STUDIES ON THE EFFECT OF PRODUCED WATER ON JUVENILE AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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CERTIFICATION

This is to certify that this research work; 'Studies on the Effect of Produced Water on Juvenile African Catfish (*Clarias gariepinus*)', was carried out by **Esie, Ngozi Georgewill** (20114842598), in partial fulfillment of the requirements for the award of Master of Science (MSc.) degree in Biotechnology in the Department of Biotechnology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

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DEDICATION

This research work is dedicated to the Almighty God for His indwelling and abiding presence in my life, and also to my parents, Mr. and Mrs. Georgewill Esie for their unflinching support.

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TABLE OF CONTENTS

CHAPTER THREE: MATERIALS AND METHODS

LIST OF TABLES

ABSTRACT

This study investigated the effect of produced water from an oil exploration company in Rivers State on the blood, gills and liver of Juvenile African Catfish (*Clarias gariepinus*). Seventy Juvenile African Catfish were separated into 7 groups (10 per treatment) and used for the study. The juveniles were exposed to three varying concentrations of fresh and stale produced water (2.5ml/l, 5.0ml/l and 10.0ml/l) for a period of 3weeks. At the end of the test period, haematological, biochemical and anti-oxidant activities were carried out on the blood and liver of the juveniles, histopathological examination of the gills and liver were also conducted. The results obtained from the physicochemical analysis of the stale produced water showed that the stale produced water was within DPR and FMENV acceptable levels, while those of the fresh produced water were slightly above the acceptable levels, although statistically not significant, except for the salinity and conductivity parameters. The results obtained showed no difference in the heamatological, biochemical and anti-oxidant parameters. Histopathological examination of the gills exposed to fresh produced water showed lesions, oedema (mucous-filled cavity), displacement of epithelial cells and filament detachment with increasing concentration of the fresh produced water, which according to previous studies indicates reduced oxygen supply, resulting to decrease in respiratory response. The results obtained from the liver showed inflammation, enlarged hepatic area and increased vacoulation of the hepatocyte suggestive of metabolic damages and signal of degenerative process possibly related to exposure to contaminated water. All the juveniles held in the control stock showed no histological degradation. The severity of damage to the gills and liver depends on the concentration of the pollutants and the period of exposure. Conclusively, this study has revealed that exposure of juvenile *Clarias gariepinus* to even low concentrations of untreated produced water may induce histological degradations in the liver and gill structures of the fish.

Keywords: *Clarias gariepinus.* Produced water, Haematology, Histopathology, Anti-oxidants.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Petroleum exploration and production in the Niger Delta region of Nigeria and the export of oil and gas resources by the petroleum sector has substantially improved the nation's economy over the past five decades. However, activities associated with petroleum exploration, development and production operations have detrimental and significant impacts on the atmosphere, soil and sediments, surface and groundwater, marine environment, biological diversity and sustainability of terrestrial ecosystems in the Niger Delta. (Nwilo *et al.*, 2006) Discharges of petroleum hydrocarbon and petroleum-derived waste streams have caused environmental pollution, adverse human health effects, and detrimental impact on regional economy, socio-economic problems and degradation of host communities in the oil producing states in the Niger Delta region. (Daniel-kalio *et al.*, 2002) Although, there are other potential anthropogenic sources of pollution, some of the major environmental consequences such as air pollution, global climate change and oil spills in the Niger Delta may be regional or global in scale. (Snowden *et al.*, 1987; Benka-

coker *et al.*, 1995; Benka-coker *et al.*, 1996; Amadi *et al.*, 1996; Eweje *et al.*, 2006 & Kamalu *et al.*, 2011.)

Apart from other anthropogenic emission sources, atmospheric pollution in the region is associated with emissions from flaring and venting of petroleum associated natural gas by petroleum industries (Ogri *et al.*, 2001; Scheren *et al.*, 2002; Ite *et al.*, 2013). Atmospheric contaminants from anthropogenic activities can be categorized into (i) Gaseous pollutants (ii) Persistent Organic pollutants (iii) Particulate matter and (iv) Trace element and /or heavy metals (Kampa *et al*, 2008). Release of petroleum hydrocarbons into the environment, whether accidentally or due to anthropogenic activities, is a major cause of controlled water and soil pollution. (Benka-Coker *et al.*, 1995; Benka-coker *et al.*, 1996; Scheren *et al.*, 2002; Kharaka *et al.,* 2007) and may also contribute to regional atmospheric pollution (Ite *et al.*, 2013).

The major environmental issues arose primarily from the inproper disposal of large volumes of petroleum-derived hazardous waste streams, such as oily and toxic sludge, equipment failure, oil spills, operational discharges and sabotage of petroleum facilities, (Scheren *et al.*, 2002) but most importantly, pollution that occurs due to the aforementioned instances all end up in the aquatic environment, as a result of rainfall and its run off which wash off all these products into rivers, lakes and ponds, thus increasing the pollution in the

aquatic ecosystem and so water bodies are especially susceptible to contamination from these petroleum products (Adam *et al.*, 2002). Damage done by pollution to the environment is irreversible (Omoregie *et al.*, 1999; Miles *et al.*, 1999). For this reason, awareness of the harm caused by several pollutants to the natural environment has led political and legislature authorities of the industrially developed countries to introduce or enhance regulations to protect the environment. This is because oil leaks, spills and operational discharges do not just affect marine-life; they have a direct impact on humans too. Since fishes are intimately associated with the aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish (Omoregie *et al.,* 1999). Fish has been used as bio-indicators to evaluate the environmental contamination levels of toxic pollutants, because these pollutants tend to accumulate more in organisms than in the environment (Anyakora *et al.*, 2005). The early life stages of fish are particularly sensitive to xenobiotics, and are often used to determine legally applicable measurements of pollutants and to estimate their effects on aquatic biota (Westernliagen *et al.*, 1989). Biochemical, haematological and histopathological changes in fish exposed to pollutants have been proposed and used as biomarkers for contaminants including petroleum products (Eseigbe *et al.*, 2013).

1.2 PROBLEM STATEMENT

Crude oil pollution is usually accompanied by large amounts of produce water which must be disposed off after separation from oil at the wellhead. Safe handling requires that the produce water is injected into a reservoir or be thoroughly treated to acceptable levels before disposal into the environment. Improper disposal of these pollutants into the aquatic environment not only affects the integrity of the ecosystem but also affects the physiological functions of aquatic animals (Sen *et al*, 2004). In recent years, there has been greater environmental awareness on the need to treate waste water (effluent) properly before discharge into water bodies. Although comprehensive system of environmental regulations is now in place, environmental pollution associated with oil exploration and production operations have continued to persist under these laws. It is of paramount importance that studies be carried out to ensure that proper treatment of effluent is done to prevent damage to the aquatic ecosystem.

1.3 AIM AND OBJECTIVES

The aim of this study is to determine the effect of treated and untreated effluent from an oil exploration company on juvenile African Catfish (*Clarias gariepinus*)

The specific objectives of the study are:

1. To determine the effect of the produced water on the haematological, biochemical and anti-oxidant parameters of Juvenile African Catfish.
2. To determine the histopathological effect of the produce water on the gills and liver of the Juvenile African Catfish.
3. To carry out the physicochemical analysis of the treated and untreated produced water.

1.4 HYPOTHESIS

The following hypothesis shall be assessed in this research.

1. Null Hypothesis ($H_0 \neq 0$): There is no significant difference in the effect of treated and untreated produce water from the petroleum exploration company on juvenile African Catfish (*Clarias gariepinus*).

   Alternative hypothesis ($H_1 = 0$): There is a significant difference in the effect of treated and untreated produce water from the petroleum exploration company on Juvenile African Catfish (*Clarias gariepinus*).
2. Disposal of both treated and untreated produced water will impair vital organs in juvenile *Clarias gariepinus*.
3. The composition of produced water is the cause of stress and poisoning in juvenile *Clarias gariepinus*.

1.5 SCOPE OF STUDY

The study will centre on investigating the effect of treated and untreated produced water on Juvenile African Catfish (*Clarias gariepinus*). Haematological and histopathological studies will be carried out on the blood, gills and liver of the fish respectively; biochemical and antioxidant activities will also be carried out, as well as the physicochemical analysis of the produced water.

1.6 JUSTIFICATION OF THE STUDY

Studies have been conducted on the exposure of African Catfish (*Clarias gariepinus*) to various pollutants. Abdel-Moneim *et al.*, (2008) conducted a study on the physiological and histopathological effects of juvenile catfish exposed to dyestuff and chemical waste water. Doherty *et al.*, (2013) conducted another study on the toxicological effect and histopathology of African Catfish exposed to water soluble fractions of Diesel and kerosene. George *et al.*, (2014) investigated the acute toxic effect of Qua Iboe light crude oil on the gills of *Clarias gariepinus* Juveniles, Nwaezeigwe *et al.,* (2011) conducted a study on the effects of spent oil on *Clarias gariepinus* amongst other studies.

So far, no study has been done on the effect of the produced water discharged from this particular oil company. The company is located close to a water body and hence it becomes necessary to investigate its effects.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE NIGER DELTA REGION OF NIGERIA

The Niger Delta region is situated at the apex of the Gulf of Guinea on the west coast of Africa and on the Nigeria's South-South goepolitical zone. (Hack *et al.*, 2000; Doust., 1990). The Niger Delta, which is home to about 31million people, occupies a total area of about 75,000km^2 and makes up 7.5% of Nigeria's land mass. The Niger Delta regions consists of 9 Oil Producing states; Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Ondo, Imo and Rivers and 185 Local government areas. This region cuts across over 800 oil producing communities with an extensive network of over 900 producing oil wells and several petroleum production related facilities (Osuji *et al.*, 2004). The ecological zones in the Niger Delta region can be broadly grouped into tropical rainforest in the Northern part of the Delta and mangroove forest in the warm coastlines of Nigeria. Mangroove forests and swamps, which are characterized by regular salt water inundation, lie at the centre of a complex and sensitive ecosystem which is vital to the local economy and accomodates inportant flora and fauna (Ugochukwu *et al.*, 2008). The Niger Delta, which is the largest in the world, is the widest part of Nigeria in terms of petroleum resources and diverse natural ecosystems supportive of numerous species of terrestrial and

aquatic fauna. Over the past five decades, a total of about 1,182 exploration wells have been drilled to date in the delta basin, and about 400 oil and gas fields of varying sizes have been documented (Obaje, 2009)

2.2 HISTORICAL PERSPECTIVE OF OIL EXPLORATION AND PRODUCTION IN THE NIGER DELTA REGION OF NIGERIA

Petroleum resources exploration in Nigeria dates back to 1908, when German surveyors for the Nigerian Bitumen Corporation, began prospecting for Tar Sand deposit in the South-Western Nigeria. These pioneering efforts ended abruptly with the outbreak of the World War I in 1914. Exploration of petroleum resources did not begin until 1938, when Shell D'Arcy, a consortium of lranian Oil company (later British petroleum) and Royal Dutch Shell) was granted sole concessionary right over the whole country. However, World War II (1939-1945) terminated the initial oil exploration activities by Shell D'Arcy. Oil exploration in the Nigeria's Niger Delta resumed in 1946 after World War II and shell D'Arcy drilled a number of oil exploratory wells in 1951. At the initial stage, shell D'Arcy later Shell-British Petroleum) enjoyed a complete monopoly of oil exploration for a considerable long time (1938-1955). Thereafter, Mobil producing Nigeria Ltd, a subsidiary of American Seconny-Mobile Oil Company, obtained license to explore for oil and began operations

in Nigeria in 1955 under the name Mobil Exploration Nigeria incorporated (which was later incorporated as mobile producing Nigeria on June 16, 1969). The first commercial oil discovery in the tertiary delta was confirmed at Olobiri field in January 1956 by Shell D'Arcy and a second oil field was later discovered at Afam (Haack *et al.*, 2000; Vassiliou., 2009). In February 1958, Shell British Petroleum (now Royal Dutch Shell) started exporting crude oil produced from Olobiri and Afam oil fields (Pearson., 1970) the giant Bomu oil field, which has estimated ultimate recovery (EUR) of 0.311 billion of barrels of oil and a total of 0.608billion of barrels of oil equivalent (BBOE) including gas, was discovered South-East of Port Harcourt, Rivers State in 1958 (Vassillou., 2009).

The petroleum sector began to play a vital role in shaping the Nigerian economy and political vesting of the country in the early 1960's. When Nigeria became an independent nation on 1st October 1960, Shell BP began to relinquish its acreage and its exploration licenses were converted into prospecting licenses that allowed development and production. (Vassilous., 2009; Bamberg., 2000) following the increase dominance of the Nigerian economy by petroleum sector, the sole concession policy was abandoned and exclusive exploration right was introduced to encourage other multinational oil companies aimed at accelerating petroleum exploration and production. Other

multinational oil companies joined oil and gas exploration in Nigeria and these include Texaco Overseas Nigeria Petroleum Company Unlimited in 1961, Amoseas in 1961, Gulf Oil Company in 1961 (Now Chevron), Societe Africane des Petroles (SAFRAP) in 1962 (which later became ELF Nigeria Limited in 1974), Tennessee Nigeria Limited (Tenneco) in 1962, Azienda Generale Italiana Petroli (AGIP) in 1962, ENI in 1964, Philips Oil Company in 1964 and Pan Ocean Oil Corporation in 1972. Most of these multinational oil companies recorded considerable successes in oil and gas exploration and production in both onshore and offshore fields in the Niger Delta.

The Federal Government of Nigeria Started its Department of Petroleum Resources (DPR) inspectorate in 1970 and Nigeria joined the Organization of the petroleum Exporting Countries (OPEC) in 1971. The first national oil company, the Nigerian National Petroleum Corperation (NNPC) was established in 1977. In order to take control of the country's petroleum industry, Nigeria nationalized Bp's holding completely in 1979, and Shell BP became Shell Petroleum Development Company of Nigeria (SPDC) (Genova, 2007). Although several other oil companies have joined in exploration and production over the past decades, SPDC has the largest average in the country from which it produces 39 percent of the nation's oil and remains the major producer in Nigeria's petroleum industry. The Niger Delta region is richly endowed with 31

giant oil and gas fields and each has an estimated ultimate recoverable oil of more than 500million barrels and produces in excess of 1 million barrel a day out of the nation's total production of about 2.1million barrels per day (Obaje, 2009). Currently, there are over 18 multinational oil companies which are involved in oil and gas exploration and production in the Niger Delta (Poindexter, 2008) and the major players include Dutch Shell, Exxon Mobil, Eni/AGIP, Total Fina Elf and US based Chevron Texaco. 17 Giant oil and gas production fields are located offshore and some of the examples include Bonu, Oso, Ubit, Assan, Meren, Abo, Bonga, Bonga Southwest and Agbami etc. Bonga and Bonga Southwest were discovered in 1996 and 2001, respectively and are operated by a joint venture led by SPDC.

2.3 ENVIRONMENTAL REGULATION OF OIL AND GAS EXPLORATION AND PRODUCTION IN NIGERIA

There are many approaches that have been developed for the safety and management of environmental impact of oil and natural gas exploration and production operations in the Niger Delta. The emergence of Niger Delta as one of the most ecologically sensitive region in Nigeria has led to the institutionalization of several statutory laws and environmental regulations. Nigerian Federal Government has promulgated laws and regulations so that oil

and gas exploration and production operations on both onshore and offshore oilfield could be controlled by systems of limits which aim at minimizing the associated environmental impacts, some of the related environmental laws and regulations in the oil and gas sector including oil pipelines Act 1956 (amended in 1965), Mineral oils (safety) Regulations (1963), Oil in Navigable waters acts (1968), petroleum Acts (1969), Associated Gas Re-injection act (1979), Federal Environmental protection Agency (FEPA) Act (1988), The Natural policy on the Environment, 1989 (revised in 1999), Natural Environmental Protection (Effluent limitations) Regulations (1991), Environmental impact Assessment (EIA) Act (1992) and Department of Petroleum Resources (DPR) Environmental Guidelines and standard for the petroleum Industry in Nigeria (EGASPIN) (2002). Most of these statutory laws and regulations provide the frameworks for petroleum recourse exploration and exploitation in Nigeria and only some of these environmental regulations give guidelines on issues of petroleum pollution.

The establishment of FEPA in 1988 significantly changed the legal status quo of environmental regulation in the Nigerian petroleum industry. Under the 1988 Act, parities and enforcement mechanisms were imposed, multinational oil companies could be held liable for costs of clean-up, restoration and multinational oil companies could pay compensation to parties injured by their

illegal practices. However, the existing statutory laws and regulations for environmental protection applicable to the Nigerian petroleum industry appear to be grossly inadequate and ineffective (Eweje, 2006., Ogri, 2001., Aghalino *et al*, 2009 and Eaton, 1997).

In the Niger Delta region of Nigeria, the participation of communities in the environmental decision making process is a relatively new process and often ineffective with little or no sustainable development goal (Adomatai *et al*, 2004). Over the past fifty years, the multinational oil companies operating in the Niger Delta region have failed to adopt sustainable exploration and production practices due to increased costs of complying with environmental regulations is now in place, environmental pollution associated with oil and gas exploration and production operations has continued to persist under these laws for several reasons. Therefore, unsustainable petroleum exploration and production practices and poor environmental management practices has impacted on the atmosphere, controlled waters, soils and sediments, biological diversity and sustainability of the natural ecosystem in the Niger Delta region for several decades.

