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Evaluating the Potentials of *Costus afer* Juice for Bioremediation of Crude Oil Contaminated Soil

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***Abstract**: Contamination of soil by crude oil has caused more harm than good to our environment. The use of locally sourced environmental remediation stimulants brings the hope of inventing such products to be reality for today's intractable environmental contamination conditions. This will make way for the subsequent usage of more quantity to restore contaminated soil. Despite the high nutrient composition of Costus afer juice, it has been an elusive to be used as biostomulants for soil remediation. This study evaluated the potentials of Costus afer juice for bioremediation of crude oil contaminated soil. The experiment was carried out in Teaching and Research Farm of Rivers State University, Port-Harcourt. Soil samples with crude oil were bulk in five (5) cells ($_{T1, T2, T3, T4, T5}$) with three replications each. The physiochemical properties of treated soil and Costus afer juice such as particle size distribution (PSD), nitrogen, phosphorus (P), potassium (K), total petroleum hydrocarbon (TPH), total polycyclic aromatic hydrocarbon (TPAH), total heterotrophic bacteria (THB) were analyzed in the laboratory before and after treatment. The results showed that the soil is a loamy sand soil, and Costus afer juice has a high NPK values that proved it as biostimulant. TPH and PAH also reduced severely in all the treatment options at the end of the remediation period of 8 weeks, and their percentage reductions were above 94% in all treatment options for the same period. Moreover, Analysis of Variance (ANOVA) result showed significant difference at 95% confidence levels. Furthermore, there were increase in the number of THB and later reduce as the stimulants reduced after gotten to its peak within eight (8) weeks of remediation. It is therefore recommended that the Costus afers juice can serve a good biostimulant that should be used for degradation of TPH and PAH in crude oil contaminated soil.*

Keywords— bioremediation; *costus afer* juice; crude oil contaminated soil; total heterotrophic bacteria; total petroleum hydrocarbon total polycyclic aromatic hydrocarbon

1. Introduction

Crude oil is a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds, when spilled on land affects the physicochemical properties of the soil such as temperature, structure, nutrient status and pH. It is primarily composed of different hydrocarbon complex mixtures [1]. The chemical compositions can have diverse effects on different micro-organisms and macro-organisms within the same ecosystem. In current years, several researchers in the world have successively conducted significant studies on the restoration of petroleum hydrocarbon contaminated soil. Studied by Ekemube *et al*. [2] revealed that bulk density, total porosity, pH, available Phosphorus, total petroleum hydrocarbon (THC), organic matter, % organic carbon, Total Nitrogen, exchangeable Ca, Na, P, mg, ECEC, TEA, and base saturation were affected due to different volume of crude oil simulated into soil. They also reported that all the characteristics studied including bulk density, pH, available P, THC, organic matter, % organic carbon, total Nitrogen, exchangeable cation (Ca, Na, P, Mg), ECEC, TEA, and base saturation increased with increase in volume of crude oil except porosity. Also, crude oil contamination can impede the normal growth of crops such that it reduces the germination rate and fertility and decline the resistance to pests and diseases [3]. Ekemube *et al*. [2] also found that crude oil alters the physicochemical characteristics of the soil, hence the more the crude oil in the soil the more physicochemical characteristics of soil change thereby causing imbalance in soil nature. Furthermore, their study suggested that remediation should be adopted to regain the originality of the soil. Han [4] stated that the common methods are physical, chemistry, microbiology, and plant remediation. The physical processes or chemical reactions to change the physical properties of the soil and effective control and regulation of the contaminants are carried out by the traditional physical and chemical methods, which include soil removal and replacement, elution method, heat treatment and thermal resolution, extraction-separation and chemical oxidation methods [1]. These methods are generally more thorough and stable, but it requires the building of some fixed processing facilities, the use chemical agents, the constant regulation of temperature, pressure, and the provision of regular power supply during the entire operation in order to handle the whole process [4 - 6]. An assessment of its high cost and secondary pollution has shown it to not be suitable for large area remediation and as such it is not widely used in the actual application [1]. Bioremediation technology in comparison, has been shown to be more suitable to be used for the remediation of petroleum contaminated soil as it has the following benefits: low cost, simple in-situ and ex-situ treatments, environmentally friendly, no secondary pollution, and removes most pollutants with high efficiency [7 – 9]. Ekemube *et al*. [10] used spent mushroom substrate (SMS) and

