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Changes in Antioxidant Enzyme Activities in *Allium cepa* Roots Cultivated in Crude Oil Contaminated Soil

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ABSTRACT: A study was carried out to evaluate the changes in Superoxide dismutase (SOD) and Glutathione peroxidase activities (GPx) in *Allium cepa* roots exposed to crude oil. Crude oil used in this study was fractionated in water (ratio 1 to 2) to produce water-soluble fractions (WSF) and water insoluble fractions (WIF). *Allium cepa* was grown in soil treated with 2%, 5%, 10% or 20% of Whole Crude (WC), WIF or WSF for 14 days, while the control was with distilled water. Antioxidant enzyme activity was evaluated after treatment for 14days using standard protocols. The result showed that there was a significant increase in SOD activity at higher concentrations for WSF and WIF treated plants. However, there was a decrease in SOD activities at 10% concentration and no root sprout was seen at 20% concentrations for WC treated plants. On the other hand, GPx activity increased at significant levels at 20% concentration for WSF while significant changes were observed in plant treatments with WIF and WC at lower concentrations. Results from this study suggest that soils contaminated with varying fractions of crude oil could portend great risk for plants, animals and humans in general.

Keywords; Crude oil, *Allium cepa*, superoxide dismutase and glutathione peroxidase

Introduction

Worldwide, crude oil is still the most significant source of fossil fuel for domestic and industrial energy generation. It is the most importance source of revenue for countries endowed with millions of barrels in reserve. Despite this, there are problems associated with crude oil exploration and exploitation in many developing countries. Foremost of these are spills caused by sabotage or old pipes which frequently burst (Agbonifo, 2016). Other causes of oil spillages include accidental leakages, theft, vandalization, terrorism, war and petroleum transportation (Hosmer *et al*., 1997; Ajagbe *et al*., 2012; Khosravi *et al*., 2013). These scenario has led to damage of various compartments of the environment with numerous incidence of oil pollution frequently reported in some regions in Nigeria especially the Niger Delta (Ahmadu and Egbodion, 2013; Oyinloye and Olamiju, 2013).

Crude oil is a complex mixture of compounds comprising of 90% of different hydrocarbons and 10% of non-hydrocarbon components such as metals, trace elements and salts (Alinnor and Nwachukwu, 2013; Yasin *et al*., 2013). It can be separated into its water soluble and insoluble components by fractionation process (Anderson *et al.,* 1974). The water-soluble fraction (WSF) is the component of crude oil that can dissolve in water; these components contain metallic ions and other volatile compounds. The WSF contains about 14 saturated hydrocarbons, from carbon 14 to paraffin and 20 aromatic hydrocarbons ranging from benzene to dimethyl phenanthrene (Edema, 2012). High molecular weight aromatics of crude oil are more toxic thanlow molecular weight aromatics; however,

unsaturated hydrocarbons with a low boiling point like Benzene, Xylene and Toluene are seen to be the most toxic components in crude oil (Noyo *et al*., 2008).

Due to the toxicity of crude oil components, oil spills have led to the massive loss of biodiversity in the aquatic and terrestrial environment. In the terrestrial environment, studies have shown that plants are greatly impacted by crude oil exposures in the following ways; inhibition of plant germination, inhibition of nutrient and water uptake by plant root causing deficiency to other parts of the plant such as leaves and affects anatomical features of leaves. It also causes oxygen starvation which could result in enlargement of cells in various tissues and chlorosis. Some of these effects has been reported for *Zea mays* (Ekundayo *et al*., 2001; Ogboghodo *et al*., 2004), *Pistia stratiotes* (water lettuce) (Akapo *et al*., 2011) and cassava (Ahmadu and Egbodion, 2013). It was observed that crude oil toxicity caused root stress which reduced leaf growth via stomata conductance, could disrupt plant water balance, which may indirectly influence plant metabolism and in severe cases lead to plant death (Ekundayo *et al*., 2001; Ogboghodo *et al*., 2004; Akapo *et al*., 2011).

