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Physicochemical Changes in Maize Plant (Zea Mays) Grown on Contaminated Soil Exposed to Sawdust Treatment

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ABSTRACT: The potential effects of sawdust for bioremediation of growing maize grown on crude oil contaminated soil was evaluated in this study. The experiment was divided into 3 groups control (soil without crude oil), polluted (soil with varying concentrations of crude oil), and sawdust treated (polluted soil with 50g sawdusts). The polluted and sawdust treated regime received four levels of treatments with crude oil (25g, 50g, 75g and 100g). Viable seeds of maize were grown on the soil beds for 35 days to assess the % germination, % survival, stem height, chlorophyll a and b, oxidative stress indicators and selected macronutrients using standard methods. Data obtained shows that the growth performance of the sawdust treated samples containing low concentration of crude oil was better in comparison to polluted groups but less than that of control. Sawdust remediation helped in overcoming the growth inhibition due to pollution to some extent. Significant increase ($p<0.001$) in the activity of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) were observed in the sawdust treated regime when compared with the polluted and control groups. The result also showed a significant decrease ($p<0.001$) in Malondialdehyde levels and a non- significant increase ($p>0.05$) in macronutrients assessed in the remediated regime when compared with the polluted regime. Remediation of the polluted soil with sawdust relieved the inhibitory effect of crude oil on the plant growth particularly in groups containing lower concentrations of crude oil.

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The soil environment is increasingly exposed to changes resulting from indiscriminate disposal of oil-based contaminants such as crude oil. Environmental pollution from these oil activities in a major oil producing country as Nigeria is inevitable (Agbogidi and Eshegbeyi, 2006). The procedures been used in the processing and storage of crude oil has resulted in the abuse of man's environment directly or indirectly. The large quantities of oil reportedly lost to agricultural lands reduces the soil's fertility (Abii and Nwosu, 2009) and also provides to the soil excessive hydrocarbon which affects soil enzymatic activities. All these negative effects of soil pollution with petroleum derivatives threaten human health and that of the organisms that are dependent on the soil (Aboribo, 2001). The adverse effects of crude oil on soil cannot be overemphasized. To improve crude oil polluted soils for enhanced and sustainable ecosystems, several effort have been employed in the remediation of the polluted soils (Erdogan and Karaca, (2011), Okoh (2006). Nevertheless, these methods are grossly inadequate and ineffective, and may even result in further contamination of the environment (Steven, 1991). Therefore, protecting the plants with vegetative components that form parts of the environment becomes necessary (Lin and Mendelssohn, 2004). As a means of remediating soils polluted with petroleum derivatives or crude oil, soil amendments such as sawdust are added to the soil in order to increase the ability of the soil matrix to supply biologically available water and nutrients to microorganisms that are capable of degrading the target compound (Tanee and Akonye, 2009). Sawdust consists chiefly of organic materials synthesized by the tree from water, and from carbon dioxide and oxygen of the air. This study therefore seeks to investigate the possible effects of sawdust as an alternative remediating agent on crude oil polluted soil by determining the influence on growth, macronutrient and oxidative stress parameters on maize plant (*zea mays*).

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MATERIALS AND METHODS

Source of plant material and soil: Maize seeds (*Zea mays*) were purchased from Uselu Market Benin City, Edo state, Nigeria. Sandy loamy soil was collected in sterile plastic containers at a depth of 10- 20 cm from a farmland in Oluku in Benin-City while Bonny Light crude oil was sourced from Nigerian National Petroleum Corporation (NNPC), Warri, Delta State.

Experimental Design: The entire experiment was divided into three (3) groups namely control groups, polluted group and sawdust amended groups. Each treatment has 3 replicates.

Control- control group comprises of control 1(C1) and control (C2)

C1 consists of 4 kg soil sample only
C2 consist of 4 kg soil sample +50g sawdust

Polluted groups- This consist 4kg soil sample mixed with 25g, 50g, 75g and 100g of crude oil only and labeled as PI, P2, P3 and P4 with the contamination level corresponding to each treatment respectively i.e.

