Cow Dung Augmentation Remediates Crude Oil Contaminated Soil Planted With Maize Seedlings

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ABSTRACT

Aim: To evaluatethe effect of cow dung augmentation on the growth, antioxidant enzymes activity and the nutrient status of maize seedlings grown in crude oil contaminated soil.

Procedure: The maize seeds were purchased from Mile 12 market in Lagos State and soaked overnight in clean water, sufficient to totally submerge all the seeds. Floating seeds were deemed to be non-viable and were discarded while the submerged seeds were considered to be viable and were used for planting.5 kg of loamy soil was weighed into each of the polythene bags used. The positive control group consisted of soil without any treatment with crude oil. The four negative control groups comprised of 5kg of soil treated with 25ml,50ml,75ml and 100ml of crude oil respectively while the experimental groups consisted of 5kg of soil without crude oil augmented with 10g of cow dung,5kg of soil with 25ml of crude oil augmented with 25g of cow dung,5kg of soil with 50ml of crude oil augmented with 50g of cow dung,5kg of soil with 75ml of crude oil augmented with 75g of cow dung and 5kg of soil with 100ml of crude oil augmented with 100g of cow dung. Each of the bags was transferred to the greenhouse to simulate an almost natural environment. The plants were left to grow for 56 days (eight weeks) during which their percentage survival,stem height, chlorophyll number, elemental nutrient analysis as well the antioxidant activity of the plants including changes in the total petroleum hydrocarbon were determined.

Results: Results revealed that the augmentation of crude oil contaminated soils led to a significant increase ($p<0.05$) in soil nutrients as well as antioxidant activity with respect to the non simulated contaminated soils.It was observed that there was a significant increase ($p<0.05$) in the growth performance of the maize seedlings in the cowdung simulated soils compared to the non augmented ones. There was also an observed significant reduction($p<0.05$) in the concentration of total petroleum hydrocarbon in cow dung augmented soil samples

Conclusions: The research results which indicated the petroleum hydrocarbons biodegradable ability of cow dung manure with improvement in soil nutrients in crude oil contaminated soils.

Keywords: Remediation, Petroleum Hydrocarbon, Crude Oil, Contamination, Soil Simulation

INTRODUCTION

Crude oil (petroleum) is a complex mixture of hydrocarbons that form from the partial decomposition of biogenic materials. It is the largest and most important source of hydrocarbons [1] and it varies in appearance and composition from one oil kind to another[2]. Crude oil when distilled yields a great variety of products which include petrol, kerosene, diesel etc.[3].

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The threat to the natural environment caused by crude oil product due to land disposal of waste, leakage from storage tanks and pipeline during distribution process as well as by car and railway transport and petrol station is rapidly increasing [4],[5],[6],[7].Oil spillage may result because of faults at any stage of production and transportation of crude oil [8].

Crude oil not only modifies the physico-chemical properties [5]and biological properties of the soil [9],[10] but also contributes to limitation of the productive ability of aerable crops.It is known that these compounds are able to affect the quality of surface and ground water and that these products are potentially dangerous for animals and human health [11].

Nigeria is a major producer and exporter of crude petroleum oil as well as an important agricultural nation in the West African sub-region [12],[13].The continuous exploration, production, processing of crude oil and its transportation exposes the environment to constant threat of oil pollution [12].Oil pollution whether acute or chronic, has deleterious effects on agricultural lands and hence significant effect on plant growth[13],[14],[15]. Crude oil spillage on soil generally retard plant growth [16],[17] reduces aeration by blocking air space between soil particles hence create condition of anaeriobiosis [18] and causes root stress in plant which also reduces leaf growth[19]. An important consequence of stress in plants is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radicals (OH^-) particularly in chloroplast and mitochondria. Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) for protection against the damaging effect of ROS [20].

Maize (*Zea mays*) is an important food fodder and industrial crop in the world [21]. It is second to wheat in the world's cereal production. In Nigeria, Maize is a major food and industrial crop grown both commercially and at subsistence level by most farmers [22]. As one of the cheapest source of food energy, maize plays a major role in meeting the rising consumption of both food and animal feed in developing countries [21]. Considering the Large quantities of oil reportedly lost to agricultural lands [23], it has become necessary to investigate the effect of oil spillage in agricultural land and crops grown in them.

