CHAPTER ONE

1.0 INTRODUCTION

The pollution of aquatic and terrestrial ecosystems by petroleum products has become a rampant occurrence due to frequent leakages, spillages and vandalisation of refined petroleum products' pipelines and blowouts. Although, Nigeria is a major producer of crude oil, it is ironic that it is also a major importer of petroleum products which are stored in large petroleum tankfarms and distributed via pipelines to some parts of the country. The storage and distribution of these petroleum products results in frequent oil spillages impacting both aquatic and terrestrial ecosystems. As a result, high concentrations of monocyclic aromatic hydrocarbon (BTEX) compounds have been observed in various components of the environment by various workers (De Oliveira *et al.*, 2007; Akpoborie *et al*., 2008; Guimaraes *et al.*, 2010; Osu *et al*., 2010; Osuji and Achugasim, 2010). BTEX are considered a major cause of environmental pollution because of widespread occurrences of petroleum products leakage from underground petroleum storage tanks and rampant spillages of petroleum products.

The monocyclic aromatic fractions, that is, Benzene, Toluene, Ethylbenzene and Xylene (BTEX), are the most toxic components of petroleum products and have higher water solubility than other organic compounds that are present in gasoline such as aliphatics (Farhadian *et al*., 2008). They also make up a significant percentage of petroleum products and about 18% (w/w) in standard gasoline essentially because they are added to unleaded petroleum products to increase fuel performance (Budavari, 1996). For BTEX compounds, the principal concern is their migration away from the source areas and consequently these compounds are some of the most common contaminants found in groundwater.

BTEX like other hydrocarbons have a high degree of lipophilicity which enables them to penetrate biological cell membranes and leading to their concentration in fatty deposits. The potential toxicity of BTEX compounds is linked to their lipophilicity. The severity of the effects depends on the organisms exposed, the concentration of the components and mode of exposure. Even though these toxic components are sometimes present at sublethal levels, they can cause carcinogenic effects, asthma, aplastic anaemia, formation of methaemoglobin (MetHb) in automobile mechanics, morphological abnormalities in earthworms, neurological effects, leukemia and decreased sperm vitality and motility (ASTDR, 2006).

Petroleum products contamination of the aquatic ecosystems can cause changes to the structure of benthic communities, which are necessary to study and quantify in order to evaluate the effects of contaminants on a particular ecosystem. Several authors have studied the distribution of benthic communities in order to assess the pollution status of water bodies (Chukwu and Nwankwo, 2003; Edokpayi *et al* ., 2010; Emmanuel and Ogunwenmo, 2010; Nkwoji *et al*., 2010; Uwadiae, 2010 and Balogun *et al*., 2011). However, there is little or no information on the impact of petroleum industry activities on benthic communities around the Atlas cove and Apapa areas of the Lagos lagoon. These parts of the Lagos lagoon are subjected to incremental levels of petroleum product spillages because the Atlas Cove, has located within it a marine receipt terminal and depot for imported petroleum products into Nigeria while the Apapa area where the Lagos harbor is located has many petroleum products especially diesel tank farms for storage and distribution of petroleum products in Nigeria. The Atlas Cove Depot marks the origin of the system 2B pipeline network of Petroleum Products Marketing Company Ltd (PPMC) and is the

largest petroleum products depot in Nigeria. Oil spill incidents are therefore a common occurrence around the Atlas Cove and Apapa areas of the Lagos Lagoon.

For adequate pollution diagnosis and control, there is also the need to identify biological responses or biomarkers which can serve as early warning signals of petroleum hydrocarbon related stress in oil impacted ecosystems. These biomarkers will complement chemical analysis of pollutant levels in environmental media during monitoring programmes. Biological responses at the cellular or biochemical levels occur at early stages of pollution impact, therefore they are used as early warning signals for assessing the influence of contaminants on organisms. Hydrocarbons are known to cause oxidative stress through the formation of reactive oxygen species (ROS). This will ordinary lead to cellular damage through protein oxidation, lipid peroxidation (LPO) and DNA damage. To prevent these injuries, enzymatic and non-enzymatic antioxidant systems are triggered to eliminate contaminant stimulated ROS, allowing the organism to overcome oxidative stress in polluted environments (Ahmad *et al*., 2004).

This defence system comprises of antioxidant molecules, such as glutathione (GSH), catalase (CAT), glutathione S- transferase (GST), and superoxide dismutase (SOD). The antioxidant systems can serve as biomarkers of oxidative stress. Evidence of oxidative stress in fish can be found in various studies observing alterations in antioxidant enzyme activities in animals exposed to pollutants in controlled laboratory experiments or contaminated sites (Sturve *et al*., 2008; Kopecka and Pempkowiak, 2008). It appears that the majority of toxicity information on pollutants have been generated for aquatic invertebrates and fish. Changes in the activities of

antioxidant enzymes in response to hydrocarbons in terrestrial invertebrates, especially, earthworms are generally lacking and require further investigation.

Additionally, the exposure of animals to chemical contaminants also induces a number of lesions in different organs which can serve as histopathological biomarkers of exposure to petroleum products related stressors. These histopathological biomarkers have been identified as definite biological endpoints of historical exposure and can thus serve as indicators of environmental stress. The histology of gill and liver are good biomarkers to evaluate the toxicity of hydrocarbons (Brand *et al*., 2001). However due to non-specific nature of most biomarkers, the current approach during biomonitoring is to use multiple biomarkers as pollutant stress indicators as opposed to the previous use of single biomarkers. Currently, there exists little information on biomarkers of toxic responses for organisms exposed to monocyclic aromatic hydrocarbons particularly the BTEX compounds. However, determining biological effects at sublethal concentrations will greatly assist in risk assessment for chronic effects associated with development, reproduction, and survival. By studying the dynamic changes of biomarkers, researchers may better understand the biological impacts of oil in a more complete manner to predict the risk of different types of petroleum compounds to different species.

Due to the potential of these monocyclic aromatic hydrocarbon compounds to cause varied health effects, they have been classified as priority pollutants by many regulatory bodies in the developed economies. Remarkably in Nigeria, there is little information available in the literature about the occurrence and effects of BTEX compounds on indigenous flora and fauna. It is therefore necessary to establish dose-response relationships where indigenous sensitive species are exposed to varying concentrations in order to establish toxicity indices which will serve as a

tool for hydrocarbon pollution diagnosis and management, particularly for the establishment of environmental safe limits and standards for BTEX fractions in Nigeria. These standards will be more relevant for the protection of indigenous species in Nigeria rather than the usually adopted foreign standards.

1.2 STATEMENT OF THE PROBLEM

Due to the activities occurring around and within the Lagos lagoon, especially the petroleum products storage around the Atlas Cove and the location of several petroleum tank farms around Apapa, the lagoon is constantly under considerable risk of anthropogenic pollution from petroleum products spillages. Although, Nigeria is a major crude oil producing country, considerable importation of petroleum products occurs. The Atlas Cove Depot is the major marine receipt terminal for storage and distribution of petroleum products in Nigeria, therefore, major and minor oil spills incidents occur frequently at the Atlas Cove and Apapa area where many petroleum tank farms are situated (Otitoloju *et. al*., 2007). This continual loading of the lagoon environment with different types of pollutants including petroleum products have been indicated as causing drastic reduction in volume of fish catches from the Lagos Lagoon from about 1,000,000kg per year in 1980 to only 100,000Kg per year in 1990 (Osae-Addo, 1995). It was also observed that seventy two fish species distributed over thirty four families which was recorded in 1994 in the Lagos lagoon, reduced to fifty-two fish species in thirty two families in 2004 (Fagade and Olaniyan, 1974; Ayoola and Kuton, 2009).

Additionally, the land portion of Lagos state contains networks of petroleum distribution pipelines, depot and petrol filling stations with underground tanks which leak petroleum products

into the terrestrial environment. Occasionally, accidental discharges of petroleum products occur leading to large releases of petroleum products into the surrounding environment causing fire outbreak and contamination of groundwater. One of such incident is the Ijegun fire incident which occurred in May 2008, following an accidental damage of NNPC Mosimi-Atlas cove system 2B pipeline carrying petroleum products. This led to a fire disaster causing human fatalities, loss of properties, contamination of groundwater and soil along the pipeline right-of-way (ROW) with petroleum products. This consistent contamination of the aquatic and terrestrial environment of Lagos with monocyclic aromatic hydrocarbons represented by Benzene, Toluene, Ethylbenzene and Xylene (BTEX) which are the most toxic components in the petroleum products requires constant monitoring and assessment of their impacts. One of the major tools for monitoring impacted ecosystems is the identification of biomarkers which can serve as early warning signals for detection of petroleum related stress in aquatic and terrestrial ecosystems.

1.3 AIM/OBJECTIVES OF THE STUDY

1.3.1 Aim of study

In view of the stated problems above, the aim of this research is to identify histopathological alterations and antioxidants defence systems in *Clarias gariepinus* (catfish) and *Eudrilus eugeniae* (earthworm) that can serve 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 use of histopathological and biochemical responses as biomarkers during environmental monitoring programmes is derived from the basis that a toxic effect manifests itself at the subcellular level before it becomes apparent at higher levels of biological

organization. The measurement of these responses will therefore serve to improve the assessment of biologically significant exposures to toxic chemicals and enhance ability to assess the risk of effects of pollutants on the health and survival of toxicant exposed population.

1.3.2 Objectives of Study

The objectives of this research are to:

1. Determine the level of occurrence and distribution of hydrocarbon in water and sediment samples collected from stations around the Atlas Cove and tank farms in Apapa areas of the Lagos lagoon over a period of two years.
2. Determine the level of residual contamination of groundwater and soil samples by hydrocarbon around impacted areas of Ijegun following a pipeline explosion and petroleum product leakage over a period of two years.
3. Investigate the impacts of the petroleum products spillages on macrobenthic community structure around Atlas Cove and Apapa areas of the Lagos lagoon over a period of two years.
4. Establish the lethal concentration (LC_{50}) of the aromatic fractions of hydrocarbons: Benzene, Toluene, Ethylbenzene and Xylene (BTEX) on an aquatic organism (*Clarias gariepinus*) and a terrestrial organism (*Eudrilus eugeniae*).
5. Identify biochemical responses especially enzymatic effects (Superoxide dimustase (SOD), Catalase (CAT), Glutathione-S-Transferase (GST), Reduced Glutathione (GH) and lipid peroxidation in *Clarias gariepinus* and *Eudrilus eugeniae* that can serve as biomarkers of exposure to Benzene, Toluene, Ethylbenzene and Xylene (BTEX) in the environment.

6. Identify histopathological biomarkers of exposure to sublethal concentrations of Benzene, Toluene, Ethylbenzene and Xylene (BTEX) on selected organs of *Clarias gariepinus* and *Eudrilus eugeniae.*
7. Establish the relevance of the laboratory identified enzymatic, non-enzymatic and histopathological biomarkers in the natural ecosystems by determination of their occurrence in selected wild fish species (*Chrysichthys nigrodigitatus* and *Tilapia zillii*) and earthworms (*Eudrilus eugeniae*) collected from impacted areas (Lagos lagoon and Ijegun).

1.4 SIGNIFICANCE OF STUDY

The field assessment of level of hydrocarbon especially the aromatic fractions (BTEX) around the Atlas Cove, Apapa and the Ijegun pipeline ROW, will provide insight into the impact of petroleum products importation and distribution on the surrounding environment. The results of the acute toxicity studies of BTEX on locally available test organisms will also constitute one of the first sets of toxicity data in Nigeria which will form the basis for the establishment of environmental safe limit and standards for BTEX and related compounds in Nigeria. The sublethal tests carried out will also provide information on the health status of the aquatic and terrestrial ecosystems in affected areas of Lagos state through determination of histopathological effects and oxidative stress level (LPO) in affected organisms. The results of the abundance and distribution of benthic organisms along Apapa and Atlas cove will provide information on the effect of petroleum product spillages on benthic organisms in the lagoon. The identification of biomarkers will also complement chemical monitoring methods and therefore afford environmental regulators with the necessary biological tools for early detection of stress in the

environment and avoid ecological disruption which are already being reported in the Lagos lagoon by several authors (Osae-Addo, 1995 and Otitoloju *et al.,* 2007b).

1.5 RESEARCH QUESTIONS

1. What is the level of THC in surface water and sediment around Atlas Cove and Apapa areas of the Lagos lagoon?
2. What is the level of THC in groundwater and soil around the oil impacted areas of Ijegun?
3. What are the effects of petroleum products spillages on the community structure of macroinvertebrates in selected areas of the Lagos lagoon?
4. What is the lethal concentration of Benzene, Toluene, Ethylbenzene and Xylene (BTEX) against *Clarias gariepinus* and *Eudrilus euginiae*?
5. Which marker enzymes and non-enzymes of oxidative stress could serve as biomarkers of exposure to BTEX compounds in the environment?
6. Which histological features most affected by the sublethal concentrations of BTEX compounds could serve as biomarkers?
7. Can the observed effects be useful biomarkers of hydrocarbon during biomonitoring and pollution control programmes?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Incidences of Petroleum Product Spillages in Nigeria

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 (Adeniyi and Afolabi, 2002). Nigeria has a wide pipeline network and depots for distributing refined petroleum products (Renner *et al*., 2008). There are about 5,000km worth of pipelines and about 20 oil depots altogether in Nigeria (Adewuyi and Olowu, 2012). The Atlas Cove Depot marks the origin of the system 2B pipeline network of Petroleum Products Marketing Company Ltd. (PPMC) and is the largest petroleum products depot in Nigeria.

There is usually major public concern expressed following pipeline accidents, in many parts of the world. This is due to the fact that pipelines run through a large number of local communities so that repercussions and public interest are not inherently restricted to the area where the accident happens (Papadakis *et al*., 1999). Most oil spillage results are followed by fire that destroys vegetation, animal and human life. Major and minor oil spills incidents have been occurring at the Atlas Cove and in communities along the pipelines Right of Way. On the 16th of September 2004, an oil pipeline in Imore village in Lagos State, Nigeria was vandalized, resulting in the leakage of petrol to the environment and followed by a massive fire outbreak that destroyed more than 5 km^2 of the area. It was observed that the oil spill and ensuing fire

incidence had a severe negative effect on the population of *T. fuscatus* at the impacted site as complete absence of this species was observed (Renner *et al*., 2008). Complete destruction of both plants and animal community following a petroleum product spillage and fire ravaged ecosystem at the Atlas cove jetty was observed by Otitoloju and Don-Pedro (2004). In April 1998, there was a reported diesel spillage along the 49 km Atlas Cove/Mosimi multiproduct pipeline at its 1km point at the mangrove ecosystem of Ebute Oko village near Takwa bay, Lagos. The spilled diesel affected about $4km^2$ of the mangrove and destroyed a total of 345 individual animals (Samuel *et al*., 2008).

The fire outbreaks associated with petroleum products spillage usually cause more damage to the environment than the petroleum products spill alone (Otitoloju and Are, 2003). The rupture and explosion of the NNPC high-pressure pipeline carrying gasoline from the Warri Refinery in Southern Nigeria to Kaduna in Northern Nigeria on October 17, 1998, killed 1078 people, induced $54 million in property damages and devastated $12km^2$ of land (Sovacool, 2008). A report was published in the Newspaper, Saturday Punch, 13th May 2006 on pipeline explosion that occured in Ilado, Lagos. This led to more than 200 people incinerated in the pipeline fire. In May 2000, an oil pipe leakage occurred at the Diebu Creek field, a freshwater environment in the Niger Delta area of Bayelsa State, many forests and agricultural land were damaged and common food crops like *Musa* spp (plantain) *Dioscorea* spp (yam), *Manihot esculenta* (cassava), and *Saccharum officinarum* (sugar cane) were also reported to be affected by the oil spill (Daniel-Kalio and Braide, 2004).

Ground water contamination is one of the most essential environmental issues confronting mankind today (Adewuyi and Olowu, 2012). Apart from the loss of lives and property through pipeline fire, the run-off from the impacted sites usually degrade the quality of the fresh water sources which serves the domestic rural water supply needs of most communities in Nigeria. Many Nigerians are drinking water with high and dangerous levels of hydrocarbons due to the problem of petroleum spillage into groundwater. For over three decades now the Baruwa community of Alimosho Local Government of Lagos State, has being experiencing lack of potable water owing to the fact that there was a leakage or perhaps vandalisation of the NNPC pipeline that runs through a part of the community (Oseni *et al*., 2013). The oil pipes have become old, rusted and leak in some locations thus polluting the groundwater and wells sunk in the area for the inhabitants' domestic water supply (Ayolabi *et al*.,2013). In May 2008, a pipeline explosion occurred in Ijegun, a suburb in Lagos, when construction workers accidentally damaged NNPC Mosimi-Atlas cove system 2B pipeline carrying petroleum products. The spill resulted in contamination of the groundwater including wells and boreholes with petroleum products. This raised concern over exposure of residents using this water. The explosion also led to a fire disaster causing loss of lives and properties along the pipeline right-of-way (ROW).

While investigating groundwater contamination in Ogoni land, the United Nations Environment Programme (UNEP) assessment found petroleum hydrocarbons, most notably benzene, in 28 drinking water wells used by Ogoni communities, at concentrations far above the threshold of acceptability according to WHO guidelines. Exposure to such high levels of hydrocarbons is certain to lead to long-term health consequences for community members (UNEP, 2011).

Recently, the Nigerian National Petroleum Corporation (NNPC) in January, 2013 confirmed on their website, several breaks in its system 2B pipeline at Arepo in Obafemi/Owode Local Government Area of Ogun State. This has resulted in explosions claiming the lives of several people in the area. In Nigeria, a total number of 2,097 oil spill incidents were recorded between 1997 and 2001. In 2005, 117 cases of fire outbreaks were recorded as a result of pipeline vandalization and rupture of Nigerian National Petroleum Corporation (NNPC) pipelines (Renner *et al.*, 2008). Thousands of barrels of oil have been spilt into the environment through oil pipelines and tanks in the country. This spillage is as a result of lack of regular maintenance of the pipelines and storage tanks (Egberongbe, 2006). According to Shell Petroleum Development Company of Nigeria Limited, in the Niger Delta region alone, 1,301 oil spill incidents have been recorded around SPDC facilities between 2007 and March 2013 resulting from equipment failure, corrosion or human error.

2.2 Hydrocarbon Contamination of the Lagos Lagoon

Nigeria's vast water resources especially Lagos lagoon are among those most affected by environmental stress imposed by human population growth, urbanization and industrialization (Adeyemi *et al*., 2009). The pollution of the Lagos lagoon by petroleum hydrocarbon is a major problem as the large population of Lagos, depend on the Lagos lagoon for potable and recreational water, as well as a source of cheap and affordable protein in form of fish. The Lagos Lagoon by virtue of the petroleum industry activities and the fact that it is surrounded by industrialized Lagos metropolis, is at risk from anthropogenic pollution. Hydrocarbons enter the lagoon from river run-off, accidental spillages at oil depots, operational shipping loss in harbor and outboard motors plying the Lagoon and from careless waste oil disposal (Ajao, 1996).

Leakages and spills associated with loading and offloading of petroleum products in depots as well as washing of oil storage tanks has adversely impacted the aquatic environment. These impacts depend primarily on the petroleum products, its concentration after release and the biotic community that is exposed. A huge bulk of waste generated from depot activities are oil discharges and since oil contains mostly hydrocarbons (petroleum hydrocarbons), these discharges has significantly increased the pollution levels of surface water near depots (Adewuyi and Olowu, 2012). High levels of petroleum hydrocarbon have been reported in the Lagos lagoon (Adekanbi, 1989; Oshibanjo and Bamgbose, 1990; Ajao, 1990; Ajao and Fagade, 1990; Adedayo *et al*., 2012)

The Lagos lagoon consists of three main segments namely the Lagos Harbour Segment, the Metropolitan and the Epe Division Segment. Lagos Lagoon empties into the Atlantic via the Lagos harbor. The principal ocean port of Lagos is located at Apapa in a broad western branch off the main channel of the harbor (Adeyemi *et al*., 2009). Apapa terminal is unarguably the busiest port in Nigeria and possibly one of the busiest in the sub-Saharan Africa. The terminal is highly polluted due to enormous activities of ships and boats that make use of hydrocarbon fuel, but more especially due to the great activities of import and export of petroleum related products. Moreover, numerous tank farms are located around this terminal which further increases the level of hydrocarbons in the water bodies around the terminal (Anyakora *et al*., 2004).

Results from the study conducted by Anyakora *et al*., (2004) reveal high level of Polycyclic Aromatic Hydrocarbons (PAHs) in the Apapa terminal which were above the minimum allowable concentration. Fifteen out of sixteen US EPA priority PAHs were detected with

concentration ranging from 0.19ug/l to 8.84ug/l. The PAHs detected in the samples include: naphthalen 2.04µg/L; acenaphthalene 0.19µg/L; acenaphthene 0.38µg/L; fluorene 1.99µg/L, phenanthrene 0.89µg/L, anthracene 0.22µg/L, fluoranthene 0.27 µg/L; pyrene 0.32µg/L; chrysene 0.69µg/L; benzo(b)fluoranthene 1.0µg/L; benzo(k) fluoranthene 0.89µg/L; benzo(a)pyrene 1.31µg/L; benzo(ghi) perylene 3.75µg/L; dibenz(a,h)anthracene 8.84µg/L and indeno (1,2,3- cd)pyrene 3.37µg/L. The concentration of PAHs in surface and coastal waters are usually around 0.05 ug/L, therefore anything above this indicates contamination.

Adedayo *et al*., (2012) assessed the levels of PAHs in surface and bottom waters from different locations of the Lagos lagoon namely: NIOMR Jetty, Bonny, Falomo, Apapa, Ijora and Oko Baba. 31.5% of samples analyzed recorded positive for the presence of PAHs, while the mean concentration of PAHs in 28.8 % of samples analyzed were sufficiently high (> 0.01µg/L) to cause acute toxicity to the exposed organisms. The mean concentration of PAHs in surface and bottom water varied from ND - 9.8 and ND - 7.9 µg/l respectively. Phenanthrene, pyrene and fluoranthene were detected in larger concentrations (1.88-5.73 µg/L) and were widely distributed in the analyzed samples, suggesting the possibility of contamination from industrial effluents. Acenaphthylene, fluorene, anthracene, chrysene, benzo[a] pyrene and benzo (g,h,i) perylene were detected in lower concentrations (0.01-0.42 µg/l), while benzo [k] fluoranthene, benzo [b] fluoranthene, dibenz [a,h] anthracene, indeno (1,2,3-cd) pyrene, chrysene, naphthalene, 2-methyl naphthalene and acenaphene were below detection limits in all the samples analyzed. Phenanthrene (4.7-9.8 µg/l) was the most predominant chemical detected at NIOMR Jetty. Studies have shown that the likely sources of such low molecular weight PAHs are petrogenic, from spillages of petroleum products and industrial effluents.

Alani *et al*., (2012) assessed the Polycyclic aromatic hydrocarbons in fish, invertebrates, water and sediment collected from different locations of the Lagos lagoon. The study showed that fish and invertebrates from Lagos Lagoon were more contaminated with PAHs. The biota from Lagos Lagoon contained mainly naphthalene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)- anthracene and chrysene. The fish and invertebrates from Unilag lagoon front were found to bioaccumulate phenanthrene to magnitudes several times higher (ranging from10ng/g to 110ng/g for fish and 22ng/g to 180ng/g for invertebrates) than the sediment concentration (20.12ng/g) at same location. The authors observed that Benzo(a)pyrene was not detected in any of the fish and invertebrate samples from Lagos Lagoon but the concentrations in sediment (881.240ng/g d. w.) obtained from Okobaba was high enough to bioaccumulate in fish and invertebrates that dwell at the location. Sum (12) PAHs in water at all the locations in the study ranged from 0.110 to 0.480ng/ml. Sum (16) PAHs in sediments at all the locations ranged from 12.320 to 955.510ng/g dry weight. All the 16 PAHs assessed were found in the sediment, 9 were found in the invertebrates and 10 in the fish samples.

2.3 Biological Effects of Benzene, Toluene, Ethylbenzene and Xylene

Monocyclic aromatic hydrocarbons such as BTEX compounds are major volatile constituents of gasoline and commonly found in gasoline and gasoline contaminated environment. BTEX have in recent years attracted much attention, since they constitute one of the most common and serious threats to groundwater reservoirs and soil. BTEX are classified as priority pollutants regulated by the US Environmental Protection Agency. Soil and groundwater becomes contaminated with petroleum hydrocarbon (BTEX) through accidents, transportation, spills from

automobile service stations, leakage from storage tanks and pipelines ruptures (Nicolotti and Eglis, 1998; Siddiqui and Adams, 2002). Elevated concentration of BTEX compounds detected in service stations was about 10 times the concentration determined in the control location (De Oliveira *et al*., 2007). Soil pollution by gasoline has become a significant environmental concern due to it's adverse ecological effects. Concentrations of BTEX compounds ranging from 0.001-0.013mg/kg have been detected in soil samples collected from automobile mechanic workshops (Osu *et al*., 2010). BTEX compounds are more toxic than liquid alkanes, and well-known toxicants to a wide range of terrestrial biota as well as aquatic organisms (An, 2004).

Animal studies show neurologic, immunologic and hematologic effects from exposure to benzene. Methaemoglobin (MetHb) was significantly higher in blood samples taken from Automobile Mechanics and Petrol station attendants as a result of exposure to benzene in petrol vapour (Udonwa *et al*., 2009). Benzene inhalation can cause aplastic anemia, a disorder where elements in the bone marrow are replaced with fat (ATSDR, 2006). The International Agency for Research on Cancer and the U.S. environmental protection agency classify benzene as a confirmed human carcinogen (ATSDR, 2006). Leukemia mortality has been associated with exposure to benzene among shoe factory workers in Italy (Costantini *et al.,* 2003). Long-term gastrointestinal and respiratory exposure to benzene in mice and rats produced liver cancer and leukemia (Budavari *et al*., 2001). Children exposed to benzene levels in their homes had increased risk of hospitalizations for asthma. The severity of asthma symptoms is associated with ambient benzene concentrations **(**Gordian *et al* 2006, 2010).

There is growing evidence that traffic-related air pollution (BTEX) reduces birth weight. A significant effect of BTEX on birth weight among pregnant women was observed by Aguilera *et*

al.,(2009). A study of women working in pathology/histology laboratories showed an association of xylene exposure with an increased risk of spontaneous abortion (Taskinen *et al*., 1994). Low daily exposure to toluene in women working in the printing industry is associated with reduced fecundity (Plenge-Bönig and Karmaus, 1999). BTEX compounds were found in the blood and semen of ex-workers at workplaces where the air concentrations of benzene, toluene, and xylene exceeded the maximum allowable concentration (Xiao *et al*.,2001). Neurotoxic symptoms were observed in gasoline station workers (Chen *et al*., 2002) and histology technicians (Kilburn *et al*., 1985) exposed to BTEX compounds. Regulatory agencies have traditionally focused on BTEX compounds, due primarily to concerns with the carcinogen benzene, and the knowledge that aromatic compounds are generally more toxic than aliphatics.

2.4 Biomarkers of Oxidative Stress

Aerobic organisms depend on oxygen (O_2) as the electron acceptor in controlled electron transfer reactions. However, during the stepwise one-electron reduction of O_2, cells continuously produce the stable O_2 intermediates superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), commonly collectively known as reactive oxygen species (ROS), and can cause damage or stress to the cell. Stress can produce a number of biochemical, physiological, and histological indicators that can potentially serve as biomarkers of exposure, stress, and adverse effects (Livingstone, 2001, van der Oost *et al*., 2003, Valavanidis *et al.*, 2006). The main reason to study oxidative stress in organisms is to understand whether animals are detrimentally affected by exposure. Oxidative stress as a result of environmental pollution has been documented in numerous organisms over the past decade (Valavanidis *et al.*, 2006; Mohamed *et al*., 2008; Padmini *et al*., 2009; Nogueira *et al*., 2010). Biomarkers of oxidative stress, such as changes in

antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure to xenobiotics. These oxidative stress parameters have been associated with various disease pathologies and organism longevity in a number of species, thereby establishing ecological relevance in these cases. Depledge in (Depledge,1994) defined biomarker as a biochemical, cellular, physiological or behavioural variation that can be measured in tissue or body fluid samples at the level of the whole organism (either individuals or populations) that provides evidence of exposure (exposure biomarkers) to and/or effects (effect biomarkers) of one or more chemical pollutants.

2.5 Biochemical Biomarkers

The need to detect and assess the impact of pollution, particularly low concentrations of increasingly complex mixtures of contaminants, on environmental quality has led to the development of molecular indicators (biomarkers) of exposure to, and effects of, contaminants on organisms. Biochemical biomarkers are increasingly used in ecological risk assessment of the ecosystem to identify the incidence and effects of xenobiotics. This is because of their potential as rapid early warning signal against potentially damaging effects caused by stressor. Ideally, biochemical biomarkers will identify effects at subcellular level before they are apparent at higher levels of biological organization (Olsen *et al*., 2001). Assaying antioxidant enzymes and non enzymes can offer an indication of the antioxidant status of the organisms and can serve as biomarkers of oxidative stress (Livingstone, 2001). Antioxidant defenses, which are generally ubiquitous in animal species and different tissue types, are found widely in organisms. Measurements of their depletion or induction can be used as biomarkers for adverse health effects by xenobiotics (Valavanidis *et al.,* 2006).

2.6 Non Enzymatic ROS Scavengers

2.6.1 Level of lipid peroxidation as indicators of oxidative damage

Lipid peroxidation has been used as bioindicator of oxidative damage in organisms exposed in polluted environmental conditions. Most toxins produce hydroxyl radicals (HO•) which react with membrane lipids (LH). Lipids containing polyunsaturated fatty acids and their esters are oxidized readily by molecular oxygen because of the high susceptibility of bis-allylic hydrogens to oxidation. Such an oxidation, called autooxidation, proceeds by a free radical chain mechanism. This process is divided into three steps, initiation, propagation, and termination (Vichnevetskaia and Roy, 1999). Initiation is a step in the formation of the lipid radical (L·) from a lipid. The carbon-centred lipid radical reacts with oxygen rapidly to give a lipid peroxyl radical (LOO·). The lipid peroxyl radical attacks another lipid molecule and abstracts a hydrogen atom to give a lipid hydroperoxide (LOOH) and another lipid radical and then continues through the propagation sequence. Thus, many molecules of lipids may be oxidized to lipid hydroperoxides for every initiation step. Termination is a bimolecular interaction of lipid peroxyl radicals to give nonradical products, and this step ends the free radical chain oxidation.

Initiation of lipid peroxidation:

LH (polyunsaturated lipid) + $R^{\bullet}$ or $HO^{\bullet}$ $\rightarrow$ $L^{\bullet}$ + RH, or HOH

Propagation of lipid peroxidation:

$L^{\bullet}$ (carbon – centered lipid radical) + O_2 $\rightarrow$ $LOO^{\bullet}$ (lipid peroxyl radical)

LH + $LOO^{\bullet}$ $\rightarrow$ $L^{\bullet}$ + LOOH (lipid hydroperoxide)

Termination of lipid peroxidation is the result of interactions of lipid radicals and/or formation of nonradical species by lipid peroxyl radicals. The resulting LOOH can easily decompose into several reactive species including lipid alkoxyl radicals ($LO^{\bullet}$), aldehydes (e.g., malondialdehyde,

HOC–CH2–CHO), alkanes, lipid epoxides, and alcohols. Most of these products are toxic and active mutagens (Valavanidis *et al.,* 2006). As the metabolic product of lipid peroxidation in organisms, MDA indirectly reflects the degree of intracellular injury. So it is a sensitive parameter by which to judge cellular oxidative injury. It is reported that MDA has a good correlation with ROS generation and it is regarded as an important indicator of the oxidative stress level (Valavanidis *et al*., 2006).

2.6.2 Glutathione (GSH) activity

Glutathione (GSH) is often used in biomarker studies, as it is an overall modulator of cellular homeostasis (Ringwood *et al.*, 1999). Glutathione (GSH) is a low molecular weight scavenger of oxygen radicals (Regoli *et al.*, 1998). The reduced form conjugates with electrophilic xenobiotics transforming them into water soluble and thus easily excretable products (Nusetti *et al.*, 2001). In addition it is a cofactor for GST activity, GSH is used as a conjugating molecule by GST to ease excretion of xenobiotics. It reacts with many oxidants such as H_2O_2 to form the oxidized form, a disulfide known as GSSG:

Hydrogen peroxide H_2O_2 + GSH --------- GSSG + H_2O

Since induction of antioxidants represents a cellular defense mechanism to counteract toxicity of (ROS), they have been extensively used in several field studies to assess the extent of pollution in rivers, lakes and coastal waters (Goksoyr, 1995; Almroth, *et al*., 2008; Kopecka and Pempkowiak, 2008; Howcroft *et al*., 2009). The level of GSH have all been proposed as biomarkers of oxidative stress in organisms (Stephensen *et al*., 2002).

2.7 Enzymatic ROS Scavenging Mechanisms

The removal of xenobiotics, and even some endogenous substances, from the cell is catalyzed by a number of different enzymes, two types of antioxidant enzymes exist: so called phase I and II enzymes. Phase I enzymes are involved in xenobiotic biotransformation via the introduction of a polar moiety which renders a lipophilic contaminant more hydrophilic. Phase II enzymes are involved in conjugating metabolised xenobiotics to endogenous molecules, thereby easing excretion. Phase I enzymes defend directly against ROS such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and phase II enzymes can inactivate the by-products of ROS, such as lipoperoxidation products and lipid hydroperoxide (Doyen *et al*., 2008).

Among the various types of biomarkers that have been used in both the field and laboratory, the following have received special attention in ecotoxicological studies: antioxidant enzymes - superoxide dismutase, catalase, glutathione transferase, lipid peroxidation and histological damage (Valavanidis and Vlachogianni, 2010). Although the activity of antioxidant enzymes may be increased or inhibited under chemical stress, there is, however, no general rule for the different enzymes (Cheung *et al*., 2001). The antioxidant enzymes tend to respond differently to different chemical compounds; therefore, the activity of an individual antioxidant enzyme cannot serve as a general marker of oxidative damage. As a result, multiple antioxidant values are often measured together to indicate the total oxyradical scavenging capacity and this has been observed to provide greater indicating value (Regoli *et al*., 2002).

2.7.1 Glutathione S-transferases (GST) activity

One of the most abundant and ubiquitous detoxification enzyme families is the glutathione-S-transferase family. These enzymes play a pivotal role in inhibiting the cellular damage produced by a wide variety of structurally diverse carcinogens and endogenous toxins (Jaiswal, 1994). The susceptibility of different fish species to chemical carcinogenesis may be modulated by the activity of GST (Van der Oost *et al*., 2003). Glutathione S-transferases are major phase II detoxification enzymes (Valavanidis *et al*., 2006). The conjugation of electrophilic compounds (or phase I metabolites) with GSH is catalyzed by the glutathione S-transferases (GSTs), a multigene superfamily of dimeric, multifunctional, primarily soluble enzymes. A critical role for GSTs is defence against oxidative damage and peroxidative products of DNA and lipids. The efficacy of GST as a biomarker has been investigated in organisms collected from various polluted sites (Filho *et al*., 2001; Fernandes *et al*., 2002; Howcroft *et al*., 2009).

2.7.2 Superoxide dismutase (SOD) activity

The SODs are the group of metalloenzymes that catalyze the conversion of reactive superoxide anions (O_2^{-o}) to yield H_2O_2 which is in itself an important ROS as well. SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Superoxide dismutases are considered to play a pivotal antioxidant role. Superoxide dismutase catalyses the dismutation of the O_2^{-o} to water and hydrogen peroxide, which is detoxified by the CAT activity.

$$2O_2^- \xrightarrow[2H+]{SOD} H_2O_2 + O_2$$

Usually a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants (Dimtrova *et al*., 1994). SOD is called the cell's first line of defense

against ROS because superoxide radical is a precursor to several other highly reactive species so that control over the steady state of superoxide concentration by SOD constitutes an important protective mechanism (Fridovich, 1997). Interestingly, SOD is induced by its own substrate, the superoxide radical and thus activation of cellular SOD may be an indication that the cell is experiencing pollutant induced superoxide radical stress (Fatima and Ahmad, 2005). SOD is inducible in mammals and the level of the enzyme increases with increased need of protection against toxic oxygen radicals (Livingstone, 2001). Recent investigations of changes in antioxidant defenses showed that they can be used as biomarkers of oxidative stress by various pollutants in organisms (Achuba and Osakwe, 2003; Avci *et al*., 2005; Jifa *et al*.,2006; Vlahogianni and Valavanidis, 2007; Vinodhini and Narayanan, 2009).

2.7.3 Catalase activity

Catalase belongs to the cellular antioxidant system that counteracts the toxicity of ROS. Catalases are haem-containing enzymes that facilitate the removal of hydrogen peroxide. As phase 1 enzymes, SOD and CAT directly scavenge ROS, SOD removes O_2^- through the process of dismutation to singlet oxygen (O_2) and H_2O_2 ($2O_2^- + H^+ \rightarrow H_2O_2 + O_2$). H_2O_2 produced by SOD is sequentially reduced to H_2O and O_2 by CAT (Kashiwagi *et al*., 1997). CAT is an oxidoreductase enzyme that breaks down two molecules of H_2O_2 to two molecules of H_2O and O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$), therefore counteracting the toxicity of H_2O_2 (Kashiwagi *et al*., 1997).

$2H_2O_2$ ------CAT--------- $2H_2O + O_2$

Unlike some peroxidises that reduce various lipid peroxides as well as hydrogen peroxide, CATs can only reduce hydrogen peroxide.

2.8 Biomonitoring using Histopathological Biomarkers

Histopathological biomarkers have been identified as definite biological endpoints of historical exposure and thus serve as indicators of environmental stress (Stentiford and Feist, 2005). A wide range of histological alterations in fish have been developed and recommended as biomarkers for monitoring the effects of pollution. For instance, extensive studies in the USA (e.g. National Oceanic and Atmospheric Administration NOAA's National Status and Trends Program) and Europe (e.g. the International Council for the Exploration of the Sea (ICES) and the North Sea Task Force Monitoring Master Plan) have established a causal relationship between fish pathology and levels of pollution in the marine environment (Au, 2004). In these programs, histopathological lesions in fish served as primary indicators of exposure to contaminants, and certain diseases and lesion types have proven to be reliable biological indicators of toxic/carcinogenic effects resulting from such exposure.

2.8.1 Histopathology of liver

The liver is the primary organ for biotransformation of organic xenobiotics, and probably also for the excretion of harmful trace metals, food digestion and storage, and metabolism of sex hormones (Akaishi *et al*., 2004). Livers of fish are sensitive to environmental contaminants because many contaminants tend to accumulate in the liver, making this organ exposed to a much higher levels (several orders of magnitude) than in the environment, or in other organs (Khan, 2003). The US National Marine Fisheries Services has conducted large scale surveys to determine the relationships between toxicopathic lesions in livers and exposure to chemical contaminants. Similar extensive surveys have been carried out in Canada, Europe and Australia to assess the impact of environmental contaminants on fish health (Au, 2004; Stehr *et al*., 2003).

2.8.2 Histopathology of gill

Fish gill is a multifunctional organ responsible for respiration, osmoregulation, acid–base balance and nitrogeneous waste excretion (Rodrigues *et al*., 2010). This organ is sensitive to chemicals in water, since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water (Dede and Kaglo, 2001). Gill histopathological changes are, in general, responsive but non-specific to pollutant exposure. Epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, edema with epithelial lifting, and epithelial desquamation are typical histopathological lesions of gills in response to a wide range of contaminants, including organochlorines, petroleum compounds, organophospates, carbamates, herbicides and heavy metals. The histology of gill and liver are good biomarkers to evaluate the toxicity of hydrocarbons (Brand *et al*., 2001; Khan 2003; Akaishi *et al*., 2004). In general, gill histopathology appears to be a promising biomarker for general environmental contamination.

2.8.3 Gonad

Chronic pollution may lead to a decrease in quality of gametes, thereby impairing reproductive success and posing a significant threat to the sustainability of fish population/community. Oocyte atresia, characterized by degeneration and necrosis of developing ova, occurs in the ovaries of fish species following exposure to xenobiotic compounds (Hinton *et al*., 1992). Oocyte atresia can be employed as a histopathological marker for xenobiotic exposure.

2.9. Ecological Indices as a Tool for Assessing Pollution Status in Water Bodies

Risk assessors and managers examine ecological health using bioindicators. Bioindicators are organisms that are used to monitor the health of the environment. The organisms are monitored for changes that may indicate a problem within their ecosystem. They are used to detect changes in the natural environment, monitor for the presence of pollution and its effect on the ecosystem in which the organism lives. Furthermore, ecological indices are used as quantitative tools in simplifying, through discrete and rigorous methodologies, the attributes and weights of multiple indicators with the intention of providing broader indication of a resource, or the resource attributes, being assessed (Pinto *et al*., 2008). Ecological indices are very useful tools in decision-making processes since they describe the aggregate pressures affecting the ecosysytem, and can evaluate both the state of the ecosystem and the response of managers.

The use of the benthic macroinvertebrates is very useful in biomonitoring because they are relatively sedentary and long-lived and can therefore be effectively used to assess the pollution status of the water body. Benthic macro-invertebrates include crustaceans such as crayfish, molluscs such as clams and snails, aquatic worms and the immature forms of aquatic insects. Several workers have studied the effect of pollutants on the macrobenthic community in the Lagos lagoon (Chukwu, 2002; Bamikole *et al*., 2009; Nkwoji *et al*., 2010; Balogun *et al*., 2011), but very few have investigated the effect of petroleum products on the macrobenthic community in the Lagos lagoon. The presence of *Pachymelania aurita* and *Neritina glabrata* (gastropods) has been observed in stations relatively less impacted by pollution (Balogun *et al*., 2011). Therefore, by examining shifts in the benthic communities' overtime, one can gain understanding of the major environmental events and processes affecting the local biota.

Low values of diversity, low numbers of species and strong dominance of a few species have been found often in lagoonal ecosystems (Reizopoulou and Nicolaidou, 2004). Dominance of polychaetes can be attributed to their high level of pollution tolerance. Polychaetes such as *Capitella capitata, Nereis sp*, *Chironomus sp* and *Polydora sp*. were found associated with sites grossly polluted with organic matter, heavy metals and petroleum hydrocarbons in the Lagos lagoon (Ajao and Fagade, 1990; Saliu and Ekpo, 2006; George *et al*., 2010; Nkwoji *et al*., 2010). Ten taxa belonging to four classes of benthic macroinvertebrates observed in the study by Balogun *et al*., (2011) reveals reduction in species diversity as compared with earlier reports of the Lagos lagoon. Ajao and Fagade (1990) recorded forty two species, Williams (1999) recorded fifteen species and Emmanuel and Ogunwenmo, (2010) reported twenty species in the Lagos lagoon. The very low species diversity is usually a good indicator of a stressed ecosystem (Edokpayi *et al*., 2010; Emmanuel and Ogunwenmo, 2010). Diversity indices in lagoons are often affected by fluctuations of the abundance of the most dominant species. Macrobenthic communities are considered good indicators of ecosystem health due to their strong link with sediments, which at the same time, are linked to water column (Daur *et al*., 2000). Benthic communities respond to improvements in habitat quality in three progressive steps: the abundance increases; species diversity increases; and dominant species change from pollution-tolerant to pollution-sensitive species (Reizopoulou and Nicolaidou, 2004).

2.10 Usefulness of Biomarkers in Environmental Monitoring

The increasing emphasis on the need for a more complete assessment and monitoring of terrestrial and aquatic ecosystems other than the mere chemical monitoring of water, soil and

sediment has highlighted the need to develop useful biomarkers for assessing the health status of ecosystems. The use of biomarkers has become an important tool for modern environmental assessment as they can help to predict the effects of some particular chemicals involved in monitoring programme. By their very nature, biomarkers can be used to predict what may happen when chemicals find their way into a particular environment, or what will happen if exposure continues to occur. The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels. Biomarkers have therefore been proposed as a method of detecting contaminant induced suborganism level stress in the biota before population or community-level responses become apparent (Adams *et al*., 2005). A wide range of biomarkers had been developed and suggested for use in monitoring programmes (van der Oost *et al*., 2003). Oxidative stress biomarkers have been used to monitor the pollution status in several coastal environments (Cajaraville *et al*., 2000; Ahmad *et al*., 2004; Ros and Nesto, 2005; Marigomez *et al*.,2006; Lehtonen and Schiedek, 2006; Kopecka and Pempkowiak, 2008; Banni *et al*.,2009).

Presently, biomarkers are not used in pollution monitoring by regulatory agencies in developing countries (UNEP, 2004). There is an increasing need to develop methods for the identification, estimation, comparative assessment and management of the risks posed by chemical pollutant discharges to the environment and national resources. The implementation of biomarkers of effects in national monitoring programmes in Nigeria is unquestionably an important near-future step. To this end, biomarkers of effects identified in this study will be an important contribution. These biomarkers can be useful tools for decision makers to improve the impact assessment of accidental pollution and they can be used to detect impacts when chemicals can not be measured

or are no longer detectable. The Biomarkers identified in this study have a huge potential for use in monitoring programmes.

Fish species are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution. Fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants (van der Oost *et al*., 2003). They can be found virtually everywhere in the aquatic environment and they play a major ecological role in the aquatic food-webs because of their function as a carrier of energy from lower to higher trophic levels. The understanding of toxicant uptake, behaviour and responses in fish may, therefore, have a high ecological relevance. Between different fish species, however, considerable variation in both the basic physiological features and the responsiveness of certain biomarkers towards environmental pollution may become apparent. Fish species are chosen as test organisms because they are more sensitive to exposure and toxicity compared to terrestrial organisms. Despite their limitations, such as a relatively high mobility, fish are generally considered to be the most feasible organisms for pollution monitoring in aquatic systems.

The *Eudrilus eugeniae* (earthworm) is an important organism at the bottom of the terrestrial trophic food chain, and it plays a key role in ecotoxicological tests as a sensitive biomonitor of soil pollution. They have been broadly used to assess environmental impact from pollution, and they are typical test organisms in standardized toxicity tests (Sanchez-Hernandez, 2006). Earthworms are common in a wide range of soils and may represent 60±80% of the total soil biomass (Saint-Dennis *et al*., 2001). This makes them one of the most suitable bioindicator

organisms for testing chemicals in soils. Earthworms have been used to assess soil health through both acute and sub-lethal ecotoxicity tests. In recent years the use of other biological responses (biomarkers) in earthworms, to estimate either exposure or resultant effects of chemicals has received increased attention. Reports have shown that pollutants can induce an increase in reactive oxygen species (ROS) in earthworms (Saint-Denis *et al*., 2001; Xiao *et al*., 2006). Induction or inhibition of the antioxidant enzymatic system is considered valid biomarkers of environmental pollution in earthworm toxicology (Song *et al*., 2009). Histopathological responses in earthworms have also been reported as valuable markers of toxicity (Kilic, 2011; Bansiwal and Rai, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 FIELD STUDIES - AQUATIC ECOSYSTEM

3.1.1 Description of study site (Lagos lagoon)

The Lagos Lagoon stretches from Cotonou in the Republic of Benin and extends to the fringes of the Niger Delta in Nigeria along its 257 km course (Webb, 1958). The sampling stations in the Lagos Lagoon were located along Atlas Cove, Apapa, Iddo with Unilag as the reference station (Fig 3.1). The main body of the Lagoon lies between longitude 3^0 22' and 3^0 40' East and latitudes 6^0 17' and 6^0 28' N. It is an expanse of shallow water, which in most areas is between 0.5-3.2 m deep with a maximum of about 5m deep in the main body of the lagoon and about 25m in some dredged parts of the Lagos harbor.

The water is brackish especially the tidal reaches surrounding an inlet from the sea. The interconnecting creeks are very shallow and are sites of active siltation and deposition of mud. The Lagos lagoon extends from the coast of Lagos to about 37 km north at Ikorodu and about 48 km east where it narrows and continues as the Lekki lagoon. It envelopes a chain of islands to the west, on which the city of Lagos spanning the Lagos Island, Ikoyi, Maroko and Victoria Island is built. On the northern and southern shores, the lagoon is bounded by low-lying marshy areas, often thickly-clad mangrove swamps, behind which is the rainforest (Ajao and Fagade, 1990).

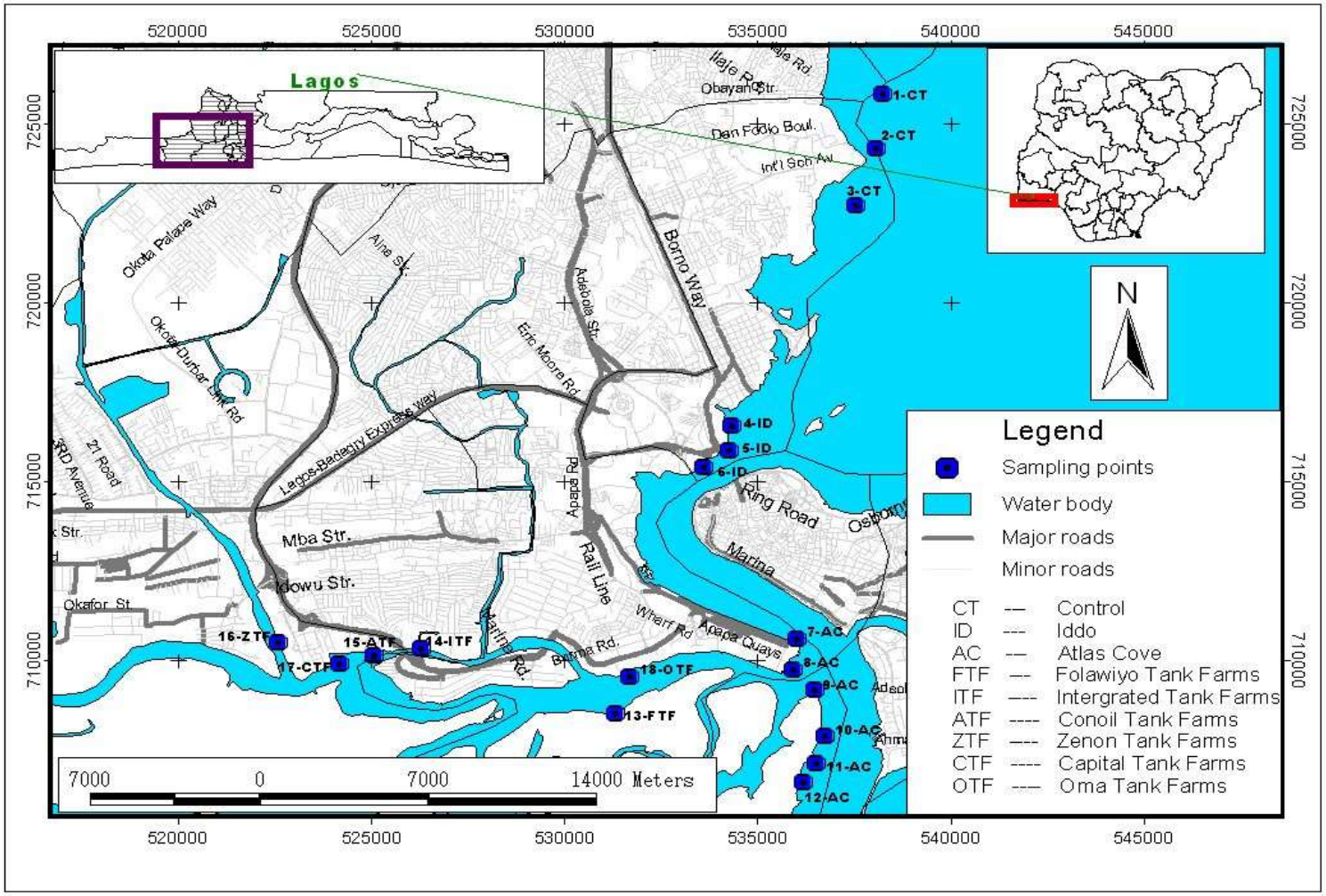


Fig 3.1: Map of a section of the Lagos lagoon showing the sample stations.

3.1.2 Sampling design

A total of 18 sampling stations were chosen for this study (Fig 3.1). The sampling locations in the Lagos lagoon were chosen due to the petroleum industry activities, specifically samples were taken around tank farms (Conoil, Zenon, Folawiyo, Capital tank farms) in Apapa and Atlas cove. The positions of the sampling stations within the lagoon were accurately located using the GPS as shown in Table 3.1. Accurate positioning was further facilitated by careful observations of permanent and semi permanent structures in the Lagoon.

3.1.3 Sampling operations

Sampling operations at the designated stations (Fig 3.1) were carried out on an open motorized research vessel. Timing of sampling was dictated by the two hydrological seasons (wet and dry) prevalent in the tropics that also bring about significant differences in the physico-chemical conditions of bodies of water (Otitoloju, 2000). The sampling regime covered two dry and two wet season samplings. Fish samples were collected for biochemical analysis, Benthic animals were collected for analysis and samples of water and sediment were taken over a two – year period and investigated for total hydrocarbon content and BTEX compounds, following the schedule given below:

2009 Dry season sampling- 14th Feb. to 18th Feb.

2009 Wet season sampling- 11th July to 14th July .

2010 Dry season sampling- 14th Feb. to 18th Feb.

2010 Wet season sampling- 11th July to 14th July .

Table 3.1: Geographical Positioning System (GPS) reading of the sampling stations

Sampling stations	Station code	GPS	
Unilag (Reference)	L1, SD1	N06 ^{o} 31'. 28.2"	E003^{o} 24'.29.3"
Unilag (Reference)	L2, SD2	N06 ^{o} 30'. 56.8"	E003^{o} 24'.25.6"
Unilag (Reference)	L3, SD3	N06 ^{o} 30'. 24.6"	E003^{o} 24'.15.2"
Iddo	L4, SD4	N06 ^{o} 28'. 13.0"	E003^{o} 23'.05.1"
Iddo	L5, SD5	N06 ^{o} 28'. 07.7"	E003^{o} 22'.01.6"
Iddo	L6, SD6	N06 ^{o} 28'. 02.8"	E003^{o} 22'.54.1"
Atlas Cove	L7, SD7	N06 ^{o} 25'. 49.0"	E003^{o} 24'.52.5"
Atlas Cove	L8, SD8	N06 ^{o} 25'. 39.1"	E003^{o} 23'.52.9"
Atlas Cove	L9, SD9	N06 ^{o} 25'. 36.8"	E003^{o} 23'.54.7"
Atlas Cove	L10, SD10	N06 ^{o} 25'.24.9"	E003^{o} 24'.54.4"
Atlas cove	L11, SD11	N06 ^{o} 25'. 13.7"	E003^{o} 23'.50.2"
Atlas cove	L12, SD12	N06 ^{o} 24'. 57.4"	E003^{o} 23'.47.4"
Apapa: Folawiyo Tank farm	L13, SD13	N06 ^{o} 25'. 25.2"	E003^{o} 23'.54.6"
Apapa: Integrated oil	L14, SD14	N06 ^{o} 26'. 15.3"	E003^{o} 24'.06.8"
Apapa: Conoil	L15, SD15	N06 ^{o} 26'. 15.2"	E003^{o} 19'.59.4"
Apapa: Zenon Oil	L16, SD16	N06 ^{o} 26'. 15.6"	E003^{o} 19'.15.8"
Apapa: Capital Oil	L17, SD17	N06 ^{o} 26'. 14.1"	E003^{o} 19'.40.4"
Apapa: Oma oil	L18, SD18	N06 ^{o} 26'. 03.2"	E003^{o} 19'.14.5"

3.1.4 Collection of Surface water samples

Surface water samples from the experimental stations were collected by dipping glass containers about 6-10cm below the surface film of the water body. Samples were taken to the laboratory for hydrocarbon analysis.

3.1.5 Measurements of Physico-Chemical parameters

Salinity was determined *in situ* using a Beckman Electrodeless Salinometer (model RS 5-3). Dissolved oxygen (DO) measurements were determined *in situ* a digital DO meter (Jenway Product model 3000), pH was determined using a pH meter (Kahl Scientific Instrument model II 4W13) calibrated with freshly prepared buffer solutions (pH 4, 7 and 9). TDS and conductivity was also measured insitu.

3.1.6 Collection of Sediment

Sediment samples from the experimental stations were collected with the aid of a stainless steel grab of the Van –Veen type ($0.1m^2$). The sediment samples each were put into clean aluminum foil and placed in polythene bags, stored in the deep freezer while awaiting analysis in the laboratory. Details of sediment particle size analysis in Appendix 7**.**

3.1.7 Collection of fish

Tilapia zillii (Gervais, 1848) and *Chrysichthys nigrodigitatus* (Lacapede, 1803) were caught from the Lagos Lagoon. The fish species were collected with the assistance of fishermen using a fish cast net (31mm – mesh size) which was used to form a complete fence, the debris within was removed and gradually the net was drawn together to entrap the fishes caught. The fishes

were transported live from their natural environment to the laboratory. The fishes were dissected and the liver, gills and gonads were quickly removed for biochemical analysis.

3.1.8 Collection of Macrobenthic invertebrates

Three grab hauls were taken from each station using $0.1m^2$ Van Veen grab from an anchored boat. The collected materials were washed through a 0.5mm mesh sieve in the field. The residue in the sieve was preserved in 10 % formalin solution and kept in labeled plastic containers for onward transportation to the laboratory.

3.1.8.1 Macrobenthic Invertebrate Analysis

3.1.8.1.1 Sorting

Preserved benthic samples were washed with tap water to remove the preservative and any remaining sediment. Smaller bits of sample were collected and put into petri dish and little amount of water added to separate clump of materials and specimens. The petri dish was then placed on a white tray and animals sorted with the aid of hand lens and a dissecting microscope.

3.1.8.1.2 Identification

The animals were identified and classified into different taxonomic groups (Phyla, class, families, species) using suitable identification manuals: Buchanan (1954); Edmunds (1978); Barnes *et al.*, (1988); Yankson and Kendall (2001). The number of species and individuals for each station were counted and recorded. Macrobenthic community structure was estimated using the Margalef's species richness index, Shannon-Weiner index and Equitability index.

3.2 FIELD STUDIES - TERRESTRIAL ECOSYSTEM

3.2.1 Description of Study Site (Ijegun)

Ijegun is located in the suburb area of Alimosho LGA, Lagos Nigeria with a population of close to a million people. It is bordered by Ijagemo and Ijedodo in the south, Isheri-Osun, Oke-rube in the west and Abaranje in the east of Alimosho Local Government. It lies between longitude 3^0 4.0' East and latitude 6^0 28.8' and 6^0 8.0' North (Fig 3.2).

3.2.2 Sampling design

The impacted area around the pipeline Right of Way (ROW) was divided into 9 buffer stations ranging from 50m to beyond 500m away from the pipeline (Fig 3.2). Within the buffer zones, a total of 20 sampling stations were chosen. Groundwater and soil samples were taken from the following streets around the impacted area in Ijegun: Kudeyibu street; Isolo road, Awolumate street, Ayinde street, Ajala road, Church street, Pipeline road, Ijedodo road and Ijagemo road.

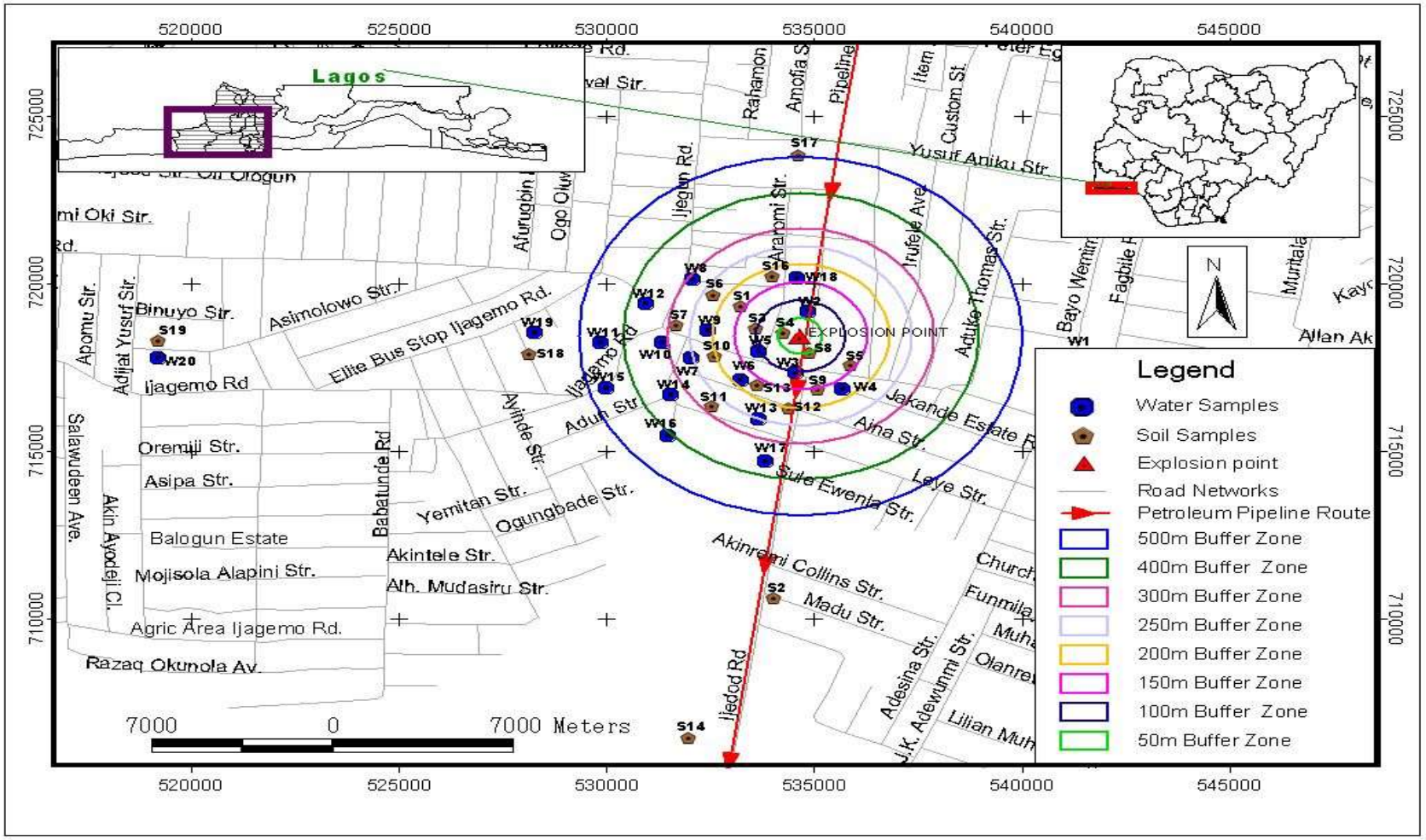


Fig 3.2: Map of Oil explosion Impacted area of Ijegun, Lagos State, showing the sampled stations.

