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Research Article

Use of Cassava Peel as Biostimulant in Bioremediation of Crude Oil-Polluted Soil

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Abstract

Background and Objective: Crude oil pollution is a serious issue in Nigeria. There is, therefore, a need to source an affordable method that is eco-friendly for remediating these polluted soils. The study investigated the use of cassava peel waste as a bio stimulating agent in an *in situ* bioremediation of crude oil polluted site. **Materials and Methods:** Treatments were as follows, H₁: Polluted soil with no bio-stimulation (Control), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel and H₄: No pollution, no bio-stimulation (double control). Soil pH, electrical conductivity, moisture content, nitrogen, phosphorus, total organic matter, total organic carbon, potassium, total petroleum hydrocarbon, total hydrocarbon content and microbial population in soil were monitored at 0, 2 and 4 months. **Results:** Results showed that the percentage reduction of THC and TPH in the soil after 4 months were as follows, H₂ (90.48 and 81.39%), H₃ (80.19 and 63.81%) and H₁ (32.90 and 14.76%), respectively. Soil properties improved with a concurrent increase in the microbial population in the bio-stimulated soil as compared to the control. **Conclusion:** This result proves that cassava peel waste is an effective bio-stimulating agent in crude oil degradation especially at 500 g m³ compared to other treatment options hence providing an alternative for the remediation of crude oil-polluted soil, especially in the Niger Delta part of Nigeria.

Key words: Petroleum, hydrocarbon, contamination, bioremediation, bio-stimulation, cassava peel, pollution, soil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Petroleum hydrocarbons are one of the most dominant soil contaminants in the Ogoni area of the Niger Delta Region of Nigeria. This is a result of crude oil exploration and other related activities such as transportation infrastructure malfunction, human inaccuracy, or natural hazards¹. This poses a concern on both local and global scales as petroleum hydrocarbon contamination can have a lethal effect on human and environmental conditions. Crude oil contaminants can persist in the environments un-degraded for decades^{2,3}. Remediation techniques are used to manage petroleum hydrocarbon contaminated soils as they involve the manipulation of soil properties that can be limiting factors for petroleum hydrocarbon degradation, including aeration, moisture content, temperature and nutrient availability. Conventional physical and chemical *in situ* and *ex situ* clean-up technologies for petroleum hydrocarbon (PHC) remediation amongst others includes excavation, air-sparging, removal and off-site treatment in biopiles, pump and treat and incineration⁴. However, with progressive research on remediation of polluted environments, these strategies have been proven expensive and observed to only result in a partial disintegration of the pollutants of concern. Thus, research focus on remediation has moved from the conventional ones towards biological methods (bioremediation) which use biologically mediated processes to degrade, reduce or transform pollutants into less toxic or harmless form⁵. Based on the principle of the total transformation of petroleum products into less toxic forms by different groups of microorganisms⁶, bioremediation is the most effective, non-invasive, least expensive and eco-friendly method⁷⁻⁹. The conservation of soil texture and characteristics are among the advantages of bioremediation. Also, physical and chemical properties of the soil, such as aeration, pH, water-holding capacity and ion exchange capacity can be improved after bioremediation¹⁰. Biostimulation can be human-induced or natural which is dependent on the activation of the microbial population through the supply of nutrients (inorganic or organic), oxygen and other environmental factors necessary for their optimal performance^{6,11}.

The principle behind biostimulation as a method to increase petroleum hydrocarbon degradation relies on the establishment of a favourable environment for hydrocarbon bacterial communities through the addition of biostimulants such as rice straw, biochar, plantain peel, yam peel and sawdust which after decomposition supplies nutrients, for instance, nitrogen and phosphorus which absence in the soil is a limiting factor in the process of bioremediation of

contaminated soils. Thus the addition of these organic materials in soil stimulates extensive growth of hydrocarbon degraders in soil¹²⁻¹⁴. The use of organic materials such as sugarcane rice coconuts and their by-product has been reported to have bio-stimulatory effects on the biodegradation of pollutants in soils^{11,13,15,16}. Studies have revealed improved degradation of petroleum hydrocarbons due to the addition of inorganic or organic fertilizers^{17,18}.

This study is aimed at evaluating the efficiency of different amounts of cassava peel as biostimulant in petroleum hydrocarbon degradation in crude oil polluted soil as a substitute for expensive physical and chemical treatments.

MATERIALS AND METHODS

Study area: The research was done at Botem Community in Tai Local Government Area of Rivers State from 2017 to 2018. Two different sites in the community were used for the experiment: A crude oil-polluted site and an unpolluted site (4 km away from the polluted site). The polluted site is an oil-impacted site from a broken oil pipe owned by shell petroleum development company (SPDC) over one year before the study was done.

