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Effect of Fermented and Non-fermented *Vernonia Amygdalina* (Bitter Leaf) Extract on Remediation of Crude Oil Contaminated Loamy Sand Soil

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Abstract

This work considered the remediation of hydrocarbon polluted soil using different volume of fermented and unfermented *Vernonia amygdalina* (bitter leaf) extracts. The NPK (Nitrogen, Phosphorus, and Potassium) value of the non-fermented and fermented *Vernonia amygdalina* extract, as well as the pretest of the of the particle size distribution/textural class of the soil conditions, were examined. Physicochemical and bacteriological parameters of the treated soil such as Total Petroleum Hydrocarbon (TPH), Total Polycyclic Aromatic Hydrocarbon (TPAH), Total Monocyclic Aromatic Hydrocarbon (TMAH), and Bacteria Count were determined. The study used Microsoft Excel software, paired t-tests and Analysis of Variance (ANOVA) for statistical analyses. The results showed that the *Vernonia amygdalina* extract, both fermented and non-fermented, had a high NPK (Nitrogen, Phosphorus, and Potassium) values, hence, making both extracts suitable for cleaning up contaminated soil. At the end of the 72-day remediation period, the results similarly demonstrated a considerable reduction in TPH, TPAH, and BTEX (TMAH) in all treatment approaches. Over the course of 72 days, the percentage reductions for TPH, TPAH, and TMAH for fermented and non-fermented extracts were 98.37, 96.94, 94.51, and 98.57, 92.02, 94.51, respectively. The microorganisms in the area had an impact on these degradations as well. Furthermore, the results of the ANOVA (Analysis of Variance) indicated a slight difference at 95% confidence intervals. Therefore, it is advised that the non-fermented and fermented *Vernonia amygdalina* extracts be utilized on loamy sand soil contaminated with crude oil for its remediation.

Keywords: *Vernonia amygdalina*; Contaminants; Remediation; Crude oil; Soil

1. Introduction

The effects of petroleum hydrocarbons pollution on the environment, plant, animals and humans have been well documented in the literatures [1]. The quantity of petroleum hydrocarbons is determined by various factors such as high viscosity, low emulsifying ability and low density. These qualities undoubtedly make it easy for hydrocarbons to be absorbed on surfaces such as soil. The absorption of hydrocarbons on such surfaces tends to affect the properties of the soil especially soil permeability and its porosity [2, 3]. Due to the rich carbon composition of the hydrocarbon and low nitrogen content, when it comes in contact with soil, it tends to change the soil components, carbon to nitrogen as well as carbon to phosphorus ratios. Other factors affected due to soil contact with hydrocarbon include conductivity, pH and salinity [4]. Petroleum compounds could also reduce inorganic nitrogen and phosphorus content in the soil thereby, limiting nitrification and phosphoric acid removal from the soil. These phenomena would result in the decrease in the absorptive capacity of crops [5, 6, 7].

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The micro flora and fauna of the soil environment are also not left out in the negative impact of petroleum hydrocarbons. Studies have shown that an environment that is polluted by hydrocarbons can have devastating effects on the ecosystem, population of microorganisms as well as the enzyme system in the soil [8, 9]. Research has revealed that micro-organisms that can live in a hydrocarbon polluted soil are not produced under normal condition. However, micro-organisms can survive in such an environment by producing a number of enzymes that can help them have dominance in terms of population with synergetic or symbiotic effect [10].

The alteration of the physiochemical and biological composition of a soil by hydrocarbon pollution can hinder crop growth and yield due to reduction in germination rate and the weakening of the resistance of crops to pests and diseases [11, 12, 13]. This could lead to food insecurity. In addition, hydrocarbons or petroleum have polycyclic aromatic hydrocarbons (PAHs) that are carcinogenic, mutagenic, teratogenic and other damaging effects [14]. It may enter into the body via breathing, diet or body contact with its attendant effects to the internal organs such as liver and kidney. This undoubtedly would have health implications. Finally, the petroleum pollutants in the soil affect the entire ecosystem especially, the low boiling point and light weight hydrocarbons. In addition, these petroleum pollutants can enter into the body through the food chain [15].

Remediation of polluted soil has been studied by different researchers. Different methods such as physical, chemical and biological are employed in the course of polluted soil remediation [16]. These methods, which are traditional, may include soil removal and replacement, heat treatment, extraction-separation and chemical oxidation methods [17, 18]. Biological method has a lot of benefits compared to the physical and chemical methods. The advantages include low cost, environment friendly, no secondary pollution, simple in-situ treatment, and removes some pollutants with higher efficiency [19, 20, 21, 22, 23].

