Sudan University of Science and Technology

College of Veterinary Medicine and Animal production

Department of Fisheries and Wildlife science

Behavioural and Some histopathological effects of chlorine on tilapia fish, *oreochromiusniloticus*

تأثير الكلور على سلوك وبعض أنسجة أسماك البلطي النيلي

A Thesis Submitted in Partial Fulfillment of

the Requirement Of B.SC. In Fisheries

Science and Wildlife(Honour)

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بسم الله الرحمن الرحيم

قال تعالى: (وهُوَ الَّذِي سَخَّرَ الْبَحْرَ لِتَأْكُلُوا مِنْهُ لَحْمًا طَرِيًّا وَتَسْتَخْرِجُوا مِنْهُ حِلْيَةً تَلْبَسُونَهَا وَتَرَى الْفُلْكَ مَوَاخِرَ فِيهِ وَلِتَبْتَغُوا مِن فَضْلِهِ وَلَعَلَّكُمْ تَشْكُرُونَ)

صدق الله العظيم

سورة النحل الايه(14)

Dedication

We dedicate this research to our

beloved mother

To our love families

To the soul of my father

Our sisters ,and special dedicate to my(aunt) .

We also dedicate this research our friend

To every person whom we love,

To everyone who help us to finish this research.

We dedicate this research and give special thanks to our supervisor

Dr. Haram Hassan.

Acknowledgments

Our deeply thanks and gratitude to almighty Allah for giving us health and patience to complete this study.

We are greatly indebted to our supervisor **Dr.Haram Hassan AbassBakhiet** for her keen guidance,assistance and continuous encouragement during this work and for reading draft manuscript.

Our deep appreciation to technical staff of College of Animal Production Science and Technology, Sudan University of Science and Technology

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ABSTRACT

Objective:

Nile Tilapia ,*oreochromiusniloticus* were exposed to different sublethal concentrations(30mg/L and 50mg/l) of chlorine to observe behavioral and histop

athological alterations in gills and liver

Methods;

Chlorine is solved (pouder) and put it different concentration(30mg/l,50mg/l).

Reuslts:

The fishes showed marked changes in their behavior when exposed to the experiment dose at solution of different concentration of chlorine.

The fish showed rapid swimming than in control ,loss of balance, increased surfacing activity.

The liver showed marked alterations, the focal areas of necrosis vacuolation and dilation and thrombosis formation in central vein, rupture in hepatocytes, cell damage, moreover,pyknosis(PY) and hemorrhages was seen.

Gills show slouging of secondary lamellae proliferation in the epithelium of gill filaments and secondary lamellae ,congestion in blood vessels of gill filaments and atrophy of secondary lamellae.

Keywords : Nile tilapia, chlorine never behaviour .

الخلاصة

أهداف التجربة:

الغرض من هذه الدراسة ملاحظة التغيرات السلوكية والتغيرات النسيجية في الكبد والغلاصم .

طريقة التجربة:

تم تذويب الكلور (بدرة) تم تعريضه بتراكيز مختلفة (30ملج و50ملج من الكلور لكل لتر من الماء).

النتيجة:

اظهرت الاسماك استجابة سلوكية واضحة بعد تعرضها لمحلور الكلور بمختلف تراكيزه فاظهرت سلوك السباحة السريع وفقدان التوازن بالاضافة للنشاط السطحي للأسماك .

اظهرت الخياشيم تضخم في النسيج الطلائي لخيوط الثانوية واحتقان دمودي في الاوعية الدموية علي الخيوط الخيشومية وضمور في الخيوط الثانوية وانسلاخ في غشاء الخيوط الثانوية.

كما اظهرت الكبد تغيرات نسيجية شملت موت بعض الخلايا مما ادي الي حدوث فجوات بالاضافة الي تكوين بعض التخثر في الوريد المركزي وتمزق وانفجار في خلايا الكبد كما اظهرت بعض الخلايا والانوية الميت.

CHAPTER ONE

1.1Background of the study

Fish is an excellent source of protein inhuman diet. The unique feature which differentiates fish food other animal protein sources is the presence of omega-3 fatty acids such as Linolenic acid, Decosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). DHA promotes learning ability in children and improved memory in adults. DHA is also essential for the fetal growth and development.

Omega fatty acid is also good for heart and helps to control diabetics by improving insulin action **(Lee and Reasner, 2000 and Lemos*et al*., 2005)**.

That's why it must be included in human diet at least 1.3 kg per week **(FAO, 1989)**. However, the fish habitats are being contaminated alarmingly through a number of aquatic pollutants.

The Nile tilapia*Oreochromisniloticus* is indigenous to Africa and introduced It is an omnivore which feeds on plankton and aquatic plants. *Oreochromisniloticus* is famous in aquaculture and it is cultured as a food source in over seventy countries. It is cultured more than any other cichlid. It is of high economic value. Each Nile tilapia can grow very fast. They are very invasive and fast breeders. They are usually dark in colour but hybridisation in commercial rearing provides a difference in colour which tends to be more appealing to consumers. Recent studies have shown that this fish could possibly be used in the fight against malaria.

Freshwater fish and crustaceans are hyperosmotic to their environment. They Actively take in ions via the gills to balance that lost in their urine and the passive outflowby gills.**(MAETZ, 1971)**. Fish gills: mechanism of salt transfer in

fresh water and sea water.(Philosophical Transactions of the Royal Society of London Series B BiologicalSciences 262, 209-249).

Among these pollutantsthe ecological effects of power plant cooling water chlorination in freshwater **(Brungs, 1973; Mattice and Zittel, 1976; Hall *et al*., 1981; Howells, 1983)**. There is an absence of a recent synthesis of the ecological and histological impacts of chlorine and chlorine residuals from laboratory and field studies on freshwater fish.

Chlorine is a chemical element with symbol (cl) and atomic number 17.It has a relative atomic mass of about 35.5 ,Chlorine is in the halogen group(17) and is the second lightest halogen,following fluorine .The element is a yellow-green gas under standard conditions, where it forms diatomic molecules. Chlorine has the highest electron affinity and the third highest electronegativity of all the reactive elements.For this reason ,chlorine is a strong oxidizing agent. Free chlorine is rare on Earth,and is usually a result of direct or indirect oxidation by oxygen.

The most common compound of chlorine ,sodium chlorine(common salt), has been known since ancient time.Around 1630,chlorine gas was first synthesized in a chemical reaction, but not recongnized as a fundamentally important substance.

The chemistry of chlorine in natural waters is complex **(White, 1972).**

Toxicological studies have primarily concentrated on the two formswhich are apparently the most toxic to aquatic life **(Heath, 1977)**. Thefirst form, commonly referred to as free chlorine, is the portion ofchlorine injected into the water that remains as molecular chlorine (Cl2),hypochlorous acid (HOCl), or a hypochlorite ion (OCl–) after chlorinedemand has been satisfied. The second form, called monochloramine orcombined chlorine (NH2Cl), is the portion of chlorine injected into thewater that remains combined with ammonia or nitrogenous compounds

After the chlorine demand has been satisfied. The sum of the free andcombined forms generally is referred to as total residual chlorine (TRC)

The relative proportion of free and combined chlorine present followinga chlorination dose depends primarily on the concentration of nitrogenousmaterials in the water. The chlorine demand is defined as thedifference between the amount of chlorine added to the water and theamount of TRC that remains at the end of a specified period **(Mattice andZittel, 1976).**

1.2 Objectives:

In the present work, an attempt was made to evaluate the short term exposureto of chlorine and to effects:

1.Behavior of the fish.

2. Histology of gill and liver of the freshwater fish *oreochromiusnilotic*.

CHAPTER TWO
LTERATURE REVIEW

2.1 Introduction

Microorganisms can be found in raw water from rivers, lakes and groundwater. While not all microorganisms are harmful to human health, there are some that may cause diseases in humans. These are called pathogens. Pathogens present in water can be transmitted through a drinking water distribution system, causing waterborne disease in those who consume it. In order to combat waterborne diseases, different disinfection methods are used to inactivate pathogens. Along with other water treatment processes such as coagulation, sedimentation, and filtration, chlorination creates water that is safe for public consumption.

Chlorination is one of many methods that can be used to disinfect water. This method was first used over a century ago, and is still used today. It is a chemical disinfection method that uses various types of chlorine or chlorine-containing substances for the oxidation and disinfection of what will be the potable water source.

2.2 The History of Chlorination

Chlorine was first discovered in Sweden in 1744. At that time, people believed that odours from the water were responsible for transmitting diseases. In 1835, chlorine was used to remove odours from the water, but it wasn't until 1890 that chlorine was found to be an effective tool for disinfecting; a way to reduce the amount of disease transmitted through water. With this new find, chlorination began in Great Britain and then expanded to the United States in 1908 and Canada by 1917. Today, chlorination is the most popular method of disinfection and is used for water treatment all over the world.

2.3Chlorine inactivate microorganisms

Chlorine inactivates a microorganism by damaging its cell membrane. Once the cell membrane is weakened, the chlorine can enter the cell and disrupt cell respiration and DNA activity (two processes that are necessary for cell survival).

Residues of organ chlorine compounds have been recorded in Fish from Lake Nubia more than 200km downstream. The Fish "kass". HydrocyonForkalii fat tissue was found to have the highest residue levels ranging from 0.4 - 3.3 ppm, followed by muscles with concentrations 0.01- 0.25 ppm (**ElZorgani*et al,* 1998**).

2.4 Applications of chlorine:

2.4.1 Production of industrial and consumer products

Principal applications of chlorine are in the production ofa wide range of industrial and consumer products.**(Euro Chlor. October 2010 andChlorine Tree. Retrieved 2007)**

For example, it is used in making plastics, solvents fordry cleaning and metal degreasing, textiles, agrochemicalsand pharmaceuticals, insecticides, dyestuffs, householdcleaning products, etc.Many important industrial products are produced

Viaorganochlorine intermediates. Examples includepolycarbonates, polyurethanes, silicones,polytetrafluoroethylene, carboxymethyl cellulose,

and propylene oxide. Like the other halogens, chlorineparticipates in free-radical substitution reactions withhydrogen-containing organic compounds. When appliedto organic substrates, reaction is often—but notinvariably—non-regioselective, and, hence, may resultin a mixture of isomeric products. It is often difficultto control the degree of substitution as well, so multiplesubstitutions are common. If the different reaction productsare easily

separated, e.g., by distillation, substitutivefree-radical chlorination (in some cases accompaniedby concurrent thermal dehydrochlorination) may be auseful synthetic route. Industrial examples of this arethe production of methyl chloride, methylene chloride,chloroform, and carbon tetrachloride from methane,allyl chloride from propylene, and trichloroethylene, andtetrachloroethylene from1,2-dichloroethane.

Quantitatively, about 63% and 18% of all elementalchlorine produced is used in the manufacture of organicand inorganic chlorine compounds, respectively.**(Greenwood 1997)**

2.4.2 Sanitation, disinfection, and antisepsisWater chlorination

Chlorine is usually used (in the form of hypochlorousacid) to kill bacteria and other microbes in drinking watersupplies and public swimming pools. In most privateswimming pools, chlorine itself is not used, butrather sodium hypochlorite, formed from chlorine andsodium hydroxide, or solid tablets of chlorinated isocyanurates.

