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The performance of *Zea mays* in crude oil polluted soil enriched with leaf litters of *Mangifera indica*

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Abstract:
Background*: The remediation of crude oil polluted sites in the Niger Delta has become a necessity owning to the increasing amount of crude oil spillage from both operational errors from oil companies, and those from vandalized crude oil pipes by illegal bunker activities. Crude oil polluted soils are characterized by the presence of Total Petroleum Hydrocarbon (TPH) and Chlorinated Hydrocarbons (VOC-Cl). The effect of these on plants and animals have been well studied. This study was done to determine the growth response or the performance of maize (Zea Mays) plant in a crude oil contaminated soil amended with leaf litters of Mangifera indica.*
Materials and Methods*. Top loamy soil collected from behind the Multipurpose Hall at the University of Port Harcourt, Nigeria was treated with different volumes of crude oil viz: 100ml, 200ml, and 400ml. 0.0ml, which represents no crude oil and served as control. In all, there were four treatments with five replicates. Five seeds of maize were planted in each perforated polythene bag filled with 24.6kg of loamy soil two weeks after amendment and later thinned to two plants in each bag. Growth parameters measured/monitored were plant heights, number of leaves, dry and fresh weight.*
Results*: Based on the result, the plant height, number of leaves, dry and fresh weights were reduced in contaminated treatments in comparison with the control and amended treatment. It was observed that maize plant's height in control and amended treatments ranged from 20cm to 100cm. The treatment with 100ml of crude oil provided the best phytoremediation response, while the treatment with 400ml of crude oil provided the worst response to phytoremediation. The highest number of leaves was recorded on the treatment with 0.0ml of crude oil amended with Mangifera indica followed by the treatment with 100ml of crude oil. Hence, the application of leaf litters of Mangifera indica as amendment can be said to have significantly increased plant height and leaf counts per plant.*
Conclusion: *This study suggests that leaf litters of Mangifera indica can be utilized to aid phytoremediation. It also shows that growth was seriously reduced at high level of crude oil contamination despite the amendment. The study equally demonstrated that maize plant can extract heavy metals from crude oil polluted soil. However, this poses a challenge to the consumption of the cobs and the disposal of the plant after harvest.*
Key Word*: Crude oil spill; Soil Pollution; Plant Growth, Environmental Degradation, Niger Delta*

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I. Introduction

Crude oil is a naturally occurring hydrocarbon containing carbon and hydrogen only. In addition to hydrocarbons, crude oils will contain a variety of other compounds where the carbon and hydrogen combines with other elements such as oxygen, Nitrogen, Sulphur, and Metals. The remediation of crude oil polluted sites in the Niger Delta has become a necessity owning to the increasing amount of crude oil spillage from both operational errors from oil companies, and those from vandalized crude oil pipes by illegal bunker activities. The peculiar nature of the Niger Delta soil, and the possibility of seasonal flooding makes it easy for crude oil spill to travel along the path of surface or ground water flow from the originating sites[1]. This creates problem to spill control and remediation. Crude oil polluted soils are characterised by the presence of Total Petroleum Hydrocarbon (TPH) and Chlorinated Hydrocarbons (VOC-Cl). The effect of these on plants and animals have been extensively studied in the literature. Studies have indicated that TPH can undergo both aerobic and anaerobic biodegradation in the soil, and the rate varies from one soil type and structure to another. Aerobic biodegradation happens in the presence of suitable species of microorganisms in soil water, temperature, and concentration of toxic elements and the content of oxygen in water. In a study carried out by Ogunkunle, et al. (2008)[2], it was reported that the topsoil of the Niger Delta contains low humus level, with higher amount of sandy and clayey layers, thus making it acidic with pH range of 3.5-5. If this is taken as representative of typical topsoil characteristics in the Niger Delta, it shows that the soil is already fragile, and further pollutants could render the soil unsuitable for plant cultivation.

Various means have been employed to remediate crude oil impacted soils. However, each of these approaches depends on the soil properties such as texture, organic matter content and pH value and the composition of contaminants which as metals, organic pollutants, and cyanides. A preliminary risk assessment is usually carried out before the implementation of remediation option(s). Such assessment takes into consideration the Source-Pathway-Receptor (SPR) approach. Remediation is the management of contaminants in impacted soils to prevent, reduce or mitigate damages to human health and the environment. Remediation can also lead to the quick recovery of affected lands to improve the social, sanitary and economic value of the land[3]. This effort has been carried out through physical, biological, and chemical means. One of such approach is phytoremediation with the advantages of being environmentally friendly, cost effective, ease of deployment, and independent of soil type[4].

While plants can be used in remediation, it is equally important that the soil is supplied with nutrients such as potassium (K), phosphate (P) and nitrates (NO_3^-) which promotes bioremediation. Also, the levels of potassium, nitrogen and phosphorus are important in determining the pH of the soil and stimulating the condition for crude oil degrading microbes. A healthy soil for normal plant growth requires high nitrate, potassium, and phosphate nutrient contents and pH range close to neutrality[5]. Crude oil reduces the amount of nutrients in the soil, hence the need to supply these nutrients back into the soil through a process known as 'soil amendment.' In this research projects, leaf litters of *Mangifera indica* (mango) was used as amendment. Empirical observations suggest that higher doses of crude oil pollution in soils reduce the vertical growth of maize plants[6,7]. This is because, crude oil pollution displaces air from pore spaces in the soil leading to poor aeration. However, it has been observed that lower doses of crude oil up to 5.2 ml tend to achieve a fertilizing effect on the luxurious growth of 5 weeks old maize plant[6], due to possible degradation of oil in the soil, releasing nitrogen and essential mineral nutrients for plant uptake[8]. Also, reduction in leaf area at higher doses of crude oil pollution has been reported[9]. It has also been indicated that the leaves can store heavy metals from crude oil polluted soil when planted a week after pollution[7], suggesting that maize plant can be used in the extraction of heavy metals from crude oil polluted soils.