It has been reported that plants and soil microbes compete for the little nutrient available in soils that are not rich like that polluted with crude oil thereby suppressing the growth of plants in such soils [24]. However it is generally known that when soils not suitable for plant growth are augmented with manure, growth and performance of plants in such soil are enhanced.[25] reported that addition of inorganic fertilizer in a crude oil polluted soil enhanced the growth and performance of *Brachiaria brizantha* in crude oil polluted soil. Although, the performance of plants as reported by Merkl and co–workers can be enhanced in crude oil polluted soil with fertilizer, it also increases the cost of crop production in crude oil polluted soil. It is therefore necessary to investigate the impact of organic manure like cow dung can make in the growth of crops in crude oil polluted soil. This is because such manure is cheaper and is more affordable to farmers than the inorganic fertilizers. This study was therefore carried out to investigate the impact of cow dung on remediation of crude oil contaminated site in relation to maize seedlings. The study covers scopes such as evaluation of effect of cow dung on the growth, antioxidant enzymes activity and the macro-element status of maize seedlings grown in crude oil contaminated soil as well as determination of total petroleum hydrocarbon contents of both crude oil simulated and non simulated experimental oil samples involved in the present study..

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Source of maize seeds

Maize seeds (*Zea mays*) were purchased from Mile 12 market in Lagos State, Nigeria.

2.1.2 Source of soil sample

The soil sample used for planting was obtained from a farmland in Ikorodu, Lagos State,Nigeria using a hand trowel at a depth of 0-20cm below soil surface, having no pollution history and devoid of hydrocarbon contamination.

2.1.3 Source of crude oil

Crude oil with specific gravity of 0.77g/cm3 was obtained from Nigerian National Petroleum Corporation (NNPC), Warri, Delta State.

2.1.4 Source of cow dung

Cow dung was collected from a cattle market along Mushin road, near the Nigerian Bottling Company Limited (makers of Coca Cola) plant in Oshodi, Lagos State, Nigeria.

2.2 Methodology

2.2.1 Seed viability test

The maize seedlings weresoaked overnight in clean water, sufficient to totally submerge all the seeds. Floating seeds were deemed to be non-viable and were discarded while the submerged seeds were considered to be viable and were used for planting.

2.2.2 Soil treatment

4 kg of loamy soil obtained from a farmland in Ikorodu, Lagos State, Nigeriawas weighed into each of the polythene bags used. Four groups consisted of soil which was treated with 25ml, 50ml, 75ml and 100ml of crude oil (w/w) per group respectively.The positive control was made up of soil, without any treatment with abioremediation agent (cow dung) or pollutionwith crude oil. The negative control consists of four

different groups of 5kg soil treated with varying volume of crude oil (25ml, 50ml, 75ml and 100ml) respectively in each of the groups.

Experimental Group	**Description**
Group 1 (Positive Control)	5kg of soil + 0g of crude oil
Group 2 (Negative Control)	5kg of soil + 25g of crude oil
Group 3 (Negative Control)	5kg of soil + 50g of crude oil
Group 4 (Negative Control)	5kg of soil + 75g of crude oil
Group 5 (Negative Control)	5kg of soil + 100g of crude oil
Group 6 (Experimental)	5kg of soil + 0ml of crude oil +10g cow dung
Group 7 (Experimental)	5kg of soil + 25ml of crude oil + 25g of cow dung
Group 8 (Experimental)	5kg of soil + 50ml of crude oil + 50g of cow dung
Group 9 (Experimental)	5kg of soil + 75ml of crude oil + 75g of cow dung
Group 10 (Experimental)	5kg of soil + 100ml of crude oil + 100g of cow dung

The cow dung manure samples collected for the purpose of bioremediation was first of all prepared by sun drying for five days followed by grinding, thorough mixing and sieving using a 2mm diameter mesh so to achieve homogeneity in terms of particle size and carefully stored in neat polythene bag for use. The treatment groups were prepared by adding 10g of cow dung to the positive control,25g of cow dung to negative control group simulated with 25ml of crude oil,50ml of cow dung to negative control group simulated with 50ml of crude oil,75g of cow dung to negative control group simulated with 75ml of crude oil and 100g of cow dung to negative control group simulated with 100ml of crude oil respectively.