The drawback of using chlorine in swimmingpools is that the chlorine reacts with the proteins in humanhair and skin (see Hypochlorous acid). Once thechlorine reacts with the hair and skin, it becomes chemicallybonded. Even small water supplies are now routinelychlorinated.(**Hammond, C. R. 2000)**

2.5 Health effects and hazards

Chlorine is a toxic gas that irritates the respiratory system.Because it is denser than air, it tends to accumulateat the bottom of poorly ventilated spaces. Chlorine

gas is a strong oxidizer, which may react with flammablematerials. Chlorine MSDS" **(PDF). 1997**

Chlorine is detectable with measuring devices in concentrationsof as low as 0.2 parts per million (ppm), and bysmell at 3 ppm. Coughing and vomiting may occur at 30ppm and lung damage at 60 ppm. About 1000 ppm canbe fatal after a few deep breaths of the gas. Winder, Chris (2001) The IDLH(immediately dangerous to life and health) concentrationis 10 ppm. Chlorine MSDS" **(PDF). 1997.**

Breathing lower concentrations can aggravatethe respiratory system, and exposure to the gas canirritate the eyes.**Winder, Chris (2001)**the toxicity of chlorine comes fromits oxidizing power. When chlorine is inhaled at concentrationsabove 30 ppm, it begins to react with water andcells, which change it into hydrochloric acid (HCl) andhypochlorous acid (HClO).

When used at specified levels for water disinfection, thereaction of chlorine with water is not a major concern forhuman health. Other materials present in the water maygenerate disinfection by-products that are associated withnegative effects on human health. **(Richardson, Susan D et al 2007)**

The chemistry of chlorine in natural waters is complex **(White, 1972).**

Toxicological studies have primarily concentrated on the two forms which are apparently the most toxic to aquatic life (Heath, 1977). The first form, commonly referred to as free chlorine, is the portion of chlorine injected into the water that remains as molecular chlorine (Cl2), hypochlorous acid (HOCl), or a hypochlorite ion (OCl–) after chlorine demand has been satisfied. The second form, called monochloramine or combined chlorine (NH2Cl), is the portion of chlorine injected into the water that remains combined with ammonia or nitrogenous compounds after the chlorine demand has been satisfied. The sum of the free and combined forms generally is referred to as total residual chlorine (TRC).

The relative proportion of free and combined chlorine present following a chlorination dose depends primarily on the concentration of nitrogenous materials in the water. The chlorine demand is defined as the difference between the amount of chlorine added to the water and the amount of TRC that remains at the end of a specified period **(Mattice and Zittel, 1976).**

Data on the relative toxicity of free and residual chlorine have been variable and contradictory. In general, however, most research reports suggest that free chlorine is the most toxic form **(Merkens, 1958; Eren and Langer, 1973)**. The biocidal efficiency of chlorine depends on the amount of hypochlorous acid (HOCl) in the water, because HOCl can penetrate cells and react with cell enzymes **(Moore, 1951)**. It is this property that makes chlorine particularly toxic to fish and other aquatic organisms.

2.6generalized behavioral response of fish to chlorine

Studies of behavioral responses of fish to chlorine are important inunderstanding if and how fish react to this toxicant **(Cherry and Cairns,1982; Gray, 1983)**. In general, fish respond to chlorine exposure in aseries of phases, each of which has more or less distinct characteristics.

In the first phase, fish become restless and frequently gulp air at thesurface.(**Basch and Truchan ,1976)** observed gulls feeding on small fishfloundering on the surface during a chlorination event at a Lake Michiganpower plant. A general lethargy usually follows and can last throughoutthe exposure period **(Zeitoun, 1978b; Seegert*et al.*, 1979)**.

When fishbecome lethargic, they may appear dead; they have shallow respirationRates and often are immobile. Fish that succumb to chlorine sink to the Bottom without convulsive movements. Their respiration slows andfinally stops. Fish killed due to chlorine exposure usually assume anatural posture. However, some fish, such as sauger (Stizostedioncanadense), die with the mouth gaping and the gills flared **(Seegert*etal.*, 1979).** High chlorine concentrations

also may result in the loss oflarge amounts of mucous, particularly for common carp (Cyprinuscarpio) **(Seegert*et al.*, 1979).**

The equilibrium loss response (EL50) seems particularly importantfor minimizing sublethal effects of chlorine exposure and reducing lossesby predation. Chinook salmon *(Oncorhynchustshawytscha)* exposed toelevated temperature and chlorine required a 55% reduction in the lethaltime (LT50) to ensure that equilibrium loss did not occur **(Stober andHanson, 1974)**. Salmon generally did not recover from chlorine exposureonce equilibrium loss occurred.

2.7Generalized physiological effects of chlorine

Zeitoun**(1977, 1978a,b)** provided insights into the biochemical responsesof rainbow trout *(Oncorhynchusmykiss)* to chlorine. Hematological

analyses suggested that chlorine was not the primary cause ofobserved stress. Of parameters measured, only hematocrit and hemoglobinconcentrations differed between caged and control fish. The generalizedresponse of increases in the proportion of red blood cells in theblood of test fish, and the subsequent increase in hemoglobin could bethe result of various stress factors and are not specifically due to chlorine.

Sprague (1971) reported the same problem with nonspecificity. Rainbowtrout exposed to sublethal concentrations of total residual chlorine hadless hepatic ribosomal translational ability **(Orvos*et al.*, 1986)**. Theseresults may provide an explanation for the altered plasma proteins notedin other studies.

The ventilation rates of bluegill *(Lepomismacrochirus)* exposed tochlorine decrease initially, at low concentrations of chlorine, but increaseat higher levels of chlorine **(Miller *et al.*, 1979)**.

Some investigators alsohave reported that the coughing rate of fish increases 15-fold in responseto intermittent chlorine concentrations of 0.4 to 0.5 mg/l **(Bass andHeath, 1977)**. These authors also reported that the arterial PO2 declined

by about 60% during the first pulse peak. Work by **Block (1977)** hasshown that the gill is the chief target organ for chlorine toxicity.

Theauthors surmised that chlorine kills fish by internal hypoxia induced by gill damage. Histopathological studies **(Bass et al., 1977)** have indicatedthat sublethal concentrations of chlorine cause moderate gill hyperplasiaand swelling of the lamellar epithelial cells. These studies highlight thenumerous sublethaleffects of chlorine that may alter the fitness of thefish. Studies investigating the long-term effects of chlorine exposure arenonexistent.

Wedemeyer and Ross (1973) suggest that the stress response andsusceptibility of fish to chlorine may be related to diet. However, fishliving in thermal discharge canals usually have abundant food resources**(Coutant, 1970)**, which may negate diet as a major cofactor in theecological effects of chlorine. Additional evidence presented by **Marking*et al.* (1984)** suggests that diet appears to have little influence on thesensitivity of young rainbow trout to chlorine. **Hettrick*et al.* (1984)**reported that chlorine did not influence the susceptibility of striped bass(Moronesaxatilis) to Vibrio anguillarum, a pathogenic bacterium.

With the industrialisation the water of the streams,lakes and rivers are receiving an increasing load ofindustrial wastes. Besides polluting waters, in many casesthese waters kills the fish and other aquatic organisms.

Freshwaters are highly vulnerable to pollution since theyact as immediate sinks for the consequences of humanactivity always associated with the danger of accidentaldischarges or criminal negligence (**Vutukuru, S.S., 2005)**. Primary effects suchas fish kills can be detected but secondary effects may gounnoticed (**Simon, D., S. Helliwell and K. Robards, 1998).**

The ability to detect, identify and properlyrespond to natural chemical stimuli is an importantcomponent of the environmental physiology of fishes.

The classic ecotoxicology approach to testing aquatictoxicity is to measure the direct effect in simpleexperiments using death, more often than not, as the Endpoint(**Cairns, J.J., 1983).** In situations where no toxicity is available,bioassays become extremely important. Developmentof acute toxicity bioassay data must be viewed as anecessary step for providing comparative toxicity

information on different toxicants and species oforganisms**(Rajendran, N., R. Rajendran, O. Matsuda and V.K. Venugopalan, 1989).**

CHAPTER THREE

Methodology

3-1 Study design

Descriptive cross bass study conducted in soba lab.

3.2 Fish:

Adult freshwater fish Tilapia (Family: Cichlidae), were obtained from the Department of fisheries and wildlife science,Hatchery in Sudan University of science and Technology. A total of 23 adult fish of both sexes were used. The average weight of the fish was 20-90 g. the fish were placed in large tanks (34 x 22 x 18 cm) withaerated tap water and were fed with commerciallypellets. Fish were acclimatized for 2 days and an average temperature of29°C. The tap water used for the experiment had a pH value of6-7 and the water was tested for chlorine .

Chlorine was added as a powder. In two dosages:

The first group was 30 mg/l with two replicates each of 5fish

The second group was 50 mg/l with two replicates each 5fish

The third was the control group

Plate: group fish

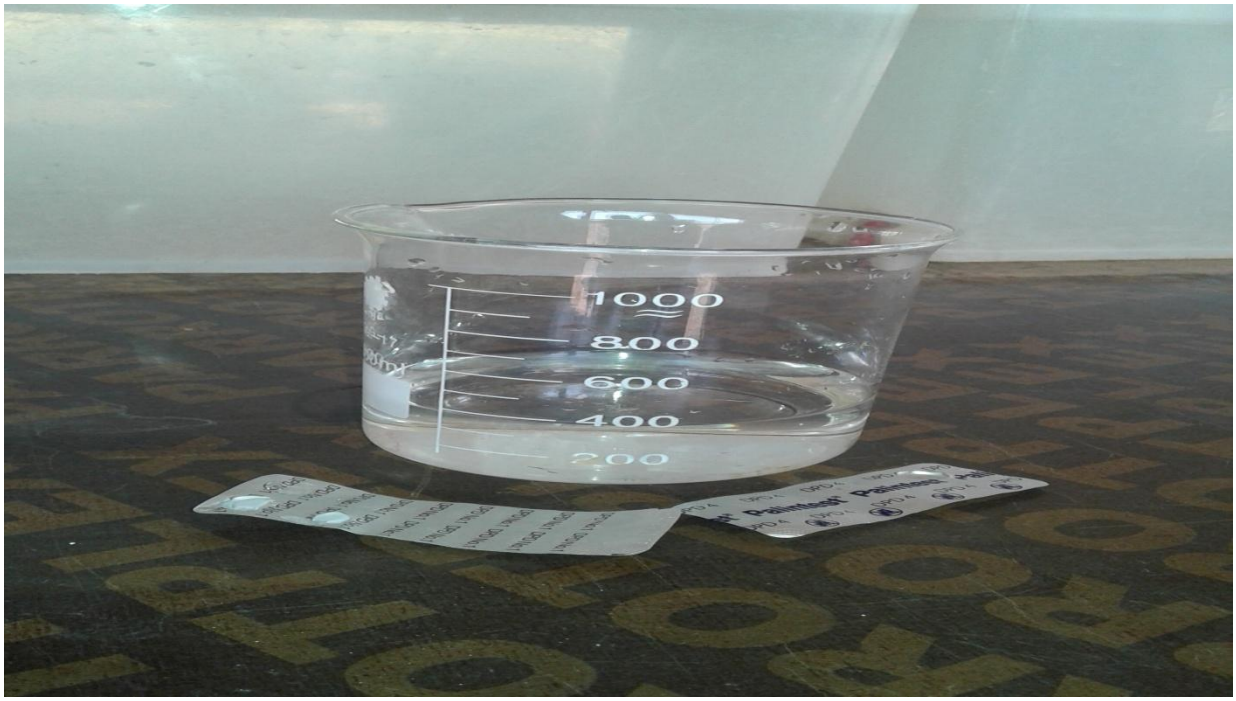

Plate: water quality parameters

3.3Preparation of Stock Solution:

Chlorine was procured from the DAL group. Ltd, Bahre Sudan. Stock solution was prepared by dissolving the and diluted with tap water.