2.4 PETROLEUM SPILLS AND ACCIDENTAL DISCHARGES IN THE NIGER DELTA

Oil spillage, which often results from accidental or operational discharges of petroleum into the environment, is a global issue that has been occuring since the discovery of crude oil. The Niger Delta has experienced a number of disasters from oil blowouts, according to estimates, over 2,567,960 barrels of crude oil has been spilled in 5733 incidents in the Niger Delta from 1976-2000 and about 549,060 barrels were recovered while 1,820 barrels were lost to the environment (Edoho, 2008). Furthermore, the causes of unreported spills by some oil exploration and production companies could be related to the Nigerian National Petroleum Corporation (NNPC) inspectorate classification guidelines whereby spillage are classified into minor, medium and major disaster. In past years, major oil spills have attracted global attention and created awareness due to the associated ecological, human health and environmental risks and/or damages that result from such spillages. The main sources of oil spill and pollution in the Niger Delta are equipment failure, oil blowouts from the flow stations, leakages from aged and corroded network of the pipelines, operational mishap, sabotage and vandalization of the oil pipelines by the local militant groups (Ogri, 2001). However, oil spill resulting from the vandalization of pipelines either as a result of civil disaffection with the political process or as a

criminal activity causes serious contamination of the environment (Nwilo *et al*, 2006). In general, oil spills and production discharges in the Niger Delta have resulted in disastrous effects on land, freshwater swamps and the marine environment as well as potential threats to human health in the affected host communities (Obaje, 2009).

The immediate effects of large scale spills and oil pollution in the coastal areas are well documented and most of the terrestrial ecosystems and shore lines in the oil producing communities are often impacted (Blumer, 1972). Defoliation and mortality of the mangroves have been observed in swamps affected by oil spills (Linden *et al*, 1980), the effects of oil pollution on the diversity and functioning of fish and turtle communities in the Niger Delta have been reported (Luiselli *et al*, 2003). In the aquatic environment, the oil slick sometimes floats on the water surface where it is dispersed to shorelines by wind and wave actions and invariably affecting the soil. When oil spillage occurs onshore or near shore, the soil and other components of the terrestrial ecosystem are inevitably affected (Osuji *et al*, 2007). Contamination of the marine environment associated with oil spills and accidental discharges of petroleum, if not effectively checked, can lead to degradation of the mangrove forests, destruction of ecosystems, drastic decline in the fish and agricultural yields that are central to the livelihoods of local communities.

2.5 DRILLING DISCHARGES

Prior to the institution of statutory laws and regulations in the 1970's, the major petroleum-derived wastes such as produced water, spent drilling needs, drilling cutting and wastes that require handling during site abandonment were commonly discharged into coastal waters, swamps, and underlined evaporation ponds. (Kharaka *et al*, 2003).

2.5.1 PRODUCED WATER

Crude oil production is usually accompanied by large amounts of produced water which must be disposed off after separation from oil at the wellhead. Safe handling requires that the water be injected into reservoir or be thoroughly treated to reduce oil to acceptable levels before disposal into the environment. Most of the oily waters arising from oil production are discharged directly or indirectly into the environment after some level of mechanical treatment as API skimmers (Beg *et al*, 2001 & Helen 2005). In most cases, the water still contains oil, in offshore production where extracted oil is stored in under-water containers, the containers are usually filled with water, which is then displaced by the introduction of oil. When the soluble fraction of the crude oil of this water is extracted, effluents and ballast waters are discharged near coastal shore before the taking on of new cargo; it results in contamination of coastal and inshore waters (Kuehn *et al*, 1995). Produced water is the largest volume water

stream associated with oil and gas exploration and production processes. The chemical composition of the oil field produced water is complex, including large amounts of dissolved salts, hydrocarbons, heavy metals, organic and inorganic components, naturally occurring radioactive materials and chemicals added in the oil extraction and separation steps (Kharaka *et al*, 2010). Produced water is either discharged into above ground storage facilities or re-injected into a subsurface formation as a permanent disposal/secondary recovery process during on-shore operations whilst it is either discharged through shore side outfalls or coastal rim releases (cutting 4 miles from shore) during offshore or coastal operations (Tellez *et al*, 2002). In many cases, the past and current disposal practices such as these have caused severe environmental contamination of coastal waters, groundwater, soils and sediments, and marine ecosystems in the Niger Delta. The improper disposal of produced water on the ground is associated with salt scares and potentially contaminated land that is difficult to remediate.

The discharges of petroleum-derived waste streams from oil and gas exploration and production are toxic to the coastal waters, soils and sediments near the discharge points. According to Roach *et al* (1992), adverse impacts to mangrove vegetation are the most obvious signs of environmental effects resulting from produced water spill or discharges. The toxicity of produced

water is directly related to high salinity, total dissolved solids (TDS) (Kharaka *et al*, 2003) and other parameters such as toxic metals, soluble organics and produced water are toxic to a wide variety of aquatic organisms or estuarine organisms (D'unger *et al*, 1996 & Roach *et al*, 1992), therefore there is no justification for the continuous disposal of produced water into ecologically sensitive areas in the Niger Delta. Although oil spills and discharges of petroleum-derived waste have plagued the natural environment for the past five decades, there is an urgent environmental concerns for effective disposal and /or remedial strategies for this highly saline water.

2.6 EFFECT OF PETROLEUM-DERIVED WASTE ON THE MARINE ECOSYSTEM

The aquatic ecosystem serves as the final destination for most contaminant (Winstom *et al*, 1991) with innumerable organic and non-organic pollutants of municipal waste, industrial, agricultural and mining industrial activities (Lenartova *et al*, 1997). Crude oil pollutant is among the most important and abundant class of pollutants found in the aquatic environment, such pollutants not only affects the integrity of the ecosystems but also affects the physiological functions of animals and humans as consumers (Perez-lopez *et al*, 2002). Marine and coastal wildlife exposed to petroleum-derived waste may suffer both immediate health problems and long-term changes to their physiology and

behaviour. In small doses, pollutants can cause temporary physical harm to animals. Types of trauma can include skin irritation, altering of the immune system, reproductive or developmental damage and liver disease. When large quantities of pollutants enter a body of water, chronic effects such as cancer become more likely, and direct mortality of wildlife can be widespread (Ben-David *et al*, 2000).

2.6.1 DIRECT EFFECTS OF PETROLEUM-DERIVED WASTE ON THE MARINE ECOSYSTEM.

Petroleum-derived waste can impact the ecosystem directly through three primary pathways.

- **Ingestion:** Ingestion of petroleum derived waste can cause gastrointestinal irritation, ulcers, bleeding, diarrhea and digestive complications (Soetan *et al,* 2013). These complications may impair the ability of animals to digest and absorb food which ultimately leads to reduced health and fitness (Ugochukwu, 2008). Ingestion may occur at multiple levels of the food chain. Herbivorous (plant-eating) wildlife, such as sea turtles, may consume vegetation that has been coated with oil particles. Carnivorous (animal-eating) wildlife, such as shore birds that feed on clamps, mussels or worms buried in the intertidal area, may

consume prey organisms that have been exposed to oil sediments washed onto the shoreline. Top predators may become vulnerable to large quantities of pollutants through bioaccumulation (the increased concentration of toxins found at higher levels of the food chain (Van-der Oast *et al*, 2003).

- **Absorption:** Absorption of petroleum-derived wastes through the skin can damage the liver and kidneys, cause anaemia, suppress the immune system, induce reproductive failure, and in extreme cases kill an animal (Wilson *et al,* 1993). Exposure to petroleum-derived waste may initiate, burns or cause infections to the skin of some species, fish and sea turtle embryos may grow more slowly than normal, leading to lower hatching rates and developmental impairments (Jensenn, 1994)
- **Inhalation:** Inhalation of volatile chemicals commonly occurs among those species of wildlife that need to breathe air. Inhalation of these harmful materials can cause respiratory inflammation, irritation, emphysema, or pneumonia (Togum *et al,* 2007). Nematodes, dolphins, whales and sea turtles all come to the surface to breathe periodically and are all susceptible to this risk (Jensenn, 1994).

2.6.2 INDIRECT EFFECT OF PETROLEUM DERIVED WASTE ON THE MARINE ECOSYSTEM.

Petroleum-derived waste can also have indirect effects on wildlife by causing changes in behaviour.

- **Changes in foraging locations:** If a spill causes direct mortality to the food resources of a particular species, many individuals of this species will need to relocate their foraging activities to regions unaffected by the spill. This leads to increased competition for remaining food sources in more localized areas. This congregation can be especially problematic for rare species which may become more susceptible to predation or to future catastrophic events, while a large proportion of the population forages on a few concentrated patches. (Van-der Oast *et al*, 2003)
- **Increase in foraging time:** This may be required to meet energetic requirements. Animals may need to make longer trips to find food in unfamiliar areas, and they may need to forage on less preferred food that takes more times to acquire or that is digested less efficiently. Decrease in diet diversity due to lower food availability may lead to reduced overall health. At the same time, the energetic requirements of these animals may be heightened due to the physiological challenges brought

on by exposure to the petroleum-derived substances. (Alonzo-Alvarez *et al*, 2007)

- **Disruption of life cycles:** This may become apparent if particular life forms are more susceptible to the effects of petroleum-derived wastes than others. Eggs, larvea, and juveniles of many species are more vulnerable to harmful effects from pollutants than adults. Changes in the relative numbers of individuals from different life stages within a species may lead to shifts in habitat used patterns which cause ripple effects up and down the food chain. Furthermore, if a particular life stage of specie is decimated, the ability of the species to rebound after the spill is greatly reduced. (Alonzo-Alvarez *et al*, 2007).

2.7 THE AFRICAN CATFISH (*Clarias gariepinus*)

2.7.1 SCIENTIFIC CLASSIFICATION

Kingdom:	Animalia
Phylum:	Chordate
Class:	Actinoptenygii
Order:	Siluriformes
Family:	Claridae
Genus:	Clarias
Species:	*Clarias gariepinus*

(Burchell, 1822)

2.7.2 NATURAL DISTRUBUTION OF THE AFRICAN CATFISH

Clarias gariepinus or African sharptooth catfish is a specie of catfish of the family Clariidae, the air breathing catfishes. They are found throughout Africa and the middle East, and live in fresh water lakes, rivers and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African sharptooth catfish was introduced all over the world in the early 1980s for agriculture purposes, so is found in countries far outside its natural habitat, such as Brazil, Vietman, Indonesia and India.

2.7.3 DESCRIPTION OF THE AFRICAN CATFISH

The African Catfish (*Clarias gariepinus*) is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. In Africa this catfish has been reported as being second in size only to the vundu (the largest freshwater species in Southern Africa) of the Zambesian waters, although Fishbase suggests the African catfish surpasses that species in both maximum length and weight (Froese *et al*, 2014). *Clarias gariepinus* has an average adult length of 1-1.5m (3ft 3in-4ft 11in). It reaches a maximum length of 1.7m (5ft-7in) and can weigh up to 60kg (130lb) (Froese *et al*, 2014). They have slender bodies, flat bony heads, notably flatter than the genus *Silunus*, and broad,

terminal mouths with four pairs of barbels. They also have large accessory breathing organs composed of modified gill archies.

The African catfish is a nocturial fish like many catfish. It feed on living, as well as dead, animal matter because of its wide mouth. It is able to swallow relatively large prey whole. It has been known to take large waterbirds such as the common moorhen. It is also able to crawl on dry ground to escape drying pools, and is able to survive in shallow mud for long periods of time, between rainy seasons (Anoop *et al*, 2009)

2.7.4 ECONOMIC IMPORTANCE OF AFRICAN CATFISH.

Fish farming is the sub-set of agriculture that focuses on rearing of fish under controlled conditions for economic and social benefit (Anthonio *et al*, 2002). Food and Agriculture Organization (2000), made a statement that fisheries products represented a major source of export revenue for developing countries, amounting to over 20 billion US Dollars per annum in late 1990s. This exceeded the values obtained from the exports of meat, diary, cereals, vegetables, fruit, sugar, coffee, tobacco and oil seeds in 1997 from developing countries (International Trade Centre, 2002). However, F.A.O. (2007), estimated that Nigeria imports about 560,000 tonnes of fish estimated at about $400million anually while annual domestic fish supply in Nigeria stands at

about 400,000 tonnes. This makes Nigeria one of the largest importers of fish in the developing world.

Catfish production is important to the Nigerian economy. It serves as a source of income, reduces the rate of unemployment in the economy and increases the Gross Domestic Product (GDP). In Most countries, it fetches a higher price than tilapia as it can be sold live at the market, as they have a market value two to three times that of tilapia (Emokaro, 2010). According to Olagunju, *et al* (2007), it requires less space, time, money and has a higher feed conserving rate. Catfish provides food for the populace, it allows for improved protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein (Anoop *et al*, 2009). Many species of fish are farmed all over the world, but catfish is taking the lead because of its uniqueness. The demand for catfish in Nigeria is unprecedented so much that no matter the quantity supplied into the market, it would be consumed by ready buyers. This is because of its low calorie value, low carbohydrate content, high protein content, low in fat and is quick and easy to prepare and above all, it tastes great.

2.8 HAEMATOLOGICAL STUDIES

Heamatology refers to the study of the numbers and morphology of the cellular elements of the blood. Heamatological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to the blood (Onyeyili *et al*, 1992 and Togun *et al*, 2007). Heamatological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Ovuru *et al*, 2004) and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions. (Ekeatte *et al*, 2013), they act as a pathological reflector of the status of exposed animals to toxicant and other conditions (Olafedehan *et al*, 2010).

Heamatological components include the red blood cells (Erythrocytes), white blood cells (Leucocytes), Mean corpuscular haemogolobin, mean corpuscular haemoglobin concentration, mean corpuscular volume and haemoglobin concentration. All these play a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004 & Doyle, 2006).

2.8.1 WHITE BLOOD CELLS (WBC)

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign

organisms and to produce or at least transport and distribute antibodies in immune responses. Animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan *et al*, 2013) and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al*, 2011; Okunlola *et al*, 2012 and Iwuji *et al*, 2013)

2.8.2 RED BLOOD CELLS (RBC)

Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this heamoglobin that reacts with oxygen carried in the blood to form oxy-haemoglobin during respiration (Chineke *et al*, 2006). According to Isaac *et al* (2013) red blood cell is involved in the transport of oxygen and carbondioxide in the body. Thus a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al*, 2013 and Isaac *et al*, 2013).

2.8.3 HAEMOGLOBIN (Hb)

Heamaglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for

the other body functions as well as transport carbondioxide out of the body of animals (Ugwuene, 2011 and Omiyale *et al*, 2012)

2.8.4 MEAN CORPUSCULAR VOLUME (MCV)

Mean corpuscular volume is a measure of the average volume of a red blood corpuscle. MCV is a part of a standard completed blood count. Its measurement is attained by multiplying a volume of blood by the proportion of blood that is cellular and dividing the product by the number of erythrocytes in that volume. Mean corpuscules is decreased in accordance with average red cell size. Low MCV indicates microcytic (Small average RBC size), normal MCV indicates normocytic (normal average RBC size) and high MCV indicates macrocytic (large average RBC size).

2.8.5 MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

Mean corpuscular heamoglobin is the average mass of heamoglobin per red blood cell in a sample of blood. It is also a part of a standard complete blood count. It is calculated by dividing the total mass of heamoglobin by the number of red blood cells in a volume of blood.

2.8.6 MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION

Mean corpuscular haemaglobin concentration is a measure of the concentration of haemoglobin in a given volume of pack. It is calculated by dividing the haemoglobin by the heamatocrit. This count is used to give a rough guide to what shade of red enythrocytes will be, that is, the paler the colour, the lower the MCHC will be than the standard.

2.9 BIOCHEMICAL PARAMETERS

Biochemical changes in fish exposed to pollutants have been proposed and used as biomarkers for contaminants including petroleum products (Eseigbe *et al*, 2013). Biochemical parameters are valid for the detection and evaluation of adverse effects and relatively early events of pollutants damage to the animals.

Biochemical tests carried out include Aspartate aminotransferase (AST), Alanine Transaminase (ALT), Total Protein and Total Cholesterol.

2.9.1 ALANINE TRANSAMINASE (ALT)

Alanine Transaminase (also called Alanine Amino-transferase) is found in plasma and in various body tissues, but is most common in the liver, it spills into the blood if the liver is injured, thereby raising the enzyme levels in the blood, which is an indication of liver damage (Ray *et al*, 2014).

ALT catalyzes the transfer of an amino group from L-alanine to L-Ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. ALT is commonly measured as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health (Wang *et al*, 2012).

2.9.2 ASPARTATE AMINOTRANSFERASE (AST)

Aspartrate aminotransferase, also known as aspartate transaminase is a pyridoxal phosphate dependents transaminase enzyme. AST catalyzes the reversible transfer of an L-amino group between aspartate and glutamate and as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain and red blood cells (Lungi *et al*, 2013). AST is similar to ALT in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart and skeletal muscles. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in disease affecting other organs. (Soetan *et al*, 2013).

