Vol. 27 (no. 4) 159–162 31 December 2015

Biokemistri

An International Journal of the Nigerian Society for Experimental Biology

Short Communication

Abattoir waste water attenuates kerosene toxicity on cowpea (*Vigna unguiculuta*) seedlings

*[1]Fidelis I. Achuba and [2]Gideon Okunbor

[1]Department of Biochemistry, Delta State University, PMB 1, Abraka, Nigeria.

[2]Department of Chemical Sciences, Novena University, Ogume, Nigeria.

Correspondence: Fidelis I. Achuba; achubabch@yahoo.com, +234(0)8035663720

ABSTRACT: **Nigeria is a major producer of crude oil resulting in spillages that contaminate both cultivated and uncultivated farmlands. This exposes the immediate biota to the deleterious effect of petroleum hydrocarbons. The aim of the present investigation is to determine the ability of cowpea seedlings to handle exposure to kerosene in the presence of abattoir waste water. Poly bags were filled sieved soil 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) kerosene while group six served as control (0.0%) Corresponding concentrations were prepared and then treated with known amount of abattoir waste water. Cowpea seedlings were planted in each bag and the activity of suphite oxidase determined in the leaves twelve days after planting. The result indicates that kerosene treatment of soil caused a progressive decrease in sulphite oxidase activity as the concentration of kerosene in soil increases. The decrease in sulphite oxidase activity was significant ($p<0.05$) at 0.5% - 2% kerosene treatment of soil. Moreover, treatment of soil with abattoir waste water caused a significant ($P<0.05$) increase in the enzyme activity relative to the corresponding kerosene in soil treatment. However, at higher concentration of kerosene in soil, the activity of the enzyme was still significantly ($P<0.05$) lower relative to the control. This is against the near restoration of the enzyme activity at low concentration of kerosene in soil. These observations indicate abattoir waste can remediate the toxic effect of kerosene at low levels of soil contamination.**

KEYWORDS: **abattoir, cowpea seedling, soil, sulphite oxidase**

INTRODUCTION

Crude oil is a complex chemical that is toxic to plants (Achuba, 2006; Peretiemo-Clarke and Achuba, 2007; Achuba 2010). Negative effect of petroleum-polluted soil on plants has been reported (Okonokhua *et al.,* 2007; Achuba, 2010; Agbogidi, 2011; Kekere et al*.,* 2011; Ojimba and Iyagba, 2012). The toxic effect of oil spill on the biota varies. It can be lethal or sublethal depending on the physical nature of the oil or its inherent toxic chemical components (Sunmonu and Oloyede, 2008).The sublethal alteration in chemical composition and enzyme changes in plants by hydrocarbon-contaminated soil was earlier reported (Achuba*,* 2006; Peretiemo- Clarke and Achuba, 2007; Achuba, 2015; Achuba and Okoh, 2015)

Hydrocarbon contaminated soil has been reported to be amendable to bioremediation because of inherent soil microorganisms that are capable of degrading petroleum hydrocarbons (Jones and Edington 2005). Moreover, simulated bioremediation of hydrocarbon polluted soil and the effects of these remediation on plants have been previously reported (Adedokun and Ataga, 2007; Nwaogu et al., 2008; Walker et al*.,* 2008). The objective of this study was to investigate the effect of abattoir waste water treatment of kerosene polluted soil on sulphite oxidase activity in the leaves of cowpea seedlings.

MATERIALS AND METHODS

The refined petroleum fractions (of known physical properties) were obtained from Warri Refining and Petrochemical Company, Warri, Nigeria. Cowpea seeds (Improved varieties) were obtained as single batch from Delta Agricultural Development Project (DTADP) Ibusa Delta State, 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.

Soil treatment and planting of seeds

One thousand six hundred grams of soil was added to hundred 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) kerosene 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 samples was manually mixed vigorously to obtain homogeneity of the mixture. The procedure was repeated for 0.25% (4.0ml), 0.5% (8.0 ml), 1.0% (16 ml), 1.5% (24 ml) and 2.0% (32 ml) of kerosene in 1600 g of soil. Each concentration was then mixed with 40 ml of pre-established tolerable volume of abattoir waste water. Each treatment including control was replicated five times and consisted of three sown seed per polythene bag. The treatments were watered every day in order to keep the soil moist. The design of the experiment was completely randomised one.

Germination (indicated by the appearance of hypocotyls above the soil level) records were taken at 4 days interval up to 12 days. Seeds that failed to sprout after 12 days were regarded as not germinable.

