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Phytonutrient Effect of Velvet Bean *(Mucuna Pruriens)* Powder on Petroleum Hydrocarbon Impacted Soil

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ABSTRACT
Crude oil stressed agricultural soil, make microbial activities less active due to contaminant concentrations which needed urgent attention for treatment. The velvet bean powder (VBP) was analyzed for its nutrients content of nitrogen 5.87 mg/kg, phosphorus 1.36 mg/kg and potassium 28.34 mg/kg. The samples contained fixed masses of soil 2000 g and crude oil of 500 g blended with different VBP proportion of 4:1, 8:2:1, 4:1:1, 8:2:3, and 4:1:2 respectively, option (A - E), and control option (A) without any form of treatment for an interval of 45 days. There was reduction under all conditions ranging from 5215 to 289 mg/kg with the most reduction in option (C, D, and E) containing 4:1:1, 8:2:3 and 4:1:2 corresponding to 96, 96, and 97.2% respectively. There was a surge in microbial growth even though there was evidence of hydrocarbons in the soil, the levels of nutrient interaction were highly significant (p<0.05). The pH levels increased with VBP application from 5.75 to 6.99 under all conditions. The observed changes in electrical conductivity (EC) were in the crude oil saturated soil control (option A) 15 and 45 days (709 and 877 µS/cm) respectively, and the treatment option C and E (613 and 692 µS/cm). The addition of VBP amendments resulted in a significant increase in TN, TP, and TK in option D and E ratios 8:2:3 and 4:1:2 respectively. This shows that VBP can provide favourable environment for the growth of microorganisms that facilitate the degradation of petroleum hydrocarbons in soil.

KEYWORDS: Bioremediation, petroleum hydrocarbon, fertility improvement, phytochemical stimulation, microbial growth.

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1.0 INTRODUCTION
Activities of oil and gas industries are considered a major source of petroleum hydrocarbon pollution in the environment due to accidental spills of crude oil or its associated products from either willfully or naturally damaged oil facilities (Okparanma *et al.*, 2022). Most of the Niger Delta's agricultural land has been damaged by the crude oil spill, and their productive areas have become wastelands. This oil pollutes the water source and creek, destroy the aquatic life and the pollution of traditional fishing ground (Osuagwu & Olaifa, 2018). The corresponding effects of crude oil on soil fertility led to low agricultural productivity by farmers (Akpokodje & Salau, 2015). The lack of food leaves the inhabitants with no other means of support.

Oil spills reported in the Niger Delta have been widely documented in addition to treatment techniques (Onyena & Sam 2020; Zabbey *et al.*, 2017). Hydrocarbon impacted soil causes undesirable change in the physical and chemical characteristic of the soil structure, that hindered biological growth components of the environment (Truskewycz *et al.*, 2019). Researchers analyzed the effect of crude oil, dispersants on epithelial cells of human airways and identified similar pathological modes of action for the development of various lung diseases (Sayed *et al.*, 2021). As established by Linden and Palsson (2013) the need for remediation depend on the degree or level of contamination and that appropriate nutrient

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would be responsible to reduce toxicity exerted on the soil to acceptable levels. Despite the toxic constituents of petroleum hydrocarbon, there is an emerging use of legume with the potential to restore soil structure, which is not only effective in the bioactivity of crude impacted soil but would be less expensive and as well environmentally friendly.

This paper systematically studies the treatability of velvet bean power (VBP) with varying dosage that have not been used in soil remediation. Furthermore, the application of VBP modulation on the activities of total petroleum hydrocarbon impacted soil has been identified to be environmentally friendly and as well less expensive. The objectives of this paper are to examine the nutrient contents of Velvet bean powder (VBP) on hydrocarbon contaminated soil and investigate the effectiveness of VBP on crude oil contaminated soil.

2.0 MATERIALS AND METHODS

2.1 Materials

The following materials were used during experiment: crude oil (contaminant), agricultural soil, velvet bean powder (nutrient), measuring cylinder, plastic containers, hand auger, hand glove, sample bottles, weighing balance, pH meter, dichloromethane, and gas chromatograph.