P1= 4kg soil + 25g crude oil
P2= 4kg soil+ 50g crude oil
P3=4kg soil +75g crude oil
P4=4kg soil +100g crude oil

Sawdust treated groups- 4kg soil was also mixed with 25g, 50g, 75g and 100g of crude oil. These were allowed to stand for 7days after which 50g of sawdust were added to each treatment level of crude oil and labeled as R1, R2, R3 and R4 i. e

R1= 4kg soil+25g crude oil+ 50g sawdust
R2=4kg soil+50g crude oil+ 50g sawdust
R3= 4kg soil+75g crude oil+ 50g sawdust
R4= 4kg soil+100g crude oil+50g sawdust

This remediated set-up was allowed to stand for 7 days before planting of seeds.

Seed viability test: Maize seeds were soaked overnight in clean water. This was done to check for viable seeds. Only viable seeds were used for this study.

*Planting and Harvesting***:** Five viable maize seeds, selected by simple flotation method were planted, evenly spaced, in each bag. The experimental setup was immediately transferred to a greenhouse to simulate a natural environment for planting. Seeds were then supplied with 50mls of water on a daily basis to ensure germination of the seeds. At the end of 35 days, maize plant from various groups were harvested. The root, stem and leaves of the plant were subsequently used for vegetative, chemical and biochemical analyses.

*Determination of growth parameters***:** The growth parameters investigated were: germination and survival percentage and stem (shoot) height. The percentage of germination of seeds was determined after 7(seven) days of planting while survival percentage of seedlings were computed after 35 days. Germination percentage was calculated as:

$$\% \text{ Germination } = \frac{NSG}{NSP} x100$$

NSG = Number of seedlings that germinated per bag;
NSP = Number of seeds planted per bag

Stem (Shoot) height determination was done by measuring the shoot of the plants in the bags from the soil level to the terminal bud using a meter rule. The rate of survival of the seedlings of the plants in each treatment group was calculated by counting the number of seedlings that were standing after 35days.

Harvesting: At the end of the 35days the plants were harvested and split up into root and shoot systems. Leaves in each group were collected for chlorophyll content determination. Roots in each group were also collected and used for antioxidant enzymes estimation (catalase, peroxidase, superoxide dismutase) and malondialdehyde and also for macronutrients determination.

Determination of chlorophyll content: Chlorophyll A and B content was measured according to the method of Dere *et al*., 1998. The amount of the pigment was calculated based on the formula of Lichtenthaler and wellburn, 1985

Determination of antioxidant enzymes and malondialdehyde levels: Assay for Malondialdehyde activity: Malondialdehyde contents of the roots were evaluated according to the method of Beuge and Aust, (1978).

Assay of superoxide dismutase activity: The roots were homogenized using 5mls of 0.05M carbonate buffer (pH 10.2) in a mortar. The homogenate was then centrifuged at 1000g for 10 minutes and the supernatant used for assay according to the method of Misra and Fridovich, (1972).

Assay of catalase activity: The roots were homogenized using 5.5mls of 0.05M phosphate buffer (pH 7) in a mortar. The homogenate was then

centrifuged at 1000g for 10 minutes and the supernatant used for assay according to the method of Cohen *et al*., (1970).

Assay of peroxidase activity: The roots were homogenized using 5.5mls of 0.05M phosphate buffer (pH 7) in a mortar. The homogenate was then centrifuged at 1000g for 10 minutes and the supernatant used for peroxidase assay according to the method of Chance and Maehly, (1995).

Determination of macronutrients (nitrogen, phosphorus, sodium, potassium and magnesium) in roots: Available nitrogen was ascertained using semi-micro Kjeldhal method (Bremner and Mulvaney,1982), the available phosphorus was determined according to the method of Bray and Kurtz, (1945), sodium and potassium, calcium values were read using atomic absorption spectrometry.