Each of the groups consisted of three bags of soil. Five seeds of maize were planted in each bag, evenly spaced. The bags were transferred to the greenhouse to simulate an almost natural environment. The plants were left to grow for 56 days (8 weeks) during which it was adequately monitored on daily basis.

2.2.3 Percentage Germination

The number of seeds that germinated in each of the soil was monitored for percentage germination. This test is done, 14 days after planting the maize seedlings. Percentage germination is calculated using the formula;

$$\text{Germination \%} = \frac{\text{number of seedlings that germinated from soil} \times 100}{\text{Total number of seeds sown.}}$$

2.2.4 Percentage Survival

Thiswas done by counting the number of seedlings that will be standing after 35 days planting period. The percentage survival for each treatment is calculated using the formula;

$$\text{Survival \%} = \frac{\text{number of crops that are standing} \times 100}{\text{Number of seeds that germinated}}$$

2.2.5 Stem Height

The stem height was determined by measuring the length of the plant stem from the stem origin at the base of the soil to the stem apex

2.2.6 Antioxidant Enzyme Activity

2.2.6.2 Superoxide Dismutase

This was carried out according to the method of [26].

2.2.6.3 Catalase

This was carried out according to the method of [27].

2.2.6.4 Peroxidase

This was carried out according to the method of [28].

2.2.7 Chlorophyll number

This was determined according to the method of [29]

2.2.8Elemental Nutrient Analysis

Total nitrogen of fresh samples of the plant roots was determined by [30].Total phosphorus in fresh samples of plant roots was determined colorimetrically using ascorbic acid method described by [31].Total potassium in fresh plant root samples was determined by flame photometer [32].Total heavy metals in plant were determined by atomic absorption spectrophotometer[32].

2.2.9 Determination of Total petroleum Hydrocarbon (TPH):

Total petroleum hydrocarbon (TPH) was determined by measuring the amount of TPH left in the soil at weekly intervals in eight weeks of crude oil treatments and amendment in order to establish the effectiveness of the bioremediation process.This was carried out gravimetrically following standard method of TPH analysis according to [33].

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2.2.9. Statistical analysis

All the data were presented as mean±SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P \leq 0.05$ was considered to be significant.

3. RESULTS

Table 1.Effect of crude oil contaminated soil (COCS) and cow dung on the germination and survival of maize seedlings.

Experimental Groups	% Germination	% Survival
5kg of soil + 0g of crude oil	100.00	100.00
5kg of soil + 25g of crude oil	73.00	60.00
5kg of soil + 50g of crude oil	62.00	40.00
5kg of soil + 75g of crude oil	45.00	25.00
5kg of soil + 100g of crude oil	32.00	18.00

5kg of soil + 0ml of crude oil +10g cow dung	100.00	100.00%
5kg of soil + 25ml of crude oil + 25g of cow dung	84.00	80.00%
5kg of soil + 50ml of crude oil + 50g of cow dung	85.00	81.50%
5kg of soil + 75ml of crude oil + 75g of cow dung	85.60	80.60%
5kg of soil + 100ml of crude oil + 100g of cow dung	84.80	81.10%

The percentage germination and survival of the maize seedlings of the non cow dung treated groups were observed to decrease with increasing concentration of crude oil in the soil. Soils augmented with cow dung had higher germination and survival percentage compared to contaminated soils that were not augmented with cow dung with comparative volume of crude oil treatment groups.

Table 2.Effect of cow dung on crude oil contaminated soil (COCS) on the height and chlorophyll content of maize seedlings.