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NPK fertilizer as biostimulants to remediate crude oil polluted soil, they found that different amount of SMS, NPK fertilizer, and changes in microbial activities were responsible for the removal of total petroleum hydrocarbon (TPH) concentration across treatment cells.

Costus afer (C. afer) is a plant commonly known as ginger lily, spiral ginger, or bush cane. It is reportedly used in traditional medicine practice (TMP) to treat and manage many ailments including diabetes mellitus, stomach ache, arthritis, inflammation, and gout. It has been reported by Boison *et al*. [11] that the plant could be used as an alternative and complementary therapy for many oxidative stress-related diseases, provided further scientific studies on the toxicological and pharmacological aspects are carried out. Results obtain by the study by Nimame *et al*. [12] clearly demonstrates the significance of monkey sugarcane (*Costus afar*) juice in preventing and controlling pipeline corrosion to the barest minimum if applied adequately. It has been abundant in the study area, lying waste in the environment and creating a bushy surrounding for that there is need for harnessing it for remediation purposes. Despite its potentials it has not be documented in literatures the usefulness of *costus afer* juice in the remediation of contaminated soils. Therefore, there is need for this study. Hence, this study aims on the evaluation of potential *Costus afer* juice in the degradation of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) in crude oil contaminated soil. The formatter will need to create these components, incorporating the applicable criteria that follow.

2. MATERIALS AND METHODS

2.1 Study Area Discription

The experimental was carried out at the Teaching and Research Farm of Rivers State University, Port Harcourt, Nigeria. Port Harcourt is the capital of Rivers State and most especially the center of crude oil exploration in Niger Delta as well as Nigeria at large. The ambient environment (i.e., Port Harcourt metropolis) have a mean monthly relative humidity of 85% and a daily minimum temperature about 230c with a mean daily maximum temperature of 320c.

2.2 Sample Collection

For this research, loamy sand soil was collected from Teaching and Research farm of Rivers State University, Port Harcourt, Nigeria. Crude oil was collected from an oil and gas exploration and exploitation company in the region. *Costus afer* juice was gotten from *Costus afe*r plants in an uncontaminated site without any history of contamination.

2.3 Experimental Design

The experimental design that adopted is completely randomized design (CRD) for single factor experiment. The design consisted of 5 experimental reactors including the control T1 with three replications. Here, randomization will be achieved using the draw lots approach as described in several literatures [14 -15]. Each reactor contained 10kg of soil with a depth of 15cm. The primary functions of the reactors are to control the surface area of the soil, the nutrients concentration, moisture content, temperature and oxygen availability.

They also serve to prevent excessive run-off of the contaminant, which is a common trend in the locality during the period the study will be conducted. Cell T1 receives 100ml of water two times per week; Cell T2 receives 100ml of *Costus afer* juice; Cell T3 receives 200 ml of *Costus afer* juice; Cell T4 receives 300 ml of *Costus afer* juice; and Cell T5 400 ml of *Costus afer* juice.