Previous empirical study also suggest that organic matter increases in polluted soils, with decreasing value of phosphorus at higher concentrations of the pollutant[10]. Again, crude oil adsorption experimentation with mango shells after cracking the seed shows that *Mangifera indica* is an effective natural adsorbent that can remove crude oil from contaminated soil[11]. As stated earlier, the leaves of *Mangifera indica* have been found to contain high potassium content[12], hence high content of potassium can be extracted when the leaves are harvested. Furthermore, research findings indicates that crude oil can reduce the level of potassium below the critical value of 0.2mg/kg (5.13E-04Cmol/kg) needed for plant production[13]. Hence, amending crude oil polluted soil with *Mangifera indica* should increase the amount of potassium above this critical value to enhance plant growth.

The aim of this work is to determine the growth response or the performance of maize plant (Oba super 4 specie) in a crude oil contaminated soil amended with leaf litters of *Mangifera indica.* The main objective is to demonstrate that crude oil contaminated soil can be amended with leaf litters of *Mangifera indica* to enhance the production of maize grown in a crude oil polluted soil and improve the economic viability of polluted soils.

II. Material And Methods

The experimental materials used for this project work include seeds of *zea-mays* collected from Agricultural Development Project (ADP) Port Harcourt, Rivers State. Crude oil used for pollution of soil was collected from Agbada 1 SPDC flow station in Aluu town and the soil was collected from a farmland beside the water factory at the University of Port Harcourt. A measuring cylinder used in determining the quantity of crude oil for each of the soil treatment was collected from the mycology laboratory at the University of Port Harcourt, Nigeria and the pollution of the soils was carried out on the 22nd of February 2018.The experimental treatments are defined as follows:

- T0-(0ml); Serves as control as maize was planted with no amendment and no pollution.
- T1-(0ml); Also serves as control with an amendment but no pollution.
- T2- (100ml), T3 (200ml) and T4 (400ml) are treatments with pollutants and amendment

Study Design: A completely randomised design (CRD) approach was employed with four (4) replicates.
Study Location: University of Port Harcourt, Nigeria
Study Duration: February 2018 to June2022.

Procedure methodology

After the application of the crude oil, it was allowed to settle properly for 14 days (two weeks), and the soil was amended with leaf litters of *Mangifera indica* thereafter. The dried leaves of *Mangifera indica* were

shredded and chopped into tiny pieces and divided into weights which was transferred into the soil divided into 5 replicates and thoroughly mixed to form a composite bulk. The shredded leaves of *Mangifera indica* weighed 3kg in total while the individual weight weighed 0.15kg. This was done on the 14th of March 2018, which was 2 weeks after the soils were polluted with crude oil as detailed above. The same size of polythene bags was used filled as standard measurement of the soil for all treatments. Seeds of *Mangifera indica* were planted on the 4th of April 2018. Five seeds were planted in each polythene bag. The thinning of the germinated plants was done on the 19th of April 2018. Fourteen days later, two plants were removed from the five plants to avoid competition for nutrients. After another two weeks, extra two plants were thinned, leaving only two healthy plants for the experimental measurements. Theoretically, plant thinning carried out in most cases is to improve the rate of growth of the remaining plants. The experimental arrangements were carried out in the Centre for Ecological Studies, University of Port Harcourt. The site at the centre was cleared and the experimental polythene bags were placed and filled with 24.6kg of soil in each of the 25 polythene bags perforated to avoid water logging. The crude oil treatments of 0.0ml, 100ml, 200ml, and 400ml were slowly dropped into the various polythene bags for contamination.

Pre-germination test: This test was performed using 40 seeds from the 'Oba super 4' maize varieties and placing them in a Petri dish that has been lined with filter paper. Four Petri dishes were used and to each Petri dish, water was added. Ten seeds were placed in each Petri dish and all the seeds grew after 5 days to give a pre-germination probability of 100%.

Total heterotrophic bacteria (THB): 1g of soil sample was weighed into 9ml sterile dilute 0.85% NaCl solution under aseptic laboratory condition. The combination was shaken vigorously and the concentration of the soil in the 0.85% NaCl solution was reduced through serial dilution. 0.1ml aliquot of the inoculums was inoculated on Nutrient Agar (NA) medium using a sterile pipette. A sterile hockey stick was used to spread the inoculum was spread evenly. Plates were incubated at 37^{o}C for 24hours. Bacteria colonies were counted and the colony forming unit per gram (cfu/g) of the soil sample obtained.

Total heterotrophic fungi (THF): 1g of soil sample was weighed into 9ml sterile dilute 0.85%NaCl solution under aseptic laboratory condition. The combination was shaken vigorously and the concentration of the soil in the 0.85% NaCl solution was reduced through serial dilution. To allow for only the growth of fungi in the soil sample, 0.1ml aliquot of inoculum was inoculated on Potato Dextrose Agar (PDA) medium acidified with 0.1% lactic acid to inhibit growth of bacteria. Inoculated plates were incubated at ambient temperature for 5-7 days. Afterwards, bacteria colonies were counted and the colony forming unit per gram (cfu/g) of the soil sample was obtained.