Biological method also known as bioremediation is the controlled use of biological agents including fungi, bacteria, and plants to transform complex or simple hazardous chemical compounds into non-hazardous forms [25, 24]. Microorganisms used for bioremediation could be indigenous or imported [26]. However, one of its major drawbacks is the lengthy remediation time and the commitment to monitoring. In order to facilitate the bioremediation process, organic and inorganic nutrients are often added [27, 28, 29, 30]. Several agro-wastes have been used as nutrients because of their availability and cost effectiveness. Some effective nutrients as reported in literature include Larva cast, saw dust and groundnut shell [31]. These nutrients are a major source of NPK which is the best microorganisms' live keeping substance. For optimal bioremediation process, nutrient supplementation must be accompanied by other environmental conditions including pH, temperature, water and oxygen to speed up microbial metabolism rate and pathway [32, 33, 34, 35]. Observably, most nutrients supplied are in the solid form and so must require the application of water [36]. But with fertigation which is a combination of fertilizer application and irrigation process, nutrient and water will be applied together in one process. One such nutrient source is the *Vernonia amygdalina* (bitter leaf) extract.

Bitter leaf extract is an agro-waste (liquid waste) commonly discharged into the environment without treatment. This effluent has the tendency of causing eutrophication because of the high content of NPK when discharged into surface water bodies without treatment. However, bitter leaf extract can be used in the bioremediation of hydrocarbon polluted soil because of its NPK content. This will not only preserve the environment, but also could save energy required to pump water for irrigating a bioremediation site. Although the bitter leaf plant has been used for phytoremediation, there is a dearth of study on the use of the extract as a fertilizer and irrigation component. This study investigates the effectiveness of bitter leaf extract as bio-stimulants of indigenous hydrocarbon utilizing bacteria (HUB) in the remediation of petroleum hydrocarbon polluted soil which is lacking in literature.

2. Materials and Methods

2.1. Materials

The materials used for the experiment include soil, crude oil, *Vernonia amygdalina* (bitter leaf), Reactors, Plastic containers, hand auger, hand glove, sample bottles, weigh balance, laptop, books, pen, ruler, pencil, eraser.

2.2. Methods

2.2.1. Sample Preparation

Soil samples were collected from different random spot per reactor using a hand dug auger capable of obtaining uniform cores of equal volumes to desired depth for the background check. The samples were put in amber coloured glass vials with no headspace to preserve the integrity of the sample in order to prevent volatilization. The sample was then stored

in a cooler containing sufficient ice blocks, and transported to the laboratory for analysis in order to achieve background data. The sample preparation was strictly adhered to in line with Environmental Guideline and Standard of petroleum industry in Nigeria for quality assurance [34]. The *Vermonia amygdalina* leaf was squeezed manually by hand with water at equal (leaf:water) ratio. The squeezed leaf with water was filtered to remove the leaf and retrieve the juice. A part of the juice was left to ferment for 5days similar to [36] while the other part was left unfermented.

2.2.2. Experimental Design

The Completely Randomized Design (CRD) was used in this study. For this experiment, the design consisted of 21 reactors, with crude oil serving as the source of contamination. The experimental reactor of volume 0.02 m^3 (20L) which was divided into 7 treatments and 3 replication each totaling 21 reactors. Each reactor contained 5 kg of soil with different treatment options and labeled T_0, T_1, T_2,T_3, T_4, T_5, and T_6 including the control T_0 with three replications as shown in Table 1. Randomization was achieved using the draw. The experimental design is as follows: T_0 is the control without any treatment;T_1 is the addition of 250 ml of non-fermented *Vermonia amygdalina* extract; T_2 is the addition of 500 ml of non-fermented *Vermonia amygdalina* extract; T_3 is the addition of 750 ml of non-fermented *Vermonia amygdalina* extract; T_4 is the addition of 250 ml of fermented *Vermonia amygdalina* extract; T_5 is the addition of 500 ml of fermented *Vermonia amygdalina* extract; T_6 is the addition of 750 ml of fermented Vermonia *amygdalina* extract.