Treatment Groups	PlantHeight (cm)	Chlorophyll Content (µg/gFW)
5kg of soil + 0g of crude oil	16.50± 0.632[a]	37.34±0.457[a]
5kg of soil + 25g of crude oil	10.67±0.441[b]	29.72±1.447[b]
5kg of soil + 50g of crude oil	9.17± 0.384[c]	24.16±0.332[c]
5kg of soil + 75g of crude oil	8.17± 0.318[d]	19.00±1.114[e]
5kg of soil + 100g of crude oil	6.77± 0.578[e]	16.74±0.452[e]
5kg of soil + 0ml of crude oil +10g cow dung	16.57±0.240 [a]	37.40±0.460[a]
5kg of soil + 25ml of crude oil + 25g of cow dung	13.37±0.328 [f]	34.23±0.802[d]
5kg of soil + 50ml of crude oil + 50g of cow dung	13.30±0.306 [f]	34.53±0.456[d]
5kg of soil + 75ml of crude oil + 75g of cow dung	13.86±0.233 [f]	34.90±0.472[f]
5kg of soil + 100ml of crude oil + 100g of cow dung	13.76±0.168 [f]	34.95±0.364[f]

Results represent mean ± SEM

The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference ($P \geq 0.05$) between the values while different alphabets down a column reflect significant differences ($P \leq 0.05$) between the values.

The groups containing maize seedlings simulated with crude oil were observed to significantly decrease ($p \leq 0.05$) in their respective plant heights and chlorophyll contents compared to their respective experimental group treated with cow dung. However, it was established that the treatment of the crude oil contaminated soil groups with cow dung was dose independent as comparative amounts of cow dung used for similar volumes of crude oil produced results that were not significantly difference ($p \geq 0.05$) from the corresponding treated and non treated groups.Similarly the same results were obtained for studies involving the chlorophyll content.

Table 3.Effect of cow dung on crude oil contaminated soil (COCS) on the macro-element concentration (mg/kg) in the root of maize seedlings.

Treatment Groups	Potassium (K) (mg/kg)	Nitrogen (N) (mg/kg)	Phosphorus (P) (mg/kg)
5kg of soil + 0ml of crude oil	96.93±0.385 [a]	0.835±0.05 [a]	31.95±0.550[a]
5kg of soil + 25ml of crude oil	11.90±0.065[b]	0.415±0.05 [b]	0.17±0.001[b]
5kg of soil + 50ml of crude oil	8.805±0.105 [c]	0.335±0.05 [c]	0.15±0.008[c]
5kg of soil + 75ml of crude oil	5.240±0.030 [d]	0.290±0.01 [d]	0.12±0.002[d]
5kg of soil + 100ml of crude oil	2.740±0.050 [c]	0.205±0.05 [e]	0.08±0.001[e]
5kg of soil + 0ml of crude oil +10g cow dung	98.07±0.540 [a]	0.840±0.01 [a]	32.10±0.395[a]
5kg of soil + 25ml of crude oil + 25g of cow dung	68.62±0.375[e]	0.550±0.01 [a]	22.04±0.190[f]
5kg of soil + 50ml of crude oil + 50g of cow dung	68.06±0.325[e]	0.549±0.05 [a]	22.78±0.160[f]
5kg of soil + 75ml of crude oil + 75g of cow dung	67.97±0.290[e]	0.551±0.05 [a]	22.71±0.110[f]
5kg of soil + 100ml of crude oil + 100g of cow dung	67.86±0.205[e]	0.550±0.05 [a]	22.92±0.090[f]

Results represent mean ± SEM
The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference (P ≥0.05) between the values while different alphabets down a column reflect significant differences (P≤0.05) between the values

Table 3 shows the effect of cow dung on crude oil contaminated soil (COCS) on the macro-element concentration (mg/kg) in the root of experimental samples of maize seedlings. There was a significant reduction ($P \leq 0.05$)in the concentration of potassium, nitrogen and phosphorus in the crude oil contaminated soil samples compared to their respective cow dung augmented experimental groups. However, there was no significant difference ($P \geq 0.05$)between all the cow dung augmented experimental groups.

Table 4.Effect of cow dung on crude oil contaminated soil (COCS) on the heavy metal concentration (mg/kg) in the root of maize seedlings.

