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TOXICOLOGICAL IMPLICATIONS OF CRUDE OIL ON THE MORPHOLOGY AND HEAVY METAL CONTENT OF *Zea mays.*

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ABSTRACT

*Zea. mays*are widely consumed world over, especially in the tropical regions of the world. Owing to its several nutritional and industrial benefits and the menace of crude oil pollution to the cultivation of maize (especially for oil-rich Nigeria), this study was conducted to investigate the toxicological implications of crude oil pollution on the morphological growth and heavy metal content of the crop. Following a completely randomized design, maize seeds planted in bags were treated with different concentrations (0ml, 100ml, 200ml, 300ml and 400ml) of crude oil and the morphological parameters (height of plant, number of leaves and dry weight) and heavy metal (Pb and Ni) content were observed, and statistically analyzed (ANOVA and OCTA). Results showed a significant depression (observable by the negative values of the OCTA indices) of the morphological growth parameters and a concomitant increase in the heavy metal (Pb and Ni) content with increase in the amount of pollutant. This therefore suggests that crude oil pollution has certain toxicological implications on the growth of *Z. mays*, bothering on water and oxygen stress conditions that trigger a hyper-accumulation of heavy metals, as a physiological response to initiate a physiological gradient as a counter measure. The toxicity of crude oil pollution to *Z. mays* does not only affect the economic returns of the farmer, but also the general health of the consumers of such plants, as the increase in heavy metal content within the plant tissue could bio-magnify in the food chain and thus leading to heavy metal poisoning.

Hence,*Z. mays* should not be planted on sites, or proximal to sites, exposed to crude oil and/or heavy metal pollution.

Key words: Crude oi, pollution, heavy metals, maize, pots

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INTRODUCTION

Maize (*Zea mays*) belongs to the family Poaceae. Despite originating from the lines of Mexico and Central America, *Z. mays* is widely consumed world over, especially in the tropical regions of West Africa and Nigeria.Globally, about 1016.73 million metric tonnes of maize is produced every year – the highest among major staple cereals (FAOSTAT, 2013). A major portion of maize produced worldwide is used for animal consumption as it serves as a vital source of proteins and calories to billions of people in developing countries, particularly in Africa, Mesoamerica and Asia (Shiferaw*et al*. 2011). Further, it is a source of important vitamins and minerals to the human body. Along with rice and wheat, maize provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries. Maize provides over 20 % of total calories in human diets in 21 countries and over 30 % in 12 countries that are home to a total of more than 310 million people (Shiferaw*et al*. 2011). At present, the developed world uses more maize than the developing world, but forecasts indicate that by the year 2050, the demand for maize in the developing countries will double owing to the rapid growth in poultry industry, the biggest driver of growth in maize production (Rosegrant*et al*. 2009; Prasanna 2014).

Maize is established to contain about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g, as compared to rice and wheat, but has lower protein content. Maize is also established to provide for a lot of vitamin and mineral requirements (Nuss and Tanumihardjo, 2010). It is estimated that in 2012, the total world production of maize was 875,226,630 tons, (FAO, 2012) with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production of maize, respectively.Maize can be processed into a variety of food and industrial products, including starch, sweeteners, oil, beverages, glue, industrial alcohol, and fuel ethanol (Ranum*et al*. 2014). Olaniyan (2015) has shown that Maize is the panacea for hunger in Nigeria, as it cuts across all the socio-economic classes in the nation, (serving as a means of foods security due to its nutritive values) and is readily available and affordable by the lower class.

For a crop as valuable as *Zea mays*, and just like most other agricultural crops, crude oil pollution has been a menace to its agricultural production, especially in Nigeria which has the crude oil as its major source of revenue. Several reports have shown that crude oil has been a menace to the production of the crop – *Zea mays*. Okonwu*et al*., (2010) has shown that crude oil pollution affects the growth and chlorophylls content of *Zea mays*. Odiyi*et al*., (2020) have shown that the growth of Z. mays have been hindered by the application of crude oil.This pollution results mainly due to accidental discharge (human error), sabotage, transportation and other natural causes (Agbogidi, 2005). Other possible causes could be traced to damaged oil tankers, storage vehicles, leakages of oil pipelines, oil tankers overflow (Nicolloti and Eglis, 1998) due to increase in crude oil exploration, exploitation, storage and transportation . These and a lot more are observable in Nigeria because crude oil is the economic back bone of the Nigerian nation (Adegeye*et al*. 1993) and generating over 90% of the national revenue (Nwilo, 1998).