The 20 sampled stations at Ijegun were divided into buffer zones away from the point of explosion in an attempt to establish a pollution gradient as shown in Table 3.2. The positions of the sampling stations were accurately located using the GPS as shown in Table 3.3.

3.2.3 Sampling operations

The pipeline explosion point was identified and sampling was done away from the epicenter of the spill at Ijegun (Fig 3.2). The sampling station at Ijagemo served as the reference station (Fig 3.2). Samples of contaminated groundwater, soil and earthworm species were taken over a two – year period (4 seasons) and investigated for total hydrocarbon content and BTEX compounds following the schedule given below:

Dry season sampling- 1st March to 6th March 2009;

Wet season sampling- 15th July to 20th July 2009;

Dry season sampling- 1st March to 6th March 2010;

Wet season sampling- 15th July to 20th July 2010.

Table 3.2: Sampling stations divided into buffer zones

BUFFER ZONES	SAMPLING STATIONS
50m Buffer zone	S4, S8
100m buffer zone	S3, W2, W3, W5
150m buffer zone	S5, S9, S1, W18
200m buffer zone	S12, S16, S10, S13,W9, W6, W4
250m buffer zone	S6, W7, W13
300m buffer zone	S7, S11, W8, W10
400m buffer zone	W14, W12, W16, W17
500m buffer zone	S17, W11, W15
Beyond 500m buffer zone	S19, S2, S14, S15, S18, W1
Control	W19, W20

Where W is groundwater and S is soil.

Table 3.3: Global Positioning System (GPS) reading of the sampling stations

Code	GPS		Code	GPS	
W1	N06 ^{o} 31'. 59.6"	E003^{o} 16'.59.6"	**S1**	N06 ^{o} 31'. 67.1"	E003^{o} 15'.96.3"
W2	N06 ^{o} 31'. 67.5"	E003^{o} 15'.88.5"	**S2**	N06 ^{o} 30'. 98.5"	E003^{o} 15'.98.5"
W3	N06 ^{o} 31'. 51.3"	E003^{o} 15'.98.0"	**S3**	N06 ^{o} 31'. 63.3"	E003^{o} 15'.95.6"
W4	N06 ^{o} 31'. 51.6"	E003^{o} 16'.06.3"	**S4**	N06 ^{o} 31'. 61.0"	E003^{o} 15'.97.3"
W5	N06 ^{o} 31'.56.6"	E003^{o} 15'.92.3"	**S5**	N06 ^{o} 31'.60.6"	E003^{o} 16'.07.0"
W6	N06 ^{o} 31'. 53.3"	E003^{o} 15'.91.6"	**S6**	N06 ^{o} 31'. 68.5"	E003^{o} 15'.86.5"
W7	N06 ^{o} 31'. 58.8"	E003^{o} 15'.81.6"	**S7**	N06 ^{o} 31'. 67.3"	E003^{o} 15'.78.6"
W8	N06 ^{o} 31'. 61.0"	E003^{o} 15'.79.6"	**S8**	N06 ^{o} 31'. 60.0"	E003^{o} 15'.96.6"
W9	N06 ^{o} 31'. 60.6"	E003^{o} 15'.78.3"	**S9**	N06 ^{o} 31'. 51.0"	E003^{o} 16'.07.3"
W10	N06 ^{o} 31'. 62.8"	E003^{o} 15'.75.8"	**S10**	N06 ^{o} 31'. 59.5"	E003^{o} 15'.86.3"
W11	N06 ^{o} 31'. 64.5"	E003^{o} 15'.71.6"	**S11**	N06 ^{o} 31'. 50.1"	E003^{o} 15'.88.3"
W12	N06 ^{o} 31'. 65.8"	E003^{o} 15'.72.8"	**S12**	N06 ^{o} 31'. 49.8"	E003^{o} 15'.89.6"
W13	N06 ^{o} 31'. 53.6"	E003^{o} 15'.82.5"	**S13**	N06 ^{o} 31'. 52.5"	E003^{o} 15'.91.8"
W14	N06 ^{o} 31'. 55.6"	E003^{o} 15'.80.1"	**S14**	N06 ^{o} 30'. 63.3"	E003^{o} 15'.81.0"
W15	N06 ^{o} 31'. 55.6"	E003^{o} 15'.79.6"	**S15**	N06 ^{o} 30'. 11.0"	E003^{o} 15'.736"
W16	N06 ^{o} 31'. 45.3"	E003^{o} 15'.84.5"	**S16**	N06 ^{o} 31'. 79.5"	E003^{o} 15'.98.3"
W17	N06 ^{o} 31'. 42.0"	E003^{o} 15'.91.1"	**S17**	N06 ^{o} 32'. 10.1"	E003^{o} 16'.03.5"
W18	N06 ^{o} 31'. 79.0"	E003^{o} 16'.03.3"	**S18**	N06 ^{o} 31'. 60.0"	E003^{o} 15'.48.8"
W19	N06 ^{o} 31'. 59.1"	E003^{o} 15'.52.3"	**S19**	N06 ^{o} 31'. 61.0"	E003^{o} 14'.72.3"
W20	N06 ^{o} 31'. 59.0"	E003^{o} 14'.73.1"			

Where W is groundwater and S is soil.

3.2.4 Collection of Soil

Nineteen (19) Soil samples were collected around the impacted area of Ijegun and Ijagemo reference station (S 19; 1.5km from Ijegun) in replicates using a soil auger. The auger was plunged into the ground and the handle turned to collect soil at 0-15cm. The latter step was repeated to collect soil from 15-30cm. Both depths were composited into one sample. The soil samples collected were packed, labeled and preserved for onward transmission to the laboratory, Department of Zoology, University of Lagos for subsequent analysis within 24hours.

3.2.5 Collection of Ground water

Twenty (20) Groundwater samples were collected in replicates from 20 sampling locations around the impacted area of Ijegun and Ijagemo reference station (W20; 1.5km from Ijegun). Existing water wells and boreholes were sampled, kept and preserved for analysis in the laboratory. The same wells and boreholes were sampled continuously over a period of two years covering two dry and two wet seasons. Samples were collected in 1.5litre plastic bottles for heavy metals and amber bottles for THC and glass vial with Teflon-lined septum for BTEX, after rinsing with the water being sampled and were properly sealed. Samples were properly labeled and stored in insulated coolers containing ice cubes at 2-6^{0}C and were transferred to the laboratory for analysis within 24 hours. Sampling, preservation and transportation of water samples were carried out under standard method (APHA, 1998).

3.2.6 Collection of earthworms

Earthworms, *Eudrilus eugeniae* (Kinberg, 1867) were collected around the oil spill impacted sites in Ijegun and from the Unilag garden; they were transported live to the laboratory for further biochemical and histopathological analysis.

3.2.7 Physico-Chemical Analysis of Samples

All the samples were analyzed for relevant physico-chemical parameters according to internationally accepted procedures and standard methods (APHA, 1998). The parameters analyzed in the groundwater samples include pH, conductivity, total dissolved solids (TDS), zinc (Zn), nickel (Ni), and lead (Pb). The concentrations of heavy metals were determined using an atomic absorption spectrophotometer. pH was determined on-site using a pH meter (Kahl Scientific Instrument model II 4W13) calibrated with freshly prepared buffer solutions (pH 4, 7 and 9).

3.2.7.1 Determination of Heavy metals

The heavy metal in the groundwater samples were determined by Varian SpectrAA 400 Plus Atomic Absorption Spectrophotometer (AAS) method. Samples are presented to the AAS for reading by inserting the Aspirator into the 100mL sample plastic containing the digestate placed on the sample compartment in front of the machine. Upon presentation of samples, the samples get aspirated (sucked) into the Flame through the capillary into nebulizer, gets atomized in the flame, impinged upon by the optimized ray from the Hollow Cathode Lamp (HCL), amplified within the AAS compartment and detected by the Detector within the compartment. The values are read out on the Monitor Display attached to the AAS.

3.2.7.2 THC and BTEX Analysis of Samples using Standard Methods ASTM D 2887-93 and US EPA Method 1664

Analysis was done for groundwater and soil samples collected from Ijegun, surface water and sediment samples collected from the Lagos lagoon. Preparation (extraction) of samples for Gas Chromatography GC. A known quantity (100ml) of the sample (water) was transferred to a 250ml separatory funnel and a 50ml (70:30) mixture of hexane: dichloromethane (DCM) was added for the extraction process. The separatory funnel was sealed with glass stopper and shaken vigorously for one to two minutes with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of five minutes. The combined extracts were concentrated to about 10ml and using a rotatory evaporator. Solvent exchange to n-hexane was carried out on the extract by adding 10ml of hexane in a round-bottomed flask. For soil and sediment samples, 20g was extracted using 50ml (70:30) mixture of hexane: dichloromethane (DCM) for 48Hours after which it was cleaned up the same way for water before analysis using Gas Chromatography GC. Samples were analyzed on Agilent 4890D Gas Chromatograph/Flame Ionization Detector (GC/FID) already calibrated. The samples for BTEX analysis were lowered into a headspace sampling vial, sealed and transferred to the headspace sampler for analysis

3.2.8 Quality assurance

Appropriate quality assurance procedures and precautions were taken to ensure the reliability of the results. Samples were carefully handled to avoid contamination. Appropriate sample preservation/labeling were ensured. Glasswares were properly cleaned, and reagents were of analytical grades. Acid digestion was carried out for heavy metal analysis. Deionized water was

used throughout the study. Reagent blank determinations were used to correct the instrument readings and repeated calibration of analytical equipment was done.

3.3 LABORATORY STUDIES

3.3.1 Test chemicals

Benzene: Puriss. P.a., ≥99.7% (GC) was obtained from Sigma Aldrich.

Toluene: Laboratory Reagent, ≥99.3% (GC) was obtained from Sigma Aldrich.

Xylene: Puriss. P.a., ≥99.0% (GC) was obtained from Sigma Aldrich.

Ethylbenzene: Laboratory Reagent was obtained from Sigma Aldrich.

The BTEX compounds were chosen because they are the toxic and major components of petroleuem products and also due to the carcinogen benzene. The interest in the monocyclic aromatic compounds is also because of the frequent spillage of petroleum products especially gasoline in waterbodies and terrestrial ecosystems in Nigeria. This frequent spillage will release these toxic chemicals into the environment.

3.3.2 Test Animals (Sources and Collection)

African catfish, *Clarias gariepinus* (Burchell, 1822) is of great commercial importance because it is the most common fresh water fish widely consumed in Nigeria. It can therefore be a good model to study responses to various environmental contaminants. The catfish were obtained from the fish farm at Nigerian Institute for Oceanography and Marine Research (NIOMR). The earthworms *Eudrilus eugeniae* (Kinberg, 1867) were collected from the Botanical and Zoological garden in the University of Lagos. All the test animals were collected from NIOMR and Unilag garden for the following reasons:

1) The areas are devoid of any industrial or facilities that could cause pollution and thus affect the biochemical responses of the control samples. Therefore, the animal stocks are shielded from the full impact of pollutants.

2) The fish in the farm were of known stock history. It is often preferred to use animals in culture for ecotoxicological bioassays, since those from the wild where the medium is contaminated may have acquired increased tolerance to pollutants over years of exposure to sublethal concentrations (Otitoloju, 2000).

(a) ***Clarias gariepinus*** **(**Class: Actinopterygii, Order: Siluriformes, Family: Clariidae)

The fishes collected were of similar sizes. The fish were collected in the morning between 8.00am and 10.00am and transported to the laboratory in aerated bags containing water from the pond from which they were collected. The animals were kept in holding glass tanks. Dechlorinated tap water was used in stocking the test organisms. This was achieved by storing water in an open barrel for 72 hours and then aerating the water by the use of cosmos aquarium pump for another two days. The juveniles (200 to 300 g- body weight) and fingerlings (1.5 to 2.0 g) were kept in separate square holding tanks in which they were allowed to acclimalize to laboratory conditions. During the acclimatization, the fishes were fed with NIOMR fish feed. The fish were acclimatized in the laboratory conditions for two weeks. Unconsumed feed and faecal were removed and water replenished regularly. Juveniles of *Clarias gariepinus* were used for toxicity tests due to the more sensitive nature of juveniles than adult for toxicity test (Solbe, 1995; Odiete, 1999; Reish and Oshida, 1987).

(b) ***Eudrilus eugeniae*** (Class: Clitellata, Order: Haplotaxida, Family: Eudrilidae)

Sexually mature earthworms as determined by the presence of the clitellum were collected from the Zoological and Botanical garden. Most of the earthworms (0.9-1.2g – body weight) were collected from under leaf litter and small logs in the early hours of the day (usually by 8am) by digging and handsorting. The earthworms were collected from the same site in order to reduce variability in biotype. Collected specimens were identified according to Segun (1998). Samples were taken immediately with good quantity of soil from its habitat to the laboratory for acclimatisation. In the laboratory, the earthworms were kept in glass containers (volume five litres) which was half filled with a mixture of loamy and humus soil supplemented with half-boiled, ground water-leaf (*Talinum triangulare)* and moistened with deoxygenated tap water after Fafioye and Owa, (2000). The glass containers were adequately covered with plastic lid with ventilation holes (OECD, 1984). The earthworms were kept in the containers for a minimum of ten days in order to allow them adapt to experimental conditions. During the period of acclimatization, the animals were fed with leaflets of lettuce *Nymphea lotus* every four days after Otitoloju (2005). Earthworms of similar sizes ranging from 80mm to 120mm- length and 0.9-1.2g body weight were carefully selected from the holding containers into pre-experimental containers from where they were randomly transferred to the bioassay containers.

3.3.3 Acute toxicity studies

(a) Fish

Rectangular glass tanks (Vol. 4.5 liters; 15cm×15cm×15cm) were used as bioassay containers for both fish and earthworms. In all bioassays for fish, test media was made up to two liters of water to hold 12 fingerlings per bioassay container, 3 replicates. Preliminary screening was

carried out to determine the appropriate concentration for testing chemical as described by Solbe (1995).

Twelve active fingerlings of similar sizes and age from pre-bioassay holding tank caught with a net and randomly distributed into bioassay tanks already holding aromatic hydrocarbon (BTEX) and untreated control medium. Each treatment had 3 replicates.

(a) Benzene against *Clarias gariepinus* fingerlings at 0.65, 1.0, 1.8, 3.24, 5.83, 10.49 ml/l and untreated control.

(b) Toluene against *Clarias gariepinus* fingerlings at 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 ml/l and untreated control.

(c) Ethylbenzene against *Clarias gariepinus* fingerlings at 0.2, 0.3, 0.5, 0.65, 0.85, 1.0 ml and untreated control.

(d) Xylene against *Clarias gariepinus* fingerlings at 0.1, 0.3, 0.75, 1.0, 1.5, 1.8 ml/l and untreated control.

Mortality assessment were carried out once every 24-hour over a 96-hour period.

(b) Earthworms

In the bioassay for the earthworms, test media were made up of 1000g of soil to hold 10 earthworms per bioassay container in 3 replicates. Active *Eudrilus eugeniae* of similar sizes from pre-bioassay holding tank were randomly distributed into bioassay tanks already holding Benzene, Toluene, Ethylbenzene and Xylene each and untreated control medium. Each treatment had 3 replicates.

(a) Benzene against *Eudrilus eugeniae* at 0.5, 1.0, 2.0, 4.0 mg and untreated control.

(b) Toluene against *Eudrilus eugeniae* at 0.3, 0.5, 1.0, 2.0 mg and untreated control.

(c) Ethylbenzene against *Eudrilus eugeniae* at 0.3, 1.0, 1.50, 2.0 mg and untreated control.

(d) Xylene against *Eudrilus eugeniae* at 0.3, 0.5, 1.0, 2.0 mg and untreated control.

Mortality assessment were carried out once every 24-hour over a 7 day period.

Preparation of substrate

A mixture of loamy and humus soil collected from the zoological and botanical garden in the University of Lagos was used as substrate. The soil was air-dried, ground and sifted through a 0.25mm (mesh size) screen in order to standardize the grain size. A 1000g portion of the prepared soil moistened with dechlorinated tap water was used as substrate in bioassays. Preliminary screening was carried out to determine the appropriate concentration for testing chemical. Pre-determined amounts of test chemicals were measured out using micropipette into bioassay containers already containing soil acting as substrate. The substrate and test chemical was properly mixed by shaking vigorously to ensure uniform distribution of the chemical.

3.3.3.1 Assessment of quantal response (mortality)

Animals were taken to be dead when no body movements were observed even when prodded with a glass rod during an observation period of fifteen minutes in a petri dish.

3.3.3.2 Measurements of Physico – Chemical Parameters of Test Media (Water)

The physico – chemical parameters of test media were measured at least 2 times (at the beginning and end for the various bioassays with the aid of digital read ouy instruments, Jenway products model 3000 series of pH meter and DO meter.

3.3.3.3 Measurements of Physico-Chemical Parameters of Test Media (Soil)

The total organic content of soil samples was determined by furnace method. The samples were oven- dried at 105^0C and allowed to cool in dessicators. A known mass (5.0g) of the oven dried soil samples were placed in a clean , dry and weighed porcelain crucible and placed in a muffle furnace and heated at 550-560^0C for 6-8 h, after which the crucible and the content were allowed to cool in a dessicator and reweighed. The loss of weight after ignition in the furnace was calculated and the percentage combustible material (total organic content) was estimated as:

$$\% \text{ Total Organic Content} = \frac{\text{Loss in weight on ignition}}{\text{Initial weight of soil before ignition}}$$

3.3.4 Chronic Toxicity Studies.

(a) Clarias gariepinus

Rectangular glass tanks (58cm by 39cm by 37cm) were used as bioassay containers. In all bioassays, test media was made up to 20 litres of water to hold five juvenile fish per bioassay containers in three replicates. Physic-chemical parameters of the test media (water) were measured. This series of bioassays went on for 60 days in order to investigate the sublethal effect of the hydrocarbons.

3.3.4.1 Semi – Static Bioassay Technique

The semi static bioassay procedure was adopted to avoid drastic changes in concentration of test media via evaporation, prevent excessive reduction in dissolved oxygen and to prevent excessive accumulation of toxic wastes. In this semi-static procedure each test media was changed into a fresh solution at exactly the same concentration of each hydrocarbons (BTEX), once every 4

days, transferring the same exposed test animals into the freshly prepared test media over the 60 day period of experimentation.

3.3.4.2 Sublethal concentrations

Sublethal concentrations of test hydrocarbons were employed, extrapolated as fractions (1/10th, 1/100th) of the 96hr LC_{50} concentrations obtained in test conducted as follows:

(a) Benzene against juvenile *Clarias gariepinus* at:

0.666ml × 1/100 = 0.006ml/l

0.666ml × 1/10 = 0.066ml/l

(b) Toluene against juvenile *Clarias gariepinus* at:

1.190 × 1/10 = 0.119ml/l

1.190 ×1/100 = 0.0119ml/l

(c) Ethylbenzene against juvenile *Clarias gariepinus* at :

0.479ml/l × 1/10 = 0.0479ml/l

0.479ml × 1/100 = 0.00479ml/l

(d) Xylene against juvenile *Clarias gariepinus* at :

0.519ml/l × 1/10 = 0.0519ml/l

0.519 × 1/100 = 0.00519ml/l

Five juvenile fish having average weight of 250g were exposed to each sublethal concentration and untreated control. There were 2 replicates per treatment meaning that 10 juvenile *Clarias sp* were exposed per treatment.

(b) Earthworm

Rectangular glass tanks (15cm×15cm×15cm) were used as bioassay containers, test media was made up of 1000g of soil substrate to hold twelve earthworms per bioassay container in three replicates. This bioassay test was carried out for 28 days according to standard procedure (OECD, 1984). Sublethal concentrations of test hydrocarbons were employed, extrapolated as fractions ($1/10^{th}$, $1/100^{th}$) of the LC_{50} concentrations obtained in test conducted as follows:

(a) Benzene against *Eudrilus eugeniae* at:

1.9 ml × 1/10 = 0.19mg/kg

1.9 ml × 1/100 = 0.019mg/kg

(b) Toluene against *Eudrilus eugeniae* at:

1.3 ml × 1/10 = 0.13mg/kg

1.3 ml × 1/100 = 0.013mg/kg

(c) Ethylbenzene against *Eudrilus eugeniae* at :

1.4ml × 1/10 = 0.14mg/kg

1.4ml × 1/100 = 0.014mg/kg

(d) Xylene against *Eudrilus eugeniae* at :

1.2ml × 1/10 = 0.12mg/kg

1.2ml × 1/100 = 0.012mg/kg

3.3.4.3 Collection of samples

(a) *Clarias gariepinus*

At pre-determined time intervals (day 0, 15, 30, 45 and 60), five live animals were randomly selected. The fish were dissected and the organs (liver, gonad and gills) were carefully removed.

(b) *Eudrilus eugeniae*

At pre-determined time intervals (day 0, 2, 7, 15, 21, 28), five live earthworms were randomly selected for further analysis.

3.3.4.4 Preparation of Homogenate

The earthworms and the organs of the fishes were quickly removed and was prepared as follows: They were washed in ice-cold 1.15%, KCl solution blotted and weighed. They were then homogenized in 4 volumes of homogenizing buffer (50mM Tris- HCl mixed with 1.15% KCl and pH adjusted to 7.4), using Teflon Homogenizer. The resulting homogenate was centrifuged at 10000 rpm for 20 min in a Beckman L5-50B centrifuge at 0-4^{0}C. The supernatant was decanted and stored at -20^{0}C until analysis.

3.3.4.5 Total Protein Estimation

The protein content of the various fractions was estimated by the method of Lowry *et al*., (1951) using Bovine Serum Albumin (BSA) as standard. 1 ml of the supernatant, 1ml of blank contaning distilled water and 1ml of the standard (BSA) were put into three test tubes labelled a, b and c respectively. Buiret reagent (4ml) was added to the test tubes containing the supernatant, blank and BSA. Absorbance was read at 540nm.

3.3.4.6. Measurement of Antioxidant Enzymes and Non Enzymes

The following antioxidant enzymes studies were carried out on:

a) *Clarias gariepinus* and *Eudrilus eugeniae* exposed to sublethal concentrations of BTEX in the laboratory

b) *Tilapia zilli*, *Chrysichthys nigrodigitatus* collected from the Lagos lagoon and *Eudrilus eugeniae* collected from Ijegun.

3.3.4.6.1 Superoxide Dismutase (SOD) Enzyme Assay

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml, 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. molar extinction for SOD at 480nm is 4020 $m^{-1}cm^{-1}$

$$\frac{\Delta A \times V_T \times 10^6}{\sum \times V_S \times \text{mg protein}}$$

ΔA = Change in absorbance

V_T = Total volume (volume of sample + reagent).

V_S = Volume of sample alone

$\sum$ = Molar extinction

3.3.4.6.2Catalase Enzyme Assay

This was determined adopting the methods of Sun and Zigma (1978). Hydrogen peroxide was prepared with phosphate buffer; 0.2 ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H_20_2) substrate in a 2 ml curvette. The phosphate buffers were used as a blank. The

absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30s interval for 1 min. The enzyme activity was calculated using the molar extinction coefficient of 40 $m^{-1}cm^{-1}$.

$$\frac{\Delta A \times V_T \times 10^6}{\sum \times V_S \times \text{mg protein}}$$

3.3.4.6.3 Determination of Glutathione S- transferase (GST)

Glutathione S- transferase (GST) activity was determined by the method of Habig *et al*.,(1974) using 1 chloro 2,4 dinitrobenzene as substrate. The reaction mixture (3ml) contained 1.7ml of 100mM phosphate buffer (pH 6.5), 0.1ml of 30 mM CDNB. After pre-incubating the reaction mixture at 37^0C for 5 min, the reaction was started by the addition of 0.1 ml diluted sample and the absorbance was followed for 5 min at 340 nm. Reaction mixture without the enzyme was used as blank. The specific activity of glutathione S-transferase is expressed as nmoles of GSH-CDNB conjugate formed /min/ mg protein using an extinction coefficient of 9.6$mM^{-1}cm^{-1}$.

$$\frac{\Delta A \times V_T \times 10^6}{\sum \times V_S \times \text{mg protein}}$$

The following antioxidant non enzymes studies were carried out on:

a) *Clarias gariepinus* and *Eudrilus eugeniae* exposed to sublethal concentrations of BTEX in in the laboratory.
b) *Tilapia zilli, Chrysichthys nigrodigitatus* collected from the Lagos lagoon and *Eudrilus eugeniae* collected from Ijegun.

3.3.4.6.4 Malondialdehyde (Lipid Peroxidation).

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100^{o}C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^{5}\ m^{-1}cm^{-1}$.

$$\frac{A \times V_T \times 10^6}{\sum \times V_S \times \text{mg protein}}$$

A = Absorbance

V_T = Total volume (volume of sample + reagent)

V_S = Volume of sample alone

$\sum$ = Molar extinction

3.3.4.6.5 Glutathione (GSH)

The reduced glutathione (GSH) content of liver tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm. Molar extinction coefficient for GSH is $1.34 \times 10^{4}\ m^{-1}cm^{-1}$.

$$\frac{A \times V_T \times 10^6}{\sum \times V_S \times \text{mg protein}}$$

A = Absorbance

V_T = Total volume (volume of sample + reagent)

V_S = Volume of sample alone

$\sum$ = Molar extinction

3.3.4.7 Histopathology

The desired parts of the earthworms *E.euginiae* and the organs (gills, liver and gonads) of *Clarias gariepinus* exposed to sublethal concentrations of BTEX were removed and prepared for histopathological observation. They were fixed in Bouin's fluid for 24 hours, washed with 70% ethanol and dehydrated through a graded series of ethanol (Schalm *et al*., 1975; Kelly, 1979; Egonmwan, 2007). They were embedded in paraffin, sectioned at 4-5µm thickness, stained with hematoxylin and eosin, dehydrated in graded alcohol, cleared in xylene and mounted in Canada balsam. The slides were left to dry on the hot plate for 2 h before observation under the microscope and photomicrography (Details of histology procedure in Appendix 24).

3.3.5 Statistical Analysis

3.3.5.1 Probit Analysis

Toxicological dose-response data involving quantal response (mortality) were analysed by probit analysis using SPSS 14.0 The indices of toxicity measurement derived from the analysis were:

LC_{50} = Median lethal concentration that causes 50% response (mortality) of exposed organisms.

LC_{95}= Lethal concentration that causes 95% response (mortality) of exposed organisms.

LC_5 = sublethal concentration that causes 5% response (mortality) of exposed organisms and their 95% confidence limits (C.L.).

$$\text{T.F Toxicity factor} = \frac{\text{96h } LC_{50} \text{ value of other chemical}}{\text{96h } LC_{50} \text{ value of most toxic chemical}}$$

$$\text{S.F Susceptibility factor} = \frac{\text{96h } LC_{50} \text{ value of other test animals}}{\text{96h } LC_{50} \text{ value of most sensitive test animals}}$$

3.3.5.2 Analysis of Data by Analysis of Variance (ANOVA)

Data was analysed with One-way analysis of variance (ANOVA). Differences at $P < 0.05$ were considered significant. This was used to compare several treatment means in appropriately designed experiments. When significant variations were detected, a post hoc test was performed. Data analysis was carried out using SPSS 15.0 computer software package and Excel 2007.

CHAPTER FOUR

4.0 RESULTS

4.1 FIELD STUDIES: AQUATIC ECOSYSTEM

4.1.1 Distribution of Physico-Chemical Parameters in the Lagos lagoon surface water during the wet seasons of 2009 and 2010 Sampling Year.

a) **Hydrogen ion concentration (pH)**

The pH values obtained during this study in the wet seasons are presented in Table 4.1. The values ranged between 7.61 and 8.48. The lowest value of 7.61 occurred in the Unilag station which was the reference station, while the highest value of 8.48 was observed in the Atlas Cove station.

The pH values obtained during this study in the dry seasons are presented in Table 4.2. The values ranged between 7.28 and 7.89. The lowest value of 7.28 was observed in the control station while the highest value of 7.89 was observed in the Atlas Cove station. The results indicate a higher pH condition in the wet seasons than in the dry seasons.

b) **Salinity**

Values of salinity recorded in the wet and dry seasons are shown in Tables 4.1 and 4.2 respectively. Values ranged from 0.23‰ (Unilag stations) to 8.41‰ (Atlas Cove station) in the wet seasons. In both years of study, the dry seasons had values ranging from 11.16‰ to 21.51‰. Maximum salinity values recorded in all the stations occurred in the dry seasons.

Table 4.1: Mean values of physico – chemical parameters of water at the study stations during the wet seasons (July) in the Lagos lagoon (2009 and 2010)

Stations	**pH**	**Salinity (‰)**	**Conductivity (μ/Scm^{-1})**	**TDS (mg/L)**	**DO (mg/L)**
Unilag	7.61 ± 0.55	0.23 ± 3.03	739.16 ± 610.13	369 ± 304.84	5.11 ± 0.99
Iddo	8.45±1.58	7.96±1.12	28061.67±15401.3	14005±7681.185	6.48±135.47
Atlas Cove	8.48±0.26	8.41±2.08	27862.5±12085.92	13244.16±7163.68	4.64±1.09
Apapa	8.05±0.17	6.26±3.79	10958.33±3334.77	4861.66±1952.05	3.98±0.92

Table 4.2: Mean values of Physico – Chemical parameters of water at the study stations during the dry seasons (February) in the Lagos lagoon (2009 and 2010)

Stations	**pH**	**Salinity (%0)**	**Conductivity (μ/Scm^{-1})**	**TDS (mg/L)**	**DO (mg/L)**
Unilag	7.28±0.28	11.16±6.17	1092.45±1564.52	335.42±337.87	5.48±1.22
Iddo	7.59±0.24	12.16±2.99	189.13±171	93.33±85.77	5.28±1.77
Atlas Cove	7.89±0.12	21.51±3.02	172.85±157.77	95.21±85.76	5.48±0.67
Apapa	7.58±0.09	21.33±2.06	158.62±165.15	102.76±116.77	5.72±0.84

c) **Conductivity**

Conductivity measured in the wet seasons was between 739.16 and 28061.67µ/Scm^{-1} while conductivity measured in the dry seasons ranged from 158.62 to 1092.45 µ/Scm^{-1}. Higher values were observed in the wet season. (Tables 4.1 and 4.2)

d) **Total dissolved solids (TDS)**

Values of TDS recorded in the wet and dry seasons are shown in Tables 4.1 and 4.2. Values ranged from 369mg/l (Unilag station) to 4861.66mg/l (Apapa station) in the wet seasons. In both years of study, the dry seasons had values ranging from 93.33mg/l to 335.42mg/l. Maximum TDS values recorded in all the stations occurred in the wet seasons.

e) **Dissolved oxygen (DO)**

Values of DO recorded in the wet and dry seasons are shown in Tables 4.1 and 4.2 respectively. Values ranged from 3.98mg/l to 6.48mg/l in the wet seasons. In both years of study, the dry seasons had values ranging from 5.28mg/l to 5.72mg/l . Maximum DO values recorded in all the stations occurred in the dry seasons , however differences in the values obtained at the different stations and months reflect the differences in environmental conditions of the stations at the time of sampling.

4.1.2 Distribution of BTEX in Water and Sediment from the Lagos Lagoon

Benzene, Toluene, Ethylbenzene and Xylene were found to occur in analysed samples of water as well as sediment in all the stations of the Lagos lagoon during the wet and dry seasons of 2009/2010 in varied concentrations. The mean concentrations of Benzene, Toluene, Ethylbenzene (BTE) in surface water in the Lagos lagoon is presented in Fig 4.1. In the water samples, the Atlas cove station, which is the major marine receipt terminal for storage and distribution of petroleum products in Nigeria, had the highest value of 6.41 µg/l and 1.34 µg/l for Benzene and Toluene respectively. The mean concentrations of Benzene ranged from 0.30 µg/l to 6.41 µg/l. The mean concentrations of Toluene ranged from 0.04 µg/l to 1.34 µg/l. The mean concentrations of Ethylbenzene ranged from 2.41 µg/l to 12.77 µg/l . The mean concentrations of Xylene and total BTEX in surface water of the Lagos lagoon is presented in Fig 4.2. Xylene had the highest concentrations followed by Ethylbenzene in all the stations. Concentrations of Xylene ranged from 396.05 µg/l (Iddo) to 583.72 µg/l (Unilag station). Total BTEX was highest in the Unilag staion with 596.98 µg/l, followed by 540.97 µg/l recorded in Apapa station.

The mean concentrations of Benzene, Toluene, Ethylbenzene (BTE) in sediment of the Lagos lagoon is presented in Fig 4.3. In the lagoon sediment, Apapa had the highest value for Ethylbenzene which was 11.06 µg/kg followed by Atlas Cove station (9.53 µg/l). The highest concentration of Toluene (8.76 µg/kg) was found in the Atlas cove station, this was followed by 8.08 µg/kg in Unilag station. The lowest concentration of Toluene occurred in Apapa station (2.85 µg/kg). The mean concentrations of Xylene and total BTEX in sediment of the Lagos lagoon is presented in Fig 4.4. Highest concentration of Xylene (436.58 µg/kg) was recorded in Apapa

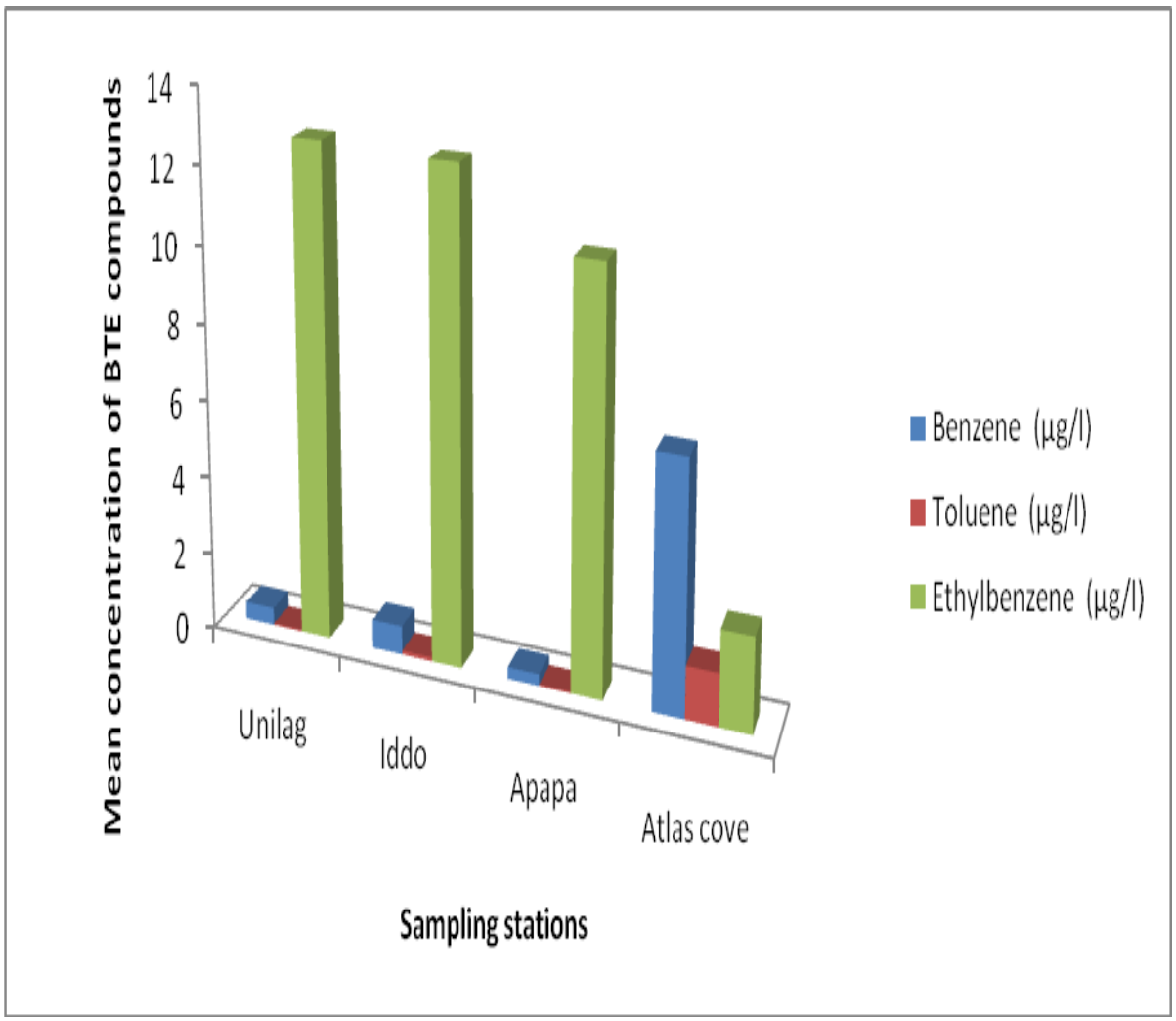


Fig 4.1: Mean concentrations of BTE compounds in surface water samples (µg/l) collected from different stations of the Lagos lagoon in 2009 and 2010.

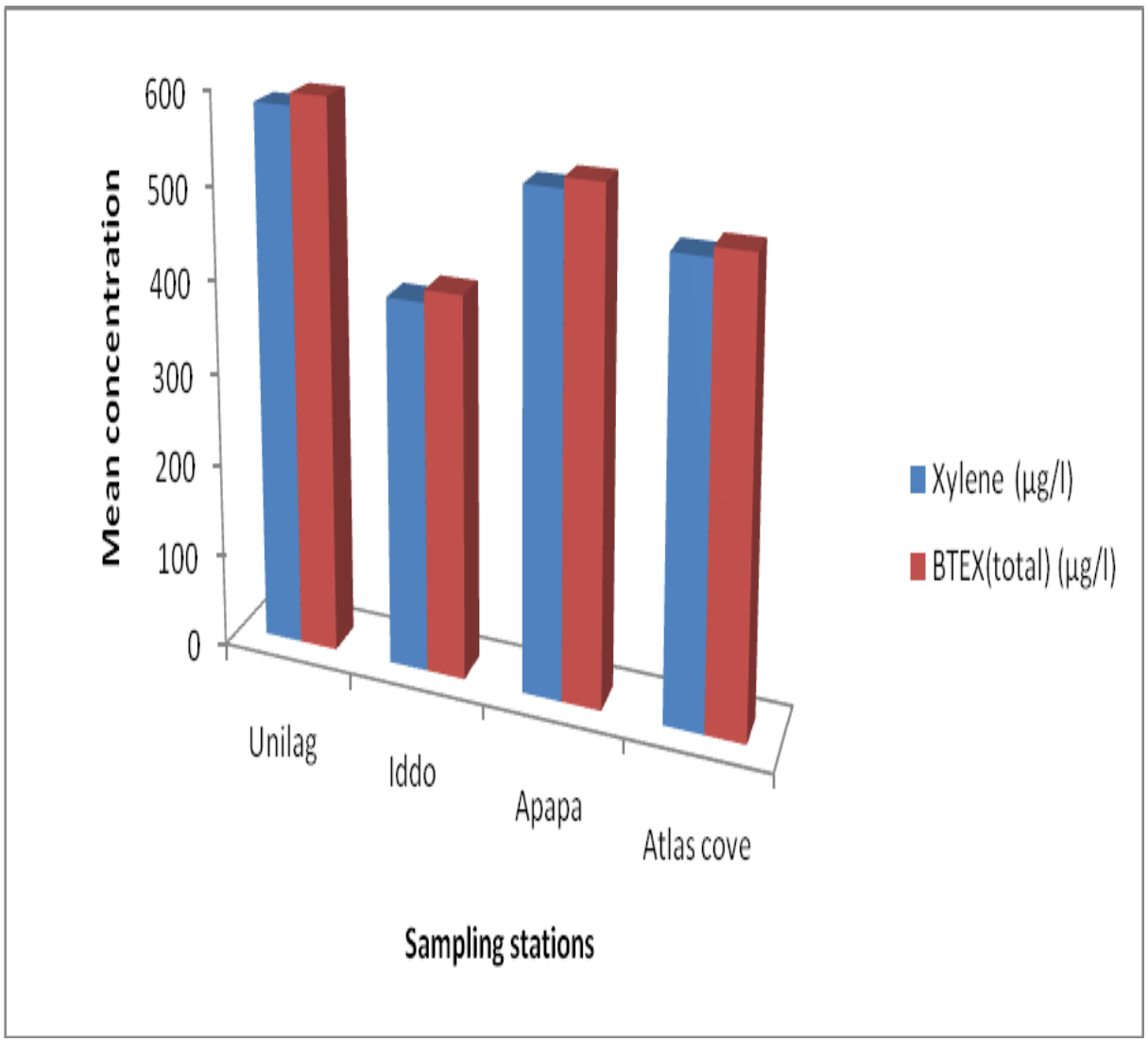


Fig 4.2: Mean concentrations of Xylene and total BTEX compounds in surface water samples (µg/l) collected from different stations of the Lagos lagoon in 2009 and 2010.

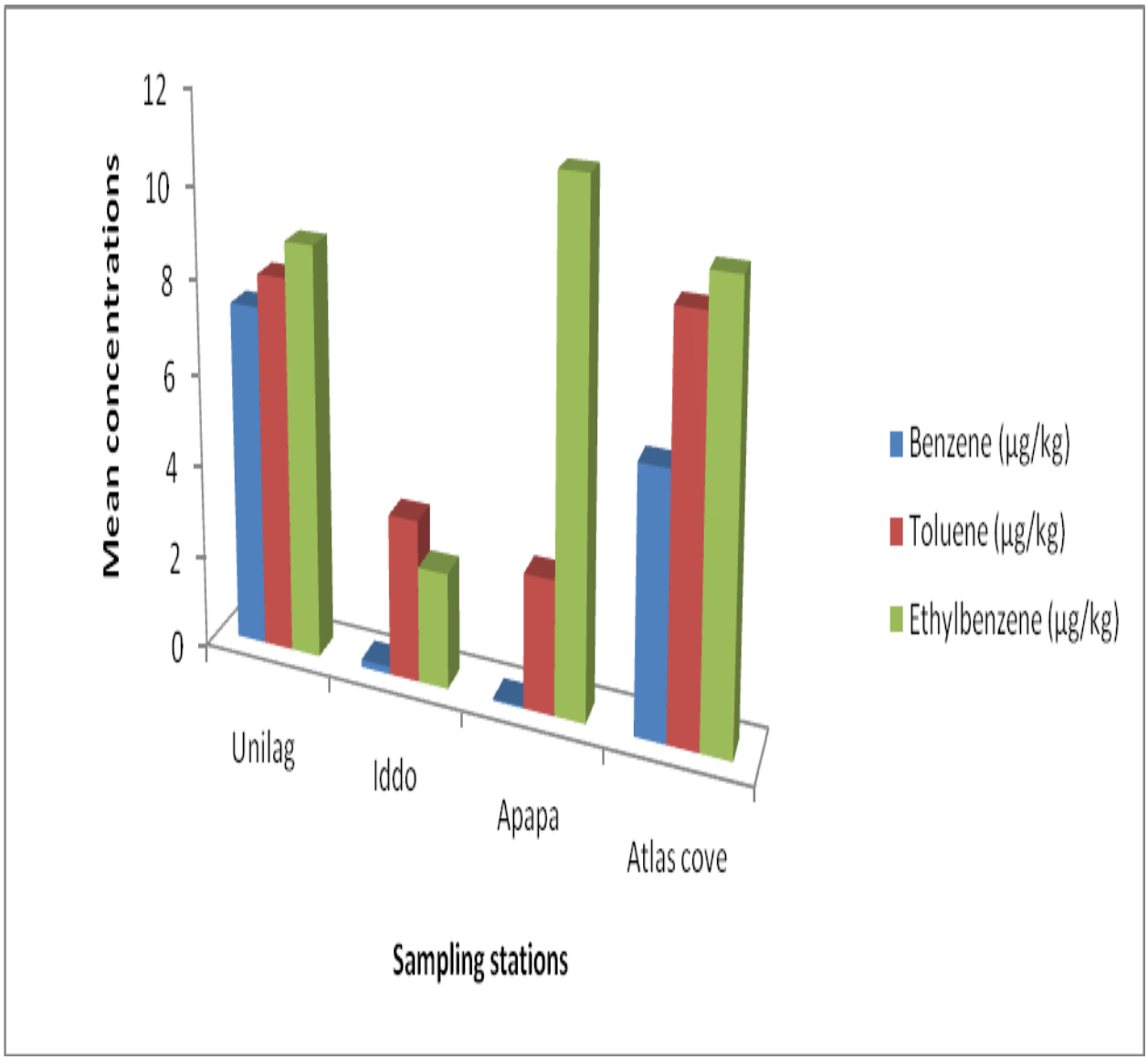


Fig 4.3: Mean concentrations of BTE compounds in sediment collected from different stations of the Lagos lagoon.

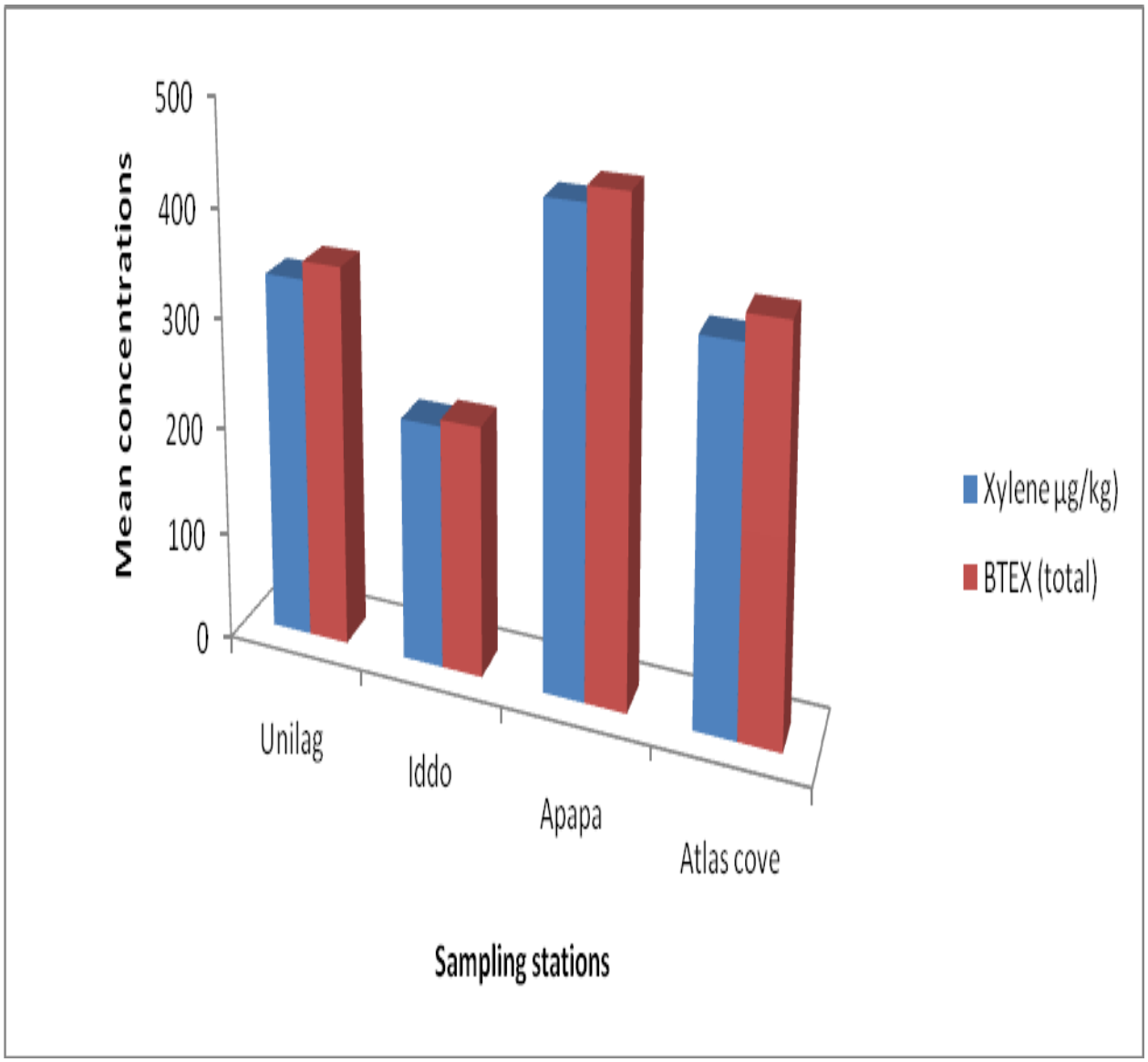


Fig 4.4: Mean concentrations of Xylene and total BTEX compounds in sediment collected from different stations of the Lagos lagoon.

station and the lowest concentration of 221.94 µg/l was recorded in Iddo. The highest value for total BTEX in the lagoon sediment was recorded in Apapa station with 450.53 µg/l, this was followed by Atlas cove stations (364.41 µg/l). Lowest concentration of 228.02 µg/l was observed in Iddo for total BTEX. Although the Unilag station which was the refernce station had high values for some of the compounds. Benzene was very high (7.38 µg/l) in the sediment collected from Unilag station.

4.1.3 Distribution of Total Hydrocarbon Content in Water and Sediment samples collected from the Lagos Lagoon

The Total Hydrocarbon Content in sediment and water samples collected from different stations of the Lagos Lagoon in wet and dry seasons 2009 and 2010 is presented in Figure 4.5 and 4.6. The mean concentration of Total Hydrocarbon (TH) in the sediment ranged from 1.74mg/kg to 267.198 mg/kg for the two years of study. In the sediment, Total hydrocarbon was highest in the year 2010, concentrations ranged from 24.01 mg/kg in Apapa to 267.19 mg/kg in Iddo station (Figure 4.5). The highest Total Hydrocarbon Content in the sediment was recorded in Iddo, 267.19 mg/kg. In the Unilag station, the THC ranged from 16.55 mg/kg to 100.71 mg/kg for year 2009 and 2010. Concentrations of TH in the sediment ranged from 1.74 mg/kg to 35.74 mg/kg in the Atlas Cove for both years. In Apapa station, concentrations of Total hydrocarbon ranged from 10.48 mg/kg to 32.55 mg/kg for both years in the sediment (Figure 4.5).

The mean values for Total hydrocarbon in the water samples ranged from 0.84 mg/l to 31.38 mg/l for both years of study (Figure 4.6). The highest mean value of Total Hydrocarbon in the

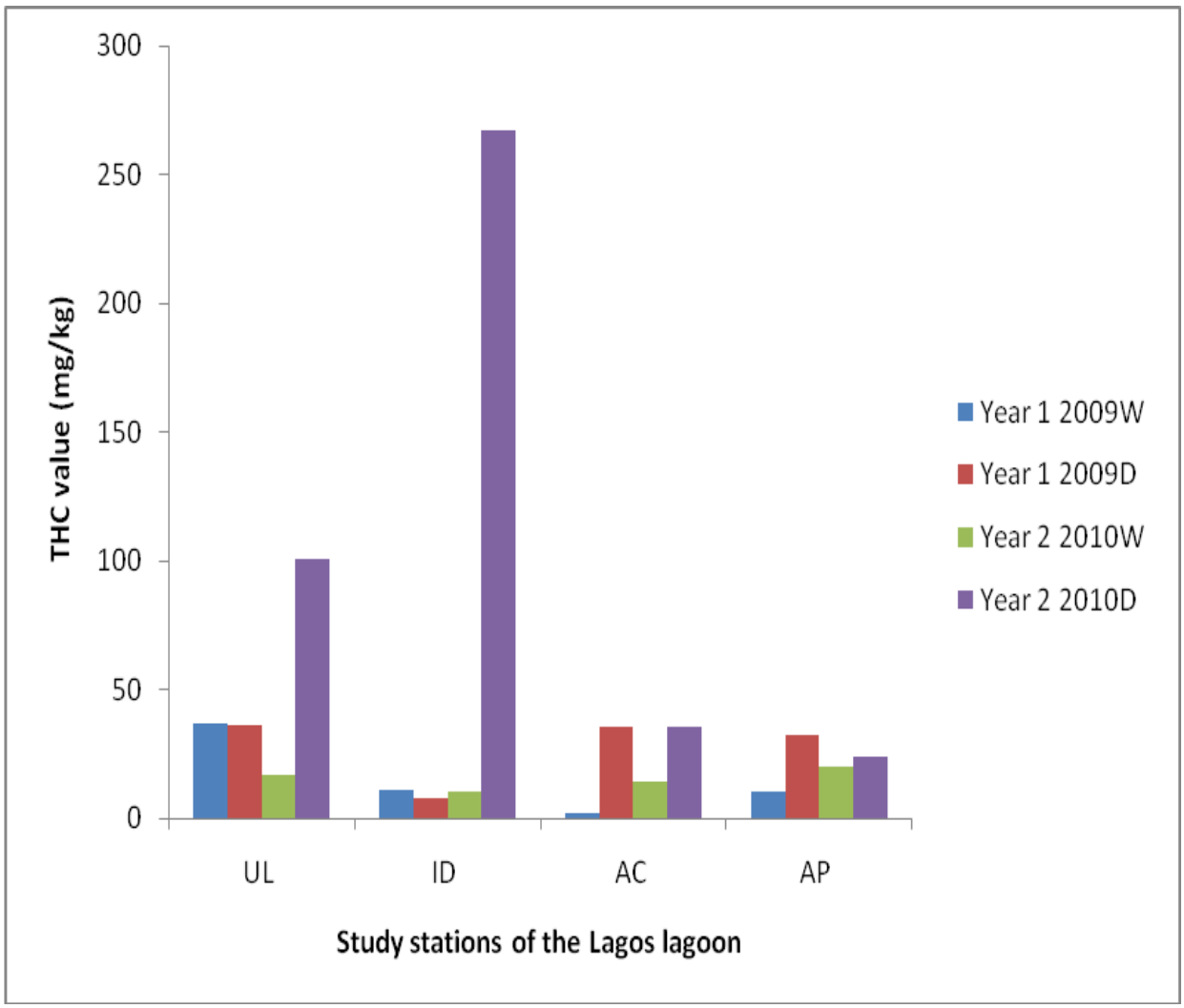


Fig 4.5: Mean value of THC (mg/kg) in sediment samples collected from different stations of the Lagos Lagoon in wet and dry seasons 2009 and 2010.

UL= Unilag; ID= Iddo; AC= Atlas cove; AP= Apapa

2009W= 2009 wet season; 2009D= 2009 dry season

2010W= 2010 wet season; 2010D= 2010 dry season

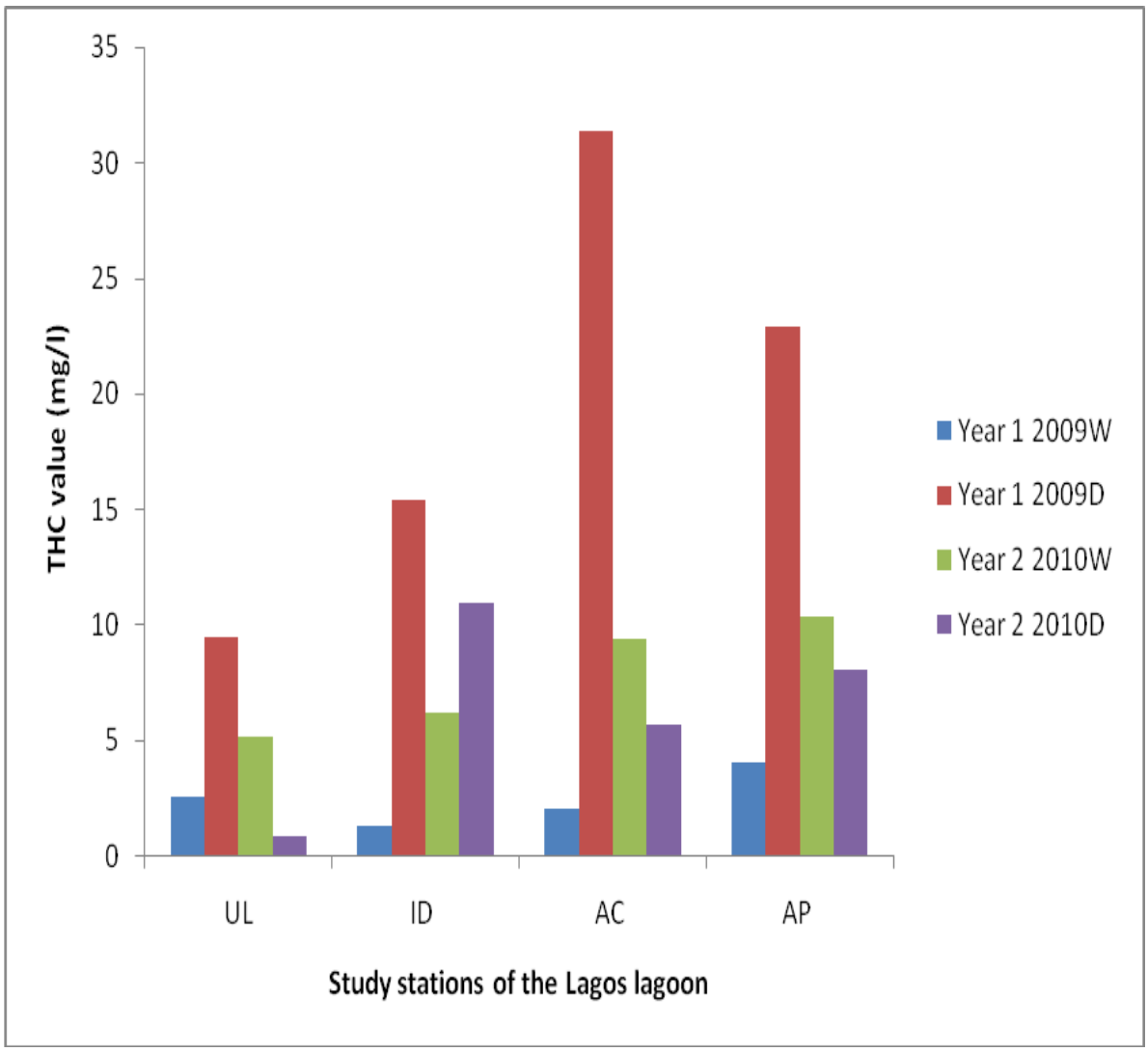


Fig 4.6: Mean value of THC (mg/l) in surface water samples collected from different stations of the Lagos Lagoon in wet and dry seasons 2009 and 2010. UL= Unilag; ID= Iddo; AC= Atlas cove; AP= Apapa

2009W= 2009 wet season; 2009D= 2009 dry season

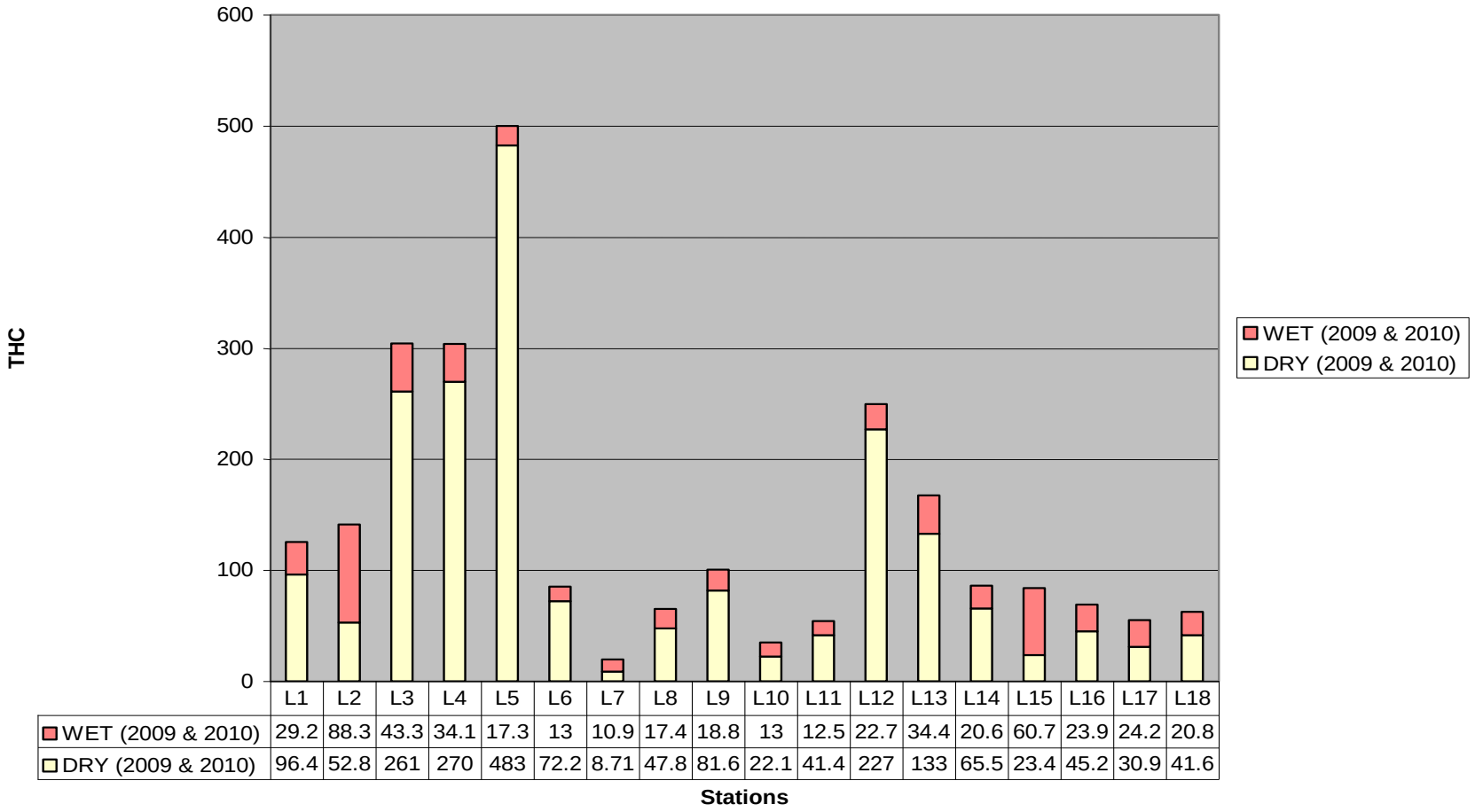
2010W= 2010 wet season; 2010D= 2010 dry season

water samples was found in Apapa station, 31.38 mg/l in 2009 (Figure 4.6). In Unilag station, THC ranged from 0.84 mg/ l to 9.41 mg/l for years 2009 and 2010. Concentration of TH ranged from 1.26 mg/l to 15.41 mg/l in Iddo station. Total Hydrocarbon Content ranging from 2.03 mg/l to 31.38 mg/l was recorded in Atlas Cove station while THC ranging from 4.04 mg/l to 22.89 mg/l was observed in Apapa station for both years (Fig.4.6). The lowest concentration of Total Hydrocarbon in water samples collected from the Lagos lagoon was recorded in Unilag station, 0.84 mg/l (Figure 4.6).