Treatment Groups	Copper (Cu) (mg/kg)	Lead (Pb) (mg/kg)	Nickel (Ni) (mg/kg)	Vanadium (V) (mg/kg)	Manganese (Mn) (mg/kg)
5kg of soil + 0ml of crude oil	0.025±0.385 [a]	0.008±0.050 [a]	0.194±0.550[a]	0.061±0.550[a]	0.205±0.550[a]
5kg of soil + 25ml of crude oil	0.091±0.065 [b]	0.385±0.050 [b]	0.276±0.001[b]	0.086±0.001[b]	0.231±0.001[b]
5kg of soil + 50ml of crude oil	0.145±0.105[c]	0.421±0.050[c]	0.389±0.005 [c]	0.092±0.005 [c]	0.240±0.005[c]
5kg of soil + 75ml of crude oil	0.260±0.030[d]	0.470±0.010[d]	0.462±0.002[d]	0.099±0.002[d]	0.251±0.002[d]
5kg of soil + 100ml of crude oil	0.340±0.050[c]	0.502±0.050[e]	0.558±0.001[e]	0.118±0.001[e]	0.264±0.007[e]
5kg of soil + 0ml of crude oil +10g cow dung	0.021±0.540[a]	0.005±0.010[a]	0.192±0.095 [a]	0.059±0.395[a]	0.203±0.395 [a]
5kg of soil + 25ml of crude oil + 25g of cow dung	0.062±0.375[e]	0.214±0.010[f]	0.239±0.090[f]	0.075±0.190[f]	0.221±0.190[f]
5kg of soil + 50ml of crude oil + 50g of cow dung	0.061±0.325[e]	0.215±0.050[f]	0.240±0.120[f]	0.074±0.160[f]	0.222±0.160[f]
5kg of soil + 75ml of	0.062±0.290[e]	0.214±0.050[f]	0.239±0.110[f]	0.075±0.110[f]	0.223±0.110[f]

crude oil + 75g of cow dung					
5kg of soil + 100ml of crude oil + 100g of cow dung	0.061±0.205^{e}	0.214±0.050^{f}	0.239±0.090^{f}	0.074±0.090^{f}	0.222±0.090^{f}

Results represent mean ± SEM
The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference (P $\geq$ 0.05) between the values while different alphabets down a column reflect significant differences (P$\leq$ 0.05) between the values

Table 4 shows the effect of cow dung on crude oil contaminated soil (COCS) on the heavy metal concentration (mg/kg) in the root of experimental samples of maize seedlings. There was a significant elevation (P$\leq$0.05)in the concentration of copper, lead, nickel, vanadium and manganese in the crude oil contaminated soil samples compared to their respective cow dung augmented experimental groups. However, there was no significant difference (P$\geq$0.05)between all the cow dung augmented experimental groups.

Table 5. Effect of cow dung on crude oil contaminated soil (COCS) on the activity of Catalase and Peroxidase in the root of maize seedlings.

Treatment Groups	Catalase (CAT) (unit/mg protein/min)	Peroxidase (POX) (unit/mg protein/min)
5kg of soil + 0ml of crude oil	0.1123±0.0112^{a}	0.9660±0.0010^{a}
5kg of soil + 25g of crude oil	0.9750±0.0373^{b}	0.8560±0.0204^{b}
5kg of soil + 50g of crude oil	0.8550±0.0653^{c}	0.7758±0.0460^{c}
5kg of soil + 75g of crude oil	0.7450±0.0205^{d}	0.6141±0.0077^{d}
5kg of soil + 100g of crude oil	0.6250±0.0056^{e}	0.5192±0.0256^{e}
5kg of soil + 10ml of crude oil +10g cow dung	0.1229±0.0224^{a}	0.9809±0.0076^{f}
5kg of soil + 25ml of crude oil + 25g of cow dung	0.1031±0.0224^{f}	0.9803±0.0257^{f}
5kg of soil + 50ml of crude oil + 50g of cow dung	0.1029±0.0630^{f}	0.9806±0.0102^{f}
5kg of soil + 75ml of crude oil + 75g of cow dung	0.1029±0.0131^{f}	0.9805±0.0037^{f}

5kg of soil + 100ml of crude oil + 100g of cow dung	0.1030±0.0037[f]	0.9804±0.0077[f]

Results represent mean ± SEM
The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference (P > 0.05) between the values while different alphabets down a column reflect significant differences (P< 0.05) between the values

Table 5 shows the effect of cow dung on crude oil contaminated soil (COCS) on the activity of Catalase and Peroxidase in the root of maize seedlings. There was a significant reduction ($P \leq 0.05$)in the concentration of catalase and peroxide in the crude oil contaminated soil samples compared to their respective cow dung augmented experimental groups. It was observed that the activity of catalase and peroxidase were volume dependent with regards to crude oil simulation. Increases in the volume of crude oil simulation of the experimental soils led to a significant reduction in the levels of catalase and peroxidase activities in the soils. However, there was no significant difference ($P \geq 0.05$)between all the cow dung augmented experimental groups.

