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Antioxidant Enzyme Activities of the Leaves of Groundnut (*Arachis hypogeae* L.) Planted on Crude Oil-Contaminated Soil

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ABSTRACT: Antioxidant enzyme activity of groundnut leaves planted in crude oil contaminated soil was evaluated. 1.5 kg of soil each was placed in black polythene bags. They were divided into four groups (A-D) of five bags each. Each group was treated with different concentrations of crude oil (0 ml, 5 ml, 10 ml and 15 ml per 1.5 kg soil respectively). five seeds of *Arachis hypogaea* were planted in each bag. The seeds were cultivated for four weeks. The leaves sample was collected at weeks 2, week 3 and week 4. The Activity of catalase, superoxide dismutase and glutathione peroxidase were assayed. Also, lipid peroxidation index was determined. Our findings show that the activity of superoxide dismutase significantly ($p < 0.05$) increases (2.0×10^{-6} to 2×10^{-6} u/mg) at all weeks. Catalase and lipid peroxidation reduced significantly (25×10^{-3} u/mg to 2×10^{-3} u/mg and 3.4×10^{-7} u/mg to 2.1×10^{-7} u/mg) at week 2 but in week 3 catalase activity increased (14×10^{-3} u/mg) but lipid peroxidation was not significant. At week 4 lipid peroxidation was significantly reduced (1.5×10^{-7} u/mg) but catalase was not significant. Glutathione peroxidase activity was significantly reduced (5.5×10^{-3} u/mg to 2.5×10^{-3} u/mg) at week 2 in contrast to weeks 3 and 4 which shows no significant difference. It was also observed that crude oil reduced the growth rate and chlorophyll content significantly. These data indicate that levels of crude oil in soil, affects negatively the oxidative and growth parameters of *Arachis hypogaea*.

Keywords: Crude oil, *Arachis hypogaea*, Antioxidant enzymes, Lipid peroxidation

Introduction

The cultivated groundnut (*Arachis hypogaea L.)* is an ancient crop, which can be traced to South America (southern Bolivia/North West Argentina region) where it was cultivated as early as 1000 B.C (Weiss *et al*., 2000). It originated in Latin America. Portuguese in the 1600's introduced this plant from Brazil to the African continent (Adinya *et al*., 2010). Groundnut is a geocarpic crop which produces fruits embedded in pods below ground. Groundnut pods are usually located at a depth of 7 - 10 cm referred to as pod zone (Ademiluyi *et al*., 2011). The duration of maturity of the groundnut crop is between 7 to 9 weeks in the soil. This is indicated by maximum levels of protein, oil, dry matter, and presence of darkened veining and brown splotching inside the pod. Groundnut is harvested when most of the leaves turned yellow and pods become hard usually 120 – 150 days after planting depending on the variety (Arakama, 2009; Oyelade *et al*., 2011). Crude oil, also known as petroleum, is the term used for the unprocessed naturally occurring oil found in the earth crust. Naturally, it is formed from decaying plants and animals living in ancient swamps, lakes and seas and buried millions of years ago. The toxicity of crude oil can be traced to the presence of toxic compounds such as polycyclic aromatic hydrocarbons, benzene and its substituent and cycloalkane rings in relatively high concentrations (Agarry and Ogunleye, 2012). Plants exposed to oil pollutions show metabolic disorder, reduced growth, yield and complete mortality (Agbogidi and Edema, 2003). In as much as studies on the effects of oil on plants have been

investigated, more scientific data are still required as food security is paramount in our world today. Crude oil pollution, arising from exploration and processing operations, is a common environmental challenge (Athar *et al*., 2015). This introduction of crude oil (via large or small spills) into the environment could arise from technical errors, deliberate human acts as well as transportation and storage faults (Khamehchiyan *et al*.,2007; Schmidt-Etkin, 2011) noted that although large spills sometimes occur with serious environmental and socioeconomic damage, small spills are more common. The impacts and damages caused by these spills generally depend on the location, oil type, volume, closeness to sensitive resources, and season, among other factors. Nonetheless, accidental large-scale oil spills make up a significant part of contaminants around the globe (Yavari, *et al.,* 2015; Ivshina *et al*., 2015) further noted that recorded cases of oil spills on land are more than those recorded in water. Thus, plant life in agricultural fields becomes exposed to petroleum hydrocarbons (PH) (Yavari, *et al.,* 2015) with both acute and chronic effects on agricultural produce (Athar *et al*., 2015). Biochemical assays of the activities of enzymes in plants can therefore serve as a credible measure of the effects of crude oil on such plants. There are a number of enzymes that are present in groundnut all of them performing specific functions. Among them are superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX) and Lipase each of which belongs to a category of enzymes known as the antioxidant enzymes. The drop or increase in the activities of these enzymes as discovered through the bioassays serve as indicators of the effects of crude oil in the plant. Superoxide Dismutase (SOD) plays a central role in the defense against oxidative stress in all aerobic organisms (Scandalios, 1993). Overproduction of SOD has been reported to result in enhanced oxidative stress tolerance in plants (Gupta *et al*., 1993). Catalase is a ubiquitous tetrameric heme-containing enzyme that catalyzes the dismutation of two molecules of H_2O_2 into water and oxygen. It has high specificity for H_2O_2, but weak activity against organic peroxides. H_2O_2 has been implicated in many stress conditions. When cells are stressed for energy and are rapidly generating H_2O_2 through catabolic processes, H_2O_2 is degraded by catalase in an energy efficient manner. Environmental stresses cause either enhancement or depletion of catalase activity, depending on the intensity, duration, and type of the stress (Moussa and Abdel-Aziz, 2008). This study was therefore tailored to evaluate the effect of crude oil on plant antioxidant enzymes activities in groundnut leaves (*Arachis hypogaea*).