3.4 Methods:

The fish were exposed to dissolved chlorine till fishes were dead. Any dead fish was dissected for its gills and liver.

3.5Water quality parameters:

Physical measurements:

Temperature (C^o): water temperature was measured using an ordinary centigrade measuring thermometer.

PH: Water pH was determined by taking water sample and measuring its pH by portable digital Ph meter

3.6Behavioural Manifestation:

The observations of the fish behavior were recorded during the experiment.

3.7Histopathological Examination:

Tissue specimens from fish of all groups were taken from (gills and livers) and fixed in 10 % buffered neutral formalin. Then processed using the normal histological techniques to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin and examined under light microscope.

CHAPTER FOUR

RESULTS

4.1 Mortality:

No mortality was observed in the control group however, mortality increased with the increase in the concentration and the exposure duration of the chlorine. The concentration at which the highest percent mortality was50mg/L and hundred percent mortality were caused in the both concentrations

Table (4.1) Fish mortality time in oreochromiusnilotius exposed to two concentrations of chlorine

Concentration cl_2^- (mgl)2	**Cumulativemortality**				
	Timeofexposition				
	5 minute	**15 minute**	**25 minute**	**35minute**	**45minute**
0.0(controlgroup)	0	0	0	0	0
30	0	2	3	3	2
50	2	3	4	1	0

4.2 Behavioural Manifestation: The behavior and condition of the fishes in both the control and test solutions were noted all the time up to completemortality (Fig 4.1,4. 2,4. 3 and4. 4). The fishes showed marked changes in their behavior when exposed to the test solution of different concentrations. In both concentration of chlorine (30mg/L, 50 mg/ L) the fishes showed rapid swimming than in control. Behavioural manifestations of acute toxicity like hyperactivity, loss of balance, rapid swimming, increased surfacing activity was also observed.

Plate(4.1):scattered shoal

plate(4.2) : vertical swimming

Plate((4.3)Loss of balance

4.3 Histopathological results:

The histopathology of different Tilapiatissues revealed that there are several histopathologicalchanges in different Tilapia organs (liver, gills) as shown in (plates 1-20).

Liver showed Focal areas of necrosis (FN) as shown in plate (2), **Dilation and thrombosis formation in central vain (CV)** as shown in plate (3, 4), **ruptured hepatocytes (RH) and vacuolation (V)** as shown in plate (5, 6) , **cell damage (CD)** as shown in plate (7),**pyknosis (PY) and hemorrhages** as shown in plate (8, 9 and 10),*(Oreochromisniloticus)* were showed histopathological alterations.

Gills showed**sloughing of secondary lamellae** as shown in plate (12,13,14,15,16,17),**proliferation and damage in the epithelium of gill filaments** as shown in plate (18,19)**congestion in blood vessels of gill filaments and atrophy of secondary lamella** as shown in plate (20).

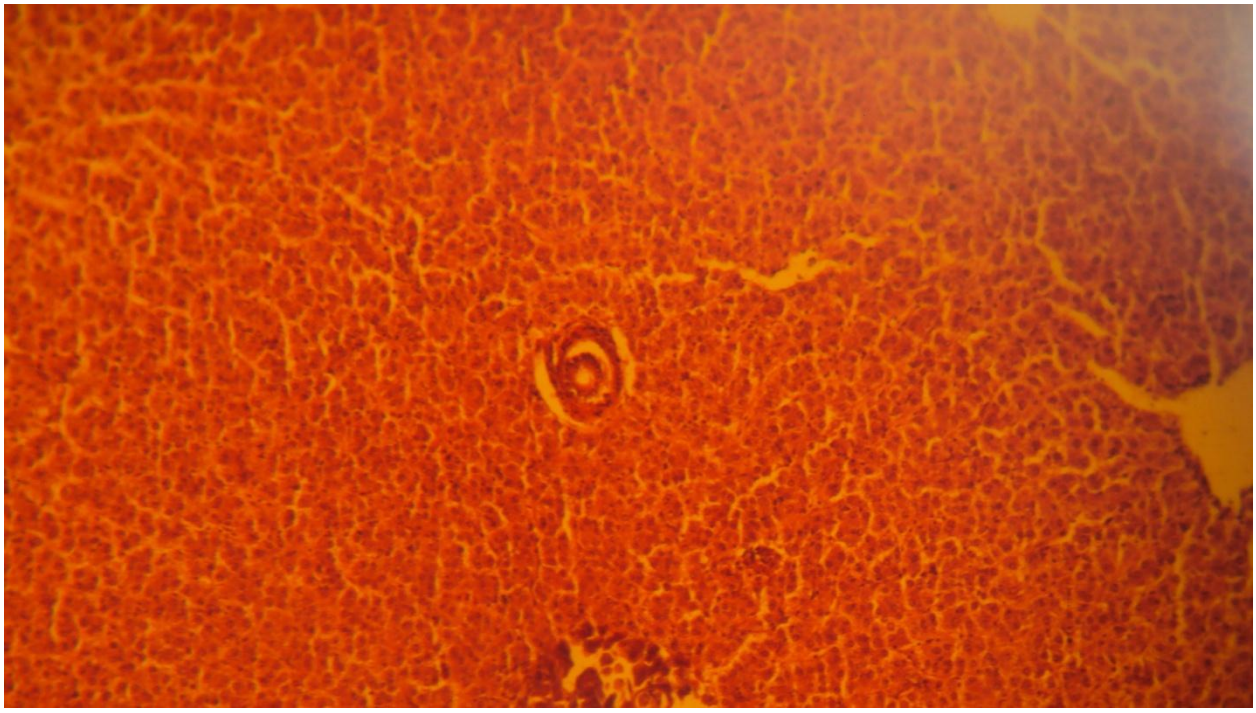

Plate 1. Section through the liver of *Oreochromisniloticus* showing the normal tissues of liver. H&E X 200.

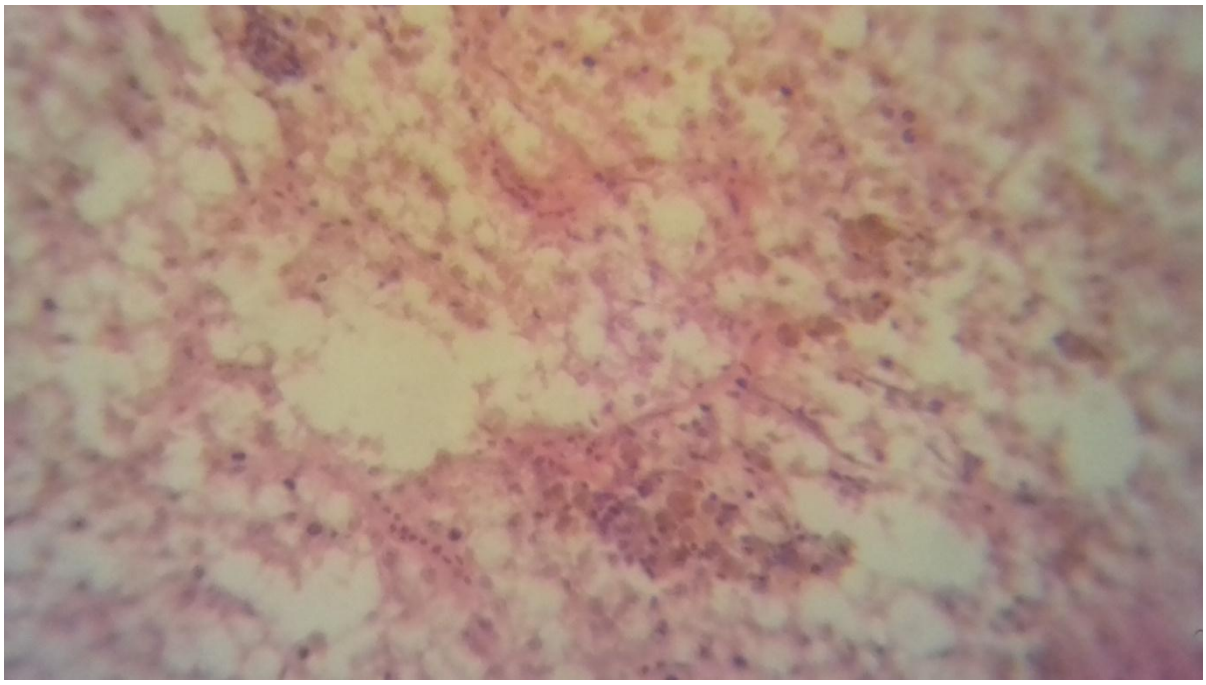

Plate 2. Section through the liver of *Oreochromisniloticus* showing Focal areas of necrosis(FN).H&E X200

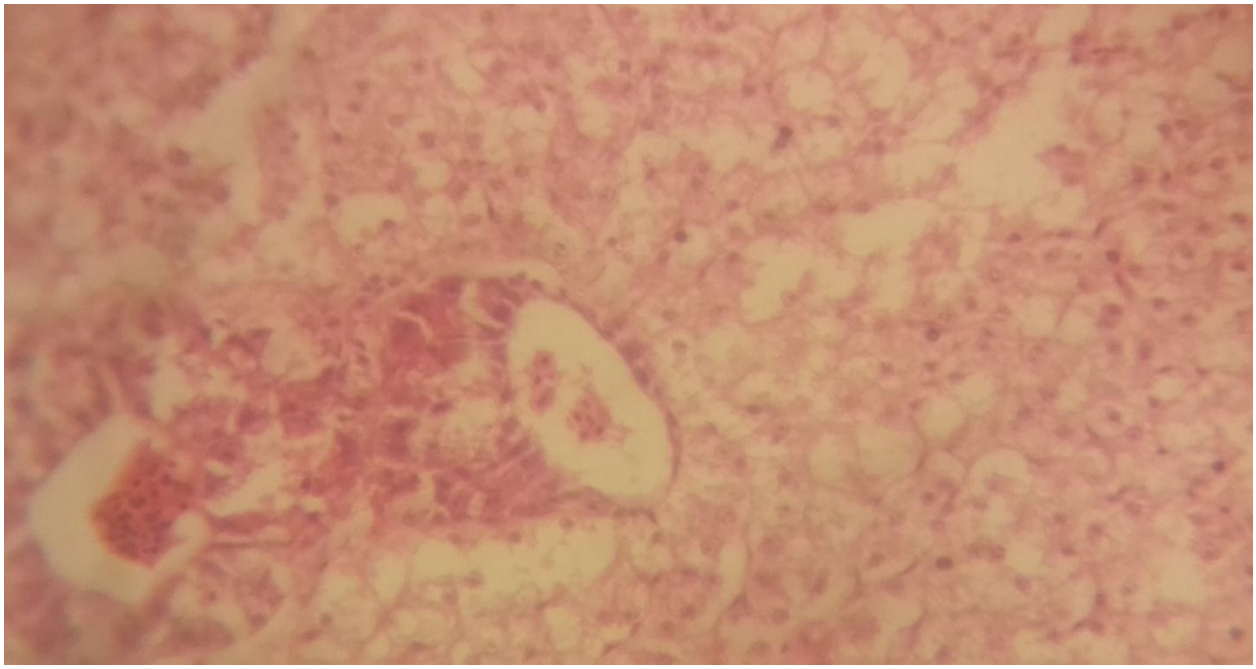

Plate 3. Section through the liver of *Oreochromisniloticus* showing Dilation and thrombosis formation in central vain (CV). H&E X200