Table 6. Effect of crude oil contaminated soil (COCS) and cow dung on the activity of Superoxide dismutase (SOD) and Malondialdehyde (MDA) content in the root of maize seedlings

Treatment Groups	SOD (unit/mg protein/min)	MDA (mmol MDA/mg FW)
5kg of soil + 0g of crude oil	1.438±0.0098 [a]	0.0885±0.00025[a]
5kg of soil + 25g of crude oil	1.100±0.0060 [b]	0.1285±0.00025[b]
5kg of soil + 50g of crude oil	0.862±0.0180 [c]	0.1352±0.00105[c]
5kg of soil + 75g of crude oil	0.765±0.0003 [d]	0.1572±0.00080[d]
5kg of soil + 100g of crude oil	0.671±0.0004 [e]	0.1767±0.00070[e]
5kg of soil + 0ml of crude oil + 10g cow dung	1.435±0.0370[a]	0.0883±0.00106[f]
5kg of soil + 25ml of crude oil + 25g of cow dung	1.346±0.0052 [f]	0.0991±0.00015[g]
5kg of soil + 50ml of crude oil + 50g of cow dung	1.345±0.0021 [f]	0.0992±0.02050[g]
5kg of soil + 75ml of crude oil + 75g of cow dung	1.346±0.0014 [f]	0.0991±0.00120[g]
5kg of soil + 100ml of crude oil + 100g of cow dung	1.344±0.0007 [f]	0.0992±0.01300[g]

Results represent mean ± SEM

The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference (P > 0.05) between the values while different alphabets down a column reflect significant differences (P< 0.05) between the values

Table 6 shows the effect of cow dung on crude oil contaminated soil (COCS) on the activity of superoxide dismutase and malondialdehyde in the root of maize seedlings. There was a significant reduction ($P \leq 0.05$)in the concentration of superoxide dismutasein the crude oil contaminated soil samples compared to their respective cow dung augmented experimental groups. It was observed that the activity of superoxide dismutase was volume dependent with regards to crude oil simulation. Increases in the volume of crude oil simulation of the experimental soils led to a significant reduction in the levels of superoxide dismutaseactivities in the soils. However, there was no significant difference ($P \geq 0.05$)between all the cow dung augmented experimental groups.

In contrast,there was a significant elevation ($P \leq 0.05$)in the concentration of malondialdhyde in the crude oil contaminated soil samples compared to their respective cow dung augmented experimental groups.However, there was no significant difference ($P \geq 0.05$)between all the cow dung augmented experimental groups.

Table 7: Changes in concentration (mg/kg) of total petroleum hydrocarbon (TPH) with time for eight weeks of remediation of crude oil treated soil and cow dung augmented soil.

Weeks After Planting	5kg of soil + 0g of crude oil	5kg of soil + 25g of crude oil	5kg of soil + 50g of crude oil	5kg of soil + 75g of crude oil	5kg of soil + 100g of crude oil	5kg of soil + 0ml of crude oil + 10g cow	5kg of soil + 25ml of crude oil + 25g of cow dung	5kg of soil + 50ml of crude oil + 50g of cow dung	5kg of soil + 75ml of crude oil + 75g of cow dung	5kg of soil + 100ml of crude oil + 100g of cow dung