Materials and methods

Sample collection: Groundnut seeds (*Arachis hypogaea*) were purchased at ring road market and crude oil was obtained from Escravos, Warri, Delta state. The soil was collected from a portion of land at the back of Faculty of Medicine, University of Benin.

Soil Treatment: Twenty polythene bags of equal size were filled with 1.5 kg of soil. The bags were perforated with holes to facilitate drainage. The soil was polluted with the following volume of crude oil: 0, 5, 10, and 15 mL to establish four levels of treatment where 0 mL treatment was the control. Each level contains five bags. Pollution of the soil was achieved by thoroughly mixing each volume of crude oil properly with soil using gloved hands. Five seeds were planted per bag at an approximate depth of 2 cm, 750 mL of water was used to keep the soil moist on a daily basis.

Sample preparation: The plants were harvested and the leaves were cut, it was homogenized using mortar and pestle and 10 mL of ice cold distilled water. The homogenized samples were placed in sample bottles and then centrifuged at 4000 g for 15 minutes. The supernatant was then used as enzyme source. The leaves sample were collected at weeks 2, 3 and 4.

Determination of chlorophyll content: This was determined according to Arnon (1949). This procedure was carried out in dim light in order to reduce photo-destruction of the pigments. fresh green leaves of the plant were collected and 1 gram of each will be weighed and ground to a fine pulp with the addition of 20 mL of 80% acetone using a mortar and pestle. This paste is centrifuged for 5 minutes at 3000 rpm and the supernatant is transferred to a 50ml beaker. The residue is grinded with 20 ml of 80% acetone, and is centrifuged for 5 minutes at 3000 rpm and the supernatant is transferred to the same beaker. This process is repeated several times till the residues became colourless. The volume is made up to 100 mL with 80% acetone. This process is repeated for all the leaf samples. The absorbance of the extract solutions was read at 645 and 663nm against the solvent (80% acetone) blank. The total chlorophyll was calculated as follows:

$$\text{Total chlorophyll } (\mu g/ml) = 20.2\ (A645) + 8.02(A\ 663)$$

Determination of total protein concentration was assayed using the method of (Henry *et al*, 1957). Cupric ions, in an alkaline medium, interact with the peptide bonds of protein resulting in the formation of a violet colored complex which is then read at 540 nm in a spectrophotometer.

Determination of catalase activity: This is based on the method of (Cohen *et al*., 1970). Plant homogenate (0.5 ml) was placed in ice-cold test tubes, the blank contained 0.5 ml distilled water. Cold phosphate-buffered H_2O_2

(30 mM, 5 ml) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 ml of 6 M H_2SO_4. The tubes were mixed thoroughly by inversion after which 7 ml of 0.01 M $KMnO_4$ was added. Absorbance was read at 480 nm within 3 minutes.

Determination of glutathione peroxidase activity: Glutathione peroxidase (GPx) activity was measured according to the method described by (Nyman, 1959). This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting in a deep brown coloration, which is read at 430 nm. To a plant homogenate (0.2 mL), 5 ml of phosphate-buffered H_2O_2 and 1.5 ml of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

Lipid peroxidation assay: Lipid peroxidation was assayed for by the method of (Buege and Aust, 1978). 1.0 ml of extract and 2.0 ml of TCA-TBA-HCl reagent was placed in a sample bottle, 3.0 mL of TCA-TBA-HCl reagent was also placed in a sample bottled labelled blank. The tubes were heated for 15 minutes in boiling water (100˚C). After cooling, it was centrifuged to give a flocculent precipitate of 1000 g for 10 minutes. The absorbance of the supernatant of the sample was read at 535 nm against the blank.

Determination of superoxide dismutase (SOD) activity: This method is well described by Mccord, and Fridovich, (1969). and can be applied for determination of antioxidant activity of a sample. It is estimated in the plant homogenate prepared. To 50 µL of the plant homogenate, 75 mM of Tris-HCL buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol are added. An increase in absorbance is recorded at 420 nm for 3 minutes by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autoxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein.

