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Study of the time-efficacy and rate of phytoremediation of crude oil polluted soil by *Vigna unguiculata* (L) Walp.

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Abstract
The use of plants for enhanced remediation of crude oil contaminated soil has been a subject of interest due to the various benefits attributed to it. However, little or no study has focused on determining the time efficacy of plants to enhance high level of remediation and the rate at which phytoremediation of crude oil takes place. This study investigated the time-efficacy of cowpea (*Vigna unguiculata*) in remediating crude oil contaminated soil and rate of remediation using TPH loss as indicator. Three kilogrammes of soil each were experimentally contaminated with 12.5ml, 25ml, 50ml, 75ml and 100ml of Bonny light crude oil. The cowpea plants were introduced into the contaminated soil and the total petroleum hydrocarbon (TPH) contents were determined on day 0, after 45 days and after 90 days of planting and the pH, moisture content and organic matter content were determined on same days. The concentration of crude oil in the soil affected the efficacy of the remediation and the soils physical and organic properties. Higher levels and faster rates of remediation were obtained in the different treatments of the soil with *V. unguiculata* than in the soil without the plant. The growth of the plant contributed to faster rates of remediation in the 12.5ml and 25ml crude oil treated soils in the first 45 days than in second 45 day while in the second 45 days, the growth of the plant contributed to faster rate of remediation in 50ml, 75ml and 100ml crude oil treated soils than in the first 45 days. The results suggest that the impact of *V. unguculata* is higher in the early period for low level of contamination and higher in the later period in the soil with higher level of contamination. The presence of cowpea in crude oil contaminated soil led to improved remediation efficacy of the soil and decrease the time required for remediation to occur thus with the growth *V. unguiculata*, it will take a shorter period restore crude oil contaminated soil to its good state. It is recommended that cowpea can be combined with other plants or bacteria or organic components that have been known to aid the remediation of crude oil to achieve a higher level of remediation.

Keywords
Crude oil, Phytoremediation, phytoremediation efficiency, physicochemical, TPH, Vigna unguiculata

Introduction

Crude oil is a complex mixture of several different structural classes of hydrocarbon compounds, which is characterized into four major segments; aliphatics, aromatics, resins and aspalthenes (Fatima *et al*, 2017). It is the major source of pollution in regions with commercial crude oil exploration with severe consequences to both humans and the environment. Crude oil pollution occurs mainly through oil spills and the major causes of oil spills include corrosion of pipelines and tankers, this accounts for 50% of oil spills, sabotage (28%), oil operations (21%)

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and inadequate or non-functional equipment accounting for 1% (Nwachukwu and Osuagwu, 2014). There is a great deal of environmental stress due to oil spills that occur as a result of the activities of oil production from exploration, extraction, transportation to refinement. Crude oil spillage has impact on various aspects of the environment such as the soil, crop yield, water quality and biodiversity and microbial flora (Njoku *et al* 2022)

There are different techniques of cleaning up polluted soil. However, the conventional traditional treatment method for cleaning polluted environment poses some problems (Ferraji *et al* 2016). Phytoremediation is a process by which green plants and their associated microbes are used to remove or extract contaminants in the soil, sediment, surface water and groundwater (Ayotamuno *et al*., 2010). Phytoremediation uses the natural processes of the plant to degrade, concentrate, sequester, bioaccumulate, stabilize and metabolize contaminants (Ossai *et al*, 2019). Phytoremediation is economically feasible, environment and eco-friendly, easily applicable and can improve soil fertility and prevents erosion and spread of pollutants (Aken *et al* 2009, Wuana and Okieimen, 2011, Jacob *et al* 2018). Phytoremediation can be used for effective remediation of organic and inorganic contaminants like heavy metals, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, halogenated hydrocarbons, petroleum hydrocarbons, dyes, pesticides and explosives using diverse plants (Tripathi *et al.,* 2019, Raiz *et al.,* 2022).

Some of the plants that have been tried in the field, pot and outdoor studies for phytoremediation of crude oil are listed in Adesipo *et al* (2020). Egharevba *et al* (2017) were of the opinion that legumes are better candidates for phytoremediation of crude oil contaminated soil than grasses. Legumes have the ability to establish a symbiotic relationship with nitrogen-fixing rhizobacteria (Velazquez *et al*., 2010). Legumes are usually considered as good options for phytoremediation due to their ability to fix atmospheric nitrogen thereby reducing the full dependence of the plant on soil nutrients (Yateem *et al.,* 2000). The ability of cowpea to fix nitrogen and not compete with the indigenous microbial community makes cowpea the ideal plant to use in phytoremediation (Ukaoma *et al*, 2015). *V. unguiculata* has been proven through various studies to be able to survive and thrive in harsh environments such as one posed by a crude oil contaminated soil. Its ability to fix its own nitrogen and supply nutrients to the soil makes it desirable for this study as the ability of a given crop to reduce the level of crude oil in oil contaminated soil can help restore polluted soil for agricultural purposes (Njoku *et al*, 2009)

Although, studies have shown that cowpea can enhance the remediation of crude oil polluted soil (Tanee and Akonye, 2009; Agbogidi, 2010; Jidere *et al*, 2012; Eze *et al*, 2013; Ukaoma *et al*, 2015; Igwebuike, 2017; Yahaya, *et al*, 2019; Manga *et al*, 2020), none of them has been able to show the best growing period where *V. unguiculata* has the highest efficacy in contributing to the remediation of crude oil from the soil. Also, little or no information has demonstrated the rate at which *V.unguiculata* enhances the remediation of crude oil contaminated soil. This study was therefore undertaken to establish the effectiveness of cowpea as a remediating agent for crude oil pollution, the period of growth that *Vigna unguiculata* enhances best remediation efficiency and the rate of remediation of crude oil contaminated soil. The results obtained can help in projecting the time that we can achieve 100% remediation crude oil polluted soil due to the growth of *Vigna unguiculata*.