The study was conducted at the Rivers State University, Port Harcourt. The experiment area is made up of flat plains with soil characterized as coastal plain sand. The soil type is Ultisol (USDA classification), and its texture is sandy loam. The climatic condition is that of a humid tropical climate with an average rainfall of about 2100 mm, of which 70% falls between May and August. The rest of the year is relatively dry with the mean air temperature varying between 24 and 30°C (Ayotamuno *et al.,* 2006). Port Harcourt is economically the most important city in the Niger Delta region of Nigeria. From this region, more than 98% of Nigeria's current economic mainstay, crude oil, is derived. Hence this research became pertinent in the study area as frequent oil-spills arising from crude-oil exploration and development activities have devastated farmlands and other agricultural settlements over the years.

2.2 Methods

Bonny light crude oil was collected from the Nigerian National Petroleum Corporation (Port Harcourt Refining Company of Nigeria), characterized for baseline value on total petroleum hydrocarbon concentration. The rationale for using Bonny Light crude was due to its wide spillage in the Niger Delta region. Agricultural soil samples were collected from the experimental site before crude oil contamination using a clean hand dug soil auger at depths of 0-30 cm. The seeds of velvet bean were collected from a known commercial market and selected to remove impurities, ground to powder using electric blender with rated power of 1500W and rated voltage of 210 ~ 220v. The ground velvet bean was allowed to pass through 300μm (No. 50 sieve size) governed by ASTMD6913.

2.2.1 Preparation of Sample

The experimental design used was randomized complete block design (RCBD) consisting of formulated velvet bean powder (VBP) blended with crude oil contaminated soil in triplicates. The treatment options (B - E) contained a fixed masses of soil 2000 g and crude oil of 500 g blended with different proportion of VBP in different ratios of 8:2:1, 4:1:1, 8:2:3, and 4:1:2 respectively, and control Option (A) without any form of treatment. Upon treatment, 0.3 L of water was sprinkled 2 times a week for an interval of 15 days during a period of 45 days of study. Prior to the application of nutrient amendment of VBP, soil and crude oil. Samples were taken to the reference laboratory to determine nutrient contents, soil physicochemical properties such as total nitrogen (TN), total phosphorus (TP), total potassium (TK), were analyzed using Hach-

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method 10072, 8048 and APHA 3111B respectively.

Soil pH and electrical conductivity (EC) was measured using portable handheld meter of Hanna instruments (HI98331 model) capable to represents the readings on the screen. Hydrocarbon degrading bacteria (HDB) count was performed on nutrient agar (Oxide), using the spread place method (Rijal, 2022). The physicochemical properties of the polluted soil samples were analyzed for 3, 15, 30, and 45 days to investigate the effectiveness of treatment. After crude oil contamination, the total petroleum hydrocarbon (TPH) of the soils in the various bioreactors were determined to establish the true concentration of the contaminant (in mg of oil per kg of soil). The analytical procedure involved the use of dichloromethane (DCM) to extract the hydrocarbon content, the absorbance of the extract was then determined at 420 nm in a Spectronic 70 spectrophotometer. Thereafter, the TPH of the soil was determined from the chromatograph of known concentrations of petroleum fractions. Details of this method of analysis are contained in the work of (Dumkhana & Ekemube 2020; Osuji *et al.*, 2006). The following remediation procedures were employed for the study.

Option A (control), Addition of 2000g of soil + 500g of crude oil ratio of 4:1
Option B, Addition of 2000g of soil + 500g of crude oil + 250g of velvet bean ratio of 8:2:1
Option C, Addition of 2000g of soil + 500g of crude oil + 500g of velvet bean in a ratio of 4:1:1
Option D, Addition of 2000g of soil + 500g of crude oil + 750g of velvet bean in a ratio of 8:2:3
Option E, Addition of 2000g of soil + 500g of crude oil + 1000g of velvet bean in a ratio of 4:1:2

2.3 Contaminant Removal Efficiency

$$\text{Contaminant Removal (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

where, C_i = Initial concentration, C_f = Final concentration

2.4 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the data obtained from the various replications of the experiment to compare the mean values of various treatment options. The analysis was carried out using Microsoft excel 2021 at 1 and 5 % significance levels and TAL for Windows to determine the textural class of the agricultural soil.