Statistical Analysis: All the analyses were carried out in triplicate and the results were expressed as mean ± SEM. The data were subjected to one-way analysis of variance (ANOVA) where applicable. P values of <0.05 were regarded as significant.

Results

Figure 1 shows catalase activity in crude oil contaminated soil. At week 2, as contamination increase, there was a significant decrease relative to the control. At week 3, there was a significant increase in the catalase activity of the 5 ml and 10 ml contamination relative to control and also a significant increase between 15 ml and 10 ml (c). No significant difference was observed at week 4.

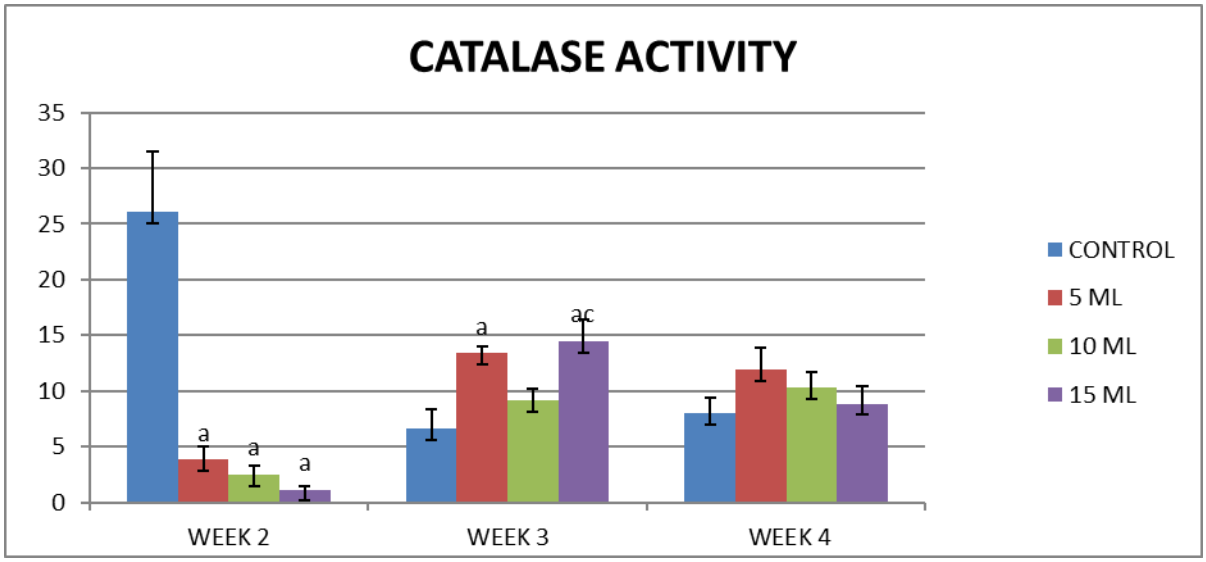

Figure 1. Catalase activity in the leaves of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (10^{-3} unit/mg). Different lower case letters represent significant difference between mean at $p < 0.05$.

Glutathione peroxidase activity of the contaminant groups is presented in Fig. 2. At week 2 there was a significant reduction in the glutathione peroxidase activity of the contaminant groups as contamination increased. At week 3 and week 4 no significance was observed.

