

Chaya Plant Extract *Jatropha tanjorensis*: The Concepts of Bioremediation of Contaminated Soil Evaluation

Ukpaka Chukwuemeka Peter^{1,*}, Ikenyiri Patience Nna², Alhassan Monday Alkali³

Abstract

This research was carried out to study the performance of sun-dried and room dried Chaya plant extract on the remediation of crude oil contaminated soil samples from Obele Community. The parameters under investigation were Total Petroleum Hydrocarbon degraded (TPHD), pH, amount of Nitrogen, Phosphate, and Potassium, Total organic carbon. Twenty-two (22) batch reactors were used for this research with each reactor containing 1kg of soils samples contaminated with 100ml of crude oil were treated with 20g, 40g, 60g, 80g, 100g of Chaya plant extract sun-dried (CPEL), Chaya plant extract dark (CPED), to each. The soil samples used for this research were loamy and sandy, the physiochemical properties of the soil samples used before and after were determined at the beginning and 84 days of the experiment. These physiochemical properties were electrical conductivity (EC), moisture content (MC), Total organic carbon (TOC), soil total organic matter (STOM), total nitrogen (TN) and phosphorus (P) in the soils increased after pollution. However, EC, MC, TOC, TOM, TN and P declined significantly with time after exposure to the treatment, while pH trend towards neutrality after the treatment. The rate of biodegradation of TPH was examined in every 14 days' interval for 84 days, the results obtained shows a high performance of the plants extracts on all the soils samples with the sample on the loamy soil performing better when compared to the sand soil, however with relatively high degradation in remediate processed in the absent of direct sun light.

Keywords: Chaya plant extract, *Jatropha tanjorensis*, concepts, bioremediation, contaminated, soil, evaluation

INTRODUCTION

*Author for Correspondence

Ukpaka Chukwuemeka Peter
E-mail: peter.ukpaka@ust.edu.ng

¹Professor, Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria

²Associate Professor, Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria

³Research Student, Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria

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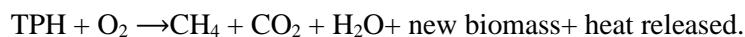
Petroleum is any mixture of natural gas, condensate and crude oil [1]. It consists of various hydrocarbons and rock material found under the earth's surface. Crude oil, a heterogeneous liquid of hydrocarbons comprised of almost entirely of hydrogen and carbon elements in a ratio of 2:1 respectively and element, such as nitrogen, sulphur and oxygen at less than 3%. There are also small trace constituents of phosphorous, vanadium and nickel with less than 1%. The composition of crude may vary with location, age, and depth within an individual well [2–3].

This crude oil can be processed to have gas, kerosene, diesel, fuel, aviation fuel, etc. these products has enhanced industrialization and increase the economy growth of the region where they are found, which leads to employment

opportunity and economic stability, however, it has been found to be hazardous to man, environment and ecosystem [4–6]. The exploration and exploitation of crude oil poses a serious environmental problem owing to its contaminations of the products in air, water and soil [7]. Human and Animal get infected directly as they drink and breathe on the polluted water and air. There is an accumulation of manganese and ferrous ions to levels which become very toxic to plants. Plant growth in crude oil contaminated soil is adversely affected due to changes in nutrient status of the soil and disruption of microbial activities [8]. In the Niger Delta region of Nigeria where exploration and exploitation of crude oil is known for, the ecosystem has suffered untold hardship due to pollution caused by oil spillage due to pipeline vandalism, rupture of oil/deteriorated pipeline and unending gas flaring by the oil companies in the region. Hence, there is hazardous effect on both flora and fauna and adverse effect on germination and growth performance of plant in the region [9, 10] by creating conditions which inhibit nutrients like nitrogen, phosphorous and oxygen needed by plants [9–12]. Pollution of soils with crude oil [delayed the period of germination velocity, plant height and leaf production [13].

However, crude oil contaminated soils are amendable to bioremediation because of degrading petroleum hydrocarbon are present in organic matter [14]. Bioremediation which is a natural process can be optimized to transform or degrade contaminants to non-hazardous chemicals [15]. It enhances the rate at which microbes biodegrade organic chemicals that have been released into the environment.

Bioremediation can also be defined as a process of adding or introducing micro-organism into an environment to breakdown larger molecules of hydrocarbon to a lesser one and eventually eliminate all the microbes in the soil without harming the environment. The concept is based on using micro-organisms to clean up environment by removing/reducing the toxicity of the environment.



The rate at which biodegradation takes place depends on the optimal living environment for the microbes. Most microbes that degrade petroleum hydrocarbon make use of the appropriate amount of oxygen, water, acidity pH and other nutrient such as nitrogen and phosphorus. Factors such as oxygen, nutrients, salinity, temperature, pH and soil moisture content or water content and assessed to determine which of these factors is limiting and thereafter it is modified by correcting it in a manner that will enhance microbial rates without causing more harm to the environment [16].

Jatropha tanjorensis or chaya plant is Agricultural plant (a plant biomass) is one of the organic matters gotten as by plants obtained during extraction processing in the plant. Chaya plant (*Jatropha tanjorensis*) refers locally as hospital too far is one of the many medicinal plants of nature. The plant is known for its super food medicinal efficacy which earned the name hospital too far. It is called Chaya plant since the word (chai-yah) originated from Spinach in Maya. The leaf of the plant has high concentration of vitamins A, B1 and C in combination with other mineral such as calcium, iron and other protein. The plant is widely grown in Africa because of its adaptability to the climatic conditions. The leaf is harvested from the plant to get the extract to be used as food supplements or as medicine, is predominantly grown in the Niger Delta region which incidentally is the worst hit by crude oil pollution in the environment. the plant leaf itches when rubbed against the skin it's a good source of iron.

The plant extracts mitigate environmental pollution problems by enhancing degradation of biodegradable substance since it is rich in calcium, iron and other minerals, It can be used to improve soil fertility [17–20].

MATERIALS AND METHODS

Collection of Samples

The soil samples that will be used for this research work will be collected from three different locations within Obelle town, in Emohua L.G.A of Rivers State and transported to Rivers State University (RSU) Chemical Engineering laboratory for onward preparations for analysis. The plant

leaves will be identified at the Forestry Department of the Rivers State University. The soil samples will be air-dried, and stored in a polyethylene bag, and kept in the laboratory before use. The Crude oil to be used will be obtained from Niger Delta refineries (NDPR) to RSU, Rivers State, Nigeria. The plants Chaya plant extract (CPE) locally known as hospital too far will be harvested from a garden in Obelle community in Emohua local government area of Rivers State. The extracts from this plant will be subdivided into two portions, one of it will be treated under atmospheric temperature while the other portion at room temperature to about 98% dryness. The extracts were further grinded and sieved to obtained uniform size particles as well as to increase the surface area, Each amendment agent was stored in a polyethylene bag and kept before use.

Characterization of Samples

The soil samples and amendment agents were characterized for total carbon (TOC), total nitrogen (N), total phosphorus, moisture content, electrical conductivity, and pH according to standard methods. The pH was determined and total organic carbon was determined by the modified wet combustion method and total nitrogen was determined by the semi-micro-Kjeldahl method. Available phosphorus was determined by the Brays method and total hydrocarbon (THC) was estimated using the Gas Chromatographic method. Furthermore, the total microbial count (TMC) was assessed using on plate count following standard microbiological method according to while the electrical conductivity meter was used to measure the electrical conductivity (EC) in the soil.

Determination of Total Petroleum Hydrocarbon (TPH)

The standard compliance level set by DPR for petroleum industries in Nigeria is within the range of less than equal to 50 ppm, contrary to this standard limit amount to environmental pollution or degradation. TPH level in petroleum prone area should not be more than 50ppm, exposure of petroleum above this standard is hazardous to the surface, subsurface of the soil. This high concentration of TPH enhances anaerobic activities of microbes, hence leading to the depletion of oxygen which in turn reduces the increases the mortality aerobic organism. The percentage of the removed can be evaluated using,

$$\% TPH = \frac{TPH_c - TPH_R}{TPH_c} \times 100 \quad (1)$$

Where the TPH_c and TPH_R are contaminated and remediated TPH concentration.

Experimental Design

1 kg of each soil sample was put into 22 different reactors and labelled A₂₀, A₄₀, A₆₀, A₈₀, A₁₀₀, to D₂₀, D₄₀, D₆₀, D₈₀, D₁₀₀, respectively. The soil in each reactor was mixed with 100ml (w/v) bonny light crude oil and 20g, 40g 60g 80g, and 100g of each of the two different bioremediation treatments and thoroughly mixed to achieve complete uniform contamination. 100ml was adopted to achieve severe contamination because contamination above 3% concentration of oil has been reported to be detrimental to soil biota and crop growth.

Furthermore, the soil C: N ratio in each reactor was adjusted by the addition CPEL, and CPED respectively, as carbon co-substrate and nitrogen source and was thoroughly mixed. The moisture content of each reactor was adjusted to 50% water holding capacity by the addition of sterile distilled water at room temperature. The content of each reactor will be tilled once a week for aeration, and the moisture content was maintained at 50% water holding capacity. The soil in the reactor that will be labelled C and D will be also contaminated with the same amount of crude oil without amendment agents and thus served as control. The experiment will be set up for the soil samples (loamy, and sandy) a total of 22 reactors will be setup for twelve weeks (84 days). Periodic sampling from each reactor will be for the total petroleum hydrocarbon (TPH) mitigation and hydrocarbon-degrading bacteria, respectively Tables 1, 2.

Also, these reactors will be kept in the chemical engineering laboratory (unit operation) with the covering removed to allowed for oxygen and humidity for the microbes in the substrate to grow. 100

ml of tap water will be added to the twenty-two reactors, A₂₀ to D₁₀₀ to maintain good moisture content for the microbes. At intervals of seven days, the polluted samples A to F was mixed to spurred even circulation of oxygen for adequate or imperial remediation to take place.