4.1.4 Seasonal Variation of Total Hydrocarbon Content in Sediment and Water samples collected from the Lagos lagoon

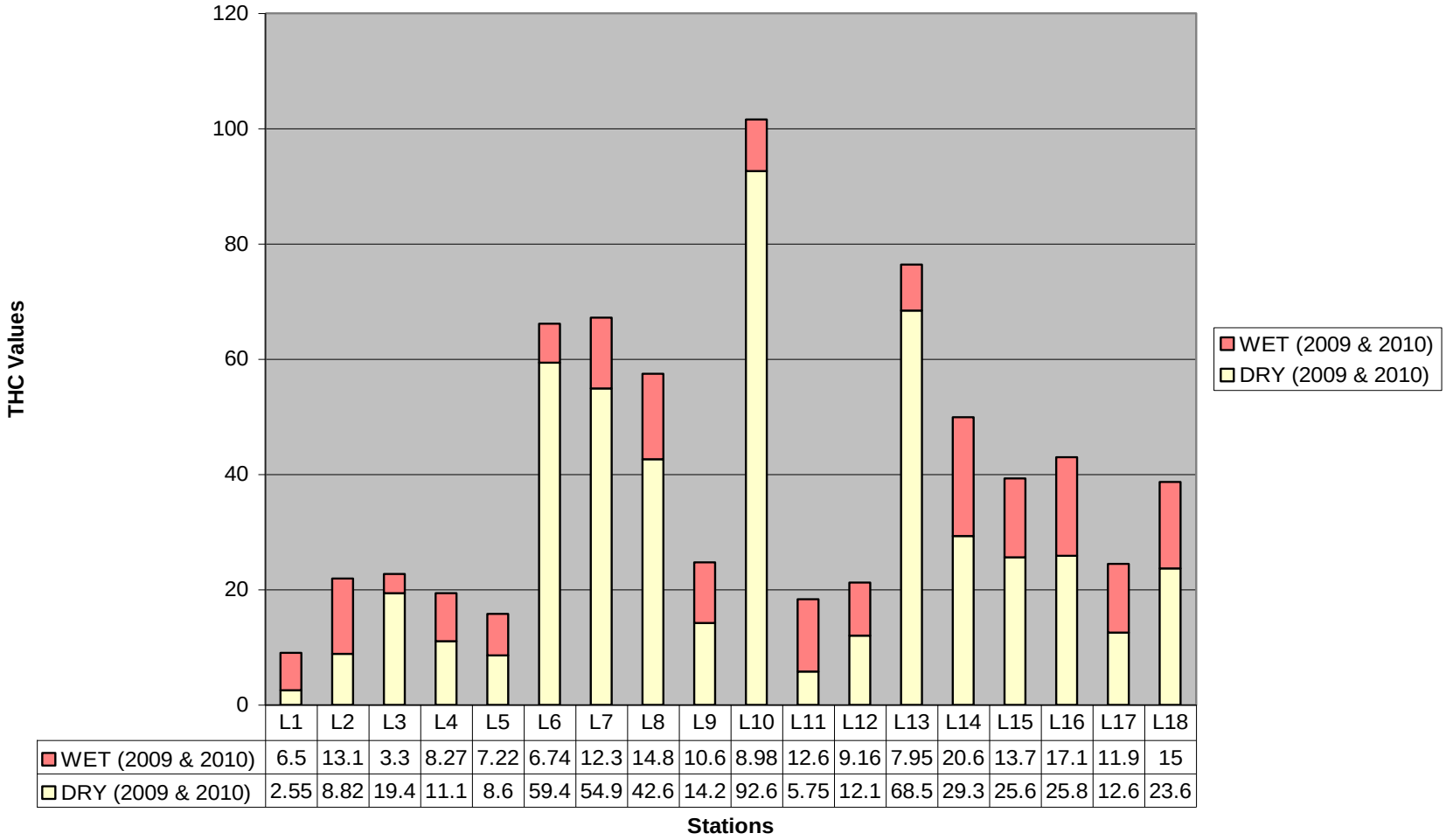
Results of seasonal variation of THC in sediment collected from the Lagos lagoon is presented in Figure 4.7. The results of THC showed consistent and distinct patterns in seasonal variations. The dry seasons had significantly ($P< 0.05$) higher values of THC in comparison with the wet season in the sediment collected from Lagos lagoon for both years (Figure 4.7). In the wet season, THC values ranged from 10.9 mg/kg to 88.3 mg/kg, while in the dry season, THC values ranged from 8.71 mg/kg to 483 mg/kg in the sediment.

Results of seasonal variation of THC in water samples collected from the Lagos lagoon is presented in Figure 4.8. The results of THC showed consistent and distinct patterns in seasonal variations. The dry seasons had significantly ($P< 0.05$) higher values of THC in comparison with the wet season in the water samples collected from Lagos lagoon for years 2009 and 2010 (Figure 4.8). In the wet seasons, THC values ranged from 3.3 mg/l to 20.6 mg/l, while in the dry



	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
WET (2009 & 2010)	29.2	88.3	43.3	34.1	17.3	13	10.9	17.4	18.8	13	12.5	22.7	34.4	20.6	60.7	23.9	24.2	20.8
DRY (2009 & 2010)	96.4	52.8	261	270	483	72.2	8.71	47.8	81.6	22.1	41.4	227	133	65.5	23.4	45.2	30.9	41.6

Fig. 4.7: Seasonal variation in the concentration of Total Hydrocarbon (mg/kg) in sediment samples collected from different sampling stations of the Lagos Lagoon in wet and dry seasons of 2009 and 2010.

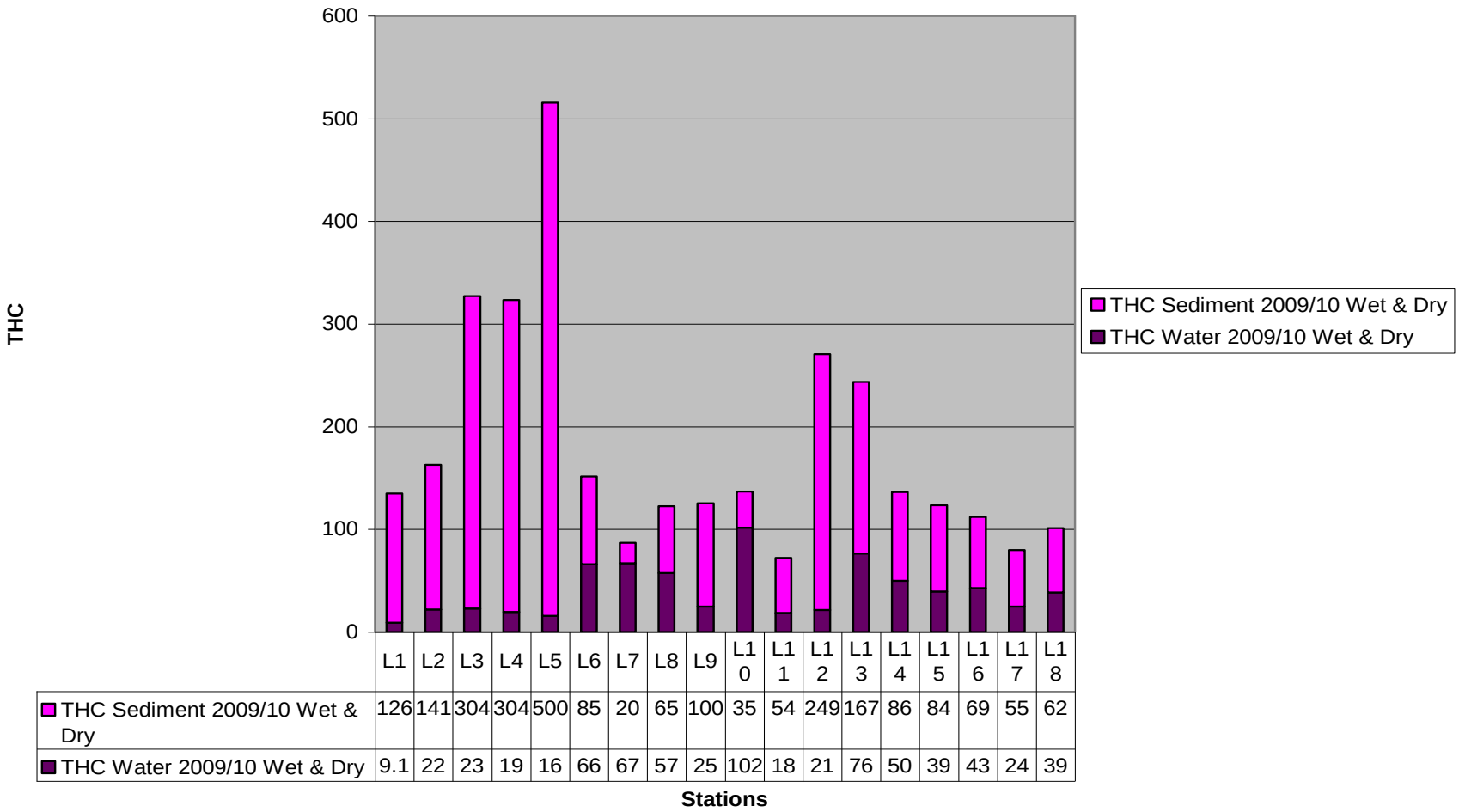


	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
WET (2009 & 2010)	6.5	13.1	3.3	8.27	7.22	6.74	12.3	14.8	10.6	8.98	12.6	9.16	7.95	20.6	13.7	17.1	11.9	15
DRY (2009 & 2010)	2.55	8.82	19.4	11.1	8.6	59.4	54.9	42.6	14.2	92.6	5.75	12.1	68.5	29.3	25.6	25.8	12.6	23.6

Fig. 4.8: Seasonal variation in the concentration of Total Hydrocarbon (mg/l) in surface water samples collected from different sampling stations of the Lagos Lagoon in wet and dry seasons of 2009 and 2010.

seasons, THC values ranged from 2.55 mg/l to 92.6 mg/l in the lagoon water. Statistical analysis by ANOVA showed no significant ($P<0.05$) difference in the THC in lagoon water within the different locations.

Figure 4.9 shows the comparison between the THC in sediment and water samples collected from the Lagos lagoon in years 2009 and 2010. Over the two year sampling period of four seasons, the THC in the sediment collected from the lagoon was significantly ($P< 0.05$) higher than the THC in the water samples collected from the lagoon (Figure 4.9). Statistical comparison by Analysis of Variance (ANOVA) of THC in water and sediment samples collected from the lagoon show that there was significant ($P< 0.05$) difference in the measured concentration of THC between the seasons (dry and wet). There was no significant ($P< 0.05$) difference in the Total Hydrocarbon Content between the years (2009 and 2010).



	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
THC Sediment 2009/10 Wet & Dry	126	141	304	304	500	85	20	65	100	35	54	249	167	86	84	69	55	62
THC Water 2009/10 Wet & Dry	9.1	22	23	19	16	66	67	57	25	102	18	21	76	50	39	43	24	39

Fig. 4.9: Comparison between the mean values THC in sediment (mg/kg) and water (mg/l) samples collected from different stations of the Lagos Lagoon in in wet and dry seasons of 2009 and 2010

4.1.5 Distribution of Macrobenthic Invertebrate Communities of the Lagos lagoon

The effect of petroleum product spillages on community structure indices such as diversity, abundance of macrobenthic fauna within the sampled stations of the Lagos lagoon was found to be pronounced. The overall species composition in the 18 sampling stations of the Lagos Lagoon during the sampling period is presented in Table 4.3. Statistical analysis which was conducted on benthic abundance to test significance based on locations was significant, ($P < 0.05$). Post Hoc test which was carried out revealed that control Unilag station differed significantly from other locations with higher benthic values. The highest number of individuals recorded was in the control (Unilag) stations 1, 2 and 3 having 52, 45 and 86 individuals respectively, followed by the Iddo, Atlas Cove and Apapa stations. The Iddo sampling stations 4, 5 and 6 had the following number of individuals; 36, 27 and 21 respectively. The Atlas Cove sampling stations 7, 8, 9, 10, 11, 12 had 9, 16, 16, 17, 29 and 21 individuals respectively. The stations with the least number of individuals were the Apapa sampling stations 13, 14, 15, 16, 17 and 18 having 11, 5, 15, 5, 1 and 9 individuals respectively (Table 4.3).

A total of 425 macrobenthic invertebrates were recorded in this study. *Tympanotonus fuscatus, Tympanotonus fuscatus* var *radula, Pachymelania aurita and Pachymelania fusca quadriseriata* dominated both in population density and range of distribution (Table 4.3). *Clibanarius africanus, Mitra fusca* and *Mytilus edulis* were species with the lowest number (1). The molluscan group was represented by *Tympanotonus sp, Pachymelania sp, Macoma cumana* which were the dominant species in the Unilag (control) stations (Table 4.3).

Table 4.3: Overall species composition in all the sampling stations at the Lagos Lagoon (2009 and 2010).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Heteromastus filiformis	1				1	11	1	5	1	2					2			1	25
Nereis lamellose			4	3		1		1	1		1	3	1						15
Nereis succinea			3		1	1		3	1				4		1		1	1	16
Nais eliguis					14													1	15
Clibanarius africanus											1								1
Penaeus notialis	1				1	1							1						4
Palaemonetes vulgaris					1		1			1				1					4
Tympanotonus fuscatus	7	4	14		2	1			2		3			1	1	2			37
Tympanotonus fuscatus var *radula*	14	20	11	1	2					2	2		1		1				54
Pachymelania aurita	21	7	10	1		2		2	2			2	2	1	2	1		1	54
Pachymelania fusca quadriseriata	3	2	5	3	2			3	1	2	1	2	1		3	1			29
Mytilus perna	1	1			1	2	1	2	2	2					2	1			15
Gryphaea gazar	3	3	16	4															26
Macoma Cumana		2	23	21						1		2						4	53
Mitra fusca							1												1
Tellina nymphalis	1	4		1					1	4									11
Aloidis trigona		1		2	1	2			5	2	3		1	2	2			1	22
Aloidis dautzenbergi											5								5
Donax pulchellus											11	7							18
Dreissena Africana							1					3							4
Dentalium sp							4								1				5
Corophium volutator					1					1	1								3
Mytilus edulis											1								1
Pera perna		1										2							3

These organisms were poorly represented in Iddo, Atlas cove and Apapa stations. The Annelids represented by *Nais eliguis, Heteromastus filiformis and Nereis sp* were found mostly in Iddo, Atlas cove and Apapa station. *Nais sp was* completely absent in Unilag (control) stations. These organisms are known as opportunistic and tolerant species (Table 4.3). The Total number of species recorded in Apapa station 17 was 1(*Nereis succinea)*, which was the lowest; while the highest number of species was found in Iddo station 5 with 11 organisms (Table 4.3).

4.1.5.1 Diversity of Macrobenthic Invertebrate in the study stations of the Lagos lagoon

High species diversity and richness was observed in the Atlas cove and Apapa stations. Table 4.4 shows the values of diversity indices calculated for the study stations in Lagos lagoon. The species richness in the control stations 1-3 ranged from 1.57 to 2.02. The species richness in the Apapa stations 13 to 18 ranged from 1.86 to 2.95. The species diversity was between 0.70 and 0.82 in the control station 1-3 and between 0.41 and 0.92 in the Apapa stations 13 to 18. The species richness was highest in Apapa station 15 with 2.95 while the lowest was 1.57 in control station 3. The diversity index was highest in Apapa station 15 with 0.92 and lowest in Apapa station 16 with 0.58. The highest number of individuals was recorded in the control stations with a total of 183 individuals. In Atlas Cove and Iddo stations, 108 individuals and 84 individuals were recorded respectively. Apapa stations had the least number of individuals of 46. In this study, some families contained small number of species, indeed most contained only a single species. Families Capitellidae, Dreissenidae, Dentaliidae, Corophiidae, Naididae contained only one species each.

Table 4.4: Diversity of macrobenthic invertebrates in the study stations of the Lagos lagoon (2009-2010)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Total number of individuals	52	45	86	36	27	21	9	16	16	17	29	21	11	5	15	5	1	9
Total number of species	9	10	8	8	11	8	6	6	9	9	10	7	7	4	9	4	1	6
Margalef's index	2.02	2.36	1.57	1.95	3.03	2.30	2.28	1.80	2.89	2.82	2.67	1.97	2.50	1.86	2.95	1.86	1.92	2.28
Shannon-wiener index	0.70	0.77	0.82	0.62	0.80	0.70	0.70	0.73	0.87	0.91	0.83	0.79	0.78	0.58	0.92	0.58	0.50	0.67
Equitability	0.32	0.33	1.70	0.30	0.32	0.33	0.40	0.41	0.40	0.41	0.36	0.41	0.39	0.42	0.42	0.42	0.31	0.38

4.1.5.2 Spatial distribution of major macrobenthic invertebrate species in the sampled stations.

Percentage composition of Mollusca, Arthropoda and Annelida in the sampled stations at the Lagos Lagoon in 2009 and 2010 is represented in Figure 4.10. Three major groups (Mollusca, Arthropoda and Annelida) were distributed in the 18 sampled stations in this study. Among the major groups recorded, mollusca have the highest total abundance with 9 families, 2 classes and 14 species. Arthropoda had the lowest total abundance of 13 with 3 families, 1 class and 3 species. Arthropoda was not present in 9 out of the 18 stations namely stations 2, 3, 4, 8, 9, 15, 16, 17, 18.

The highest percentage (100%) of mollusca was found in station 2 (Unilag), followed by station 4 (97%), station 1 (95%), station 11(90%), station 3 (85%) . The lowest percentage of mollusca was found in station 17 (0%), followed by station 5 (30%), 6 (36%) and 8 (47%) (Figure 4.10**).**

The highest percentage of Annelida was found in station 17 (100%), followed by station 5 (60%), 6 (59%), 8 (52%) and 13 (45%). The lowest percentage was recorded in station 1 at (2.5%), followed by station 4 (3%), station 11(3%), 7 (10%) and station 3 (12%). The highest percentage of Arthropoda was recorded in station 14 with 20% followed by stations 7, 5, 13 while in Staion 1, 2.5% was recorded for Arthropoda (Figure 4.10**)**. Phylum Mollusca ranked highest in the distribution. They occurred in all the stations sampled except station 17. The families; Melaniidae, Mytilidae, Ostreidae, Potamididae, Donacidae, Mitridae, Tellinidae, Aloididae, Dreissenidae were recorded in the sampled stations (Figure 4.11).

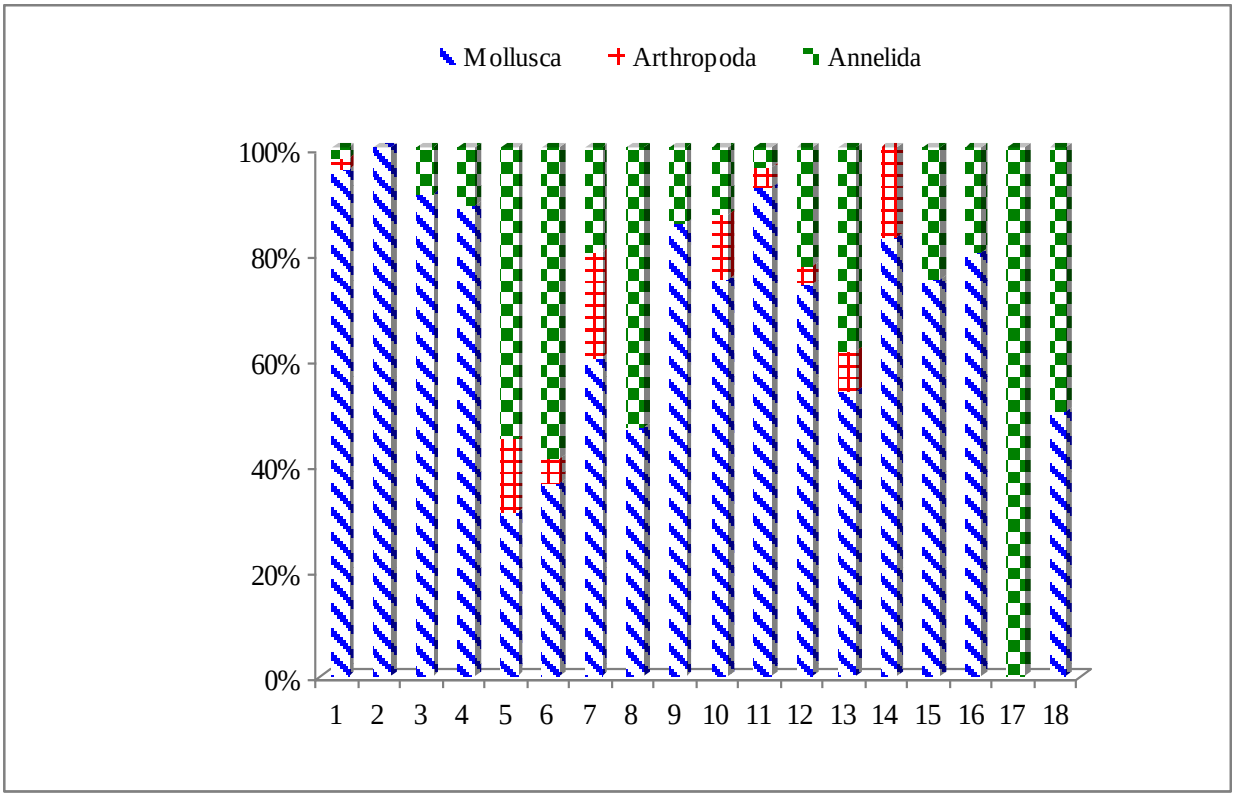


Fig.4.10: Percentage composition of Mollusca, Arthropoda and Annelida in the study stations at the Lagos Lagoon in 2009 and 2010.

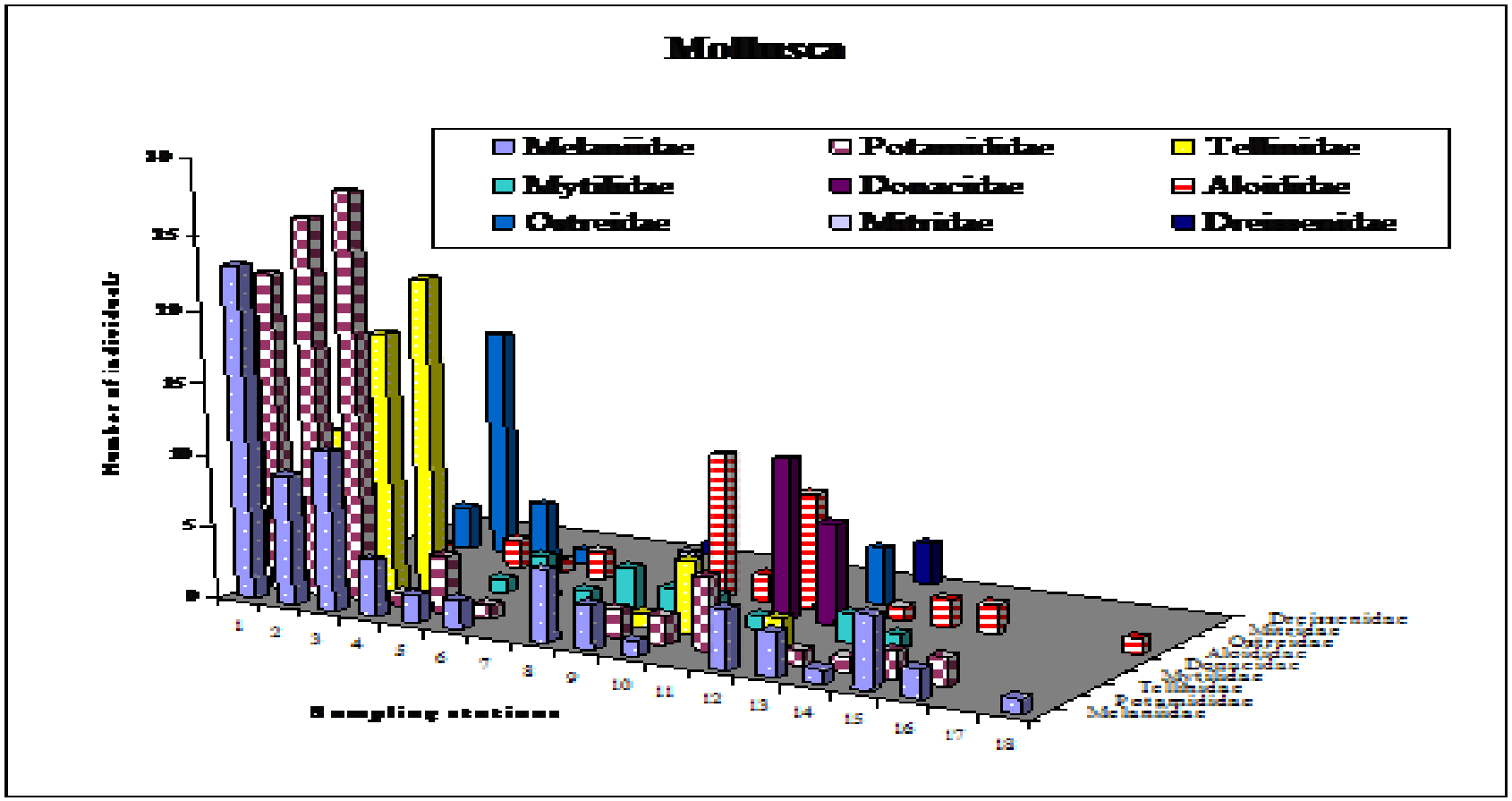


Fig.4.11: Spatial variation in the density of major Molluscan groups in the study stations at the Lagos Lagoon in 2009 and 2010.

The highest number of individuals for Melaniidae, Potamididae, Tellinidae and Osreidae were recorded in Unilag stations 1, 2 and 3. Melaniidae was recorded in all the stations except stations 7, 11 (Atlas Cove) and station17 (Apapa). Family Potamididae was recorded in all stations except the Atlas Cove stations 7, 8, 12 and Apapa station 17. Ostreidae was found only in the Unilag stations and Atlas Cove station 12. Aloididae was recorded in stations 6, 7, 8, 10, 11, 12, 14, 15, 16 and 18 . Donacidae was recorded in only two stations namely Atlas Cove stations 12 and 13. The family Dreissenidae was also found in only two stations, 8 and 12 (Figure 4.11).

The phylum Annelida ranked second after Mollusca in the extent of Spatial distribution. They occurred in most of the stations sampled though in low numbers compared to the Molluscs. Three families were recorded; Nereidae, Capitellidae, Naididae (Figure 4.12). Nereidae had the largest distribution, was recorded in 13 stations, Capitellidae was next, occurring in 8 stations while the Naididae had the least distribution, was recorded in only two stations.

The Arthropods were recorded in very few sampling stations represented by the families Corophidae, Penaeidae and Diogenidae. Corophidae and Diogenidae were only recorded in two stations each, while Penaeidae was found in 7 stations (Figure 4.13). In the Apapa stations 15, 16, 17 and 18, no Arthropods were found.

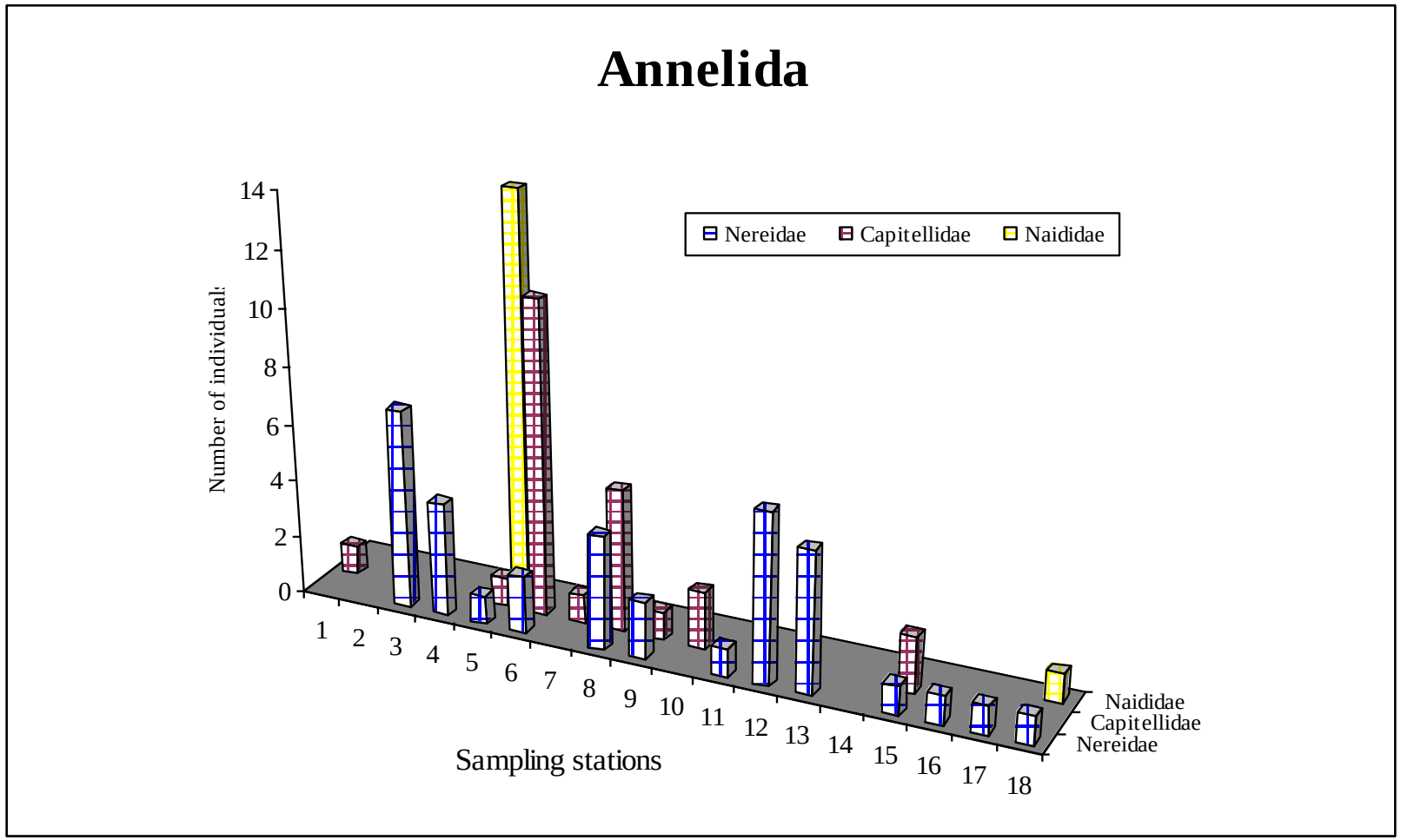


Fig.4.12: Spatial variation in the density of major Annelida groups in the study stations at the Lagos Lagoon in 2009 and 2010.

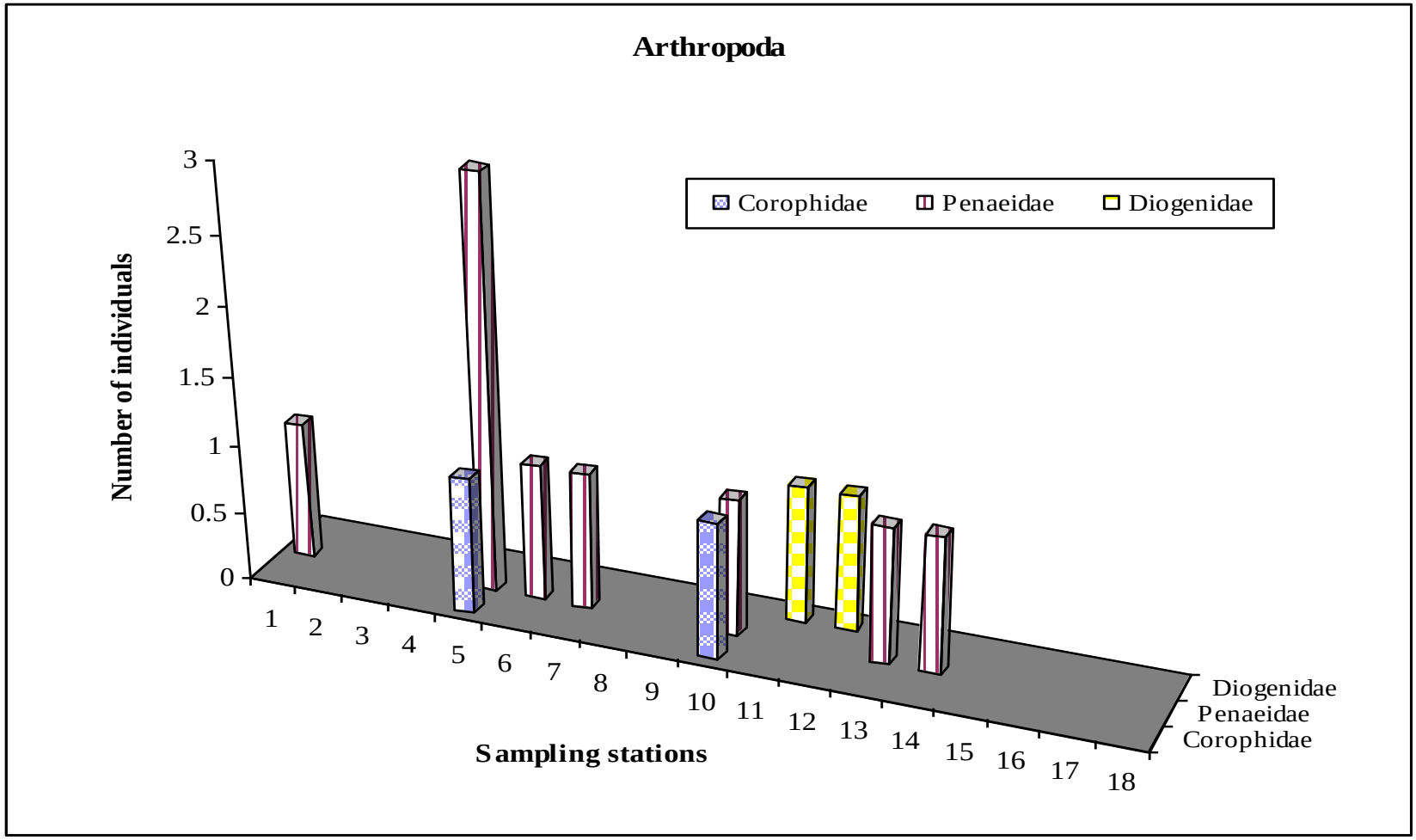


Fig.4.13: Spatial variation in the density of major Arthropoda groups in the sampled stations at the Lagos Lagoon in 2009 and 2010.

4.1.5.3 Seasonal variations in the composition of macrobenthic invertebrates in the sampled stations of the Lagos Lagoon.

The seasonal variations in the number of species of macrobenthic invertebrates collected during the sampling periods are graphically represented in Figure 4.14. The abundance of benthic invertebrates between seasons was not significant ($P < 0.05$). In the dry season of 2009, 138 individual macrobenthic invertebrates were recorded while in the wet season of the same year, 141 individuals were recorded. In the dry season of 2010, 77 individuals were recorded while in the wet season, 70 individual macrobenthic invertebrates were recorded which was the lowest. In the dry seasons of 2009, 2010 and wet season 2010, there was absence of macrobenthic invertebrates in all the Apapa stations, except in station 18 (2010 wet season), station 13 and 18 (2009 dry season) and station 15 (2010 dry season) (Figure 4.15). The year 2009 had the highest number of individual macrobenthic invertebrates with a total of 261 individuals than the year 2010 with 147 individuals (Figure 4.15). A one-sample t test conducted to test abundance of benthic in lagoon stations between years differed significantly ($P<0.05$) with year 2010 having low abundance.

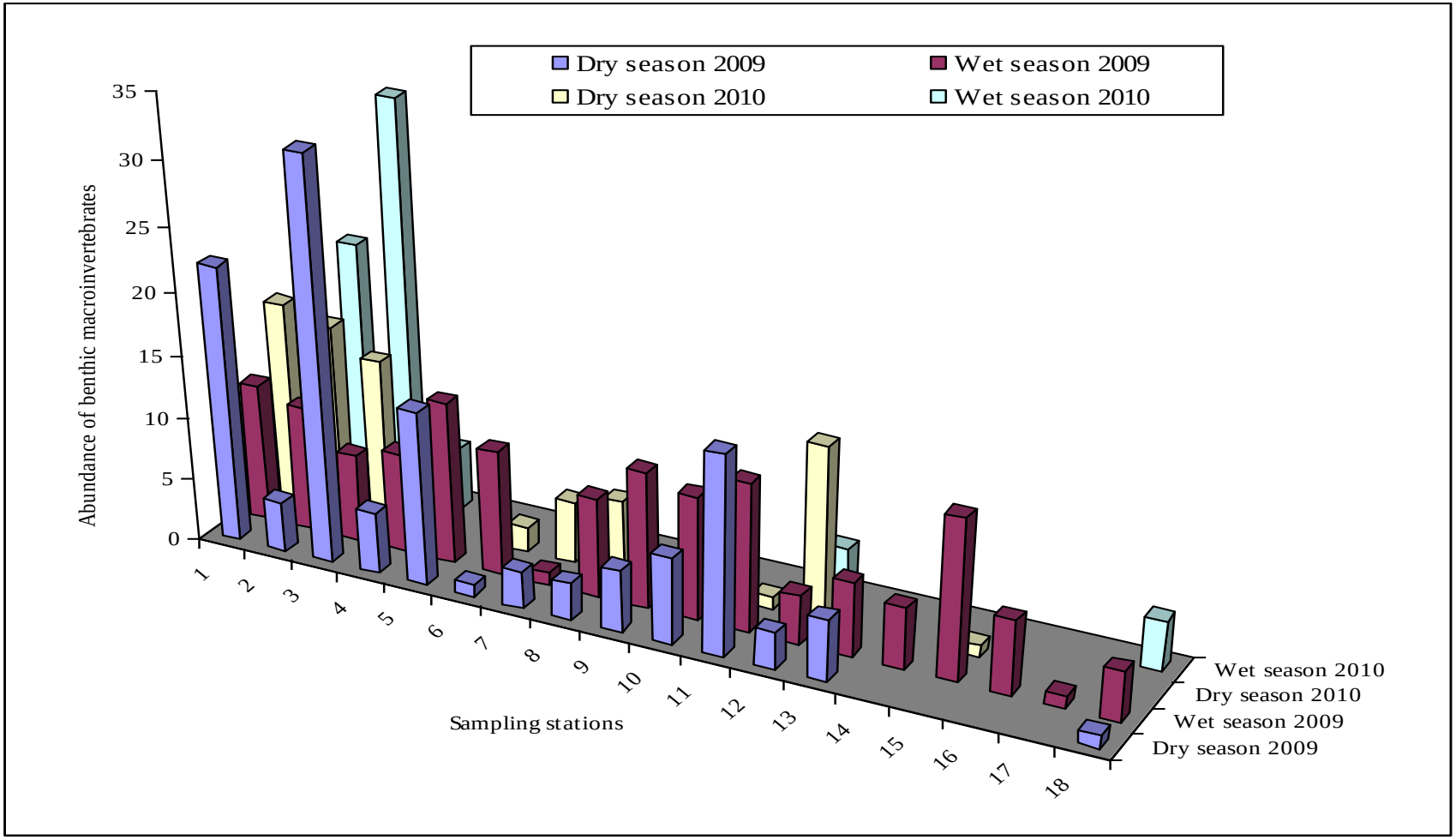


Fig. 4.14: Variations in seasonal density of macrobenthic invertebrates species in the study stations of the Lagos Lagoon.

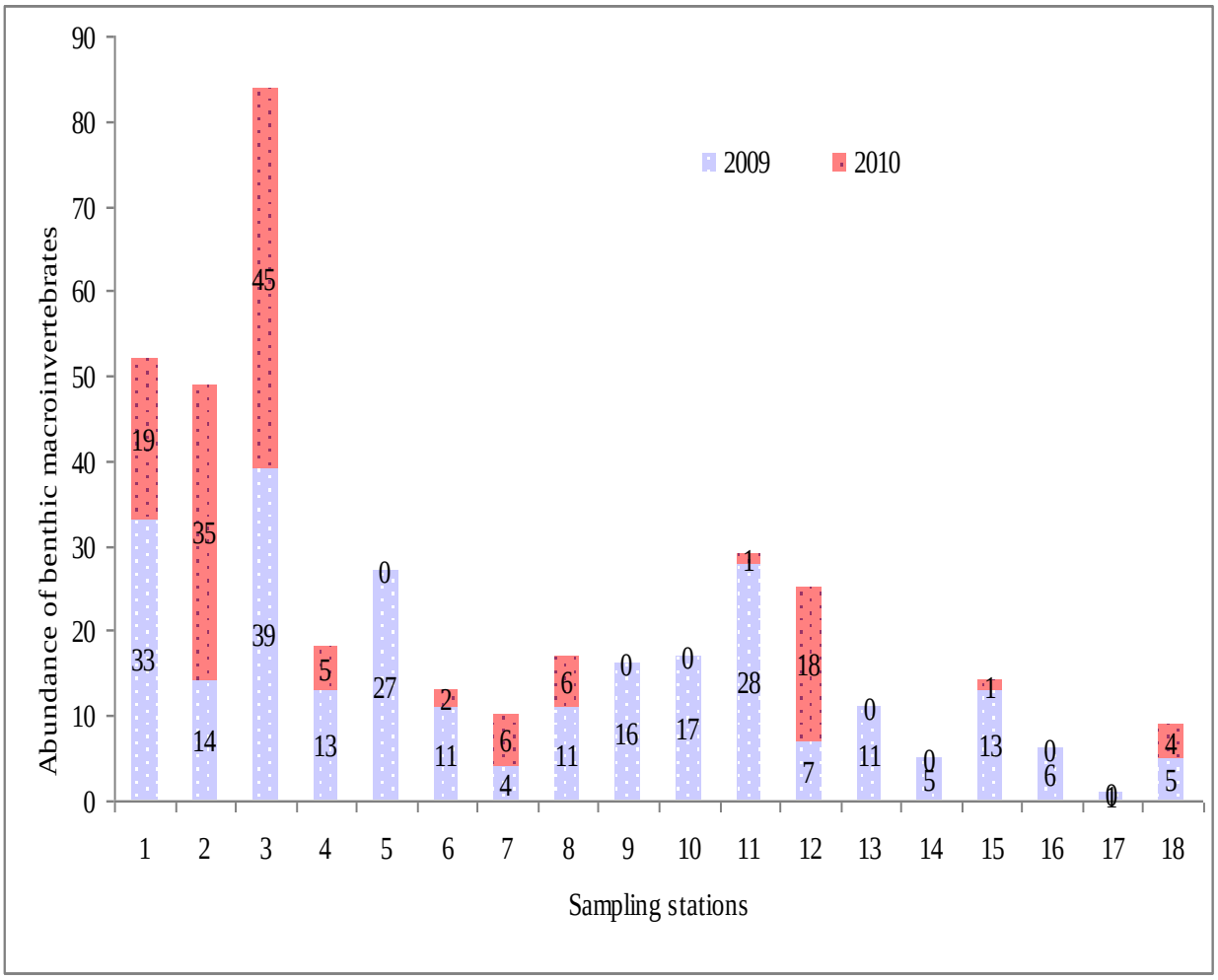


Fig. 4.15: Variation in the abundance of benthic macroinvertebrates in the sampled stations in 2009 and 2010.

4.1.5.4 Relationship between hydrocarbon concentration and faunal indices

In this study, the abundance of macroinvertebrates correlated positively with hydrocarbon concentration in the sediment of the Lagos lagoon in years 2009 and 2010 with R values of 0.31 and 0.45 respectively (Figures 4.16 and 4.17). It could be inferred that the concentrations of hydrocarbons was not significant, from the results of the regression of abundance of macrobenthic invertebrates on hydrocarbons in the sediment. The concentrations of hydrocarbons in the water samples of the Lagos lagoon was found to be negatively correlated with the abundance of macrobenthos in 2009 and 2010 with R values of -0.25 and - 0.71 (Figures 4.18 and 4.19). The abundance of macrobenthic invertebrates was not affected by the concentration of hydrocarbons in the Lagos lagoon water.

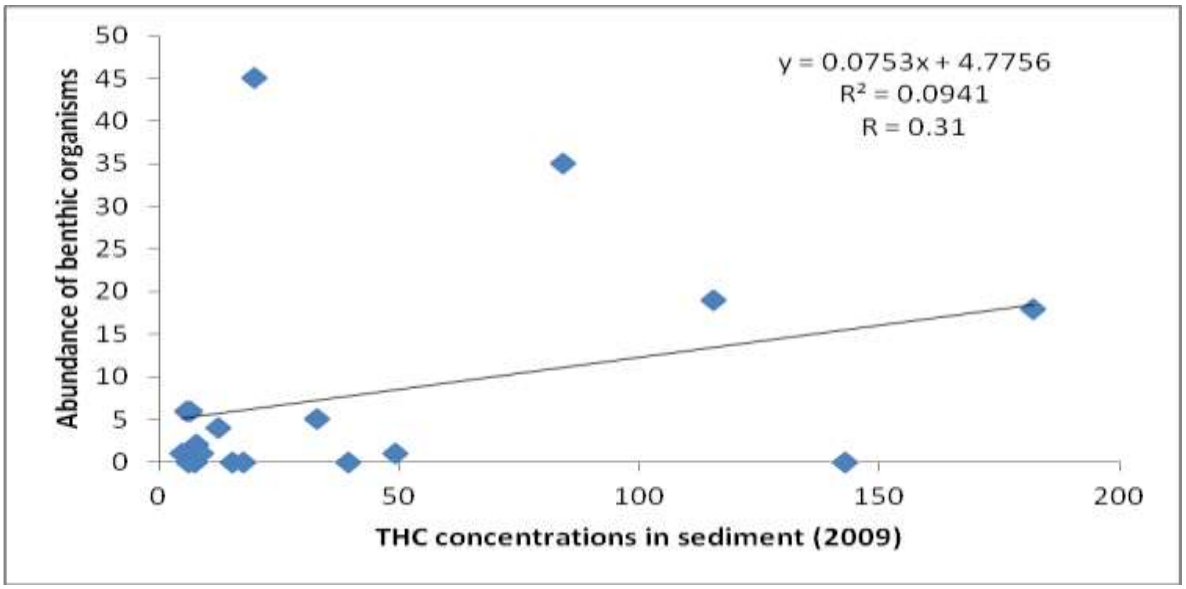


Fig 4.16: Relationship between abundance of macrobenthic invertebrates and THC concentration in sediment in 2009.

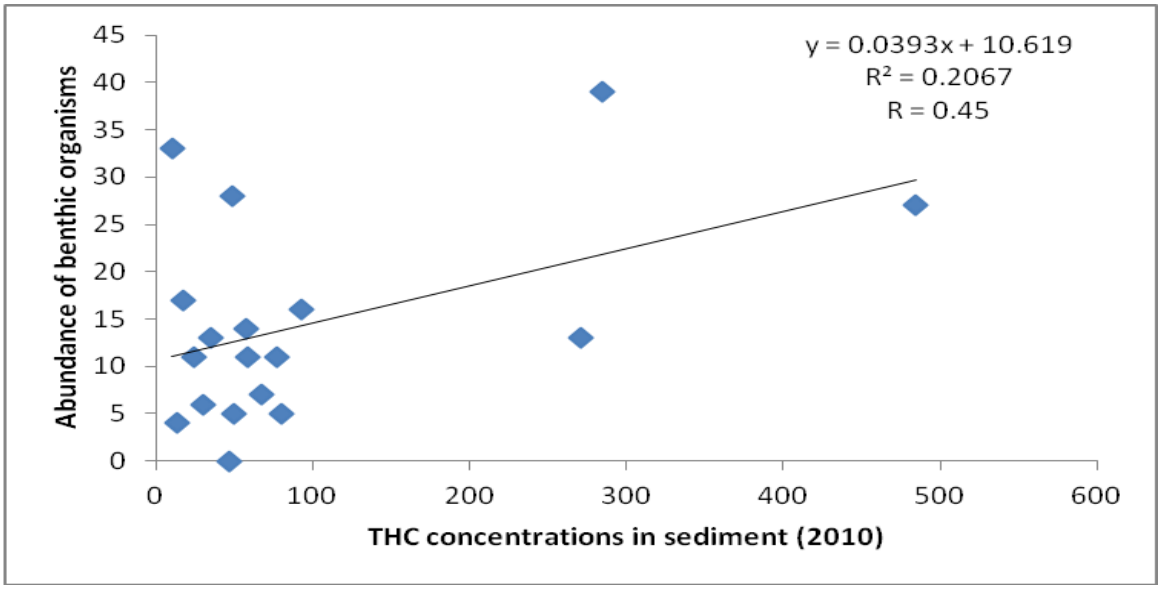


Fig 4.17: Relationship between abundance of macrobenthic invertebrates and THC concentration in sediment in 2010

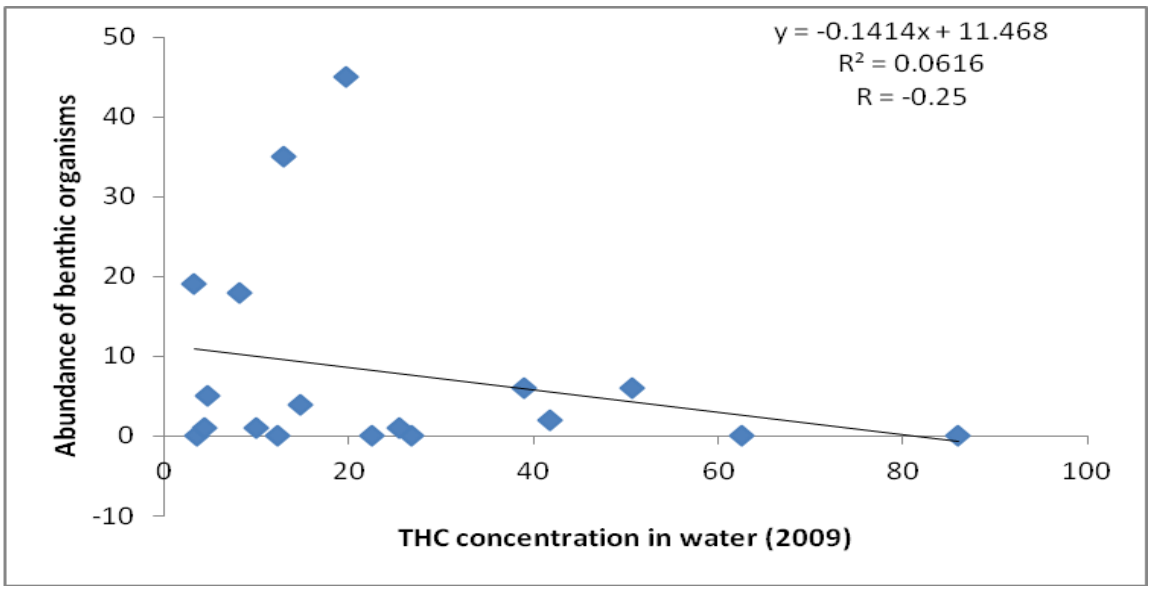


Fig 4.18: Relationship between abundance of macrobenthic invertebrates and THC concentration in water in the Lagos lagoon in 2009.

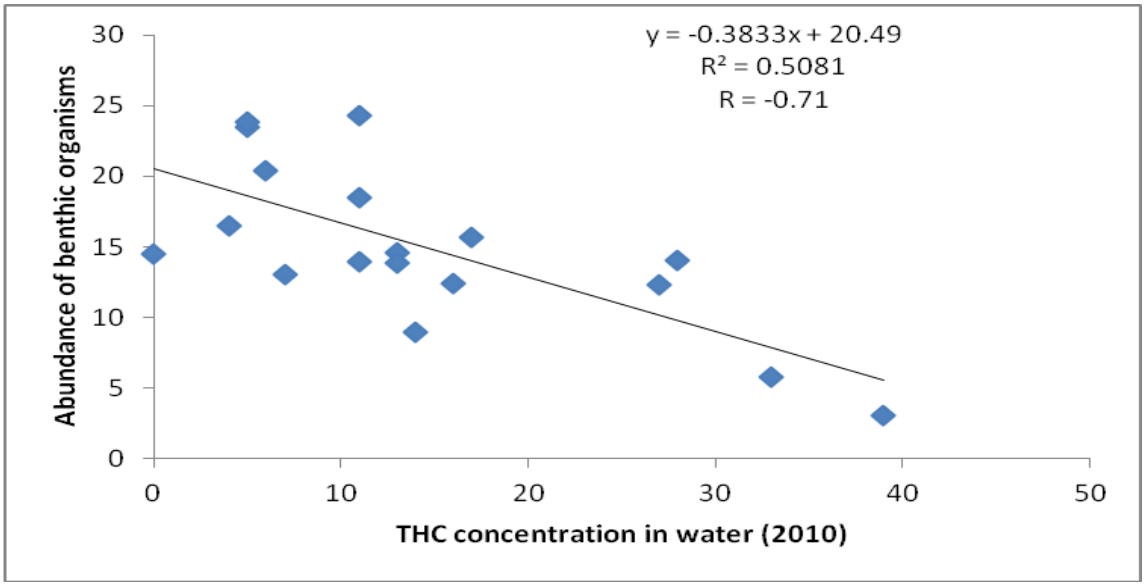


Fig 4.19: Relationship between abundance of macrobenthic invertebrates and THC concentration in water in the Lagos lagoon in 2010

4.1.6 Particle Sizes of Sediment from the Lagos lagoon

The percentage sand, silt and clay for the study stations in 2009 and 2010 are illustrated in Figures 4.20 and 4.21 respectively. The study area was predominantly sand intermixed with varying proportions of clay and silt in the stations sampled. The highest value of sand fraction (94.81%) recorded occurred in station 9 (Atlas cove) in sampling year 2009, while the least (30.17%) fraction of sand was recorded in station 14 (Apapa). The highest (37.24%) and least (1.97%) fraction of silt occurred in station 14 and 10 respectively in 2009. The highest (41.00%) and least (2.40%) fraction of clay occurred in station 8 and 9 respectively in year 2009 (Figure 4.20).

The highest value of sand fraction (98.42%) recorded occurred in station 12 (Atlas cove) in sampling year 2010, while the least (20.46%) fraction of sand was recorded in station 1(Unilag). The highest (42.30%) and least (0.97%) fraction of silt occurred in station 1(Unilag) and 12 (Atlas Cove) respectively in 2010. The highest (43.17) and least (0.01) fraction of clay occurred in station 13 (Apapa) and 6(Iddo) respectively (Figure 4.21).

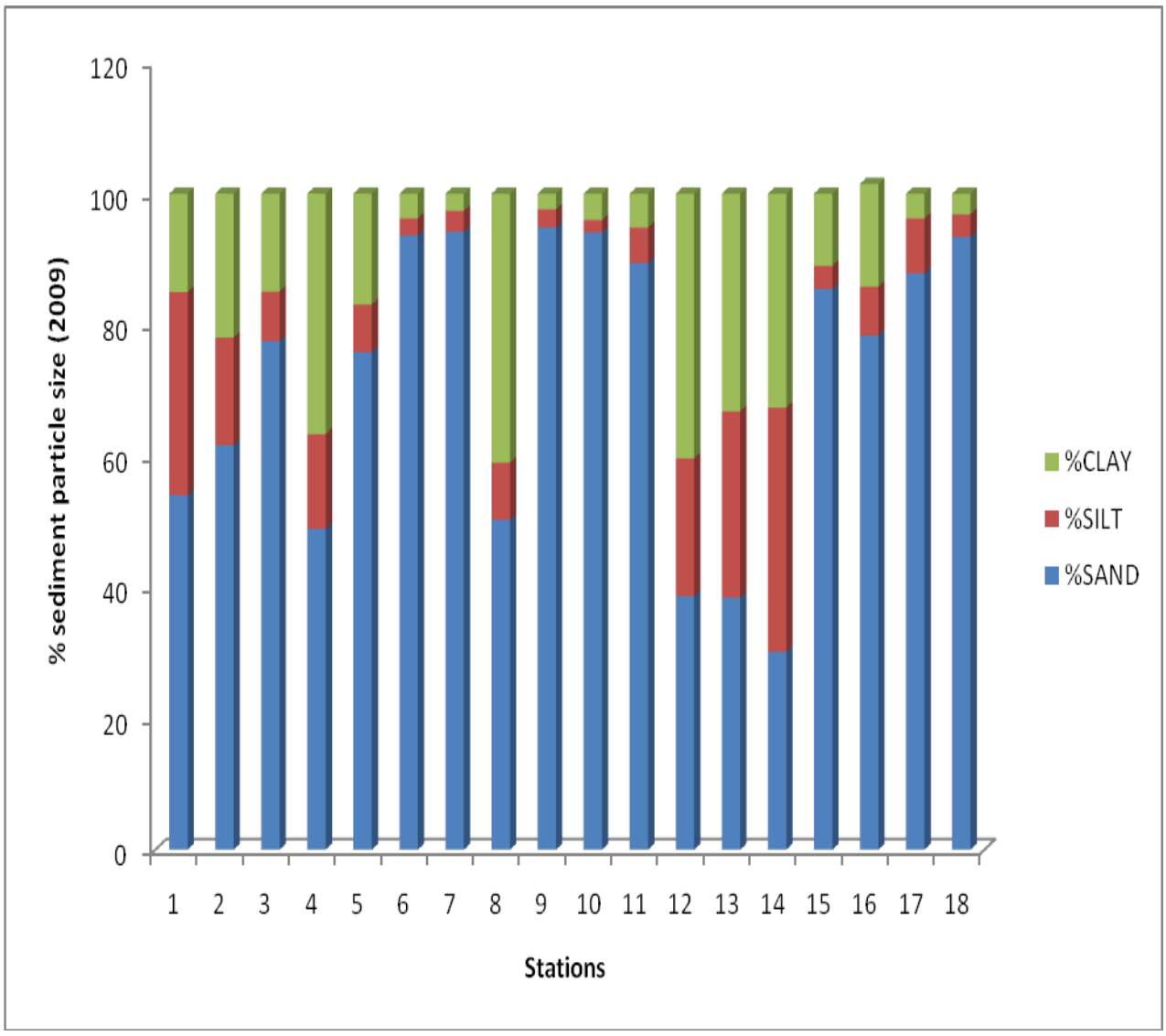


Fig.4.20: Percentage Sand, Silt and Clay at the study stations in the Lagos Lagoon in 2009

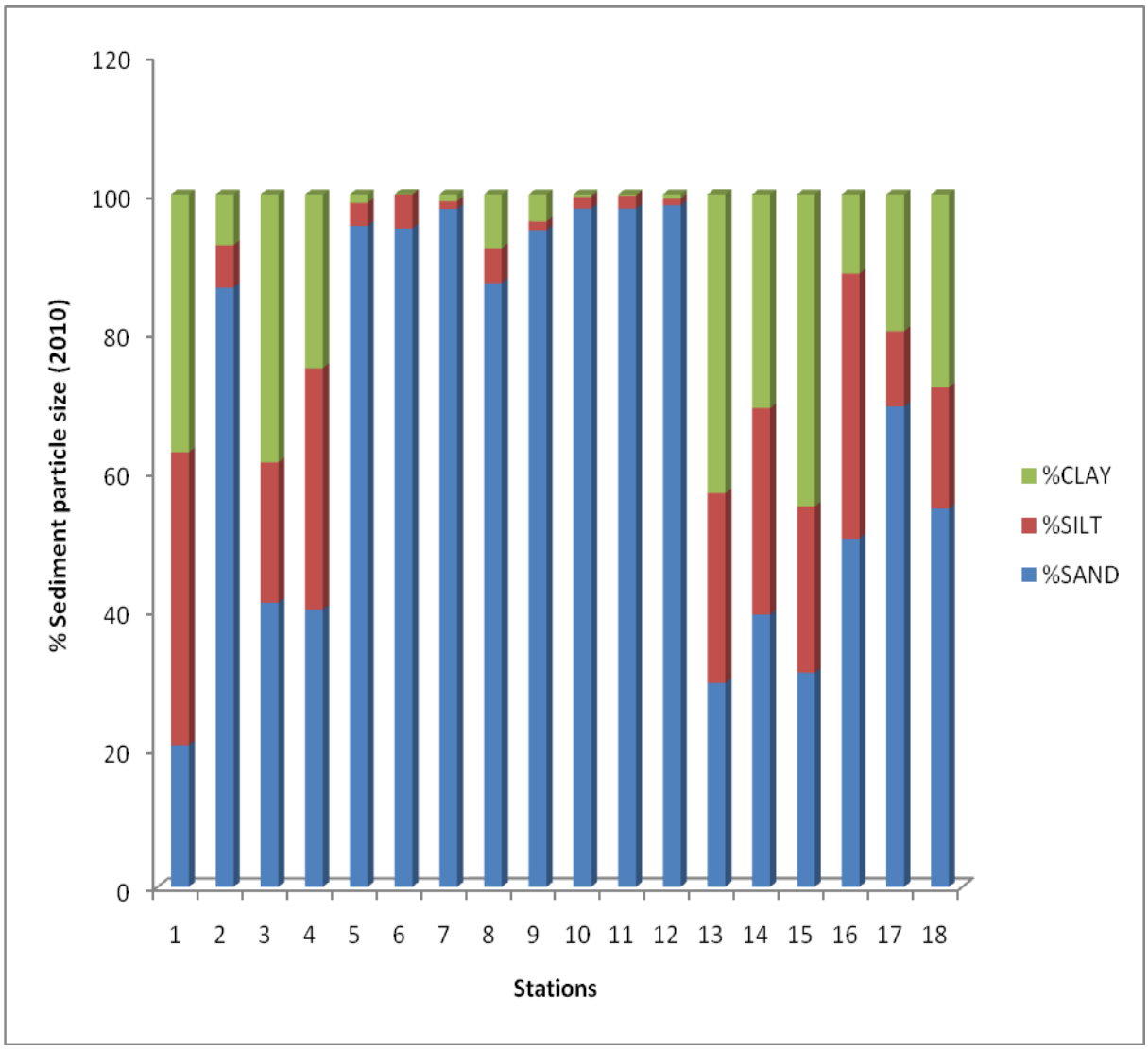


Fig. 4.21: Percentage Sand, Silt and Clay at the study stations in the Lagos Lagoon in 2010

4.2 FIELD STUDIES: TERRESTRIAL ECOSYSTEM

4.2.1 Distribution of Physico-Chemical Parameters of Groundwater Samples in Ijegun during the 2009 and 2010 Sampling Year

a) Hydrogen ion concentration (pH)

In 2009, the pH value ranged from 6.32 to 7.46. The pH of the groundwater samples vary between 6.50 and 7.48 in 2010. The pH for the two years was within the WHO standard for drinking water (Tables 4.5 and 4.6).

b) Conductivity

There were marked variations in conductivity. In 2009, conductivity values varied between 202.00 and 721.00 µS/cm. In 2010, the value ranged from 125.50 to 354.00 µS/cm. All were within the WHO standard of 500 µS/cm except samples collected beyond the 500m in 2009 which was 721.00 µS/cm (Tables 4.5 and 4.6).

c) Total dissolved solid (TDS)

Total dissolved solids were within WHO standard of 500mg/l in year 2009 and 2010. In 2009, TDS values ranged from 110.30 to 396.50mg/l. Values between 64.00 and 184.00mg/l was observed in 2010 (Tables 4.5 and 4.6).

