Nigerian Research Journal of Engineering and Environmental Sciences 6(1) 2021 pp. 274-285

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

Original Research Article

Remediation of Hydrocarbon Contaminated Soil Using Selected Tropical Plants

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ARTICLE INFORMATION

Article history:
Received 18 Jan, 2021
Revised 18 Apr, 2021
Accepted 02 May, 2021
Available online 30 Jun, 2021

Keywords:
Remediation
Degradation
Plant
Contamination
Phytoremediation

ABSTRACT

This study evaluated the phytoremediation potentials of selected tropical plants, Glycine max, Panicum maximum, Sorghum bicolor, Tephrosia bracteolata and Vigna unguiculata, on 0%, 3% and 5% hydrocarbon concentration contamination. Top soil was collected from department of crop science garden, University of Benin at depth ranging from 15 cm to 20 cm using hand auger. Soil samples (5 kg) were placed in perforated buckets (cells) in different concentrations of 0% (control), 3% (equivalent of 2530 mg/kg) and 5% (equivalent of 4562 mg/kg). This was replicated into total of 87 cells. Pre-planting of selected plants was done and transplanted into the cells two weeks after planting with 2 kg cow dung as augment. The study lasted for duration of 16 weeks; height of plants, number of leaves, roots length and leaves area were measured every fortnight. Soil samples were taken to laboratory every two weeks for total petroleum hydrocarbon (TPH) degradation monitoring. The result revealed that the plant grew and increased in height. The number of leaves, root length and leaves area all increased with about 70%. The degradation result also shows that the plants reduced the residual TPH with an average of 77% in 3% concentration and 81% in 5% concentration. The order of the plants remediation is in the range of Panicum maximum > Glycine maximum > Tephrosia bracteolata > Vigna unguiculata > Sorghum bicolor. The plants are therefore recommended for remediation practice in contaminated sites within the Niger Delta region of Nigeria with cow dung as augment.

1. INTRODUCTION

The Nigerian economy has been sustained for over five decades arising from the proceeds of crude oil exploration, exploitation, processing and sale (Sam and Zabbey, 2018). It accounts for more than 90% of foreign exchange earning of the nation (Tukur and Hajj, 2017). The Organization of Petroleum Exporting

Countries (OPEC) recently ranked Nigeria as the sixth crude oil exporting country in the world and first in Africa, with the Niger Delta accounting for over 37.4 billion barrels of crude oil reserve in addition to hosting one of the most bio-diverse ecosystems found globally (Atubi, 2015; Albert *et al.,* 2018). Interestingly, the Niger Delta area is host to the third largest mangrove forest in the world with extensive fresh and saltwater swamps and a rich variety of plant and animal species (Kuenzer *et al.,* 2014; Anejionu *et al.,* 2015). Despite being the financial driver of the Nigeria economy, the Niger Delta region is one of the most environmentally devastated regions in the world which is fallout of crude oil exploitation coupled with weak clean-up regulations and the activities of illegal refiners (Dominic, 2016; Gundlach, 2018).

Since crude oil was discovered in Niger Delta region of Nigeria, more than 13million tonnes have been reportedly spilled into the environment causing severe harm and unquantifiable contamination of coastal regions and land (Lopes *et al.,* 2009). The release of petroleum products into land (onshore) and marine (offshore) environments is damaging to the ecosystem and toxic to marine life (Eneh, 2011; Ojewumi *et al.,* 2018). Hydrocarbon contamination can result from divergent sources which maybe anthropogenic or occur naturally (Aislabie *et al.,* 2004; Brakstad *et al.,* 2017; Marinescu *et al.,* 2011Mahjoubi *et al.,* 2018). Accidental leakages, improper handling from exploitation sites, pipe rupture and damages in storage facilities contaminates the soil and water in the immediate environment (Ojewumi *et al.,* 2017). Also, untreated wastes generated during illegal mining are dumped by the miners into near-by streams causing considerable damage to aquatic life and surrounding vegetation (Asimiea and Omokhua, 2013).