Preparation of plant extract for the determination of sulphite oxidase

The cowpea seedlings were uprooted after it was allowed to grow for the period of 12 days and washed to remove soil particles. The leaves of the seedling from each concentration of kerosene were pooled together and 0.5g of the leaves was then homogenize in 10 ml of Buffer solution using mortal and pestle. The homogenate was filtered using muslin cloth to obtain the extract, which was used as the crude enzyme source.

Sulphite oxidase activity was determined using a modified method of Ahmad *et al*., (2008). Sulphite oxidase activity was determined by measuring the decrease in reduction of ferricyanide at an absorbance of 420 nm. An extinction coefficient of 1.02 X 10^{-3} was used for the calculation. Potassium phosphate buffer, pH 8.0 (2.5 ml) containing 0.1 mM EDTA was measured into labelled test tubes using a pipette. This was followed by 0.5 ml of potassium ferricyanide and 0.8ml of sodium azide. The plant extract, 0.2 ml was added to the test tubes. The total volume of reactants was 4.0 ml for each test tube and absorbance was read at 420 nm. Digital photo colourimetric readings were taken at 30 seconds, 60 seconds, 90 seconds and 120 seconds respectively.

Statistical analysis

All the results were expressed as means ±SD and all data were analyzed using Analysis of variance (ANOVA) using SPSS version 19. Significant difference between the control and treatment means were determined at 5% ($P < 0.05$) confidence level using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The effect of kerosene treatment of soil on sulphite oxidase activity in cowpea seedlings is shown in table 1. The result indicates that kerosene treatment of soil caused a progressive decrease in sulphite oxidase activity as the concentration of kerosene in soil increases. The decrease in sulphite oxidase activity was significant ($p<0.05$) at 0.5%−2% kerosene treatment of soil. Moreover, treatment of soil with abattoir wastewater caused a significant ($P< 0.05$) increase in the enzyme activity relative to the corresponding kerosene in soil treatment.

Table 1: The effect of abattoir waste water treatment of soil on sulphite oxidase activity in the leaves of cowpea seedlings grown in kerosene polluted soil

	Sulphite oxidase activity (unit/gfwt)	
Concentration of kerosene in soil (% v/w ml/g)	Kerosene	Kerosene + AWW
0.00	3.31 ± 0.21 [a]	3.31 ± 0.21 [a]
0.10	2.56 ± 0.43 [a]	3.03 ± 0.51 [a]
0.25	2.11 ± 0.11 [b]	2.81 ± 0.11 [a]
0.50	1.56 ± 0.68 [b]	2.31 ± 0.66 [a]
1.0	1.33 ± 0.14 [b]	2.32 ± 0.23 [a]
1.5	1.20 ± 0.62 [b]	1.81 ± 0.45 [b]
2.0	1.13 ± 0.71 [b]	1.80 ± 0.50 [b]

Values are means ±SD of five determinations. Means with different superscript letters in the same column are significantly different at $P<0.05$ level.

However, at higher concentration of kerosene in soil, the activity of the enzyme was still significantly ($P<0.05$) lower relative to the control. This is against the near restoration of the enzyme activity at low concentration of kerosene.

DISCUSSION

Plant sulphite oxidase has a detoxifying function (Brychkova *et al*., 2007). Sulphite is a toxic metabolite. It is assumed that sulphite oxidase could possibly serve as a safe way for detoxifying excess amount of sulphite and protecting the cell from sulphitolysis (Ahmad and Ahmad 2010).

In the present study, the presence of kerosene in soil positively decreased the activity of sulphite oxidase in the leaves of cowpea seedlings (Table 1). Petroleum products mediated changes in plant enzymes have been reported previously (Achuba, 2015; Achuba and Okoh 2015). The decrease in the enzyme activity indicates that the presence of kerosene in soil compromises the ability of exposed plants to detoxify sulphite in the plant. Sulphur and its oxidases have been reported to be present in petroleum (Achuba 2006). Therefore, one of the mechanisms of petroleum-mediated toxicity is the decrease in the activity of sulphite oxidase, thereby predisposing exposed plants to sulphitolysis. However, treatments of soil with abattoir wastewater tend to restore the activity of the enzyme to control value at lower concentration of kerosene in soil (Table1). This observation agrees with the report of various authors (Nwadinigwe and Onyeidu, 2012; Jayasree *et al*., (2013) who reported that remediation of petroleum contaminated soil improved the performance of several crop species

Kerosene is toxic to plant by decreasing the activity of sulphite oxidase in plants. However, treatment of polluted soil with abattoir waste water tend to restore the activity of the enzyme to control value at lower concentration of kerosene in soil Thus, giving credence to the fact that abattoir waste water can attenuate kerosene toxicity in exposed plants

REFERENCES

Achuba F.I. (2006) The effects of sublethal concentrations of crude oil on the growth and metabolism of cowpea (Vigna unguiculata) seedlings. *The Environmentalist.* 26:17-20.

