



## Effects of Crude Oil Pollution on the Morphology, Growth and Heavy Metal Content of Maize (*Zea mays* Linn.)

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**ABSTRACT:** This study was designed to investigate the effect of crude oil pollution on the morphology, growth and heavy metal content of maize (*Zea mays* L.) by analyzing selected physicochemical parameters and heavy metals in soil samples and plant parts using standard field experimental methods. Results from this study show that all the growth parameters of *Zea mays* planted in the polluted soil were adversely affected by crude oil pollution. The highest mean height for treatments 50 ml, 100 ml and 150 ml were 33.54 cm, 31.34 cm and 27.44 cm respectively while that of the control was 87.58 cm. The pH of the soil increased as the volume of the crude oil increased. Chlorophyll content of the *Zea mays* leaves reduced with increase in the volume of crude petroleum. Of the plant parts examined, root had the highest content of chromium (Cr) with 0.22 ppm (150 ml), nickel (Ni) with 0.46 ppm (150 ml), lead with 0.06 ppm (150 ml) and cadmium (Cd) with 0.02 ppm (150 ml) while the highest copper (Cu) value of 0.28 ppm (150 ml) was recorded in the leaves (150 ml). Heavy metals concentration significantly increased ( $P < 0.05$ ) with the increase in volume of crude oil pollution. This study has shown that crude oil polluted soil brings about reduction in the growth, yield and leaf chlorophyll of maize plant. Coupled with increasing heavy metal concentration, this could lead to scarcity and safety concerns in maize consumption in areas impacted by crude oil pollution.

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Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds and at high concentrations can be toxic to living organisms (Nelson-Smith, 1973; Anderson and Labelle, 2000). Over the years, there has been an increase in the demand for crude oil as the major source of energy both for personal and industrial use. As a result of this increasing use, there is a commensurate increase in the production, transportation and refining of crude oil leading to gross pollution of the environment majorly resulting from oil spillages (Rowell, 1977). The occurrence of oil spillage is a global phenomenon occurring in both land and water causing serious problems and hazards to the environment. Nigeria as a major producer and exporter of crude oil has had its own fair share of oil spillage. Soil pollution by crude oil and petroleum products are presently a menace in Nigeria, particularly in the oil producing areas. Anoliefo and Vwioko (1995) noted that oil spillage leads to a general increase in the heavy metal content of soils. This heavy metal pollution is caused by various metals especially copper, nickel, cadmium, zinc, chromium and lead (Hinojosa *et al.*, 2004). Contamination of the soil by crude oil has been reported to adversely affect germination, reduce crop yield and also lead to premature death of plants (Udom *et al.*, 2012). Maize (*Zea mays* L.) is the most

important and most widely distributed cereal in the world after wheat and rice (Nafziger, 2006). It is used for three main purposes, as a staple food crop for human consumption, as feed for livestock and also as raw material for many industrial uses, including bio-fuel production, starch, flour and alcohol production (Agoda *et al.*, 2011; Oyewo, 2011). It is one of the main staple cereals in Nigeria and many African countries (Oyewo, 2011). Nigeria being a major exporter of crude oil has experienced several oil spills which affected agricultural lands and agricultural produce. Maize, as one of the staple food in Nigeria is adversely affected. There is a need therefore to investigate the effect of crude oil on the growth and performance of maize grown in a crude oil polluted soil. The objective of this paper is to evaluate the effects of crude oil on the morphology, growth and heavy metal content of maize (*Zea mays* Linn.)

### MATERIALS AND METHODS

**Study Site:** The experiment was carried out in a screen house.

**Sources of Material:** Loamy soil was collected from a pristine vegetation in University. Maize seeds were purchased at a local market in Akure metropolis while

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the crude oil used was collected from Warri Refining and Petrochemical Company.

*Experimental Design:* The experiment involved polluting 10 kg of loamy soil with 50ml, 100ml and 150ml of crude oil. The oil-soil mixture was turned thoroughly and left for 2 days to allow for proper mixing. Another pot with no crude oil (0ml) served as the control. This set up was replicated five times and was arranged in a completely randomized design (CRD). Three weeks old seedlings of maize earlier raised in a nursery was transplanted into the experimental pots and left for a total duration of 14 weeks.