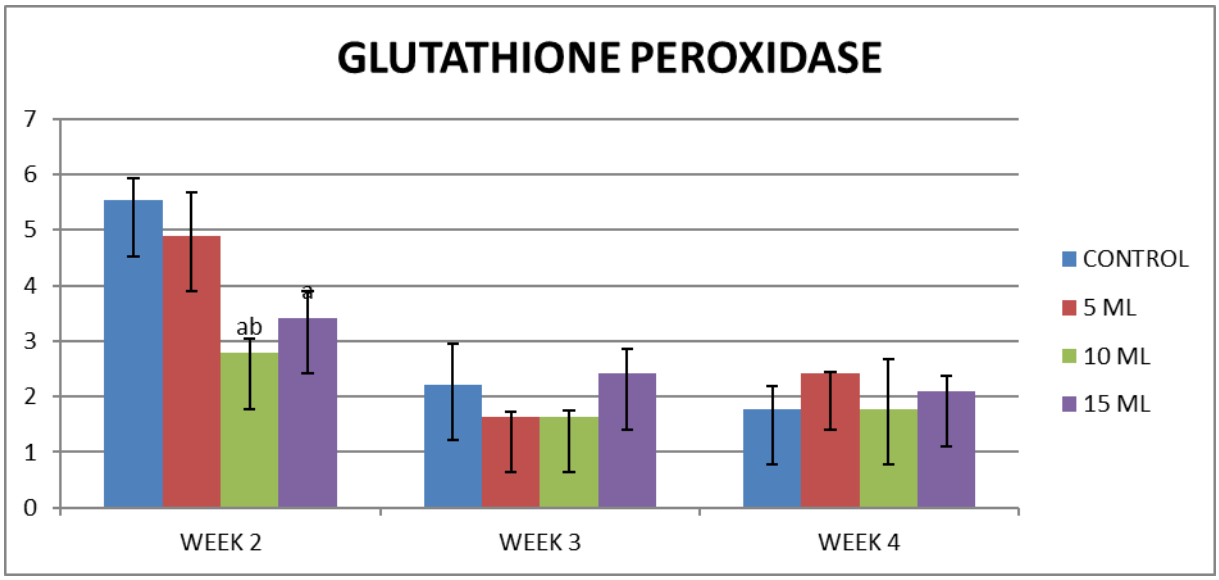

Figure 2: Glutathione peroxidase activity in the leaves of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (10^{-3} unit/mg). Different lower case letters represent significant difference between mean at $p < 0.05$.

The lipid peroxidation activity in the leaves of groundnut plant cultivated on crude oil contaminated soil is presented in Fig. 3. At week 2 and week 4, there was a significant reduction in the lipid peroxidation activity of the contaminant groups as contamination increased. No significance was observed at week 3.

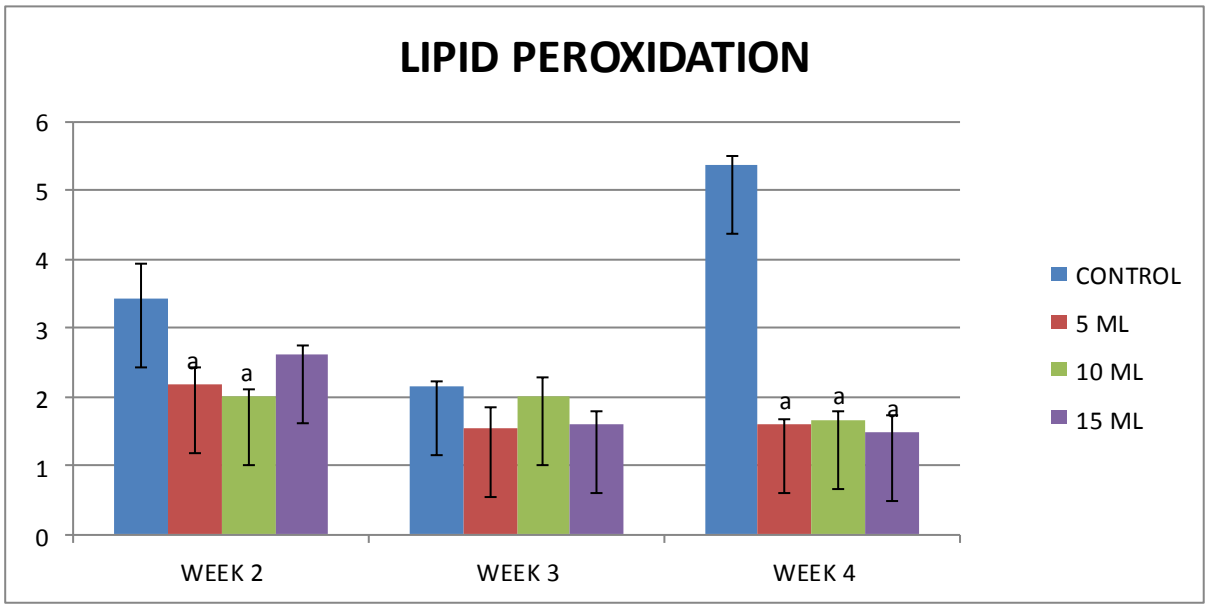

Figure 3. Lipid peroxidation activity in the leaves of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (10^{-7} unit/mg). Lower case letter represent significant difference between mean at $p < 0.05$.

Fig. 4 shows the superoxide dismutase activity in the leaves of groundnut plant cultivated on crude oil contaminated soil. At week 2, week 3 and week 4, there was a significant increase in the superoxide dismutase activity of the contaminant groups as contamination increased.