Achuba F.I. (2010). Spent engine oil mediated oxidative stress in cowpea (Vigna unguiculata) seedlings. *EJEA FChe. 9: 910-917*

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

Achuba F.I (2015) Effect of petroleum products treatment of soil on succinate dehydogenase and lactate dehydogenase activities in cowpea (*Vigna unguiculata*) and Maize (*Zea mays*) seedlings. *American Journal of Experimental Agriculture 5: 498-508.*

Achuba F.I., Okoh, PN (2015).Effects of Petroleum Products in Soil on α- amylase,starch phosphorylase and peroxidase activities in cowpea and maize seedlings. *American Journal of Experimental Agriculture 6(2): 112-120.*

Adedokun O.M, and Ataga A.E (2007) Effects of amendments and bioaugumentation of soil polluted with crude oil, automotive gasoline oil, and spent engine oil on the growth of cowpea (Vignaunguiculata L. Walp). *Science Research Essay* 2: 147-149.

Agbogidi O.M. (2011) Effects of crude oil contaminated soil on Biomass accumulation in Jatropha curcas L. seedlings. *Journal of Ornamental and Horticultural Plants 1: 43-49*

Ahmad A., Ahmad S. and Baig M.A (2008) Purification and characterization of sulfite oxidase from goat liver. *Indian Journal of Biochemistry and Biophysics* 45: 379-386

Ahmad A. and Ahmad S (2010) Screening and partial immunochemical characterization of sulphite oxidase from plant source. *Indian Journal of Experimental Biology 48: 83-86.*

Brychkova G, Xia Z, Yang G, Yesbergenova Z, Zhang Z, Davydov O, Fluhr R and Sagi M (2007) Sulfite oxidase protects plants against sulfur dioxidase toxicity. *Plant Journal* 50: *696*-709.

Jayasree S., Deepa S., Bincy B., Krishnapriya M., Remya P.K., Anusha C., Deepthi V., Gayathri V. and Elna V. (2013). Bioremediation studies of petroleum contaminated soil by vermicompost for sustainable agriculture in Hibiscus esculentus (L) Moench. *Acta Biologica Indica,* 2: 300-309.

Jones T.G. and Edington M.A. (2005). An ecological survey of hydrocarbon oxidizing micro-organisms. *Journal of General Microbiology 52: 389-393.*

Kekere O, Ikhajiagbe B and Apela B.R. (2011). Effects of crude petroleum oil on the germination, growth and yield of Vigna unguiculata walp L. *Journal of Agriculture and Biological Sciences 2: 158−165.*

Nwadinigwe A.O and Onyeidu E.G. (2012). Bioremediation of crude oil-polluted soil using bacteria and poultry manure monitored through soybean productivity. *Polish Journal of Environmental Studies* 21: 171−176

Nwaogu L.A, Onyeze G.O.C and Nwabueze R.N (2008). Degradation of diesel oil in polluted soil using Bacillus subtilis. *African Journal of Biotechnology 7: 1939−1943.*

Ojimba,T. P and Iyagba A.G.(2012). Effects of crude oil pollution on Horticultural crops in Rivers State, Nigeria. Global Journal of Science Frontier *Research in Agriculture and Biology 12: 37−43*

Okonokhua B.O, Ikhajiagbe B., Anoliefo G.O and Emede T.O (2007). The Effects of Spent Engine Oil on Soil Properties and Growth of Maize (Zea mays L.). *Journal of Applied Science and Environmental Management 11: 147−152.*

Sonmonu T.O and Oloyede O.B (2007). Biochemical assessment of the effects of crude oil contaminated catfish (Clarias gariepinus) on the hepatocytes and performance of rat. *African Journal of Biochemistry Research 1: 083-089*

Walker R.L, Burns I.G and Moorby J. (2001). Response of plant growth rate to nitrogen supply: A comparison of relative addition and nitrogen interruption treatments. *Journal of Experimental Botany 52: 309-317.*