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Petroleum Products in Soil Mediated Oxidative Stress in Cowpea (*Vigna unguiculata*) and Maize (*Zea mays*) Seedlings

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Abstract

The effects of petroleum products (kerosene, diesel, engine oil and petrol) treatment of soil at various sublethal concentrations (0.0%, 0.1%, 0.25%, 0.5%, 1.0%, 1.5% and 2.0%) on oxidative stress markers (lipid peroxidation, superoxide dismutase activity, catalase activity and xanthine oxidase) were studied in cowpea and maize seedlings. The results indicated that the petroleum products caused a significant increase in lipid peroxidation and a significant decrease in the activities of the antioxidant enzymes: Superoxide dismutase, catalase and xanthine oxidase activities. Kerosene had a greater effect on these indicators of oxidative stress than did the other petroleum products. The effects on lipid peroxidation and antioxidant enzymes were more pronounced in cowpea seedlings than in maize seedlings.

Keywords

Cowpea Seedlings, Maize Seedlings, Catalase Activity, Superoxide Dismutase Activity, Xanthine Oxidase Activity

1. Introduction

In photosynthetic plants, two forms of activated oxygen are formed from superoxide anion. These are hydrogen peroxide and hydroxyl radicals. Activated oxygen is often formed as a component of metabolism to enable "complex" chemical reactions such as the oxidation of xenobiotics or the polymerisation of lignin [1]. It is also formed by the dysfunctioning of enzymes or electron transport systems as a result of perturbations in the meta-

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bolism caused by chemical or environmental stress [1]. Activated oxygen species are produced in various sites in plants. These include the chloroplast, peroxisomes and mitochondria [2]-[4]. Various Fe-S proteins and NADH dehydrogenase have also been implicated as possible sites of superoxide and hydrogen peroxide formation [1]. The activated oxygen species generated either during normal metabolic activity or during metabolism of xenobiotics, when produced more than the body can accommodate leads to lipid peroxidation.

This process of lipid peroxidation precedes oxidative damage in plants and animals. However, living organisms are endowed with antioxidant defence systems. The defense mechanisms against oxidative damage include enzymes such as superoxide dismutase, catalase as well as non enzymes such as ascorbic acid. Alterations in the level of these antioxidants represent a measure of oxidative stress. In addition, the activity of xanthine oxidase is also an example of defense mechanism as well as a measure of oxidative stress.

Crude petroleum has been reported to alter oxidative stress indices [5]-[8]. These oxidative stress markers include lipid peroxidation and changes in the activities of anti-oxidant enzymes such as superoxide dismutase, catalase as well as xanthine oxidase The aim of this study was to investigate the effects of petroleum products: Kerosene, diesel, engine oil and petrol contaminated soil on some oxidative stress markers in cowpea and maize seedlings.

2. Materials and Methods

2.1. Refined Petroleum Products and Planting Materials

The refined petroleum products of known specific gravities (kerosene = 0.81; diesel = 0.85; engine oil = 0.87; petrol = 0.75) were obtained from Warri Refining and Petrochemical Company, Warri, Nigeria. Improved varieties of maize (*Zea mays*) were obtained as single batch from Delta Agricultural Development Project (DTADP) Ibusa Delta State, Nigeria. Improved varieties of *Vigna unguiculata* (L.) Walp were obtained from International Institute of Tropical Agriculture IITA, Ibadan, Nigeria. The soil (sand 84%, silt 5.0%, clay 0.4% and organic matter 0.6%, pH 6.1) was obtained from a fallow land in Delta State University, Abraka. The nutrient content of the soil used is shown in **Table 1**. The experiment was carried out in a laboratory condition of temperature 28˚C and 12 hr day/night regime.

2.2. Soil Treatment and Planting of Seeds

One thousand six hundred grams of soil was added to each small size planting bags (1178.3 cm^3, 15 cm deep) and divided into six groups of five replicates. Groups 1 to 5 contained 0.1%, 0.25%, 0.5%, 1.0% and 2.0% (v/w) respectively of each of the petroleum products while group six served as control (0.0%). To the first bag, 1.6 ml of kerosene, corresponding to 0.1%, was added. The petroleum product treated soil sample was mixed vigorously with hand to obtain homogeneity of the mixture. The procedure was repeated for 0.25%, 0.5%, 1.0%, 1.5% and 2.0%. This same procedure was applied to diesel, engine oil and petrol. Each treatment including control was replicated five times. The treatments were watered every day in order to keep the soil moist. The design of

Table 1. Physicochemical properties of test soil.

Parameters	Value
pH	6.09
Total organic carbon, %	2.90
Phosphorus, mg/kg	<0.01
Nitrogen, mg/kg	8.47
Nitrate, mg/kg	9.86
Cation exchange capacity, meq/100g	0.74
Sodium, mg/kg	9.06
Potassium, mg/kg	6.72
Calcium, mg/kg	2.98
Magnesium, mg/kg	0.31

the experiment was completely randomized design (CRD).

Damaged seeds were determined by floatation. All seeds that floated on water were discarded and others that remained at the bottom of water were deemed potentially plantable. Three seeds were planted in each test bag to an approximate depth of 2 cm immediately after pollution and kept under partial shade. During the experiment 80 cm^3 of water was supplied to the set up as at when needed to keep the soil moist. Germination [which is indicated by the appearance of epicotyls (for cowpea) and hypocotyls (for maize) above the soil level] records was taken at 4 days interval up to 12 days. Seeds, which failed to sprout after 12 days were regarded as not germinable. At the end of each experimental period, the seedlings were carefully removed from the bags by destroying the bags while the bulk soil containing the seedling was placed under slow running tap water to wash off the soil particles.

2.3. Preparation of Extracts for the Determination of Oxidative Stress Markers in the Leaves of Cowpea and Maize Seedlings

The leaves (0.5 g) of four day old cowpea seedlings was measured and homogenized in pre-hilled mortar with pestle in the presence of 0.05 M phosphate buffer pH 7.5 and few drops of butylated hydroxyl toluene (BHT), filtered with double layered cheese cloth and then centrifuge at 5000 g for 10 min. The supernatant obtained was finally used for the determination of oxidative stress markers. The same procedure was adopted in the preparation of extract for eight and twelve-day-old cowpea seedlings respectively. This same procedure was followed in the preparation of leave extract of four, eight and twelve day's old maize seedlings.

2.4. Determination of Lipid Peroxidation Markers in the Leaves of Cowpea and Maize Seedlings

This assay is based on the reaction of malondialdehyde (MDA) with thiobabituric acid (TBA); forming a MDA-TBA_2 adduct that absorbs strongly at 532 nm. Acetic acid (1.0 ml) was placed in a test tube and to the test tube 1.0 ml of 10% TBA was added followed by 0.1 ml of the supernatant. The test tube was covered and immersed in boiling water for 15 min. the mixture was cool, thereafter centrifuged at 5000 g for 10 min. The spectrophotometer was zeroed and absorbance of test sample was read at 532 nm against the reagent blank.

2.5. Determination of Superoxide Dismutase (SOD) Activity in the Leaves of Cowpea and Maize Seedlings

SOD inhibits the auto-oxidation of epinephrine to adrenochrome [9]. To 2 ml of the homogenate, 2.5 ml of 0.05 M phosphate buffer, pH 7.4 was added. The reaction was initiated by the addition of 0.5 ml of freshly prepared 0.3 nm epinephrine to the buffer-supernatant mixture. This was mixed by inversion. The reference cuvette contained 2.5 ml of the buffer 0.5 ml of epinephrine and 2 ml of deionized water. The increase in absorbance at 480 nm was monitored every second for 150 seconds. One unit of superoxide dismutase activity is defined as the amount of enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome at 480 nm per minute [10]. The enzyme activity was assayed with an Sp 1800 UV/VIS Spectrophptometer.

2.6. Determination of the Activity of MnSOD and Cu/Zn SOD in the Leaves of Cowpea and Maize Seedlings

Manganese dependent superoxide dismutase (MnSOD) was analysed in the presence of 1 mM NaCN, to suppress Cu/Zn SOD activity. The cytosolic copper/zinc superoxide dismutase (Cu/Zn SOD) activity was determined, as the difference between total SOD and cyanide sensitive enzyme activity [11]. The enzyme activity was assayed with a spectrometer S22.

The assay mixture contained 1.0 ml of the homogenate, 10 ml of 0.05 M phosphate buffer pH 7.4 and 0.05 ml of 1 mM NaCN. The reaction was initiated by adding 0.5 ml of 0.3% freshly prepared epinephrine. This was mixed by inversion. The absorbance was monitored at 480 nm for every 30 seconds for 150 seconds.

2.7. Determination of Catalase Activity in the Leaves of Cowpea and Maize Seedlings

Catalase breaks down hydrogen peroxide to give oxygen that oxidizes potassium dichromate. The oxidation of chromate gives a chromophore that absorbs maximally at 610 nm. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.05 M phosphate buffer (pH 7.5), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period t_1, t_2 and t_3 for 1 minute, 2 minutes and 3 minutes respectively. The reaction was terminated after each time interval by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes in boiling water and the absorbance was read at 610 nm with an Sp 1800 UV/VIS Spectrophptometer. Catalase activity was expressed in terms of moles of H_2O_2 consumed/min [12].