2.4 Sample Preparation

50,000mg of Bonny light crude oil was poured on each treatment cells (including the control cell, T1) through a perforated can at the rate of 50,000mg of crude oil per 10kg of soil and the surface of the soil in each cell will be completely covered with a thin layer of oil. The objective is to stimulate condition of a major spill. The cells were left undisturbed for 72 hours. After the 72 hours, treatment options were applied. *Costus afer* plants was harvested from uncontaminated site without any history of contamination, pounded and its juice extracted undiluted to be used for cell T2, T3, T4, and T5, respectively. The extracted *Costus afer* juice was applied after 72 hours of bulking, once for the 8 weeks of remediation period. Different random spots were augured using hand dug soil auger capable of obtaining uniform cores of equal volume to desired depth, bulked together (composite soil samples) and put in well labeled sample bottles. This was carried out at the interval of two (2) weeks each for eight (8) weeks. The physiochemical properties analysed for in the laboratory were total petroleum hydrocarbon (TPH), total polycyclic aromatic hydrocarbon (TPAH), and total heterotrophic bacteria (THB) in crude oil contaminated soils, respectively.

2.5 Laboratory Analysis

The following soil physicochemical parameters: particle size distribution, total petroleum hydrocarbon (TPH), and total heterotrophic bacteria (THB) was analyzed using standard method.

2.5.1 Particle Size Distribution (PSD)

The hydrometer method was used to determine the soil textural class in the laboratory. It was also determined using soil textural triangle according to United States Department of Agriculture (USDA), soil textural classification. Finally, the textural class was determined using soil triangle according to United State Department Agriculture (USDA) soil textural classification scheme using TAL for Windrows.

1.5.2 Nitrogen, Phosphorus and Potassium

Nitrogen was determine using the modified Kjeldahl method. Ascorbic acid method was used to estimate available phosphorus. While potassium content in the *Costus afer* juice samples were analysed by flame atomic absorption spectrometry using a UNICAM-969 atomic

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spectrophotometer by measuring the light absorbance of the sample at the wavelength range of 357.9 – 228.8µm according to APHA method 3111C.

2.5.3 Petroleum Hydrocarbon (TPH)

TPH of the samples were analysed in line with USEPA 8015 method using Gen Tech master G equipped with a split / split less injector, J and W 30-meter DB-5column and an FID detector.

2.5.4 Total Polycyclic Aromatic Hydrocarbon ((TPAH)

PAH analysis was carried out in line with EPA 8270 method on an Agilent 6890 GC /MSED 5973 equipped with a split/splitless injector, J and W 30-meter DB-5 column and mass selective detector

2.5.5 Bacteria Count/Microbial Analysis

THB cultivation and enumeration was carried out using the method by Harrigan and McCane (1990), in addition the isolation, characterization, identification of bacteria in the soil was done with reference to Cowan [16]; Buchanam and Gibbons [17].

2.6 **Statistics Analysis**

The statistical method that was employed to analyze the data is analysis of variance (ANOVA). ANOVA is based on the F-test and it help to obtained an appropriate error term with single probability risk determined if the means considered are totally different and if the difference are beyond what is attributed to chance or experimental error sand difference will be considered as significant at $p \leq 0.05$.

3. Results and discussion

3.1 Physicochemical Composition of Uncontaminated Soil, *Costus afer* Juice

Table 1 shows the physicochemical and THB composition of the soil, and *costus afer* juice. The physicochemical compositions analyzed and used as indices for evaluation of level of contamination before crude oil contamination (original state) and after crude oil contamination. Among the options, O (soil) and C (Custus afer juice). It was found that C has higher concentration of the physicochemical parameter followed than O. Hence it was adopted to be used as a remediating agent.

Table 1: Physicochemical Composition of the Initial Soil Condition and *Costus afer* Juice

Parameters	Treatment Options	
	O	M
N (mg/Kg)	12.65	6.21
P (mg/Kg)	1.89	0.79
K (mg/Kg)	6.768	4.351
TPH (mg/Kg)	84.23	N/A
PAH (mg/Kg)	<0.01	N/A
THB X10^5(cfu/mg)	2.3	2.7

3.1.1 Soil Textural Classification

Soil samples were collected using soil corer for particle size distribution (PSD) analysis, from the soils studied prior to contamination of the soil by crude oil. The result of the uncontaminated soil indicated the relative content of soil particles of various sizes such as sand, silt, and clay in the soil (Table 1). From the results, it was revealed that the soil textures were Loam according to United State Department Agriculture (USDA) textural classification of soil using TAL for windows a software used by the USDA to classify soil type.

