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# Effects of Different Levels of Plantain Peel Waste on The Amelioration of Crude Oil Polluted Soil

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**Abstract:**
The effect on different levels of plantain peel waste in the amelioration of crude oil polluted site was investigated. This experimental research was set up at the Department of Forestry and Environment Ecological Centre Rivers State University. Two kilograms (2kg) of soil were introduced into each of 5 experimental planting bags (EPB) of 5 replications with the following treatment options displayed as experimental planting bag 1, 2 and 3 (EPB1, 2, 3) had spent diesel soil plus 120g, 140g and 160g of plantain peel waste respectively, while EPB 4 had spent diesel soil sample only (control) and EPB5 had unpolluted soil sample alone which was represented as a double control. Result showed that with the application of different levels of plantain peel waste, Increased microbial growth from 1.23 x106 -8.9 x106 cfu/g, 4.8 x106 -5.11 x106 cfu/g, 1.03 x105 -7.6 x104 cfu/g and 2.1 x104 -6.5 x106 cfu/g for THBC, TFC, HUBC and HUFC respectively. Soil physicochemical properties was also influenced with the addition of plantain peel waste. Total nitrogen, phosphorus, potassium and total organic carbon increased from 3.8-5.9%, 0.04-2.1 mg/kg, 13.0-50.2mg/kg and 1.81-5.6% respectively. THC decreased from 2520-485 mg/kg/day, TPH decreased from 1205-329 mg/kg/day while pH decreased from 8.9-6.3. The results suggest that toxic nature of spent diesel and nutrient depletion can be ameliorated with the addition of different concentration of plantain peel waste and should be harnessed as efficient treatment for the restoration of crude oil polluted soil.

## INTRODUCTION

In Nigeria, crude oil exploration has a great positive impact on its economic this includes revenue generation, employment opportunities, foreign exchange earnings and much more. Crude oil production process has significant environmental effects throughout its lifecycle, including extraction, transportation, refining, and combustion [1]. The effects of crude oil pollution can be detrimental to ecosystems, wildlife, and contribute to climate change[2]. The soil is an essential component of the biosphere which is referred as *solum* in Latin and it is the top most layer of the earth crust. However, presence of crude oil on soil result in adverse environmental degradation of the ecosystem. The benefits of soil in the survival of living things especially plants and microorganisms is overwhelming. Soil is a basic medium in which plants are anchored, it is an upper parts of earth surface which consist of geological and biological attributes. These layers different of soil play immense function in water and nutrients availability to plants [2,3]. Growth of a plant to its full potential is depends on the suitable environment conditions. Soil properties sensitive to environmental and anthropogenic perturbation serve as indicator to soil quality and these indicators plays crucial roles in supporting plant life. Soil major components include nutrient, essential minerals and innumerable microorganism and its significant functions include:

1. To supply required plant nutrients.
2. The soil stores water necessary in converting elements to ions.
3. It also a habitat for microorganisms.

4. The soil interfaces with lithosphere, hydrosphere, atmosphere, and biosphere [4].

The functionality of a given ecosystem depends on the stability of it soil. However, crude oil spills can have a significant impact on soil which could lead to various environmental and ecological consequences [3]. The presence of some toxic compounds such as benzene and toluene present crude have adverse effects on the overall functioning of the ecosystem [5]. The presence of crude oil on soil also alters the physical properties of soil, impacting on its structure and composition, the hydrophobic nature of crude disrupts oxygen supply and exchange in soil atmosphere and also capable of repelling water, leading to increased soil compaction, decrease in porosity and decrease in nutrient availability for plants [5].

Soil microorganisms play crucial role in maintaining the health and fertility of soil. Crude oil pollution is capable of disrupting the essential role and soil processes of microorganisms through decrease in microbial communities (decline in microbial abundance and diversity) [3]. Crude oil exposure can harm plants in various ways such as plant growth and seed germination inhibition, stunt root development and decrease in photosynthetic rate and leaf transpiration [5]. Numerous remedial techniques adopted in restoration of crude oil impacted soil include physical, chemical, and biological techniques [6]. Microbial degradation is seen as the most environmentally friendly techniques suitable for crude oil mitigation. This form of biological remedial method is faced with numerous challenges such as decrease in soil structure and fertility, reduction in microbial diversity and population, toxicity nature of pollutant and decrease in nutrient availability and uptake [3]. In the quest to address this pitfall face in polluted soil resulted in the use of soil amendments. The addition of any material either organic or inorganic with the objective of improving soil physical, chemical and biological properties especially in a polluted soil is known amendment [2].

