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Role of *Pentaclethra macrophylla* Benth in crude oil-polluted soil remediation

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ABSTRACT

This study evaluated the role *Pentaclethra macrophylla* plays in the bioremediation of crude oil-contaminated soil in a controlled environment. Viable seeds *P. macrophylla* were planted in plant bags containing 3000 of clay-loamy soil to which varying amounts (0, 25, 50, 75 and 100 ml) of crude oil were added. Plant growth parameters (plant heights, girths, number of leaves, nodulation and germination rate) and soil physicochemical parameters (pH, organic matter, sodium, potassium, calcium, magnesium phosphorous, and nitrogen) were determined using standard methods. Results showed that the germination rate of *P. macrophylla* reduced progressively, 68%, 68%, 52%, 47% and 38% in that order, with increasing concentrations of crude oil, 0 ml, 25 ml, 50 ml, 75 ml and 100 ml. A similar pattern was observed for plant height, 16 weeks after planting, with values 52.80cm, 43.50 cm, 42.60 cm, 42.50 cm and 35.80 cm in that order; 0.37 mm, 0.26 mm, 0.24 mm, 0.22 mm and 0.21 mm in that order for girth; 15, 10, 8, 8 and 2 in that order for number of nodules and 16.00, 14.00, 14.00, 11.00 and 8.00 in that order for number of leaves. The physicochemical characteristics of the soil decreased corresponding to the concentration of crude oil, from 0-100 ml. Nevertheless, the plant exhibited the capability to endure the suppressive effect of crude oil with recorded relative growth rates ranging from 0-0.23 and percentage growth suppression of 0.00% - 32.20%. Being a native nitrogen-fixing plant, *P. macrophylla* could be utilised for the bioremediation of crude oil-contaminated soil in the Niger Delta.

***Keywords*:** Crude oil pollution, nitrogen-fixing plant, *Pentaclethra macrophylla*, soil

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1. INTRODUCTION

Crude oil, a product of worldwide significance and one of the major merchandises in the world market today is the mainstay of Nigeria's economy, as the country has large reserves and has been a foremost exporter of this commodity in Africa since the middle of the 20th century [1]. Crude oil like natural gas, is among the major energy resource from a global perspective, with both accounting for about 50% of total global energy demands [2].

With the availability of crude oil comes the inevitability of spillage in Nigeria, which is prevalent in the Niger Delta and areas within the grid of pipelines and installations, owing to several reasons but chiefly because of cannibalistic vandalization for profit [3-5]. Crude oil pollution has been accounted for to debase biological systems bringing about harm to soil properties, obliteration of biota and very likely to prompt adversative health outcomes in humans and mortality [6, 7]. The presence of crude oil in the environment has caused several adverse impacts on the environment [8]. As reported by Aigberua *et al*. [9] and Onwuna *et al*. [7] crude oil could lead to variations in microbial, physiochemical, heavy metal and hydrocarbon content of the soil which in turn may affect the biological components of the ecosystem including microbes, insects, vegetation and wildlife. Crop plants and some forest species have been reported to be impacted by crude oil pollution [10-12], but information on the response of *Pentaclethra macrophylla*, a plant vulnerable to environmental pollution in the Niger Delta, is still scanty.

Pentaclethra macrophylla commonly referred to as the African oil bean, a leguminous tree species in the subfamily Mimosoideae and family, Fabaceae, is a widely grown plant from the Middle Belt down to the Southern, part of Nigeria, for food and timber when fully grown [13]. Ladipo and Boland [14] describe it as a plant with excellent soil improvement properties while Enujiugha and Agbede [15], and Asoegwu *et al*. [13] posited that it is a minor food supplement owing to its nutritive richness in protein (30-36%). It is a tropical tree with many uses including beekeeping, its flowers are attractive to bees [16].

Though the tree is generally called the oil bean tree, it has local names - "Apara apagha" in Yoruba, "Okpaghan" in Bini, "Okpagha" in Urhobo, "Ugba" in Idoma and Igbo, "Ugbe" in Esan, "Ukana" in Efik. This plant is famed in ethnomedicine as its leaves, bark and root are believed to provide relief from fever, and stomach-ache, and can be a soothing lotion against skin maladies and sores [17].