*Measurement of Plant Growth Parameters:* During the course of the experiment, growth parameters were measured on a two weekly basis. Plant height was measured with the aid of a measuring tape, number of leaves was physically counted and leaf area measured following Agbogidi and Ofuoko (2005). It was done by measuring the length and breadth of the maximum leaf per plant and multiplying by the correction factor 0.75. At harvest, fresh and dry weight was determined. Fresh weight was obtained by weighing the uprooted and rinsed maize plant using Ohaus Analytical balance (Valour500 3dp). Dry weight was determined by first drying the plant in Gallenhamp drying cabinet at 105° C for 24 hours and then followed by weighing in an Ohaus Analytical balance (Valour500 3dp).

*Determination of total chlorophyll content:* The chlorophyll of plant samples was determined using the method of Heidcamp (2003) which involves the extraction of the chlorophyll of 1g of each leaf with 10ml of 80% acetone, the mixture of each extract was sieved using muslin cloth poured in a curvette. The optical density (OD) of each extract was read off at 652 nm using Uv-vis spectrophotometer (spectrum Lab. 7555). The chlorophyll content (mg/l) of each leaf was determined by dividing the optical density reading with 34.5.

*Determination of plant heavy metals:* Heavy metal analysis was carried out on plant leaves, stems and roots. 2g of pulverized sample was put into a conical flask. 10ml of HNO<sub>3</sub> was added, then the mixture was boiled with steady heat till it almost dried. This was allowed to cool before 50 ml distilled water was added and boiled for 10 minutes. Mixture was allowed to cool again and then filtered. The mixture was made up to a known volume. Heavy metal analysis was done using Atomic Absorption Spectrophotometer Buck Scientific 210 VGP model. The instrument was first calibrated with already prepared working standard of corresponding elements to be analyzed after which the

concentration of the element in each sample was determined.

$$\text{Conc. mg/kg} = \frac{\text{AAS reading} \times \text{Dilution Factor}}{\text{Weight of sample}}$$

*Soil analysis:* Soil samples were analysed before and after the experiment. Analysis carried out to determine soil includes. Soil pH was determined using a pH meter (pH-2 Hanna) which was standardized with buffer 4 solution. Soil organic carbon was determined using the Walkley-Black wet oxidation method (Walkley and Black, 1934). Total Nitrogen was determined using Total N-Kjeldahl procedure (Bremner and Keeney, 1966). Available Phosphorus was determined by using Bray method (Bray and Kurtz, 1945). Soil particles sizes were estimated using the Hydrometer method (Bouyoucos, 1962).

*Data analysis:* All data obtained was subjected to Analysis of Variance (ANOVA), where significant differences exist, treatment means were compared at 0.05 significant level using Duncan's new Multiple Range Test SPPS 21.0 software was used for all statistical analysis.

## RESULTS AND DISCUSION

The results of this study are presented in figures 1-8 and tables 1-5. Poor growth and slender stems were observed in most of the treated *Z. mays* plants. Plants in both 100ml and 150ml pots had stunted growth, chlorosis and wilting of leaves, necrosis, the soil was dried, hardened and compacted. There was 40% death in treatment 150 ml. The growth parameters of *Zea mays* L. plants were adversely affected by the crude oil pollution. The effects were concentration dependent as the growth parameters were reduced as concentration of crude oil applied increased. Plant height was highest in control plant when compared to the treatments. The most affected by crude oil pollution were those treated with 150ml crude oil. In the treatments, the highest mean height observed for plant with treatment 50 ml, 100 ml and 150 ml were 33.54 cm (week 10), 31.34 cm (week 8) and 27.44cm (week 8) respectively (see figure 1). Similar trend was observed by Etukudoh and Chukwumati (2016) that plants height were higher in the soil without pollution as compared to the plants in polluted soil. The reduction in height of the plants could be due to unfavourable soil conditions mainly due to insufficient aeration following a decrease in the air filled pore spaces (Atuanya, 1987), effects on soil microbes (Benka-Coker and Ekundayo, 1995), presence of toxic oil components (Siddiqui and Adams, 2002), reduced biochemical activities as well as presence of heavy metals (Agbogidi and Egbuchua,

2010) and a disruption in the soil water-plant interrelationship (Agbogidi, 2011).