Despite all the dangers crude oil pollution poses on agricultural profitability, none is as lethal as heavy metal accumulation. Kumar *et al*. (2019) explained that heavy metals play a vital role in nature as they are essential for the plant's normal growth as they are involved in redox reactions, transferring electrons, basic functions in nucleic acid metabolisms, and being an integral part of several enzymes as a direct participant. Further stating, Kumar *et al*. (2019), explained that the availability at a certain concentration of these essential metals in growing medium is very important, but their excess concentration results in several toxic effects. Several reports have shown that crude oil pollution leads to increase in heavy metal content of the plant. While reviewing the incidence of different oil spills in the world, Dasuki*et al*., (2015) realized that certain heavy metals were usually associated with the incidence of crude oil spills all over the world. While delineating them as Pb>Ni>V>Zn>Cd, they explained that these metals could have many effects to human health especially cancer.

Researchers such as Essiett*et al*., (2010), working with *Dissotiserecta*, *Urenalobata*, *Selaginellamyosurus*, *Diodiascandens* and *Pityrogramma*calomelanos, have shown that the concentration of heavy metals in plants increases with an increase in the amount of crude oil pollution. Owing to the fact that heavy metals accumulated in a plant tissue (*Zea mays*, in this case) can be consumed and biomagnified in humans and/or animals who serve as direct or indirect consumers of these plants could lead to several plausible cases of human ailments, and thus calls for alarm (Khan *et al*., 2015).

The plethora of implications of growing a major staple food in a crude oil polluted environment thus give objective to this study to determine the effect of crude oil pollution on agricultural soils, looking at the morphological growth (economic returns to farmers) and the toxicological implications to humans who serve as the consumers of these plants.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Center for Ecological Studies in the Department of Plant Science and Biotechnology, University of Port-Harcourt, Rivers state, Nigeria. It is on geographical coordinates: latitude 4° 52'N and 4° 55'N Longitudes 6° 54'E and 6° 56'E in Obio/Akpor Local Government Area (LGA), Rivers State. It is situated in the Niger Delta wetland of Southern Nigeria.The climatic weather condition of the area is characterized by tropical monsoon climate with mean annual temperature of 25°C to 28°C and annual rainfall of over 3000mm. The relative humidity is very high with an annual mean of 85% while the soil is usually sandy or sandy loam underlain by a layer of impervious pan.

PLANTING MATERIALS USED

The seeds of the test crop (*Zea mays* L. Hybrid II yellow variety) were obtained from the Agricultural Development Program (ADP) Rumuodumaya, Obio-Akpor L.G.A., Rivers State, Nigeria. The soil samples were obtained behind the University of Port-Harcourt Farm House, Faculty of Agriculture. Planting bags were bought from an open market while the crude oil was obtained from Mbodo-Aluu, Ikwerre L.G.A Rivers State.

PRE-GERMINATION TEST

Pre-germination test was carried to ensure the seeds were viable. Ten seeds of the test crop(*Zea mays*) were plated in Petri dishes lined with the Whatman No. 1 filter paper and kept for 7 days with 10ml of distilled water every 3 days. 9 seeds out of the ten seeds sprouted (plumule and radicle) showing 90% viability, thus showing that the seeds are good enough for planting as against <70% viability which would have indicated that the seeds may not be viable.

POLLUTION OF SOIL

Different concentration (0ml, 100ml, 200ml and 400ml) of crude oil were added to a measured quantity (21.5kg) of soil; properly tagged as T1, T2, T3 and T4 respectively. These were thoroughly mixed before being transferred into a perforated planting bag. The polluted soil samples were allowed to precondition with the oil for two (2) weeks before remediation.