Table 1 Layout of the Completely Randomized Design for Crude Oil Contaminated Soil Treatments (T_0, T_1, T_2, T_3, T_4, T_5, T_6) each replicated into three times)

Treat-ment	**Levels of Treatment**		
	Replicate 1	**Replicate 2**	**Replicate 3**
T_0	$T_{0,1}$	$T_{0,2}$	$T_{0,3}$
T_1	$T_{1,1}$	$T_{1,2}$	$T_{1,3}$
T_2	$T_{2,1}$	$T_{2,2}$	$T_{2,3}$
T_3	$T_{3,1}$	$T_{3,2}$	$T_{3,3}$
T_4	$T_{4,1}$	$T_{4,2}$	$T_{4,3}$
T_5	$T_{5,1}$	$T_{5,2}$	$T_{5,3}$
T_6	$T_{6,1}$	$T_{6,2}$	$T_{6,3}$

2.2.3. Experimental Procedure

For the experiment, about 5000g of uncontaminated soil was mixed with 250ml of crude oil. The crude oil sample of 250ml was administered into each of the 5000g of soil samples in the reactors through a perforated can and mixed using a hand auger and kept undisturbed for 3 days. On the 3rd day the reactors were fertigated with non-fermented and fermented bitter leaf extract of 250ml, 500ml, 750 ml respectively. Following the fertigation process, the treated crude oil polluted soils were aerated by tilling using the hand auger thrice every week. Soil samples were collected from each reactor every two weeks after aeration for laboratory analysis. Soil samples were analyzed for Hydrocarbon Utilizing Bacteria count (HUB), total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbon (PAH) and BTEX. Particle size distribution (PSD) analysis for soil textural class determination for contaminated soil was also carried out using the hydrometer method.

3. Results and Discussion

3.1. Particle Size Analysis of the Uncontaminated and Contaminated Soils

The result of the uncontaminated and contaminated soils showed the relative content of soil particles of various sizes such as sand, silt and clay in the soil (Table 2). The results as seen, shows that the soil texture was loamy sand for uncontaminated and contaminated soils, according to United State Department of Agriculture for textural classification of soil [34]. As was expected, crude oil contamination did not alter the soil texture.

Table 2 Particle Size Analysis of soil samples

Treatment	Sand	Silt	Clay	Textural Class
Uncontaminated	79.00	8.40	12.60	Loamy Sand
T_0	79.90	9.90	10.20	Loamy Sand
T_1	80.00	8.30	11.70	Loamy Sand
T_2	78.60	9.80	11.60	Loamy Sand
T_3	76.60	12.80	10.60	Loamy Sand
T_4	75.60	10.80	13.60	Loamy Sand
T_5	77.60	9.80	12.60	Loamy Sand
T_6	80.40	9.20	10.40	Loamy Sand

3.2. NPK Values of *Vermonia amygdalina* extract

Table 3 shows the NPK (Nitrogen, Phosphorus and Potassium) composition of the non-fermented and fermented *Vermonia amygdalina* extract. The results showed that (Nitrogen, Phosphorus, Potassium Content in thefermented and non-fermented *Vermonia amygdalina* extract were similar with N content being the lowest and K content being the highest.The trend of the nutrient (NPK) values in the fermented extract differs from other works [37, 36]. In the work of [36] the K and P showed the highest and least values while in [37], P and K showed the highest and least values. These works suggest that the NPK values of plant fermented extract is dependent on the source plant as well as duration of concentration.

Table 3 *Vernonia amygdalina* Extract and NPK Values

BiostiMulants	Nitrogenmg/kg	Phosphorusmg/kg	Potassiummg/kg
NF	0.52	24.41	37.46
F	0.57	24.40	38.03

NF = Non-fermented *Vernonia amygdalina* extract, and F Fermented *Vernonia amygdalina* extract.

3.3. Effect of fertigation on HUB count with time of incubation

The application of fermented and unfermented bitter leaf extract to the contaminated soil showed stimulation of HUB. Figures 1, 2 & 3 show the graphical presentation of hydrocarbon utilization bacterial count for the control experiment and that of the crude oil polluted soil treated with different quantities of fermented and non-fermented *Vermonia amygdalina* extract. The HUB count in the reactor varied according to the amounts of fermented and non-fermented *Vernonia amygdalina* extract added to the reactors, and it climbed progressively until it reached its peak as seen in Figures 1, 2 & 3 below. The growth of the HUB may be due to favorable ecological conditions found in the soil [38]. One advantageous tactic for increasing the metabolic activity of microorganisms is the provision of suitable nutrients [39]. After about 35 days of the experiment, there was a decline in the growth of the bacteria which continued till the end of the experiment. The decline in bacterial growth is because the bacteria have used up the contaminant (hydrocarbon) in the soil. This is consistent with literature [40, 41].