3.0 RESULTS AND DISCUSSION

3.1 Soil Textural Classification

The textural classification was determined using TAL for Windows of sample soil consisting of 59.0 % sand, 28.6 % silt and 12.4 % clay, indicating that the soil texture was sandy loam as displayed in Figure 1. Prior to contamination, the pH level of the unpolluted soil (5.86) and crude oil contaminated soil (5.76) were slightly different as presented in Table 2. The analysis on velvet bean powder (VBP) shows the richness on nutrient contents of TN 5.87 mg/kg, TP 1.36 mg/kg, and TK 28.336 mg/kg respectively, as displayed in Table 1. Prior to remediation, nutrient contents of the unpolluted soil were TK 4.86 mg/kg, TN 0.902 mg/kg, and TP 0.851 mg/kg (TK) respectively, with the highest proportion of nutrient in TK. However, hydrocarbon-degrading bacteria (HDB) in the unpolluted soil was 1.32 x 10^4 cfu/g, and crude oil was not determined. The initial concentration of total petroleum hydrocarbon (TPH) before remediation was 10,499 mg/kg as presented in Table 2

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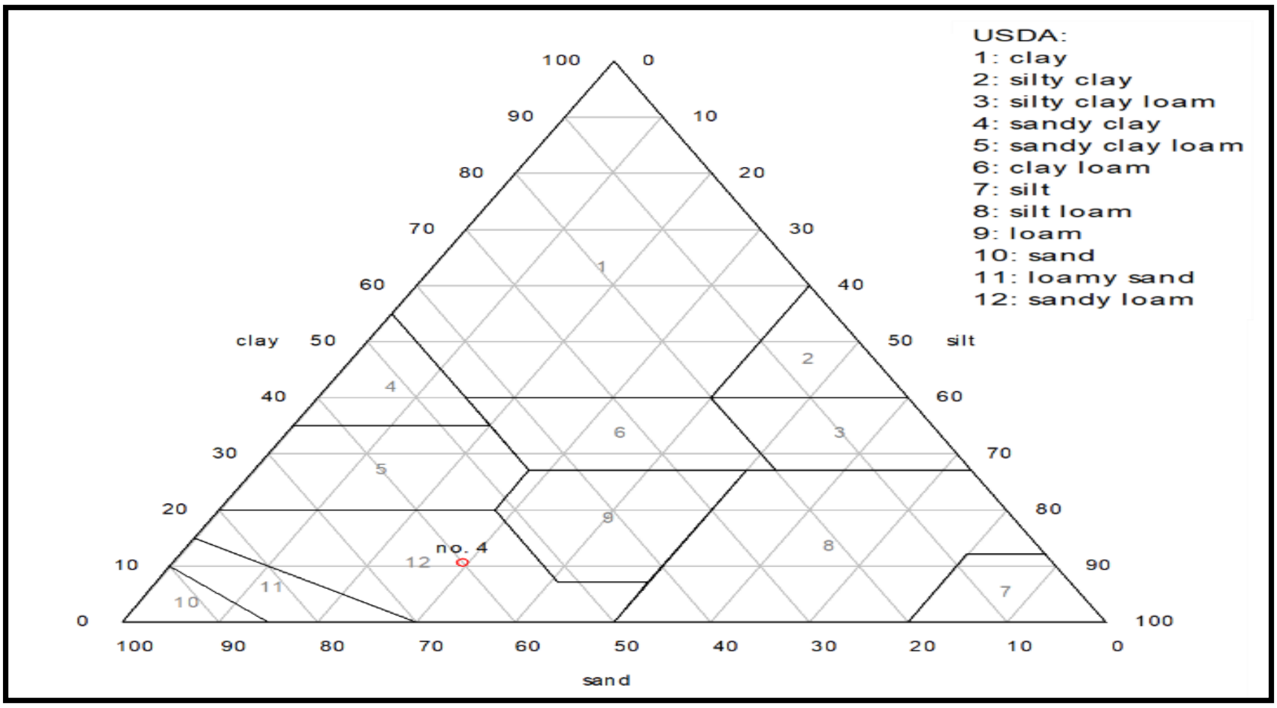