PLANTING

Planting was done two weeks after pollution. 5 seeds of *Z. mays* hybrid II yellow variety were planted per bag and watered daily. The 5 plants were reduced to 2 plants per bag 2 weeks after planting. Growth parameters were observed and recorded every 3 weeks for a period of 9 weeks.

Experimental Design

The seeds were planted in bags arranged in a completely randomized design with each bag weighing an average of 21.5kg with 5 treatments (0ml, 100ml, 200ml, 300ml and 400ml) and 4 replicates giving a total of 20 bags.

Measurement of Growth Parameters

The growth parameters examined during the duration of the study were plant height (cm), number of leaves (NOL), and dry weight of plant.

- **PLANT HEIGHT**

  Each plant was measured from the base to the apex of the plant using a meter rule. The average plant height of the two plants per bag was determined.

- **NUMBER OF LEAVES**

  The number of leaves was obtained by visual counting of the leaves of each plant in the planting bag. The average number of leaves of two plants per bag was determined.

- **DRY WEIGHT**

  The harvested plants were oven-dried at 80^0C for 72 hours. The individual dry weight of the plants was weighed using an electronic digital weighing balance, and the average of the two plants in a bag was taken as the dry weight for the replicate.

ANALYSIS OF HEAVY METALS (NIKEL AND LEAD) IN PLANT LEAVES

Leaves of the subject plant were analyzed for the presence of the heavy metals Nickel (Ni) and Lead (Pb) using the method of Malomo et al., (2013) with some slight modificatons as stated by the following procedures:

- **Nikel (Ni)**

  The machine was stabilized for 15 minutes, wavelength of 23.0mm was selected. Air and Acetylene flow were adjusted; other necessary settings as recommended for the instrument were adjusted. Hollow cathode lamp was allowed adequate time to stabilize. Standard Nikel solutions of known concentration were aspirated and were corresponding absorbance were recorded. The sample solution was aspirated after flushing instrument system with deionized water. The absorbance displaced on the measurement system was recorded. The concentration of Nikel in the sample was interpolated from the standard graph.

- **Lead (Pb)**

  Lead ion was analyzed by an atomic spectrophotometer at 283.3mm wave length. The wavelength was selected with a narrow slit, air and acetylene gas flow was adjusted. Other setting as recommended for the instrument employed was attended to and regulated. Hallow cathode lamp was given adequate time to stabilize before aspirating standards for equipment calibration. After calibrating the equipment with standard lead concentrations, the aspiration tubing and system were flushed with distilled water severally before aspirating the test sample solution on the sample experimental condition used for the standard. The concentration of Lead ion in the sample was extrapolated from the standard graph of Lead ion plotted. The concentration was expressed in mg/l or ppm from the equipment corrections were made necessary in units of choice.

Soil Physiochemical Analysis

The soil physiochemical characterization was carried out at the International Institute for Tropical Agriculture, Analytical Service Laboratory, Ibadan, Oyo State, Nigeria (Table 1)

Table 1: Physiochemical characterization of the soil

Id No.	pH	O	N	P	Ca	Mg	K	Na	Al	ECEC	Clay	Silt	Sand
Unit	(1:1) H_2O	%		Cmol/kg							%		
Value	4.40	2.51	0.260	42.711	20.362	0.662	0.091	0.692	0.00	22.126	14.0	21.4	64.6

Statistical Analysis

Mean values were taken and ANOVA (Analysis of Variance) was calculated for each treatment using Data analysis on Microsoft Excel sheet

The effects of the various treatments were compared parametrically using the Ochekwu Comparative Treatments Average (OCTA) Trend (Ochekwu*et al*., 2020).

RESULTS

The results of the study on the effect of the different treatments (0ml [control], 100ml, 200ml, 300ml, 400ml) of crude oil on plant height (HOP), number of leaves (NOL), dry weight (DW) of *Zea mays* within a period of 9 weeks are presentedin figures 1, 2 and 3 respectively.