The uncontaminated samples from the control reactors will be taken for an onward analysis for the pH and Total Petroleum Hydrocarbon (TPH). Analyses of pH and Total Petroleum hydrocarbon (TPH) polluted soil samples was repeatedly analyzed at intervals of 14 days for eighty-four days. Results from the concentration of Total Petroleum Hydrocarbon (TPH) and pH will be plotted with the time taken. These graphs were analyzed and compared to determine how effective plant extract is, in remediating crude oil polluted sites.

Table 1. Demonstration of Experimental Set-up for Reactors A and B.

S/No	Reactors	Descriptions	Mass of PE
1	A ₂₀	Polluted Loamy soil +CPED	20g
2	A ₄₀	Polluted Loamy soil +CPED	40g
3	A ₆₀	Polluted Loamy soil +CPED	60g
4	A ₈₀	Polluted Loamy soil + CPED	80g
5	A ₁₀₀	Polluted Loamy soil + CPED	100g
6	B ₂₀	Polluted Loamy soil +CPEL	20g
7	B ₄₀	Polluted Loamy soil + CPEL	40g
8	B ₆₀	Polluted Loamy soil + CPEL	60g
9	B ₈₀	Polluted Loamy soil + CPEL	80g
10	B ₁₀₀	Polluted Loamy soil + CPEL	100g

Table 2. Demonstration of Experimental Set-up for C and D.

S/No	Reactors	Descriptions	Mass of PE
1	C ₂₀	Polluted Sandy soil +CPED	20g
2	C ₄₀	Polluted Sandy loamy soil +CPED	40g
3	C ₆₀	Polluted Sandy soil +CPED	60g
4	C ₈₀	Polluted Sandy soil + CPED	80g
5	C ₁₀₀	Polluted Sandy soil + CPED	100g
6	D ₂₀	Polluted Sandy soil + CPEL	20g
7	D ₄₀	Polluted Sandy soil + CPEL	40g
8	D ₆₀	Polluted Sandy soil + CPEL	60g
9	D ₈₀	Polluted Sandy soil + CPEL	80g
10	D ₁₀₀	Polluted Sandy soil + CPEL	100g

RESULTS AND DISCUSSION

This chapter explained the results obtained from the experimental analysis of bio kinetics of Chaya leaf extract upon crude oil polluted soils. The results obtained were reported in line with the study aims and objectives as were presented on Table 3 as ands Figures below.

Physiochemical Characterization of Soil Sample and Plant Extract Physiochemical Characterization of Soil Sample

The physicochemical properties of the uncontaminated and contaminated soil samples that were used in this research work are tabulated in Table 3. The above illustrate the two soil samples used for the analysis and the result obtained shows a slightly acidic for the polluted soil with a pH value of 6.18 and 6.27 for sandy and loamy soil respectively. Whereas after contamination the acidic level of the soil

reduces to near neutrality and slightly alkaline for the unpolluted soil with loamy soil been more acidic. The value for the unpolluted indicate a normal and optimal range for a soil pH range which lie between 4.5 to 8.2. this pH range allows microbial and plant growth the mic for the growth of microbes and other plants. Soil electrical conductivity for the polluted sandy and loamy were 148.36 and 183.82(μ /cm) respectively, while there was an increase for electrical conductivity of the polluted soil samples. The soil total organic carbon (TOC) for both uncontaminated and contaminated soil samples were found to be 3.2483 and 4.524 for contaminated while 4.35, and 5.45 for the contaminated sandy, and loamy soil respectively. Also, the TOM, TBC, and the macronutrients; primarily required mostly for normal growth of microbes and plant species, such as nitrogen (N), phosphorous (P), potassium (K), were within limits for optimum plant and microbial growth as shown Table 3. this result agrees with the investigation carried out by.

Table 3. Physicochemical properties of contaminated and uncontaminated soil samples.

	pH	E.C	TOC	TOM	T.N.	P	K	TBC
Sample		μ s/cm	mg/kg	mg/kg	%	mg/kg	mg/kg	cfu/ml
Loamy(contaminated)	6.84	946.57	5.45	9.4	0.131	12.473	26.21	1.43×10^3
Sandy(contaminated)	7.13	776.21	4.35	7.5	0.104	18.68	21.96	2.15×10^2
Loamy(uncontaminated)	6.27	183.82	4.524	7.8	1.58	321.78	452.18	4.13×10^5
Sandy((uncontaminated)	6.18	148.36	3.2483	5.6	1.03	127.56	249.02	1.93×10^4

Physiochemical Characterization of Plant Extract

Some physicochemical properties of the chaya plants extract used in the experiment are shown in Table 4 shows that all the two extracts used for the biodegradation have macronutrient that will spur the growth of microbes that are capable for effective bio-remediation of TPH. This was possible due to the presence of micro- nutrients such as N, P, and K. The nitrite concentration varies from each of the plant extracts with the dark sample having the lower concentration of 1.01% and 0.86% for CPED while the light plant extract has the highest concentration of nitrate (1.05% and 0.91%). Also, the TOC and TOM followed the trend as that of the nitrate but the P and K are more in dark samples. The total bacteria count for both light and dark samples also indicate a high population of microbes indicating the viability for the remediation with CPED and CPEL. It is observed that CPED has the highest TBC followed by CPEL as shown in Table 4.

Table 4. Physicochemical property of plant extract.

	N	TOC	TOM	P	K	TBC
Sample	%	%	%	(mg/kg)	(mg/kg)	(cfu/ml)
CPEL	1.05	2.56	4.413	153.61	298.54	6.29×10^8
CPED	1.01	2.21	3.81	168.42	315.19	7.096×10^8

Physiochemical Properties of Remediated Soil (Crude Oil Contaminated Soil and Treated with Plant Extract)

Table 5 Demonstrate the changes in the physiochemical properties of the polluted Loamy soil sample treated with *Chaya* plant extract (CPED) dried in the without direct sunlight as a degradative stimulant, it is observed that the electrical conductivity (EC) of the polluted sandy soil decreases across the different mass of the CPED in the as following $A_{20} > A_{40} > A_{60} > A_{80} > A_{100}$ (148.62, 184.64, 127.24, 109.43, and 107.52) respectively. Likewise, there is a drop in concentration of TPH in all the reactors treated with a different mass of CPED, the higher the dosage of the bio stimulator the lower the TOM. Also, the Soil Total Carbon (TOC) followed the same trend as the TOM with reactor A_{100} having the lowest TOC while A_{20} has the highest. The decreased in concentration of the TOC and TOM is a clear indication that the carbon content has been fully used up by the microbes for the biodegradation activity. In addition to the changes in the TOM and TOC, the microbial analysis in each of the remediated

reactors containing loamy soil showed an increase in the total microbial population as showed in Table. 5 the result indicates a reasonable growth substrate degradative microbe such as the bacterial and fungi for 84 days. At the end of the investigation, the reactor that measured the highest bacterial count was A₁₀₀ from 35.1×10^6 cfu/ml to 3.69×10^8 cfu/ml against A₁ with 7.1×10^6 to 8.9×10^8 cfu/ml. the result shows that contaminated soil sample with crude oil can be remediated by the used of CPED.

Table 5. Variation in the Physiochemical Parameters of Remediated loamy Soil with CPED for 84days.

Reactor	TBC (cfu/ml)		TOC (%)		TOM (%)		Electrical Conductivity(μ s/cm)	
Type	initial* 10^6	final* 10^8	initial	final	initial	final	Initial	final
A ₂₀	7.1	0.89	5.77	5.25	9.95	9.05	610.03	148.62
A ₄₀	15.1	1.69	5.78	4.58	9.96	7.89	610.64	1 84 .64
A ₆₀	21.5	2.3	5.78	4.53	9.97	7.81	611.25	127.24
A ₈₀	28.7	3	5.79	4.46	9.98	7.68	611.86	109.43
A ₁₀₀	35.1	3.69	5.79	4.31	9.99	7.41	612.47	107.52

Table 6. Variation in the Physiochemical Parameters of Remediated loamy Soil with CPEL for 84 days.

Reactor	TBC (cfu/ml)		STOC (%)		STOM (%)		Electrical Conductivity(μ s/c)	
Type	initial* 10^6	final* 10^8	initial	final	Initial	Final	Initial	Final
B ₂₀	6.9	0.87	6.124	6.02	10.56	10.37	610.03	221.98
B ₄₀	13.2	1.5	6.13	4.94	10.56	8.52	610.64	205.11
B ₆₀	20.5	2.23	6.14	4.95	10.57	8.53	611.25	178.41
B ₈₀	27.3	2.91	6.14	4.83	10.58	8.33	611.86	122.67
B ₁₀₀	33.7	3.55	6.15	4.73	10.61	8.17	612.47	120.91

Table 6 reveals the variations in the physiochemical properties of the contaminated clay soil sample treated with fine particles of *chaya* plant extract (CPEL) dried under sunlight as a degradative stimulant, it is observed that the electrical conductivity (EC) of the polluted sandy soil decreases across the different mass of the CPEL in the following order of magnitude B₂₀>B₄₀>B₆₀>B₈₀>B₁₀₀ (221.98, 205.11,178.41,122.67 and,120.91) respectively. Also, there is a decline in all the reactors treated with a different mass of CPEL, the higher the mass of the bio activator the lower the STOM. Likewise, the Soil Total Carbon (TOC) followed the same trend as the TOM with reactor B₅ having the lowest TOC while B₁ has the highest. The decreased in concentration of the TOC and TOM is a clear indication that the carbon content has been fully used up by the microbes for the biodegradation activity. In addition to the changes in the TOM and TOC, the microbial analysis in each of the remediated reactors containing loamy soil revealed an increase in the total microbial population as shown in Table 6 the result indicates a significant growth of petroleum degradative microbes such as the bacteria and fungi during the period of exposure. (84 days). At the end of the investigation, the reactor that measured the highest bacterial count is B₁₀₀ from 33.37×10^6 cfu/ml to 3.55×10^8 cfu/ml against B₂₀ with 6.9×10^6 to 8.7×10^8 cfu/ml. the result shows that contaminated soil with Petroleum hydrocarbon can be remediated by CPEL.

