TOXICITY OF TEXTILE EFFLUENT ON *OREOCHROMIS NILOTICUS*

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BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN BIOLOGY

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JANUARY, 2018

DECLARATION

I hereby declare that the work in this thesis entitled "TOXICITY OF TEXTILE EFFLUENT ON *OREOCHROMIS NILOTICUS*" has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any Institution.

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CERTIFICATION

This thesis entitled "TOXICITY OF TEXTILE EFFLUENT ON *OREOCHROMIS NILOTICUS*" by ENI, VICTORIA MARTIN meets the regulations governing the award of the degree of Master of Science in Biology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The toxicity of textile effluent on the fingerlings of *Oreochromis niloticus* was investigated. The physico-chemical parameters and concentration of heavy metals in the textile effluent was investigated. Acute and chronic effects of the textile effluent on behaviour and morphology, haematology, histopathology, growth and nutrient utilization parameters were also investigated. Fingerlings of *Oreochromis niloticus* of average weight (167.55-172.52) were exposed to varying concentrations (25ml/L, 30ml/L, 35ml/L, 40ml/L and 45ml/L) of the textile effluent for 96hrs in glass tanks of 25L in a static bioassay. Data were analysed using Microsoft Excel for Windows 2007. The LC_{50} value was found to be 33.50. The result of physico-chemical parameters analysed from the Wastewater were include DO (3.10-4.30mg/l), EC (68-315µS/cm), temperature (29.10-31.20°C), BOD (0.30-3.40mg/l), pH (7.10-10.00), TDS (75-1770mg/l), alkalinity (0.6-3.93mg/l) and hardness (2.11-33mg/l) nitrite (0.021-4.70mg/l) showed significant difference ($p<0.05$) across the treatments. The following metals were assessed in the textile effluent, Aluinium(Al), Chromium (Cr), iron (Fe), copper (Cu), lead (Pb) and Zinc (Zn) and comparing their mean values with those of WHO (1984)/FEPA (1991), metals like Cr, Fe, Cu and Pb posed significant ($p<0.05$) environmental threat because their mean values were above the WHO, (1884)/FEPA (1991). There were some behavioural and morphological observations made during the acute bioassay which includes vertical positioning, erratic swimming, deformities, incessant gulping of air, accumulation of mucus on the body surface and gill filament which usually proceed to death. The acute and chronic exposure of the toxicant to the fingerlings of *Oreochromis niloticus* elicited significant changes in some haematological parameters; RBCC was fairly constant in all the treatment tanks, WBCC had its lowest value in the control group of 3.60 and PCV was also fairly constant in all the treatment tanks and higher

concentrations of mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and white blood cell indicating that test fish suffered haemolyticanaemia and leucocytocis. Different histopathological studies showed attenuated primary lamellae, marked loss of secondary lamellae, loss of epithelial cells, erosion of gill villi, oedema mild fatty change, moderate steatosis, necrosis and hyperemia as it was observed in all treatments except in the control tank which showed no any change on the tissues.

The textile effluent showed high significant ($p>0.05$) effect on the growth with initial mean growth of 167.55 and final mean of 180.35 which showed increased growth and all the nutrient utilization parameters (LWG, SGR, FCR, GFCE, FE and NM). Based on the results obtained from this work, it is recommended that textile effluents should be treated before being released because the result showed that they are lethal to fish and other organisms.

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CHAPTER ONE

1.0 INTRODUCTION

One of the most challenging problems encountered in Nigeria is the improper disposal of industrial effluents into water bodies. These effluents from industries have a great deal of influence have a great deal of influence on the pollution of the water body, these effluents can alter the physical, chemical and the nature of the receiving water body (Sangodoyin, 1995).

Water bodies which are major receptacles of treated and untreated or partially treated industrial wastes have become highly polluted. The resultant effects on this on public health and the environment are usually great in magnitude (Osibanjo, *et al.,* 2011). Textile industries form part of these prominent industries with attendant's indiscriminate discharge of effluent into the surrounding water bodies. They constitute severe environmental problems because the effluent are made up of complex composition such as chemical reagents, which are very diverse in chemical composition ranging from inorganic compounds to polymers and organic products such as dyes plasticizers, detergents (Zagal and Mazmanci, 2010).

They form one of the main sources with severe pollution problems world wide and its dye-containing wastewaters (i.e. 10,000 different textile dyes with an estimated annual production of 7.105 metric tones are commercially available worldwide; 30% of these dyes are used in excess of 1,000 tones per annum, and 90% of the textile products are used at the level of 100 tones per annum or less) (Robinson *et. al*.2001 Solomon *et. al.* 2009 Baban *et al*.2010).The discharge of dye-containing effluents into the water environment is undesirable, not only because of their colour, but also because many of dyes released and their breakdown products are toxic, carcinogenic or mutagenic to life forms mainly because of carcinogens, such as benzidine, naphthalene and other aromatic compounds. Without adequate treatment these dyes can remain in the environment for a long period of time affecting terrestrial and

aquatic environments. The physico chemical parameters of water are thus affected such as temperature, pH, hardness, dissolved oxygen, Biological Oxygen Demand (Auta, 2001).Water pollution is the resultant effect of these textile discharges and these pollutants build up in the foodchain. They accumulate in the organism and persist in the environment due to their stability or poor biodegradability (Zagal and Mazmanci, 2010).

Mohsen *et al*., (2011) reported that two poisoning mechanisms may take place, one occuring at high concentrations and provoking a rapid suffocation by destruction of the gill epithelium, the other prevailing at low concentrations and consisting of an inhibition of the main metabolic pathways. Histological examination of *Oreochromis niloticus* gave significant indication of toxicity of *Morinda lucida* (Oyedapo and Akinduyite, 2011). Olufayo and Jatto, (2011) reported on the adverse effects of tobacco leaf dust on the blood of *Oreochromis niloticus*.

Aquatic organisms are affected by pollutants especially fish which is affected in form of lipid content, growth rate, physical activity, physiological and nutritional states which all play important roles in determining the rate and extent of biotransformation and bio-concentration observed in fish (Huckle *et al.,* 2012).

Akhila *et al.,* (2007) reported that the determination of toxicity is a valuable assessment and evaluation of the toxic characteristics of all compounds. Mahabub *et al.,* (2008) discovered a high lipid peroxidase level in fishes exposed to textile effluent. They opined that if these fishes are consumed by man they may cause physiological problems.

1.1 Statement of Research Problem

The textile effluent constitutes severe environmental problems because they are made up of complex compositions. Such as chemical reagents which are very diverse chemical compositions, ranging from inorganic compounds to polymers and organic products, such as

dyes, plasticizers and detergents (Zagal and Mazmanci, 2011). The effluent contains selected environmental pollutants that tend to accumulate in the organism and persist in the aquatic environment due to chemical stability or poor biodegradability (Karthikeyan *et al,* 2011).

1.2 Justification of the Study

The Nile Tilapia (*Oreochromis niloticus*) is one of the most commonly farmed tilapia species in the world. It is also commonly used for hybridization to create tilapia variants even more suitable for farming in various conditions (Zagal and Mazmanci, 2011). It is a fast growing species that can live in many types of waters from lakes and rivers to sewage canals. It is highly adaptable and can make use of a wide range of different food sources (including plants), but feeds primarily on phytoplankton and benthic algae. Since Nile Tilapia is such a popular food fish it has been deliberately or accidentally introduced to the wild in many different parts of the world and as such, the Nile tilapia should be protected due to these special qualities. *Oreochromis niloticus* is commonly found in Nigerian rivers, it is a highly proteinous fish which contains essential amino acid, minerals, vitamins and some lipids all of Swhich are very useful to human growth.

Toxicity evaluation is an important cost effective tool in waste water quality monitoring, as it provides the complete response of test organism to all the compounds in a cumulative way. Also the studies are very useful for determining the safe concentration of waste water to be discharged into aquatic water bodies. Textile effluent has been proved to impact adverse effects on humans, animals and plants (Adeogun and Chukwuka, 2013).

1.3 Aim of the Study

This study is aimed at examining the effect of textile effluents on *Oreochromis niloticus* .

1.4 Objectives of the Study

To determine

i. The physico-chemical parameter and metal composition of textile effluents from textile industry
ii. The acute and chronic toxicity of the textile effluents on *Oreochromis niloticus*
iii. The acute and chronic effects on some blood parameters
iv. The effects of the textile effluent on the histopathology of the *Oreochromis niloticus*
v. The sub lethal toxicity on the feeding and growth of *Oreochromis niloticus*

1.5 Research Hypotheses

i. The physico-chemical parameters and metal composition of textile effluent do not differ significantly
ii. Acute and chronic exposure of *Oreochromis niloticus* to textile effluent do not differ significantly
iii. Acute and chronic effect of textile effluent do not have any significant difference on blood parameters
iv. Acute and chronic effect of textile effluent do not have any significant difference on the histopathology of the *Oreochromis niloticus*
vi. Chronic exposure of *Oreochromis niloticus* does not significantly affect feeding and growth.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Composition of Textile Effluent

Waste water generated by different production of textile mill have high pH, temperature, detergents, oil, suspended and dissolved solids, dispersants, leveling agents, toxic and non-biodegradable matter, color and alkalinity (Kumar, *et al.* 2007). Heavy metals pose a serious concern, (Shaw, 2006) to developing nations.

Heavy metals induce biomagnifications, (Endorh, 2007). They accumulate gradually in living organisms due to their poor biodegradability. The consumption of contaminated fish is one factor which constitutes bioaccumulation. Heavy metals are hazardous to humans even at low concentration (Adakole, 2000). The presence of heavy metals in textile effluents which are being discharged into water bodies reduces the developmental growth of fish and other aquatic organisms, (Tuberose, 2007). Farkas, *et al*. in 2000 argued that levels of heavy metals in water bodies and fish are as a result of uptake and release processes with characteristic kinetics for elements in their biological half life time, which are also influenced by the age of the fish, feeding habits and by season. Textile effluent is made up of divergent composition which is hazardous to the ecosystem. The effect of textile effluent on aquatic organisms is an issue which has attracted international concern (Fakayode, 2005) The receiving water bodies into which this effluent is dumped is daily used for domestic activities and there is little awareness on the hazardous effects of using these water bodies for domestic activities.

Eutrophication is the rapid growth of algae in water bodies. This results in the change of the water body from clear low biological productivity to a turbid and high productivity. It could occur naturally in stagnant water bodies like ponds and lakes. The dumping of textile effluent

into water bodies has accelerated biologic of productivity through the sedimentation of biologic of debris. It has been known to be one of the causes of eutrophication. Eutrophication reduces light penetration into aquatic habitats and restricts atmospheric reoxygenation of water. The death of the algae also has an adverse effect on the water. Its biodegradation results in anaerobic condition and the death of many aquatic organisms (Hodgson, 2004). Fish as, an aquatic organism is one of the worst hit of all the aquatic organisms. The heavy metals accumulate in the fish due to their poor bio degradability. The public awareness created on the presence of heavy metals in fish and its feed is low and as such, there is the possibility of having adverse effects on humans when consumed (Tacon,2007).

2.2 Effect of Textile Effluent on Living Organisms in the Environment

The effect of effluents from textile industries on fish, plants and other aquatic organism in the exposed water has been studied at various levels. Studies have also been extended to the plants and vegetables grown in the vicinity exposed to effluents from textile industries. The levels of Lead (Pb), Cadmium (Cd) and Chromium (Cr) in the tissue of *Talinum triangulare*, an economic plant grown around a textile industry in Ikorudu area of Lagos State was studied. The level of these metals in soil from the industrial area and the plants grown on the soil were found to be higher than its non-industrial area counterparts. The results obtained further confirm the increased danger of growing vegetables around industries, Akinola *et al*. (2008). Heavy Metals and Anion Levels in Some Samples of Vegetable Grown Within the Vicinity of Challawa Industrial Area, Kano State has been studied, concentration of heavy metals which include, Cu, Zn, Co, Mn, Mg, Fe, Cr, Cd As, Ni and Pb, were determined and were found to be higher than the FAO, WHO/EU and FAO/WHO allowed limit. The concentrations of the anions were also higher than the permissible limit (Akan, *et al.*, 2009). Adeogun and Chukwuka (2010) also worked on the differential sensitivity of saggital otolith

growth and somatic growth in *Oreochromis Niloticus* exposed to textile industry effluent, the effluent samples for this study they reported was collected from a textile company (Sunflag PLC) at Eric Moore, Lagos, Nigeria. Their result was subjected to statistical analysis and it shows that there was serious weight loss in the exposed fish as compared with the control fish. Olusegun *et al*, (2010) carried out studies on the comparison of cytogenotoxicity effects of "treated" industrial effluents discharge from textile and paint industries in Lagos metropolis *using A. cepa* root-tip assay. They reported that textile effluent was found to be 4.5 times more toxic than the paint effluent.

2.3 Bioassay Studies in Fishes

Bioassay plays a crucial role in the understanding of ecosystem functioning, but only as a tool, and more comprehensive studies of contaminated sites should be performed to understand the influence of chemical speciation and environmental factors on the toxicity of environmental pollutants. Toxicity tests have been conducted using various toxicants and reported by many authors.

Shaw, (2006) observed the morphological responses of *Oreochromis niloticus* exposed to textile effluent during acute toxicity .He observed that, the fishes introduced to different test tanks with different concentrations responded differently. Maruthi and Subba, (2000) recorded the LC_{50} of distillery effluent in the fish, *Channa punctaus*. Ganeshwade *et al*. (2000) studied the effect of acute toxicity of copper on three life stages of common carp, *Cyprinus carpio* and worked out the 96 hour LC_{50} value. Variations in the acute toxicity of Endosulfan and monocrotophos to *Labeo rohita, Mystus vittutus* and *Channa punctatus* was evaluated by Rao and Ramaneswari, (2000).

Toxicity levels of industrial effluents in Taiwan were studied using *Tilapia mossambica* by Chen *et al*. (2001). Srivastava, (2002) recorded LC_{50} of Cadmium chloride in the fish, *Heteropneustes fossilis.* According to Datta and Kaviraj, (2003) the determination of toxic

compounds presents in the aquatic environment and its effect on aquatic organisms is a basic issue in aquatic toxicology.

Mathivanan, (2004) reported on the toxicity of Quinalphos on alkaline phosphatase activity in *Oreochromis mossambicus.* Venkataramana *et al*. (2004) reported on the gobiid fish, *Glossogobius giuris* which was exposed to sub lethal concentrations of (0.05, 0.25 and 0.5 ppm) Organophosphorus pesticide, Malathion for short duration (24 to 96 hr). A Bioassay study for effluents from waste water treatment plants provides a complete response of test organisms to compounds present in waste water and to understand the discharge capacity of the raw and treated wastewater as reported by Movahedian *et al*. (2005).

Pandey *et al*. (2005) conducted 96-hour acute toxicity tests in flow-through systems to determine the lethal toxicity of Mercuric chloride and Malathion to air breathing teleost, C*hanna punctatus.* Verma *et al*. (2005) conducted the bioassay of dyeing effluent for 96 hour LC_{50} in *Clarias lazera* according to the graphical interpolation method. Naskar *et al*. (2006) and Madoni and Maria, (2006) measured the susceptibility and surviving potential of fish to particular toxic substances such as heavy metals. According to them to establish the discharge standards for pollution in the environment, it is important to study their toxicity on common flora and fauna of the area.

Murat *et al*. (2006) studied the acute toxicity of Organophosphorous pesticide, diazinon and its effects on fingerlings of European catfish, *Silurus glanis.* L. Muley *et al*. (2007) carried out acute toxicity (96 hours) experiment in fingerlings of fresh water fish, *Labeo rohita* which was exposed to tannery, electroplating and textile mill effluents. Yadav *et al*. (2007) reported on the toxicity of fertilizer‟s industrial waste water on snake head fish, *Channa striatus* (Bloch) previously named as *Ophiocephalus sp.*, at different concentrations viz., 20, 40, 60, 80 and 100 percent on the behavioural changes and mortality. In the acute toxicity of contaminants in static bioassays, the use of 96-hour LC_{50} has been widely recommended as a

preliminary step in toxicological studies on fishes, which was reported by Moreira *et al*. (2008). Zodape, (2010) studied on the effect of *Aloe vera* juice and chromium on *Labeo rohita* fingerlings exposed to sub – lethal concentration of Chromium and *Aloe vera* juice for 21 days. According to Ogundiran *et al*. (2010) the toxicity of commercial detergent effluent in the median lethal concentration (LC_{50}) values for lethal and sublethal tests were (0.0166 mg/L and 0.0038 mg/L) present in the juvenile of African Catfish, *Clarias gariepinus*. The toxicity of sodium cyanide to the freshwater fish, *Labeo rohita* was studied using static bioassay method of LC50 in 96 hour by Prashanth *et al*. (2011). Ganeshwade, (2011) studied on the freshwater fish, *Puntius ticto* which was exposed to lethal (5.012 ppm) and sublethal (2.50 ppm and 1.253ppm) concentration of Dimethoate for 96 hours and 60 days durations. Alireza *et al*. (2012) observed the sub-lethal effects of a herbicide, Paraquat (96 hours) on LC_{50} concentration in the fish, *Mesopotamichthys Sharpeyi*. Nikalje *et al*. (2012) reported on the fingerlings of *Labeo rohita* which were exposed to acute (96 hour) and chronic (30 days) dose of textile mill effluent. Acute toxicity of pesticide (Monocrotophos and Lambda cyhalothrin) on the fresh water fish, *Labeo rohita* was studied by Muthukumaravel *et al*. (2013).

2.4 Effects of Textile Effluent on the Histopathology of Fish

Gills histopathology has been recognized to be a reliable biomarker in water quality and related stress. (Vander Oost *et al*., 2003). Livers of exposed fish exposed to toxicant also exhibited hepatocellular swelling and necrosis (Auta, 2004). Increased prevalence of skin pathologies, epithelial and chloride cells were also associated to long term exposure of three to ten months to reclaim sites containing elevated levels of oil and constituent Naphthenic acids. The moderate histopathological alternation in gonads of *Channa punctatus* exposed to paper mill effluent was reported by Sayeed *et al*. (2000). Alterations in the size of nucleus in *Brachydanio rerio* exposed to sublethal concentrations of copper sulphate were studied by

Paris-Palacios *et al*. (2000). Erkmen *et al.* (2000) reported on the lifting of epithelial layer from gill lamellae, necrosis and degeneration of secondary lamellae, shortening of secondary lamellae and club-shaped lamellae in the gills of *Lepistes reticulatus* exposed to cyphenothrin.

Dass and Mukherjee, (2000) reported on the dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis in the nuclei of affected cells of *Labeo rohita* exposed to hexachlorocyclohexane. Peris and Kalaiarasi, (2001) studied on the histopathological response of catfish to chronic and acute toxicity of an organophosphate pesticide and found that the histopathological changes revealed that defense reactions occur in the fish to resist the pesticide. Koponen *et al*. (2001) stated that histopathological study of bream (*Abramis brama*) and asp (*Aspius aspius*) living in a PCB (Polychlorinated biphenyl)-polluted freshwater lake revealed abnormal cellular changes in the renal corpuscle of both species, dilation of glomerular capillaries, mesangial edema, an adhesion between visceral and parietal layers of Bowman`s capsule and filling of Bowman`s space.

Histopathological effects of sublethal concentration of monocrotophos on the gills of *Anabas testadineus* was reported by Santhakumar *et al*. (2001).

According to Esther *et al*. (2001) gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gill for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress.