Bio-stimulating agent (cassava peel waste): The cassava peel (organic amendment) used in the study was collected from local farmers who removed these peels during garri processing. The peel collected was sun-dried for 2 weeks and ground. Its chemical composition analyzed were, pH 5.3, phosphorus 0.12 mg kg⁻¹, nitrogen 1.686%, potassium 2459.5 mg kg⁻¹, sodium 636.52 mg kg⁻¹, magnesium 409.38 mg kg⁻¹ and calcium 193.77 mg kg⁻¹. The concentration levels (amounts) of cassava peel used were 500 and 1000 g/1 m².

Experimental design/layout: A Randomized Complete Block Design (RCBD) consisting of four treatments in four blocks was used for the experiment. Each treatment was represented in each block and the treatments were replicated three times. An area of 16×10 m of the contaminated (polluted) site was mapped out. The area was subdivided into nine sub-plots of 1×1 m (1 m²) each with an interval of 1 m between them. Ridges were made at the demarcations to prevent the interchange of materials between plots. Six sub-plots of the polluted site, H₂ and H₃ were tilled and 500 g and 1000 g of dried ground cassava peel were added to each (1 m²), respectively. The other three sub-plots were without any treatment (H₁). Another sub-plot (unpolluted site) was mapped out. This was about 4 km away from the polluted plots and was also subdivided into three sub-plots.

Number of blocks	Treatments		
Block 1	H ₁ : Polluted soil with no bio-stimulation	H ₂ : Polluted soil bio-stimulation with 500 g cassava peel	H ₃ : Polluted soil bio-stimulation with 1000 g cassava peel
Block 2	H ₃ : Polluted soil bio-stimulation with 1000 g cassava peel	H ₁ : Polluted soil with no bio-stimulation	H ₂ : Polluted soil bio-stimulation with 500 g cassava peel
Block 3	H ₂ : Polluted soil bio-stimulation with 500 g cassava peel	H ₃ : Polluted soil bio-stimulation with 1000 g cassava peel	H ₁ : Polluted soil with no bio-stimulation
4 km interval			
Block 4	H ₄ : No pollution, no bio-stimulation	H ₄ : No pollution, no bio-stimulation	H ₄ : No pollution, no bio-stimulation

Fig. 1: Experimental layout with treatment description

The four treatments were as follows:

- H₁: Control (polluted soil with no bio-stimulation)
- H₂: Polluted soil bio-stimulated with 500 g cassava peel
- H₃: Polluted soil bio-stimulated with 1000 g cassava peel
- H₄: Double control (no pollution, no bio-stimulation)

The experimental layout was as shown in Fig. 1.

Soil collection and analysis: Soil samples were collected from the blocks (sampled sites) using a soil auger at three different times for the analysis of soil properties (pH, electrical conductivity, moisture content, total organic matter, total organic carbon, total nitrogen, phosphorus, potassium, Total Petroleum Hydrocarbon (TPH), Total Hydrocarbon Content (THC) and microbial population. The first was done before the addition of amendment (0 months) and then 2 and 4 months after the application of cassava peel (waste).

Determination of soil properties and microbial population:

Soil pH and electrical conductivity were determined electronically using a glass electrode pH metre (PHS. 25 Model) and conductivity metre (Labtech Model), respectively. The air oven method was used to determine soil moisture content. The API-RP45 Colorimetric method used by Aigberua *et al.*¹⁹ was used to determine the Total Hydrocarbon Content (THC) of soil. The TNRC Tx Method 1005 cited by Jude *et al.*²⁰ was used to determine the total petroleum hydrocarbon in the soil. Walkley-Black method cited by Tanee and Jude²¹ was used to determine total organic carbon in soil and potassium. The total organic matter content of the soil was determined by calculation, using the formula outlined by Osuji *et al.*²². Bray No.1 method, Jude *et al.*²³ were used to determine available phosphorus in the soil while

Kjeldahl method as outlined by Jude and Tanee²⁴ was used to determine total nitrogen of soil. Microbial population (total heterotrophic bacteria, total fungi, hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi) of soil was determined using the method by Baath and Anderson²⁵.

Statistical analysis: From the data obtained, treatment means and standard deviations were calculated. Two-way Analysis of Variance (ANOVA) was used to determine the significant difference(s) between means of treatments. The results were further subjected to Duncan's New Multiple Range Test to determine specific means with significant difference(s) at $p = 0.05$.