Table 2: Particle Size Analysis Results

Sand %	Silt %	Clay %	Textural Class
12.60	6.80	80.60	Sandy loam

3.1.2 Fertilizing Value *of Costus afer* Juice

The initial NPK characteristics determined were used as indices for evaluation of the fertilizing value (i.e., remediation potential) of the *Costus afer* Juice. Table 2 shows the NPK values of the *Costus afer* Juice. Compared this *Costus afer* Juice NPK value with the recommended fertilizer value of 3: 2.5: 0.5 (N: P: K) for materials, *Costus afer* Juice is within the acceptable values. This suggests that Costus afer Juice is an excellent contender for remediation purposes, which justifies the use of the products for remediation works.

Table 3: NPK Values of Costus afer Juice

Properties	***Costus afer* Juice**
N (mg/kg)	6.21
P (mg/kg)	0.79
K (mg/kg)	4.35

3.2 Effect of *Costus afer* Juice on TPH

The effect of *Costus afer* juice on TPH concentration in the crude oil contaminated soil is shown in Table 4. Figure 1 also shows the graphical variation of TPH concentration in crude oil contaminated soil with time for different cells used in this study remediated with *Costus afer* juice. An examination of the Fig. 1 showed that the TPH concentrations in different cells (T1, T2, T3, T4, and T5) decreased with increased in *Costus afer* juice and number of weeks the study was carried out. The cells were amended with *Costus afer* juice that was not diluted with water at different application rates to the cells (T2, T3, T4, and T5). The TPH concentrations reduction contained in the various cells (T1, T2, T3, T4, and T5, respectively) ranged from 16,382.12 to 8,858.53, 16,38.10 to 863.13, 16,38.15 to 638.31, 1638.11 to 418.13, and 16,38.19 to 208.31 mg/kg for 0, 2, 4, 6, and 4 weeks, respectively according to the order of reduction. Table 5 shows the percentage reduction of the TPH due to the presence of *Costus afer* juice with increase in the number of weeks. The TPH percentage reductions ranged from 0.00 to 45.93, 0.00 to 94.73, 0.00 to 96.10, 0.00 to 97.45, and 0.00 to 98.73%, for week 0, 2, 4, 6, and 8, respectively as shown in Table 5 and Fig. 2. This is due to the presence of *Costus afer* juice applied to the crude oil contaminated soil, which has high content of nutrients (NPK). This is study on bioremediation of crude oil contaminated soil using *Costus afer* juice a locally sourced material has TPH degradation potential efficiency of 94.73, 96.10, 97.45, and 98.73% for eight (8) weeks of remediation period. This carried was carried out using four (4) different application rate (100, 200, 300, and 400 ml) of *Costus afer* juice with high degradation efficiency of above 90 %. In addition, the TPH results revealed that the volume of *Costus afer* juice of 400 ml degraded TPH in the soil to 98.73%. This confirmed that that the degradation of TPH was more efficient with the treatment T5 because of higher quantity of *Costus afer* juice applied. The ANOVA result for the effect of *Costus afer* juice on the TPH concentration showed that that there was significant difference in the treatment means at 5% significance levels. This suggests that with 95% confidence, the difference in treatment means was due to the different proportion in volume of *Costus afer* juice applied.