Jiraungkoorskul *et al*. (2002) reported on the histopathological changes in the liver and gills of Nile tilapia, *Oreochromis niloticus* exposed to glyphosate herbicide. In the gills, filamentous cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were observed. In liver, there were vacuolations of hepatocytes and nuclear pyknosis. Velmurugan *et al*. (2003) reported on the congestion, cloudy swelling of hepatocyte and focal necrosis in liver of *Cirrhinus mrigala* exposed to fenvalerate. Ortiz *et al.*

(2003) showed tubular necrosis, desquamation and vacuolization of tubular epithelial cells in kidney of fishes exposed to Lindane. Roy and Bhattacharya, (2006) also reported similar results in fish exposed to arsenic.

Van Dyk *et al*. (2005) reported that the sublethal levels of metal mixtures of cadmium and zinc to have influence on the histological responses in exposed specimens with the most histological characteristics identified being hyalinization of hepatocyte, increase vacuolation associated with lipids accumulation, congestion of blood vessels and cellular swelling. Sarkar *et al*. (2005) observed hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocyte, focal coagulative necrosis, disorganised hepatic canaliculi in the fish of *Labeo rohita* exposed to cypermethrin.

Koca *et al*. (2005) studied on the water quality and the distribution of some heavy metals in gills of *Lepomis gibbosus* from the Cine stream and observed a significant decrease in mean length of primary and secondary lamellae. Moreover, cellular proliferation developed with secondary lamellae fusion, ballooning degenerations or club deformation of secondary lamellae, distribution of necrotic, hyperplastic and clavate secondary lamellae.

These alterations at the cellular level may explain fish behaviour in terms of kidney tubule pathology and relative amounts and conditions of organelles within affected cells.

Epithelial hyperplasia, aneurism, curling and fusion of secondary lamellae were noticed in *Gambusia affinis*, after 30 days of exposure to deltamethrin, which was observed by Cengiz and Unlu, (2006). Wangsongsak *et al*. (2007) found out prominent tubular and glomerular damage in the kidney of common silver barb, *Puntius gonionotus* when exposed to the nominal concentration of 0.06 mg L-1 Cd for 60 days.

Abdel-Moneim *et al*. (2008) reported on the histopathological changes in catfish, *Clarias lazera* exposed to dyestuff and chemical waste water. Histological analysis of gill samples revealed a range of lesions including lamellar fusion due to hyperplasia and

hypertophy of epithelial cells, sub-epithelial cell oedema, collapsed pillar cell system and extensive lamellar aneurism. Liver pathologies included extensive necrosis of hepatocytes, cytoplasmic vacuolation, distended sinusoids with massive congestion and infiltration of inflammatory cells throughout the liver parenchyma.

Osman *et al*. (2009) studied on the two environmental pollutants namely copper sulfate and lead acetate that induced histopathological changes in the fish, *Oreochromis niloticus.* Swarna and Tilak, (2010) reported severe changes in the histology of gills, which led to the disturbance in basement membrane, degeneration of gill lamellae, cyst formation, swelling of base, increased lamellar space in the fish, *Ctenopharyngodon Idella,* when exposed to sublethal concentration of Nuvan 76% EC.

Jayaseelan *et al*. (2011) suggested that the gill damage along with thick mucous deposition in the gill filaments resulted due to the exposure of sublethal concentration of herbicide glyphosate, which lead to decreased efficiency for gas exchange and breakdown of vital function of *Labeo rohita* and finally leads to the death of animals.

Palanisamy *et al*. (2011) observed that the electroplating industrial effluent nickel induced hyperplasia, multiple telangiectases (aneurysms), desquamation of the epithelial cells, complete fusion of secondary gill lamellae and congestion of blood sinuses were the significant histological lesions in the gill of *Mystus cavasius.* Liver showed abnormal fatty degeneration. Enlargement of liver was observed in few cases.

Necrosis, congestion and fatty degeneration were also observed. Histopathological alterations induced by diazion on the gills and kidney of Rainbow trout (*Oncorhynchus mykiss*) were reported by Banaee *et al*. (2013).

2.5 Effects of Textile Effluent on Heamatology

Blood is important as a supplier of essential nutrients, ions. It also functions as a reservoir of excretory products. Of metabolism, meaning alteration in blood parameters are often reflective of overall toxic impacts of environmental contamination, (Dietrich *et al.* 2006). Heamatological variables remain veritable tools in determining the sub-lethal concentration of pollutants such as heavy metals in fish, (Witeska, 2003). Stress is a general and non specific response to any fact disturbing homeostasis. The white blood cells were increased during stress while the hemoglobin and red blood cells decreased. The reduction in red blood cells and heamoglobin was probably responsible for the anaemic nature of the fish.

Significant increases in the haemoglobin concentration and the number of the haematocrit were found in *Carassius auratus gibelio* and they were attributed to the toxic effects of textile dyes which were studied by Al-Sabti, (2000). Haematological changes in fish may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants as reported by Affonso *et al.* (2002). Saxena and Seth, (2002) showed a significant change in the haematology of the common fresh water fish, *Channa punctatus* on exposure to pyrethroid. Gautam and Gautam, (2002) observed decrease in RBC, Hb content and increase in WBC of blood in *Channa striatus* treated with endosulfan and diazinon. Davids *et al.* (2002) observed significant haematological changes in two tilapine species, *Sarotherodon melanotheron* and *Tilapia guineensis*, from adjourning rivers receiving treated effluents from the National Fertilizer Company, NAFCON anda nearby petrochemical company.

Navaraj and Kumaraguru, (2003) observed that the fish, *Oreochromis mossambicus* exposed to electroplating effluent induced haematological changes with the increase in time of exposure and concentration of the effluent. Shah and Altindag, (2004) have reported decrease in Hb, RBC count and haematocrit in the fish, *Tinca Tinca* exposed to mercuric chloride and lead. Effluents released from various industries have been a major concern in causing aquatic pollution and a considerable number of researchers have been done on blood parameters of

fish to determine the effects of effluents from petroleum refinery in *black Goby* as reported by Selma and Hatice, (2004). Ramesh, (2006) studied haematological parameters in the *Clarias batrachus* exposed to treated sago effluent and revealed the elevation and declining trend in RBC,HCT, MCH and MCV.

Shasikumar and Nagarajan, (2007) reported on the alterations in the haematological parameters in the fish, *Clarias batrachus* exposed to different concentration of treated dairy effluent which may be due to physiological stress caused by the presence of high TDS, alkalinity, chloride and sodium in the effluent.

According to Maheswaran *et al*. (2008) the blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants. The decrease in the RBC, Hb, PCV and MCV concentration was reported by Benarjee *et al*. (2009) in the fish, *Channa puntatus* on exposure to Rayon industrial effluents. A decreased blood cholesterol of *Tilapia mossambica* exposed to detergent surf excel was observed by Shanthi *et al*. (2009). Majumdar *et al*. (2010) studied on the effect of galvanizing industry effluent on the haematological parameters of *Heteropneustes fossilis.* Analysis of haematological and biochemical profiles of blood are widely used as indicators to assess the toxic stress, functional status of the animal health and the internal environment of the organism as reported by Li *et al*. (2011) and Lavanya *et al*. (2011).

Alireza *et al*. (2012) recorded on the sublethal effects of herbicide paraquat on haematological parameters of benny fish, *Mesopotamichthys Sharpeyi.* It is suggested that the paraquat exposure might produced adverse effect on blood parameters of fish, which resulted in anaemia. This condition may affect normal growth, reproduction, immunity and survival of fish in both natural and culture conditions. Olaniyi *et al*. (2013) studied the effect of cassava mill effluent on haematological characteristics of Adult African cat fish, *Clarias gariepinus* which showed decreased levels of Hb, RBC and PCV when compared to control.

2.6 Effects of Textile Effluent on the Feeding of Growth and Fish

Adakole, (2000) reported that the fishes were fed three times daily on $3^0/_0$ biomass at $35^o/_o$ crude protein, this was done at 7.00am, 1.00am and 5.00pm, the experimental tanks were washed thoroughly two times a week. The retardation in growth and poor feed utilization was due to the introduction of toxicant into the water. Fishes are noted to increase their metabolic activities for the excretion of toxicants, hence, making more energy available for homeostatic maintenance than storage, which could be used for growth (Gbem *et al*., 2003). Shaw and Handy (2006) reported a reduction in growth associated with reduced food intake after feeding Nile tilapia with 2000 CU mg/kg food. The effects of sub-lethal doses of dimethoate (20, 10 and 5mg/L) and malathion (2.0, 1.0 and 0.5mg/L) on growth parameters of Nile tilapia (12.0cm and 40.0g weight) was investigated by Sweilum (2006). These in turn stimulated the release of amino acids, glycerol and fatty acids, present in the blood and increased the synthesis enzymes in the liver, which converted amino acids and glycerol into glucose (gluconeogenesis) (Ogueji and Auta, 2008). Shallangwa and Auta (2008) reported a reduction in growth of *Clarias gariepinus* exposed to sub-lethal effects of 2, 4-Dichlorophenoxy – acetic acid. The authors attributed this to lower feeding rate and or the toxicant made the feed unsuitable for consumption. They further opined that it could be due to an increased expenditure of energy on chemical detoxification and tissue repair.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Effluent and Fish

The textile effluent was collected from Funtua textile industry, Katsina state. Effluent was collected as whole effluent at the point of discharge. The effluent was collected into five gallons of 25ltrs of capacity, tightly capped and transported to the Department of Biological Sciences, Ahmadu Bello University, Zaria. The effluent was preserved in the fridge. *Oreochromis niloticus* used for the experiment was collected from Kano State Fisheries Institute Bagauda, Kano State. This was collected into oxygenated plastic bags and carefully transported to the Department of Biological Sciences. Fish was acclimatized for two weeks before the commencement of the experiment.

3.2 Physico-Chemical Parameters of Textile Effluent

3.2.1 Dissolved Oxygen

Dissolved oxygen was determined by collecting the effluent into 250 dissolved oxygen bottle (APHA 1985; 1990). The bottle was filled to the brim; 2mls of manganese sulphate solution and the alkaline iodine were added. The cork of the bottle was removed. 3m of the effluent was removed and titrated with Sodium Thiosulphate, it gave a pale blue color. Then 1ml of starch solution was added and titration continued until a colourless end point was obtained. The 1mL of the titrant is equivalent to the Mg dissolved oxygen Mg/L.

3.2.2 Electrical Conductivity

The electric conductivity was determined using Hanna Model H113952 instrument. This instrument was pre rinsed and set at a zero point. The electrode was then dipped into the textile effluent and the reading noted and recorded in µS/cm.

3.2.3 Temperature

Mercury in glass thermometer was used to determine the temperature of the effluent. The thermometer was inserted into the effluent and allowed to stabilize. The temperature was then taken at the initial and final stages of the experiment.

3.2.4 Biological Oxygen Demand

This was determined by using EPA method. 100MLS of the effluent was placed in 300mls of BOD bottles. 1ml each of phosphate buffer was added to $MgSO_3$, $CaCl_2$, $NaSO_3$, and $FeCl_3$ solutions per litre of water. The mixture was then filled to 300mls mark and incubated for five days.

$$\text{BOD, } \frac{D1 - D2}{P}$$

Where D1 = D0 of dilute sample immediately after preparation.

D2 = D0 of dilute sample after five days of incubate

P= decimal volumetric fraction of sample used.

3.2.4 pH

pH was determined using Hanna multi parameter instrument model (H13592). It was immersed into the textile effluent for one minute to obtain a stable reading of the pH.

3.2.5 Total Dissolved Solids

This was analyzed using Hanna conductivity meter. It was pre rinsed in water and set at zero point, it was then dipped into the textile effluent and reading noted accordingly.

3.2.6. Total Alkalinity

About 100ml of the water sample was poured into a conical flask after which 2 drops of methyl red solution and 2 drops of Bromo Creasol green were added, a faint greenish color was observed then Shake well. This was then titrated against 0.02N standard sulphuric acid solution until a pink colour was observed which served as the end point APHA (1989). Total alkalinity = mg/L = ml of titrant × 10.

3.2.7 Total Hardness

About 25ml of the water sample and 25ml of distilled water was poured into a beaker after which 2ml of buffer solution of pH 10.4 was added and chips of Errochrome black T dye were added, this was then titrated against ethylenediamine tetracetic acid (EDTA) titrant(0.01M) until it changed to blue. The titrant value was multiplied by 40, as CaCO3/L APHA (1989).

3.3 Dechlorination of Water

The test water was dechlorinated by aeration. This was done during the period of acclimatization. The water was changed twice weekly. This was done by siphoning the water from the pond. About three quarter of the water was removed and replaced with chlorinated water.

3.4 Heavy Metals Analysis

This was analyzed using Energy Dispersive X-ray Florescence (EDXRF), at NARICT. The elements determined were aluminium, chromium,iron, copper, lead, and zinc .

Water samples were collected for 2 months and preserved by adding 1.0ml of conc. HNO_3 to about 150ml of the sample. This was stored under refrigeration to stabilize the metals. The wastewater sample was digested using the procedure below. Samples were placed in a pre-acid rinsed tubes (PP, LDPE, Teflon, etc.), carefully recording its volume or weight. To the sample, Optima grade nitric acid was added and the mixture was mixed by repetitive pipetting. The volume of nitric acid added depends on sample size. Sample was placed in a hot-block set to 90 degrees Centigrade for approximately two hours. When no further color change was seen and sample particulates were no longer visible, the sample is removed from the hot block and allowed to cool. The digestate was diluted to a final acid concentration that was similar to that of the calibration standards. This digestion protocol would take approximately 4 hours for 15 sample repetitive pipetting.

10% of APDC (Ammonium Pyrrolidine Dithiocarbamate, $C_6H_{12}N_2S_2$) was added to 100ml of acidified water sample and allowed for 15 to 20 minutes. The sample was then filtered using Millipore membrane filter in a filtration unit by the aid of a vacuum pump. The precipitated on filter was then measured. Measurements were performed using an annular 25mCi Cd-109 as the excitation source, that emits Ag-K X-rays (22.1KeV) in which case all elements with characteristic excitation energies were accessible for detection in the samples. The system consists furthermore of a Si(Li) detector, with a resolution of 170eV for the 5.90KeV line, coupled to a computer controlled ADC-card.

Quantitative analysis of the samples was carried out using the Emission-Transmission (E-T) method for which a number of quantification methods have been developed and applied (Leroux and Mahmoud, 1966, Giauque *et al*, 1979, Markowicz, 1979, Markowicz and Van Grieken, 1993, Kump, 1996, Bernasconi *et al.*, 1996, Tang *et al.*, 1986). This work quantification was carried out using a modified version of E-T method (Angeyo *et al.,* 1998, Funtua, 1999a, Funtua, 1999b) and it involved the use of pure target Material (Mo) quantification methods provide different approaches to correct the matrix absorption as well as enhancement effects, in this is to measure the absorption factors in the sample.

The Mo target serves as a source of monochromatic X-rays, which are excited through the sample by primary radiation and then penetrate the sample on the way to the detector. In this way, the absorption factor was experimentally determined which the program used in the quantification of concentration of the elements.

Sensitivity calibration of the system was performed using pure metal foils (Ti, Fe, Co, Ni, Cu, Zr, Nb, Mo, Sn, Ta, Pb) and stable chemical compounds (K_2CO_3, $CaCO_3$, Ce_2O_3, WO_3, ThO_2, U_3O_8). The spectra for the samples were collected for 3000s with the Cd-109 source.

3.5 Pilot Study

Pilot study was carried out to determine the definitive concentration of the effluent to be used for the experiment. This was done according to APHA (1985). According to this method, the concentration of toxicant in each treatment should be at least fifty percent of that in the next higher one also one concentration other than the control should be less than thirty seven percent. The percentage capable of killing more than sixty three percent, should be included in the concentrations.

3.6 Experimental Design

The renewal method of bioassay 96h were conducted in the laboratory following methods by Spraque (1973) and APHA (1985) to determine the toxicity of the textile effluent against *Oreochromis niloticus.* The bioassay test was carried out in 12 glass tanks each of size 30.5 X 30.5 by 92.5cm into which approximate quantity of the textile effluent was taken to give a final volume of 25.0L. The nominal concentrations of wastewater used were 0.0, 5, 25, 50, 75 and 100.

The mixture of the toxicant and dilution water was allowed to stand for 30minutes before the introduction of test fishes.

The fishes were randomly distributed to each glass tank as suggested by Spraque (1973). Each tank contained 10 fish. Each assay was replicated simultaneously and repeated once to determine reproducibility. Aeration of the test water was suspended a few minutes prior to addition of the toxicant and during the test. The toxicant and the test water were renewed after 48hours during each series to test. The fish were starved 24hours prior to the start and during each assay test.

Acute toxicity was conducted according to APHA (1985). The acute toxicity was conducted to determine the toxicity of the textile effluent on *Oreochromis niloticus*. This was done according to APHA (1985). Twelve tanks of the aquaria of size 30.5 x 30.5 by 92cm were

filled with 25L or 15L textile effluent of concentration $25^{0}/_{0}$, $30\ ^{0}/_{0}$, 35 40 and 45. These concentrations were allowed to stand for 30minutes before the introduction of fish. The fish were starved 24hours before the commencement of the experiment. Ten fish were randomly distributed into the five glass tanks and this was done in duplicates. The effluents were renewed 48hours during each series of test.

Fig.3.1 showing Species of *Oreochromis niloticus* used for the experiment.

3.7 Behavioural Studies

In the course of the experiment, behavioural studies of the fish were carried out. The morphological and behavioral responses of the fish were made at 12hrs, 24hrs, 48hrs, 72hrs and 96hrs. Symptoms of toxicosis in the fish were scored in ascending order of severity and recorded.

Each of the tanks was observed for fifteen minutes in order to enhance accurate evaluation.

3.8 Opercular and Tail Fin Movement

This was counted at intervals of 12, 24, 48, 72, and 96hrs, and the number of deaths per minutes was recorded. This was done with the help of stop watch.

3.9 Mortality

The mortality of the fish was determined through the observation that was carried out at 12, 24 48,72and 96hrs and the fish was considered dead when there was no response to gentle probing. The dead fish were then removed in order to avoid oxygen depletion in the water. (Schrech and Brouna,1975).

3.10 LC_{50}

The lethal concentration was calculated using minitab version16.0. The percentage mortality was converted to probit scale through probit table (Finey, 1964). The concentration was transformed into logarithm. Mortality analysis was then plotted against concentration. Regression analysis was then used to fix the straight line in the graph. The value of x when Y=5 was calculated by using a straight line equation (Y = a+bx). This was used to determine the $LC_{50.}$

3.11 Feeding and Growth Studies

Oreochromis niloticus was fed on commercial fish feed at $3^0/_0$ biomass on $35^0/_0$ crude protein diet per day. Daily ration was shared into three and fed to the fish three times a day. Feeding was done at 7.00am, 1.00pm and 5.00pm.The quality of diet was adjusted weekly based on

fish weight increment. The experimental tanks were washed thoroughly twice a week and the waste from the fish was drilled out with the help of flexible rubber tube.

3.11.1 Analysis of Fish Growth and Nutrient Utilization

Data was collected based on the initial and final weight of the fish, this was computed thus

I. Weight Gain(WG)

The fresh weight gain was calculated as the difference between the initial weight and the final weight.