Few scientists have investigated the efficacy of organic biostimulants such as poultry manure, pig and goat dung and the use of inorganic biostimulants such as NPK fertilizer. This work seeks to explore the potential of plantain peel wastes as bio stimulating agents in the cleanup of hydrocarbon polluted soil.

## MATERIALS AND METHODS

This experimental research was set up at Department of Forestry and Environment Ecological Centre Rivers State University, this area is located in the tropical rainforest region of southern Nigeria which is referred as Niger Delta region. The region has a daily and annual temperatures of $36^{oc}$ and $28^{oc}$ [7]. This area shows two main seasons- The rainy season starts from April – October while the dry season starts from November – March. The climatic pattern of this area negatively influences the nutrient pattern with an annual rainfall of 2400mm and high humidity and sunshine [8, 9].

### Collection of Soil and Processing of Amendment

A spent diesel oil polluted soil was collected from depth 0-10cm using soil auger behind the central generator plant house while an unpolluted soil was obtained from a farm land situated 300m from the suspected spent diesel polluted site in Rivers State University. The soils were air dried and sieved through a 2mm wire mesh to obtain a homogenous fine fraction of soil composites. The ripe plantain used was source from Kaiama in Kolokuma/Opukuma L.G.A, Bayelsa State is popularly known as 'Beribe'. The plantain peel waste was generated mechanically by hand peeling. The peels (waste) generated from this mechanical process was dried and processed into

powder form. The soils and plantain peels waste were analyzed for physicochemical and microbiological properties.

**Table 1. Microbial and physicochemical properties of soil**

| S/N | Parameter | Control | polluted |
|---|---|---|---|
| 1 | TOC | 2.5 | 4.61 |
| 2 | TPH | 0.8 | 1450 |
| 3 | pH | 8.4 | 8.9 |
| 4 | P | 0.05 | 0.35 |
| 5 | N | 13 | 4.6 |
| 6 | K | 345 | 432 |
| 7 | THBC (cfu/g) | 2..1 x $10^5$ | 1.22 x $10^5$ |
| 8 | HUBC (cfu/g) | 3.0 x $10^5$ | 1.01 x $10^5$ |
| 9 | TFC (cfu/g) | 4.2 x $10^5$ | 1.2 x $10^5$ |
| 10 | HUFC (cfu/g) | 3.56 x $10^5$ | 1.1 x $10^5$ |

**Table 2. Microbial and physicochemical properties of plantain peels waste**

| S/N | Parameter | Plantain peels waste |
|---|---|---|
| 1 | Phosphorus (mg/kg) | 36.84 |
| 2 | Potassium (mg/kg) | 26,743 |
| 3 | Nitrogen % | 11 |
| 4 | pH | 9.08 |
| 5 | THBC (cfu/g) | 5.0x $10^5$ |
| 6 | HUBC (cfu/g) | 6.8 x $10^5$ |
| 7 | TFC (cfu/g) | 7.2 x $10^5$ |
| 8 | HUFC (cfu/g) | 8.9 x $10^5$ |

**Experimental Design and Treatment Application**

Two kilograms (2kg) of soil were introduced into each of 5 experimental planting bags (EPB) of 5 replications with the following treatment options displayed as. Experimental planting bag 1, 2 and 3 (EPB1,2,3) had spent diesel soil plus 120g, 140g and 160g of plantain peel waste respectively, while EPB 4 had spent diesel soil sample only (control) and EPB5 had unpolluted soil sample alone which stands as double control. This experiment was monitored for 60 days and weeding was done by handpicking method when necessary. At 60$^{th}$ day, the soil samples obtained from each treatment application were sieved using 2 mm mesh before the determination of microbiological and physiochemical properties the soil which was carried out as follows:

**Determination of Soil pH**

pH was measured by meter method from slurry of 50/50 (W/V) of sample soil mixed with distilled water in a beaker which was stirred with a stirrer for 5 minutes to homogenize and pH meter (Jennway 3015 model) electrodes were dipped into solution and the pH value displayed in the meter was recorded.

**Determination of Phosphorus**

Soil samples (2g) were added with 40 ml of Olsen's extracting solution was added which was then filtered using whatmann filter paper. Five (5) ml of the filtrate was measured into 25 ml measuring cylinder and 10 ml of distilled water was added along with 4 ml of B-reagent which was added to 25 ml mark with distilled water. This was allowed for 10 minutes and the absorbance readings was taking using the T60 UV visible spectrophotometer.