HEAVY METALS

d) Nickel

In 2009, most of the groundwater sampled had concentrations of Nickel which were higher than the WHO standard of 0.07mg/l. The level of nickel in the contaminated groundwater within the 100m, 150m, 200m, 250m and beyond 500m buffer zones in 2009, was higher than the WHO

Table 4.5: Physico – chemical parameters measured in the groundwater samples in Ijegun in 2009

Parameters	**Buffer Zones**									**WHO**
	100m	**150m**	**200m**	**250m**	**300m**	**400m**	**500m**	**B500m**	**Control**	
pH	6.99± 0.49	7.31± 0.34	7.46± 0.27	7.14± 0.34	6.62± 0.29	6.72± 0.69	6.32± 0.37	6.73± 0.92	7.38± 0.95	6.5-8.5
Conductivity(µS/ cm)	202.00± 154.50	224.50± 21.29	433.67± 34.65	384.00± 110.31	330.00± 62.23	360.00± 66.53	336.00± 52.33	721.00± 59.4	421.00± 20.44	500.00
TDS (mg/l)	110.30± 84.91	123.50± 10.61	226.00± 27.5	211.00± 60.81	161.50± 28.99	196.3± 36.85	184.50± 28.90	396.50± 33.23	420.00± 34.27	500.0
Nickel (mg/l)	0.15± 0.06	0.19± 0.12	0.12± 0.02	0.09± 0.02	0.07± 0.09	0.06± 0.06	0.01± 0.00	0.15± 0.01	ND	0.07
Lead (mg/l)	0.09± 0.07	0.14± 0.09	0.11± 0.13	0.42± 0.42	0.18± 0.13	0.12± 0.11	0.22± 0.29	0.01± 0.00	ND	0.01
Zinc (mg/l)	0.07± 0.05	0.06± 0.08	0.04± 0.004	0.01± 0.02	0.04± 0.004	0.04± 0.004	0.01± 0.02	0.02± 0.01	0.01± 0.00	5.00

Table 4.6: Physico – chemical parameters measured in the groundwater samples in Ijegun in 2010

Parameters	**Buffer Zones**									**WHO**
	100m	**150m**	**200m**	**250m**	**300m**	**400m**	**500m**	**B500m**	**Control**	
pH	6.50± 0.32	7.39± 0.57	7.17± 0.35	7.48± 0. 04	6.81± 0.71	6.52± 0.21	6.68± 0.44	7. 01± 0.48	7.51± 0.89	6.5-8.5
Conductivity(µS/cm)	354.00± 65.55	332.50± 161.93	282.00± 64.21	274.00± 96.17	125.50± 14.85	137.50± 132..23	131.00± 123.04	243.67± 180.38	206.01± 18.43	500.00
TDS (mg/l)	184.00± 26.0	173.00± 84.0	86.00± 75.00	142.00± 49.00	64.00± 7.10	72.00± 69.0	68.00± 64.0	127.00± 115.00	107± 15.96	500.0
Nickel (mg/l)	0. 01±0	0. 01±0	ND	ND	ND	ND	ND	ND	ND	0.07
Lead (mg/l)	0. 01±0	0. 01±0	ND	ND	ND	ND	ND	ND	ND	0.01
Zinc (mg/l)	0. 09±0	ND	ND	ND	ND	ND	ND	ND	ND	5.00

ND: Not detected

WHO: World Health Organisation.(2008). *Guidelines for Drinking-water Quality*. Third Edition Incorporating the First and Second Addenda, Volume 1: Recommendations. Geneva.

limit of 0.07mg/l. The highest value of 0.19mg/l was recorded in the 150m buffer zone. In 2010, Nickel was not detected in most buffer zones. Concentration of 0.01mg/l was observed in 100m and 150m buffer zones in 2010. In the control station, nickel was not detected (Tables 4.5 and 4.6).

e) Lead

Concentration of Lead was found to be very high ranging from 0.01mg/l to 0.42mg/l in 2009, which was higher than the WHO standard of 0.01mg/l. In year 2010, values of lead in groundwater samples were within the WHO standard and lead was not detected in some buffer zones. In the control station (B500m), lead was not detected (Tables 4.5 and 4.6).

f) Zinc

In 2009 and 2010, the concentration of zinc in the groundwater samples was within the WHO standard of 5.00mg/l. Values ranged from 0.01 to 0.07mg/l in 2009. Zinc was not detected in most of the buffer zones in 2010 (Tables 4.5 and 4.6).

4.2.2 Distribution of BTEX Compounds in Ijegun Groundwater and Soil in 2009 and 2010

Mean concentrations of BTEX compounds in groundwater samples collected from stations in different buffer zones away from the point of explosion is presented in Figures 4.22 and 4.23. The groundwater samples collected within the 100m and 150m buffer zones away from the explosion point had very high values for the BTEX compounds (monoaromatic hydrocarbons). The highest concentrations of Benzene (16.65 µg/l), Toluene (2.08 µg/l) and Xylene (4864.79 µg/l) were found in stations within the 100m buffer zone. Total BTEX was highest within the

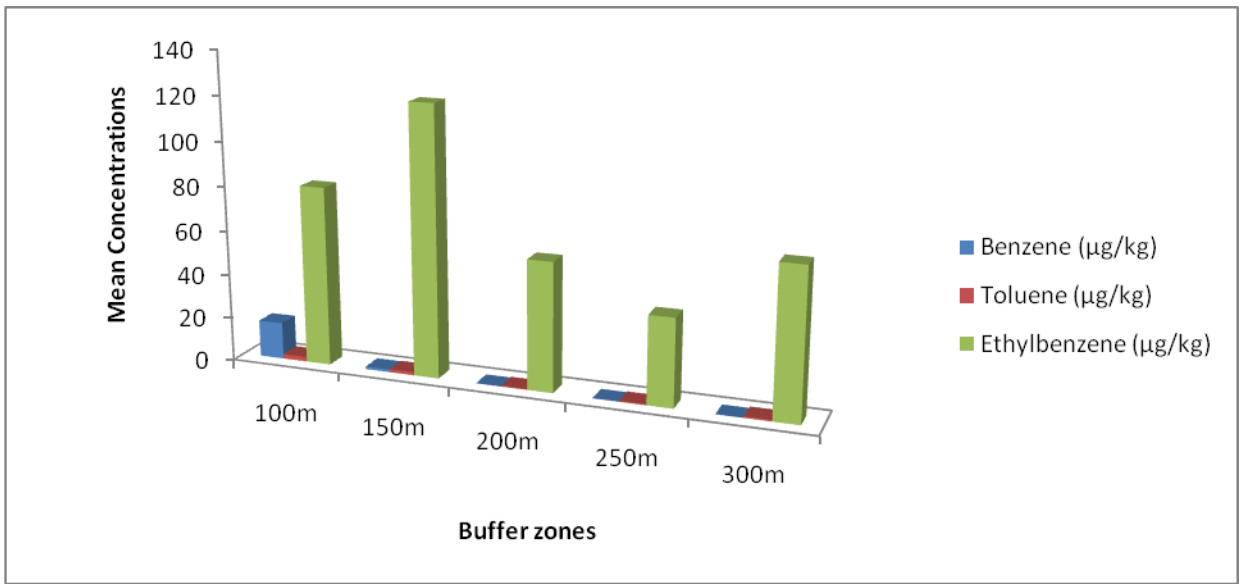


Fig 4.22: Mean concentrations of BTE compounds in groundwater samples collected from stations in different buffer zones away from the point of explosion in 2009 and 2010.

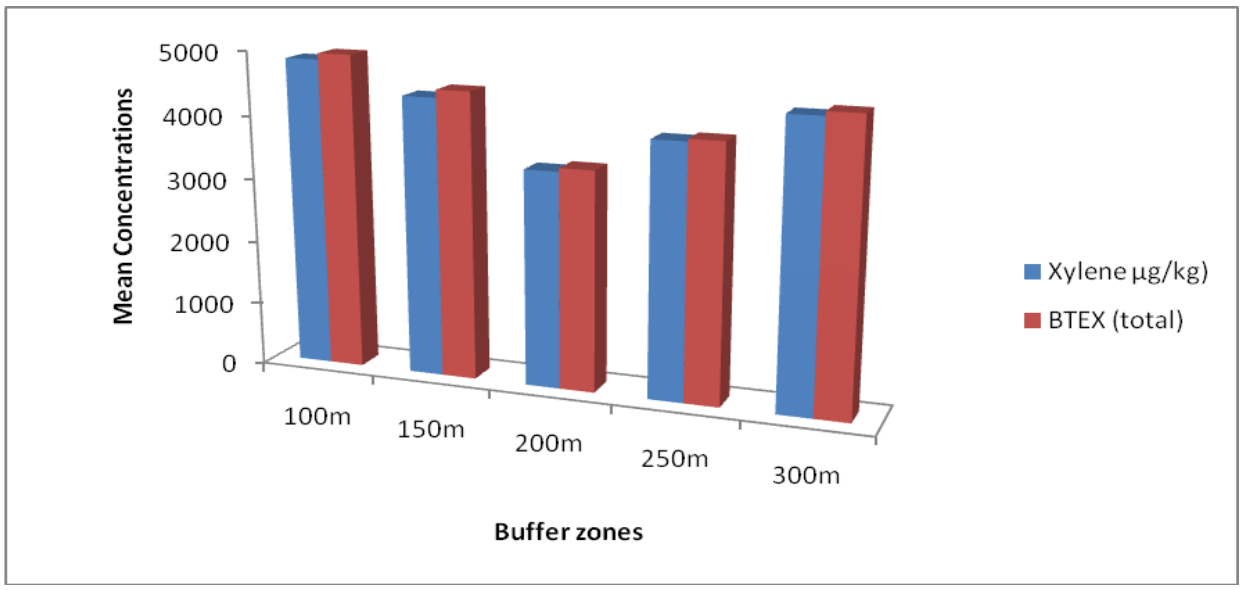


Fig 4.23: Mean concentrations of Xylene and total BTEX compounds in groundwater samples collected from stations in different buffer zones away from the point of explosion in 2009 and 2010

100m buffer zone, 4964.33 µg/l (Figure 4.23). Concentrations of BTEX compounds in the groundwater were higher than concentrations in the soil samples.

Mean concentrations of BTEX compounds in soil samples collected from stations in different buffer zones away from the point of explosion is presented in Figures 4.24 and 4.25. Lowest concentrations of BTEX compounds were observed within the 250m buffer zones. Soil samples analysed had concentrations ranging from 0.02 µg/kg to 0.15 µg/kg for Benzene. Concentrations of Toluene ranged from 0.39 µg/kg (250m) to 0.77 µg/kg (150m). Concentrations of Ethylbenzene ranged from 0.12 µg/kg (250m) to 7.49 µg/kg (150m) (Figure 4.24). Concentrations of Xylene ranged from 408 µg/kg (250m) to 1167 µg/kg (150m). Concentrations of total BTEX ranged from 409.07 µg/kg (250m) to 1176 µg/kg (150m) (Figure 4.25).

4.2.3 Distribution of THC in Groundwater samples collected around the oil explosion impacted area of Ijegun.

During the 2009/2010 sampling years, the levels of THC measured in the ground water samples collected from the impacted areas of Ijegun were found to have varying concentrations of THC (Figures 4.26). Statistical comparison by Analysis of Variance (ANOVA) of THC in groundwater samples from the locations sampled show that there was significant ($P< 0.05$) difference between the years (2009 and 2010). It was observed that the THC values for year 2009 were higher than the values for year 2010. The concentrations of Total Hydrocarbon in groundwater around buffer zones in 2009 ranged from 2.00 mg/l to 689.12 mg/l (Figure 4.26) while in 2010, the mean THC values in groundwater around the buffer zones were lower and ranged from 5.10 mg/l to 15.35mg/l (Figure 4.26). The highest THC values observed during the two year sampling period occurred in 2009 dry season.

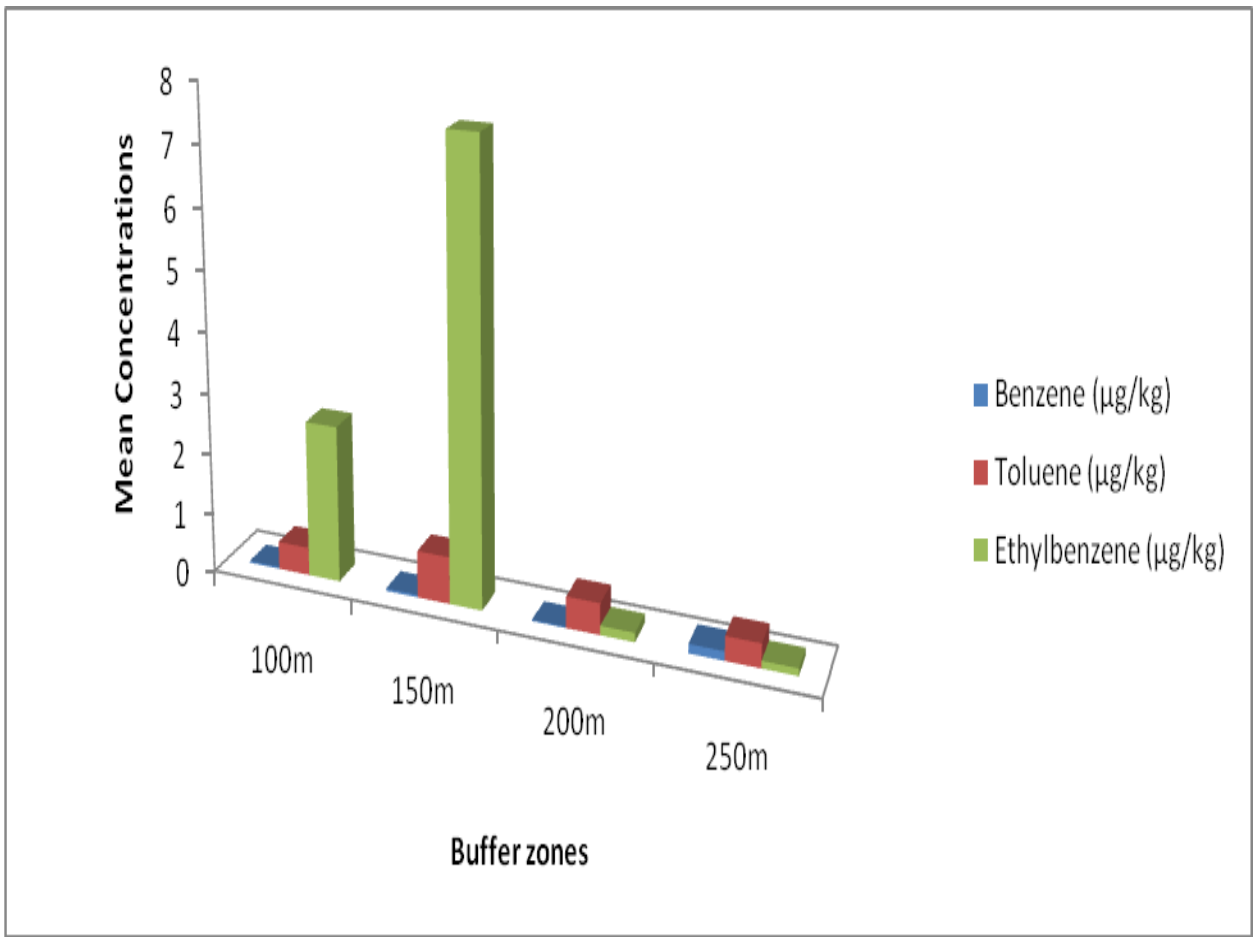


Fig 4.24: Mean concentrations of BTE compounds in soil samples collected from stations in different buffer zones away from the point of explosion in 2009 and 2010.

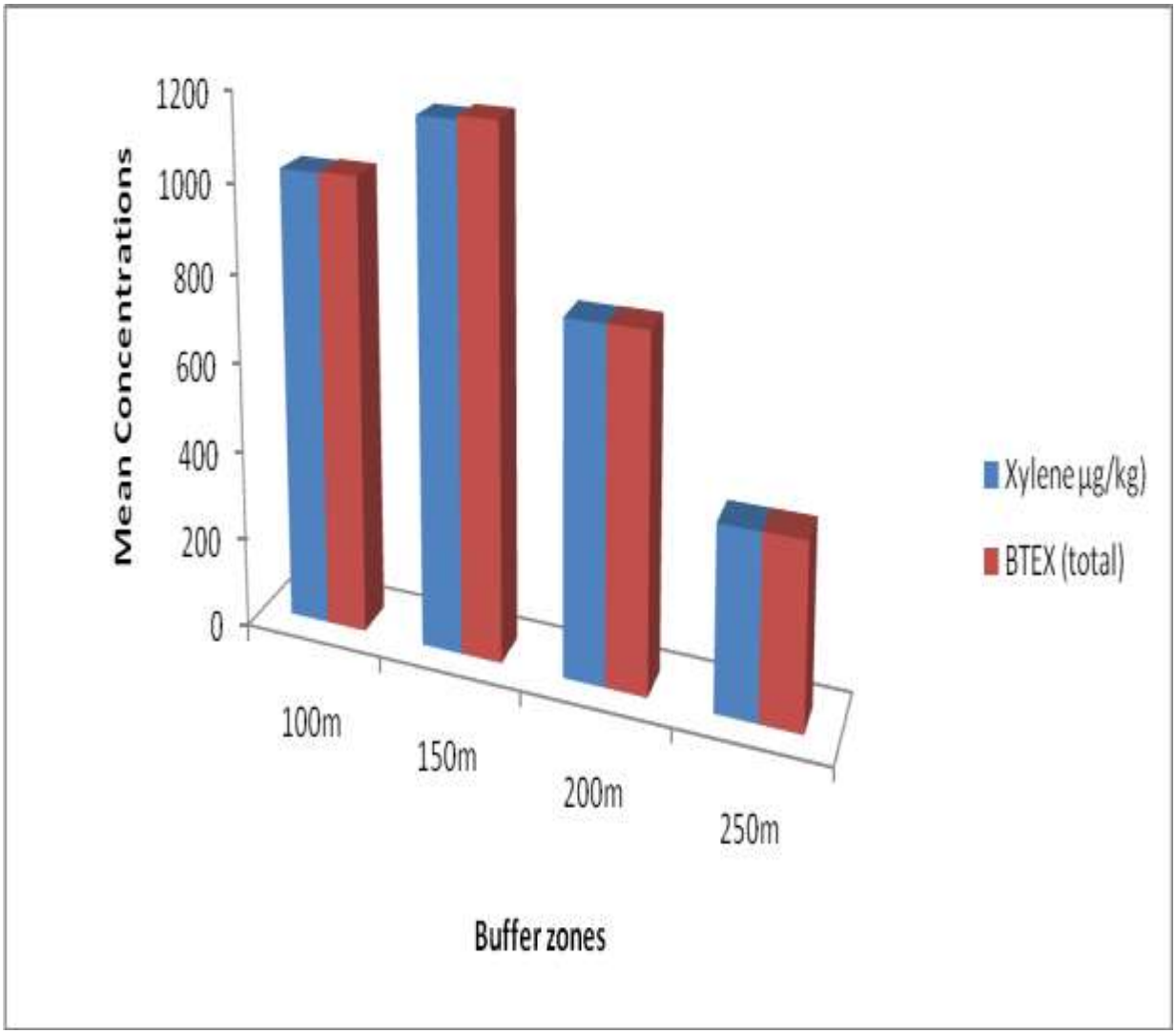


Fig 4.25: Mean concentrations of Xylene and total BTEX compounds in soil samples collected from stations in different buffer zones away from the point of explosion in 2009 and 2010.

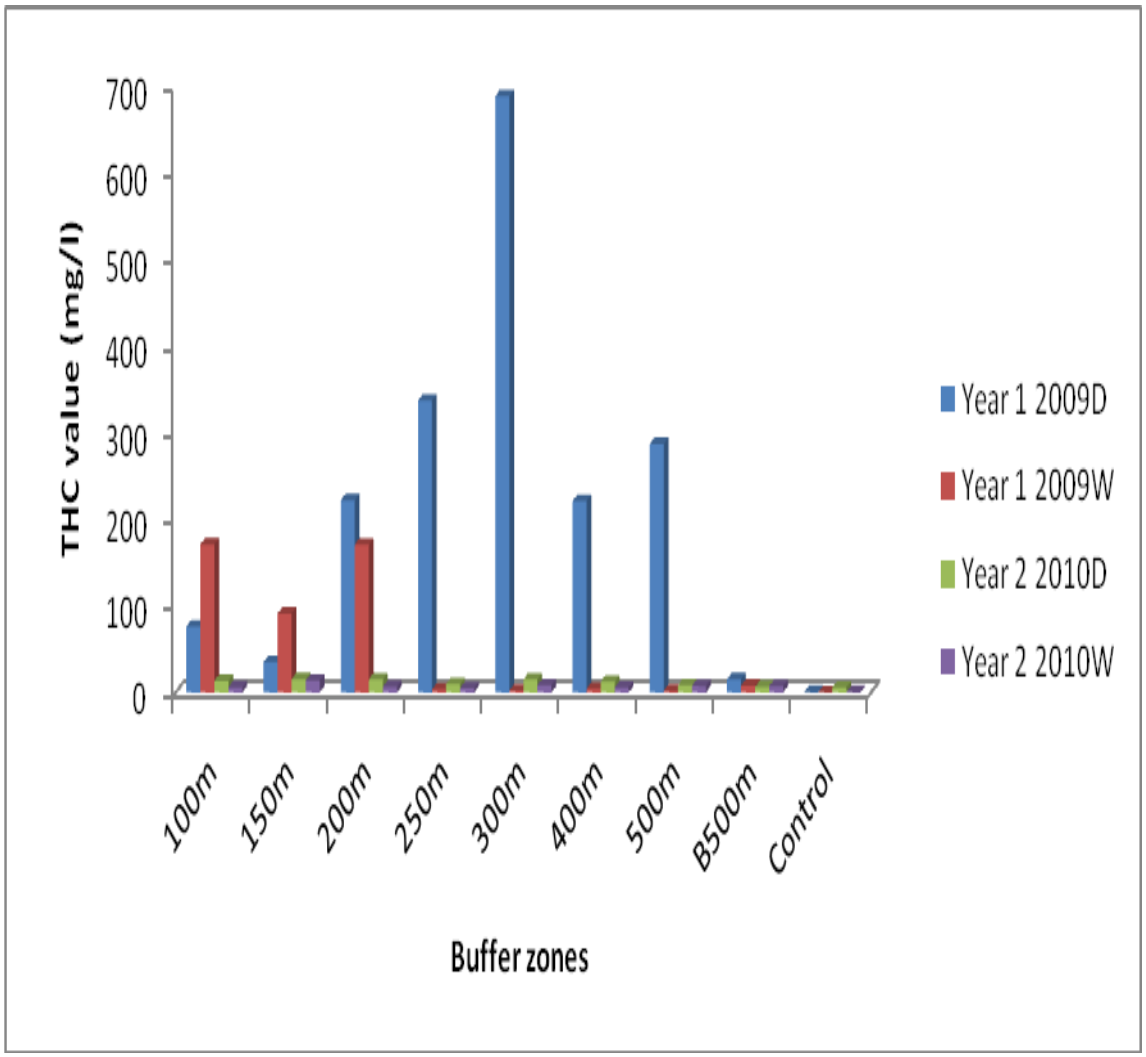


Fig 4.26: Mean value of THC (mg/l) in groundwater samples collected from different buffer zones away from the explosion point in wet and dry seasons of 2009 and 2010. W- wet season; D- dry season

Comparing the control station with the other stations within the oil impacted area, the control station was observed to have lower values of THC. During the two year sampling period, the value of THC in the control station ranged from 0.38 mg/l to 5.75 mg/l. In the 100m, THC values ranged from 5.97 mg/l to 171.12 mg/l. In the 150m buffer zone, concentrations ranged from 13.49 mg/l to 91.25 mg/l. Values ranged from 6.57mg/l to 222.17mg/l in the 200m buffer zone. Concentrations of Total Hydrocarbon in the 250m buffer zone was between 3.58 mg/l and 337.35 mg/l for the two year period. There were varying degrees of THC contamination in all the groundwater sampled. There was no significant difference in THC concentrations among the buffer zones but there was significant difference between the buffer zones and the control station. Comparing the results of THC with the World Health Organisation (WHO) maximum admissible value of 0.1mg/l for groundwater. All the ground water sampled during the study were above the WHO limit of 0.01mg/l. Two years after the incident in 2010, all the groundwater were still contaminated and above the limit

4.2.4 Distribution of THC in soil samples collected around the oil explosion impacted area of Ijegun

During the 2009/2010 sampling years, the levels of THC measured in soil samples collected from the impacted areas of Ijegun were found to have varying concentrations of THC (Figures 4.27). Statistical comparison by Analysis of Variance (ANOVA) of THC in soil samples from the locations show that there was significant ($P< 0.05$) difference between the years (2009 and 2010). It was observed that the THC values for year 2009 were higher than the values for year 2010. The concentrations of Total Hydrocarbon in soil around the oil impacted area in 2009 ranged from 4.06 mg/kg to 401.41 mg/kg (Figure 4.27) while in 2010, the mean THC values

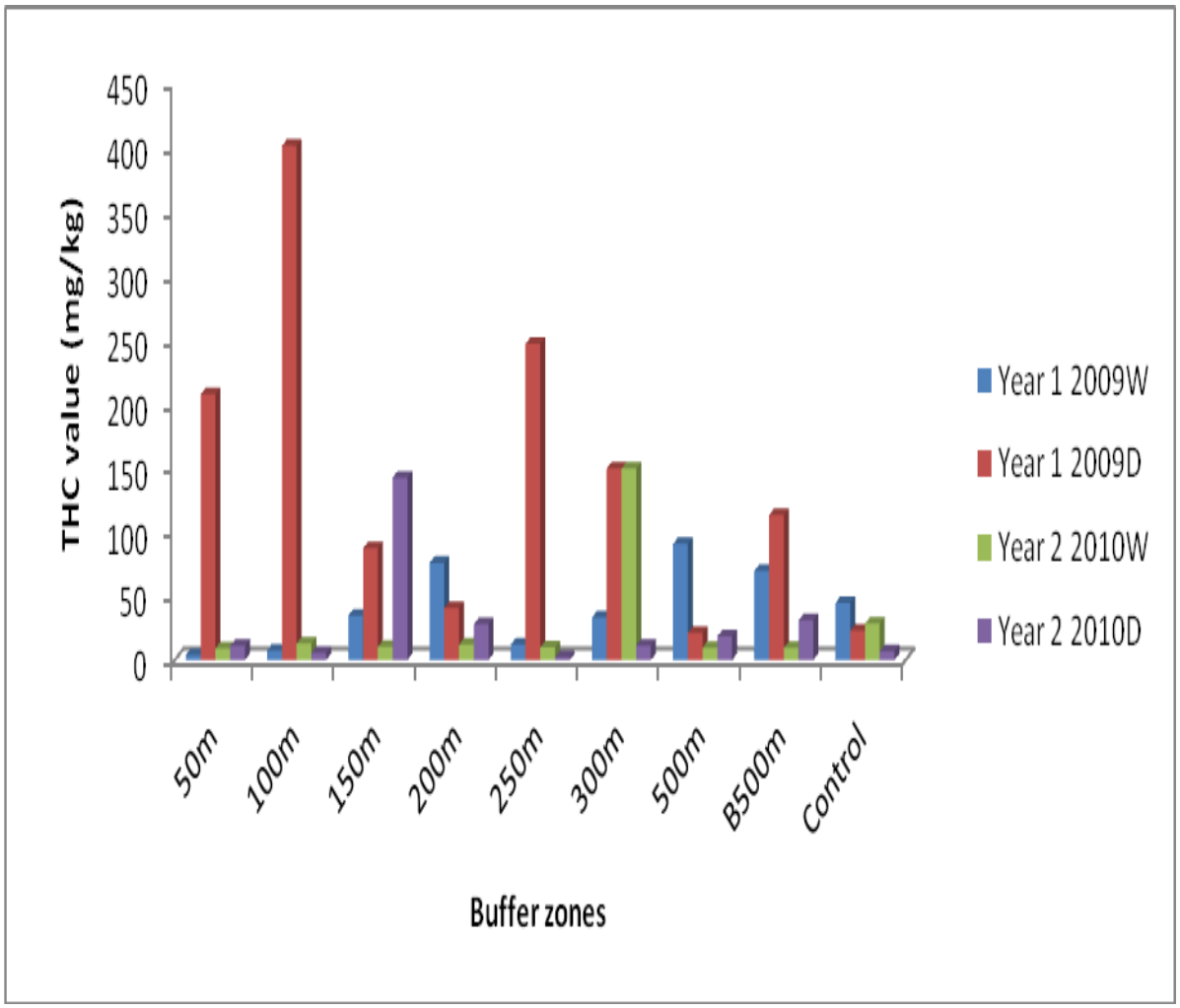
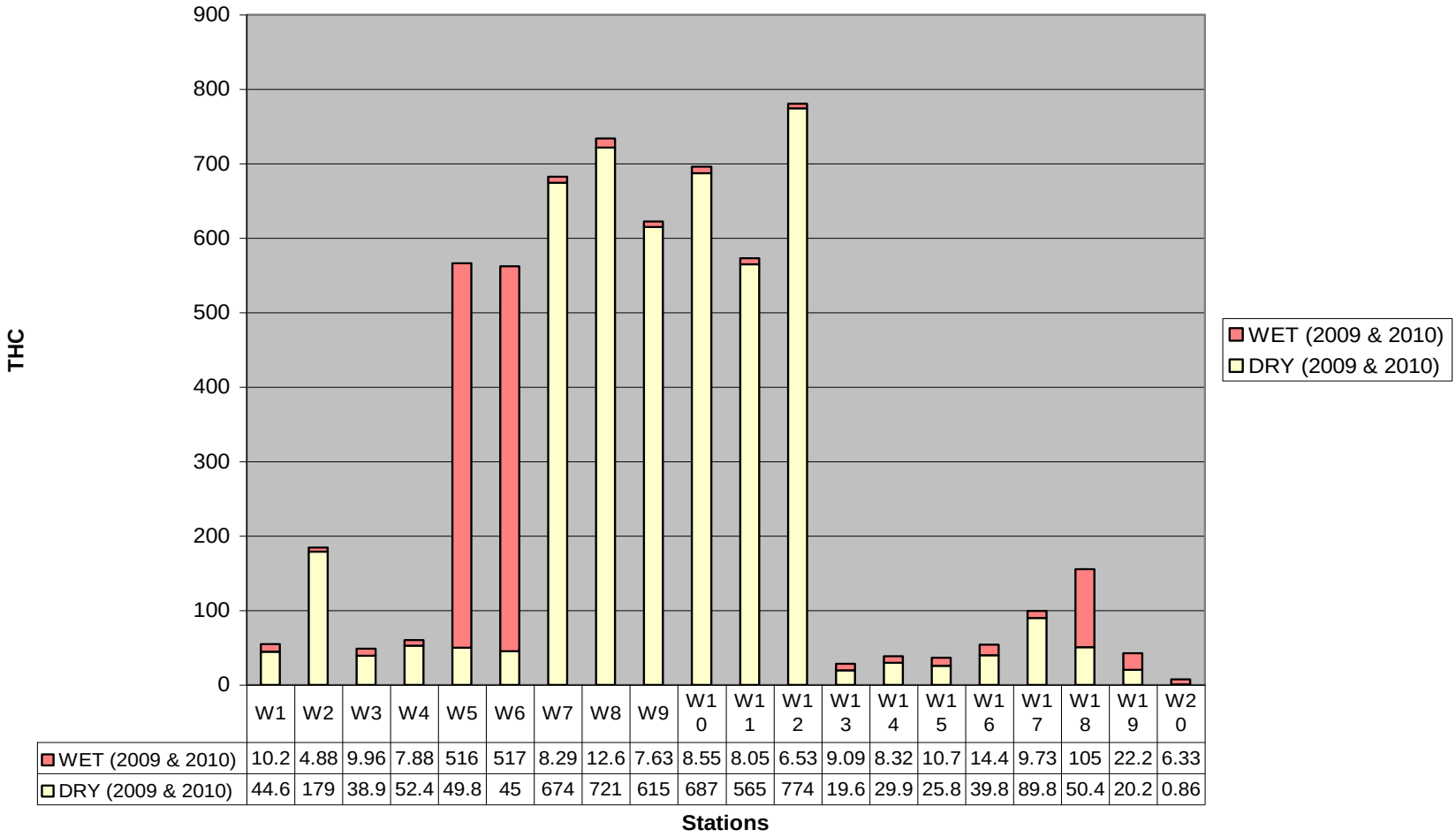


Fig 4. 27: Mean value of THC (mg/kg) in Ijegun soil samples collected from different buffer zones away from the explosion point in wet and dry seasons 2009 and 2010. W-wet season; D- dry season

in soil samples around the impacted area were lower and ranged from 2.16 mg/kg to 149.48mg/kg (Figure 4.27). The highest THC values observed during the two year sampling period occurred in 2009 dry season. Comparing the control station with the other stations within the impacted area, there were varying degrees of THC contamination in all the soil sampled. There was no significant difference in THC concentrations among the buffer zones and between the buffer zones and the control station. During the two year sampling period, the value of THC in the control station ranged from 7.03 mg/kg to 44.61 mg/kg. In the 50m buffer zone, THC values ranged from 4.06 mg/kg to 207.48 mg/kg. In the 100m buffer zone, concentrations ranged from 5.55 mg/kg to 401.41mg/kg. Values ranged from 10.43 mg/kg to 142.39 mg/kg in the 150m buffer zone. Concentrations of Total Hydrocarbon in the 200m buffer zone was between 11.91 mg/kg and 75.84 mg/kg for the two year period (Figure 4.27).

4.2.5 Seasonal Variation of Total Hydrocarbon Content in groundwater and soil samples collected from the Lagos lagoon

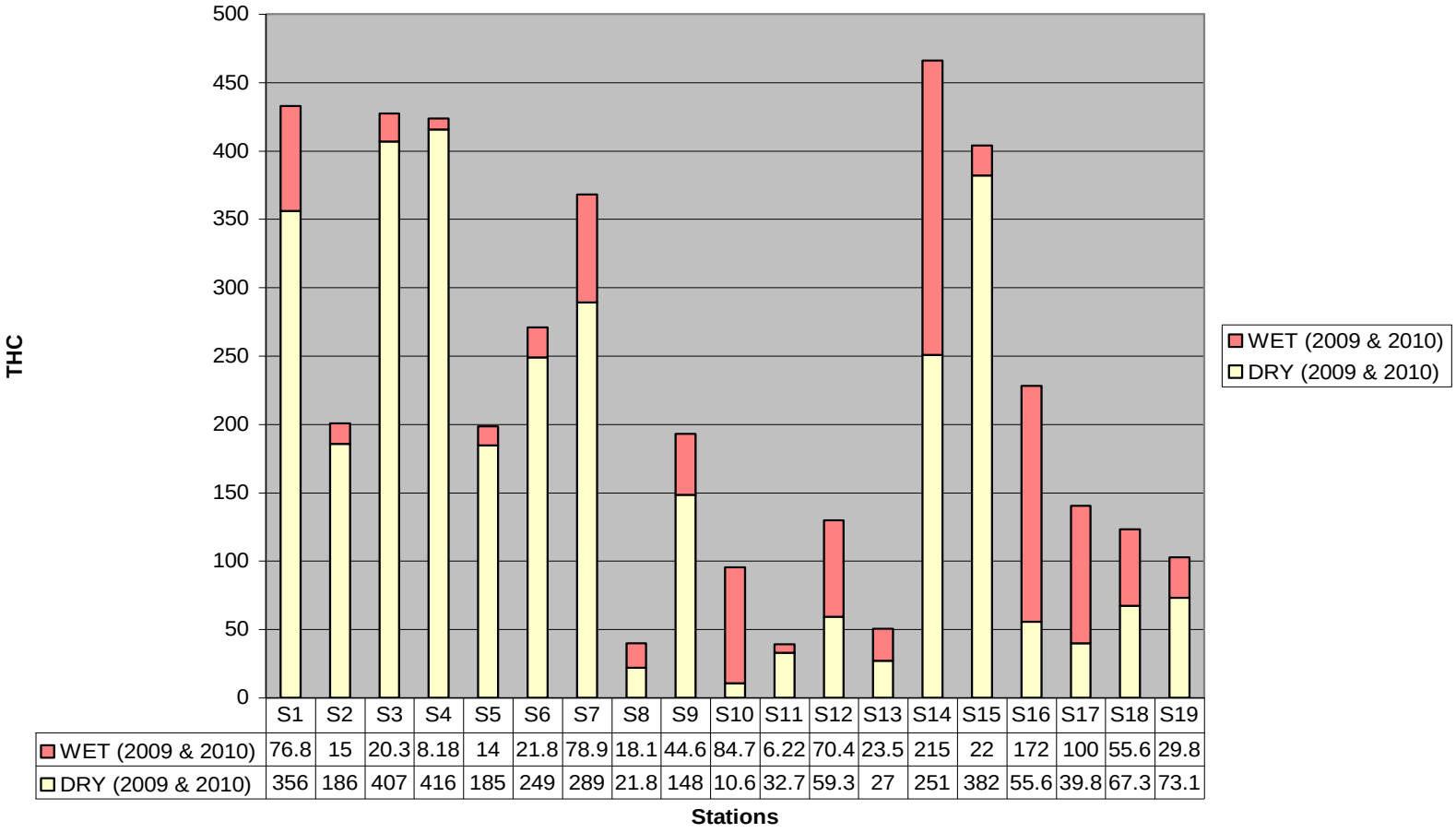
Seasonal variation in the THC in groundwater samples collected from different locations around the oil impacted area in 2009 and 2010 is presented in Figure 4.28. There was significant ($P < 0.05$) difference in the THC in groundwater, between the seasons (dry and wet) with the THC values for dry seasons being significantly higher (Figure 4.28). Stations W7 to W12 (Figure 4.28) had very high values of THC in the dry seasons which were between 565.00 mg/l and 774.00 mg/l, while lower values between 6.53mg/l and 12.6mg/l were recorded in the wet seasons over the two year sampling period. Although there were instances where the THC for the wet seasons were higher than THC for the dry seasons, specifically, stations W5 and W6 had higher values in the wet seasons than in the dry seasons (Figure 4.28).



	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20
WET (2009 & 2010)	10.2	4.88	9.96	7.88	516	517	8.29	12.6	7.63	8.55	8.05	6.53	9.09	8.32	10.7	14.4	9.73	105	22.2	6.33
DRY (2009 & 2010)	44.6	179	38.9	52.4	49.8	45	674	721	615	687	565	774	19.6	29.9	25.8	39.8	89.8	50.4	20.2	0.86

Fig.4.28: Seasonal variation in the values of THC (mg/l) in groundwater samples collected from different locations around the oil impacted area in Ijegun (2009/2010).

Seasonal variation in the values of THC in soil samples collected from different locations around the oil impacted area in 2009 and 2010 is presented in Figure 4.29. There was significant ($P < 0.05$) difference in the THC in soil, between the seasons (dry and wet) with the THC values for dry seasons being significantly higher (Figure 4.29). Stations S1 to S4 had very high values of THC in the dry seasons, which were between 186.00 and 416.00 mg/kg, while lower values between 8.18 and 76.80 mg/kg were recorded for the wet seasons (Figure 4.29).



	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
WET (2009 & 2010)	76.8	15	20.3	8.18	14	21.8	78.9	18.1	44.6	84.7	6.22	70.4	23.5	215	22	172	100	55.6	29.8
DRY (2009 & 2010)	356	186	407	416	185	249	289	21.8	148	10.6	32.7	59.3	27	251	382	55.6	39.8	67.3	73.1

Fig. 4.29: Seasonal variation in the values of THC (mg/kg) in soil samples collected from different locations around the oil impacted area in Ijegun (2009/2010).

4.3 LABORATORY STUDIES

4.3.1 ACUTE TOXICITY OF BTEX COMPOUNDS (TOXICITY RANKING ORDER OF TEST COMPOUNDS) AGAINST EACH TEST SPECIES

4.3.1.1 Acute toxicity of BTEX compounds against *Clarias gariepinus*

On the basis of $96hLC_{50}$ values, Ethylbenzene with value of 0.479 ml/l was the most toxic compound tested against *C. gariepinus* followed by Xylene (0.519ml/l), Benzene (0.666ml/l) and Toluene (1.190 ml/l) in a descending order of toxicity (Table 4.7). Computed toxicity factor ($96hLC_{50}$ ratios) showed that Ethylbenzene was about 1.08x, 1.40x, 2.48x, more toxic than Xylene, Benzene and Toluene respectively when tested against *C. gariepinus* (Table 4.7).

4.3.1.2 Acute toxicity of BTEX compounds against *Eudrilus eugeniae*

On the basis of $96hLC_{50}$ values, xylene with value of 1.212 mg/kg was the most toxic compound tested against *E. eugeniae* followed by Toluene, Ethylbenzene and Benzene ($96hLC_{50}$ = 1.896 mg/kg) in a descending order of toxicity (Table 4.8). Computed toxicity factor ($96hLC_{50}$ ratios) showed that xylene was about 1.10x, 1.13x, 2.41x, more toxic than toluene, ethylbenzene and benzene respectively when tested against *E. eugeniae* (Table 4.8).

4.3.1.3 Relative susceptibility (responses) of test animal species to BTEX compounds

A. **Responses of test species to Benzene**

C. gariepinus was the most susceptible/sensitive test animal to benzene, with a response level based on $96hLC_{50}$ value of 0.666ml/l. *E. euginiae* was more tolerant with $96hLC_{50}$

Table 4.7: Relative acute toxicity of benzene, toluene, ethylbenzene and xylene acting singly against *C. gariepinus*

Test chemicals	**LC_{50} (95% CL)**	**Slope±S.E.**	**Probit line equation**	**T.F.**
Benzene	0.666(1.047-0.332)	1.385± 0.276	Y = 0.244 + 1.385x	1.40
Toluene	1.190(1.309-1.068)	5.499 ±1.249	Y = -0.416 + 5.499x	2.48
Ethylbenzene	0.479(0.619-0.375)	2.064 ±0.448	Y = 0.659 + 2.064x	1.00
Xylene	0.519(0.708-0.380)	1.752 ±0.273	Y = 0.499 + 1.752x	1.08

C.L. = 95% confidence limit

S.E = standard error

D.F = degrees of freedom

T.F = toxicity factor

$$\text{T.F Toxicity factor} = \frac{\text{96h } LC_{50} \text{ value of other chemical}}{\text{96h } LC_{50} \text{ value of most toxic chemical chemical}}$$

Table 4.8: Relative acute toxicity of benzene, toluene, ethylbenzene and xylene acting singly against *E. eugeniae*

Test chemicals	**LC_{50} (95% CL)**	**Slope±S.E.**	**Probit line equation**	**T.F.**
Benzene	1.896(4.600-1.046)	1.792±0.669	Y= -0.498 + 1.792x	2.41
Toluene	1.335(4.565-0.824)	2.129±0.755	Y= -0.267+ 2.129x	1.10
Ethylbenzene	1.366(2.632-0.861)	2.502±0.874	Y= -0.338 + 2.502x	1.13
Xylene	1.212(3.292-0.758)	2.191±0.751	Y= -0.183+ 2.191x	1.00

C.L. = 95% confidence limit

S.E = standard error

D.F = degrees of freedom

T.F = toxicity factor

$$\text{T.F Toxicity factor} = \frac{\text{96h } LC_{50} \text{ value of other chemical}}{\text{96h } LC_{50} \text{ value of most toxic chemical chemical}}$$

value of 1.896ml/l in a descending order of susceptibility (Table 4.9). On the basis of the computed susceptibility factor, *C. gariepinus* was found to be about 2.85 times more susceptible to the toxic effect of benzene than *E. eugeniae.*

B. **Responses of test species to Toluene**

C. gariepinus was the most susceptible/sensitive test animal to toluene, with a response level based on 96hLC_{50} value of 1.190ml/l. *E. eugeniae* was more tolerant with 96hLC_{50} value of 1.335ml/l in a descending order of susceptibility (Table 4.9). On the basis of the computed susceptibility factor, *C. gariepinus* was found to be about 1.12 times more susceptible to the toxic effect of toluene than *E. eugeniae.*

C. **Responses of test species to Ethylbenzene**

C. gariepinus was the most susceptible/sensitive test animal to ethylbenzene, with a response level based on 96hLC_{50} value of 0.479ml/l. *E. eugeniae* was more tolerant with 96hLC_{50} value of 1.366ml/l in a descending order of susceptibility (Table 4.9). On the basis of the computed susceptibility factor, *C. gariepinus* was found to be about 2.85 times more susceptible to the toxic effect of ethylbenzene than *E. eugeniae.*

D. **Responses of test species to Xylene**

C. gariepinus was the most susceptible/sensitive test animal to xylene, with a response level based on 96hLC_{50} value of 0.519ml/l. *E. eugeniae* was more tolerant with 96hLC_{50} value of 1.212ml/l in a descending order of susceptibility (Table 4.9).

On the basis of the computed susceptibility factor, *C. gariepinus* was found to be about 2.34 times more susceptible to the toxic effect of xylene than *E. eugeniae.*

Table. 4.9: Relative susceptibility of the test animals against BTEX compounds.

Animals	**LC_{50} (95% CL)**	**Slope±S.E.**	**Probit line equation**	**S.F.**
Benzene				
Clarias gariepinus	0.666(1.047-0.332)	1.385 ±0.276	Y = 0.244 + 1.385x	1.00
Eudrilus eugeniae	1.896(4.600-1.046)	1.792±0.669	Y= -0.498 + 1.792x	2.85
Toluene				
Clarias gariepinus	1.190(1.309-1.068)	5.499 ±1.249	Y = -0.416 + 5.499x	1.00
Eudrilus eugeniae	1.335(4.565-0.824)	2.129±0.755	Y= -0.267+ 2.129x	1.12
Ethylbenzene				
Clarias gariepinus	0.479(0.619-0.375)	2.064 ±0.448	Y = 0.659 + 2.064x	1.00
Eudrilus eugeniae	1.366(2.632-0.861)	2.502±0.874	Y= -0.338 + 2.502x	2.85
Xylene				
Clarias gariepinus	0.519(0.708-0.380)	1.752 ±0.273	Y = 0.499 + 1.752x	1.00
Eudrilus eugeniae	1.212(3.292-0.758)	2.191±0.751	Y= -0.183+ 2.191x	2.34

C.L. = 95% confidence limit. S.E = standard error. D.F = degrees of freedom

T.F = toxicity factor

S.F Susceptibility factor = $\frac{\text{96h } LC_{50} \text{ value of other test animals}}{\text{96h } LC_{50} \text{ value of most sensitive test animals}}$

4.3.1.4. Physico-chemical Conditions in Bioassay during Toxicity Testing:

a) **Toxicity test (Aquatic)**

In all the bioassay, the dissolved oxygen contents of test media remained greater than 5.5mg/l over the test period during the acute and sublethal toxicity evaluations. The measured pH, temperature and dissolved oxygen values remained fairly constant at 7.5±0.3; 26 ± 3^{0}C and 7.0mg/l respectively from the beginning to the end of the bioassays.

b) **Toxicity test (Soil)**

The Physic-Chemical characteristics of soil used as substrate in the bioassays are given in Table 4.10. pH was 6.5.

Table 4.10: Physico-chemical characteristics of the soil substrate used for the earthworm bioassay

Parameters	**Mean values**
Phosphorus	0.68mg/kg
TOC (total organic carbon)	0.02%
Nitrogen	0.001%
pH	6.5
Conductivity	115 $uscm^{-1}$
Potassium	30.04mg/kg
Magnesium	282.72mg/kg
Calcium	518.32mg/kg
Sodium	55.58mg/kg

4.3.2 CHRONIC TOXICITY STUDIES

4.3.2.1 Antioxidant Enzymes and Non Enzymes Activity in the Organs of *C. gariepinus* Exposed to Sublthal Concentrations of Benzene, Toluene, Ethylbenzene and Xylene.

4.3.2.1.1 Superoxide dismutase activity in the gills

The activity of SOD in the gills of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 60days is presented in Figures 4.30. The results show that the activity of the enzyme SOD was inhibited in the gills of fishes exposed to BTEX compounds. Measurements of SOD activity in gills of fish exposed to sublethal concentration of the test chemicals over a 60-day period decreased significantly ($P<0.05$) when compared to day 0 (control animals) (Figure 4.30).

In the gills, level of SOD activities decreased from 102.42 ± 10.14 u/mg protein (day 0) to 12.12± 2.82 (benzene $1/10^{th}$), 6.48± 2.09 (benzene $1/100^{th}$), 7.37 ± 0.76 (toluene $1/10^{th}$), 12.0 ± 0.04 (toluene $1/100^{th}$), 30.00 ± 3.76 (ethylbenzene $1/10^{th}$), 20.28 ± 1.12 (ethylbenzene $1/100^{th}$), 16.73 ± 2.81 (xylene $1/10^{th}$) and 16.73 ± 2.43 u/mg protein (xylene $1/100^{th}$) in the exposed group of day 60. The percentage change (decrease) in SOD activities in the gills after exposure to low concentration ($1/100^{th}$) of Benzene, Toluene, Ethylbenzene and Xylene were 93.67 %, 88.28 %, 80.21% and 83.67% respectively when compared to control. The percentage decrease in SOD activities in the gills after exposure to high concentrations ($1/10^{th}$) of Benzene, Toluene, Ethylbenzene and Xylene were 88.17 %, 92.80%, 70.71% and 83.67% respectively when compared to control**.**

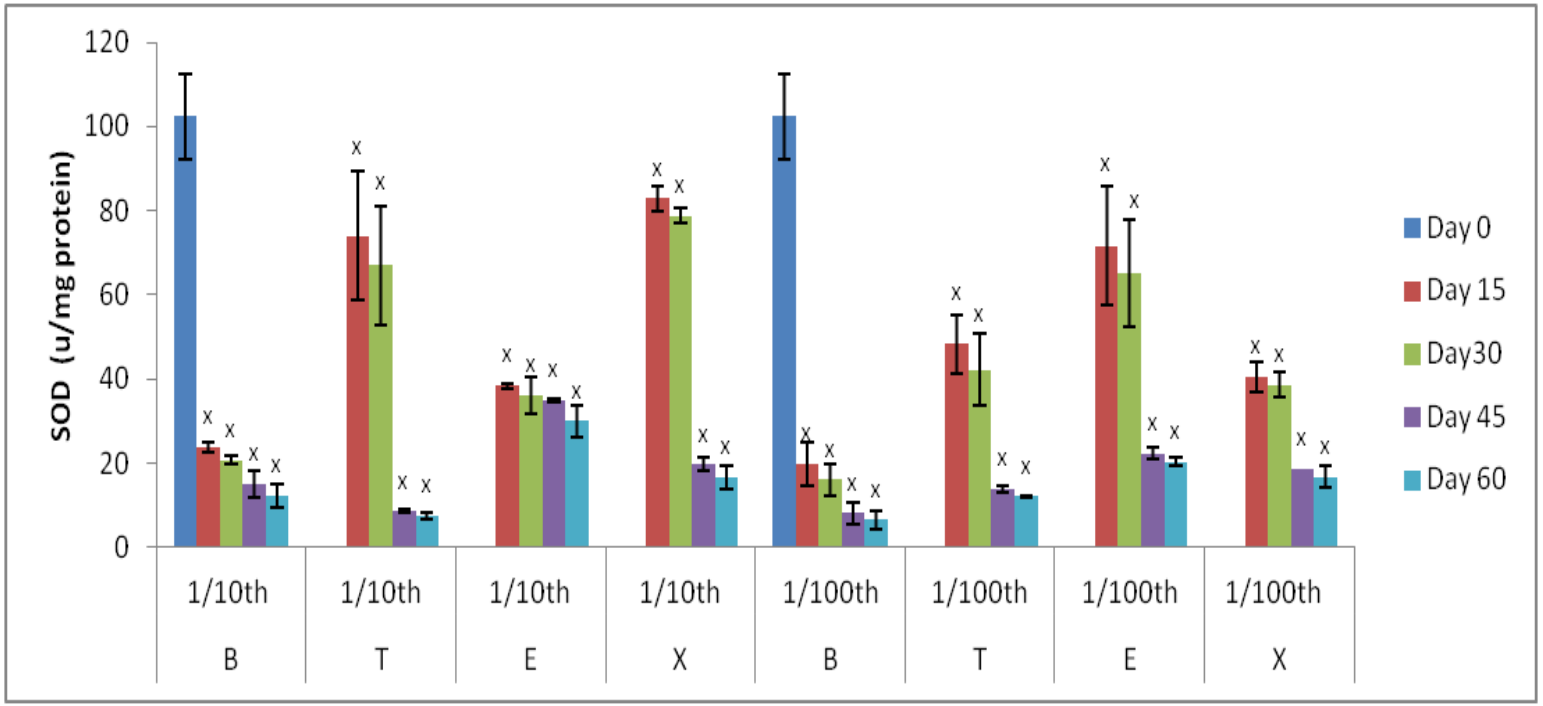


Fig 4.30: The effect of sub lethal concentrations of BTEX compounds on the activity of Superoxide dismutase (SOD) in the gills of *Clarias gariepinus*. Values are mean±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.2 Superoxide dismutase activity in the liver

The activity of SOD in the liver of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.31. The results show that the activity of the enzyme SOD was inhibited in the liver of fishes exposed to BTEX compounds. Measurements of SOD activity in liver of fish exposed to sublethal concentration of the test chemicals over a 60-day period decreased significantly ($P<0.05$) when compared to day 0 (control animals) (Figure 4.31). The activity of SOD was higher in the liver compared to the gill. In the liver, SOD activities decreased from 216.23 ± 9.10 u/mg protein (day 0) to 40.64 ± 1.07 (benzene $1/10^{th}$), 22.58 ± 2.22 (benzene $1/100^{th}$), 14.02 ± 2.41 (toluene $1/10^{th}$), 7.40 ± 1.34 (toluene $1/100^{th}$), 42.90 ± 5.17 (ethylbenzene $1/10^{th}$), 50.42 ± 2.33 (ethylbenzene $1/100^{th}$), 10.23 ± 0.48 (xylene $1/10^{th}$), 24.36 ± 4.44 u/mg protein (xylene $1/100^{th}$) in the exposed group of day 60 (Figure 4.31).

In the liver, the percentage decrease in SOD activities after exposure to low concentrations ($1/100^{th}$) of Benzene, Toluene, Ethylbenzene and Xylene were 89.56%, 96.58%, 76.68% and 88.73% respectively when compared to control. The percentage decrease in SOD activities in the liver after exposure to high concentrations ($1/10^{th}$) of Benzene, Toluene, Ethylbenzene and Xylene were 81.21%, 93.52%, 80.16% and 95.27% respectively when compared to control.

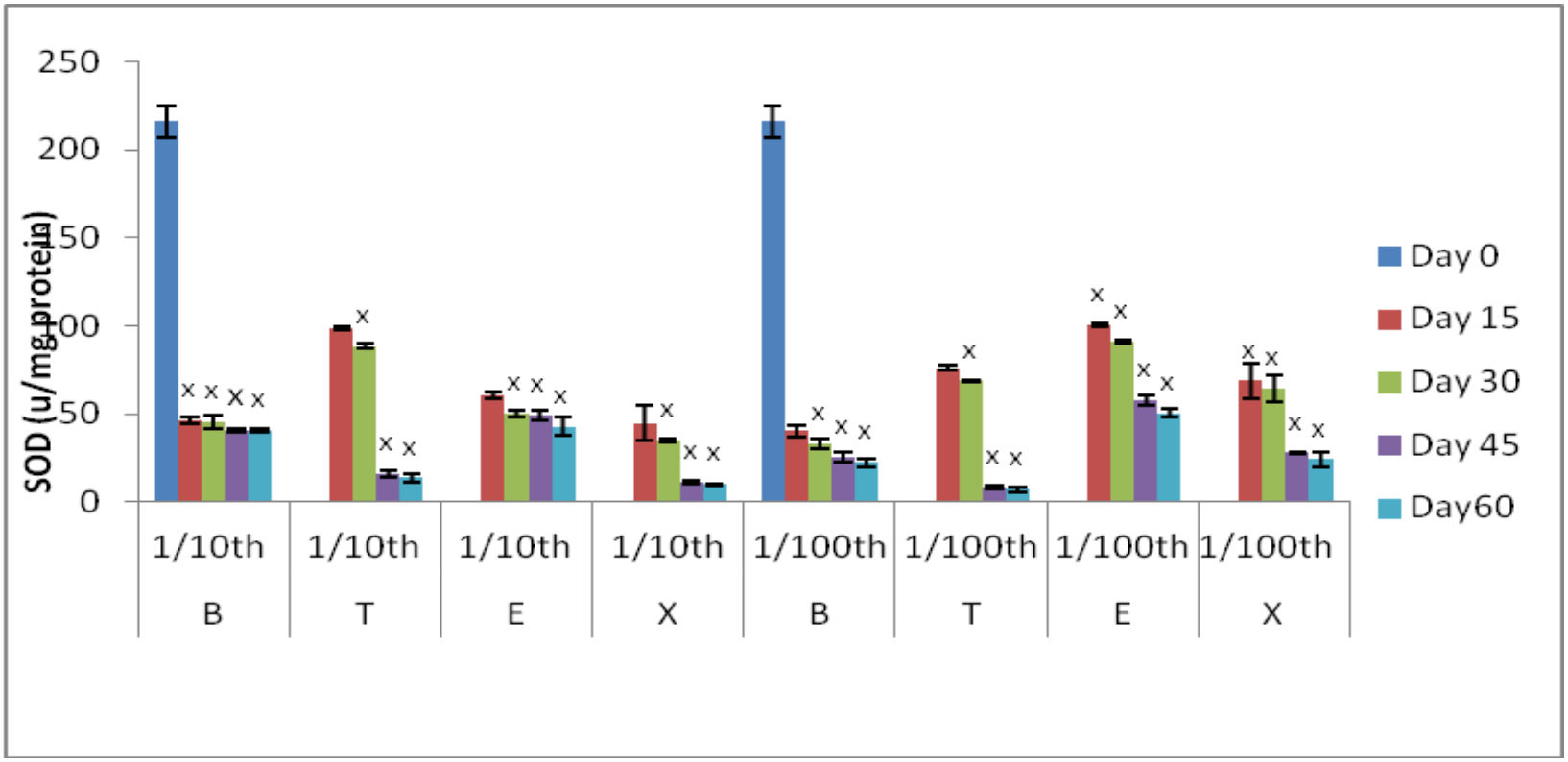


Fig 4.31: The effect of sub lethal concentrations of BTEX compounds on the activity of Superoxide dismutase (SOD) in the liver of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.3 Catalase activity in the gills

The activity of CAT in the gills of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 60days is presented in Figures 4.32. The results show that the activity of the enzyme CAT was inhibited in the gills of fishes exposed to BTEX compounds. Measurements of CAT activity in gills of fish exposed to sublethal concentration of the test chemicals over a 60-day period decreased significantly ($P<0.05$) when compared to day 0 (control animals) (Figure 4.32). In the gills, CAT activities decreased significantly ($P < 0.05$) from 717.5 ± 49.49 u/mg protein (day 0) to 118.88 ± 4.75 (benzene $1/10^{th}$), 121.48 ± 2.27 (benzene $1/100^{th}$), 182.71 ± 19.66 (toluene $1/10^{th}$), 168.43 ± 8.79 (toluene $1/100^{th}$), 168.89 ± 8.63 (ethylbenzene $1/10^{th}$), 70.53 ± 37.87 (ethylbenzene $1/100^{th}$), 165.62 ± 11.49 (xylene $1/10^{th}$), 183.61 ± 12.16 u/mg protein (xylene $1/100^{th}$) in the exposed group of day 60.