RESULTS

The result for Total Petroleum Hydrocarbon (TPH) and Total Hydrocarbon Content (THC) of soil remediated with different concentrations of cassava peel is shown in Fig. 2 and 3, respectively. Reductions in TPH and THC were observed in polluted soil bio-stimulated with cassava peel (H₂ and H₃) than in the control (H₁: Polluted soil with no bio-stimulation). The highest reduction was recorded in polluted soil bio-stimulated with 500 g cassava peel (H₂) while the least was observed in the control (H₁) at both 2 and 4 months. There was a significant difference ($p = 0.05$) in hydrocarbon reduction between bio-stimulated soil (H₂ and H₃) and the control (H₁: Polluted soil with no bio-stimulation) at both 2 and 4 months. Within bio-stimulated soil (H₂ and H₃), higher reductions in TPH and THC were obtained in polluted soil bio-stimulated with 500 g cassava peel (H₂). There was also a significant difference ($p = 0.05$) in hydrocarbon reduction between bio-stimulated soils at 2 and 4 months.

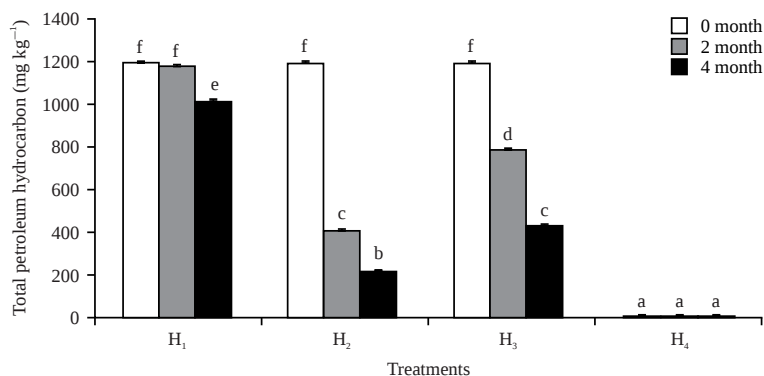


Fig. 2: Effects of treatments on total petroleum hydrocarbon of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

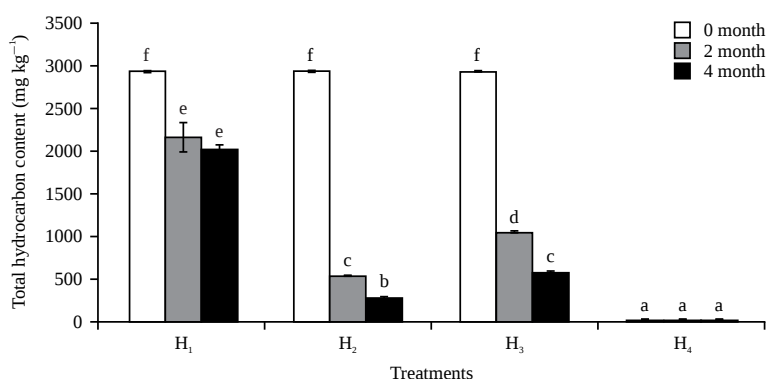


Fig. 3: Effects of treatments on total hydrocarbon content of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

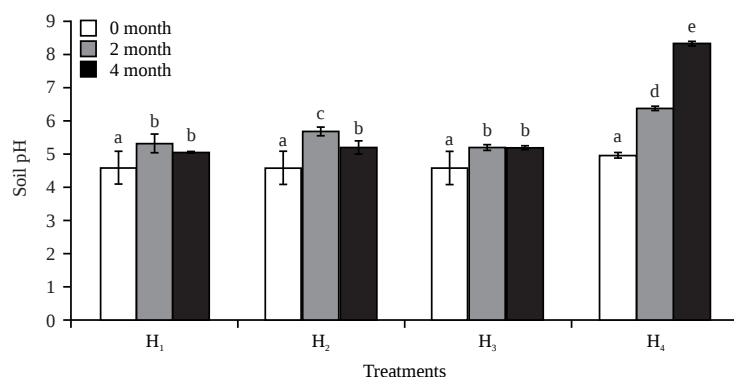


Fig. 4: Effects of treatments on soil pH

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

Results showed an increase in soil pH in both bio-stimulated soil and the control (Fig. 4). Between polluted soil (either bio-stimulated or no bio-stimulation), polluted soil bio-stimulated with 500 g cassava peel recorded the highest

increase in pH at 2 months. There was a significant difference ($p = 0.05$) between polluted soil bio-stimulated with 500 g cassava peel (H₂) and both H₁ (control: Polluted soil with no bio-stimulation) and H₃ (polluted soil bio-stimulated with