2.8. Determination of Xanthine Oxidase Activities in the Leaves of Cowpea and Maize Seedlings

Xanthine oxidase catalyses the conversion of methylene blue to the reduced clourless forms. Enzyme activity is proportional to the reciprocal of time taken for methylene blue to change to colourless. Two test tubes labeled control and test were placed in a test tube rack, one milliter of 0.05% neutral formaldelyde was pipetted into each test tubes. The 0.02% methylene blue solution was added in the test tube labeled test and followed by the addition of 1 ml of the supernatant to the respective test tube. 1 ml of distilled water was added to the control test tube and 2 drops of liquid paraffin was also added in both test tubes to prevent atmospheric oxidation.

2.9. Statistical Analysis

The results were expressed as mean ± SEM. All results were compared with respect to the control. Comparisons between the test and control were made by using Analysis of Variance (ANOVA), Least Significant Difference (LSD) was used to conduct Post Hoc test for the significant difference. Differences at $p < 0.05$ were considered as significant.

3. Results

Lipid peroxidation in the leaves of cowpea and maize seedlings grown in kerosene, diesel, and engine oil and petrol treated soil after four days of germination are shown in **Figure 1**. Lipid peroxidation in the leaves of cowpea seedling grown in kerosene treated soil was found to increase significantly ($p < 0.05$) from 0.25% through 2%. In maize seedlings grown in kerosene treated soil , there was a significant ($p < 0.05$) increase in lipid peroxidation in the leaves at 1.5% and 2% concentrations relative to control. Comparing lipid peroxidation in the leaves of cowpea and maize seedlings grown in kerosene treated soil; lipid peroxidation in the leaves was significantly higher in cowpea seedlings than in maize seedlings at 1.5% and 2% concentrations. Lipid peroxidation in the leaves of cowpea seedlings grown in diesel treated soil was found to increase significantly ($p < 0.05$) from 0.25% through 2% concentration compared with control. In maize seedlings grown in diesel treated soil, there was a significant ($p < 0.05$) increase in lipid peroxidation in the leaves from 0.25% to 2% concentration when compared with control. Lipid peroxidation was significantly ($p < 0.05$) lower in the leaves of cowpea seedlings than in the leaves of maize seedlings at 0.1% and slightly higher in the leaves of cowpea seedling than in the leaves of maize seedling at all other levels of concentrations tested. Also, lipid peroxidation in the leaves of cowpea seedlings grown in engine oil treated soil were found to increase significantly ($p < 0.05$) from 0.25% to 2% concentration when compared with control. Lipid peroxidation in the leaves of maize seedling grown in engine oil treated soil was also found to increase significantly from 0.25% to 2%. In comparison, lipid peroxidation was lower in the leaves of cowpea seedlings than in the leaves of maize seedlings. In all the concentrations tested, petrol treated soil resulted in significant ($p < 0.05$) increase of lipid peroxidation from 0.25% to 2% in the leaves of cowpea seedling when compared with control. Lipid peroxidation in the leaves of maize seedlings grown in petrol treated soil also exhibited significant increase from concentration level of 0.5% to 2% when compared with control. When compared, lipid peroxidation in the leaves of cowpea and maize seedlings grown in petrol treated soil was not significantly different. Lipid peroxidation in the leaves of cowpea and maize seedlings grown in kerosene, diesel, and engine oil and petrol treated soil after eight days of germination are shown in **Figure 1**. Lipid peroxidation in the leaves of cowpea seedlings grown in kerosene treated soil after eight days was found to increase significantly from 0.25% through 2% when compared with control. In maize seedlings grown in kerosene treated soil, there were significant ($p < 0.05$) increases in lipid peroxidation in the leaves at 1.5% and 2% concentrations compare to control. Comparing lipid

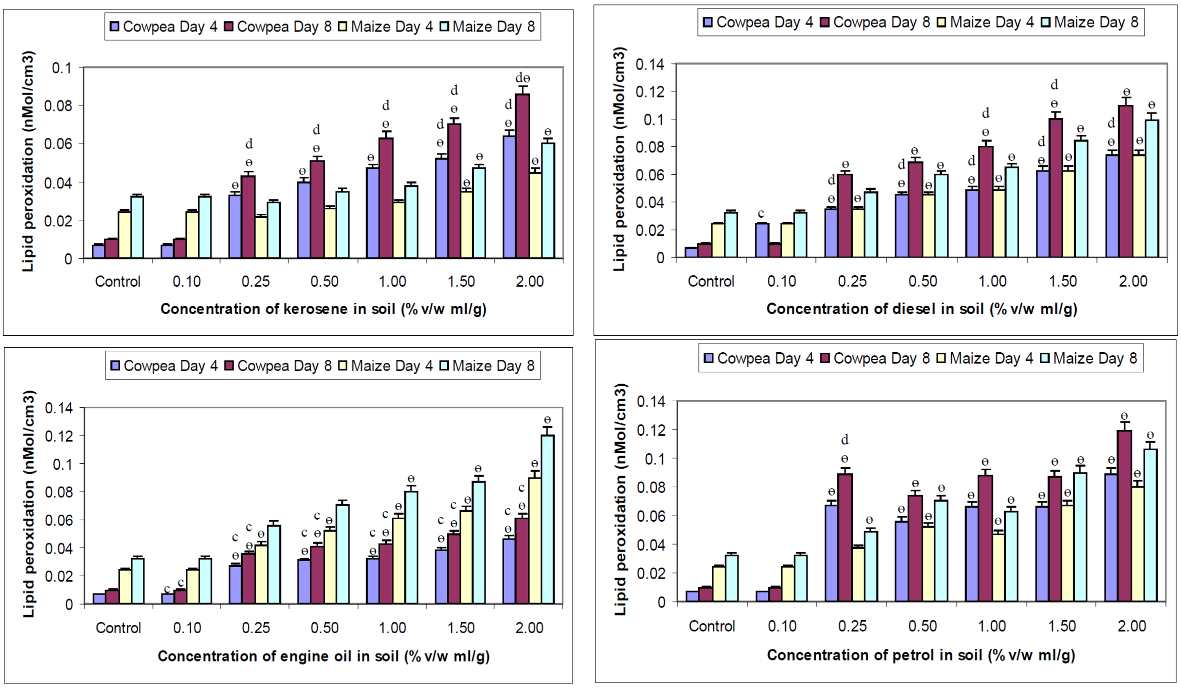

Figure 1. Effect of concentration of petroleum products on lipid peroxidation in the leaves of cowpea and maize after four and eight days of germination. [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [e]Significantly lower relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

peroxidation in the leaves of cowpea and maize seedlings grown in kerosene treated soil; lipid peroxidation was significantly ($p < 0.05$) higher in the leaves of cowpea seedling than in the leaves of maize seedlings from 0.25% through 2% concentrations. Lipid peroxidation in the leaves of cowpea seedlings grown in diesel treated soil were found to be significantly ($p < 0.05$) higher from 0.25% to 2% when compared with control. In maize seedlings grown in diesel treated, lipid peroxidation in the leaves showed a significant ($p < 0.05$) increase from 0.5% through 2% concentrations. Lipid peroxidation were significantly ($p < 0.05$) higher in the leaves of cowpea seedling at 1% and 1.5% concentration than in the leaves of maize seedling. Also, lipid peroxidation in the leaves of cowpea seedling grown in engine oil treated soil were found to increase significantly ($p < 0.01$) from 0.25% to 2% engine oil contaminated soil relative to control. Lipid peroxidation in the leaves of maize seedlings grown in engine oil treated soil showed a significant ($p < 0.05$) increase from 1% to 2% concentrations relative to control. In comparison, lipid peroxidation was significantly lower in the leaves of cowpea seedling than in the leaves of maize seedlings. Lipid peroxidation in the leaves of cowpea seedling grown in petrol treated soil was found to increase significantly ($p < 0.05$) from 0.25% to 2% concentrations compared with control. Lipid peroxidation in the leaves of maize seedlings grown in petrol treated soil showed a significant ($p < 0.05$) increase from 0.25% to 2% compared to control. When lipid peroxidation in the leaves of cowpea seedlings and maize seedlings grown in petrol treated soil are compared, lipid peroxidation level was found to be significantly ($p < 0.05$) higher at 0.25% in the leaves of cowpea than in the leaves of maize seedlings. Lipid peroxidation in the leaves of cowpea and maize seedlings grown in kerosene, diesel, and engine oil and petrol treated soil after twelve days of germination are shown in **Figure 2**. Lipid peroxidation in the leaves of cowpea seedlings grown in kerosene treated soil was found to increase significantly at 0.25% through 2%. In maize seedlings grown in kerosene treated soil, there was a significant increase in leaves lipid peroxidation at 1.0% to 2.0% concentrations respectively of kerosene in soil. Comparing lipid peroxidation in the leaves of cowpea and maize seedlings grown in kerosene treated soil; lipid peroxidation was significantly higher in the leaves of cowpea seedlings than in the leaves of maize seedlings at 0.25% and 2% concentrations. Similarly, lipid peroxidation in the leaves of cowpea seedlings grown in diesel treated soil was found to increase significantly at 0.25% to 2% concentrations compared to control. In maize seedlings