Table 7. Variation in the Physiochemical Parameters of Remediated Sandy Soil with CPED for 84 days.

Reactor	TBC (cfu/ml)		TOC (%)		CTOM (%)		Electrical Conductivity(μ s/cm)	
Type	initial* 10^6	final* 10^8	Initial	final	initial	final	Initial	final
C ₂₀	7.1	0.925	6.76	6.24	11.65	10.75	776.21	219.41
C ₄₀	15.1	1.73	6.77	5.57	11.67	9.59	776.99	203.91
C ₆₀	21.5	2.3	6.77	5.51	11.68	9.51	777.76	186.27
C ₈₀	28.7	3.09	6.78	5.49	11.69	9.39	778.54	181.25

C ₁₀₀	35.1	3.73	6.79	5.29	11.71	9.11	779.32	151.68
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Table 7 shows the variations in the physiochemical properties of the contaminated sandy soil sample treated with fine particles of *Chaya* plant extract (CPED) dried in the absence of sunlight as a degradative stimulant was added, the electrical conductivity (EC) is observed declining across the different mass of the CPEL in the order of C₂₀>C₄₀>C₆₀>C₈₀>C₁₀₀. Also, there is a decline in all the reactors treated with a different dosage of CPED, the higher the dosage of the bio activator the lower the TOM. Likewise, the Soil Total Carbon (TOC) followed the same trend as the TOM with reactor C₁₀₀ having the lowest TOC while C₂₀ has the highest. The decreased in concentration of the TOC and STOM is clear measured that the carbon content has been fully utilized by the microbes for the biodegradation activity. In addition to the changes in the TOM and TOC the microbial evaluation or analysis in each of the remediated reactor containing sandy soil showed an increase in the total microbial population as showed in Table 7, the result indicates a significant growth of petroleum degradative microbes such as the bacteria and fungi for 84 days. At the end of the investigation, the reactor that measured the highest bacterial count is B5 from 35.1×10^6 cfu/ml to 3.73×10^8 cfu/ml against B₂₀ with 7.1×10^6 to 9.25×10^8 cfu/ml, the result shows that contaminated soil with Petroleum hydrocarbon can be remediated by CPED.

Table 8. Variation in the Physiochemical Parameters of Remediated Sandy Soil with CPEL for 84 days.

Type	TBC (cfu/ml)		TOC (%)		TOM (%)		Electrical Conductivity(μs/cm)	
	initialx10 ⁶	finalx10 ⁸	Initial	Final	initial	Final	Initial	Final
D ₂₀	6.9	0.95	6.91	6.81	11.91	11.73	776.21	237.85
D ₄₀	13.2	1.54	6.92	5.73	11.89	9.85	776.99	215.79
D ₆₀	20.5	2.27	6.92	5.74	11.91	9.87	777.76	198.51
D ₈₀	27.3	2.95	6.93	5.62	11.92	9.67	778.54	184.91
D ₁₀₀	33.7	3.59	6.94	5.53	11.93	9.51	779.31	170.16

Table 8 illustrates the variations in the physiochemical properties of the contaminated sandy soil sample when fine particles of *Chaya leaf* plant extract (CPEL) dried under sunlight were added, to a crude oil contaminated soil sample reactor D₂₀ to D₁₀₀ as shown in presented in Table 8, the values shown in the indicate a decline in the electrical conductivity(EC) across the different dosage of the CPEL correspondently with the smaller mass (20g) having the highest value of EC while the lease value to the 100g mass. The order of reduction follows as indicated D₂₀> D₄₀> D₆₀> D₈₀> D₁₀₀. Also, there is a decrease in TOM for all the reactors activated with different dosage CPEL, it was observed the higher the dosage of the bio activator the lower the TOM. Likewise, the Soil Total Carbon (TOC) followed the same trend as the TOM with reactor A5 having the lowest TOC while A₂₀ has the highest. The decreased in the concentration of the TOC and TOM is a clear indication that the carbon content has been fully utilized by the microbes for the biodegradation activity. In addition to the changes in the

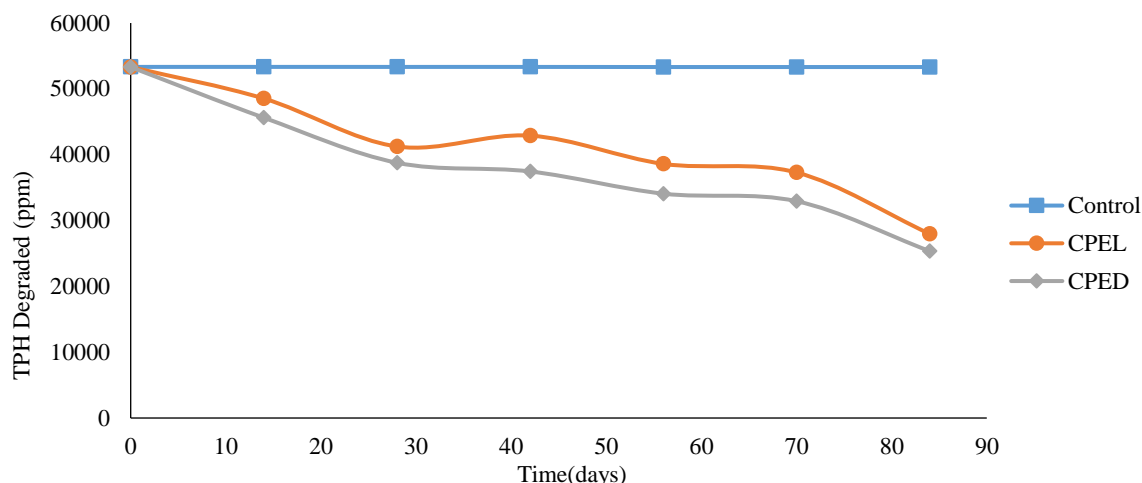


Figure 1. Degradation of TPH in sandy soil using 20 g of CPED and CPEL.

TOM and TOC, the microbial analysis in each of the remediated reactors containing sandy soil showed an increase in the total microbial population as showed in Table 8 the result indicates a significant growth of petroleum degradative microbes such as the bacteria and fungi for 84 days. At the end of the investigation, the reactor that measured the highest bacterial count is A5 from 33.7×10^6 cfu/ml to 3.59×10^8 cfu/ml against A1 with 6.9×10^6 to 9.5×10^8 cfu/ml. the result shows that contaminated soil with Petroleum hydrocarbon can be remediated by CPEL.

Performance of 20 g of CPED and CPEL on Substrate exposure for 84 days in sandy soil

Figure 1 shows the rapid degradation of substrate (TPH) upon the action of 20g of CPED and CPEL on sandy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 1, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

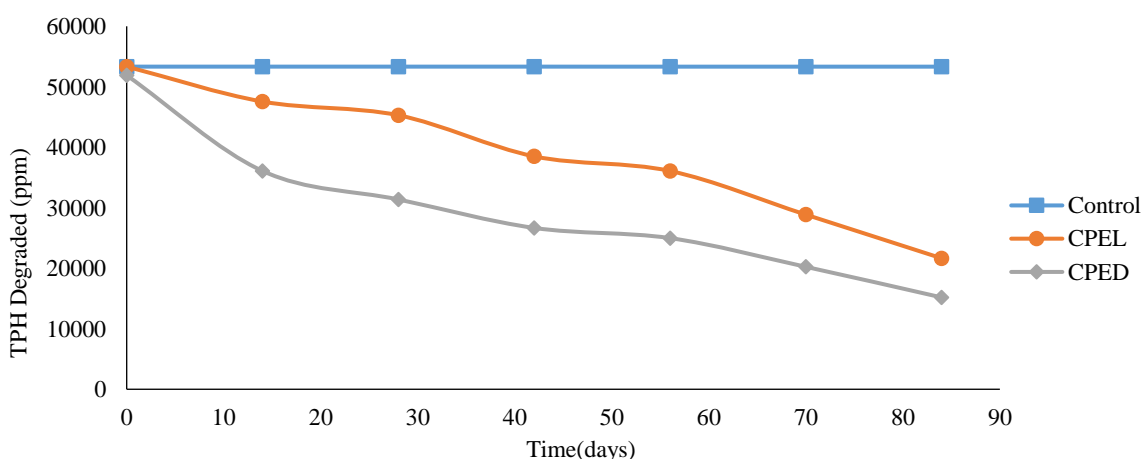


Figure 2. Degradation of TPH in Sandy Soil using 40g of CPED and CPEL.