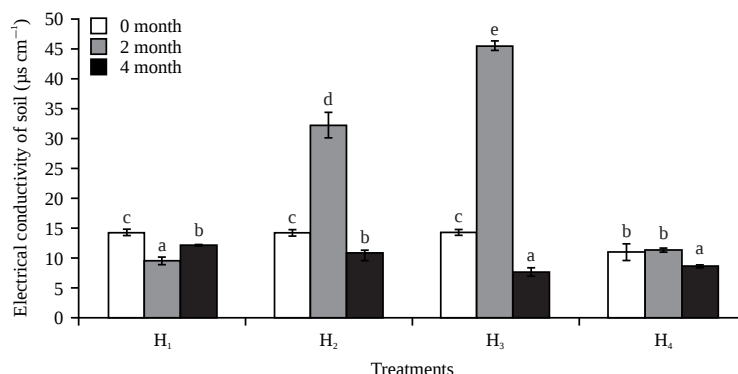


Fig. 5: Effects of treatments on soil electrical conductivity

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean ± SEM and different alphabet shows significance between means of treatments

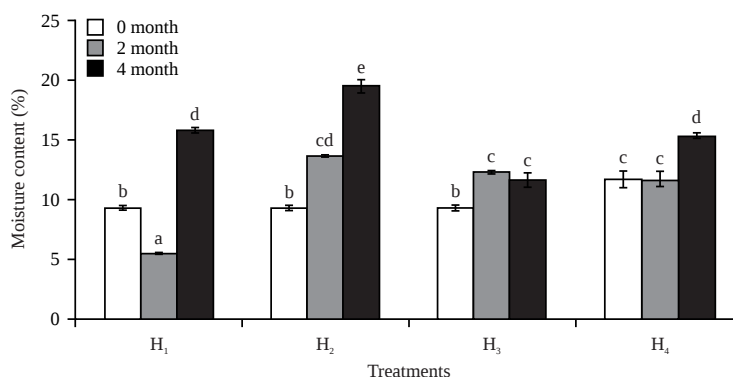


Fig. 6: Effects of treatments on moisture content of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean ± SEM and different alphabet shows significance between means of treatments

1000 g cassava peel). However, the double control (no pollution, no bio-stimulation) recorded a significant ($p = 0.05$) increase in pH compared to the polluted soil stimulated with cassava peel and the control at both 2 and 4 months.

Figure 5 shows the result for soil electrical conductivity in the course of the study. Fluctuation in soil conductivity was observed. At 2 months, increases were observed in polluted soil bio-stimulated with cassava peel (H₂ and H₃) and the double control (H₄: No pollution, no bio-stimulation) while the control (H₁: Polluted soil with no bio-stimulation) recorded a decrease in soil conductivity compared to the baseline result obtained at 0 months. At 4 months the reverse was the case, decreases were observed in polluted soil bio-stimulated with cassava peel (H₂ and H₃) and the double control (H₄) while an increase was observed in the control (H₁) compared to the result obtained at 2 months. There was a significant difference

in the increase of soil conductivity between bio-stimulated soils (H₂ and H₃) and the double control (H₄) and also between the bio-stimulated soils (H₂ and H₃).

The result showed an increase in soil percentage moisture content of bio-stimulated soil (H₂ and H₃) while in the control (H₁) decrease in moisture content was observed at 2 months (Fig. 6). However, at the end of the study (4 months), an increase in moisture content of the soil was observed in all treatments except in polluted soil bio-stimulated with 1000 g cassava peel (H₃) where a decrease in moisture content of soil compared to that obtained at 2 months was observed. The highest increase in moisture content of the soil was obtained in polluted soil bio-stimulated with 500 g cassava peel at 4 months. There was a significant difference ($p = 0.05$) in an increase in soil moisture content between polluted soil bio-stimulated with 500 g cassava peel (H₂) and H₁ (control) and H₄ (double control) at 4 months.

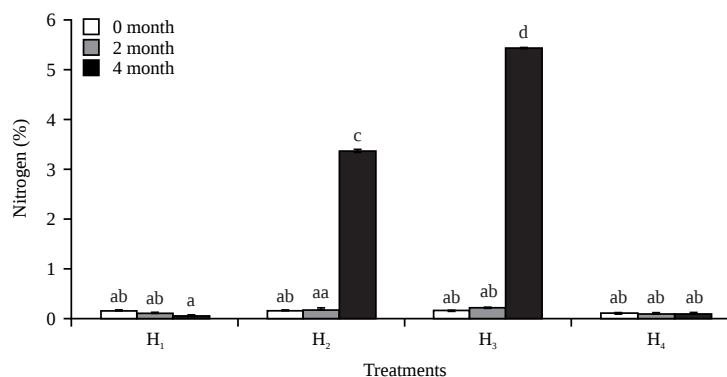


Fig. 7: Effects of treatments on total nitrogen of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