Figure 1: Textural Classification of Experimental Sample Soil

Table 1: Nutrient Contents of Velvet Bean Powder (VBP)

Properties	Method	Velvet Bean Powder
Total Nitrogen (mg/kg)	Hach-method 10072	5.87
Total Phosphorus (mg/kg)	Hach-method 8048	1.36
Total Potassium (mg/kg)	APHA 3111B	28.336

Table 2: Physicochemical Characteristics of the Soil Before Crude Oil Contamination

Parameter	Unpolluted Soil	Contaminant Crude oil
Ph	5.86	5.76
EC (*u*S/cm)	156	1.20
TOC (%)	0.958	_
TPH (mg/kg)	NR	10,499
Total Nitrogen (mg/kg)	0.902	_
Potassium (mg/kg)	0.851	-
Phosphorus (mg/kg)	4.86	-
Hydrocarbon-degrading Bacteria (HDB) (cfu/g)	1.32×10^4	_

Note: Electrical conductivity (EC), Total Organic Content (TOC), Total Nitrogen (TN), Total Phosphorus (TP), Hydrocarbon-degrading bacterial (HDB), Total Petroleum Hydrocarbon (TPH), Not Reported (ND)

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3.2 Variation of pH Levels and EC under Crude Oil Concentrations in VBP Treated Soil

The pH value in the oil saturated control option A was 5.76 and the blend with 8:2:3 amendment ratio treated with VBP option D at 5.49 rank the highest at 3 days. There was a surge in pH values on the 15 days ranging from 5.70 to 6.41 across control and treatment (A – E) and further decrease experienced for 30 days treatment ranging from 5.42 to 5.76. The changes in pH values ranging from 5.75 to 6.99 across control and treatment options during remediation period of 45 days of treatment in each reactor as presented in Fig 2a were due to degradation interaction of VBP treatment. This explains how slightly acidic and alkaline soil pH (6.5 and 8.0) are considered optimum for biodegradation, within this range specific enzymes function within a particular pH spectrum (Vidali, 2001; Neina, 2019). The difference in pH could be attributed to temperature variation that can alter the concentration under soil redox conditions. According to (Neina, 2019) pH influences biodegradation through its effect on microbial activity, microbial community and diversity, enzymes that aid in the degradation processes as well as the properties of the substances to be degraded. However, the difference in nutrient interaction was not significant $p<0.05$ due to increased application of VBP. These results were mainly due to the variable uptake of nitrogen and phosphorus during degradation process. This indicates good performance of velvet bean powder as a strong oxidant that can degrade organic pollutant by maintaining acid medium. The observed change in electrical conductivity (EC) were in the crude oil saturated soil control option A, 15 and 45 days (709 and 877 µS/cm) respectively, and the treatment option C and E (613 and 692 μS/cm) as described in Figure 2b. The results indicate that crude oil affected the stability of the soil, which could have contributed to the increase in EC. Despite the high conductivity of electron sharing in the mixtures, the blend with fixed masses of soil, crude oil, and different ratio of VBP were able to degrade hydrocarbon contents. The difference in EC at $p<0.05$ was due to VBP immobilization by microbes leading to soil fertility.

