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Effect of Soil Contamination With Diesel Oil on The Growth Characteristics and Epidermal Features of Zea Mays L.

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Abstract

Oil pollution is undoubtedly one potential consequence of industries at large, and oil pollution and contamination occurs in countries around the world. However, only a few research have examined the deleterious effect of diesel oil on plant anatomy. Therefore, the major objectives include: to determine the impacts of diesel oil on the growth parameters, physiology and the anatomical features of Zea mays L. Experimental plots were treated with four different percentage of diesel oil (4.64mg/kg, 35.91mg/kg, 48.39mg/kg, 65.89mg/kg and 67.64mg/kg) and compared to control plots (where no oil was applied). The effect of the treatment (diesel oil) on the growth parameters (leaf area, number of leaves, plant height and plant biomass) and anatomical features (lower epidermis) were investigated at 2, 4, 6, 8 and 10 weeks after planting. Results of this experiment showed that for the parameters studied (leaf area, number of leaves and plant biomass), the values obtained for the treatments were reduced compared to the control, thereby establishing that diesel oil had a negative impact on Z. mays. Also, the diesel oil impacted some of the Physiological characters (plant biomass and chlorophyll content) and the anatomical features (stomata length and epidermal cell length). Hence, it is advised that Z. mays should be grown on soils with little or no diesel content to enable optimum growth and development of the plant. Further analysis should be carried out to ascertain the effect of diesel oil on other anatomical features (root and petiole) of Z. mays for future research and decision making for sustainable agro-ecosystem.

Keywords: Ze Mays, Diesel Oil, Soil, Epidermal, Biomass.

1.0: INTRODUCTION

Oil spillage is a global issue that has been occurring since the discovery of crude oil, which was part of the industrial revolution. The total spillage of petroleum products into the oceans, seas and rivers through human activities is estimated at 0.7 – 1.7 million tons per year. Oil spills have posed a major threat to the environment of the producing areas, which if not effectively checked can lead to total destruction of ecosystems. The Niger Delta is among the ten most important wetland and marine ecosystems in the world. The oil industry located within this region has contributed immensely to the growth and development of the country which is a fact that cannot be disputed but unsustainable oil exploration activities has rendered the Niger Delta region one of the five most severely petroleum damaged ecosystems in the world. Studies have shown that the quantity of oil, spilled over 50 years was at least 9-13 million barrels. Petroleum is one of the dominant sources of the economic and social development of a country. Soil contamination by diesel fuel poses a great threat to the farm lands and the environment as a whole. The choice of maize as the test crop for this study is because maize is one of the cheapest sources of food energy and it plays a major role in meeting the rising consumption of both food and animal feed in developing countries; Food and Agricultural Organization, (FAO, 2002). FAO maintained that each part of maize including the stalk, leaves, silk, cob and kernels has a commercial value, the kernel being the most useful; hence maize is a universal crop specie. Maize is grown in every important Agricultural area of the world (Russell and Halluauer, 1980) including the southern part of Nigeria where oil industries activities are dominant. According to FAO, maize is an important food, fodder and industrial crop. It is only second to wheat in terms of cereal production. It is widely grown and consumed in Nigeria and also serves as good raw materials for industrial activities. Environmental pollution from oil activities in a major oil producing country as Nigeria is inevitable (Agbogidi and Eshegbeyi, 2006). It has become necessary to investigate the effects of oil spillage on agricultural lands and the crops grown in them. Maize is a widely grown crop among farmers in rural areas and is therefore suitable as test crop for experimental study as this. On the other hand, diesel fuel, at least as traditionally formulated, produces greater quantities of certain air pollutants such as sulfur and solid carbon particulates, and the extra refining steps and emission-control mechanisms put into place to reduce those emissions can act to reduce the price advantages of diesel over gasoline.