WG = FW-IW

Where

WG = Weight

FW = Final Weight

IW = Initial Weight

II. Percentage Live Weight Gain ($LWG^{0}/_{0}$)

The initial live weight gain was computed as the difference between the initial and final weight divided by the initial weight expressed as percentage (Wanningamma *et al*., 1985)

$$LWG = \frac{Wt - W_O \, X100}{Wo}$$

Where Wo = initial fish Weight (g)

Wt = Final fish weight (g)

III. Specific Growth Rate (SGR)

This was calculated according to Herper (1988)

$$SGR = \log Wt - \frac{\log Wo \times 100}{t - to}$$

Where Wt = final Weight

Wo = initial Weight

t – to time interval between the initial and final weight(days)

IV. Feed Conversion Ratio (FCR)

This was calculated by computing the dry weight of the of feed offered divided by the weight gain of fish (Shallaby *et al*., 2006)

$$FCR = \frac{\text{Feed supplied(g)}}{\text{Net fish produced}}$$

V. Gross Feed Conversion Efficiency(GFCE)

This was done according to (Shallaby *et al*.,2006)

1. $GFCE = \frac{I \times 100}{FCR}$

3.12 Histopathology

This was done according to Allen *et al*. (1983). The tissues (gills and livers) were removed from the dissected fish. These were fixed in $10^0/_0$ formalin. The tissues were removed and washed in running water for 2hours to remove the traces of fixatives. The tissues were then removed and dehydrated in successive percentages of alcohol such as such as 30%, 50%, 70%, 90% and100%. The tissues were then infiltrated in chloroform and blocked in paraffin wax 58-60^{0}C melting point. Samples were embedded in fresh molten wax using L shaped embedding moulds. Sections were removed and stained in heamatoxylin and eosin.

Permanent slides were prepared with these sections and micrographs taken with a magnification of X400.These slides were compared with that of control.

3.13 Collection and Analysis of Blood

This was done according to Blanxhall *et al.* (1973). Six samples of fish were collected from the tank containing the textile effluent and some from the control tank. Their caudal peduncles were severed and blood obtained from their caudal arteries. The blood was collected into heparin zed micro heamatocrit tubes. This was done bi weekly 40 minutes after the exposure of the fish (Klont and Smith 2008).

3.13.1 Total Erythrocyte Count

The Neubaucers chamber was prepared and blood drawn at 0.5mls. The pipette was filled to 101 mark with dilution fluid. It was thoroughly shaken for proper mixing. The dilute suspension of cells was drawn into the chamber. The heamocytometer was placed under the microscope. The cells at the boundaries of the small squares were counted and viewed with microscope. The cell were then multiplied 106 this gave the total number of cells per cubic milliliter, (Babatunde, 1997).

3.13.2 Total Leucocytes Count

Leucocytes were counted by using Shaw‘s solution A (neutral red (25mg), sodium chloride (0.9g), distilled water (100mls) and B (crystal violet (12mg), sodium citrate (3.8g), distilled water (100mls)). The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shake the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed in to the haemocytometer. The cells in the four large squares of the chamber (Hesser, 1960) were counted with 4mm objective and X40

eyepiece microscope. The number of cells was multiplied by 500 to obtain the total number of leucocytes per cubic millimeter (mm3) of blood (Hesser, 1960).

3.13.2 Haematocrit (Packed Cell Volume)

Blood samples were collected in the heparinised capillary tube to determine the haematocrit, the end of the tube was sealed with plasticine and the tubes spun for 4 minutes in a micro haematocrit centrifuge (Gallen Kamp D-7200 model) at the speed of 12500ppm. The haematocrit was measured with a micro capillary reader to obtain the value for the packed cell volume (PCV).

3.13.3 Haemoglobin

0.02mls of well mixed blood was added to 0.4mls of Drabkins reagent. The solution was mixed gently by the method of inversion and allowed to stand for conversion of haemoglobin to cynanomethaemoglobin. Small wooden steel was used to remove any coagulating formed. A well mixed blood was placed in the curved of the colorimeter and reading recorded using optical density from a standard haemoglobin table. The method used was the cyanomethaemoglobin method (Klonz, 1972).

3.13.4. Leucocytes Differential Count

Two drops of blood was placed on a slide, made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with giemsa‘s stain and 170 buffered distilled water. It was allowed to stand for about 20-30 minutes after which the slide was rinsed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope and the parameters counted include neutrophils, lymphocytes, basophils, eosinophils, monocytes. This was applicable for both acute and sub-lethal bioassay.

3.13.4 Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

The values for mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) they were calculated based on the equations given below (Dacie and Lewis 1975, Adewuyi, 2007),

Mean Cell Volume (MCV)

$$MCV\ (m^3) = \frac{Ht\ (\%)\ X\ 10}{RBCC\ (cells/mm3)}$$

Where,

Ht= Haematocrit

RBCC= Red Blood Cell Count

Mean Corpuscular Haemoglobin (MCH)

$$MCH\ (g/cell) = \frac{Hb(g100m\ x10}{RBC(cells/mm3)}$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

$$MCHC\ (G/100ml) = \frac{Hb(g/100ml)\ x\ 100}{PCV\%}$$

3.14 Statistical Analysis

Data that was obtained from behavioural, heamatological and concentration of heavy metals in wastewater was subjected to Analysis of Variance (ANOVA) using Microsoft Excel for Windows 2007 and Duncan Multiple Range Test (DMRT) were used to test differences between levels of treatment and to separate means respectively. Probit Analysis was computed using Minitab Statistical tool to determine 96h LC_{50} for the concentration of the toxicants.

CHAPTER FOUR

4.0 RESULTS

4.1 Physico-chemical Analysis of textile effluent

The following values were recorded for the physico-chemical parameters in the textile effluent in the test tanks during the research. Some of the parameters with high values affected the behavior of the fish in the test tank.

4.1.1 Total Dissolved Oxygen (TDO)

The values obtained for total dissolved oxygen in the treatment tanks is presented in Table 4.1. The result showed a significant difference between the control and the treatments ($p<0.05$) the control had values higher than those of the treatments and the values remained fairly constant during the exposure period.

4.1.2 Electrical Conductivity (EC)

Table 4.2 represents values obtained for electrical conductivity in the treatment tank and the control tank. The result showed that there was a significant difference between the control and the treatments ($p<0.05$) the control had values lower than those of the treatments and at higher concentration electrical conductivity had higher values, the control had lower values which remained fairly constant throughout the study period.

Table 4.1 Effects of textile effluent on Dissolved Oxygen (mg/l) during the acute study.

Concentration(mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	4.30^{ab}	4.20^{b}	3.70^{c}	3.10^{d}
40	3.90^{c}	3.80^{c}	3.90^{b}	3.40^{d}
35	3.70^{d}	3.90^{c}	4.20^{ab}	4.30^{b}
30	3.80^{c}	3.30^{d}	3.60^{c}	3.70^{c}
25	4.00^{b}	3.30^{c}	3.40^{d}	4.10^{bc}
0.0	5.70^{a}	5.60^{a}	5.75^{a}	5.65^{a}
Mean	4.23	4.02	4.09	4.04
S.E.M	0.31	0.35	0.35	0.37

S.E.M=Standard Error of Mean

Dissolved Oxygen (mg/L) across the column with different superscripts are significantly different ($p<0.05$)

Table 4.2 Effects of textile effluent on Electric Conductivity (µS/cm) during the acute study.

Concentration(mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	933^{a}	920^{a}	925^{a}	923^{a}
40	726^{b}	720^{ab}	730^{b}	745^{b}
35	490^{cb}	450^{b}	505^{c}	510^{c}
30	395^{c}	410^{b}	460^{c}	440^{c}
25	310^{c}	290^{c}	285^{cd}	315^{cd}
0.0	176^{d}	175^{d}	178^{d}	170^{d}
Mean	505	494.17	513.83	517.17
S.E.M	114.11	113.27	113.12	113.12

S.E.M=Standard Error of Mean

Electric conductivity (ms) across the column with different superscripts are significantly different ($p<0.05$)

4.1.3 Temperature

Figure 2, Present results for temperature during the acute study and values obtained showed that control and the treatment tanks shows variation with significant difference ($p<0.05$) recorded between 72Hr and 96Hr of the exposure period. Values of the control tank were fairly constant except in the 72Hr.

4.1.4 Biological Oxygen Demand (BOD)

The values obtained for Biological Oxygen Demand in the treatment tank is shown in Table 4.3. The treatment had higher level when compared with control which had lower levels and this is due to fact that the treatment tanks had concentration of the toxicant and exposure time, there was significant difference ($p<0.05$) between the treatments and the control group during the study period.

4.1.5 pH

The mean values of pH of different effluents concentration and exposal time are summerised in Table 4.4. The pH of different concentrations of effluent was significantly higher ($p<0.05$) than the control. The toxicant showed increase in with increase in concentration. There was significant difference ($p<0.05$) between the lowest concentration, 25mg/L and the highest concentration of 45mg/L.

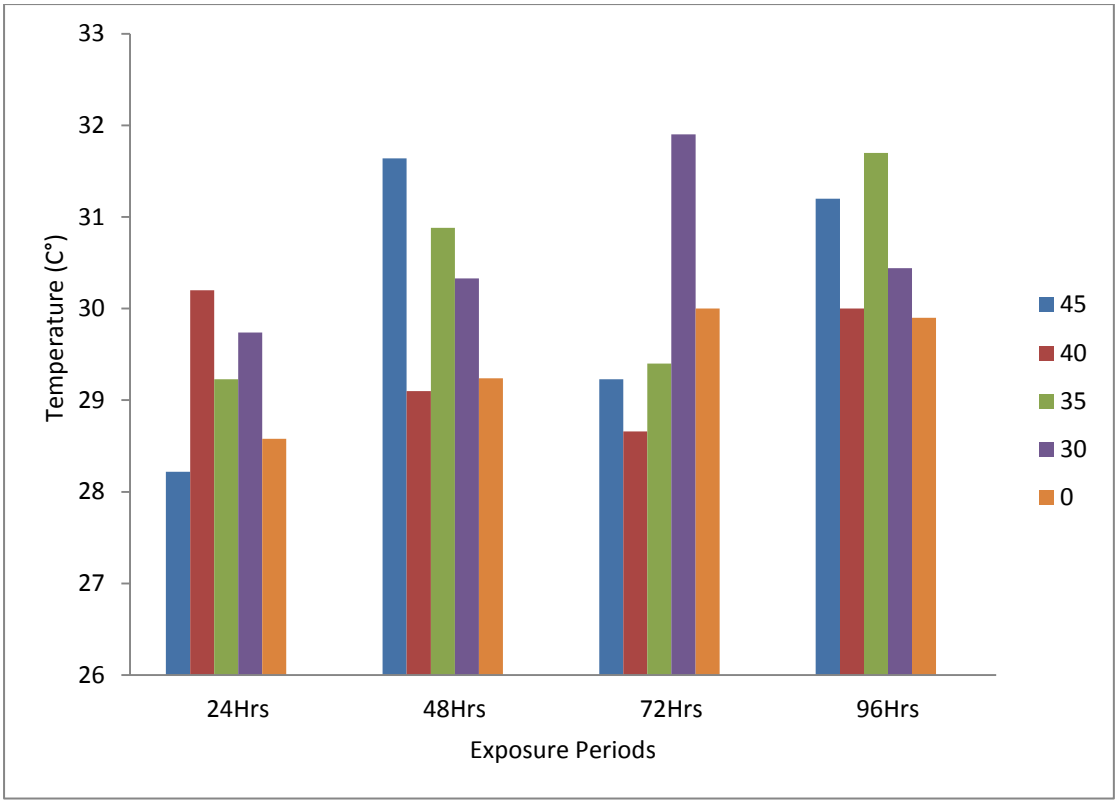

Fig.4.2 Graph of Mean±SE showing effects of textile effluent on Temperature (^{0}C) during the acute study.

Table 4.3 Effects of textile effluent on Biological Oxygen Demand (mg/l) during the acute study.

Concentration (mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	3.20^{a}	3.10^{a}	3.20^{a}	3.40^{a}
40	2.80^{b}	2.90^{ab}	2.70^{bc}	3.10^{a}
35	2.60^{b}	2.70^{b}	2.80^{b}	2.60^{b}
30	2.50^{c}	2.30^{b}	2.40^{c}	2.30^{c}
25	1.90^{cd}	2.10^{c}	2.20^{c}	2.30^{c}
0.0	0.30^{d}	0.40^{d}	0.20^{d}	0.40^{d}
Mean	2.27	2.25	2.25	2.35
S.E.M	0.42	0.40	0.43	0.42

S.E.M=Standard Error of Mean

Biological Oxygen Demand (mg/L) across the column with different superscripts

Table 4.4 Effects of textile effluent on pH during acute study.

Concentration mg/L	24Hrs	48Hrs	72Hrs	96Hrs
45	8.80^{ab}	9.30^{a}	9.80^{a}	10.00^{a}
40	8.70^{b}	8.60^{bc}	9.50^{b}	9.90^{a}
35	8.85^{a}	8.95^{b}	9.10^{b}	9.60^{c}
30	8.72^{b}	8.50^{cd}	8.90^{c}	9.80^{b}
25	8.50^{c}	8.90^{b}	8.70^{c}	9.70^{c}
0.0	7.15^{d}	7.10^{d}	7.20^{d}	7.10^{d}
Mean	8.45	8.56	8.87	9.35
S.E.M	0.27	0.31	0.37	0.45

S.E.M=Standard Error of Mean

pH along the column with different superscripts are significantly different ($p<0.05$)

4.1.6 Total Dissolved Solids (TDS)

Figure 3 represent values for total dissolved solids the result shows that there was variation between the treatments and the control. The control with no toxicant had lower values while the treatments had higher values and there was significant difference at ($p<0.05$) between the group with concentrations and the control group.

4.1.7 Total Alkalinity

The values presented in Table 4.5 showed variation between the treatments and the control, concentration and exposure period had effect on total alkalinity and between treatments and the control group there was significant difference ($p<0.05$). Exposure time had no effect on total alkalinity**.**

4.1.8 Total Hardness

Table 4.6, present the results for total harness and it shows that there was variation between the treatments and the control. The control had lower levels compared to the treatment that had higher level and there was significant difference ($p<0.05$) between the group with concentrations and the control group.

4.1.9 Nitrite

The values obtained for nitrite in the test tank are shown in Table 4.7; the result shows that there is variation between the treatments and the control. The control had lower levels compared to the treatment that had higher levels and there was significant difference ($p<0.05$) between the group with concentrations and the control group.

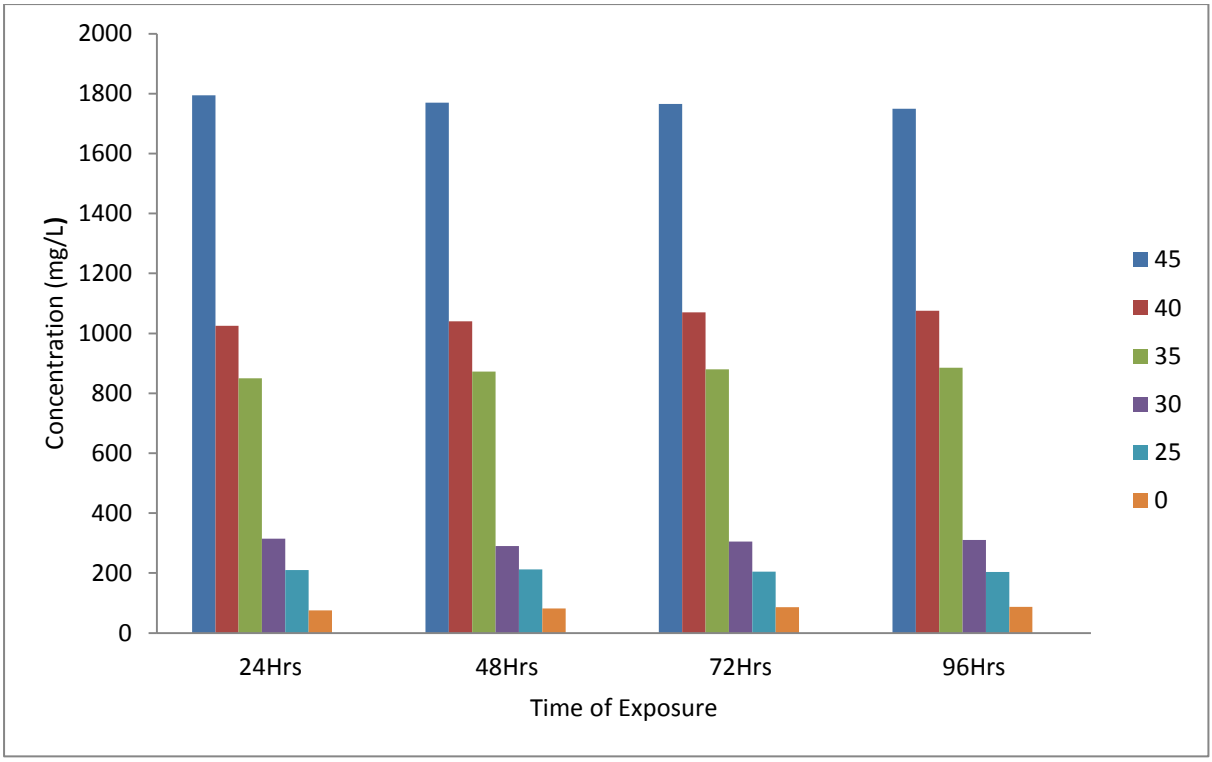

Fig.4.3 Graph of Mean±SE showing effects of textile effluent on TDS during the acute study.

Table 4.5 Effects of textile effluent on Total Alkalinity (mg/l) during the acute study.

Concentration (mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	3.68^{a}	3.76^{a}	3.80^{a}	3.93^{a}
40	2.98^{ab}	3.00^{b}	3.15^{ab}	3.20^{b}
35	2.90^{b}	2.70^{b}	2.55^{b}	2.75^{c}
30	2.50^{c}	2.40^{c}	2.48^{b}	2.60^{c}
25	2.20^{c}	2.33^{c}	2.30^{c}	1.90^{cd}
0.0	0.8^{d}	0.6^{d}	0.5^{d}	0.8^{d}
Mean	2.51	2.47	2.46	2.53
S.E.M	0.40	0.43	0.45	0.44

S.E.M=Standard Error of Mean

Total alkalinity across the column with different superscripts are significantly different ($p<0.05$)

Table.4.6 Effects of textile effluent on Total Hardness (mg/l) the acute study.

Concentration (mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	27^{a}	29^{a}	29^{a}	33^{a}
40	25^{ab}	26^{b}	28^{b}	30^{a}
35	23^{b}	25^{c}	28^{b}	29^{ab}
30	22^{b}	24^{c}	23^{c}	25^{b}
25	14^{c}	16^{cd}	17^{c}	19^{c}
0.0	2.11^{d}	2.42^{d}	2.55^{d}	2.73^{d}
Mean	18.85	20.40	21.26	23.12
S.E.M	3.81	4.01	4.17	4.53

S.E.M=Standard Error of Mean

Total Hardness (mg/L) across the column with different superscripts are significantly different ($p<0.05$).

Table 4.7 Effects of textile effluent on Nitrite during the acute study.

Concentration (mg/L)	24Hrs	48Hrs	72Hrs	96Hrs
45	4.10^{a}	4.25^{a}	4.60^{a}	4.70^{a}
40	3.40^{b}	3.50^{ab}	3.97^{a}	4.05^{ab}
35	2.95^{bc}	3.08^{b}	3.15^{b}	3.20^{b}
30	2.46^{c}	2.70^{c}	2.90^{b}	3.00^{b}
25	2.22^{c}	2.30^{c}	2.66^{d}	2.80^{c}
0.0	0.013^{d}	0.016^{d}	0.017^{d}	0.021^{d}
Mean	2.52	2.64	2.88	2.96
S.E.M	0.57	0.59	0.64	0.66

S.E.M=Standard Error of Mean

Nitrite (mg/L) across the column with different superscripts are significantly different ($p<0.05$).

