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Original Research Paper

Toxicological Evaluation and Usefulness of Lipid Peroxidation as Biomarker of Exposure to Crude Oil and Petroleum Products Tested against African Catfish, *Clarias gariepinus* and Hermit Crab, *Clibanarius africanus*

Michael A. King, Temitope O. Sogbanmu, Adesola O. Osibona*, F. Doherty and Adebayo A. Otitoloju**
Environmental Toxicology and Pollution Management Unit, Department of Zoology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
*Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
**Department of Biological Sciences, Yaba College of Technology, Yaba, Lagos, Nigeria

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ABSTRACT

The toxicological evaluations of crude oil, petrol, kerosene and diesel were carried out against the African Catfish (*Clarias gariepinus*) fingerlings and Hermit crab (*Clibanarius africanus*). On the basis of 96hr LC_{50} value, petrol (LC_{50} = 2.449 mL/L) was found to be the most toxic followed by diesel (LC_{50} = 7.839 mL/L), kerosene (LC_{50} = 8.095 mL/L), and crude oil (LC_{50} = 9.355 mL/L) to *Clarias gariepinus*. For *Clibanarius africanus* also, petrol (LC_{50} = 4.569mL/L) was the most toxic followed by kerosene (LC_{50} = 8.705 mL/L), diesel (LC_{50} = 13.852 mL/L) and (LC_{50} = 35.955 mL/L). On the basis of the computed susceptibility factor, hermit crab was found to be 2x, 2x, 6.1x and slightly more tolerant than catfish when exposed to petrol, diesel, crude oil and kerosene respectively. The results of the lipid peroxidation assay against juveniles of *C. gariepinus* showed that the level of malondialdehyde (MDA) in the liver of fish exposed to sublethal concentrations of all the test chemicals increased significantly when compared to control animals. The observed increase in MDA levels in the liver tissues of test animals exposed to crude oil and refined petroleum products was recommended as a good biomarker for early detection of oil related pollution during biomonitoring programmes.

INTRODUCTION

Pollution by crude oil is widespread and a common problem, and particularly endemic in countries whose economies are dependent on the oil industry. Such pollution arises either accidentally or operationally wherever oil is produced, transported, stored, processed or used. The constituents of crude oil are complex. It contains aliphatic, alicyclic, polyaromatic hydrocarbons, oxygen, nitrogen and sulphur containing substances. Refined petroleum products are derived from crude oil through the process of catalytic cracking fractional distillation. The refined products have physical and chemical characteristics that differ according to the type of crude oil and subsequent refining processes. There are four main refined petroleum products and they include: premium motor spirit (PMS/Petrol); automotive gas oil (AGO/Diesel) which is less volatile than gasoline and is used mainly in compression ignition engines, road vehicles, agricultural tractors, locomotives, small boats and stationary engines; dual purpose kerosene (DPK) and aviation kerosene, which is a colourless, thin, flammable liquid. Aviation kerosene is a high quality aviation turbine fuel (Avtur) used by the jet engines.

Varieties of crude oil and oil products differ widely in toxicity. In general, it appears that severe toxic effects are associated with low boiling compounds and aromatics (Akpofure et al. 2000). Diesel and kerosene are petroleum products that contain mainly hydrocarbons and other additives all of which have well established biological activities. The most biologically active are the low molecular weight hydrocarbons like benzene and toluene. These hydrocarbons apart from having high acute toxicity, are also known to be carcinogenic. These hydrocarbons have differential toxicity to different animal species including fishes and shell fishes. It is well established that petroleum products, like other oily materials, form a film on water surfaces that act as a barrier reducing the rate of gaseous exchange therefore leading to reduced dissolved oxygen in water bodies, especially those that are stagnant. Oily films also bring about reduced penetration of sunlight in water bodies thereby reducing the rate of photosynthesis or productivity of the green aquatic plants. It is therefore expected that the continual exposure of these animals to sublethal concentrations of petroleum products over time would have some adverse biological effects on the exposed animals (Otitoloju 2003). A number of earlier researchers