In addition, diesel fuel emits more carbon dioxide per unit than gasoline, offsetting some of its efficiency benefits with its greenhouse gas emissions. Diesel oil is one of the major products of crude oil and it constitutes a major source of pollution to the environment (Nwaogu et al., 2008). Diesel oil is a complex mixture of hydrocarbons such as low molecular weight alkanes and polycyclic aromatic hydrocarbons (PAHs). It also contains sulfur, nitrogen and oxygen in low concentrations as well as metals such as lead, nickel, sodium, calcium, copper and uranium. Diesel oil can cause chronic or acute effects in the plants. In addition to direct and indirect toxicity, the oil causes interference in the hydric relations of the plants. This interference, accompanied by the anaerobic and hydrophobic conditions, has been found to be the most important effect of diesel oil contaminated soil on seed germination and plant growth (Racine, 1994). It is known that the hydrocarbons, in particular the Polycyclic Aromatic Hydrocarbons (PAHs), are toxic to the plants, but little research has been done on plant responses to the stress caused by the aromatic hydrocarbons. Alkio et al., (2005). At the cellular level, plants showed signs of oxidative stress, including the production of hydrogen peroxide (H_2O_2) and cellular death. Diesel fuel can enter into the environment through spillage from tankers, leakages from pipelines, off shore wells, trucks and even from underground storage tanks. The symptoms caused by the contamination with oil are countless and are generally not specific. The same substance can induce different effects in different species, and conversely, the same symptoms can arise from exposure to different substances. The most common and important symptoms observed in the plants contaminated with oil and its byproducts include the erosion of the epicuticular wax (Baker, 1970), degradation of chlorophyll alterations in the stomatal mechanism, reduction in photosynthesis and respiration etc.

Chevron Corporation (2007), reported that the chemical composition of diesel oil is 86% carbon, 14% hydrogen, 15 – 500 ppm Sulfur, 75% saturated, 25% aromatics, and <18.0% olefin while the hydrocarbon of Isoparaffins (10-80 %), Naphthalene (40-70%), Aromatics (0-60%). 2 – 3 rings of aromatic (< 20%)

Table: 1: Chemical properties of diesel oil.

86%	Carbon(%)
14%	Hydrogen(%)
15_500ppm	Sulfur(%)
75%	Saturated(%)
25%	Aromatics(%)
<18.0	Olefin(%)

Source: Chevron corporation (2007).

2.0: MATERIALS AND METHODS

Study Site: The experiments were carried out at the center for ecological studies and research University of Port Harcourt. The university is located at longitude 6^o 55 26''E and latitude 4^o 54 15''N. Port Harcourt records a mean annual temperature of 28^{o}C; relative humidity is generally high over Port Harcourt with a mean annual figure of 85%. The peak of rainy season usually occurs from June to October, with the total annual rainfall of more than 2500m.

2.01: Seed Collection: The planting material used for the experiment was clean, healthy and viable seeds of maize. The improved variety (OBA SUPER -6) was obtained from ADP Rumuodomaya.

2.02: Soil Collection: The soil used for the experiment was collected from Agricultural farm land in University of Port Harcourt. The soil was sifted to remove debris and unwanted materials, then it was packed into 20 polythene bags, each bucket weighed 7kg. These buckets were perforated at the bottom and sides in order to aid easy drainage.

2.03: Soil Analysis: Soil analysis is a set of various chemical processes that determine the amount of available plant nutrients in the soil, but also the chemical, physical and biological soil properties important for plant nutrition or "soil health". Analysis are carried out on the soil before contaminating with additional contaminant, this is so that one is aware of the level of contaminant present already in soil. This will enable you trace the effect of the outcome.

Table 2: Soil Preliminary Test

NO	VALUES
PH (1.1) H2O	4.79
% Oc	1.815
%N	0.188
Mg/gp	107.573
Cmol|/kg	18.625
Cmol/kg Mg	0.852
Cmol/kg K	0.463
Cmol/kg Na	0.709
Cmol/kg Acidity	0.40
Cmol/kg Al	0.00
Cmol/kg ECEC	24.552
% Silt	19.4
% Sand	66.6

2.1: Experimental Design: The experiment carried out was Completely Randomized Design (C. R. D) of five treatments and four replicates making it a total of 20 buckets. A measuring cylinder well calibrated I milliliters was used to measure the concentration of diesel oil; 4.64, 35.91, 48.39, 65.89 and 67.64.