4.1.10 Metals Studies in the textile effluent

Mean metal content (mg/L) obtained from the textile effluent are shown in the Table 4.8. Six metals which were analysed in the textile effluents at the beginning and at the end of the sub-lethal exposure time. The means of the values obtained were compared with those of WHO (1984)/FEPA (1991). The following metals were analysed using EDXRF, Aluminium, Chromium, Iron, Copper, Lead and Zinc. Chromium, Iron, Copper and Lead had values greater than the level recommended by WHO (1984)/FEPA (1991). The value for Zinc was lower than the recommended standard as presented in Table 4.8.

4.2 Acute toxicity test

4.2.1 Behavioural Responses

Behavioural and morphological responses of *Oreochromis niloticus* exposed to acute toxicity in test tanks containing the different concentrations of textile effluents the fishes behave differently in different concentrations of the textile effluents as shown in Table 4.9. Immediately after transferred into the test solution, the fishes became hypersensitive and showed a rapid rate of opercular movement accompanied by occasional gulping of air. They also exhibited restlessness erratic swimming, agitation and rapid respiration rate, loss of equilibrium, flashing and crowding at the water surface. After long periods of motionlessness, it was also observed that the fish lied down on the bottom of the test tanks and suddenly started to move. At the time of death, the mouth and operculum were open. The dead fish showed swollen abdomen and there were accumulation of copious mucus observed in the gill filaments and body surface of dead fish. The intensity of the mucus increased with the concentration of the toxicant, comparing the fish with those of the control tanks, they were calm and showed no sign of restiveness or restlessness.

Table 4.8 Mean Metal content of Textile Effluent

Parameter	Wastewater (mg/L)	Mean ±S.E.M	WHO(1984)/ FEPA (1991
Aluminium	0.16	0.08±0.01	NS
Chromium	0.18	0.09±0.01	0.05
Iron	2.82	1.41±0.04	0.3-1.0
Copper	0.12	0.06±0.02	0.05
Lead	0.22	0.11±0.04	0.05
Zinc	0.36	0.18±0.01	1.50

WHO (1984)/FEPA (1991) limit for effluent discharge into surface water,

NS: Not Stated

Table 4.9 Behavioural and morphological effects Textile effluents on fingerlings of *Oreocchromis niloticus*

Behavioural and Morphological Response	Diagnosis
General activity	Vertical position. Hyperactive to hypoactive(hyperactive- fish swim faster than the control fish, hypoactive-fish swim slower than the control fish with increased exposure of the toxicant).
Loss of equilibrium	It was observed that fish roll over on side or back
Startle response	Over reactive to underactive i.e. darts away from stimuli faster than the control of initial exposure period, under reactive- fish darts away from stimuli slower than the control fish with increase to the exposure time).
Haemorrhage	None

4.2.2 Opercular ventilation count (OVC) and Tail fin movement rate (TMR)

Table for 4.10 shows the mean of OVC and TMR, there was significant difference ($p<0.05$) between the control and the different concentrations of the textile effluent. A marked reduction of OVC was observed at 12hours. A high increase was recorded at 48 and 72hours. On the other hand, results of TMR showed a marked decrease with increase in concentration of the toxicant.

4.2.3 Mortality Rates

Table 4.11 shows mortality of the fish during the acute bioassay was recorded in all the treatment tanks except in the control tanks. The first mortality was observed at the first 12hours of exposure of fingerlings of *Oreochromis niloticus* to the textile effluent. Mortality was observed in the 25ml/L, 30ml/L, 35ml/L, 40ml/L and 45ml/L of the toxicant. The highest mortality was recorded in the treatment tank with 45ml/L within the first 48hours of exposure while the lowest was recorded was recorded in the treatment tank with 25ml/L of the toxicant. Mortality rate was dose dependent and time of exposure too played a role in the mortality of the fish, with increase in exposure rate of exposure also increases.

4.2.4 Determination of 96 hours LC_{50}

The 96 hour lethal concentration LC_{50} was estimated to be 33.520ml/L by probit analysis (fig.4.1). The dose response relationships were transformed in probit mortality and transformed into probit mortality and plotted against log concentration of the toxicant.

Table 4.10 Time effect of acute nominal concentrations of textile effluent on some behavioural Parameters of *Oreochromis niloticus*

Exposure Period (Hrs)						
Parameters	0	12	24	48	72	96
OVC	88.2±0.15^{d}	78.8±1.35^{a}	85.4±1.57^{a}	93.2±0.64^{c}	91.2±0.84^{ab}	88.4±0.46^{c}
TMR	112.6±1.03^{a}	107.7±0.41^{d}	104.7±0.40^{d}	101.3±0.64^{c}	74.1±0.89^{b}	55.7±0.69^{c}

Table 4.11. Mortality Rates and Log of Conc. in *Oreochromis niloticus* exposed to Acute Concentration of Textile effluents

Concentration (ml/L)	N0 of exposed fish	Mortality Rates	Log of con.
45	20	20	2.0000
40	20	12	1.9911
35	20	10	1.9751
30	20	6	1.9500
25	20	4	1.9201
0.0	20	0	0.0000

Values representing mortality rates and values of log conc. at different concentration

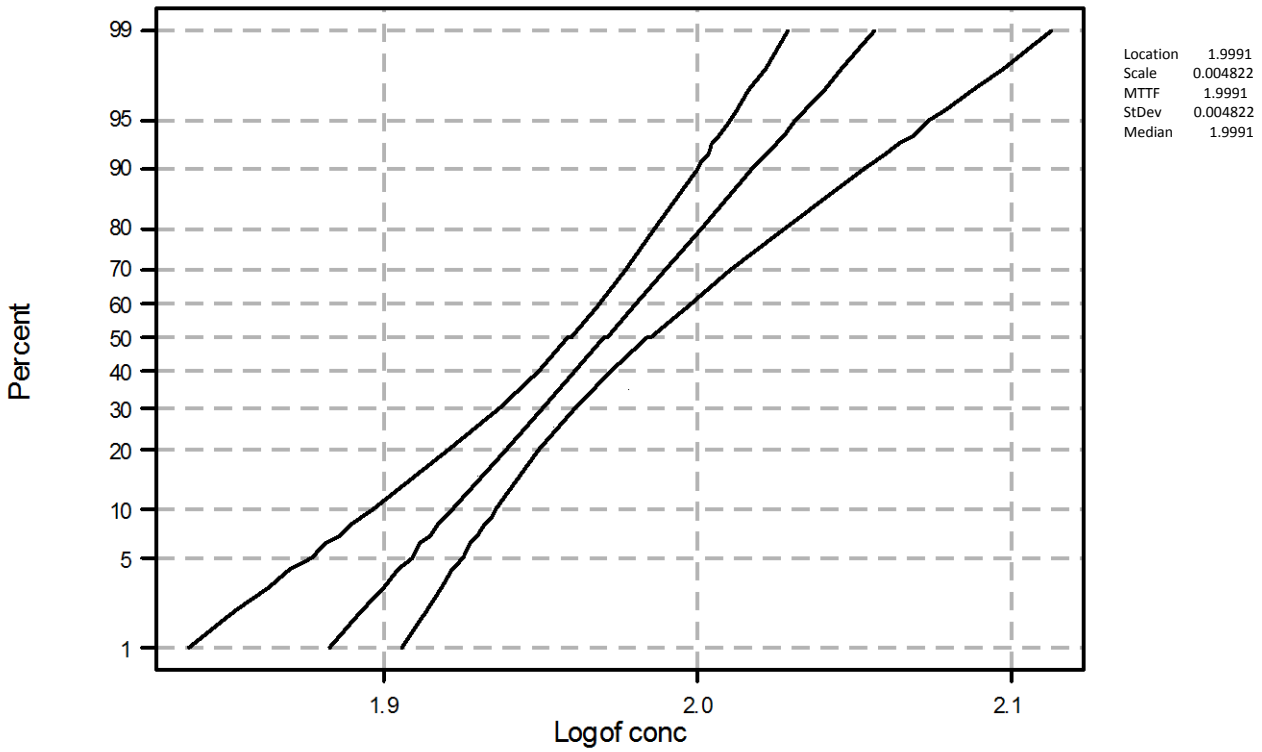

Fig.4.4. 96hour of toxicant from textile effluent on fingerlings of *Oreochromis niloticus*

4.3.0 Histopathology: Acute Exposure

4.3.1 Gill

Histological sections of the gill are presented in Plates I, II, III and IV. Gill of control fish showed a normal structure with the filament and lamellae consisting of filaments attached to cartilaginous gill bar with fingerlike projections (secondary lamellae) on each side of the gills (Plate. I). Sections of gill from different concentration of the textile effluents were the most damaged as observed in Plate II. Plate. III shows disappearance of the primary lamella. Plate IV shows fish exposed to 45ml/L wastewater there was oedema of gills lamella, it also shows filament erosion and complete filament detachment. Observations made showed proliferative lesions in the gill epithelium and mutilation of the gill rakers. Damage done to the gills was dose-dependent.

4.3.2 Liver

Plate. IV shows the features of liver sections of control fish. Observations showed hepatocytes and other cells systematically arranged. The epithelium of the veins is lined with various epithelial cells. Plate. V that is fatty degeneration of the liver. In fish exposed to 35ml/L of wastewater, necrotic hepatocytes were observed. Oedema of the liver was dose dependent. Plate VI shows extension of the areas of necrosis due to effect of the textile effluent it was similar too in Plate VII. Plate VIII showed the central vein being stretched due to the effect of the textile effluent.

4.4.Histopathology:AcuteExposure

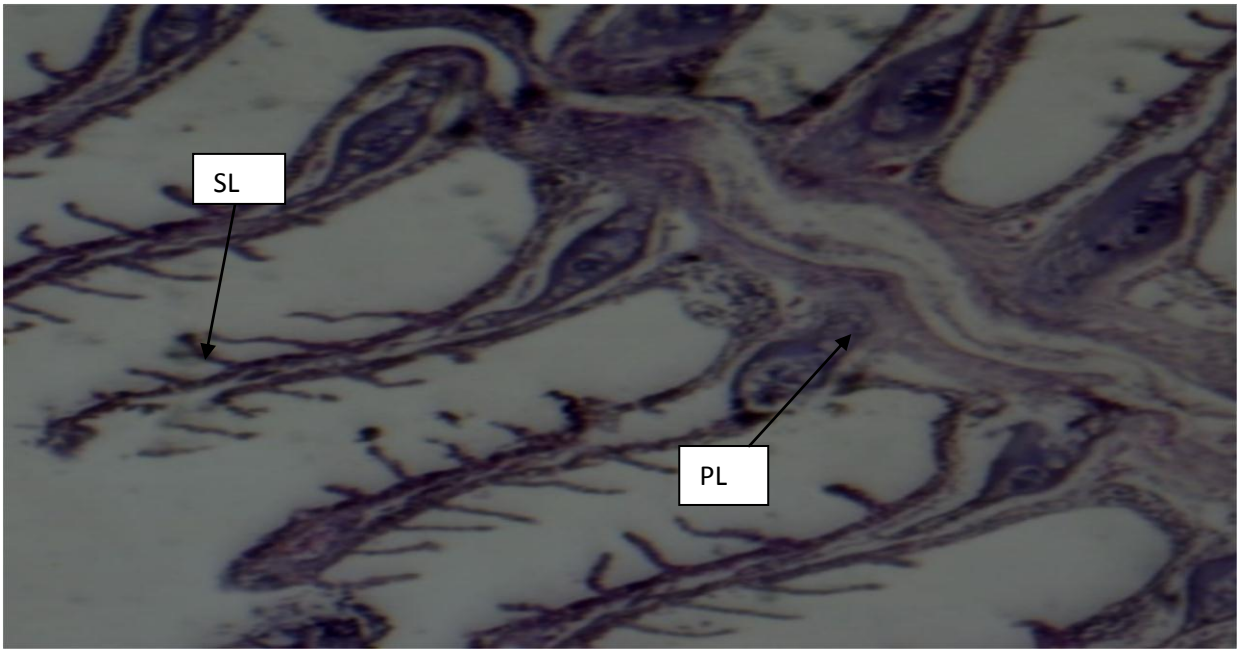

Plate I: Photomicrograph of Gill cells of control Fish, PL and SL intact (PL= primary lamellae, SL=secondary lamellae). X40

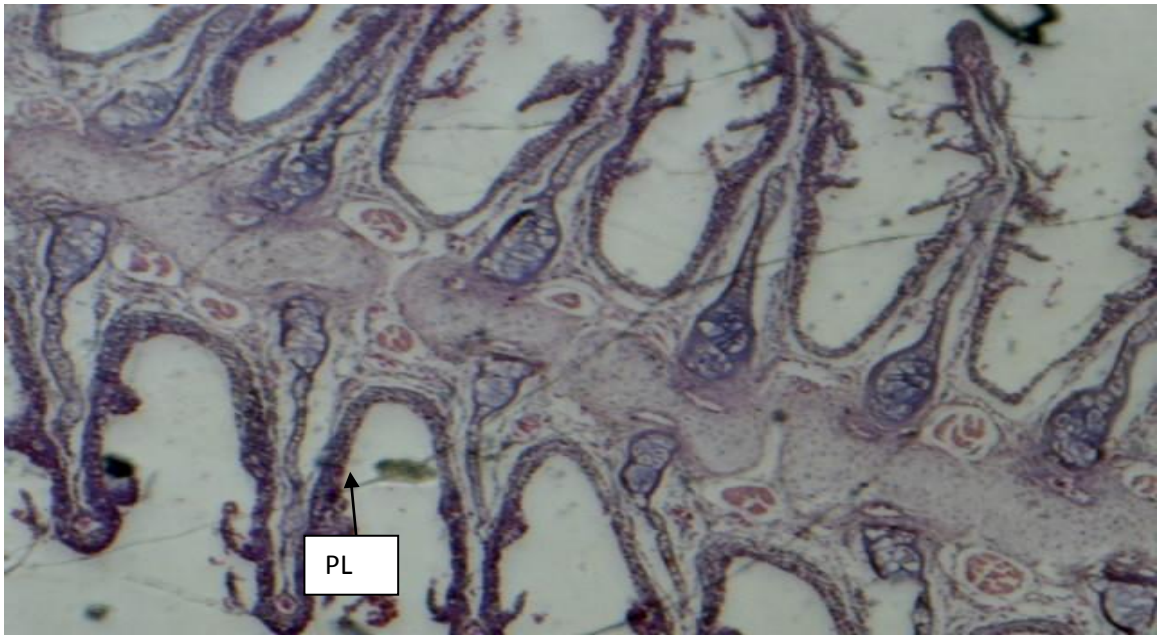

Plate II: T.S of gill of *Oreochromis niloticus* exposed to acute concentration (25ml/L). (PL=primary lamellae), disappearance of secondary lamellae. X40

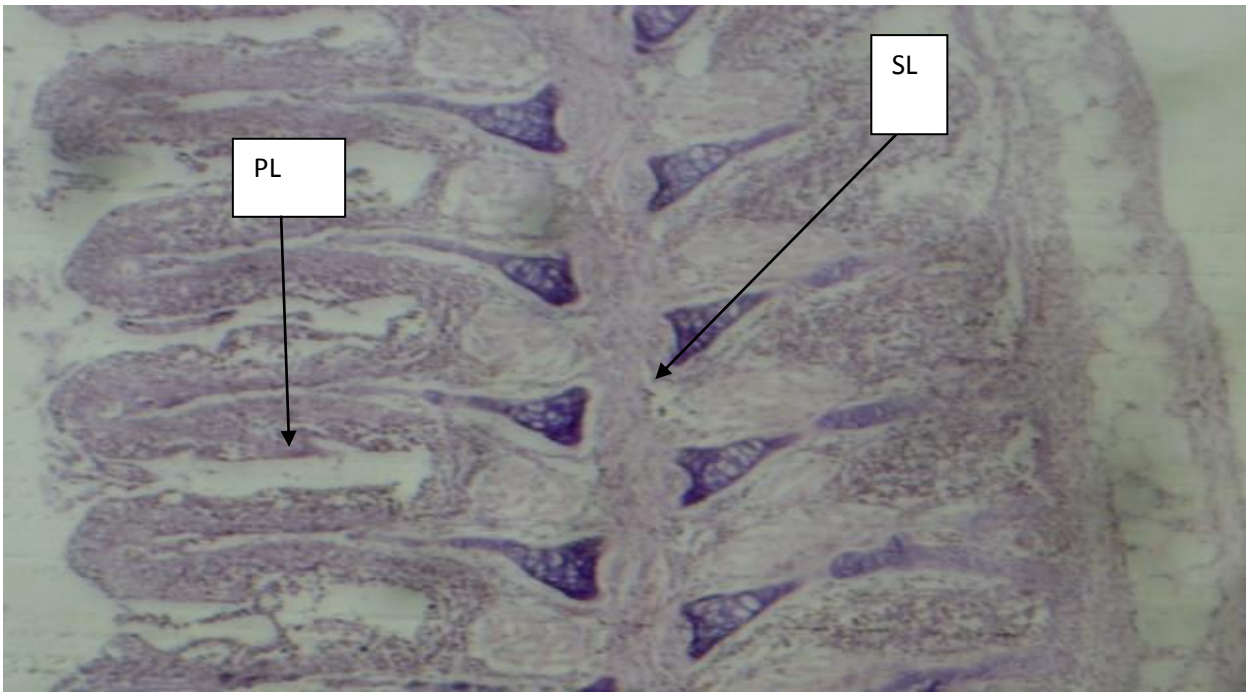

Plate III: T.S of gill of *Oreochromis niloticus* exposed to acute concentration (40ml/L). (PL=primary lamellae), complete filament detachment. X40