have demonstrated the negative effects of water soluble extracts of petroleum products on different fish species. For example, Davidson et al. (1993) demonstrated that when the Antarctic fish *Pagothenia borschgrevinki* was exposed to water-soluble extracts of diesel fuel, it showed reduction in the rate of ventilation, secretion of mucus from the opercula, enlarged spleen and increase in liver. It has also been that water soluble fractions of crude oil have a highly significant effect of reducing the growth performance of *Heterobrancuhus bidorsalis.* Lopes et al. (2001) reported that water soluble fractions of crude oil can stunt fish growth. A radical attack on lipids leads to the formation of lipid peroxides, which can decompose to yield alkanes, ketones and aldehydes. The most extensively studied aldehydes are 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal and malondialdehyde (MDA) (Zielinski & Portner 2000). The variety of lipid peroxidation (LPO) by-products can also exert adverse biological effects in exposed organisms (Catala 2009). The quantification of the diverse products of peroxidation, especially level of MDA, is now being exploited as biomarker of oxidative stress. This quantification has enabled early detection of stress in exposed organisms before lethal or pathological effects are observed.

In this study, toxicological evaluations and estimation of lipid peroxidation in *Clarias gariepinus* and *Clibarius africanus* were carried out to assess their importance as biological markers of environmental stress related to crude oil and refined petroleum products. The interest in crude oil and refined petroleum products is because of the frequent spillage of petroleum products, especially gasoline in water bodies and terrestrial ecosystems in Nigeria and many other petroleum products importing countries.

MATERIALS AND METHODS

Test Animals and Acclimatization

Fingerlings and Juveniles of *Clarias gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) also known as African Catfish were used in the bioassays. Fingerlings (weight range: 6-10g, length range: 4.8-6.0cm) and Juveniles (weight: 17 to 25g, length: 14.5 to 17.1cm) were purchased from a fish farm and transported in an oxygen bag to the laboratory. *Clibanarius africanus* (Arthropoda, Malacostraca, Decapoda, Diogenidae) also known as Hermit crab were collected from the edge of the Lagos lagoon at low tide and transported to the laboratory in holding tanks (30cm × 30cm × 30cm) which contained lagoon water. The habitat mud was collected from the same site and placed in the holding tank as substrate. These animals were also used in the bioassays.

C. gariepinus were kept in a plastic tank (28 × 51 × 29 cm) which was three-quarter filled with dechlorinated water obtained by aerating tap water in a plastic tank with an aerator for at least 24 hours. During acclimatization, the fingerlings and juveniles were fed with Coppens fish feed twice daily in the morning and evening. *C. africanus* were kept in holding tanks with a thin layer of sediment serving as substrate and food source. Both the animal species were allowed to acclimatize to the laboratory environment (Temp. 27-28°C) for 14 days and the stock water was changed every other day to prevent the accumulation of waste metabolites and food particles.

The quantal response (mortality) was assessed every 24 hr over a period of 96 hrs (4 days). Test organisms were considered dead when they showed no response to mechanical stimulation (prodding with a rod).

Test Compounds

Forcados light crude oil was obtained from Shell (SPDC) production platform in Forcados, Delta State, Nigeria. Some of the physico-chemical properties of the Forcados light brand of crude oil include: sulphur content = 0.2%; API gravity = 60/60 F; rapid vapour pressure = 2.5 psi and pour point = 25.

The three petroleum products; petrol, kerosene and diesel were purchased from AP University of Lagos filling station. They were put in 1 litre containers separately covered tightly with a screw top cap to ensure that the contents do not evaporate.

Relative Acute Toxicity

Four active catfish fingerlings and hermit crabs of similar sizes in duplicate were exposed to varied concentrations of the test compounds as described below:

C. gariepinus

Crude oil: 5.0, 10.0, 15.0, 20.0, 30.0 mL/L and untreated control
Petrol: 0.5, 1.5, 2.0, 2.5, 3.0, 5.0, 7.0 mL/L and untreated control
Kerosene: 4.0, 6.0, 8.0, 9.0, 10.0, 15.0 mL/L and untreated control
Diesel: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0 mL/l and untreated control

C. africanus

Crude oil: 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 mL/L and untreated control
Petrol: 0.3, 0.5, 1.5, 3.0, 5.0, 7.0 mL/L and untreated control
Kerosene: 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mL/L and untreated control
Diesel: 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0 mL/L and untreated control

Mortality was assessed once every 24hr for 4 days.

Sublethal Effects

In this series of experiment, the test animals were exposed to sublethal concentrations of the test compounds. The sublethal concentrations ($1/10^{th}$ and $1/100^{th}$ of 96hr LC_{50}) were derived from the acute toxicity tests results. A semi static bioassay test protocol was adopted in which the test media were changed once every 4 hours to fresh media of the same concentration and untreated control. At predetermined post-commencement periods (0, 14, and 28 days), fish samples were removed and sacrificed to obtain liver tissue required for biochemical assays.