2.9.3 TOTAL PROTEIN

The total protein test measures the total amount of two classes of proteins found in the fluid portion of the blood. These are albumin and globulin. Proteins are important parts of all cells and tissues. Albumin helps prevent fluid from leaking out of blood vessels, while globulins are important part of the immune system (Aderemi, 2004).

2.9.4 TOTAL CHOLESTEROL

Cholesterol is required to build and maintain membranes within the cell, it is a lipid molecule and is biosynthesized by all animal cell. Total cholesterol is defined as the sum of HSL (High Density Lipoprotein), LSL (Low Density Lipoproteins) and VLDL (Very Low Density lipoproteins). Usually, only the total HDL and triglycerides are measured, while the VLDL is usually estimated as one-fifth of the triglycerides. Total cholesterol is therefore the measurement of the total amount of cholesterol in the blood (Olafedehan *et al*, 2010).

2.9.5 GLUTATHIONE-S-TRANSFERASE (GST)

Glutathione-S-transferase is an antioxidant enzyme, which plays a critical role in mitigating oxidative stress in all life forms and GST activity has been widely used as a biomarker to detect stress. As an antioxidant enzyme, a GST activity either has a significant increase or decrease with different patterns according to

the exposed elements or exposure conditions (Farombi *et al*, 2007). Glutathione –S-transferases are family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism.

2.9.6 GLUTATHIONE (GSH)

Glutathione is an important antioxidant in plants, animals, fungi and some bacteria and archaea, preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine (Poompella *et al*, 2003). In healthy cell and tissues, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). And increased GSSG to GSH ratio is considered indicative of oxidative stress. (Kabir *et al*, 2011).

2.9.7 LIPID PEROXIDATION

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals “steal” electrons from the lipids in cell membranes, resulting in cell damage. The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), the

latter being known also as "Second messenger of free radicals" and major bioactive marker of lipid peroxidation due to its numerous biological activities resembling activities of reactive oxygen species. In addition, end-products of lipid peroxidation may be mutagens and carcinogenic (Marnett, 1999)

2.9.8 HISTOPATHOLOGICAL STUDIES

Histopathology is the microscopic examination of biological tissues to observe the appearance of diseased cells and tissues in very fine detail. Following sampling, fish tissues are placed in an aqueous fixative, this fixative preserves the morphology (structure and chemical constituents) of the tissues. It is essential that tissues are fixed within a very short time after death to avoid disintegration of tissues or cells by the action of their own enzymes (Ugwene, 2011). Following fixation, tissues are gradually dehydrated using series of graded alchohols, cleared, and then embedded in molten paraffin wax and cooled to harden the wax so that the sections can be cut for staining (Isaac *et al*, 2013). Histological techniques enable the description of tissue pathology and highlight the sequence of cellular changes and their progression caused by pollutants or infectious/non-infectious disease. Therefore by examining stained sections of tissues various abnormalities can be detected and identified. (Okunlola *et al*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SPECIMEN COLLECTION/ACCLIMATIZATION

Seventy healthy 6 weeks old juveniles of *Clarias gariepinus* with an average weight of 45g were purchased from a local fish farm in Imo State (AB farms, Irette) and transported in an open gallon half filled with clean tap water. The juveniles were kept in a holding tank of 200 litres capacity containing 100litres of water to acclimatize for a period of seven (7) days before being used in the experimental study.

During acclimatization, the juveniles were fed with Coppens fish feed (2mm in size) at 3% of their body weight twice daily, between the hours of 8:00-9:00hr and 17:00-18:00hrs and the water changed daily between the hours of 6-7am when the temperature was low enough to prevent heat stress, and also to avoid accumulation of waste material which could become toxic to the fishes.

TABLE 3.1 COMPOSITION OF COPPENS FEED

COMPOSITION	PERCENTAGE
Crude Protein	45%
Ash	9.55%
Crude Fibre	12%
Crude Fat	1.5%
Phosphorus	2%

3.2 EXPERIMENTAL DESIGN

After the period of acclimatization, the juvenile were separated into 7 groups of 10 juveniles per group and kept in containers of 20 litre capacity with 10 litres of clean tap water of pH 7.3 and varying levels of treated and untreated petroleum exploration produced water. The control had no produced water (0.0ml), while the other treatments had varying levels of 2.5ml, 5.0ml and 10.0ml of treated and untreated produced water per litre of water.

The juveniles were fed with measured quantities of coppens fish feed and their weight measured once in a week for the 3weeks period of study using an electronic scale of model KERN 572. At the end of the test period, juveniles (3

fishes per treatment) were taken from all the groups to the laboratory for haematological, biochemical, antioxidant and histopathological studies.

3.2.1 COLLECTION OF PETROLEUM PRODUCED WATER

The petroleum produced water used in the study was obtained from total E &P Nigeria Ltd, Ogbogu Flow Station, Egi Land in Ahoada, Rivers State. The produced water was collected in clean containers, transported properly and refrigerated prior to usage at 4^0C.

3.3 PHYSICOCHEMICAL ANALYSIS OF PRODUCED WATER

The physicochemical analysis of the produced water was carried out using standard American Public Health Association (APHA) methods.

3.3.1 ODOUR/COLOUR/TURBIDITY

The odour, colour and turbidity of the produced water were determined through physical observation.

3.3.2 DETERMINATION OF pH

Measurements were carried out by means of a Win Lab pH meter (Win Lab 192363, Germany), which was calibrated in the laboratory. Calibration was checked by measuring standard buffer solutions (4, 7 and 10).

3.3.2 DETERMINATION OF SALINITY

Salinity was determined using the motive method as described in APHA 4500B. This titration method was based on the reaction of silver with chloride ions using potassium chromate as indicator. Silver chloride was precipitated quantitatively before red silver chromate was formed. Salinity was reported in mg/l after calculations.

3.3.4 DETERMNATION OF CHEMICAL OXYGEN DEMAND

Chemical Oxygen Demand was determined using the open reflux method (APHA 1992) where a sample was refluxed and digested in a strongly acidic solution with a known amount of excess of potassium dichromate ($K_2C_2O_7$). After digestion the excess un-reacted potassium dichromate was determined with a spectrophotometer (Lamotte Smart 3, U SA) at 6000mm and results were reported in Mg/l. Results were further verified by titrating with a standard solution of ferrous ammonium sulphate (FAS).

3.3.5 DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND & DISSOLVED OXYGEN

Biological Oxygen Demand (BOD_5), which depends on oxygen uptake by bacteria, was determined using the dilution method according to APHA 5210B (APHA, 1992). Dissolved oxygen of the samples was first determined using the

Win Lab dissolved Oxygen meter (Win Lab 196363m Germany) and then incubated for 5 days at 20^0C. DO was again measured after a period of 5 days and BOD_5 in mg/l was determined, calculated and reported accordingly.

3.3.6 DETERMINATION OF HEAVY METALS

The concentrations in mg/l of heavy metals (Fe, Zn, Cu, Ni, Cd) in the collected samples were determined (after nitric acid digestion) by means of an atomic absorption spectrophotometer (Biotech Engineering, Phoenix AA-986 UK) About 100ml of the water sample was measured and 5ml of the nitric acid was added (Nitric acid digestion) into a beaker. The sample was placed on a hot plate and covered in fume hood until white fumes evolved.

The digested sample was allowed to cool and filtered in to a 100ml volume flask and made up to mark with de-ionized water. The sample was then transferred to 100ml plastic Container for AAS analysis.

Specific metal standards (Accu Standards, USA) in the linear range of the metal were used to calibrate the equipment. The concentrated and digested samples were then aspirated and the actual concentrations were obtained by referring to the calibration graph and necessary calculations and the result was reported in mg/l.

3.4 HAEMATOLOGICAL STUDIES

Blood samples were collected via the caudial vein puncture as described by Kori-Srakpere *et al*, (2005). Blood samples were collected with sterile 5ml syringe and 21G needle. The needle was introduced on the ventral midline, between the anal opening and the beginning of the anal fin to assess the caudal vein beneath the vertebral column. The collected blood was then dispensed into a plastic tube containing EDTA (Ethyl Diamine Tetraacetic Acid) as anticoagulant for haematological analysis.

The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time (Smith *et al.*, 1952).

3.4.1 DETERMINATION OF HAEMOGLOBIN (Hb)

Hb was determined using the cyanmethaemoglobin method by Darcie *et al*, (1984). Blood is mixed with 1ml of Drabkin's solution (a solution containing ferricyanide and cyanide) and left to stand for 10mins. The fericyanide oxidizes the iron in the haemoglobin thereby changing haemoglobin to methaemoglobin. Methaemoglobin then unites with the cyanide to form cyanmethaemoglobin, this then produces a colour which is measured in a colorimeter. The colour relates to the concentration of haemoglobin in the blood. The absorbency of the

mixture was then read at 540nm using the photoelectric colorimeter where the amount of Hb was then calculated.

3.4.2 RED BLOOD CELL COUNT

Blood sample was collected with an erythrocyte pipette and diluted (1/20) with the Hayem solution. One drop of haemolized blood was then transferred into a counting chamber and examined under a light microscope with a magnification of 40x using the Neubauer counting chamber (haemocytometer).

3.4.3 WHITE BLOOD CELL COUNTS

Blood sample was collected with a leucocyte pipette and diluted with the WBC diluting fluid (Turk's solution) one drop of hemolized blood was then transferred on to the counting chamber, and examined under a light microscope with a 40x magnification.

3.4.4 RBC INDICES (MEAN CORPUSCULAR HEAMOGLOBIN (MCH) MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) AND MEAN CORPUSCULAR VOLUME (MCV)

The red blood cell indices of MCHC, MCH AND MCV were calculated using the equations given by Anderson et al, (1965)

$$\text{MCV (fl)} = \frac{\text{Hematocrit}}{\text{RBC}} \times 10$$

$$\text{MCH (Pg)} = \frac{\text{Haemoglobin}}{\text{RBC}} \times 10$$

$$\text{MCHC (\%)} = \frac{\text{Haemoglobin}}{\text{RBC}} \times 100$$

3.5 BIOCHEMICAL ANALYSIS

The second portion of the collected blood was dispensed into a tube containing no anti-coagulant. The sample was centrifuged at 1,006rpm for 5minutes to obtain the serum.

3.5.1 DETERMINATION OF TOTAL PROTEIN

Total protein was measured using the standard buiret method which is based on the reaction between the peptide bonds of protein and Cu^{2+} (forms copper sulphate solution) that produces a blue-violet coloured complex in alkaline solution.

Measured 5.0ml of Buiret reagent was pipetted into labelled tubes, 0.1ml each of distilled water, standard, sample and control were pipetted into their

respective tubes, mixed and incubated for 30minutes at 25^0C. The absorbances were measured against the blank at wavelength of 546nm. To prepare the blank, 2ml of 0.85% sodium chloride solution was mixed with 8ml of buiret reagent. The concentration of total protein was calculated by dividing the absorbance of sample against absorbance of standard, multiplied by concentration of standard (Kapale et al., 2008).

3.5.2 DETERMINATION OF TOTAL CHOLESTEROL

Total cholesterol estimation was carried out using the method described by Zlatkis *et al*, (1953)

About 0.lml of serum was pipette into a cornical flask and 10ml of ferric chloride reagent was added, mixed well and kept for 10mins at room temperature. It was then centrifuged for 10mins at 3000pm. 6ml of the supernatant was pipetted out into a test tube and 3ml of concentrated sulphuric acid was added and mixed well.

To prepare the standard, 10ml of working standard was mixed with 0.1ml of sodium chloride and kept for 10mins and centrifuged. Measured 3ml of concentrated sulphuric acid was added. The two tubes were kept for 30mins at room temperature.

To prepare the blank, 6ml of ferric chloride was mixed with 3ml of concentrated sulphuric acid. This was also kept for 30minutes at room temperature. Test and standard were read against the blank at 560mm.

3.5.3 ESTIMATION OF ALBUMIN

The bromocresol green (BCG) method by Doumas *et al*, (1971) was used for albumin estimation.

Into labeled tubes, 3ml of bromocresol green reagent was pipetted and 0.1ml each of distilled water, standard sample and control was pipetted into their respective tubes mixed and incubated at 25^0C for 5minutes. The absorbances were measured at 578nm against the reagent blank. The concentration of albumin was determined by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard.

3.5.4 DETERMINATION OF ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE TRANSAMINASE (ALT)

Determination of AST and ALT activity is based on monitoring the concentrations of pyruvate hydrazone formed with 2,4 dinitrophenyl hydrazine.

About 0.5ml of buffer solution was dispensed in to test tubes labelled blank, sample and control for AST and ALT respectively. 0.1ml of sample and control was then added into their respective test tubes. All the tubes were incubated at

37^0C for 30 minutes. Again, 0.5ml of 2, 4 dinitrophenyl hydrazine was dispensed into all test tubes. The contents of each test tube was mixed and allowed to stand for 20minutes at 25^0C and 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550mm against the respective blank prepared. (Reitman *et al*, 1957).

3.6 DETERMINATION OF ANTIOXIDANT ACTIVITIES

The antioxidant activities determined in the study included Glutathione, Glutathione S-transferase and Lipid peroxidation.

3.6.1 GLUTATHIONE S-TRANSFERASE (GST) ACTIVITY

Glutathione S-transferase activity was determined by the method of Habig *et al*, (1974). The Glutathione S-transferase activity assay was based on the fact that all Glutathione S-transferase demonstrate a relatively high activity with 1-chlor-2, 4- dinotrobenzene as the second substrate. The subsequent conjugation of the substance with reduced glutathione results in a shift of its absorption miximum to a longer wave length. The absorption increase at the new wavelength of 340nm provides a direct measurement of enzymatic reaction.

The medium was allowed to run for 60 seconds each time before the absorbance was read against the blank at 340nm. The temperature was maintained at

approximately 31^0C. The absorbance was measured using the spectrophotometer. The molar extinction coefficient of CDNB=9.6mm/cm.

3.6.2 GLUTATHIONE (GSH) CONCENTRATION

The reduced glutathione (GSH) content of the tissue was estimated according to the method described by Sedlak *et al*, (1968).

To the tissue homogenate, 10% TCA was added and centrifuged. Then 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent and 3.0ml of phosphate buffer (0.2m, pH 8.0). The absorbance was read at 412nm.

The molar extinction coefficient of DTNB = 1.34 X 10^4mm/cm.

3.6.3 LIPID PEROXIDATION DETERMINATION

Malondialdehyde (MDA) is an index of lipid peroxidation and was determined using the method of Buege *et al*, 1978.

1.0ml of the supernatant was added to 2ml of TCA-TBA-Hcl reagent in a 1:1:1 ratio and boiled at 100^0C for 15minutes and allowed to cool, flocculent materials were removed by centrifuging at 3000 rpm for 10min. The supernatant was removed and the absorbance read at 532nm against a blank.

MDA was calculated using the molar extinction coefficient for MDA TBA-Complex of 1.56 x 10^5mm/cm.

3.7 HISTOPATHOLOGICAL EXAMINATION

The histopathological examination was done using the standard tissue examination techniques (Avwioro, 2002; Ochei *et al.*, 2004; Mohammed, 2009)

Fixation of the specimens (liver and gills) was done in 10% neutral-buffered formalin for 72hours. After fixing, the tissues were dehydrated by treating with ascending grades of alcholol solutions (70% to absolute). The tissues were then cleared in xylene, impregnated and embedded in molten paraffin wax using embedding moulds which confer rigidity to the pieces of tissue for easy cutting of sections.

Sectioning

Sections were cut with the use of a rotary microtone to section thickness of 4um from the paraffin wax blocks. The cut sections were placed onto 20% alcohol on a large slide, from where they were gently floated on water bath preheated to about 45^0C, after which they were picked from the water and mounted on clean grease-free microscopic slides. Slides with sections were dried for about 30 minutes before staining.

Haematoxylin and Eosin (H and E) staining for General Tissue structure

Haematoxylin and eosin staining were done as described by Avwioro (2002). The cut-out sections were dewaxed in xylene and hydrated through descending grade of alcohol (absolute 80%-70%). The slides were then stained in Harris haematoxylin for 10minutes and then rinsed in water. The slides were dipped in 1% HCl in 70% alcohol for 1minute for differentiation and then rinsed in water. Blueing was done for 10minutes in tap water after which counter staining was done with 1% eosin for a minute. The slides were then rinsed in water, dehydrated, cleared and mounted for examination on the microscope followed by photomicrography.

3.8 STATISTICAL ANALYSIS

Student T-test was carried out on the physicochemical parameters of the treated and untreated produce water at 90 and 95%confidence level while Analysis of variance (ANOVA) was carried out on the haematological and biochemical parameters of the Juvenile African Catfish.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. RESULTS

The present study investigated the effect of treated and untreated produce water on the haematological, biochemical and anti-oxidant parameters of the blood of juvenile *Clarias gariepinus.* Histopathological examination of the gills and liver was also conducted, as well as the physicochemical analysis of the treated and untreated produce water.

The Weight (g) of Individual group taken weekly

The weight of the juveniles was recorded at the end of every week for the 3weeks period of study, and is presented in Fig. 4.1. From the chart, it is observed that the weight of the juveniles increased slightly in all the groups, the control group had increased weight compared to the exposed groups.

