Sudan University of Science and Technology

College Of Animal Production Science and Technology

Department of Fisheries and Wildlife Science

Histopathological and behavioral alterations in Nile Tilapia (*Oreochromious niloticus)* by Cypermethrin pesticide

التغيرات النسيجية والسلوكية في اسماك البلطي النيلي بواسطة مبيد السايبرمثرين

Thesis submitted in partial fulfillment of the requirements of the B.Sc. degree in fisheries and wildlife science (Honor)

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الآية

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ قُلْ هُوَ ٱللَّهُ أَحَدٌ ۝١ ٱللَّهُ ٱلصَّمَدُ ۝٢ لَمْ يَلِدْ وَلَمْ يُولَدْ ۝٣ وَلَمْ يَكُن لَّهُۥ كُفُوًا أَحَدٌۢ ۝٤ ﴾

صدق الله العظيم

سورة الإخلاص الآيات من : ۝١ – ۝٤

Dedication

This is dedicated to our
Mothers fathers brothers and sister.

and
Our friends

ACKNOWLEDGMENT

Firstly thanks to allaha almighty, the most merciful Who gave us the health, strength and patience to conduct this study

Grateful thanks to our supervisor Dr. Haram hassan Abbas for her guidance and supervision of scientific knowledge

Also our appreciations go to all teaching staff in the Facultyof Science and Technology of Animal Production in Department of Fisheries College and Wildlife Science

Finally our thanks to our colleagues, relatives and friends

Abstract

This study was conducted during (May-2018) to study the effects and toxicity of short term expose we insecticide Cypermethrin on (*Oreochromious niloticus*) tissuses including: the gills, liver, kidney, and intestine and to study the effects of insecticide Cypermethrin on the fish behavior.

Fish sampl stuayc (Tilapia) were obtained from the fish Hatchery of the Department in fisherieS and wildlife Science. 30 adult fish of both sexes were used in this experiment. average weight was (50 –100 g.) fish were divided in to five groups and subjected to Cypermethrin insecticide solution in 4 dosages as follow :

The first group was exposed to 0.0001ppm Cypermethrin insecticide, the total amount of solution was 3 ml Cypermethrin insecticide / 30 liter water.(D)

The second group was exposed to 0.0002ppm Cypermethrin insecticide, the total amount of solution was 6 ml Cypermethrin insecticide / 30 liter water. (B)

The third was group was exposed to 0.0003ppm Cypermethrin insecticide, the total amount of solution was 9 ml Cypermethrin insecticide / 30 liter water.(E)

The fourth group was exposed to 0.0004ppm Cypermethrin insecticide, the total amount of solution was 12ml/ 30 liter water (A) Results of histology indicates several kinds of damages in all studied organs,in liver showed Focal areas of necrosis , Dilation and thrombosis formation in central vain and ruptured hepatocytes and vacuolation , in gills we found sloughing of secondary lamellae and congestion in blood vessels of gill filaments while the kidney notice shrinking of glomeruli and Blood congestion, and intestine notice mucosa and submucosa damage and hemorrhages. Many behavioral observation were notice ,rapid swimming and lose of balance were observed in different dosages.

key words: Cypermethrin - histopathology - fish O.niloticus – fish behavior .

الملخص

اجريت هذه الدراسة خلال مايو 2018 لدراسة اثار وسمية السايبرمثرين علي المدي القصيرعلي انسجة اسماك البلطي النيلي (الكبد ، الكلية ، الغلاصم ، الامعاء) ودراسة تاثيره علي سلوك الاسماك .

تم استجلاب عينات اسماك الدراسة من فقاسه الاسماك في جامعة السودان للعلوم والتكنولوجيا ، قسم علوم الاسماك والحياة البرية. تم استخدام 30 سمكة من كلا الجنسين في هذه التجربة كان متوسط الوزن (50ـــ100)جرام من الاسماك مقسمة الي خمس مجموعات وعرض لمبيد الثايبرمثرين في 4 جرعات علي النحو التالي :

المجموعة الاولي تعرضت للمبيد الحشري الثايبرمثرين 0.0001 جزء من المليون كان الكمية الكلية المستخدمة 3 مل من المبيد في 30 لتر ماء الحوض (د) .

المجموعة الثانية تعرضت للمبيد الحشري الثايبرمثرين 0.0002 جزء من المليون كان الكمية الكلية المستخدمة 6 مل من المبيد في 30 لتر ماء الحوض (ب).

المجموعة الثالثة تعرضت للمبيد الحشري الثايبرمثرين 0.0003 جزء من المليون كان الكمية الكلية المستخدمة 9 مل من المبيد في 30 لتر ماء الحوض (ع) .

المجموعة الرابعة تعرضت للمبيد الحشري الثايبرمثرين 0.0004 جزء من المليون كان الكمية الكلية المستخدمة 12 مل من المبيد في 30 لترماء.

اوضحت نتائج الدراسة عدد من انواع التلف لانسجة الاسماك في الكبد مثل التنخر والتجاويف وانفجار خلايا الكبد كما اظهرت الغلاصم نزع في الشعيرات الثانوية واحتقان دموي في الخيوط الخيشومية اما الكلي فقد اظهرت تقلص واحتقان دموي كما اظهرت الامعاء تاثيرات في الانسجة متمثلة في تلف ونزيف دموي في الغشاء المخاطي للامعاء .

كما اظهرت نتائج السلوك بعض الملاحظات السلوكية مثل السباحة السريعة وفقدان التوازن في جميع جرعات التجربة .

كلمات مفتاحية : مبيد ثيبرمثرين – انسجة مريضة – سمك بلطي - سلوك الاسماك

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CHAPTER ONE

INTRODUCTION

Contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades; information about the biological effects of pollution on the aquatic organism is lacking and show many gaps. Fish have been the most popular choice as test organism and for monitoring aquatic toxicity because they are presumably the best-understood organisms in the aquatic environment, their large mobility allows them to assess large –scale regional affect, and also due to their importance to man as a protein source **(Alaa G. M, *etal* 2010).**

Pollution may be defined is the introduction of contaminants into the natural environment that cause adverse change **(Merriam web ster,2010).**

Water pollution is the contamination of water bodies (eg. lakes rivers oceans and ground water).water pollution affects plants and organisms living in these bodies of water and in almost all causes the effect is damaging not only to individual species and population but also natural biological communities **(Fewtrell and Colford,2004).**