The percentage decrease in CAT activities in the gills after exposure to Benzene, Toluene, Ethylbenzene and Xylene (low concentration) were 83.43%, 76.53%, 90.17% and 74.41% respectively compared to control. The percentage decrease in CAT activities in the gills after exposure to Benzene, Toluene, Ethylbenzene and Xylene (high concentration) were 83.07%, 74.53%, 76.46% and 76.92% respectively when compared to control.

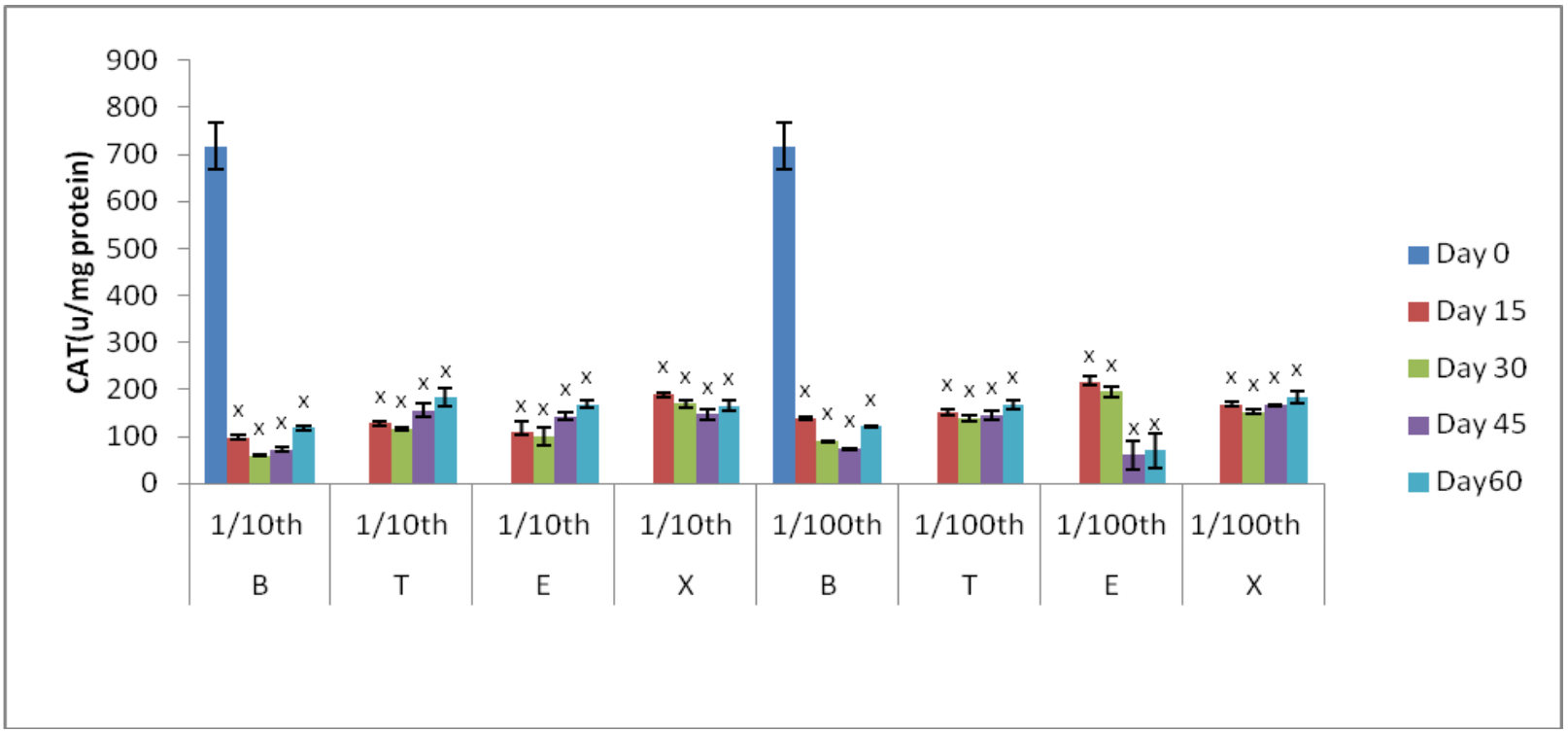


Fig 4.32: The effect of sub lethal concentrations of BTEX compounds on the activity of Catalase (CAT) in the gills of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.4 Catalase activity in the liver

The activity of CAT in the liver of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.33. The results show that the activity of the enzyme CAT was inhibited in the liver of fishes exposed to BTEX compounds. The activity of CAT was slightly higher in the liver compared to the gill. In the livers, CAT activities decreased significantly($P< 0.05$) from 740.75 ± 126.34 u/mg protein (day 0) to 187.5 ± 10.28 (benzene $1/10^{th}$), 101.98 ± 3.76 (benzene $1/100^{th}$), 289.79 ± 10.64 (toluene $1/10^{th}$), 188.84 ± 5.16 (toluene $1/100^{th}$), 140.34 ± 26.51 (ethylbenzene $1/10^{th}$), 87.1 ± 4.38 (ethylbenzene $1/100^{th}$), 153.23 ± 1.19 (xylene $1/10^{th}$), 108.53 ± 1.19 u/mg protein (xylene $1/100^{th}$) in the exposed group of day 60.

The percentage decrease in CAT activities in the livers after exposure to Benzene, Toluene, Ethylbenzene and Xylene (low concentration) were 86.24%, 74.51%, 88.24% and 85.35% respectively compared to control. The percentage decrease in CAT activities in the livers after exposure to Benzene, Toluene, Ethylbenzene and Xylene (high concentration) were 74.69%, 60.89%, 81.06% and 79.32% respectively compared to control.

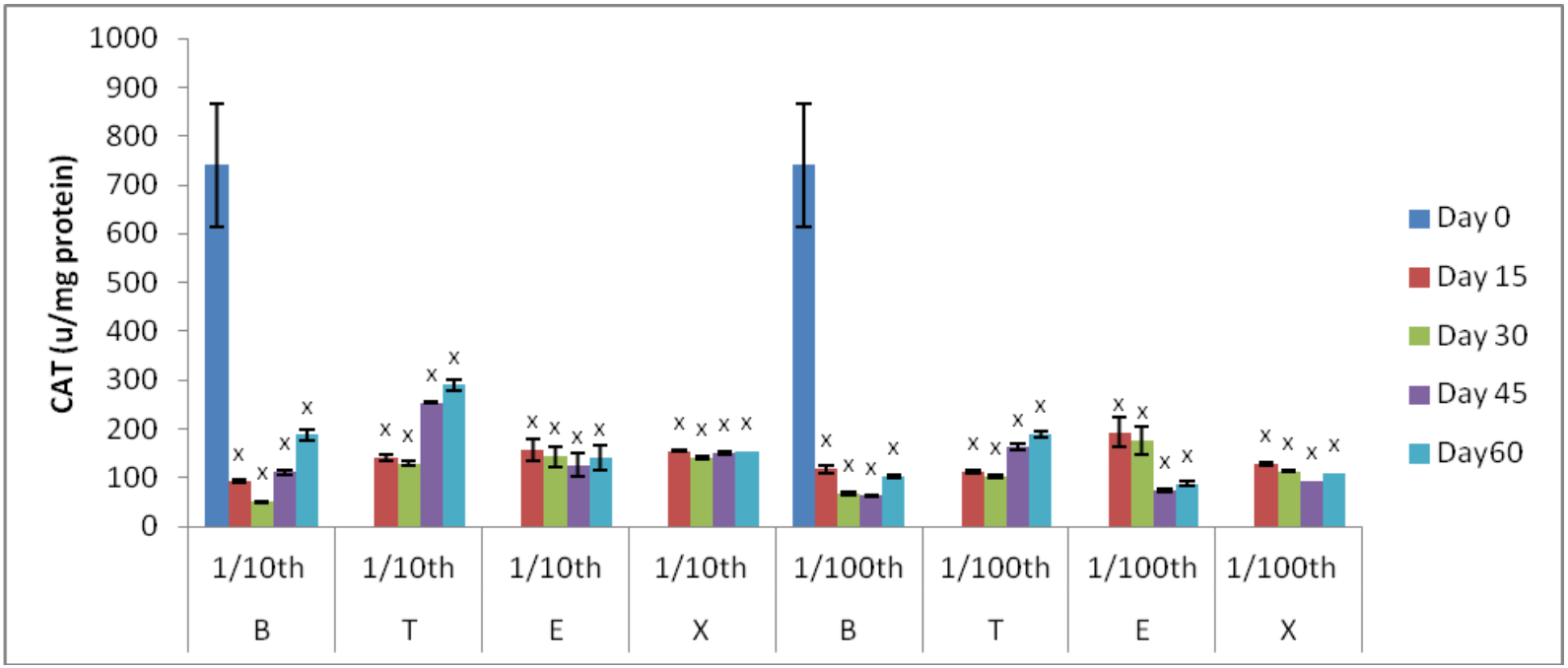


Fig 4.33: The effect of sub lethal concentrations of BTEX compounds on the activity of Catalase (CAT) in the livers of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.5 GST activity in the gills

The activity of GST in the gills of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.34. The activity of the enzyme GST was inhibited by Benzene, Ethylbenzene, and induced by Xylene and Toluene in the gills (Figure 4.34). In the gills, GST activities decreased from 422.43 ± 51.09 nm/mg protein (day 0) to 308.45 ± 12.09 (benzene $1/10^{th}$), 312.85 ±10.81 (benzene $1/100^{th}$), 176.38 ± 24.58 (ethylbenzene $1/10^{th}$), 143.9 ±11.55 (ethylbenzene $1/100^{th}$) and 398.94 ± 18.97 (xylene $1/100^{th}$); was significantly induced to 455.28 ± 8.13 (toluene $1/10^{th}$), 602.56 ± 19.26 (toluene $1/100^{th}$), and 508.92 ±14.28 nm/mg protein (xylene $1/10^{th}$), in the exposed group of day 60.

The percentage decrease in GST activities in the gills after exposure to Benzene, Ethylbenzene and Xylene (low concentrations) were 25.94%, 65.94% and 5.56% respectively when compared to control. GST activities increased significantly by 42.64% in the gills exposed to low concentration of Toluene. The percentage decrease in GST activities in the gills after exposure to Benzene and Ethylbenzene (high concentrations) were 26.98% and 58.25% respectively compared to control. GST increased by 7.78% and 20.47% in the gills exposed to high concentrations of Toluene and Xylene respectively. The activity of GST was slightly higher in the gill compared to the liver.

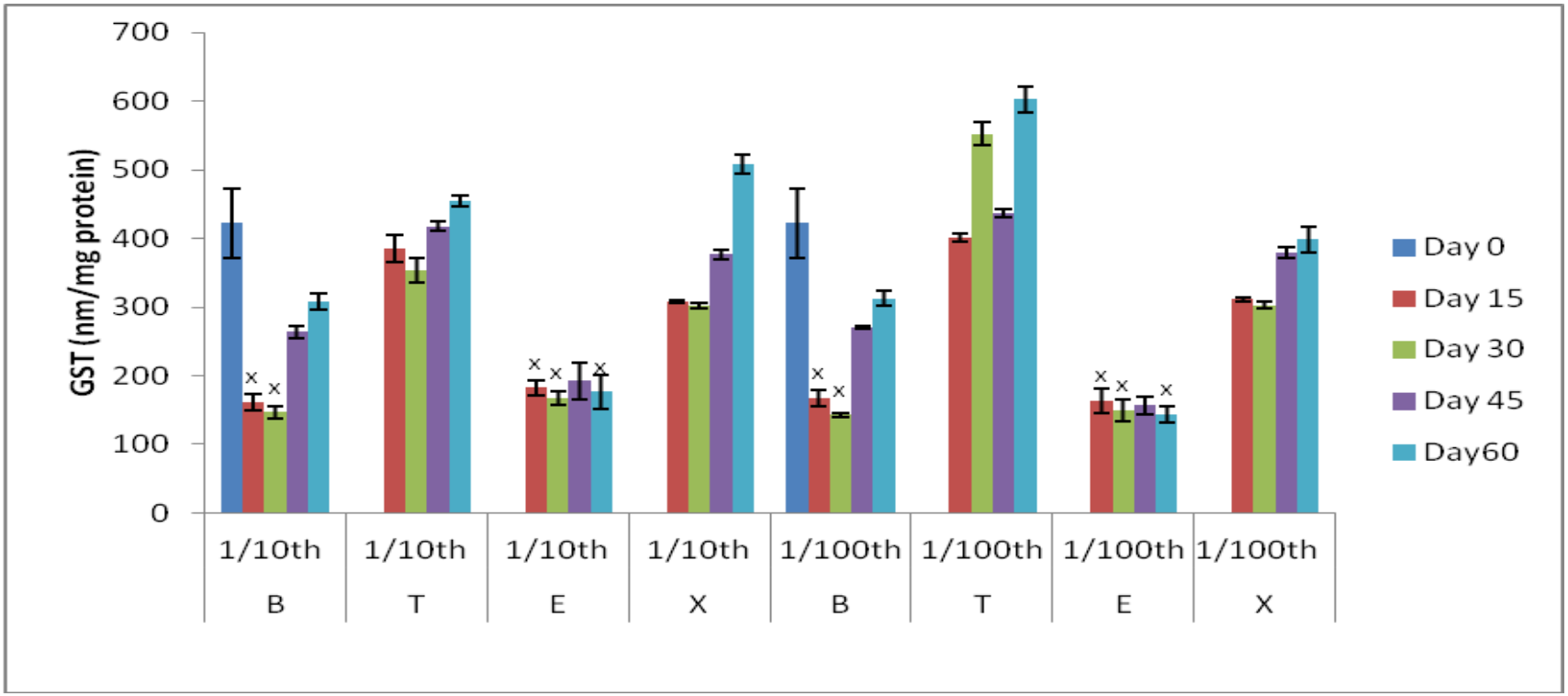


Fig 4.34: The effect of sub lethal concentrations of BTEX compounds on the activity of Glutathione S-transferase (GST) in the gills of *Clarias gariepinus*.Values are mean±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.6 GST activity in the livers

The activity of GST in the liver of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.35. In the liver, GST activities increased significantly ($P<0.05$) from 243.71 ± 16.20 nm/mg protein (day 0) to 436.72 ± 5.65 (benzene $1/10^{th}$), 355.33 ± 19.96 (benzene $1/100^{th}$), 436.18 ± 100.93 (toluene $1/10^{th}$), 391.4 ± 10.45 (toluene $1/100^{th}$), 553.9 ± 23.27 (xylene $1/10^{th}$), 499.82 ± 23.27 (xylene $1/100^{th}$) and was significantly reduced to 235.56 ± 28.39 (ethylbenzene $1/10^{th}$), 266.13 ± 27.96 nm/mg protein (ethylbenzene $1/100^{th}$) in the exposed group of day 60. Based on day 0, the activity of GST was slightly higher in the gill compared to the liver.

Activities of GST increased by 45.80%, 60.60%, 9.20% and 105.09% in the livers exposed to high concentrations of Benzene, Toluene, Ethylbenzene and Xylene respectively (Figure 4.35). Activities of GST increased by 79.20%, 78.97% and 127.28% in the livers exposed to low concentrations of Benzene, Toluene and Xylene respectively. The percentage decrease in GST activities was 3.34% in liver exposed to Ethylbenzene (low concentration).

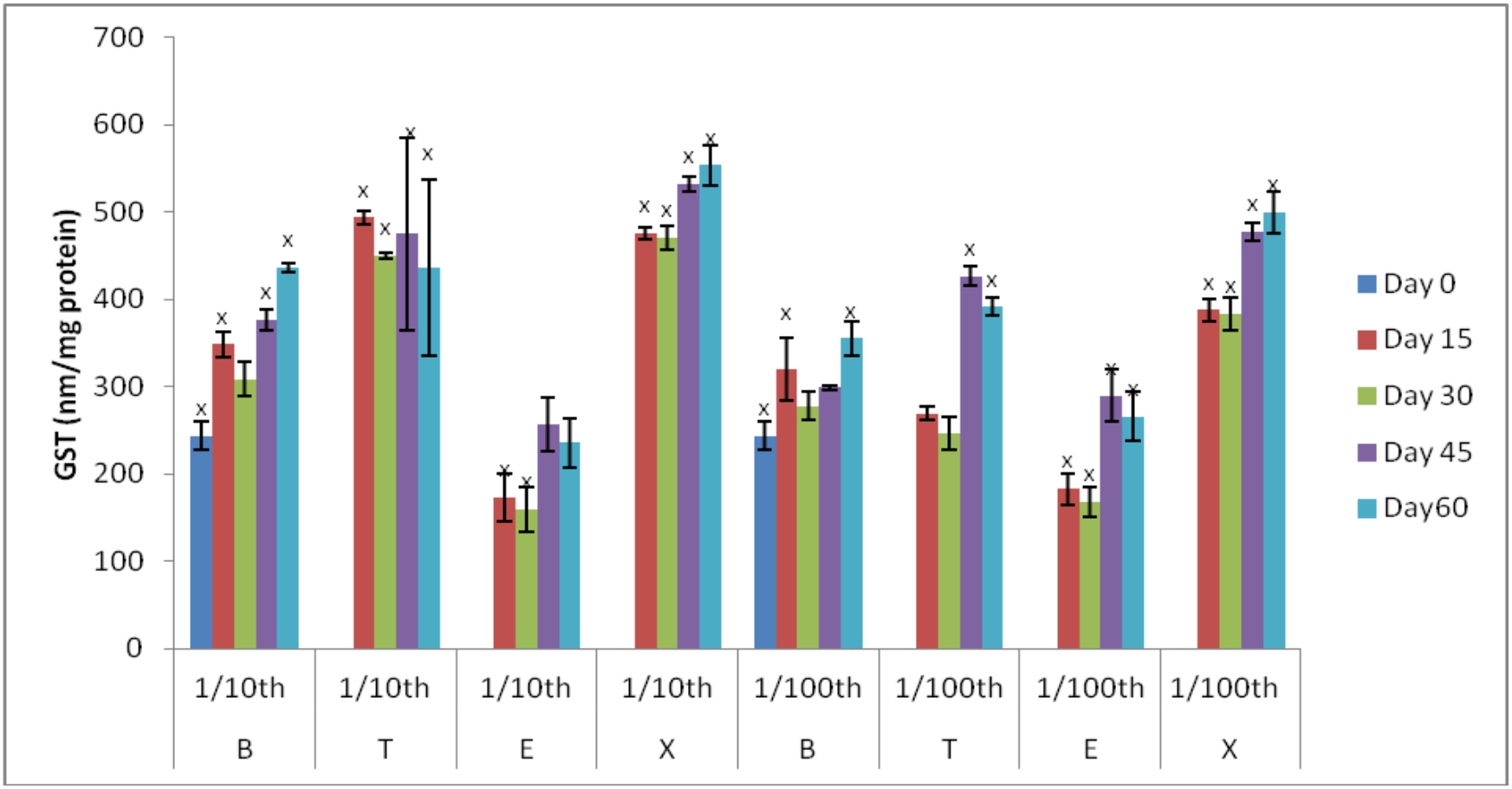


Fig 4.35: The effect of sub lethal concentrations of BTEX compounds on the activity of Glutathione S-transferase (GST) in the livers of *Clarias gariepinus*. Values are mean±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.7 Level of GSH in the gills

The level of GSH in the gills of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 60days is presented in Figures 4.36. The levels of GSH was significantly ($P<0.05$) reduced in the gills of fishes exposed to BTEX chemicals. Measurements of GSH levels in gills of fish exposed to sublethal concentration of the test chemicals over a 60-day period decreased significantly ($P< 0.05$) when compared to day 0 (control animals) (Figure 4.36). In the gills, level of GSH decreased significantly ($P < 0.05$) from 51.76 ± 7.14 nm/mg protein (day 0) to 2.45 ± 0.63 (benzene $1/10^{th}$), 1.7 ± 0.97 (benzene $1/100^{th}$), 15.71 ± 1.20 (toluene $1/10^{th}$), 3.06 ± 0.50 (toluene $1/100^{th}$), 15.85 ± 1.14 (ethylbenzene $1/10^{th}$), 14.08 ± 2.40 (ethylbenzene $1/100^{th}$), 7.54 ± 0.96 (xylene $1/10^{th}$), 4.94 ± 1.01 nm/mg protein (xylene $1/100^{th}$) in the exposed group of day 60.

The percentage decrease in the level of GSH in the gills exposed to Benzene, Toluene, Ethylbenzene and Xylene (low concentrations) were 96.72%, 94.08%, 72.80% and 90.46% respectively compared to control. The percentage decrease in the level of GSH for Benzene, Toluene, Ethylbenzene and Xylene (high concentrations) were 95.27%, 69.66%, 69.38%, and 85.43% respectively compared to control. The level of GSH was slightly higher in the gill compared to the liver.

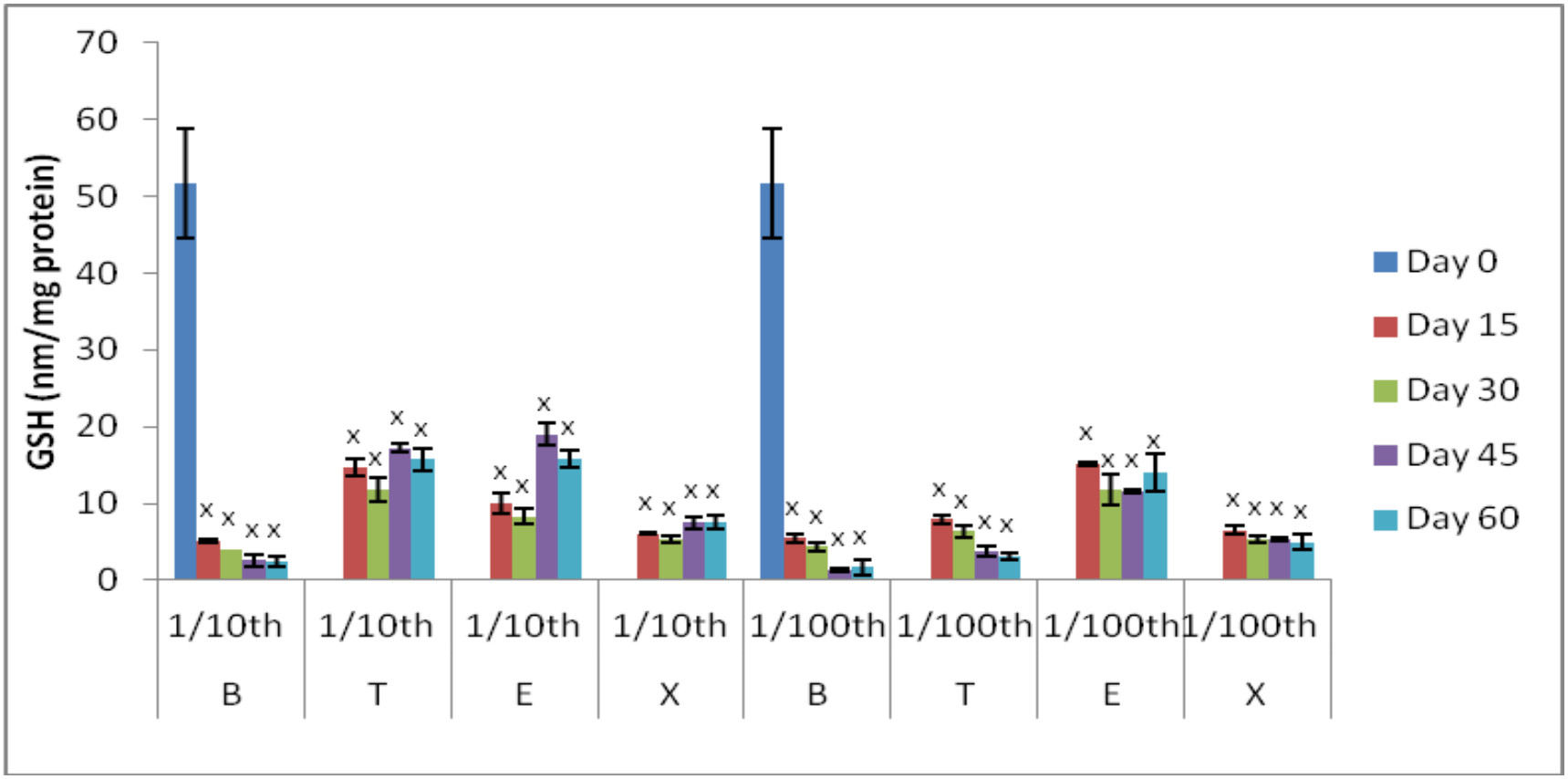


Fig 4.36: The effect of sub lethal concentrations of BTEX compounds on the levels of Glutathione(GSH) in the Gills of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.8 Level of GSH in the liver

The level of GSH in the liver of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 60days is presented in Figures 4.37. The levels of GSH was significantly ($P<0.05$) reduced in the liver of fishes exposed to BTEX chemicals. Measurements of GSH levels in gills of fish exposed to sublethal concentration of the test chemicals over a 60-day period decreased significantly ($P< 0.05$) when compared to day 0 (control animals) (Figure 4.37). In the liver, level of GSH decreased significantly($P< 0.05$) from 37.41 ± 3.92 nm/mg protein (day 0) to 5.08 ± 0.09 (benzene $1/10^{th}$), 10.32 ± 0.79 (benzene $1/100^{th}$), 8.72 ± 0.67 (toluene $1/10^{th}$), 7.52 ± 0.36 (toluene $1/100^{th}$), 14.03 ± 3.78 (ethylbenzene $1/10^{th}$), 11.90 ± 1.19 (ethylbenzene $1/100^{th}$), 9.78 ± 0.93 (xylene $1/10^{th}$), 6.76 ± 0.93 nm/mg protein (xylene $1/100^{th}$) in the exposed group of day 60. The level of GSH was slightly higher in the gill compared to the liver.

The percentage decrease in the level of GSH in the livers exposed to Benzene, Toluene, Ethylbenzene and Xylene (high concentrations) were 72.42%, 79.89%, 68.18% and 81.93% respectively when compared to control. The percentage decrease in the level of GSH in the livers exposed to Benzene, Toluene, Ethylbenzene and Xylene (high concentrations) were 86.42%, 76.69%, 62.51% and 73.85% respectively compared to control.

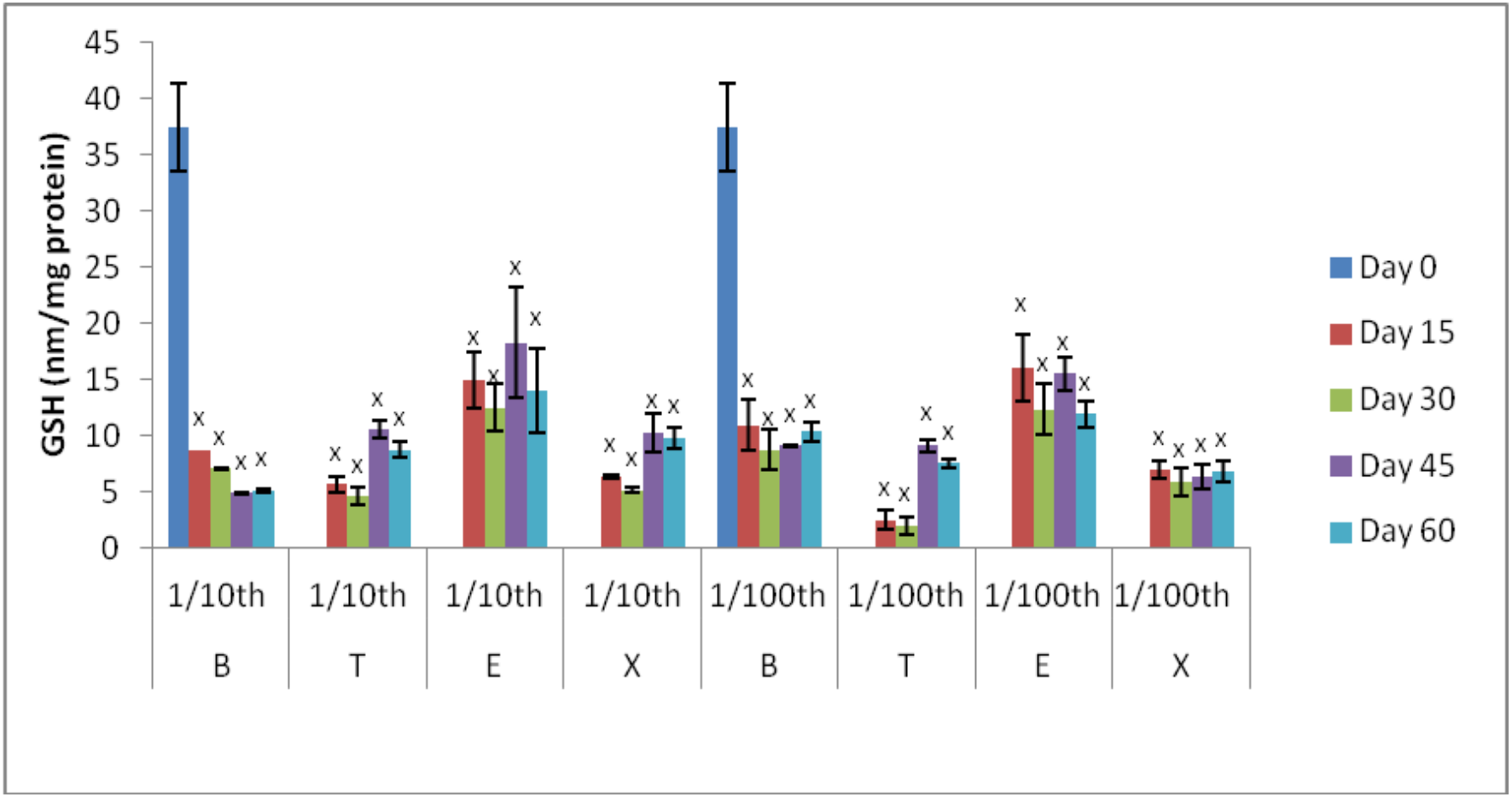


Fig 4.37: The effect of sub lethal concentrations of BTEX compounds on the levels of Glutathione (GSH) in the Livers of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05.

4.3.2.1.9 MDA in the gills

The level of MDA in the gills of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.38. The levels of MDA were significantly ($P<0.05$) increased in the gills of fishes exposed to BTEX chemicals. Measurements of MDA levels in liver and gills of fish exposed to sublethal concentration of the test chemicals over a 60-day period increased significantly ($P< 0.05$) when compared to day 0 (control animals) (Figure 4.38). In the gills, level of MDA increased significantly ($P < 0.05$) from 0.62 ± 0.14 nm/mg protein (day 0) to 25.65 ± 4.56 (benzene $1/10^{th}$), 16.65 ± 2.35 (benzene $1/100^{th}$), 16.90 ± 3.75 (toluene $1/10^{th}$), 12.92 ± 2.22 (toluene $1/100^{th}$), 5.04 ± 0.55 (ethylbenzene $1/10^{th}$), 2.59 ± 0.43 (ethylbenzene $1/100^{th}$), 14.85 ± 0.33 (xylene $1/10^{th}$), 6.16 ± 1.19 nm/mg protein (xylene $1/100^{th}$) in the exposed group of day 60.

The percentage increase in MDA in gills exposed to Benzene, Toluene, Ethylbenzene and Xylene (low concentrations) were 2360.50%, 1808.71%, 282.63% and 810.78% respectively compared to control. The percentage increase in MDA in the gills exposed to Benzene, Toluene, Ethylbenzene and Xylene (high concentrations) were 3689.36%, 2397.43%, 645.31% and 2093.84% for respectively when compared to control. The level of MDA was slightly higher in the gill compared to the liver.

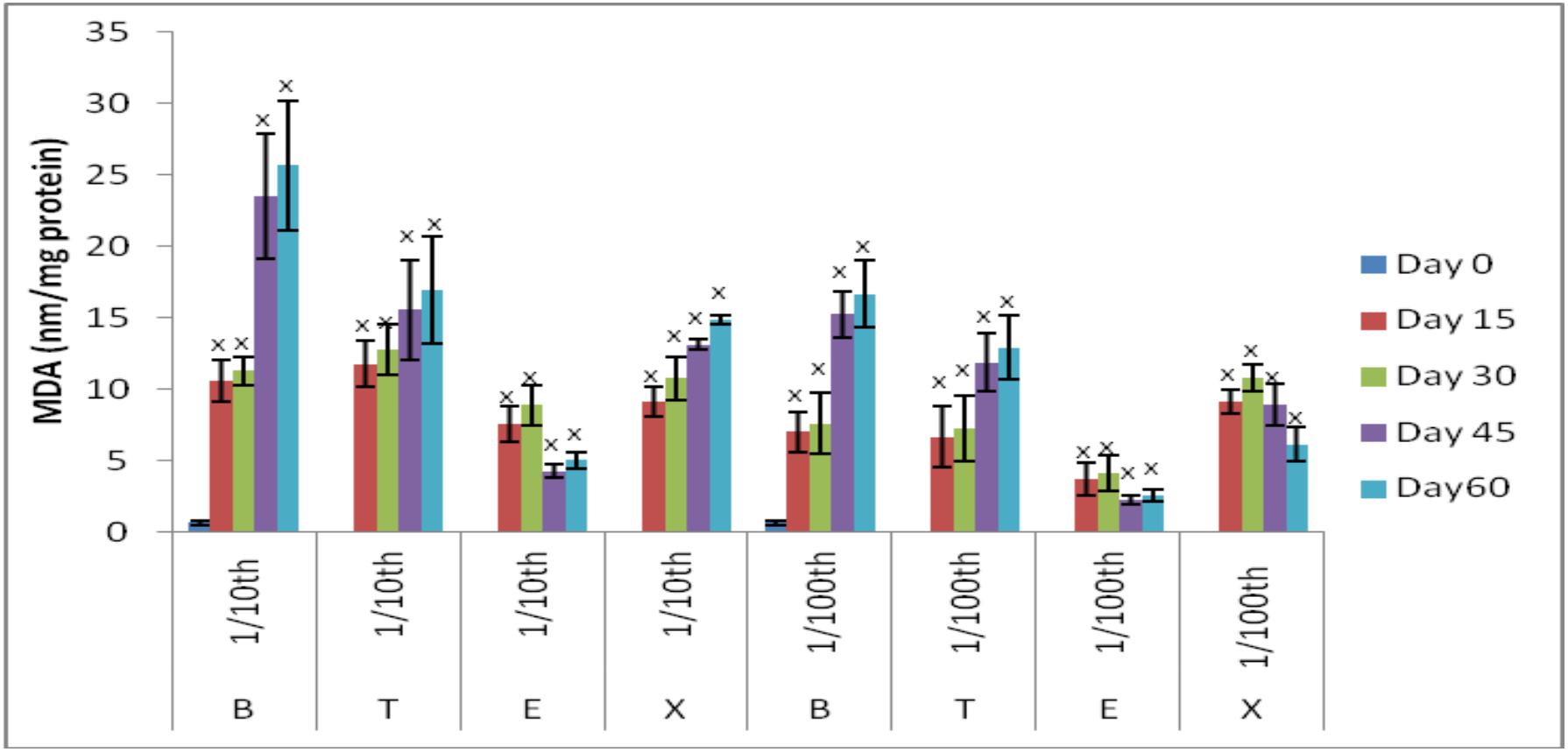


Fig 4.38: The effect of sub lethal concentrations of BTEX compounds on the levels of malondialdehyde (MDA) in the gills of *Clarias gariepinus*. Values are mean±SD. Significantly different from control (day 0), * p<0.05

4.3.2.1.10 MDA in the livers

The level of MDA in the liver of *C. gariepinus* exposed to sub lethal concentrations of $1/10^{th}$ of the 96hrLC_{50} (high) and $1/100^{th}$ of the 96hrLC_{50} (low) over a period of 60days is presented in Figures 4.39. In the livers, level of MDA increased significantly ($P< 0.05$) from 1.22 ± 0.38 nm/mg protein (day 0) to 29.02 ± 3.63 (benzene $1/10^{th}$), 20.85 ± 2.43 (benzene $1/100^{th}$), 13.19 ± 0.69 (toluene $1/10^{th}$), 7.17 ± 0.12 (toluene $1/100^{th}$), 5.29 ± 0.18 (ethylbenzene $1/10^{th}$), 3.49 ± 0.28 (ethylbenzene $1/100^{th}$), 7.05 ± 2.36 (xylene $1/10^{th}$), 4.13 ± 2.36 nm/mg protein (xylene $1/100^{th}$) in the exposed group of day 60.

The percentage increases in MDA for livers exposed to Benzene, Toluene, Ethylbenzene and Xylene (low concentrations) were 1558.59%, 486.25%, 185.36%, and 237.69% respectively. The percentage increases in MDA for gills exposed to Benzene, Toluene, Ethylbenzene and Xylene (high concentrations) were 2272.80%, 978.47%, 332.53%, and 476.44% for respectively.

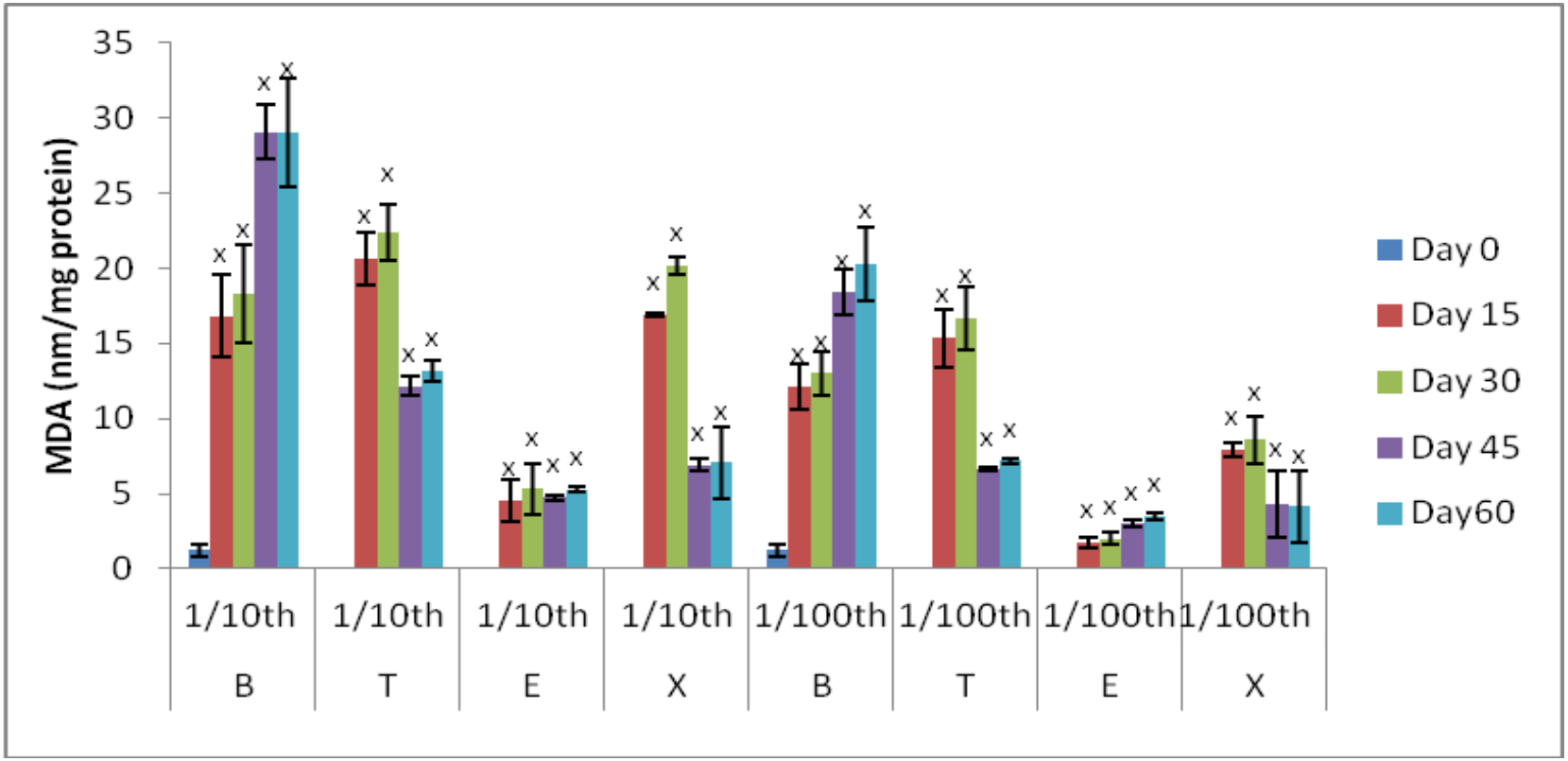


Fig 4.39: The effect of sub lethal concentrations of BTEX compounds on the levels of malondialdehyde (MDA) in the livers of *Clarias gariepinus*. Values are mean ±SD. Significantly different from control (day 0), * p<0.05.

4.3.2.2 Antioxidant Enzymes and non Enzymes in *Eudrilus eugeniae* Exposed to Sublethal Concentrations of Benzene, Toluene, Ethylbenzene and Xylene

4.3.2.2.1 Superoxide dismutase (SOD) activity

The level of SOD in the earthworms exposed to sub lethal concentrations $1/10^{th}$ of $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 28days is presented in Tables 4.11 and 4.12. SOD activity decreased significantly($P< 0.05$) from 235.20±30.41 u/mg protein (day 0) to 97.11± 9.52 (benzene $1/10^{th}$), 96.05 ± 7.41 (benzene $1/100^{th}$), 39.23±11.92 (toluene $1/10^{th}$), 170.71 ± 24.88 (toluene $1/100^{th}$), 223.29 ± 12.77 (ethylbenzene $1/10^{th}$), 135.73 ±9.17 (ethylbenzene $1/100^{th}$), 196.20 ± 16.2 (xylene $1/10^{th}$) , 41.12±3.24 (Xylene $1/100^{th}$, day 21) in the exposed group of day 28.

The percentage decrease of SOD activities from day 0 to day 28 in earthworms exposed to Benzene high ($1/10^{th}$) and low ($1/100^{th}$ concentrations were 58.71% and 59.16% respectively (Table 4.11). The percentage decrease of SOD activities at day 28 in earthworms exposed to Toluene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 83.32% and 27.42% respectively. The percentage decrease of SOD activities at day 28 in earthworms exposed to Ethylbenzene high ($1/10^{th}$) and low ($1/100^{th}$ concentrations were 5.06% and 42.29% respectively (Table 4.12). The percentage decrease of SOD activities at day 28 in earthworms exposed to Xylene high concentration ($1/10^{th}$) was 16.58%.

Table 4.11: Biochemical responses of *Eudrilus eugeniae* exposed to benzene and toluene contaminated soil. Results are expressed as means ± standard deviations (n=5). *CAT*, catalase; MDA, Malondialdehyde; GSH, total glutathione; GST, glutathione - S-transferase; SOD superoxide dismutase; statistical significance, treated versus control group:*, (p< 0.05)

Biochemical Measurements	Duration of exposure (days)	Benzene Concentration		Toluene concentration	
		High ($1/10^{th}$)	Low ($1/100^{th}$)	High ($1/10^{th}$)	Low ($1/100^{th}$)
GSH	0	28.64±1.47	28.64±1.47	28.64±1.47	28.64±1.47
	2	12.27±1.22*	20.29±1.30	10.73±0.84*	13.81±0.91*
	7	12.51±4.03*	16.70±1.23	31.84±1.1*	32.77±1.08*
	15	33.58±2.89*	62.32±32.13*	31.47±1.04*	32.98±1.35*
	21	27.04±1.18	29.36±1.04	13.08±1.31*	19.11±1.04
	28	34.52±1.48*	37.02±0.77	16.22±1.25*	16.97±1.15
SOD	0	235.20±30.41	235.20±30.41	235.20±30.41	235.20±30.41
	2	105.46±8.65*	195.75±7.87*	81.94±27.7*	69.88±9.16*
	7	94.84±5.36*	45.52±11.06*	50.07±12.41*	68.28±8.48*
	15	94.84±5.36*	45.52±11.06*	182.54±2.9*	64.64±5.11*
	21	115.70±6.00*	124.73±9.47*	64.11±4.37*	72.08±6*
	28	97.11±9.52*	96.05±7.41*	39.23±11.92	170.71±24.88
CAT	0	693.00±78.85	693.00±78.85	693±42.15	693±42.15
	2	449.75±84.29*	585.90±94.28*	470.05±64.89*	489.3±86.27
	7	288.75±19.57*	93.45±7.38*	196±38.12*	248.5±64.77*
	15	288.75±19.57*	93.45±7.38*	175.35±16.94*	214.55±24.52*
	21	399.00±33.66*	506.10±28.93	156.8±12.53*	225.4±29.58*
	28	354.90±30.75*	430.85±39.02	485.8±62.04*	429.8±85.49
MDA	0	2.46±0.12	2.46±0.12	2.46±0.12	2.46±0.12
	2	7.81±0.11*	4.74±0.28	6.8±0.72*	4.05±0.52*
	7	3.23±0.06*	1.78±0.07*	2.95±0.13	2.32±0.16
	15	3.23±0.06*	1.78±0.07*	3.03±0.16	2.32±0.15
	21	0.42±0.10*	0.23±0.04*	0.52±0.12*	1.37±0.15*
	28	2.75±0.15*	1.74±0.11*	1.62±0.82	1.78±0.13
GST	0	395.77±20.30	395.77±20.30	395.77±20.30	395.77±20.30
	2	395.77±20.30	418.27±6.32*	274.96±16.6*	246.21±15.55
	7	280.37±17.95*	169.56±16.83*	234.55±13.06*	190.8±12.55*
	15	363.69±14.17	667.39±29.70*	434.93±14.31*	453.68±19.4*
	21	373.90±16.20*	405.77±10.66*	181.22±17.34*	264.12±14.31
	28	477.01±20.46	511.58±14.40*	224.13±18.8*	234.55±15.84

Table 4.12: Biochemical responses of *Eudrilus eugeniae* exposed to ethyl benzene and xylene contaminated soil. Results are expressed as means ± standard deviations (n=5). CAT, catalase; MDA, Malondialdehyde; GSH, total glutathione; GST, glutathione-S-transferase; SOD superoxide dismutase; statistical significance, treated versus control group:*, (p< 0.05)

Biochemical measurements	Duration of exposure (days)	Ethylbenzene Concentration		Xylene concentration	
		High ($1/10^{th}$)	Low ($1/100^{th}$)	High ($1/10^{th}$)	Low ($1/100^{th}$)
GSH	0	28.64±1.47	28.64±1.47	28.64±1.47	28.64±1.47
	2	16.1±2.22*	13.87±0.77*	10.76±0.74*	16.97±0.94*
	7	12.84±0.97*	19.29±1.08*	16.91±1.92*	16.4±1.24*
	15	57.52±1.55*	21.74±4.69*	16.91±1.92*	16.67±1.13*
	21	22.43±1.6*	13.93±1.08*	10.25±1.79*	10.25±1.34*
	28	15.5±1.05*	22.88±1.28*	17.94±0.94*	16.85±1.05*
SOD	0	235.20±30.41	235.20±30.41	235.20±30.41	235.20±30.41
	2	92.56±7.39*	139.15±134.34*	78.3±20.33*	103.18±10.59*
	7	77.92±6.07*	66.77±11.25*	98.63±10.04*	100.15±5.09*
	15	41.88±2.85*	63.96±4.43*	98.63±10.04*	98.48±5.16*
	21	110.77±9.82*	114.41±15.91*	26.72±13.3*	41.12±3.24*
	28	223.29±12.77*	135.73±9.17*	196.2±16.2*	243.54±41.68
CAT	0	693±42.15	693±42.15	693±42.15	693±42.15
	2	341.25±29.02*	413±56.16*	447.65±67.05	324.45±33.24*
	7	569.8±44.85	599.55±55.97*	371±22.82	605.5±109.01
	15	315.7±5.62*	390.6±34.86*	371±22.82	431.2±48.22
	21	208.08±44.87*	220.85±26.38*	392±36.29	542.5±41.04
	28	371±41.28*	444.15±47.68*	451.15±62.22	482.3±26.21
MDA	0	2.46±0.12	2.46±0.12	2.46±0.12	2.46±0.12
	2	7.12±0.17*	4.87±0.15*	5.26±0.33*	4.42±0.14*
	7	1.1±0.14*	1.2±0.17*	3.27±0.08*	1.65±0.11*
	15	1.12±0.12*	1.31±0.15*	3.27±0.08*	1.69±0.16*
	21	8.4±0.16*	4.56±0.16*	0.88±0.08*	0.69±0.15*
	28	1.98±0.2*	1.48±1.4*	7.83±0.16*	3.7±0.15*
GST	0	395.77±20.30	395.77±20.30	395.77±20.30	395.77±20.30
	2	390.77±12.2*	400.77±10.88	428.26±11.47*	281.62±13.12
	7	148.73±10.27*	148.31±11.54*	191.64±10.62*	222.46±30.73*
	15	794.87±21.48*	280.79±88.16*	225.8±11.65*	225.38±9.92*
	21	309.95±22.16*	192.47±14.99*	141.64±24.78*	141.64±18.51*
	28	214.13±14.46*	316.2±17.64*	247.88±13.01*	232.88±14.54*

4.3.2.2.2 Catalase (CAT) activity

The activity of CAT in the earthworms exposed to sub lethal concentrations $1/10^{th}$ of $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 28days is presented in Tables 4.11 and 4.12. CAT activity decreased significantly ($P< 0.05$) from 693±42.15 u/mg protein (day 0) to 354.90±30.75 (benzene $1/10^{th}$), 430.85±39.02 (benzene $1/100^{th}$), 485.8±62.04 (toluene $1/10^{th}$), 429.8±85.49 (toluene $1/100^{th}$), 371±41.28 (ethylbenzene $1/10^{th}$), 444.15±47.68 (ethylbenzene $1/100^{th}$), 451.15±62.22 (xylene $1/10^{th}$), 482.3±26.21 (Xylene $1/100^{th}$) in the exposed group of day 28.

The percentage decrease of CAT activities at day 28 in earthworms exposed to Benzene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 48.78% and 37.83% respectively**.** The percentage decrease of CAT activities at day 28 in earthworms exposed to Toluene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 29.90% and 37.98% respectively. The percentage decrease of CAT activities at day 28 in earthworms exposed to Ethylbenzene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 46.50% and 35.94% respectively. The percentage decrease of CAT activities at day 28 in earthworms exposed to Xylene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 34.94% and 30.45% respectively.

4.3.2.2.3 Glutathione S-transferase (GST) activity

The activity of GST in the earthworms exposed to sub lethal concentrations $1/10^{th}$ of $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 28days is presented in Tables 4.11 and 4.12. GST activity decreased significantly($P< 0.05$) from 395.77±20.30 nm/mg protein (day 0) to 280.37±17.95 (benzene $1/10^{th}$), 169.56±16.83 (benzene $1/100^{th}$) at day 7, and 224.13±18.8

(toluene 1/10th), 234.55±15.84 (toluene 1/100th), 214.13±14.46 (ethylbenzene 1/10th), 316.2±17.64 (ethylbenzene 1/100th), 247.88±13.01 (xylene 1/10th), 232.88±14.54 nm/mg protein (Xylene 1/100th) in the exposed group of day 28.

The percentage decrease of GST activities at day 28 in earthworms exposed to Toluene high (1/10th) and low (1/100th) concentrations were 43.37% and 40.74% respectively**.** The percentage decrease of GST activities at day 28 in earthworms exposed to Ethylbenzene low (1/100th) concentrations was 20.11%. The percentage decrease of GST activities at day 28 in earthworms exposed to Ethylbenzene high (1/10th) was 45.90%. The percentage decrease of GST activities at day 28 in earthworms exposed to Xylene high (1/10th) and low (1/100th) concentrations were 37.37% and 41.16% respectively**.**

4.3.2.2.4 Glutathione level (GSH)

The level of GSH in the earthworms exposed to sub lethal concentrations 1/10th of 96hrLC_{50} (high) and 1/100th of the 96hrLC_{50} (low) over a period of 28days is presented in Tables 4.11 and 4.12. The level of GSH decreased significantly($P< 0.05$) from 28.64±1.47 nm/mg protein (day 0) to 12.51±4.03 (benzene 1/10th), 16.70±1.23 (benzene 1/100th) at day 7, and 16.22±1.25 (toluene 1/10th), 16.97±1.15 (toluene 1/100th), 15.5±1.05 (ethylbenzene 1/10th), 22.88±1.28 (ethylbenzene 1/100th), 17.94±0.94 (xylene 1/10th), 16.85±1.05 nm/mg protein (Xylene 1/100th) in the exposed group of day 28.

Benzene had a significant effect ($p< 0.05$) on the level of GSH. At day 28, there was a decrease of 48.37% after exposure to high concentration of Toluene and a decrease of 40.75% after exposure to low concentration of Toluene. The decrease at day 28 was 45.87% for the high concentration and 20.11% for low concentration of Ethylbenzene. The percentage decrease in the

level of GSH (day 0 to 28) in earthworms exposed to Xylene high ($1/10^{th}$) and low ($1/100^{th}$) concentrations were 37.36% and 41.16% respectively.

4.3.2.2.5 Malondialdehyde activity

The level of MDA in the earthworms exposed to sub lethal concentrations $1/10^{th}$ of $96hrLC_{50}$ (high) and $1/100^{th}$ of the $96hrLC_{50}$ (low) over a period of 28days is presented in Tables 4.11 and 4.12. The level of MDA increased significantly ($P< 0.05$) from 2.46±0.12 nm/mg protein (day 0) to 7.81±0.11 (benzene $1/10^{th}$), 4.74±0.28 (benzene $1/100^{th}$), 6.8±0.72 (toluene $1/10^{th}$), 4.05±0.52 (toluene $1/100^{th}$), 7.12±0.17 (ethylbenzene $1/10^{th}$), 4.87±0.15 (ethylbenzene $1/100^{th}$), 5.26±0.33 (xylene $1/10^{th}$), 4.42±0.14 nm/mg protein (Xylene $1/100^{th}$) in the exposed group of day 2.

Benzene had a significant effect ($p< 0.05$) on the level of malondialdehyde (MDA). The level of MDA significantly increased by 217.48% and 92.68% in earthworms exposed at day 2 to high and low concentrations of benzene, respectively. The level of MDA significantly increased by 176.42% and 64.63% at day 2 in earthworms exposed to high and low concentrations of Toluene, respectively. The level of MDA significantly increased by 189.43% and 97.97% at day 2 in earthworms exposed to high and low concentrations of Ethylbenzene, respectively. The level of MDA significantly increased by 113.82% and 79.67% at day 2 in earthworms exposed to high and low concentrations of Xylene, respectively.

4.3.2.3 Histopathological studies in Eudrilus Euginiae

4.3.2.3.1 Control

Histopathological examination of the body of the control animals and the exposed groups of earthworms is shown in photomicrographs in Plates 1 – 9. The normal architecture of the earthworm, *E. euginiae* consisting of the cuticle, epidermis, intact nature of circular and longitudinal muscles, subneural vessels is shown in Plate 1. No changes were observed: No lesion, no necrosis, no pigments, no malignancy, no inflammation and no inclusion bodies were observed in the control animals

4.3.2.3.2 Hispathological changes in the body of *Eudrilus euginiae* exposed to low concentration of BTEX compounds after 28 days.

The histological changes after exposure to low sublethal concentrations of $1/100^{th}$ 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 2-5. The sublethal concentrations of BTEX compounds have induced marked pathological changes in the body of the earthworm. The changes include cellular degeneration, moderate to severe areas of lesion, necrosis, area of inflammation, inclusion bodies, pigments, distortion of the shape of circular and longitudinal muscle. Expansion of spaces between longitudinal muscles, enlargement of ectoderm cells, ruptured cuticle, erosion of ectoderm of body wall, internal and external tissue erosion leading to total damage of body wall was observed.

Benzene: Mild necrosis with hypertrophic nerve bundle , endothelial degeneration were noted with expansion of spaces between longitudinal muscles after exposure of *E. euginiae* to low concentration of benzene.

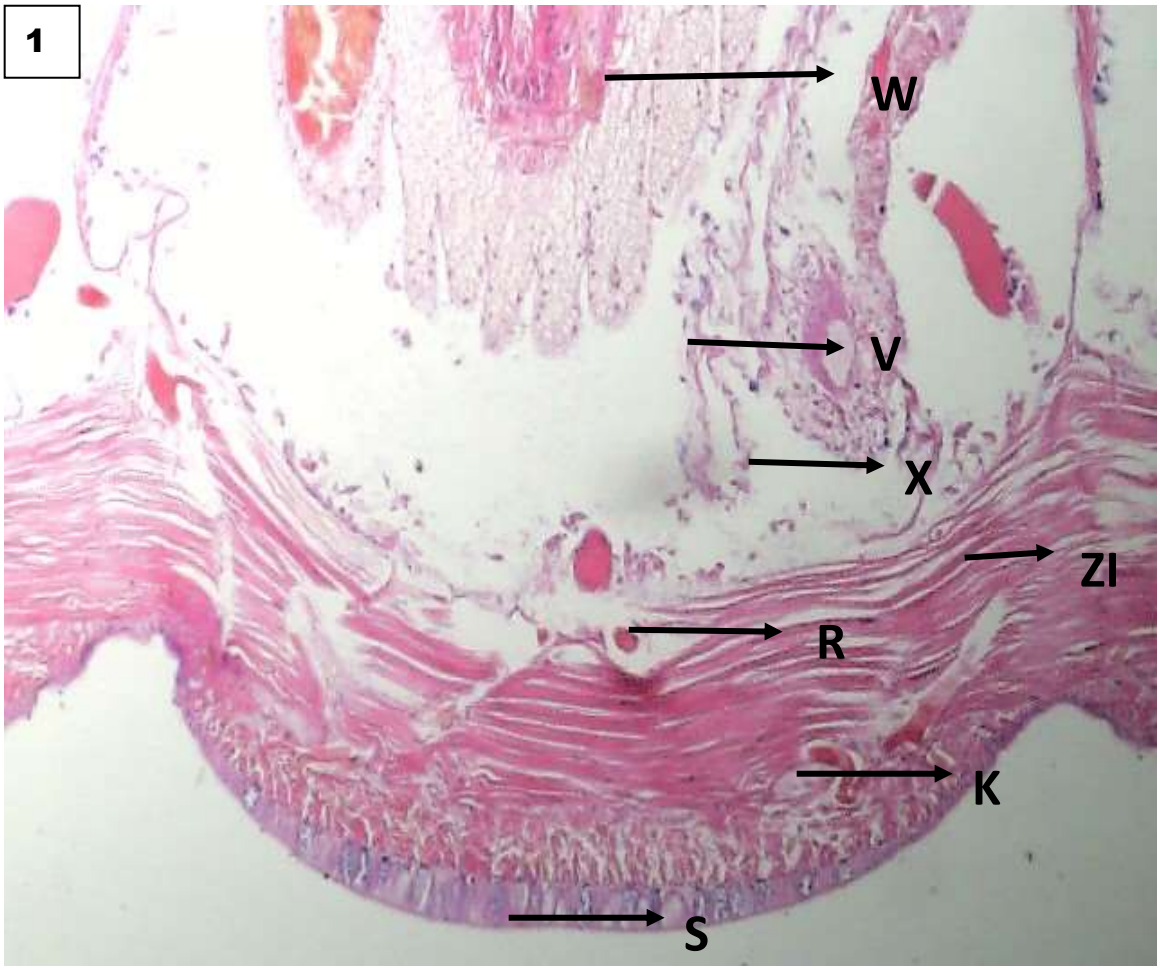


Plate 1: Control: Transverse section through the middle part of the earthworm reveal segmented body cavities, subphenogeal ganglion (K), subneural vessels (R), lining of the oesophagus (S), intestine (V), longitudinal and circular muscle (ZI), metanephridium (W) and blood vessels (X). No lesion, no necrosis, no pigments, no malignancy, no inflammation and inclusion bodies seen in the control group. Mag X 400

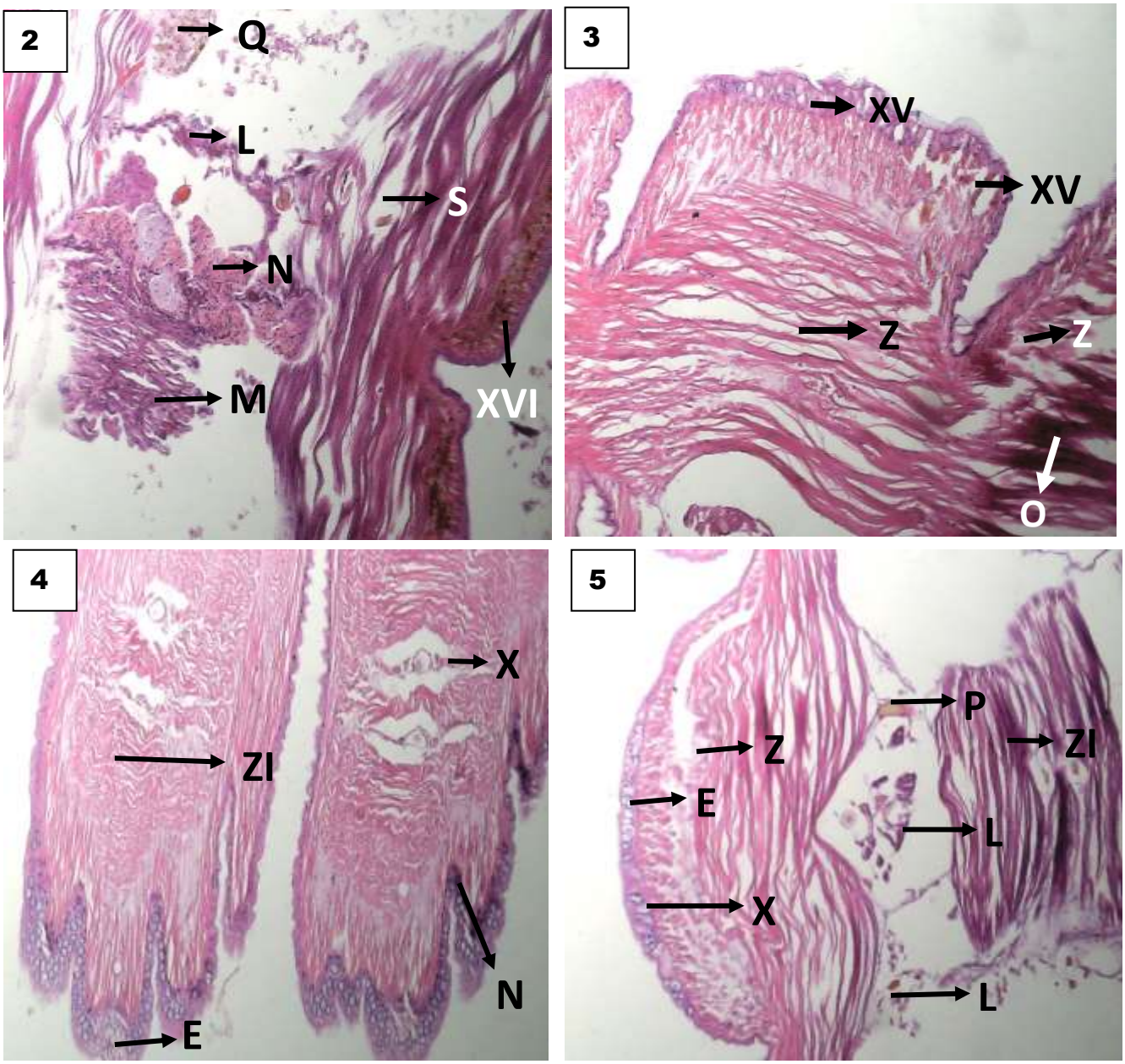


Plate 2-5: Histopathological (T.S) effects of BTEX (1/100th 96hLC$_{50}$) after exposure of *Eudrilus euginiae* for 28 days (H & E X400). 2 (Benzene), 3 (Toluene), 4 (Ethylbenzene), 5 (Xylene). Moderate area of necrosis (N), Cellular degeneration (X), Cloudy swelling of longitudinal and circular muscles (ZI), Enlargement of ectoderm cells (E), Dark brown pigment (O), Spaces between longitudinal and circular muscles (Z), Cellular degeneration with spaces in muscles (XV), Enlargement of ectoderm cells (E), Moderate area of lesion (L), Inclusion bodies (P), Enlargement of ectoderm cells E.