Preparation of Tissue Homogenates

Fish samples were harvested on pre-determined day and sacrificed. The liver was removed and washed free of blood in ice cold isolation medium (0.25M sucrose, 5Mm tris HCL), lightly blotted and weighed. The liver was cut into fragments and homogenized (9% w/v) in 100% methanol and centrifuged at 10,000 rpm for 15min at 4°C by the method described by Hermes-Lima et al. (1995). The supernatant was then collected for assays.

Lipid Peroxidation (LPO) Assay

The levels of homogenized tissue malondialdehyde (MDA), as an index of lipid peroxidation were determined by thiobarbituric acid reaction (TBARS Assay) using the method of Yagi (1998). In this method, malondialdehyde is measured spectrophotometrically at absorbance levels of 535nm to assay for the extent of lipid peroxidation in the sample.

Statistical Analysis

Toxicological data involving quantal response (mortality) were analysed by probit after Finney (1971). Toxicity indices derived from these analyses were LC_{50}, TF and their 95% confidence limits were employed as follows.

LC_{50}: Median lethal concentration that will bring about 50% mortality of the exposed population.

TF: Toxicity factor for relative potency measurements, e.g. ratio of 96hr LC_{50} of a compound to LC_{50} values at equivalent time intervals.

The lipid peroxidation data were subjected to analysis by t-Test.

RESULTS AND DISCUSSION

Relative acute toxicity and Comparison of Susceptibility Factor (SF) of crude oil, petrol, kerosene and diesel acting singly against *C. gariepinus* and *C. africanus*: On the basis of 96hr LC_{50} values of test chemicals tested against *C. gariepinus*, petrol (LC_{50} = 2.449 mL/L) was found to be the most toxic followed by diesel (LC_{50} = 7.839 mL/L), kerosene (LC_{50} = 8.095 mL/L), and crude oil (LC_{50} = 9.355 mL/L). On the basis of toxicity factor, petrol was found to be 3.2x, 3.3x and 3.8x more toxic than diesel, kerosene and crude oil respectively (Table 1).

On the basis of 96hr LC_{50} values of test chemicals tested against *C. africanus*, petrol (LC_{50} = 4.569 mL/L) was also found to be the most toxic, followed by kerosene (LC_{50} = 8.705 mL/L), diesel (LC_{50} values = 13.852 mL/L), while crude oil (LC_{50} = 35.955 mL/L) was the least toxic. On the basis of toxicity factor, petrol was found to be 1.9x, 3.0x and 7.9x more toxic than diesel, kerosene and crude oil respectively (Table 1).

This study showed that crude oil, diesel, petrol and kerosene are differentially toxic to the test the animals, *C. gariepinus* and *C. africanus.* The differential response of organisms to chemicals can be attributed to several factors such as the permeability of the body membrane or cuticles, metabolism, excretory capacity, age, sex body size, site of action and behaviour (Don-Pedro 1996). The toxicity of petroleum products was discovered to increase with time of exposure. This is expected because longer the exposure period, the higher the possibility of absorption of more toxicant and suffering from changes in the environment like decrease in dissolved oxygen. This agrees with the findings of other researchers such as Adeoye (2000), Ajileye (2002) and Ihedike (2002). The differential toxicity observed can be attributed to the fact that there are differences in the physicochemical characters of the different chemical substances and this influences the mechanisms of their action on the organisms, their penetration ability into the organisms and the ability of the organisms to metabolize and excrete them.

On the basis of the 96hr LC_{50} values, Hermit crab was found to be more tolerant than the Catfish when tested against the petroleum products. For instance, Hermit crab (LC_{50} = 4.569mL/L) was found to be about 2x more tolerant than Catfish (LC_{50} = 2.449mL/L) when exposed to petrol based on the computed susceptibility factor (SF). Hermit crab (LC_{50} = 35.955mL/L) was found to be about 6.1x more tolerant than Catfish (LC_{50} = 9.355mL/L) when exposed to crude oil. When tested against diesel, the Hermit crab (LC_{50} = 13.852mL/L) was also found to be about 2x more tolerant as compared to the Catfish. In kerosene, the Hermit crab (LC_{50} = 8.705mL/L) was slightly more tolerant than Catfish (LC_{50} = 8.095mL/L) (Table 1).