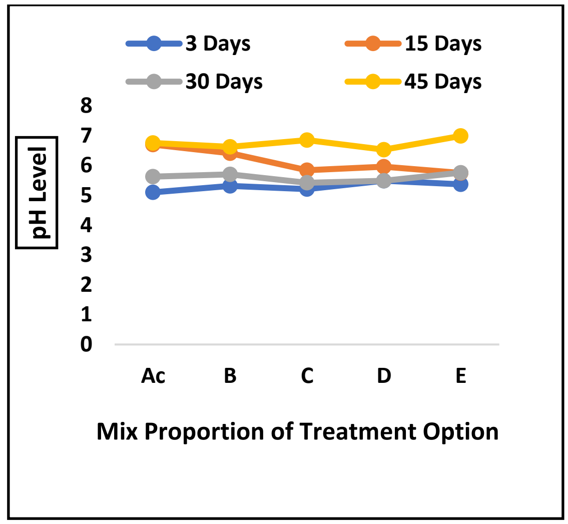

Figure 2a: pH level in VBP Treated Soil

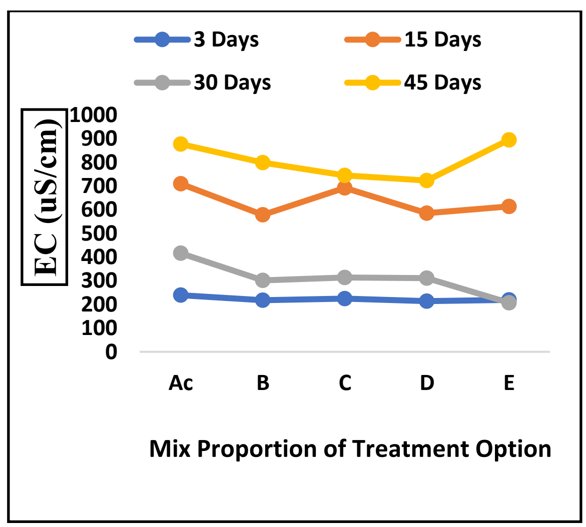

Figure 2b: EC in VBP Treated Soil

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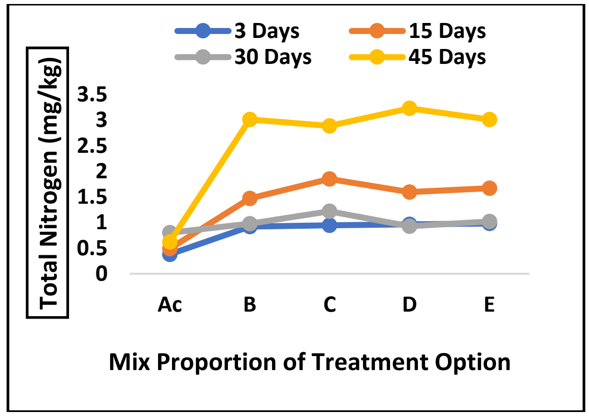
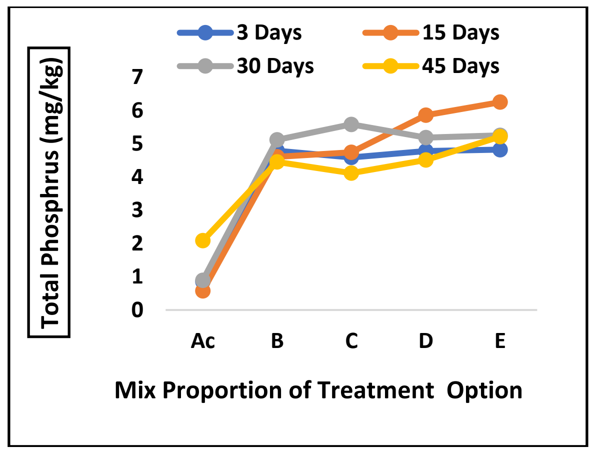
3.3 Variation of Total Nitrogen (TN), Total Phosphorus (TP) and Total Potassium (TK) Levels under Crude Oil Concentrations in VBP Treated Soil

