

**ANTIOXIDANT ENZYMES AND HISTOPATHOLOGICAL
BIOMARKERS OF EXPOSURE TO MONOCYCLIC AROMATIC
HYDROCARBONS IN CLARIAS GARIEPINUS (CATFISH) AND
EUDRILUS EUGENIAE (EARTHWORM).**

BY

DOHERTY, VICTORIA FUNMILAYO

B.Sc Zoology (1999) ABU, M.Sc Zoology (2003) UNILAG

Matriculation Number 019077002

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES OF THE
UNIVERSITY OF LAGOS, AKOKA, LAGOS, NIGERIA, FOR THE AWARD OF
DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN ENVIRONMENTAL TOXICOLOGY
AND POLLUTION MANAGEMENT.**

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DECLARATION

This work, titled “Antioxidant Enzymes and Histopathological Biomarkers of Exposure to Monocyclic Aromatic Hydrocarbons in *Clarias gariepinus* (Catfish) and *Eudrilus eugeniae* (Earthworm)”, submitted to the School of Postgraduate Studies, University of Lagos, Lagos, Nigeria, for the award of Doctor of Philosophy in Environmental Toxicology and Pollution Management, is an original research carried out by DOHERTY, Victoria Funmilayo in the Department of Zoology, University of Lagos, under the supervision of Dr A.A. Otitolaju and Dr J.K. Saliu, Department of Zoology, University of Lagos.

This work has not been submitted previously, in whole or part, to qualify for any other academic award.

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DEDICATION

This work is dedicated to God the Father, God the Son and God the Holy Spirit who made this possible; and to my husband and children for their support and encouragement.

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DEFINITION OF TERMS

Antioxidant – It is a substance capable of preventing or slowing the oxidation of other molecules, protect against toxicity by trapping free radicals and preventing reaction with reactive oxygen species.

Aromatic hydrocarbons - Hydrocarbon that contains one or more benzene rings that are characteristic of the benzene series of organic compounds.

Atresia – the process of oocyte and follicle resorption altering the oocyte structure as an indicator for the destruction and resorption of oocytes.

Biochemical biomarkers- Biochemical changes associated with pollutant exposures which include: (1) the inhibition or induction of enzymes, (2) induction of specific enzymes involved in the metabolism of pollutants and the appearance of associated metabolites.

Bioindicators - An organism giving information on the environmental conditions of its habitat by its presence, its absence or its behavior.

Biomarkers – A biochemical, cellular, physiological or behavioural variation that can be measured in tissue or body fluid samples at the level of the whole organism that provides evidence of exposure to and/or effects of one or more chemical pollutants.

Biomarkers of effects- includes measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease;

Biomonitoring – The monitoring of the organisms that live in a particular environment in order to assess the continuing quality of the ecosystem.

BTEX- Is the term used for Benzene, Toluene, Ethylbenzene and Xylene, volatile aromatic compounds typically found in petroleum product such as gasoline and diesel fuel.

Histopathology - The microscopic study of abnormal tissue and organs at the cellular level.

Hyperplasia- An abnormal increase in the number of cells in an organ or a tissue with consequent enlargement.

LC₅₀ - Experimentally derived concentration of test substance that is estimated to kill 50 percent of a test population during continuous exposure over a specified period of time.

Oil depots - An industrial facility for the storage of oil and or petrochemical products and from which, they are transported to end users or further storage facilities

Oxidative stress - The imbalance between the generation and the neutralization of ROS by antioxidant mechanisms within an organism.

Reactive oxygen species - Species such as superoxide, hydrogen peroxide, and hydroxyl radical molecules and ions of oxygen that have an unpaired electron, thus rendering them extremely reactive.

Right(s) of way - Designated land around oil pipelines or oil industry installations to facilitate access to and protection of oil industry assets.

Tank farms – Large tanks used exclusively for storing petroleum in an area.

Water soluble fraction - Is the aqueous solution which is formed when oil and water are brought into contact or the fraction of oil that dissolves into water that contacts aquatic organisms.

Xenobiotics - Is a chemical which is found in an organism but which is not normally produced or expected to be present in it. It can also cover substances which are present in much higher concentrations than are usual.

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AAS	Atomic Absorption Spectrophotometer
ATSDR	Agency for Toxic Substances and Disease Registry
APHA	American Public Health Association
EGASPIN	Environmental Guidelines and Standards for the Petroleum Industry in Nigeria
GSH	Glutathione
LC ₅₀	Lethal Concentrations
MDA	Malondialdehyde
Mg/kg	Milligramme/ Kilogramme
mM	Millimolar
O ₂ [·]	Oxygen radical
.OH	Hydroxyl radical
pH	Potency of hydrogen
ROS	Reactive Oxygen Species
SPSS	Statistical Package for the Social Sciences
U	Unit of activity
µg/l	Microgram per Litre
WHO	World Health Organisation
BTE	Benzene, Toluene and Ethylbenzene
BTEX	Benzene, Toluene Ethylbenzene and Xylene
NIOMR	National Institute for Oceanography and Marine Research
ROW	Right of way