Heterotrophic utilization bacteria (HUB): 1g of soil sample was weighed into 9ml sterile dilute 0.85% NaCl solution under aseptic laboratory condition. The combination was shaken vigorously and the concentration of the soil in the 0.85% NaCl solution was reduced through serial dilution. Using the spread plate technique,0.1ml aliquot of inoculum was inoculated on Mineral Salt Agar (MSA). Sterile filter paper was soaked with crude oil and placed in the lid of Petri dish as a source of carbon. Plates were incubated in inverted ambient temperature for 5-7 days. Thereafter, colonies were counted and the colony forming unit per gram (cfu/g) of soil sample was obtained.

Heterotrophic utilization fungi (HUF): 1g of soil sample was weighed into 9ml sterile dilute (0.85% NaCl) under aseptic laboratory condition. The combination was shaken vigorously and the concentration of the soil in the 0.85% NaCl solution was reduced through serial dilution. To permit only the growth of hydrocarbon utilizing fungi, the growth of bacteria was inhibited through0.1ml aliquot of inoculum inoculated on Mineral Salt Agar (MSA) acidified with 0.1ml lactic acid. Sterile filter paper soaked with crude oil was placed in the lid of the Petri dish. Plates were incubated in inverted position at ambient temperature for 5-7 days. Thereafter, plates were observed, and colonies were counted and the colony forming unit per gram (cfu/g) of soil sample was obtained.

Physical parameters measured: Plant heights were measured from the soil level up to the terminal bud using a meter rule. Measurement was done at 2-6 weeks after planting (2-6 WAP). The total leaf counts were recorded for each plant per treatments for 2-6 WAP intervals. The plant was weighed immediately after harvest to avoid water loss. The fresh weight was obtained using weigh balance. The dry weights were conducted after drying in an oven.

Statistical analysis

The data collected were subjected to Analysis of Variance (One -Way ANOVA) in GraphPad Prism version 9.0.0 for Mac. *Post hoc* pair-wise comparison of the mean plant height and leaf count using Least Significance Difference (LSD) at 5% level of probability was carried out when the p value of the ANOVA F statistic was less or equal to alpha level of 0.05. Tables and graphs were also used to present the values of measured paraments.

III. Result and Discussion

The results obtained from the study are graphically represented in Figure no 1and Figure no 2. InFigure no 1the height of the plants in the control (T0) increased to 100cm at the 6th week after planting (WAP), while the treatment with 400ml of crude oil pollution (T4) had the least height of 60cm within the same period. This observation is also similar in Figure no 2, where the leaf counts reduce as the volume of pollutants increased. The difference in mean height and leaf counts across the treatments was determined using ANOVA, and a significant difference in the mean heights and leaf accounts was observed. A significant difference in mean heights of the plant across the treatments was supported during the post hoc LSD test, however only the leaf count in T4 indicated a difference from the mean leaf count in the control, T0. This suggest that higher dose of crude oil pollution can inhibit plant growth. Thus, *Mangifera indicia* improved the performance of the plant in the amended soils up to 300 ml volume of pollutant in the soil. The effect was noticed as the level of phosphorus increased by WAP 8, potassium was relatively stable which should have been depleted by the presence of crude oil pollution and a reduction in the acidity of the amended soils. Again, bacteria and fungi activities increased in the amended soils by WAP 8, suggesting improvement in biodegradation. The measured values of plant heights and leaf counts are presented in Table no 1 and Table no 2, and the sections that follows presents details of the results and discussion.

Table no 1. Mean values of plant heights of two plants in each treatment

WAP	Plant Height (cm)				
	T0	T1	T2	T3	T4
2	40.90	37.02	28.40	29.60	20.41
3	57.10	49.80	42.90	38.90	25.80
4	73.40	66.30	59.10	50.80	34.10
5	89.30	83.80	73.10	66.00	50.90
6	100.40	92.70	89.40	76.90	60.20

Table no 2. Mean values of leaf counts of two plants in each treatment

WAP	Leaf Counts (numbers)				
	T0	T1	T2	T3	T4
2	5.20	4.80	4.20	4.50	3.50
3	5.00	6.00	4.80	5.30	4.50
4	7.30	7.60	7.20	6.20	4.40
5	6.60	7.90	8.00	6.10	5.50
6	8.30	8.40	7.80	7.20	6.30