Toluene: Moderate necrosis and oedematous muscles with spaces between longitudinal and circular muscles and acanthotic epidermis were noted after exposure of *E. euginiae* to low concentration of toluene.

Ethylbenzene: Sections showing moderate area of necrosis with obvious cellular degeneration , cloudy swelling of longitudinal and circular muscles, enlargement of ectoderm cells were observed after exposure of *E. euginiae* to low concentration of ethylbenzene.

Xylene: Sections show moderate area of necrosis, longitudinal and circular muscles and enlargement of ectoderm cells were observed after exposure of *E. euginiae* to low concentration of xylene.

4.3.2.3.3 Hispathological changes in the body of *Eudrilus euginiae* exposed to high concentration of BTEX compounds after 28 days.

The histological changes after exposure to high sublethal concentrations of $1/10^{th}$ 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 6 – 9. The high sublethal concentrations of BTEX compounds also induced marked pathological changes in the body of the earthworm. The changes include cellular degeneration, severe area of lesion, necrosis, area of inflammation, inclusion bodies, dark brown pigments, distortion of the shape of circular and longitudinal muscle. The changes induced as a result of the exposure to high sublethal concentrations is more severe compared to exposure to low sublethal concentrations of BTEX compounds. Expansion of spaces between longitudinal muscles, enlargement of ectoderm cells, ruptured cuticle, erosion of ectoderm of body wall, internal and external tissue erosion leading to total damage of body wall was observed (Plates 6-9).

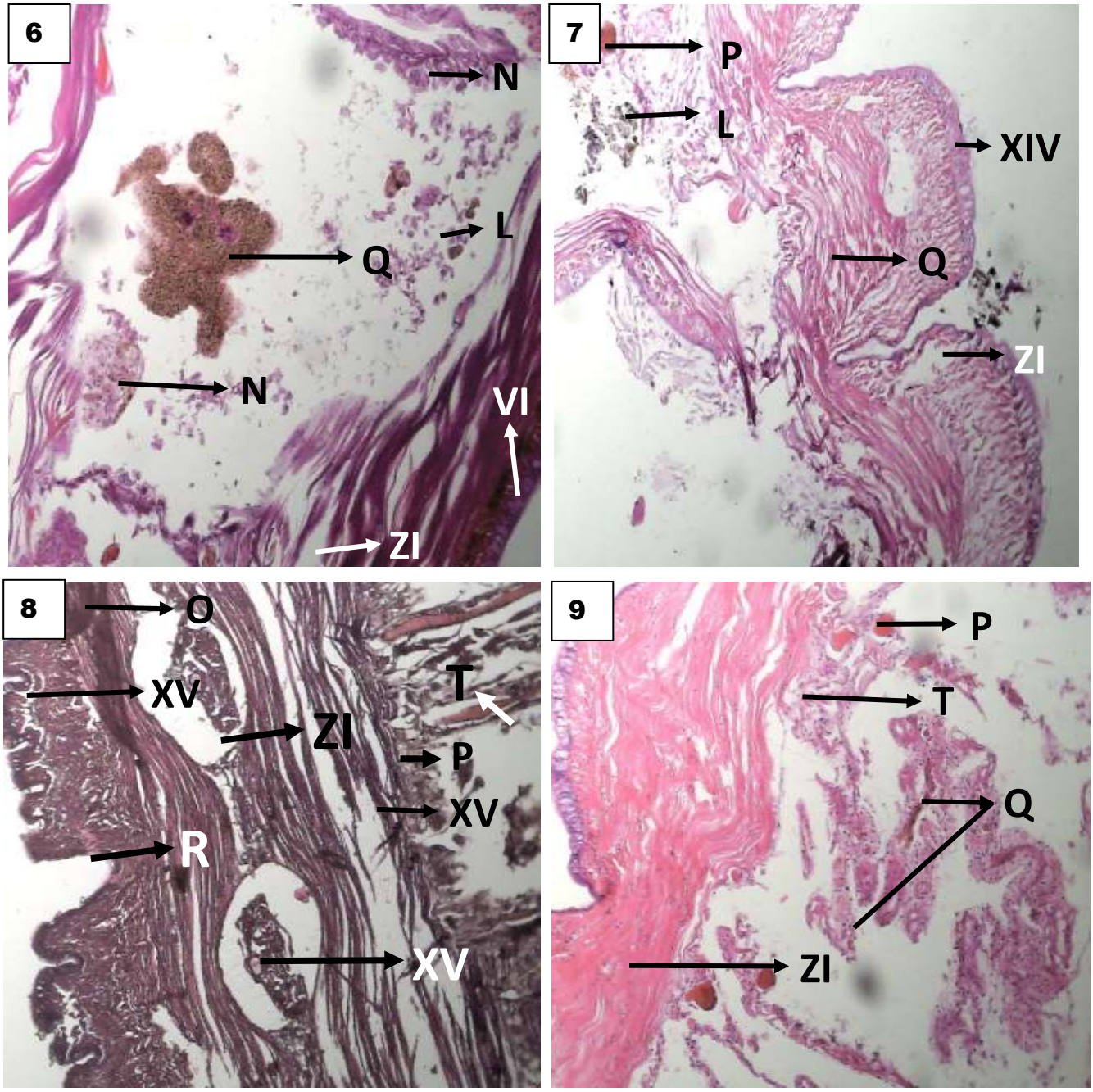


Plate 6-9: Histopathological (T.S) effects of BTEX (1/10th 96hLC$_{50}$) after exposure of *Eudrilus euginiae* for 28 days (H & E X400). 6 (Benzene), 7 (Toluene), 8 (Ethylbenzene), 9 (Xylene). Severe area of lesion (L), Necrosis (N), Inclusion bodies(P), Epidermis(VI), Area of inflammation (Q), Enlargement of ectoderm cells (XIV), Dark brown pigment (O), Cellular degeneration(XV), Ruptured body wall R, Loss of structural integrity of longitudinal and circular muscles (ZI), Tissue erosion leading to total damage (T).

Benzene : Severe necrosis, acanthotic epidermis, spaces between muscles, were noted with area of inflammation after exposure of *E. euginiae* to high concentration of benzene.

Toluene: Severe necrosis were noted with area of inflammation, spaces between longitudinal muscles, enlargement of ectoderm cells after exposure of *E. euginiae* to high concentration of toluene.

Ethylbenzene: Obvious cellular degeneration, ruptured body wall, loss of structural integrity of longitudinal and circular muscles, tissue erosion leading to total damage were observed after exposure of *E. euginiae* to high concentration of ethylbenzene.

Xylene : Mild necrosis, with obvious cellular degeneration showing enlargement of ectodermal cells, longitudinal and circular muscles, tissue erosion leading to total damage, detached digestive epithelium were observed after exposure of *E. euginiae* to high concentration of xylene.

4.3.2.4 Histopathological studies in gills of *Clarias gariepinus*

4.3.2.4.1 Control:

Histopathological examinations of the gills of the control and the exposed groups of fish are shown in photomicrographs in Plates. 10 – 18. Plate 10 shows the normal structure of the gills of fish C. *gariepinus* consisting of gill arch, filament, cartilaginous support, ceratobrachial bone of the arch and nucleus. The gill is made up of primary lamellae. Secondary lamellae are found on the lateral sides of primary lamellae. The surface of the gill lamella is covered with simple squamous epithelial cells and capillaries separated by pillar cells parallel along the surface. In between the secondary lamellae, the primary is lined by a thick stratified epithelium. This region contains the mucous cells and chloride cells. No pathological effects were observed: No lesion, no necrosis, no pigments, no malignancy, no inflammation and no inclusion bodies were observed in the control animals.

4.3.2.4.2 Histopathological changes in the gills of C*larias gariepinus* exposed to 1/100th 96hr LC_{50} low sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to low sublethal concentrations of 1/100th 96hr LC_{50} of benzene, toluene, ethylbenzene and xylene are shown in Plates 11-14. The sublethal concentrations of BTEX compounds have induced marked pathological changes in the gills of the fish. The observed changes include moderate to severe area of lesion, necrosis, and inclusion bodies, pigment, nuclear abnormality and area of inflammation. The result of the histological examinations also showed histopathological changes such as loss of regular shape, separation of lamellar epithelium, atrophy of secondary gill lamellae, loss of secondary lamellae, fusion of secondary lamellae and curving of primary lamellae in the gills.

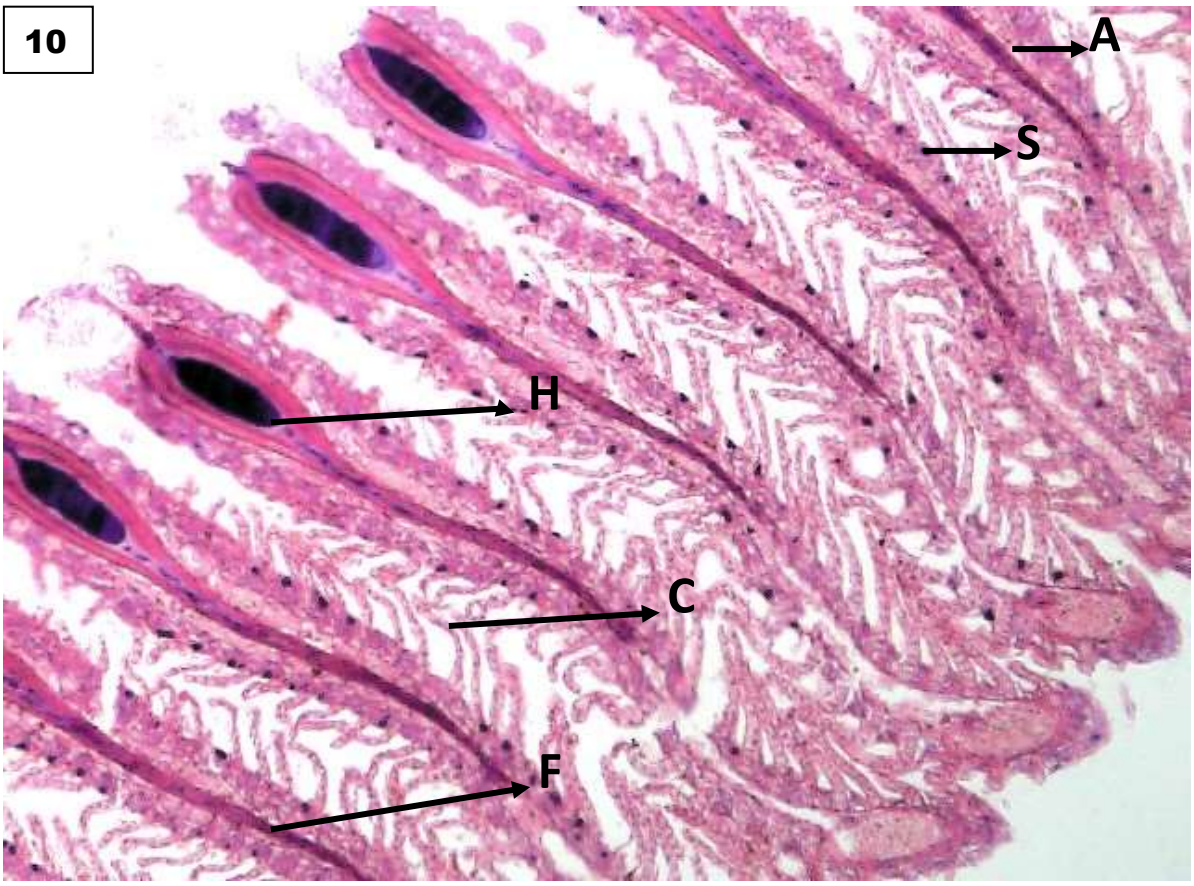


Plate 10 Control: Transverse section through the gills of *Clarias gariepinus* shows normal cellular pattern, ranging from gill arch (A), filament (C), ceratobrachial bone of the arch (H), and nucleus (S). No lesion, no necrosis, no pigments, no malignancy, no inflammation

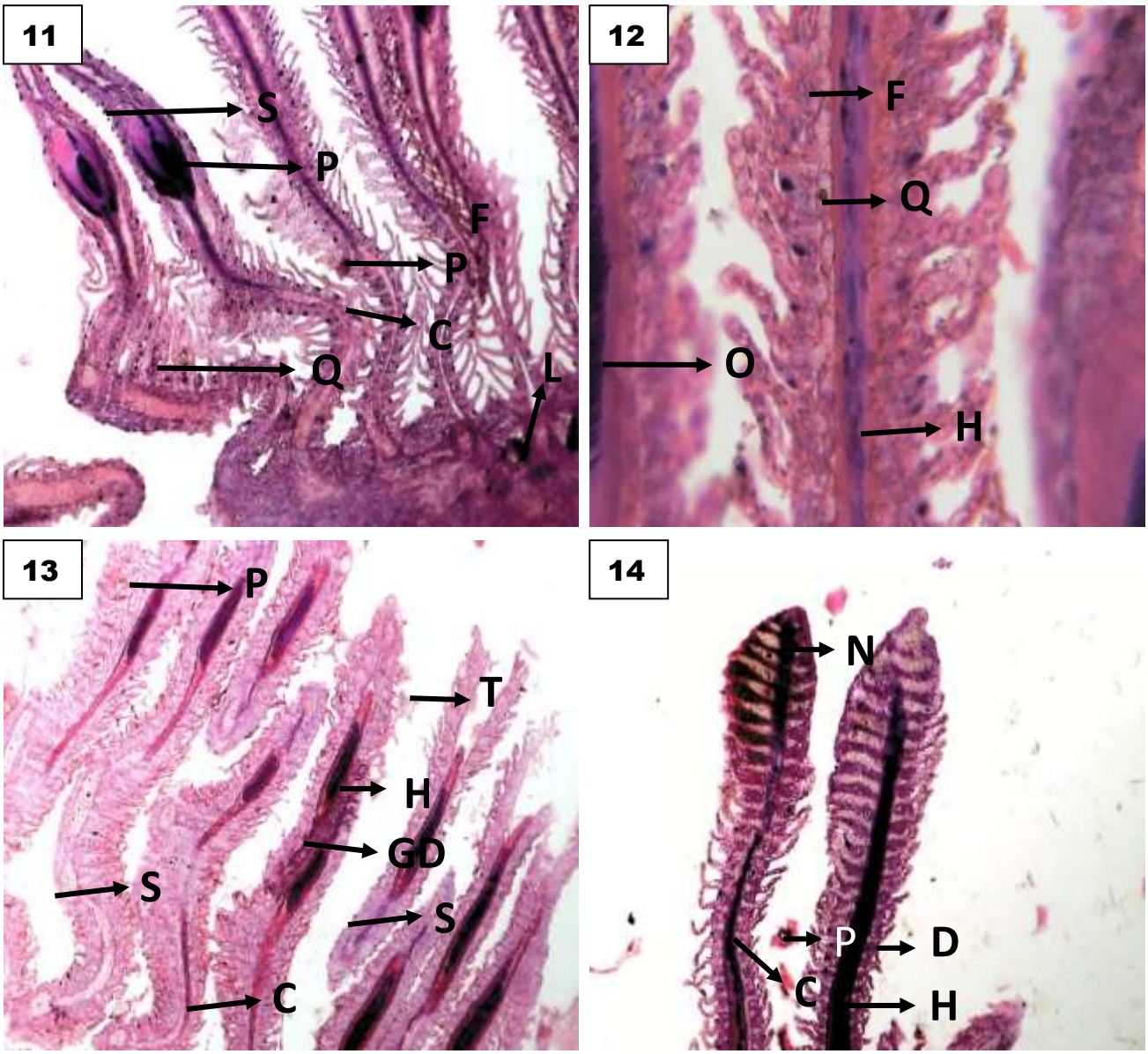


Plate 11-14: Histopathological (T.S) effects of BTEX (1/100th 96hLC$_{50}$) after exposure of C*larias gariepinus* for 60 days (H & E X400). 10 (Benzene), 11 (Toluene), 12 (Ethylbenzene), 13 (Xylene). Inclusion bodies (P), Nuclear abnormality (Q), Inflammation (L), Fusion of secondary lamellae (F), Pigment (O), Ceratobrachial bone of the arch (H), Curving of primary lamellae (C), Loss of secondary lamellae (S) Gill deformation and degeneration (GD), Decrease in length of secondary lamellae (D), Severe area of necrosis (N)

Benzene: Mild necrosis, loss of regular shape and fusion of primary and secondary lamellae, curving of primary lamellae and loss of the secondary lamellae were observed after exposure of fish gills to low sublethal concentration of benzene for 60 days.

Toluene: Nuclear clumping and fusion of secondary lamellae were noted after exposure to low concentration of toluene for 60 days.

Ethylbenzene: Severe necrosis, fusion of secondary lamellae, loss of regular shape, curving of primary lamellae, Loss of secondary lamellae, gill deformation and degeneration were noted after exposure to low sublethal concentration of ethylbenzene.

Xylene: Severe necrosis, decrease in length of secondary lamellae, curving of primary lamellae were noted after exposure to low concentration of xylene.

4.3.2.4.3 Hispathological changes in the gills of C*larias gariepinus* exposed to 1/10th 96hr LC_{50} (high) sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to sublethal concentrations of 1/10th 96hr LC_{50} of benzene, toluene, ethylbenzene and xylene are shown in Plates 15 – 18. The sublethal concentrations of BTEX compounds also induced marked pathological changes in the gills of the fish. The changes include: Severe areas of lesion, severe necrosis, pigment (slightly present), nuclear abnormality (highly present), inclusion bodies (slightly present) and area of inflammation (highly present). The result of the histological examinations also showed histopathological changes such as loss of regular shape, separation of lamellar epithelium, atrophy of secondary gill lamellae, loss of secondary lamellae, fusion of secondary lamellae and curving of primary lamellae in the gills. The changes induced as a result of the exposure to high sublethal concentrations were more severe compared to exposure to low sublethal concentrations

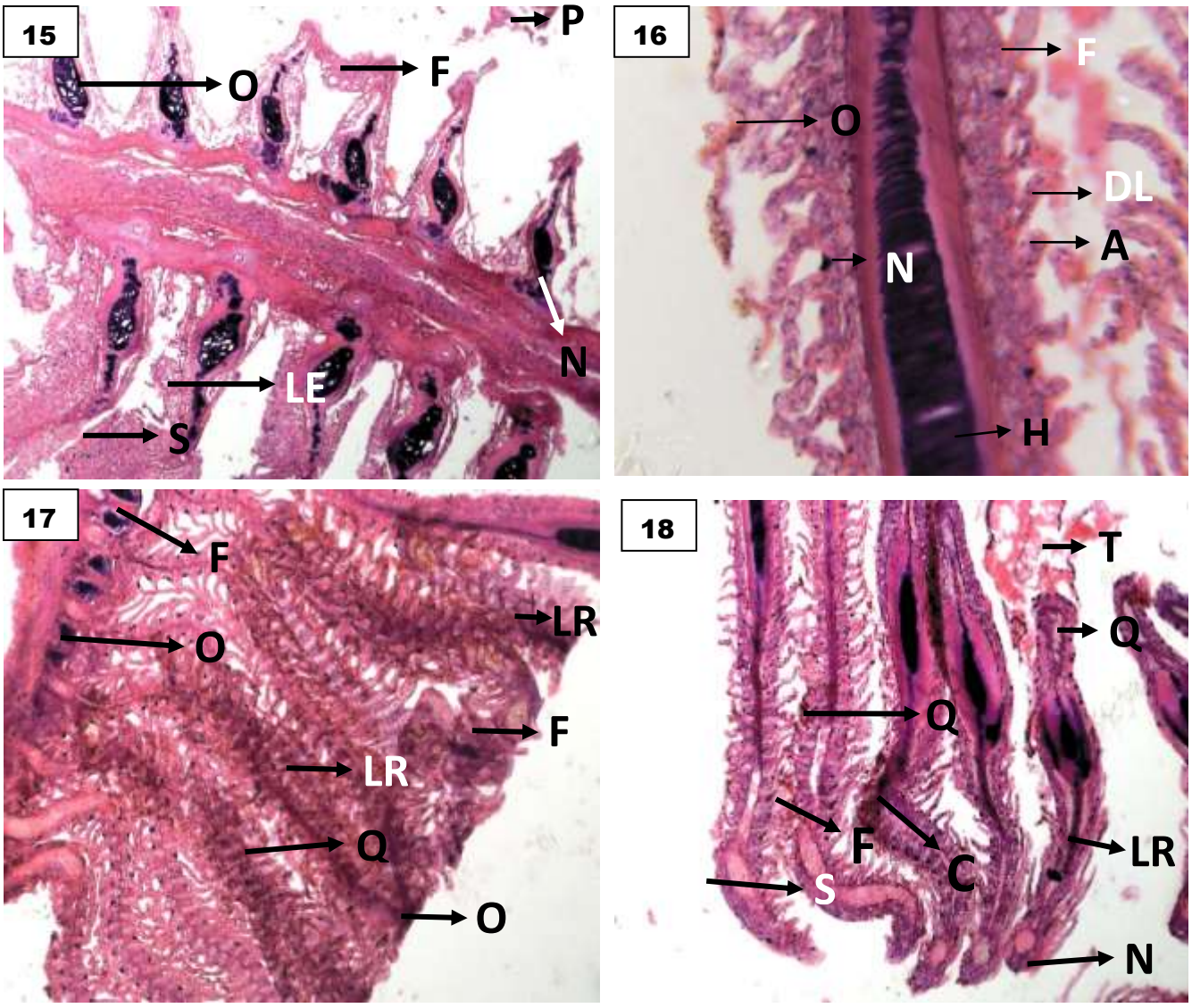


Plate 15-18: Histopathological (T.S) effects of BTEX ($1/10^{th}$ $96hLC_{50}$) after exposure of C*larias gariepinus* for 60 days (H & E X400). 15 (Benzene), 16 (Toluene), 17 (Ethylbenzene), 18 (Xylene). Loss of primary and secondary lamellae (S), Fusion of secondary lamellae (F), Severe area of lesion (LE), Necrosis (N), Pigment (O), Inclusion bodies (P), Loss of regular shape of secondary lamellae (LR), Atrophy of lamellae (A), Necrosis (N), Nuclear abnormality (Q), Connective tissue (T). Decrease in the length of lamellae (DL) and Curving of primary lamellae (C).

of BTEX compounds.

Benzene: loss of regular shape, loss of primary and secondary lamellae, fusion of secondary lamellae, Severe area of lesion, necrosis , pigment and inclusion bodies were noted with exposure of gill to high concentration of benzene.

Toluene: fusion, loss of regular shape of secondary lamellae, atrophy of lamellae, Necrosis , Lesion and pigment were noted after exposure of gill to high concentration of toluene.

Ethylbenzene: Severe areas of necrosis and pigment were noted with nuclear abnormality, loss of regular shape and fusion of secondary lamellae after exposure of gills to high concentration of ethylbenzene.

Xylene: Nuclear abnormality, area of inflammation, decrease in the length of lamellae, loss of regular shape, fusion of secondary and primary lamellae, loss of secondary lamellae and curving of primary lamellae were noted after exposure of gills to high concentration of xylene

4.3.2.5 Histopathological studies in livers of *Clarias gariepinus*

4.3.2.5.1 Control:

Histopathological examination of the liver of the control and exposed groups of fish is shown in photomicrographs in Plates 19 – 27. Plate 19 shows the normal structure of the liver of fish C*larias gariepinus* consisting of normal cellular pattern, normal central vein, bile duct, hepatic vein, hepatic artery and hepatocytes. No changes were observed: No lesion, no necrosis, no pigments, no malignancy, no inflammation and no inclusion bodies were observed in the control animals, as the hepatic organization was intact.

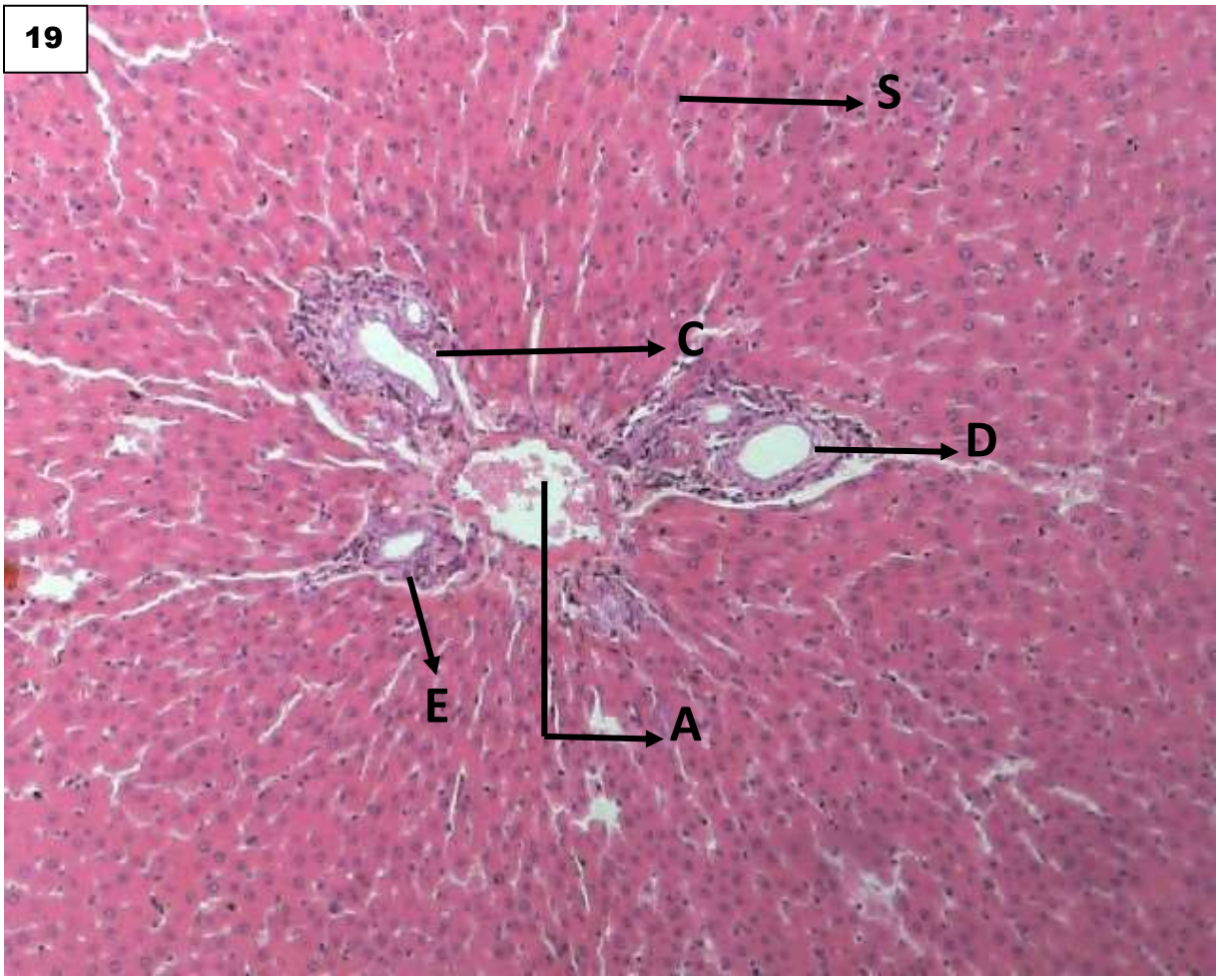


Plate 19. Transverse section through the liver of C*larias gariepinus* shows normal cellular pattern, normal central vein (A), bile duct(C), hepatic vein (D), hepatic artery (E) and hepatocytes. No lesion, no necrosis, no pigments, no malignancy, no inflammation and no inclusion bodies. Mag x400.

4.3.2.5.2 Histopathological changes in the livers of C*larias gariepinus* exposed to 1/100th 96hr LC_{50} low sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to low sublethal concentrations of 1/100th 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 20 – 23. The sublethal concentrations of BTEX compounds has induced marked pathological changes in the liver of the fish. The histological changes include moderate area of inflammation, nuclear abnormality, pigment and inclusion bodies, irregular shaped hepatocytes, increased nuclear vacuolation and bile stagnation identified as brownish –yellow granules in the cytoplasm.

Benzene: Central vein surrounded by degenerating hepatocytes, nucleus degeneration, increased vacoulation with excess fat storage and bile stagnation identified as brownish –yellow granules in the cytoplasm were noted after exposure of liver to low concentration of benzene for 60 days.

Toluene: Hepatocytes with distorted architecture, nuclear abnormality and vacuolization were observed after exposure of liver to low concentration of toluene for 60 days.

Ethylbenzene: Severe hepatic necrosis, nuclear abnormality, spotted structures melano-macrophages and bile stagnation identified as brownish –yellow granules in the cytoplasm were observed after exposure of liver to low sublethal concentration of ethylbenzene.

Xylene: Severe hepatic necrosis with spotted structures, macrophages, vacuolization and bile stagnation identified as brownish –yellow granules in the cytoplasm were observed after exposure of liver to low concentration of xylene.

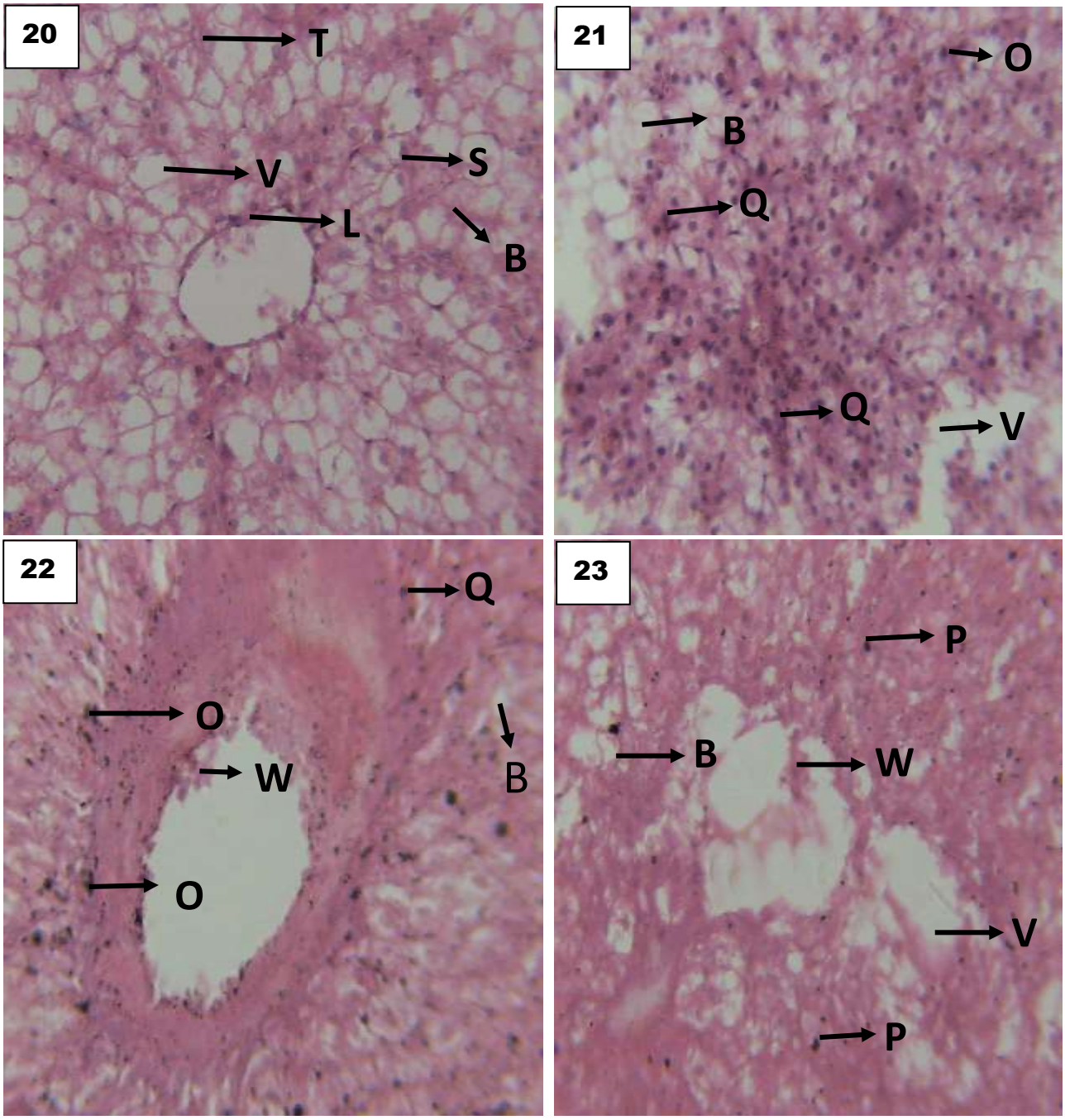


Plate 20-23: Histopathological (T.S) effects of BTEX (1/100th 96hLC$_{50}$) after exposure of C*larias gariepinus* liver for 60 days (H & E X400). 20 (Benzene), 21 (Toluene), 22 (Ethylbenzene), 23 (Xylene). Inflammation(L), Nucleus (S), Connective tissue(T), Irregular shaped nucleus and nucleus degeneration ( ), Increased vacoulation with excess fat storage (V), Brownish-yellow granules (B), Nuclear abnormality (Q), Pigment (O) and Erythrocyte (W) and Spotted structures, melano-macrophages (P).

4.3.2.5.3 Hispathological changes in the livers of C*larias gariepinus* exposed to 1/10th 96hr LC_{50} high sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to high sublethal concentrations of 1/10th 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 24 – 27. The high sublethal concentrations of BTEX compounds also induced marked pathological changes in the liver of the fish. The changes include moderate to severe areas of lesions, nuclear abnormality, necrosis, pigment, inclusion bodies, area of inflammation, irregular shaped hepatocytes, increased nuclear vacuolation and bile stagnation identified as brownish–yellow granules in the cytoplasm and necrosis. The excessive deposition of fat is especially evident. The large central fat vacuole forces the nucleus of the hepatocyte to the edge of the cell and may even flatten it. These fatty deposits are obvious macroscopically as a motthng or even total whitening of the liver.

Benzene: Moderate area of lesions, nuclear abnormality and area of inflammation, irregular shaped hepatocytes, nuclear vacuolisation, bile stagnation as brownish- yellow were observed after exposure of liver to high concentration of benzene.

Toluene: Severe area of lesion, nuclear abnormality, nuclear degeneration, vacuolization, bile stagnation as brownish-yellow granules and area of inflammation were observed after exposure of liver to high concentration of toluene.

Ethylbenzene: Severe area of lesion, necrosis, pigment, inclusion bodies, irregular shaped nucleus, vacuolization , bile stagnation and area of inflammation were observed after exposure of liver to high concentration of ethylbenzene.

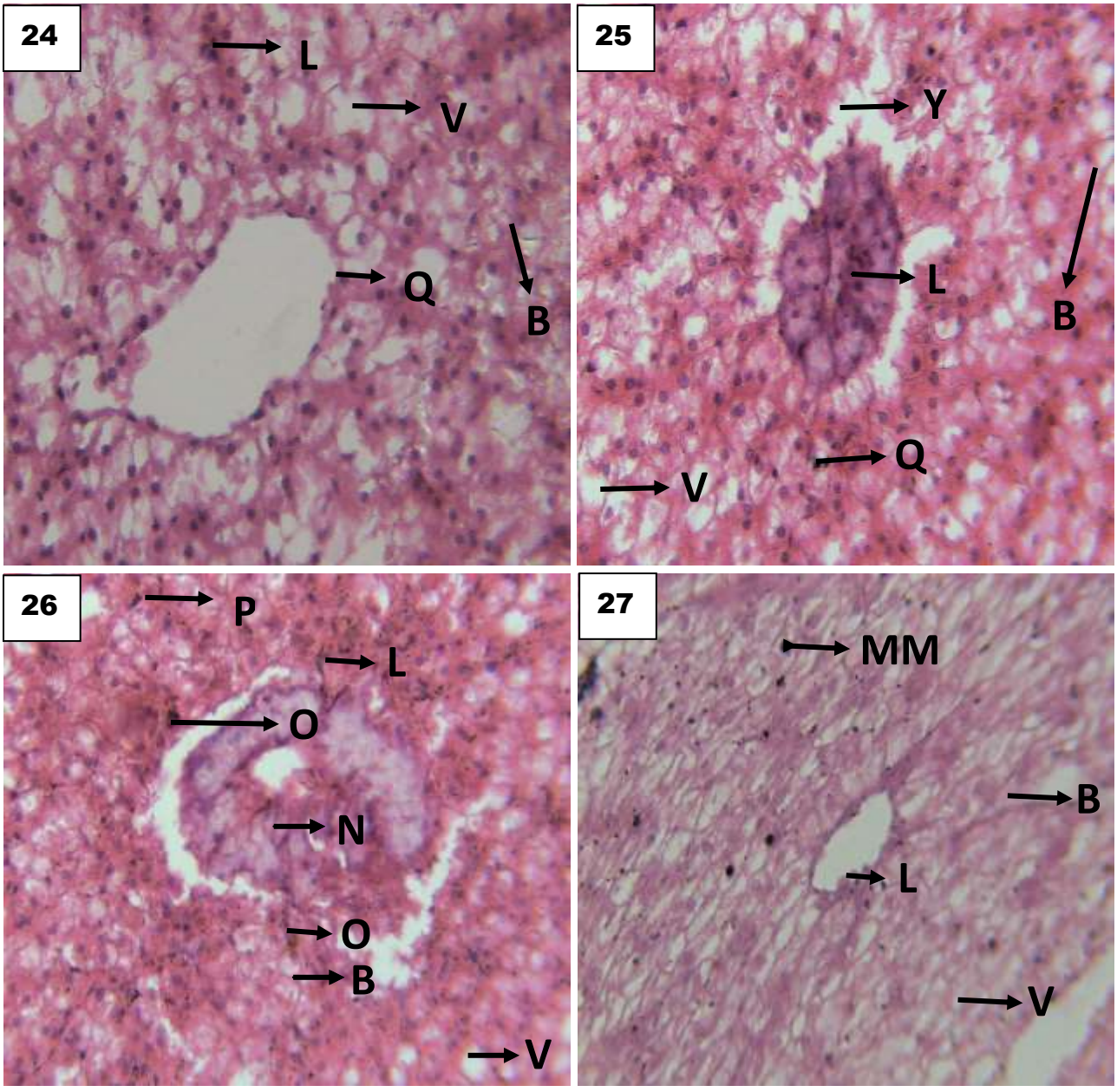


Plate 24-27: Histopathological (T.S) effects of BTEX (1/10th 96hLC$_{50}$) after exposure of C*larias gariepinus* for 60 days (H & E X400). 24 (Benzene), 25 (Toluene), 26 (Ethylbenzene), 27 (Xylene). Nuclear abnormality (Q), Inflammation (L), Vacuolisaion (V), Brownish- yellow granules (B), Interstitial space (Y). Necrosis (N), Pigment (O), inclusion bodies (P), Moderate area of lesion and area of inflammation (L). Melano-macrophages (MM).

Xylene: Area of inflammation, vacuolization, spotted structures melano- macrophages and bile stagnation were observed after exposure of liver to high concentration of xylene.

4.3.2.6. Histopathological studies in gonads of *Clarias gariepinus*

4.3.2.6.1 Control:

Histopathological examination of the gonad of the control and exposed groups of fish is shown in photomicrographs in Plates 28 – 36. Plate 28 shows the normal structure of the gonads of female fish C*larias gariepinus* consisting of different developmental stages namely oogonia and primary oocytes. No changes were observed: No lesion, no necrosis, no pigments, no malignancy, no inflammation and no inclusion bodies were observed in the control animals.

4.3.2.6.2 Histopathological changes in the gonads of C*larias gariepinus* exposed to 1/100th 96hr LC_{50} low sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to low sublethal concentrations of 1/100th 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 29 – 32. The sublethal concentrations of the hydrocarbons, has induced marked pathological changes in the gonads of the fish. The changes include necrosis, wrinkling of the membrane, deformed oocytes, contraction of oocytes, shrinkage of nucleus, presence of melanomacrophages and presence of oocytes either shrunk or vacuolated.

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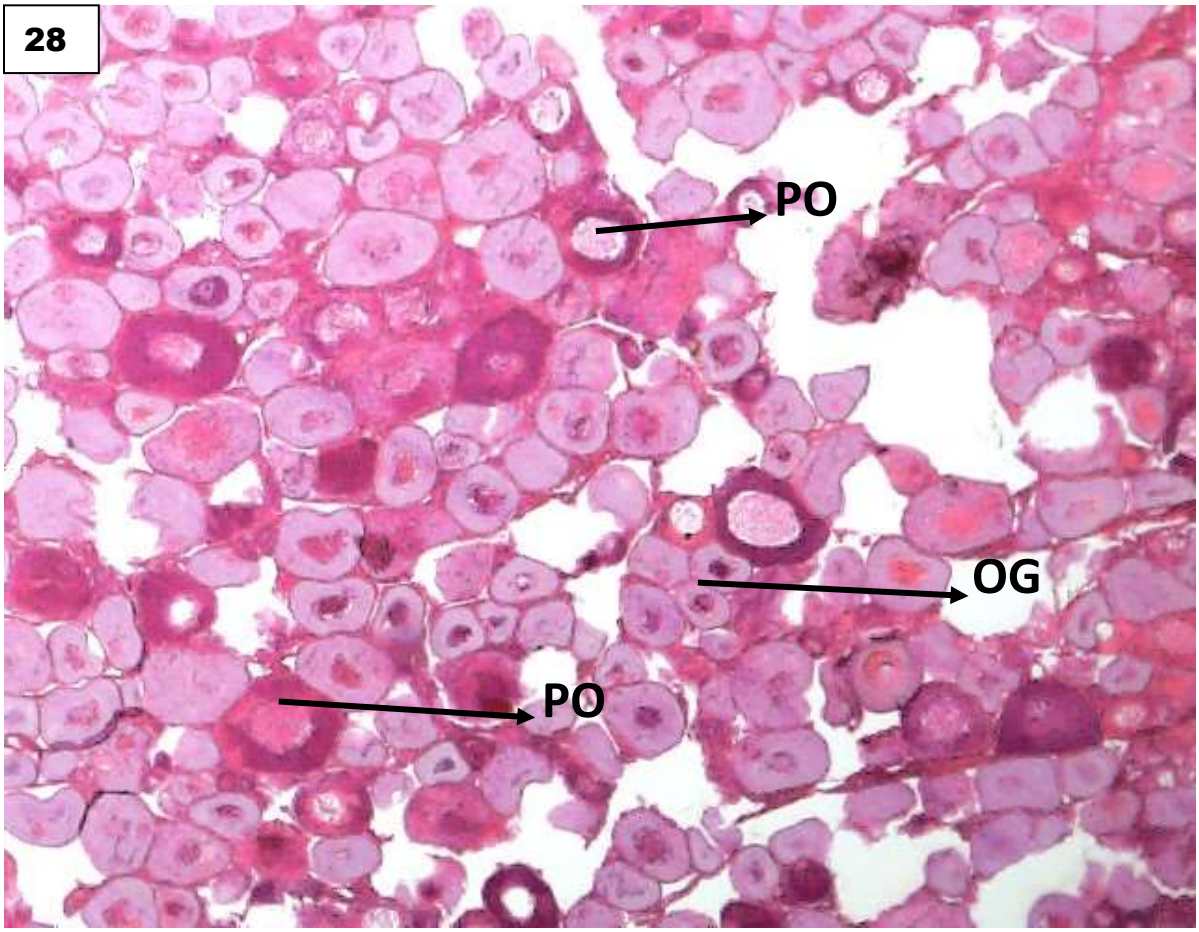

Plate 28. Transverse section through the Gonad of shows normal cellular pattern of the female gonad of control *Clarias gariepinus* showing large number of Oogonia (OG) and primary Oocytes (PO). No malignant, no necrosis, no lesion, no pigment and inclusion bodies seen. Mag. X400.

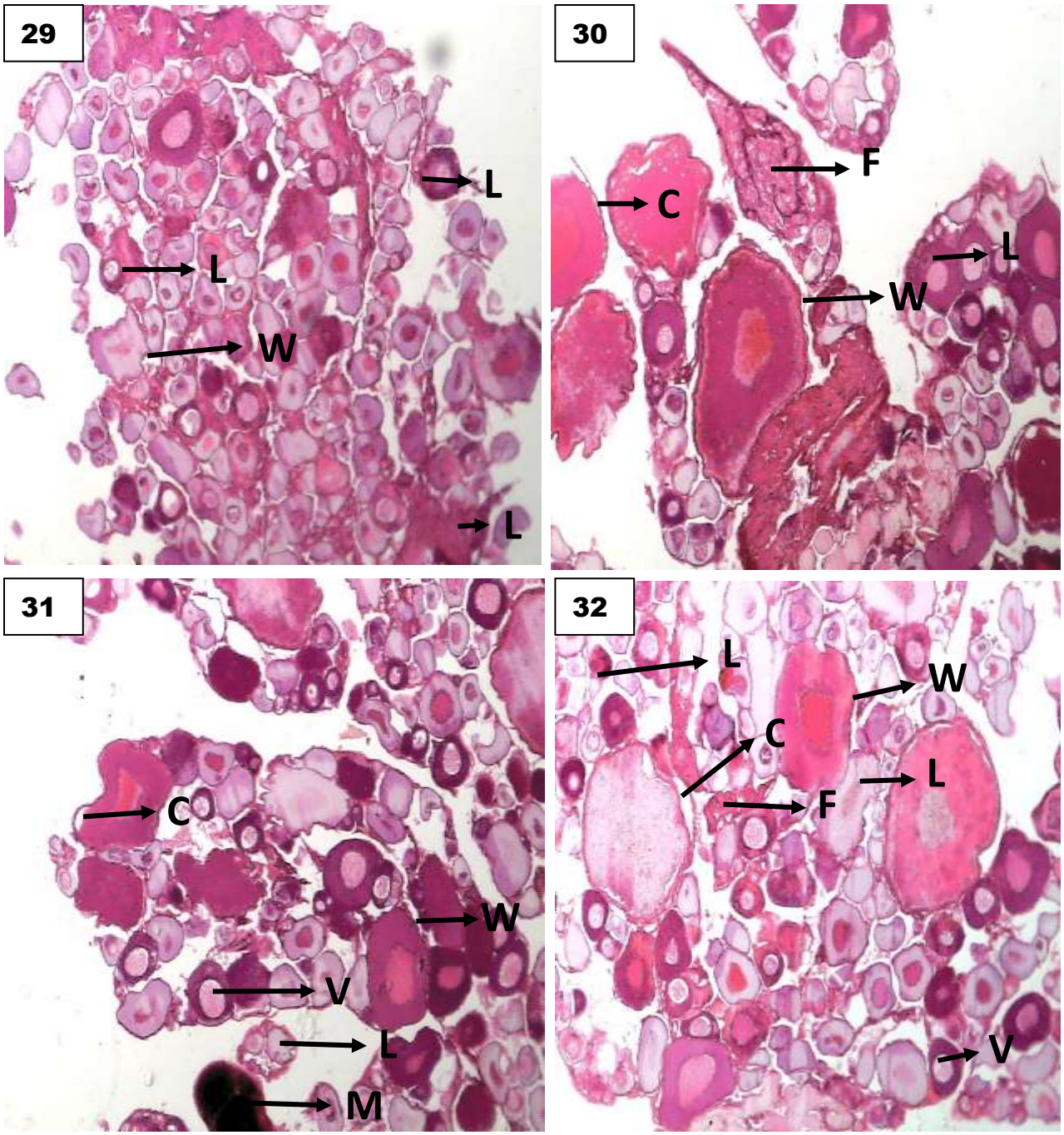

Plate 29-32: Histopathological (T.S) effects of BTEX (1/100th 96hLC$_{50}$) after exposure of C*larias gariepinus* gonads for 60 days (H & E X400). 29 (Benzene), 30 (Toluene), 31 (Ethylbenzene), 32 (Xylene). Wrinkling of oocyte membrane (W), Deformed oocytes (L), Atresic follicles (F), Contraction of oocyte (C), Vacuolated oocytes (V) and Melano-macrophages (M).

Benzene: Moderate area of necrosis, wrinkling of oocyte membrane, deformed oocytes were observed after exposure of gonad to low concentration of benzene for 60 days.

Toluene: Moderate area of necrosis, wrinkling of oocyte membrane, deformed oocyte, contraction of oocyte, were observed after exposure of gonads to low concentration of toluene.

Ethylbenzene: Moderate area of necrosis with wrinkling of oocyte membrane, deformed oocyte, contraction of oocyte, vacuolated oocytes and macrophages, were observed after exposure of gonad to low concentration of ethylbenzene.

Xylene: Wrinkling of oocyte membrane, deformed oocyte, contraction of oocytes and vacuolated oocytes, were observed after exposure of gonad to low concentration of xylene.

4.3.2.6.3. Hispathological changes in the gonads of C*larias gariepinus* exposed to $1/10^{th}$ 96hr LC_{50} high sublethal concentration of BTEX compounds after 60 days.

The histological changes after exposure to high sublethal concentrations of $1/10^{th}$ 96hr LC_{50} of Benzene, Toluene, Ethylbenzene and Xylene are shown in Plates 33 – 36. The high sublethal concentrations of BTEX compounds induced marked pathological changes in the gonads of the fish. The changes include necrosis, wrinkling of the membrane, deformed oocytes, atretic follicles, contraction of oocytes, shrinkage of nucleus, presence of melanomacrophages and oocytes either shrunk or vacuolated.

Benzene: Moderate area of lesion and necrosis with wrinkling of oocyte membrane, deformed oocyte , contraction of oocytes and melano-macrophages, were observed after exposure of gonad to high concentration of benzene.

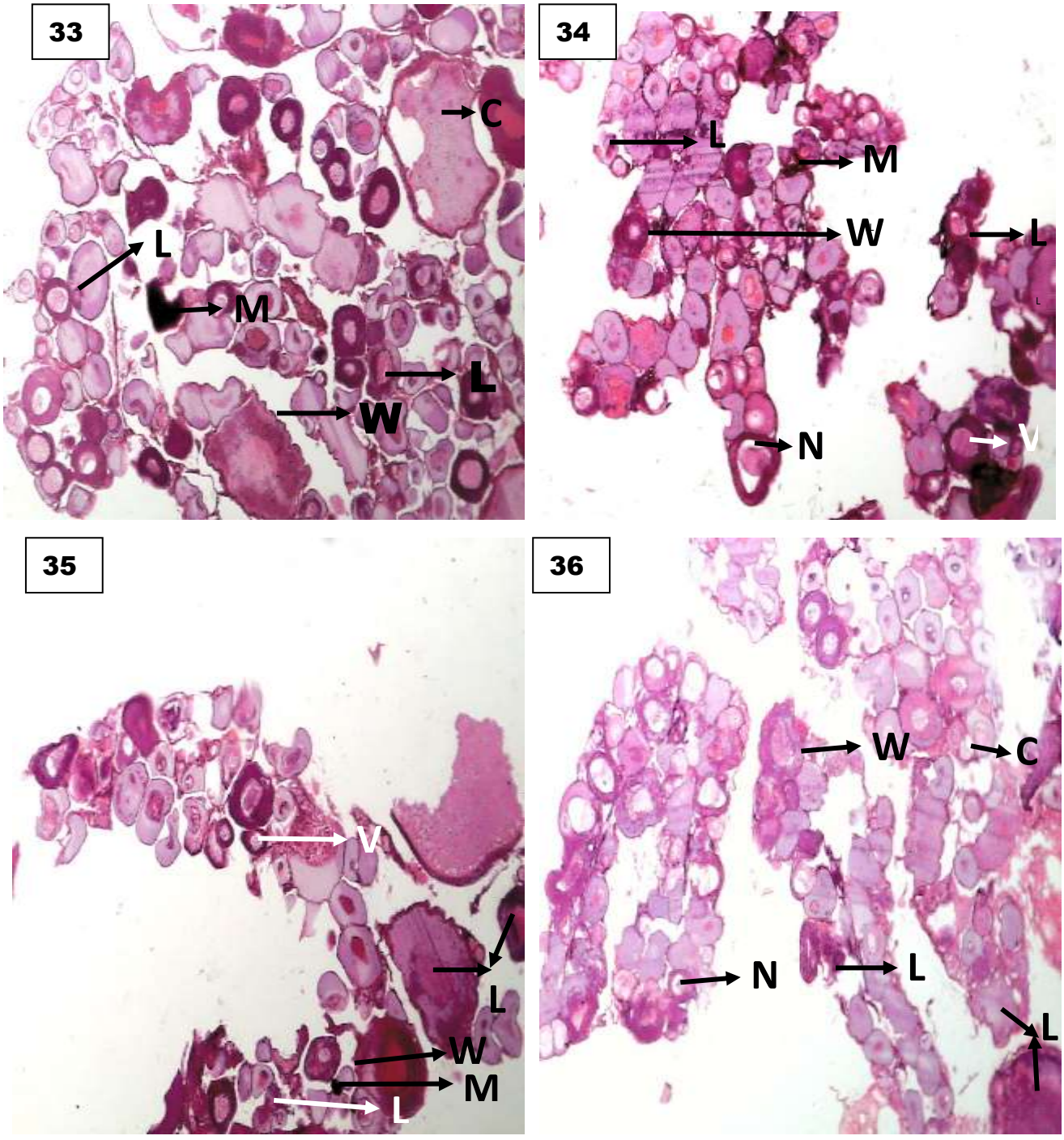

Plate 33-36: Histopathological (T.S) effects of BTEX (1/10th 96hLC$_{50}$) after exposure of C*larias gariepinus* gonads for 60 days (H & E X400). 33 (Benzene), 34 (Toluene), 35 (Ethylbenzene), 36 (Xylene). Wrinkling of oocyte membrane (W), Deformed oocyte (L), Contraction of oocytes (C), Macrophages (M), Vacuolated oocyte (V) and Shrinkage of nucleus (N)

Toluene: Wrinkling of oocyte membrane, deformed oocyte, vacuolated oocyte, shrinkage of nucleus and macrophages, were observed after exposure of gonad to high concentration of toluene.

Ethylbenzene: Wrinkling of oocyte, deformed oocyte, vacuolated oocyte and melano-macrophages, were observed after exposure of gonad to high concentration of ethylbenzene.

Xylene: Moderate area of lesion, necrosis with wrinkling of oocyte membrane, deformed oocyte, contraction of oocyte, shrinkage of nucleus, were observed after exposure of gonad to high concentration of xylene for 60 days.

4.4 Identified enzymatic and non enzymatic biomarkers in *Chrysichthys nigrodigitatus* and *Tilapia Zillii* collected from different stations of the lagos lagoon

The activities of antioxidant enzymes and non enzymes in the liver and gills of *C. nigrodigitatus* (1) and *T. zillii* (2) collected from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG,) stations of the Lagos lagoon are presented in Figures 4.40- 4.44. The results from the laboratory studies were confirmed in the field studies. The activities of antioxidant enzymes and non enzymes were higher in the gills than in the livers of *C. nigrodigitatus* and *T. zillii* sampled from the lagoon. Comparing the results of the activities of antioxidant enzymes and non enzymes in gills and livers of fishes from the Lagos lagoon and the control fish, day 0 (Figures 4.30 – 4.39), the activities of SOD, GST, CAT and GSH were inhibited in fishes collected from the Lagos lagoon compared with the control fish from the laboratory. It was observed that the levels of GSH, CAT, SOD and GST in the gills and livers of fish from the Lagos lagoon were significantly lower than the levels of SOD (gill - 102.42±10.14, liver- 216.23u/mg protein), CAT (gill- 717.5, liver- 740.95u/mg protein), GSH (gill-51.76, liver-37.41nm/mg protein), and

GST (liver- 243.71, gill- 422.43nm/mg protein) in the control fishes, day 0 (Figs 4.30 – 4.39). The level of MDA was significantly higher in gills and livers of fishes from the Lagos lagoon than the control fish, day 0 (gill- 0.68, liver- 1.22 nm/mg protein).

4.4.1 SOD in the liver and gills of fish from the lagoon

SOD decreased from 216.23u/mg protein in the control fish to 5.34 u/mg protein (Iddo), 8.09 u/mg protein (Atlas Cove), 14.48 u/mg protein (Apapa), 24.15u/mg protein (Unilag) in the liver of *C. nigrodigitatus* from the lagoon. SOD decreased from 216.23u/mg protein in the control fish to 26.92 u/mg protein (Iddo), 23.45 u/mg protein (Atlas Cove), 51.42 u/mg protein (Apapa), 4.1u/mg protein (Unilag) in the liver of *T. zillii* from the lagoon. The percentage decrease in SOD levels in the livers of *C. nigrodigitatus* were 97.53% (IDDO1), 96.26% (AC1), 93.30% (AP1), 88.83% (ULAG1) compared to the control fish. The percentage decrease in SOD levels in the livers of *T. zillii* were 87.55% (IDDO2), 89.16% (AC2), 122.19% (AP2), 98.10% (ULAG2) compared to the control fish (Figure 4.40).

SOD decreased from 102.42 u/mg protein in the control fish to 24.51 u/mg protein (Iddo), 62.88 u/mg protein (Unilag) in the gills of *C. nigrodigitatus* from the lagoon. SOD decreased from 102.42 u/mg protein in the control fish to 53.05 u/mg protein (Iddo), 49.33 u/mg protein (Atlas Cove), 55.86 u/mg protein (Apapa), 15.94 u/mg protein (Unilag) in the gills of *T. zillii* from the lagoon. The percentage decrease in SOD levels in the gills of *C. nigrodigitatus* were 76.07% (IDDO1), 38.61% (ULAG1) compared to the control fish. The percentage decrease in SOD levels in the gills of *T. zillii* were 48.21% (IDDO2), 51.84% (AC2), 45.47% (AP2) , 84.44% (ULAG2) compared to the control fish (Figure 4.40).