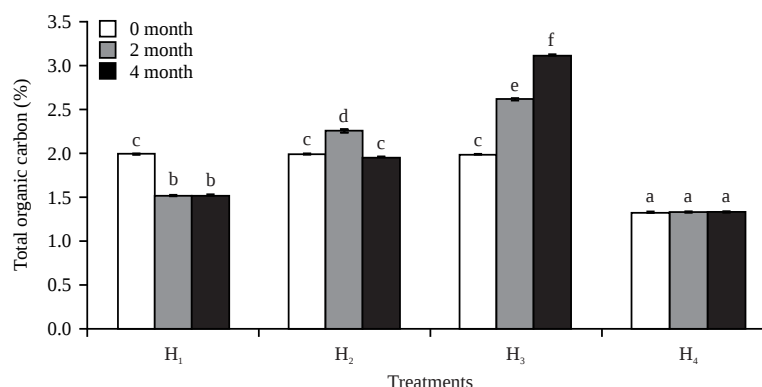


Fig. 8: Effects of treatments on total organic carbon in soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

The result for soil total nitrogen in soil is presented in Fig. 7. A significant increase ($p = 0.05$) in nitrogen were recorded in the cassava peel bio-stimulated soils (H₂ and H₃) while the control (H₁: Polluted soil with no bio-stimulation) recorded a decrease in nitrogen at the end of the study (4 months). Within bio-stimulated soil, the highest increase in Nitrogen was obtained in polluted soil bio-stimulated with 1000 g cassava peel (H₃) at 4 months. There was a significant difference ($p = 0.05$) between the nitrogen of cassava peel bio-stimulated soil (H₂ and H₃) and the control (H₁) at 4 months. The least nitrogen content of the soil was obtained in the double control (H₄: No pollution, no bioremediation).

Figure 8 and 9 shows results for total organic carbon (TOC) and total organic matter (TOM) of soil. The result showed an increase in TOC and TOM in bio-stimulated soil (H₂ and H₃) while the decrease was observed in the control (H₁: Polluted soil with no bio-stimulation). Within cassava peel

bio-stimulated soils (H₂ and H₃), the highest increase in TOC and TOM was observed in polluted soil bio-stimulated with 1000 g cassava peel at both 2 and 4 months. There was a significant difference in TOC and TOM of bio-stimulated soils and the control and also the double control (H₄: No pollution, no bio-stimulation). Results also showed a significant difference ($p = 0.05$) in TOC and TOM between bio-stimulated soil (H₂ and H₃) at 2 months and 4 months.

Figure 10 showed that phosphorus in soil increased in both cassava peel bio-stimulated soils (H₂ and H₃) and polluted soil with no bio-stimulation (H₁: Control) with the control having the highest increase in phosphorus at 2 months. There was a significant difference ($p = 0.05$) between an increase in phosphorus in the control (H₁) and the bio-stimulated soils (H₂ and H₃) at 2 months. However, a decrease in phosphorus was observed in the control (H₁) at the end of the study (4 months).

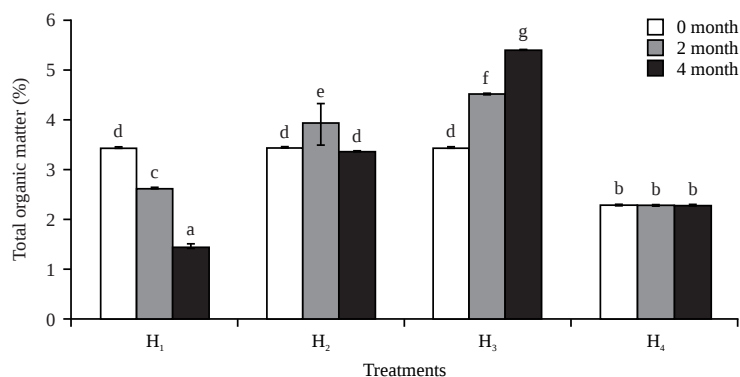


Fig. 9: Effects of treatments on total organic matter of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

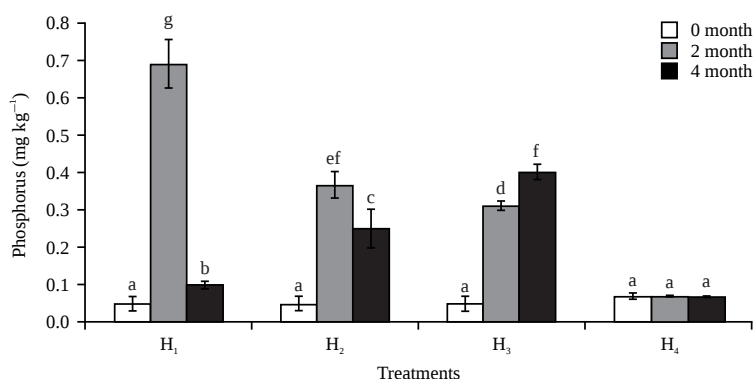


Fig. 10: Effects of treatments on phosphorus of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