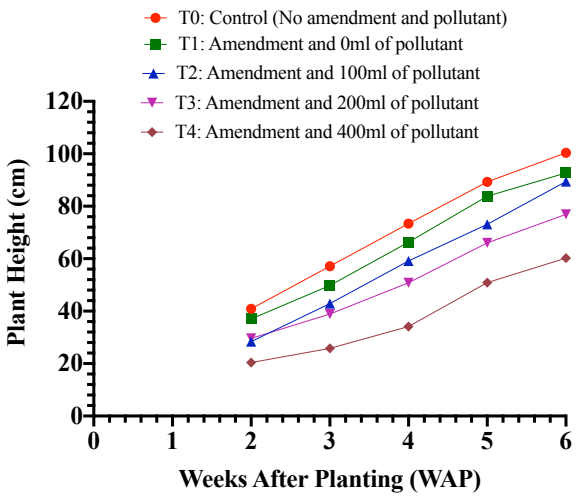

Figure no 1. Plant mean height in different soil treatments

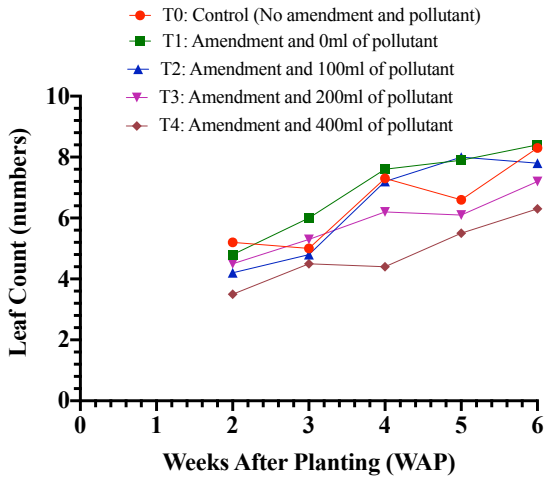

Figure no 2. Plant leaf counts in different soil treatments

Difference in Plant Heights

Plant growth performance can be established weekly through height measurement. Figure no 3 provide the plant height as percentage of the total plant heights across the treatments. The purpose is to appraise the relative height of the plants in each treatment. In Figure no 3, the plants in T0 increased in growth rate from WAP2 to WAP 3 and gradually retards in additional weekly height from WAP 4 to WAP 6. The plants in T1 had similar growth trend compared to T0 except for the drop in WAP 3. Also, the plants in T2 increased in growth response positively from WAP 2 to WAP 4, with a drop in WAP 5 and finally rising in WAP 6. The plants in T3 dropped in growth rate from WAP 2 to WAP 3 and remained almost stable in growth rate from WAP 4 to WAP 6. In T4, the plants had a downward growth response trend from WAP2 to WAP 4 and a positive trend thereafter. Thus, the plots in Figure no 3 provides a means to understand the effect of crude oil on growth response and the effect of the amendment with leaf litters of *Mangifera indica.* For T2, the effect of crude oil was minimal because of the volume (100ml), and the increase in growth response can be attributed to the presence of the amendment. The effect of the amendment can be easily noticed in T4 from WAP 4 to WAP 6 which is the period when the nutrients from the decomposing leaf litters of *Mangifera indica* was released to overcome the effect of the pollutant. Also, the positive effect of the amendment can be noticed in Figure no 4in treatments T1, T3 and T4, as the weekly additional heights increased between WAP 3 and WAP 5, and between WAP 2 to WAP 3 in T2. The weekly additional plant height in the control(T0) declined within this period.

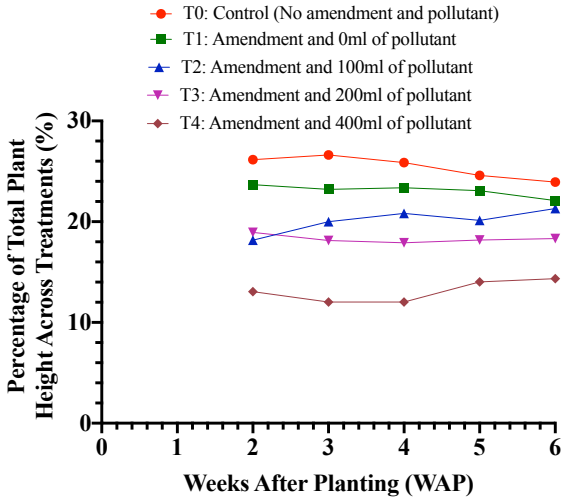

Figure no 3. Plant height as percentage of the total plant heights across treatments

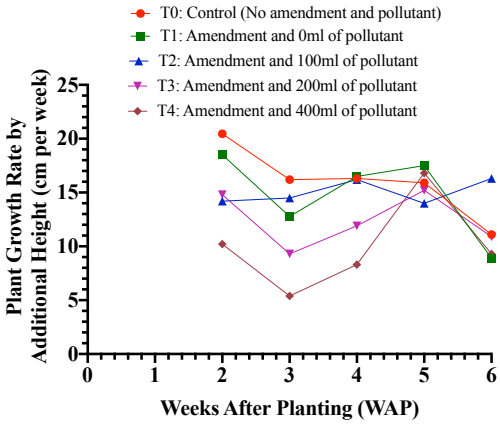

Figure no 4. Plant growth rate by weekly additional height across treatments

A one-way ANOVA was conducted to ascertain if the means of the plants are significantly different as stated inresearch hypothesis H1, below.

H1: There is a significant difference in plant height across the treatments for the duration of the experiment.

To conduct the ANOVA, it is important to confirm if to assume equal variance of the means across the treatments. The measure of central tendency is provided in Table no 3.Although, the variance across T0, T1 and T2 can be assumed to be relatively equal, the same is not true for T3 and T4, hence unequal variability of the difference in the mean across the heights of the plants (no sphericity) in the experimental treatments was assumed in the analysis of variance (ANOVA) in Table no 5.

Table no 3. Measure of central tendency for plant heights

Descriptive Statistics	Experimental Treatments				
	T0	T1	T2	T3	T4
Mean	72.220	65.924	58.580	52.440	38.282
Variance	574.197	533.018	579.417	372.463	283.078
Std. Deviation	23.962	23.087	24.071	19.299	16.825

Since the experimental arrangement, plants thinning and weekly rearrangement of the treatments were randomized, Gaussian distribution was assumed. However, the assumption for sphericity (equal variance) was confirmed using the Mauchly's test for sphericity in JASP® software [14] in Table no 4. The within treatments effect size test returned omega squaredω^2 value of 0.223, indicating a large effect size[15].Estimating effect size with omega squared provides an unbiased effect size measure, especially when the sample size is less than 30 as in this study. Mauchly's test for sphericity in Table no 4, also corroborates the earlier assumption of no sphericity.