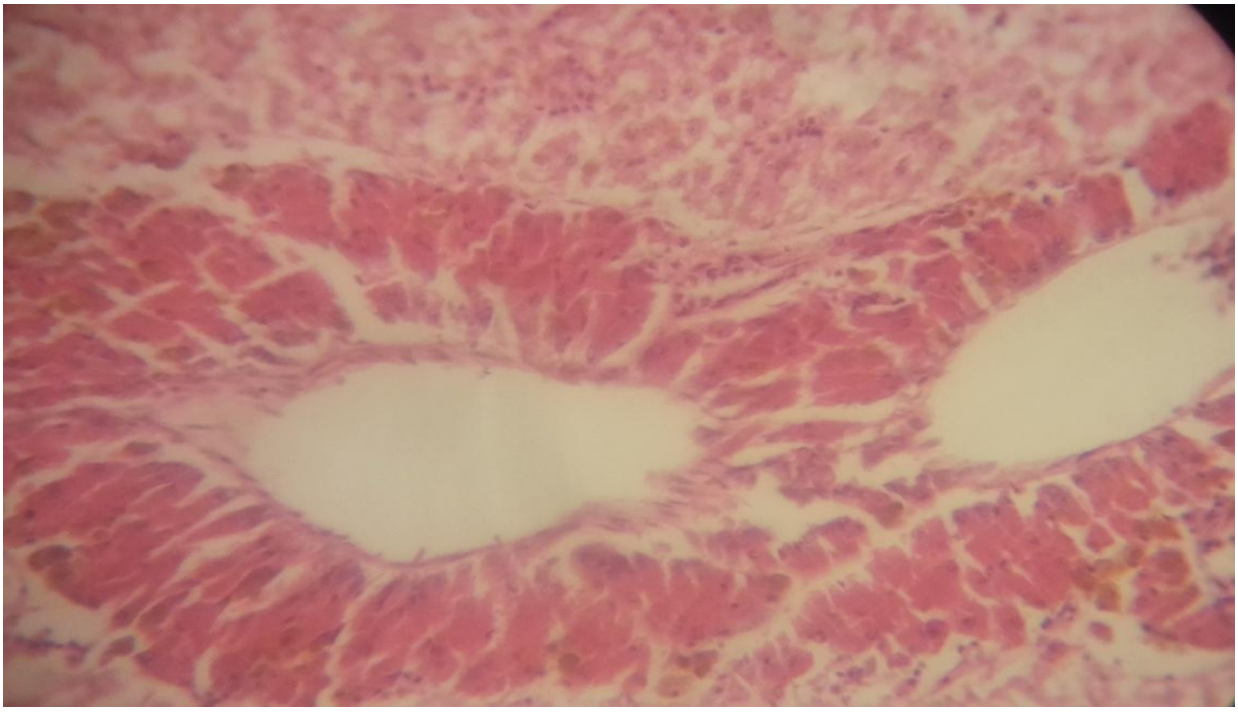

Plate 4. Section through the liver of *Oreochromisniloticus* showing Dilation and thrombosis formation in central vain (CV). H&E X200

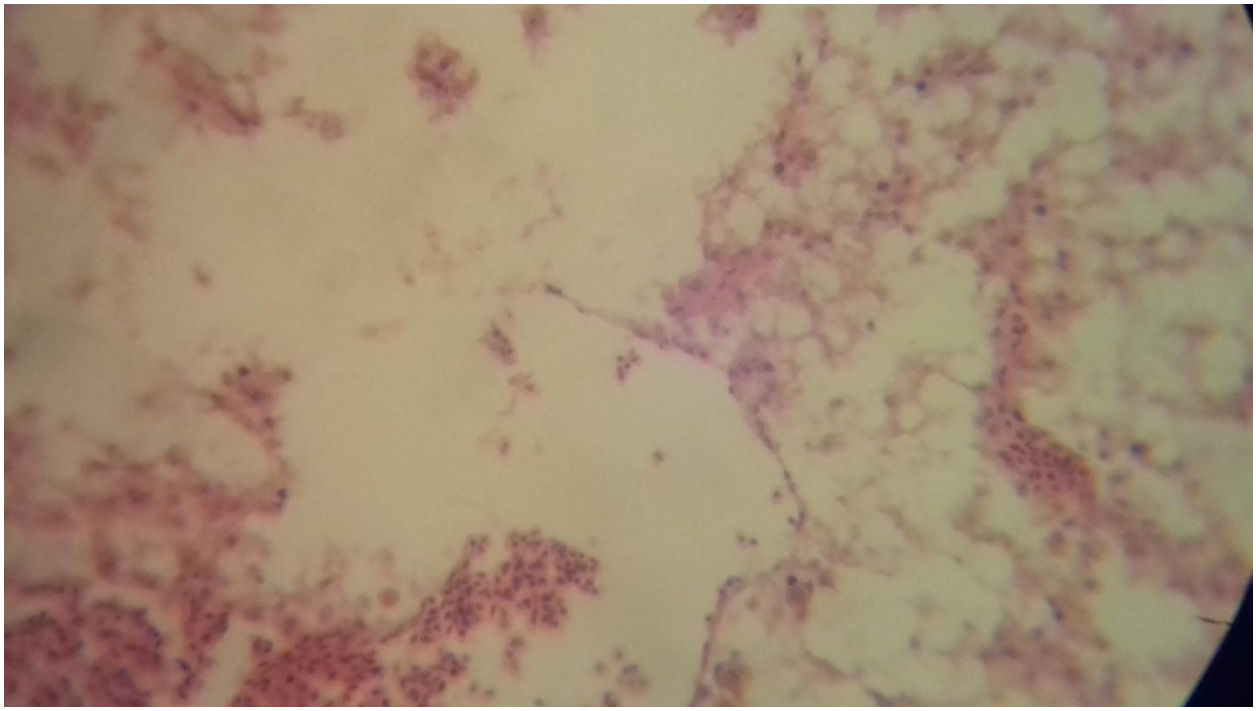

Plate 5. Section through the liver of*Oreochromisniloticus*showing ruptured hepatocytes (RH) and vacuolation (V) H&E X200

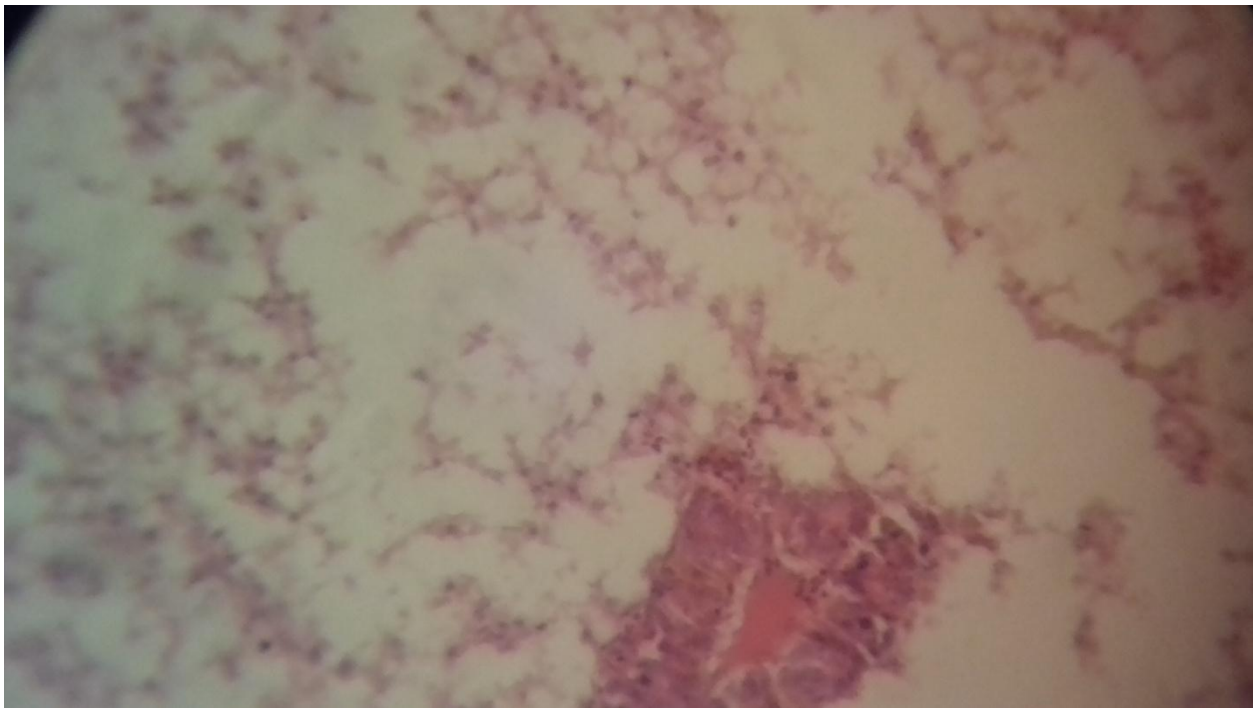

Plate 6. Section through the liver of*Oreochromisniloticus*showing ruptured hepatocytes (RH) and vacuolation (V) H&E X200

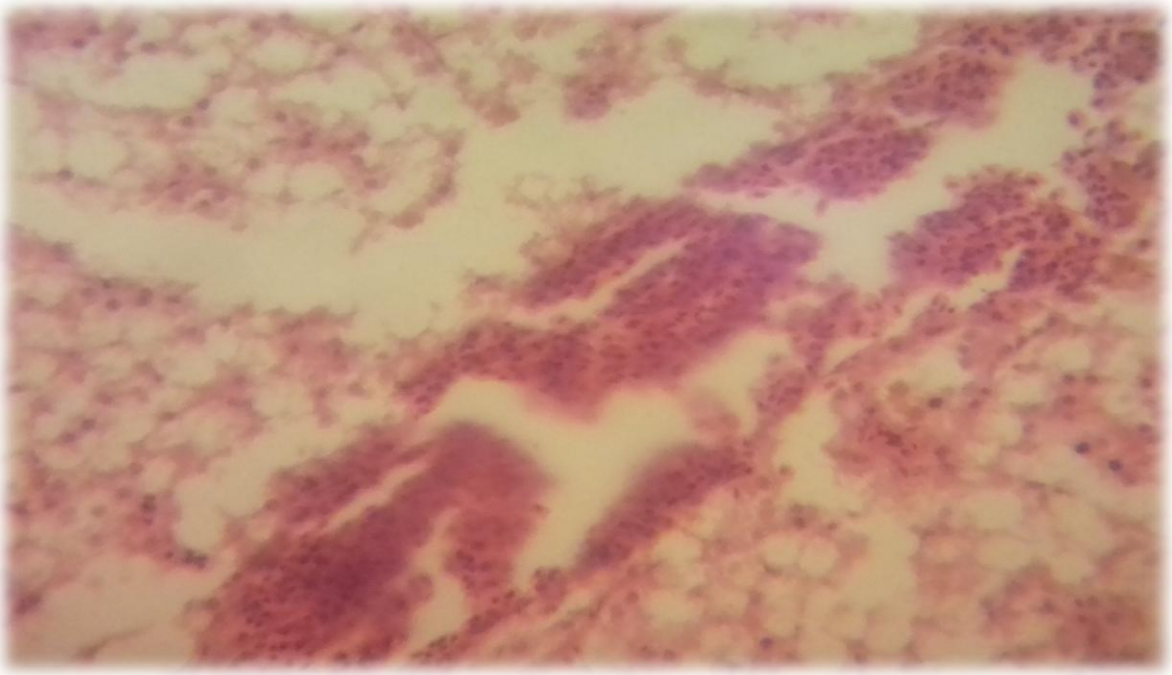

Plate 7. Section through the liver of *Oreochromisniloticus*showing cell damage (CD).H&E X200