At the termination of the study (4 months) increase in potassium was observed in the cassava peel bio-stimulated soils (H₂ and H₃) while a decrease was observed in the control (H₁: Polluted soil with no bio-stimulation), (Fig. 11). There was a significant difference in potassium increase between H₃ (polluted soil bio-stimulated with 1000 g cassava peel) and H₂ (polluted soil bio-stimulated with 500 g cassava peel) at $p = 0.05$.

The addition of cassava peel significantly increased the microbial population in polluted soil as shown in Table 1. Total heterotrophic bacteria (THB) population increased in the cassava peel bio-stimulated soil (H₂ and H₃) and decreased in the control (H₁: Polluted soil with no bio-stimulation) while in the double control (H₄: No pollution, no bio-stimulation) the THB population was constant at 2 months compared to that obtained at 0 month. Within bio-stimulated soil at 2 months, the increase in THB observed in the 500 g cassava peel bio-stimulated soil was significantly higher than that of 1000 g cassava peel bio-stimulated soil. There was a significant

difference between the THB population of polluted soil bio-stimulated with cassava peel and the double control at 4 months, $p = 0.05$. An increase in total fungi (TF) of soil was also observed in all polluted soil (either bio-stimulated or with no bio-stimulation) at 2 months. Between polluted soil with no bio-stimulation (H₁) and polluted soil bio-stimulated with the different concentrations of cassava peel (H₂ and H₃), the highest TF population was observed in H₁ (control). There was a significant difference ($p = 0.05$) between the TF population of the control (H₁) and bio-stimulated soil (H₂ and H₃). Within bio-stimulated soil, H₃ (polluted soil bio-stimulated with 1000 g cassava peel) showed a higher TF population than H₂ (polluted soil bio-stimulated with 500 g cassava peel). No increase in the TF population was observed in H₄ (double control). At 4 months, TF increase in bio-stimulated soil while the decrease was observed in the control and the double control. There was a significant difference between the TF of the bio-stimulated soil (H₂ and H₃), the control (H₁) and the double control (H₄), $p = 0.05$.

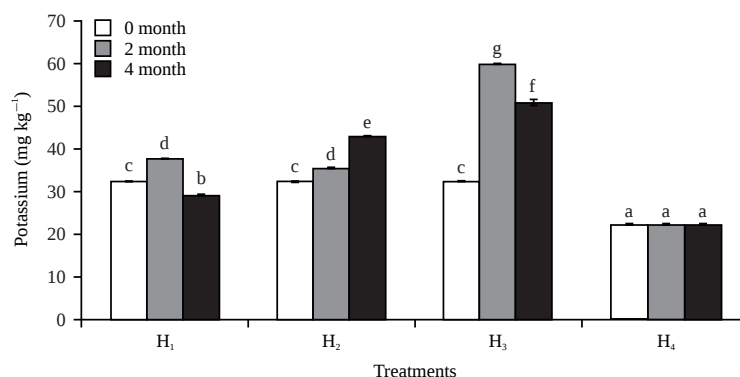


Fig. 11: Effects of treatments on potassium of soil

H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel, H₄: Double control (no pollution, no bio-stimulation), the chart represents Mean $\pm$ SEM and different alphabet shows significance between means of treatments