Oxidative stress has been reported to be associated with plant exposure to crude oil contamination. Oxidative stress is induced by the release of free radicals; reactive oxygen species (Gill and Tuteja, 2010). In photosynthetic reactions, activated oxygen is formed as a result of enzymatic dysfunction due to perturbation in metabolism caused by an environmental stressor (Olubodun and Eriyamremu, 2013). When activated oxygen is produced above physiological levels, it leads to lipid peroxidation. Lipid peroxidation results in oxidative damage in plants (Olubodun and Eriyamremu, 2013) and animals (Odo *et al*., 2012). However, antioxidant defence system mop up the free radicals thus preventing the damage caused by oxidative stress. Some enzymes that are antioxidants include superoxide dismutase, glutathione peroxidase and catalase (Achuba, 2014). The uncontrollable increase in oxidative stress and a decrease in antioxidant defence system will eventually result in cell death (Gill and Tuteja, 2010).

The use of *Allium cepa* in this study emanates from the fact that it shows an excellent correlation with animal test *in vivo* and thus can be extrapolated reliable for human studies (Grant, 1994). Also, it is not expensive and the duration for root sprout is short compared to other seed germinating plants and it is a model plant in abiotic stress research (Kumari *et al*., 2009; Geremias *et al*., 2011). *Allium cepa* has been used in a number of toxicity studies (Olorunfemi and Lolodi, 2011; Cvjetko *et al*., 2017), however its use for toxicity research of crude oil and its fraction is scarce. This study reports the influence of crude oil and its fractions on superoxide dismutase and glutathione peroxidase activity in *Allium cepa* roots cultivated in crude oil contaminated soil.

Materials and Methods

Study location and soil sample collection: The *ex situ* study was carried out at the general laboratory of Biochemistry Department, Faculty of Life sciences, University of Benin, Benin City Nigeria, from the month of April to July 2016. Soil samples were collected from "Capitol" area (a portion of the University that is relatively quiet with little or no vehicular traffic) of the University and the region is characterized with sandy loam soil.

Experimental design: 700 g of sandy loam soil (0-15cm depth) was weighed into 104 polythene bags. They were grouped into three treatments; water soluble, water insoluble fractions and whole crude. Eight (8) of the polythene bags were treated with 2%, 5%, 10% and 20% for each group and control daily. They were grouped as shown below:

Table 1: Experimental Groups

Groups	% Contamination	Number of Nursery Bags
Control	No treatment (%)	8
Whole crude	2	8
	5	8
	10	8
	20	8
Water insoluble fraction	2	8
	5	8
	10	8
	20	8
Water soluble fraction	2	8
	5	8
	10	8
	20	8

Crude oil and fractionation: Bonny light Crude oil (American petroleum institute gravity= 37) was obtained from Warri Refinery and Petrochemical company in Delta state, Nigeria. A portion was fractionated into water soluble and water insoluble fractions according to the method of Anderson *et al*. (1974). 1:2 dilution was used for fractionation; 300ml of crude oil and 600mls of distilled water was put in a 1 litre conical flask and constantly stirred with a magnetic stirrer for 48h. A separating funnel was then set up for 48hrs and Water soluble Fraction (WSF) was separated from Water insoluble fraction (WIF) and collected in a conical flask.

Planting: The purple variety of onions (*Allium cepa* Linn) was purchased from a local market in Benin City, Edo state, Nigeria. Onion bulbs were planted in the soil according to the method of Jha *et al*. (2009) with a slight modification.

Soil treatment: Control soil contained only sandy loam soil. The soil in the bags contaminated with water WSF, WIF and whole crude (WC) were mixed thoroughly in their respective polythene bags containing 700g sandy loam soil. Soil of 700g was treated with 2, 5, 10% and 20% v/w WC, WIF or WSF. The treatment was carried out at the same percentage for all treatment concentration while distilled water was used to water the control on a daily basis after planting to keep the soil moist for effect growth.