Although there have been various attempts made by the government and exploration companies to engage in clean up exercises, the result is yet to register significant success (Dominic, 2016). Most of the techniques utilized were inappropriate as they impact negatively on the environment (Giadom and Tse, 2015). Zabbey *et al*., (2017) reported that failures of previous remediation attempts were subjected to erroneous operational conclusions coupled with the adoption of unsuitable and ineffective approaches that ought to be site specific. The inability of the government to effectively remediate contaminated sites within the Niger Delta region has made the host communities very hostile hence resulting in several conflicts (Koos and Pierskalla, 2016). About 16 years ago, 7 workers and military men were murdered in Delta state which was also accompanied by kidnapping and hostage taking. The prompted the federal government of Nigeria to flag off the clean-up ogoniland project in Port Harcourt on the shores of the creeks (Sam, 2016; UNEP, 2016; Zabbey *et al,* 2017). Though previous efforts have suffered setbacks, however, the present one is optimistically positive, and the success of any viable efforts can stand out as a benchmark in Nigeria. Therefore, clean-up technique liable to receive public acceptance against any unpredictable communal challenges; cheap; easy to be employed; environmentally friendly; and potentially efficient to render the agricultural soil productive and restore livelihood within the affected regions is highly recommended.

Thermal processes, chemical and physical clean-up procedures have been applied in contaminated sites; these processes have adverse effects on the environment and are also prohibitively expensive. In recent clean up exercise in developing countries, biological procedures such as phytoremediation have proved very effective (Frick *et al.,* 2009; Aprill and Sims 2009). Phytoremediation is the cultivation of plants and/or associated microbes in order to curtail or completely mineralize harmful substances in soil or water (Schwabs and Banks, 2009). Its effectiveness has been reported in abating contaminants like heavy metal, organic pollutants and radionuclides. According to Njoku *et al.,* (2009), plants to be cultivated for phytoremediation should be suitable for the climate and also meet the contaminated site conditions. The plants must be able to withstand induced stress in the contaminated site. Phytoremediation has the advantage of not distorting the original ecosystem of the contaminated site during clean-up and it is less expensive coupled with the less technical-know-how needed to initiate the procedure (Frick *et al.,* 2009).

The aim of this work is to evaluate the remediation potentials of some selected tropical plants in decontaminating crude oil polluted soil with a view to recommending some for future clean-up exercise. The tropical plants selected for the study based on root morphology and climatic adaptation includes: *Glycine max, Panicum maximum, Sorghum bicolor, Tephrosia Bracteolata and Vigna unguiculata.*

2. MATERIALS AND METHODS

This study was carried out in the screen house located in the Department of Crop Science, Faculty of Agricultural Sciences, University of Benin, Edo state, Nigeria.

2.1. Pre-planting of Selected Plants

Rhizomes for grasses and seed for legumes of the selected plants were obtained from the Department of Crop Science farm house and planted in sandy loamy soil for an incubation period of two weeks and watered daily. The incubated plants will then be transplanted into the various experimental cells for the treatment of the contaminated soil.

2.2. Soil Sample Collection

Topsoil was collected from Department of Crop Science garden at 25 cm depth using hand trowel; the samples were placed in cellophane bags and transported to the laboratory for air drying, pulverization, sieving and characterization. The physico-chemical analyses of all the test samples were carried out using solvent extraction method which is in accordance with standard test method (ASTMD) (Okonofua *et al.,* 2020).

2.3. Collection of Crude Oil Samples and Organic Fertilizer

Crude oil samples were collected from a flow station in Ologbo, Edo State and were analyzed to determine its physical and chemical properties. Organic fertilizer (cow dung) was collected from the abattoir along Upper Mission Road in Benin City and taken to the laboratory for air drying, pulverization and characterization.

2.4. Soil Sample Treatment

Crude oil contaminated soil samples were simulated in the laboratory with three different rates of crude oil application: 0, 3 and 5% w/w. The 0% was the control while the 3% and 5% were contamination equivalents of 2530 mg/kg and 4562 mg/kg crude oil respectively. This was replicated three times and that gave a total of 87 cells (perforated buckets). Sieved soil (10 kg), were homogenized and placed in each of the cells after thoroughly mixed. After 14 days incubation period, the seedlings were transplanted into the cells at 4 seedlings per pot but were later thinned to two stands 2 weeks after sowing. Organic fertilizer (5% cow dung) was applied one week after transplanting; the cells were placed in a screen house with ambient temperature of about 25 ^{o}C to 30 ^{o}C. The cells were watered twice weekly to provide sufficient oxygen and suitable environment for bacteria growth. During the 16 weeks' period of the experiment, data collected fortnightly included: growth parameters (plant height, leaf area and numbers of leaves) and collection of soil samples for TPH analysis.