Materials and Methods

Sources of materials and study design

The Bonny light crude oil used for this study was obtained from the Port-Harcourt Refining Company (PHRC), Port-Harcourt, Rivers State, Nigeria. The soil used was obtained from a farm in the Ikorodu Local Government Area of Lagos state, Nigeria.Ikorodu is geograhically located between Longitudes 6°31′*N* and 6°41′*N* and Latitude 3°26′*E* and 3°42′*E* (Epuh *et al*,

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2020). Figure 1 shows the map of Ikorodu Local Government Area in Lagos state, Nigeria.The cowpea seeds purchased from a local market in Aguda, Surulere, Lagos state, Nigeria. A total of twenty containers were filled with 3000g of air-dried soil and divided into five groups each having four containers. Each group represented a particular treatment (12.5ml ≡ 3666.67 mg/kg, 25ml ≡ 7666.67 mg/kg, 50ml ≡ 14666.67 mg/kg, 75ml ≡ 22000 mg/kg and 100ml ≡ 29333.33 mg/kg). Each group was then sub-divided into two sub-groups representing contaminated soil with cowpea (vegetated soil) and contaminated soil without cowpea (non-vegetated soil). The soil in each group were contaminated with different treatments of crude oil and kept for 24 hours before the introduction of ten cowpea seeds each into two containers in each group that had been labeled cowpea. The cowpea seeds were allowed to germinate and the plants watered once every day for 90 days.

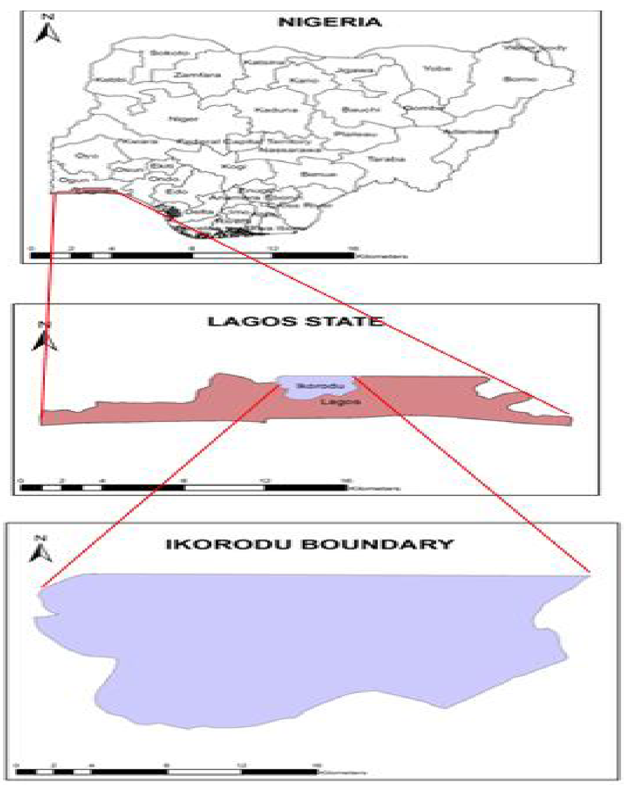

Figure 1: Map of Ikorodu Local Government Area (Source: Epuh *et al*, 2020).

Sample collection

The samples were collected on the day of contamination (initial), after forty-five days (midway) and after ninety days (final) of *Vigna unguiculata* growth. 100g of soil samples were collected from each container at each time. The topsoil and soil at a depth of 10cm were collected and weighed on an electric weighing balance and sealed in Ziplock bags.

Determination of Total Petroleum Hydrocarbon (TPH) contents of the soil samples

The TPH contents of the soil samples were determined after extracting the petroleum hydrocarbon in the soil using the procedures outlined in U. S. EPA Method 3550C (2007).5g of each air-dried soil sample was weighed into centrifuge tubes separately, 5ml of acetone: hexane (1:1) mixture was then added to the sample and mixed using a vortex for 1 minute after which it was subjected to ultrasonic treatment for 15 minutes for Total Petroleum Hydrocarbon (TPH) extraction. The mixture was then centrifuged at 2000 revolutions per minute for 10 minutes. The organic layer was transferred to a beaker and the residue re-extracted once with 5ml of acetone-hexane mixture. The extracts were combined and dried over anhydrous sodium sulfate, concentrated to 1 ml with a rotary evaporator and stored in capped Gas Chromatography (GC) vials for the determination of TPH.

The TPH content of the soil was quantified using the gas chromatography and mass spectrophotometer (GC-MS). Agilent 7820A gas chromatograph coupled to 5975C Inert Mass Spectrometer, with triple axis Mass Selective Detector and an electron-impact source manufactured by Agilent Technologies was used to quantify TPH (Hassan *et al*, 2018). The data obtained was handled with the Agilent Mass Hunter chromatography Software to determine the quantity of hydrocarbon present based on Retention Time (RT) and the abundance of the quantification ions. The percentage remediation of TPH on different days was calculated using the following formula:

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$$\text{Percentage of TPH lost} = \frac{\text{initial amount of TPH} - \text{amount of TPH at a given day}}{\text{initial amount of TPH}} \times 100 \quad [1]$$

$$\text{Rate of TPH loss} = \frac{\text{percentage TPH lost for each treatment}}{90 \text{ days}} \quad [2]$$

The contribution of *V. unguiculata* to the percentage TPH loss was calculated as the difference between the percentage of TPH lost in the soil with the plant (vegetated soil) and the percentage TPH lost in the soil without the plant (non-vegetated).