2.11: Treatment Application: Treatments were applied by incorporating the diesel oil at the rate 4.64, 35.91, 48.39, 65.89 and 67.64. The liquid was thoroughly mixed with the soil using a big bowl and a stick to ensure proper circulation of the diesel oil before turning it back to the planting buckets. The contaminated soil was left to settle for about 9 days while it was watered daily.

2.12: Total Petroleum Hydrocarbon: Total Petroleum Hydrocarbon are important environmental contaminants which are toxic to human and environmental receptors. TPH is the sum of volatile petroleum hydrocarbons and extractable petroleum hydrocarbons. VPH is also known as petrol (or gasoline) range organics and includes hydrocarbons from C6-C10. Diesel range organics includes hydrocarbons from C10-C28. Since diesel are among the petroleum hydrocarbons, we had to check for total quantity of diesel present in the soil after contaminating it before planting. The TPH levels in contaminated soils were specifically quantified through infrared spectrometry (IC) and gas chromatography (GC). Below is the result gotten from the TPH analysis that was conducted on the soil.

Table 3: Results of TPH Test Analysis

S/N	Sample identity	TPH (nC8 – nC40) (mg/Kg)
1	TP1 (Control)	4.64
2	TP2 (20%) Soil	35.91
3	TP3 (40%) Soil	48.39
4	TP4 (60%) Soil	65.89
5	TP5 (80%) Soil	67.64

2.13: Planting: The loamy top soil collected was thoroughly rated in order to remove non biodegradables and unwanted materials. The soil was weighed with a weighing balance using the bucket and they were properly arranged. No fertilizer was added, instead the diesel oil was added at varying concentrations (4.64, 35.91, 48.39, 65.89 and 67.64). Each concentration was poured into four perforated buckets of sand. The buckets were properly labelled according to the treatment each contained using a plastic spoon. The maize seed were tested to know if they are viable. So using the floating method, 75 seeds were poured into a small bowl of water and after five minutes, only one seed floated from the bowl. The seed that floated was removed or thrown away and then the viable maize seeds were sowed 4 per bucket totaling 74 seeds at a depth of 1inches. The seed were then watered and monitored continuously. The seedlings were not thinned down but they were taken every two weeks for sectioning. The number of seedlings that emerged from each bucket was counted ten days after planting and the percentage germination was calculated using the formula:

Germination Percentage: $\frac{Number\ of\ seedlings\ that\ emerged\ from\ soil}{Number\ of\ seeds\ sown} \times 100$……………..... (1)

2.2: COLLECTION OF DATA

Data collection for the following parameters was conducted at 2 weeks' interval. These parameters are a measure of growth and development as shown in the assessment variables below.

2.21: Plant Height (cm): The height (shoot length) of the plant in different treatments and its replicate were measured by placing a meter rule from the base of the terminal bud of the main stem to the apex of the plant.

2.22: Number of Leaves: The leaves of the plant in different treatments and its replicate were carefully counted.

2.23: Leaf Area (cm^2): Leaf area (cm^2), was calculated using the method field et al., (1976) leaf × breath × 0.7. For easy measurement of the leaf area, a constant was obtained through the relationship between actual leaf area measurement on graph sheets and the estimated area obtained by the multiplication of leaf length and width. In this relationship 0.7 was obtained as a constant which when multiplied by the estimated leaf area gives the true leaf area.

For example, the leaf area was obtained as 32cm^2 while the approximate leaf area was 43cm^2. This will be $\frac{Actual}{Approximate} = \frac{32cm2}{43cm2}$ = 0.7cm^2……………………………………..…(2)

2.24: Chlorophyll Content: The chlorophyll content in the leaves of the Zea mays from the diesel contaminated soils and uncontaminated soil was determined using a modified form of Saupe, (2004) method. The chlorophyll pigments of the plant were extracted using 80% acetone. The optical density of the chlorophyll was determined with spectrophotometer set at 660nm and 643nm against 100% acetone solvent blank. We take a leaf from the mother plant and weigh it to make sure it's up to 0.1g before fixing it. The amount of chlorophyll in the extract on the basis of milligram of chlorophyll per gram of leaf tissues extracted was determined using the equation below;

T.CL Mg/L = 7.12× OD at 660 + 16.8× OD at 643…………………………… (3)

2.25: Plant Biomass (Dry Weight): The mother plant in each concentration were taken at the middle and end of planting. Then they placed inside an envelope and left to dry for weeks. After they were completely dried, they were weighed on a sensitive weighing balance. The weight of each of this plant were recorded.