The result of the effect of crude oil on the stem girth measurements of *Zea mays* are presented in “figure 2”. Mean girth size followed the trend control>50ml>100ml>150ml. The highest mean stem girth obtained for control and treatments were 3.72 cm (control), 2.84 cm (50 ml), 2.22 cm (100 ml) and 2.04 cm (150 ml). Reports from various oil contaminants on soil by researchers revealed that stem girth values were found to decrease as the concentration increased. This current study follows this trend and agrees with the findings of Okonokhua *et al.*, (2007). They reported a reduction in stem girth of maize as concentration of pollutant increases. Crude oil pollution in all treatments resulted in reduction in the mean number of leaves when compared with control (Figure 3). Control and 50 ml had their highest mean number of leaves of 8.6 and 5.8 at week 10 while 100 ml and 150 ml had their highest mean number of leaves at week 8 (5.2 and 4.5 respectively). Generally, for all the treated maize plants (from week 6) the leaves showed chlorosis and wilting. At week 12 to 14 the result showed that the number of leaves reduced drastically when compared to the previous weeks and this could be because some leaves turned brown, withered and collapsed, mostly in treated plants. According to (Udo and Fayemi, 1975), this could be as a result of reduction in soil aeration due to thin film layer formation on the topsoil by the applied crude oil thereby reducing air passage through the soil pores, leading to the suffocation of the maize plants and hence, reduction in the number of leaves. The fresh and dry weights of (g) of *Z. mays* were reduced by the application of the crude oil (Figure 4 and 5 respectively). Fourteen weeks after transplanting, the lowest mean value for fresh weight among the treated plants was observed in 150 ml (21 g) while the control had the highest mean weight of 85 g. The 50 ml and 100 ml had the same mean dry weight of 30 g and 29.6 g respectively. There were significant lower yield and growth in plants grown in polluted soils compared to those of the control. This is in agreement with the findings of Okonokhua *et al.* (2007) and Anoliefo *et al.* (2006) in maize sown in oil polluted soils. In their studies, they reported a reduction in growth parameters and yield as concentration of pollutant increased. Ojimba and Iyagba (2012) reported the decreased output of horticultural crops in crude oil polluted farms as compared with the unpolluted farms.

Results of the effect of crude oil on the leaf area of *Zea mays* is presented in “figure 6”. Leaf area was significantly ( $P>0.05$ ) affected by the crude oil application. The highest mean leaf area for all the treatments and the control was at week 10, 50 ml was

228.5 cm<sup>2</sup>, 100 ml (185.5 cm<sup>2</sup>), 150 ml (144.5 cm<sup>2</sup>) and control (424.5 cm<sup>2</sup>). Chlorophyll content of the *Zea mays* leaves was significantly ( $P>0.05$ ) affected by the application of crude oil to the soil (figure 7). Control had the highest mean chlorophyll content (72.01 mg/l) and it was significantly different ( $P < 0.05$ ) from 50 ml (66.02 mg/l), 100 ml (58.10 mg/l) and that of 150 ml which had the least mean chlorophyll content (43.50 mg/l). The reduction of the chlorophyll content of the plant could be due to the interference of the oil on the ability of the plant to absorb some of the mineral nutrients. Minerals like magnesium, iron, boron, and manganese are essential for chlorophyll synthesis (Campbell, 1996; Kent, 2000; Taylor *et al.*, 2001). The results of the effect of crude oil on the soil physicochemical parameters are presented in “figure 7” and “table 1”. pH of the soil increased with increasing concentration of crude oil following the trend 150ml>100ml>50ml>control (figure 7). Control soil had the least pH value (6.7) and 150 ml treatment had the highest mean pH value (7.64) while 50 ml and 100 ml treatments had intermediate values (7.05 and 7.38 respectively). Other parameters are presented in “table 1”. The organic carbon content of the soil was significantly different in all treatments at  $p < 0.05$ . The control had the lowest organic carbon (3.41 cmol/kg) while 150ml had the highest mean organic carbon content (8.02cmol/kg). The available phosphorus (P) decreased as the concentration of crude oil increased. There was no significant difference ( $P > 0.05$ ) between control (156.4 ppm) and 50 ml (151.4 ppm) while 150 ml (99.9 ppm) was significantly different from all other treatments including the control. The application of crude oil to soil was observed to increase the total nitrogen (N) in the soil. Control (0.01%) had the least nitrogen while 150 ml (0.027%) had the highest. There was no significant difference in the nitrogen between 50 ml and 100 ml (0.019% and 0.02% respectively) at  $P > 0.05$ . The silt, clay and sand constituents were not significantly different ( $P>0.05$ ) in both treated and control soil. These results indicated that crude oil in the soil has a significant effect on soil properties like nitrogen content, phosphorus, carbon and heavy metals. This observation is in agreement with earlier reports by Agbogidi and Egbuchua (2010) who noted that oil in soil has deleterious effects on the biological, chemical and physical properties of the soil depending on the dose, type of the soil and other factors. Soil pH which is a major factor influencing the availability of elements in the soil for plant uptake (Okonokhua *et al.*, 2007) was observed to increase with increasing concentration of crude oil. This is in agreement with Vwioko *et al.*, 2008. He reported that contamination of soil increases its pH from acidic to neutral.