2.5. Total Petroleum Hydrocarbon

TPH in the contaminated soil was evaluated according to USEPA 3550 (Okonofua *et al.,* 2020). TPH was first extracted from 5 g petroleum contaminated soil which has been sieved and transferred to a 40 mL glass centrifuge tube, chloroform (25 mL) was added, and the tube closed. The tubes were ultrasonically extracted for 1hour duration while some cold water was added to keep the temperature below 40^{o}C. After extraction, the samples were centrifuged for 10minutes at 3000 rpm and the extracts were transferred into an Erlenmeyer flask, dried to a constant weight, and wetted at 65^{o}C to evaporate volatile chloroform. After evaporation of the solvent, the amount of the residual TPHs was gravimetrically determined. The percentage removal was calculated using Equation 1.

$$Q = \frac{M_2 - M_1}{M_2} x100 \tag{1}$$

Where M_2 is the concentration (mg/kg) of TPHs before remediation, M_1 is the concentration (mg/kg) after remediation while Q is the percentage TPH removal.

Statistical analysis was carried out using Excel XP and GraphPad Prism 7. Sampling and chemical analyses were carried out in triplicates in order to decrease the experimental errors and to increase the reproducibility. The confidence of data generated during the investigation was analysed by standard statistical methods to determine the mean and standard error. One-way analysis of variance (ANOVA) was also carried out at 0.05 level of significance to determine significant variation in the contaminant concentrations before and after treatment. The following hypothesis was developed for the one-way ANOVA analysis.

Null $H_0 = \mu_1 = \mu_2$: There is no significant difference in concentrations before and after treatments.

Alternate $H_1 = \mu_1 \neq \mu_2$: There is significant difference in concentration before and after treatments.

3. RESULTS AND DISCUSSION

The result obtained from the characterization of the soil samples and organic fertilizer used is presented in Table 1. The physical and chemical properties of the crude oil used for the study and the chromatogram of the crude oil are shown in Table 2 and Figure 1 respectively. Figure 1 reveals that the crude content contains high Pristane, phytane and more of nC_{10} to nC_{19}. Although higher and some other lower carbon chains were present, the main toxic carbon content highlighted above were the main focus during treatment.

Table 1: Physical and chemical properties of the soil and organic fertilizer used for the study

Properties	Soil values	Organic fertilizer values
pH (1:1 salt –water)	5.21	
Organic carbon (g/kg)	13.64	462.6
Total nitrogen (mg/kg)	6.71	62.7
Available phosphorous (mg/kg)	5.6	29
Exchangeable cations (mg/kg)		
Ca^{2+}	536	35.3
Mg^{2+}	788	11.8
K^{-}	2,016	9.2
Gradation (%)		
Sand	81.04	
Silt	10.56	
Clay	8.43	
Textural classification	Sandy loamy	

The soil being predominantly sand (81.04%) with organic content was texturally classified as sandy loamy soil. The organic fertilizer used for the study was found to be very rich in organic carbon and total Nitrogen content; this makes it suitable as substrate for microbes in the remediation procedure (Okonofua *et al.,* 2020). The physical and chemical properties of the crude oil presented in Table 2 are typical of Bonny light majorly found in the Niger Delta.

Table 2: Physical and chemical properties of the crude oil used for the study

Parameter	Value
Water content (% Vol.)	0.47
Specific gravity @15/15^{0}C	0.8862
Dry specific weight @15/15^{0}C	0.8916
*API @ 15/15^{0}C	26.4
Kinetic Viscosity @ 15/15^{0}C	12.18
Appearance	Dark brown liquid

*API is American Petroleum Institute

Figure 1 reveals that the crude content contains high Pristane, phytane and more of nC_{10} to nC_{19}. Although higher and some other lower carbon chains were present, the main toxic carbon content highlighted above were the main focus during treatment.