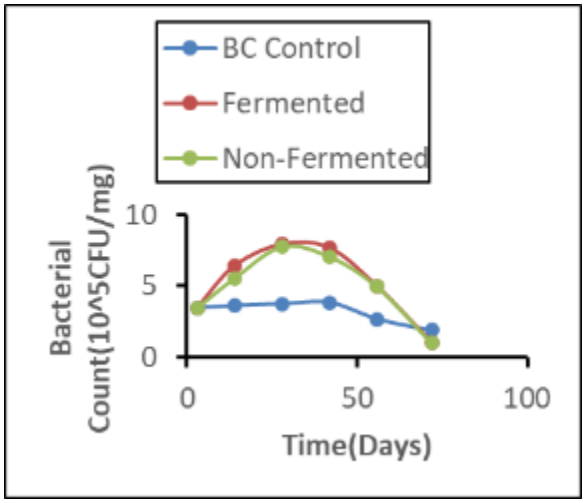

Figure 1 Effect of 250 ml of Fermented and Non-Fermented *Vermonia amygdalina* extract on Bacterial Count in Contaminated Soil

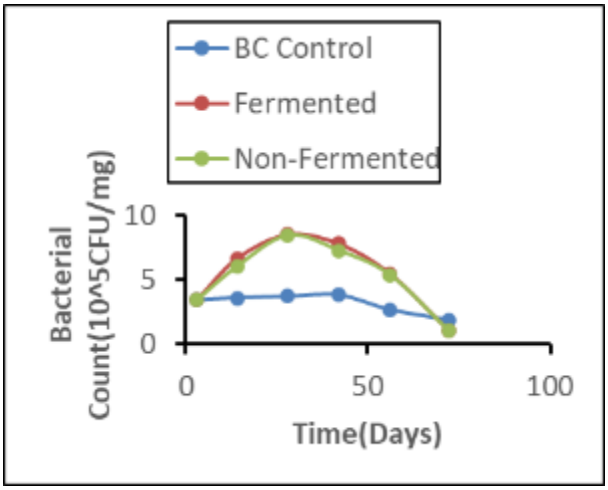

Figure 2 Effect of 500 ml of Fermented and Non-Fermented *Vermonia amygdalina* extract on Bacterial Count in Contaminated Soil

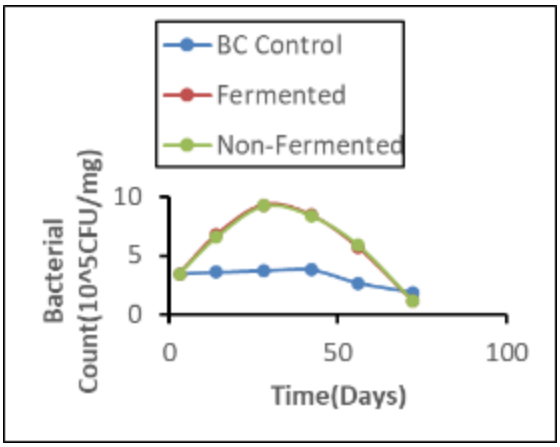

Figure 3 Effect of 750 ml of Non-Fermented *Vermonia amygdalina* extract on Bacterial Count in Contaminated Soil

3.4. Comparison of the Effectiveness of Non-Fermented and Fermented *Vermonia amygdalina* extract in the Remediation of Petroleum Contaminated Soil

3.4.1. TPH

The variability of TPH concentration measured during soil remediation with non-fermented (T_1, T_2 and T_3) and fermented (T_4, T_5 and T_6) *Vermonia amygdalina* extract are presented graphically in Figure 5. It is observed that TPH concentration decreased steadily with treatment duration and volume of bitter leaf extract for both the naturally attenuated (T_0) as well as the non-fermented (T_1, T_2, and T_3) and the fermented (T_4, T_5, and T_6) bitter leaf extract treatments. Though a significant difference ($P < 0.05$) in TPH reduction was observed between the naturally attenuated and the bio-stimulated treatment, the reduction level between the fermented extract and non-fermented extract treatment were similar. This can be attributed to the similarity in the NPK values of both extracts (Table 3).