Water pollution is one of the most serious environmental problems and it's a major problem in the global context **(Yang and Yongguan, 2004).**

it is the major source of water born disease and other health problems, Pollution of aquatic environmental can be happen because of algae. A serious problem in many lakes and reservoir as sources of water is the growth of algae which undesirable because they causes bad odors and flavors in water and may produce toxic materials of potential danger to human **(Palmer 1980, Lali berte *et al* 1994, Kamarudin *et al* 2015).**

Aquatic toxicology is the study of the effects of environmental contaminants on aquatic organisms, such as the effect of pesticides on the health of fish or other aquatic organisms. A pesticide's capacity to harm fish and aquatic animals is largely a function of its (1) toxicity, (2) exposure time, (3) dose rate, and (4) persistence in the environment **(Louis A. Helfrich, 2009).**

Development activities such construction transport and manufacturing not only deplete the natural but also produce large amount of wastes that lead to pollution of air water soil and ocean. Human industrial activites resulting discharge of various pollutants into the aquatic enviroment threatening the health of the population and damaging the quality of the enviroment by rendering water bodies **(Abowei and Sikoki ,2005).**

The term pesticide includes all of the following: herbicide, insecticides (which may include insect growth regulators, termiticides , *etc.*) nematicide, molluscicide , piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide disinfectant (antimicrobial) ***(Carolyn Randall (ed.), Core Manual (2013).***

In recent years, synthetic pyrethroids have been developed for major uses in agriculture and public health purposes. Cypermethrin were evolved from the natural pyrethrins, which possess high insecticidal potency, low mammalian toxicity and very short persistence. It is highly toxic to fish and some aquatic invertebrates ,

Cypermethrin is being increasingly used as the active ingredient in many dips that are used to prevent and treat ticks, lice, and scrab on sheep and as a treatment against infestation by the parasitic sea louse in aquaculture. The sources of

contamination of river courses occur as a result of the direct use of pyrethroid-based dips and also from the processing of sheep skin, wood industry and knitwear manufacture. The environmental concentrations of cypermethrin are often below those that are lethal to many freshwater teleost **(Khalid Abdullah Al-Ghanim ,2014).**

Objectives:

1-The aim of this research was to study the effects and toxicity of short term expose of insecticide Cypermethrin on (*Oreochromious niloticus*) tissuses including: the gills, liver, kidney and intestine and estimate the mortality rate.

2-to study the effects of insecticide Cypermethrin on the fish behavior .

CHAPTER TWO
LITERETURE REVEW

2.1 Environmental pollution

They are six types of pollution air pollution, radiation pollution, noise pollution, soil pollution, thermal pollution and water pollution.

In the most recent decades, most of the countries are undergoing a rapid industrial development, urbanization, construction, mining activities and deforestation. These activities may leads to the environmental problem such as land, air and water pollution. Water pollution is a major problem across the globe with the presence of harmful contaminants in the environment that had increased much concerns because of the green revolution **(Sabullah, m. k *et al*, 2015).**

Nowadays, the widespread use of pesticides in agricultural areas all over the world poses a threat to all living creatures and environment due to their toxic effects and accumulation of their hazardous features. Furthermore, pesticides rapidly spread around through various agents such as water, air, and food chain (**P. G. rasgele1 *et al* ,2015).**

2.1.1Water pollution

Aquatic organisms are bathed in solutions of trace metals at dissolved concentrations ranging from nanograms per liter in the open ocean, through level approximating micrograms per litre in coastal seas, to even higher concentrations approaching or exceeding milligrams per liter in estuaries and acid rich streams and salt lakes **(Oti, Egwu Emmanuel *et al*, 2007).**

Most pesticides are intended to serve as plant protection products (also known as crop protection products), which in general, protect plants from weeds, fungi, or insects. In general, a pesticide is a chemical or biological agent (such as a virus, bacterium, or fungus) that deters, incapacitates, kills, or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other species.

The Food and Agriculture Organization (FAO) has defined any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport ***(GRACE Communications. Retrieved February ,2017*** **).**

2.2.Pesticides pollution

Pesticides safety education and pesticide applicator regulation are designed to protect the public from pesticide misuse, but do not eliminate all misuse. Reducing the use of pesticides and choosing less toxic pesticides may reduce risks placed on society and the environment from pesticide use **(*LA, DL, P, ER***, etall, ***(June 1996),)*** **.**

Natural pyrethroids have proved of great value for use indoors for public hygiene, medicine and animal health. Uses include the control of lice and fleas in the homes and public buildings and the control of houseflies, mosquitoes and other insects that spread diseases of animals and humans importance.

Pyrethroids are ideal for home uses because they are of low toxicity to man and other warm-blooded animals. Moreover they are readily destroyed by heat during cooking or by digestive juices should trace get into food, onto fingers of children or onto the feet of domestic animals. Their outdoor use is severely restricted reason being that they are rapidly decomposed by light , Although safe to higher animals.

pyrethroids, both natural and synthetic are toxic to fish **(Hassall, 1990).** Pyrethroids are remarkable effective insecticides because of their ability to disrupt the insect nervous system at concentration that result in no mammalian toxicity **(Oti, Egwu Emmanuel ,2007).**

substances used to control organisms, including insects, water weeds, and plant diseases .Pesticides usage in agricultural fields to control pests is extremely toxic to non target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality**(Shankar KM, Kiran BR 2013).**

pesticides and many organic compounds, heave greatly contributed to massive fish death of aquatic ecosystems(**Dhasarathan P, Palaniappen R, Ranjit SJA., et al 2000).**

2.2.1. **Cypermethrin toxicity**

Over 90% of the cypermethrin manufactured world wide is used to kill insects on cotton in the use on cotton is important in 5 states **(U.S dept,service 1996, D.C (march).**

In california where pesticide use reporting is more comprehensive than other states use of cypermethrin in homes and other buildings is the predominant use **(1993 ca (jane).**

Cypermethrin like all synthetic pyerthroid kills insects by disrupting normal function of the nervous system , insect as well as all other animals including humans ,nervous become momentarily permeable to sodium atoms allowing

sodium to following into the nerve pyrethroid delay the closing of the (gate) that allows the sodium flow **(Vijverberg ,h.p.m and j.Van denbercken 1990).**

Cypermethrin has other effects on the nervous system. It inhibits the amino butyric acid receptor, causing excitability and convulsions In addition it inhibits calcium up take by nerves **(ramadan,a.A. Et al 1988)**.And inhibits mono amine oxidase **(Rao ,g.v and K.S.J. Rao, 1993).**