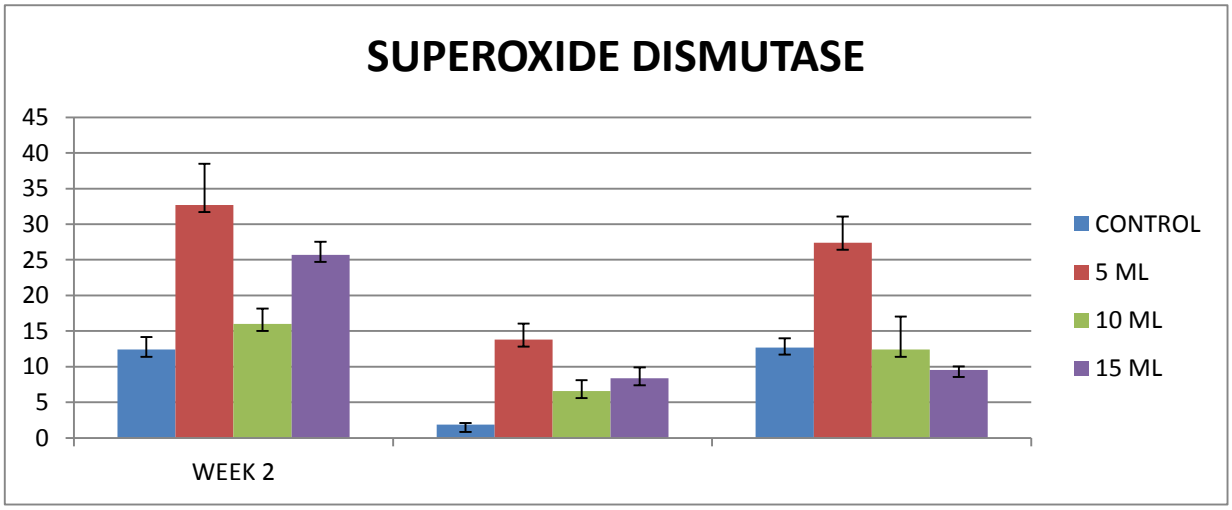

Figure 4: Superoxide dismutase activity in the leaves of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (10^{-6} unit/mg). Lower case letter represent significant difference between mean at $p < 0.05$.

The number of leaves of groundnut plant cultivated on crude oil contaminated soil is presented in Fig 5. There was a significant reduction in the leaf number of the contaminant groups as contamination increased at week 2, week 3 and week 4.

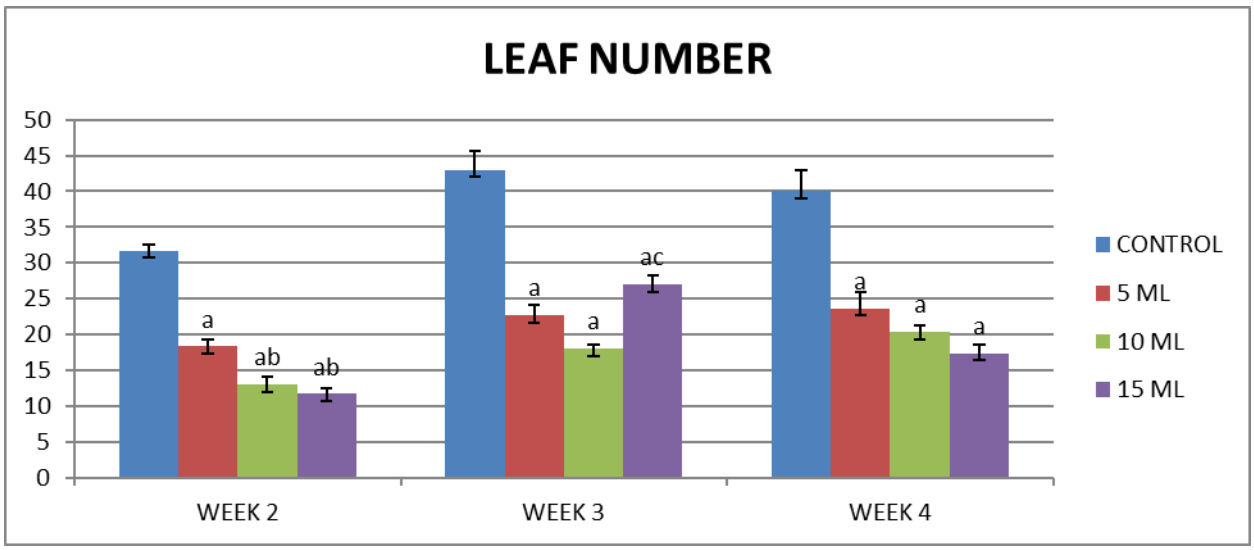

Figure 5: Leaf number of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM. Different lower case letters represent significant difference between mean at $p < 0.05$.

Leaf weight of groundnut plant cultivated on crude oil contaminated soil is presented in Fig. 6 while leaf area of groundnut plant cultivated on the same soil is presented in Fig. 7. There was a significant increase in the leaf weight of the contaminant groups as contamination increased at week 2 and week 3.No significance was recorded in week 4. Similarly, there was a significant reduction in the leaf area of the contaminant groups as contamination increased at week 2, week 3, however, there was also a significant reduction in the leaf area in week 4.