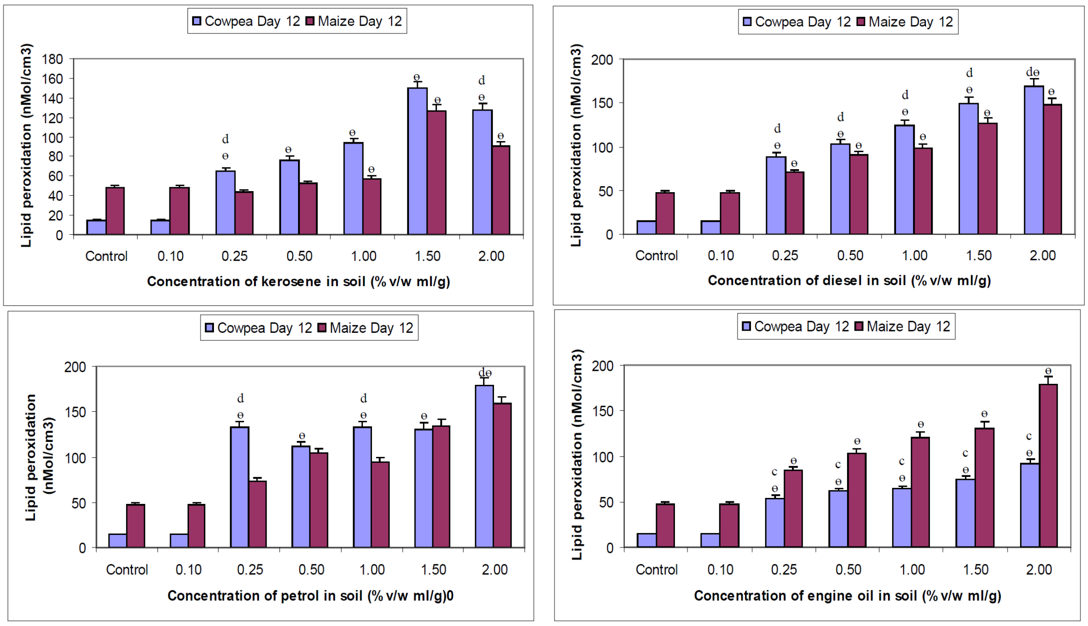

Figure 2. Effect of concentration of petroleum products on lipid peroxidation in the leaves of cowpea and maize after twelve days of germination. [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [e]Significantly higher relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

grown in diesel treated soil, there was a significant increase in lipid peroxidation in the leaves at 0.25% to 2% concentrations compared to control. Lipid peroxidation was significantly higher from 0.25% to 2% concentrations in the leaves of cowpea seedlings compared to that in the leaves of maize seedling. Also, lipid peroxidation in the leaves of cowpea and maize seedlings grown in engine oil treated soil were found to increase significantly from 0.25% to 2% relative to their respective control values. Maize seedlings grown in engine oil treated soil showed a significant increase in lipid peroxidation in the leaves from 0.25% to 2% concentrations relative to the control. In comparison, lipid peroxidation was significantly lower in the leaves of cowpea seedlings grown in engine oil contaminated soil than in the leaves of maize from 0.25% to 2% concentrations. Lipid peroxidation in the leaves of cowpea and maize seedlings grown in petrol treated soil were found to increase significantly from 0.25% to 2% concentrations. When lipid peroxidation in the leaves of cowpea and maize seedlings are compared in petrol treated soil, lipid peroxidation was found to be significantly higher at 0.25%, 1.0%, and 2.0% concentrations in cowpea seedlings over maize seedlings.

The activities of SOD in the leaves of cowpea and maize after four days of germination in kerosene, diesel, and engine oil and petrol treated soil are presented in **Figure 3**. Kerosene treated soil resulted in significant decrease ($p < 0.05$) in SOD activity in the leaves of cowpea seedlings compared with control. Maize seedling grown in kerosene treated soil resulted in significant ($p < 0.05$) decrease in SOD activity in the leaves in all the concentrations tested except at 1.5% where a slight decrease was observed. When the activities of SOD in the leaves of cowpea and maize seedlings grown in kerosene treated soil are compared, the enzyme was found to be significantly higher at 0.5% and 1% concentration in the leaves of cowpea seedlings than in the leaves maize seedlings. SOD activities in the leaves of cowpea seedlings grown in diesel treated soil showed a significant ($p < 0.05$) decrease across all the concentrations tested except at 1.5% compared with control. In maize seedlings grown in diesel treated soil, SOD activities in the leaves decreased significantly ($p < 0.05$) across all the concentrations tested relative to the control. When the activities of SOD in the leaves of cowpea and maize seedlings grown in diesel treated soil are compared, it was found to be slightly higher in cowpea seedling than maize seedlings. The activities of SOD in the leaves of cowpea seedling exposed to engine oil treated soil caused a significant ($p < 0.05$) increase in the activity of the enzyme above 1% levels of concentration compared with control. It only caused a slight decrease at 0.25% concentration. In maize seedlings

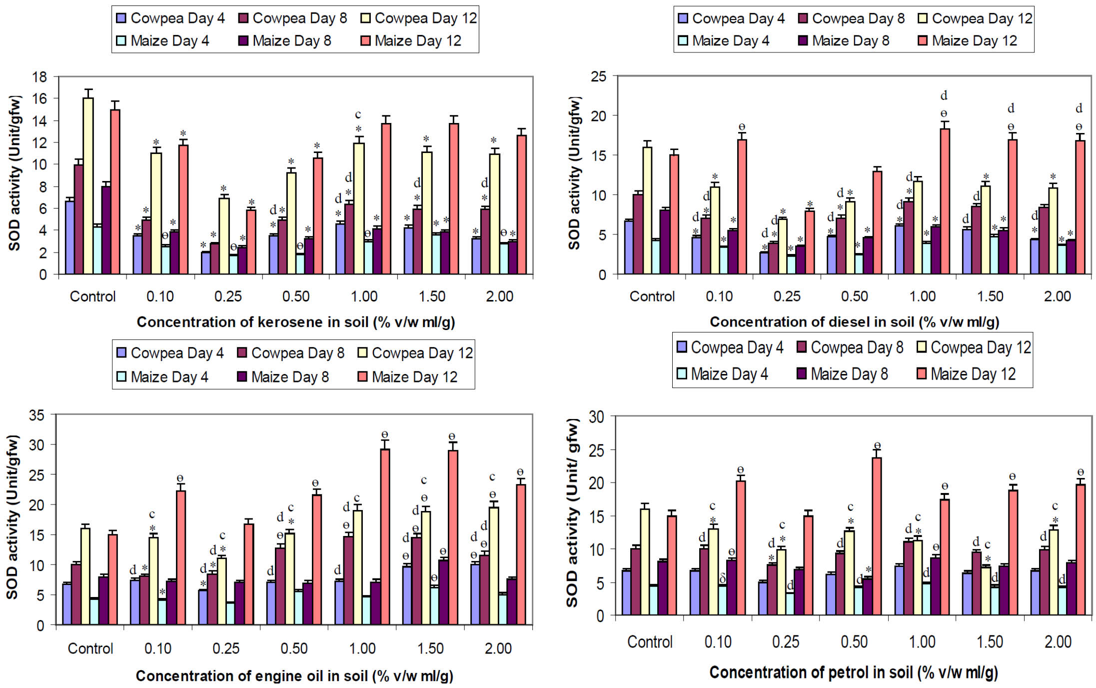

Figure 3. Effect of concentration of petroleum products on total SOD activities in leaves of cowpea and maize after four, eight and twelve days of germination. [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [o]Significantly higher relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