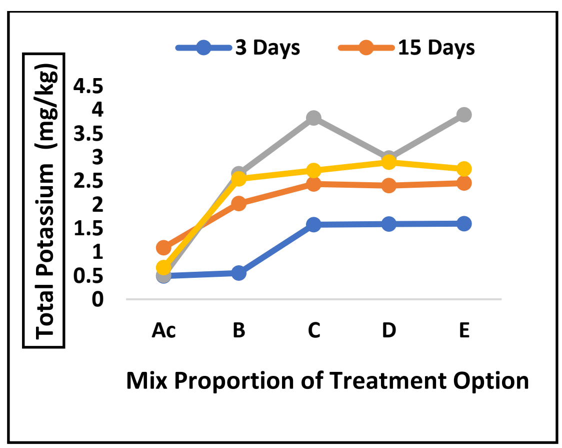
The initial TN contents in the unpolluted soil and VBP were 0.902 and 5.87 mg/kg respectively Tabel 1 and 2. The addition of VBP amendments resulted in a significant increase in nitrogen mineralization in ratios 8:2:3 and 4:1:2 option found to be 3.323 and 3.010 mg/kg, respectively at 45 days treatment Figure 3a. These means increased in TN contributed to increase in microbial population thereby decreasing the concentration of hydrocarbons. According to (Egobueze *et al.,* 2019), nutrient addition to crude oil contaminated soil is required to increase microbial population for enhanced degradation of petroleum hydrocarbons. However, the interaction of TN that reduced the toxic nature of hydrocarbon contamination level shows no significant difference at $p<0.05$. The addition of VBP contributed to a surge in TP ranging from 4.580 to 4.820 mg/kg across treatment in 3 days compared to unpolluted soil. TP at 30 days of treatment were found to have the highest range from 5.110 to 5.580 mg/kg across treatment (B - E), while the 45 days showed a slight decrease as shown in Fig 3b. The effect of VBP on TP indicates difference of $p<0.05$ due to variations in ratios of 8:2:1, 4:1:1, 8:2:3, and 4:1:2 option (B – E).



(3a)



(3b)



(3c)

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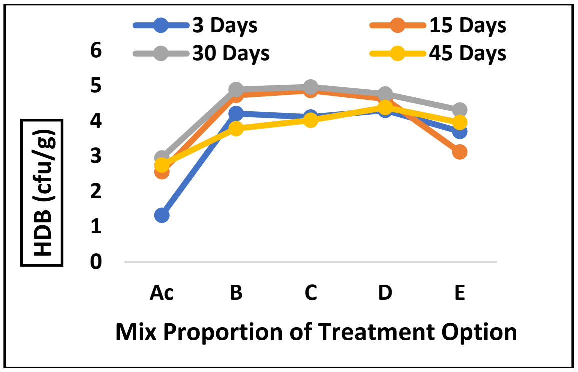

(3d)

Figure 3: (3a) TN (mg/kg) content (3b) Total Phosphorus (mg/kg) and (3c) TK (mg/kg) (3d) HDB under Crude Oil Concentrations in VBP Treated Soil

The soil potassium concentrations of the remediated soils are presented in Fig 3c. The amended soil with VBP showed statistically significant ($p<0.05$) value higher than the unpolluted soil option A. The range of different concentrations of TK in the biodegradation assay range from 0.491 to 1.597 mg/kg (option A - E) during the 3-day period. At the 45 days of study, there was a surge in the treatment option A – E ranging from 0.671 to 2.890 mg/kg due to the degrading efficiency of VBP on petroleum hydrocarbon. The extent of hydrocarbon reduction at 45 days of treatment indicates the efficacy of TK nutrients needed by bacteria for the degradability of contaminated environment. The highest TK concentration occurred at 30 days compared to 45 days of treatment which was slightly lower. Elsewhere, a study by Ule *et al.* (2021) established that crude oil contaminated soils amended with cow dung influence the decreased of potassium after two weeks of study.

The application of VBP enhanced bacterial in the bioremediation of crude oil polluted soil. The extant of HDB in the degradation over time was more abundant in oil polluted soil amended with different blend of VBP than that of unamended polluted soil Fig 3d. Statistical analysis revealed that there is significant difference in the counts of HDB between the amended and the unamended soil ($p<0.05$).