The chlorophyll content in the leaves of groundnut plant cultivated on crude oil contaminated soil is presented in Fig.8. At week 2, week 3 and week 4, there was a significant reduction in the total chlorophyll content of the contaminant groups as contamination increased.

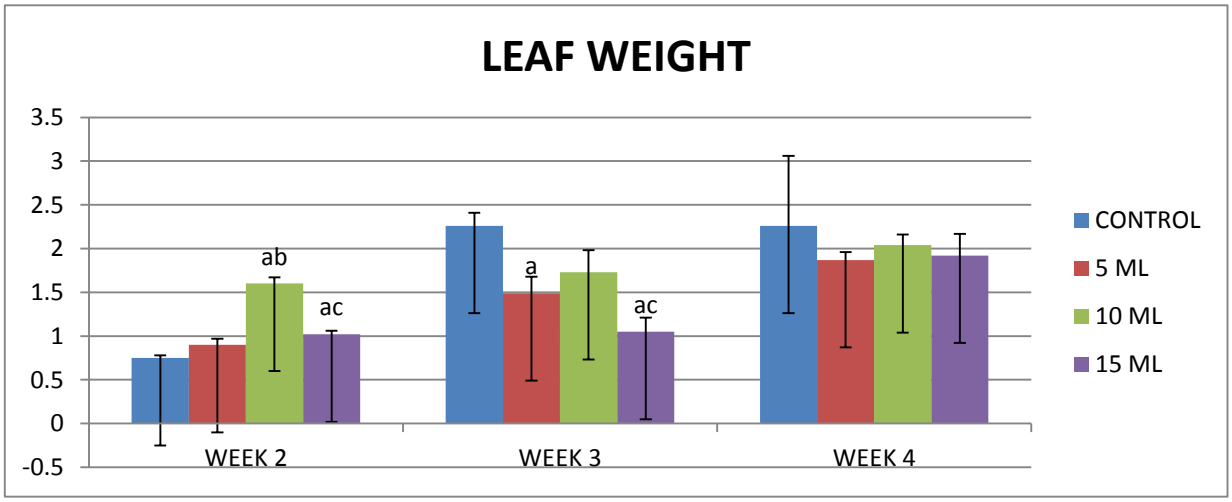

Figure 6: Leaf weight of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (kg). Different lower case letters represent significant difference between mean at $p < 0.05$.

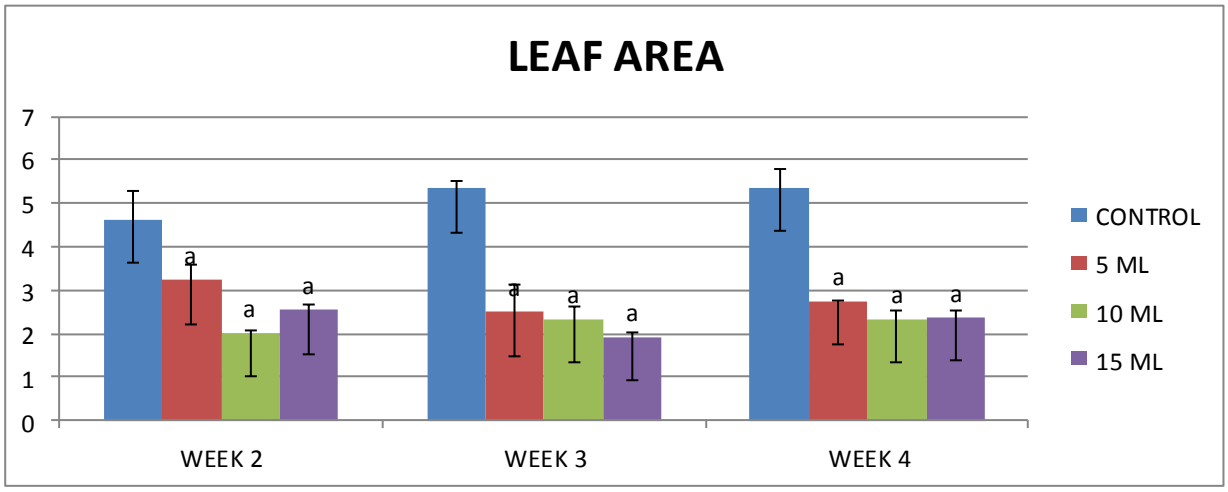

Figure 7: Leaf area of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (cm^2). Different lower case letters represent significant difference between mean at $p < 0.05$.