Performance of 20 g of CPED and CPEL on Substrate exposure for 84 days in sandy soil

Figure 2 shows the rapid degradation of substrate (TPH) upon the action of 40g of CPED and CPEL on sandy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 2, with CPED indicating faster degradation when compared to CPEL

though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

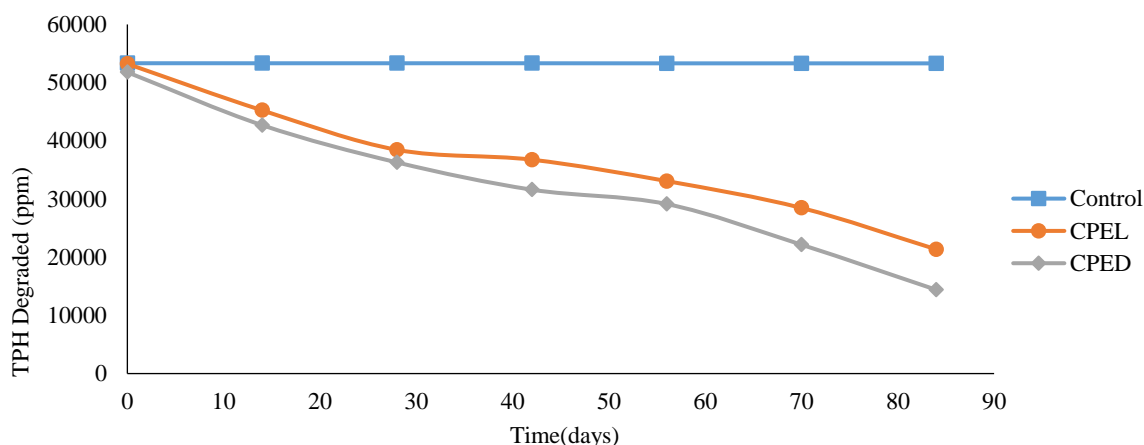


Figure 3. Degradation of TPH in Sandy Soil using 60 g of CPED and CPEL.

Performance of 60 g of CPED and CPEL on Substrate Exposure for 84 Days in Sandy Soil

Figure 3 shows the rapid degradation of substrate (TPH) upon the action of 60g of CPED and CPEL on sandy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 3, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

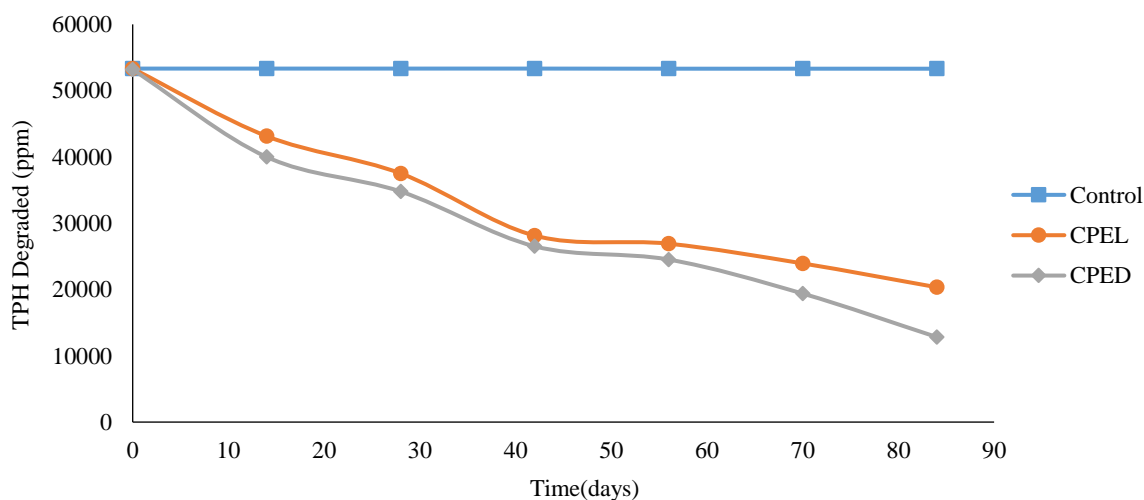


Figure 4. Degradation of TPH in Sandy Soil using 60 g of CPED and CPEL.

Performance of 60 g of CPED and CPEL on Substrate exposure for 84 days in sandy soil

Figure 4 shows the rapid degradation of substrate (TPH) upon the action of 60 g of CPED and CPEL on sandy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 4, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

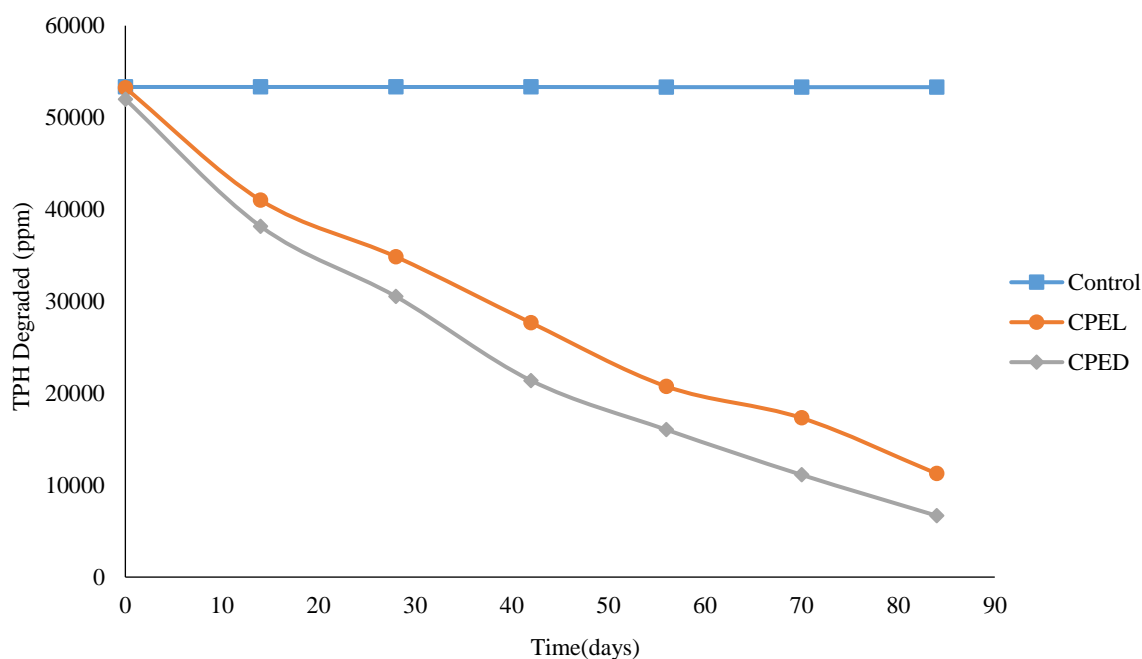


Figure 5. Degradation of TPH in sandy soil using 100 g of CPED and CPEL.

Performance of 60 g of CPED and CPEL on Substrate exposure for 84 days in sandy soil.

Figure 5 shows the rapid degradation of substrate (TPH) upon the action of 60g of CPED and CPEL on sandy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 5, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

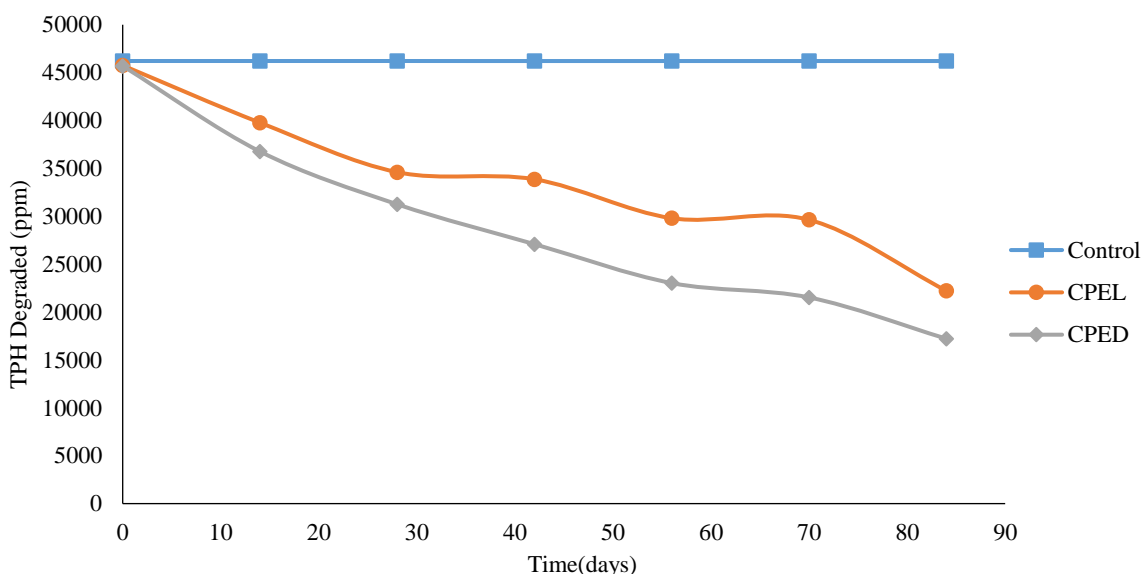


Figure 6. Degradation of TPH in loamy soil using 20 g of CPED and CPEL.