Cypermethrin also affect an enzyme not directly involved with the nervous system adenosine tri phosphatase it is involved in cellular energy production, transport of metal atoms and muscle contraction **(el .Toukhy ,m.A and r.s .Girgis 1993).**

Effects on beneficial insects, spider and mites bees: cypermethrin kills honey bees as well as leave cutter bees (used to pollinate seed alfalfa crops) residuesion leaf surfaces are toxic (killing at least 25% of bees tested) for more than 3 days following treatment **(Johansen ,c.A et al 1983).**

In addition bees exposed to cypermethrin learned more slowly and less successfully than un exposed bees **(taylor ,K.S. Et al 1987).**

Spider: spider desirable predators in many agriculture system because of their appetite for insects that would other wise be agricultural pests **(vorley ,v.T ,1993).**

Aquatic insects: while not usually considered beneficial insects are important components of aquatic eco system. cypermethrin is toxic to caddisflies mayflies , damselflies, and diving beetles.**(Siegfried, B.D..1993).** Cypermethrin has been found in ground water in France **(M.F Lengrand et al 1991).**and in river water sediment in the United Kingdom **(House , W.A et al 1991) .**

2.3 Fish as a biomarker tool

With exploding population and increasing industrialization and urbanization, water pollution by agricultural, municipal and industrial sources has become a major concern for the welfare of humanity. Water soluble toxicants from industrial and municipal wastes, leached soils and the atmosphere have rapidly transferred to natural bodies of water. While some of the pollutants decompose or volatilize, others form insoluble salts, which precipitate and get incorporated into the sediment .Uptake of such toxicants by aquatic organisms like fish may be followed by metabolism of the toxicants into more toxic derivatives. **(Ali M.Y et *al*, 2011).**

Fish has turned into a favorite subject biomarker research caused by its sensitivity to temperature changes, natural surroundings and water quality deterioration and additionally aquatic contamination antagonistically influence the fish health, which might bring mortalities and ecosystem degradation. Fish biomarker including the assessment of biomolecular, cellular and physiological alteration that were utilized for monitoring the biological effect of toxicant especially metal exposure**.(Sabullah, M. K., *et al*, 2015).**

2.3.1 histopathological incidences

Several fish organs such as gill, liver, kidney, muscle and brain were selected to visualize the cellular alteration due to pesticides and metal toxicity.

Gill is the first direct contact with the external environment and changes in fish gill around the most usually distinguished reactions to environmental toxins

,Normal and affected gill tissues was visualized by **Campagna *et al*. (2008).**

which the affected gill showed structural deformation such as epithelial lifting at secondary lamella, hypertrophy of primary epithelium, fusion of secondary lamella, aneurisms, necrosis and infiltration of inflammatory cells with the

disintegrate of epithelial cells of secondary lamellae including mucus secretion and swollen mucocyte. Fish gill defense mechanism and its potential as biomarker **(Sabullah, m. k., et al, 2015).** Fish liver and kidney also are an alternative biomarker tool to evaluate toxicity level. At the beginning of toxicity level, morphology of parenchyma cell shows the abnormalities such as cytoplasmic vacuolation along with dilation and congestion of sinusoid depending on duration and toxicant concentration exposure,

At high toxicity level, other abnormalities appeared such as macrophage activity, hyalinization, hemorrhage, binucleai, apoptosis and necrosis development Unlike liver, affected kidney shows an additional impairment such as the damage to the epithelium of some renal tubules and increased Bowman's space in the kidney while affected brain tissues showed the swelling of blood vessels on the ventral surface of the cerebellum, alteration in nerve cell bodies in the telencephalon and the thickness of the mesencephalon layers**(Sabullah, m. k., et al, 2015).**

2.3.2Enzymatic biomarker

Enzyme-based biomarker was considered as the simplest estimation for toxicant existence. This method gave multiple advantages such as rapid determination and it is also considered sensitive even the toxicant exist in low concentration, and low technical application. These properties together had made it to become a highly promising method to be use in pharmacology, agriculture and environmental protection. Various sources of enzyme from bacteria, plant and animal was reported to be a sensitive biomarker with toxicant especially heavy metals. Fish is considered as a biomarker tool and a highly sensitive enzyme as sentinel species allows the detection of lower contamination levels. **(Sabullah, m. k., et al, 2015).**

2.3.3 behavioral incidences

Since behaviour serves as the link between physiological and ecological processes, it may be ideal for studying environmental pollutant effects. Fish are an excellent model in this regard, since many ecologically relevant fish behaviours are easily observed and quantified in a controlled setting. Furthermore, a great deal is known about fish physiology, a necessary consideration for integrative studies. Indeed, many researchers have proposed using behavioural indicators in fish for ecologically relevant monitoring of environmental contamination The performance of normal behaviour by individual fish follows specific physiological sequences, which are triggered by external stimuli acting via neural networks Disruption of these sequences before completion is likely to result in detrimental behavioural alterations. Initiation of these sequences is also affected by numerous physiological and environmental influences Inappropriate behavioural responses to environmental and physiological stimuli due to toxic effects of aquatic contaminants can have severe implications for survival (**Graham r. Scott, et al,2004).**

The studies on fish behaviors provide a lots of knowledge and information because, any behavior alteration can be related to physiological biomarker in aquatic species ,For example, the monitoring of behavioral response becomes an impending option to environmental change, disease, stress and the presence of toxic compound in water, which most of this condition initiates the variation of fish behavior ,Fish behavior represents the fish physiological response towards the environmental factor**(Sabullah, m. k., et al, 2015).** The median lethal concentration (the concentration that kills 50% of population of test animals ,L C50) for most fish is less than (5 ppm) **(W H O 1989).**

This is partly because are unable to break down pyrethroids as efficiently as mammals and birds and partly because fish nervous system are particularly sensitive to pyrethroids (**Brad bury** .**S.P and j.r. coats 1989).**

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Site

This study was conducted at Sudan University of Science and Technology, College of Science and Technology of Animal Production, department of Fisheries and Wildlife Science.