grown in diesel treated soil SOD activity significantly ($p < 0.05$) increased at 1.5% concentration and also showed a slight decrease at 0.1% and 0.25% concentration when compared with control. In all the concentrations, the activities of the SOD in the leaves were significantly higher in cowpea seedlings when compared to maize seedlings. Petrol treated soil had a slight increase in the activity of SOD in the leaves of cowpea seedlings at 1% concentration but showed slight reduction at 0.25% and 0.5% concentrations when compared with control. In maize seedlings however, only slight decrease in the activity of leaves SOD was observed at 0.25% concentration. No reasonable change was shown at all other levels of concentration. In all the concentrations tested, the activities of the enzyme were found to be significantly ($p < 0.05$) higher in the leaves of cowpea seedlings compared to maize seedlings. After eight days, kerosene treated soil resulted in significant decrease in SOD activity in the leaves of cowpea seedlings compared with control. Similarly, kerosene treated soil caused a significant decrease in SOD activity in the leaves of maize seedlings. When the activities of SOD in the leaves of cowpea and maize seedlings grown in kerosene treated soil are compared, the enzyme was found to be significantly higher in the leaves of cowpea seedlings at kerosene concentration of 1% to 2% than in the leaves of maize seedlings. The activities of SOD in the leaves of cowpea seedlings grown in diesel treated soil showed a significant ($p < 0.05$) decrease at 0.1% to 0.5% concentrations when compared with control. Slight decrease of leaves SOD activity was observed at other levels of concentration except 1%. Kerosene treated soil resulted in significant ($p < 0.05$) decrease of SOD activity in the leaves of maize seedlings in all the concentrations tested compared with control. When the activities of SOD in the leaves of cowpea and maize seedlings grown in diesel treated soil are compared; it was found to be significantly ($p < 0.05$) higher in the leaves of cowpea seedlings than in the leaves of maize seedlings. SOD activity in the leaves of cowpea seedling grown in engine oil treated soil showed a significant ($p < 0.01$) decrease up to 0.25%. However, further increase in engine oil concentration led to significant ($p < 0.05$) increase of leaves SOD activity. However, in maize seedlings grown in engine oil treated soil, there was a significant ($p < 0.05$) increase in the activity of leaves SOD at 1.5% concentration while a slight decrease of leaves SOD activity at 0.5% concentration was observed. In all the concentrations, the activities of leaves SOD were significantly higher in cowpea seedlings at concentration levels above 0.25% when compared to the leaves SOD of maize seedlings. Petrol treated soil had a slight increase in the activities of total SOD in the leaves of cowpea seedlings at 0.1% and 1% levels of soil contamination. However, a significant decrease of leaves SOD activity in cowpea seedling at 0.25% concentration was observed. In maize seedlings, significant ($p < 0.05$) increases of leaves total SOD activity was observed at 0.1% and 1% concentrations; while a significant ($p < 0.05$) decrease was recorded at 0.5%. When compared, leaves total SOD activity in cowpea seedlings was significantly ($p < 0.05$) higher than in maize seedlings. The activities of superoxide dismutase (SOD) in the leaves of cowpea and maize seedlings after twelve days of germination in kerosine, diesel, engine oil and petrol treated soil are shown in **Figure 3**. In all the concentrations tested, kerosene treated soil resulted in significant decrease ($p < 0.05$) of SOD activity in the leaves of cowpea compared with control. However, in maize seedling, kerosene treated soil only resulted in significant decrease ($p < 0.05$) up to 0.5% concentration; thereafter further increase in kerosene concentration did not result in significant decrease in SOD activity. When the activities of SOD in the leaves of cowpea and maize seedlings grown in kerosene treated soil are compared, the enzyme was found to be significantly lower in the leaves of cowpea seedlings at kerosene concentration of 1.0% than in the leaves of maize seedlings. Similarly, in all the concentrations tested, diesel treated soil resulted in significant decrease ($p < 0.05$) of SOD in the leaves of cowpea seedlings compared with control. However, in diesel treated soil, there was significant decrease in the activity of SOD in the leaves of maize seedlings at 0.25%. Moreover, there was a significant increase in the activity of SOD in the leaves of maize seedlings at 0.1, 1.0% 1.5% and 2% diesel concentrations. When the activities of total SOD in the leaves of maize seedlings are compared with those of cowpea seedlings grown in diesel treated soil, the enzyme was found to be significantly ($p < 0.05$) higher in the leaves of maize seedling at diesel concentrations above 0.5%. Unlike kerosene treated soil, the changes in the activities of the enzyme in diesel treated soil are not concentration dependent.

The activities of superoxide dismutase (SOD) in the leaves of cowpea seedling grown in engine oil treated soil significantly ($p < 0.05$) decreased up to 0.5%. At higher concentrations of engine oil above 1.0% there was a significant increase in the activity of the enzyme in the leaves of cowpea seedlings. However, in maize seedling grown in engine oil treated soil, there was a significant ($p < 0.05$) increase in the activity of leaves SOD in all the concentrations except at 0.25% engine oil in soil. In all the concentrations, the activities of the enzymes were significantly ($p < 0.05$) lower in the leaves of cowpea seedlings when compared to maize seedling. Petrol treated soil had a significant ($p < 0.05$) reduction in the activities of (SOD) in the leaves of cowpea seedlings grown in all the concentrations tested relative to the control. In maize seedlings grown in petrol treated soil, there was a significant ($p < 0.05$) increase in the activity of leaves SOD at 0.1%, 0.5%, 1.0%, 1.5% and 2.0% petrol in soil. However, there was no change in the activity of the enzyme in maize seedlings grown in 0.25% petrol contaminated soil. In all the concentrations tested the activity of leaves SOD was found to be significantly lower in the leaves of cowpea seedlings compared to that in the leaves of maize seedlings.

The activities of Cu/Zn SOD in the leaves of cowpea and maize seedlings after four days of germination in kerosene, diesel, and engine oil and petrol treated soil are shown in **Figure 4**. In all the concentrations tested, kerosene treated soil showed a significant decrease of Cu/Zn SOD activities in the leaves of cowpea and maize seedlings when compared with their respective control values. When the activities of Cu/Zn SOD in the leaves of cowpea seedlings grown in Kerosene treated soil were compared to that in maize seedlings, it was found to be significantly higher in the leaves of cowpea seedlings at 1% to 2.0% than in the leaves of maize seedlings. In cowpea seedlings grown in diesel treated soil, the activities of Cu/ZnSOD in the leaves showed a significant ($p < 0.05$) decrease in all the concentrations tested compared with control. However, in maize seedlings a significant ($p < 0.05$) decrease in Cu/ZnSOD activity in the leaves at 0.1%, 0.25%, 0.5% and 2% concentrations was observed. However, leaves Cu/ZnSOD exhibited a slight decrease at 1% and 1.5% concentrations compared with control. In contrast, the activities of Cu/ZnSOD in the leaves of cowpea seedlings grown in diesel treated soil was significantly ($p < 0.05$) lower than the activity in the leaves of maize seedlings in all the concentrations above 0.5%. At the concentrations tested the activities of Cu/Zn SOD in the leaves of cowpea seedlings grown in engine oil treated soil slightly decreases at 0.25% and 0.5% concentrations. Maize seedlings grown in engine oil treated soil exhibited a significant ($p < 0.01$) increase of Cu/Zn SOD activities in the leaves at 1.5% concentration. A slight decrease however, was observed at 0.25% and 1% concentrations. By way of contrast, the activities of Cu/Zn SOD in the leaves of cowpea seedlings grown in engine oil treated soil appeared to be significantly ($p < 0.05$) higher than in the leaves of maize seedlings. In all the concentrations tested, cowpea seedlings grown in petrol treated soil showed a slight decrease of Cu/Zn

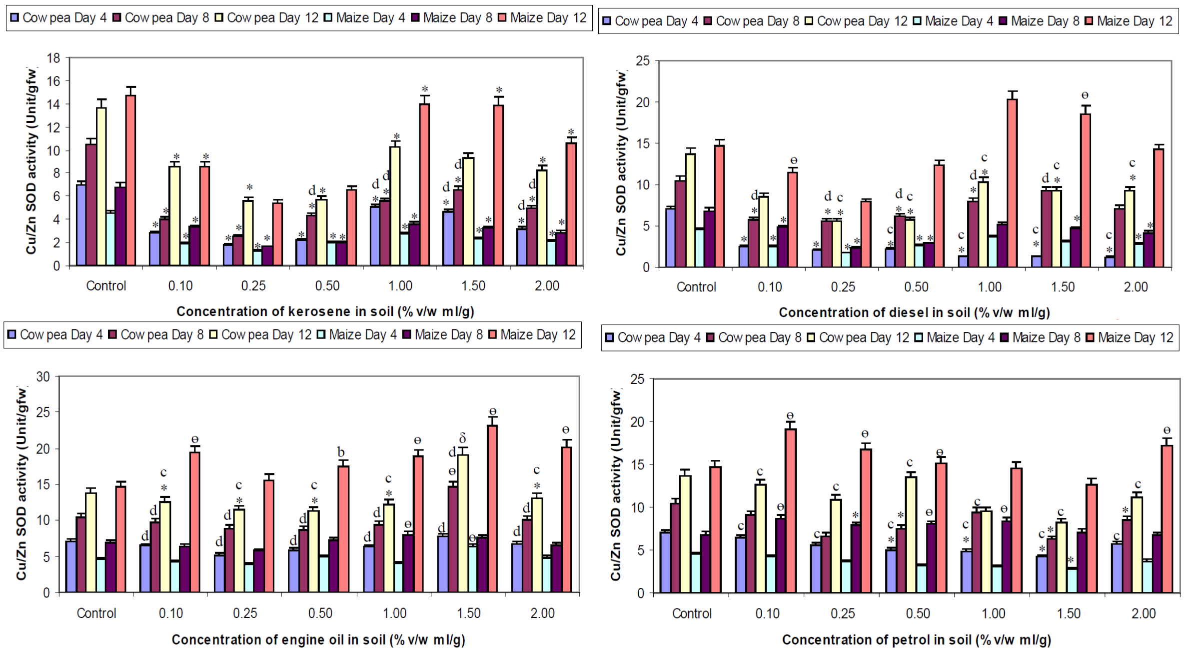

Figure 4. Effect of concentration of petroleum products on Cu/Zn SOD activities in leaves of cowpea and maize after four, eight and twelve days of germination [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [o]Significantly higher relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