2.3: ANATOMICAL STUDIES : Anatomical sections were obtained using the method of Peacock (1973). 14 days after germination, one plant was carefully excised from the first bucket of each treatment into a small poly bag to retain the fresh state of the plant. Then it was taken to the laboratory for fixing. We prepared Formalin Acetic Acid (FAA) using ethanol and acetic acid. The plants were well identified and cut into three parts using a razor blade. The plants were inserted into sample bottles and (FAA) was added to it. The plants were left inside the sample bottle for 24 - 48 hours. When the 24 hours was completed, the FAA was decanted and washed in distilled water, then ethanol was added into it. After the FAA was removed the plants were bleached. The ethanol was left inside until the day of sectioning. The required plant parts obtained includes the leaf and root of Zea mays which was used for anatomical studies.

3.0: RESULTS AND DISCUSSION.

The result of our work shows that Control (T1) had the maximum plant height from week 2 to week 10 (Fig.1). This is followed by T2 and T4 at week 2, the least Plant height at week 2 was recorded in T5. Control (T1) is statistically significant to other treatments (Table 1) from week 2 – week 10. Among the other treatments (T2 - T5) there was no significant difference between T2 and T4 although significant difference was recorded among the other two treatments (T3 and T5). The control had the highest mean plant height which was 52.43cm at 10 weeks after planting then followed by T2 which was 21.23cm, T3 was 15.13cm, T4 which was 14.58 and the shortest plant height was T5 which was 6.45cm.