3.1.1 Sample collection:

Adult freshwater fish Tilapia (Family : Cichdae) were obtained from the fish hatchery the Department of Fisheries and Wildlife Science. Total of 30 adult fish of both sexes were used in this experiment. The average weight of the fish was (50-100 g.) The fish were divided in five groups and placed in large tanks (30 liter water) with aerated tap water and were fed with commercially pellets. Fish were acclimatized for 7 days. Water temperature was measured using aquarium thermometer and pH values were measured using portable Ph /meter . After that fish were subjected to Cypermethrin insecticide solution in 4 dosages as follow :

The first group was exposed to 0.0001ppm Cypermethrin insecticide, the total amount of solution was 3 ml Cypermethrin insecticide / 30 liter water.(D)

The second group was exposed to 0.0002ppm Cypermethrin insecticide, the total amount of solution was 6 ml Cypermethrin insecticide / 30 liter water. (B)

The third was group was exposed to 0.0003ppm Cypermethrin insecticide, the total amount of solution was 9 ml Cypermethrin insecticide / 30 liter water.(E)

The fourth group was exposed to 0.0004ppm Cypermethrin insecticide, the total amount of solution was 12ml/ 30 liter water (A)

The fifth was the control group without any addition of Cypermethrin insecticide (C)

3.1.2 stock solution:

Cypermethrin insecticide was procured from veterinary pharmacy hilat Koko Bahre Sudan.

3.2 Methods:

The fish were exposed to liquid Cypermethrin till fishes were dead. Any dead fish was dissected for its gill, liver , intestine and kidney and preserve on formalin 10% for histological analysis, physiochemical parameters of water were measured during the experiment.

3.3 water quality parameters:

3.3.1 Physical measurements:

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

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

3.3.4 phosphorous : water phosphate was measured using liquid water quality kits

3.4 Behavioural Manifestation:

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

3.5 Histopathological Examination:

Tissue specimens from fish of all group were taken from (gill, liver , intestine and kidney) 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.

Experiment aquariums

Cypermethrin insecticide

Fish dissection

3.6 Statistical analysis

The data obtained were analysed using SPSS software (ANOVA test) and(LSD) to compare the mean difference and to explain the water quality parameters

CHAPTER FOUR

RESULTS

4.1 Mortality rate:

No mortality was observed in the control group however, mortality speed increased with the increase of the concentration of Cypermethrin. The concentration at which there the highest percent mortality was 12ml/ 30 liter water, aquarium (A) however the lowest percent was 3 ml Cypermethrin / 30 liter water (D), hundred percent mortality were caused in the all concentrations in different time.

Table (1) shows the number of dead fish in different concentration of the pesticide.

Concentrations of Cypermethrin ppm	**Cumulative mortality**				
	Time of exposition				
	5 minute	**20 minute**	**45minute**	**55minute**	**65minute**
0.0 (control group)	0	0	0	0	0
0.0001ppm			1	1	3
0.0002ppm		1	1	1	2
0.0003ppm		1	1	1	2
0.0004ppm		1	2	2	

4.2 Water quality measurements

There were significant differences in water quality parameters in different experiment aquariums.

Table (2) shows the water quality parameters in experiment aquariums M±SD.

Aquariums / Water parameters	A	B	C Control	D	E	sig
pH	7.1 [a]	7.1 [a]	7.0[b]	7.1 [a]	6.8[c]	*
Temperature	28.3 [b]	26.4 [e]	27.8 [d]	29.4 [a]	28 [c]	**
Phosphate	0. 5 [b]	0.25 [b]	0.25 [b]	0.25 [b]	0.5 [a]	*

M=mean ,SD=stander deviation

A, b, c, d..Litters within the same raw followed by different superscript were significant difference.

4.3 Behavioural Manifestation

The behavior and condition of the fishes in all aquariums, control and other test group were noted all the time up to mortality was complete (Fig 1, and 2). The fishes showed marked changes in their behavior when exposed to Cypermethrin in different concentrations. The fishes in the different aquariums showed rapid swimming than in control aquarium . Behavioral manifestations of acute toxicity like hyperactivity, loss of balance, rapid swimming, and increased surfacing activity were noted.

Figure (1) fish showed loss of balance

Figure (2) fish showed surfacing activity

4.4 Histopathological results

Remarkable structural changes were detected in the tissues of the fish subjected to different Cypermethrin dosages, in different Tilapia organs (liver, gills , kidney and intestine) as shown in (plates 1-20).

Gills: showed sloughing of secondary lamellae as shown in plate (8), proliferation and damage in the epithelium of gill filaments as shown in plate (9) congestion in blood vessels of gill filaments and atrophy of secondary lamella as shown in plate (10, 11).

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) , pyknosis (PY) and hemorrhages(6), *(Oreochromis niloticus)* were showed liver histopathological alterations.

Kidney: showed shrinking of glomeruli as shown in plate (13) , edema in Bowman's capsule and Blood congestion as shown in plate (14)

Intestine: showed mucosa (m), submucosa (ms) damage, as shown in plate (16, 17, 18) , Notice mucosa (m), submucosa (ms) hemorrhages as shown in plate(19

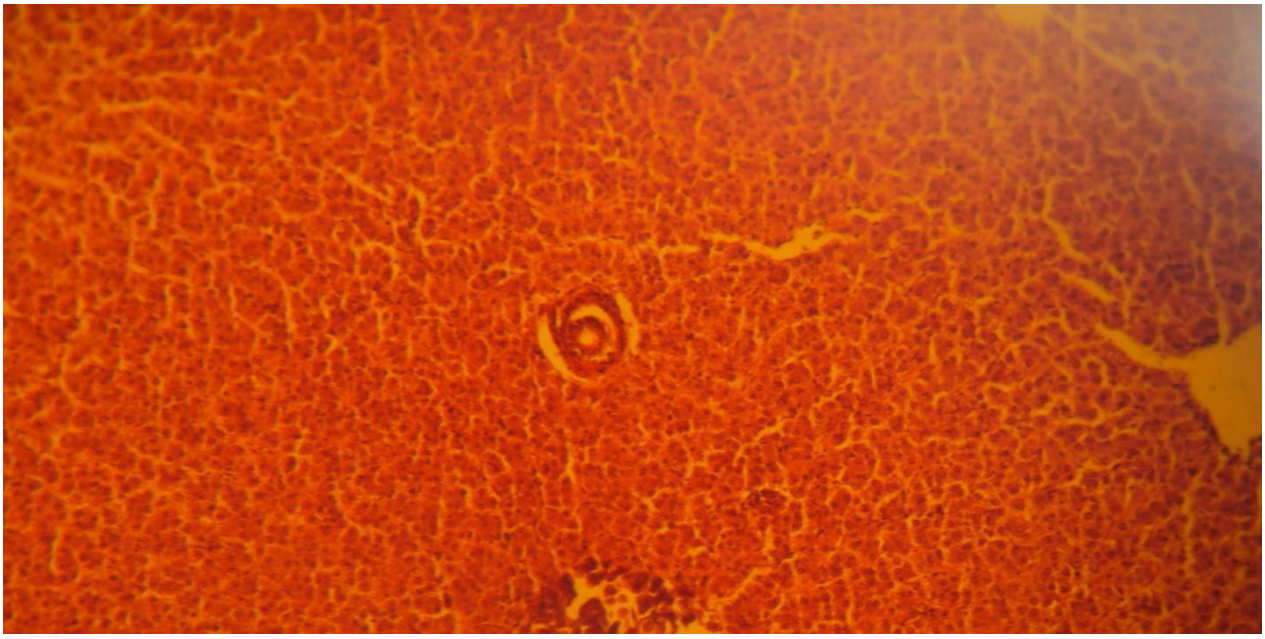

Plate 1. Section through the liver of *Oreochromis niloticus* Notice the normal tissues of liver. H&E X 200.