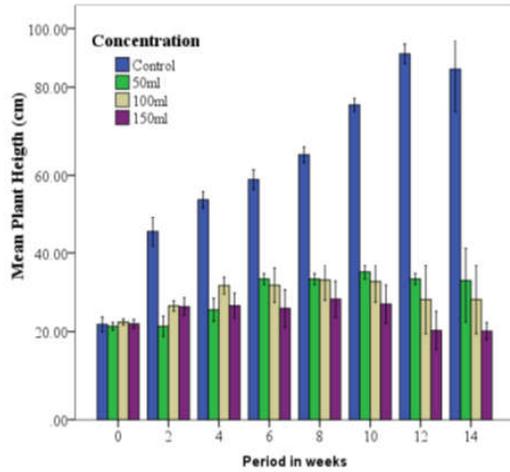


Fig 1: Effect of crude oil at various volumes on plant height (cm) of *Zea mays* L during the growing period

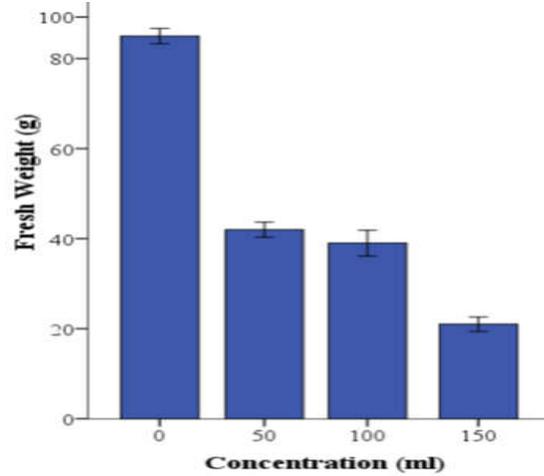


Fig 4: Effect of crude oil on fresh weight (g) of *Zea may* L at harvest.

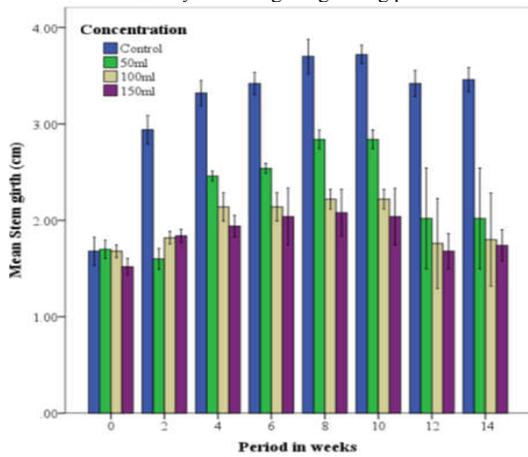


Fig 2: Effect of crude oil at various volumes on plant stem girth (cm) of *Zea mays* L during the growing period

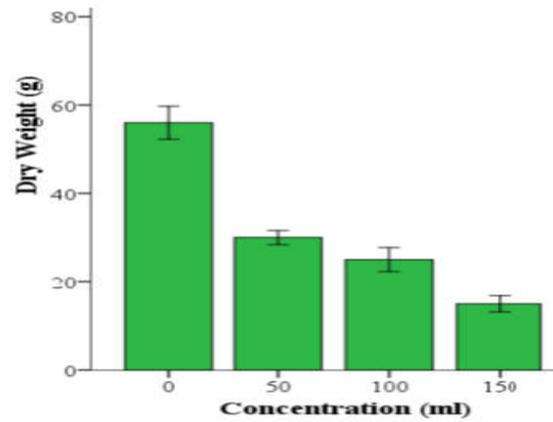


Fig 5: Effect of crude oil on dry weight (g) of *Zea may* L at harvest.