Determination of pH, moisture and organic matter content of Soil Samples

The pH of the soil samples was determined using the procedures described by Latimer and AOAC International (2016). The organic matter content was determined using the method described by Bernaldo *et al* (2019). The moisture content of the soil was determined using the procedures outlined in Latimer and AOAC International (2016).The percentage moisture was calculated as:

$$\text{Percentage Moisture content} = \frac{\text{Initial (fresh) weight} - \text{Final (oven-dried) weight}}{\text{Initial (fresh) weight}} \times 100 \quad [3]$$

The overall change in the pH, moisture and organic matter content was calculated by subtracting the value of each parameter at the beginning from the value at the end of the study

Statistical Analysis

Data obtained from laboratory analysis of the different experiments were subjected to inferential statistics (correlation) and Analysis of Variance (two-way ANOVA) at 95% confidence interval using Microsoft Excel 2013.

Results

Impact of *Vigna unguiculata* on the Total Petroleum Hydrocarbon (TPH) content of crude oil contaminated soil

Table 1 shows the total petroleum hydrocarbon content and percentage remediation of the soil treated with various levels of crude oil while figure 2 show the chromatograms of the petroleum hydrocarbons. The initial TPH values were high and decreased steadily on day 45 and on day 90 in both the vegetated and non-vegetated soils. The vegetated soil had a higher reduction in TPH and higher percentage remediation than the non-vegetated soil for all the different treatments. The percentage remediation on day 90 was higher than on day 45. The rate of TPH loss was faster in the vegetated soil than in the non-vegetated soil. In the soil with *V. unguiculata*, the highest percentage TPH loss and the fastest rate of TPH loss occurred in soil with 12.5ml crude oil (97.50% and 1.083% loss/day respectively) and 100ml crude oil treated soil had the slowest TPH loss rate (0.997% loss/day) and lowest percentage TPH loss (89.70%). In the non-vegetated soil, the highest percentage TPH loss and fastest rate of TPH loss occurred in the soil with 100ml crude oil (66.60% and 0.740% loss/day respectively) and the soil with 50ml crude oil had the slowest TPH loss (0.503% loss/day) and the lowest percentage TPH loss (45.70%). There was a significant difference in the percentage remediation of the vegetated soil and the non-vegetated soil for the different days ($p<0.05$) and also a significant difference between the percentage remediation at day 45 and at day 90 ($p<0.05$).

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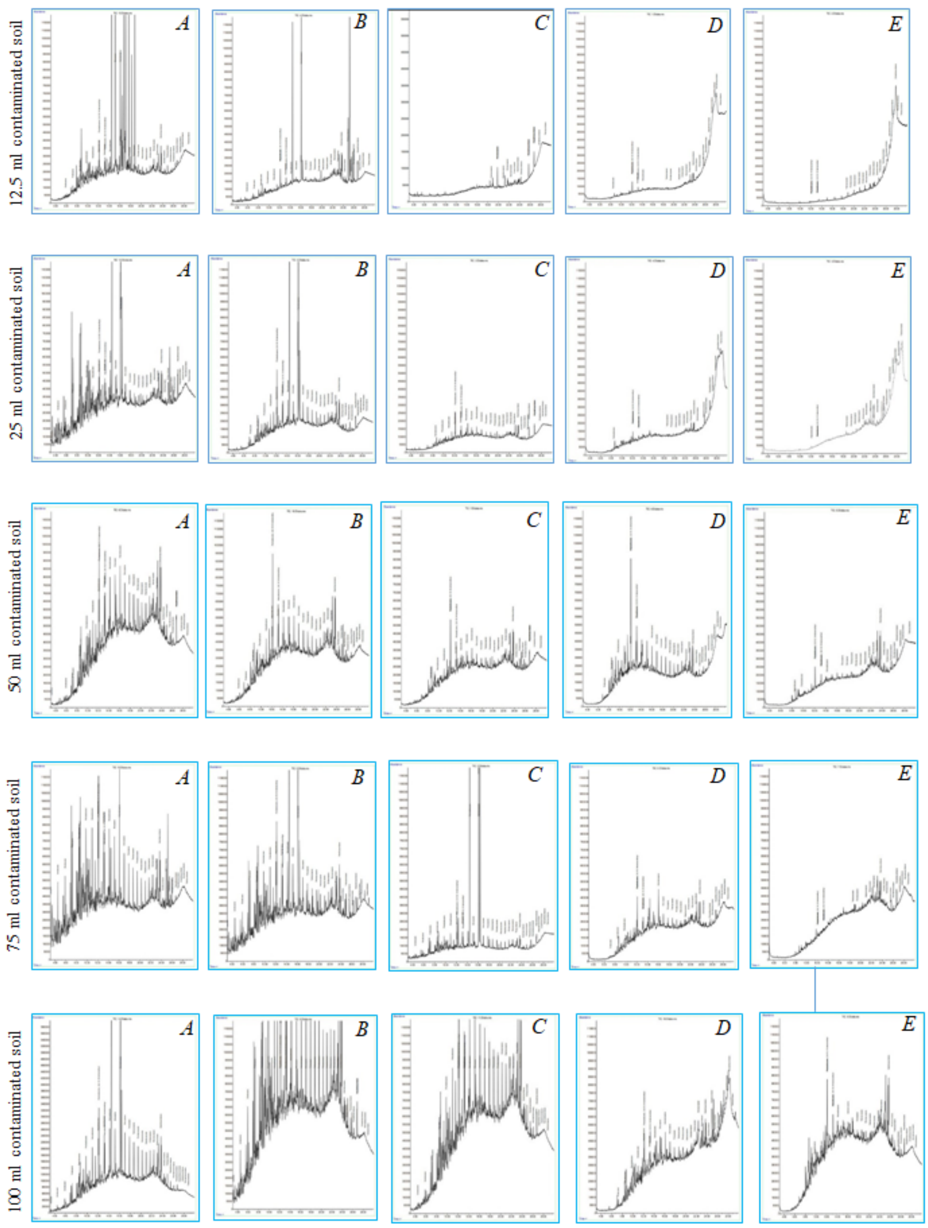