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						dung				
1	**0**	**2150**	**2150**	**2150**	**2150**	**0**	**2150**	**2150**	**2150**	**2150**
2	**0**	**2231**±0.07^{a}	**2350**±0.05^{a}	**2411**±0.05^{a}	**2530**±0.01^{a}	**0**	**1802**±0.05^{a}	**1800**±0.05^{a}	**1801**±0.05^{a}	**18002**±0.05^{a}
3	**0**	**2290**±0.01^{b}	**2420**±0.05^{b}	**2460**±0.01^{b}	**2602**±0.02^{b}	**0**	**1650**±0.01^{a}	**1649**±0.02^{a}	**1651**±0.05^{a}	**1652**±0.04^{a}
4	**0**	**2320**±0.02^{c}	**2490**±0.02^{c}	**2513**±0.02^{c}	**2659**±0.07^{c}	**0**	**1542**±0.03^{a}	**1541**±0.01^{a}	**1539**±0.05^{a}	**1540**±0.03^{a}
5	**0**	**2342**±0.02^{d}	**2550**±0.01^{d}	**2564**±0.01^{d}	**2711**±0.02^{d}	**0**	**1420**±0.05^{a}	**1421**±0.04^{a}	**1422**±0.05^{a}	**1420**±0.02^{a}
6	**0**	**2350**±0.04^{e}	**2620**±0.05^{e}	**2613**±0.09^{e}	**2762**±0.05^{e}	**0**	**1301**±0.07^{a}	**1302**±0.02^{a}	**1299**±0.03^{a}	**1301**±0.05^{a}
7	**0**	**2411**±0.02^{f}	**2690**±0.03^{f}	**2671**±0.03^{f}	**2801**±0.01^{f}	**0**	**1181**±0.07^{a}	**1182**±0.01^{a}	**1180**±0.05^{a}	**1181**±0.05^{a}
8	**0**	**2452**±0.09^{g}	**2750**±0.01^{g}	**2720**±0.02^{g}	**2852**±0.03^{g}	**0**	**1021**±0.02^{a}	**1020**±0.02^{a}	**1021**±0.05^{a}	**1021**±0.05^{a}

Results represent mean ± SEM

The various alphabets in the table indicate significant differences. Similar alphabets down a column reflect no significant difference (P > 0.05) between the values while different alphabets down a column reflect significant differences (P< 0.05) between the values

Table 7 shows Changes in concentration (mg/kg) of total petroleum hydrocarbon (TPH) with time for eight weeks of remediation of crude oil treated soil and cow dung augmented soil.

There was a significant elevation ($P\leq0.05$)in the concentration of Total Petroleum Hydrocarbon in the all the crude oil simulated experimental soil samples progressively in-between the eight weeks compared to the Total Petroleum Hydrocarbon in week one.

There was also a significant increase in the concentration of Total Petroleum Hydrocarbon with in all the crude oil simulated experimental soil samples with respect to their cow dung augmented soil experimental groups.However, there was no significant difference ($P\geq0.05$)between all the cow dung augmented experimental groups.

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4. DISCUSSION

Contamination of soil arising from spills is one of the most limiting factors in soil fertility and hence crop productivity [34]. Considerable changes in soil properties usually occur when a soil is polluted by oil spill, the changes include the water-holding capacity of the soil, loss of soil structure, introduction of anaerobic condition and reduction in activities of aerobic microorganism and fauna such as earth worms [35], and these changes affect plant growth and yield.

Percentage germination of maize seeds were observed to decrease as the quantity of the crude oil in the soil increased. The low rate of germination of the seed observed in this study could be due to the general undesirable condition that crude oil creates in the soil; this conforms to the findings of [36] and [37]. Contaminated soils augmented with cow dung had higher percentage germination compared to those sown in contaminated soils without cowdung. This is due to improved soil condition by the cow dung, similar to the finding of [38].

The seedlings survival also decreased as the contamination of crude oil increased in the soil. The longer exposure of the maize plant to crude oil in the soil prolonged the toxic effect to the plants hence death of crops. [39] were of the view that oil causes rapid damage of soil water leading to limited moistening effect in the root area. This could also lead to death of the crops after germination as was noticed in this study.

The plant grown in soil without crude oil contamination grew better than those from the contaminated soil irrespective whether cow dung was added to soil or not. This shows that crude oil contamination inhibits plant growth and it is similar to the findings of [40],[41] and [36].