In this study, petrol was found to be more toxic to both *C. gariepinus* and *C. africanus* than crude oil, diesel and

Table 1: Relative acute toxicity and comparison of susceptibility factor (SF) of crude oil, petrol, kerosene and acting singly against *C. gariepinus* and *C. africanus* respectively.

Chemicals	Test Animal	LC_{50} (95% CL)	SE	Probit line equation	DF	TF	SF
Petrol	*C. gariepinus*	2.449 (1.820-3.292)	5.287	Y= -2.997 + 7.706X	1	1	1
	C. africanus	4.569 (2.406-9.829)	0.913	Y= -1.376 + 2.058X	2	1	1.9
Kerosene	*C. gariepinus*	8.095 (7.143-9.165)	13.291	Y= -18.457 + 20.326X	1	3.3	1
	C. africanus	8.705 (5.270-14.633)	2.001	Y= -2.991 + 3.183X	1	1.9	1
Diesel	*C. gariepinus*	7.839 (6.047-8.801)	4.330	Y=-9.140 + 10.589X	1	3.2	1
	C. africanus	13.852 (10.983-17.490)	3.860	Y=-7.827 + 6.857X	1	3.0	1.8
Crude oil	*C. gariepinus*	9.355	1.350	Y=-2.869 + 2.954X	2	3.8	1
	C. africanus	35.955	2.570	Y= -3.323 + 3.110X	1	7.9	6.1

Key: SE = Standard Error; DF = Degree of Freedom

$$\text{Toxicity Factor (TF)} = \frac{96\text{hr}LC_{50} \text{ value of other chemicals}}{96\text{hr}LC_{50} \text{ value of most toxic chemical}}$$

$$\text{Susceptibility Factor (SF)} = \frac{96\text{hr}LC_{50} \text{ value of other test animal}}{96\text{hr}LC_{50} \text{ value of most sensitive animal}}$$

kerosene. On the basis of the 96hr LC_{50} of crude oil and the other petroleum products, petrol was found to be the most toxic followed by diesel, kerosene and crude oil when tested against *C. gariepinus*. Petrol was also found to be the most toxic followed by kerosene, diesel and crude oil when tested against *C. africanus*. The susceptibility of the test animals can be related to the volatility of the petroleum products because toxicity reduces with increasing density of the test compounds. More volatile components penetrate the skin/cuticle much easier than less volatile ones. On the basis of sensitivity response, *C. gariepinus* and *C. africanus* have varied physiological processes. *C. gariepinus* is more sensitive than *C. africanus* when tested against petroleum products. This can be attributed to the fact that *C. africanus* resides in a shell which protects its body from direct exposure to the petroleum products unlike *C. gariepinus* which has its body exposed to the direct impact of the toxicants at all times.

The importance of toxicity studies is the effect they have on the protection of the aquatic ecosystems considering the socio-economic activities. It is also important in deriving water quality criteria or safe limits for protection of aquatic lives from effects of petroleum products pollution.

Lipid peroxidation assay on *Clarias gariepinus* and *Clibanarius africanus*: The exposure of *Clarias gariepinus* and *Clibanarius africanus* to sublethal concentrations of crude oil, petrol, kerosene and diesel consistently led to an increase in the level of lipid peroxides, which indicates oxidative damage in the liver of the test animals irrespective of concentration (Figs. 1 and 2). Level of lipid peroxidation was found to be highest in test animals exposed to petrol followed by kerosene, diesel and crude oil. Level of lipid peroxidation was, in general, low in the control animals. Statistical analysis (t-test) of the mean concentration values of MDA levels also indicated that lipid peroxidation was significantly ($P<0.05$) higher in exposed animals when compared to control group. The pattern of lipid peroxidation in both test animals was found to be similar, although higher level of lipid peroxidation was observed in the Catfish, *C. gariepinus* than in the Hermit crab, *C. africanus*. It is important to note that the significantly ($P<0.05$) higher level of lipid peroxidation in test animals exposed to petrol when compared to other test compounds correlated positively with a significantly (no overlap in 95% confidence limits) higher level of toxicity for this test compound against both test organisms. This result is, therefore, indicative that the mechanism of action of the test compound may be as a result of generation of reactive oxygen species (ROS) in the tissues of exposed animals leading to damage of endogenous molecules such as lipids and proteins (Otitoloju & Olagoke 2011).