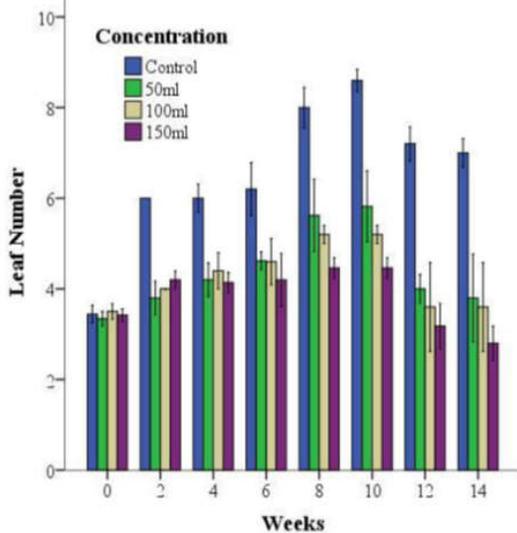


Fig 3: Effect of crude oil at various volumes on number of leaves of *Zea mays* L during the growing period

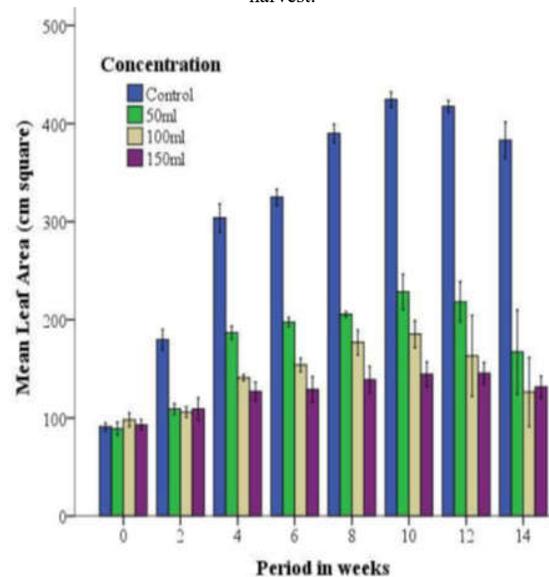


Fig 6: Effect of crude oil application at various volumes on the leaf area of *Zea mays* L during the growing period

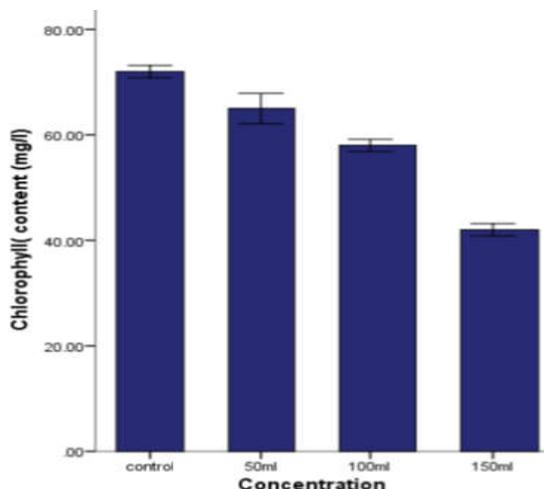


Fig 7: Effect of crude oil pollution on chlorophyll content of *Zea mays* L. leaves