Oyedeji et al. [18] averred that leguminous plants growing in crude oil-bearing regions in Nigeria can tolerate crude oil and should be explored for effective restoration of polluted soil in the Niger Delta. Phytoremediation is a green technology with tremendous advantages for use in Nigeria where remediation by enhanced natural attenuation is chiefly practised [19]. With the reported usage of *P. macrophylla* in agro-forestry, and its viability as a leguminous tropical plant, there is a need to study its suitability for phytoremediation of crude oil.

2. MATERIALS AND METHODS

2. 1. Study sites

Seed germination and greenhouse experiments were carried out in the Research and Experimental Farm and the Screen House of the Niger Delta University, Nigeria.

The physicochemical analysis of soil samples was conducted at the Central Research Laboratory, Federal University of Technology, Akure, Nigeria.

2. 2. Source of experimental samples

Clay-loamy topsoil collected at a depth of 0 – 10 cm from a 5-year-old fallowed plot in the Research and Experimental Farm was utilised for growing the plants. Furthermore, Bonny light crude oil collected from the Oporoma Flow Station of Shell Petroleum Development Company was used for spiking the soil. *Pentaclethra macrophylla* seeds were sourced from the Department of Forestry, University of Uyo, Nigeria.

2. 3. Seeds viability test

Adopting the previously described floating method by Anoliefo and Vwioko [20], about 500 seeds of *P. macrophylla* were steeped for 30 minutes in distilled water inside a beaker, to sort out seeds that floated from those that were submerged in the water. A total of 300 viable seeds were randomly selected for use afterwards.

2. 4. Germination test

Clay-loamy soil weighing 3000 g was stacked in five medium-sized plant bags ($2000 cm^3$), and arranged in the greenhouse. The same mass of soil was spiked with varying concentrations of crude oil reflecting uncontaminated-0 ml, low contamination -25 ml, average contamination -50 ml, high contamination -75 ml and very high contamination-100 ml. Two hundred grammes (200 g) of soil samples were measured out from each treatment, dissolved in 1000 ml of distilled water and left to sop up water for 72 hours. Afterwards, the mixtures were filtered to obtain the aqueous extract filtrates, which were then collected in 500 ml conical flasks and labelled correspondingly.

The germination test was carried out in Petri dishes as described by Kayode and Oyedeji [21]. Ten seeds of the plant were sown in each petri dish and moistened daily using filtrates of the respective concentrations at 0700 for 10 days (in five replicates). Germination counts were made daily and percentage germination was ascertained 10 days after sowing using the formula:

$$\text{Germination test Percentage (Gt \%)} = \frac{\text{Number of seedlings that emerged/dish}}{\text{Total number of seeds sown}} \times 100$$

2. 5. Growth response test

Ten (10) planting bags for each treatment were set in the greenhouse and were watered for two weeks at an interval of 72 hours at 0700 before seeding with 3 viable seeds per bag. Two weeks after planting (2WAP) plants per bag were pruned to one. Growth parameters (plant height and girth) were carried out every two weeks until the 16^{th} week, while the number of leaves and nodules was counted at 16 WAP. The seedling height girth was determined with the aid of a meter rule and girth with the aid of Vanier Calliper. The relative growth rate (RGR) and growth suppression (GS) were determined according to the method previously described by Kayode and Tedela [22].

2. 6. Analysis of the physical properties of the soil samples

The various physicochemical parameters of the soil were determined following standard procedures as reported in the literature including bulk density, soil organic matter, soil pH [23], moisture content [24], the volume of air in soil, soil water capillarity and porosity [25], soil nitrogen, calcium and magnesium [26], and available phosphorous [27].

3. RESULTS

The percentage germination of *P. macrophylla* contaminated with crude oil is presented in Figure 1. At varying concentrations of 0 ml, 25 ml, 50 ml, 75 ml and 100 ml of crude oil, germination of *P. macrophylla* was 68% (60 COV), 68% (55 COV), 52% (46 COV), 47% (41 COV) and 38% (30 COV) in that order.