Performance of 20 g of CPED and CPEL on Substrate exposure for 84 days in Loamy soil

Figure. 6 shows the rapid degradation of substrate (TPH) upon the action of 20g of CPED and CPEL on loamy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 6, with CPED indicating faster degradation when compared to CPEL

though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

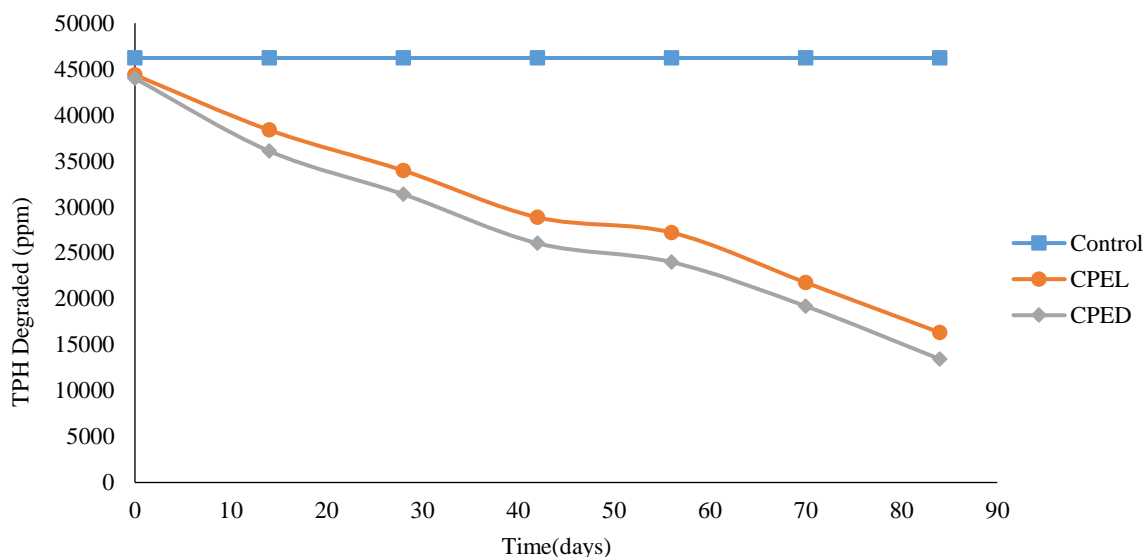


Figure 7. Degradation of TPH in loamy soil using 40g of CPED and CPEL.

Performance of 40 g of CPED and CPEL on Substrate exposure for 84 days in Loamy soil

Figure 7 shows the rapid degradation of substrate (TPH) upon the action of 40g of CPED and CPEL on loamy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 7, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

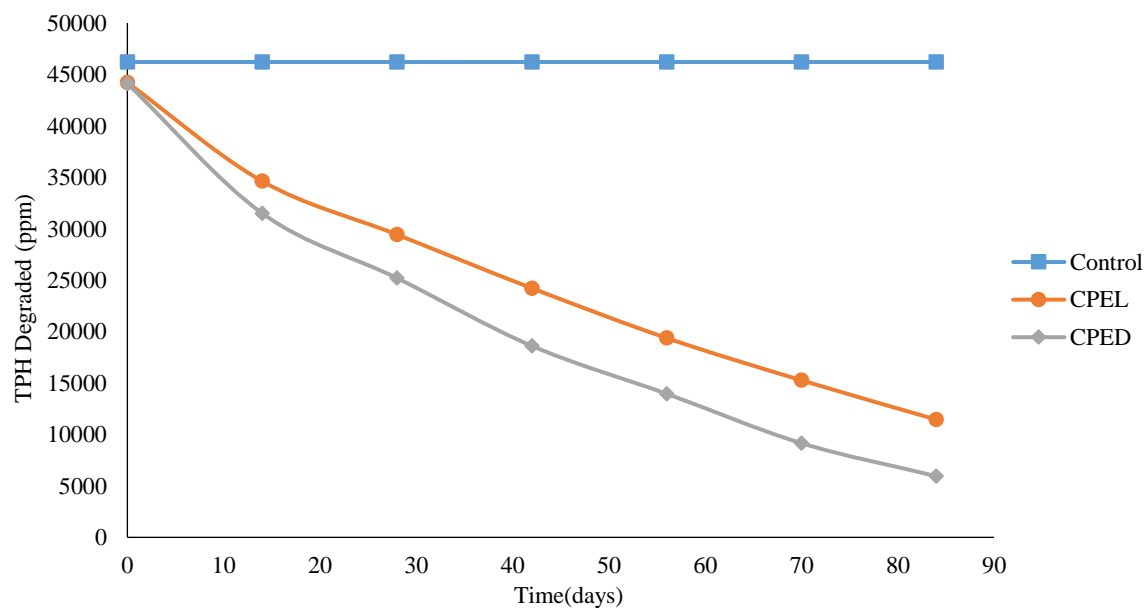


Figure 8. Degradation of TPH in Loamy Soil using 60 g of CPED and CPEL.

Performance of 60 g of CPED and CPEL on Substrate exposure for 84 days in Loamy soil

Figure. 8 shows the rapid degradation of substrate (TPH) upon the action of 60g of CPED and CPEL on loamy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same

soil sample is shown in Figure 8, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

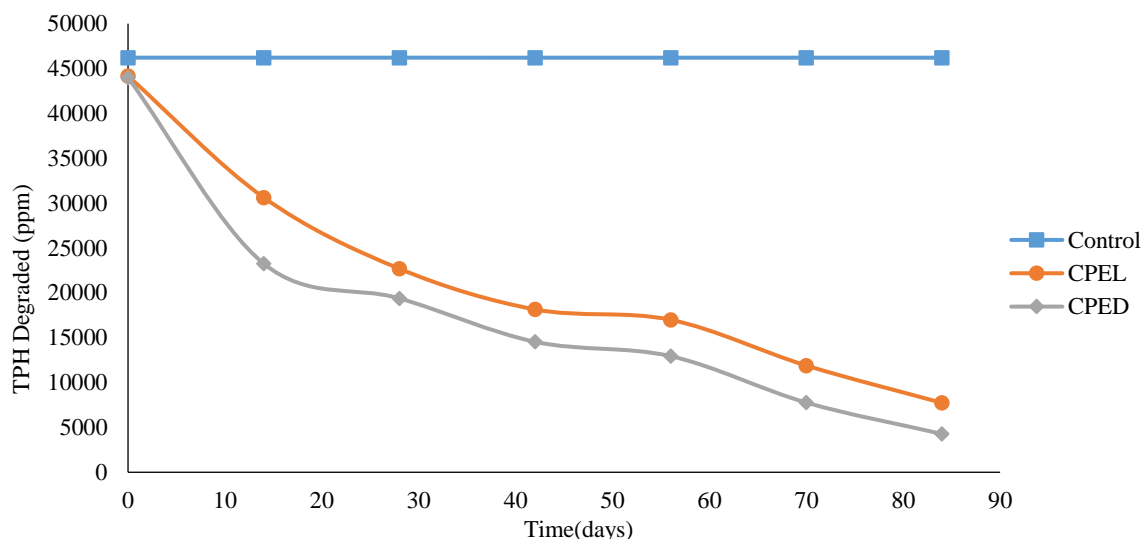


Figure 9. Degradation of TPH in Loamy Soil using 80g of CPED and CPEL.

Performance of 80 g of CPED and CPEL on Substrate exposure for 84 days in Loamy soil

Figure 9 shows the rapid degradation of substrate (TPH) upon the action of 80g of CPED and CPEL on loamy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 9, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

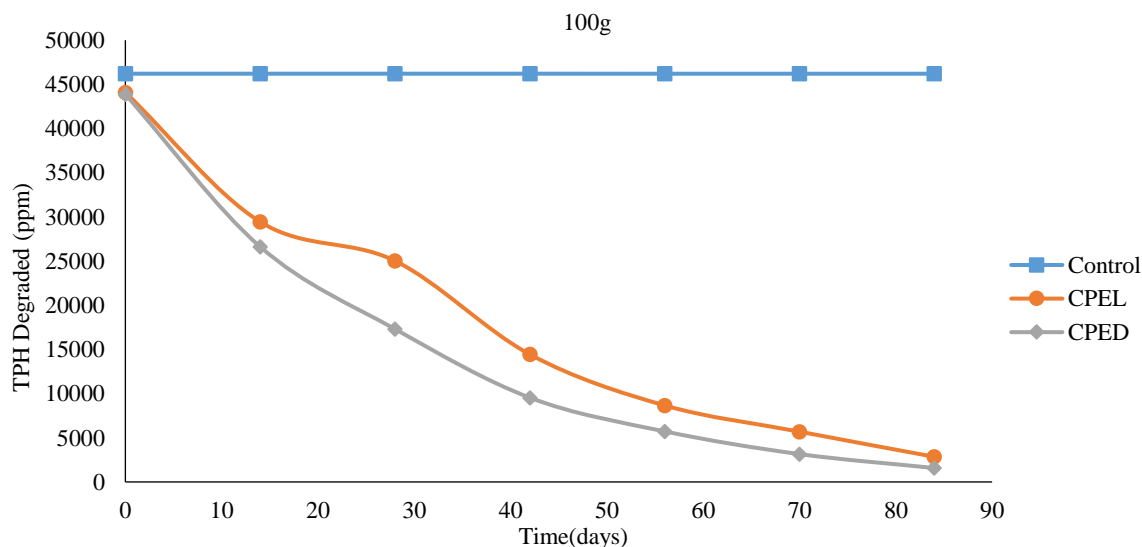


Figure 10. Degradation of TPH in Loamy Soil using 100g of CPED and CPEL.

Performance of 100 g of CPED and CPEL on Substrate Exposure for 84 days in Loamy Soil

Figure 10 shows the rapid degradation of substrate (TPH) upon the action of 100 g of CPED and CPEL on loamy soil respectively, the reduction in the concentration of TPH is due to the supply of micro-nutrient from the stimulant (CPED and CPEL). The performance of the different stimulant on the same soil sample is shown in Figure 10, with CPED indicating faster degradation when compared to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

to CPEL though with the dosage. The performance of CPED is more active compared to CPEL due to the mode of treatment. The dark treatment has more degradative microbes than the light phase.