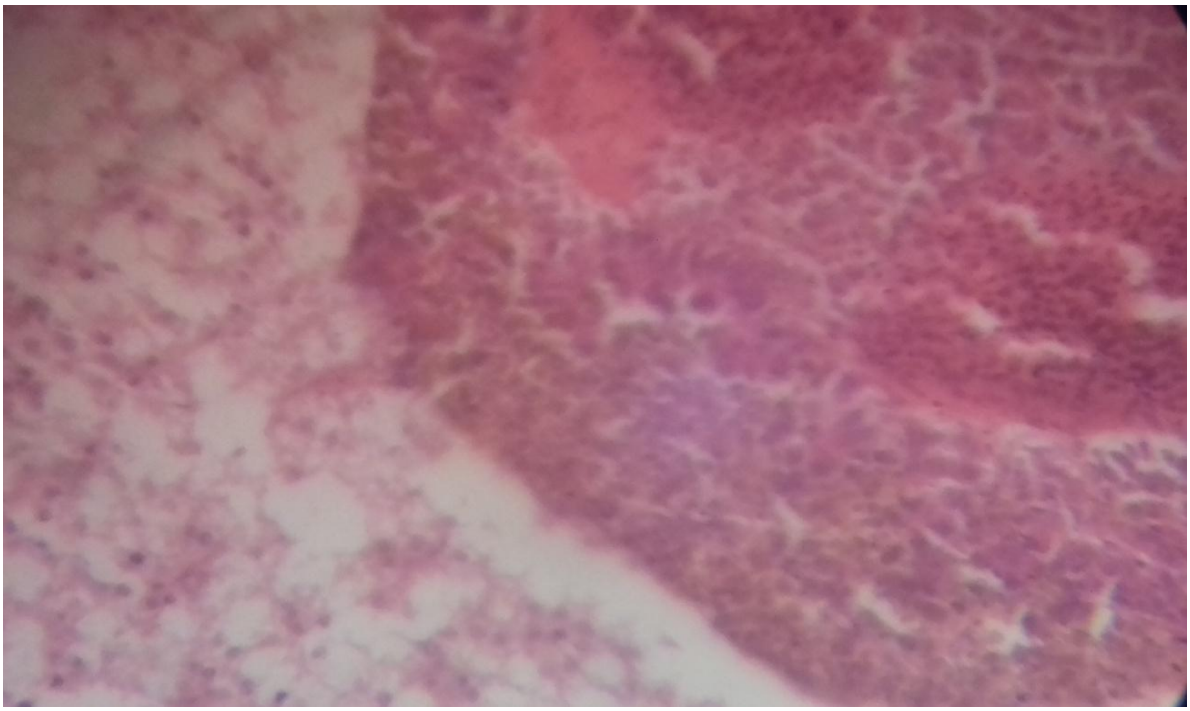

Plate 8. Section through the liver of *Oreochromisniloticus*showingpyknosis (PY) and hemorrhage .H&E X400

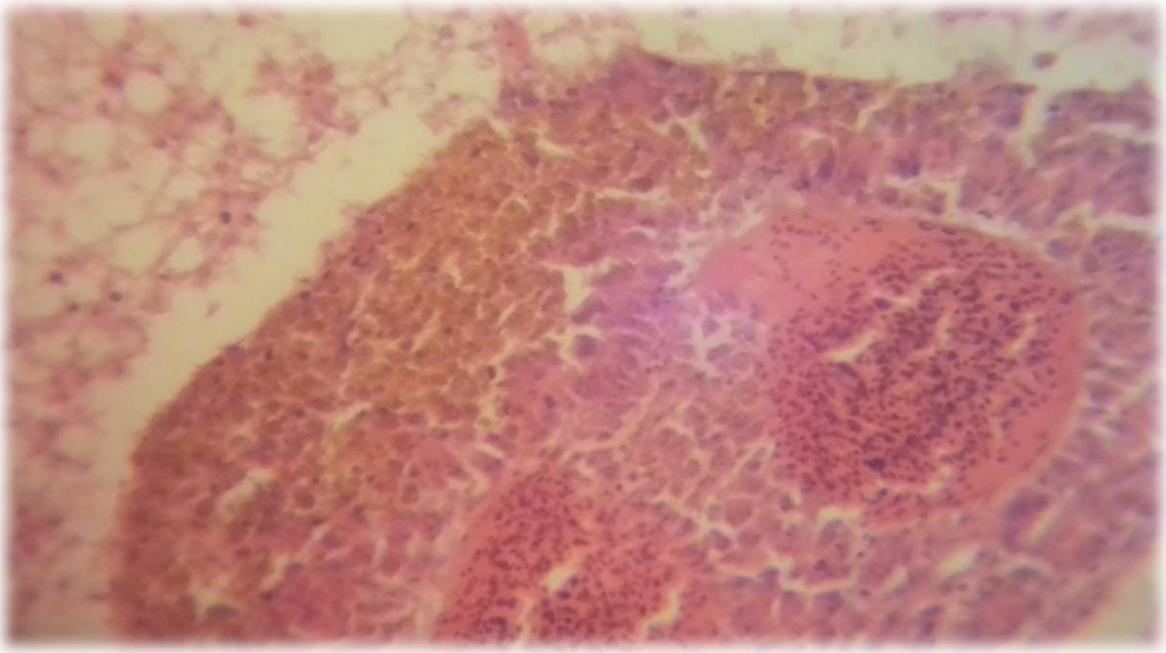

Plate 9. Section through the liver of *Oreochromisniloticus*showingpyknosis (PY) and hemorrhage .H&E X400

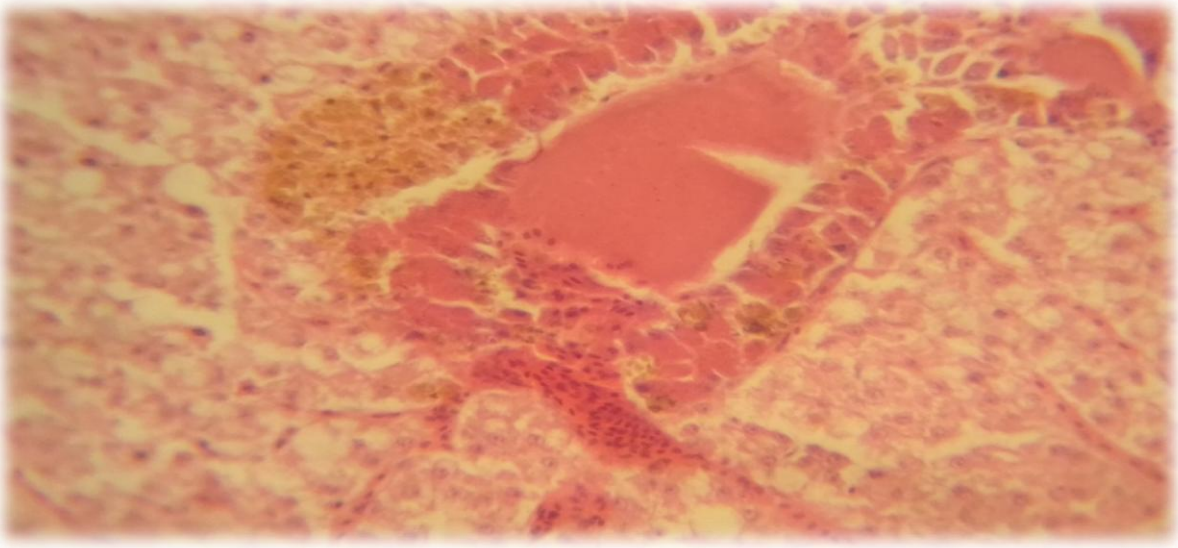

Plate 10. Section through the liver of *Oreochromisniloticus*showingpyknosis (PY) and hemorrhage .H&E X400