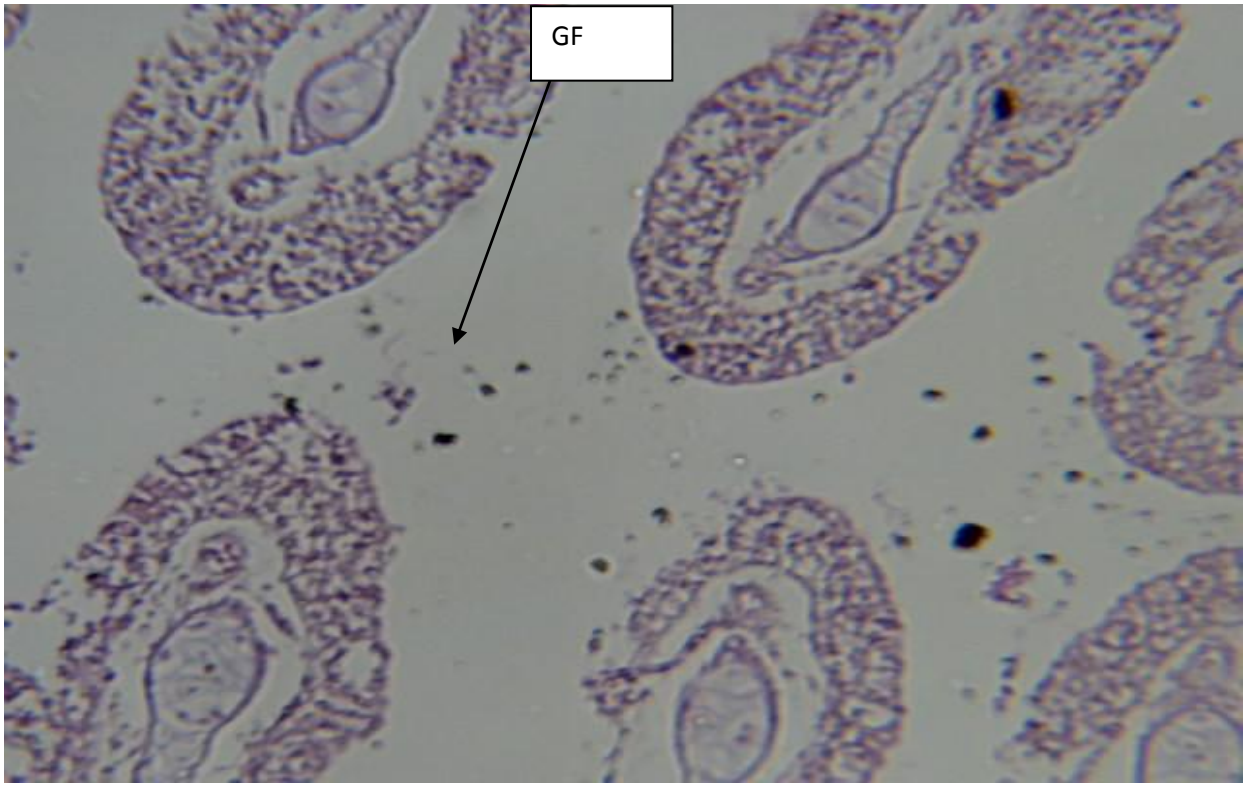

Plate IV: T.S of gill of *Oreochromis niloticus* exposed to acute concentration (45ml/L). (PL=primary lamellae), complete filament detachment. X40

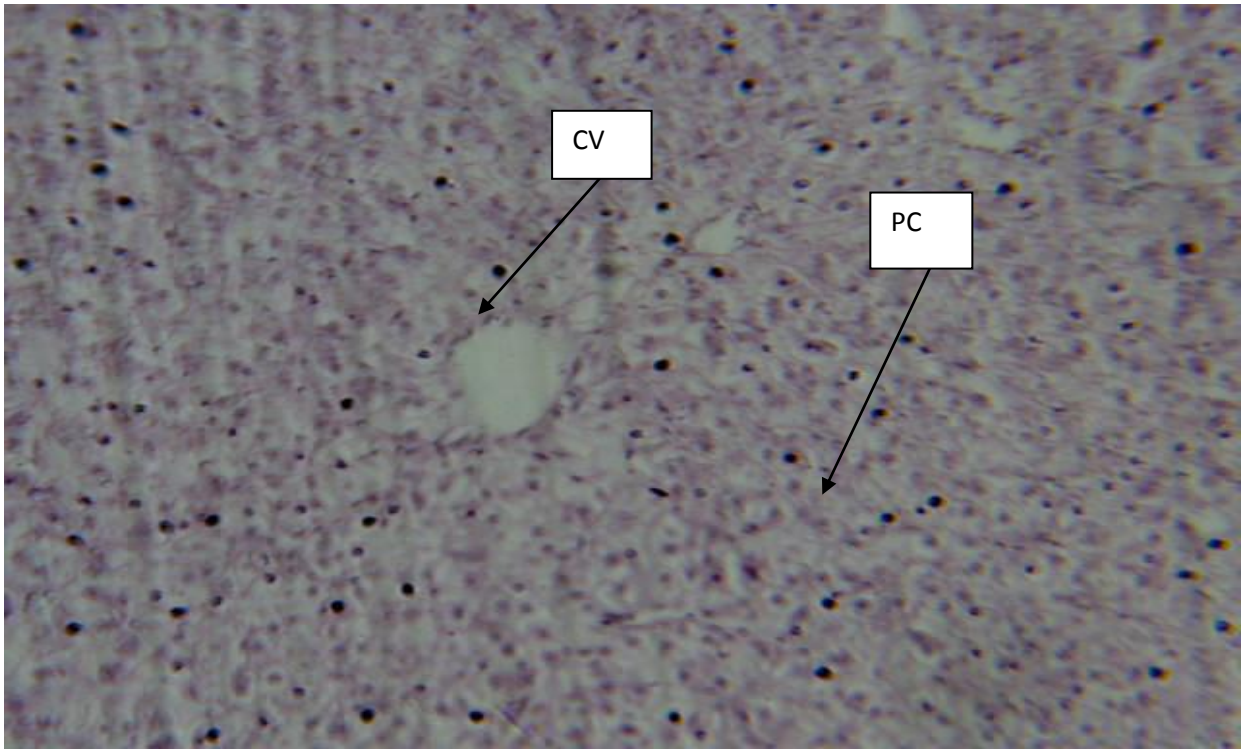

Plate V: T.S of the cells of control fish with homogenous cytoplasm. (CV= central vein, PC parenchyma cells) X100

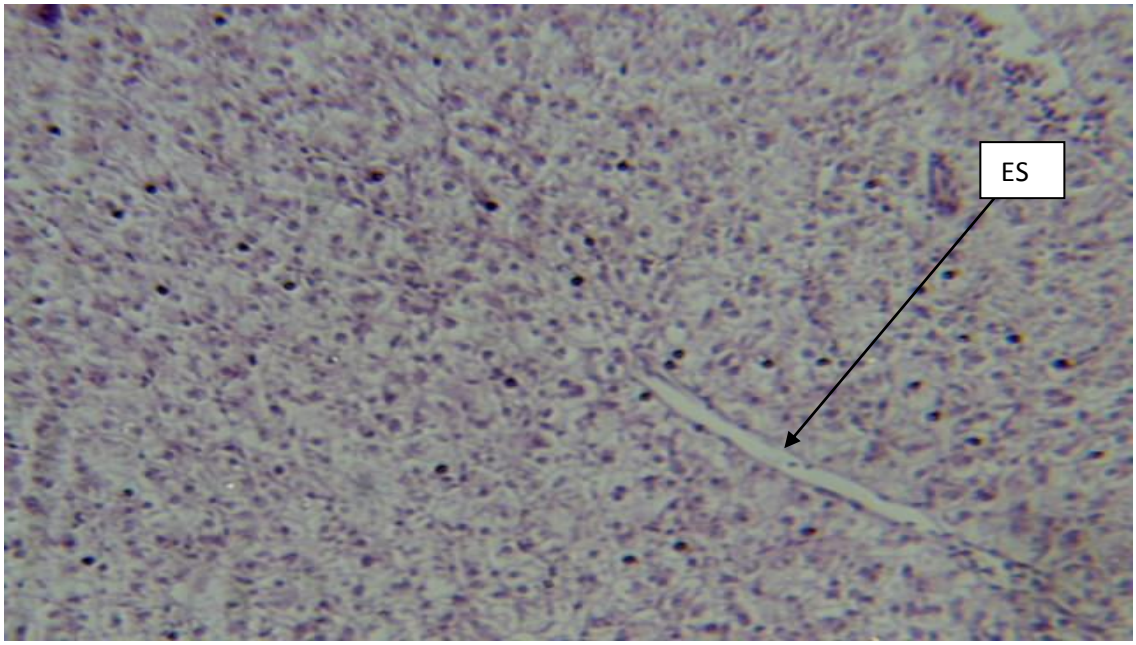

Plate VI: T.S of the liver cells of *Oreochromis niloticus*, extensive areas of necrosis exposed to 25ml/L (ES=Sinoids) X100

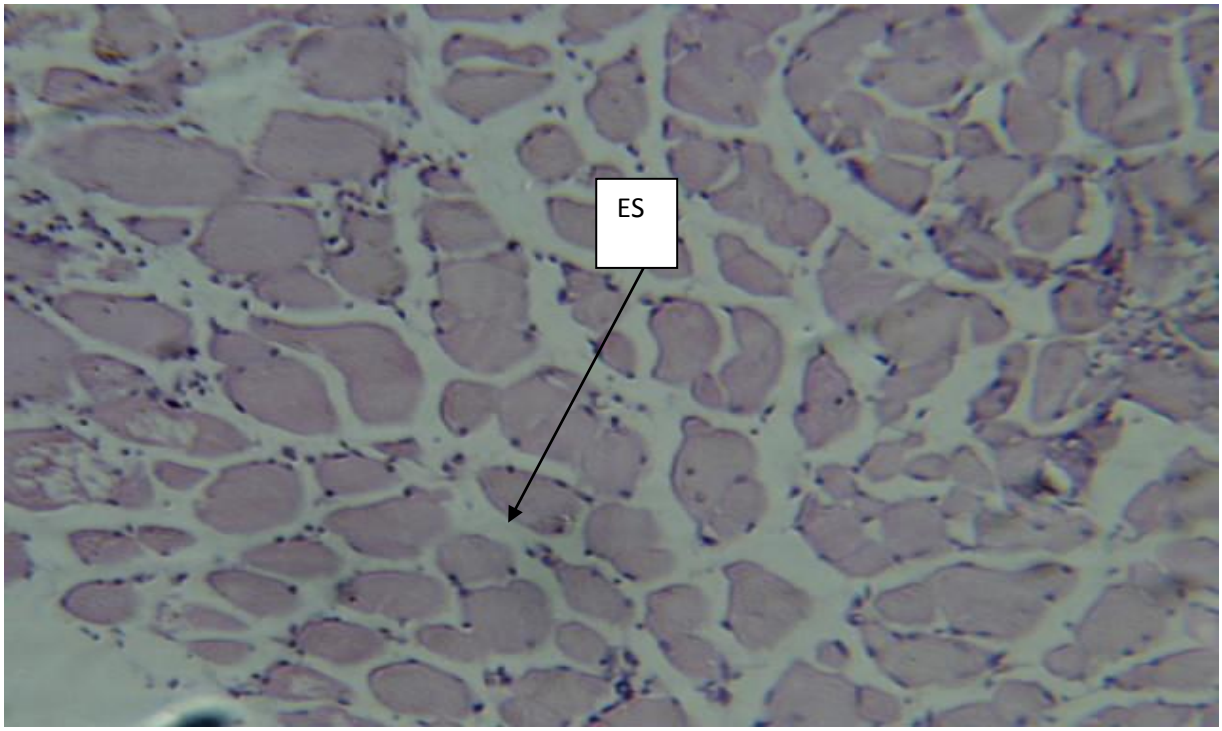

Plate VII: T.S of the liver cells of *Oreochromis niloticus*, extensive areas of necrosis exposed to 40ml/L (ES= extensive sinoids) X100

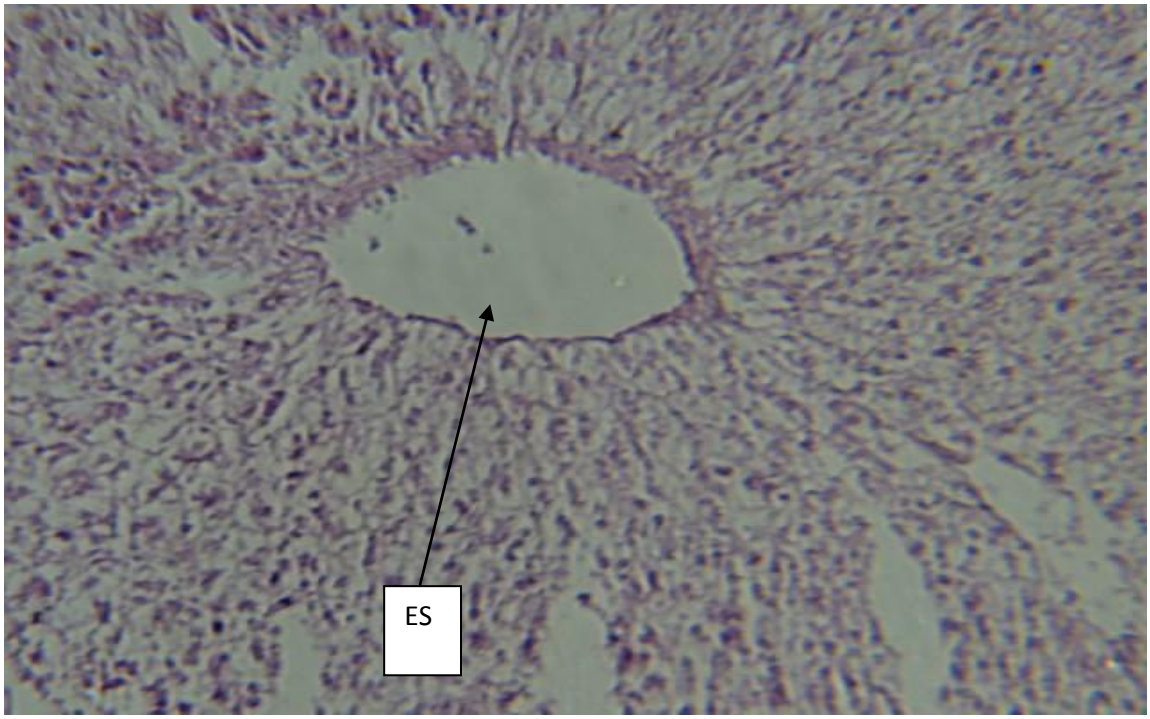

Plate VIII: T.S of the liver cells of *Oreochromis niloticus,* extensive areas of necrosis exposed to 45ml/L (ES= extensive sinoids) X100

4.4 Effect of Textile effluent on fingerlings of *Oreochromis niloticus* on some Haematological Parameters exposed after 96hours.

Studies on haematology carried on the fingerlings of *Oreochromis niloticus* exposed to acute level of the toxicant after 96hours are presented in Table 4.12. The result obtained showed a significant different between the control group and the groups of fish exposed to the toxicant ($P<0.05$). There was reduction in the level of RBCC with decrease in concentration of the toxicant while PCV remained fairly constant. Hb increases with decrease in the concentration of the toxicant, while WBC increases significantly with increase in concentration of the toxicant and later dropped toward the termination of the experiment.

The Mean Corpuscular Volume increased significantly with decrease in concentration except concentration (45ml/L) that showed a drastic decrease. In Mean Corpuscular Heamoglobin Concentration there was a significant increase as the concentration increases but at concentration (35ml/L) there was a decrease with a slight increase in the other concentrations above it.

Table 4.13 present Leucocytes Differential Count of *Oreochromis niloticus*, the result shows that neutrophil increases with decreases in concentration while lymphocyte increased with increase in the concentration. The result showed significant different ($P<0.05$) the different concentrations.

Table 4.12 Haematological Responses of *Oreochromis niloticus* exposed to acute concentration of toxicant from after 96hours

Conc. (Ml/L)	RBCC (10^6xmm)	WBCC ($500xm^3$)	PCV (%)	Hb (g/ml)	MCV (g/dl)	MCH (g/dl)	MCHC (%)
45	2.45^{a}	3.60^{a}	25.50^{d}	5.85^{d}	69.86^{d}	16.03^{d}	22.94^{d}
40	2.50^{d}	3.55^{ab}	27.00^{c}	6.90^{cd}	108^{c}	27.60^{c}	25.56^{c}
35	2.55^{c}	3.45^{b}	28.75^{c}	7.20^{c}	112.75^{c}	28.24^{c}	25.04^{c}
30	2.60^{bc}	3.40^{c}	30.50^{b}	8.20^{ab}	117.31^{b}	30.08^{b}	28.20^{a}
25	2.70^{b}	3.10^{c}	31.00^{b}	8.35^{b}	114.81^{b}	30.93^{bc}	26.94^{b}
0.0	2.75^{b}	2.60^{d}	34.65^{a}	10.00^{a}	126^{a}	36.36^{a}	28.86^{a}
Mean	2.79	3.28	29.57	7.82	108.12	28.71	26.26
S.E.M.	0.18	0.15	1.32	0.60	8.03	2.85	0.89

S.E.M=Standard Error of Mean

Column values with different superscripts are significantly different ($p<0.05$)

Table 4.13 Response of *Oreochromis niloticus* exposed to acute concentration of toxicant from after 96hours on some Leucocytes Differential Count.

Concentration (ml/L)	Neutrophil	Lymphocyte
45	25.33^{a}	86.64^{a}
40	22.27^{b}	80.54^{b}
35	23.67^{ab}	77.00^{b}
30	21.55^{c}	72.60^{c}
25	18.90^{d}	55.10^{c}
0.0	15.50^{d}	36.44^{d}
Mean	21.20	68.05
S.E.M.	1.44	7.68

S.E.M=Standard Error of Mean

Column values with different superscripts are significantly different ($p<0.05$)

4.5. Water Quality Analysis for Sub-Lethal Exposure

4.5.1 Total Dissolved Oxygen (TDO)

During the sub-lethal exposure, dissolved oxygen decreases with increase in the concentration as shown in Table 4.14 but increases with increase in exposure period as observed in week 4 and 6; there was significant difference ($p<0.05$) between the exposed group and the control. As the time of exposure increases DO also increases.

4.5.2. Electrical Conductivity (EC)

During the sub-lethal exposure, values of the Electrical Conductivity are shown in Table 4.15 Electric conductivity shows that with increase in the concentration of the toxicant EC also increased, but this was not the case with the control group which showed significant difference ($p<0.05$).

4.5.3 Water Temperature

During the sub-lethal exposure, temperature increases with increase in the concentration from week 2 to week 4 except in week 6 and 8 which maintain a fairly constant temperature as presented in Figure 5. There was a significant difference ($p<0.05$) between the exposed group and the control.

Table 4.14 Effects of Sub-lethal Concentration on Total Dissolved Oxygen

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	2.70^{c}	2.65^{c}	2.25^{c}	2.15^{d}
3.35	2.80^{c}	3.20^{c}	3.50^{b}	3.70^{c}
1.68	3.00^{b}	3.30^{b}	3.40^{b}	3.90^{b}
0.0	5.70^{a}	5.40^{a}	5.30^{a}	5.50^{a}
Mean	3.55	3.64	3.61	3.81
S.E.M	0.72	0.60	0.63	0.69

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

Table 4.15 Effects sub-lethal on Electric Conductivity (µS/cm)

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	270	290	303	315
3.35	215	209	210	225
1.68	124	130	155	190
0.0	69	72	74	68
Mean	169.50	175.25	185.50	199.50
S.E.M	45.04	47.45	48.10	51.13

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

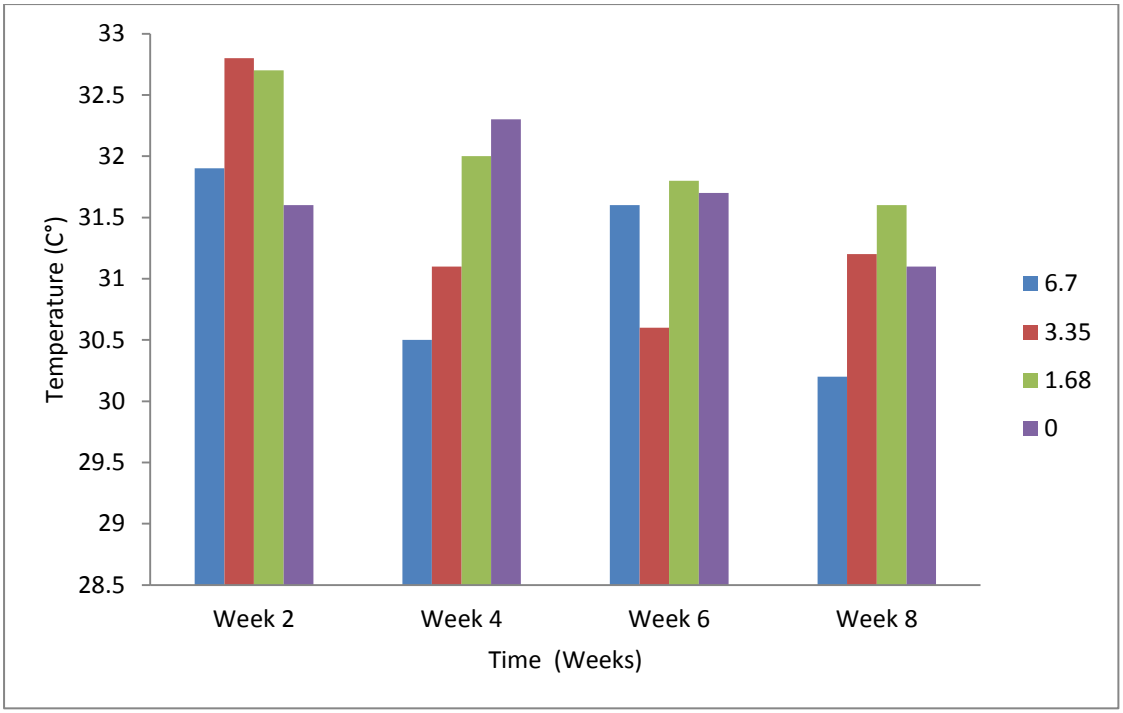

Fig.4.5. Graph of Mean±SE showing effects of textile effluent on Temperature (^{0}C) during the sublethal study.