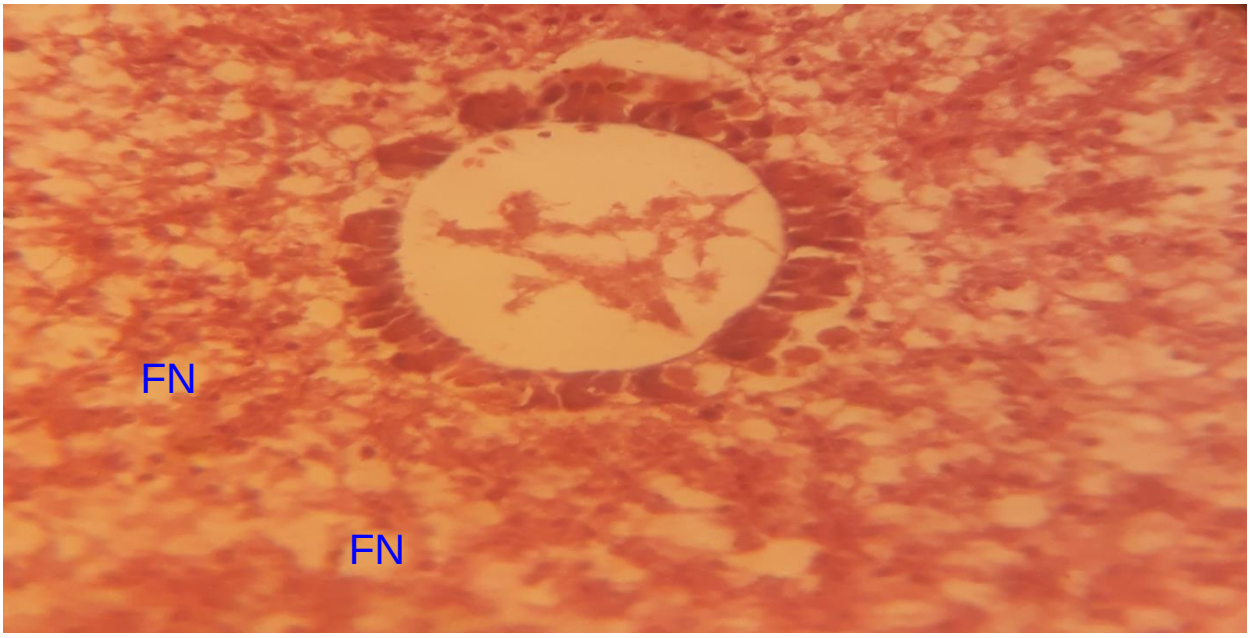

Plate 2. Section through the liver of *Oreochromis niloticus* Notice the Focal areas of necrosis (FN)**. H&E X 200.**

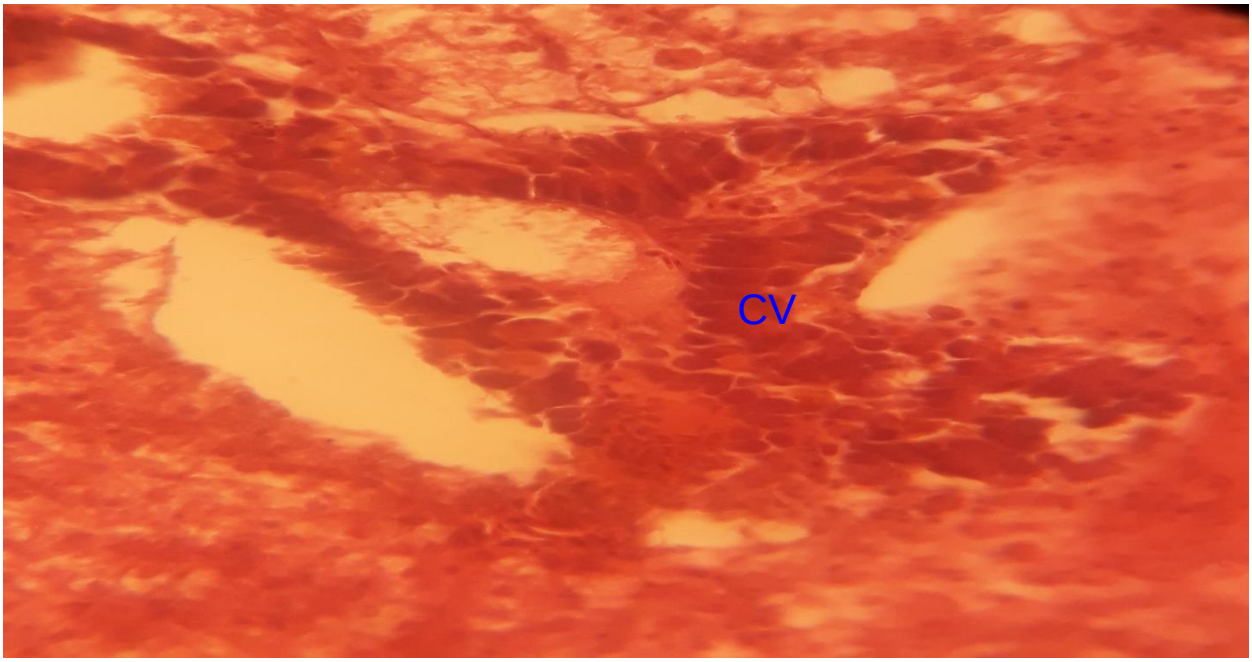

Plate 3. Section through the liver of *Oreochromis niloticus* Notice the Dilation and thrombosis formation in central vain (CV). H&E X 200.