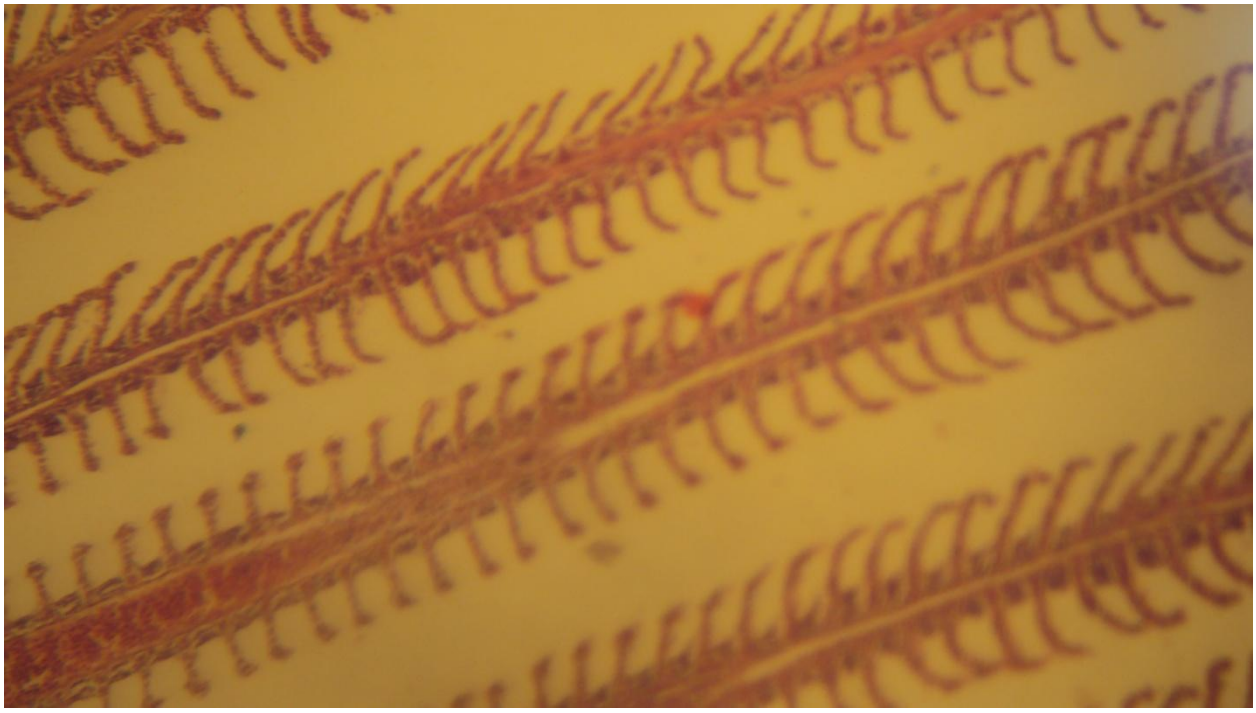

Plate 11. Section through the control gills of *Oreochromisniloticus*.H&E X200

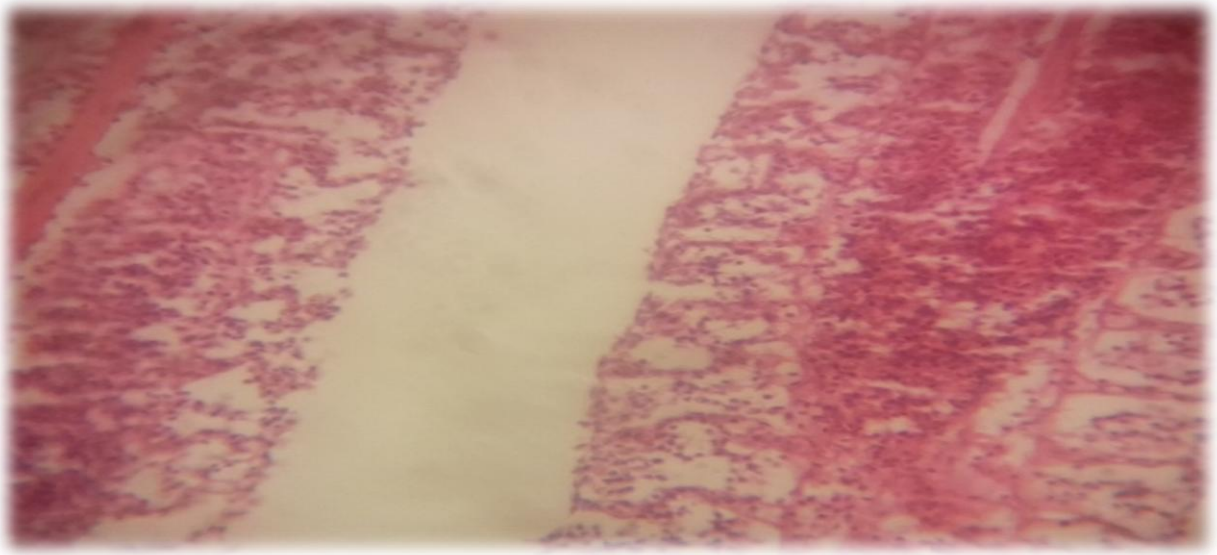

Plate 12. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

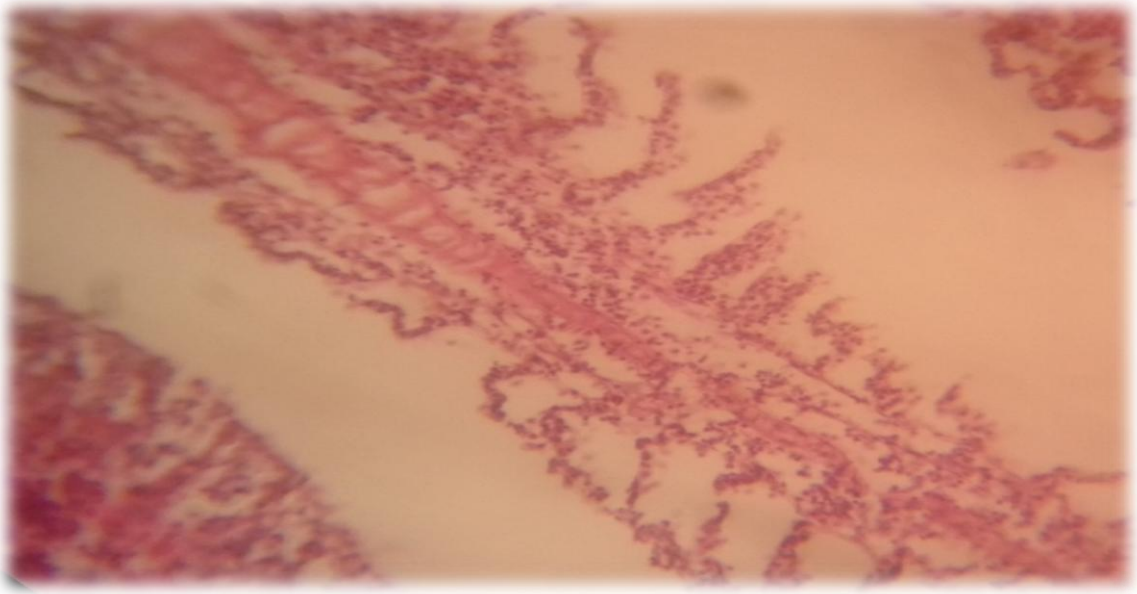

Plate 13. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

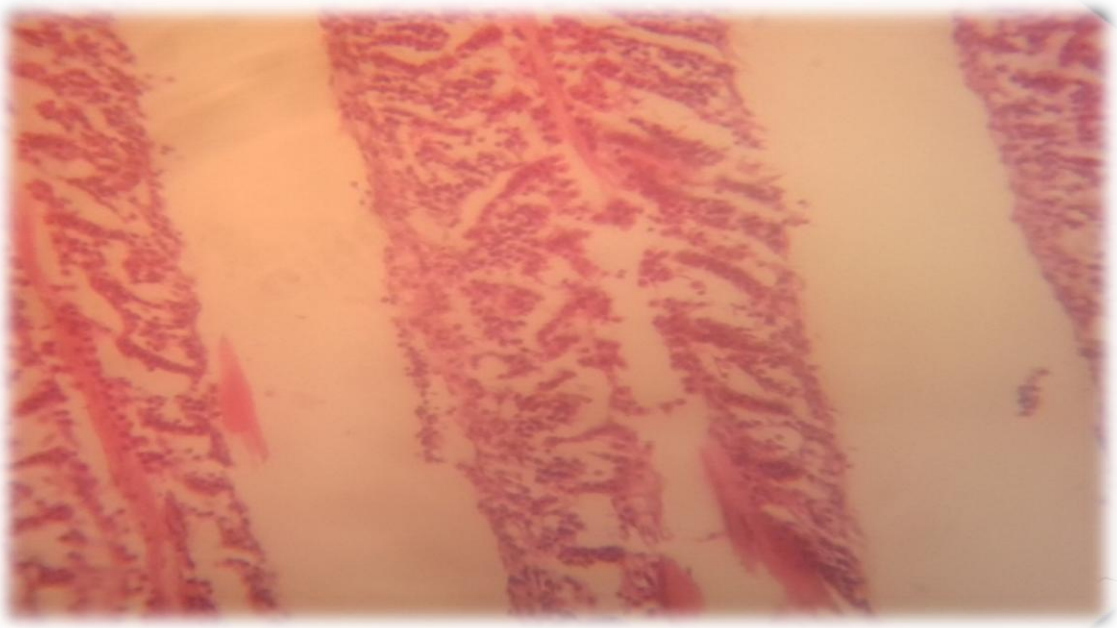

Plate 14. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

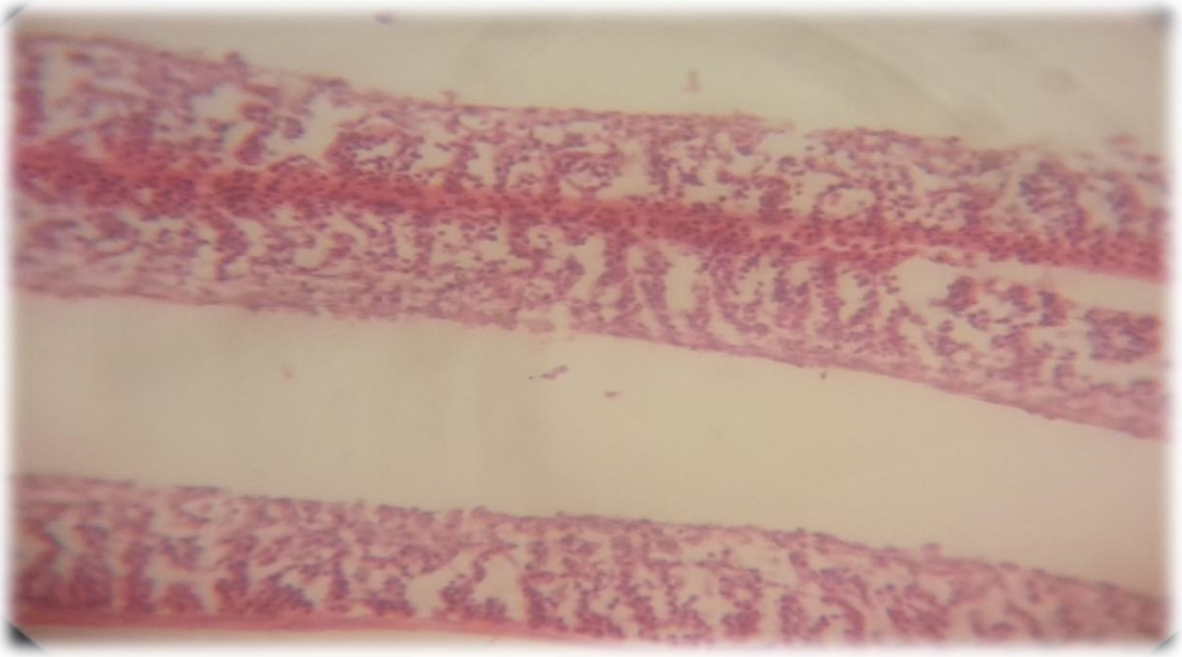

Plate 15. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

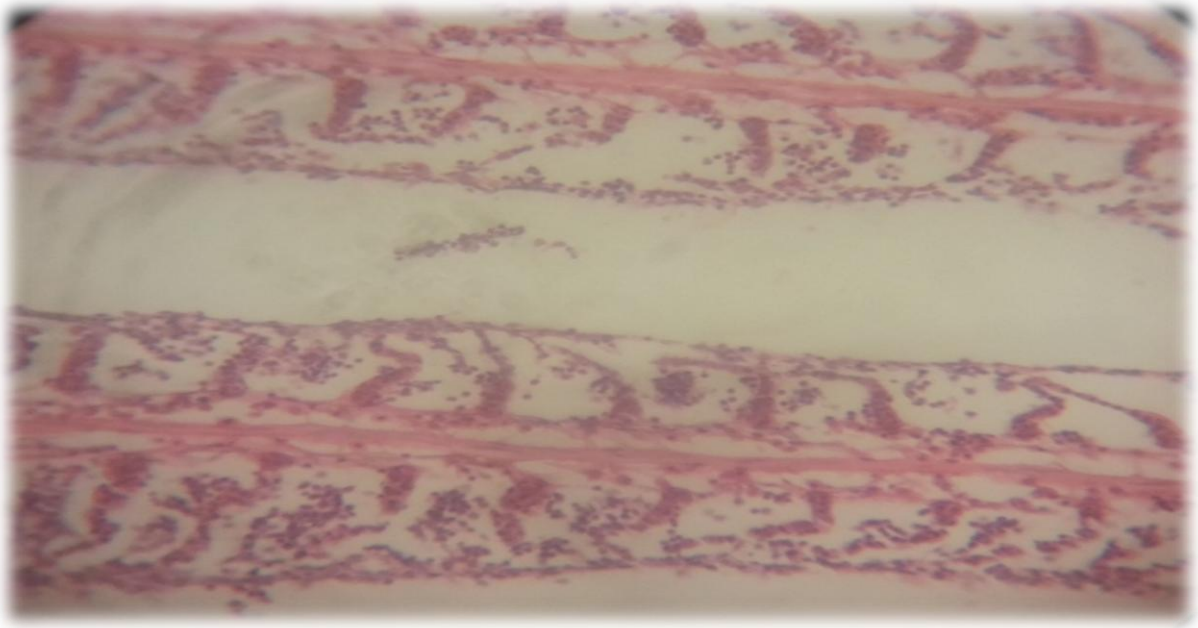

Plate 16. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

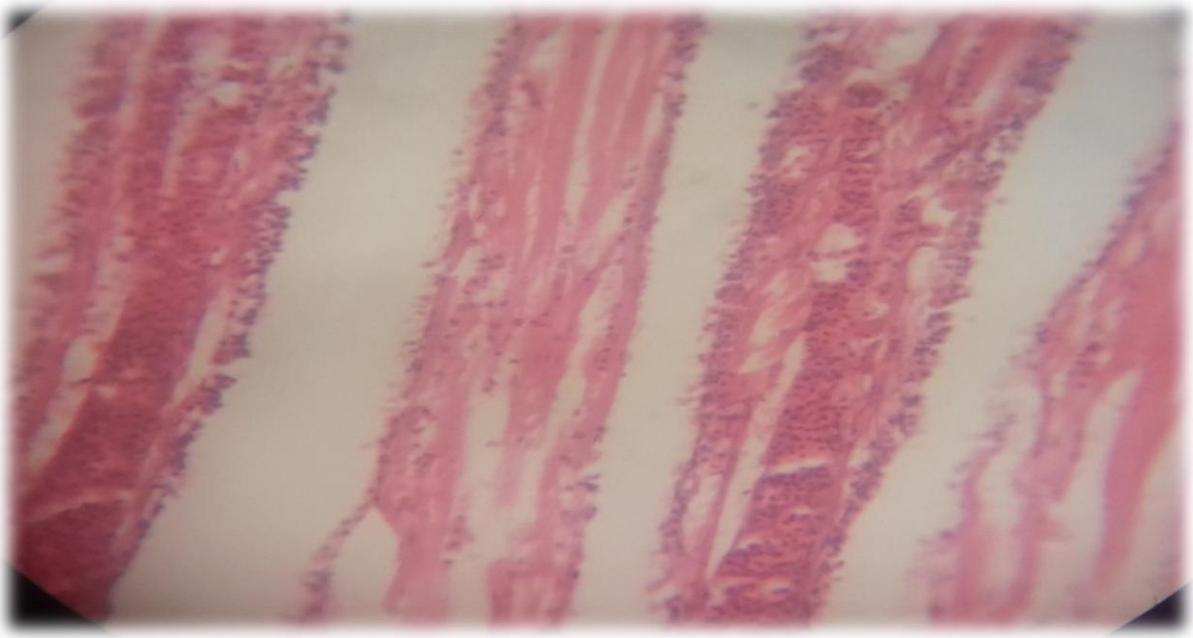

Plate 17. Section through the gills of *Oreochromisniloticus* showing sloughing of secondary lamellae .H&E X400