**Determination of Potassium**
Soil samples (5g) were weighed in 100 ml conical flask containing 25 ml of 1 N NHAOAc solution. The mixture was shaken for 5 minutes using mechanical shaker then filtered with whatman No 1. Filter paper. Potassium extract was obtained by Flame Industrial Photometer model 410.

**Soil Total Petroleum Hydrocarbon (TPH):**
TPH was determined by means of gas chromatograph (HP 5890), fitted with flame ionization detection gadget using soil sample. The soil sample was air dry for 5 days, 2g of the air-dried soil sample was added 10 ml of dichloromethane. The mixture was stirred and allowed to settle and the resultant was filtered through extraction column to obtain a clear filtrate in extraction bottles and concentrated to 2 ml by evaporation. The amount of different petroleum hydrocarbon fractions present in the extract filtrate solution were determined by gas chromatography with Gas Chromatograph (HP 5890 series 11) fitted with flame ionization detection instrument. TPH was obtained as
TPH content = Dilution (if any) x Reading (TPH) x Volume (2ml)
Weight of sample (2g)

**Total Organic Carbon, (TOC) %**
This was determined by titrimetric method (Walkley-Black techniques). One gram of soil was placed in each of two separate flasks containing 10 ml of 1 N$K_2Cr_2O_7$ solution. The flasks were gently stirred to disperse the sample soil into solution, 20 ml of $H_2SO_4$ and 100 ml of distilled water were added to each flask after 30 minutes. Four (4) drops of ferroin indicator were added to the solution. The resultant solutions were titrated with 0.5N ferrous sulphate solution. Blank solution (i.e. solution without soil sample) was also prepared and titrated with 0.5N ferrous sulphate solution
TOC = Blank- Titre Value
Wt. of soil sample used (1g)

**Soil Total Nitrogen Content**
Soil total nitrogen (TN) was determined by spectrophotometry. A blank solution (25 ml of sample supernatant) and sample solution (25 ml of supernatant + nitraver -5 + nitrate reagent powder) were prepared. The blank solution was first placed in a spectrophotometer (HACH, DR/890 colorimeter model) cell holder and the reading of the blank solution as displayed by the spectrophotometer was zero. The blank solution was substituted with the sample solution, and its nitrate content as displayed by the machine was read and recorded.

**Microbial Load Determination**:
The medium nutrient agar was used for the analysis of total heterotrophic bacteria count (THBC). In preparation of nutrient agar, 28g of the powder was added in 1L of distilled water and characterized by autoclaving at 121°C for 15 minutes and allow to stand for about 45 minutes. It was poured into sterilize Petri dishes and allow to solidify and excess moisture was eradicated from agar by drying in hot air oven set at 60°C. Then 1g of the soil sample was weighed into 9mL sterile diluent (normal saline), to carry out serial dilution. Exactly 0.l ml aliquot of inoculum was aseptically inoculated on duplicated agar surface using a sterile pipette and flame sterilized glass–rod was used to spread the inoculum uniformly and incubate at 37°C for 24 hours followed by colony to obtain colony forming unit (CFU/g)

## CALCULATION

### Cultivation, Characterization and Identification of Fungal Isolate

A ratio of 1:10 of sample to diluents was prepared. The mixture was shaken and then serially diluted to 10-4. Exactly 0.1 ml was plated on potatoes dextrose agar (PDA) impregnated with 1.0 % lactic acid. The spread plate technique was employed in the cultivation. The plates were incubated for seven (7) days then the probable fungal isolates were characterized.

Microscopically, features like cell shape, type of hypha, presence of spores and spore arrangement were also observed. The cells were first stained prior to microscopic examination. Yeast-like fungal isolates were emulsified on clean, grease free slides with a loop full of water smeared and allowed to dry before fixing. The smears were stained with crystal violet and after 1 min of dryness, the stain was gently washed off using 70 % alcohol. The smear was then gently rinsed with water and allowed to dry. The slide was then examined under oil immersion objective. The isolates were aseptically cut and placed on a clean slide, flooded cotton blue lacto phenol dye and mounted under a cover slip and viewed with the x 40 objective lens. The number of times each fungi occurred divided by the total number of fungi per plate.