Table 4: Mean Results of TPH Concentrations (mg/kg) in Crude Oil Contaminated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	16382.12	16384.10	16384.15	16384.21	16384.19
2	12489.27	6145.49	5134.98	4043.48	2132.83
4	10892.85	3452.52	2342.25	1224.52	1030.32
6	9852.63	1726.26	1562.62	1232.26	867.62
8	8858.53	863.13	638.31	418.13	208.31

Table 5: Mean Results of TPH Concentrations Reduction (%) in *Costus afer* Remediated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	0.00	0.00	0.00	0.00	0.00
2	23.76	62.49	68.66	75.32	86.98
4	33.51	78.93	85.70	92.53	93.71
6	39.86	89.46	90.46	92.48	94.70
8	45.93	94.73	96.10	97.45	98.73

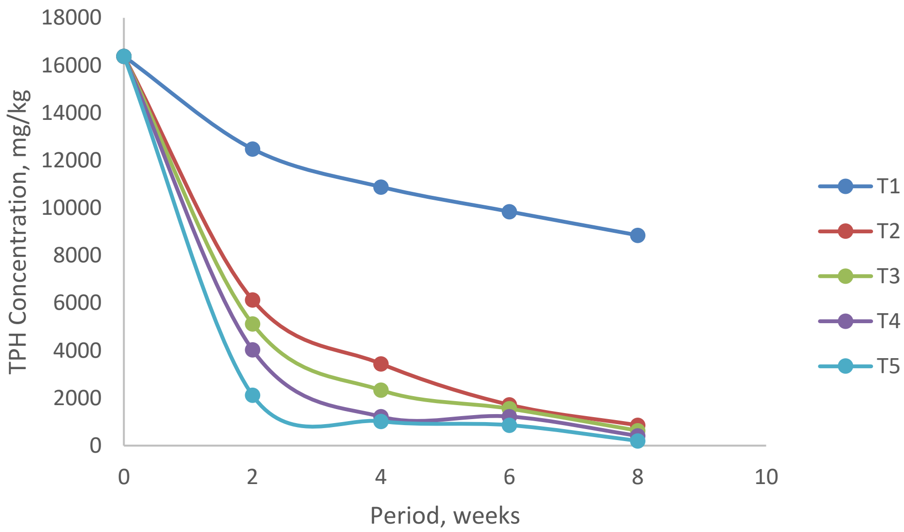

Fig. 1. Potential of *Costus afer* Juice on TPH Degradation in Crude Contaminated Soil

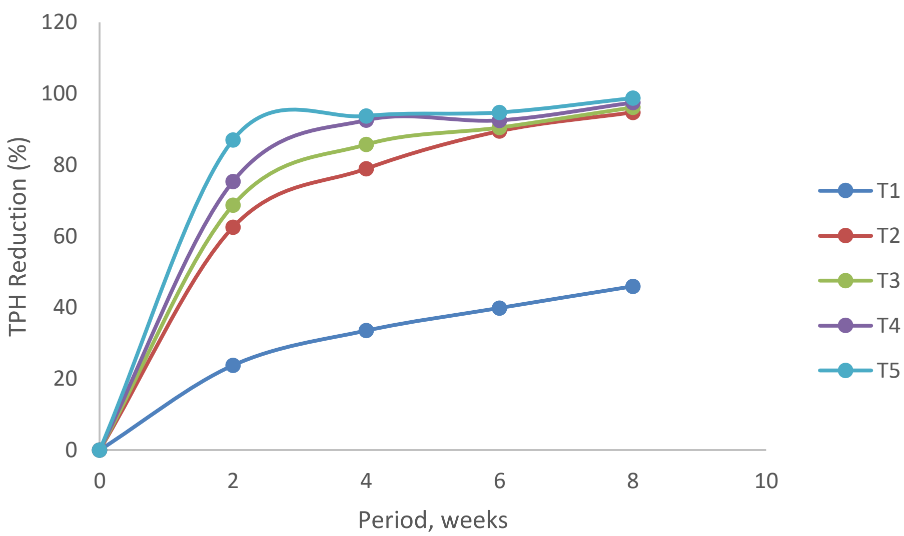

Fig. 2. TPH Reduction on Influence of *Costus afer* Juice in Crude Contaminated Soil