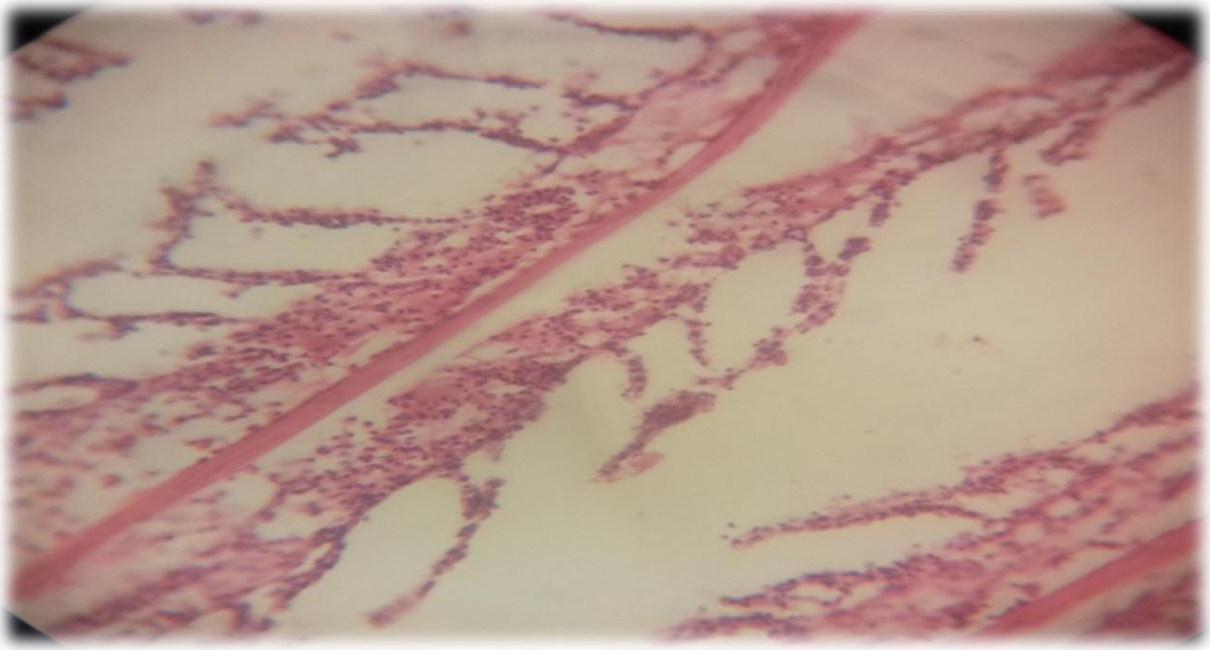

Plate 18. Section through the gills of *Oreochromisniloticus* showing proliferation and damage in the epithelium of gill filaments (EPC)H&E X400

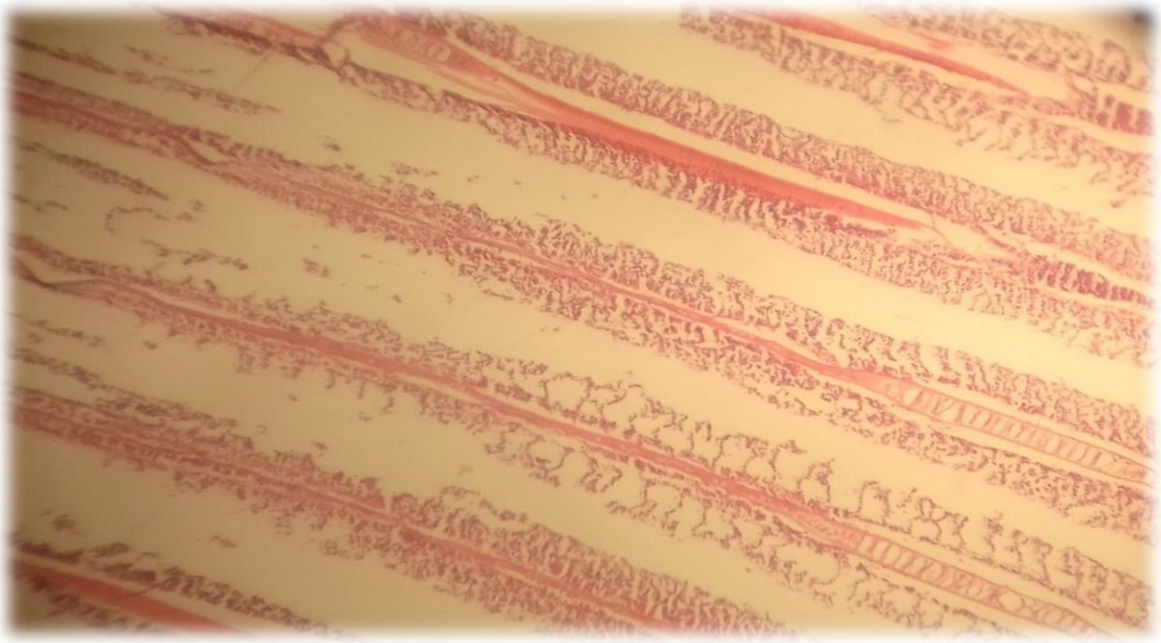

Plate 19. Section through the gills of *Oreochromisniloticus* showing proliferation and damage in the epithelium of gill filaments (EPC)H&E X400

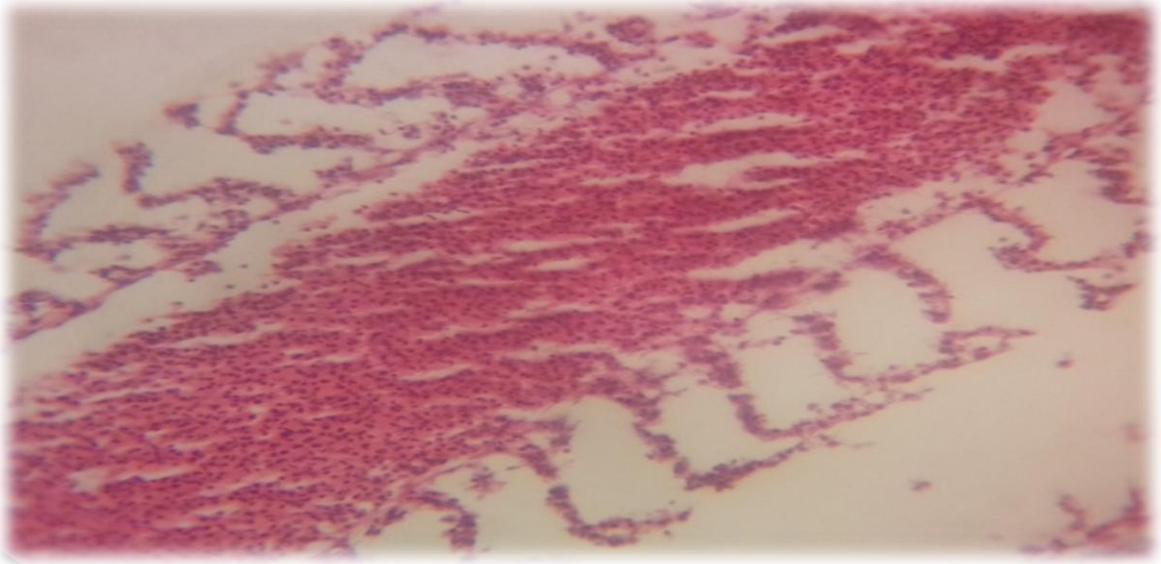

Plate 20. Section through the gills of *Oreochromisniloticus* showing congestion in blood vessels of gill filaments and atrophy of secondary lamella .H&E X400

Table (2). Showing the main types of histopathological changes detected In *Oreochromisniloticus*exposed to chlorine.

Organ	*Histopathological changes*	*Numberof fish in which t effect was detected*	
		30 ppm	*50 ppm*
Liver	Focal areas of necrosis	9	*10*
	Dilation and thrombosis formation in central vein	*10*	*10*
	Ruptured hepatocytes and vacuolation	*8*	*10*
	Cell damage	*10*	*10*
	Pyknosis (PY) and hemorrhages	*9*	*9*
Gills	Sloughing of secondary lamellae	10	*10*
	Proliferation and damage in the epithelium of gill fila	*10*	*10*
	Congestion in blood vessels of gill filaments and secondary lamella	*10*	*10*

CHAPTER FIVE

DISCUSSION

Behavioral changes are the most sensitive indication of potential toxic effects studied. In the control group the behavioral and the swimming patterns of the fishes were normal and there was no mortality. In the initial period of exposure to chlorine, the fish stayed motionless and settled to the bottom. This may be attributed to the fact that, the sudden shock caused by the toxicant. The fishesbehavioral response started appearing after 5 minute of treatment.

The shoaling behaviour was disrupted in the first minutes itself and they were spread out and appeared to be swimming independent of one another. The disturbance in the shoaling behaviour of the fish in the treated media indicates the loss of group hydrodynamic effect of fish**(Zuyev, G.V. and V.V. Bolyayen, 1970)** increased swimming activity and entails high expenditure of energy **(Zuyev, G.V. and V.V. Bolyayen, 1970)**. Erratic swimming of thetreated fish indicates the loss of equilibrium. chlorine has profound effect on the central nervous system.

The histopathological alterations attributed to theprolonged exposure to chlorine resulted inrespiratory, osmoregulatory and circulatory impairment.

These findings were demonstrated by **Fernandes*et al.*(2008)**. Moreover, **Alvarado *et al.* (2006)** reported that,the dramatic increase of chloride cells in the gills thatproduces epithelial thickening of the filamentepithelium enhances migration of chloride cells up tothe edge of the secondary lamellae and provokes thehypertrophy and fusion of secondary lamellae. Thesecould be considered as unspecific biomarker responsesof chloride exposure and disturbed health of fish.