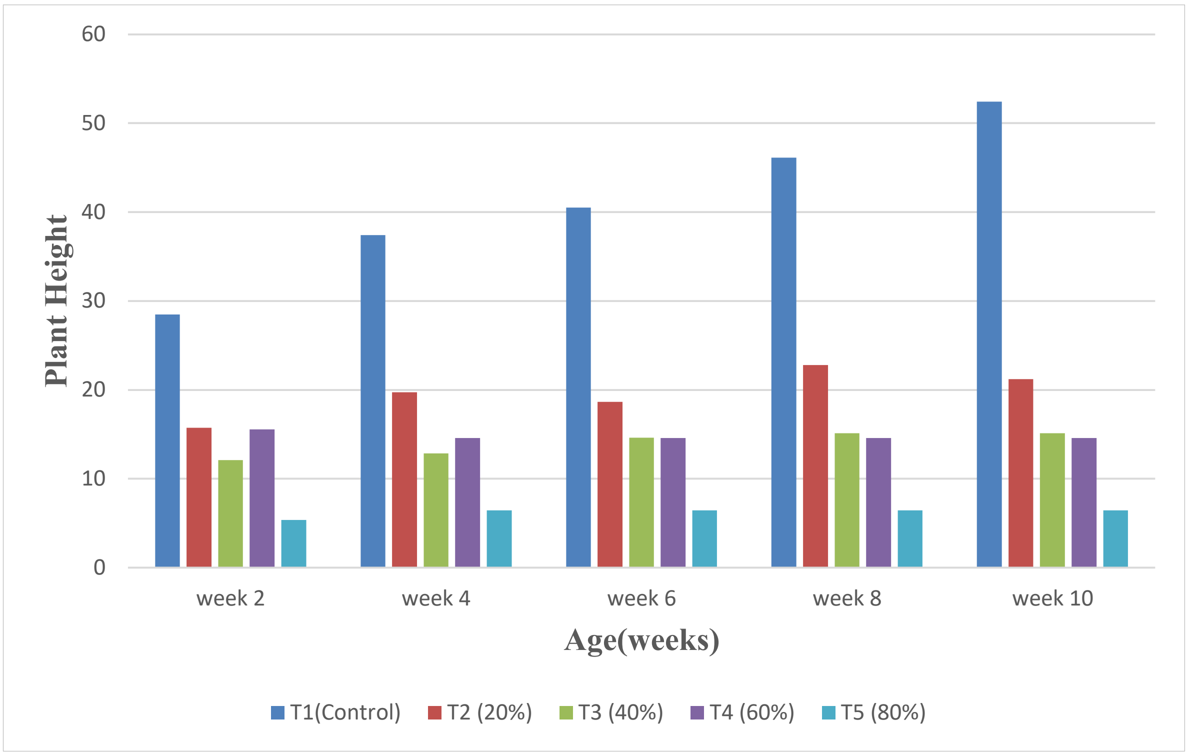

Fig 1: The Effect of Diesel Oil on Plant Height

Table 4: LSD for plant height

Treatment	Week2	Week4	Week6	Week8	Week10
T1	28.48±3.90a	37.40±14.42a	40.25±14.59a	46.13±15.49a	52.43±22.86a
T2	15.73±0.54b	19.75±1.66b	18.65±1.50b	22.8±3.79b	21.23±2.38b
T3	12.10±2.14c	12.88±1.44c	14.63±2.56c	15.13±2.46c	15.13±2.46c
T4	15.58±3.40b	14.58±3.12b	14.58±3.12b	14.58±3.12b	14.58±3.12b
T5	5.38 ± 6.22d	6.45 ± 7.74d	6.45 ± 7.74d	6.45 ± 7.74d	6.45 ± 7.74d
LSD	1.33	2.66	2.69	3.20	3.89

Note: values on the same column with the same alphabet are not significantly different.

The maximum number of leaves from week 2 – week 10 was recorded in control (T1). This is followed by T2 and T4 at week 2 and T2 and T3 at week 4, 6, 8 and 10. The least number of leaves from week 2 – week 10 was recorded in T5. There was no statistically significance differences among the varieties (Table 3.2). The control (T1) had the highest value for number of leaves which was 9.25 at10 weeks after planting when compared to the other treatments. While the least Value was recorded by T5 which was 1.5 at 10 weeks after planting.

Table 5: LSD for Number of leaves

		Week4	Week6	Week8	Week10
T1	4.25 ± 0.96	4.25 ± 1.5	6.25 ± 3.09	8.0 ± 4.32	9.25 ± 4.99
T2	3.0 ± 0	3.25 ± 0.5	5.75 ± 0.50	6.25 ±0.96	7.0 ± 2.45
T3	2.25 ± 0.50	3.5 ± 1.0	3.75 ± 1.26	3.75 ± 1.26	3.75 ±1.26
T4	2.5 ±0.58	1.5 ± 0.58	2.25 ± 0.50	2.25 ± 0.50	2.25 ± 0.5
T5	1.5 ± 1.73	1.5 ± 1.73	1.50 ± 1.73	1.50 ± 1.73	1.50 ±1.73

Note: The calculated F value for number of leaves was less than the tabulated F. This reveals that there is no statistically significant difference among the varieties. Hence there is no need to calculate LSD. The maximum leaf area from week 2-week 10 was recorded in control (Fig3.3). This is followed by T2 and T4 at week 2, the least leaf area was recorded in T5 from week 2 – week 10. There was no significant difference among the treatments (T2-T5) at week 2, although control was statistically significant to other treatment from week 4 - week 10. The untreated pot (control) had the highest value at 10 WAP which was 74.1825 cm2 followed by T2 with a leaf area of 4.6025cm2, T3 which was 3.871cm2, T4 which was 3.50875cm2 and the least of all was T5 with a leaf area of 3.185cm2.