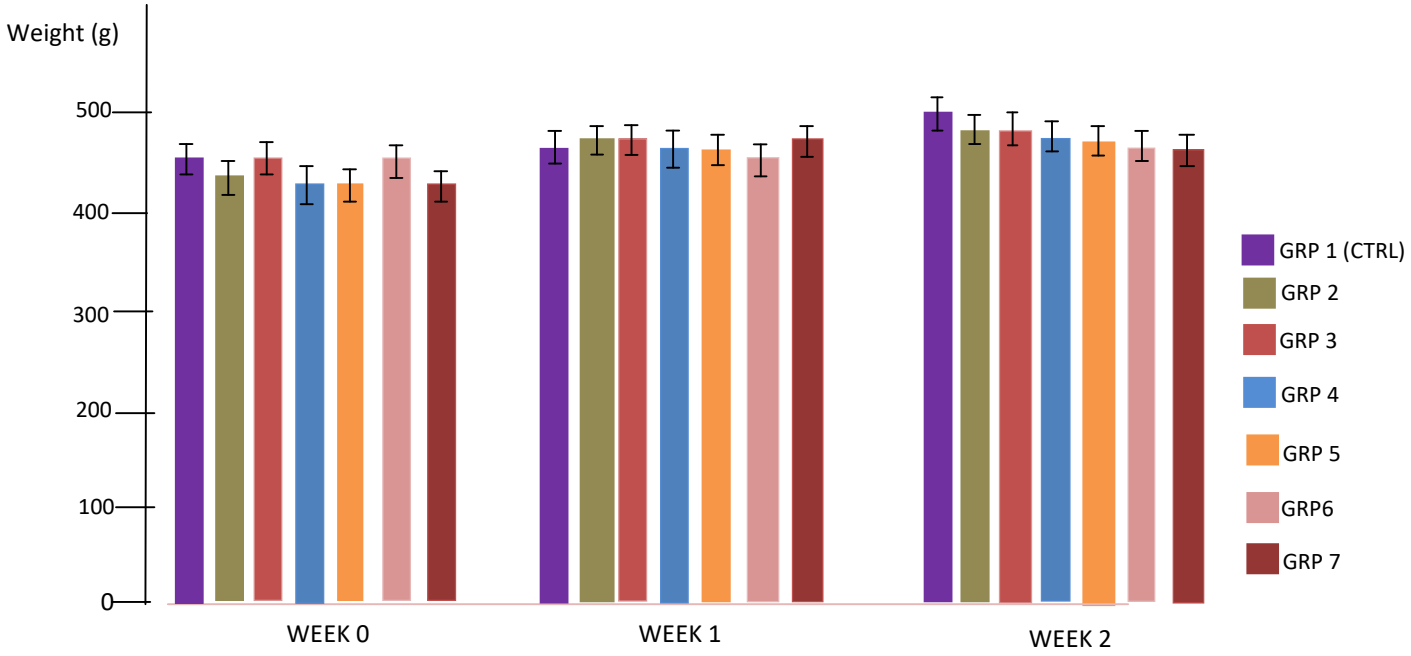

Fig 4.1. The weight (g) of individual group taken weekly.

The effect of treated and untreated produce water on the live performance of *Clarias gariepinus* juveniles is shown in Table 4.1. From the Table it is observed that the feed intake of the groups reduced slightly with increase in the concentration of the produced water, although the difference in feed intake and weight increase between the treated and untreated samples were not significant ($p>0.05$). The study recorded no mortality of juveniles throughout the test period.

Table 4.1 Effect of treated and untreated produced water on the live performance of *Clarias gariepinus.*

PARAMETER	CONTROL	TREATED			UNTREATED		
	0.0ml/l	2.5ml/l	5.0ml/l	10.0ml/l	2.5ml/l	5.0ml/l	10.0ml/l
Feed Intake(g)	13.5	13.0	13.3	13.1	13.7	13.5	13.0
Initial weight(g)	450.00	450.31	450.12	451.01	450.01	451.10	450.12
Final weight(g)	451.92	452.00	451.80	453.01	452.87	453.01	451.67
Change in weight (g)	9.89	9.20	7.99	6.36	6.02	6.32	6.02
Mortality (%)	0	0	0	0	0	0	0

PHYSICOCHEMICAL AND HEAVY METALS ANALYSIS

Physicochemical and heavy metals analysis of the treated and untreated produce water are presented in Table 4.2. From the results, it is observed that the physicochemical analysis of the treated produced water is within the DPR and FMENV permissible limit, while some of the parameters of the untreated produce water such as Total Dissolved Solid, Salinity, Biological Oxygen Demand, Zinc and Copper tend to be above the permissible limit. However, statistical analysis of the results studied were not significantly different at 90 and 95% confidence level, excluding the Salinity and Conductivity parameter which is significant at both 90 and 95% confidence level.

Table 4.2 Physicochemical and heavy metals analysis of treated and untreated produce water used in the study.

PARAMETERS	UNTREATED EFFLUENT	TREATED EFFLUENT	DPR LIMIT	FMENV LIMIT
Colour	Brownish	Colourless	-	-
Odour	Offensive	Less offensive	-	-
Turbidity(mg/l)	Not Clear	Clear	-	-
pH	5.56	6.98	6.5-8.5	6.5-9.0
Conductivity(µS/cm)	7510	299.8	NA	NA
Total Dissolved Solid(mg/L)	3980	158.89	2000	2000
Salinity(mg/L)	814.30	32.56	600	600
Dissolved Oxygen(mg/L)	3.15	5.14	NA	NA
BOD(mg/L)	19.58	6.10	10	10
COD(mg/L)	29.80	7.25	10	40
Iron(mg/L)	7.55	0.967	1.0	20
Zinc(mg/L)	2.36	0.722	1.0	<1
Copper(mg/L)	2.84	0.561	1.5	>1
Nickel(mg/L)	1.59	0.156	NA	<1
Cadmium(mg/L)	3.98	0.11	NA	<1

Key: NA=Not Available, DPR=Department of Petroleum Resources, FMENV=Federal Ministry of Environment

HAEMATOLOGICAL INDICES

The results obtained from the haematological examination of the blood samples collected from the juveniles exposed to treated and untreated produce water and control are presented in Table 4.3. From the results of haematological studies presented, it is observed that the haematological indices of both the treated and untreated groups were unchanged compared to control.

Table 4.3. Mean result for the effect of treated and untreated produce water on the haematological parameters of *Clarias gariepinus.*

PARAMETERS	CONTROL	TREATED EFFLUENT			UNTREATED EFFLUENT		
	0.0mg/l	2.5mg/l	5.0mg/l	10.0mg/l	2.5mg/l	5.0mg/l	10.0mg/l
Hb(g)	9.24± 0.29	9.24± 0.29	9.24± 0.32	9.24± 0.27	9.25± 0.28	9.24± 0.22	9.22± 0.27
RBC($x10^6$)	4.35± 1.24	4.36± 1.07	4.35± 1.30	4.35± 1.24	4.36± 1.30	4.35± 1.09	4.33± 1.21
WBC($x10^3$)	9.66± 1.32	9.66± 1.37	9.66± 1.29	9.63± 1.29	9.67± 1.37	9.67± 1.30	9.70± 1.26
MCV(μm^3)	38.22± 2.05	38.22± 2.00	38.23± 2.05	38.22± 2.03	38.22± 2.10	38.21± 2.17	38.02± 2.11
MCH(pg)	21.20± 2.09	21.21± 2.11	21.21± 2.13	21.22± 2.07	21.21± 2.00	21.24± 2.17	21.26± 2.17
MCHC (%)	5.15± 0.72	5.15± 1.31	5.15± 0.82	5.14± 0.41	5.15± 0.82	5.14± 0.73	5.14± 0.72

Key: Hb = Haemoglobin, RBC = Red blood cell, WBC = White blood cell,MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

BIOCHEMICAL INDICES

The results obtained from the biochemical analysis of the blood samples of the juveniles exposed to both treated and untreated produce water and control is presented in Table 4.4. From the results, it is observed that there's an increase in AST and ALT activities, from 155.93U/L to 181.19U/L and 37.07 U/L to 44.00U/L respectively, with increasing concentration of the effluent compared to control. The albumin and total protein levels decreased in juveniles exposed

to untreated produce water with increasing concentration of the produce water, from 7.60mg/dl to 6.80mg/dl and 20.24g/l to 17.99g/l respectively, while the juveniles exposed to the treated produce water was unaffected compared to the control.

Table 4.4. Mean result for the effect of treated and untreated produce water on the hepatic parameters of *Clarias gariepinus.*

PARAMETERS	CONTROL	TREATED EFFLUENT			UNTREATED EFFLUENT		
	0.0ml/l	2.5ml/l	5.0ml/l	10.0ml/l	2.5ml/l	5.0ml/l	10.0ml/l
AST(U/L)	155.93± 14.03	152.12± 14.00	154.00± 14.07	155.00± 14.17	170.39± 14.21	173.03± 14.00	181.19± 14.32
ALT(U/L)	37.07± 2.00	37.10± 2.31	37.32± 2.01	37.21± 2.31	39.00± 2.00	40.10± 2.21	44.00± 2.37
Alb(Mg/dl)	7.60± 0.33	7.60± 0.41	7.60± 0.52	7.57± 0.41	7.38± 0.32	7.19± 0.56	6.80± 0.32
Total Cholesterol(mg/dl)	165.17± 12.01	165.17± 12.31	165.17± 11.92	165.16± 12.40	165.20± 12.03	166.71± 12.32	167.00± 12.00
Total Protein(g/l)	20.24± 1.32	20.22± 1.31	20.23± 1.30	20.23± 1.21	20.00± 1.22	19.55± 1.30	17.99± 1.32

Key: AST = Aspartate aminotransferase, ALT = Alanine Transaminase, Alb = Albumin

ANTI-OXIDANTS

In the present study, anti-oxidant activity such as Gluthathione-S-transferase (GST); antioxidant concentration such as Glutathione (GSH) and Lipid peroxidation concentration (Malondialdehyde) were analyzed in the liver of the juveniles exposed to treated and untreated produce water and control. The

biomarkers of oxidative stress (GST, GSH and MDA) analyzed are presented in Figs 4.2, 4.3 and 4.4 respectively. Form the chart, it is observed that there is a reduction in GST and GSH activities with subsequent increase in MDA in all the groups compared to the control.

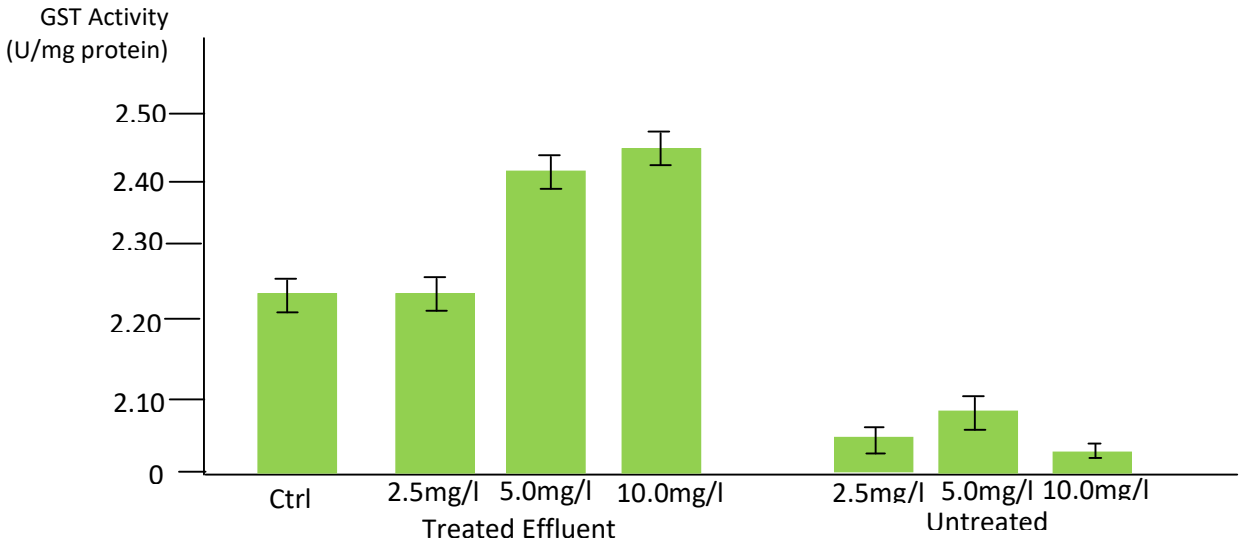

Fig 4.2 GST activity (U/mg protein) in *Clarias gariepinus* exposed to treated and untreated produce water.

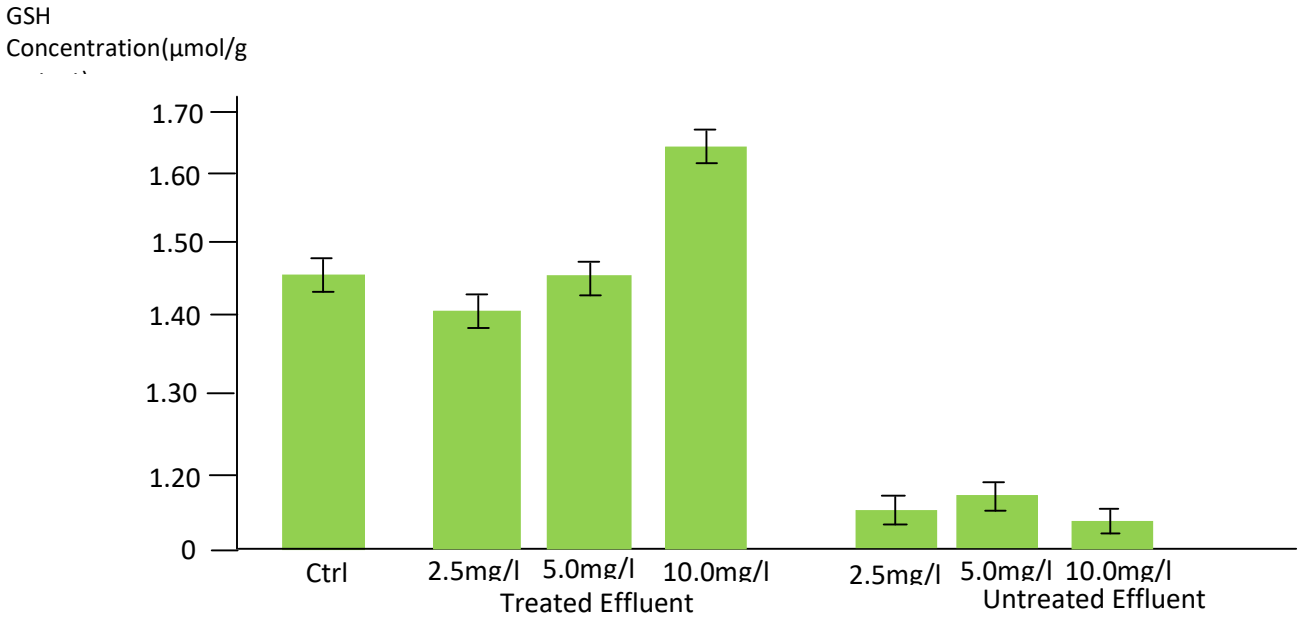

Fig 4.3 GSH Concentration (µmol/L/g wet weight) in *Clarias gariepinus* exposed to treated and untreated produce water.

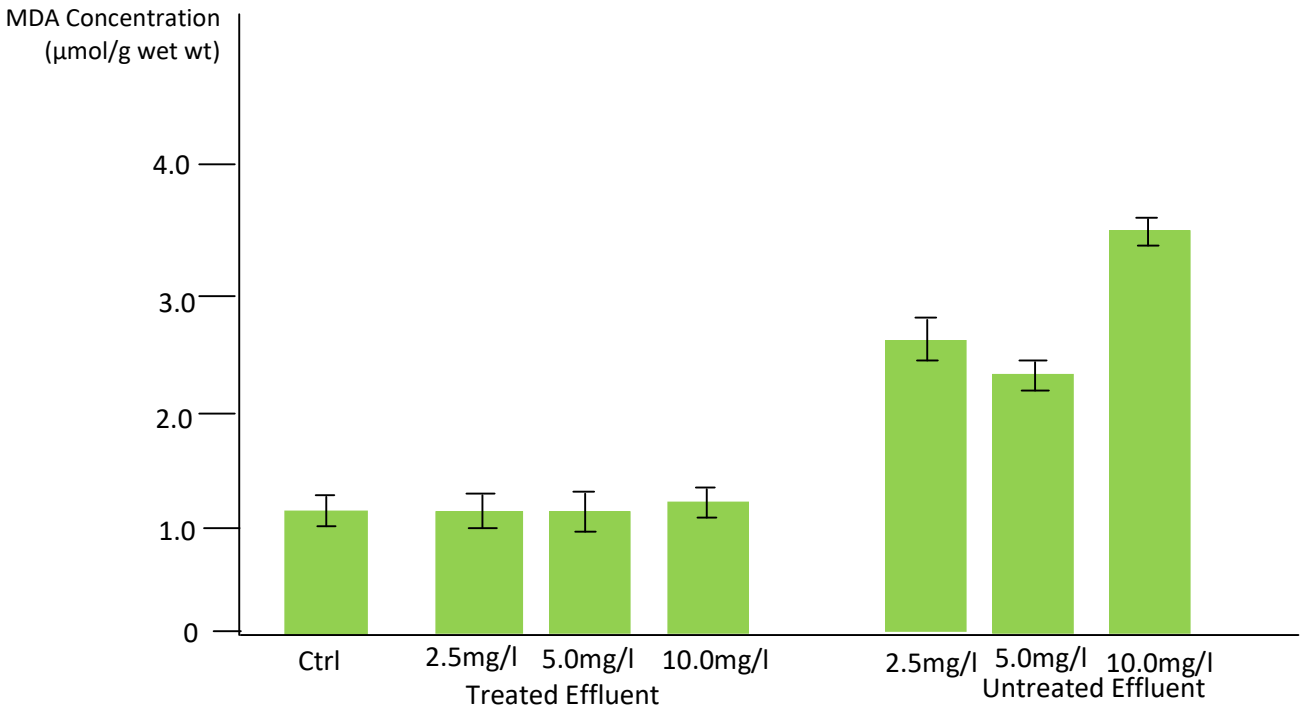

Fig 4.4 MDA Concentration (µmol/L/g wet weight) in *Clarias gariepinus* exposed to treated and untreated produce water.