3.3 Effect of *Costus afer* Juice on TPAH

The effect of *Costus afer* juice on TPAH concentration in the crude oil contaminated soil is shown Table 5 displays the impact of Costus afer juice on the TPAH levels in the crude oil contaminated soil. The graphical change of TPAH concentration in crude oil contaminated soil over time for the several cells employed in this investigation and remedied with *Costus afer* juice is also shown in Fig. 3. According to Figure 3, the TPAH concentrations in the various cells (T1, T2, T3, T4, and T5) dropped as the amount of *Costus afer* juice and the number of weeks the study was conducted increased. *Costus afer* juice that was not diluted with water was added to the cells (T2, T3, T4, and T5) with various amounts. According to the order of reduction, the TPAH concentrations reduced in the different cells (T1, T2, T3, T4, and T5) ranged from 32.23.12 to 16.68, 32.20 to 1.92, 32.23 to 1.29, 32.18 to 1.03, and 32.22 to 0.93 mg/kg for 0, 2, 4, 6, and 4 weeks, respectively. Table 7 displays the percentage decrease in TPAH caused by the presence of *Costus afer* juice as the number of weeks increases. According to Table 7 and Figure 4, the TPAH percentage decreases for weeks 0, 2, 4, 6, and 8 varied from 0.00 to 48.25, 0.00 to 94.04, 0.00 to 96.00, 0.00 to 96.80, and 0.00 to 97.11% in treatment cell T1, T2, T3, T4, and T5, respectively. This is caused by the presence of *Costus afer* juice, which has a high level of nutrients (NPK), that was applied to the crude oil contaminated soil. This study examines the bioremediation of soil contaminated with crude oil utilizing *Costus afer* juice, a locally available material with TPAH degradation potential efficiencies of 94.04, 96.00, 96.80, and 97.11% in treatment cells (T2, T3, T4, and T5,

International Journal of Academic and Applied Research (IJAAR)
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Vol. 7 Issue 6, June - 2023, Pages: 58-66

respectively) over the course of eight (8) weeks. *Costus afer* juice with a high degrading efficiency of above 90% was applied at four (4) distinct application rates (100, 200, 300, and 400 ml) in this experiment. Additionally, the TPAH results showed that 400 ml of *Costus afer* juice reduced the contaminated soils TPAH to 97.11%. This demonstrated that the treatment T5's application of a greater amount of *Costus afer* juice resulted in a more effective breakdown of TPAH. The *Costus afer* juice effect on TPAH concentration was examined using an ANOVA, and the results demonstrated a significant difference between the treatment averages at 5% levels of significance. This shows that there is a 95% chance that the variation in the volume of applied *Costus afer* juice is what caused the variance in treatment means.

Table 6: Mean Results of TPAH Concentrations (mg/kg) in *Costus afer* Remediated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	32.23	32.20	32.23	32.18	32.22
2	29.76	13.64	10.46	6.28	4.46
4	21.59	7.67	6.36	3.62	2.29
6	18.80	3.84	2.48	1.26	1.02
8	16.68	1.92	1.29	1.03	0.93

Table 7: Mean Results of TPAH Concentrations Reduction (%) in *Costus afer* Remediated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	0.00	0.00	0.00	0.00	0.00
2	7.66	57.64	67.55	80.48	86.16
4	33.01	76.18	80.27	88.75	92.89
6	41.67	88.07	92.31	96.08	96.83
8	48.25	94.04	96.00	96.80	97.11