Table no 4. Within treatments effects for plant heights

Cases	Sphericity Correction	Sum of Squares	df	Mean Square	F	p	ω²
Treatments	Greenhouse-Geisser	3418.716	1.152	2968.134	60.579	< .001	0.223
Residuals	Greenhouse-Geisser	225.737	4.607	48.996			

Note. Type III Sum of Squares

**Mauchly's test of sphericity indicates that the assumption of sphericity is violated (p < .05).*

A Type III sum of squares estimate was selected in JASP because it takes interactions among the treatments into account[16]. Thus, Greenhouse-Geisser correction for the F-statistic was adopted to adjust the degrees of freedom in each treatment. This approach is adequate when the epsilon value is less than 0.75. The calculated Greenhouse-Geisser correction epsilon value was 0.2880 with a p-value of 0.028, hence the adoption of the Greenhouse-Geisser correction is in order. The result in Table no 5 suggests a significant difference in the means of the plant heights between all the treatments F (1.152, 4.607) = 60.579, p=<0.001, ω^2= 0.223). Since

the ANOVA outcome is significant, a *post hoc* test will be carried out using Fisher's LSD test because each treatment is treated as stand-alone.

Table no 5. One-Way ANOVA table to compare plants heights

ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3419	4	854.7	F (1.152, 4.607) = 60.58	P=0.0007
Individual (between rows)	9143	4	2286	F (4, 16) = 162.0	P<0.0001
Residual (random)	225.7	16	14.11		
Total	12787	24			

For a degree of freedom (DF) between treatments of 4, $t_{.95}$ is 2.132, LSD is estimated in Equation (1):

$$LSD = t_{0.95/df(4)} \sqrt{MS_{residual}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} \quad (1)$$

Where n_1 and n_2 represent the 5rows (WAP 2 to WAP 6) of each of treatment (Table no 1). The LSD table is generated as follows using the results from GraphPad Prism version 9.0.0 for Mac.

Table no 6. Least significant difference (LSD) test for the mean difference of plant heights across treatments

Fisher's LSD Test	Mean Difference	95% CI of difference	LSD ($t_{.95/4}$)	p	t-value	LSD<Mean Difference
T0 vs.T1	6.296	4.323 to 8.269	5.065	0.0009	8.861	Yes
T0 vs.T2	13.64	11.19 to 16.09	5.065	0.0001	15.460	Yes
T0 vs. T3	19.78	13.31 to 26.25	5.065	0.0011	8.489	Yes
T0 vs. T4	33.94	23.63 to 44.25	5.065	0.0008	9.141	Yes

The results of the *post hoc* LSD testing shows that there are significant differences in plant heights between all treatments. Hence, hypothesis H1 is fully supported.

Difference in Leaf Count

A further analysis to investigate the effect of the soil amendment with leaf litters of *Mangifera indica* was carried out using the plant Leaf count during the duration of the project. Figure no 5 provide the leaf counts as percentage of the total leaf counts across the treatments.

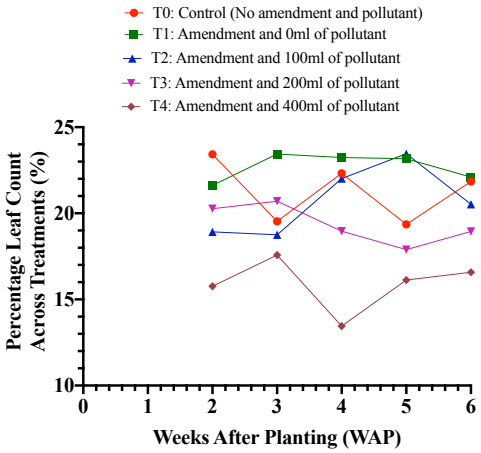

Figure no 5. Leaf counts as percentage of the total leaf counts across treatments

As seen from the above figure, the Leaf count demonstrated the positive effect of the soil amendment without pollution when T1 is compared with T0 visually, as the percentage leaf counts in T1 from WAP 3 to WAP 5was consistently above T0. Figure no 6present the additional weekly leaf counts. The purpose is to provide insight into how the amendment influenced the addition of new leaves across the treatments. This effect can be noticed in T4 as the leaf count increased in WAP 5 after experiencing a decline from WAP 2 to WAP 4.

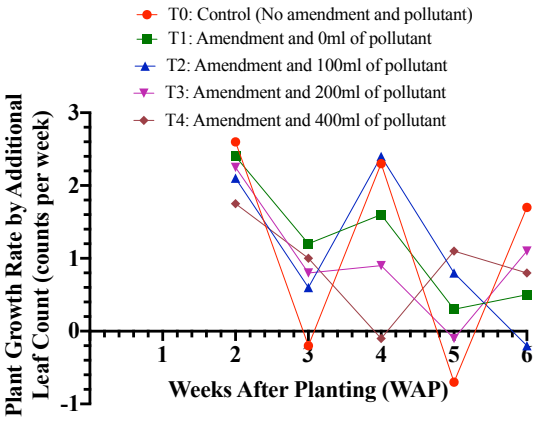

Figure no 6. Leaf count growth rate by weekly additional leaf counts across treatments

A one-way repeated measure ANOVA was conducted to ascertain if the means of the plants are significantly different as stated in research hypothesis H2, below.

H2: There is a significant difference in the mean of the plant leaf counts in all treatments for the duration of the experiment

Again, 'no sphericity has been assumed,' as the variance in T3 and T4 and relatively different from the variances of the means in T0, T1 and T2 in Table no 7.

Table no 7. Measure of central tendency for leaf counts

Descriptive Statistics	Experimental Treatments				
	T0	T1	T2	T3	T4
Mean	6.480	6.940	6.400	5.860	4.840
Variance	1.957	2.238	3.140	1.033	1.168
Std. Deviation	1.399	1.496	1.772	1.016	1.081

A Type III sum of squares adopted in the estimation of within treatments effects. The calculated Greenhouse-Geisser correction epsilon value was 0.508 which is less that 0.75, and a p-value of 0.05. Hence, the adoption of the Greenhouse-Geisser correction is also in order. The result in
Table no **8** suggests a significant difference between the means of the differences in leaf counts between all the treatments F (2.031, 8.128) = 10.875, p=<0.005, ω^2= 0.198). The effect size estimated with omega squared as discussed earlier is large (0.198), but less than the effect size of the plant heights (0.223).