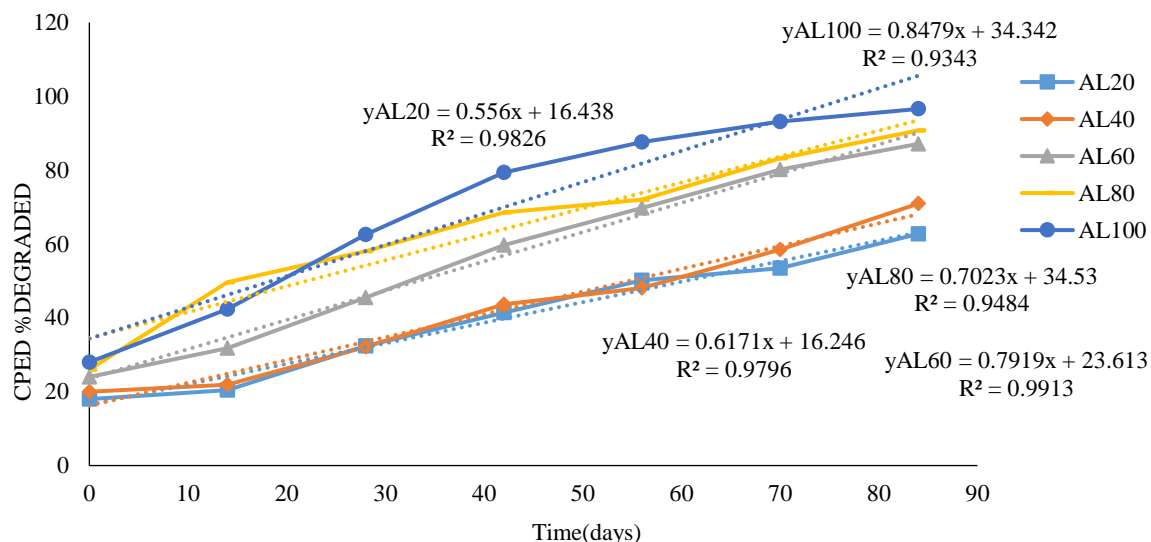


Figure 11. Percentage Performance of Different Dosage of CPED on Degradation TPH in Loamy Soil for 84 days

Effect of Dosage of CPED on TPH Contaminated Loamy Soil Exposed for 84 days

Figure 11 Demonstrate the effect of dosage of CPED on the degradation of TPH in loamy soil, the % of TPH degraded increases as the contact or exposure time increases, this increment is attributed to the present of biodegradable microbes that were fund in the substrate. These microbes were responsible for the degradation of the TPH, activeness of the microbes was seen to increases as the CPED spurred the grow of these organism by supplying the need micro-nutrient needed for the multiplication of the organisms. Also, from Figure 11 is observed that as the dosage increases the rate of degradation increases. The reduction in the TPH is highly carried out by the bio- degradative microbes in conjunction with other favorable physicochemical and physical parameters.

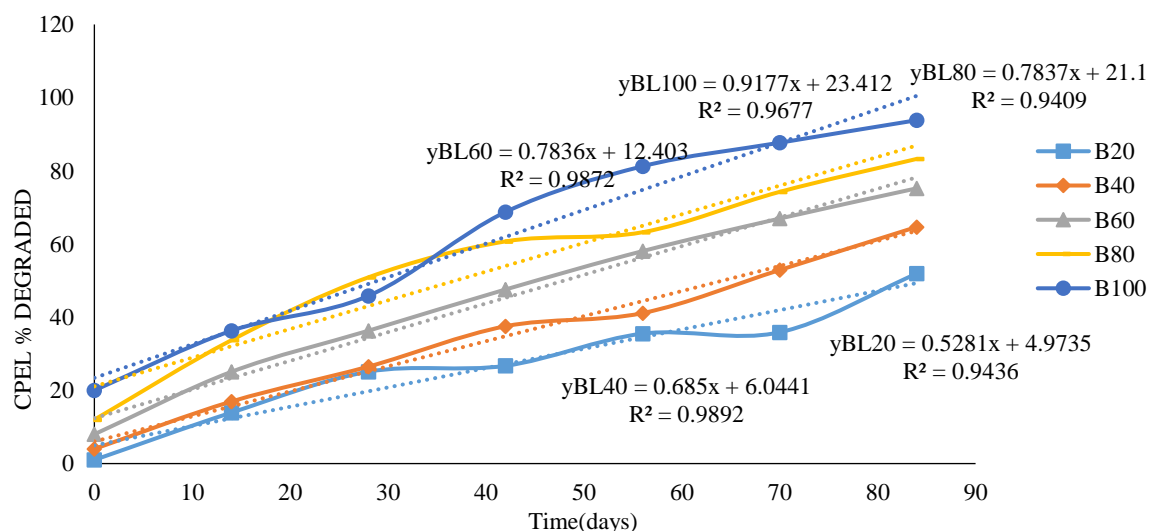


Figure 12. Percentage Performance of Different Dosage of CPEL on Degradation TPH in Loamy Soil for 84 days.

Figure 12, Demonstrate the effect of dosage of CPEL on the degradation of TPH in loamy soil, the % of TPH degraded increases as the contact or exposure time increases, this increment is attributed to the present of biodegradable microbes that were fund in the substrate. These microbes were responsible

for the degradation of the TPH, activeness of the microbes was seen to increases as the CPEL spurred the grow of these organism by supplying the need micro-nutrient needed for the multiplication of the organisms. Also, from Figure 12 is observed that as the dosage increases the rate of degradation increases in this order BL100>BL80>BL60>BL40>BL20. The reduction in the TPH is highly carried out by the bio- degradative microbes in conjunction with other favorable physicochemical and physical parameters.

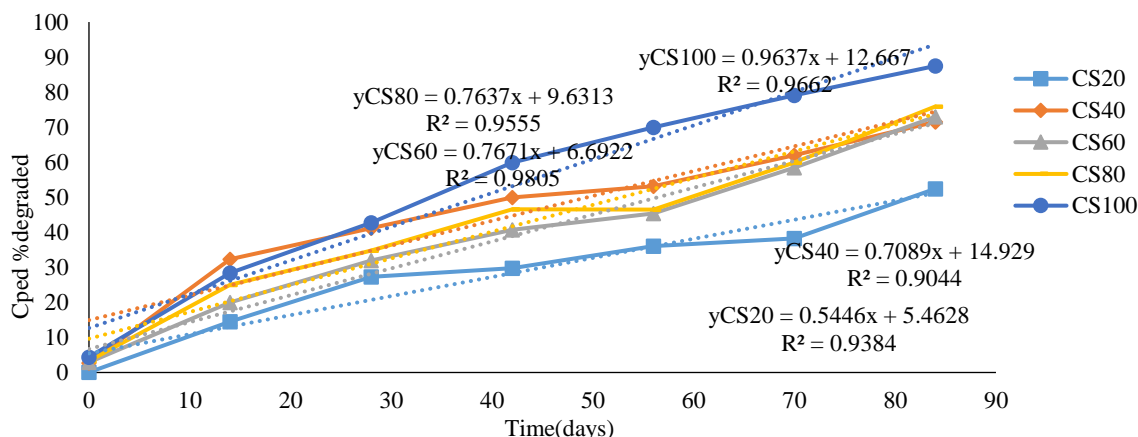


Figure 13. Percentage Performance of Different Dosage of CPED on Degradation TPH in Sandy Soil for 84 days

Figure 13, Demonstrate the effect of dosage of CPED on the degradation of TPH in sandy soil, the % of TPH degraded increases as the contact or exposure time increases, this increment is attributed to the present of biodegradable microbes that were fund in the substrate. These microbes were responsible for the degradation of the TPH, activeness of the microbes was seen to increases as the CPED spurred the grow of these organism by supplying the need micro-nutrient needed for the multiplication of the organisms. Also, from Figure 13 is observed that as the dosage increases the rate of degradation increases in this order CS100>CS80>CS60>CS40>CS20. The reduction in the TPH is highly carried out by the bio- degradative microbes in conjunction with other favorable physicochemical and physical parameters.

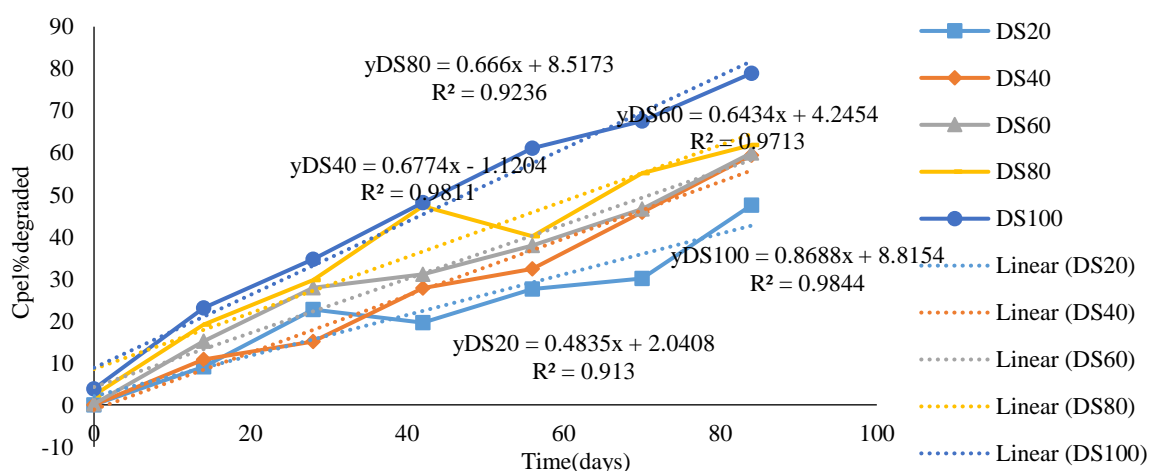


Figure 14. Percentage Performance of Different Dosage of CPEL on Degradation TPH in Sandy Soil for 84 days.