Statistical analysis: Data were expressed as mean ± SEM. The results were computed statistically (SPSS software package version 20). Analysis of variance and Tukey's multiple comparison tests was used to compare all treatment groups. Values of $p>0.05$ were considered non-statistically significant while Values of $p<0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

The result of germination rate (fig 1) shows that a high percentage rate of germination was seen in the sawdusts treated group containing high amount of crude oil (R3 and R4) that is 93.30% and 86.70% respectively when compared with the polluted groups (P3 and P4), 86% and 80% respectively this is because Some plants did not germinate. Inhibition of germination occurred as the concentration of crude oil increases indicating that at higher concentrations, crude oil exert toxic effects on the germination of seeds(Agbogidi *et al*., 2006). Chaineau, *et al*. (1997) has demonstrated in their investigations that the formation of oil coat on seeds could prevent the uptake of oxygen and water which are essential requirements for germination. The inverse relationship between the rate of germination and the concentration of crude of crude oil observed in this study is in conformity with earlier studies (Merckl *et al*., 2004; Salanitro *et al*., 1997; Onuh *et al*., 2008). The figure 1 shows a decrease in germination rates as the level of contamination increases in the polluted and remediated groups. The result of fig 2 shows that there was a low percentage survival rate in all groups treated with crude oil when compared to the controls. Although the sawdust treated groups showed a higher percentage rate of survival of 98%, 89%, 71%, 53% as against the 75%, 50%, 45% and 45% recorded in the polluted groups. This is possible as the sawdust may have supplied nutrients which are always limited in a crude oil polluted soil (Akujobi *et al*., 2011). Our findings also conforms to that reported by Oyedeji *et al*., (2012) who reported death of *C. mucunoides*, *C. brasilianum* and *S. capitata* grown in crude oil soils after six weeks of germination.

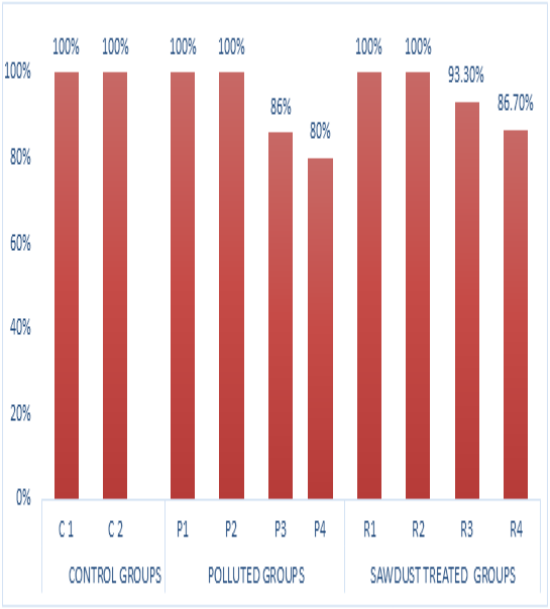

Fig 1: Germination rates of *Zea mays* seedlings grown on polluted and sawdust remediated soils Values represent mean ± standard error of mean in cm, n=3.

The result of mean stem height (fig 3) shows that a significant decrease in stem height was seen in the sawdust treated and polluted groups (except for R1) when compared with the control.

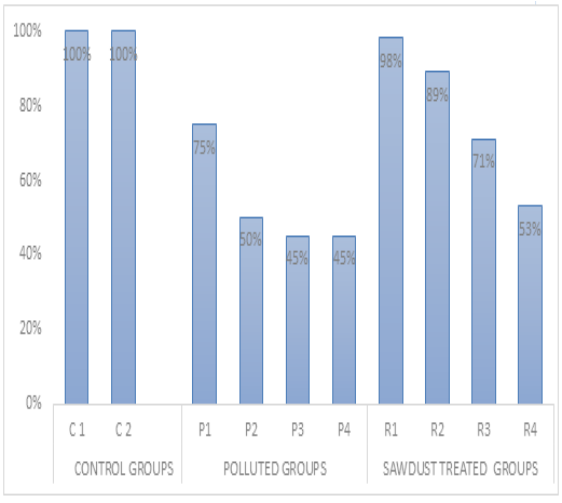

Fig 2: survival rates of *Zea mays* seedlings grown on polluted and sawdust remediated soils Values represent mean ± standard error of mean in cm, n=3