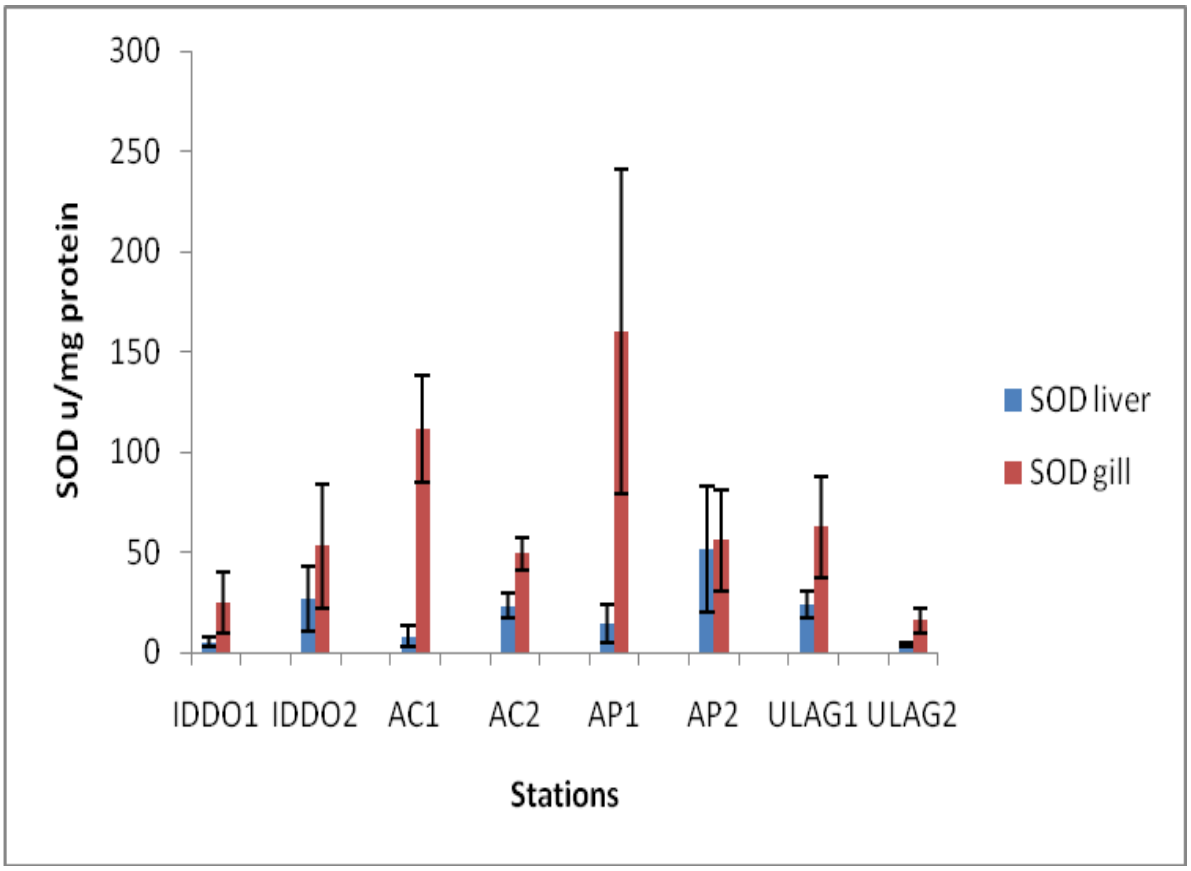

Figure 4.40: The activity of SOD in the liver and gills of *C. nigrodigitatus*(1) and *T. zillii* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon. Values are mean±SD.

4.4.2 CAT in the liver and gills of fish from the lagoon

CAT decreased from 740.95 u/mg protein in the control fish to 20.8 u/mg protein (Iddo), 48.59 u/mg protein (Atlas Cove), 262.56 u/mg protein (Apapa), 97.17 u/mg protein (Unilag) in the liver of *C. nigrodigitatus* from the lagoon. SOD decreased from 740.95 u/mg protein in the control fish to 59.02 u/mg protein (Iddo), 179.64 u/mg protein (Atlas Cove), 86.44 u/mg protein (Apapa), 19.72u/mg protein (Unilag) in the liver of *T. zillii* from the lagoon. The percentage decrease in CAT levels in the liver of *C. nigrodigitatus* were 97.19% (IDDO1), 93.44% (AC1), 64.56% (AP1), 87.02% (ULAG1) compared to the control fish. The percentage decrease in CAT levels in the liver of *T. zillii* were 92.04% (IDDO2), 75.76% (AC2), 88.33% (AP2) , 97.34% (ULAG2) compared to the control fish (Figure 4.41).

CAT decreased from 716.5 u/mg protein in the control fish to 159.2 u/mg protein (Iddo), 220.99 u/mg protein (Atlas Cove), 528.93 u/mg protein (Apapa), 273.51 u/mg protein (Unilag) in the gills of *C. nigrodigitatus* from the lagoon. CAT decreased from 716.5 u/mg protein in the control fish to 244.72 u/mg protein (Iddo), 515.7 u/mg protein (Atlas Cove), 172.72 u/mg protein (Apapa), 218.45 u/mg protein (Unilag) in the gills of *T. zillii* from the lagoon. The percentage decrease in CAT levels in the gills of *C. nigrodigitatus* were 77.81% (IDDO1), 69.2% (AC1), 26.28% (AP1), 61.88% (ULAG1) compared to the control fish. The percentage decrease in CAT levels in the gills of *T. zilli* were 65.89% (IDDO2), 28.13% (AC2), 75.93% (AP2) , 69.55% (ULAG2) compared to the control fish (Figure 4.41).

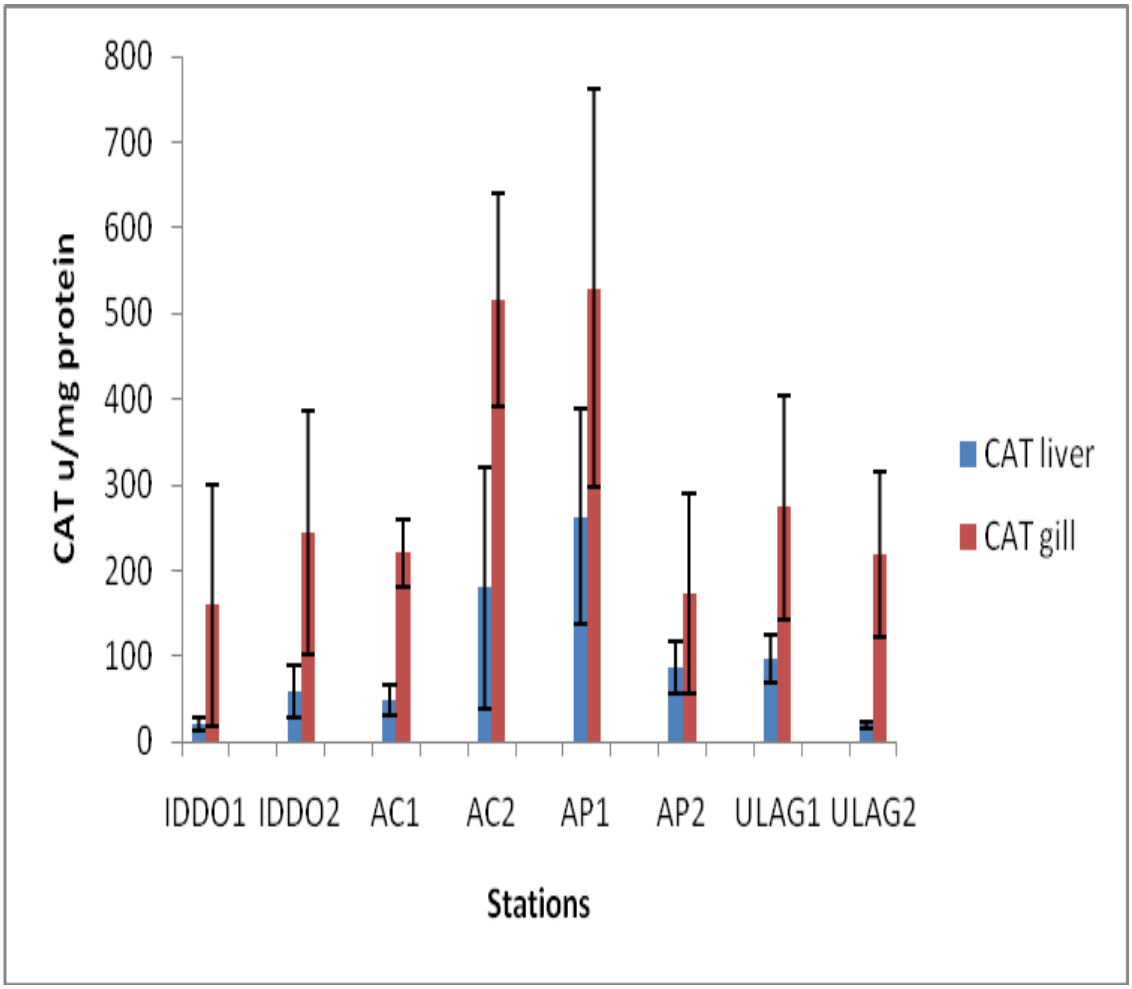

Figure 4.41: The activity of CAT in the livers and gills of *C. nigrodigitatus*(1) and *T. zillii* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon. Values are mean±SD.

4.4.3 GST in the liver and gills of fish from the lagoon

GST decreased from 243.71 u/mg protein in the control fish to 2.42 u/mg protein (Iddo), 4.52 u/mg protein (Atlas Cove), 1.74 u/mg protein (Apapa), 1.06 u/mg protein (Unilag) in the liver of *C. nigrodigitatus* from the lagoon. SOD decreased from 243.71 u/mg protein in the control fish to 6.76 u/mg protein (Iddo), 1.79 u/mg protein (Atlas Cove), 4.13 u/mg protein (Apapa), 1.19 u/mg protein (Unilag) in the liver of *T. zillii* from the lagoon. The percentage decrease in GST levels in the livers of *C. nigrodigitatus* were 99.00% (IDDO1), 98.15% (AC1), 99.29% (AP1), 99.56% (ULAG1) compared to the control fish. The percentage decrease in GST levels in the livers of *T. zillii* were 97.23% (IDDO2), 99.27% (AC2), 98.31% (AP2) , 99.51% (ULAG2) compared to the control fish (Figure 4.42).

GST decreased from 422.43 u/mg protein in the control fish to 4.88 u/mg protein (Iddo), 19.06 u/mg protein (Atlas Cove), 28.82 u/mg protein (Apapa), 3.85 u/mg protein (Unilag) in the gills of *C. nigrodigitatus* from the lagoon. SOD decreased from 422.43 u/mg protein in the control fish to 4.08 u/mg protein (Iddo), 6.77 u/mg protein (Atlas Cove), 2.02 u/mg protein (Apapa), 6.14 u/mg protein (Unilag) in the gills of *T. zillii* from the lagoon. The percentage decrease in GST levels in the gills of *C. nigrodigitatus* were 98.85% (IDDO1), 95.49% (AC1), 93.18% (AP1), 99.09% (ULAG1) compared to the control fish. The percentage decrease in GST levels in the gills of *T. zillii* were 99.03% (IDDO2), 98.40% (AC2), 99.52% (AP2), 98.53% (ULAG2) compared to the control fish (Figure 4.42).

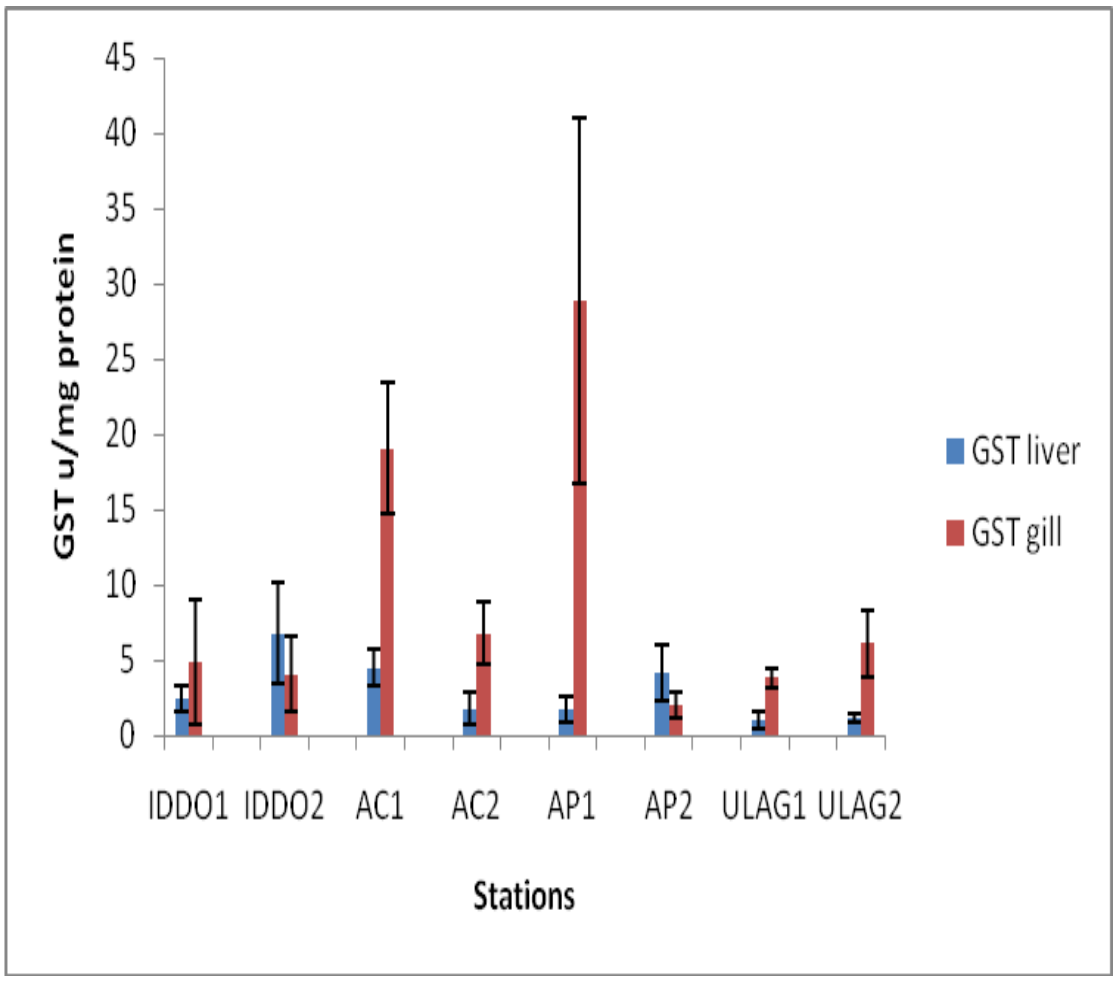

Figure 4.42: The activities of GST in the liver and gills of *C. nigrodigitatus*(1) and *T. zillii* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon. Values are mean±SD.

4.4.4 GSH in the livers and gills of fish from the lagoon

GSH decreased from 37.41nm /mg protein in the control fish to 0.17 u/mg protein (Iddo), 0.33 u/mg protein (Atlas Cove), 0.13 u/mg protein (Apapa), 0.14 u/mg protein (Unilag) in the liver of *C. nigrodigitatus* from the lagoon. SOD decreased from 37.41nm /mg protein in the control fish to 0.49 u/mg protein (Iddo), 0.13 u/mg protein (Atlas Cove), 0.30 nm/mg protein (Apapa), 0.07 u/mg protein (Unilag) in the liver of *T. zillii* from the lagoon. The percentage decrease in GSH levels in the livers of *C. nigrodigitatus* from the lagoon were 99.54% (IDDO1), 100.21% (AC1), 99.65% (AP1), 99.63% (ULAG1), compared to the control fish . The percentage decrease in GSH levels in the livers of *T. zillii* from the lagoon were 98.69% (IDDO2), 99.65% (AC2), 99.20% (AP2) , 99.81% (ULAG2) compared to the control fish (Figure 4.43).

GSH decreased from 51.76 nm /mg protein in the control fish to 0.51 nm/mg protein (Iddo), 1.45 u/mg protein (Atlas Cove), 1.52 u/mg protein (Apapa), 0.28 nm/mg protein (Unilag) in the gills of *C. nigrodigitatus* from the lagoon. SOD decreased from 51.76 nm /mg protein in the control fish to 0.25 u/mg protein (Iddo), 0. 49 nm/mg protein (Atlas Cove), 0.16 u/mg protein (Apapa), 0.39 u/mg protein (Unilag) in the gills of *T. zilli* from the lagoon. The percentage decrease in GSH levels in the gills of *C. nigrodigitatus* from the lagoon were 99.01% (IDDO1), 97.20% (AC1), 97.06% (AP1), 99.45% (ULAG1), compared to the control fish. The percentage decrease in GSH levels in the gills of *T. zillii* from the lagoon were 99.52% (IDDO2), 88.76% (AC2), 99.69% (AP2) , 99.24% (ULAG2) compared to the control fish (Figure 4.43).

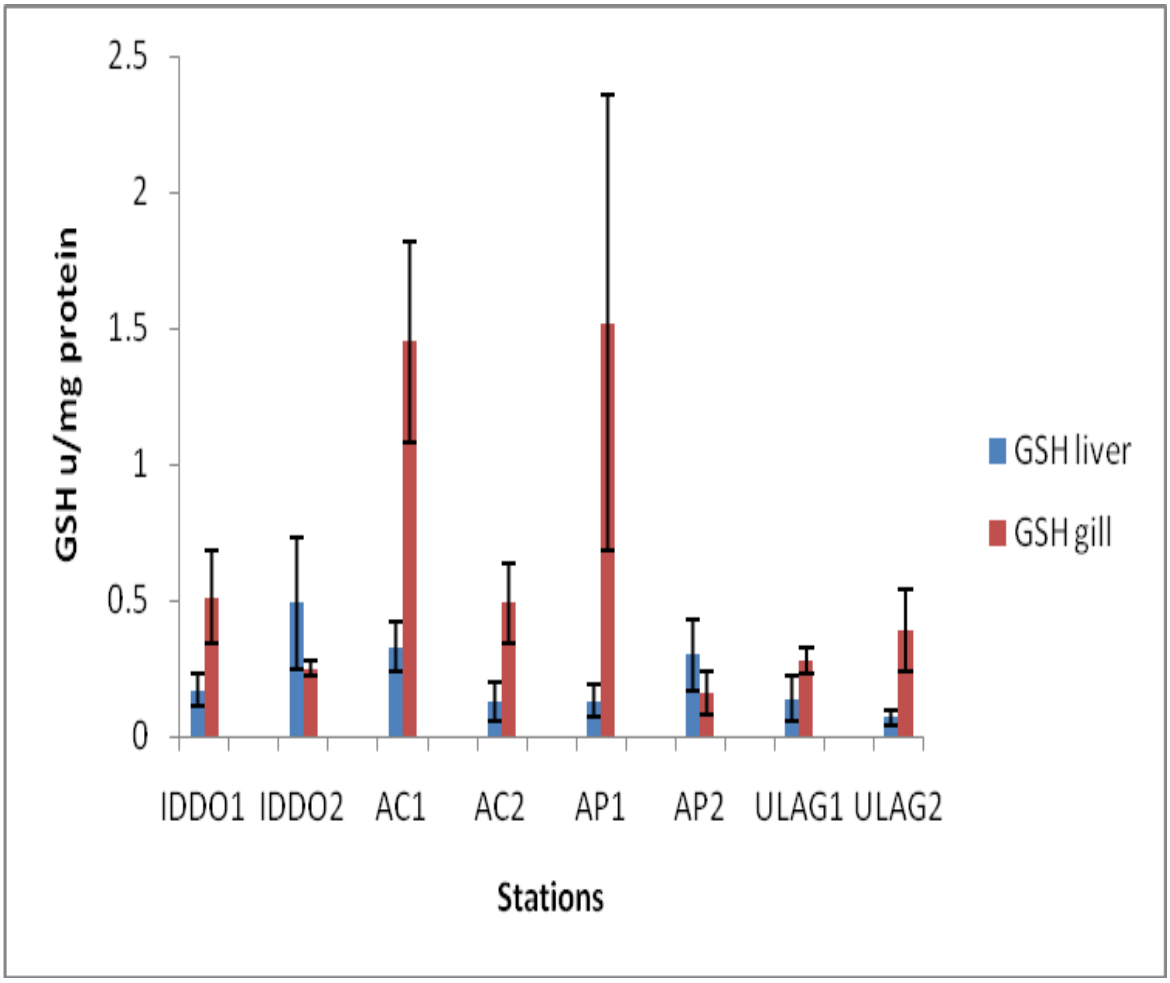

Figure 4.43: The levels of GSH in the liver and gills of *C. nigrodigitatus*(1) and *T. zillii* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon. Values are mean±SD.

4.4.5 MDA in the livers and gills of fish from the lagoon

MDA increased from 1.22nm /mg protein in the control fish to 21.18 u/mg protein (Iddo), 11.91 u/mg protein (Atlas Cove), 16.94 u/mg protein (Apapa), 6.01 u/mg protein (Unilag) in the liver of *C. nigrodigitatus* from the lagoon. MDA increased from 1.22nm /mg protein in the control fish to 10.99 u/mg protein (Iddo), 27.29 u/mg protein (Atlas Cove), 31.59 nm/mg protein (Apapa), 13.81 u/mg protein (Unilag) in the liver of *T. zillii* from the lagoon (Figure 4.44). The percentage increase in MDA levels in the livers of *C. nigrodigitatus* were 1636.07% (IDDO1), 876.23% (AC1), 1288.53% (AP1), 392.62% (ULAG1) compared to the control fish. The percentage increase in MDA levels in the livers of *T. zilli* were 800.82% (IDDO2), 2136.89% (AC2), 2489.34% (AP2), 1031.97% (ULAG2) compared to the control fish.

MDA increased from 0.68nm /mg protein in the control fish to 10.89 u/mg protein (Iddo), 5.41 u/mg protein (Atlas Cove), 5.92 u/mg protein (Apapa), 5.37 u/mg protein (Unilag) in the gills of *C. nigrodigitatus* from the lagoon. MDA increased from 0.68nm /mg protein in the control fish to 11.35 u/mg protein (Iddo), 5.02 u/mg protein (Atlas Cove), 12.17 nm/mg protein (Apapa), 3.78 u/mg protein (Unilag) in the gills of *T. zillii* from the lagoon. The percentage increase in MDA levels in the gills of *C. nigrodigitatus* were 1501.47% (IDDO1), 695.59% (AC1), 770.59% (AP1), 689.71% (ULAG1) compared to the control fish. The percentage increase in MDA levels in the gills of *T. zillii* were 1569.12% (IDDO2), 638.24% (AC2), 1689.71% (AP2), 455.89% (ULAG2) compared to the control fish.

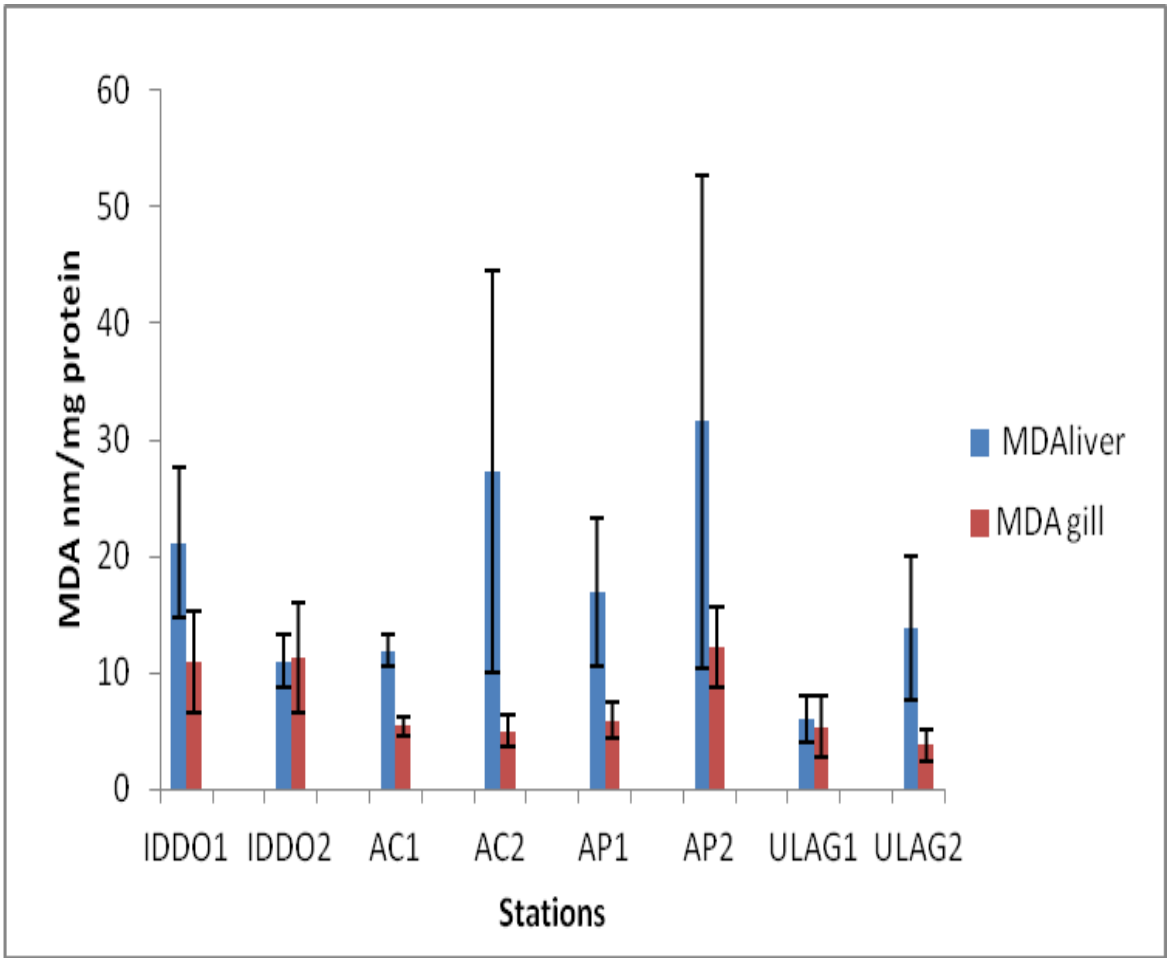

Figure 4.44: The levels of MDA in the liver and gills of *C. nigrodigitatus*(1) and *T. zillii* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon. Values are mean±SD.

4.5 Identified histopathological biomarkers in *Chrysichthys nigrodigitatus* and *Tilapia Zillii* collected from different stations of the Lagos lagoon.

Plates 37- 40 show the histopathological alterations seen in the gonad of *C. nigrodigitatus* and *T. zillii* collected from different stations of the Lagos lagoon. The pathological findings include aggregation of inflammatory cells, vascular congestion, cellular degeneration and wrinkling of oocyte membrane. Contraction of ovum, cytoplasmic vacuolation, lesion and pigment were also seen in the gonads.

Plates 41-44 show the histopathological changes observed in the gills of *C. nigrodigitatus* and *T. zillii* collected from different stations of the Lagos lagoon. Cellular degeneration, fusion of secondary lamellae, pigment, inflammation, loss of secondary lamellae, necrosis, inclusion bodies, cytoplasmic vacuolation were observed in the gills.

Plates 45-48 show the histopathological alterations seen in the liver of *C. nigrodigitatus* and *T. zillii* collected from different stations of the Lagos lagoon. The pathological changes observed were vascular congestion and degeneration, cellular degeneration, inflammation.

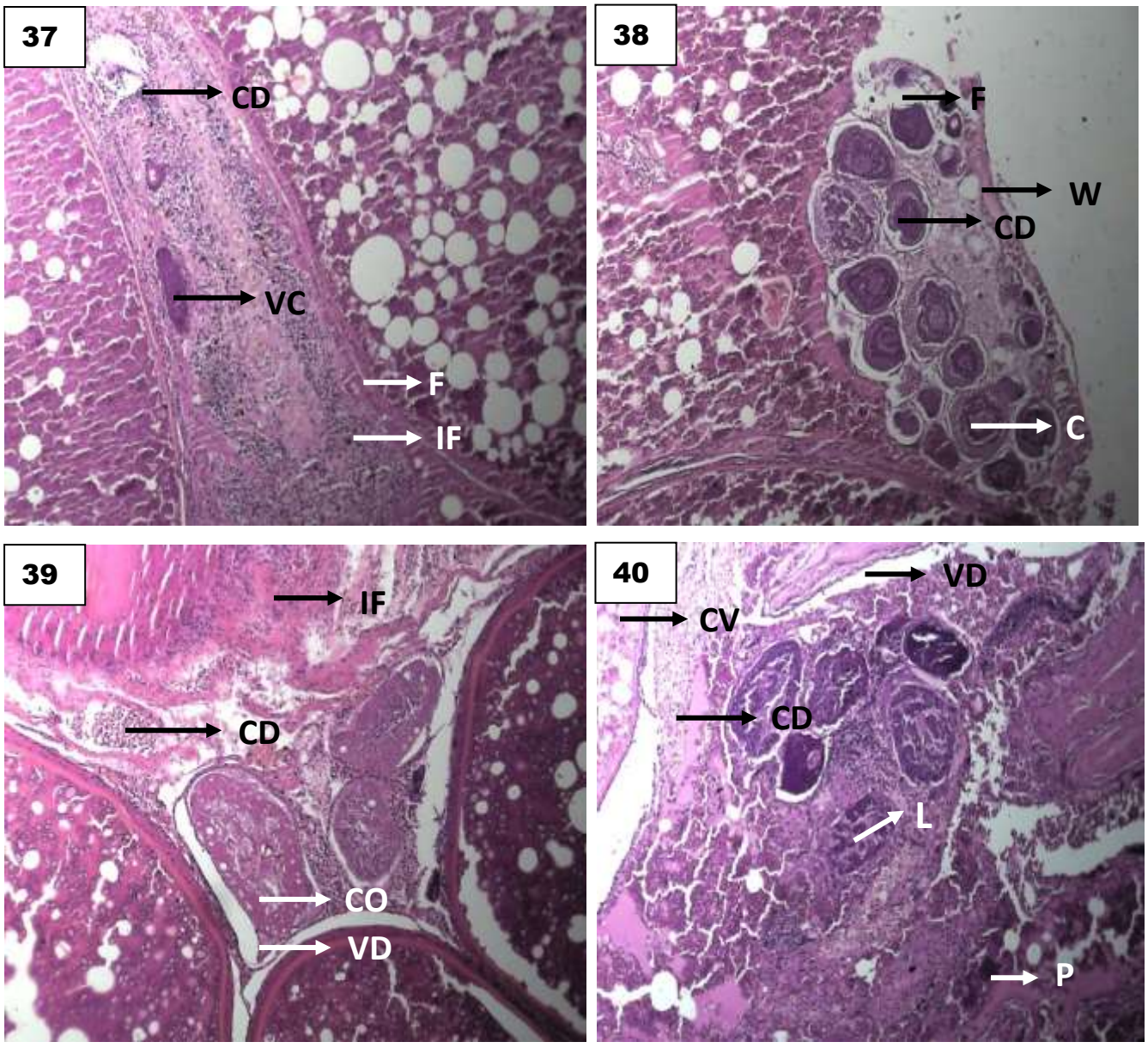

Plate 37-40: Histopathological (T.S) alterations in fish gonads collected from the lagoon. (H & E X400). Cellular degeneration CD, Vascular congestion (VC), Inflammatory cells (IF), Fibrous layer (FL), Wrinkling of oocyte membrane (W), Contraction of Oocyte (C), Vascular degeneration (VD) and contraction of ovum (CO), Cytoplasmic vacuolation (CV), Lesion (L) and Pigment (P).

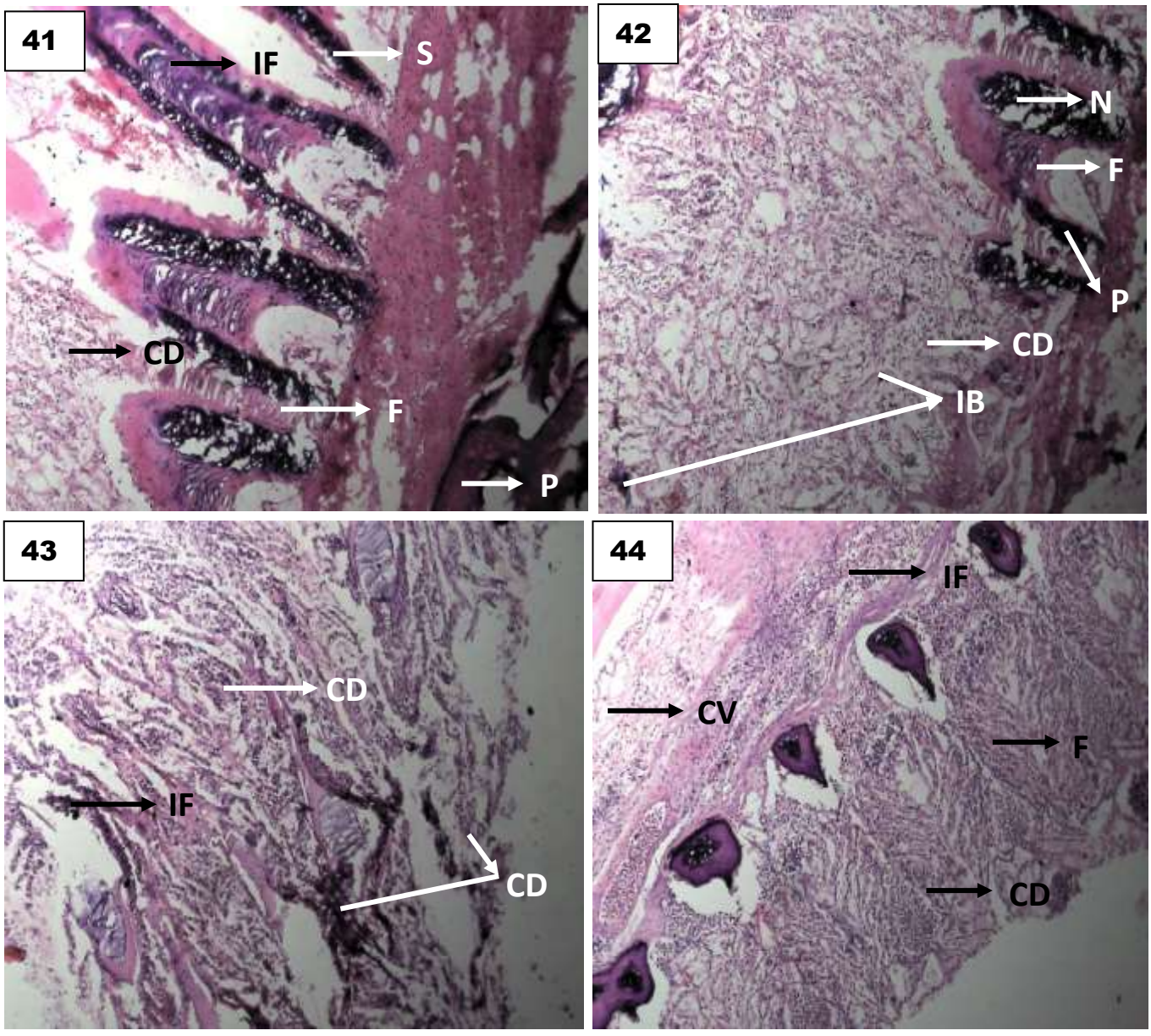

Plate 41-44: Histopathological (T.S) alterations in fish gills collected from the lagoon. (H & E X400). Cellular degeneration (CD), Fusion of secondary lamellae (F), Pigment (P), Inflammation (IF), Loss of secondary lamellae (S), Necrosis (N), Inclusion bodies (IB) and Cytoplasmic vacuolation (CV).

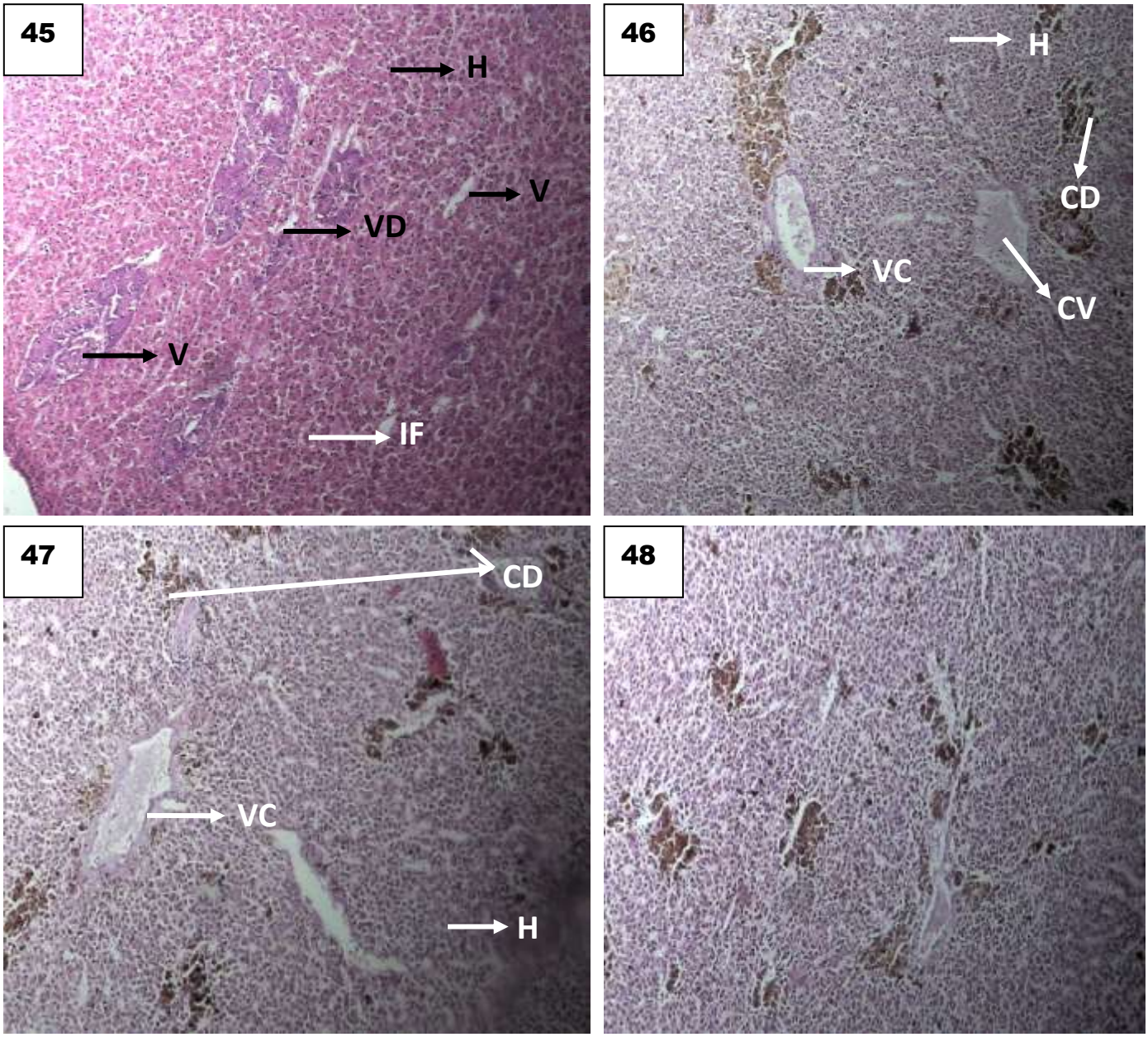

Plate 45-48: Histopathological (T.S) alterations in fish livers collected from the lagoon. (H & E X400). Vascular congestion (VC), Vascular degeneration (VD), Inflammation (IF), Vein (V), Hepatocytes H, Cellular degeneration (CD), Central vein (CV).

4.6 Identified enzymatic, non enzymatic and histopathological biomarkers in *Eudrilus eugeniae* collected from different stations around impacted areas of ijegun.

The activities of antioxidant enzymes and non enzymes in *E. eugeniae* collected from the impacted area of Ijegun are presented in Figures 4.45- 4.49. The results from the laboratory studies were confirmed in the field studies. Comparing the results of the activities of antioxidant enzymes and non enzymes in *E. eugeniae* from the impacted area of Ijegun (Figures 4.45- 4.49) and the control earthworm, day 0 (Tables 4.11 and 4.12), the activities of SOD, GST, CAT and GSH were inhibited in earthworms collected from Ijegun. It was observed that the levels of GSH, CAT, SOD and GST in earthworms collected from Ijegun were significantly lower than the levels of SOD (235.20u/mg protein), CAT (693.00 u/mg protein), GSH (28.64nm/mg protein), and GST (395.77nm/mg protein) in the control earthworms, day 0 (Tables 4.11 and 4.12). The level of MDA was significantly higher in earthworms collected from Ijegun than the control earthworm, day 0 (2.46 nm/mg protein) (Tables 4.11 and 4.12).

4.6.1 SOD activity

SOD activity decreased from 235.20 u/mgprotein in earthworms collected from the control station (Unilag garden) to 0.76, 1.94, 0.82, 2.1, 0.34, 0.69, 0.21 and 0.49 nm /mg protein in earthworms collected from the impacted area of Ijegun. The percentage decreases were 99.68%, 99.18%, 99.65%, 99.11%, 99.86%, 99.70%, 99.91%, 99.79% for stations S5, S6, S8, S9, S12, S13, S18 and S19 respectively (Figure 4.45).

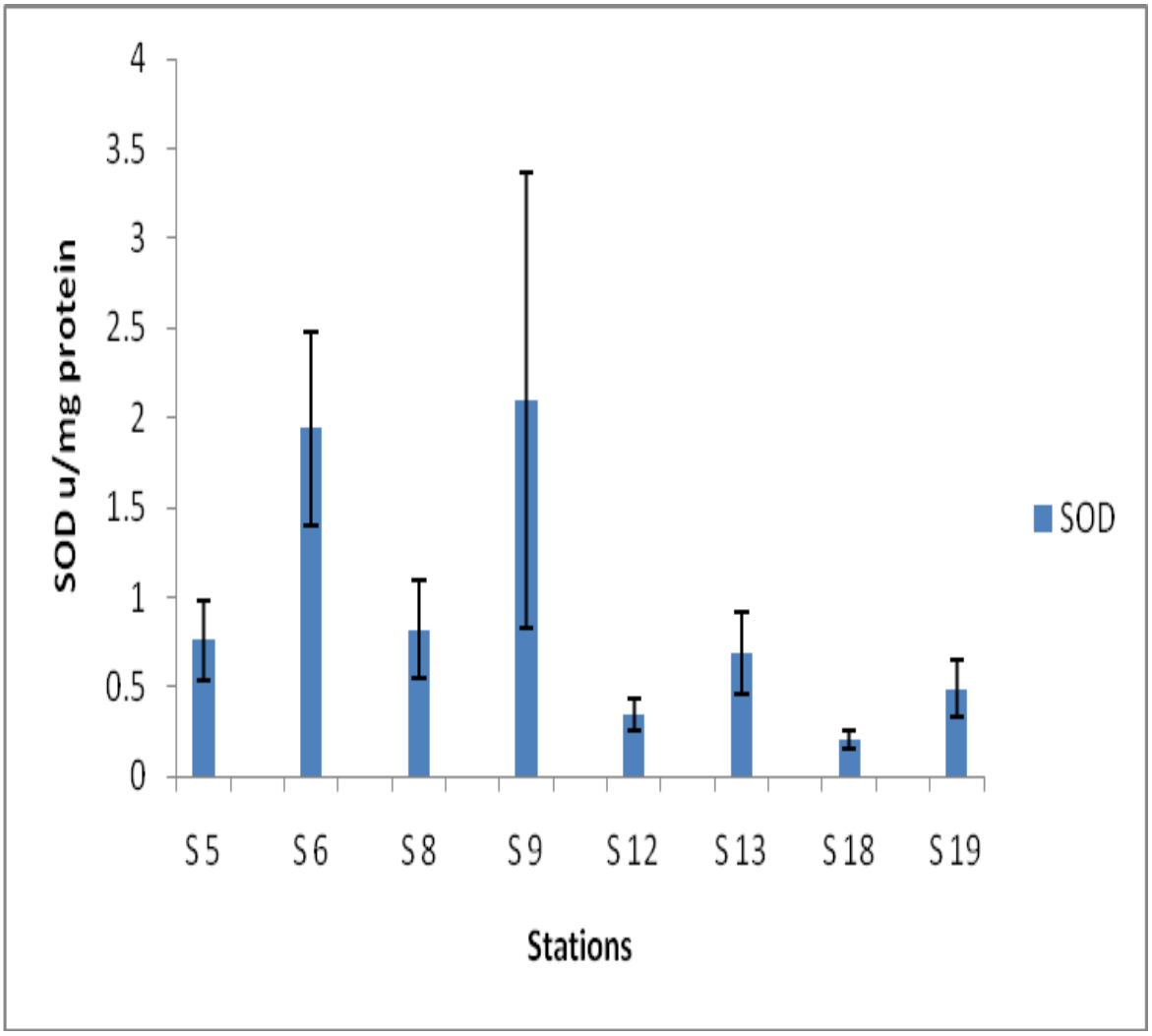

Fig4.45: SOD activity in the earthworms collected from impacted areas of Ijegun .

4.6.2 CAT activity

CAT activity decreased from 693.00u/mg protein in earthworms collected from the control station (Unilag garden) to 4.61, 11.51, 5.09, 15.91, 2.48, 11.73, 1.5, 3.84 um /mg protein in earthworms collected from the impacted area of Ijegun. The percentage decreases were 99.33%, 98.34%, 99.27%, 97.70%, 99.64%, 98.31%, 99.78%, 99.45% for stations S5, S6, S8, S9, S12, S13, S18 and S19 respectively compared (Figure 4.46).

4.6.3 GST activity

GST activity decreased from 395.77nm/mgprotein in earthworms collected from the control station (Unilag garden) to 0.5, 1.47, 0.57, 2.36, 0.36, 3.98, 0.11, 1.28nm/mg protein in earthworms collected from the impacted area of Ijegun. The percentage decreases were 99.87%, 99.63%, 99.86%, 99.40%, 99.91%, 98.99%, 99.97 %, 99.68% for stations S5, S6, S8, S9, S12, S13, S18 and S19 respectively (Figure 4.47).

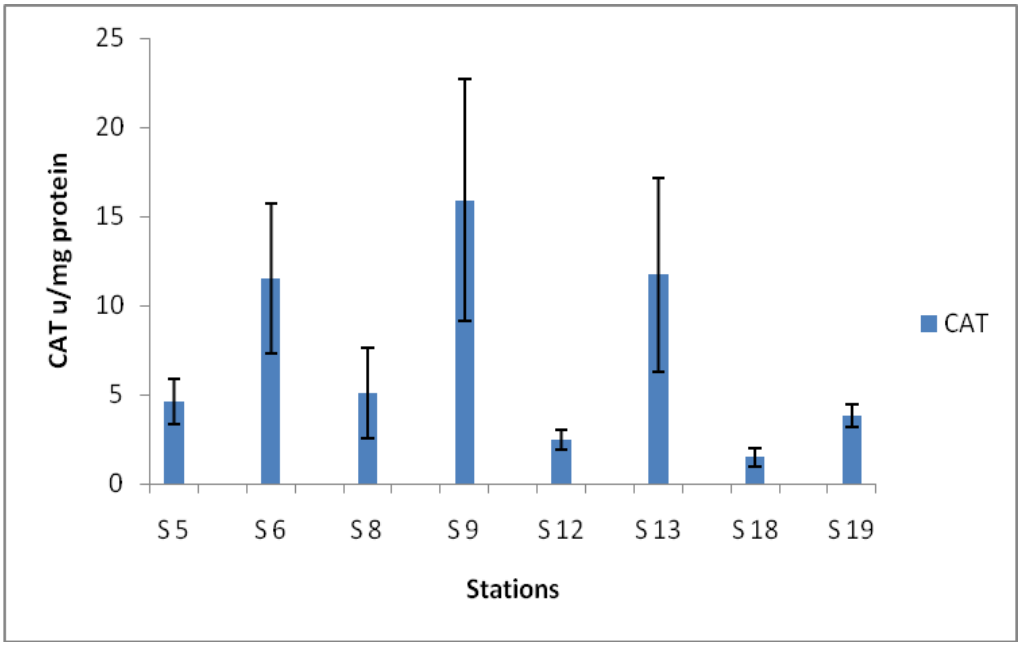

Fig 4.46: CAT activity in the earthworms collected from impacted areas of Ijegun.

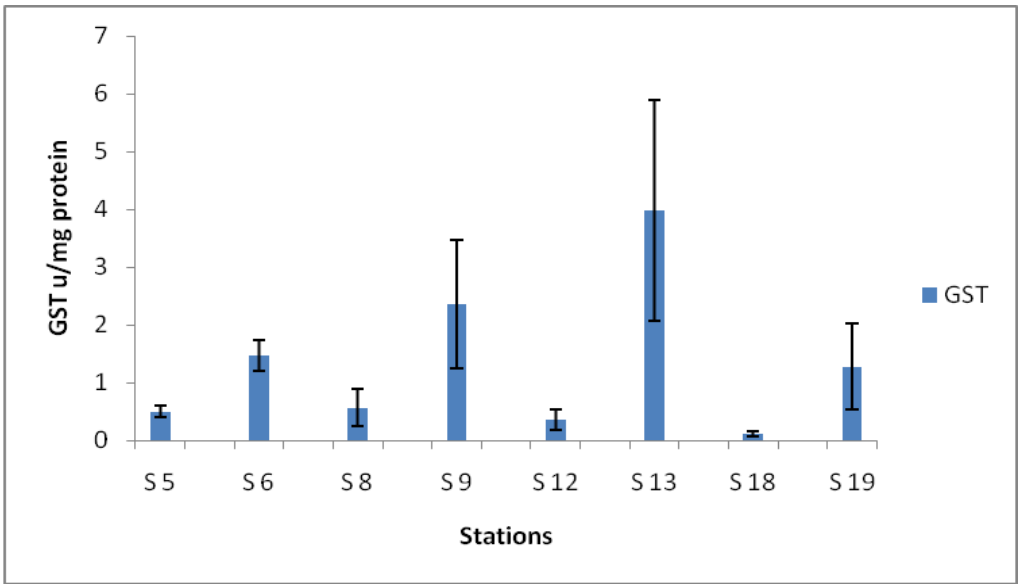

Fig 4.47: GST activity in the earthworms collected from impacted areas of Ijegun

4.6.4 Level of GSH

The level of GSH decreased from 28.64 u/mg protein in earthworms collected from the control station (Unilag garden) to 0.04, 0.1, 0.05, 0.21, 0.02, 0.2, 0.01, 0.09nm/mg protein in earthworms collected from the impacted area of Ijegun. The percentage decreases were 99.86%, 99.65 %, 99.83%, 99.27%, 99.93%, 99.30%, 99.97%, 99.69% for stations S5, S6, S8, S9, S12, S13, S18 and S19 respectively (Figure 4.48).

4.6.5 Level of MDA

The level of MDA increased from 2.46 nm/mgprotein in earthworms collected from the control station (Unilag garden) to 2.68, 9.44, 15.45, 11.38, 17.22nm/mg protein in earthworms collected from the impacted area of Ijegun.The percentage increases were 8.94%, 283.73%, 523.05%, 362.60%, 600% for stations S9, S12, S13, S18 and S19 respectively (Figure 4.49).

4.6.6 Histopathological alterations observed in earthworms collected from Ijegun

Plates 4.49-4.52 show the histopathological alterations seen in the internal structures of *E.eugeniae*. The pathological findings include cellular and epidermal degeneartion, presence of pigment and inclusion bodies. Enlargement of ectoderm cells and expansion of spaces between longitudinal muscles were also observed.

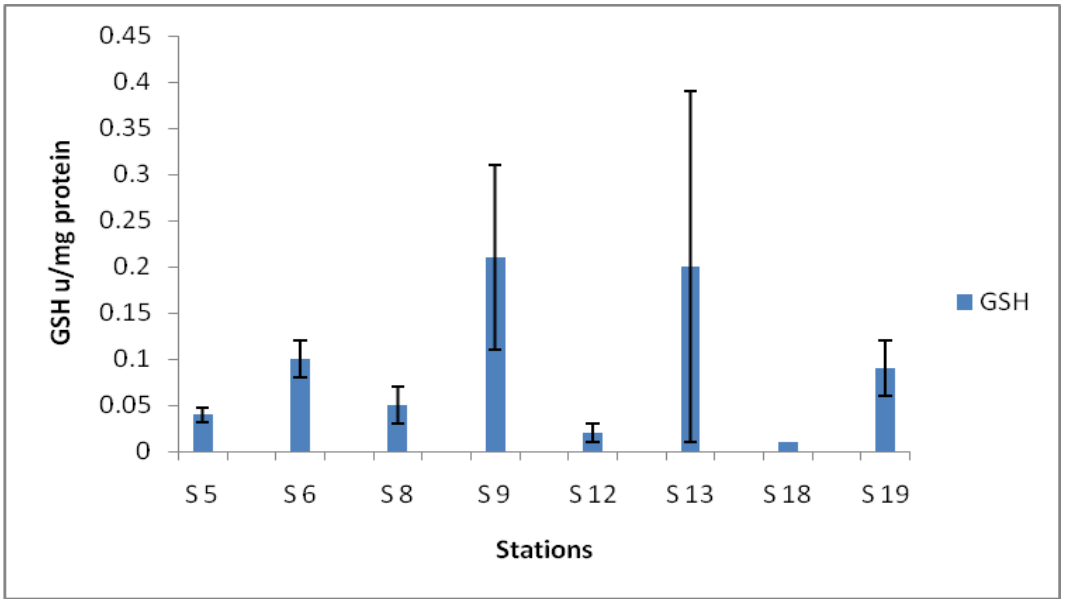

Fig 4.48: level of GSH in the earthworms collected from impacted areas of Ijegun.

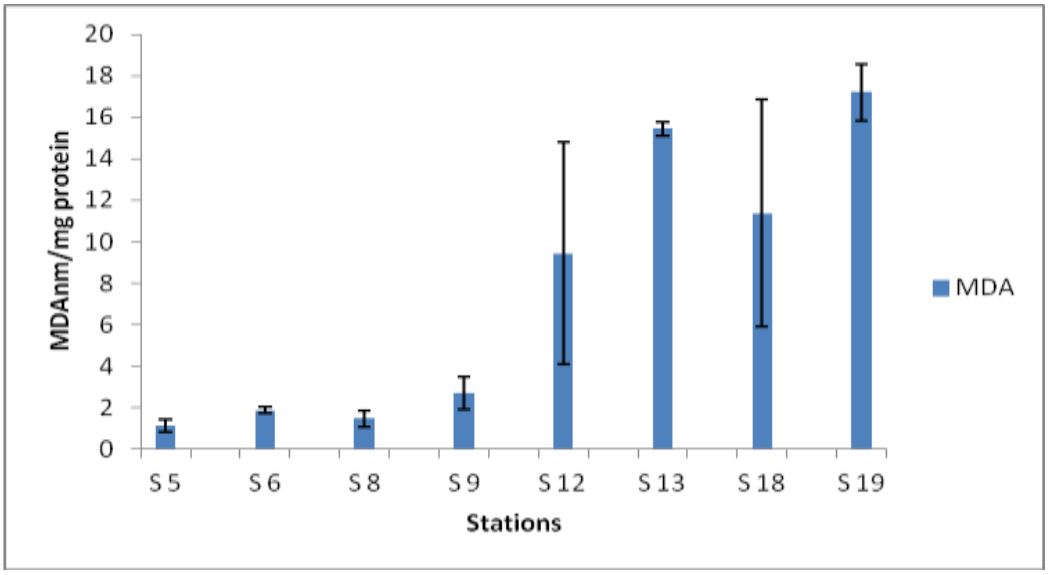

Fig 4.49: level of MDA in the earthworms collected from impacted areas of Ijegun.

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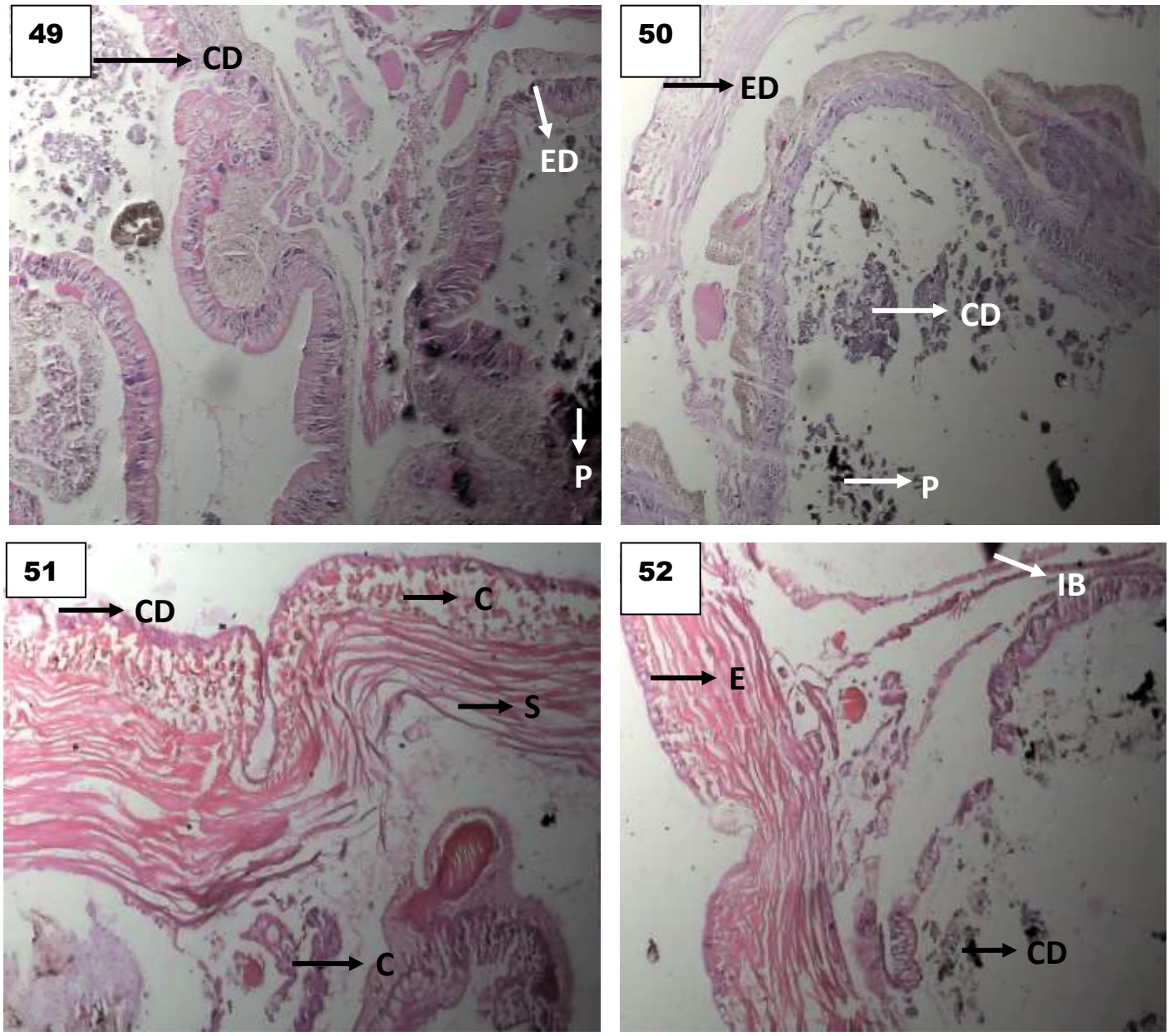

Plate 49-52: Histopathological (T.S) alterations in *E.eugeniae* collected from the impacted area of Ijegun. (H & E X400). Cellular degeneration (CD), Epidermal degeneration (ED), Pigment (P), Expansion of spaces between longitudinal muscles (S), Enlargement of ectoderm cells (E) and Inclusion bodies (IB).

CHAPTER FIVE

5.0 DISCUSSION

The information on physical and chemical parameters of the water samples of the study stations of the Lagos lagoon was similar to results observed by earlier researchers. The pH of the water throughout the study period was slightly basic. This pH range (7.61-8.48) was similar to those reported earlier by Nkwoji *et al*., (2010) for Lagos lagoon. Chukwu and Nwankwo (2005) recorded a similar pH range (7.2-8.2) at an adjacent creek to the Lagos lagoon. The salinity values of the study stations were relatively higher during the dry months and fell within range reported by other workers (Otitoloju, 2000; Nkwoji *et al*., 2010). This may be due to dilution of the water by the increase freshwater input during the raining months.

In this study, BTEX was detected in all the sampled stations of the Lagos lagoon including the Unilag station. Higher concentrations of BTEX compounds were however observed in Atlas Cove and Apapa stations. These elevated levels of BTEX around the Atlas Cove and Apapa can be attributed to the petroleum related activities in addition to other sources in those areas. The concentrations of BTEX (Benzene- 6.41µg/l, Toluene- 1.34µg/l, Ethylbenzene- 2.41µg/l and Xylene- 480.81µg/l) in the Atlas cove station of the Lagos lagoon were higher than concentrations obtained from the study conducted by An *et al*., (2002), who reported concentrations ranging from 1.0 to 2.0µg/l for Xylene and Toluene, while Benzene and Ethylbenzene were not detected in lake Texoma in the United States. The higher value of Xylene recorded in comparison with other BTEX compounds in this study can be due to the fact that Xylenes are widely used as organic solvents and are present in numerous consumer products (paints, vanishes, cigarette smoke, rust preventives and shellac) in addition to its use in unleaded

fuel. Xylene also has the highest percentage in unleaded petrol compared to other BTEX compounds (Benzene-1-2%, Toluene- 5-8%, Ethylbenzene- 1-2% and Xylene-7-10%) (Lin *et al*., 2007). According to Lokhande *et al*., (2009), high concentrations of BTEX compounds ranging from 0.1 to 1.5 ppm have been found to be responsible for the fish kills in the Savitri River in India. Releases of BTEX to water are mainly related to spills of petrol and the principal concern is their migration away from the source areas (Brigmon *et al.* 2002). Dinerman *et al*., (2011) also reported that BTEX concentration is mostly affected by recreational boating activity in waterbodies. Their results show that BTEX concentrations were generally below 0.7 ug/l but reached as high as 8.61 ug/l during Jewish holidays and weekends, with high recreational boating activities. This could explain the detection of BTEX in all the stations of the Lagos lagoon including the Unilag station which was chosen as the reference station since the different parts of the Lagos lagoon is used for several purposes including recreation, water transportation, shipping and other oil related activities, which could be responsible for the hydrocarbon pollution.