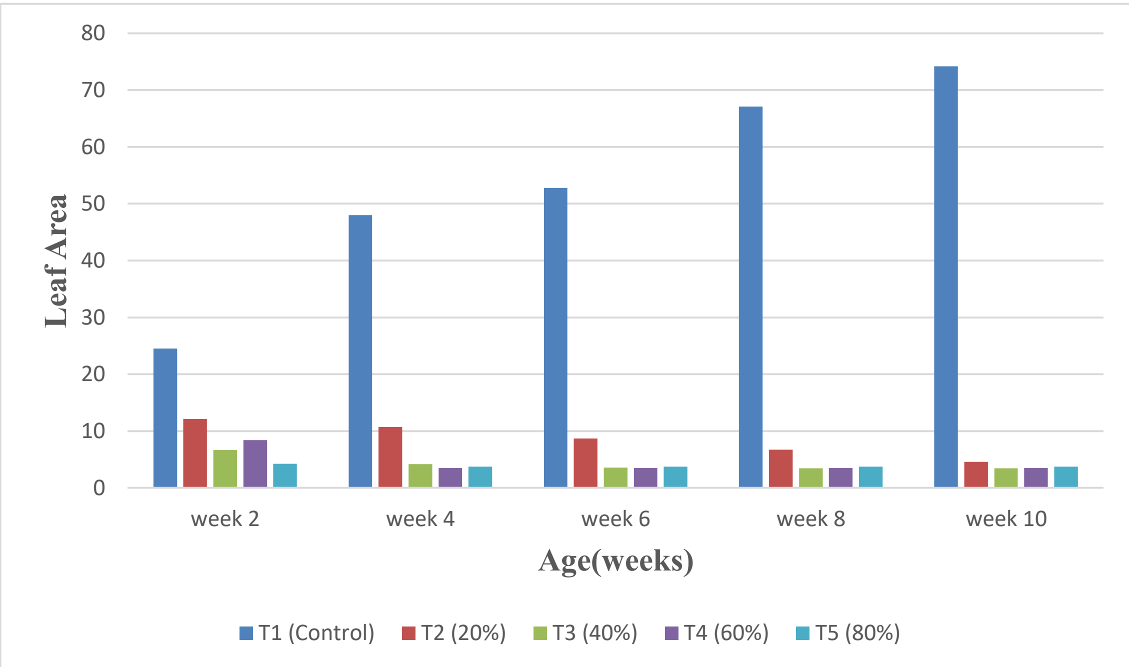

Fig 2: The Effect of Diesel Oil on Leaf Area

Table 6: LSD for leaf area

	Week2	Week4	Week6	Week8	Week10
T1	24.52 ± 4.95a	47.97±6.93a	52.81 ± 7.27a	67.11 ± 8.19a	74.18 ± 8.61a
T2	12.12 ± 3.48b	10.72 ± 3.28a	8.68 ± 2.95a	6.72 ± 2.59a	4.45 ± 2.10a
T3	6.65 ± 2.58c	4.17 ± 2.04a	3.56 ±1.89a	3.87 ± 1.96a	3.87 ± 1.97a
T4	9.31 ± 3.05d	3.51 ± 1.87b	3.51 ±1.87a	3.51 ± 1.87a	3.50 ± 1.87a
T5	3.73 ± 1.93e	3.19 ± 1.78c	3.19 ±1.78b	3.19 ± 1.78b	3.19 ± 1.78b
LSD	1.84	4.66	3.89	6.79	7.33

Note: values on the same column with the same alphabet are not significantly different.

3.01: Total Chlorophyll Content

The plant grown in the untreated pot contains more chlorophyll than those in the treated pots (Fig3.4). Among the plants grown in the untreated pots, T2 contained more chlorophyll followed by the T4 and T3 and T5 containing less chlorophyll.