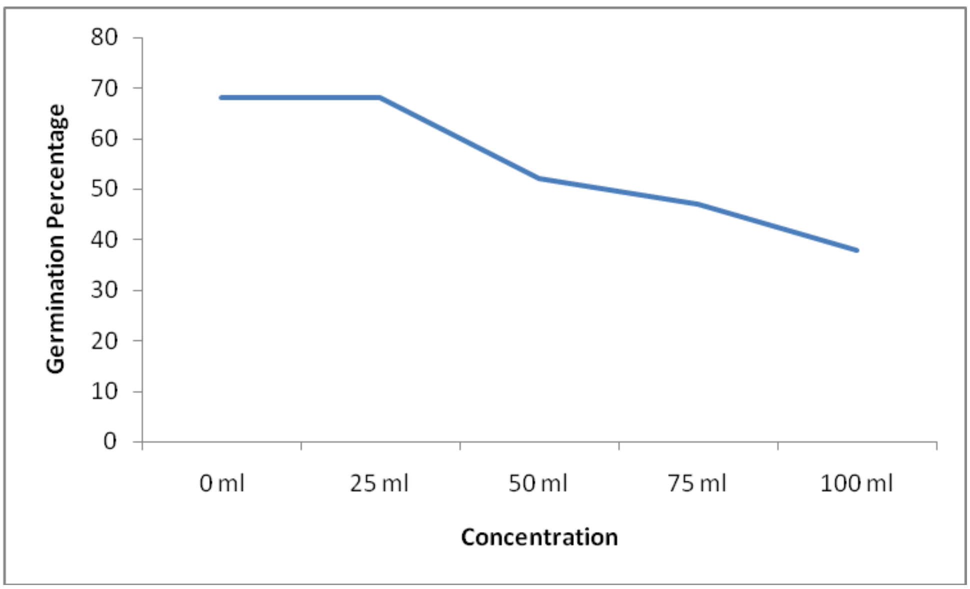

Figure 1. Percentage germination of *P. macrophylla* in crude oil-polluted soil water extracts

The mean heights of *P. macrophylla* 2 WAP were 4.80±1.18 cm, 3.60±2.80c m, 3.20±1.90 cm, 1.80±2.50 cm and 0.00±0.00 cm in increasing order of crude oil contamination, and 52.80±3.15 cm, 43.50±2.65 cm, 42.60±0.65 cm, 42.50±2.10 cm and 35.80±4.10 cm, 16 WAP (Table 1).

Table 1. Mean heights of *P. macrophylla* in crude oil-polluted soil

Time (WAP)	Plant height (cm)/Crude oil concentration (ml)				
	0	25	50	75	100
2	4.80±1.18	3.60±2.80	3.20±1.90	1.80±2.50	0.00±0.00
4	9.40±1.90	9.20±1.15	6.40±1.60	4.60±2.05	3.10±1.80

6	18.60±3.20	15.70±1.90	10.30±0.95	7.10±2.90	8.30±1.90
8	27.30±2.60	20.50±2.20	15.40±3.35	14.00±2.80	13.50±2.30
10	34.80±2.40	25.10±1.75	21.40±1.80	22.40±1.45	18.60±1.90
12	40.10±1.60	30.40±2.30	30.10±1.60	30.70±1.20	24.70±2.05
14	46.20±2.80	36.10±1.90	37.10±1.10	34.50±1.85	30.10±3.10
16	52.80±3.15	43.50±2.65	42.60±0.65	42.50±2.10	35.80±4.10
∑X±SD	234.00±18.83	184.10±16.65	166.50±12.95	157.60±16.85	134.10±17.15
$\Delta H = H_F - H_I$	48.00±1.97	39.90±0.15	39.40±1.25	40.70±0.40	35.80±4.10
RGR	0.17	0.18	0.18	0.23	0
GS	0.00	0.176	0.193	0.195	0.322
%GS	0.00	17.60	19.30	19.50	32.20

- RGR = Relative growth rate; GS = growth suppression
- H_I = Initial Height
- H_F = Final Height
- ΔH = Change in height
- $\overline{X}$ = Mean
- (±) = Standard deviation

The mean girths of *P. macrophylla* 2 WAP were 0.15±0.02 mm, 0.13±0.01 mm, 0.12±0.03 mm, 0.11±0.05 and 0.00±0.00 mm in increasing order of crude oil contamination, and 0.37±0.03 mm, 0.26±0.02 mm, 0.24±0.03 mm, 0.22±0.03 mm and 0.21±0.04 mm, 16 WAP (Table 2).