Although increase in Stem height was recorded in the sawdust treated groups containing lower concentration of crude oil (R1 and R2) when compared to the polluted groups but was less than those of control (Figure 3). Gill *et al.*(1992) observed a positive relationship between degree of growth retardation and concentration in crude oil contaminated soil. Plant height as a plant growth parameter and yield index is important for maize. This is because; the taller a plant, the more the amount of light energy absorbed by the plant leading to a higher the rate of photosynthesis and therefore a higher yield from the plant. Improved vegetative yield of plants in crude oil remediated soil has also been reported (Akinpelumi and Olatunji, 2015; Tanee and Albert, 2011; Jonathan *et al*., 2013).

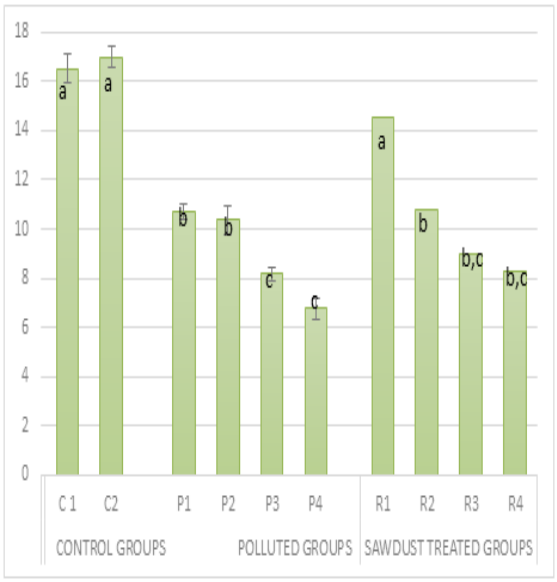

Fig 3: Mean stem height of maize plants grown on crude oil contaminated and sawdust remediated soils Values represent mean ± standard error of mean in cm, n=3. Values with the same alphabet are not significantly different (p>0.05), while different alphabet is significantly different (p<0.001).

Chlorophyll is vital for photosynthesis. However, stress in a plant may cause loss of chlorophyll. The result of our study showed a significant decrease in chlorophyll a and b contents (except for P1 and R1) for both polluted and sawdust treated groups when compared with the controls (table 1).

This therefore shows that treatment of polluted soil with sawdusts did not alleviate the loss of chlorophyll caused by the stress factor crude oil.

Reduction in chlorophyll content as an indication of environmental contamination has been reported for maize seedling grown on hydrocarbon polluted sites (Agrawal, 1992; Sharifi *et al*., 2012; Opeolu, 2000).

Table 1: Chlorophyll contents of maize plant grown on crude oil contaminated and sawdust remediated soils.

Groups		Chlorophyll a	Chlorophyll b
Control	C 1	28.21 ± 0.012^{a}	9.12 ± 0.46^{a}
Groups	C 2	28.90 ± 0.06^{a}	9.55 ± 0.34^{a}
Polluted	P1	25.35 ± 0.258^{b}	$6.37\pm1.34^{a,b}$
Groups	P2	24.59 ± 0.29^{b}	1.57 ± 0.54^{c}
	P3	14.90 ± 1.04^{c}	$4.09\pm0.53^{b,c,d}$
	P4	13.82 ± 0.09^{c}	$2.93\pm0.47^{b,c,d}$
Sawdusts	R1	28.12 ± 0.10^{a}	$7.15\pm0.85^{a,b,d}$
Treated	R2	25.63 ± 0.28^{b}	$2.33\pm0.81^{c,d}$
Groups	R3	16.40 ± 0.12^{c}	$4.91\pm0.66^{b,c,d}$
	R4	14.09 ± 0.39^{c}	$3.02\pm0.36^{b,c,d}$

F value = 270.3; 16.42; P value: = .0001; <0.0001; Values represent mean ± standard error of mean (in µg/ml). Values with the same alphabet are not significantly different (p>0.05), while different alphabet is significantly different (p<0.001).