HISTOPATHOLOGICAL EXAMINATION

Histopathological sections of the gills and the liver of juveniles exposed to the different concentrations of treated and untreated produce water and control are shown in Plates 4.1a-4.2c and Plates 4.3a-4.4c respectively. Section through the liver of the control fish (Plate 4.1a) showed normal central vein, normal cellular pattern, no pigments, lesion, inflammation or malignancy were seen. Section through the liver of juveniles exposed to treated produce water also showed

normal cellular pattern, no pigmentations or lesions were seen, the central vein appeared normal as well (Plate 4.1b, c, d). Section through the liver of the juveniles exposed to untreated produced water shows pigments, enlarged hepatic area (Plate 4.2a and b), inflammation and increased vacuolation of the hepatocyte is also observed especially at higher concentration of the untreated produce water (Plate 4.3c).

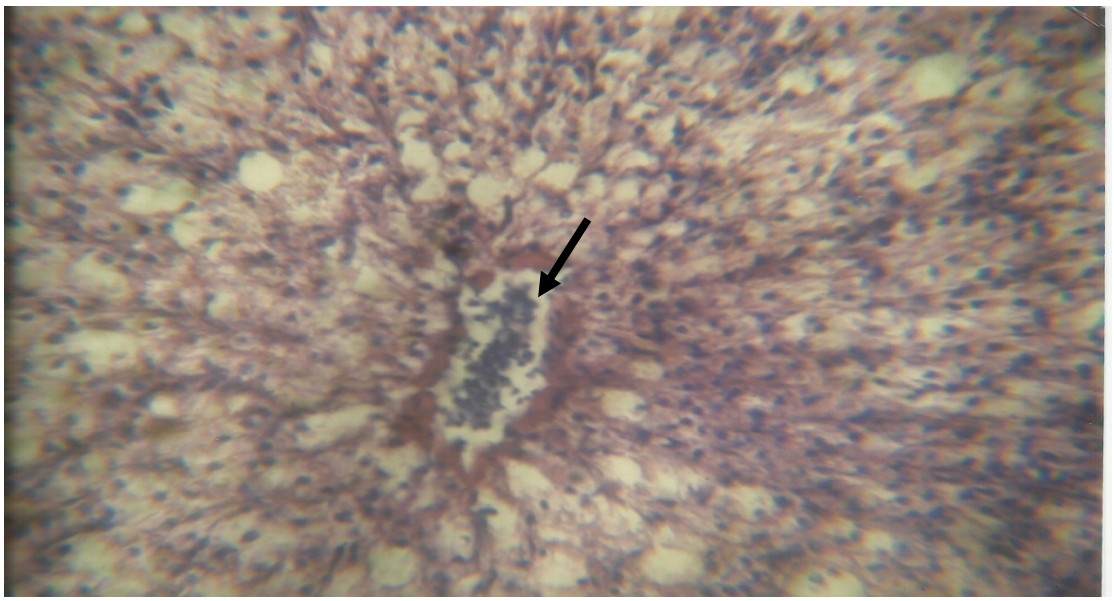

Plate 4.1a: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* (control group) stained with H & E. x400mag. Note the Central vein (black arrow)

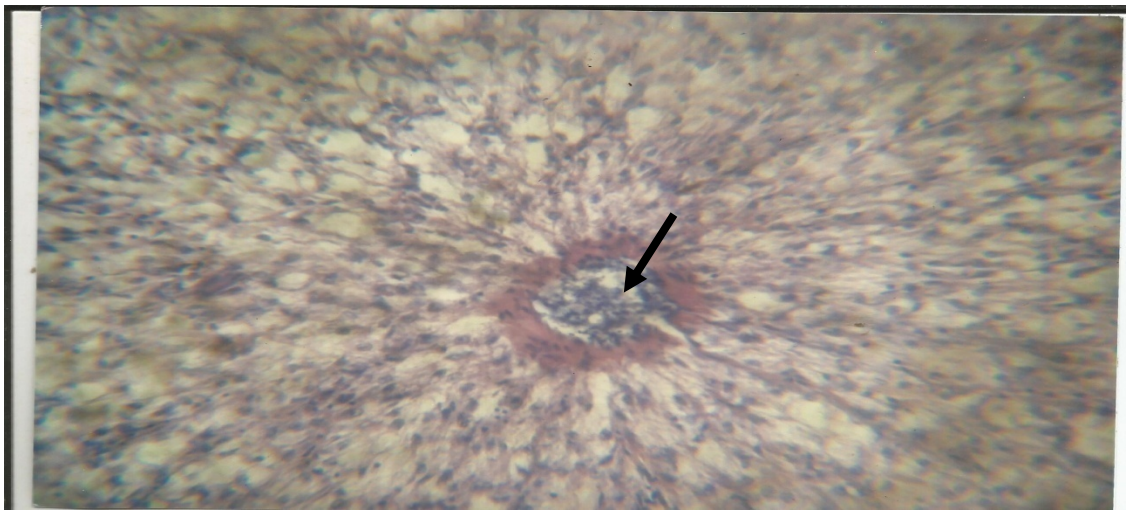

Plate 4.1b: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 2.5ml/l of treated produce water, stained with H & E. x400mag. Note the normal central vein, although slightly congested (black arrow), cellular pattern appears normal.

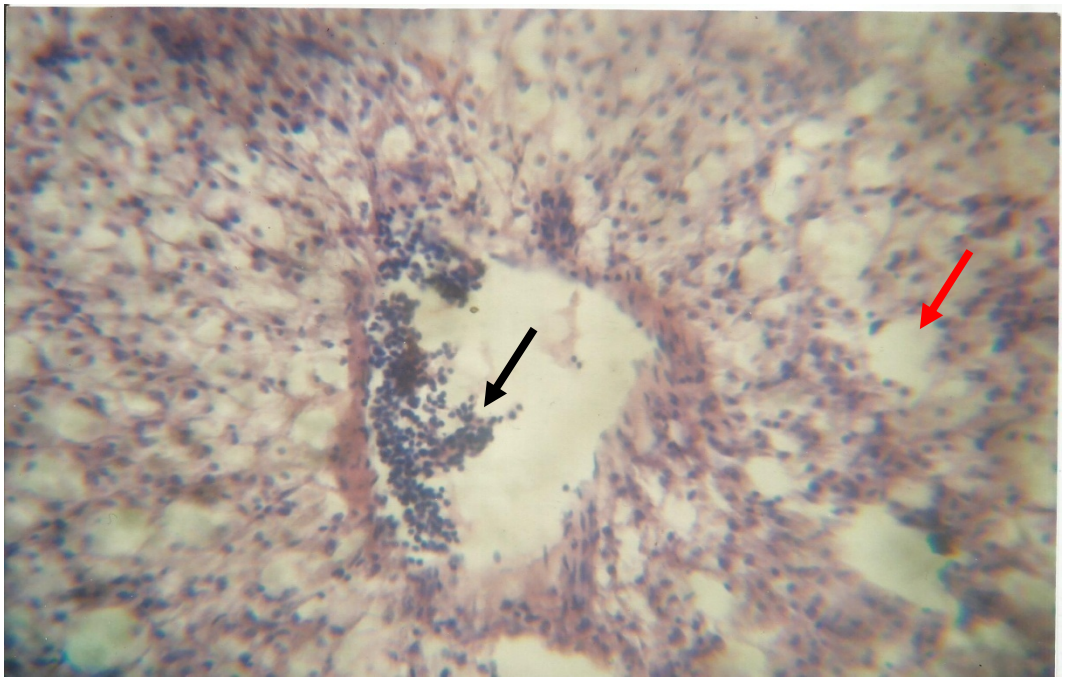

Plate 4.1c: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 5.0ml/l of treated produce water, stained with H & E. x400mag. Note irregular central vein, slight vacoulation of the hepatocyte (red arrow).

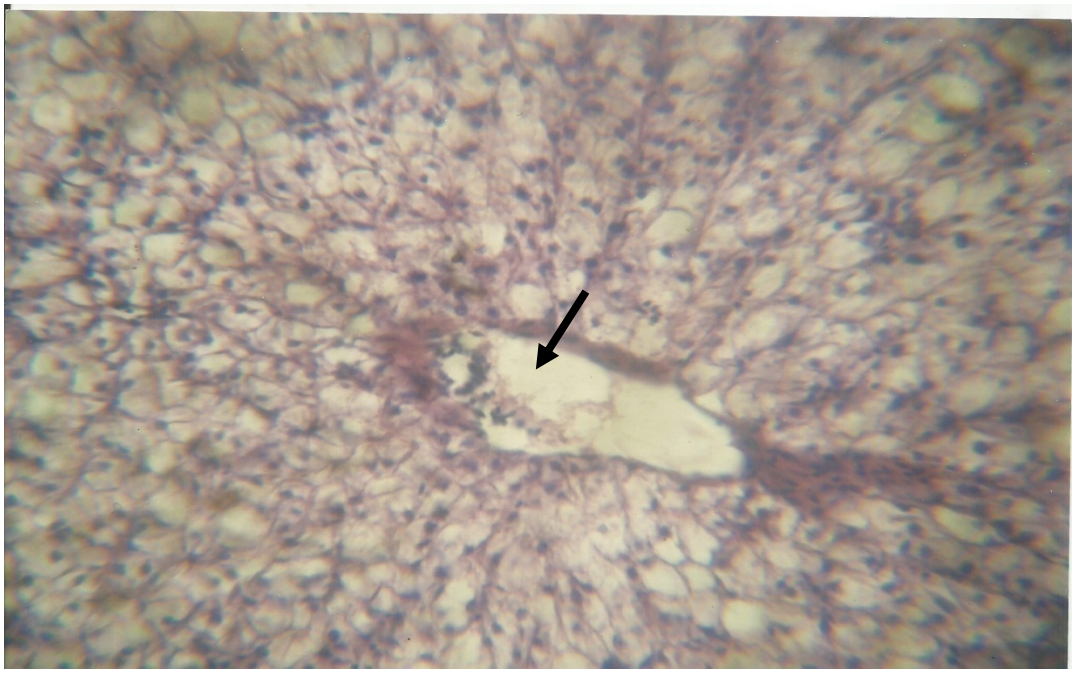

Plate 4.1d: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 10.0ml/l of treated produce water, stained with H & E. x400mag. Note the disappearance of the central vein

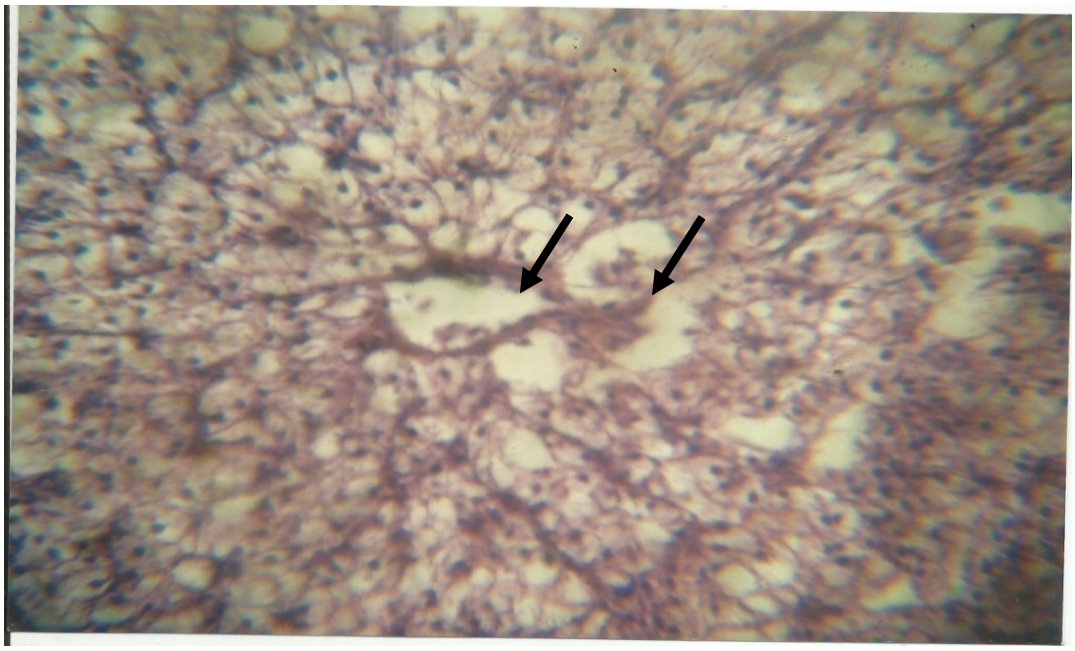

Plate 4.2a: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 2.5ml/l of untreated produce water, stained with H & E. x400mag. Note irregularity of the central vein.

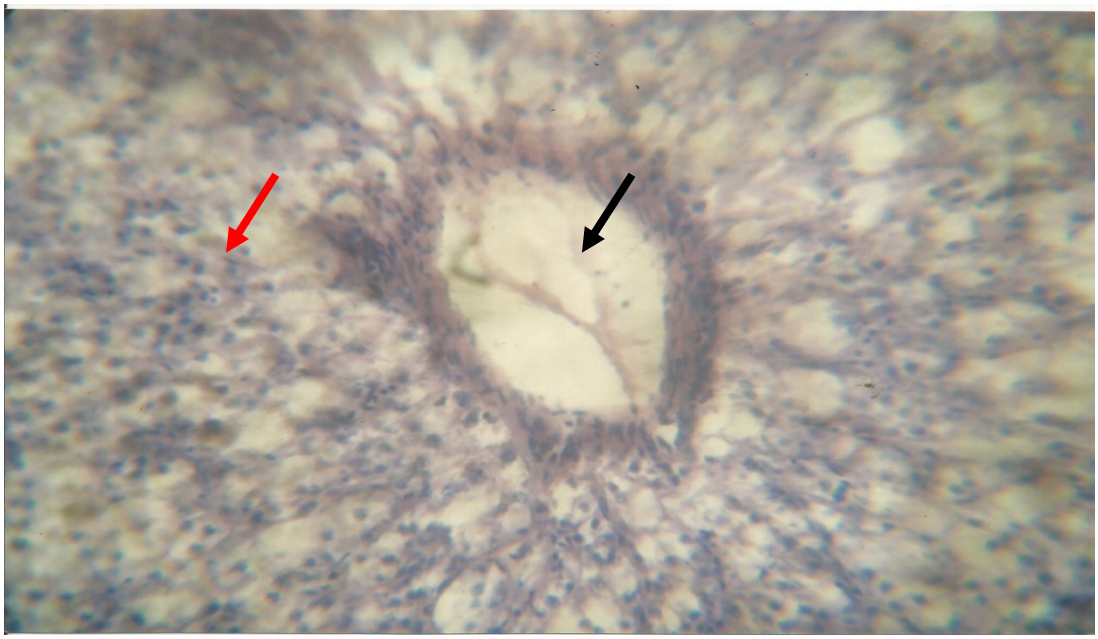

Plate 4.2b: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 5.0ml/l of untreated produce water, stained with H & E. x400mag. Note irregular central vein(black arrow) and necrosis (red arrow).