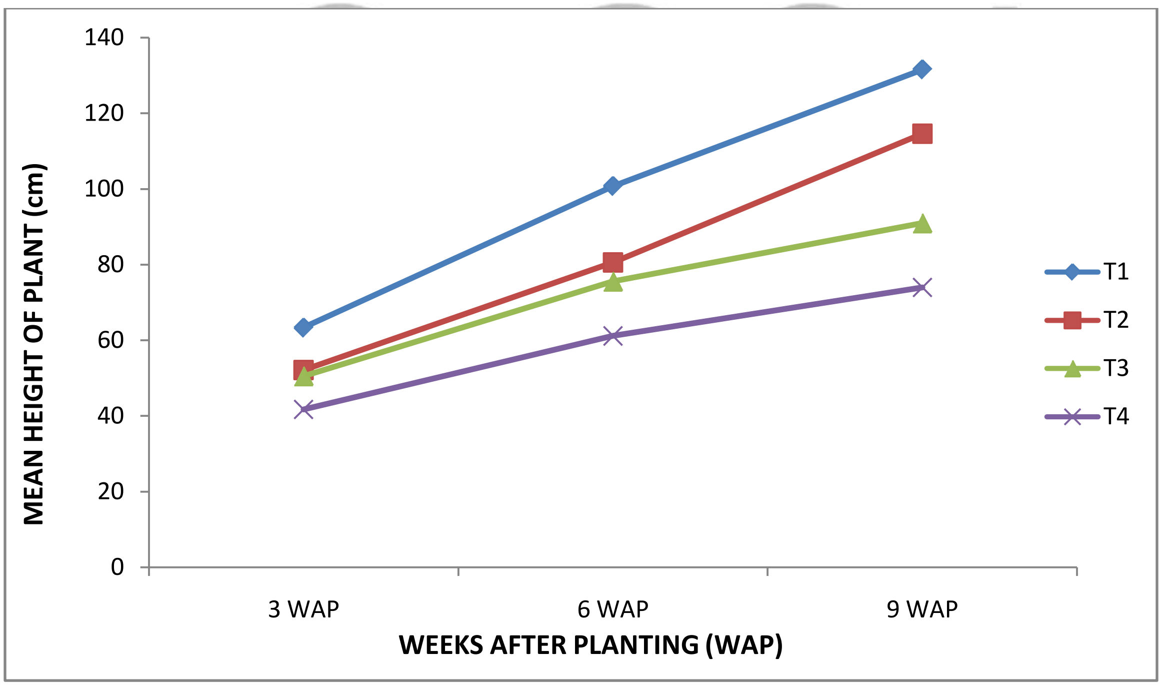

Figure 1: Mean plant height of *Z. mays* at different treatments over 9 weeks after planting (WAP)

Crude oil pollution showed a negative impact on the height of plant of *Z. mays*. TheT4 showed the most effect of the crude oil pollution, observing the least values compared to the control (T1). The highest plant height was observed for the T1 (for all the weeks under study) while the lowest was observed for the T4, with all other treatments being lower than the control, thus indicating a continuous decrease with increasing treatment (Figure 1).