Figure 2. *Gas chromatogram of hydrocarbon compounds present in contaminated soil (A= Initial; B= Control day 45; C= Cowpea day 45; D= Control day 90; E=Cowpea day 90).*

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There was no significant difference in the mean percentage remediation of different treatments (p>0.05). There is a strong positive correlation (r=0.95) between the TPH content of the vegetated soil and the TPH content of the non-vegetated soil.

Table 1. *Concentration of total petroleum hydrocarbon, percentage remediation and rate of percentage TPH loss in soil treated with various concentration of crude oil on different days for the vegetated soil and the non-vegetated soil. (Values in bracket = percentage remediation).*

Concentration of crude oil added	Initial TPH level	Final TPH level in Non- vegetated Soil			Final TPH level in Vegetated Soil		
		Day 45	Day 90	Rate of %TPH loss (%loss/day)	Day 45	Day 90	Rate of %TPH loss (%loss/day)
12.5ml	7856.25	4223.85 (46.24%)	915.03 (59.70%)	0.663	3169.40 (88.35%)	196.60 (97.50%)	1.083
25ml	14034.74	11816.56 (15.80%)	3350.00 (49.80%)	0.553	7039.60 (76.13%)	977.00 (93.00%)	1.033
50ml	20511.90	14053.82 (31.48%)	6901.36 (45.70%)	0.508	11137.50 (66.35%)	1583.00 (92.30%)	1.026
75ml	39497.42	24389.48 (38.25%)	14844.10 (62.50%)	0.728	14802.20 (62.42%)	2399.60 (93.90%)	1.043
100ml	139585.84	72266.52 (48.23%)	44901.95 (66.60%)	0.740	46805.00 (67.83%)	14338.00 (89.70%)	0.997

Contribution of *Vigna unguiculata* to Percentage TPH loss from the soils

Table 2 shows the contribution of *V.unguiculata* to the percentage remediation of crude oil contaminated soil. The percentage remediation contributed by the plant on day 45 was 42.12% in the 12.5ml treatment, 60.33% in the 25ml treatment, 34.87% in the 50ml treatment, 24.17% in the 75ml treatment and 19.60% in the 100ml treatment while on day 90 the percentage remediation contributed by the plant was 37.80% in the 12.5ml treatment, 43.20% in the 25ml treatment, 46.60ml in the 50ml treatment, 31.40% in the 75ml treatment and 23.10% in the 100ml treatment. The growth of *V. unguiculata* contributed highest remediation in the 25ml treated soil on day 45(60.33%) and in the 50ml treated soil on day 90 (46.60%). The contribution of *V. unguiculata* growth on the percentage loss of TPH was the least in 100ml treated soil was 19.60% on day 45 and 23.10% on day 90. The contribution by the plant decreased in the last 45 days in the 12.5ml and 25ml by 4.32% and 17.13% respectively while it increased in the 50ml, 75ml and 100ml by 11.73%, 7.23% and 3.50% respectively. . On the different days (day 45 and day 90), the growth of the plant led to higher rate of TPH loss in the first 45 days for 12.5ml (0.94% loss/day) and 25ml (1.34% loss/day). However in the last 45 days (between day 45 and day 90), the higher rate of TPH loss was noticed in 50ml (1.04% loss/day), 75ml (0.698% loss/day) and 100ml (0.51% loss/day) than in the first 45 days.

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Table 2. *Percentage remediation done by the cowpea plant on various concentration of crude oil contaminated soil. (Values in bracket = rate of percentage TPH loss (%loss/day)*

Days of sample collection	12.5ml	25ml	50ml	75ml	100ml
Day 45 (1st 45 days)	42.12% (0.94%/day)	60.33% (1.34%/day)	34.87% (0.78%/day)	24.17% (0.54%/day)	19.60% (0.44%/day)
Day 90 (2nd 45 days)	37.80% (0.84%/day)	43.20% (0.96%/day)	46.60% (1.04%/day)	31.40% (0.70%/day)	23.10% (0.51%/day)
Overall change between day 45 and day 90	-4.32%	-17.13%	11.73%	7.23%	3.50%

Impact of *Vigna unguiculata* on the pH of crude oil contaminated soil

The pH values of the soil contaminated with different amounts of crude oil are shown in Table 3. The initial pH (Day 0) was generally the lowest.

Table 3: *pH values of soil samples for different treatments on different days for the soil without cowpea and the soil with cowpea.*

Sampling days	12.5ml		25ml		50ml		75ml		100ml	
	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil
Day 0	8.72		8.61		8.68		8.66		8.55	
Day 45	9.01	8.61	9.15	8.86	8.55	8.94	8.86	8.8	8.89	8.91
Day 90	8.52	9.18	8.64	9.19	8.85	9.24	8.84	9.01	8.78	8.95
Overall change after 90 days	-0.20	+0.46	+0.03	+0.58	+0.17	+0.56	+0.18	+0.35	+0.23	+0.4

The pH values of the soil without cowpea showed an increase compared to the initial on day 45 and dropped further on day 90 in the soils treated with 12.5ml, 25ml, 75ml and 100ml but for the 50ml treatment, the pH dropped on day 45 and increased on day 90.

