

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 80⁰C 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 modifications 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 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 Nickel 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 ₂ O	%			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 (Ochekwuet *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 presented in figures 1, 2 and 3 respectively.

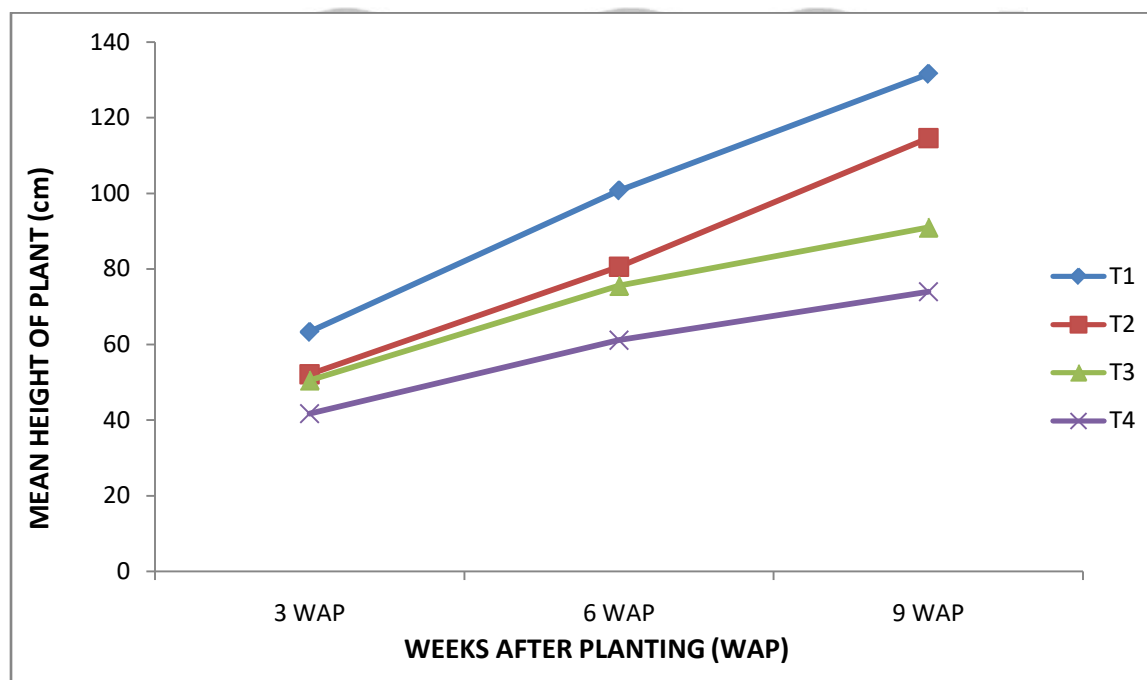


Figure 1: Mean plant height of *Z. mays* at different treatments over 9 weeks after planting (WAP)

Crude oil pollution showed a negative impact on the height of plant of *Z. mays*. The T4 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).

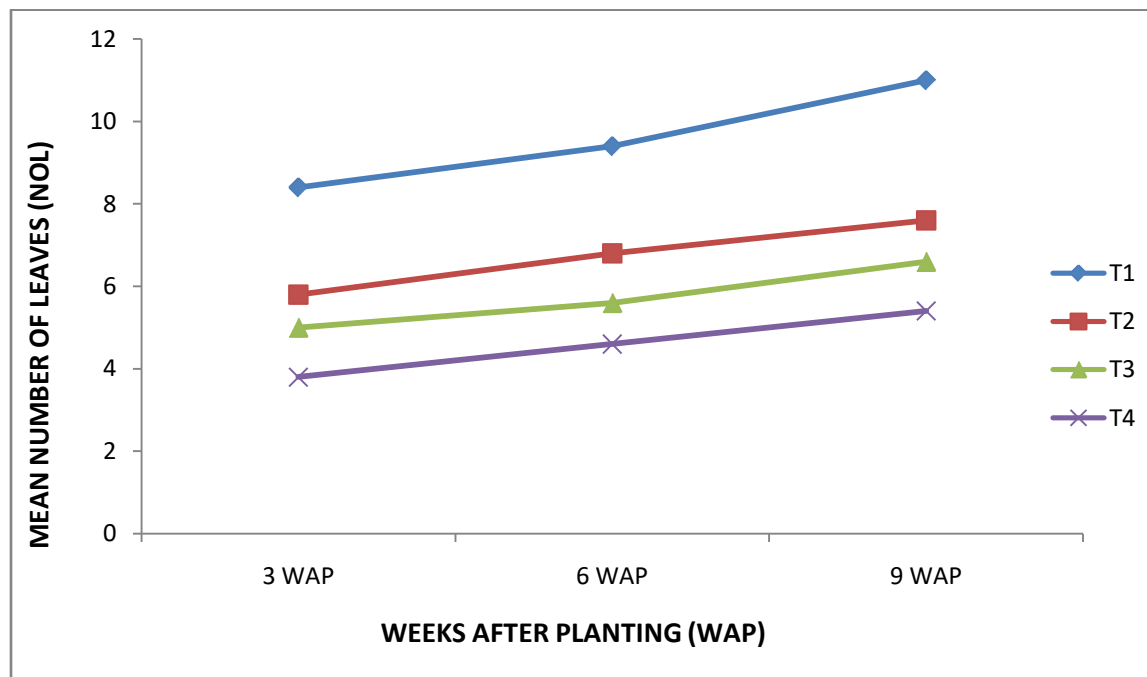


Figure 2: Mean number of leaves of *Z. mays* at different treatments over 9 weeks after planting (WAP)

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.