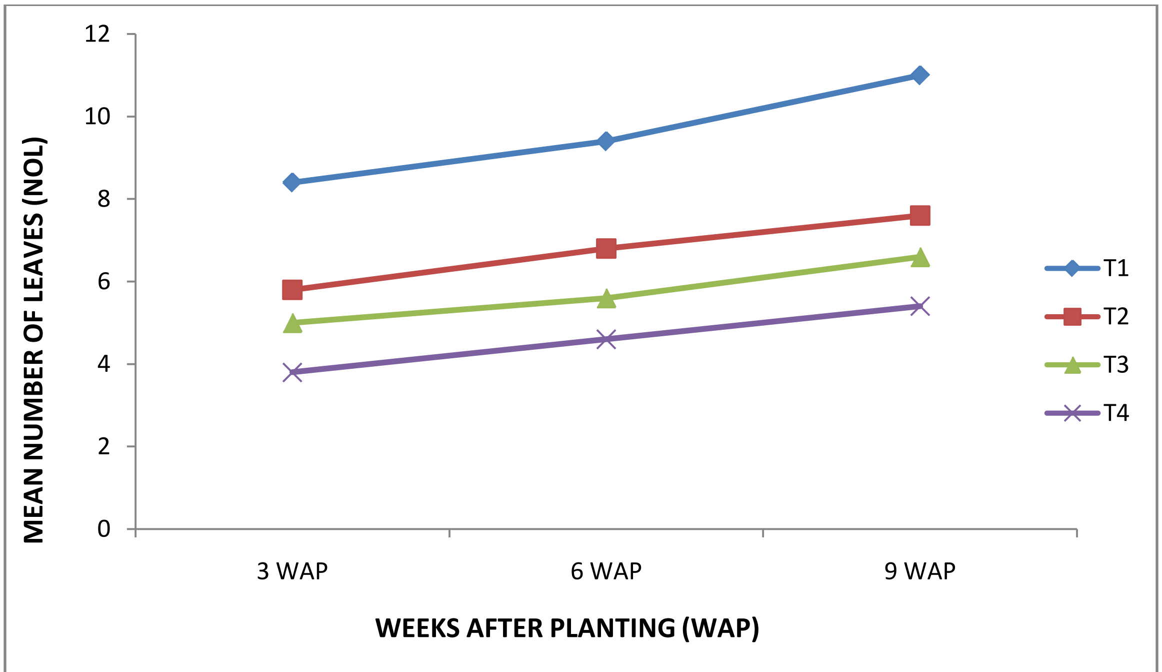

Figure 2: Mean number of leaves of *Z. mays* at different treatments over 9 weeks after planting (WAP)

The highest number of leaves was recorded at 9 weeks after planting (Figure 2). The highest number of leaves of *Z. mays* was observed at T1, with a mean of 11. The number of leaves of the plant decreased as the concentration of the pollutant increased. The lowest number of leaves was observed at week 9 for T4 with a mean of 5.4.

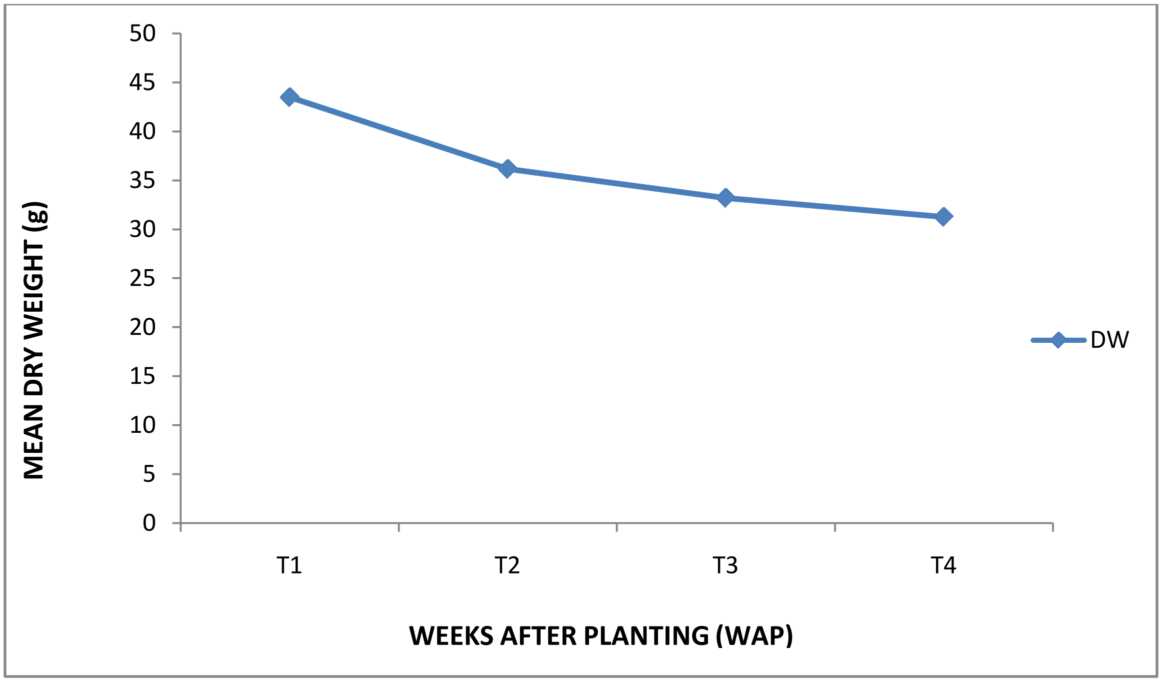

FIGURE 3: Mean dry weight of *Z. mays* at different treatments over 9 weeks after planting