There was an overall increase (positive change) in the pH for all treatment in the soil with *V. unguiculata* and soil without the plant with the exception of the 12.5ml treatment of the soil without cowpea which showed an overall decrease (negative change) in the pH. The pH of the soils with *V. unguiculata* were generally higher than the pH of the soils without the plant on day 90 but on day 45, the pH of the soils with *V. unguiculata* were only higher than that of the soils without the plant only in 50ml and 100ml treated soil. There is an observable significant difference in the pH values for the different days across the different treatments for both the soil without cowpea and the soil with *V. unguiculata* ($p< 0.05$). There is a strong negative correlation ($r= -0.58$) between the pH values of the soil with *V. unguiculata* and the pH values of the soil without cowpea. There is also a weak negative correlation ($r= -0.23$) between the pH of the soil with cowpea and the TPH in the soil with *V. unguiculata* while there is a weak positive correlation ($r= 0.15$) between the pH of soil without *V. unguiculata* and the TPH in the soil without *V. unguiculata*.

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Impact of *Vigna unguiculata* on the moisture content of crude oil contaminated soil

The percentage moisture content of the different treatment is shown in Table 4. The initial percentage moisture content (on day 0) was the lowest in the 50ml treatment (4.83%) and was highest in the 100ml treatment which had the highest value (22.50%). The percentage moisture content of the non-vegetated soil in all treatments except for the 100ml treatment increased on day 45 and dropped sharply on day 90. The moisture content value for 100ml dropped steadily for the non-vegetated soil on different days from 22.50% on day 0 to 11.71% on day 45 and 6.12% on day 90. The percentage moisture content for the soil with cowpea increased on day 45 and dropped slightly on day 90. The moisture content of the soils was more in the vegetated soils on days 45 and 90 in 12.5ml to 75ml crude oil treated soil than in the soil without the plant but in 100ml crude oil treated soils, the moisture content in the soil with the plant on day 45 was lower than that in the soil without the plant. There was an overall reduction of the moisture content after 90 days in the 25ml and 75ml treated soils without *V. unguiculata* and in 100ml treated soils (with and without the plant). In the other treatments, there was a general overall increase of the percentage moisture content of soil on day 90 compared to day 0 (the initial moisture content) overall increase in percentage moisture content was observed in the 12.5ml and 50ml treatments for the soil with cowpea and soil without the plant while an overall decrease in the moisture content was observed in the 100ml treatment. There is an significant difference in the percentage moisture content for the different days across the different treatments for both the contaminated soil without cowpea and that with cowpea ($p < 0.05$) but there is no significant difference in the percentage moisture content for the different levels of contamination ($p > 0.05$). There is a positive correlation ($r = 0.52$) between the percentage moisture content of the soil with *V. unguiculata* and the percentage moisture content of the soil without *V. unguiculata*. There is a negative correlation ($r = -0.26$) between the moisture content of the soil with cowpea and the TPH in the soil with *V. unguiculata* and there is a weak positive correlation ($r = 0.20$) between the moisture content of soil without cowpea and the TPH in the soil without *Vigna unguiculata*.

Table 4. Moisture content (%) in soil samples for different treatments on different days for the soil without cowpea and the soil with cowpea.

	12.5ml		25ml		50ml		75ml		100ml	
Sampling Days	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil
Day 0	5.21		5.16		4.83		6.15		22.50	
Day 45	13.24	15.65	11.48	13.83	10.92	15.04	12.05	18.82	11.71	10.90
Day 90	5.93	15.12	5.05	12.62	5.12	12.19	5.88	11.35	6.12	10.42
Overall change after 90 days	+0.72	+9.91	-0.11	+7.46	+0.29	+7.36	-0.27	+5.20	-16.38	-12.08

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Impact of *Vigna unguiculata* on the organic matter content of crude oil contaminated soil

The percentage organic matter content values of the different treatments are shown in Table 5. The initial (day 0) organic matter content was generally the highest except for 75 ml treated soils that had *V. unguiculata* in which the organic matter content in the soil on day 45 and day 90. In the soil without cowpea, 12.5ml and 100ml treatments there was gradual decrease in the organic matter from the initial to day 45 and then to day 90. The 25ml and 50ml treatments showed a decrease in the organic matter content on day 45 when compared to the initial but increased on day 90 when compared to day 45. There was an increase in the organic matter content in 75ml treated soils in on day 45 and which decreased on day 90. In the soil with cowpea, 12.5ml, 25ml and 50ml treatments showed a gradual decrease in the organic matter content from the initial to day 45 and then day 90. 75ml and 100ml concentrations both showed an increase in the organic matter content from the initial (day 0) to day 45 but reduced on day 90. An overall decrease in the percentage organic matter was observed across all treatments for the soil with cowpea and the soil without cowpea. The 75ml treatment of the soil with cowpea was the exception as an overall increase in percentage organic matter content was observed. There is a no significant difference in the percentage organic matter content for the different days across the different treatments for both the vegetated soil and non-vegetated soil ($p>0.05$) but there is a significant difference in the percentage organic matter content across the different treatments ($p<0.05$). There is a positive correlation ($r= 0.67$) between the percentage organic matter content of the soil with cowpea and the percentage organic matter content of the soil without cowpea. There is strong positive correlation ($r= 0.92$) between the organic matter content in the soil with cowpea and the TPH in the soil with cowpea and there is also a strong positive correlation ($r= 0.81$) between the organic matter content in the soil without cowpea and the TPH in the soil without cowpea.