Enzyme Extraction: Enzyme extraction was carried out according to the procedure of Kaur *et al*. (2009). 1g of *Allium cepa* roots were homogenized in 0.1M phosphate buffer (pH 7.4) that contained1mM EDTA, 1% polyvinylpyrrolidone (PVP) and 10µM β-mercaptoethanol. All homogenates were centrifuged at 3,000g for 30 min and the supernatant obtained was used for allenzyme assays.

Superoxide dismutase (SOD) activity determination: The enzyme activity was assayed according to the method described by Misra and Fridovich (1978) and was expressed as units/mg tissue weight. One unit of the enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute.

Glutathione peroxidise: The estimation of Glutathione peroxidase activity was carried out according to the method of Nyman (1959). The principle is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting in the formation of a deep brown colouration. The amount of purpuragallin formed was read at 430nm and was expressed as unit/mg protein.

Statistical Analysis: The result of the study was expressed as mean ± standard error of mean (SEM). The test of statistical significance between control and percentage treatments (comparing means) within each group was with post hoc (tukey) test in a one-way ANOVA version 20.0 for window 2007. Significant differences were set at $p< 0.05$.

Results

Result showed that there were noticeable changes in the antioxidant enzyme activities in *Allium cepa* roots. The effect of whole crude on superoxide dismutase activity is presented in Figure 1. Superoxide activity for whole crude was observed to increase significantly for 2% (1.36 ± 0.03) and 5% (1.56 ± 0.03) v/w treatments. A decrease in activity below control levels was observed for 10% (1.12 ± 0.09) v/w treatments.

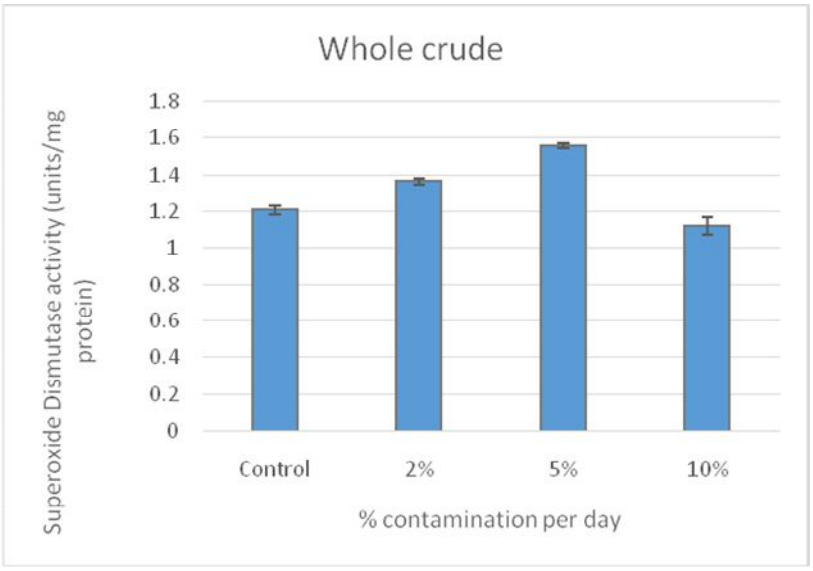

Figure 1: Superoxide dismutase activity of *Allium cepa* roots on treatment with whole crude (WC).Data are presented in mean and standard error of mean..

Figure 2 shows the enzymatic activity of superoxide dismutase in *Allium cepa* roots treated with water insoluble fraction. Similarly, superoxide activity decreased significantly for 2% contamination and increased significantly in water insoluble fraction, the increase was observed for 5% (1.49 ± 0.03), 10% (1.67 ± 0.02) and 20% (1.53 ± 0.03) v/w treatments.