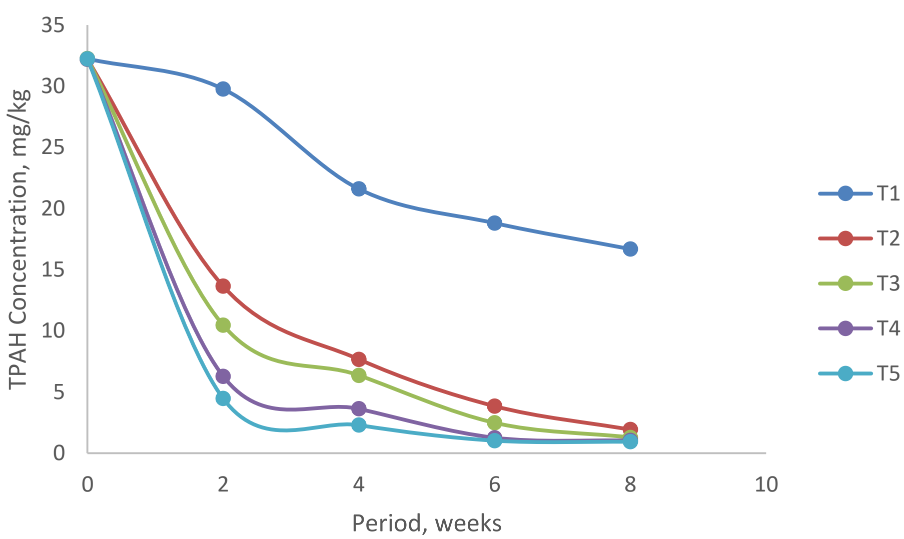

Fig. 3. Potential of *Costus afer* Juice on TPAH Degradation in Crude Contaminated Soil

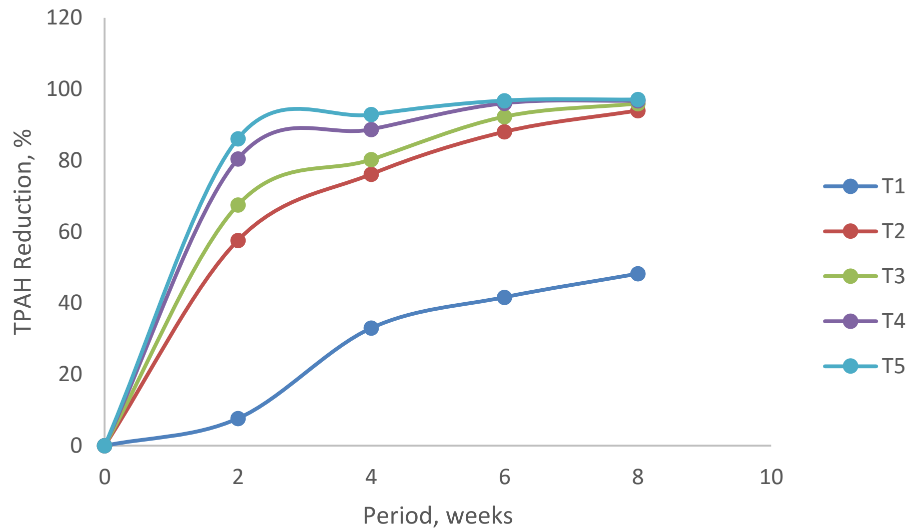

Fig. 4. TPAH Reduction on Influence of *Costus afer* Juice in Crude Contaminated Soil

3.4 Effect of THB on Crude Oil Contaminated Soil

The graphical representation of the THB in the soil treated with various amounts of *Costus afer* juice, which have a high nitrogen, phosphorus and potassium content is shown in Fig. 5. THB increased in the treatment cells with increase application rate because the cell (T5) with the highest volume of *Costus afer* juice contained the highest population of THB. The number of THB in the cells (T1, T2, T3, T4, and T5) fluctuated with the amounts of *Costus afer* juice s supplied to the cells (T1, T2, T3, T4, and T5) until it reached its peak (Table 8). This is in line with the findings of Ekemube *et al.* [10] that changes in microbial activities were responsible for the removal of total petroleum hydrocarbon (TPH) concentration across treatment cells. The fact that the soil THB therein were strongly favored by the environment is likely what led to the increase. As a result, the number of THB reduced as the experiment's time period came to a conclusion. This might be caused by a decrease in nutrients in the soil that has been modified with crude oil. This supports the conclusions reached by Chibuike *et al.* [18]. According to Onakaughoto *et al.* [19], providing the right nutrients is a good technique for increasing the metabolic activity of microorganisms.