The addition of VBP to hydrocarbon contaminant helps alleviate the negative impact and stress on soil properties at 45 days of treatment. The highest effect was observed for option C and D containing 4:1:1 and 8:2:3 which indicates the efficacy of VBP nutrients needed by bacterial for the degradability of contaminated environment. The percentage reduction of hydrocarbon could be attributed to high nutrient content, particularly nitrogen and potassium that make up vast arrays of microbial activities within the contaminated medium. Almost similar findings were noted by Wei *et al.* (2020) reporting that rapid degradation of organic fertilizer amendment was because of increase in nitrogen nutrient. This explains that considerable quantities of VBP were beneficial in biodegradation of petroleum hydrocarbon. According to Lui *et al.* (2016) the preference for removal of certain hydrocarbon classes is related to the microbial population in the soil.

3.4 Effect of VBP Amendments on Total Petroleum Hydrocarbon (TPH) Removal from Soils

Fig 4a shows the effectiveness of VBP interactions on TPH contaminated soil. The concentration of TPH after 3 days in all treatments (Ac, B, C, D, and E) varied with the amendment of VBP ranging from 9755 to 4715 mg/kg in all reactors. At 45 days of treatment, there was reduction in TPH concentration ranging from 5215 to 289 mg/kg across treatment options detailed in Fig 6b. The effectiveness of various amendment ratio after 45 days to bioremediate hydrocarbon contamination from soils was observed under all conditions with the most reduction in option C, D, and E containing 4:1:1, 8:2:3, and 4:1:2 corresponding to 96, 96, and 97.2% respectively. This study compared favourably with Coulon *et al.* (2012) reporting that amendment of nitrogen and phosphate can

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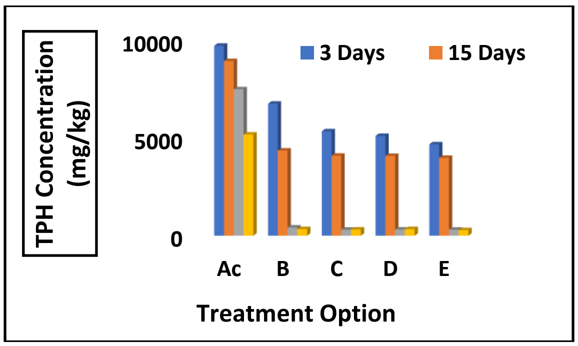
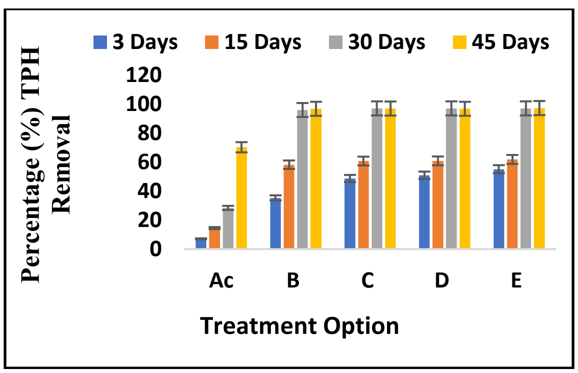
enhance biodegradation in oil contaminated soils. As explained in (Brown *et al.,* 2017) after 45 days of augmented VBP observed reduction in TPH concentration compared to the no VBP control option. The degradation obtained may be contributed to higher nutrient content in option C, D, and E corresponding to increase microbial growth. Most increase in microbial growth were observed even though there was evidence of hydrocarbons in the soil, the levels of nutrient interaction were highly significant ($p<0.05$). The application of VBP amendment has shown high nutrient availability, particularly nitrogen and potassium that enhanced degradation.



(4a)



(4b)

Figure 4: (4a) TPH Concentration after Velvet Bean Powder Addition Days after Treatment (4b) TPH Percentage Reduction after Velvet Bean Powder Addition Days after Treatment.

4.0 Conclusion

The application of velvet bean powder (VBP) amendments on crude oil was investigated for its NKP values and found to be an effective method for treatment of petroleum hydrocarbons contaminated soils. The remediation performance of VBP amended soils contributed to high reduction of TPH removal in all treatment despite the different mix ratios with the most reduction in option C, D, and E containing 4:1:1, 8:2:3, and 4:1:2 respectively, during bioremediation of hydrocarbon contaminated soils. The application of VBP on oil contaminated soil showed better performance due to an increase in microbial communities that reduce petroleum hydrocarbon contaminated hazardous soil.

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