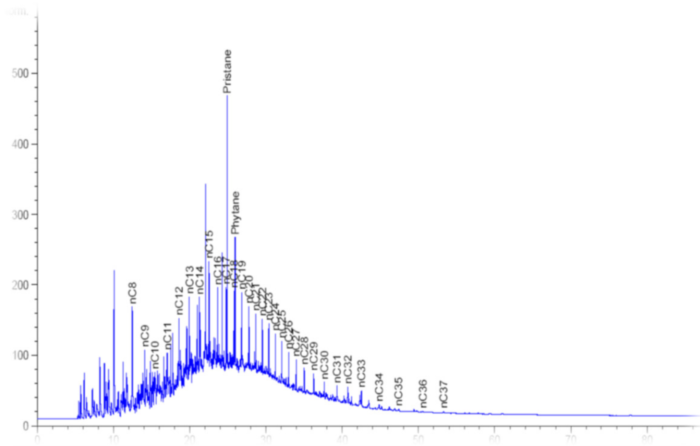

Figure 1: Chromatogram of crude oil used for the study

The treatment plants utilized for the study (*Glycine max, Panicum maximum, Sorghum bicolor, Tephrosia bracteolata and Vigna unguiculata),* were first cultivated and transplanted into the cells after two weeks of planting. The results of the survival rate (%) of the plants after transplanting for duration of 16 weeks in different hydrocarbon concentrations are presented in Table 3. *Panicum maximum* had 100% survival rate in all the hydrocarbon concentrations while *Glycine maximum* had 100% survival rate in 0% concentration but dropped to between 72 – 98% in 3% and 5% concentrations respectively. *Sorghum bicolor, Tephrosia bracteolata* and *Vigna unguiculata* had survival rate similar to *Glycine maximum* which had 100% survival rate in 0% hydrocarbon concentration but lower survival rate ranging from 55 – 96% as hydrocarbon concentration increased from 3% to 5%. The results agree with the findings of Akinpelumi and Isichei, 2015. The responses obtained from the investigation of cultivation of tropical plants in crude oil contaminated sites shows the potency of *Panicum maximum* and *Glycine maximum*. These plants also show resistance to severe hydrocarbon contamination and degraded the contaminants better than other plants (Okonofua *et al.,* 2020).

Table 3: Survival rate (%) of treatment plants in 16 weeks in different concentrations

Plants	Conc. (%)	Week 4	Week 8	Week 12	Week 16
Glycine maximum	0	100	100	100	100
	3	100	98	86	86
	5	92	84	84	72
Panicum maximum	0	100	100	100	100
	3	100	100	100	100
	5	100	100	100	100
Sorghum bicolor	0	100	100	100	100
	3	75	62	53	53
	5	86	81	76	61
Tephrosia bracteolata	0	100	100	100	100
	3	92	87	62	55
	5	96	81	76	63
Vigna unguiculata	0	100	100	100	100
	3	92	88	62	57
	5	81	81	66	56

According to Sam and Zabbey (2018), the root morphology of such plants has the ability to expand the pores in soil which makes it receptive to more oxygen and air and the root nodes can also fix atmospheric nitrogen which serves as source of nutrient to the plant. These factors play dominant role in hydrocarbon degradation.

The shoot heights of the selected plants used for the study were measured using meter rule and the results of the computed mean and the standard error are shown in Figures 2 – 5. In all the plants used for the treatment, there was increase in height of the shoots with less hydrocarbon concentration and decrease in shoot height as the concentration of hydrocarbon increased. Increase in shoot height was recorded in all the tropical plants as the study period increased with higher percentage shoot increase of about 83% recorded in *Vigna unguiculata* in 5% 0% concentration and least increase of 49% recorded in Sorghum Bicolor in 3% hydrocarbon concentration. Njoku *et al.,* (2009) reported that higher concentrations of crude oil and trace elements in soil affect shoot growth as the toxic constituents of hydrocarbon attack stem cells which are mainly responsible for shoot development. The stem cells of these tropical plants transmit soil nutrients to the leaves and other part of the plants but are weakened when these nutrients are contaminated by toxic substances. Plants with less resistance to higher concentration of hydrocarbon are completely destroyed within few weeks of the contamination (Schwabs and Banks, 2009).