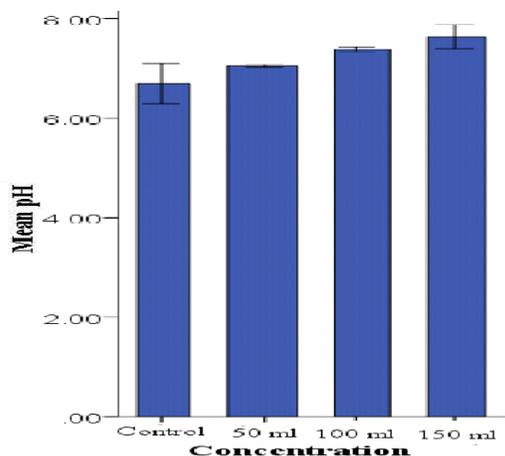


Fig 8: Effect of crude oil pollution on soil pH

Table 1: Physicochemical properties of polluted and unpolluted soil

Treatment (ml)				Heavy Metals (ppm)			Type
	OC, cmol/kg	P, ppm	N, %	Sand	Clay	Silt, %	
00	3.41 ± 0.12 <sup>a</sup>	156.40 ± 2.48 <sup>c</sup>	0.010 ± 0.000 <sup>a</sup>	13.3±1.67 <sup>a</sup>	7.3± 0.06 <sup>a</sup>	79.4 ± 3.46 <sup>a</sup>	Loamy
50	4.08 ± 0.09 <sup>b</sup>	151.40 ± 0.70 <sup>c</sup>	0.019 ± 0.0003 <sup>b</sup>	12.4± 0.23 <sup>a</sup>	7.3± 0.12 <sup>a</sup>	80.3± 0.17 <sup>a</sup>	Loamy
100	7.03 ± 0.02 <sup>c</sup>	132.40 ± 0.63 <sup>b</sup>	0.020 ± 0.0032 <sup>b</sup>	12.9± 0.58 <sup>a</sup>	7.4± 0.23 <sup>a</sup>	79.7± 0.58 <sup>a</sup>	Loamy
150	8.02 ± 0.04 <sup>d</sup>	99.90 ± 2.62 <sup>a</sup>	0.027 ± 0.0032 <sup>c</sup>	13.2± 1.67 <sup>a</sup>	7.3± 0.13 <sup>a</sup>	79.4± 2.54 <sup>a</sup>	Loamy

Mean ± standard error represent 5 (five) replicates. Means value with the same alphabet down the column are not significantly different from each other using Duncan’s New Multiple Range Test (DNMRT) at P>0.05.

Table 2: Heavy metals concentration (ppm) in *Zea mays* L. leaves in the polluted and unpolluted soil

Treatments (ml)	Heavy Metals (ppm)		
	Cr	Cu	Ni
00	0.03 ±0.01 <sup>a</sup>	0.20 ±0.00 <sup>a</sup>	0.05 ±0.01 <sup>a</sup>
50	0.05 ±0.01 <sup>ab</sup>	0.20 ±0.00 <sup>a</sup>	0.08 ±0.01 <sup>a</sup>
100	0.06 ±0.01 <sup>b</sup>	0.26 ±0.02 <sup>a</sup>	0.11 ±0.01 <sup>ab</sup>
150	0.07 ±0.01 <sup>b</sup>	0.28 ±0.02 <sup>a</sup>	0.37 ±0.18 <sup>b</sup>

Mean ± standard error represent 5 (five) replicates. Mean values with the same alphabet down the column are not significantly different from each other using Duncan’s New Multiple Range Test (DNMRT) at P > 0.05

Table 3: Heavy metals concentration (ppm) in *Zea mays* stem in the polluted and unpolluted soil.

Treatments (ml)	Heavy Metals (ppm)				
	Cr	Cu	Ni	Pb	Cd
00	0.06 ±0.01 <sup>a</sup>	0.03 ±0.01 <sup>a</sup>	0.02 ±0.00 <sup>a</sup>	0.01 ±0.00 <sup>a</sup>	0.01 ±0.00 <sup>a</sup>
50	0.09 ±0.01 <sup>b</sup>	0.03 ±0.01 <sup>a</sup>	0.04 ±0.01 <sup>a</sup>	0.01 ±0.01 <sup>a</sup>	0.01 ±0.00 <sup>a</sup>
100	0.10 ±0.01 <sup>b</sup>	0.03 ±0.00 <sup>a</sup>	0.10 ±0.00 <sup>b</sup>	0.02 ±0.01 <sup>b</sup>	0.01 ±0.00 <sup>a</sup>
150	0.12 ±0.01 <sup>c</sup>	0.05 ±0.01 <sup>a</sup>	0.15 ±0.00 <sup>b</sup>	0.03 ±0.01 <sup>c</sup>	0.02 ±0.00 <sup>b</sup>

Mean ± standard error represent 5 (five) replicates. Mean values with the same alphabet down the column are not significantly different from each other using Duncan’s New Multiple Range Test (DNMRT) at P > 0.05.

Table 4: Heavy metals concentration (ppm) in *Zea mays* L. root in polluted and unpolluted soil.