The exposure of the test organisms to sublethal concentrations of crude oil, petrol, diesel and kerosene was found to cause an increase in level of lipid peroxides, which indicates oxidative damage to the liver of exposed *C. gariepinus* compared to control individuals of the same species. This result is in agreement with findings of Achuba & Osakwe (2003) and Avci et al. (2005) who reported an increase in LPO in tissues of fishes exposed to petroleum hydrocarbons. The increase in lipid peroxidation levels in the liver of the test animals exposed to the test chemicals can, therefore, serve as a good biomarker for early detection of pollution associated with crude oil and petroleum products and its inclusion in related biomonitoring programmes is recommended.

REFERENCES

Achuba, F.I., Osakwe, B.P., Theresa, O.C. 2003. Oxidative stress induced in the brain of rabbits with petroleum-induced hypoglycaemia. Biochem. Physiol., 64: 253-382.

Adeoye, O.A. 2000. Acute Toxicity and Substantial Effects (Tainting and Weight Loss) of Crude Oil (Forcados Light) Against *Tilapia guineensis.*

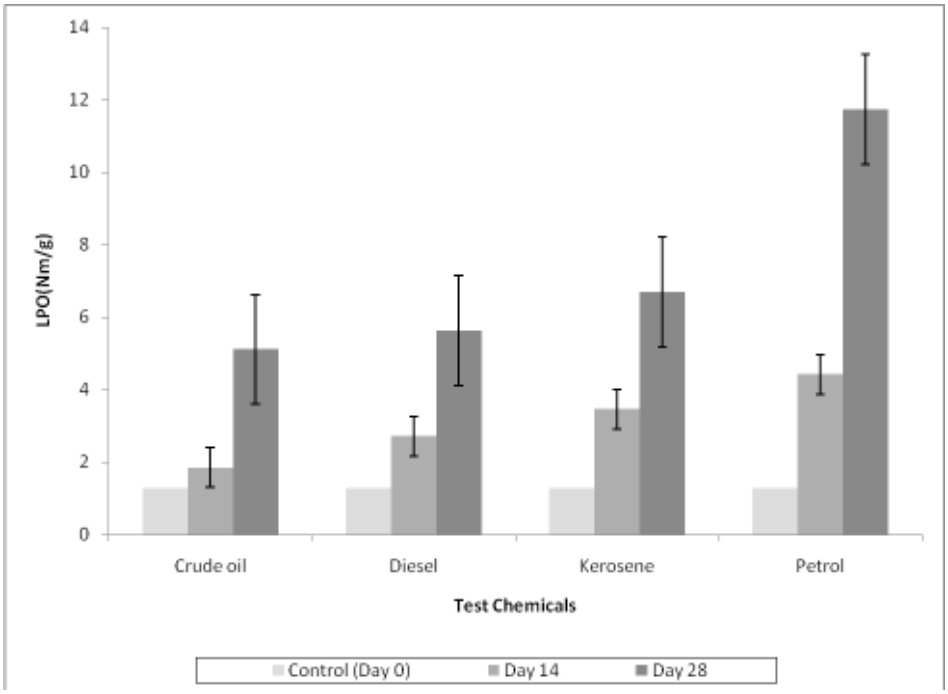

Fig 1: Comparison of the level of lipid peroxidation (MDA) in *Clarias gariepinus* exposed to 1/10th LC_{50} values of crude oil, petrol, kerosene and diesel after control (0), 14 and 28 days of exposure.