Measured only at the 9th week, the dry weight of the plants decreased with increase in the amount of crude oil pollution in the soil. The highest value for dry weight was observed at T1 while the lowest was observed at T4, following a partially linear trend.

OCTA Analyses

Using the Ochekwu Comparative Treatments Average (OCTA) to determine the Relative OCTA Trend which would show how the respective treatments compared against each other for the respective parameters over the duration under study, the following plots were deduced:

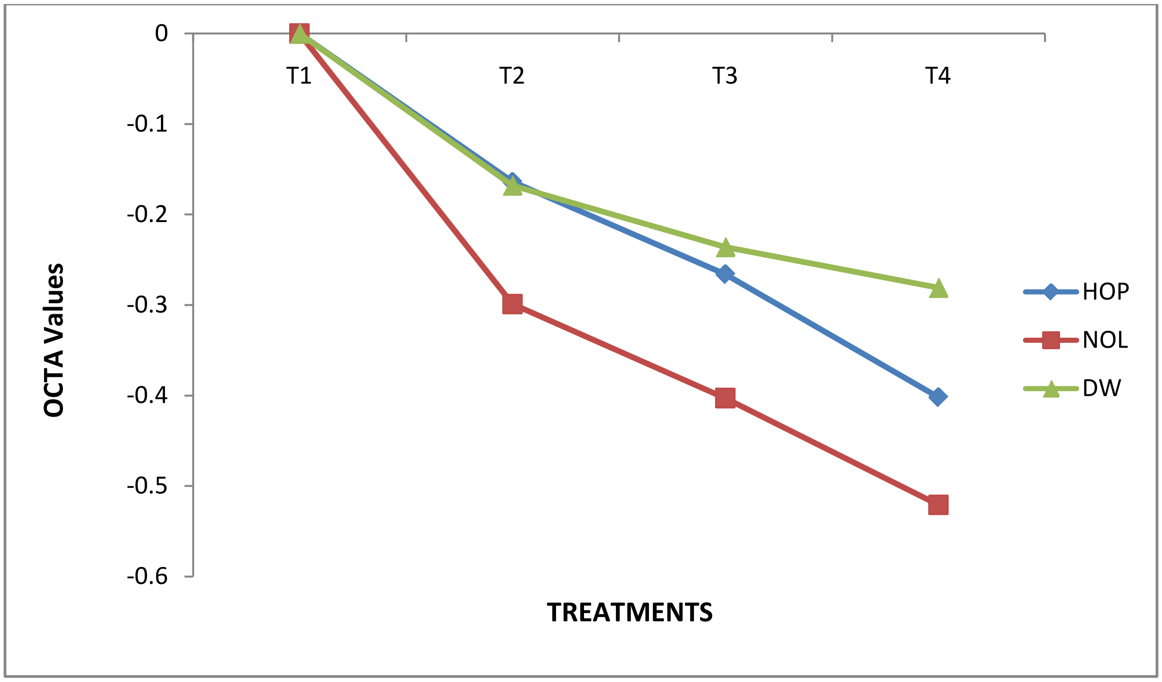

Figure 4: OCTA Trend for HOP, NOL and DW.

The Relative OCTA Trend shows how the treatments faired for the respective parameters under study. The OCTA trend for the three parameters under study (HOP, NOL and DW) showed a general decrease in all the parameters with increase in the treatment over the study period. (Figure 4).