Table no 8. Within treatments effects for leaf counts

Cases	Sphericity Correction	Sum of Squares	df	Mean Square	F	p	ω²
Treatments	Greenhouse-Geisser	12.926	2.031	6.364	10.875	0.005	0.198
Residuals	Greenhouse-Geisser	4.754	8.124	0.585			

In the ANOVA table the treatments were paired and compared with the control T0 (Table no 9).

Table no 9. One-Way ANOVA table to compare leaves count

ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	12.93	4	3.231	F (2.031, 8.124) = 10.87	P=0.0050
Individual (between rows)	33.39	4	8.347	F (4, 16) = 28.09	P<0.0001
Residual (random)	4.754	16	0.2972		
Total	51.07	24			

Since the ANOVA outcome is significant, a *post hoc* test will be carried out using Fisher's LSD test because each treatment is treated as stand-alone. Details of how LSD test is conducted using Equation (1) was explained earlier

Table no 10. Least significant difference (LSD) test for the mean difference of leaf counts across treatments

Fisher's LSD Test	Mean Difference	95% CI of difference	LSD ($t_{.95/4}$)	p	t-value	LSD<Mean Difference
T0 vs.T1	-0.46	-1.314 to 0.3940	0.735	0.2091	1.496	No
T0 vs.T2	0.08	-1.035 to 1.195	0.735	0.8519	0.199	No
T0 vs. T3	0.62	-0.09544 to 1.335	0.735	0.0739	2.406	No
T0 vs. T4	1.64	0.5102 to 2.770	0.735	0.0157	4.030	Yes

The results of the *post hoc* LSD testing shows that there are no significant differences in leaf counts between T0 and T1, T2 and T3 treatments except for T0 versus T4. Hence, hypothesis H2 is partially supported, indicating that a significant difference in the mean of plant leaf counts exist at higher crude oil pollution level when compared with the control without amendment and pollution.

Difference in Mean Wet and Dry Weight of the Plants

The wet and dry weight of a plant can assist in assessing the overall health of the plant. These parameters can provide growth performance information on the response of the plant to various doses of crude oil in the experimental soil. Table no 11presents both wet and dry weights for the 2nd and 8th week after planting. results in Table no 11 shows that the treatment in T1. The recorded the highest wet weight of 94.32 kg, implying that the amendment improved the growth of the plant. For the polluted soils, the highest wet weight of 82.55 kg was recorded for T2, which had 100 ml of the pollution while T4 with 400 ml of the pollution had the lowest wet weight. The dry weights of the plants across the treatments are relatively equal for T0 (21.49kg), T1 (20.06kg) and T2 (21.13kg), while the plants in T4 had the lowest dry weight. The results further suggest that increasing the volume of the pollutant retards plant growth and performance, which corroborates earlier position of this study.Figure no 7 represent both wet and dry weights as bars for ease of comparison.

Table no 11. Wet and dry weights of harvested plants at WAP 2 and WAP 8

WAP	Wet and Dry Weights (g)									
	T0		T1		T2		T3		T4	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
2	3.30	0.8	2.63	0.56	1.09	0.28	1.83	0.46	0.51	0.16
8	174	42.188	186	39.562	164	41.978	134	33.728	106	33.49
Mean	88.65	21.49	94.32	20.06	82.55	21.13	67.91	17.09	53.25	16.83

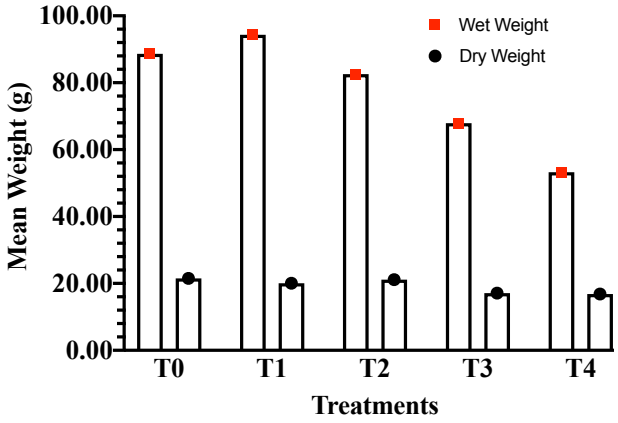

Figure no 7. Wet and dry weights of plant at WAP 8 in different soil treatments

Level of Hydrocarbon Utilization Bacteria (HUB)

The level of the activity of microbes and fungi during the experiments is presented in Table no 12, Figure no 8 and Figure no 9. The results suggests that the increased in the activities of heterotrophic bacteria and fungi in T1 is because of the amendment. Hence, it can be said that the amendment improved biodegradation across the treatments except for the soil in T0 which was not amended with leaf litters of *Mangifera indica*. The highest value for HUB count was recorded in T4 (7.2E05 cfu/g); suggesting that increasing the volume of the pollutant can also increase the bacteria count in the soil. The highest level of THF was also recorded in T4 (7.5E04 cfu/g). When compared with T0, higher level of biodegradation can be observed in all the treatments with the amendment which increased the needed nutrient for the activities of heterotrophic bacteria.