Table 2. Mean girths of *P. macrophylla* grown in crude oil-polluted soil

Experimental Time (WAP)	Plant girth (mm)/Crude oil concentration (ml)				
	0	25	50	75	100
2	0.15±0.02	0.13±0.01	0.12±0.03	0.11±0.05	0.00±0.00
4	0.17±0.01	0.14±0.02	0.13±0.01	0.01±0.03	0.11±0.02
6	0.18±0.04	0.16±0.05	0.15±0.02	0.14±0.01	0.12±0.04
8	0.20±0.03	0.17±0.02	0.16±0.01	0.15±0.01	0.13±0.01
10	0.27±0.05	0.15±0.01	0.14±0.04	0.14±0.02	0.15±0.03

12	0.31±0.04	0.22±0.05	0.18±0.02	0.16±0.03	0.17±0.01
14	0.35±0.04	0.23±0.04	0.22±0.01	0.21±0.05	0.19±0.06
16	0.37±0.03	0.26±0.02	0.24±0.03	0.22±0.03	0.21±0.04
$\Delta G=G_F-G_I$	0.22±0.01	0.13±0.01	0.12±0.00	0.11±0.02	0.21±0.04
$\sum X \pm SD$	2.00±0.26	1.46±0.22	1.34±0.17	1.14±0.23	1.08±0.21

Table 3 shows the mean number of leaves and nodules of *P. macrophylla* grown in crude oil-polluted soil. The highest number of leaves (16) was noticed in uncontaminated soil treatment (0 ml) and the lowest (8) was observed in highly contaminated (100 ml) soil treatment. The highest number of nodules (15) was noticed in uncontaminated soil treatment (0 ml) and the lowest (2) was observed in highly contaminated soil treatment (100 ml).

Table 3. Mean number of leaves and nodules of *P. macrophylla* grown in crude oil-polluted soil

Crude oil concentration (ml)								SD
Parameter	0	25	50	75	100	Mean	Variance	
Leaf	16.00	14.00	14.00	11.00	8.00	12.60	9.80	3.13
Nodule	15.00	10.00	8.00	8.00	2.00	8.60	21.80	4.67

The physiochemical characteristics of crude oil-contaminated soil that *P. macrophylla* was used for remediation are presented in Table 4. The contaminated soil pH was in the range of 5.18 (at 0 ml) and 4.77 (at 100 ml). However, the control soil was in the range of 5.51 – 5.18. The organic matter concentration decreased as the concentration of the crude oil increased i.e. 1.91% (0 ml) - 1.35% (100ml), whereas the control had organic matter in the range of 2.58 – 2.95% (Table 4).

Table 4. Physiochemical characteristics of crude oil-contaminated soil

Parameters	Groups	Crude oil concentration (ml)				
		0	25	50	75	100
pH	Contaminated	5.18	5.08	5.02	5.85	4.77
	Control	5.51	5.42	5.36	5.18	5.18

Organic matter, %	Contaminated	1.91	1.80	1.60	1.54	1.35
	Control	2.25	2.09	2.04	2.01	2.01
% N	Contaminated	0.52	0.46	0.44	0.41	0.40
	Control	0.81	0.75	0.60	0.60	0.60
P, mg/kg	Contaminated	7.85	7.55	7.15	7.10	6.00
	Control	13.50	12.68	12.38	11.25	7.45
K, mg/kg	Contaminated	4.38	4.27	4.18	4.05	4.05
	Control	9.27	9.15	9.08	9.02	9.00
Na, mg/kg	Contaminated	2.25	2.15	2.05	2.05	2.05
	Control	3.85	3.50	3.19	9.02	8.00
Ca, mg/kg	Contaminated	21.50	20.54	20.32	20.20	20.20
	Control	33.50	31.70	30.58	30.45	30.45
Mg, mg/kg	Contaminated	1.58	1.55	1.45	1.38	1.38
	Control	2.70	2.60	2.41	2.38	2.38