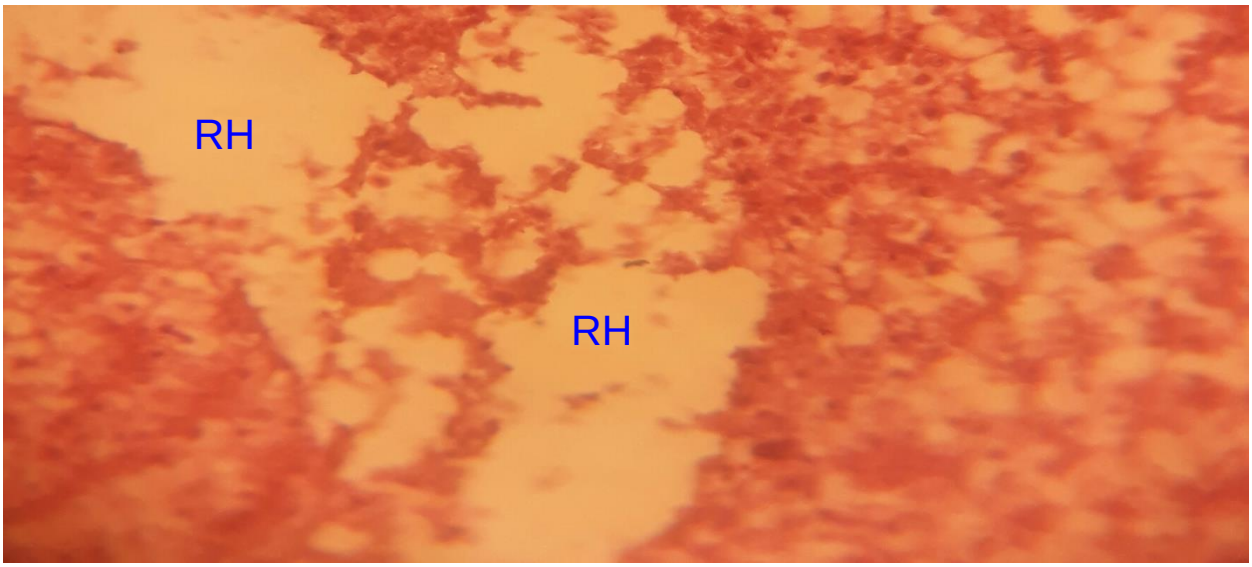

Plate 4. Section through the liver of *Oreochromis niloticus* Notice ruptured hepatocytes (RH) and vacuolation (V)H&E X 200.

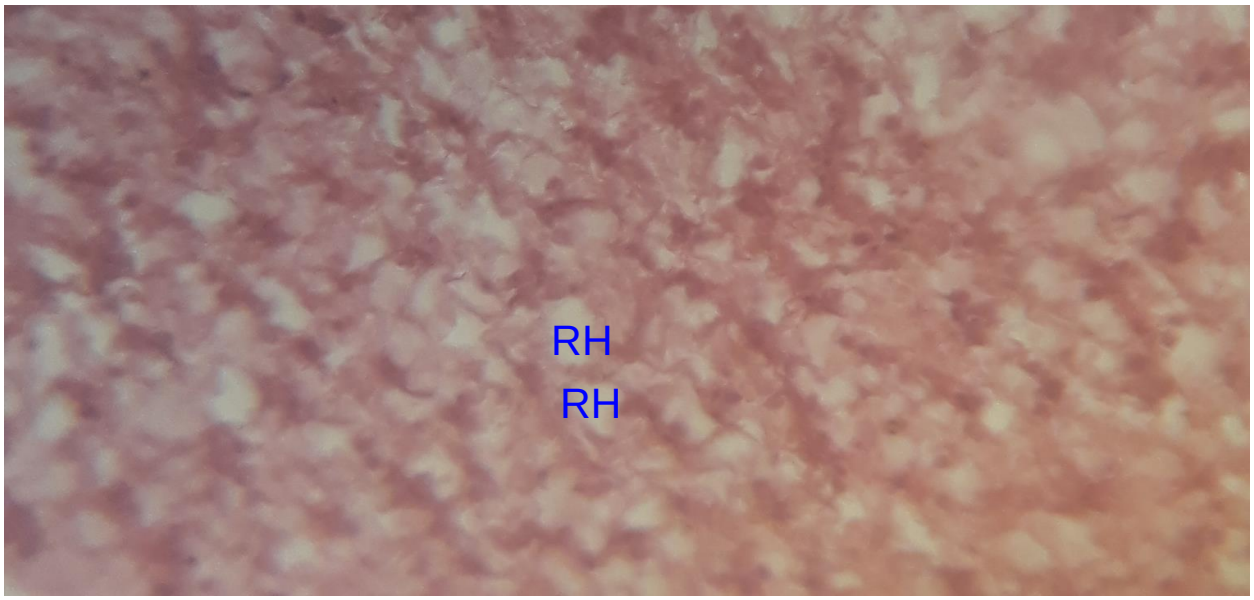

Plate 5. Section through the liver of *Oreochromis niloticus* Notice ruptured hepatocytes (RH) and vacuolation (V). H&E X 200.

