, and NPK + PSA and the control sample, the THB increased from  $6.0 \times 10^5$  cfu/g to  $3.3 \times 10^8$  cfu/g,  $3.95 \times 10^8$  cfu/g and  $3.02 \times 10^8$  cfu/g respectively after 60 days of the experimentation process.

In the same vein, HUB also increased from  $2.74 \times 10^4$  cfu/g to  $3.33 \times 10^7$  cfu/g,  $4.45 \times 10^7$  cfu/g and  $2.59 \times 10^7$  cfu/g for NPK, NPK + PSA and control respectively after 60 days of the experimentation process.

Total Petroleum Hydrocarbon (TPH) reduced from 58,200 mg/kg to 34,891 mg/kg for control sample, 29,183 mg/kg for sample amended with

100g NPK, 27,103 mg/kg for sample with 100g NPK + 100g PSA; 25,636 mg/kg for sample with 100g NPK + 200g PSA, 24,857 mg/kg for sample with 100g NPK + 300g PSA, 18,045 mg/kg for sample with 100g NPK + 500g PSA and 18,640 mg/kg for sample with 500g PSA respectively at the end of the 90 days experimentation period.

The soil PH decreased as a result of the pollution of the soil with crude oil, increased with the addition of the biostimulants, and hence improved the soil properties.

Finally, remediation of crude oil polluted soil with periwinkle shell ash and NPK fertilizer have proven to be effective in improving the physicochemical properties of the soil.

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