SOD activities in the leaves at 0.1%, 0.25% and 2% concentrations. However, a significant ($p < 0.01$) decrease in the activity of leaves Cu/Zn SOD was observed at other levels of concentrations tested. In maize seedlings grown in petrol treated soil the activities of leaves Cu/Zn SOD decreased slightly at all concentrations tested except at petrol concentration of 1.5% where it showed a significant ($p < 0.05$) decrease in the activities of the enzyme. When the activities of Cu/Zn SOD in the leaves of cowpea seedlings are compared with the activities of the enzyme in maize seedlings, it was observed to be significantly ($p < 0.05$) lower in the leaves of cowpea seedling. The activities of Cu/Zn SOD activity in the leaves of cowpea and maize seedlings after eight days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 4**. In all the concentration tested, kerosene treated soil showed a significant ($p < 0.05$) decrease of Cu/Zn SOD activities in the leaves of both cowpea and maize seedlings compared with control. When the activities of Cu/Zn SOD in the leaves of cowpea and maize seedlings grown in kerosene treated soil are compared, it was found to be significantly ($p < 0.05$) higher in cowpea seedlings at levels of soil contamination above 0.25%. Diesel treated soil, resulted in significant ($p < 0.05$) decrease of Cu/Zn SOD activity in the leaves of cowpea seedlings in all the concentrations tested except 1.5% compared with control. Similarly, maize seedlings grown in diesel treated soil showed a significant ($p < 0.05$) decrease in the activities of Cu/Zn SOD in the leaves at all levels of concentration tested except 1% diesel concentration. When compared, diesel treated soil resulted in a significantly ($p < 0.05$) higher activity of Cu/Zn SOD in the leaves of cowpea seedlings than in maize seedlings. At the various concentrations; the activity of Cu/Zn SOD in the leaves of cowpea seedlings grown in engine oil treated soil significantly ($p < 0.05$) increase at 1.5% but slight decreases at concentration levels below 1.5% compared with control were observed. The activity of Cu/Zn SOD in the leaves of maize seedlings grown in engine oil treated soil significantly ($p < 0.05$) increase at 1% concentration. By way of contrast, the activities of Cu/Zn SOD were significantly higher in the leaves of cowpea seedlings than in maize seedling. In all the concentrations tested, cowpea seedlings grown in petrol treated soil had Cu/Zn SOD activities in the leaves slightly lower at 0.1%, and 1% concentrations, but significantly ($p < 0.05$) decreased at other levels of concentration when compared with control. In maize seedlings grown in petrol treated soil, a significant ($p < 0.05$) increase in the activities of Cu/Zn SOD in the leaves at 0.1%, 0.5% and 1% concentration was observed but minor increases were recorded at 0.25% and 1.5%. When compared, there was no significant difference in the activities of Cu/Zn SOD in the leaves of both cowpea and maize seedlings grown in petrol treated soil. The activity of copper/zinc superoxide dismutase (Cu/ZnSOD) in the leaves of cowpea and maize seedlings after twelve days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 4**. In cowpea seedlings grown in kerosene treated soil a significant decrease of Cu/ZnSOD activity in the leaves was observed at all concentrations tested. Similarly, in maize seedlings grown in kerosene contaminated soil a significant decrease of Cu/ZnSOD activity in the leaves at 0.1%, 1.0%, 1.5% and 2.0% was observed. When the activities of Cu/Zn SOD in the leaves of cowpea seedlings grown in kerosene treated soil were compared to those of maize seedlings, non significant lower values in cowpea across the various levels of concentrations was observed. In cowpea seedlings grown in diesel treated soil, the activities of Cu/Zn SOD in the leaves were found to be significantly lower at all concentrations tested compared to the control. At the various concentrations, the activities of Cu/Zn SOD in the leaves of maize seedling grown in diesel treated soil exhibited an increase at 0.1%, and 1.5%; a decrease in the activities of Cu/Zn SOD in the leaves of maize seedlings grown in diesel contaminated soil was shown at other concentrations except at 0.25% concentration. The activities of Cu/Zn SOD in the leaves of cowpea seedling grown in diesel treated soil exhibited decreases at various concentrations compared to the activity in the leaves of maize seedlings. The activities of Cu/ZnSOD in the leaves of cowpea seedling grown in engine oil treated soil significantly ($p < 0.05$) decreased at 0.1%, to 2%, but increased at 1.5%, compared to control. However, in maize seedling grown in diesel treated soil, very high increases of Cu/ZnSOD activities in the leaves at 0.1%, 0.5% to 2% concentrations were observed. The activities of Cu/Zn SOD in the leaves of cowpea seedlings grown in engine oil treated soil, were significantly ($p < 0.05$) lower across the various concentrations than in maize seedlings. Cowpea seedlings grown in petrol treated soil had Cu/Zn SOD activities in the leaves relatively lower at 0.1%, and 0.5% concentrations respectively. However, petrol treated soil resulted in a decrease in Cu/Zn SOD in the leaves activities at 0.25%, 1%, 1.5% and 2.0% concentrations. Maize seedlings grown in petrol treated soil only resulted in significant increase in the activities of Cu/Zn SOD in the leaves at all concentrations except at 1% and 1.5% relative to control. When compared, the activities of the enzyme in the leaves of cowpea seedlings significantly ($p < 0.05$) than in the leaves of maize seedlings.

The activities of MnSOD in the leaves of cowpea and maize seedlings after four days of germination in kerosene, diesel, and engine oil and petrol treated soil are shown in **Figure 5**. Kerosene treated soil resulted in significant ($p < 0.05$) decrease of MnSOD activities in the leaves of cowpea seedlings except at kerosene concentration of 1%. Similarly, kerosene treated soil gave rise to a significant decrease of MnSOD activities in the leaves of maize seedlings at all the levels of concentrations tested except 1.5% where a slight increase was observed. There was a higher increase in MnSOD activities in the leaves of cowpea seedlings than in the leaves of maize seedlings when compared. The activities of MnSOD in the leaves of cowpea seedlings grown in diesel treated soil significantly ($p < 0.05$) decreased at 0.1% 0.25% and 0.5% concentrations compared to control. However, a slight decrease was recorded at concentration levels above 1%. The activities of MnSOD in the leaves of maize seedlings exposed to diesel showed a slight decrease at all levels of concentrations tested except at 0.25% and 0.5% where significant ($p < 0.05$) reduction was observed. At 1.5% concentration MnSOD activity in the leaves of maize seedling showed a slight increase when compared with control. Nonetheless, there was a higher increase in MnSOD activities in the leaves of cowpea seedlings than in the leaves of maize seedlings. Engine oil treated soil resulted in significant ($p < 0.05$) increase in MnSOD activities in the leaves of cowpea seedlings at concentration levels above 1%. It was also found that MnSOD activities in the leaves activities exhibited a significant ($p < 0.05$) decrease at 0.1% and 0.25% concentrations. Also, MnSOD activities in the leaves of maize seedlings grown in engine oil treated soil significantly ($p < 0.05$) increased at 1.5% and 2% concentrations. However, a slight decrease was recorded at 0.25% concentration. In addition, the activities of MnSOD were significantly ($p < 0.05$) higher in the leaves of cowpea seedlings than in the leaves of maize seedlings when compared. Petrol treated soil resulted in slight decrease of MnSOD activities in the leaves of cowpea in all the concentrations except at 0.1% and 0.5% concentrations where significant ($p < 0.05$) decreases were observed. However, it showed a significant decrease at 0.25%, 1% and 1.5% concentrations. Also, there was an insignificant increase of MnSOD activities in the leaves at 2% level of petrol concentration. In maize however, petrol treated soil only gave rise to slight increase in MnSOD activities in the leaves in all the levels of concentrations tested except at 1.5% where a minor decrease was observed. When compared, MnSOD activities were slightly higher in the leaves of cowpea seedlings than in the leaves of maize seedlings. The activities of MnSOD in the leaves of cowpea and maize seedlings after eight days of germination in kerosene, diesel, engine