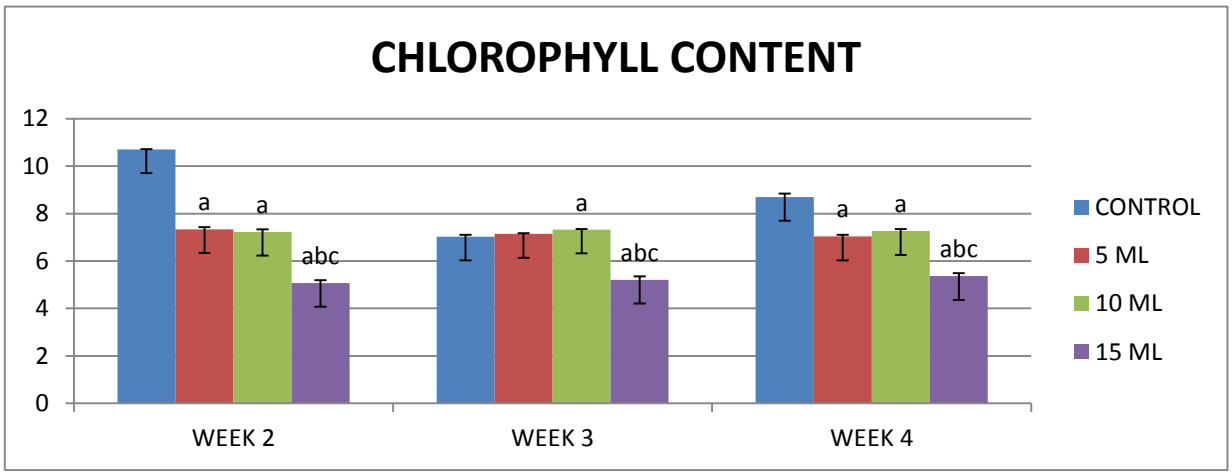

Figure 8. Chlorophyll content in the leaves of groundnut plant cultivated on crude oil contaminated soil. Values are expressed in mean ± SEM (10^{-5}unit/mg). Different lower case letters represent significant difference between mean at $p < 0.05$.

Discussion

Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD) and catalase that guide against oxidative damage. The present study showed that exposure of groundnut to crude oil contaminated soil negatively altered the activity of catalase. However, studies have shown that increased catalase activity in response to contamination suggests a prominent role for this enzyme in the protection of leaf tissue against oxidative damage. But in this case, there was a reduction in catalase activity. Environmental stresses cause either enhancement or depletion of catalase activity, depending on the intensity, duration, and type of the stress (Moussa and Abdel-Aziz, 2008). Superoxide dismutase activity was significantly increased during this study. Superoxide dismutase activity has been reported to increase in plants exposed to various environmental stresses, including drought and metal toxicity (Mishra *et al*, 2011). Increased activity of SOD is often correlated with increased tolerance of the plant against environmental stresses. Overproduction of SOD has been reported to result in enhanced oxidative stress tolerance in plants (Gupta *et al*., 1993). Different levels of superoxide dismutase activity might occur depending on stress intensity, species or genotype, growth conditions, stress period and plant age (Sgherri *et al*., 2000). A decrease in glutathione peroxidase activity was observed at higher concentrations, this was in agreement with Liubov *et al*., (2021) and they recorded a slight decrease in glutathione peroxidase as the concentration of contamination increased. In this study, malondialdehyde (MDA) was reduced although this does not correlate with Liubov *et al*., (2021). This may be due to environmental factors such as mineral nutrients high intensity of light, soil contamination with heavy metals. The systematic decrease in the germination of groundnut as crude oil contamination increased corresponds with the reports of earlier studies by (Merkl *et al*., 2004). The soil-root-plant interaction causes the stress induced by oil pollution to indirectly affect the physiological state of plants (Odukoya *et al*., 2019). These effects are attributable to the interference of the plant uptake of nutrients by crude oil. The growth parameters, such as, leaf number and leaf area reduced as the concentration of crude oil increased but the leaf weight increased with increase in contamination. It has been reported that plants and soil microbes compete for the little nutrient available in soils that are not rich and worse still, polluted with crude oil thereby suppressing the growth of plants in such soils (Kelechi *et al*., 2008). The lower levels of chlorophyll content reported in this study are in agreement with a similar work reported by Peretiemo-Clarke and Achuba, (2007). The reduction in chlorophyll content is likely due to the environmental and oxidative stress induced by crude oil pollution.

Conclusion

These findings seem to suggest that crude oil induced environmental stress in seedlings. Crude oil also affects antioxidant enzymes in different ways. These negative effects limit food production in affected areas causing diseases and hunger, therefore Government agencies and multinational companies should maintain a consistence effort in reducing crude oil spill in agricultural lands.

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References

Ademiluyi YS, Oyelade OA, Jaes D, Ozumba IC: Performance evaluation of a tractor drawn groundnut digger/shaker for agricultural productivity. Tillage for Agriculture Productivity and Environmental Sustainability Conference. pp15 – 27. 2011. http://iworx5.webxtra.net/~istroorg/download/Nigeria_conf_downloads/FPM/Oyelade%20et%20al

Adinya IB, Enun EE, Ijoma JU: Exploring profitability potentials in groundnut production through agroforestry practices: a case study in Nigeria. J Anim Plant Sci, 20(2):123 – 131. 2010.