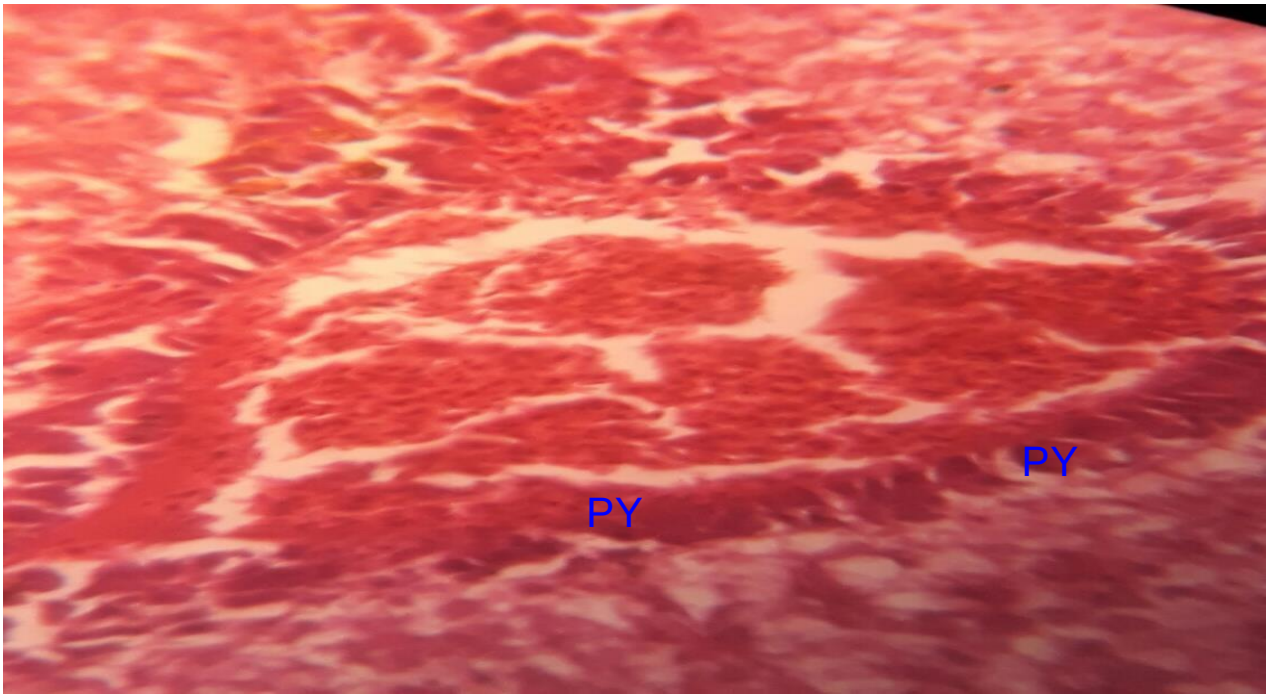

Plate 6. Section through the liver of *Oreochromis niloticus* Notice pyknosis (PY) and hemorrhages H&E X 200.

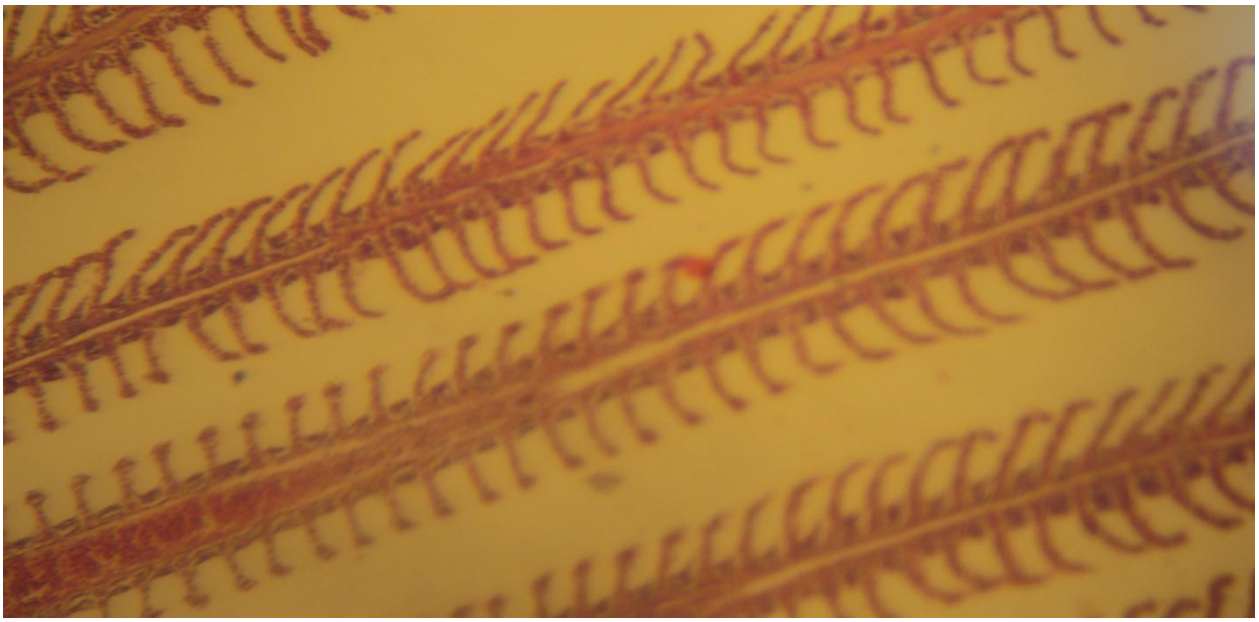

Plate 7. Section through the gills of *Oreochromis niloticus* Notice normal tissues of gills. H&E X 200.