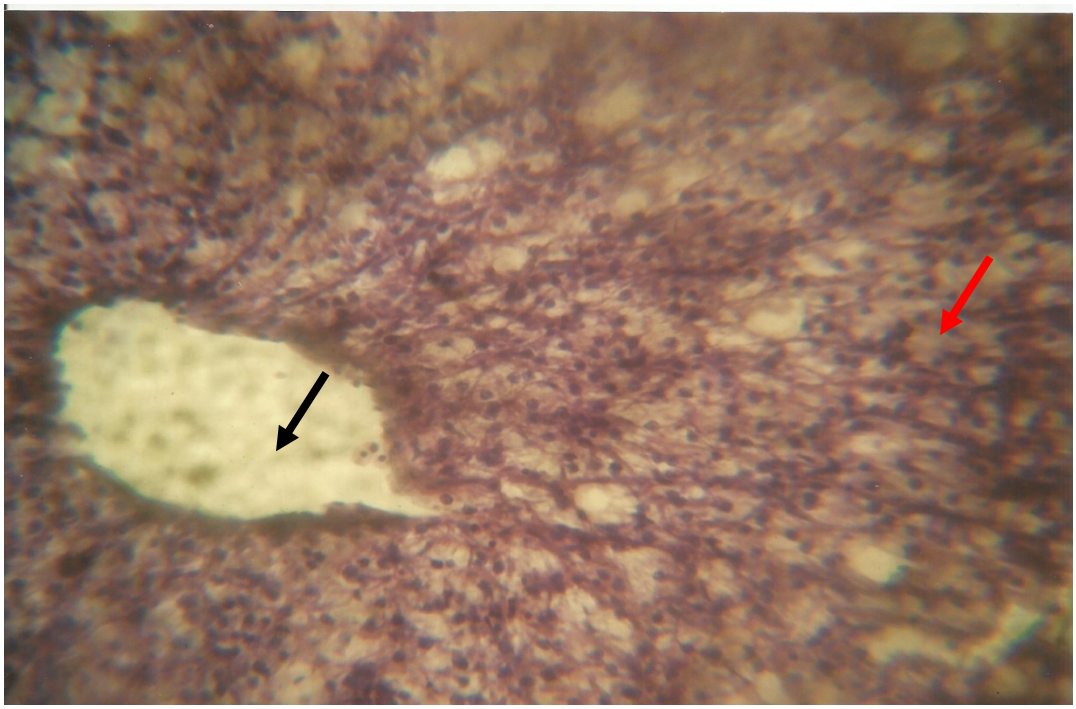

Plate 4.2c: Photomicrograph of section of the liver of juvenile *Clarias gariepinus* exposed to 10.0ml/l of untreated produce water, stained with H & E. x400mag. Note the disappearance of the central vein (black arrow), hepatocyte necrosis can also be seen (red arrow).

Section through the gills of the control juveniles showed no recognizable changes in the gill structure. Each gill consists of primary filament and secondary lamellae and rakers, normal cellular patterns was observed, the chondrocytes appeared numerous and tiny with prominent nuclei, no lesion, pigmentation or vacuolization of the gill lamella were observed (Plate 4.3a).

Section through the gills of the juveniles exposed to the treated produce water as presented in Plate 4.3b, c and d showed no lesions, pigmentation or vacuolation of the lamellae and epithelia cells. Section through the gills of the juveniles exposed to untreated produce water showed moderate area of lesion

(Plate 4.4a), irregular cellular pattern, as well as slight separation of epithelium from gill lamellae (Plate 4b and c)

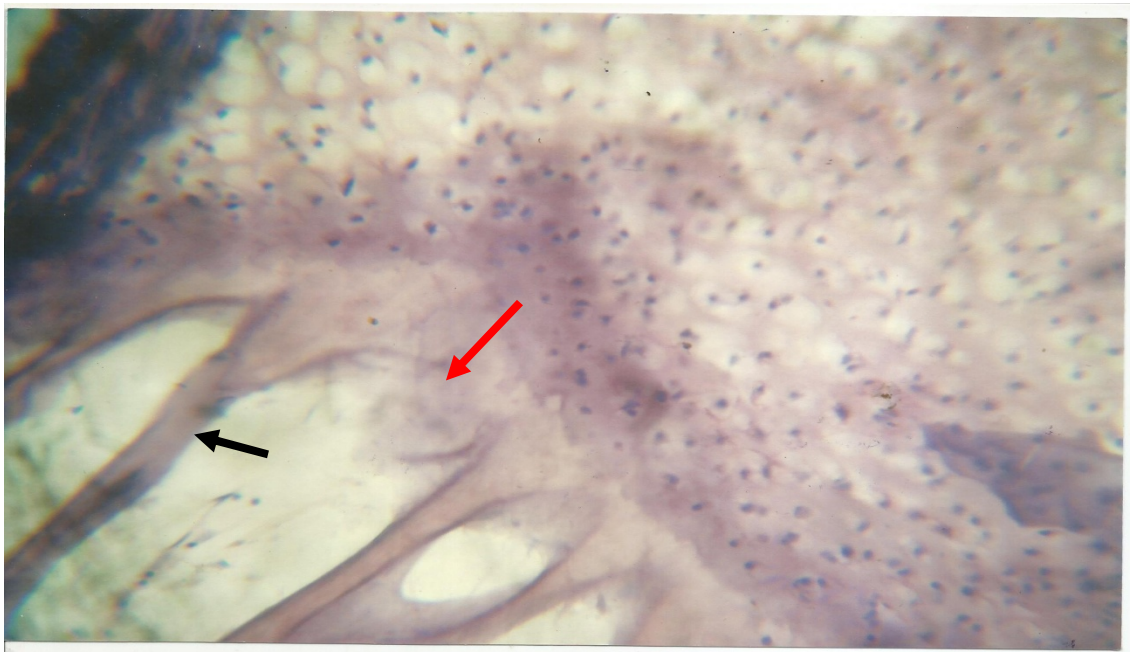

Plate 4.3a: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* (control group) stained with H & E. x400mag. Note the Secondary lamellae (black arrow) and Primary lamellae (red arrow)

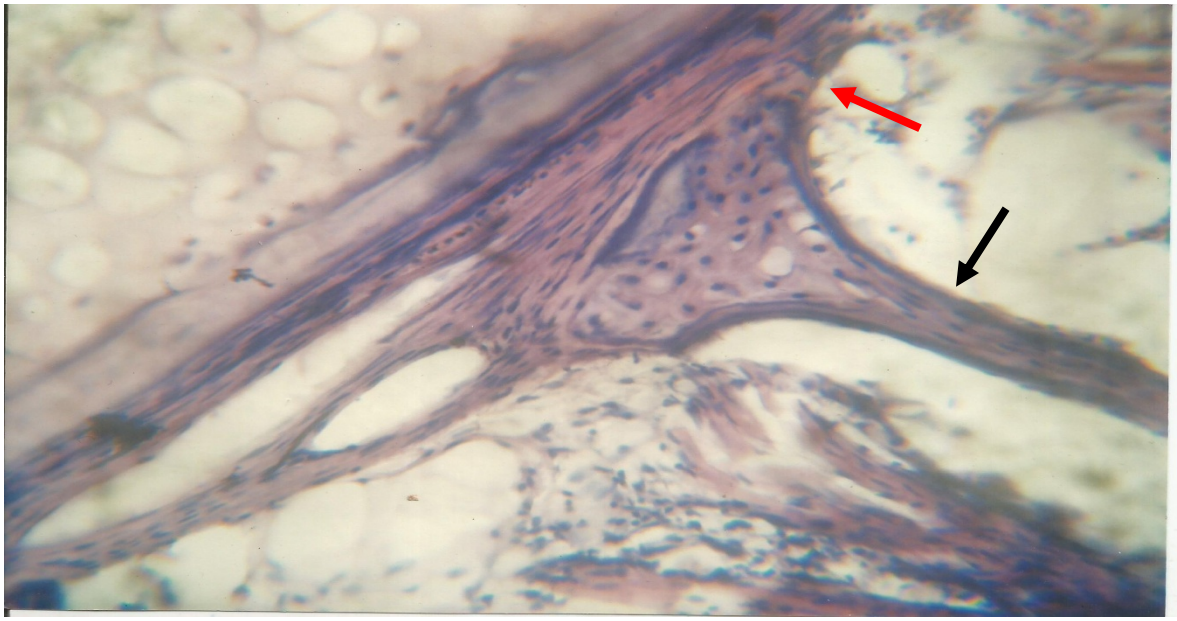

Plate 4.3b: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 2.5ml/l of treated produce water, stained with H & E. x400mag. Note the Secondary lamellae (black arrow) and Primary lamellae (red arrow) still appears normal.

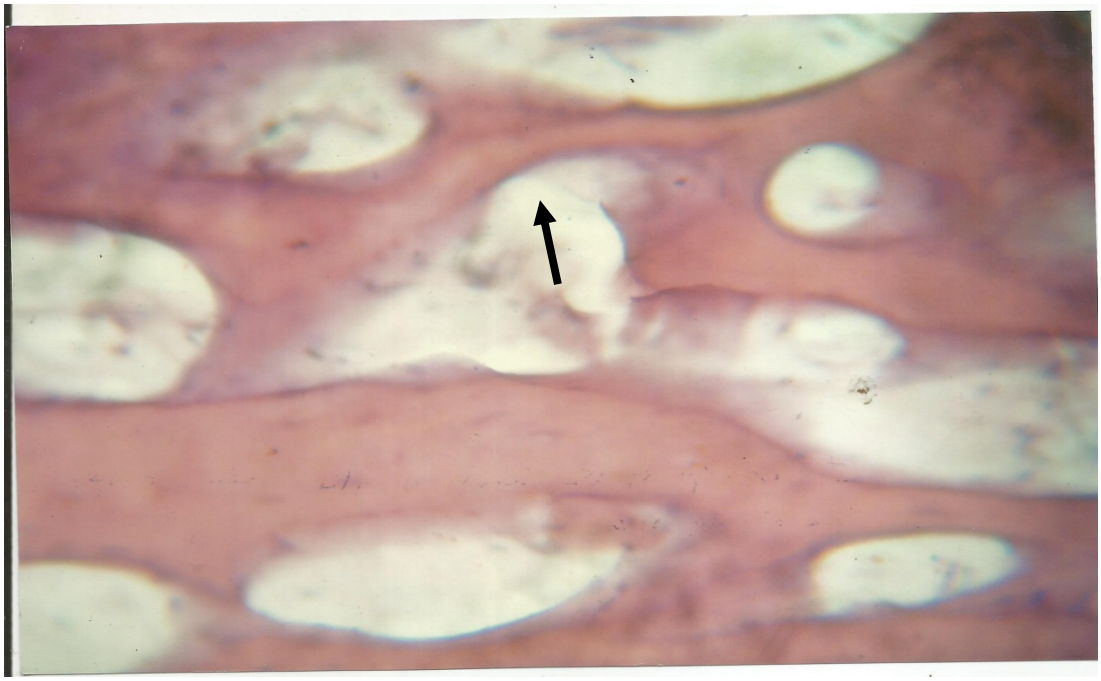

Plate 4.3c: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 5.0ml/l of treated produce water, stained with H & E. x400mag. Note slight fusion of lamellae.

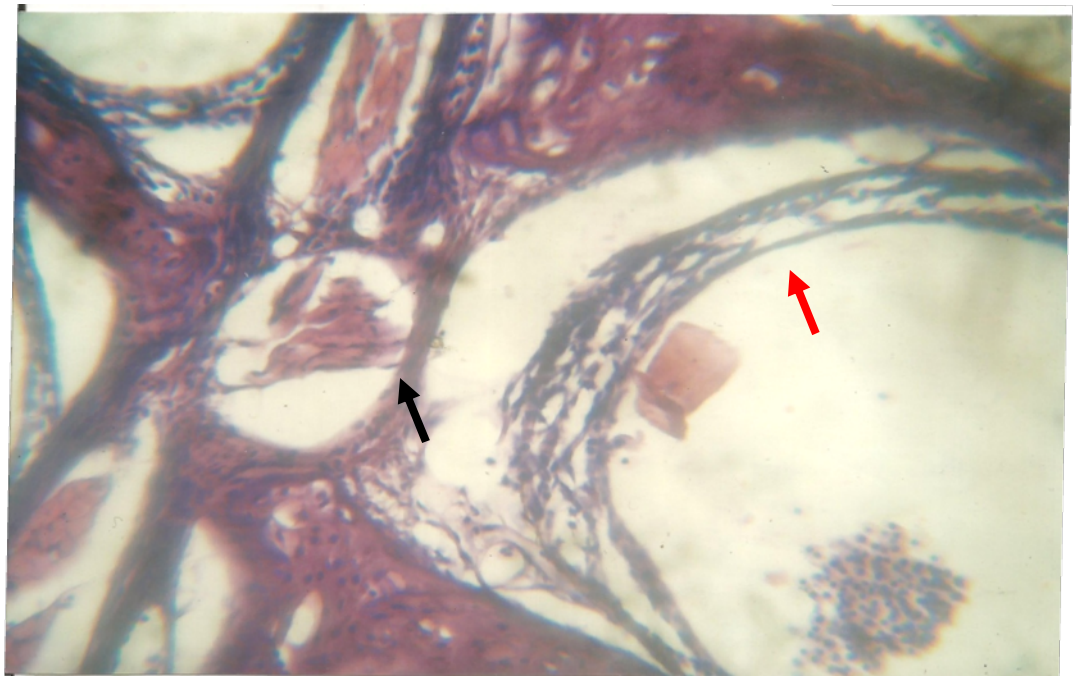

Plate 4.3d: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 10.0ml/l of treated produce water, stained with H & E. x400mag. Note epithelial lifting (black arrow) and filament detachment (red arrow)

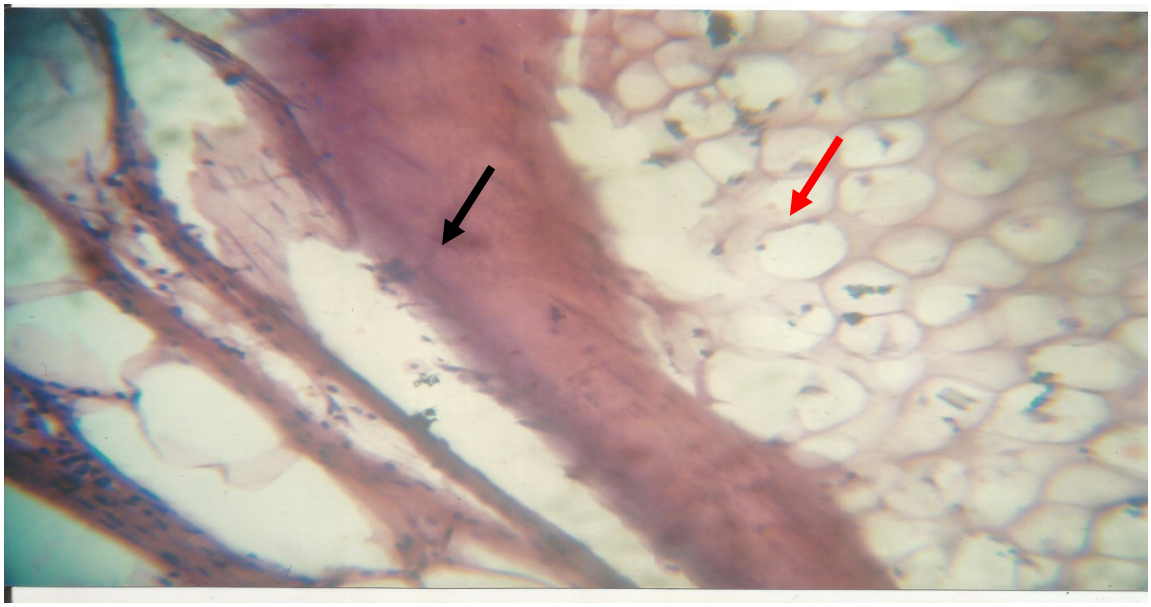

Plate 4.4a: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 2.5ml/l of untreated produce water, stained with H & E. x400mag. Note oedema(red arrow) and necrosis (black arrow)

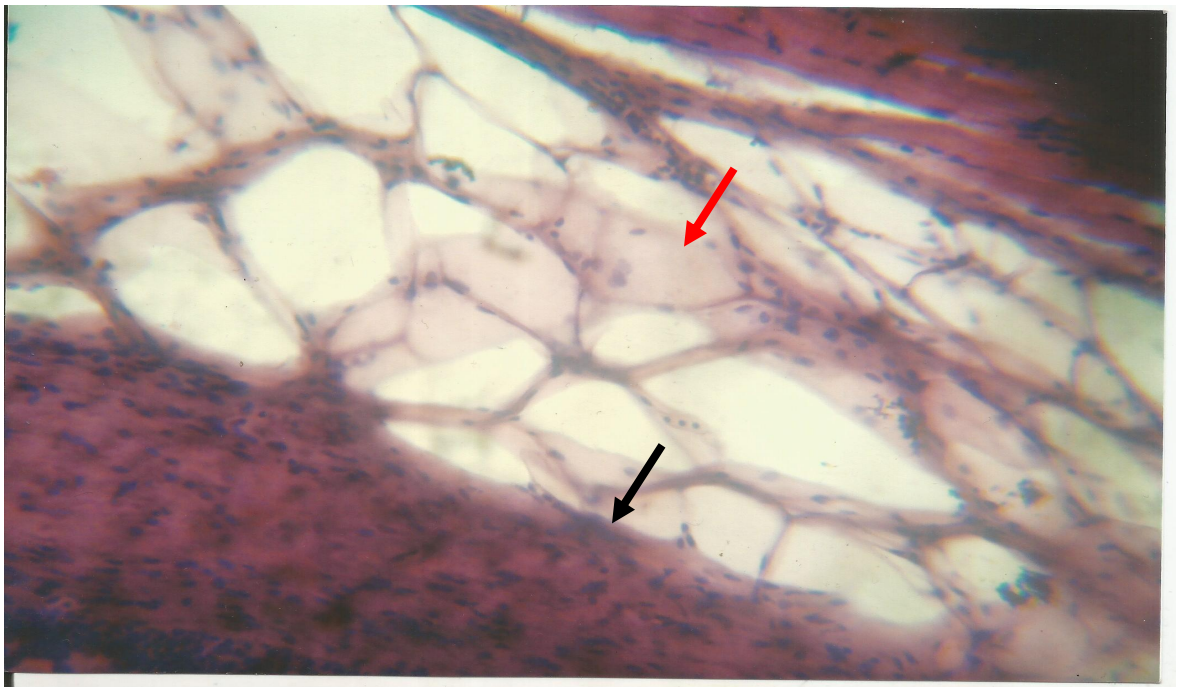

Plate 4.4b: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 5.0ml/l of untreated produce water, stained with H & E. x400mag. Note lamellae necrosis and cell infiltration (black arrow) and Oedema (red arrow)