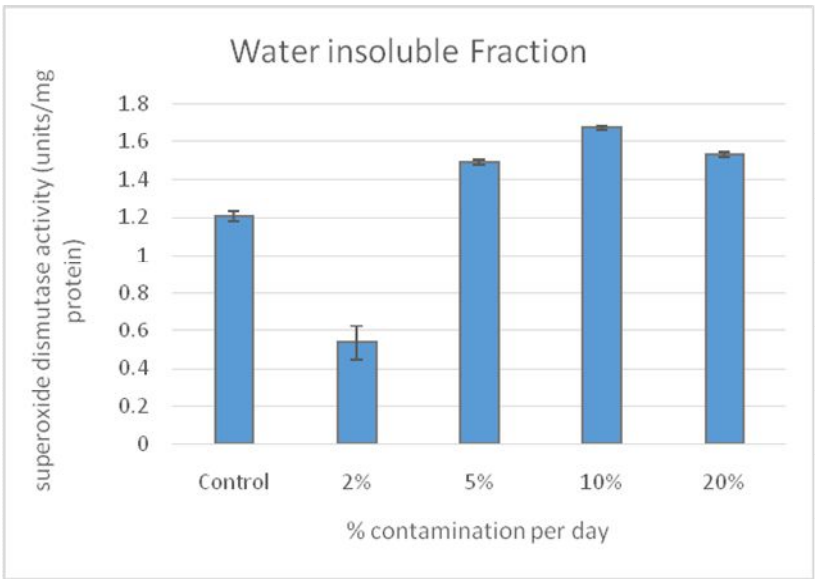

Figure 2: Superoxide dismutase activity of *Allium cepa* roots on treatment with water insoluble fraction (WIF) of crude oil. Data are presented in mean and standard error of mean.

The activity of superoxide dismutase in *Allium cepa* roots grown in soil contaminated with varying concentrations of water soluble fraction of crude oil is presented in Figure 3. The result showed a significant decrease in enzyme activity at 2% and a significant increase in the enzyme activity within treatment group in comparison with control. The increase was observed with 5% (1.71 ± 0.02), 10% (1.56 ± 0.02) and 20% (1.66 ± 0.02) v/w treatments.

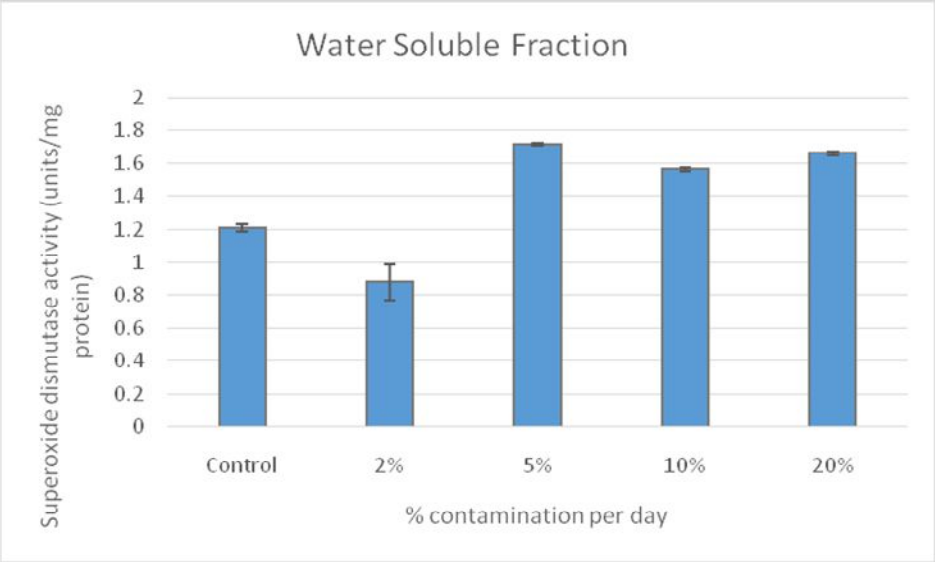

Figure 3: Superoxide dismutase activity of *Allium cepa* roots in soil treated with water soluble fraction (WSF) of crude oil. Data are presented in mean and standard error of mean.

Changes in the activity of Glutathione peroxidase in *Allium cepa* roots is as presented in figure 4. A significant increase was observed for WC treatment, 2% (7.37 ± 0.02), 5% (5.10 ± 0.02) and 10% (4.52 ± 0.03). A decrease was observed for 5% and 10% but above control levels.