Agarry SE, Ogunleye OO: Factorial desin application to study enhanced bioremediation of soil artificially contaminated with weathered Bonny Light crude oil through biostimulation and bioaugmentation strategy. J Environ Prot, 3: 748-759. 2012.

Agbogidi MO, Edema NE: Effects of crude oil and its water soluble fraction on the growth parameter of Panicum repen "Linn". Nig J Ecol, 6:1-4. 2003.

Arakama NK: Profitability of intercropping corn with mungbean and peanut. Agric Res & Dev J 17(1): 65-70. 2009.
Arnon DI: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaria. Plant Physiol, 24(1):1-15. 1949.
Athar H, Ambreen S, Javed M, Hina M, Rasul S, Zafar ZU, Manzoor H, Ogbaga CC, Afzal M, Al-Qurainy F: Influence of sub-lethal crude oil concentration on growth, water relations and photosynthetic capacity of maize (*Zea mays* L.) plants. Environ Sci Pollut Res, 23:18320–18331. 2015.
Buege JA, Aust SD: Microsomal lipid peroxidation. Meth Enzymol, 52:302-310. 1978.
Cohen G, Dembiec D, Marcus J: Measurement of catalase activity in tissue extracts. Anal Biochem 34:30-38. 1970.
Gupta AS, Heinen JL, Holaday AS, Burke JJ, Allen RD: Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci.. 90(4): 1629-1633. 1993.
Henry RJ, Charles S, Berkman S: Interferences with biuret methods for serum proteins use of Benedict's qualitative glucose reagent as a biuret reagent. Anal Chem, 29:1491-1495. 1957.
Ivshina IB, Kuyukina MS, Krivoruchko AV, Elkin AA, Makarov SO, Cunningham CJ, Peshkur TA, Atlas RM, Philp JC: Oil spill problems and sustainable response strategies through new technologies. Environ Sci Process Impacts, 17:1201–1219. 2015.
Kelechi L, Modupe O, Bola O: Growth and performance of *Glycine max* L. (Merril) grown in crude oil contaminated soil augmented with cow dung. Life Sci J, 6:48-55. 2008.
Khamehchiyan M, Hossein Charkhabi A. Tajik M: Effects of crude oil contamination on geotechnical properties of clayey and sandy soils. Eng Geol. 89:220–229. 2007.
Liubov S, Pavel M, Anastasia N, Mikhail, K: Effect of crude oil on growth, oxidative stress and response of antioxidative system of two rye (*Secale cereale* L.) varieties. Plants. 10 (1):157. 2021.
Mccord JM, Fridovich I: Superoxide dismutase, an enzyme function for erythrocuprein (hemocuprein). J Biol Chem, 244(22): 6049-6055. 1969.
Merkl N, Schultze-Kraft R, Infante C: Phytoremediation in the tropics—the effect of crude oil on the growth of tropical plants. Bioremediat J. 8:177–184. 2004.
Mishra S, Jha AB, Dubey RS: Arsenite treatment induces oxidative stress, unregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma, 248(3): 565-577. 2011.
Moussa R, Abdel-Aziz SM: Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust J Crop Sci, 1(1): 31-36. 2008.
Nyman M: Serum hetoglobin; methodological and clinical studies. Scand J Clin Lab Invest Supp 11(39):1-169. 1959.
Odukoya J, Lambert R, Sakrabani R: Understanding the impacts of crude oil and its induced abiotic stresses on agrifood production: a review. Horticulturae, 5:47. 2019.
Oyelade OA, Ademiluyi YS, James D, Ozumba IC: Performance evaluation of a modified tractor drawn groundnut digger/shaker for agricultural productivity. Tillage for Agriculture Productivity and Environmental Sustainability Conference. pp. 7 – 12. 2011.
Peretiemo-Clarke BO, Achuba FI: Phytochemical effects of petroleum on peanut (*Arachis hypogea*) seedlings. Plant Pathol J, 6(2): 179-182. 2007.
Scandalios JG: Oxygen stress and superoxide dismutases. Plant Physiol, 101(1): 7-12. 1993.
Schmidt-Etkin D: Spill ocurrences: a world overview. Spill Sci. Technol. Bull, 10: 7-48. 2011.
Sgherri C, Stevanovic B, Navari-Izzo F: Role of phenolic acids during dehydration and rehydration of *Ramondia serbica*. Plant Physiol Biochem, 38:196. 2000.
Weiss VL, Bernardi M: Communicating agrometeorological information to farming communities. Agric For Meteorol, 103: 185–196. 2000.
Yavari S, Malakahamad A, Sapari NB: A review on phytoremediation of crude oil spills. Wat Air Soil Poll, 226(8): 279. 2015.