Table 5. *Percentage Organic matter content of soil samples for different treatments on different days for the soil without cowpea and the soil with cowpea.*

	12.5ml		25ml		50ml		75ml		100ml	
Sampling Days	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil	Non-vegetated Soil	Vegetated Soil
Day 0	6.25		6.80		10.52		7.30		10.60	
Day 45	5.04	5.05	4.56	5.28	3.60	8.04	10.12	7.86	10.42	14.94
Day 90	4.98	2.20	5.14	4.40	6.04	5.54	7.26	7.64	9.87	7.78
Overall change after 90 days	-1.27	-4.05	-1.66	-2.40	-4.48	-4.98	-0.04	+0.34	-0.73	-2.82

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Correlation between soil physical and chemical properties

There is a negative correlation (r= -0.40) between pH of the soil with cowpea and the moisture content of the soil with cowpea and also a strong negative correlation (r= -0.87) with the moisture content of the soil without cowpea.

Table 6. *Results of correlation analysis between the physico-chemical and biological parameters and the total petroleum hydrocarbon of the diffrent days in the soil with cowpea and the soil without cowpea.*

	pH (cowpea)	pH (natural)	Moisture content (%) (cowpea)	Moisture content (%) (natural)	%Organic matter (cowpea)	%Organic matter (natural)	TPH content (cowpea)	TPH content (natural)
pH (cowpea)	1							
pH (natural)	-0.58	1						
Moisture content (%) (cowpea)	-0.40	-0.03	1					
Moisture content (%) (natural)	-0.87	0.50	0.52	1				
Organic matter (%) (cowpea)	-0.29	0.20	-0.33	0.38	1			
Organic matter (%) (natural)	-0.18	0.20	-0.20	0.07	0.67	1		
TPH (cowpea)	-0.23	0.14	-0.26	0.37	0.92	0.73	1	
TPH (natural)	-0.17	0.15	-0.45	0.20	0.89	0.81	0.95	1

There is also a weak negative correlation between the pH of the soil with cowpea and the organic matter content in the soil with cowpea (r = -0.29) and the organic matter content of soil without cowpea (r = -0.18). There is a weak negative correlation (r = -0.03) between the pH of the soil without cowpea and the moisture content of the soil with cowpea while there is a positive correlation (r = 0.50) with the moisture content of the soil without cowpea. There is a weak positive correlation (r = 0.02) between the pH of the soil without cowpea and the organic matter content of the soil with cowpea and also with the organic matter content of the soil without cowpea.There is a weak negative correlation (r = -0.33) between the moisture content of the soil with cowpea and the organic matter content of the soil with cowpea and also a weak negative correlation (r = -0.20) with the organic matter content of the soil without cowpea. There is a weak positive correlation (r=0.38) between the moisture content of the soil without cowpea and the organic matter content in the soil with cowpea and also a weak positive correlation (r=0.07) with the organic matter content in soil without cowpea.

Discussion

Petroleum hydrocarbon contamination of soils is a major threat to environmental health (Aliku *et al* 2021). Clean up of crude oil polluted soil is important as a lot of land that could be used

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for farming activities is being lost due to crude oil pollution. These lost lands contribute to the livelihood of the residents around the area and also contribute to the availability of food for the country. The lands are also a source of revenue to the community and the country. Pollution of the soil can also result in the accumulation of the pollutants in the tissues of plants through uptake and thus can pose health risk to humans who feed on the crops directly or indirectly. Thus, remediation of polluted soil not only makes the lands available for farming but reduces risks associated with bioaccumulation and biomagnification of the pollutant along the food chain

The higher percentage loss of TPH in the soil with cowpea than the soil without cowpea agrees with several studies such as Egharevba *et al* (2017) and Njoku *et al* (2016) which both reported that growth of *Glycine max* which is also a legume enhanced the loss of TPH from crude oil contaminated soil. Fatima *et al* (2018) also reported higher loss of crude oil in the vegetated than non-vegetated soil. Remediation in higher concentration crude oil contaminated soil occurred more in vegetated than non-vegetated soil. TPH loss in soil is mainly connected with the kind, quantity and type of crude oil, organic matter content, soil moisture and season of spill (Kisic *et al*, 2009). Generally, the higher remediation we observed within the first 45 days agrees with Polyak *et al* (2018) who reported that degradation rates were higher in the first three years and remained stagnant in the last years of the experiment. However, the greater level of remediation in the 50ml, 75ml and 100ml in the 2nd 45 day of the study is similar to the report by Milić *et al* (2009) where the highest reduction in TPH occurred from day 49 to day 96. The reduced rate of decontamination of the soils after 45 days which we reported in this study could be that most of the available hydrocarbons had removed leaving only the recalcitrant and non-bioavailable ones as was stated by Polyak *et al* (2018).

However, in this study, we observed that percentage TPH lost from the soil due to the growth of cowpea was higher in the first 45days for the 12.5ml and 25ml treatments but higher in the second 45 days for the 50ml, 75ml and 100ml treatments. The contribution of the plant to the percentage TPH lost in the first 45 days was highest in the 25ml treatment while in the second 45 days, it was greatest in the 50ml treatment. These may imply that for low level of soil contamination, cowpea functions best in reducing TPH in soil in the first 45 days after which the efficiency drops. However, when the soil is moderately contaminated, cowpea will lead to higher loss of TPH in the second 45 days than in the first 45 days. In the soil with higher level of crude oil, plant and microbial activities are usually limited or inhibited (Nwaichi *et al*, 2021). However, such limitation or inhibition can reduce with time and thus the impact of the microbes or plant on the soil improves when such happens thus leading to higher rate of remediation rate. Also, the presence of *V. unuguiculata* helps to shorten this adjustment period by providing nutrients required by the microbes which could have led to greater contribution of the plant to TPH loss and higher rate of TPH loss in the 12.5ml and 25ml treatments in the first 45 days than in the second 45 days. The slower rate of TPH loss in the 12.5 and 25ml treatment in the second 45 days compared to the first 45 days could be attributed to possible switching over of the hydrocarbon utilizers to other carbon sources when the TPH level drastically reduced after 45 days and possible presence of less bioavailable and less biodegradable forms of the hydrocarbons after the first 45 days leaving only the recalcitrant and non-bioavailable ones as was opined by Polyak *et al* (2018). Al-Obaidy *et al* (2018) also observed greater TPH removal rate in 30% crude oil treatment than in 10% crude oil treatment and that growth of the plant increased the TPH removal compared to the soils without cowpea. The higher remediation in the first 45 days in soil without cowpea could be explained by the presence of hydrocarbon utilizing bacteria and fungi already present in the soil, the addition of crude oil provided a new energy source for their metabolic process while in the last 45 days, the