Table 8: Mean Results of THB Population (10^5cfų/mg) in Crude Oil Contaminated Soil with *Costus afer* Treatment

Period, weeks	**Total Heterotrophic Bacteria, THB** (10^5cfų/mg)				
	T1	T2	T3	T4	T5
0	3.47	3.47	3.42	3.51	3.4
2	3.74	7.03	7.98	8.14	8.48
4	3.83	9.14	10.23	11.16	11.63
6	2.68	5.4	5.89	6.04	6.39
8	1.88	4.48	4.89	5.16	5.21

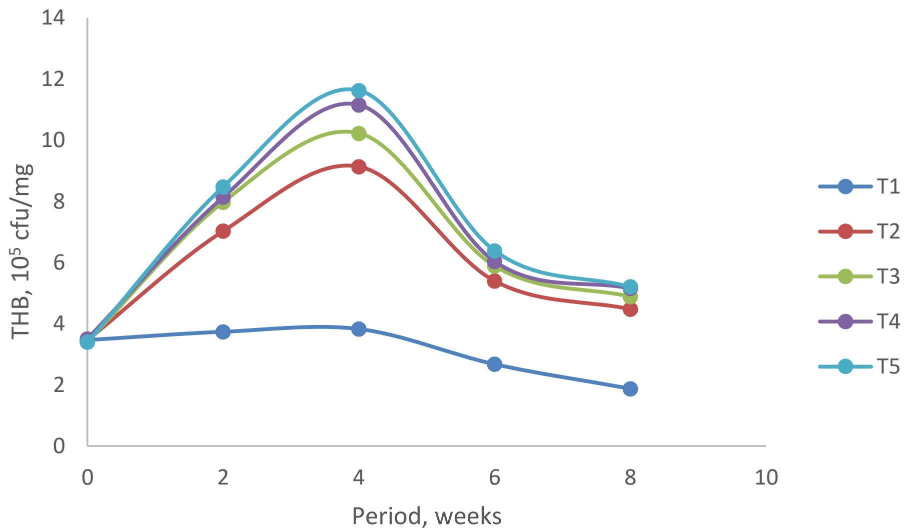

Fig. 5. Influence of *Costus afer* Juice on THB in Crude Oil Contaminated Soil

4. CONCLUSION

The potentiality *of Costus afer* juice in the bioremediation of crude oil contaminated soil was studied using investigational approach. Test results of the laboratory analysis supports the following conclusions:

i. The uncontaminated was confirmed to be loamy sand soil type according to United State Department Agriculture (USDA) textural classification of soil. In addition, *Costus afer* juice has the potential for remediation of crude oil contaminated soil because of its high content of NPK (6.21, 0.69, and 4.35 mg/kg, respectively).

ii. *Costus afer* juice influenced the biodegradation of total petroleum hydrocarbon (TPH) in all the cells (T1, T2, T3, T4, and T5) of the crude oil contaminated soil. This is because of its high nutrient composition (NPK). This Costus afer juice degraded TPH to 94.73, 96.10, 97.45, and 98.73% in all treatment (T2, T3, T4, and T5, respectively) at eight (8) weeks of remediation.

iii. Total petroleum hydrocarbon (TPAH) degradation was influenced by *Costus afer* juice in all the cells (T1, T2, T3, T4, and T5) of the crude oil contaminated soil. This is because of its high nutrient composition (NPK). This *Costus afer* juice degraded TPAH to 94.04, 96.00, 96.80, and 97.11% in all treatment (T2, T3, T4, and T5, respectively) at eight (8) weeks of remediation.

iv. Total heterotrophic bacteria were influenced by the stimulant (*Costus afer* juice) in the biodegradation of TPH and PAH in the crude oil contaminated soil due to its high nutrient composition (NPK). These THB reduce as the stimulants reduced after gotten to its peak within eight (8) weeks of remediation.

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