### Hydrocarbon Utilizing Bacterial and Fungal Count (HUB / HUF)

Bushnell Haas media was prepared by dissolving 3.2g of the salt in a liter of distilled water, and 15grams of agar, the medium was preheated and allowed to cool. The prepared medium was autoclaved at 121$^{o}$C, 15psi for 15mins. The vapour phase culturing technique was adopted by using a pre-sterilized whattman filter paper, impregnated with Bonny light crude oil and placed on the lid of the petri dishes. The plates were incubated at 37$^{o}$C for 48 hours. Fungal flora was determined by inhibiting bacterial contamination by addition of 1.0% (v/v) lactic acid to the medium, was poured and allow to solidify at conditions of vapor-phase under ambient temperatures for 7 days.

### Data Analysis

The data generated were subjected to statistical analysis of variance (ANOVA) using Statistical Analysis System [10] to test the significant of bio-stimulant on soil characteristics

## RESULT AND DISCUSSION

**Table 3: Soil microbiological properties.**

| Treatment | THBC ((cfu/g)) | TFC | HUBC | HUFC |
|---|---|---|---|---|
| EPB1 | $8.9 \times 10^4$ | $4.9 \times 10^4$ | $6.1 \times 10^4$ | $6.5 \times 10^6$ |
| EPB2 | $8.2 \times 10^6$ | $5.11 \times 10^6$ | $7.2 \times 10^6$ | $3.2 \times 10^4$ |
| EPB3 | $8.4 \times 10^4$ | $5.0 \times 10^4$ | $7.6 \times 10^4$ | $3.8 \times 10^4$ |
| EPB4 | $1.23 \times 10^5$ | $4.8 \times 10^4$ | $1.03 \times 10^5$ | $2.1 \times 10^4$ |
| EPB5 | $1.36 \times 10^6$ | $4.4 \times 10^4$ | $5.32 \times 10^4$ | $4.3 \times 10^4$ |

The effect of different levels of plantain peel treatments on the abundance of soil bacterial and fungi count was observed in table 3. Increment in soil fungal and bacterial population was found in soil amended with plantain peel waste. Total heterotrophic bacterial (THBC) was highest in EPB1 while the least decrease was recorded for EPB4 sampled soil. However, highest increase in total fungal count (TFC) was recorded in EPB2 amended soil with EPB5 (double control) showing the least decrease. Highest increase in hydrocarbon utilizing bacterial count (HUBC) was found in EPB3 soil while least decrease in HUBC was recorded in EPB4. It was also found that plantain peel influenced the concentration of soil hydrocarbon utilizing fungal count (HUFC). The highest in HUFC was recorded in EPB1 treated soil and the least was recorded for EPB4 (polluted soil with

og amendment). Increase in HUBC, HUFC, THBC and TFC experienced in soil with various levels of plantain peels waste may be attributed to the treatments added. The treatment contains essential nutrient needed by microbial which is capable of increasing its activities hence leading to an increase in microbial population. This result obtained from this investigation agreed with the finding of [3] that organic amendment has the capacity to influence microbial population and enhanced their activity in a given environment. [11] also explained that biostimulants has the potential to increase microbial growth through mitigating toxicity effect. These results also agreed with [12] who reported that using plant materials as treatment can sustenance higher microbial population since they serve as a source of carbon.

**Table 4: Soil physicochemical properties.**

| Treatment | TN % | P mg/kg | K mg/kg | THC mg/kg/day | TPH mg/kg/day | TOC % | pH |
|---|---|---|---|---|---|---|---|
| EPB1 | 5.9 | 2.1 | 50.2 | 485 | 329 | 5.6 | 6.3 |
| EPB2 | 5.2 | 1.42 | 42.2 | 512.0 | 444.3 | 2.12 | 6.8 |
| EPB3 | 5.6 | 2.41 | 49.5 | 511.1 | 473.8 | 2.5 | 6.6 |
| EPB4 | 3.8 | 0.04 | 13.0 | 2520 | 1205 | 1.81 | 8.9 |
| EPB5 | 2.1 | 1.13 | 29.5 | 0.88 | 8.87 | 2.5 | 5.6 |

**Soil Total Nitrogen Content (TN)**

Total nitrogen content was influenced with the addition of different levels of plantain peel waste. There was significant different between and within treatment at (p=0.05). Increase in soil total nitrogen was reported in treated soil and the highest in TN level was in EPB1 amended soil and EPB4 (polluted control soil with og amendment) showed a significant decrease in TN while the least decrease in TN was recorded in EPB5 (unpolluted control soil with og amendment) sampled soil as shown in Table 4. The highest increase in nitrogen content could be attributed to mineralization process obtained through the degradation mechanisms of the plantain peel waste by microorganisms triggered the availability of soil nitrogen. The organic amendment added provided the require energy and carbon required to increase the propensity of microbial activity making them more efficient in converting organic nitrogen into solution phase hence increasing its accessibility. This increase recorded in total nitrogen content could be attributed to the concentration of TN present in the added amendment. The TN content was made available in the soil through microbial metabolism. This finding agreed with Amajuoyi and Wemedo (2015) who reported an increase in biostimulated soil.