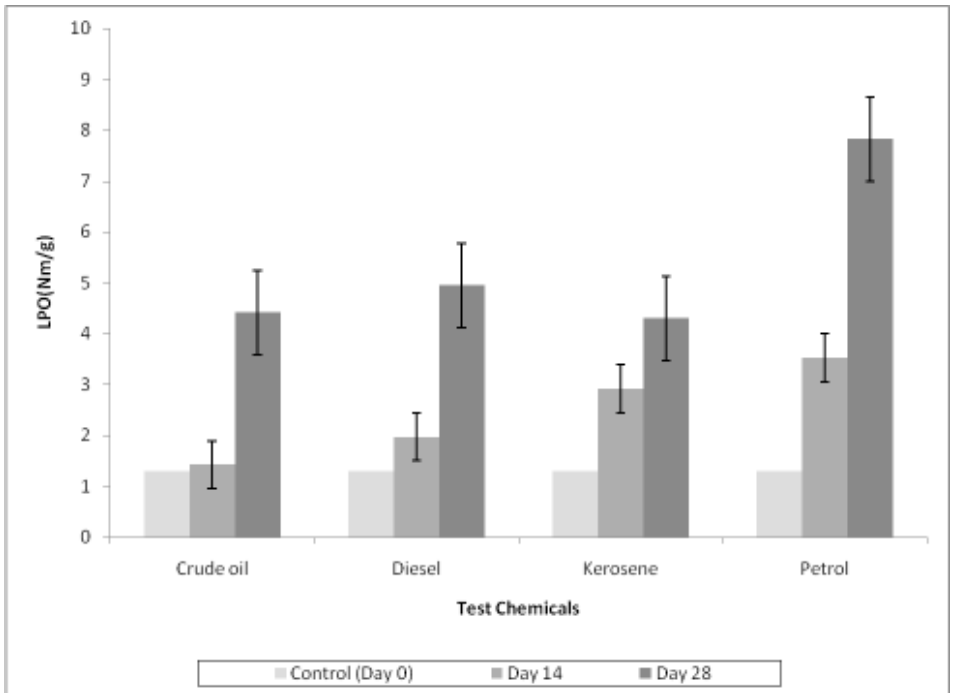

Fig 2: Comparison of the level of lipid peroxidation (MDA) in *Clibanarius africanus* exposed to 1/10th LC_{50} values of crude oil, petrol, kerosene and diesel after control (0), 14 and 28 days of exposure.

B.Sc Dissertation, Department of Zoology, Marine Biology and Fisheries, University of Lagos, Akoka, Nigeria, pp. 3-4.

Ajileye, D.A. 2002. Studies on the Single and Joint Action Toxicity of Cypermethrin and Dichlorvos against *Clibanarius africanus.* B.Sc. Dissertation, Department of Zoology, Marine Biology and Fisheries, University of Lagos, Akoka, Nigeria, pp. 16.

Akpofure, E.A., Efere, M.L. and Ayawei, P. 2000. The Adverse Effects of Crude oil Spills in the Niger Delta. Urhobo Historical Society.

Avci, C.A., Hildago, C.M. and Alberto, D. 2005. Antioxidant enzymes and lipid peroxidation on sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss.* A comparative study. Archive of Hydrobiology, 60: 432-461.

Catala, A. 2009. Lipid peroxidation of membrane phospholipids generates hydroxyl-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem. Phys. Lipids, 157: 1-11.

Davidson, W., Franklin, C.E., Mckenzie, J.C. and Carey, P.W. 1993. The effects of chronic exposure to the soluble fractions of fuel oil on an Antarctic fish *Pagothenia borchgrevinki.* Biochem. Physiol., C 104: 67-70.

Don-Pedro K.N. 1996. Investigation of single and joint fumigal insecticidal action of Citrus peel oil components. Pesticide Science, 46: 78-84.

Finney, D.J. 1971. Probit Analysis, 3rd Edition. Cambridge University Press, pp. 38.

Hermes-Lima, M., Willmore, W.G. and Storey, K.B. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III) xylenol orange complex formation. Free Radical Biol. Med., 19: 271-280.

Ihedike, C.C. 2002. Laboratory Evaluation of Single and Joint action toxicity of Petroleum Products Against *Clibanarius africanus,* M.Sc. Dissertation, Department of Zoology, Marine Biology and Fisheries, University of Lagos, Akoka, Nigeria, pp. 16.

Lopes, P.A., Pinheiro, T., Santos, M.C., Da Luz Mathias, M., Collares-Pereira, M.J. and Viegas-Crespo, A.M. 2001. Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Sci. Total Environ., 280: 153-163.

Otitoloju, A.A. 2003. Tolerance: A useful biological parameter for identifying contaminated sites. Bull. Environ. Toxicol., 71: 1139-1144.

Otitoloju, A.A. and Olagoke, O. 2011. Lipid peroxidation and antioxidant defense enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. Environmental Monitoring & Assessment, DOI: 10.1007/s10661-010-1870-0.

Yagi, K. 1998. Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. Methods Mol. Biol., 108: 107-110.

Zielinski, S. and Portner, H.O. 2000. Oxidative stress and antioxidative defense in cephalopods: A function of metabolic rate or age? Comp. Biochem. Physiol., B, 125: 147-160.