The results of THC levels from these field observations showed the same trend with the detected levels of BTEX in the sampled stations. The detection of higher THC values in Atlas Cove and Apapa area of the Lagos lagoon in this study also corroborated the findings of Adewuyi and Olowu (2012) and Anyakora *et al*., (2008). They observed high level of PAHs ranging from 0.19ug/l to 8.84ug/l in the Apapa terminal. High values of Total Petroleum Hydrocarbon (20.34 to 27.40 mg/l) were recorded for surface water within the vicinity of NNPC oil depot in the study carried out by Adewuyi and Olowu (2012). These contaminations maybe as a result direct discharges of untreated stormwaters and effluent from the oil depots due to petroleum product tank washings, petroleum products spills, activities of import and export of petroleum related

products and other cleaning activities that take place in the depot which are channeled into lagoon. According to Shahbazi *et al.,* (2010), oil pollution sources originate from urbanization, motorization, heavy industrial activities, heavy supertankers traffic and international shipping. High pollution levels of PAHs were found in mussels near Penang Bridge in Malaysia, although, there is no oil refinery in the vicinity of Penang Bridge, the oil pollution sources were shown to have originated from shipping activities and industrial activities in the catchment (Shahbazi *et al.,* 2010).

The detection in this study of higher concentrations of BTEX and THC in sampling stations located around the Atlas Cove and Apapa provides evidence that the terminal / oil tank farms activities are the major contributor of BTEX and THC in the Lagos lagoon. There is therefore an urgent need for the regulatory agencies to ensure that all the oil depots and tankfarms situated along the Lagos lagoon treat all their stormwaters and effluents in order to reduce its hydrocarbon level before discharge into the lagoon. The other implication is that the regulatory standards and guidelines of the oil industries which are well stipulated in EGASPIN (2002) have not been effectively enforced to reduce the high values of THC and BTEX compounds observed.

Comparisons of the concentrations of THC detected in the various media (sediment and water) revealed that generally, the highest concentrations of THC were detected in the sediment. Sediments are a pollutant sink and an important factor to establish the assimilative capacity of the environment. They have been proven an efficient tool to identify environmental impacts (Guo *et al*., 2007). The detection of higher concentrations of THC in sediment samples have been reported by several authors including Ololade and Lajide, (2009) and Naidu and Kelly,

(2003). Several studies involving the use of sediment for the assessment of hydrocarbon pollution in coastal aquatic environment have shown that hydrocarbons from spilled petroleum persist in sedimentary environment (Zheng and Van vlect, 1988; Pendoley, 1992; Fernandes and Tronczynski, 1997).

Furthermore, the comparison of THC detected in the sediment and water samples of the Lagos Lagoon during the 2009/2010 sampling years to that of other water bodies around the world revealed that the concentrations of THC in the Lagos Lagoon were of similar values to that in estuaries and coastal regions such as Arabian sea along the Indian coast (0.6- 5.8mg/l), Straits of Johor in Malaysia (0.7-36.7mg/l), Arabian Gulf (5.4-92.0mg/l), Changjiang estuary in China (2.2-11.82mg/l), Fraser River Basin in Canada (1.6-20.6mg/l), Bassein-Mumbai coast in India (7.0-38.2mg/l), Bizerte lagoon in Tunisia (0.05-8.12mg/l), Jiaozhou Bay in China 90.54-8.12mg/l), Gulf of Fos in France (7.8-180mg/l) and Tamilnadu coast in India(1.48- 4.23mg/l). However the THC observed in the Lagos lagoon were much lower than the values detected in Shetland Island in the UK (8816mg/l), Victoria Harbour (5277mg/l), Kitimat Harbour (66700mg/l), Casco Bay (2900mg/l) and UAE coast (51,000mg/l) (Veerasingam *et al*., 2010; Guo *et al*., 2007). Based on this, it has become necessary for the relevant regulatory agencies in Nigeria to initiate stricter control measures if we are to avoid such situation whereby the THC concentrations in the Lagos Lagoon will reach the levels attained in more polluted estuaries such as the Shetland Island in the UK.

The results of species composition, abundance and diversity of macrobenthic invertebrates observed in this study showed that petroleum hydrocarbon contamination can affect structures of

the ecosystems as indicated by other benthic ecologists including Ogbeibu and Victor (1989), Uwadiae, (2009) and Ogbeibu and Oribhabor, (2002). The relatively lower number of individuals recorded in the Apapa, Iddo and Atlas cove stations may be due to the cumulative toxic action of these contaminants over the years. Macrobenthic communities have been seen as effective tools for assessing organic pollution (Nkwoji and Igbo, 2010). Their sedentary nature makes it possible for them to be highly impacted by any xenobiotic compounds and other stressors released into the water body.

Early works on the benthic macroinvertebrates of the Lagos Lagoon were on genus Pachymelania (Oyenekan, 1979) and *Iphigenis truncate* (Yoloye, 1977; Oyenekan and Bolufawi, 1986; Oyenekan, 1988). Ajao and Fagade (1990) worked on the seasonal and spatial distribution of the population of benthic macroinvertebrate, *Capitella capitata* in Lagos lagoon and observed that the abundance of these organisms was influenced by sediment, metals and hydrocarbon content. The Pachymelania community encountered in the stations studied was a reflection of the sandy nature of the sediment. Balogun *et al*., (2011) in the study of benthic macro-faunal communities in the Lagos lagoon reported that the Pachymelania community is characterized by sand and muddy sand deposit.

The abundance and distribution of benthic macroinvertebrates such as *Heteromastus sp* and *Nais sp* in this study can be said to be influenced by the presence of hydrocarbon in the water. This is in agreement with the findings of Edokpayi *et al*., (2004) that recorded significant number of *Nais sp* in polluted waters. Naidid worms have been known to respond to pollution by increasing in abundance. The high abundance of pollution sensitive species like *Pachymelania sp*

in the control stations and low abundance of these same organisms in Atlas cove, Apapa and Iddo stations is a reflection of the pollution status in these areas of the lagoon. The high abundance in the control stations indicate relatively healthy communities. This is in agreement with the findings of Nkwoji and Igbo (2010) that recorded low abundance of this animal in Iddo, Okobaba and Ogudu due to the high load of biodegradable wastes in that part of the lagoon. The molluscan group was the most abundant of the macroinvertebrates recorded in this study, this is in agreement with the result of Balogun *et al*., (2011).

Benthic communities are sensitive to oil spills, but the effects of oil pollution depend on the proportion of hydrocarbon – sensitive species in the affected areas. The result of high species diversity and richness recorded in the hydrocarbon polluted stations Apapa, Atlas cove and Iddo is in agreement with the study of Gomez Gesteir *et al*., (2003). They reported increase in Shannon-weiner index after an oil spill. Likewise investigations near marine oil terminal facilities show increased abundances of opportunistic polychaetes in association with increased sediment hydrocarbons (Blanchard *et al*., 2003). Changes in Shannon weiner index is not usually sufficient to assess pollution impact as pointed out by Gomez Gesteir *et al*., (2003).

Petroleum hydrocarbons are adsorbed to silt and clay particles and carried to the bottom sediments where they accumulate. If accumulations reach a sufficient concentration, destruction or reduction of macrobenthic population occurs. The decline in benthic macroinvertebrates observed in this study is a negative trend, as the benthic macroinvertebrates are important in the aquatic ecosystem because they not only form part of the aquatic food chain but are also used to assess water quality as pollution indicators. Similar observations were made by Balogun *et al*.,

(2011). In Nigeria, there is no nationally- generated safe limits of pollutants for the protection of our aquatic organisms, what is available is an adopted interim industrial effluent limitation guidelines (FEPA, 1991), which most earlier workers have described as an importation form western countries guidelines (Otitoloju, 2000). There is need for a review of the existing national effluent limitation guidelines and setting up of water body protection standards.

Spillages of petroleum products are not restricted to aquatic ecosystems alone, especially since the transportation of these products is carried out via pipelines which may leak due to accidents, corrosion and vandalisation. The leakage of petroleum products into the terrestrial environment following the Ijegun incident indicate that two years after the spill, there was still high level of hydrocarbon contamination of the soil and groundwater in the area. Infact concentrations of BTEX in the groundwater were found to be higher than in the soil samples during this study. The safety limits for groundwater (0.2 µg/l) and soil (50µg/kg) pollution are set out in EGASPIN as target values for BTEX. Most of the groundwater sampled were above the target value of 0.2 µg/l set for the BTEX compounds. The detection of higher concentrations of BTEX in the groundwater may be due to their polarity and high water solublity characteristics. Therefore, BTEX compounds are able to enter the groundwater systems and cause serious pollution problems (Brigmon *et al*. 2002). Consequently, these chemicals are some of the most common contaminants found in drinking water and have been linked with a variety of health issues in humans (Yadav and Reddy 1993; Budavari 1996).

The detection of THC and concentrations of BTEX in groundwater and soil samples from the oil impacted area of Ijegun corroborates the findings of Osuji and Achugasim (2010) and Osu *et al*.,

(2010), that detected concentrations of BTEX, two months after an oil spillage. According to Kamal and Klein (2010), BTEX compounds are usually found in soils and groundwater samples polluted by leaks from underground fuel tanks and pipelines. The lower THC values in year 2010 than year 2009 in Ijegun, suggests that the natural attenuation occurring may be attributed to dilution due to rainfall and dispersion by the facilitated groundwater flow. Degradation of petroleum hydrocarbon may have also occasioned the significant reduction in the concentration of hydrocarbon in 2010.

Result from this study reveal seasonal variation in the concentration of THC in the groundwater samples from impacted area of Ijegun, which revealed higher THC in the dry seasons than in the wet seasons. Based on these results, it can also be inferred that dilution due to rainfall during the wet season contributed greatly to the decrease in the contaminant concentrations in this petroleum contaminated site. Although natural attenuation has not successfully reduced the hydrocarbon level below WHO limit as at the time of sampling during this field survey. Consequently, immediate treatment of the groundwater resources around the impacted areas of Ijegun would be required in order to ameliorate some of the adverse effects of pipeline accident in the study area.

It is also important to note that hydrocarbon contamination was observed in soil samples from all sampled stations including control station. This indicates that there are other contributory sources of THC in the soil samples collected from the study area. Some of these other sources are likely to be leakages from underground tanks of petrol stations and vehicular emissions. Some authors have observed lower concentrations of THC in contaminated soils and groundwater than the

values observed in this study. The level of THC in contaminated soil as a result of oil spill was investigated by Osu and Asuoha (2010) with concentrations of soil samples ranging from 0.601 – 3.678 mg/kg. In another study, Nganje *et al*.,(2007) recorded mean THC concentrations of 0.66mg/l in contaminated groundwater near petrol stations and mechanic workshops in Calabar. The THC found in soil samples around the impacted area of Ijegun in this study were lower than that reported in some studies on the distribution of soil hydrocarbons (Johnsen *et al*., 2006; Ye *et al*., 2006; Zuo *et al*., 2007; Ping *et al*., 2007). For example, the mean concentration of total hydrocarbons from 188 soil samples from Tianjin municipal area in China was found at less than 1,000 μg/kg (Ye *et al*., 2006). In Detroit, the average total hydrocarbon concentrations range from 2,299 μg/kg for open space soils to 20,900 μg/kg for soils along busy streets (Wang *et al*., 2007). Several authors have focused on urban soils as a media for deposition of hydrocarbons (Takada *et al*., 1991; Mielke *et al*., 2001; Manta *et al*., 2002; Madrid *et al*., 2002; Krauss and Wilcke, 2003; Wang *et al*., 2004; Zhang *et al*., 2005; Iqbal *et al*., 2007; Wang *et al*.,2007). These studies show that hydrocarbons are present at higher concentrations in urban soils than in rural soils.

In the laboratory toxicological evaluations, ethylbenzene was found to be the most toxic when tested against *C. gariepinus* while xylene was the most toxic when tested against *E. eugeniae.* This result is similar to the 96hr LC50 report from the US EPA ECOTOX (2007), ethylbenzene was the most toxic chemical to *Pimephales promelas*, a freshwater fish. On the basis of the characteristics of the different compounds, the high toxicity of Ethylbenzene and Xylene can be as a result of their low vapour pressure, 9.5 mm Hg and 6.6 mm Hg, resulting in high concentration that will remain in the test media.

Results from ecotoxicology data can be used in the establishment of water and soil quality criteria for BTEX compounds for the protection of aquatic and terrestrial organisms in Nigeria, as this is the practice in other developed countries. The sensitivity of *C. gariepinus* (aquatic organism) than *E. eugeniae* (terrestrial organisms) to the test chemicals can be attributed to the fact that the BTEX compounds are the water soluble fractions of petroleum products, as a result they are readily available to the aquatic animals. On the other hand, the less sensitivity of *E. eugeniae* (terrestrial organisms) to BTEX compounds is primarily due to the adsorption of BTEX compounds to soil particles, leading to less bioavailability of the monoaromatic hydrocarbons to the earthworm in soil.

According to Depledge, (1996), different responses to hydrocarbons may also be a consequence of variable tolerances within and among species. Similar observations were made by An and Lee, (2008) and Ogeleka *et al*., (2010) after exposure of the earthworm, *Aporrectodea longa* and fish (*Tilapia guineensis*) to toxicants. The sensitivity of the fish can also be attributed to the physiology of fresh water organisms, which have a body fluid concentration (about one-third) their surrounding environment, they are constantly taking in water by diffusion through their gills and skin for osmotic balance. Thus, there is an influx of not only water but also the test chemical leading to a high lethal toxicity of the chemical (Ogeleka *et al*., 2010). Counter current blood flow (direction of blood flow is opposite the direction of water flow over the lamellae) promotes not only the efficient exchange of oxygen but also the absorption of pollutants through the lamellae in a polluted environment.

Several studies on aquatic and terrestrial animals have demonstrated the importance of enzymatic antioxidants defenses in protecting cellular systems from oxidative stress induced by xenobiotics (Valavanidis *et al*., 2006; Saint-Dennis *et al*., 1999; Song *et al*., 2009). The activity of antioxidant enzymes may be enhanced or inhibited under chemical stress depending on the intensity and the duration of the stress applied, as well as, the susceptibility of the exposed species. In normal situations, a balance exists between the production of ROS and antioxidant processes, but the balance is not perfect.

A disturbance in the balance between the prooxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress and has become an important subject for terrestrial and aquatic toxicology (Livingstone, 2001). Indicators of oxidative stress include changes in antioxidant enzyme activity and lipid peroxidation products. The inhibition in the activities of SOD and CAT in the gills and livers of fish reported in the laboratory studies after 60 days exposure may be a response to oxidative stress caused by the BTEX compounds leading to the production of superoxide radical and hydrogen peroxide radical.

The enzyme SOD is known to provide cytoprotection against free radical induced damage by converting superoxide radicals (O_2^-) generated in peroxisomes and mitochondria to hydrogen peroxides. The hydrogen peroxide is then removed from the system by the enzyme CAT, which converts it to water and molecular oxygen (O_2). The inhibition of the enzyme SOD by the test chemicals will, therefore, lead to increased oxidative stress in the gill and liver tissues as a result of the damaging activities of the superoxide radicals (O_2^-). However, the inhibition of the

enzyme SOD will expectedly result in a reduction in the activity of the enzyme CAT, due to a decrease in H_2O_2 generation from SOD activities. This indeed proved to be the case in this study as there was a significant reduction in CAT activity in the exposed fishes. The decreased CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity. The findings of this study are in agreement with the investigations of Farombi *et al*., (2007), Fatima and Ahmad (2005) and Otitoloju and Olagoke (2010).

GSH is a sensitive indicator of oxidative stress. It conjugates with electrophilic xenobiotics transforming them into water soluble and thus easily excretable products (Nusetti *et al.*, 2001). Decreased level of GSH was observed in the gills and liver of fish in this study after exposure to BTEX. The reduction in the liver was probably due to the liver's attempt to detoxify the body from the toxicant; as GSH can offer protection against toxicants by active subsequent conjugation. The gills are also more exposed to contaminated water and as such the dissolved hydrocarbons can penetrate through their thin epithelial cells. The apparent decrease in glutathione detoxification system in the gills, the first point of contact with environmental xenobiotics indicates that this system is a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982). Results from this study are in agreement with the findings of Elnwishy *et al*., (2007), Farombi *et al*., (2007) and Farombi *et al*., (2008).

The enzyme GST plays an important role in protecting tissues from oxidative stress. In this study, the activity of GST was also inhibited in the gill and liver of fish tissues after exposure to BTEX for 60 days. The enzyme GST has been reported to respond differently to different compounds, for example, Hamed *et al*., (1999) reported that the enzyme was strongly inhibited

by dimethoate, while Zhang *et al*., (2005) reported statistically significant enhancement in GST in animals exposed to oxidative stress of 2,4 dichlorophenol. Recent investigations of changes in antioxidant defenses showed that they can be used as biomarkers of oxidative stress by various pollutants in aquatic organisms (Manduzio *et al*., 2003; Gorinstein *et al*., 2003; Brown *et al*., 2004). As protective antioxidants, it plays an important role in detoxification and elimination of xenobiotics including hydrocarbons. The inhibition of the enzyme, GST after 60 days exposure in this study is similar to the findings of Farombi *et al*., (2008) and Kaur *et al*., (2005). The decrease in GST activity in the fish suggests an overproduction of reactive species which inactivated GST enzyme. The decreased levels of antioxidant enzymes and GSH with lowered level of GST in the gills could account for the marked lipid peroxidation observed (Farombi *et al*., 2008). Indeed, the gills are more exposed to the water being the first area of contact with aquatic xenobiotics like BTEX and as such the compounds can penetrate through their thin epithelial cells to produce reactive species, thereby inactivating the antioxidant defenses.

Bocchettii *et al*., (2004) have confirmed the role of antioxidant enzymatic systems under polluted environmental conditions and their importance in sensitive ecotoxicological studies. This inhibition or low level of enzymatic antioxidant defense in this study was found to be correlated with an increase in oxidative damage, as reflected by the increased level of MDA in gill and liver tissues of exposed fishes. This is not surprising because generation of superoxide radicals is considered as the central component of the signal transduction which triggers the genes responsible for antioxidant enzymes, therefore any chemical with capabilities to inhibit SOD may ultimately have an inhibitory effect on a cascade of enzymes involved in antioxidant defenses. The observed inhibition of the antioxidants defense enzymes such as SOD, CAT, GSH

and GST, in conjunction with an increase in LP levels in the gill and liver tissues of test animals exposed to hydrocarbon compounds can therefore serve as a good battery of biomarkers for early detection of pollution associated with petroleum hydrocarbon.

Malondialdehyde (MDA) is a clinical marker of oxidative stress, specifically lipid peroxidation (LPO) which occurs in hydrocarbons. Significant increase of MDA in the liver and gill tissues of *Clarias gariepinus* was observed in both laboratory and field studies which agree with findings of Achuba and Osakwe (2003), Avci *et al*., (2005) and Otitoloju and Olagoke (2010) who reported an increase in MDA in tissues of fishes exposed to petroleum hydrocarbons. The increase in MDA is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular reactive oxygen species ROS (Stohs *et al*., 2001).

The increase in MDA indicate that reactive oxygen species may be associated with the metabolism of BTEX leading to peroxidation of membrane lipids of the respective organs. The observed lipid peroxidation resulting possibly from ROS generated by the compounds may lead to cell apoptosis. ROS and oxidative stress have been shown to be triggers of apoptosis (Shen and Liu (2006). Exogenous ROS at moderate levels induce apoptosis in many cell types. Endogenously produced ROS have also been found to be important in the apoptotic cell death triggered by many other stimuli including environmental chemicals (Shen and Liu (2006).

Elevated reactive oxygen species level in tissues lead to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems. The

measurements of lipid peroxides levels in animal tissues exposed to different pollutants have been recognized as reliable early warning signal of exposure to environmental stress and integrated to environmental monitoring programmes (Avci *et al*., 2005; Fatima and Ahmad, 2005; Valavanidis *et al*., 2006). The use of biochemical responses as biomarkers during environmental monitoring programmes is derived from the basis that a toxic effect manifests itself at the subcellular level before it becomes apparent at higher levels of biological organization. The measurement of these biochemical responses may therefore serve to improve the assessment of biologically significant exposures to toxic chemicals and enhance ability to assess the risk of effects of pollutants on the health and survival of toxicant exposed population.

Earthworms are continuously exposed to soil chemicals through their alimentary surfaces and skins. Thus, they are dependent on efficient detoxification systems for their survival (Kilic, 2011). Decrease in GSH, SOD and CAT activity was observed after days of exposure to Benzene, Toluene, Ethylbenzene and Xylene. This result is in agreement with the results of Song *et al*.,(2009), they reported overall inhibition of the activity of SOD activity of earthworms and significant stimulation of the activity on the 28^{th} day after exposure to atrazine. The decrease in the SOD activity may result from the elimination of the highly reactive O_2^- which was converted to H_2O_2 by SOD, however, increase in the SOD activity may be due to the production of O_2^- which stimulated the SOD activity, with the continuation in the exposure time.

The increase in the CAT activity may be stimulated by the production of H_2O_2, while the decrease in the CAT activity may result in H_2O_2 accumulation. CAT is responsible for breaking

down the free radical by-product hydrogen peroxide into water and molecular oxygen. GST also serves as an important phase II detoxification enzyme to excrete and eliminate the products of phase I metabolism (Otitoju and Onwurah, 2007; Zhang *et al*., 2009). Increase in the GST activity was observed for Benzene, however, there was decrease in GST activity when the earthworm was exposed to Toluene, Ethylbenzene and Xylene. This decrease in GST activity is in agreement with the report of Ribera *et al*., (2001).

BTEX compounds have been known to bind to soil molecules therefore, the hydrocarbon is unavailable to the earthworm for uptake unlike in water bodies where they dissolve, becoming water soluble and available for uptake by aquatic organisms. This might be responsible for the changes observed in the enzymes activities in *E.eugeniae* after exposure to BTEX compounds observed in this study, and previous reports like Song *et al*.,(2009) mentioned earlier. The importance of using a suite of biomarkers to evaluate the effect of pollutant on organisms have been confirmed (Van der Oost *et al*., 1997). This study shows that MDA levels were higher in the *E. eugeniae* after 2 days of exposure to Benzene, Toluene, Ethylbenzene and Xylene. This is in agreement with the results of Saint-Dennis *et al*., (2001), who reported higher levels of MDA in *E. fetida* after 14 days of exposure to Pb. This suggests that ROS have been formed. The increase was followed by a decrease thereafter, suggesting the elimination of ROS**.**

Histopathological responses in earthworms have been reported as valuable markers of toxicity. Kilic, (2011) investigated histopathological and biochemical alterations of the earthworm (*Lumbricus Terrestris*) and its use as biomarker of soil pollution along Porsuk River Basin

(Turkey). The study investigated biomarker responses of the earthworm in order to evaluate the soil pollution along River Basin. The results reflected the biological effects of soil pollution around the Porsuk River Basin on the indicator organism *L. terrestris* and constitute an early warning of ecological change in relation to human health. Earthworm skin has direct contact to the contaminated soils and is considered as a significant route of uptake of toxicants. Earthworms are affected by hydrocarbons either through skin contact or by feeding on contaminated litter in soil. Primarily, these toxicants passing through the skin reach the coelomic fluid and are thus transported throughout the body.

In this study, sublethal concentrations of BTEX compounds induced marked pathological changes in the body of the earthworm including cellular degeneration, moderate to severe area of necrosis, area of inflammation, inclusion bodies, pigments, distortion of the shape of circular and longitudinal muscle. Bansiwal and Rai, (2010) confirmed this result as they reported marked pathological changes including ruptured cuticle and distortion of shape of muscles after exposure of earthworm to Malathion. Kilic, (2011) reported enlargement of epithelial cell lining, necrosis and loss of structural intergrity of circular and longitudinal muscles.

Reactive Oxygen Species (ROS) produced by the hydrocarbons may be responsible for the various histopathological changes noted in this study. Peroxidative damage to membrane lipids has long been regarded as the critical initiating event causing cell injury. Cellular necrosis can be induced by a number of external sources, including ROS and inflammation. In addition to lack of chemical signal to the immune system, cells undergoing necrosis can release harmful chemicals into the surrounding tissue (Saint-Dennis *et al*., 2001). Necrosis typically begins with cell

swelling and distruption of the plasma membrane. The core events of necrosis are bioenergetic failure and rapid loss of plasma membrane intergrity.

In this study, the gills were affected by the BTEX compounds and histopathological changes such as necrosis, inflammation, loss of regular shape, loss of secondary lamellae, fusion of secondary lamellae and curving of primary lamellae in the gills were observed. Gills are vulnerable to pollutants in water because of their large surface area and external location. Previous histopathological studies of fish exposed to pollutants have shown that fish gills are efficient indicators of water quality (Cengiz and Unlvy 2006). This study is in agreement with the findings of Raskovic *et al*., (2010), Rudnicki *et al*., (2009) and Fatma, (2009). Histological alterations constitute excellent biomarkers of exposure to toxicants because they represent the result of biochemical and physiological changes (Rudnicki *et al*., 2009).

According to Skidmore and Tovell (1972) toxicants appear to work by reducing adhesion between the epithelial cells and the underlying pillar cell system, accompanied by a collapse of the structural integrity of the secondary lamellae, causing failure and distortion in the respiration, osmoregulation, excretion of nitrogenous waste products, and acid–base balance. The severity of gill damage depends on the concentration of toxicant and on time of exposure (Oliveira *et al*., 1996). The histopathological changes observed in tissues are the reactions to the pollutants in the histological level.

Environmental toxicity can result in two types of structural changes. One is the direct toxic effect of the pollutant leading to tissue degeneration and necrosis, and the other is the development of

compensatory mechanisms such as cellular hyperplasia to deal with the stressor. Generally, hyperplasia of epithelium and inflammation could be a defense response of the circulatory system against the pollutants. The gill lamellae help increase their surface area for oxygen exchange therefore the fusion of secondary lamellae could cause a decrease in free gas exchange thus affecting the general health of fish. Cell proliferation of secondary lamellar filaments and lamellar cell hypertrophy decreases the space between lamellae and causes fusion. (Nowak, 1992; Jiraungkoorskul *et al*., 2002).

Gill morphology is an important biomarker providing a rapid method for detection of effects of pollutants such as hydrocarbons. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i. e. histopathological biomarker on a tissue level.

Liver histopathology and macrophage aggregates have been recommended for use by the WHO-FAO (Au, 2004). They have been used successfully by the US National Marine Fisheries Services on the Pacific Coast, employed by the NOAA marine monitoring programmes and the US national programme in Virginia estuaries (Au, 2004). The changes in the liver observed in this study such as inflammation, nuclear abnormality, irregular shaped hepatocytes, increased nuclear vacuolation and bile stagnation identified as brownish – yellow granules in the cytoplasm have been associated with fish exposed to hydrocarbons by Pacheco and Santos,

(2002) and Camargo and Martinez, (2007). The liver necrosis observed can be a response to the hydrocarbon. Camargo and Martinez, (2007) described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water.

The hepatic tissue in this study appears spotted. Between the normally formed polygonal hepatocytes, slightly enlarged, cells of especially dark coloration were observed. Melanomacrophages are known by their dark pigments and are the spotted structures observed in this study. Melano-macrophages are pigment (melanin) containing cells and develop in association with chronic inflammatory lesions. Melano-macrophage centres increase in size or frequency in conditions of environmental stress and have been suggested as reliable biomarkers for water quality (Agius and Roberts, 2003).

Incidence of melano-macrophage in the liver of fish from polluted water has been reported (Agius and Roberts, 2003). The liver plays a key role in the metabolism and biochemical transformations of pollutants from the environment, which inevitably reflects on its integrity by creating lesions and other histopathological alterations of the liver parenchyma. The liver is one of the organs most affected by contaminants in water.

Similar effects in gonads such as wrinkling of the germinal epithelium, deformed oocytes, contraction of oocytes, shrinkage of nucleus, presence of macrophages observed in this study have been noticed by several authors including Au (2004), Akpu and Chinda, (2009) and Lehman *et al*., (2007). Marwa and Hatem (2009) observed higher incidence of atretic and

deformed oocytes in fish collected from a polluted river in Egypt. Oocyte atresia in fish after chronic sublethal exposure to aromatic compounds have been demonstrated by Au, (2004). *Tilapia guineensis* exposed to sublethal concentrations of parateq produced deformed oocytes (Akpu and Chinda, 2009). Melanomacrophages were observed by Lehman *et al*., (2007) in the ovaries of *Corbicula fluminea* exposed to Polychlorinated biphenyl suggesting that the melanomacrophages probably serve as the "clean-up crew" which are involved in innate immunity and usually associated with atretic oocytes.

Chronic pollution may lead to a decrease in quality of gametes, thereby impairing reproductive success and posing a significant threat to the sustainability of fish population. Histological response of the fish gonads to environmental stress has shown to be a biomarker indicative tool to assist in the bio-monitoring process of aquatic ecosystems (Bhuiyan and Nessa, 2001). Similar findings to results from this study on the effects of pollutants on fish have been reported (Koca *et al*., 2008; Yasser and Naser 2010). Following the *Amoco Cadiz* spill (Arabian and Iranian crude) histopathological lesions were observed in the ovaries, kidneys, and gills of plaice (*Pleuronectes platessa*) collected in low-energy tidal estuaries that had very high hydrocarbon concentrations (up to 28 mg/g) of petroleum hydrocarbons in the sediment (Anderson and Lee, 2006). Research has demonstrated a positive link (by association) between the presence of certain xenobiotic chemicals in sediments, seawater or food organisms and the onset of histopathological changes in fish species (Brand *et al.*, 2001).

Liver histopathology, gill histopathology, macrophage aggregates and oocyte atresia have been developed and recommended as biomarkers by the International Council for the Exploration of

the Sea ICES in Europe and National Oceanic and Atmospheric Adminitration NOAA marine monitoring programmes in the USA for monitoring the effects of pollution. The information provided in this study reveals that fish histopathology could be used as biomarkers to provide information on the effects of hydrocarbons on fish health. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs that are responsible for vital functions such as respiration, and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.*, 2001). Furthermore, the alterations found in these organs are normally easier to identify and serve as warning signs of damage to animal health.

CONCLUSION

The results obtained in this study have established that petroleum products importation, storage and distribution are the major sources of monocyclic aromatic hydrocarbon contamination of the Lagos lagoon environment. Therefore, there is the need to enforce the treatment of stormwaters and effluents from the oil distribution and storage facilities located around the Lagos lagoon. The inclusion of the identified biomarkers such as malondialdehyde level, inhibition of SOD, CAT, GST, GSH and histopathological markers in environmental monitoring programmes will also ensure early detection of the adverse impacts of petroleum hydrocarbon contamination in the Lagos lagoon. The results of the residual level of BTEX and THC in groundwater and soil at Ijegun clearly show that there is widespread contamination of groundwater and soil in the impacted area even two years after the incident. There is therefore an urgent need to treat the groundwater so as to avert public health risk that may be associated with drinking such polluted waters.

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RECOMMENDATIONS

It is recommended that:

- Waste treatment plants should be installed in all petroleum depots and tank farms located around the Atlas cove and Apapa in order to treat their stormwaters and effluents before discharge into aquatic environment.
- There is the need for the relevant government agencies such as the Department of Petroleum Resources (DPR) and Lagos State Environmental Protection Agency (LASEPA) to enforce relevant sections of EGASPIN (2002) to ensure reduction in the hydrocarbon related pollution level of the Lagos lagoon.
- Increase in MDA level in conjunction with inhibition of CAT, SOD, GST and histopathological biomarkers which can serve as early warning signals should be included in environmental monitoring programmes involving petroleum hydrocarbon.
- With regards to the Ijegun oil impacted areas, there is the need to identify all impacted water wells and carry out public awareness campaign to inform the people about the dangers of using such water for domestic purposes.
- Immediate treatment of the groundwater in Ijegun is required to reduce the level of monocyclic aromatic hydrocarbons (BTEX) below the WHO recommended safe limits in drinking water.

SUMMARY OF FINDINGS

1. The two year survey programme in the Lagos lagoon established that BTEX compounds were detected in varying concentrations in all the sampling stations.

   The mean value of Total Hydrocarbon Content recorded in 2010 (water and sediment) was higher than the mean value of Total Hydrocarbon Content observed in 2009. The level of THC in the sediment was 3 times higher than the level of THC in the water samples from the Lagos lagoon.

2. The two year survey programme in the oil impacted area of Ijegun established that the Total Hydrocarbon Content of groundwater samples (2.00–689.12mg/l) around the impacted areas were higher than the WHO limit of 0.1mg/l. It was observed that the THC in year 2009 were higher than the values obtained in year 2010. The dry seasons had signific**a**ntly ($P<0.05$) higher THC in comparison with the wet season. Concentrations of BTEX compounds in the groundwater were higher than concentrations in the soil samples.

3. Investigation on the impact of petroleum product spillages on macrobenthic community structure in the study stations of the Lagos lagoon reveal that Benthic organisms were low in abundance in Apapa station and high in abundance in Unilag station. Indicator organisms represented by *Nais eliguis, Heteromastus filiformis and Nereis sp* were found mostly in Iddo, Atlas cove and Apapa station*s*. Three major groups (Mollusca, Arthropoda and Annelida) were distributed in the Lagos lagoon.

4. In the Acute Toxicity studies, Ethylbenzene was the most toxic followed by Xylene, Benzene and Toluene against *C. gariepinus*. Xylene was the most toxic followed by

Toluene, Ethylbenzene and Benzene against *E. eugeniae*. Comparatively, *C. gariepinus* was found to be more susceptible/sensitive to all the hydrocarbons than *E. eugeniae.*

5. The studies on antioxidant enzymes and non enzymes activities in fish, *C. gariepinus* and earthworm, *E. eugeniae* after exposure to BTEX compounds identified changes in the activities of Superoxide dimustase (SOD), Catalase (CAT), Glutathione-S-Transferase (GST) and levels of Glutathione (GSH) and lipid peroxidation (LP) as biomarkers of exposure to BTEX.

6. The studies on histopathology revealed as follows:

   - BTEX compounds induced histopathological changes such as cellular degeneration, necrosis, distortion of shape of circular and longitudinal muscles, enlargement of ectoderm cells in *E. euginiae* that can be used as biomarkers.
   - BTEX compounds induced histopathological changes such as loss of regular shape, loss of secondary lamellae, fusion of secondary lamellae and necrosis in the gills of fish, *C. gariepinu*s that can be used as biomarkers.
   - BTEX compounds induced histopathological changes such as necrosis, increased nuclear vacuolation, nuclear degeneration and inflammation in the livers of fish, *C. gariepinu*s that can be used as biomarkers.
   - BTEX compounds induced histopathological changes such as necrosis, wrinkling of the membrane, deformed oocytes and contraction of oocytes in the gonads of fish, *C. gariepinu*s that can be used as biomarkers.

7. The determination of levels of SOD, CAT, GST, LP and GSH in the selected wild fish species, *C. nigrodigitatus, T. Zillii* and Earthworm, *E. euginiae* confirmed their relevance

as useful biomarkers of hydrocarbon. The level of antioxidant enzymes in fish from the Lagos lagoon and earthworm from Ijegun were found to be significantly reduced than that in control fish and earthworm.

CONTRIBUTIONS TO KNOWLEDGE

1. This study has established the extent and residual level of hydrocarbon (THC and BTEX) in the ground water and soil in Ijegun over a period of two years after the pipeline explosion, therefore, indicating the need for the relevant regulatory agencies to carry out additional post impact evaluation of the incident in order to safeguard the lives of the people in the area.
2. The study established inhibition of SOD, CAT, GST & GSH; increase in Malondialdehyde, MDA; and tissue histopathology (lesion, inflammation, endothelial degeneration) as useful battery of general biomarkers for identifying hydrocarbon induced stress which can serve as early warning signals during pollution monitoring programmes by regulatory agencies in Nigeria.
3. The decrease in abundance and changes in species composition of benthic animals were established in Atlas Cove (marine receipt terminal) and Apapa (Tank farms location) as major ecological impacts of petroleum products' loading, offloading and storage activities in these areas.
4. The laboratory toxicity studies and level of occurrence of BTEX determined in the study area can form a basis for the establishment of environmental safe limits and standards for BTEX against indigenous animal species which are currently not available.

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APPENDICES

Appendix 1: Physic – chemical parameters measured at Lagos lagoon sampling stations during the July 2009 sampling year

Stations	pH	Salinity (%)	Cond (µ/Scm)	TDS (ppm)	DO (mg/L)
L1	8.25	5	260	130	3.88
L2	8.44	6	308	154	3.27
L3	8.78	6.1	266	133	3.06
L4	9.00	7	1270	635	3.98
L5	5.70	15	19000	9500	337
L6	8.83	22	30400	15200	4.08
L7	8.39	24	31400	15700	3.78
L8	8.82	20	24600	12300	3.98
L9	8.75	20	26900	13450	3.47
L10	8.77	18	23700	11850	3.67
L11	8.75	20	10000	5000	3.47
L12	8.63	10	7540	3770	3.37
L13	8.18	11	8190	4095	3.57
L14	8.23	8	6080	3040	3.98
L15	8.13	10	7820	3910	3.78
L16	8.09	10	8990	4495	3.27
L17	8.14	10	8350	4175	3.88
L18	8.02	10	8880	4440	3.98

Appendix 2: Physic – chemical parameters measured at Lagos lagoon sampling stations during the February 2009 sampling year

Stations	pH	Salinity (%)	Cond (µ/Scm)	TDS (ppm)	DO (mg/L)
L1	6.90	21	177.70	16.30	6.04
L2	7.00	26	291.50	134.30	5.34
L3	7.30	34	288.20	64.90	3.09
L4	7.30	34	263.10	182.30	2.36
L5	7.90	38	414.00	203.00	4.31
L6	7.90	43	340.00	116.00	4.99
L7	8.00	40	389.20	205.20	5.00
L8	8.00	44	340.90	204.40	4.89
L9	8.00	46	279.20	181.60	5.10
L10	8.00	42	132.50	114.80	4.89
L11	8.10	42	376.90	204.00	4.99
L12	7.70	35	345.30	126.60	4.51
L13	7.70	40	269.00	377.00	4.88
L14	7.40	40	365.00	166.10	4.99
L15	7.50	35	117.00	132.90	5.00
L16	7.50	36	362.40	180.50	4.99
L17	7.60	35	366.70	166.00	4.89
L18	7.50	35	340.20	170.10	4.88

Appendix 3: Physic – chemical parameters measured at Lagos lagoon sampling stations during the July 2010 sampling year

Stations	pH	Salinity (%)	Cond (µ/Scm)	TDS (ppm)	DO (mg/L)
L1	7.61	0.20	1079	539	5.11
L2	7.42	0.10	731	363	2.69
L3	7.61	0.33	1791	895	4.90
L4	8.28	7.38	37800	18800	6.15
L5	8.32	7.96	41200	20600	6.42
L6	10.57	7.48	38700	19300	5.25
L7	8.35	8.14	42100	21000	5.83
L8	7.91	3.49	18010	200	5.69
L9	8.33	8.30	42900	21500	5.82
L10	8.34	7.13	36700	18800	5.82
L11	8.38	8.41	43000	21500	5.58
L12	8.34	5.35	27500	13860	5.29
L13	7.78	2.67	13750	6870	4.67
L14	7.98	2.91	14110	7080	3.95
L15	8.01	2.91	15130	7530	3.54
L16	7.77	2.11	10850	5220	3.44
L17	7.99	2.60	13400	6710	6.65
L18	8.33	2.98	15950	775	3.15

Appendix 4: Physic – chemical parameters measured at Lagos lagoon sampling stations during the February 2010 sampling year

Stations	pH	Salinity (%)	Cond (µ/Scm)	TDS (ppm)	DO (mg/L)
L1	7.50	35	3999	539	6.23
L2	7.62	35	7.31	363	5.86
L3	7.37	36	1791	895	6.31
L4	7.49	40	37.80	18.8	7.00
L5	7.50	40	41.20	20.6	6.42
L6	7.48	40	38.70	19.3	6.62
L7	7.84	40	42.10	21	5.70
L8	7.91	43	18.01	9.2	5.69
L9	7.88	44	42.90	21.5	5.88
L10	7.84	45	36.70	18.8	6.39
L11	7.75	45	43	21.5	6.42
L12	7.77	44	27.55	13.86	6.39
L13	7.54	37	13.75	6.87	6.00
L14	7.65	39	14.11	7.08	6.67
L15	7.66	39	15.13	7.53	6.72
L16	7.65	35	10.85	5.22	6.82
L17	7.66	39	13.4	6.71	6.65
L18	7.59	38	15.95	7.15	6.16

Appendix 5: Concentrations of THC measured in the Lagos lagoon surface water at sampling stations during the 2009/2010 sampling year

Stations	2009wet season	2009 dry season	2010wet season	2010 dry season
L1	1.32	1.93	5.17	0.62
L2	5.14	7.83	7.99	0.99
L3	1.14	18.48	2.15	0.91
L4	1.08	3.67	7.18	7.41
L5	1.20	2.29	6.01	6.32
L6	1.50	40.29	5.23	19.10
L7	1.53	49.16	10.71	5.74
L8	4.98	34.02	9.86	8.59
L9	2.15	10.18	8.42	4.01
L10	1.41	84.56	7.56	8.07
L11	1.66	2.64	10.89	3.11
L12	0.41	7.76	8.74	4.29
L13	2.90	59.58	5.04	8.87
L14	5.86	20.54	14.74	8.77
L15	2.89	22.54	10.83	3.03
L16	7.83	14.70	9.28	11.14
L17	1.16	8.85	10.73	3.72
L18	3.64	11.14	11.35	12.51

Appendix 6: Concentrations of THC measured in the Lagos lagoon sediment at sampling stations during the 2009/2010 sampling year

Stations	2009wet season	2009 dry season	2010wet season	2010 dry season
L1	19.01	96.35	10.23	0.01
L2	78.59	5.37	9.69	47.42
L3	13.51	6.35	29.74	254.71
L4	22.71	10.12	11.43	259.43
L5	6.77	8.55	10.58	474.04
L6	3.81	4.01	9.15	68.16
L7	1.01	4.92	9.94	3.79
L8	1.51	4.92	15.89	42.85
L9	4.08	3.48	14.75	78.12
L10	0.38	17.30	12.59	4.84
L11	0.73	4.01	11.81	37.44
L12	2.73	179.26	19.93	47.42
L13	14.89	127.87	19.54	4.91
L14	1.45	4.55	19.19	60.97
L15	37.48	11.81	23.18	11.59
L16	6.85	32.47	17.01	12.73
L17	1.35	7.29	22.84	23.64
L18	0.89	11.32	19.94	30.23

Appendix 7: Sediment particle size determination

S/N	Reagent/Apparatus	Description of Procedures
1	15g air-dried sediment	Weigh accurately into a conical flask
2	10mL H_2O_2 (Hydrogen Peroxide)	Add 10mL of H_2O_2 (5mL at a time -progressive additions) to remove the binding organic matter in the sediment. Hydrogen peroxide oxidizes organic matter which binds soil particles into aggregates
3	45mL HMP (Sodium Hexametaphosphate [HMP, $(NaPO_3)_n$],) - 5% Calgon	Add 45mL 5% Calgon to the soil/sediment sample. The function of HMP is to complex any Ca^{2+} in solution and to replace Ca^{2+} with Na^+ on the ion-exchange complex of soil particles, resulting in the dispersion of individual soil particles and causing breakdown of soil aggregates
4	Shake mixture	Shake mixture vigorously for 2hours. Mechanical agitation enhances the dispersion
5	0.053mm Sieve	Sieve out clay and silt with 0.053mm sieve till water clears.
6	Residue	Collect residue in aluminum foil and dry in oven at 55^0C to constant weight. This represent the sand content.
7	Filtrate	Collect the filtrate in a beaker and allow to stand for 48hours
8	Decantation	Decant off supernatant layer which represents the clay content of the soil/sediment sample.
9	Precipitate	The precipitate representing the silt content of the soil/sediment sample is oven dried at 105^0C to constant weight
10	Weigh	Weigh accurately the dry mass of both the sand and silt.
Calculations		
Clay quantity in the sediment sample is given by the difference of the sum of the dry mass of sand and the dry mass of silt from the total sample:		

Weight of clay = Soil sample weight – (weight of dry mass sand + weight of dry mass silt) Weight of clay = 15g – (weight of sand + weight of silt)
% Sand = $\frac{\text{Wt. of sand}}{\text{Wt. of Soil sample}} \times \frac{100}{1}$
% Silt = $\frac{\text{Wt. of silt}}{\text{Wt. of Soil sample}} \times \frac{100}{1}$
% Clay = $\frac{\text{Wt. of clay}}{\text{Wt. of Soil sample}} \times \frac{100}{1}$

Appendix 8: Physic – chemical parameters measured at Ijegun sampling stations during 2009 sampling year

Stations	pH	Conductivity	TDS	Nickel	Lead	Zinc
W1	7.33	720	400	0.15	0.01	0.02
W2	7.07	240	131	0.19	0.14	0.12
W3	6.47	32	17	0.19	0.01	0.03
W4	7.52	401	220	0.11	0.01	0.00
W5	7.43	334	183	0.09	0.13	0.05
W6	7.70	470	256	0.11	0.25	0.00
W7	6.88	462	254	0.10	0.71	0.03
W8	6.83	286	141	0.13	0.08	0.01
W9	7.16	430	202	0.15	0.06	0.01
W10	6.41	374	182	0.01	0.27	0.01
W11	6.58	299	164	0.01	0.42	0.01
W12	6.79	362	199	0.12	0.04	0.01
W13	7.39	306	168	0.08	0.12	0.01
W14	5.79	327	175	0.01	0.19	0.01
W15	6.05	373	205	0.01	0.01	0.03
W16	6.86	452	247	0.09	0.01	0.03
W17	7.44	299	164	0.01	0.22	0.03
W18	7.55	209	116	0.06	0.07	0.05
W19	6.08	679	373	0.16	ND	0.03
W20	7.38	763	420	0.14	ND	0.03

Appendix 9: Physic – chemical parameters measured at Ijegun sampling stations during 2010 sampling year

Stations	pH	Conductivity	TDS	Nickel	Lead	Zinc
W1	6.37	44	23	ND	ND	ND
W2	6.22	311	162	0.01	<0.01	0.02
W3	6.42	427	177	0.01	<0.01	0.01
W4	6.82	288	145	ND	ND	ND
W5	6.85	324	177	0.01	<0.01	0.01
W6	7.16	215	111	ND	ND	ND
W7	7.45	342	177	ND	ND	ND
W8	7.31	115	59	ND	ND	ND
W9	7.52	343	1.79	ND	ND	ND
W10	6.31	136	69	ND	ND	ND
W11	6.99	218	113	ND	ND	ND
W12	6.67	231	120	ND	ND	ND
W13	7.51	206	107	ND	ND	ND
W14	6.37	44	23	ND	ND	ND
W15	6.36	45	23	ND	ND	ND
W16	6.65	229	119	ND	ND	ND
W17	6.66	230	118	ND	ND	ND
W18	7.79	447	232	0.02	<0.01	0.022
W19	7.51	206	107	ND	ND	ND
W20	6.37	44	23	ND	ND	ND

Appendix 10: Concentrations of THC measured (mg/l) in Ijegun soil at sampling stations during the 2009/2010 sampling year

Stations	2009wet season	2009 dry season	2010wet season	2010 dry season
S1	68.67	99.89	8.17	256.02
S2	3.63	131.64	11.35	53.92
S3	7.43	401.41	12.85	5.55
S4	1.89	402.52	6.29	12.98
S5	4.33	138.12	9.68	46.42
S6	12.04	246.87	9.77	2.16
S7	65.49	286.55	13.42	2.52
S8	6.24	12.44	11.82	9.36
S9	31.21	23.61	13.43	124.72
S10	69.63	9.29	15.08	1.30
S11	0.82	12.41	5.39	20.30
S12	55.28	1.87	15.08	57.43
S13	18.14	9.35	5.39	17.64
S14	207.16	234.54	7.93	16.30
S15	11.31	380.72	10.68	1.25
S16	160.29	20.23	12.08	35.32
S17	90.72	21.25	9.68	18.55
S18	44.43	41.69	11.22	25.65
S19	22.72	44.62	7.03	28.43

Appendix 11: Concentrations of THC measured (mg/l) in Ijegun groundwater at sampling stations during the 2009/2010 sampling year

Stations	2009wet season	2009 dry season	2010wet season	2010 dry season
W1	0.91	29.59	9.33	14.97
W2	0.91	164.90	3.97	14.44
W3	2.54	23.35	7.41	15.53
W4	1.14	33.31	6.73	19.11
W5	509.91	39.91	6.51	9.90
W6	509.48	39.88	7.62	5.08
W7	2.29	660.38	5.99	13.76
W8	2.30	708.93	10.27	12.39
W9	2.27	593.31	5.35	21.23
W10	2.28	669.29	6.26	17.84
W11	2.38	554.75	5.66	10.19
W12	2.28	757.97	4.23	16.21
W13	4.87	14.31	4.21	5.30
W14	4.88	20.02	3.44	9.92
W15	1.62	19.64	9.11	6.11
W16	6.28	23.54	8.13	16.21
W17	3.08	83.13	6.64	6.61
W18	91.25	35.08	13.49	15.35
W19	15.28	15.97	6.95	4.24
W20	0.57	0.38	5.75	0.47

Appendix 12: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of MDA in the livers of *Clarias gariepinus*

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	1.22±0.37				1.220.37			
Day 15	16.80±2.72	20.62±1.70	4.54±1.42	16.91±0.14	12.13±1.53	15.38±1.93	1.79±0.35	7.91±0.47
Day 30	18.29±3.23	22.42±1.85	5.34±1.68	20.19±0.57	13.02±1.42	16.71±2.09	1.99±0.39	8.57±1.61
Day 45	29.04±1.80	12.13±0.64	4.76±0.16	6.91±0.37	18.38±1.52	6.59±0.12	2.97±0.24	4.34±2.24
Day60	29.02±3.63	13.19±0.69	5.29±0.18	7.05±2.36	20.28±2.43	7.17±0.12	3.49±0.28	4.13±2.36

Appendix 13: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of MDA in the gills of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	0.67±0.14				0.67±0.14			
Day 15	10.62±1.48	11.75±1.61	7.57±1.20	9.10±1.03	7.03±1.41	6.68±2.09	3.70±1.14	9.16±0.81
Day 30	11.32±0.98	12.77±1.75	8.90±1.40	10.79±1.49	7.62±2.15	7.26±2.27	4.11±1.27	10.79±0.96
Day 45	23.51±4.36	15.55±3.45	4.28±0.47	13.14±0.40	15.24±1.65	11.88±2.04	2.25±0.28	8.94±1.48
Day 60	25.65±4.56	16.90±3.75	5.04±0.55	14.85±0.33	16.65±2.35	12.92±2.22	2.59±0.43	6.16±1.19

Appendix 14: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of GST in the gills of *Clarias gariepinus*

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	422.43±51.09				422.43±51.09			
Day 15	161.04±12.14	385.30±19.94	182.52±10.68	308.64±2.02	166.97±11.58	401.59±5.29	163.57±17.09	311.36±3.30
Day 30	146.86±8.42	353.48±18.34	167.45±9.80	302.9±4.08	142.64±3.01	552.81±17.67	150.07±15.68	302.9±4.87
Day 45	263.95±8.54	417.69±7.46	192.3±26.74	376.55±6.83	270.42±1.70	437.74±5.76	156.85±12.60	379.88±8.43
Day60	308.45±12.09	455.28±8.13	176.38±24.58	508.92±14.28	312.85±10.81	602.56±19.26	143.9±11.55	398.94±18.97

Appendix 15: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of GST in the livers of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	243.71±16.20				243.71±16.20			
Day 15	348.72±14.18	493.84±7.44	173.375±27.93	475.62±6.68	320.77±35.76	269.48±7.44	183.24±18.10	388.05±12.71
Day 30	308.86±20.27	450.78±3.59	159.36±26.041	470.31±14.14	277.7±16.26	247.23±19.04	168.11±16.60	383.89±18.87
Day 45	376.5±12.28	475.4±110.02	256.76±30.95	532.19±8.19	298.97±3.00	426.65±11.38	290.08±30.47	477.15±10.16
Day60	436.72±5.65	436.18±100.93	235.56±28.39	553.9±23.27	355.33±19.96	391.4±10.45	266.13±27.95	499.82±23.27

Appendix 16: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of GSH in the livers of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	37.41±3.92				37.41±3.92			
Day 15	8.69±0.01	5.63±0.65	14.97±2.52	6.34±0.12	10.88±2.28	2.46±0.81	16.01±2.92	6.96±0.77
Day 30	7.04±0.03	4.53±0.77	12.48±2.10	5.14±0.18	8.74±1.78	1.99±0.76	12.31±2.24	5.89±1.24
Day 45	4.83±0.10	10.50±0.75	18.23±4.92	10.20±1.76	9.06±0.11	9.10±0.54	15.48±1.55	6.33±1.11
Day 60	5.08±0.09	8.72±0.67	14.02±3.78	9.78±0.93	10.32±0.79	7.52±0.36	11.90±1.19	6.76±0.93

Appendix 17: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of GSH in the gills of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	51.76±7.14				51.76±7.14			
Day 15	5.11±0.24	14.69±1.14	9.97±1.28	6.09±0.04	5.46±0.58	7.91±0.46	15.12±0.23	6.52±0.58
Day 30	4.03±0.07	11.8±1.58	8.31±1.07	5.35±0.52	4.34±0.46	6.35±0.73	11.73±2.00	5.35±0.48
Day 45	2.59±0.78	17.17±0.55	19.02±1.37	7.52±0.74	1.38±0.17	3.71±0.65	11.64±0.17	5.32±0.30
Day 60	2.45±0.63	15.70±1.52	15.85±1.14	7.54±0.96	1.7±0.97	3.06±0.50	14.08±2.40	4.94±1.01

Appendix 18: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of CAT in the gills of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	717.5±49.49				717.5±49.49			
Day 15	98.59±4.08	128.32±3.50	109.22±21.69	190.89±2.84	140.25±1.85	151.56±7.09	214.95±13.58	169.03±5.96
Day 30	60.23±2.87	117.2±3.95	100.20±19.90	169.57±7.53	90.78±1.61	138.44±7.36	195.41±12.35	153.24±4.82
Day 45	73.16±5.44	156.15±15.52	143.56±7.33	147.41±10.22	74.34±1.56	144.91±9.94	60.48±29.85	167.41±1.64
Day60	118.88±4.75	182.71±19.66	168.89±8.63	165.62±11.49	121.48±2.27	168.43±8.79	70.53±37.87	183.61±12.16

Appendix 19: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of CAT in the livers of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	740.95±126.34				740.95±126.34			
Day 15	92.09±2.93	140.94±5.81	156.48±22.30	154.38±1.84	118.21±8.16	111.74±3.50	193.33±31.48	127.54±2.65
Day 30	51.02±1.42	129.31±5.33	143.56±20.46	141.51±3.53	67.76±2.17	102.47±3.15	175.87±28.78	112.36±1.64
Day 45	111.46±4.87	253.37±0.92	126.31±23.86	150.54±2.35	62.76±1.66	162.44±7.11	74.03±3.72	93.57±0.70
Day60	187.5±10.28	289.79±10.64	140.34±26.51	153.23±1.19	101.98±3.76	188.84±5.16	87.1±4.38	108.53±1.19

Appendix 20: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of SOD in the gills of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	102.42±10.14				102.42±10.14			
Day 15	23.72±1.17	73.95±15.25	38.38±0.62	82.91±3.04	19.78±5.28	48.24±7.01	71.62±13.98	40.45±3.55
Day 30	20.57±0.98	66.97±14.23	36.01±4.51	78.74±1.71	16.01±3.90	42.21±8.69	65.11±12.71	38.62±3.11
Day 45	15.11±3.19	8.44±0.35	34.89±0.56	19.56±1.59	8.02±2.50	13.79±0.79	22.30±1.23	18.51±0.07
Day 60	12.12±2.82	7.37±0.76	30.01±3.76	16.73±2.81	6.48±2.09	12.00±0.04	20.27±1.12	16.73±2.43

Appendix 21: Data on the effect of sub lethal concentrations of BTEX compounds on the activity of SOD in the livers of *Clarias gariepinus*.

	B	**T**	**E**	**X**	**B**	**T**	**E**	**X**
	1/10th	**1/10th**	**1/10th**	**1/10th**	**1/100th**	**1/100th**	**1/100th**	**1/100th**
Day 0	216.23±9.10				216.23±9.10			
Day 15	46.63±2.17	98.38±0.81	60.57±1.66	44.83±9.82	40.47±3.23	76.18±1.66	100.7±1.06	68.92±9.82
Day 30	45.73±3.76	88.67±1.68	50.47±1.88	35.55±0.92	33.05±2.82	68.91±0.76	91.54±0.96	64.34±7.72
Day 45	40.55±1.08	16.03±1.78	49.15±2.92	11.39±0.67	25.88±2.67	8.46±1.02	57.98±2.68	28.06±0.70
Day60	40.64±1.07	14.02±2.41	42.9±5.17	10.23±0.48	22.58±2.22	7.4±1.34	50.42±2.33	24.36±4.44

Appendix 22: Data on the levels of antioxidants defence systems in the liver and gills of *C. nigrodigitatus*(1) and *T. zilli* (2) from Iddo, Atlas Cove (AC), Apapa (AP) and Unilag (ULAG) stations of the Lagos lagoon.

		Iddo 1	**Iddo2**	**AC1**	**AC2**	**AP1**	**AP2**	**ULAG1**	**ULAG2**
GSH	Liver	0.17±0.1	0.49±0.2	0.33±0.1	0.13±0.1	0.13±0.1	0.3±0.1	0.14±0.1	0.07±0.0
	Gill	0.51±0.17	0.25±0.03	1.45±0.37	0.49±0.15	1.52±0.84	0.16±0.08	0.28±0.05	0.39±0.15
SOD	Liver	5.34±2.49	26.92±15.95	8.09±5.17	23.45±6.22	14.48±9.13	51.42±31.28	24.15±6.84	4.1±0.67
	Gill	24.51±15.21	53.05±30.68	111.79±26.76	49.33±8.02	160.23±80.64	55.86±25.06	62.88±25.17	15.94±6.38
CAT	Liver	20.8±8.19	59.02±29.53	48.59±16.92	179.64±140.73	262.56±125.99	86.44±29.65	96.17±27.69	19.72±4.09
	Gill	159.2±141.57	244.72±141.56	220.99±39.07	515.7±123.46	528.93±232.09	172.72±117.55	273.51±129.91	218.45±95.8
MDA	liver	21.18±6.49	10.99±2.3	11.91±1.37	27.29±17.27	16.94±6.29	31.59±21.19	6.01±1.97	13.81±6.15
	Gill	10.89±4.39	11.35±4.73	5.41±0.77	5.02±1.39	5.92±1.5	12.17±3.48	5.37±2.59	3.78±1.39
GST	Liver	2.42±0.88	6.76±3.37	4.52±1.24	1.79±1.03	1.74±0.85	4.13±1.83	1.06±0.54	1.19±0.33
	Gill	4.88±4.19	4.08±2.46	19.06±4.37	6.77±2.06	28.82±12.14	2.02±0.86	3.85±0.66	6.14±2.22

Appendix 23: Data on the levels of antioxidant defence systems in earthworms collected from different stations of Ijegun

	Stations							
	S5	**S6**	**S8**	**S9**	**S12**	**S13**	**S18**	**S19**
GSH	0.04±0.01	0.1±0.02	0.05±0.02	0.21±0.1	0.02±0.01	0.2±0.2	0.01±0	0.09±0.1
SOD	0.76±0.22	1.94±0.5	0.82±0.27	2.1±1.27	0.34±0.09	0.69±0.2	0.21±0.1	0.49±0.2
CAT	4.61±1.26	11.51±4.22	5.09±2.5	15.91±6.78	2.48±0.55	11.73±5.43	1.5±0.5	3.84±0.6
MDA	1.11±0.3	1.86±0.2	1.47±0.4	2.68±0.8	9.44±5.4	15.45±0.3	11.38±5.5	17.22±1.4
GST	0.5±0.1	1.47±0.3	0.57±0.3	2.36±1.1	0.36±0.1	3.98±1.9	0.11±0.1	1.28±0.7

Appendix 24: Histology procedure

Collection of Tissues

About 3-5mm in thickness samples were selected from various organs(Liver, Kidney, Heart and Testis) for histological demonstration.

Fixation

The selected tissues were fixed in Bouin's fluid for 5-10hours to ensure proper fixation which prevent the tissue from autolysis and putrefaction.

Dehydration and Clearing

The fixed tissues were placed in different grades of alcohol (70%- 95%- 100%) to ensure complete removal of water from the fixed tissues. The dehydrated tissues were treated with xylene to remove excess alcohol from the tissues and to form the link between the tissue and parraffin wax.

Infiltration or Impregnation

The cleared tissues were infilterated using using molten paraplast melted at 56°C to remove excess clearing agent from the tissues.

Embedding

Infilterated/Impregrated tissues were dipped in individual embedding medium to creat support for the tissue to enhance sectioning.

Moulding

Embedded tissues were mounted on the block.

Trimming

The blocked tissues were trimmed with trimming knife to make sectioning easy.

Microtomy

This is the use of microtome machine (Rotary microtone) to cut the embedded tissue at 3- 5. The sections were floated out using 20% ethanol. The sectioned tissues that were floated out in warm water at about 35°C were placed on a glass slide and drained. Mounted slides were put on hot plate for proper adhesion of tissue sections to the microscope slide.

Staining

The prepared slides were stained using haematoxylin and eosin.