4.5.4 Biological Oxygen Demand (BOD)

Biological Oxygen Demand observed during the sub-lethal exposure is presented in Table 4.16.The table presents the values of BOD observed during the sub-lethal studies. BOD increases with increase in concentration of the toxicant and it shows a significant difference ($p<0.05$) when compared with the control group. The values through out the period of study varied.

4.5.5 Water pH

During the sub-lethal exposure, it was observed that the pH increased with increase in the concentration of the toxicant, from week 4 – 8 there was significant difference between the exposed groups to the toxicant, there was also significant difference ($p<0.05$) between the exposed group and that of the control. Values obtained for the control remained within the neutral zone through out the period of study as shown in Table 4.17.

4.5.6 Total Dissolved Solids (TDS)

During the sub-lethal exposure of the fish during the experimental period the result for the total dissolved solids is presented in Fig.6. The result shows that with increase in concentration, there was increase in total dissolved solid while decrease in concentration revealed decrease in TDS but comparing treatment with the control group which showed a significant difference($p<0.05$) between the concentrations and the control group and this was also similar with the time of exposure.

Table 4.16 Effects of Sub-lethal Concentration on Biological Oxygen Demand

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	2.85	2.90	3.20	2.40
3.35	2.60	2.40	2.10	2.70
1.68	1.15	1.26	2.12	2.20
0.0	0.80	1.10	1.50	1.40
Mean	1.85	1.92	2.23	2.18
S.E.M	0.51	0.44	0.35	0.28

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

Table 4. 17: Effects of Sub-lethal Concentration on Water pH

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	9.40^{b}	10.15^{a}	9.90^{a}	10.10^{b}
3.35	9.85^{a}	9.52^{b}	10.00^{a}	10.20^{a}
1.68	8.45^{c}	8.60^{c}	9.10^{b}	8.50^{c}
0.0	7.15^{d}	7.17^{d}	7.10^{c}	7.20^{d}
Mean	8.71	8.86	9.03	9.00
S.E.M	0.60	0.65	0.67	0.72

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

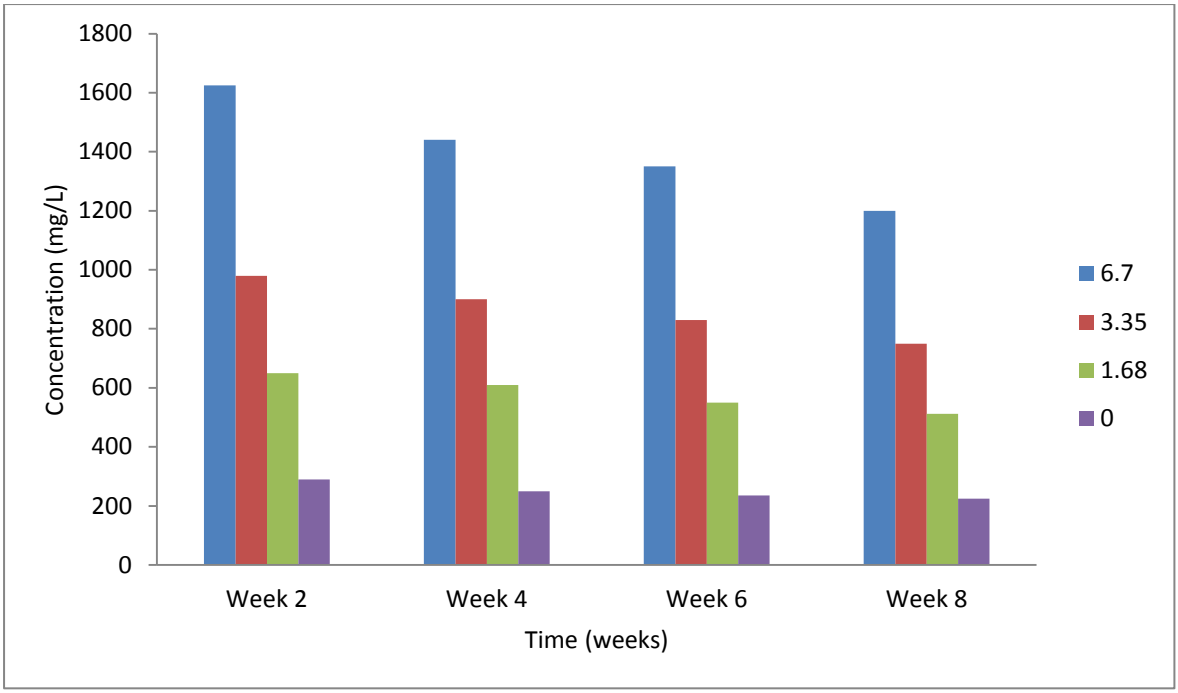

Fig.4.6. Graph of Mean±SE showing effects of textile effluent on TDS during the sub-lethal study

4.5.7 Total Alkalinity

Total alkalinity observed during the sub-lethal exposure is presented in Table 4.18. Increase in concentration shows increase in total alkalinity, comparing this figures with the different concentrations it showed significant difference ($p<0.05$) with the control group. Exposure time also had an effect on total alkalinity.

4.5.8 Total Hardness

The result for total hardness is presented in Table 4.19; it was observed that total hardness was increasing with increase in the concentration of the toxicant, while it showed significant difference ($p<0.05$) when compared with the control group. Total hardness also increases with increase in exposure period.

4.5.9 Nitrites

Nitrites observed during the sub-lethal exposure are presented in Table 4.20.The values revealed that the treatments had higher results and this may be due to the fact that there was a high concentration of Nitrite in the treatments thereby giving it a high value and it also showed a significant difference ($p<0.05$) between the exposed group and the control group. With the time of exposure the values remain fairly constant through the period of the sub-lethal studies.

Table 4.18 Effects of Sub-lethal Concentration on Total Alkalinity

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	2.70^{a}	2.60^{a}	2.45^{a}	2.30^{a}
3.35	2.40^{b}	2.36^{b}	2.30^{b}	2.22^{b}
1.68	2.25^{c}	2.20^{c}	2.15^{c}	1.80^{c}
0.0	0.7^{d}	0.8^{d}	0.6^{d}	0.8^{d}
Mean	2.01	1.99	1.86	1.78
S.E.M	0.45	0.41	0.43	0.35

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

Table 4.19 Effects of Sub-lethal Concentration on Total Hardness

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	15.00[a]	18.05[a]	20.11[a]	24.00[a]
3.35	9.00[b]	10.20[b]	14.18[b]	16.50[b]
1.68	6.15[c]	8.00[c]	11.60[c]	13.17[c]
0.0	1.90[d]	2.05[d]	2.15[d]	2.20[d]
Mean	8.01	9.56	12.01	13.97
S.E.M	2.75	3.31	3.74	4.53

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

Table 4.20 Effects of Sub-lethal Concentration on Nitrite

Concentration (ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	0.250	0.370	0.320	0.400
3.35	0.090	0.100	0.150	0.200
1.68	0.013	0.017	0.022	0.027
0.0	0.004	0.006	0.005	0.008
Mean	0.089	0.123	0.242	0.159
S.E.M	0.057	0.085	0.073	0.091

Means with the same superscript along columns are not significantly different ($p<0.05$)

(Mean values ±S.E.M.)

4.6.0 Sub-Lethal Toxicity Test

During the sub-lethal studies the textile effluent caused some behavioural changes in the fish, these changes were not pronounced like that of the acute study maybe its due to the fact that an LC_{50} was established from the acute studies. The activities of the fish decreased with increase in duration of the exposure. The control fish fed vigorously at the water surface and consumed all the food supplied, less of that was observed in the test tanks with different concentrations of the textile effluent. Those with higher concentration fed less as compared with those with lower concentration.

4.6.1 Effect of textile effluent on Fingerlings of *Oreochromis nilotcus* to Growth Response and Feed Utilization

Table 4.21 present growth studies of the fingerlings of *Oreochromis niloticus* cultured in different concentration of textile effluents. The estimation of the growth was done from the beginning of the sub-lethal studies, sum of individual wet weight of the fish were recorded at 2, 4, 6 and 8 weeks of the exposure to the textile effluent. The result obtained was expressed as percentage cumulative wet weight gain of the fish. The initial mean weight for 6.7ml/L, 3.35ml/L, 1.68ml/L and 0.0ml/L concentrations were 172.52g, 169.98g, 170.10g and 167.55g respectively. The result showed that growth was dose – dependent and it decreased with increase in concentrations as can be seen in the results for final growth rate for 6.7ml/L, 3.35ml/L, 1.68ml/L and 0.0ml/L concentrations 177.62g, 176.53g, 178.30 and 180.35g respectively, average weight gain for 6.7ml/L which had a value of 5.10g while the control which had 0.0ml/L which had a value for average weight gain of 12.80g which is significantly different ($p<0.05$) as shown in Table 4.21. and Figure 7 shows the increase in growth rate during the sub-lethal bioassay. The wet weight gain in the control tank was recorded too and this was dependant on the exposure period. Similarly, LWG, SGR, FCR, GFCR, FE, PER and NM were dose – dependent.

Table 4.21 Growth Studies of *Oreochromis niloticus* exposed to sub-lethal concentrations of Textile effluent

Concentrations (ml/L)						
Growth Parameters	6.70	3.35	1.68	0.0	Mean	S.E.M
N0 of fish	10	10	10	10		
Av. Initial Weight (g)	172.52	169.98	170.10	167.55	170.04	1.01
Av. Final Weight (g)	177.62	176.53	178.30	180.35	178.20	0.80
Av. Weight Gain (g) (%)	5.10	6.55	8.20	12.80	8.16	1.67
LWG (g)	3.00^{d}	3.90^{c}	4.82^{b}	7.64^{a}	4.84	1.00
SGR (g)	0.02^{d}	0.03^{c}	0.04^{b}	0.06^{a}	0.04	0.01
FCR (g)	2.82^{b}	2.83^{a}	2.80^{c}	2.77^{d}	2.81	0.01
GFCR (g)	35.46^{c}	35.33^{d}	35.71^{b}	36.10^{a}	35.65	0.17
FE (g)	0.18^{d}	0.23^{c}	0.29^{b}	0.46^{a}	0.29	0.06
PER (g)	0.15^{d}	0.19^{c}	0.23^{b}	0.37^{a}	0.24	0.05
NM (g)	78.40^{d}	100.69^{c}	126.05^{b}	196.76^{a}	125.48	25.68

Means with the same superscript along rows are not significantly different ($p<0.05$)

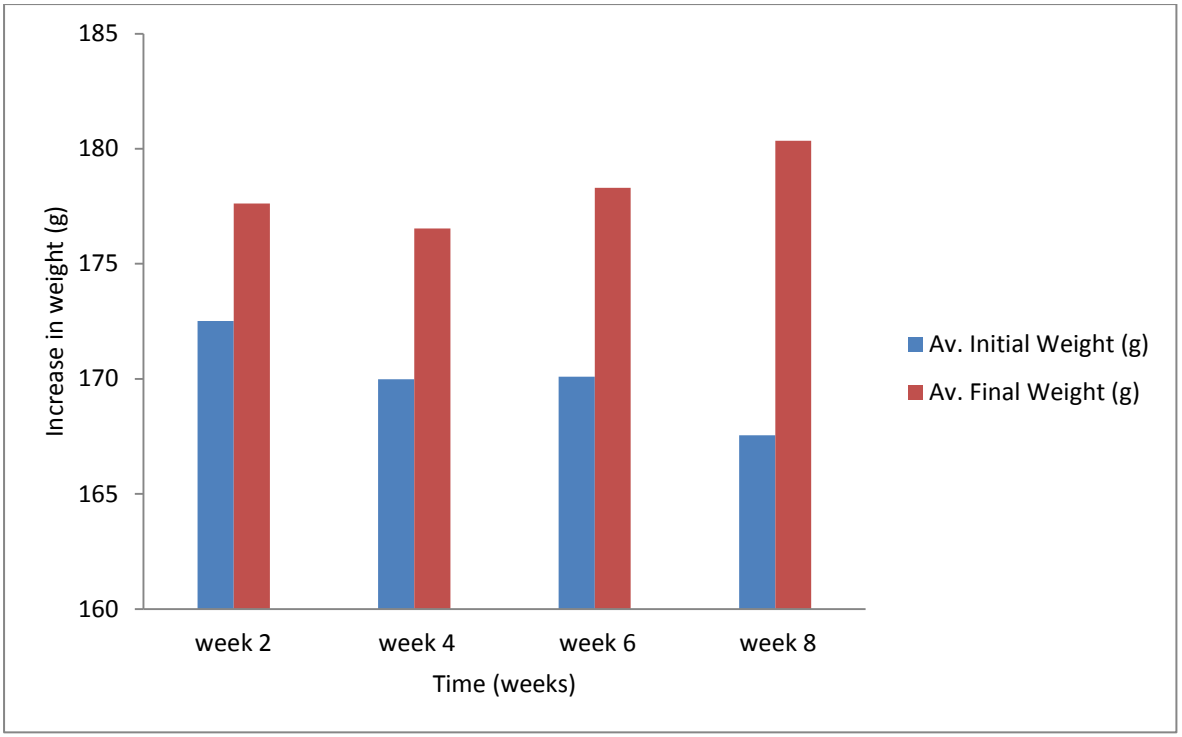

Fig. 4.7 Graph showing increase in weight rate

4.6.2 Histopathological Studies for the Sub-Lethal

A histopathological study was carried on the gills and liver of fish exposed to different concentration of textile effluents. Plate IX shows gills from control fish. In other tanks with lower concentrations, Plate X there was mild differences in the gills when compared with the gills of control fish. Plate XI shows sections of gills after 6weeks of exposure showing Complete erosion of filament. Gills exposed for the 8 weeks showed marked differences of secondary and primary lamellae and erosion of the primary lamellae in the tanks with the highest concentration of 6.7ml/L as shown in Plate XII

Plate XIII which is the control with a concentration of 0.0ml/L shows the liver intact. There was mild to moderate fatty change and inflammation of periportal veins in the tank with 3.35ml/L as seen in Plate XIV. The liver of the fingerlings from tanks with the highest concentration of the textile effluent (6.7ml/L) showed the cytoplasm of the liver scattered with enlarged sinusoids and necrotic hepatocytes as seen in Plate XV.

4.6. HISTOPARTHOLOGICAL STUDIES FOR THE SUB-LETHAL

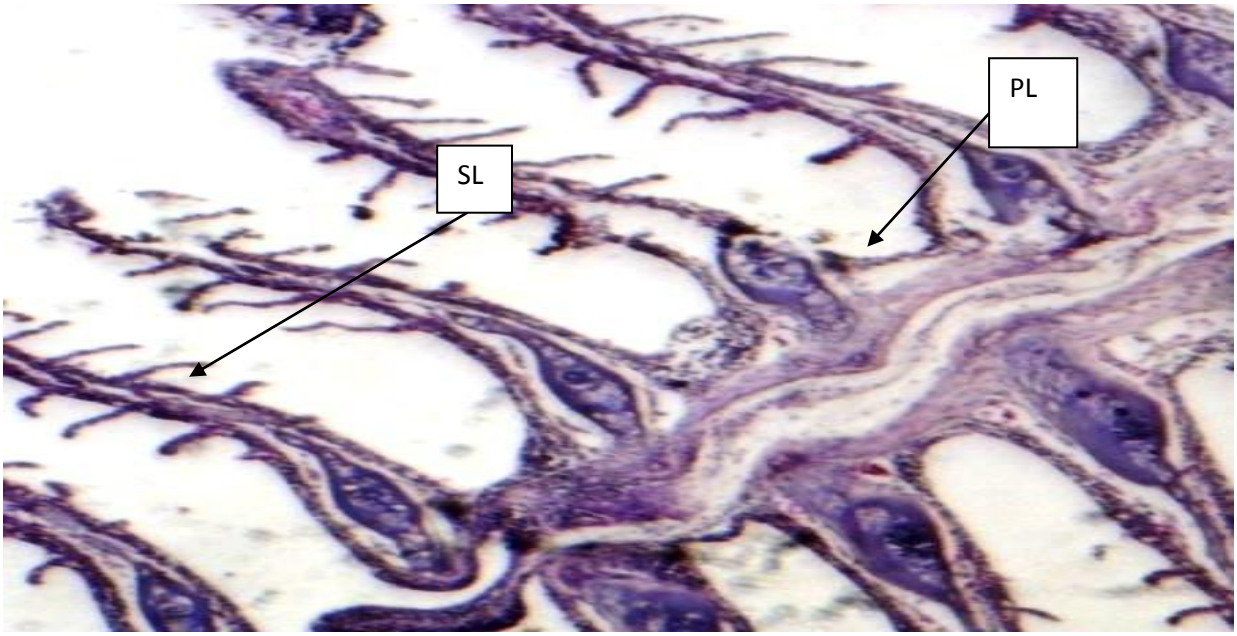

Plate IX: Gill filament of *Oreochromis niloticus* from control fish

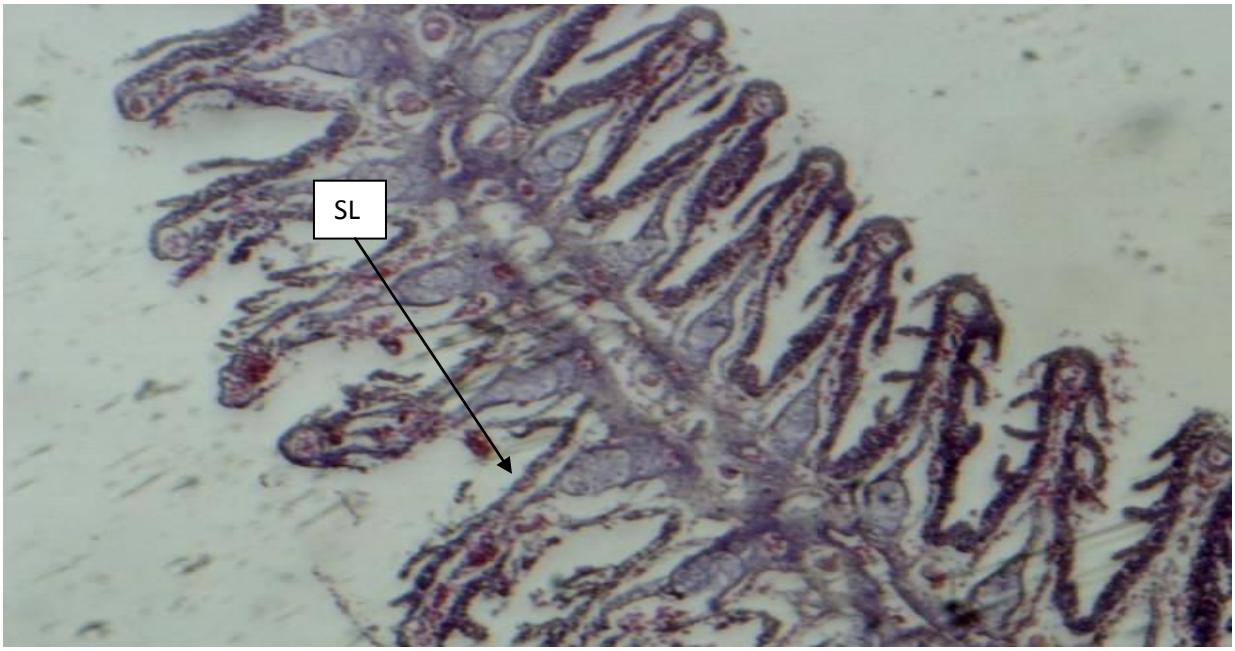

Plate X: Gill filament *Oreochromis niloticus* from 1.68ml/L after 2 weeks of sub-lethal Exposure (SL=secondary lamellae) X40