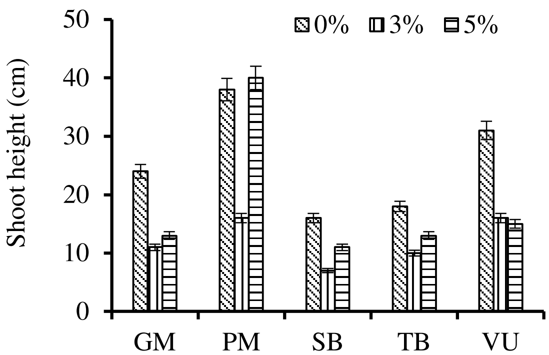

Figure 2: Shoot Height at different hydrocarbon content at 4 weeks

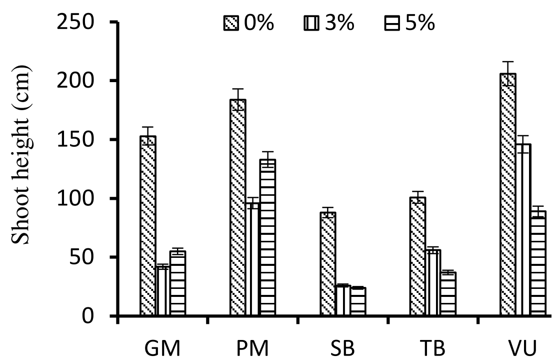

Figure 3: Shoot Height at different hydrocarbon content at 8 weeks

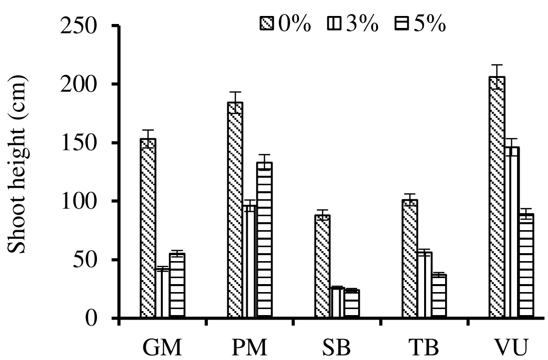

Figure 4: Shoot Height at different hydrocarbon content at 12 weeks

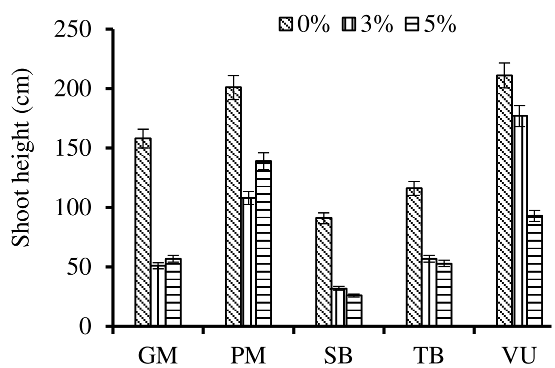

Figure 5: Shoot Height at different hydrocarbon content at 16 weeks

GM = *Glycine max,* PM = *Panicum maximum,* SB = *Sorghum bicolor,* TB = *Tephrosia bracteolate,* VU = *Vigna unguiculata*

The number of leaves on each plant used for the treatment were counted and recorded fortnightly; the results are presented in Figures 6 – 9. The number of leaves on each of the treatment plants increased progressively in the 16 weeks of the study with *Vigna unguiculata* being the exception. After 10 weeks of treatment, *Vigna unguiculata* shed off some leaves in hydrocarbon concentration 3% and 5%. This indicates least resistance to hydrocarbon contamination which is common with some tropical plants (Njoku *et. al.,* 2009). A similar result was obtained by Okonofua *et. al.* (2019), Akinpelumi and Isichei, (2015), as well as Schwabs and Banks, (2009) who all reported that tropical plants tend to shed leaves in the phase of continuous contamination which is due to high accumulation of hydrocarbon contaminants and other toxic substances. The result was the same pattern with the leaves area of the treatment plants presented in Figures 10 - 13. The area of the leaves increased as the duration of the study increased with *Vigna unguiculata* as the exception. The shedding of the leaves due to high concentration of hydrocarbon affected the leaves area as similar results were reported by Frick *et. al.* (2019) in their study. Although this did not affect the remediation potentials of the plant, it reduced the process due to near absence of volatilization. Zabbey (2018) reported that *Vigna unguiculata* stores up contaminants in the leaves and when the concentration of the contaminants increase, the rate of photosynthesis decreases sharply, this occurrence diminishes the full development of the leaves. The other plants store up contaminants in the roots, stems and branches hence leaf area showed better results.

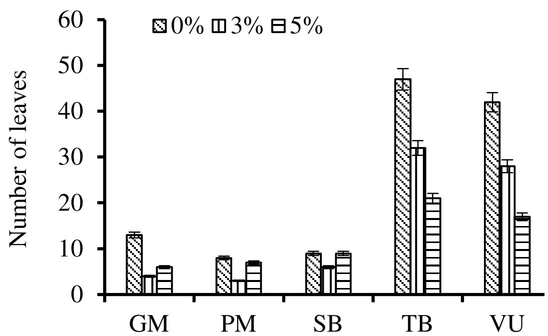

Figure 6: Number of leaves per plant in different hydrocarbon concentration at 4 weeks