MetHb	Methaemoglobin
USEPA	United States Environmental Protection Agency
CAT	Catalase
GST	Glutathione -S- Transferase
TDS	Total Dissolved Solid
Zn	Zinc
Ni	Nickel
Pb	Lead
ASTM	American Society for Testing and Materials
OECD	Organisation for Economic Cooperation and Development
THC	Total hydrocarbon content
TCA	Tricarboxylic acid
TBA	Thiobarbituric acid

ABSTRACT

The widespread distribution of petroleum products arising from the rapid growth of the petroleum industry in Nigeria has resulted in the pollution of the environment through oil spills involving leakages from tankers, pipelines, tank farms and dumping of waste petroleum products. This study was aimed at identifying histopathological alterations and antioxidants defence systems in *Clarias gariepinus* (catfish) and *Eudrilus eugeniae* (earthworm) that can be used as a good battery of biomarkers for early detection of pollution associated with petroleum hydrocarbon and that can be used in monitoring programmes in Nigeria. The impacts and distribution of major toxic components (BTEX - Benzene, Toluene, Ethylbenzene and Xylene) of petroleum products in water and sediment samples collected from sampling stations in the Lagos lagoon and in groundwater and soil around hydrocarbon impacted areas in Ijegan, Lagos, following a pipeline explosion in May 2008 was investigated over a two- year period (February, 2009 – July, 2010). The distribution of benthic communities in the different sampling stations of the Lagos lagoon was assessed. The toxicological evaluations of BTEX were carried out against juvenile stages of *C. gariepinus* and *E. eugeniae*. The effect of BTEX on antioxidant enzyme system, lipid peroxidation formation and different organs was investigated in *C. gariepinus*, *Tilapia zillii*, *Chrysichthys nigrodigitatus* and *E. eugeniae*. The determination of hydrocarbon levels in the samples showed that the levels of Total Hydrocarbon Content (THC) in the water samples around the Atlas cove and Apapa were high with values ranging from 2.03mg/l - 31.38 mg/l and 4.04mg/l - 22.89mg/l, respectively. The highest value of total BTEX in the lagoon sediment was also recorded in the Apapa station (450.53µg/kg) where oil depots and tank farm facilities are located. The study of the macrobenthic community structure showed that the species richness ranged from 1.57 to 2.02 in the reference station, Unilag while in the Atlas Cove, Iddo

and Apapa stations, it ranged from 1.80 to 2.89, 1.95 to 3.03 and 1.86 to 2.95 respectively. The highest number of organisms (183) was recorded in the reference stations while the least number (46) was recorded in Apapa. The main hydrocarbon pollution indicator species identified in the impacted aquatic stations were *Nais eliguis* and *Heteromastus filiformis*. Results showed elevated THC levels (2.00mg/l–689.12mg/l) in groundwater compared to the World Health Organization maximum permissible value of 0.1 mg/l. Higher values of THC in the soil ranging from 2.16 to 401.41mg/kg were also observed around the impacted area of Ijegan in comparison with 7.03 to 44.61mg/kg observed in the control station. The level of BTEX in the groundwater sampled within the 100m and 150m buffer zones ranged from 1.19 to 4964.33 µg/l compared to the safe limit of 0.2 µg/l set for BTEX compounds in the Environmental Guidelines and Standards for Petroleum Industry in Nigeria (EGASPIN). Toxicological evaluations of the monocyclic aromatic components (BTEX) on *Clarias gariepinus* showed that ethylbenzene (0.479ml/l) was the most toxic compound tested followed by xylene (0.519ml/l), benzene (0.666ml/l) and toluene (1.190ml/l). Toxicity evaluations against *Eudrilus eugeniae* showed that xylene (1.212 mg/kg) was the most toxic compound followed by toluene (1.335mg/kg), ethylbenzene (1.366mg/kg) and benzene (1.896mg/kg). The biomarker study revealed an increase in malondialdehyde level and inhibition of the activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione-S-Transferase (GST) in the liver and gills of *Clarias gariepinus*, *Tilapia zillii* and *Chrysichthys nigrodigitatus*, which can serve as good biomarkers for early detection of BTEX and related hydrocarbon pollution. Similar results of consistent increase in MDA levels and inhibition of SOD, CAT, GSH and GST activities in *Eudrilus eugeniae* exposed to sublethal concentrations of BTEX and petroleum hydrocarbon were observed, therefore confirming the relevance of these antioxidant enzymes as good general

biomarkers of hydrocarbon related stress. The histological study identified necrosis and deformation of the gills, inflammations in the liver, wrinkling of the oocyte membrane in the gonads of fish, and endothelial degeneration in earthworms as good histopathological biomarkers of hydrocarbon related stressors. The levels of hydrocarbon observed in the aquatic and terrestrial environment showed that there is widespread contamination as a result of petroleum product importation, storage and distribution. The combination of chemical analysis with these identified biomarkers will therefore provide important tools for early detection, diagnosis and management of hydrocarbon pollution.