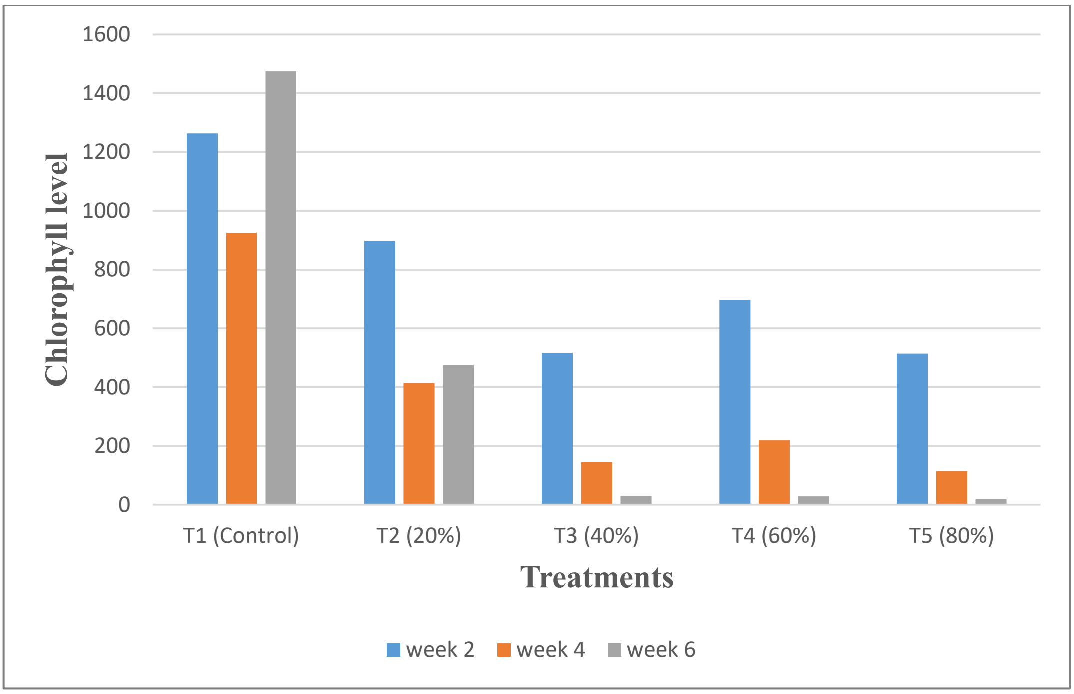

FIG 3: The effect of diesel oil on the chlorophyll content.

The highest value was recorded in control (0.55g & 0.62), which was followed by T2 (0.165g & 0.13), T3 (0.16g & 0.8), T4 (0.13g & 0.66) and T5 had the least value (0.12g & 0.5).