**Soil Phosphorus**

In Table 4, it was also observed that increase in soil phosphorus was more in soil amended with different concentrations of plantain peel waste. There was significant different between and within treatment at (p=0.05). Highest in P concentration was found in EPB3 amended soil while least decrease in P was recorded in untreated polluted soil (EPB5). Soil potassium was also found to increase in its concentration with plantain peel waste amendment. The highest increment in soil P was found in EPB1 while EPB4 showed the least decrease. The increase in soil phosphorus is understandable as the phosphorus content of the plantain peel waste added was above 36 mg/kg. The phosphorus contained in the organic amendment was released after microbial degradation and became available for plant uptake. The result obtained from this investigation agreed with the finding of [11] who reported high phosphorus content in organic amended soil which resulted in the amelioration of crude oil polluted soil.

**Soil THC and TPH Content**

Decrease in soil concentration of THC and TPH was found in amendment soil with EPB1 showing the least decrease in THC and TPH while the highest increment was found in EPB4 sampled soil (polluted soil with og amendment). There was significant different between and within treatment at (p=0.05).as shown in table 4. The reduction in total hydrocarbon and total petroleum hydrocarbon levels could be attributed to the organic amendment added.

The organic amendment added is a soil enhancer which may have stimulated and promoted microbial diversity and due to the increased microbial activity leads to enhanced breakdown and degradation of THC and TPH. The increase in THC and TPH levels recorded in the control soil (polluted with og amendment) could be attributed to the decrease in microbial activity recorded, the soil was devoid of essential nutrient required to promote microbial growth and enhanced their degradation process. In addition, increase in the concentration of THC and TPH showed the presence of crude oil contamination. This result agreed with [6] who reported that crude oil is composed of 83-89% carbon and therefore its presence in soil is capable of influencing the presence of TPH and THC. The decrease in TPH and THC recorded in the polluted treated soil could be attributed to the organic amendment added. The added amendment contains essential nutrient which has the potential to stimulant microbial growth when incorporated into soil resulting in decrease in soil TPH and THC. This finding agreed with [13] who reported similar result on the effect of microbial population in crude oil biostimulated soil.

**Soil Total Organic Carbon Content & pH**

Soil total organic carbon was found to increase with treatment addition. There was significant different between and within treatment at (p=0.05). Highest increase in TOC was found in EPB1 sampled soil because the least was recorded in EPB4. Decrease in pH level was recorded with amendment addition of various concentrations. The least decrease in pH was in EPB1 treated soil while highest increment in pH was recorded in EPB4.

The increase in TOC content recorded in the amended soil could be attributed to the type and concentration of amendment used. The TOC content in the plantain peel waste treatment was not readily decomposed by microorganisms. The finding corroborated with the report of [14] who reported an increase in TOC in sawdust amended soil and concluded that TOC embedded in the sawdust treatment was not made available as readily source of food energy for microorganisms hence increase in its concentration was found. This also disagrees with the report of [15] who recorded a significant decrease in TOC content in soil containing readily degradable soil enhancers. The addition of amendment influenced pH levels; optimal pH was achieved with addition of amendment. pH values decreased from 8 to 6. Decrease in pH level could be attributed to mineralization process achieved during degradation of plantain peels waste. This result agrees with [3] who reported a reduction in the level of soil pH in an enhanced soil.

## CONCLUSION

This research investigated the capacity of plantain peel of different levels to reduce TPH, TOC, THC and to increase microbial and fungi population. This study demonstrated that plantain peel waste amelioration was effective in the restoration of crude oil polluted soil since it stimulated microbial growth leading to decrease in hydrocarbon content. Therefore, the use of plantain peel waste to facilitation polluted soil restoration process is recommended.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## DECLARATIONS

Each of the authors confirms that this manuscript has not been previously published by another international peer-review journal and is not under consideration by any other journal. Additionally, all of the authors have approved the contents of this paper and have agreed to the submission policies of the journal.

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