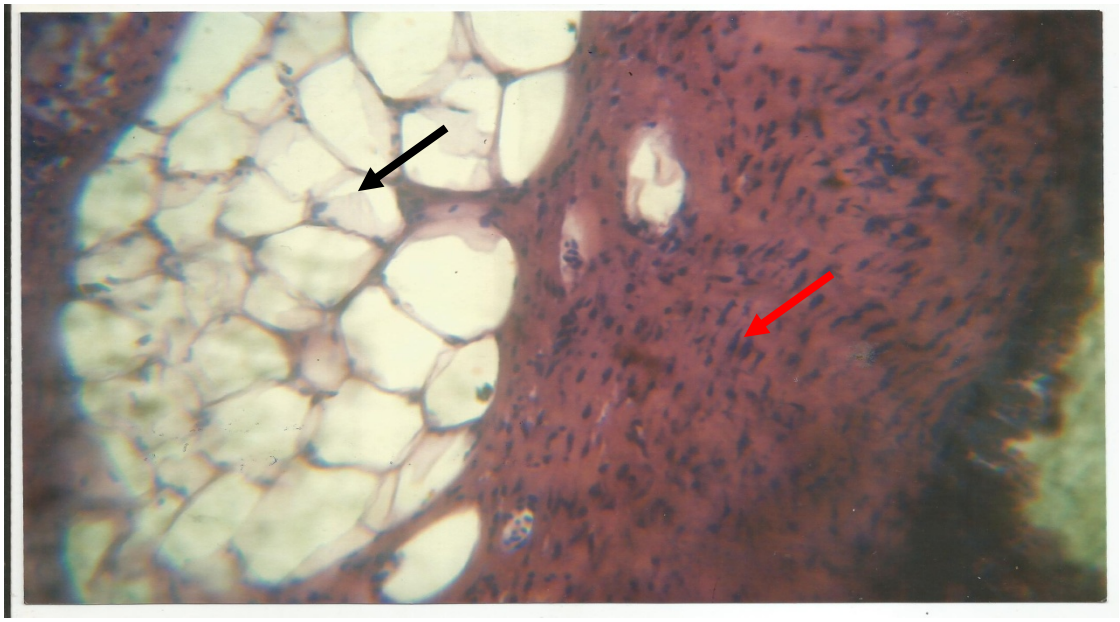

Plate 4.4c: Photomicrograph of section of the gills of juvenile *Clarias gariepinus* exposed to 10.0ml/l of untreated produce water, stained with H & E. x400mag. Note mononuclear cellular infiltration (red arrow), lamellae necrosis and oedema (black arrow)

BEHAVIOURAL OBSERVATION OF THE JUVENILES

Erratic swimming, projecting of their heads to gasp for air, sudden quick movement and summersaulting were observed in the juveniles exposed to untreated produced water.

STATISTICAL RESULTS

Tables 4.5 shows the student t-Test results of physicochemical parameters of the treated and untreated produced water, which was not significant at 90 and 95% confidence level, except for the salinity and conductivity parameters.

Table 4.5 Student t-Test result of the Physical properties of Treated and Untreated Produced water

Parameters	Treated Effluent	Untreated Effluent	MD	ESDD	SEM	T-test
pH	6.98	5.56	-1.42	1.73	0.86	-1.64
Conductivity	299.8	7510	7209	79.01	39.51	182.46
TDS	158.89	3980	3821.11	4679.88	2339.94	1.63
Salinity	32.56	814.30	781.74	302.78	151.39	5.16
DO	5.14	3.15	-1.99	2.43	1.21	-1.64
BOD	6.10	19.58	13.48	16.50	8.25	1.63
COD	7.25	29.80	22.55	27.61	13.80	1.63

Key: TDS = Total Dissolved Solids; DO = Dissolved Oxygen; BOD = Biological Oxygen Demand; COD = Chemical Oxygen Demand; MD = Mean Difference; ESDD = Estimated Standard Deviation Difference; SEM = Standard Error of Mean.

Table 4.6 Student t-Test result of Heavy Metal ions of Treated and Untreated Produced water

Parameters	Treated Effluent	Untreated Effluent	MD	ESDD	SEM	T-test
Iron	0.96	5.55	4.59	31.60	15.8	0.29
Zinc	0.72	2.36	1.64	4.03	2.0	0.81
Copper	0.56	2.84	2.28	7.79	3.89	0.58
Nickel	0.15	2.59	2.44	8.90	4.46	0.54
Cadmium	0.11	3.98	3.87	22.46	11.23	0.34

MD = Mean Difference; ESDD = Estimated Standard Deviation Difference; SEM = Standard Error of Mean.

4.2 DISCUSSION

PHYSICOCHEMICAL PROPERTIES OF THE TREATED AND UNTREATED PRODUCED WATER SAMPLES.

pH

Various studies carried out on the effect of pH of fish include those of Saha *et al*, (2002), Fashina-Bombata *et al*, (2003) and Norm (2001) which were made to study the effects of various levels of pH on the survival of Juveniles of *Clarias gariepinus,* this is with a view to determine the safe levels of these parameters to the juveniles of *Clarias gariepinus*. It was concluded that the safe level for pH is between 5 to 9. The range of pH values obtained in this study is in agreement with the work of Wurts and Durborow (1992) and Bhatnagar *et al* (2013), who recommended optimum pH levels in ponds to be within 6.0 to 9.0. The pH of the treated and untreated produced water was within the standards suitable for the aquatic ecosystem.

SALINITY

In the present study, the salinity of the treated produced water was shown to be within the DPR and FMENV permissible limit, whereas the untreated produced water was far above the permissible limit. Unusual salinity levels are usually indicative of pollution, which can result in the death of edible species. Increase

in salinity has been shown to cause shifts in biotic communication, limit biodiversity, exclude less tolerant species and cause acute or chronic effects at specific life stages. (Wetzel, 2001)

DISSOLVED OXYGEN (DO) AND BIOCHEMICAL OXYGEN

According to Chapman (1997), the stipulated standard of dissolved oxygen for sustaining aquatic life is from 4-5mg/l, a concentration below this value adversely affects fishes while concentrations below 3mg/l may lead to actual death of the fish population. In the present study, the dissolved oxygen of the treated effluent was 5.14mg/l which is within the acceptable limit, whereas the dissolved oxygen of the untreated effluent was 3.15mg/l.

The result revealed a BOD of the treated effluent at 6.10, while the BOD of the untreated effluent at 19.58mg/l. The subsequent increase of BOD and decrease of DO in the untreated effluent as observed is probably as a result of microbial content and organic matters in the effluent as described by Castro *et al*, (2011) and Hossain *et al*, (2012) in a related study.

TOTAL DISSOLVED SOLIDS (TDS) AND CONDUCTIVITY

Conductivity, along with TDS serves as a general indicator of change in water quality and affects the taste and freshness of the water (Kharaka, 2003). In the

present study, the total dissolved solids and conductivity of the treated produced water was shown to be within the EPA, FMENV and DPR permissible limits (Table 4.3) whereas the TDS and Conductivity of the untreated produced water was above the permissible limit. In a similar study carried out by Stekoli *et al* (2003), embryos, fry and juveniles of *Clarias gariepinus* were exposed to elevated TDS and they found no significant increase in mortalities with higher concentrations of TDS and concluded that these life stages were unaffected by TDS exposure on either the short or long term. The measurement of TDS integrates all anions and cations in the sample and some ions or combination of ions are substantially more toxic than other ions or combination of ions. Some species might be more sensitive to TDS toxicity at certain life stages, as many fishes are during fertilization, thus water quality for TDS can take several approaches. The standard can be low enough to protect all species and life stages exposed, or the standard can be set to protect most species and life stages for most ions or combination of ions. Different limits can be defined for different categories of ions as well as different species of fish. *Clarias gariepinus* has proven to be resistant to various toxicants (Datta *et al.*, 2002; Olaifa *et al.*, 2004 & Okomoda *et al.*, 2010). Conductivity outside the acceptable range could indicate that the water is not suitable for certain species of fish.

CHEMICAL OXYGEN DEMAND (COD)

COD analysis is a measurement of the oxygen depletion capacity of a water sample contaminated with organic waste matter. Specifically, it measures the equivalent amount of oxygen required to chemically oxidize organic compounds in water (Ambrose *et al*, 1993). The discharge of wastes with high levels of COD and BOD can cause water quality problems such as severe dissolved oxygen depletion and may cause mortality in fish in receiving water bodies. (Edenfelder, 2000)

In the present study, the COD of the treated and untreated produce water was within the permissible limit.

HEAVY METALS (Cd, Ni, Fe, Cu and Zn)

In the present study, the level of heavy metals in the treated water was within the DPR and FMENV permissible limit, while the untreated produced water had higher levels of heavy metals. Heavy metals such as Cadmium, Nickel, Iron, Copper and Zinc as analysed in the study are the most important pollutants which affect the aquatic environment and are extremely dangerous to fishes. Most of these metals are characterized by being accumulated in tissues and lead to the poisoning of fish, thereby influencing the vital operations and reproduction of fish; they also weaken the immune system and induce

pathological changes. According to Jarup (2003) Cadmium has been shown to accumulate mainly in kidney, liver and gills of freshwater fish, but it can also be deposited in the hearts and other tissues (Chowdhury *et al.,* 2004) and also cause pathological changes in these organs (Melgar *et al.,* 1997) which can be said to be the reason for the histological changes observed in the present study. Histological alterations in liver of fishes exposed to Cadmium have also been documented by (Thophon *et al.,* 2003).

Liao (2004) reported that Copper-induced histological alterations are found in the gill, kidney hematopietic tissue and other tissues. Histological alterations in liver of fishes exposed to copper have been documented by Figueiredo et al., 2007. Higher level of copper caused visible external lesions such as discoloration and necrosis on livers of fishes (Thophon et al., 2003) as observed in the present study. Arellano *et al* (2006) reported vacoulation of endothelial cells, hepatocyte vacoulation, necrosis, shrinkage and increase of sinusoidal spaces as a result of the exposure of the fishes to copper. High concentration of copper have been reported o inhibit catalase enzyme in liver, gills and muscle after 24hr exposure (Grosell *et al.,* 2004). The present study is also in agreement with Sanchez *et al* (2005) who observed in a study that Cu is able to induce oxidative stress in fish even before significant metal accumulation occurs in the liver as seen in the present study. A recent study by Omar *et al*

(2014) proved that the fish liver is the target organ for Iron (Fe), because the gills surface of the fish tends to be alkaline, this then covers the gill lamellae and inhibits respiration as observed in the present study. Abas *et al* (2002) also stated in a recent study that Iron caused necrosis of the secondary lamellae.

In the present study Zn level in the untreated produced water was above the permissible limit and can be said to be a joint cause of the increase in mucous cell and vacoulation of the hepatocyte. Farombi *et al* (2007) recorded gill proliferation, stimulation of mucous cells and an increase in mucous production of Clarias gariepinus exposed to zinc. Abd El-Gawad (2009) also recorded similar changes in Oreochromis niloticus fish exposed to zinc sulphate. According to Nebaker *et al* (2004), Nickel has been shown as moderately toxic to *Clarias gariepinus* and aquatic invertebrates when compared to other metals, but accumulation of Nickel in the body through chronic exposure can lead to cardiovascular as well as liver disease in fish. (Denkhaus *et al*, 2002).

As shown in the present study (Table 4.3), the Ni level of the treated and untreated effluent is within the permissible limit and thus cannot be said to have had deleterious effect on the juveniles.

HAEMATOLOGICAL EXAMINIATION

Hematological parameters are good indicators of the physiological status of animals (Khan *et al*, 2005). They act as a pathological reflector of the status of exposed animals to pollutants (Olafedehan *et al*, 2010). Haematological components which include Red blood cells, white blood cells, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and haemoglobin all play a vital role in the physiological status of the organism (Doyle, 2006).

There was no significant change ($P<0.05$) of haematological parameters of *Clarias gariepinus* exposed to treated and untreated produce water. This is in agreement with Joshi *et al* (2002) who reported the effect of toxicant on blood parameters in fresh water teleost *Clarias batrachus*. Gabriel *et al* (2004) also reported a non significant difference in hematological values for *Clarias gariepinus* exposed to toxicants which is similar to the observation in this study.

The physicochemical parameters of the treated and untreated effluent were within acceptable levels for the survival of *Clarias gariepinus*, as earlier reported by Viveen *et al*. (1985) and Peteri *et al* (1992), especially as the juveniles had wide tolerance for high salinity as observed in the present study. This might have accounted for the insignificant changes in the oxygen transport

vehicles (Hb, RBC, MCH, MCHC) of *Clarias gariepinus* exposed to both treated and untreated produce water.

HEPATIC PARAMETERS

Bioichemical parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferases (AST), total proteins, total cholesterol etc, are valid for physiopathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutants damage (Juneja *et al.*, 2002; Atmeida *et al.*, 2002; Matos *et al.,* 2007; Osman *et al.*, 2010).

From the results obtained, the blood samples collected from the juvenile exposed to untreated effluent showed an increase in AST and ALT level from 155.93 to 181.19U/L and 37.07 to 44.00U/L respectively. According to the submission of Atalaka *et al* (2011), increased levels of AST and ALT activities in the exposed fishes observed in this study are suggestive of hepatic cellular damages leading to their leakage into circulation. These findings are in agreement with the reports of Neskovic *et al* (1996) who recorded high level of AST and ALT activities in adult *Clarias gariepinus* exposed to aqueous and ethanol extracts of *parkia biglobosa* pods. Even though these findings disagreed with the reports of Sadlu *et al* (1985) and Okechukwu *et al* (2007) who also used sub-lethal doses of toxicants and reported significant decrease in both AST and ALT activities which may have resulted from the type of toxic compound

in the solution used. A significant increase in albumin was also observed in the present study from 1.60 to 6.80mg/dl. According to Sugio (1991) decrease in albumin may be caused by dehydration while increase may be as a result of liver disease or mal-absorption. The total cholesterol level in the present study was unchanged while the total protein level decreased from 20.24 in the control to 17.99g/l. Sudansu *et al* (2008) reported that biochemical parameters such as total protein are sensitive to pollutant in exposed organism. Therefore, a decrease in protein value observed may be attributed to higher energy demand for metabolic purposes.

ANTIOXIDANT ACTIVITIES

Fish tissues are endowed with antioxidant defense systems which include GST enzyme to protect them from oxidative stress caused by pollutants. (Basha *et al*, 2003). GST plays a critical role in mitigating oxidative stress in all life forms and its activity also has been widely used as a biomarker to detect stress. As an antioxidant enzyme, a GST activity either has a significant increase or decrease with different patterns according to the exposed elements or exposure conditions. (Farombi *et al*, 2007). The present study revealed a decrease in GST activities of the liver of juveniles exposed to treated and untreated produce water, although not significant. Malondialdehyde, which is one of the oxidative damage products of lipid per oxidation and whose presence in tissues indicates

oxidative stress, was found to increase from 1.03g/l to 3.46g/l. GSH, a major non-protein thiol in living organism, plays a central role in coordinating the body‘s antioxidant defense processes (Cerebrum *et al*, 2009). The insignificant change observed for juveniles exposed to treated and untreated produce water is not in agreement with Olagoke (2008) who studied antioxidant defense enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons and reported decreased level of GST and GSH in exposed fish. The antioxidant results is not also in agreement with Faramobi (2007) who studied the biomarkers of oxidative stress and heavy metal levels in *Clarias gariepinus* and reported an increased activity of GST and GSH levels in the liver of the fish. The results obtained in this study perhaps depends on the intensity and duration of stress applied, the physicochemical properties of the effluent used as well as the susceptibility of the target species.

HISTOPATHOLOGY OF THE LIVER OF THE EXPOSED JUVENILES AND CONTROL

Anormalies such as irregular shaped central vein, cellular vacoulation and infiltration may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the toxicants. This is in conformity with the submission of Fanta *et al* (2003) who worked with Siluriform

Corydoras paleatus contaminated by organophosphate pesticides which are related to the normal metabolic function of the *Clarias gariepinus* liver. Machando *et al* (2003) noted that exposure of fish to toxicants may result in the accumulation of fats (Vacuolation) as recorded in the study. This is also in line with the findings of Pacheco *et al* (2003) and Liu *et al* (2006) who also described increased level of vacuolation of the hepatocytes as a signal to the degenerating process that suggests metabolic damage, possibly related to exposure to contaminated water.

HISTOPATHOLODY OF THE GILLS OF THE EXPOSED JUVENILES AND CONTROL.