Table 1: Microbial population in the different treatments

Treatments	THB (CFU g ⁻¹)			TF (CFU g ⁻¹)			HUB (CFU g ⁻¹)			HUF (CFU g ⁻¹)		
	0 month	2 month	4 month	0 month	2 month	4 month	0 month	2 month	4 month	0 month	2 month	4 month
H ₁	8.4 $\times$ 10 ⁵	8.0 $\times$ 10 ⁴	3.8 $\times$ 10 ⁶	3.7 $\times$ 10 ⁴	4.5 $\times$ 10 ⁶	4.0 $\times$ 10 ⁵	4.0 $\times$ 10 ⁴	4.4 $\times$ 10 ⁵	8.5 $\times$ 10 ⁵	3.2 $\times$ 10 ⁴	7.5 $\times$ 10 ⁵	6.3 $\times$ 10 ⁴
H ₂	8.4 $\times$ 10 ⁵	6.8 $\times$ 10 ⁶	3.4 $\times$ 10 ⁶	3.7 $\times$ 10 ⁴	4.6 $\times$ 10 ⁴	9.0 $\times$ 10 ⁵	4.0 $\times$ 10 ⁴	6.5 $\times$ 10 ⁶	6.5 $\times$ 10 ⁵	3.2 $\times$ 10 ⁴	1.95 $\times$ 10 ⁵	1.1 $\times$ 10 ⁵
H ₃	8.4 $\times$ 10 ⁵	3.4 $\times$ 10 ⁶	3.4 $\times$ 10 ⁶	3.7 $\times$ 10 ⁴	1.98 $\times$ 10 ⁵	1.9 $\times$ 10 ⁵	4.0 $\times$ 10 ⁴	9.6 $\times$ 10 ⁵	8.9 $\times$ 10 ⁵	3.2 $\times$ 10 ⁴	1.7 $\times$ 10 ⁶	9.0 $\times$ 10 ⁴
H ₄	1.26 $\times$ 10 ⁶	1.26 $\times$ 10 ⁶	2.6 $\times$ 10 ⁶	4.3 $\times$ 10 ⁴	4.3 $\times$ 10 ⁴	2.7 $\times$ 10 ⁴	5.3 $\times$ 10 ⁴	5.3 $\times$ 10 ⁴	8.5 $\times$ 10 ⁵	4.2 $\times$ 10 ⁴	4.2 $\times$ 10 ⁴	2.3 $\times$ 10 ⁴

THB: Total heterotrophic bacteria, TF: Total fungi, HUB: Hydrocarbon utilizing bacteria, HUF: Hydrocarbon utilizing fungi, H₁: Control (polluted soil with no bio-stimulation), H₂: Polluted soil bio-stimulated with 500 g cassava peel, H₃: Polluted soil bio-stimulated with 1000 g cassava peel and H₄: Double control (no pollution, no bio-stimulation)

Results showed an increase in hydrocarbon utilizing bacteria (HUB) in soil. Between the control (H₁) and bio-stimulated soil, the highest HUB population was observed in the cassava peel bio-stimulated soil. There was a significant difference ($p = 0.05$) between the HUB population of cassava peel bio-stimulated soils (H₂ and H₃) and the control (H₁) at 2 months. Within cassava peel, bio-stimulated soils, the polluted soil bio-stimulated with 500 g cassava peel (H₂) recorded a higher HUB population than the polluted soil bio-stimulated with 1000 g cassava peel (H₃) at 2 months while at 4 months the polluted soil was bio-stimulated with 1000 g cassava peel (H₃) recorded highest HUB population. There was a significant difference ($p = 0.05$) between the HUB population of bio-stimulated soil (H₂ and H₃) at 2 months and 4 months.

Results showed an increase in the hydrocarbon utilizing fungi (HUF) population of both cassava peel bio-stimulated soils (H₂ and H₃) and the control (H₁: Polluted soil with no bio-stimulation) while the HUF of the double control (H₄: No pollution, no bio-stimulation) was constant at 2 months. Between bio-stimulated soil (H₂ and H₃) and the control (H₁), bio-stimulated soils had a higher HUF population

than the control. There was a significant difference ($p = 0.05$) between the HUF population of bio-stimulated soils and the control. Within bio-stimulated soils (H₂ and H₃), the 1000 g cassava peel bio-stimulated soil recorded higher HUF than the 500 g bio-stimulated soil. There was a significant difference between the HUF of 1000 g cassava peel bio-stimulated soil and the 500 g cassava peel bio-stimulated soil, at 2 months. The significant difference was at $p = 0.05$.

At 4 months, a HUF decrease was observed in all the soil. However, the highest HUF population was obtained in 1000 g cassava peel bio-stimulated soil. There was a significant difference between HUF populations in 500 g cassava peel bio-stimulated soil and other treatment H₃, the control and double control.

DISCUSSION

In this study, nutrient addition (cassava peel) as bio stimulating agent to crude oil-polluted soil in the bioremediation process helped in speedy reduction of hydrocarbon of the soil to as low as a reasonably practicable condition.

Higher reduction in Total Petroleum Hydrocarbon (TPH) and Total Hydrocarbon Content (THC) observed in treated polluted soil against the polluted soil without treatments could be attributed to the addition of cassava peel to polluted soil. The cassava peel acted as a bio-stimulating agent accelerating the biodegradation of the hydrocarbon contaminant. The rapid degradation of hydrocarbon observed in the treated soil could also be because the cassava peel added to the polluted soil stimulated the catabolic process of indigenous microorganisms thus enhancing the utilization of the contaminant as a source of energy and carbon^{26,27}. Agbor *et al.*²⁸ reported cassava peel as an agricultural waste with the potential for enhancement of reduction of crude oil in polluted soil. Similar observations have been reported using other organic amendments²⁹.