Gills showed edema of the primary lamellae;severe edema, hyperplasia, fusion and focaldesquamation of the epithelial lining of the secondary

lamellae were observed. According to **Mallatt (1985),**the edema of the gill epithelium is one of the mainstructural changes caused by the exposure to chlorine. Our results confirm this lesion of chlorine exposure. These alterations have been reported forother species exposed to heavy metals particularly Cd **(Gardner and Yevich 1970; Karlsson-Norrgren*et al*.1985; Pratap and WendelaarBonga 1993; Thophon*etal.* 2003)** and sometimes referred as a first sign ofpathology **(Thophon*et al*. 2003).** Cellular proliferationin the gill epithelium is also observed in fish exposedto different pollutants as described by **Gardner andYevich 1970 and Thophon*et al*. 2003**. Lifting,swelling, and hyperplasia of the gill epithelium couldserve as a defense function.

The liver showed congestion of central vein and nuclear pyknosis in themajority of hepatic cells. These findings were apparentas the liver considered the organ of detoxification,excretion and binding proteins such as metallothionein(MTs).

Thecell damages. Similar resultswere observed by **Van Dyk (2003)** and **Mela*et al*.(2007)**.

Liver of fish is sensitive to environmentalcontaminants because many contaminants tend toaccumulate in the liver and exposing it to a muchhigher levels than in the environment or other Organs**(Heath 1995).**

Pandey*et al*., (1994) described the alterations inliver and intestine of *Liza parsia*exposed to Hg Cl2(0.2 mg Hg l– 1) for 15 days. Similarly (**Oliveira Ribeiro**

***et al*. 2002)** reported serious injuries in gills liver of *Salvelinusalpinus*exposed to0.15 mg Hg l– 1.

CONCLUSIONS AND RECOMMENDATIONS:

CONCLUSIONS:

- Chlorine in water ponds of fish affect the fish behavior clearly

- Chlorine in water ponds of fish cause the fish mortality

- Many histological impacts were found in the gills and liver of the studied fish species exposed to different dosage of chlorine.

- Many histological impacts were found in the gills and liver of the studied fishspecies exposed to different dosage of chlorine.

- The analysis of observations and tissues from the present investigation evidenced that chlorine is highly toxic and had profound impact on behaviour and respiration in *oreochromiusniloticus*

- Tap water may contains small amount of chlorine to safeguard our health , which can be harmful to your fish

RECOMMENDATIONS:

- The general research environment in fisheries sector must be improved, through technical support; instruments availability, trained technicians, and laboratory equipment's.

- Further investigation on bioaccumulation and the impacts of chlorine in different organs and different fish species is needed.

- Sudanese standards and metrology organization must have their own critical limits (permitted concentration) of toxicants,Sanitation, disinfection, and antisepsis Water chlorination in fish ponds and to put in consideration human health.

CHAPTER SIX

REFERENCES

Alvarado NE, Quesada K, Hylland I, Marigómez, L, Soto M. 2006. Quantitativechanges in metallothionein expression in target cell-types in the gills of turbot (Scophthalmusmaximus) exposed to Cd, Cu, Zn and after a depuration treatment. Aquatatic Toxicology 77: 64-77.

Basch, R. E. and J. G. Truchan. Toxicity of chlorinated power plant condensing cooling waters to fish. Environmental Protection Agency Report EPA-600/3–76–009. East Lansing, Michigan. 105 pp. (1976).

Bass, M. L., C. R. Berry, Jr., and A. G. Heath. Histopathological effects of intermittent chlorine exposure on bluegill (Lepomismacrochirus) and rainbow trout (Salmogairdneri). Water Res. 11: 731–735 (1977).

Block, R. M. Physiological responses of estuarine organisms to chlorine. Chesapeake J. Sci. 18: 158–160 (1977).

Cairns, J.J., 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia, 100: 47-57.

Cherry, D. S. and J. Cairns. Biologial monitoring. Part V — preference and avoidance studies. Water Res. 16: 263–301 (1982).

Coutant, C. C. Biological aspects of thermal pollution I. Entrainment and discharge canal effects. Crit. Rev. Environ. Control. 1: 341–381 (1970).

El Zorgani, G.A, Abbass, I.A and Ali, M.T (1998): Pollution of surface waters withpesticides in udan.The Fish "kass" HydrocyonForskalii (cur) as monitor for the contamination of the Nile with Organochlorine pesticides, scientific conference on: Dimension of Environmental stress in the Region. "Yarmouk University. Irbid.Jordan Oct.**26**-27, 1998".

Eren, Y. and Y. Langer. The effect of chlorination on Tilapia fish. Bamidgeh 25: 56–60 (1973).

Fernandes C, Fernandes AF, Ferreira M, Salgado M. 2008. Oxidative stress response in gill and liver of Liza saliens , from the Esmoriz-Paramos coastal lagoon, Portugal. Archives of Environmental Contamination and Toxicology 55: 691-700.

Gardner GR, Yevich PP. 1970. Histological and hematological responses of an estuarine teleost to cadmium.Canadian Journal of Fish Research 27:2185-2196.

Gray, R. H. Behavioral response of fish to altered water quality: a review of selected examples with emphasis on salmonids. Environ. Impact Assess. Rev. 4: 84–96 (1983).

Hammond, C. R. (2000). The Elements, in Handbook of Chemistry and Physics (81st ed.). CRC press. ISBN 0- 8493-0481-4.

Heath AC. 1995. Water pollution and fish physiology. 2nd edn., Lewis Publishers, Boca Raton. pp. 125-140.

Heath, A. G. Toxicity of intermittent chlorination to freshwater fish: influence of temperature and chlorine form. Hyrdobiologia 56: 39–47 (1977).

Hetrick, F. M., L. W. Hall, Jr., S. Wolski, W. C. Graves, and B. S. Roberson. Influence of chlorine on the susceptibility of striped bass (Moronesaxatilis) to Vibrio anguillarum. Can. J. Fish. Aquat. Sci. 41: 1375–1380 (1984).

In: Water Chlorination: Environmental Impact and Health Effects. Vol. 3. (Jolley et al., Eds.) Ann Arbor Science, Inc., Ann Arbor, MI (1979)

Karlsson-Norrgren L, Runn P, Haux C, Förlin L. 1985. Cadmium-induced changes in gill morphology of zebra fish Brachydaniorerio (Hamilton -Buchanan) and rainbow trout Salmogairdneri Richardson. Journal of Fish Biology 27: 81–95.

Mallatt J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Aquatic

Science 42:630-648

Marking, L. L., T. D. Bills, and J. R. Crowther. Effects of five diets on sensitivity of rainbow trout to eleven chemicals. Prog. Fish-Cult. 46: 1–5 (1984).

Mattice, J. S. and H. E. Zittel. Site-specific evaluation of power plant chlorination. J. Poll. Control Fed. 48: 2284–2308 (1976).

Mela MR, Ventura F, Carvalho DF, Pelletier CE, Ribeiro CA. 2007. Effects of dietary methylmercury on liver and kidney histology in

the neotropical fish Hopliasmalabaricus. Ecotoxicology and Environmental Safety 68: 426 - 35.

Merkens, J. C. Studies on the toxicilty of chlorine and chloramines to the rainbow trout. J. Water. Waste. Treat. 7: 150–151 (1958).

Miller, W. R., D. Gruber, J. Cairns Jr., A. C. Hendricks, and R. C. Hoehn. Biologicalmonitoring of chlorine-induced ventilatory changes in bluegill sunfish (Lepomismacrochirus).pp. 589–597.

Moore, E. W. Fundamentals of chlorination of sewage and waste. Water Sew. Works. 98: 136–139 (1951).

NOAA Office of Response and Restoration, US GOV. "CHLORINE". noaa.gov. Retrieved 25 August 2015.

Oliveira-Ribeiro CA, Belger L, Pelletiter E, Rouleau C. 2002. Histopathological evidence of inorganic mercury and methylmercury toxicity in the arctic charr (Salvelinusalpinus). Environmental Research 90: 217-225.

Orvos, D. R., C. M. Sellers, Jr., and N. E. Garrison. Effect of a single residual chlorine pulse upon subsequent in vitro protein synthesis by rainbow trout (Salmogairdneri) liver. Hydrobiologia 131: 249–255 (1986).

Pandey AK, Mohamed MP, George KC. 1994. Histopathological alterations in liver and intestine of Liza parsia (Hamilton-Buchanan) in response to mercury toxicity. Journal of Advanced Zoology

Pratap HB, WendelaarBonga SE. 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells, and sodium, potassium -ATPase

activity in the gills of the freshwater teleost Oreochromismossambicus at normal and high calcium levels in the ambient water. Aquatic Toxicology 26: 133–150.

Rajendran, N., R. Rajendran, O. Matsuda and V.K. Venugopalan, 1989. Acute toxicity of organochlorine pesticides to fishes and shell fishes of tropical estuary. J. Fac. Appl. Biol. Sci. Hiroshima University 28: 37-48.

Richardson, Susan D.; Plewa, Michael J.; Wagner, Elizabeth D.;Schoeny, Rita; DeMarini, David M. (2007).

Seegert, G. L., A. S. Brooks, J. R. Vande Castle, and K. Gradall. The effects ofmonochloramine on selected riverine fishes. Trans. Am. Fish. Soc. 108: 88–96 (1979).

Simon, D., S. Helliwell and K. Robards, 1998. Pesticide toxicity endpoints in aquatic ecosystems. J. Aquatic Ecosystem Stress and Recovery, 6: 159-177.

Sprague, J. B. Measurement of pollutant toxicity to fish – III. Sublethal effects and safe concentrations. Water Res. 5: 245–266 (1971).

Stober, Q. J. and C. H. Hanson. Toxicity of chlorine and heat to pink (Oncorhynchusgorbuscha) and chinook salmon (O. tshawytscha). Trans. Am. Fish. Soc. 103: 569– 576 (1974).

Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan, S.2003. Histopathological alterations of white

seabass, Latescalcarifer, in acute and subchronic cadmium exposure. Environmental Pollution 121: 307–320.

Van Dyk JC. 2003. Histological changes in the liver of Oreochromismossambicus (Cichlidae) after exposure to cadmium and zinc. MSc thesis,

Rand Afrikaans University, South Africa.

Vutukuru, S.S., 2005. Acute Effects of Hexavalent Chromium on Survival, Oxygen Consumption, Haematological Parameters and Some Biochemical Profiles of the Indian Major Carp, Labeorohita. Int J. Environ. Res. Public Health, 2(3): 456-462.

Wedemeyer, G. and A. J. Ross. Nutritional factors in the biochemical pathology oforynebacterial kidney disease in the coho salmon (Onchorhynchuskisutch). J. Fish. Res. Bd. Can. 30: 296–298 (1973).

White, G. C. Handbook of Chlorination. Van Nostrand Reinhold, New York.

(1972)

Winder, Chris (2001). "The Toxicology of Chlorine". Environmental Research 85 (2): 105–114. Bibcode:2001ER85. 105W. doi:10.1006/enrs.2000.4110. PMID 11161660.

Zeitoun, I. H. Assessment of intermittently chlorinated heated effluents on survival of adult rainbow trout (Salmogairdneri) at power

generating facilities. **Environ. Sci. Technol. 12: 1173–1179 (1978a).**

Zeitoun, I. H. The effect of chlorine toxicity on certain blood parameters of a d u l trainbow trout (Salmogairdneri). Environ. Biol. Fishes 1: 189–195 (1977).

Zeitoun, I. H. The recovery and hematological rehabilitation of chlorine stressed adult rainbow trout (Salmogairdneri). Environ. Biol. Fishes 3: 355–359 (1978b).

Zuyev, G.V. and V.V. Bolyayen, 1970. An experimental study of the swimming of fish in groups as exemplified by the horse mackerel, Trachurus