Figure 14, Demonstrate the effect of dosage of CPEL on the degradation of TPH in sandy soil, the % of TPH degraded increases as the contact or exposure time increases, this increment is attributed to the present of biodegradable microbes that were fund in the substrate. These microbes were responsible for

the degradation of the TPH, activeness of the microbes was seen to increase as the CPED spurred the growth of these organisms by supplying the needed micro-nutrient for the multiplication of the organisms. Also, from Figure 14 is observed that as the dosage increases the rate of degradation increases in this order DS100>DS80>CS60>DS40>DS20. The reduction in the TPH is highly carried out by the bio-degradative microbes in conjunction with other favorable physicochemical and physical parameters.

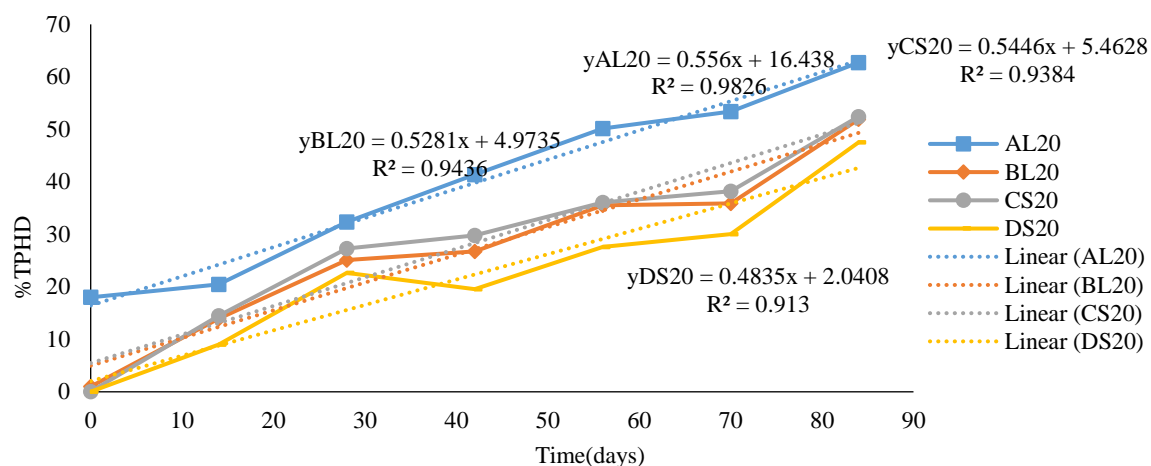


Figure 15. Degradation of TPH with 20g Dosage of CPED and CPEL on Different Soil Samples for 84 days

Effect of 20 g of CPED and CPEL on Substrate Degradation on different Soil samples

In Figure 15 illustrate the decline in substrate concentration of TPH degradation as examined with varying period of contact (time). The decreased in substrate concentration of TPH was significant with an increase in the period of exposure (time) for the different soil samples used for the research. The dark processed plant extract (CPED) for both sandy and loamy soil environments performed significantly than the light processed plant extract (CPEL). These extracts were used to enhance the performance of the microbes that were responsible for the breaking down of the higher molecular toxic hydrocarbon to lighter nontoxic component through bioremediation, the loss in the TPH concentration was due to the period of exposure (time) as well as the activities of the enhance microbes and other factors such as physiochemical parameters.

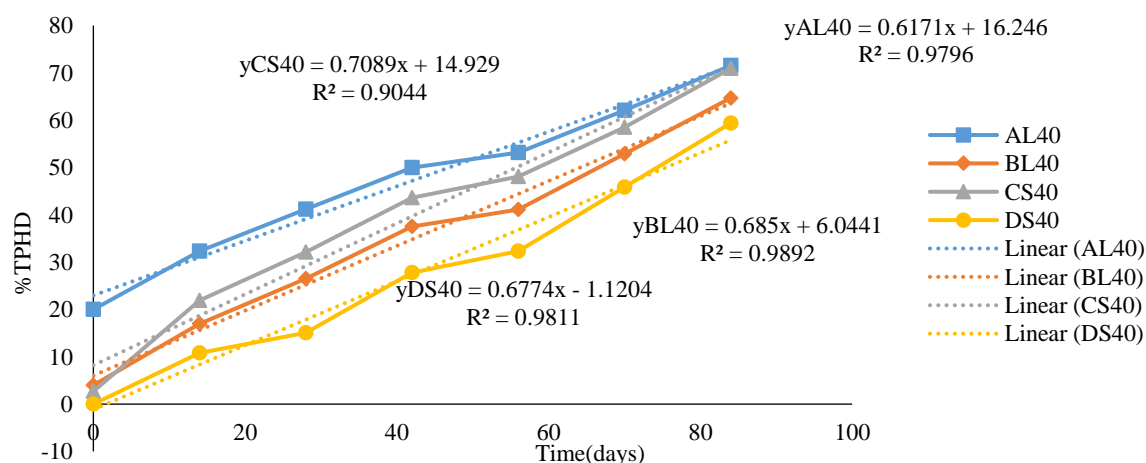


Figure 16. Degradation of TPH with same Dosage(40g) of CPED and CPEL on different Soil Samples 84 days.

Effect of 40 g of CPED and CPEL on Substrate Degradation on Different Soil samples

In Figure 16 revealed the reduction in substrate concentration of TPH degradation as examined with varying period of contact (time). The decreased in substrate concentration of TPH was substantial with

a surge in the period of exposure (time) for the different soil samples used for the research. The dark processed plant extract (CPED) for both sandy and loamy soil environments performed significantly than the light processed plant extract (CPEL). These extracts were used to improve the performance of the microbes that were liable for the breaking down of the complex molecular toxic hydrocarbon to simple nontoxic component through bioremediation, the loss in the TPH concentration was due to the period of exposure (time) as well as the activities of the enhance microbes and other factors such as physiochemical parameters.

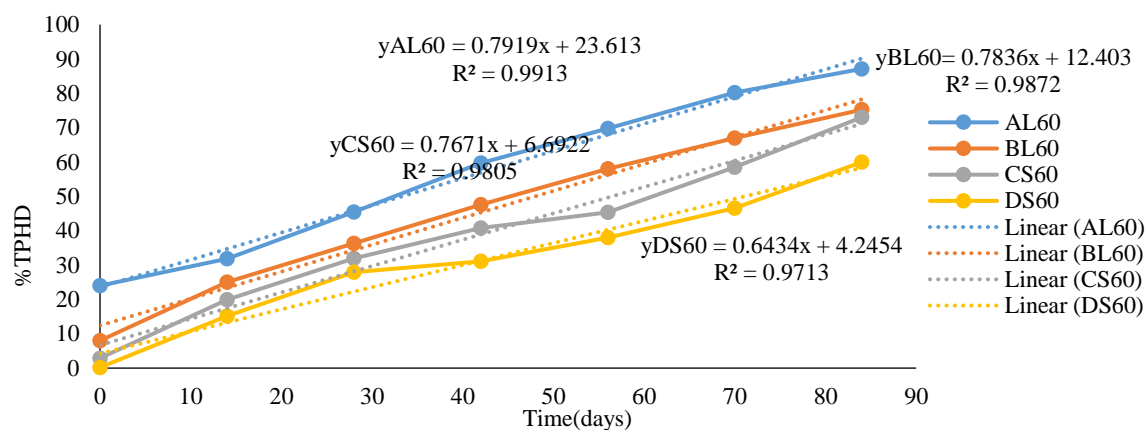


Figure 17. Degradation of TPH with same Dosage (60 g) of CPED and CPEL on different soil samples 84 days.

Effect of 60 g of CPED and CPEL on Substrate Degradation on Different Soil Samples

In Figure 17 shown the decrease in substrate concentration of TPH degradation as examined with varying period of contact (time). The declined in substrate concentration of TPH was sizeable with a increased in the period of contact (time) for the different the soil samples used for the research. The dark processed plant extract (CPED) label as AL₆₀ and CS₆₀ for both sandy and loamy soil environments performed brilliantly than the light processed plant extract(CPEL) label BL₆₀ and DS₆₀ These extract were used to advance the performance of the microbes that were responsible for the degradation of the complex hydrocarbon to simple nontoxic component through bioremediation, the loss in the TPH concentration was due to the period of exposure (time) as well as the activities of the enhance microbes and other factors such as physiochemical parameters.

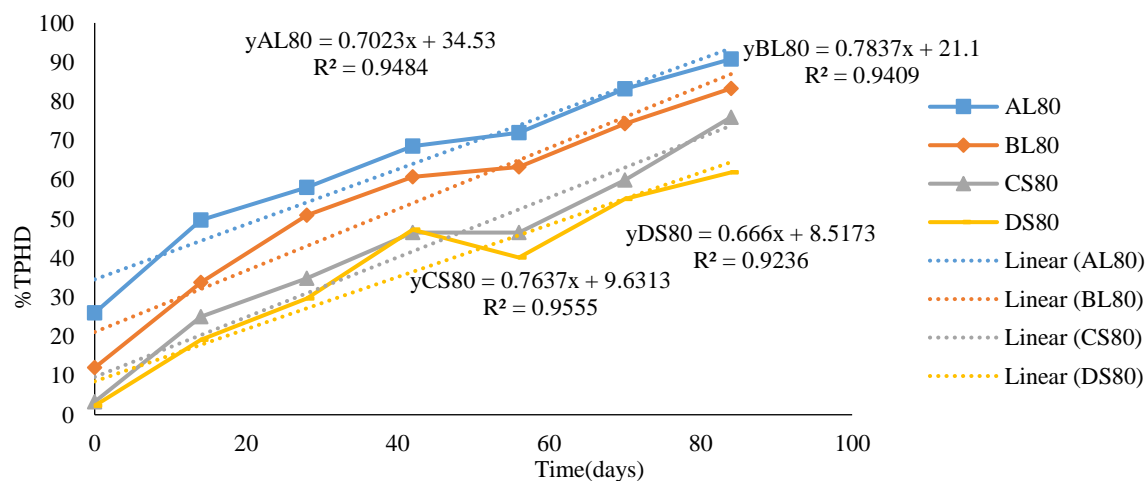


Figure 18. Degradation of TPH with same Dosage(80g) of CPED and CPEL on different soil samples

Effect of 80 g of CPED and CPEL on Substrate Degradation on different Soil Samples

In Figure 18 shown the decreased in substrate concentration of TPH degradation as examined with different period of exposure (time). The declined in substrate concentration of TPH was more significant as the time of contact increases for the different the soil samples used for the research. The dark processed plant extract (CPED) label as AL₈₀ and CS₈₀ for both sandy and loamy soil environments, performed brilliantly than the light processed plant extract (CPEL) label as BL₈₀ and DS₈₀ for loamy and sandy soil. These extracts were used to advance the performance of the microbes that were responsible for the degradation of the complex hydrocarbon to simple nontoxic component through bioremediation, the loss in the TPH concentration was due to the period of exposure (time) as well as the activities of the enhance microbes and other factors such as physiochemical parameters.