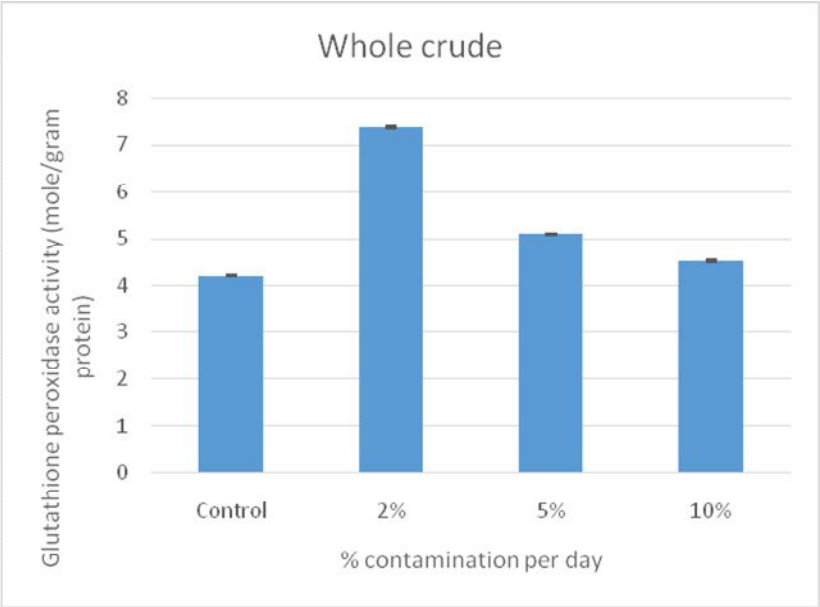

Figure 4: Glutathione peroxidase activity of *Allium cepa* roots on treatment with Whole crude (WC). Mean and SE are presented.

Glutathione peroxidase activity in *Allium cepa* roots is as shown in Figure 5. It was observed that there was a significant increase for WIF treatment, 2% (6.30 ± 0.04), 5% (6.45 ± 0.03), 10% (4.87 ± 0.01) and 20% (4.35 ± 0.04). A slight decrease was observed for 10% and 20% but it was above control levels.

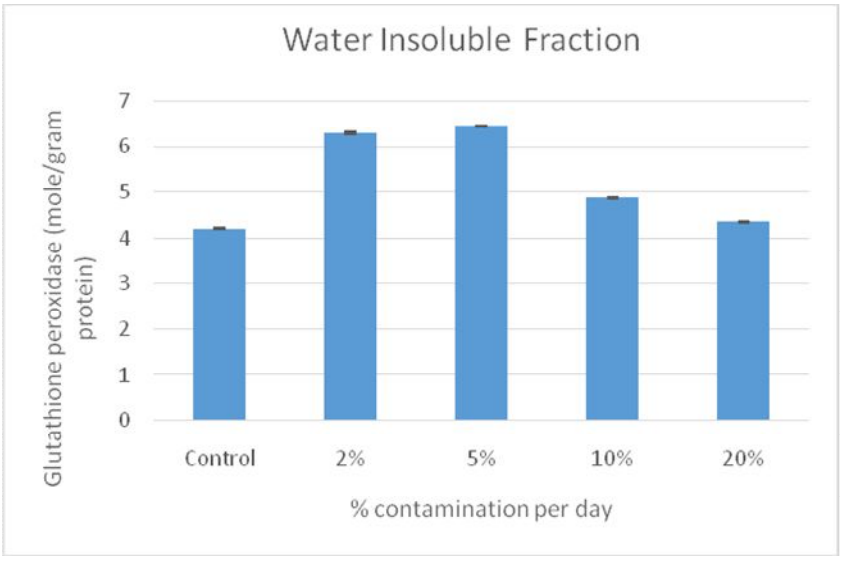

Figure 5: Glutathione peroxidase activity of *Allium cepa* roots treated with water insoluble fraction (WIF) of crude oil. Data are presented in mean and standard error of mean.

There was a significant increase in the enzyme activity within treatment groups in comparison with control for 5% (5.24 ± 0.03) and 20% (12.82 ± 0.05) v/w treatments for WSF, This is as presented in Figure 6.