Table no 12. Bacteria and Fungi counts in the soil at WAP 2 and WAP 8 across treatments

Samples	THB (10^6CFU/g)		TF(10^4CFU/g)		HUB(10^5CFU/g)		HUF (10^4CFU/g)	
	WAP 2	WAP 8	WAP 2	WAP 8	WAP 2	WAP 8	WAP 2	WAP 8
T0	1.8	4.8	1.6	4.8	3.3	6.8	1.2	4.2
T1	2.4	5.4	2.2	5.2	2.7	5.7	2.0	5.0
T2	2.5	5.5	3.2	6.2	3.1	6.1	2.3	5.3
T3	4.9	7.9	3.0	6.9	3.7	6.7	3.0	6.0
T4	3.6	6.6	4.5	7.5	4.2	7.2	2.4	5.4

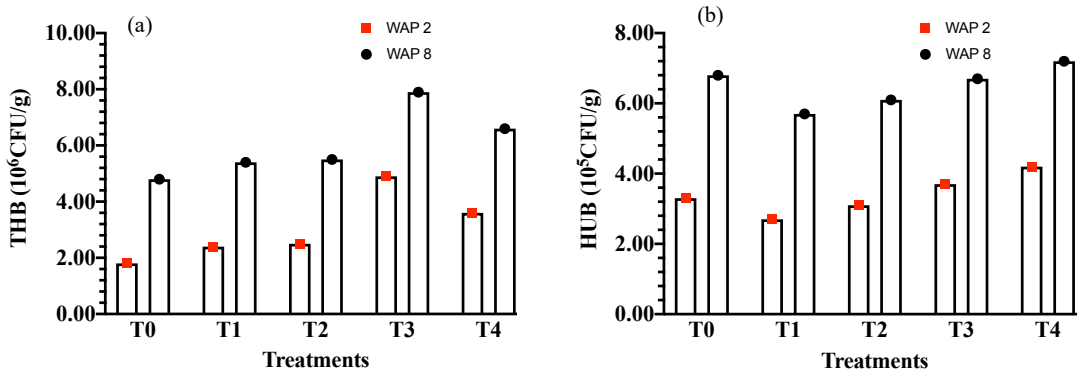

Figure no 8. Bacteria countat WAP 2 and WAP 8 across soil treatments. (a) Total heterotrophic hydrocarbon bacteria (THB), (b) Hydrocarbon utilization bacteria (HUB).

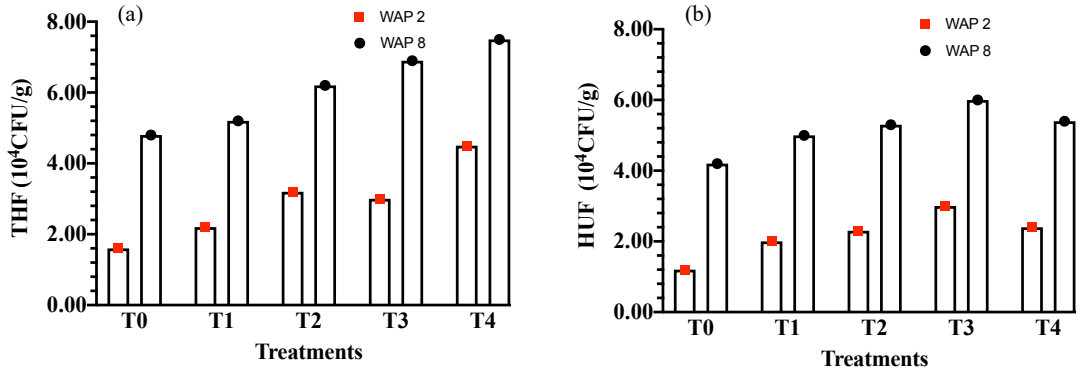

Figure no 9. Fungi count at WAP 2 and WAP 8 across soil treatments. (a) Total heterotrophic hydrocarbon fungi (THF), (b) Hydrocarbon utilization fungi (HUF).

Level of Physicochemical Properties

There is an increase in the level of potassium at WAP 2 and WAP 8 in the amended soil (T1) compared to the control soil (T0) within the same period in Table no 13.This increase is attributed to the release of potassium into the soil from the leaf litters of *Mangifera indica*. While the increase in potassium was 9.09% from WAP 2 to WAP 8 in T0, the treatments recorded the following percentage increase in potassium: T1 (8.97%),T2 (9.09%),T3 (8.82%), and T4 (8.78%). Crude oil pollution depletes the level of potassium in the soil[17], but the change in the level of potassium is relatively stable, indicating that the amendment positively improved the potassium level. The same is true for phosphorus, calcium, and magnesium. Thus, the increase in the effective cation exchange capacity (ECEC) from WAP 2 to WAP 8, in the polluted soil can be attributed to the presence of the amendment, because crude oil reduces the ECEC level in the polluted soils[17].The acidity level also increased minimally in T4 (12.5%)across the treatments Figure no 10, instead of the higher increase in acidity (up to 4%) as observable in crude oil polluted soils when compared with the control[17]. No extractable lead and nickel were detected in the plants grown in the control soils T0 and T1without the pollution (Table no 14). Nickel was detected only in the plants grown in the soil with the highest volume of pollutant T4. While the level of extractable lead in the plants increased with the volume of the pollutant, which is also supported in the literature [17]. Hence, the consumption of cobs harvested from crude oil polluted soils should be discouraged to prevent ingestion of heavy metals.