The result of oxidative stress marker assay (table 2) shows that variations in antioxidant enzyme activities were observed in this study. Catalase (CAT) activity was observed to be significantly increased (P< 0.001) in the polluted treatment as the concentration of crude oil was increased however the activities of CAT was markedly elevated (P<0.001) in groups treated with sawdust when compared with the polluted group. In environmental stress conditions, higher activity of CAT is important for plants to tolerate stresses. Therefore, the increase in catalase activity observed in this study may infer a role of this enzyme in the protection of root tissue against oxidative damage. In this study, Significant increase (P<0.001) in superoxide dismutase (SOD) were observed in the polluted groups containing higher concentration of crude oil(75g and 100g) (Table 2) whereas significant increase(p< 0.001) in peroxidase (POX) activities was observed at all concentrations of crude oil in the polluted groups when compared with the controls. However the activities of these enzymes were markedly higher in the remediated groups. Mafakheri *et al*.,(2011) stated that even in an ideal growth environment, many metabolic processes produce reactive oxygen species(ROS) in plants, such as superoxide (O_2-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (-OH)(Sudhakar *et al*., 2001). Meanwhile, plants possess effective antioxidant defense systems for scavenging ROS (Zhu *et al*., 2004). The intercellular level of H_2O_2 produced under stress conditions is controlled by catalases and peroxidases. SOD is considered as a key enzyme in the antioxidant defense system as it regulates the concentration of O_2- and H_2O_2.The graph shows that the antioxidant activity of the plant was seen to be higher in the sawdust treated groups when compared with the polluted groups. Measurement of malondialdehyde levels in tissues is widely used as an indication of lipid peroxidation (Lin and Kao, 2000).

Table 2: Level of oxidative stress markers (superoxide dismutase, catalase, and peroxidase) in roots of maize plant (Zea mays) grown on crude oil and remediated soils

Groups		Superoxide dismutase (units/mg protein)	Catalase(units/mg protein)	Peroxidase(units/mg protein)
Control Groups	C 1	1.438±0.009[a]	0.57±0.005[a]	0.78±0.005[a]
	C 2	1.442±0.004[a]	0.60±0.338[a]	0.80±0.003[a]
Polluted Groups	P1	1.371±0.006[a]	0.87±0.04[a]	0.966±0.020[b]
	P2	1.562±0.018[b]	1.09±0.065[a]	1.214±0.008[c]
	P3	1.965±0.0003[c]	1.23±0.02[a]	1.339±0.026[d]
	P4	1.971±0.0004[c]	1.32±0.006[a]	0.780±0.005[a]
Sawdust Treated Groups	R1	1.863±0.013[d]	2.889±0.339[b]	1.083±0.027[e]
	R2	1.705±0.034[e]	3.118±0.002[b]	2.305±0.003[f]
	R3	1.989±0.012[e, f]	3.22±0.062[b]	2.368±0.012 [f]
	R4	1.991±0.006[e, f]	2.51±0.069[b]	2.521±0.027[g]

F value = 338.74; 46.832; 1766.0; P value = <0.0001; <0.0001; <0.0001; Values represent mean ± standard error of mean, n=3; Values with the same alphabet are not significantly different (p>0.05), while different alphabet is significantly different (p<0.001).

Lipid peroxidation in membranes results in loss of fluidity, fall in membrane potential, increased permeability to H^+ and other ions and eventual rupture leading to release of cell and organelle content (Gutteride, 1995). Malondialdehyde levels were significantly elevated (p<0.001) (Table 3) as a result of exposure to crude oil signifying perturbation of physiological balance.