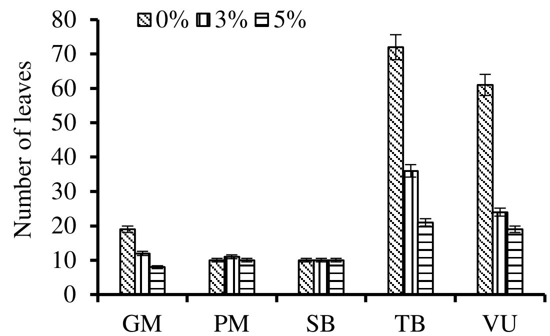

Figure 7: Number of leaves per plant in different hydrocarbon concentration at 8 weeks

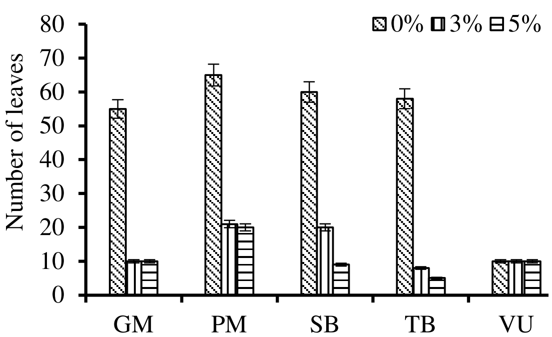

Figure 8: Number of leaves per plant in different hydrocarbon concentration at 12 weeks

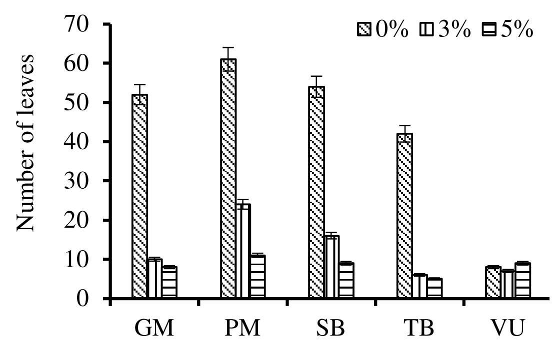

Figure 9: Number of leaves per plant in different hydrocarbon concentration at 16 weeks

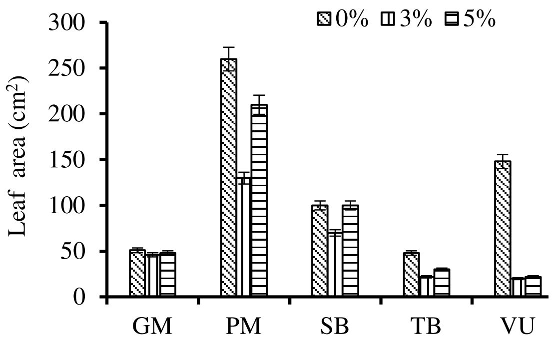

Figure 10: Leaf area per plant in different hydrocarbon concentration at 4 weeks

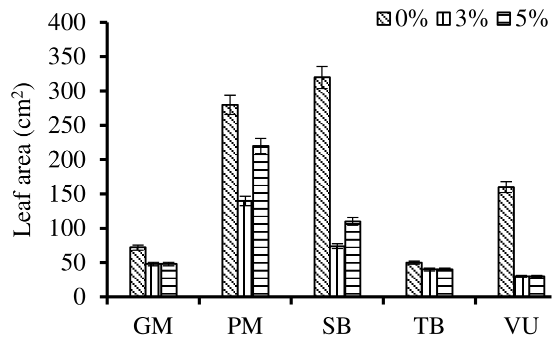

Figure 11: Leaf area per plant in different hydrocarbon concentration at 8 weeks

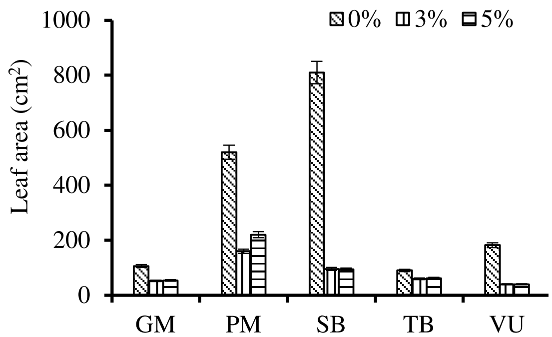

Figure 12: Leaf area per plant in different hydrocarbon concentration at 12 weeks