The concentration of fertilizer monitoring parameters (nitrogen, phosphorous and potassium) also decreased as the concentration of the crude oil increased. The concentrations were in the range of 0.52 % (0 ml) – 0.40% (100 ml) (nitrogen), 7.85 mg/kg (0 ml) – 6.00 mg/kg (100 ml) (phosphorous) and 4.38 mg/kg (0 ml) – 4.05 mg/kg (100 ml) (potassium) among the contaminated soil (Table 4).The concentration of sodium was in the range of 2.25 mg/kg (0 ml) – 2.05 mg/kg (100 ml), calcium was in the range of 21.60 mg/kg (0 ml) – 20.20 mg/kg (100 ml) and magnesium was in the range of 1.58 mg/kg (0 ml) – 1.38 mg/kg (100 ml) (Table 4).

Table 5. Physical properties of unpolluted and crude oil-polluted soil

Treatment (ml)	Bulk Density (g/cm^3)	Moisture content (%)	Soil air (%)	Water Holding capacity (ml)	Soil porosity (ml)
0	5.8	72	72.5	58.4	86.4
25	6.4	44.5	38.6	50	81.5
50	6.4	40	30.5	34.5	60.4
75	6.8	28.5	40.4	24.1	48.3
100	7.7	18.2	43.6	13.7	32.8

Table 5 shows the effect of the physical properties of unpolluted and crude oil-polluted soil used in the experiment. Bulk densities of 5.80, 6.40, 6.40, 6.8 and 7.70 g/cm^3 were observed in the treatments, 0 ml, 25 ml, 50 ml, 75 ml and 100 ml crude oil-polluted soil in that order. Soil moisture content was reduced in the crude oil-polluted soil samples, particularly in the 100 ml crude oil-contaminated soil. Similarly, the presence of crude oil in the soil samples affects the soil air, 72.50, 38.60, 30.50, 40.40 and 43.60 % were observed in 0, 25, 50 75 and 100 ml in that order. Water holding capacity was also reduced in the crude oil-polluted soil.

4. DISCUSSION

The effect of crude oil on the germination and growth response of *P. macrophylla* as determined in this study, revealed that crude oil decreased germination potentials of polluted soil. The decrease is a function of the concentration of oil in the soil, as the coefficient of velocity decreased with an increase in contamination from uncontaminated to highly contaminated. This is in congruence with the study of Osuji and Adesiyan [28]; Kayode and Oyedeji [21] and Oyedeji and Kayode [29] who reported that crude oil harms soil conditions, microorganisms and plants, and invariably germination. The seed emergence rate was >30 which was higher than the rate for *Afzelia africana* (>25) reported by Oyedeji and Kayode [29].

The result showed that the heights *P. macrophylla* decreased corresponding to the concentration of crude oil in the soil. Thus, the percentage growth suppression increased with an increase in the concentration of the crude oil in the soil, similar to reports by Oyedeji and Oyedeji [30]; Anoliefo and Okoloko [31] and Kayode et al [32, 33]. This study also showed a decrease in girth with increased concentrations of crude oil in the soil as earlier reported by Oyedeji and Kayode [29] and Oyedeji and Oyedeji [30].

The biological performance of *P. macrophylla* in crude oil-polluted soil as indicated by the number of leaves and number of nodules showed a declining pattern which is indicative of the inhibitory effects of pollutants on plant growth and development. Crude oil affects nitrogen-fixing bacteria and symbiotic nodulation by plant-microbe interaction [34].