Table 3: Malondialdehyde (MDA) concentration of maize plants grown on contaminated soil and sawdust remediated soil

Groups		MDA (mMol/gmfresh root)
Control Groups	C 1	0.00854±0.0002[a]
	C 2	0.00860±0.0008[a]
Polluted Groups	P1	0.1285±0.00025[b]
	P2	0.1352±0.00011[c]
	P3	0.1572±0.00080[d]
	P4	0.1777±0.0007[e]
Sawdust Treated Groups	R1	0.0754±0.001[f]
	R2	0.050±0.0005[g]
	R3	0.0544±0. 0020[g]
	R4	0.033±0.0010[h]

F value = 4755.1; P value = <0.0001; Values represent mean ± standard error of mean, n=3 (in mMol MDA/gm fresh root)
Values with the same alphabet are not significantly different (p>0.05), while different alphabet is significantly different (p<0.001)

However, a significant decrease in malondialdehyde level was observed in the sawdusts treated groups when compared with the polluted groups signifying that the sawdusts helped reduce the formation of lipid peroxidation byproducts. The result of mineral contents analysis is shown in table 4. The result shows that a significant decrease (P<0.001) in the levels of sodium, potassium, and phosphorus was observed in both sawdust treated and polluted groups when compared with the controls (Table 4). Also, a significant decrease was also observed for calcium (except for R1) and a non -significant decrease in nitrogen for sawdust treated groups and polluted groups when compared with the controls. However, there was significant increase in sodium, potassium, and calcium levels in sawdust treated group contaminated with 25g crude oil (R1). The significant increases in these macronutrients seen in this group may come from the added sawdust. Akujobi, (2011) observed that sawdust supplied nutrients to plants which are always limited in crude oil polluted soil.

Table 4: Mineral content (Na, K, Ca, N, and P) of *Zea mays* grown on contaminated and remediated soil.

Groups		Na (mg/kg)	K (mg/kg)	Ca(mg/kg))	N_2 (mg/kg)	P (mg/kg)
Control Groups	C 1	77.65±0.55[a]	96.93±0.385[a]	18.73±0.10[a]	0.835±0.05[a]	31.95±0.55[a]
	C 2	77.04±0.44[a]	97.50±0.25[a]	18.78±0.28[a]	0.830±0.002[a]	31.96±0.08[a]
Polluted Groups	P1	26.39±0.3[b]	11.90±0.07[b]	14.61±0.49[b]	0.415±0.005[a]	0.17±0.001[b]
	P2	18.98±0.07[c]	8.81±0.11[c]	11.74±0.53[c]	0.535±0.05[a]	0.11±0.0005[b]
	P3	7.98±0.105[d]	5.24±0.18[d]	11.0±0.495[c]	0.620±0.01[a]	0.112±0.002[b]
	P4	7.19±0.09[d]	8.740±0.05[c]	4.67±0.49[d]	0.705±0.006[a]	0.08±0.001[b]
Sawdust Treated Groups	R1	29.73±0.79[e]	15.20±0.22[e]	18.40±0.65[a]	0.84±0.014[a]	2.75±0.05[c]
	R2	18.99±0.17[c]	10.05±0.49[c]	11.94±0.48[c]	0.595±0.02[a]	1.48±0.03[d]
	R3	8.33±0.41[d,f]	6.51±0.31[d]	11.52±0.06[c]	0.500±0.02[a]	1.16±0.04[d]
	R4	7.57±0.02[d,f]	9.37±0.17[c]	6.695±0.12[e]	0.795±0.03[a]	1.11±0.01[d]

F value=5181.6; 4755.1; 135.1; 0.6255; 4755.1; P value = <0.0001; <0.0001; <0.0001; 0.7621; <0.0001; Values represent mean ± standard error of mean, n=3; Values with the same alphabet are not significantly different (p>0.05), while different alphabet is significantly different (p<0.001).

*Conclusion***:** This study has shown that crude oil soil may have adverse effects on plant growth and that Sawdust augmentation of the soil relieved the inhibitory effect of crude oil on the plant growth only in groups containing lower concentrations of crude oil

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