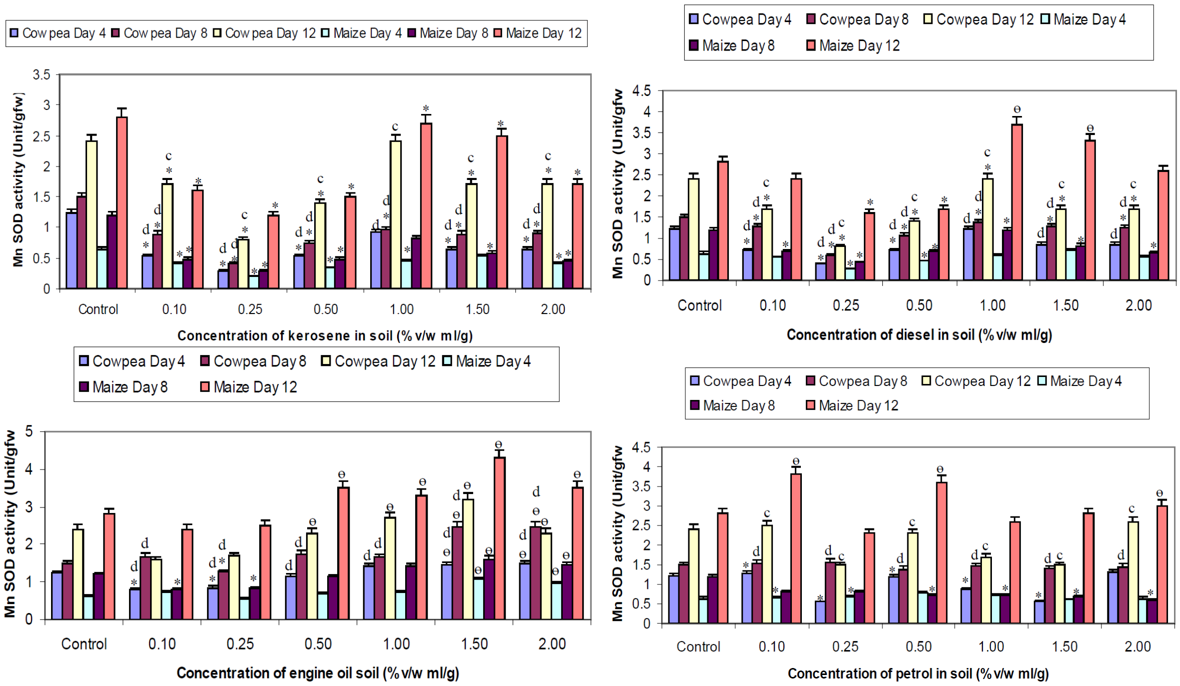

Figure 5. Effect of concentration of petroleum products on MnSOD activities in leaves of cowpea and maize after four, eight and twelve days of germination. *Significantly lower as compared to control; +Significantly lower as compared to engine oil; ++Significantly lower as compared to kerosene; eSignificantly higher relative to control; aSignificantly lower relative to other petroleum products; bSignificantly higher relative to other petroleum products; cSignificantly lower in cowpea relative to maize seedlings; dSignificantly higher in cowpea relative to maize seedlings.

oil and petrol treated soils are shown in **Figure 5**. Kerosene treated soil resulted in significant ($p < 0.05$) decrease of MnSOD activities in the leaves of cowpea seedlings grown in kerosene treated soil compared with control. In maize seedlings, however, MnSOD activities in the leaves decreased significantly ($p < 0.01$) at all the levels of concentrations tested except 1% where a slight reduction was observed. In comparison, the activities of MnSOD were significantly ($p < 0.05$) higher in the leaves of cowpea seedling than in the leaves of maize seedlings. At the concentrations tested, the activities of Mn SOD in the leaves of cowpea seedlings grown in diesel treated soil resulted in a significant ($p < 0.05$) decrease in the activity of the enzyme relative to control value. In maize seedlings exposed to diesel treated soil. MnSOD activity in the leaves decreased significantly ($p < 0.05$) in all the concentrations tested. However, at 1% concentration a slight decrease in MnSOD activity in the leaves was recorded. When the activities of MnSOD in the leaves of cowpea and maize seedlings grown in diesel treated soil were compared, it was found to be significantly ($p < 0.05$) higher across the concentrations tested in cowpea seedlings than in maize seedlings. The activities of MnSOD in the leaves of cowpea seedlings grown in engine oil treated soil significantly ($p < 0.05$) increased at 1.5% and 2% concentrations compared to control value. However, MnSOD activities in the leaves of cowpea seedlings grown at 0.1%, 0.5% and 1% concentration showed a slight increase compared to control. Nevertheless, a significant decrease was observed at 0.25%. In maize seedlings grown in engine oil treated soil, MnSOD activities in the leaves activities showed a significant ($p < 0.05$) increase at 1.5% and 2%. However, there was significant ($p < 0.05$) decrease at 0.1% and 0.25%. Moreover, a slight increase of MnSOD activities in the leaves was also observed at 0.5% and 1% concentrations when compared to the control. When MnSOD activities in the leaves of cowpea and maize seedlings grown in engine oil treated soil were compared, it was observed to be significantly ($p < 0.05$) higher at all the levels of concentrations tested in cowpea than in maize seedlings. Petrol treated soil gave rise to a slight decrease in MnSOD activities in the leaves at concentration levels above 0.25% in cowpea seedlings when compared with control. However, in maize seedling grown in petrol treated soil, a significant decrease of MnSOD activities in the leaves was exhibited in all the concentrations tested. When compared, MnSOD activities were significantly higher in the leaves of cowpea seedlings than in maize seedlings. The activities of MnSOD in the leaves of cowpea and maize seedlings after twelve days of germination in kerosene, diesel, engine oil and petrol treated soils are shown in **Figure 5**. Kerosene treated soil gave rise to a significant ($p < 0.05$) decrease in Mn SOD activities in the leaves of cowpea seedling grown in all the concentrations except 1% kerosene contaminated soil. The results also show that MnSOD activities in the leaves of cowpea seedlings grown in kerosine contaminated significantly ($p < 0.05$) decreased at 0.25% and 0.5% level of contamination. In maize seedlings grown in kerosene contaminated soil, the MnSOD activities significantly ($p < 0.05$) decreased at concentrations corresponding to those of cowpea seedlings and vice-versa. Nonetheless, MnSOD activities in the leaves of cowpea seedlings were lower than in the leaves of maize seedlings. Diesel treated soil resulted in a significant ($p < 0.05$) decrease in MnSOD activities in the leaves of cowpea seedlings at 0.1%, 1%, 1.5% and 2%. It was also found that MnSOD activities exhibited a significant decrease at 0.25% and 0.5%. Also, MnSOD activities in the leaves of maize seedlings grown in diesel treated soil significantly increased at 1% and 1.5% but decreased at 0.25% and 0.5%. When compared MnSOD activities were lower in the leaves of cowpea than in the leaves of maize seedlings, MnSOD activities in the leaves of cowpea seedling in engine oil treated soil showed a significant increase from 0.5% - 2% concentrations. On the other hand, the activities of Mn SOD in the leaves of maize seedling grown in engine oil treated soil showed a sharp increase at 0.5% - 2% concentrations. The activities of Mn SOD in the leaves of cowpea seedling grown in petrol treated soil resulted in a slight increase at 0.1%, 0.5% and 2% concentrations but decreases were recorded at 0.25%, 1% and 1.5%. The activities MnSOD in the leaves of maize seedling showed a sharp increase at concentrations levels of 0.1%, 0.5% and 2%. Conversely, the result shows a decrease in Mn SOD activities in the leaves at 0.25%, 1% and 1.5% concentrations. However, MnSOD activities were lower in the leaves of cowpea seedlings than in the leaves of maize seedling.

The activities of catalase in leaves of cowpea and maize seedling in kerosene, diesel, and engine oil and petrol treated soil after four days of germination are shown in **Figure 6**. At the various concentrations tested, kerosene treated soil brought about a successive decrease of catalase activity in the leaves of cowpea seedlings as well as in the leaves of maize seedlings compared with control. In comparison, however, there was no significant difference in the decrease across the various concentrations tested between the activities of catalase in

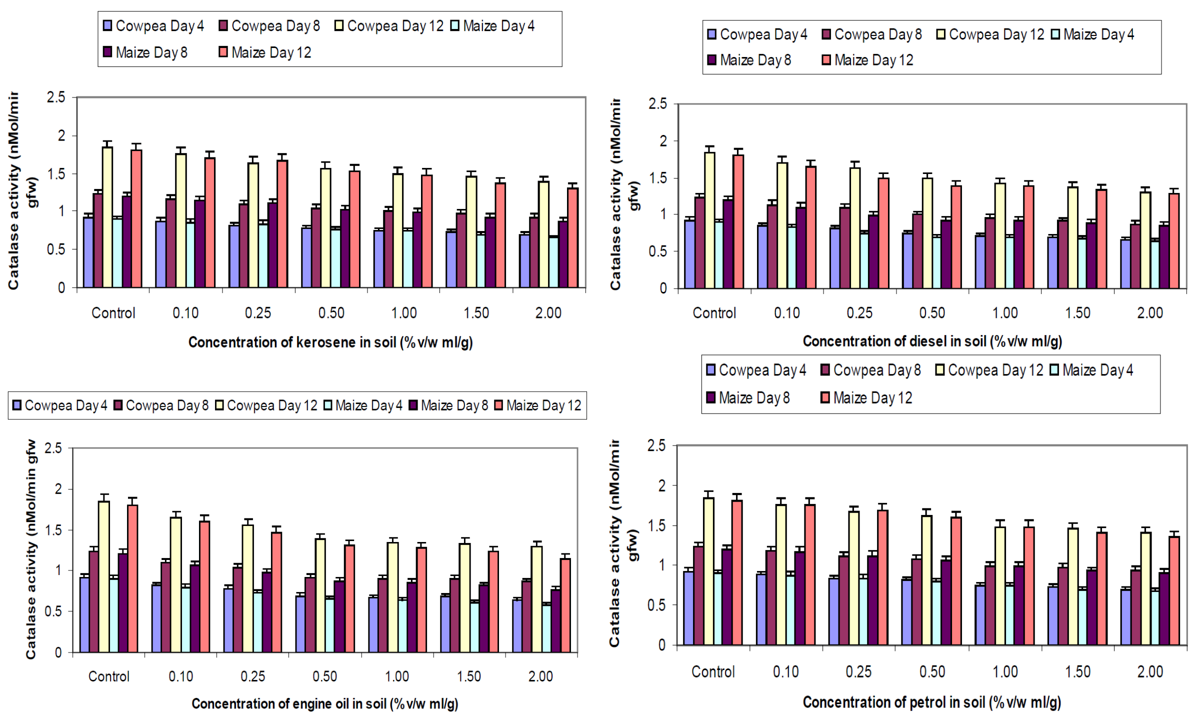

Figure 6. Effect of concentration of petroleum products on catalase activities in leaves of cowpea and maize after four, eight and twelve days of germination. [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [e]Significantly higher relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

the leaves of cowpea and maize seedlings. Catalase activities tested in the leaves of cowpea seedlings grown in diesel treated showed a decrease in activities at all levels of soil contamination compared to control. Similarly, catalase activity in the leaves of maize seedlings grown in diesel treated indicated a progressive decrease across all levels of concentrations tested. However, catalase activity appeared to be slightly higher in the leaves of cowpea seedlings than in the leaves of maize. In all the concentrations tested, engine oil treated soil resulted in successive decrease of catalase activity in the leaves of cowpea seedlings as well as in the leaves of maize seedlings relative to control. Nonetheless, the decrease of catalase activity was slightly higher in the leaves of cowpea seedlings compared to that of maize seedlings. Petrol treated soil resulted in slight decrease of catalase activity in the leaves of cowpea seedlings at all levels of concentration compared with control. Similarly, in maize seedlings, catalase activity in the leaves showed a minor decrease in all the concentration tested compared to control. There was no significant difference in the activities of catalase in the leaves of cowpea and maize seedlings.