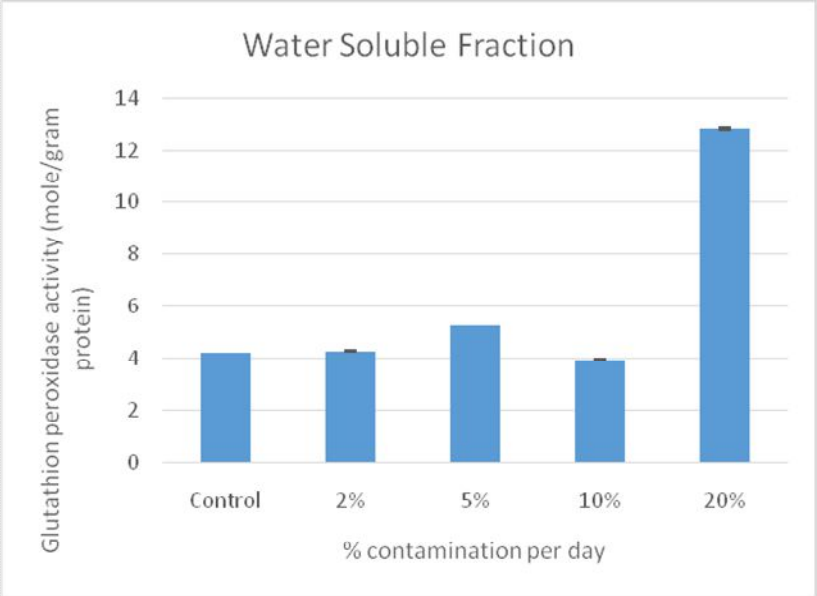

Figure 6: Glutathione peroxidase activity of *Allium cepa* roots treated with Water soluble fraction (WSF) of crude oil. Data are presented in mean and standard error of mean.

Discussion

The release of free radicals is a natural phenomenon that occurs normally in every living organism and often leads to oxidative stress. However, antioxidant defence mechanism is inbuilt in living organisms to prevent cell damage due to stress. Antioxidant defence mechanism consists of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and ascorbate peroxidase (Gill and Tuteja, 2010). These enzymes binds to the free radicals (reactive oxygen species), thus mopping them up from the system and preventing the free radicals from binding to biomolecules in the cells, that could result in cellular damage (Gill and Tuteja, 2010).

The study was done to determine the effect of crude oil, its water soluble and insoluble fraction on antioxidant enzymes enzyme activity in *Allium cepa* roots. Antioxidant enzyme activities were not measured for whole crude fraction at 20% contamination, because there was no root sprout and the onion bulb got rotten. This implies death inducement which may have arisen from the direct effect of crude oil on the water absorption ability and oxygen intake by the plant. It may have also resulted from severe case of oxidative damage by the whole crude. SOD activity increased significantly for 2% and 5% treatments but at higher concentration, 10%, a significant decrease in activity was observed, as seen in figure 1. This may infer that whole crude caused oxidative stress in *Allium cepa* roots at low concentrations of 2% and even 5%, hence the increase in antioxidant activity. On the other hand at 10% treatments, the decrease in enzyme activity may result from increased oxidative stress and thus reduction in the availability of SOD enzymes to mop up the reactive oxygen species, it may also be that increased oxidative stress to a level that inhibited the synthesis of the enzyme, hence the reduced enzyme activity. Similar results were reported by Olubodun and Eriyamremu (2013) and Odjegba and Badejo (2013).