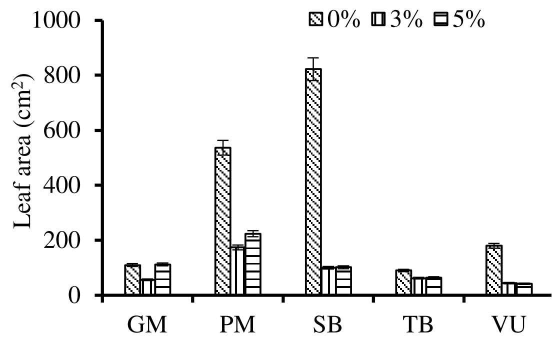

Figure 13: Leaf area per plant in different hydrocarbon concentration at 16 weeks

The root length of the treatment plants was measured at the end of the 16 weeks duration of the study; the result is shown in Figure 14. *Sorghum bicolor* had the longest root length of about 48cm while the other plants had roots length ranging from 2 – 5cm. All the plants had longer roots in 0% crude oil concentration (control) as against higher concentration of 3% and 5% respectively. Frick *et. al.,* (2019), reported that the roots of plants for phytoremediation treatment are vital quality required for its application. Plants with root nodes are more suitable for contamination remediation than others without nodes; this is because of its ability to fix atmospheric nitrogen which serves as additional substrates for microbes within the soil (Schawbs and Banks, 2009). The root of such plants tends to penetrate and elongates more in uncontaminated soil than contaminate soils as the presence of toxic substances in soil retards root growth and development. *Sorghum bicolor's* roots have nodes which enable it to fix atmospheric nitrogen and penetrate soil further than other plants used for the study (Frick *et al.,* 2019). Toxic substances such as heavy metals and hydrocarbon constituents in soil also affect the rate of nutrient absorption by the roots and this impacts negatively on plant growth.

After treatment by tropical plants, a drastic reduction in hydrocarbon content in both concentrations (3% and 5%) was observed during the 16 weeks of study (Figures 15 and 16). The percentage removal ranged from 76.3% to 81.7 for 3% concentration and 81.7% to 96.9% for 5% concentration respectively (Figures 15 and 16). Enhanced degradation rate was observed in the contaminated soil when compared with the control. The growth of these plants generally reduced the acidity of the hydrocarbon contaminated soil. Crude oil among other things causes low permeability and low infiltration of water into the soil. This was observed in 5% concentration as the condition lead to the accumulation of water on the soil surface. This can lead to difficulty in the roots to absorb water and nutrients which in the water as the roots usually grow deeper into the subsurface layer (Andrade *et al.,* 2004). The inhibition of root growth can lead to low penetration of water and higher accumulation of water on the soil surface. Also, the release of organic carbon to the soil due to degradation of crude oil can possibly lead to carbon accumulation (Baker, 2015). Figures 15 and 16 show the degradation of hydrocarbon concentration (3% and 5%) using tropical plants in 16weeks.