It has been earlier reported that crude oil-polluted soil amended with the organic matter may stimulate the growth of the indigenous oil-degrading microbiota in it^{30,31}.

The pH values of crude oil polluted soil were lower as compared to the unpolluted soil (double control) at 0 months, a finding which is in line with Benson *et al.*³². The increase in pH values (decrease in acidity) may be due to the increased degradation of crude oil by microorganisms in the soil. Electrical conductivity increased in the cassava peel-treated soil at 2 months, this infers that the cassava peel helped in the release of dissolved solutes and hence, increases the EC. The moisture content of polluted soil was lower than that of the cassava peel treated polluted soil unpolluted soil at 2 months. This lower moisture observed in polluted soil may be since the pollutant coats the soil and consequently prevented the penetration of water. This is in line with Nte *et al.*³³, who observed a reduction in water infiltration in soil polluted with spent engine oil. This is also in agreement with the work of Onifade *et al.*³⁴, who observed the same trend of result when working on bioremediation of crude oil-polluted soil using enhanced natural attenuation. However, the result is in contrast with the work of Benson *et al.*³². The increase in nitrogen observed in the cassava peel treated soil may be linked to nutrient supply from the cassava peel (organic waste) which improved soil properties and subsequently its fertility. Cassava peel (an organic waste addition, especially at the higher concentration (1000 g) was effective in increasing the total nitrogen of soil resulting in accelerated biodegradation of hydrocarbon. Jude and Tanee²⁴ observed increased nitrogen in crude oil polluted soil amended with sawdust (organic waste). Jude *et al.*^{20,23} also reported an increase in nitrogen in cassava peel amended soil. The decrease in TOC and TOM of polluted soil without treatment (control) may be a result of carbon utilization by soil microorganisms as an energy source for hydrocarbon degradation. This agrees

with the findings of Shahi *et al.*¹², who reported that the microorganisms in the soil can utilize crude oil as a source of carbon and energy. The increase in TOC and TOM in the cassava peel treated soil infers that cassava peel after decomposition supplies nutrients (organic matter) This is following Agbor *et al.*²⁸, who reported cassava peel efficiency in stimulating hydrocarbon degradation by increasing the total heterotrophic microbial growth and activity.

The decrease in phosphorus and potassium in the control (polluted soil) at the end of the study is an indication that these nutrients can be used as fertilizers by the microorganisms that degrade the petroleum hydrocarbon. An increase in soil THB and TF, hydrocarbon utilizing and hydrocarbon utilizing fungi in the treated soils may be due to the addition of the cassava peel (organic waste) which improved soil properties previously damaged by a crude oil spill, increased the population of both bacteria and fungi in the polluted soil to speed up remediation rate resulting in the great reduction in THC and TPH observed in the cassava peel treated soil. The suppression of microbial count in polluted soil compared to the unpolluted soil (double control) at 0 month could be attributed to the selective destruction of the microorganisms by the crude oil. This is because the crude oil produces an anaerobic condition as it is introduced into the soil and it automatically eliminates most of the aerobic organisms. This is in agreement with the work of Kayode *et al.*³⁵ and Al-Sayegh *et al.*³⁶, who in their separate studies observed that when crude oil is introduced into the soil, it affects soil physico-chemical properties and causes damage to the soil biota.

The method used for remediation of crude oil-polluted soil in the study is an eco-friendly method with minimal effect compared to the chemical methods. The method in the study can be used in the remediation of mild crude oil-polluted soil. The method should be extended to other polluted sites to verify its efficacy. It was a microcosm trial and has not been done on a large-scale.

CONCLUSION

From the result of this study, it could be concluded that the biodegradation of petroleum hydrocarbon was significantly enhanced by the addition of cassava peel to the crude oil polluted soil as higher hydrocarbon (TPH and THC) reductions and improved soil properties as well as increased microbial population (hydrocarbon-degrading bacteria and fungi) were observed in polluted soil treated with the different amounts of cassava peel than in the control. Thus, the usage of cassava peel in the bioremediation of crude oil-polluted soil is hereby recommended.

SIGNIFICANCE STATEMENT

The study establishes cassava peel waste as an effective biostimulant in the biodegradation of hydrocarbon. The use of cassava peel (which is a major waste produced during garri processing with lots of disposal problems) in bioremediation of crude oil-polluted soil will be an affordable eco-friendly and alternative method of soil remediation. Thus, increasing the remediation options for crude oil pollution and improving agricultural activities, especially in Ogoni where crude oil spill/pollution is a common phenomenon.

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