The observed significant concentration dependent increase in GPx activity at 2%, 5% and 10% treatment for WC, could infer again that WC contamination induced oxidative stress in plant at low concentration, 2%. A decrease in GPx activity was observed as concentration increased (10%). This may infer that oxidative stress increased to a level that reduced superoxide activity. Similar reports have been seen in previous research (Olubodun and Eriyamremu, 2013; Odjegba and Badejo, 2013). It was also observed that superoxide dismutase and glutathione peroxidase activity in *Allium cepa* roots increased significantly for WSF, this is in conformity with previous studies (Olubodun and Eriyamremu, 2013). Water soluble fraction of crude oil is the fraction that dissolves in water and has the ability to be retained in the soil even after a prolong period of crude oil pollution (Noyo *et al.*, 2008). However,

its ability to induce oxidative stress is minimal compared to the WIF and WC. The increase was observed at high percentage contamination, 5%, 10% and 20% for SOD activity while GPx activity had the greatest activity increase at 20% concentration. This has been similarly reported in Olubodun and Eriyamremu (2013). It has also been reported that WSF contains some soluble metallic ions, heavy metals and hydrocarbons capable of inducing oxidative stress depending on duration of exposure and concentration resulting in reduced chlorophyll and cell damage in plants (Noyo *et al*., 2008; Yasin *et al*., 2013).

It was reported that ascorbate peroxidase and catalase activity increased significantly on contamination (Odjegba and Badejo, 2013). Elevated levels of other antioxidants; catalase, xanthine oxidase and superoxide dismutase for treatments with crude oil has also been reported by Achuba (2014). Similar findings on the elevation of antioxidant enzymes due to crude oil contamination have also been reported in animals (Odo *et al*., 2012). Although there is paucity of data for examining the effect of crude oil and its fractions on antioxidant enzyme activity in *Allium cepa*, the result from this study in conformity to previous studies (where other plants were used) infers that crude oil, its water insoluble and soluble fractions may induce oxidative stress in *Allium cepa* roots resulting in a corresponding increase in antioxidant enzyme activity which may result in cell damage in severe cases.

Conclusion

The result from this study suggests that crude oil, its water insoluble and soluble fractions may induce oxidative stress in *Allium cepa,* which may cause cellular damage in severe cases. This finding adds/improves on the existing data on the use of plants as a model, as studies on crude oil and its fractions have not been carried out using *Allium cepa*.

References

Achuba FI: Petroleum products in soil mediated oxidative stress in cowpea (*Vigna unguiculata*) and maize (*Zea mays*) seedlings. Open J Soil Sci 4: 417- 435. 2014.

Agbonifo P: Oil Spill Injustices in the Niger Delta region: reflections on oil industry failure in relation to the United Nations Environment Programme (UNEP) report. Int J Petrol Gas Explor Manag 2(1): 26-37. 2016.

Ahmadu J, Egbodion J: Effect of oil spillage on Cassava Production in Niger Delta region of Nigeria. Am J Exp Agric 3(4): 914-926. 2013

Ajagbe WO, Omokehinde OS, Alade GA, Agbede OA: Effect of crude oil impacted sand on compressive strength of concrete. Const Build Mater 26: 9-12. 2012.

Akapo AA, Omidiji SO, Otitoloju AA: Morphological and anatomical effects of crude oil on *Pistia statiotes*. J Environ 31(3): 288-298. 2011.

Alinnor IJ, Nwachukwu MA: Determination of total petroleum hydrocarbon in soil and groundwater samples in some communities in Rivers State, Nigeria. J Environ Chem Ecotox 5(11): 292-297. 2013.

Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM: Characteristics of dispersions and water-soluble extracts of crude oils and their toxicity to estuarine crustaceans and fish. Mar Biol. 27: 75 – 88. 1974.

Cvjetko P, Milošića A, Domijanb A, Vrčekc IV, Tolićd S, Štefanića PP, Letofsky-Papste I, Tkaleca M, Balena B: Toxicity of silver ions and differently coated silver nano particles in *Allium cepa* roots. Ecotox Environ Safety 137:18-28. 2017.

Edema N: Effects of crude oil contaminated water on the environment. *In*: Crude oil emulsions- composition stability and characterization. ME Abdul-Raouf (ed.) InTech - Open Access Company 9:170-180. 2012.

Ekundayo EO, Emede TO, Osayande DI: Effects of crude oil spillage on growth and yield of maize (*Zea mays* L.) in soils of Midwestern Nigeria. J Plant Food Human Nutr 56(4):313-324. 2001.