Treatments (ml)	Heavy Metals (ppm)				
	Cr	Cu	Ni	Pb	Cd
00	0.11 ±0.00 <sup>a</sup>	0.04 ±0.01 <sup>a</sup>	0.24 ±0.02 <sup>a</sup>	0.01 ±0.00 <sup>a</sup>	0.01 ±0.00 <sup>a</sup>
50	0.14 ±0.01 <sup>b</sup>	0.05 ±0.02 <sup>ab</sup>	0.25 ±0.02 <sup>a</sup>	0.03 ±0.01 <sup>ab</sup>	0.01 ±0.00 <sup>a</sup>
100	0.20 ±0.01 <sup>c</sup>	0.05 ±0.01 <sup>ab</sup>	0.38 ±0.01 <sup>b</sup>	0.05 ±0.01 <sup>c</sup>	0.01 ±0.00 <sup>a</sup>
150	0.22 ±0.01 <sup>c</sup>	0.07 ±0.02 <sup>b</sup>	0.46 ±0.02 <sup>c</sup>	0.06 ±0.01 <sup>c</sup>	0.02 ±0.00 <sup>b</sup>

Mean ± standard error represent 5 (five) replicates. Mean values with the same alphabet down the column are not significantly different from each other using Duncan’s New Multiple Range Test (DNMRT) at P > 0.05.

Results of the heavy metal concentration in leaves, stems and roots are presented in tables “2, 3 and 4” respectively. Copper (Cu), Nickel (Ni), Chromium (Cr), Cadmium (Cd) and Lead (Pb) contents in leaves,

stem and root were higher in polluted soil than unpolluted soil. The higher concentrations of heavy metals in leaves, stem and root of the treated *Zea mays* plants could be as a result of the roots absorbing the

heavy metals present in treated soil and translocating to other parts of the maize plant. This has been shown by Baker et al. (2000) that certain plants do not only accumulate metals in the roots but also translocate from roots to the leaves or shoots. Although the concentration of heavy metals in the leaves stems and roots of treated plants were below the toxic level of 100 ppm (Charman and Murphy, 1992), heavy metals have a great tendency of accumulating in human

organs over time. Heavy Metals present in the polluted soil was slightly higher than that of the control soil. This suggests that heavy metals are present in crude oil and their concentration depend on the intensity of pollution, this observation is in line with earlier report of Atuanya (1987) which states that the concentration of heavy metals present in soil polluted with crude oil depends on the quantity of crude oil present in the soil.

**Table 5:** Heavy metals concentration in polluted and unpolluted soil

Treatment (ml)	Heavy Metals (ppm)							
	Pb	Fe	Cd	Zn	Mn	Cu	Ni	Cr
00	0.30 ± 0.09 <sup>a</sup>	60.00 ± 2.89 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	0.15 ± 0.03 <sup>a</sup>	2.16 ± 0.44 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>	0.14 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>
50	0.30 ± 0.01 <sup>a</sup>	61.00 ± 0.64 <sup>a</sup>	0.02 ± 0.00 <sup>a</sup>	0.25 ± 0.01 <sup>b</sup>	2.10 ± 0.06 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>	0.17 ± 0.01 <sup>a</sup>	0.22 ± 0.06 <sup>b</sup>
100	0.30 ± 0.01 <sup>a</sup>	62.50 ± 0.29 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	0.34 ± 0.02 <sup>c</sup>	3.30 ± 0.17 <sup>b</sup>	0.10 ± 0.01 <sup>b</sup>	0.17 ± 0.02 <sup>a</sup>	0.24 ± 0.02 <sup>b</sup>
150	0.31 ± 0.03 <sup>a</sup>	68.88 ± 0.46 <sup>b</sup>	0.03 ± 0.01 <sup>a</sup>	0.35 ± 0.01 <sup>c</sup>	4.00 ± 0.11 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	0.18 ± 0.01 <sup>a</sup>	0.24 ± 0.01 <sup>b</sup>

Mean ± standard error represent 5 (five) replicates. Means value with the same alphabet down the column are not significantly different from each other using Duncan's New Multiple Range Test (DNMRT) at P>0.05.

**Conclusion:** Various contaminants including crude oil, spent engine oil and heavy metals have been found to significantly affect the growth and performances of various plant species. This study has shown that crude oil polluted soil has a significant effect on the growth and yield of maize. In a country like Nigeria where maize cultivation is very high, incidences of crude oil pollution can led to significant loss in yield of this important crop thereby exacerbating the already existing food security challenges of the Nation.

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