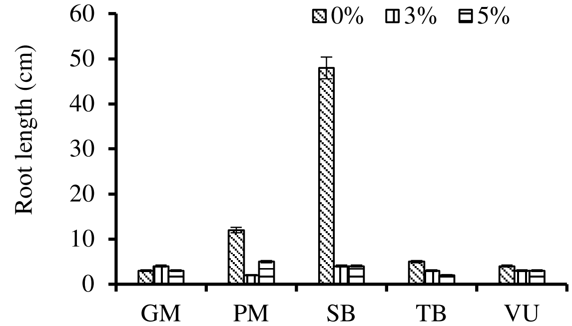

Figure 14: Root length of plants in different concentration at 16 weeks

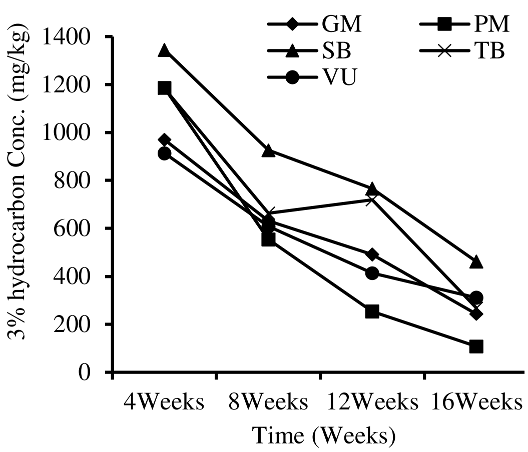

Figure 15: 3% Hydrocarbon degradation with treatment in 16 weeks

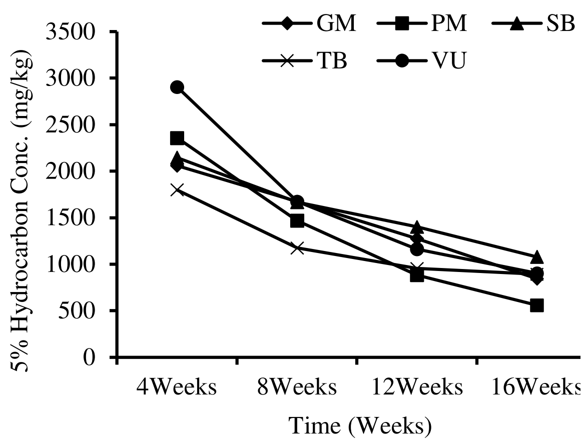

Figure 16: 5% Hydrocarbon degradation with treatment in 16 weeks

Table 4 and 5 shows the line equations of each of the plots and the respective percentage degradation of each tropical plant used for the study. This was to establish the relationship between the contaminants treatment and duration of treatment. The R^2 values were also determined in order to select the most suitable plant for remediation on a commercial scale.

Table 4: Line equation and R^2 values for 5% hydrocarbon concentration

Plant	Equation of Line	R^2	% Degradation
Glycine maximum	Y = 4548.4 – 822x	0.7946	81.5
Panicum maximum	Y = 4809.2 – 947.4x	0.8731	81.7
Sorghum bicolor	Y = 4483.6 – 770.6x	0.7662	76.3
Tephrosia bracteolata	Y= 4330.9 – 817.9x	0.7025	80.4
Vigna unguiculata	Y = 4958.1 – 905.7x	0.7014	80.18

Table 5: Line equation and R^2 Values for 3% hydrocarbon concentration

Plant	Equation of Line	R^2	% Degradation
Glycine maximum	Y = 2488.9 – 505.3x	0.7722	90.4
Panicum maximum	Y = 2515.2 – 541.6x	0.8761	96.9
Sorghum bicolor	Y = 2620.2 – 471.4x	0.8557	81.7
Tephrosia bracteolata	Y = 2569.2 – 498.9x	0.8090	89
Vigna unguiculata	Y = 2436.9 – 493.9x	0.7367	87.7

Where *Y* is contaminant concentration and *x* is duration.

The R^2 values for 5% concentration ranges from 0.7025 to 0.9014 while that of 3% concentration ranges from 0.7722 to 0.8761. The order of plant remediation is in the range of *Panicum maximum* (0.8731, 0.8761) > *Glycine maximum* (0.7946, 0.7722) > *Tephrosia bracteolata* (0.7025, 0.8090) > *Vigna unguiculata* (0.7014, 0.7367) > *Sorghum bicolor* (0.7662, 0.8557). The result of ANOVA analysis for the respective concentration and treatments are presented in Tables 6 and 7.

Table 6: ANOVA of TPH response to phytoremediation treatment (3%)

Source of variation	Sum of squares	df	MSS	Variance ratio	F Pr. < 0.05
Treatment	82	964.15	11.78		
Error	4	7.62	1.91	6.168	3.89
Total	86				

Table 7: ANOVA of TPH response to phytoremediation treatment (5%)

Source of variation	Sum of squares	df	MSS	Variance ratio	F Pr. < 0.05
Treatment	82	1062.72	12.96		
Error	4	13.03	3.26	3.98	3.89
Total	86				

In the ANOVA analysis, the whole product analysis method which gives the yielding concentration of TPH was used in determining the hydrocarbon degradation as opposed to the individual hydrocarbon fractionalization. The results presented in Tables 6 and 7 revealed that the calculated variance ratio values (6.168 and 3.98) are higher than the tabulated value (3.89) at 0.05. The null hypothesis was therefore rejected and the alternate hypothesis accepted. It was concluded that there is a significant difference in hydrocarbon concentration before and after treatment.

4. CONCLUSION

This research work outlined the prospect of phytoremediation in the clean-up of oil contaminated soil in Nigeria. The findings of the study revealed the growth of the five selected tropical plants utilized for the remediation exercise. The study has enhanced the understanding of the effectiveness of phytoremediation on soil contaminated with crude oil at concentrations of 0%, 3% and 5%. It was therefore inferred from the findings that the growth of the plants in crude oil contaminated soil reduced the toxicity of crude oil in the soil. The study recommends that soil augments like cow dung should be added to crude oil contaminated soil to enhance the increased efficacy of using tropical plants in remediation study

5. ACKNOWLEDGEMENT

The authors wish to acknowledge the assistance and contributions of the Department of Crop Science and Department of Civil Engineering, University of Benin, Benin City toward the success of this work.

6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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