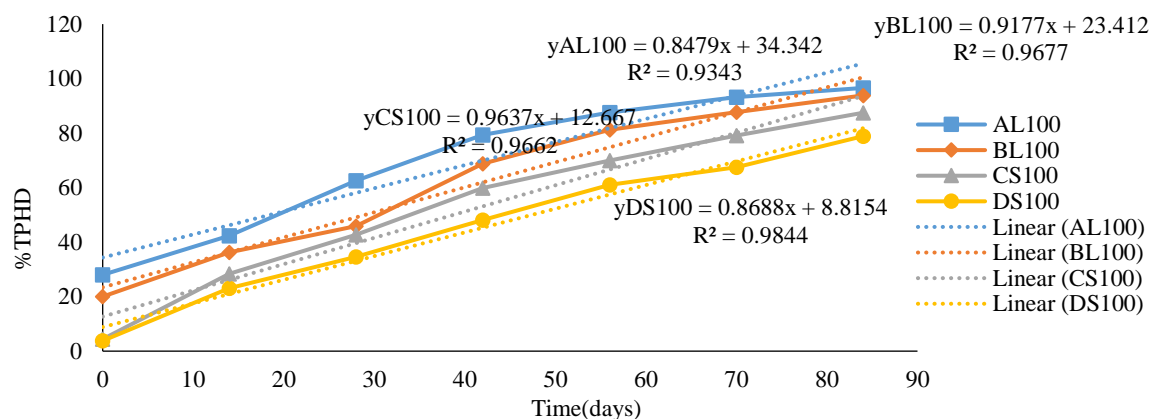


Figure 19. Degradation of TPH same Dosage(100 g) of CPED and CPEL on different Soil samples 84 days.

In Figure 19 shown the decreased in substrate concentration of TPH degradation as examined with different period of exposure (time). The declined in substrate concentration of TPH was more significant as the time of contact increases for the different the soil samples used for the research. The dark processed plant extract (CPED) label as AL₁₀₀ and CS₁₀₀ for both sandy and loamy soil environments, performed brilliantly when compared the light processed plant extract (CPEL) label as BL₁₀₀ and DS₁₀₀ for loamy and sandy soil. These extracts were used to advance the performance of the microbes that were responsible for the degradation of the complex hydrocarbon to simple nontoxic component through bioremediation, the loss in the TPH concentration was due to the period of exposure (time) as well as the activities of the enhance microbes and other factors such as physiochemical parameters.

CONCLUSION

This research work was carried out to investigate the degradation rate of TPH on polluted soil environment with the effect of dosage of locally sourced plant extract (Chaya) leaf which served as source of nutrient to the bio-degradative micro-organisms. The remediand used in this research is rich in micro- nutrients, these nutrients spurred the microbial activities of the bio-degrader in each of the bioreactors.

In this work the physiochemical properties of the samples used for the research were examined, in other to know the constituent of each sample and its significant in the degradation of the TPH in the soil polluted environment. The samples that were examined include the soil, crude oil, and the plant extract (Chaya leaf) for both room and sun-dried which served as the remediand and biostimulant. The examined physiochemical properties played a vital role in enhancing the biodegradation process of the TPH in each of the bioreactor. As the exposure (contact) time increases the percentage rate of TPH degraded was monitored and evaluated with the aid of a Gas Chromatophy (GC) analyzer for fourteen days' interval, likewise this reduction in the TPH concentration varies with remediand dosage(mass) as well as the time of exposure. As the dosage of the remediand increases the degradation rate also increase which led to a reduction in TPH concentration level within the polluted soil environment.

This work was also able to showcase that the remediant processed both in the room and sun-dried has shown a significant performance in the degradation of the TPH with the soil environment at the exposure time interval. The performance of the extract in the dark(room) revealed a high degree of remediation when compared with the sun-dried extract. Also, the soil environment used in this research were sandy and loamy, during this research the remediant in the loamy soil environment performed brilliantly when compared to that of the sandy soil this is due to the enhanced nature of the loamy soil environment to support bio-degradation, the water retention capacity of the loamy soil was also an attribute for the significant performance evaluated.

REFERENCES

1. Oyedeji, A.A., Kayode J., Besenyei, L. & Fullen, M. A. (2015c). Phytoremediation potential of selected Nigerian indigenous leguminous tree species on crude oil- contaminated soils: early growth response and vegetative establishment. In: Book of Abstracts of the Botanical Society of America and other related Societies Conference, Edmonton, Alberta, Canada, 131
2. Parrish ZD, Banks MK, Schwab AP. Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil. *International Journal of Phytoremediation*. 2004 Apr 1;6(2):119–37.
3. Prince RC, Clark JR, Lee K. Bioremediation effectiveness: Removing hydrocarbons while minimizing environmental impact. In 9th International Petroleum Environmental Conference, IPEC (Integrated Petroleum Environmental Consortium), Albuquerque, NM 2002 May 30.
4. Rahman KS, Thahira-Rahman J, Lakshmanaperumalsamy P, Banat IM. Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource technology*. 2002 Dec 1;85(3):257–61.
5. Rowland AP, Lindley DK, Hall GH, Rossall MJ, Wilson DR, Benham DG, Harrison AF, Daniels RE. Effects of beach sand properties, temperature and rainfall on the degradation rates of oil in buried oil/beach sand mixtures. *Environmental pollution*. 2000 Jul 1;109(1):109–18.
6. Alamri SA. Use of microbiological and chemical methods for assessment of enhanced hydrocarbon bioremediation. *Journal of Biological Sciences*. 2009;9(1):37–43.
7. Sublette KL. Fundamentals of bioremediation of hydrocarbon contaminated soils. The University of Tulsa, Continuing Engineering and Science Education. Houston, TX. 2001 Oct 11.
8. Tanee, F. B. G. & Albert, E. (2011). Post-remediation assessment of crude oil polluted site at Kegbara-Dere community, Gokana L.G.A. of Rivers State, Nigeria. *Nigeria Journal of Bioremediation and Biodegradation*, 2, 122–123.
9. Tanee, F. B. G. & Kinako, P. D. S. (2008). Comparative Studies of Biostimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. *J. Appl. Sci. Environ. Manage.* 12(2), 143 – 147.
10. Tian, W., Wen, X. & Qian, Y. (2004). Using a zeolite medium biofilter to remove organic pollutants and ammonia simultaneously. *Journal of Environmental Sciences- China*, 16(1), 90–93.
11. Udoye, M. C., Okpala, K.O., Osoka, E. C., Obijiaku, J.C., Ogah, A.O. & Chukwu, M. M. (2017). Modeling a bioremediation process of a petroleum contaminated soil enhanced with NPK Fertilizer and animal/plant derived organic manure. *International Research Journal of Advanced Engineering and Science*, 2(4), 87–97
12. Ukpaka, C. P. (2016). Development of model for bioremediation of crude oil using moringa extract. *Chemistry International* 2(1), 19–28
13. Ukpaka, C. P. & Itabeni, C. (2016). Characteristics concept for the examination of total hydrocarbon content and total organic carbon in contaminated soil zone. *Current Science Perspectives*, 2(3), 69–77
14. Ukpaka, C. P. (2017). Modelling the Methodology for Crude Oil Bioremediation Decision Tree for an Integrated Environmental Management System. *J Chem Eng Process Technol*, 8(2), 325–231.
15. Ukpaka, C. P., Lezorghia, S. B. & Nwosu, H. (2020). Crude oil degradation in loamy soil using Neem root extracts: An experimental study. *Chemistry International* 6(3), 160–167.

16. Ukpaka, C. P., Amadi, S. A. & Umesi, N. (2009). Modeling the physical properties of activated sludge biological wastewater treatment system in a plug flow reactor. *The Nigeria Journal of Research and Production. A Multidisciplinary Journal*, 15(1), 37–56.
17. Ukpaka, C. P., Oboho, E. O. (2006). Biokenetics for the production of Nitrogen in a natural aquatic ecosystem polluted with crude oil. *Journal of Modeling, Simulation and Control*, 67(2), 39–58.
18. Venosa, A. D. & Zhu, X. (2003). Biodegradation of crude oil contaminating marine shore lines and fresh water wet land. *Spill sci. Tech Bull*, 8(2), 163–197
19. White, P. M. Jr., Wolf, D. C., Thoma, G. J. & Reynolds, C. M. (2003). Influence of organic and inorganic soil amendments on plant growth in crude oil-contaminated soil. *International Journal of Phytoremediation*, 5(4), 381–397.
20. White, P. M. Jr., Wolf, D. C., Thoma, G. J. & Reynolds, C. M. (2006). Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil- contaminated soil. *Water, Air and Soil pollution*, 169(1–4), 207–220.