The physiochemical characteristics of crude oil-contaminated soil in that *P. macrophylla* was used for remediation differed from the control. The contaminated soil pH was in the range of 5.18 (at 0 ml) and 4.77 (at 100 ml). However, the control soil was in the range of 5.51 – 5.18. The pH of soils helps in determining the number and kind of soil organisms, such that substantial changes in valuable soil microbial population may adversely affect soil health [35].

The organic matter concentration was reduced in the crude oil relative to the concentration of the pollutant i.e. 1.91% (0 ml) - 1.35% (100 ml), whereas the control had organic matter in the range of 2.58 – 2.95%. Typically, the organic matter content of soils is a great factor in soil fertility and is used as a pollution indicator. The organic matter content has momentous implications for mineralization as it is, that carbon mineralizing in the soil is directly proportional to the organic carbon content, which is tied to microbial metabolism. The low level of organic matter in the crude oil-contaminated soils might be explained by the absorptive and metabolism dimensions of *P. macrophylla* as well its transport systems that permit selective suck up of contaminants from the growth milieu.

This is supportive of the report by Oyedeji [5] that leguminous trees have good root uptake mechanisms and aided by their symbiont microbes, they can lead to a reduction in soil organic matter via hydrocarbon degradation.

The concentration of fertilizer monitoring parameters (nitrogen, phosphorous and potassium) also decreased as crude oil concentration increased. The concentrations were in the range of 0.52 % (0 ml) – 0.40% (100 ml) (nitrogen), 7.85 mg/kg (0 ml) – 6.00 mg/kg (100 ml) (phosphorous) and 4.38 mg/kg (0 ml) – 4.05 mg/kg (100 ml) (potassium) among the contaminated soil. Total nitrogen, phosphorus and potassium were altered in the remediated soil samples at 16 WAP compared to their controls, but concentrations were fairly constant. This is indicative that *P. macrophylla* is effective in maintaining the soil NPK. Typically, leguminous plant species such as *P. macrophylla* have system mechanisms in their root systems that support microbial populations [36], and the nitrogen fixed in the soil could help in hydrocarbon degradation [34].

The results of the soil analyses show that calcium ion had the highest concentration among measured exchangeable cations in the crude oil-polluted soils, followed by sodium ion and the least cation measured was magnesium. *P. macrophylla* reduced the concentrations of the cations, in the impacted soil relative to the control. Soil particles carry plant nutrients which exist within the rhizosphere as ions. The concentrations of the cations generally decreased with increasing crude oil concentration, consistent with the reports by Onyeike *et al.* [37] and Oyedeji and Oyedeji [30]. Bulk densities of 5.80, 6.40, 6.40, 6.8 and 7.70 g/cm^3 were observed in the treatments with 0 ml, 25 ml, 50 ml, 75 ml and 100 ml of crude oil concentration in that order. Soil moisture content was reduced in the crude oil-polluted soil samples, particularly in the 100 ml crude oil-tainted soil. Similarly, the presence of crude oil in the soil samples affects the soil air, 72.50, 38.60, 30.50, 40.40 and 43.60 % were detected in the treatments with 0 ml, 25 ml, 50 ml, 75 ml and 100 ml of crude oil concentration in that order. Water holding capacity was also reduced in the crude oil-polluted soil. This aligns with the previous investigation by Ewetola [38] and Oyedeji and Oyedeji [30] in which it was reported that crude oil could block pore spaces within soil and as a result interfere with soil properties and function which may have adverse effects on plant physiology and productivity.

5. CONCLUSION

It was observed that tainting soil with crude oil affects germination and growth parameters of *P. macrophylla*, although the plant was able to tolerate crude oil as evidenced by a decrease in germination, height, leaf number and nodulation, as the concentration of the crude increased. Crude oil in soil tends to reduce soil physicochemical characteristics, especially nitrogen, phosphorous, calcium, sodium, magnesium, and potassium organic matter among others. It was observed that as the concentration of the crude oil increased, the soil bulk density, moisture content, soil air, water holding capacity and porosity reduced in the crude oil-polluted soil. The tolerance level of *P. macrophylla* is a pointer to its suitability in the phytoremediation of crude oil-contaminated soil in the Niger Delta.

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