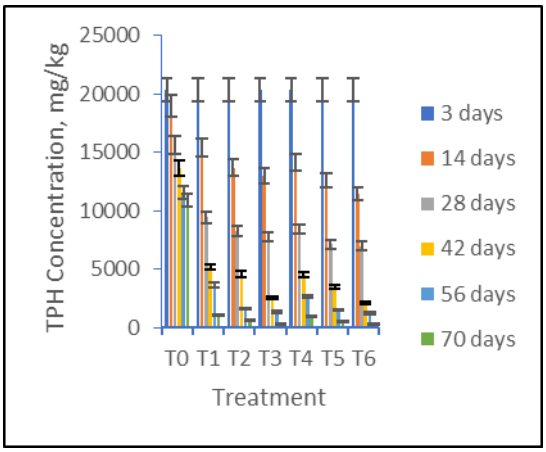

Figure 4 Variation in TPH during Remediation of Crude Contaminant Soil using Non-fermented and Fermented *Vermonia amygdalina* extract

3.4.2. PAH

Figure 6 depicts the variation in PAH concentrations measured during the remediation of crude oil contaminated soil using non-fermented (T_1, T_2, and T_3) and fermented (T_4, T_5, and T_6) *Vermonia amygdalina* extract. The graphical analysis (Figure 6) revealed difference in PAH concentrations in different reactors (T_1 and T_4, T_2 and T_5, T_3 and T_6) during the remediation period. The results of the soil samples taken at the different reactors (T_1 and T_4, T_2 and T_5, T_3 and T_6) agreed closely, indicating their slight significant discrepancy. A statistically significant difference was clear in the graphical observation of the plot of PAH concentration data throughout the remediation period at 5% standard error bars in Figure 6.

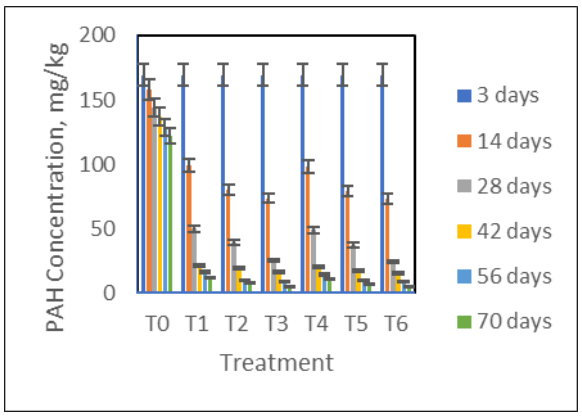

Figure 5 Variation in PAH during Remediation of Crude Contaminant Soil using Non-fermented and Fermented *Vermonia amygdalina* extract

3.4.3. BTEX

The variation in BTEX concentrations measured throughout the period of remediation is represented in Figure 6 below. Different bar associations were found at the various treatment with non-fermented (T_1, T_2 and T_3) and fermented (T_4, T_5 and T_6) *Vermonia amygdalina* extract, according to the graphical analysis (Figure 6). The soil samples taken at the same place were recorded, and the results showed a considerable variance. In Figure 6, the graphical observation of the plot of BTEX concentration data over the course of the *Vermonia amygdalina* extract at 5% standard error bars revealed a statistically significant difference.

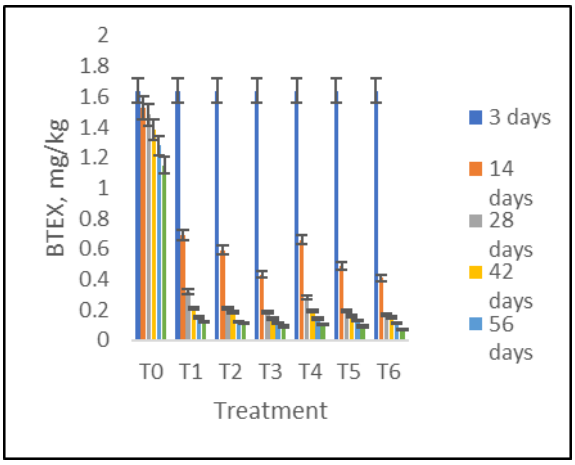

Figure 6 Variation in BTEX during Remediation of Crude Contaminant Soil using Non-fermented and Fermented *Vermonia amygdalina* extract

4. Conclusion

The effect of fermented and non-fermented *Vernonia amygdalina* (bitter leaf) extract on remediation of crude oil contaminated loamy sand soil was studied using experimental approach. Test results of the analysis revealed that *Vernonia amygdalina* extract is efficient stimulants to be used for remediation of crude oil polluted soil and *Vernonia amygdalina* extract yielded positive degradation of TPH, PAH, BTEX (TMAH).

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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