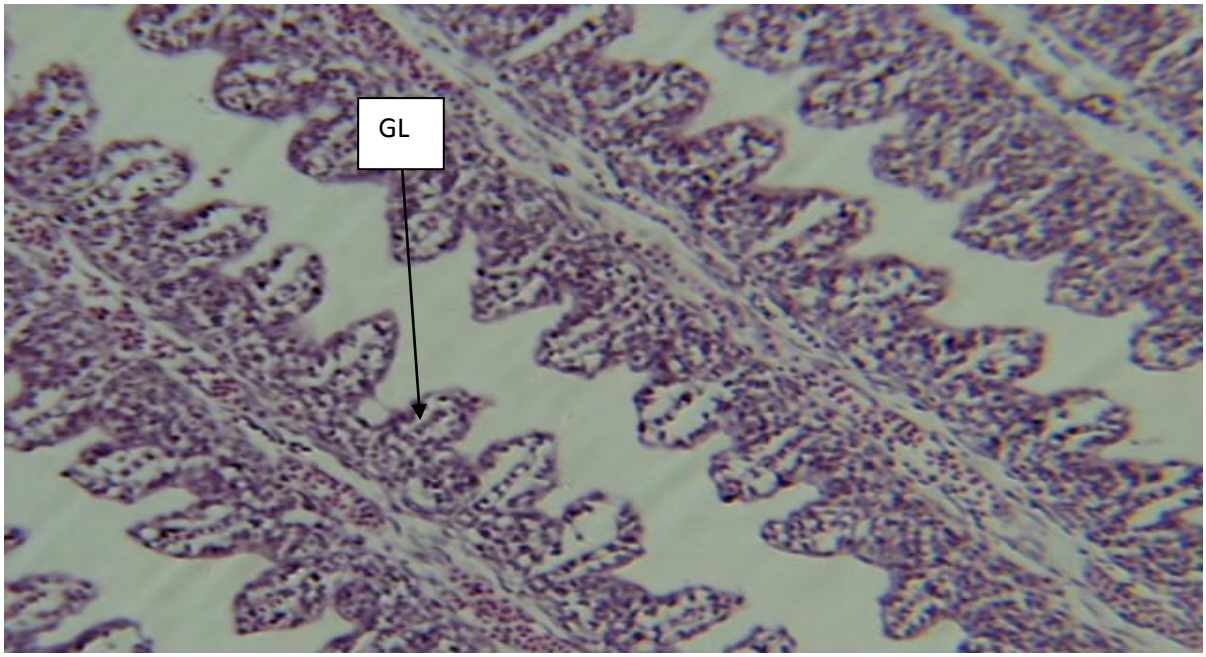

Plate XI: Gill filaments of *Oreochromis niloticus* from 3.35ml/L after 6weeks of exposure showing Complete erosion of filament(GL= gill filament)X40

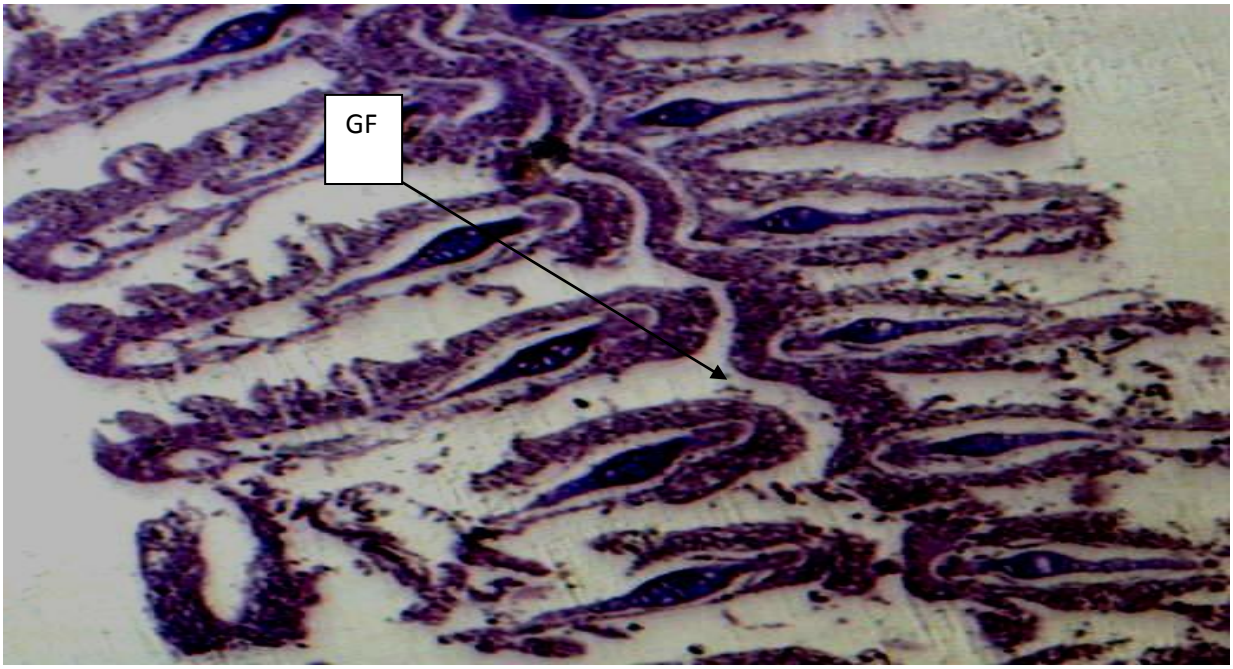

Plate XII: Gill filaments of *Oreochromis niloticus* from 6.7ml/L after 8weeks of exposure showing Complete erosion of filament(GL= gill filament)X40

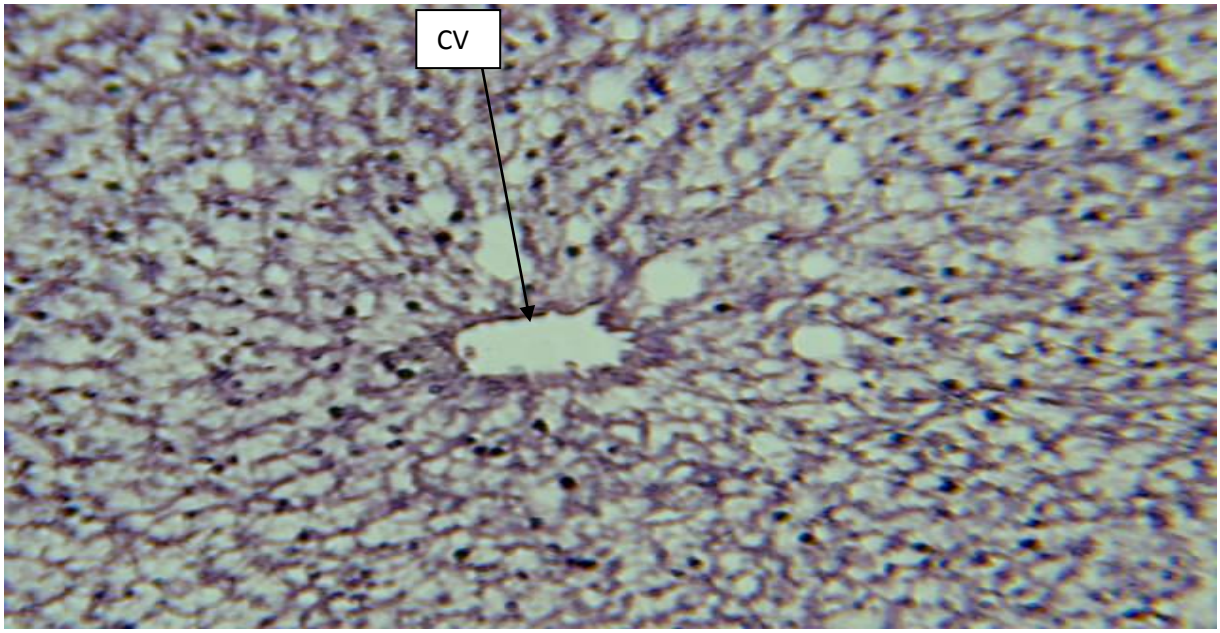

Plate XIII: T.S of the cells of control fish showing homogenous cytoplasm (CV= central vein) X 100

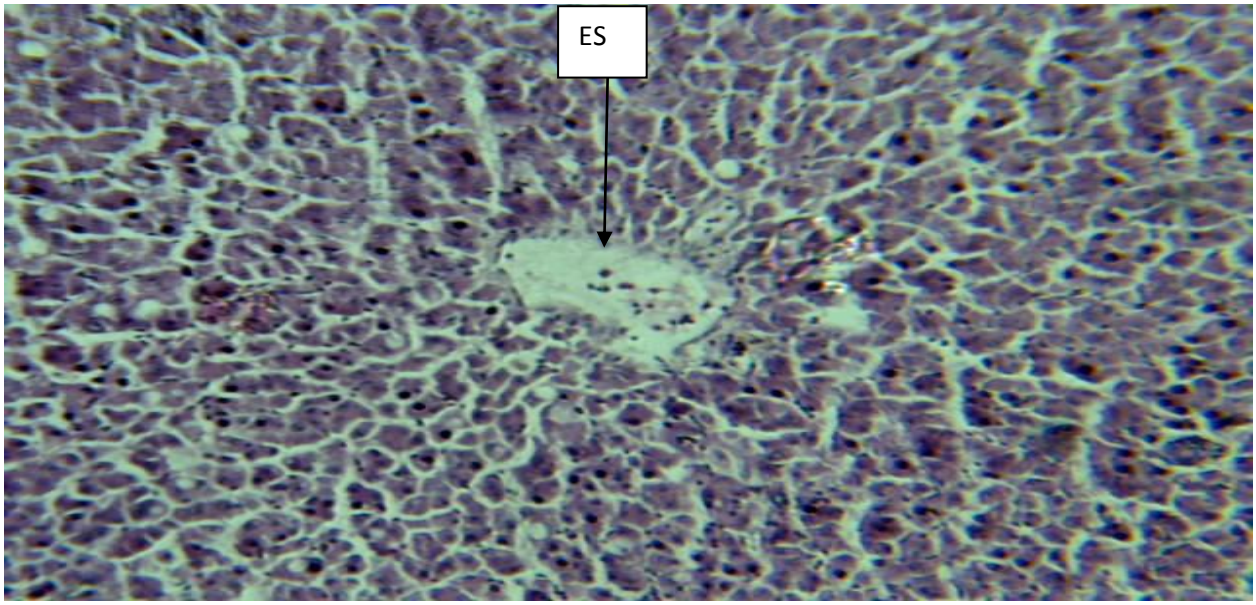

Plate XIV: T.S. of liver cells of *Oreochromis niloticus* showing extensive areas of necrosis exposed to 3.35ml/L after 4wks of exposure (ES= Extensive sinusoids) X10

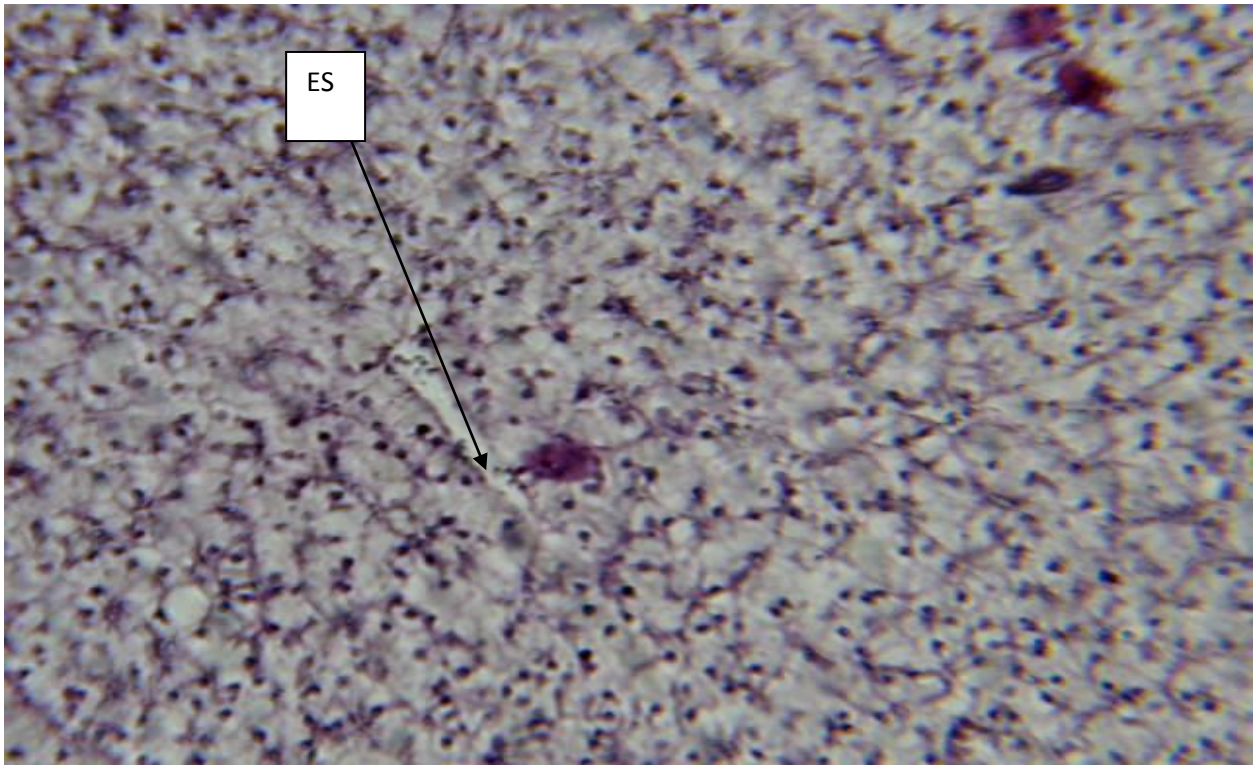

Plate XV: T.S. of liver cells of *Oreochromis niloticus* showing extensive areas of necrosis exposed to 9ml/L after 8wks of exposure (ES= Extensive sinusoids) X10

4.6.3 Heamatological Parameters for sub-lethal exposure

The result for haematology is recorded in Table 4.22 to Table 4. 28. For the 8-weeks of study, the result shows that there was decrease in haematocrit and haemoglobin. The haemoglobin of the control tank were higher than those of the exposed tanks. Haematoogical studies of *Oreochromis niloticus* conducted on the fingerlings during the sub-lethal period are shown below:

4.6.3.1 Red Blood Cell Count (RBCC)

The result for RBCC during the sub-lethal studies is presented in Table 4.22; the result showed increase in values with increased in exposure period but with decrease in concentration there was decrease in RBCC value. The control varies partially. Results of the treatment and the control showed significant difference ($p<0.05$).

4.6.3.2 White Blood Cell Count (WBCC)

Result in Table 4.23 showed that there was an increase in WBCC through out the study period and this drop with decreased in concentration and in the control the WBCC increased during the duration of the study period. The values often from treatments showed significant difference ($p<0.05$) with that of the control as the values from treatments increased with increase in concentration.

4.6.3.3 Packed Cell Volume (PCV)

The data for PCV as presented in Table 4.24 revealed that the highest concentration of 6.7ml/L of the textile effluent PCV dropped in week 4 and increases in weeks 6 and 8 but it is not the same in the lower concentrations as you go down the group and as the exposure period increased there was increase in PCV in the treatments tanks and comparing this with the control tanks it showed significant difference ($p<0.05$).

Table 4.22 Red Blood Cell Count of *Oreochromis niloticus* exposed to sub-lethal textile effluent Concentration for 8 weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.7	3.22^{a}	3.55^{a}	3.60^{a}	3.63^{a}
3.35	3.10^{b}	3.30^{b}	3.28^{b}	3.50^{b}
1.68	2.50^{c}	2.10^{d}	3.00^{c}	3.45^{c}
0.0	2.30^{d}	2.43^{c}	2.40^{d}	2.50^{d}
Mean	2.78	2.85	3.07	3.27
S.E.M	0.22	0.35	0.25	0.26

S.E.M= Standard Error of Mean

RBC along the columns with different superscripts are significantly different. ($p<0.05$)

Table 4.23 White Blood Cell Count of *Oreochromis niloticus* exposed to sub-lethal textile effluent for 8 weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.7	3.84^{a}	3.20^{a}	3.75^{a}	4.13^{a}
3.35	2.97^{c}	3.15^{b}	3.22^{c}	3.60^{c}
1.68	3.06^{b}	3.10^{c}	3.30^{b}	3.80^{b}
0.0	2.63^{d}	2.48^{c}	2.55^{d}	2.58^{d}
Mean	3.13	3.00	3.21	3.53
S.E.M	0.26	0.17	0.25	0.33

S.E.M= Standard error of mean
WBCC along the columns with different superscripts are significantly different. ($p<0.05$).

Table 4.24 PCV of *Oreochromis niloticus* exposed to sub-lethal textile effluent for 8 Weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	24^{c}	22^{c}	26^{c}	28^{c}
3.35	25^{b}	27^{a}	29^{a}	30^{a}
1.68	22^{d}	25^{b}	26^{c}	29^{b}
0.0	26^{a}	25^{b}	27^{b}	27^{d}
Mean	24.23	24.75	27	28.50
S.E.M	0.86	1.03	0.70	0.65

S.E.M= Standard error of mean
PCV along the columns with different superscripts are significantly different. ($p<0.05$)

4.6.3.4 Haemoglobin (Hb)

Haemoglobin (Hb) values revealed that with high concentration of the textile effluent there is decrease in the Hb as observed in week 2- week 6, with increase in the exposure time Hb also increases and this decrease in week 8 which showed a significant difference ($p<0.05$) with that of the control as presented in Table 4.25

4.6.3.5 Mean Cell Volume (MCV)

Table 4.26 showed that MCV values generally increases with the decrease in concentration and exposure period ranging from 92.48-99.80, this showed that there is a significant difference ($p<0.05$) between the different treatment and the control as shown in the Table 4.29.