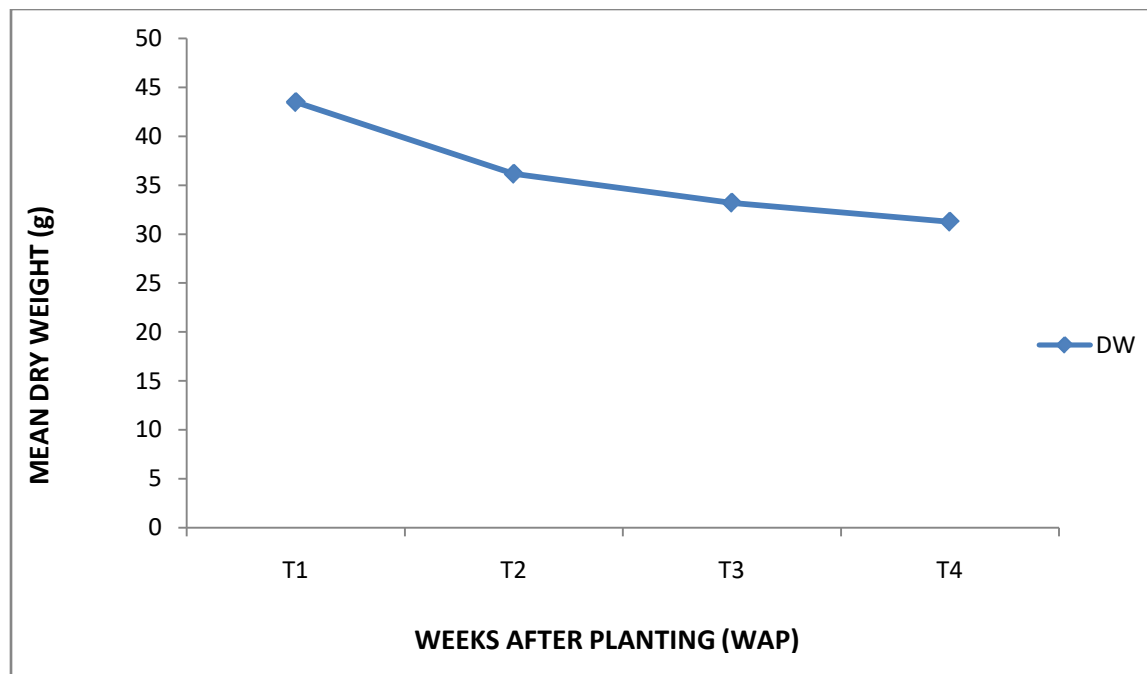


FIGURE 3: Mean dry weight of *Z. mays* at different treatments over 9 weeks after planting

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:

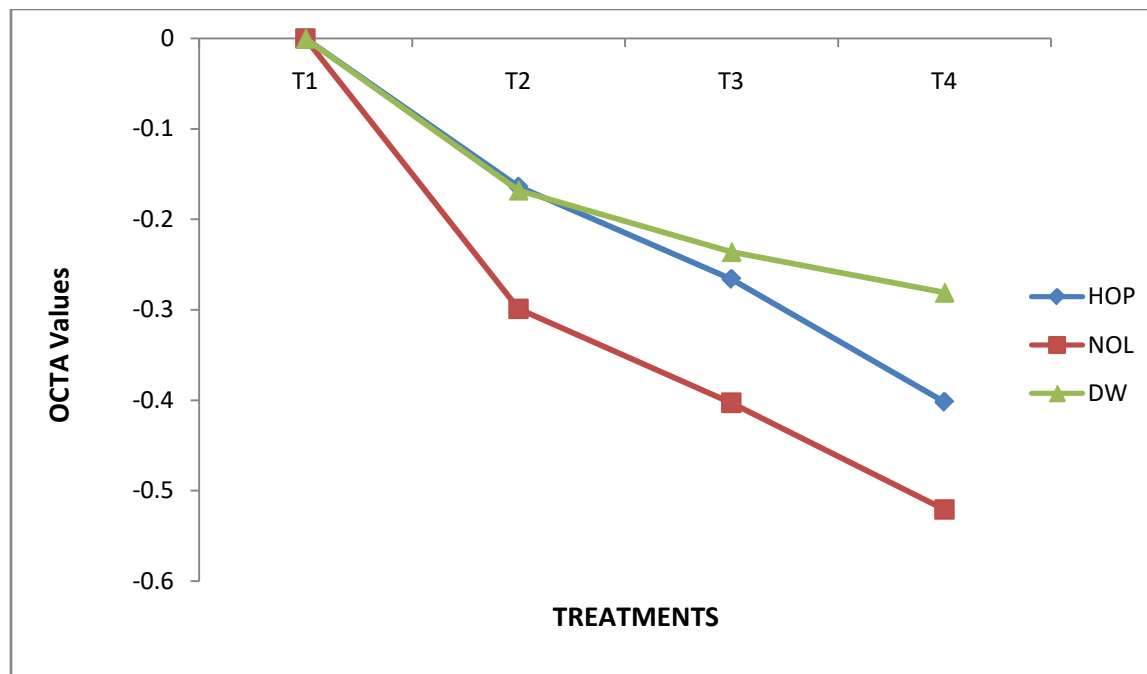


Figure 4: OCTA Trend for HOP, NOL and DW.

The Relative OCTA Trend shows how the treatments fared 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 Okonwuet *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. Okonwuet *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 Dasukiet *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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