For all parameters, T1 had the highest OCTA value (0), while T4 had the lowest value for all parameters (-0.402 for HOP, -0.521 for NOL, and -0.281 for DW) which signifies a decrease of 40.2%, 52.1% and 28.1% for HOP, NOL and DW respectively, as compared to the contro

Heavy Metals Analysis

The amount of heavy metals in the leaves of the plant is presented in table 1

Table 1: HEAVY METAL (Nickel and Lead) CONTENT OF *Z. mays* LEAVES AT VARIOUS TREATMENTS

Sample identity	Nikel (mg/kg)	Lead (mg/kg)
T1	**ND**	**5.17**

T2	**ND**	**6.69**
T3	**0.05**	**6.86**
T4	**0.07**	**9.17**

ND = NOT DETECTED

It was observed that the control (T1) and T2 showed no detectable trace of Nickel. T1 and T2 were not detected while there was a slight increase in the amount of Nickel in T3 and T4. Lead was detected in all the treatments, and it followed an increase with increase in the concentration of crude oil, ranging from T1 to T4.

DISCUSSION

The study has shown that, for all parameters studied (HOP, NOL and DW), the increasing amount of crude oil pollution in the soil greatly reduced the growth parameter of *Zea mays*. Graphically, all parameters reduced with increasing amounts of the pollutant. This claim is evidenced by the negative values the respective growth parameters scored on the OCTA trend. Crude oil, being hydrophobic in nature does not allow the exchange of gases and uptake of water by the roots, this may trigger certain stress responses in the plant that could lead to the conservation of available resources that would have otherwise been used in to support aerial growth. This goes in line with the works of Okonwu*et al*., (2010) who explained that the reduction in growth and development may be attributed to loss of photosynthetic ability; stating that plants grow well only when they are able to carry out photosynthesis efficiently. Okonwu*et al*., (2010) further explained that the differential changes in the rate of aerial growth may be associated with anatomical and morphological alterations caused by the oil, resultant of Oxygen stress.

It was also observed that the heavy metal (Pb and Ni) content of the plant increased with increasing amounts of the pollutant – crude oil. This increment may be due to the accumulation of the trace amounts of the respective heavy metals that are associated with crude oil in minute amounts. Several reports have shown that the occurrence of crude oil spills leads to a concomitant increase in the heavy metal content of both the plant and the soil at the spill site. This goes in line with the work of Dasuki*et al*., (2015), who, while reviewing the incidence of different oil spills in the world, realized that certain heavy metals were usually associated with the incidence of crude oil spills all over the world. He delineated some of them as Pb>Ni>V>Zn>Cd.

There is also the possibility of hyper-accumulation of heavy metals being triggered as a sort of stress response due to the consequent shortage of gas (Oxygen) and water uptake to and/or through the roots. This hyper-accumulation brings in more solute into the plant cells, and may serve, as a response to environmental assault, to create a sort of physiological gradient that will draw in water and gas into the plant where there seems to be a high concentration of the minerals (heavy metals, in this case). This supposition is supported by the work of (Rucinska-Sobkowiak, 2016) who established that heavy metals influence water delivery to the shoot due to the inhibition of transpiration as they decrease the size of the leaves and the thickness of the lamina, reduce intercellular spaces, affect the density of stomata and decrease stomatal aperture. He further stated that stomatal closure is induced by direct interaction of toxic metals with guard cells and/or as a consequence of the early effects of metal toxicity on roots and stems. Jamal *et al*., (2013) have also explained that plants exposed to heavy metals develop injury in terms of chlorosis along with toxic effects in the form of reduced photosynthesis, browning of root tips, growth inhibition, and finally death.

CONCLUSION

Crude oil pollution negatively affects the growth of *Z. mays*, inhibiting all the growth parameters and increasing its heavy metal content. The increase in the heavy metal content of the plant is regarded as being toxic to the plant as it triggers certain physiological responses in the plant that bothers around senescence. The effect of crude oil pollution on *Z. mays* does not only affect the economic returns of the farmer but also the overall health of the consumers of such crops grown in such conditions, as the increase in the heavy metal content of the plant tissues could lead to certain lethal implications in the health of the consumer.

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