Reduction in chlorophyll content has been an indicator of environmental contaminant [42]. Chlorophyll pigments exist in a highly organized state and under stress they may undergo several photochemical reaction such as oxidative reduction, pheophytinization and reversible bleaching [43]. In this study there was a significant decrease in the chlorophyll content of the maize plant with increasing concentration of crude oil in the soil. Pollution induced degradation in photosynthetic pigment was also observed by a number of workers [44] and [45].However the effect was ameliorated in soils augmented with cow dung. The cow dung augmented samples recorded an increase in chlorophyll content although plant grown on the 25g and 50g crude oil contaminated samples augmented with cow dung had significant difference compared with those grown on soil contaminated with crude oil only. This difference may only be due to improved soil condition by the cow dung.

Crude oil spillage on soils has been shown to cause root stress in plant [46]. An important consequence of stress in plant is the excessive generation of reactive oxygen species (ROS) O^-, OH^-, H_2O_2 particularly in chloroplasts and mitochondria [47]. Meanwhile, plants posses' efficient antioxidant defense system for scavenging ROS which include superoxide dismutase (SOD), catalase (CAT) and peroxidases (POX) [48]. Studies have established the fact that these antioxidant enzymes (SOD, CAT and POX) work together in scavenging ROS [49]. The activity of these antioxidant enzymes was observed to be significantly reduced in maize seedlings grown in crude oil contaminated soil (25g, 50g, 75 and 100g of crude oil) but was significantly elevated in all the cowdung augmented soil pots.This correlates with the findings of [50] who showed increased antioxidant enzyme activities in canola (*Brassica Napus L.*) under stress.

However treatment with crude oil augmented with cow dung showed higher activity of these enzymes than those grown in soils contaminated with crude oil but without cow dung. This difference may be due to enhanced physico-chemical properties of soil by addition of cow dung which increased the adaptive ability of the plant. In several cases transgenic plants overexpressing SOD showed increased tolerance to oxidative treatments and became more resistant to photo inhibition when exposed to different abiotic stresses [51].

It is well known that ROS induced lipid peroxidation of membrane is a reflection of stress induced damage at cellular level [52].The malondialdehyde (MDA) content is often used as an indicator of lipid peroxidation in plant tissues that results from oxidative stress induced by various abiotic stresses [53]. The MDA content of maize seedlings was observed to increase with increasing contamination of the soil with crude oil. However there was a significant reduction in the MDA content of plants grown on the 50g and 25g soils augmented with cow dung compared with contaminated soil without cow dung. Because of higher antioxidant activities, less ROS accumulates in these plants and, as a result, the oxidative damage is reduced.

Oil pollution has been reported to create some conditions in the soils, which make some essential minerals unavailable to plants and make some non-essential ones either readily available or cause them to rise to toxic level [54], [55], [56]. In this study the concentration of sodium (Na), potassium (K), calcium (Ca), nitrogen (N) and Phosphorus (P) in maize seedlings was observed to decrease with increasing concentration of crude oil in the soil (0g to 100g).[56] reported shortage of available nitrogen and phosphorus in soils contaminated with crude oil for plant. [57] also showed that the presence of oil in soil significantly decreased the available forms of phosphorus and potassium to plants. However contaminated soils augmented with crude oil showed a high level of significant increase in these macro nutrients when compared with contaminated soils without cow dung.

The TPH was poorly reduced in simulated soil sample weekly until after ninth week of planting as observed (1499.39 mg/kg/7.90 %). This may be due to the microbial activities of the indigenous petroleum utilizing microbes that may have been present or found in the crude oil polluted soil [58].

The appreciable total petroleum hydrocarbon reduction (mg/kg) observed in every other amended planting pots in eight week for cow dung application respectively and this is likelydue to the elevation in petroleum utilizing microbes population and biomass in cow dung manure [59] which utilized the crude oil for carbon and energy source to degrade crude oil in cow dung amended soil [60]. Organic manure like cow dung improves the rate of biodegradation of the petroleum pollutants [61] and some of the products of biodegradation are useful plants nutrients, organic matter and organic fertilizers which do not destroy beneficial microorganisms and earthworms [62].

5. CONCLUSION

Crude oil contamination of the soil alters the physicochemical and biological properties of the soil and also induces stress in plants grown on them generating reactive oxygen species which are toxic to plants. Crude oil contaminated soil augmentation with cow dung was able to ameliorate these effects caused by the presence of the crude oil in the soil. This therefore provides yet another proof of the remediation ability of the cow dung on crude oil contaminated soil.

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