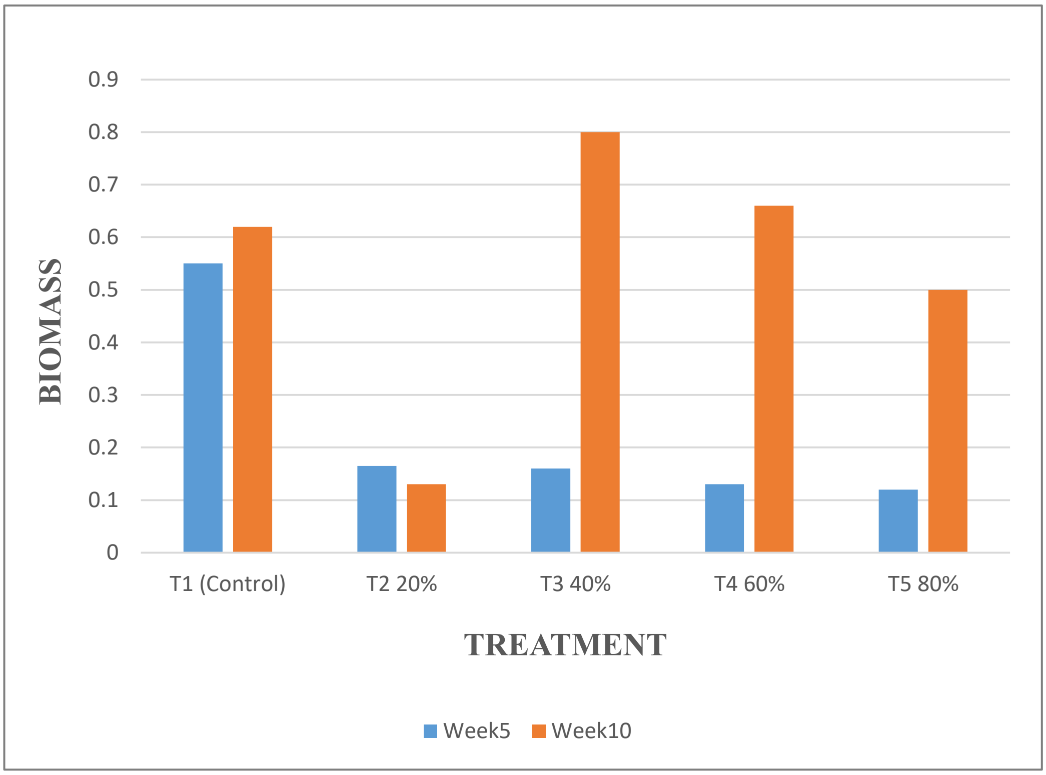

Fig 4: The effect of diesel oil on plant biomass.

3.1: DISCUSSION

Diesel fuel like every other petroleum products adversely affects the growth and performance of plants as indicated in the results. In this study, diesel oil significantly affected the growth characteristics (leaf area, number of leaves and plant height) of Zea mays plant. In plant height, control (T1) was statistically significant to the other treatments. Significant difference was also observed in T3 and T5, while T2 and T4 showed no significant difference. In the number of leaves, there were no statistical significant differences among the treatments because the F calculated was less than the F tabulated which implies that LSD calculation would not be needed. Finally, in leaf area, there was no significant difference among the treatments at week 2, whereas at week 4, control was statistically significant to other treatments. This was followed by T2 which was also statistically significant to the other treatments, but there was no significant difference among T3, T4 and T5. At week 6 – week 10, T1 was statistically significant to other treatments, whereas there was no significant difference among the other treatment (T2- T5). Significant difference existed in the effect of diesel oil pollution levels on plant height. Reduction in the height of maize plant subjected to higher levels of the oil; retardation is possible with oil pollution of soil due to insufficient aeration caused by displacement of air from pores spaces. Leaves are the sources or sites of assimilate where food materials are manufactured and transported to the sinks (the seeds and other part of the plant body). If the conditions in the sources are favourable, the rate of photosynthesis is enhanced and consequently, the yield of the plant. For example, increased number of leaves and larger surface area allow for greater photosynthetic surfaces, which can lead to higher photosynthetic rates and consequently, better yield. Therefore, the reduction of the leaf area as observed in this study implies that there would be low photosynthetic efficiency of the plant as much of the solar energy would not be absorbed by plant for photosynthesis. Thus, this can lead to low yield of the plant with subsequent low availability of food and poor economy due to low sales of this plant produce. The low value obtained in terms of dry matter yield may be due mainly to the difficulties in the absorption of water and nutrients by roots as well as toxicity of the oil components. Significant differences also existed among the number of leaves of the maize plant treated with diesel oil. It was observed that 4 weeks the leaves were already withering while still attached to the mother plants, eventually they wilted and fell out. Although the plants were watered twice in a week aside the usual rainfall, it seems that the leaves did not get enough water which lead to wilting. So it is possible that diesel causes low soil moisture. In the soil treated with higher levels of oil, water logging was observed which also could be responsible for wilting. We can deduce that diesel oil do not allow easy drainage of water in soil thus depriving the young roots of oxygen which in turn lead to yellowing and wilting of leaves due to lack of water. The root rot, stem rot, drying of lower leaves, growth stunting and super stunting, leaf burnt and falling of leaves from the base differential changes in the rate of leaf initiation and leaf growth as well as reduced root activity in maize plants exposed to higher oil levels could be attributed to the adulterated structure of the soil or higher oil assimilation by plants of higher concentrations levels applied to the soil.

4.0: CONCLUSION AND RECOMMENDATION

So far, this study has demonstrated that diesel oil levels have a significant effect on the growth of maize and diesel oil can contaminate soil which may result in low soil fertility. Maize plants that happen to survive in this contaminated soil actually became stunted. Farmlands and their surroundings must be protected against indiscriminate disposal of oil. Also, dispensing units, oil servicing company and oil exploration and production site should be cited in locations away from arable lands so as to prevent or minimize the flow of diesel oil to such soil. Doing so will reduce the poor yield and high mortality of plants due to petroleum pollution. Also farm lands that have being polluted by oil, should be remediated as soon as possible so that farming activity can begin.

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