The activities of catalase in the leaves of cowpea and maize seedling after eight days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 6**. At the various concentrations tested, kerosene treated soil brought about a successive decrease of catalase activity in the leaves of cowpea seedlings as well as in maize seedlings compared with control. In comparison, however, the decreases across the various concentrations tested were more pronounced in the leaves of cowpea seedlings than in the leaves of maize seedlings. Similarly, catalase activity in the leaves of both cowpea seedlings and maize seedlings grown in diesel treated soil decreased throughout the various levels of concentrations tested relative to control. No significant differences existed in the activities of catalase in the leaves of cowpea seedlings and maize seedlings grown in diesel treated soil. In all the concentrations tested, engine oil treated soil resulted in a slight decrease of catalase activity in the leaves of cowpea seedlings compared with control. In maize seedlings, catalase activity in the leaves insignificantly reduced across all the concentrations tested relative to control. By contrast, the decrease of catalase activity was slightly more in the leaves of maize seedling than cowpea seedlings. In all the concentrations tested, petrol treated soil gave rise to a decrease of catalase activity in the leaves of cowpea seedling compared with control. Similarly, petrol treated soil brought about decrease of catalase activity in the leaves of maize seedlings. When compared, the decrease in the activities of catalase was more in the leaves of maize seedlings than in the leaves of cowpea seedlings

The activities of catalase in the leaves of cowpea and maize seedlings after twelve days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 6**. At the various concentrations tested, kerosene treated soil brought about a successive decrease of catalase activity in the leaves of cowpea seedlings as well as in the leaves of maize seedlings compared with control. There were no distinct differences between the activities of the enzyme across the various concentrations in the leaves of cowpea seedlings as well as in the leaves of maize seedlings. Catalase activities in the leaves of cowpea seedlings grown in diesel treated soil showed a decrease in activities in all the concentrations tested. Similarly, catalase activities in all the concentrations tested in the leaves of maize seedling indicated a progressive decrease. However, catalase activities decreased less in the leaves of cowpea seedlings relative to that in the leaves of maize seedlings. Also in all the concentrations tested, engine oil treated soil resulted in significant decrease of catalase activity in the leaves of cowpea seedlings compared with control. Similarly, catalase activities in the leaves of maize seedling decreased successively as concentration of engine oil increase. However, the decrease in catalase activity in the leaves of cowpea seedlings was slightly lower compared to that of maize seedlings. In all the concentrations tested, petrol treated soil gave rise to a significant ($p < 0.05$) decrease in catalase activity in the leaves of cowpea seedling. Similarly, catalase activities decreased in the leaves of maize seedling in all the concentrations tested relative to control. However, the reduction in the catalase activity at 0.1%, 1% and 2% concentrations was less in the leaves of cowpea seedlings than in the leaves of maize seedlings. On the contrary, the reduction of catalase activities in the leaves of cowpea seedlings grown in petrol treated soil at 0.25% was more than that of maize seedlings.

The activities of xanthine oxidase in the leaves of cowpea and maize seedlings after four days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 7**. In kerosene treated soil, of the various concentrations tested, it was found that xanthine oxidase activities in the leaves of cowpea and maize seedlings decreased significantly ($p < 0.05$) compared with control. Comparing xanthine oxidase activities in the leaves of cowpea and maize seedlings, there was no significant difference between the activities of the enzyme at each level of concentration. In diesel treated soil, xanthine oxidase activity decreased significantly ($p < 0.05$) across all the concentrations tested in the leaves of both cowpea and maize seedlings. By comparison, the

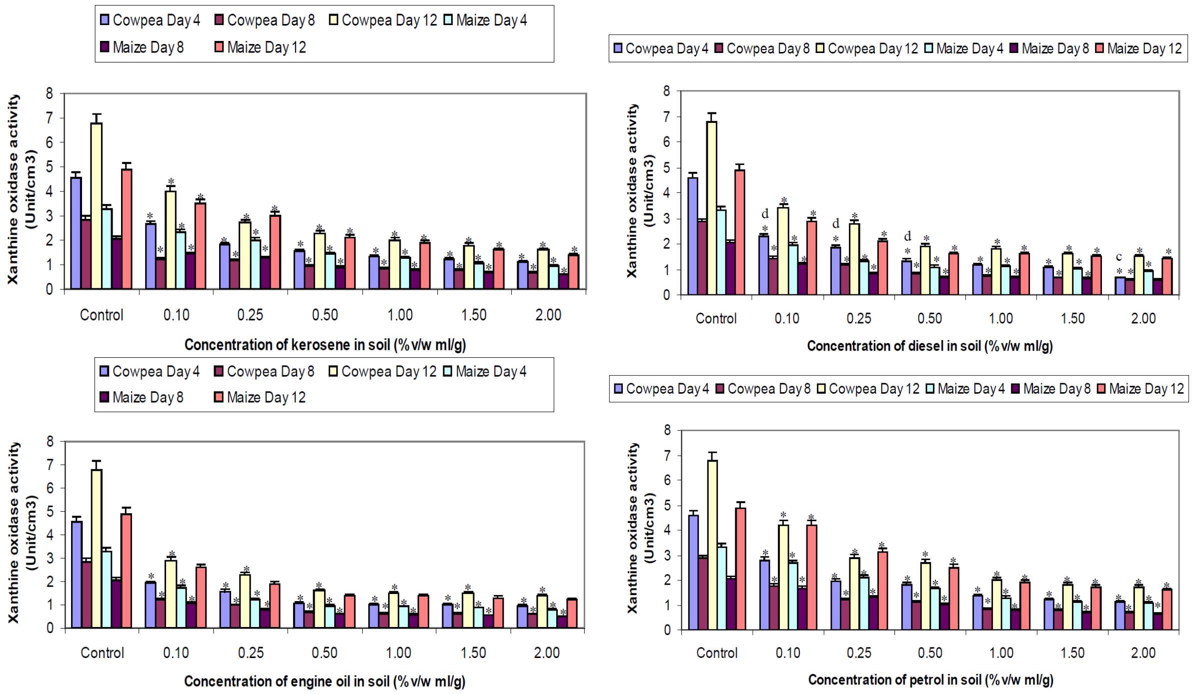

Figure 7. Effect of concentration of petroleum products on xanthine oxidase activities in the leaves of cowpea and maize after four, eight and twelve days of germination. [*]Significantly lower as compared to control; [+]Significantly lower as compared to engine oil; [++]Significantly lower as compared to kerosene; [o]Significantly higher relative to control; [a]Significantly lower relative to other petroleum products; [b]Significantly higher relative to other petroleum products; [c]Significantly lower in cowpea relative to maize seedlings; [d]Significantly higher in cowpea relative to maize seedlings.

activity of xanthine oxidase in the leaves of cowpea seedlings appeared to be higher at 0.1% - 0.5% and lower at 2% concentrations in relation to the xanthine oxidase activity in the leaves of maize seedling. In all the concentrations tested, engine oil treated soil resulted in significant decrease in xanthine oxidase activity in the leaves of cowpea seedlings. In same vain, xanthine oxidase activity in the leaves of maize seedlings decreased significantly across all concentrations tested compared with control. In comparison, xanthine oxidase activity was slightly higher in the leaves of cowpea seedlings than in the leaves of maize seedlings, but a significant difference in the enzyme activity was not observed. Petrol treated soil caused a significant ($p < 0.05$) decrease in the activities of xanthine oxidase activity in the leaves of both cowpea and maize seedlings compared with control. In comparison, xanthine oxidase activity was slightly higher in the leaves of cowpea leaves but not significantly different from the activity in the leaves of maize seedlings.