4.6.3.6 Mean Corpuscular Haemoglobin (MCH)

MCH values decreases with decrease in concentration of the textile effluent and increases with increase in exposure period ranging from 14.56-16.10 which shows a significant difference ($p<0.05$) between the different concentrations in the tanks and this is presented in Table 4.27.

4.6.3.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

Table 4.28 showed that at higher concentration there is low MCHC and as the concentration reduces there is high MCHC and this is also seen as duration of the experiment increases, MCHC also shows significant difference ($p<0.05$), with the control group

Table 4.25 Hb of *Oreochromis niloticus* exposed to sub-lethal concentration of textile effluent for 8 Weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	7.00^{c}	7.44^{c}	8.02^{c}	7.90^{c}
3.35	8.24^{a}	8.11^{a}	8.14^{b}	8.12^{a}
1.68	7.46^{b}	7.68^{b}	8.33^{a}	7.95^{b}
0.0	6.73^{d}	6.20^{d}	7.00^{d}	6.89^{d}
Mean	7.36	7.36	7.87	7.72
S.E.M	0.33	0.41	0.30	0.28

S.E.M= Standard Error of Mean

Hb along the columns with different superscripts is significantly different. ($p<0.05$)

Table 4.26 MCV of *Oreochromis niloticus* exposed to sub-lethal textile effluent concentration for 8 Weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	79.02^{d}	83.04^{d}	90.67^{d}	88.02^{d}
3.35	88.80^{c}	86.50^{c}	96.00^{b}	107.10^{a}
1.68	92.11^{b}	94.70^{b}	98.30^{a}	101.60^{b}
0.0	110^{a}	105^{a}	95.26^{c}	100.00^{c}
Mean	92.48	92.31	95.06	99.8
S.E.M.	6.47	4.89	1.60	4.02

S.E.M= Standard Error of Mean
MCV along the columns with different superscripts are significantly different. ($p<0.05$)

Table 4.27 MCH of *Oreochromis niloticus* exposed to sub-lethal concentration of textile effluent for 8 Weeks

Concentration(mol/L)	Week 2	Week 4	Week 6	Week 8
6.70	15.36[a]	16.60[a]	16.73[a]	17.45[a]
3.35	15.47[a]	15.00[b]	16.02[b]	16.88[b]
1.68	14.25[b]	13.69[c]	14.66[c]	16.06[c]
0.0	13.17[c]	12.55[d]	13.99[d]	14.02[d]
Mean	14.56	14.46	15.35	16.10
S.E.M	0.54	0.87	0.62	0.75

S.E.M= Standard Error of Mean

MCH along the columns with different superscripts are significantly different. ($p<0.05$)

Table 4.28 MCHC of *Oreochromis niloticus* exposed to sub-lethal concentration for 8 weeks

Concentration(ml/L)	Week 2	Week 4	Week 6	Week 8
6.70	53^{b}	56^{c}	56^{c}	57^{b}
3.35	67^{a}	62^{b}	58^{b}	52^{c}
1.68	54^{b}	67^{a}	71^{a}	61^{a}
0.0	44^{c}	45^{d}	53^{d}	49^{d}
Mean	58	61.67	61.67	56.67
S.E.M.	4.51	3.18	4.70	2.60

S.E.M= Standard Error of Mean
MCHC along the columns with different superscripts are significantly different. ($p<0.05$)

CHAPTER FIVE

5.0 DISCUSSION

5.1Pysico-Chemical Parameters of Textile Effluent

Characterization of Textile Effluents was carried out bi-weekly for pH, Temperature, Total Dissolved Oxygen, Biological Oxygen Demand, Electrical Conductivity, Total Dissolved Solids, Alkalinity, Hardness and Nitrite. Mean values were compared with WHO (1984)/FEPA (1991). The values for the pH varied between 7.10-7.15 in the control group while the test group was alkaline in nature. The exposure period had effect on the pH because during the acute and sub-lethal bioassay, the longer the period of exposure the higher the pH value. Temperature during the period of study remained fairly constant, changes in the temperature in the test tanks during the period of acute and sub-lethal may be due to changes in atmospheric temperature.

Low levels of DO reported in this work in the test tanks may be due the presence of the Toxins as compared with the control group, similar report was made by Gabriel *et al*. (2005) that the DO level in the control was significantly higher than those in the treatment while high BOD was also recorded in both the acute and sub-lethal studies, Hari *et al*. (1994) reported similar result where he stated that low level of 3.81-4.10 of dissolved oxygen (DO) and 2.35 and 2.18 of high level of Biological Oxygen Demand (BOD) will makes the waste unsuitable for drinking and irrigation and other uses. These values are more than the permissible limit of WHO (1984)/FEPA (1991).

High values of DO and BOD reported in this study will make oxygen depletion and may also cause sulfocation of fish and other aquatic organisms if released into a nearby stream. Adewoye and Fawole (2002) and Adewoye *et al*. (2005) reported that indiscriminate deposition of effluent into an aquatic system might decrease the dissolved oxygen

concentration, which stand to impair respiration leading to asphyxiation (which is an indication of unconsciousness or death produced by failure of the blood to become properly oxygenated in the lungs) and may ultimately results into organ architectural degradation such as liver dysfunction. In Electrical Conductivity the result showed that with higher concentrations EC was high while with lower concentration EC was low in value similar observations were made in Total Dissolved Solid (671.75), Alkalinity (1.7) and Nitrite (0.159) had higher values indicating the presence of more toxic components than the control group.

5.2. Studies of Heavy Metals from Textile Effluent

The metals that were analysed from the textile effluents included Aluminium, Chromium, Iron, Copper, Lead and Zinc. Values obtained were compared with those of WHO (1984)/FEPA (1991) for maximum allowable concentration of metals for wastewater released into the environment, Aluminium had no value for comparison while Chromium, Iron, Copper and Lead had values greater than the level recommended by WHO (1984)/FEPA (1991) as such posed great threat to man and his environment similar reports was made by Giguere *et al.* (2004) in their report finding that heavy metal concentration in fish increased with age that exerted significant impact on the tolerance limits of fish also but value for Zinc was lower than the recommended standard. Gbem *et al*., (2001) also reported that in heavy metal pollution (e.g. Pb), organs such as the gills and liver have been identified as storage sites in *C. gariepinus*.

5.3 Behaviour Studies of *Oreochromis niloticus*

Some abnormalities were observed during the acute exposure of the fingerlings of *Oreochromis niloticus* to textile effluent**.** Observation was made on what happened to the fish immediately after been exposed to the acute and sub-lethal concentration showed

hyperactivity and loss of equilibrium at the initial exposure of the fish during the bioassay, other observations made were loss of equilibrium, frequent surfacing, agitated and erratic swimming, loss activity and finally death.

Effluent capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate and persistence in the environment. Toxicity of the industry waste refers to how poisonous it is. Immediately after the fingerlings of *Oreochromis niloticus* were transferred into the test solution, the fishes became hypersensitive and showed a rapid rate of opercular movement accompanied by occasional gulping of air, there was also vertical positioning of the fish and this agrees with the work of Bobmanuel *et al*, (2006). A short duration of exposure to some chemicals may have little effect on fish, whereas longer exposure may cause harm extremely. The fishes behave differently in different concentrations of the textile effluent such as restlessness, respiratory distress, erratic swimming, loss of balance, vertical movement and death, similar report was made by Auta *et al*. (2006). The higher the concentration, the more pronounced was this behaviour. After several minutes of exposure the fishes lost their equilibrium. They were suspended with complete cessation of movements and finally dead.

Behavioural abnormalities as observed in this study may have been attributed to nervous impairment as a result of blockage of nervous transmission between the nervous systems and various effectors sites that may induce paralysis of respiratory muscles and/or depression of respiratory centre and disturbances in energy or metabolic pathways which results in depletion of energy. No adverse behavioural changes or any mortality were recorded in the control experiment throughout the period of the bioassay but in the tanks containing the textile effluents and fishes, several abnormal behavioural response were observed such as incessant jumping and gulping of air, restlessness, frequent surface to bottom movement, sudden change of direction during movement, resting at the bottom, loss of skin colouration,

loss of equilibrium and gradual onset of inactivity and such observations were similar to those of Omoregie *et al*., (1990); Okwuosa and Omoregie, (1995); Avoajah and Oti (1997); Omoniyi *et al*., (2002); Rahman *et al*., (2002); Aguiwo (2002) and Ogundiran *et al*., (2010).

In the present study it was observed that the opercular beats increased with the increasing concentration of the textile effluents similar reports were made by Baskaran and Palaniswamy (1990) reported that the opercular beats increased with the increasing concentrations of fertilizer in *Oreochromis mossambicus*. Ramamurthy *et al*., (2009) observed the same in *Channa punctatus* when exposed to pesticide monocrotophos.

5.4 Growth Studies and Feed Utilization

Growth of the fish was dose dependent as the lowest concentration with the textile effluent gave the best result of growth while those with the highest concentration of the textile effluent gave worst result when compared with the control group. Specific growth rate was observed to be reducing with increased in exposure period increased in concentration. Those that did not show best growth rate was due to avoidance of feed intake by the fish due to presence of the toxicant which agrees with the work of Auta *et al*., (2002) that reduction of growth by the fish might be attributed to increase activity associated with attempt to avoid contaminated water, or an increased expenditure of energy on chemical detoxification and tissue repairs. Presence of the toxicant in the test tank can also lower feeding rate with an attempt to avoid the polluted water and sometimes their taste receptors, similar report was made by Nwanna *et al*., (2003) that destruction of the taste receptors and pharyngeal wall by detergents prevented the fish from recognizing their food. Feed utilization was recorded high in the control fish as those of the tanks with the concentration had lower values which were dose dependent. Feed conversion ratio was highly significant in the control group of fish ($p<0.05$) as seen in the treatment tank with 6.7ml/L with a value of 2.82 and this showed that the fish utilized the feed supplied to them. Other parameters such as life weight gain (LWG)

increased with decrease in concentration; the results of Specific growth rate (SGR) tend to be fairly constant based on the result presented. Gross feed conversion efficiency (GFCE) showed increase in value as the concentration decreases, feed efficiency (FE) and nitrogen metabolism (NM) also increased with decreases in concentration as obtained in the results which showed a significant increase as observed with fish exposed to sub-lethal concentrations of the toxicant.

5.5 Histopathology of Gills of *Oreochromis niloticus*

Gills are the most delicate structure of the telesost body, their vulnerability is thus considerable because of their external location and necessarily intimate contact with water which means that they are liable to damage by any irritant materials whether dissolved or suspended in the water.

Gill sections of normal fish with 0.0ml/L concentration of the textile effluent showed the primary gill lamellae are laterally compressed flat leaf like structures situated alternately on either side of the interbranchial septum. Each one of them bears a row of secondary lamellae on both sides perpendicular to the long axis of primary gill lamella. They gill structures during the acute and sub-lethal studies were found to be intact without any destruction as seen in other gill sections with different concentrations of the textile effluents.

During the acute and sub-lethal studies there was rupture and necrosis of gill epithelium of fish may be due to direct toxic effect of the textile effluent. However, hyperplasia, hypertrophy, lamellar fusion and mucus secretion and sloughing of gills may be defense responses of the fish to the effluent toxicity. The above finding agrees with the work of Mallatt (1985), who reported that the rupture and necrosis of the gill epithelial cells occur in acute toxic conditions caused by low pH. Fafioye *et al*., (2004) also reported histopathological response of *Clarias gariepinus* to plant extracts under brief exposure. Plate XIII represents a long exposure of the gills of *Oreochromis niloticus* to textile effluent at

6.7ml/L showed complete erosion of gill filament which is in agreement with report by Ramesh, (1994), that toxicants at lower levels given for a prolonged time causes severe damage to the branchial system of fish than to short term treatment.

The histopathological changes observed in the gills of *Oreochromis niloticus* in the present study are in good agreement with the report of Rao *et al*. (2003). Rao *et al*. (2003) observed that the bulging of secondary lamellae at the terminal ends, lesions and erosions at the base of lamellae on 12th day of exposure of *O. mossambicus* to chlorpyrifos. A thick coat of mucus on the gill filaments was persisting on 18th day of exposure. Jauch (1979) reported that 96-hour fenthion exposure induced gill lesions, including hyperplasia and desquamation of the epithelium and thrombosis in the secondary gill lamellae.

Liver in organisms function as an organ for detoxification and in this present study the liver was affected by textile effluent in both acute and sub-lethal bioassay as shown in the microphotographs and this agree with the report that the parenchymatous hepatic tissue in teleosts, has many important physiological functions and also detoxification of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides (Roberts and Rodger, 2001).

The liver tissues of textile effluent treated fishes showed structural alteration unlike those from control group. Sinusoids which are irregularly distributed between the polygonal hepatocytes were fewer in number and are lined by endothelial cells with very prominent nuclei as presented in plates VII and XV. Hepatocytes are often swollen with glycogen or neutral fat.

In this study *Oreochromis niloticus* treated with sub-lethal concentration of textile effluent for a period of 8 weeks after observation, hepatocytes appeared swollen with granular cytoplasm. Detachment of hepatic cells in the present study indicates its non functional condition. The cells became more rounded-off showing acute necrosis and also glycogen

depletion. The above observations are in good agreement with reports by Singh *et al.* (1998); Tilak *et al.* (2005) and Kunjamma *et al.* (2007). A Study by Singh *et al*. (1998) reported clubbing, vacuolation and also necrosis of pancreatic tissue by the exposure of endosulfan and carbaryl on *Nandus nandus*. Intercellular spaces and spaces around pancreatic mass were also seen. They observed that after a long-term exposure of both endosulfan and carbaryl, the liver tissues were converted into spongy mass. The pancreatic tissues were seen shrunken and scattered due to heavy necrosis of hepatic mass. Tilak *et al.* (2005) observed the same changes in liver of *Catla catla*. The pathological changes included degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis and hepatocyte cell membrane disposition. Hepatic cords appeared in decreased size, nucleus became pyknotic. Radhaiah and Jayantha, (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood vessels among hepatocytes, pyknotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate.

The histological sections of control fish liver revealed normal typical paranchymatous appearance, the liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus similar observation was made by Risbourg and Bastide (1995), the exposure of fish to atrazine herbicide increased in the size of lipid droplets, vacuolization in the liver.

Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis.

5.6. Toxicity Studies of Wastewater on Haematological Parameters of *Oreochromis niloticus*

During the bioassay in both acute and sub-lethal the following parameters were analysed in the blood of the fish: red blood cell count (RBCC), white blood cell count (WBCC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). The composition of blood of fishes varies with the changing conditions of the environment and responds immediately to any change in water quality because of intimate contact. In this study, haematology showed increased in the RBCC with increase in concentration of the textile effluent in both the acute and sub-lethal concentration. The control had the lowest value of RBCC because of absence of the textile effluent in the tank out of varied haematological parameters differential red blood cells counts are of immense physiopathological importance. WBCC also had values increasing with increase in concentration. WBCC major function is to fight infection, to defend the body by phagocytosis against invasion by foreign organisms and to produce or transport and distribute antibodies in immune response, so it increase with increase in concentration maybe attributed to it ability to as a defense mechanism triggered by the immune system of the fish. PCV and Hb had a different trend because at higher concentration PCV and Hb had lower values during the acute and sub-lethal concentrations. MCV had a mean value of 108.12 and 99.8 during the acute and chronic bioassay, MCH during the acute study had a high value of 28.71 while during the sub-lethal studies there was a low value of 16.10 and MCHC obtained from this study showed that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting aneamic condition.

Acute and sub-lethal concentrations of textile effluent resulted in a significant decrease in RBC's count leading to anemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organ similar reports have

been made by Ramamurthy *et al.* (2009) in the freshwater fish, *Channa punctatus* exposed to tannery effluent. Sampath *et al*., (1998) on *Heteropneustes foessilis* and *Oreochromis mossambicus* after exposure to zinc and copper. Sindhu and John Thomas (2002) have shown that exposure to sub-lethal concentration of Lead produced haemolytic anemia leading to the lysis of erythrocytes, decrease in Hb content and PCV value in *Colisa fasciatus*. RBC count and PCV values resulting in hypochronic anaemia due to deficiency of iron and decreased utilization for Hb synthesis.

In the present research the reduction in number of RBC and Hb content and decrease in MCV values might have caused microcytic anemia as suggested by Haider and Inbaraj (1986) in *Glossogobicus giuris* after exposure to sublethal concentration of Malathion. Varadharaj *et al*. (1993) have observed that a reduction in the number of RBC and Hb content with an increase in MCV and MCHC values might cause macrocytic anemia in *O. mossambicus*. Since the Hb and RBCs are oxygen carrying devices, the quantitative decrease in their levels might have led to the rearrangement of the oxidative metabolism with a concomitant decrease in the tissues of respiratory potential.

Leucocytes count showed an increase in their number in the higher concentrations, but at lower concentration, there is a gradual decrease in their number, in this study only neutrophils and lymphocyte were recorded. In the present investigation at higher concentration and longer exposure period produces an increase in total WBCC and MCHC which agrees with reports suggested by Venkataraman *et al*., (2004) in *G.giuris*. Nair *et al*., (2000) suggested that the leucocytosis was the result of direct stimulation of the immunological defenses due to the presence of toxic substance or may be associated by induced tissue damage.

The constant increase in the differential count clearly indicates that the effluent stress certainly stimulate the white blood cells to produce more at all times of exposure. It has been

suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary

During the bioassay observations were made on behavioural responses which included erratic swimming, restlessness, agitation, gulping of air, loss of equilibrium and death. High mortality rate was recorded during the acute than during the sub-lethal. Physico-chemical parameters such as pH, Temperature, Total Dissolved Oxygen, Biological Oxygen Demand, Electrical Conductivity, Total Dissolved Solids, Total Alkalinity, Total Hardness and Nitrite. The above physico-chemical parameters varied in different test tanks during the bioassay.

Haemato cal parameter includes RBCC, WBCC, PCV, Hb, MCV, MCH and MCHC. RBCC decreases with increased concentration while PCV and increased with increased in concentration. Histopathological sections of gills and liver showed marked changes when compared with those gotten from the control fish in both acute and sub-lethal bioassay. Growth studies and feed utilization was dose-dependent, as those with the low concentration gave best growth against that with the highest concentration.

6.2 Conclusion

In conclusion, this study showed that the composition of textile effluent is toxic to fingerlings of *Oreochromis niloticus* which objective one tries to address. From the result it showed that textile effluent is highly toxic as it is seen in the value of $LC_{50.}$ Result on histopathology showed complete filament detachment and necrosis with increase in concentration effluent, it was also seen that blood parameters increased with increase in concentration when compared with the control group. Results on growth studies as presented in Table 4.30 showed that the control group had a better growth rate when compared those exposed to the textile effluent. From the results obtained from this study, it revealed that all

the objectives outlined in this research had been addressed. It is advisable that the textile should not be used near any water body or for irrigation and fish from such water bodies should not be consumed.

6.3 RECOMMENDATIONS

- ✓ Textile effluent should be treated before being released into the environment because they are lethal to fish and other organisms.
- ✓ Further research should be carried out to investigate the effect of this effluent to other aquatic organisms.
- ✓ There should be continue check on the quality of the textile effluents to know whether they are within acceptable limits.
- ✓ Studies should be carried to find the lethal level of other biochemical parameters of the fish.

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