Table no 13. Physicochemical properties of the soils across different soil treatments

Treatments	PH (1:1) H_2O	O (%)	N (%)	P (mg/g)	Ca (Cmol/kg)	Mg (Cmol /kg)	K (Cmol /kg)	Acidity (Cmol /kg)	ECEC (Cmol/kg)
2 Weeks After Planting (WAP)									
T0	5.250	2.360	0.245	40.660	21.015	1.155	0.154	0.160	23.136
T1	5.180	2.178	0.226	48.095	21.070	1.124	0.156	0.320	23.106
T2	4.980	2.251	0.233	46.044	18.917	1.070	0.165	0.320	21.251
T3	4.930	1.888	0.196	56.042	18.942	1.117	0.136	0.320	21.176
T4	5.240	2.468	0.256	47.838	21.073	1.234	0.148	0.240	23.456
8 Weeks After Planting (WAP)									
T0	5.580	3.085	0.320	34.708	23.379	1.276	0.168	0.120	25.627
T1	5.506	2.847	0.295	41.054	23.440	1.242	0.170	0.240	25.594
T2	5.293	2.943	0.304	39.304	21.045	1.182	0.180	0.240	23.539
T3	5.240	2.468	0.256	47.838	21.073	1.234	0.148	0.240	23.456
T4	5.569	3.226	0.334	40.835	23.444	1.363	0.161	0.180	25.981

Table no 14. Total extractable lead (Pb) and nickel (Ni) in the plant

Treatments	Pb (mg/kg)	Ni (mg/kg)
T0	N.D	N.D
T1	N.D	N.D
T2	2.42	N.D
T3	2.56	N.D
T4	3.66	0.178

N.D: None detected

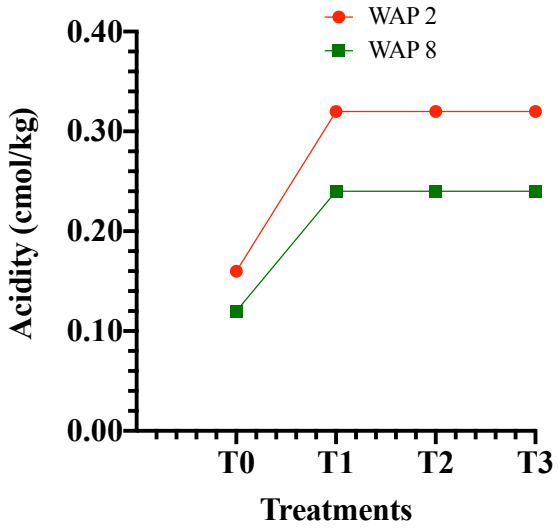

Figure no 10. Acidity level of the soil WAP 2 and WAP 8 across the experimental treatments

Discussions

The study recorded no significant difference in the mean plant height across the amended and polluted treatments, but there was significant reduction in the leaf counts for the treatment with 400 ml of the pollutant. Robustness in plant growth was observed in the unpolluted control treatment with the amendment through the recorded wet weights, suggesting a positive influence of *Mangifera indica* on plant growth. The activities of heterotrophic utilization bacteria also increased across the treatments with the pollution and amendment, indicating accelerated biodegradation because of the availability of nutrient, which agrees with previous findings in bioremediation[18]. The availability of nutrients for biodegradation was aided by the amendment carried out on the soil using the leaf litters of *Mangifera indica.* The level of potassium for the unpolluted amended soil was above that of the control, also suggesting a positive effect of *Mangifera indica* on the potassium level. The amendment enabled the level of potassium level to remain above the critical value of 0.2mg/kg (5.13E-04cmol/kg) for plant productivity in literature[13]. The average pH level also remain relatively high with a reduced acidity effect across all the polluted treatments when compared with the control [19].Also, the level of nickel and lead extracted from the maize plant in the polluted treatments indicated values of 2.42mg/kg to 3.66mg/kg, and 0.178mg/kg for nickel in the treatment with the highest volume of the pollutant. This shows that the uptake of heavy metals by maize plant is possible, which also corroborates a previous study elsewhere[17]. While nickel is essential in small quantity for healthy body metabolism, higher uptake into the human body can be toxic to human health [20], with a cancer-inducing effect[21]. The indicated value of 0.178 mg/kg show that the amount of nickel present in the experimental plant was lower than the highest level of tolerable level of Ni (7.6 mg/kg) in the body[20]. Lead (Pb) poisoning is dangerous to human health with high cancer risk. Lead exposure can affect nearly every system in the body, to the extent that 0.1mg/kg in human blood can lead to Pb poisoning[22].Since the minimum level of Pb extracted (2.43mg/kg) is higher than the maximum tolerable in human blood, the cobs harvested is not fit for human consumption. As a result of the poising effect of the Pb, the experimental plants were properly disposed after dry weighing in line with the disposal regulations of the Centre for Ecological Studies, University of Port Harcourt.

IV. Conclusion

This project work was carried out to demonstrate the positive effect of the leaves of Mangifera indica on crude oil polluted soil. The literature review gave indications of the effect of crude oil on polluted sites and the impact of plant growth. The experimental set-up was designed with 24.60 kg soil amended with 0.15kg leaves of Mangifera indica except for the control. Another control with 0 ml of the polluted was set up to initially establish the effect of the amendment. The growth of zea mays in three polluted soils with leaf litters of Mangifera indica were compared with the control without the pollutant. The measured variables include plant heights, leaf counts, wet and dry weight of the harvested plant, bacteria and fungi activities and physicochemical properties of the soils. The results indicated that the plants grown in the crude oil polluted soils had no significance reduction in plant leaf counts except for the soil with the highest volume of the pollutant. The experimental evidence in this study suggests that leaf litters of Mangifera indica can aid biodegradation, increase the level of potassium available for plant performance and improve the effective cation exchange in the soil. This study recommends the use of leaf litters of Mangifera indica in bioremediation of oil impacted soils in

the Niger Delta while waiting for enhanced remediation activity from the responsible government institutions to enhance early return of the impacted soils to their natural state. However, it is important to note that crude oil properties vary from location to location, hence for optimum field results, a laboratory experimental trial of the impacted soil with the amendments should be carried out, and the results used to indicate the weight of leaf litters of Mangifera indica needed to amend the estimated concentration of crude oil pollution in the impacted soil.

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