The activities of xanthine oxidase in the leaves of cowpea and maize seedlings after eight days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 7**. In kerosene treated soil, of the various concentrations tested, it was found that xanthine oxidase activities in the leaves of maize seedlings decreased significantly ($p < 0.05$) compared with control. Similarly, xanthine oxidase activity in the leaves of cowpea seedlings grown in kerosene treated soil significantly ($p < 0.05$) decrease in all the concentrations tested. Comparing xanthine oxidase activities in the leaves of cowpea and maize seedlings, it was found to be slightly lower in cowpea seedlings at all concentration except at 0.1% and 0.25%, but no significant difference existed in xanthine oxidase activity between cowpea and maize seedlings. In diesel treated soil, xanthine oxidase activities decreased significantly ($p < 0.05$) across all the concentration tested in the leaves of both cowpea and maize seedlings. By comparison the activities of xanthine oxidase increased slightly in the leaves of cowpea seedlings than in the leaves of maize seedlings across all the concentrations tested. In all the concentrations tested, engine oil treated soil resulted in significant ($p < 0.05$) decrease in xanthine oxidase activity in the leaves of cowpea seedlings. Similarly, xanthine oxidase activity decreased significantly in the leaves of maize seedlings grown in engine oil treated soil compared with control. In comparison, the activities of xanthine oxidase in the leaves of cowpea seedlings are higher than in maize seedlings. However, there is no significant difference between the xanthine oxidase activity in the leaves of cowpea and maize seedlings. Petrol treated soil gave rise to significant

($p < 0.05$) decrease in xanthine oxidase activity in the leaves of cowpea seedlings compared with control. Similarly, the leaves of maize seedlings grown in petrol treated soil witnessed a significant ($p < 0.05$) decrease in xanthine oxidase activities compared with control. When compared, the activities of xanthine oxidase activities in the leaves of cowpea seedlings were slightly higher than in the leaves of maize seedlings at 0.1%, 0.5% and1.5%, but slightly lower at 0.25%. The activities of xanthine oxidase in the leaves of cowpea and maize seedlings after twelve days of germination in kerosene, diesel, engine oil and petrol treated soil are shown in **Figure 7**. In kerosene treated soil, of the various concentrations tested, it was found that xanthine oxidase activities in the leaves of cowpea and maize seedlings decrease significantly ($p < 0.05$) compared with control. Comparing xanthine oxidase activities in the leaves of cowpea and maize seedlings, there was no significant difference between the activities of the enzyme at each level of concentration. In diesel treated soil, xanthine oxidase activity decreased significantly ($p < 0.05$) across all the concentration tested in the leaves of both cowpea and maize seedlings. By comparison, the activity of xanthine oxidase in the leaves of cowpea seedling grown in diesel treated soil was higher than in the leaves of maize seedling. But at each concentration there was no significant difference between the activity of the enzyme in the leaves of both cowpea and maize seedlings. In all the concentrations tested, engine oil treated soil resulted in a significantly ($p < 0.05$) decrease in xanthine oxidase in the leaves of cowpea seedling. Similarly, it was found that the activity of xanthine oxidase in the leaves of maize seedling was significantly ($p < 0.05$) inhibited across all concentrations relative to control. In comparison, xanthine oxidase activity was slightly higher in the leaves of cowpea seedling but not statistically different from the activity in the leaves of maize seedling. Petrol treated soil caused a significant ($p < 0.05$) decrease of xanthine oxidase activity in the leaves of both cowpea and maize seedlings. In comparison, xanthine oxidase activity was slightly higher in the leaves of cowpea seedling except at 0.25% but not statistically different from the activity in the leaves of maize seedlings.

4. Discussion

The formation of lipid peroxidation products in plants exposed to adverse environmental conditions is an indication of free radical formation in tissue and it may be used as index of lipid peroxidation in biological system [13] [14]. Previous report indicated that metal exposure results in the generation of reactive oxygen species in plant [15]. The present results show that an increasing level of refined petroleum products in soil increased the level of lipid peroxidation in the leaves of both cowpea and maize seedlings exposed to petroleum products treated soil for four and eight days after germination (**Figure 1**) and twelve days (**Figure 2**). An increase in the level of lipid peroxidation has been reported for plants exposed to metal ion [14] [16]-[18]. Elevated levels of reactive oxygen species initiate lipid peroxidation [19] [20] that culminate in oxidative stress [21]. An increase in the level of lipid peroxidation is the evidence most frequently cited in support of the involvement of oxidative stress in tissues [22]-[24].

This study shows that refined petroleum products (Kerosene, diesel, and petrol and engine oil) could induce lipid peroxidation in exposed plants (**Figure 1** and **Figure 2**). This is because refined petroleum toxicity could result in the production of reactive oxygen species (ROS); which in turn can cause membrane damage. The relationship between lipid peroxidation and ROS has been reported [25] [26]. The process of lipid peroxidation was more pronounced in cowpea seedlings compared to maize seedlings (**Figure 1** and **Figure 2**), which also lends further credence to the report that monocotyledonus seeds are less affected by toxicants than dicotyledonous seeds.

The production of reactive oxygen species during stress has been reported to results from pathways such as photorespiration, from the photosynthetic apparatus and mitochondria respiration. Environmental stresses have been shown to trigger the active production of reactive oxygen species by NADPH oxidase [27]-[30]. The enhanced production of reactive oxygen species during stress can pose threat to cells but plant have inbuilt mechanism to counteract the reactive oxygen species. The scavenging mechanisms of plant include superoxide dismutase (SOD) and catalase [31]-[33]. The present investigation indicated that the exposure of cowpea and maize seedlings to refined petroleum products, kerosene, diesel engine oil and petrol affected superoxide dismutase activity after four, eight days and twelve days of germination (**Figure 3**). After twelve days of exposure to kerosene, diesel and petrol treated soil the activity of total SOD decreased relative to control but there was increase in the activity of the enzyme in the leaves of cowpea and maize seedlings grown in engine oil treated soil compared to the other petroleum products (**Figure 3**). Decrease in the activity of the enzyme portends reduction in the capacity of the exposed plant to handle reactive oxygen species. This is because increase in the activity of the antioxidant enzyme may have a role in imparting tolerance against any type of environmental stress [33]. Moreover, the petroleum products caused a reduction in Cu/Zn-dependent superoxide dismutase after four, eight days and twelve days of germination (**Figure 4**) as well as Mn-containing superoxide dismutase (MnSOD) after four, eight days and twelve days of growth in contaminated soil (**Figure 4**). These enzymes are involved in the general defense system against natural or chemically induced production of reactive oxygen species [34]. It has been suggested that a decrease in the activity in these detoxification mechanisms can generate severe cell damage due to imbalance in the production of toxic oxygen radicals. Conversely, increased activity could contribute to cell protection from chemical toxicants [35]-[38]. Smirnoff [39] established that an increase in the capacity of antioxidant defenses in response to an increased level of reactive oxygen represents an indirect measure of oxidative stress.

The results indicated that kerosene affected the seedlings more than the other refined petroleum products. Similarly, the activity of the antioxidant enzymes in the leaves of maize seedlings appears to be less affected by the petroleum products compared to cowpea seedlings. This is exhibited by the effects of petroleum products on CuZnSOD (**Figure 4**) and MnSOD (**Figure 5**) in cowpea and maize seedlings. This is in agreement with previous reports in which maize seedling has been shown to be resistant to crude oil contamination [40] while beans possess the ability to uptake crude oil [41]. However, this observation is in contrast to the report of Baek *et al.* [42] in which *Zea mays* was found to be more sensitive to oil exposure than red beans. Previous reports of interspecies differences in sensitivity to petroleum hydrocarbon, which may be related to differences in systemic uptake of hydrocarbon and cell wall structure, had been documented [43] [44].

Catalase is another important enzyme that protect living system against oxidative stress, being able to scavenge hydrogen peroxide, which is the major product produced by superoxide dismutase [45]. The inhibition of catalase activity as concentration of petroleum product increases after four, eight days and twelve days of germination (**Figure 6**) corroborates the effect of petroleum hydrocarbon on superoxide dismutase activity in the leaves of both cowpea and maize seedlings. The similarity of catalase to superoxide dismutase is not suprising because the two enzymes have been reported to act in tandem [5] [46]. However, there is no marked difference in the activity of catalase in the leaves of both cowpea and maize seedlings at each of the concentration tested (**Figure 6**).

A wealth of information is available confirming that xanthine oxidase is involved in the metabolism of heterocyclic and polycyclic aromatic hydrocarbons [47] [48] as well as the involvement of the enzyme in the conversion of hypoxanthine via xanthine into uric acid. The role of uric acid as an antioxidant has been documented [49] [50]. The significant ($p < 0.05$) decreases in the activity of the enzyme as the concentration of petroleum products increased after four, eight and twelve days of germination (**Figure 7**) portends that petroleum mediated decrease in xanthine oxidase activity in cowpea and maize seedlings could contribute to oxidative stress by its decreased ability to metabolize aromatic hydrocarbon as well as its reduced ability to generate uric acid, a potent antioxidant. This effect is more pronounced in the leaves of cowpea seedlings compared to maize seedlings (**Figure 7**). The decrease in the activities of superoxide dismutase, catalase and xanthine oxidase as a result of increased formation of reactive oxygen species was earlier reported [51]-[54]. Therefore, it is pertinent to note that refined petroleum products could impose oxidative stress in exposed plant.

5. Conclusion

This study has indicated that exposure of plant to refined petroleum products in soil could impose oxidative stress on plant. The effect was more on cowpea relative to maize seedlings. Moreover, kerosene affected the exposed plant more than the other petroleum products

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