Geremias R, De Fávere VT, Pedrosa RC, Fattorini D: Bioaccumulation and adverse effects of trace metals and polycyclic aromatic hydrocarbons in the common onion *Allium cepa* as a model in ecotoxicological bioassays. Chem Ecol 27 (6):515–522. 2011.

Gill SS, Tuteja N: Reactive Oxygen specie and Antioxidant machinery in abiotic stress tolerance in crop plants. J Plant Physiol Biochem 48(12): 909-930. 2010.

Grant WF: The present status of higher plant bioassays for the detection of environmental mutagens. Mutat Res 310: 175-185. 1994.

Hosmer AW, Stanton CE, Beane JL: Intent to Spill: Environmental effects of oil spills caused by war, terrorism, vandalism, and theft. Int Oil Spill Conference. Am Petroleum Instit.157-163. 1997.

Jha SK, Nayak AK, Sharma YK: Fluoride toxicity in onion (*Allium cepa*L.) grown in contaminated soil. Chemosphere 76:353-356. 2009.

Kaur H, Gupta AK, Kaur N, Sandhu JS: Differential response of the antioxidant system in wild and cultivated genotype of chickpea. Plant growth Regul 57:109-114. 2009.

Khosravi, Ghasemzadeh H, Sabour MR, Yazdani H: Geotechnical properties of gas oil-contaminated kaolinite. Eng Geol 89: 220-229. 2013.

Kumari M, Mukherjee A, Chandrasekaran N: Genotoxicity of silver nano particles in *Allium cepa*. Sci Total Environ. 407 (19): 5243–5246. 2009.

Misra HP, Fridovich I: Inhibition of superoxide dismutase by azide. Arch Biochem Biophys 189(2):317-322. 1978.

Noyo, EE., Okoloko, GE, Agbogidi, OM:. Physico-chemical characteristics of the water-soluble fraction of Ogini Well Head crude oil and the effect of *Pistia stratiotes* Linn (water lettuces). Am Eur J Agric Environ 2 (6):633 – 638. 2007

Noyo EE, Okoloko GE, Agbogidi, OM: Physical and ionic characteristics in water soluble fraction (WSF) of Olomoro well-head crude oil before and after exposure to *Azolla africana* Desv Afr J Biotech 7 (1): 35-40. 2008.

Nyman M: Serum haptoglobin. Methodological and clinical studies. Scand J Clin. Lab Invest (Suppl.39) 11:1-169. 1959.

Odjegba VJ, Badejo JO: Crude oil induced oxidative stress in *Capsicum annum* L. J Nat Sci 11(2):46-50. 2013.

Odo CE, Nwodo OF, Joshua PE, Ubani CS, Etim OE, Ugwu OP: Effects of bonny light crude oil on anti-oxidative enzymes and total proteins in Wistar rats. Afr J Biotech, 11(98): 16455-16460. 2012.

Ogboghodo IA, Iruaga EK, Osemwota IO, Chokor JU: An Assessment of the Effects of crude oil pollution on soil properties, germination and growth of maize (*Zea mays*) using two crude types- Forcados Light and Escravos Light. J Environ Monit Assess 96 (1-3): 143-152. 2004.

Olorunfemi DI, Lolodi O: Effect of cassava processing effluents on antioxidant enzyme activities in *Allium cepa* L. Biokemistri 23(2): 49-61. 2011.

Olubodun OS, Eriyamremu EG: Effect of different crude oil fractions on growth and oxidative stress parameters of maize radicle. Int J Plant Soil Sci, 2(1): 144 – 154. 2013.

Oyinloye, MA, Olamiju OI: An Assessment of the physical impact of oil spillage using GIS and remote sensing technologies: Empirical evidence from Jesse Town, Delta State, Nigeria. British J Arts Soc Sci. 12(2):2046-9578. 2013.

Yasin G, Bhanger MI, Ansari TM, Naqvi, Ashraf M, Ahmad K, Talpur, FN: Quality and chemistry of crude oils. J Petrol Tech Alter Fuels 4(3):53-63. 2013.