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reduction in TPH content lead to reduced activity or inactivity of these microbes. The higher level of percentage remediation in the soils with lower concentration of crude oil observed in this study agrees with the findings of Uwidia and Uwidia (2021) with similar percentage remediation's as observed in this study because the lower the concentration of crude oil present in the soil the easier it is for the cowpea plant to thrive and carry out remediation processes.

A closer look at the percentage losses on day 45 and day 90 indicate that the percentage losses were more in the first 45 days than in the second 45 days for 12.5ml and 50ml treatments and that more TPH losses occurred in the soils with *V. unguiculata* than in the non-vegetated soils. This trend is similar to the impact of the growth of *V. unguiculata* on TPH where the impact was more in first 45 days and second 45 days for soils contaminated with 12.5ml and 25ml and those contaminated 50ml, 75ml and 100mls crude oil respectively. This can suggest that the impact *V. unguiculata* on TPH is more in the first 45 days for lower level of contamination but more in second 45 days in higher level of contamination. This can further suggest that in soils contaminated with crude oil, *V. unguiculata* can be uprooted after the first 45 days and replanted to achieve higher level of remediation within 90 days.

pH is a very important physical property of the soil which has a great influence on solute concentration and absorption and also microorganisms present in the soil (Tale and Ingole, 2015). Soil pH plays an important role in controlling various physico-chemical reactions in the soil (Njoku *et al*, 2016). High pH (alkaline) reduces uptake of micronutrients such as iron, calcium, magnesium by the plant while low pH increases the solubility of micronutrients which are toxic to plants in excess but decreases the availability of macronutrients therefore the increase in pH observed in this study may have led to increase in the absorption of macronutrients (Tale and Ingole, 2015; Uwidia and Uwidia, 2021). The negative correlation between pH and TPH we observed in this study may be due to reports that increase in pH decreases the solubility of hydrocarbon contaminants in pot experiments (Nwaichi *et al*, 2015). Increase in the soil pH of crude oil contaminated soil due to growth has been reported in various previous studies (Efe and Elenwo, 2014; Njoku *et al*, 2016). Increases in pH from acidic to alkaline have been reported to increase the degradation of crude oil in response to organic waste addition (in this study presence of cowpea) (Nwaugo *et al*, 2015). The initial pH of the soil in strongly alkaline agreeing with the report of Masakorala *et al* (2014) that in soil contaminated with petroleum hydrocarbon the pH ranged from slightly alkaline to strongly alkaline. The overall change in the pH of the soil agrees with Njoku *et al* (2016) who reported an increase in soil pH due to the growth of *Glycine max* in soil contaminated with crude oil. The increase in pH has been observed in similar studies as higher pH range (6-9) provides better conditions for the mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH conditions to neutrality (Obasi *et al*, 2013; Osazee *et al*, 2014; Yunaiati, 2018). The increase in pH of the soil during the experiment could be a sign that the soil was becoming more favorable for biodegradation to occur (Njoku, 2008). Chemical reactions between petroleum hydrocarbons and soil elements maybe the reason for the variation in pH (Masakorala *et al*, 2014) noticed in 12.5ml treatment in soil without cowpea.

Moisture affects the overall rate of enzyme production by microorganisms (Maphuhla *et al*, 2021) as most microorganisms do best in a warm and moist environment and when the microorganism does well the rate of enzyme production also increases. The negative correlation between TPH and moisture content of soil with cowpea suggests that increasing moisture content leads to reduction of the TPH indicating that remediation is enhanced by the presence of cowpea. The increase in the overall percentage moisture content in the soil with cowpea and in 12.5ml and 50ml treatments in the soil without cowpea is similar to what was observed by Osazee *et al* (2014). The severe low moisture content in the 100ml treatment in contaminated

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soil with cowpea and contaminated soil without cowpea and low moisture content of the 25ml and 75ml treatments in soil without cowpea could be as a result of low permeability, low porosity and high resistance to water penetration as a result of adherence of water droplets to the hydrophobic layer of the crude oil polluted soil which prevented the penetration of water into the soil and wetting of the soil aggregates (Njoku, 2008). The high moisture content in soil with cowpea agrees with Njoku (2008) who observed that the presence of *Glycine max* in crude oil polluted soil increased the percentage moisture content of the soil, possibly because the growth of the cowpea plant increases the number of pores in the soil and in the process improves permeability of water into the soil. The increase in moisture content can be an indicator of removal of crude oil during the period of the study, this is due to the nonpolar nature of crude oil which causes it to absorb the moisture in the soil reducing the moisture content (Devatha *et al*, 2019), so an increase in moisture content means that there is less crude oil present to create an hydrophobic coating of oil around the soil thereby reducing the moisture in the soil.