Owing to the direct and continuous contact with the aquatic environment, fish gills which are organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous wastes products and acid base regulation are directly affected by contaminants. The histological alterations such as gill epithelial proliferation and detachment with lamellar oedema and fusion of the primary and secondary lamellae observed in the present study were also reported by Van Heerden *et al* (2004). Similar lesions were reported in the gills of *Clarias gariepinus* exposed to ethanolic extract by Abalaka *et al* (2010). The epithelial proliferation observed in this study was a regenerative response of stressed fish to epithelial damages (Banerjee *et al*., 2005). However, when this is not controlled, it might

become degenerative in nature resulting in loss of morphological and functional efficiency of the affected gill structures in the juveniles exposed, as observed in the present study. The observed oedematous changes in the gill lamellar of the juvenile exposed may be due to increased capillary permeability of the blood vessels of affected gills (Olurin *et al,* 2006) leading to epithelial detachment (Schwaigner *et al,* 2004) as shown in this study. Adeogun *et al* (2012) reported similar oedematous gills in *Clarias gariepinus* exposed to sub-lethal concentration of methanolic extract. The observed lamellar mononuclear cellular infiltrations were inflammatory responses to tissue damage (necrosis) in the affected gills. Similar cellular infiltrations were reported in the gills of *Clarias gariepinus* exposed to Malathion (Sharpudin *et al*, 2010). Irregular cellular pattern as well as separation of epithelium from gill lamellae, loss of secondary lamellae, attenuated primary lamellae, oedema (mucous-filled cavity), necrosis and hyperaemia (vascular congestion) shown in this study agrees with reports by Dhanapalkiam *et al* (2004), Harper *et al* (2009) & Pathan *et al* (2010). Au (2004) and Nordberg *et al* (2005) reported that gill lesions such as oedema formation and shortened gill filament did not only indicate possibilities of impaired respiratory functions but impaired osmo-regulatory functions too. The changes recorded in the gill structure of the test fish exposed to the untreated produce water were indicative of reduced oxygen supply to the

test fish, resulting also in hypoxic respiratory response. Such histopathological changes of gills exposed to different toxicants have been reported by Ayoola (2008) after the exposure of *Clarias gariepinus* to phenols and heavy metals in water. Similar observations of gill conditions had been made by Ufodike (1990) in *Clarias gariepinus* exposed to inorganic fertilizers.

BEHAVIOURAL CHANGES IN THE JUVENILES

Observed behavioural changes, such as erratic swimming, gasping for air, sudden quick movement and summersaulting are similar to changes linked to the toxicity of xenobiotics in fish and has been reported in previous studies (Ferrando et al, 1991 & Sarikaya *et al,* 2003). The observed repeated attempts to jump out of the holding containers will be described as adaptation behavioural responses to escape from the toxic environment (Abalaka *et al,* 2010). For example at higher concentration of untreated produced water (10.0ml/l), it was observed that most of the juvenile were projecting their heads out of water to gasp for fresh air, indicating that they were apparently having dissolved oxygen problems, however, such behavior could also be attributed to damage to the gills, which impairs oxygen uptake and leads to toxicity induced hypoxia, this is in line with the observation of Barbieri (2008) in a recent study. The damage induced on the gill of the test fish was found to be concentration and exposure period dependent.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The effects of the treated and untreated produced water were more pronounced in the gills and liver of the juveniles than in the hematological and biochemical responses of the exposed juveniles. However, such measurable changes depend upon the biological status of exposed fish as well as upon the type and duration of their exposure to toxicants within that aquatic environment. Stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic response. If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing. Even though individual concentrations of any toxicant might be low, the combined concentrations could be fatal to aquatic health. Though the concentrations of heavy metals as well as other physiochemical parameters of the effluent fell within the DPR and FMENV specifications, their residual effects which may impair organs like the gills and liver should not be ruled out. These toxicants no matter how little the concentration could be biomagnified in a water body, with the resultant effect being gradual accumulation of the toxicants in water, which in turn becomes toxic to aquatic organism. Although, *Clarias gariepinus* have a high adaptive ability, higher concentration of treated

and untreated produce water may be detrimental to their health as shown in this study.

5.2 RECOMMENDATIONS

Oil and gas operations usually generate significant quantities of water from reservoirs globally. The average ratio is three barrels of produced water for one barrel of oil. There is need to:

1. Establish a beneficial reuse matrix

Knowledge on how best to recycle and reuse produced water in a cost-efficient manner will elevate Nigeria's intellectual profile and support its goal to both improve its water security and become a knowledge-based economy.

2. Tighten Treatment Standards

The government needs to provide more stringent standards that must be met in the treatment and management of produced water in terms of local legislation and international best practice.

3. Minimizing the volume of produced water generated

Presently, the oil and gas operation generates significant quantities of water globally; the average ratio is three (3) barrels of produced water to one (1) barrel of oil. Reduction in the volume of water produced from oil production wells will go a long way in minimizing water pollution. This could be achieved by modifying the existing processes, adapting technologies, both old and new to ensure that less water is generated from the onset.

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APPENDIX

APPENDIX A: APPARATUS

1. Win Lab pH meter
2. Beakers
3. TDS meter(200363, Germany)
4. Retort stand
5. Measuring cylinder
6. Lamotte spectrophotometer (Smart 3)
7. Atomic Absorption Spectrophotometer
8. Photo-electric colorimeter
9. Light microscope
10. Rotary microtone
11. Water bath

APPENDIX B: STATISTICAL ANALYSIS OF THE PHYSICOCHEMICAL PARAMETERS (T-test)

Parameters	**Treated Effluent**	**Untreated Effluent**	**MD**	**ESDD**	**SEM**	**T-test**
pH	6.98	5.56	-1.42	1.73	0.86	-1.64
Conductivity	299.8	7510	7209	79.01	39.51	182.46
TDS	158.89	3980	3821.11	4679.88	2339.94	1.63
Salinity	32.56	814.30	781.74	302.78	151.39	5.16
DO	5.14	3.15	-1.99	2.43	1.21	-1.64
BOD	6.10	19.58	13.48	16.50	8.25	1.63
COD	7.25	29.80	22.55	27.61	13.80	1.63

Note:

MD: Mean Difference

ESDD: Estimated Standard Deviation Difference

SEM: Standard Error of Mean

Degree of freedom = 3-1 = 2

P = 0.05 = 2.92

At 95% confidence level, it is not significant except the salinity and conductivity parameter.

pH

Untreated	Treated	Deviation	Variance
5.56	6.98	-1.42	2.0164
5.55	6.99	-1.44	2.0736
5.57	6.97	-1.40	1.96
16.68	**20.94**		**6.05**

MD = 16.68 - 20.94 ÷ 3 = -1.42

ESDD = √6.05 ÷ 3-1 = 1.73

SEM = 1.73 ÷ 2 = 0.865

T-test = -1.42 ÷ 0.865 = -1.64

TDS

Untreated	Treated	Deviation	Variance
3980	158.89	3821.1	14600881.63
3979	158.90	3820.1	14593164.01
3981	158.88	3822.1	14608601.29
11940	**476.67**		**43802646.93**

MD = 1190 – 476.67 ÷ 3 = 3821.11

ESDD = √43802646.9 ÷ 3-1 = 4679.88

SEM = 4679.88 ÷ 2 = 29339.94

T-test = 3821.11 ÷ 2339.94 = 1.63

SALINITY

Untreated	Treated	Deviation	Variance
814.30	32.56	781.74	611117.42
814.29	32.55	781.74	611117.42
814.31	32.57	781.74	611117.42
2442.9	**97.68**		**1833352.28**

MD = 2442.9 – 97.68 ÷ 3 = 781.74

ESDD = √183352.28 ÷ 3-1 = 302.78

SEM = 302.78 ÷ 2 = 151.39

T-test = 781.74 ÷ 151.39 = 5.16

DO

Untreated	Treated	Deviation	Variance
3.15	5.14	-1.99	3.9601
3.14	5.15	-2.01	4.0401
3.16	5.13	-1.97	3.8809
9.45	**15.42**		**11.8811**

MD = 9.45 – 15.42 ÷ 3 = -1.99

ESDD = √11.8811 ÷ 3-1 = 2.43

SEM = 2.43 ÷ 2 = 1.21

T-test = -1.99 ÷ 1.21 = -1.64

BOD

Untreated	Treated	Deviation	Variance
19.58	6.10	13.48	181.71
19.57	6.11	13.46	181.17
19.59	6.09	13.50	182.25
58.74	**18.30**		**545.13**

MD = 58.74 – 18.3 ÷ 3 = 13.48

ESDD = √545.132 ÷3-1 = 16.50

SEM = 16.50 ÷ 2 =8.25

T-test = 13.48 ÷ 8.25 = 1.63

COD

Untreated	Treated	Deviation	Variance
29.80	7.25	22.55	508.50
29.79	7.26	22.53	507.60
29.81	7.24	22.57	509.40
89.40	**21.75**		**1525.50**

MD = 89.4 – 21.75 ÷ 3 = 22.55

ESDD = √1525.5083 ÷ 3-1 = 27.61

SEM = 27.61 ÷ 2 = 13.80

T-test = 22.55 ÷ 13.80 = 1.63

CONDUCTIVITY

Untreated	Treated	Deviation	Variance
7510	299.8	7210.2	51986984.04
7509	299.7	7209.3	51974006.49
7507	299.5	7207.5	51948056.25
22526	**899.0**		**155909046.8**

MD = 22526 – 899 ÷ 3 = 7209

ESDD = √155909046.8 ÷ 3-1 = 79.01

SEM = 79.01 ÷ 2 = 39.51

T-test = 7209 ÷ 39.51 = 182.46

APPENDIX C: STATISTICAL ANALYSIS OF THE HEAVY METALS

Fe

Untreated	Treated	Deviation	Variance
5.55	0.967	4.583	21.003889
5.54	0.968	4.572	20.903184
5.56	0.966	4.594	21.104836
16.65	**2.901**		**63.011909**

MD = 16.65 – 2.901 ÷ 3 = 4.583

ESDD = 63.2043 ÷ 3-1 = 31.60

SEM = 31.60 ÷ 2 = 15.8

T-test = 4.583 ÷ 15.8 = 0.29

Degree of freedom = 2

Zn

Untreated	Treated	Deviation	Variance
2.36	0.722	1.638	2.6830
2.35	0.723	1.527	2.3317
2.37	0.721	1.649	2.7192
7.08	**2.166**		**8.0688**

MD = 7.08 – 2.166 ÷ 3 = 1.638

ESDD = 8.0688 ÷ 3-1 = 4.03

SEM = 4.03 ÷ 2 = 2.0

T-test = 1.64 ÷ 2.0 = 0.81

Cu

Untreated	Treated	Deviation	Variance
2.84	0.561	2.27	5.1938
2.83	0.562	2.26	5.1438
2.85	0.560	2.29	5.2441
8.52	**1.683**		**15.5817**

MD = 8.52 – 1.683 ÷ 3 = 2.279

ESDD = 15.5817 ÷ 3-1 = 7.79

SEM = 7.79 ÷ 2 = 3.89

T-test = 2.279 ÷ 3.89 = 0.58

Ni

Untreated	Treated	Deviation	Variance
2.59	0.15	2.44	5.9536
2.58	0.14	2.44	5.9536
2.60	0.16	2.44	5.9536
7.77	**0.45**		**17.8608**

MD = 7.77 – 0.45 ÷ 3 = 2.44

ESDD = 17.8608 ÷ 3-1 = 8.934

SEM = 8.9304÷ 2 = 4.46

T-test = 2.44 ÷ 4.6 = 0.54

Cd

Untreated	Treated	Deviation	Variance

3.98	0.11	3.87	14.9769
3.97	0.10	387	14.9769
3.99	0.12	3.87	14.9769
11.94	**0.33**		**44.9307**

MD = 11.94 – 0.33 ÷ 3 = 3.87

ESDD = 44.9307 ÷ 3-1 = 22.46

SEM = 22.46 ÷ 2 = 11.23

T-test = 3.87 ÷ 11.23 = 0.34

APPEDIX E: STATISTICAL ANALYSIS OF THE EFFECT OF TREATED AND UNTREATED PRODUCE WATER ON THE HAEMATOLOGICAL PARAMETERS OF JUVEVILE *CLARIAS GARIEPINUS.*

	Control	Treated Effluent(mg/l)			Untreated Effluent(mg/l)		
Parameters	0.0	2.5	5.0	10.0	2.5	5.0	10.0
Hb	9.24	9.24	9.24	9.24	9.25	9.24	9.22
RBC	4.35	4.36	4.35	4.35	4.36	4.35	4.33
WBC	9.66	9.66	9.66	9.66	9.66	9.67	9.70
MCV	38.22	38.22	38.23	38.22	38.22	38.21	38.02
MCH	21.20	21.20	21.21	21.21	21.21	21.24	21.26
MCHC	5.15	5.15	5.15	5.15	5.15	5.14	5.14
Y	87.82	87.83	87.84	87.82	87.85	87.85	87.87
N	6	6	6	6	6	6	6

1. GT = 87.82 + 87.83 + 87.84 + 87.82 + 87.85 + 87.85 + 87.67

= 614.68

2. S.S Total = 2134.22 + 214.3 + 2135.42 + 2134.51 + 2134.89 + 2135.3 + 2121.64
   =14390.28
3. S.S Among = $\frac{87.82^2}{6} + \frac{87.83^2}{6} + \frac{87.84^2}{6} + \frac{87.82^2}{6} + \frac{87.85^2}{6} + \frac{87.85^2}{6} + \frac{87.67^2}{6}$
4. CT = $\frac{614.68^2}{42} = 8995.9$
5. New S.S Total = S.S total – CT
   = 14930.28 – 8995.9 = 5934.38
6. New S.S Among = S.S among – CT
   = 8995.97 – 8995.9
   =0.07
7. New S.S Within = New S.S total – New S.S among
   =5934.38 – 0.07
   =5934.31

ANOVA TABLE

Source of variation	Degree of	Sum of Squares	Mean Square	F.S
$y - \bar{y}$ among groups	Freedom	0.07	$\frac{0.07}{6}$=0.01166	$\frac{0.01166}{169.55}$=
	7-1=6			0.000068
$\bar{y} - \bar{\bar{y}}$ within groups		5934.31	$\frac{5934.31}{35}$=169.55	
	42-7=35			
$\bar{y} - \bar{\bar{y}}$ total		5934.38		
	41			

F.05 (6, 35) = 2.42

F.01 (6, 35) = 3.47

At 95 and 99% Confidence levels, the result is not significant. Because the calculated value (0.000068) is less than the critical or table values (2.42 and 3.47).

Note:

GT = Grand Total

S.S Total = Sum of squared total

S.S Among= Sum of squared among

CT= Correction Term

APPEDIX F: STATISTICAL ANALYSIS OF THE EFFECT OF TREATED AND UNTREATED PRODUCE WATER ON THE BIOCHEMICAL PARAMETERS OF JUVEVILE *CLARIAS GARIEPINUS.*

	Control	Treated Effluent(mg/l)			Untreated Effluent(mg/l)		
Parameters	0.0	2.5	5.0	10.0	2.5	5.0	10.0
AST	155.9	152.11	154.00	155.00	170.39	173.03	181.19
ALT	37.01	37.10	37.32	37.21	39.00	40.10	44.01
ALB	7.60	7.60	7.60	7.56	7.38	7.19	6.79
T.Cholesterol	165.16	165.16	165.16	165.16	165.21	166.70	166.99
T.Protein	20.24	20.22	20.22	20.22	20.20	19.55	17.99
Y	385.91	382.19	384.30	385.15	401.98	406.57	416.97
N	5	5	5	5	5	5	5

1. GT = 385.91 + 382.19 + 384.30 + 385.15 + 401.98 + 406.57 + 416.97

= 2763.07

2. S.S Total = 53419.79 + 52258.29 + 52853.21 + 53153.41 + 58302.56 + 59770.17 + 63022.10

   =392779.55

3. S.S Among = $\frac{385.91^2}{5} + \frac{382.19^2}{5} + \frac{384.30^2}{5} + \frac{385.15^2}{5} + \frac{401.98^2}{5} + \frac{406.57^2}{5} + \frac{416.97^2}{5}$

   = 218354.72

4. CT = $\frac{2763.07^2}{35}$ = 218130.16

5. New S.S Total = S.S total – CT

   = 392779.55 – 218130.16 = 174649.39

6. New S.S Among = S.S among – CT

   = 218354.72 – 218130.16

   =224.56

7. New S.S Within = New S.S total – New S.S among

   =174649.39 – 224.56

   =174424.83

ANOVA TABLE

Source of variation	Degree of	Sum of	Mean Square	F.S
$y - \bar{y}$ among groups	Freedom	Squares	$\frac{224.56}{6}$=37.42	$\frac{37.426}{6229.458}$ =
	7-1=6	224.56		
$\bar{y} - \bar{\bar{y}}$ within groups			$\frac{174424.83}{28}$=6229.4	0.006
	35-7=28	174424.83		
$\bar{y} - \bar{\bar{y}}$ total				
	34	174649.39		

F.05 (6, 28) = 2.45

F.01 (6, 28) = 3.53

At 95 and 99% Confidence levels, the result is not significant. Because the calculated value (0.006) is less than the critical or table values (2.45 and 3.53).

Note:

GT = Grand Total

S.S Total = Sum of squared total

S.S Among= Sum of squared among

CT= Correction Term