Organic matter maintains soil fertility and prevents soil degradation, erosion and desertification (Yunaiati, 2018; Maphuhla *et al*, 2021). In this study, we observed that the percentage organic matter increased with increase in concentration of crude oil which agrees with the observations by Obasi *et al* (2013) and Osazee *et al* (2014). This could be because crude is majorly made up of hydrocarbons which have organic carbon as the major component and that organic matter is derived mainly from organic carbon. Thus, the higher the organic carbon component due to high level of crude oil, the more the organic matter content. The increase in the percentage organic matter content could be due to the mineralization of the crude oil by microorganisms (Obasi *et al*, 2013); making soils with higher concentration of crude oil have a higher percentage organic matter content. The decrease in the percentage organic matter in some treatments in this study agrees with Njoku *et al* (2012 and 2016) who reported reduction of organic matter content of contaminated soil due to plant growth. The overall increase in percentage organic matter in the 75ml treatment of the soil with cowpea could be as a result of fallen dead leaves from the cowpea plant which was observed to be more than the amount of fallen leaves observed in other treatments which is a source of organic matter (Tale and Ingole, 2015). The lower organic matter content in soil with cowpea than in soil without cowpea could be due to increased rhizosphere activities which might have enhanced the mineralization of organic matter (Stephen *et al*, 2013). Organic matter commonly increases the water content and infiltration rate of nutrients (Tale and Ingole, 2015) but in this study while there is a positive correlation between percentage organic matter and moisture content in the soil without cowpea while there is a negative correlation between the organic matter and moisture content in the soil with cowpea. The relationship between percentage organic matter and total petroleum hydrocarbon could be the reason for the observation.

The significant difference in the percentage remediation of the in the soil with cowpea and soil without cowpea for the different days ($p<0.05$) suggests that the presence of cowpea in the soil is an important factor that affects the remediation of crude oil as its ability to fix its own nitrogen and supply nutrients to the soil makes it desirable for remediation. This quality enables its survival in extreme environment; its survival improves soil quality by creating pores, improving aeration and permeability of nutrients and water in the soil. The significant difference between the percentage remediation at day 45 and at day 90 ($p<0.05$) shows that time is another important factor that affects the remediation process as the percentage remediation results obtained on different days showed varying degrees of remediation irrespective of the crude oil concentration. The absence interaction between the percentage remediation on different days and the percentage remediation of the different experiments (soil with cowpea and soil without cowpea), suggests that time and the presence or absence of cowpea are two factors acting alone to

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remediate the soil. The non-significant difference in the mean percentage remediation of different treatments ($p>0.05$), could imply that the level of crude oil in the soil had little to no effect on the level of remediation.

The drop in the concentration of crude oil in the soil shows that given more time the concentration level can drop further. Leewis *et al* (2013) suggested that natural attenuation (no amendments), land farming or phytoremediation are all capable of achieving cleanup limits for hydrocarbon contaminated soils with initial concentration of up to 8300mg/kg within -15 years. This means that if cowpea plant can drop the concentration of crude oil in the soil by an average of 39671.91mg/kg in just 90 days given more time or in combination with other methods of remediation we can achieve higher levels of remediation within a short period of time. That is to say that without the presence of cowpea as long as the soil is left alone remediation will still occur and that period of the remediation can be shortened by the presence of cowpea and its associated systems. This fact is corroborated with the rate of TPH loss we recorded in this study. For instance, if one goes by the 0.663% loss/day in 3000g soil contaminated with 12.5ml crude oil without *V. unguiclata*, it may take about 157.98 days for such soil to have 100% clean up through natural attenuation whereas with the growth of the plant it will take about 92.34 days to have a 100% clean of such (1.083% loss per day).

Conclusions

From the study we can slowly start to disregard time as a limiting factor to the application of phytoremediation as the study has shown results that with the presence of cowpea alone we can achieve high levels of remediation especially in lower concentration of crude oil but the effect of crude oil in the soils with higher concentration cannot be disregarded as we got some positive results and with the level of remediation observed in this study, the time taken will be greatly reduced even with crude oil concentrations greater than 8300mg/kg. Removal of crude oil from the soil was higher in the first 45 days than in the last 45 days across all the treatments. Soils with lower concentration of crude oil showed more remediation than those with higher concentration of crude oil. Most of the shorter chain compounds were totally lost and the number of compounds in which a total loss was observed decreased as the concentration of crude oil increased. The work by the plant decreased during the last 45 days of the study for the 12.5ml and 25ml treatments while in the 50ml, 75ml and 100ml treatments it increased slightly. The use of cowpea as phytoremediator will not be a problem as cowpea seeds are readily available for cultivation and cowpea plants are resilient and tolerant to harsh conditions.

Reccomendation

Although some levels of remediation were achieved in this study with the soil with some of the treatments having over 90% remediation, the amount of TPH lost did not meet the maximum acceptable limit recommended. Planting cowpea together with a plant with shallow roots known to be able to remediate crude oil polluted soil such as maize will improve the remediation of the soil without adversely affecting the amount nutrients in the soil while increasing the economic value of remediation. Also replanting of cowpea can be done after 45 days to improve the cleanup activity of the cowpea plant if acceptable limits are yet to be achieved as the results from this study show that remediation began to stagnate after the first 45 days. The cowpea plant removed from the field should be disposed of as the hydrocarbons accumulate in the tissues of the plant. The cowpea plant can be amended with organic manure such as cow dung and poultry droppings or augmentation with microorganisms known to aid in the breakdown of hydrocarbons in the soil such as *Acinetobacter, Pseudomonas, Staphylococcus, Streptobacillus, Enterobacter* e.t.c. This will increase remediation of crude oil and enhance the conditions in the soil and prevent the cowpea plant from undergoing a lot of stress.

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