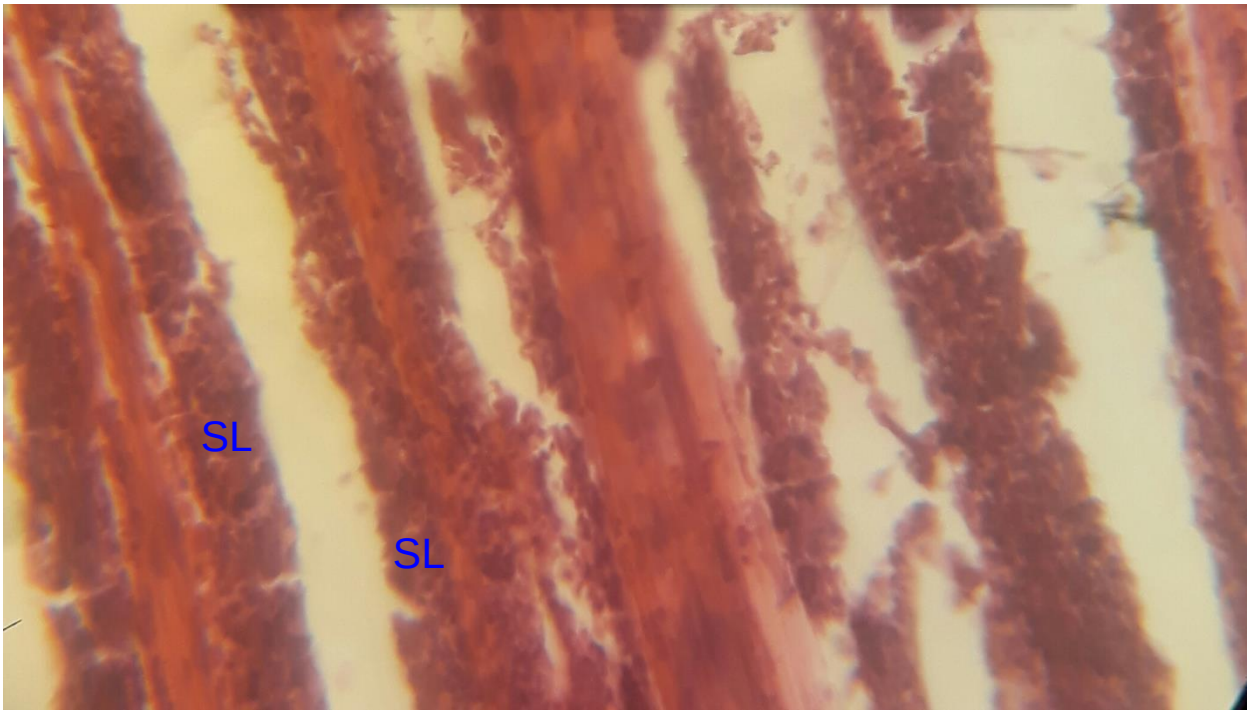

Plate 8. Section through the gills of *Oreochromis niloticus* Notice sloughing of secondary lamellae (SL) H&E X 200.

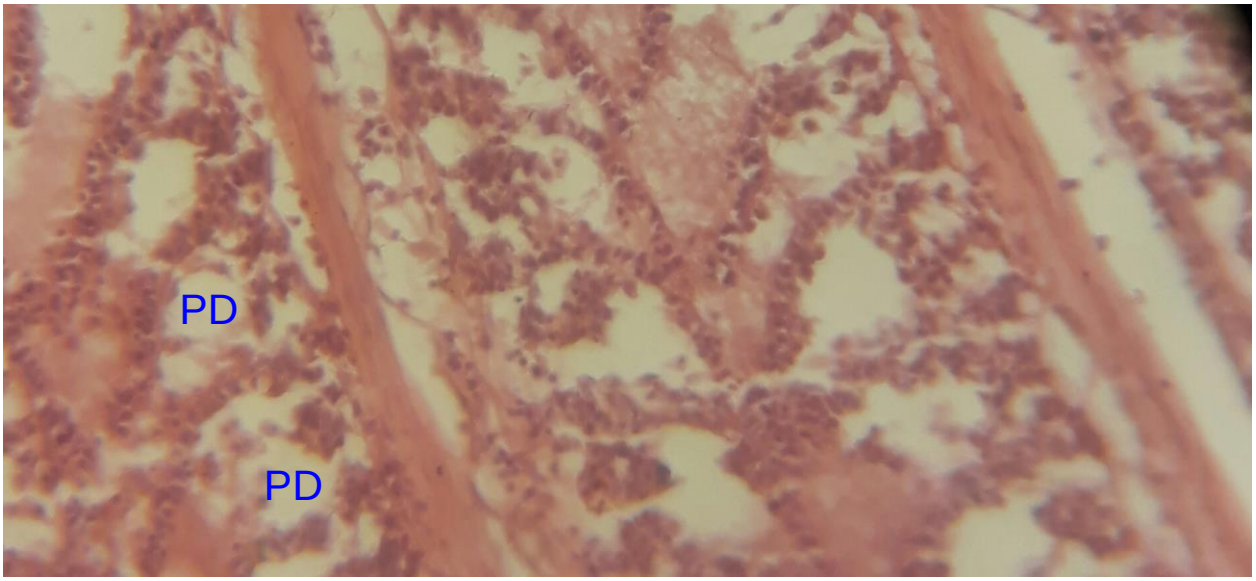

Plate 9. Section through the gills of *Oreochromis niloticus* Notice proliferation and damage (PD) in the epithelium of gill filaments. H&E X 200.

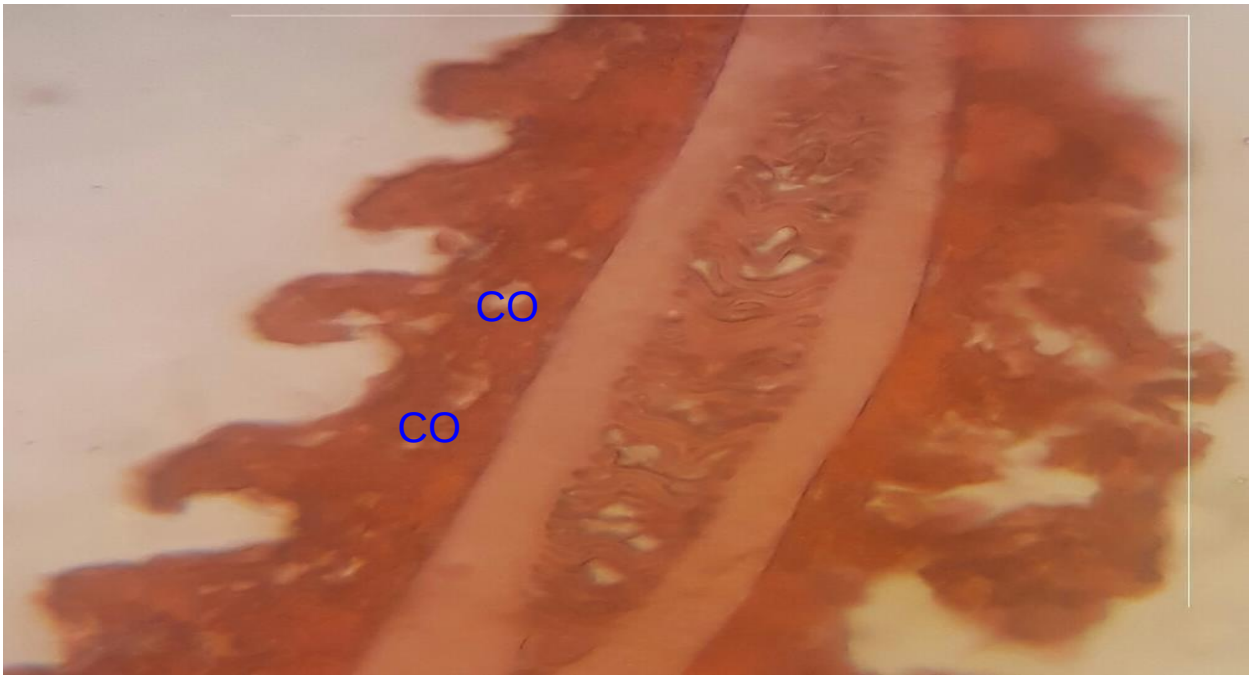

Plate 10. Section through the gills of *Oreochromis niloticus* Notice congestion (CO) in blood vessels of gill filaments and atrophy of secondary lamella H&E X 200.

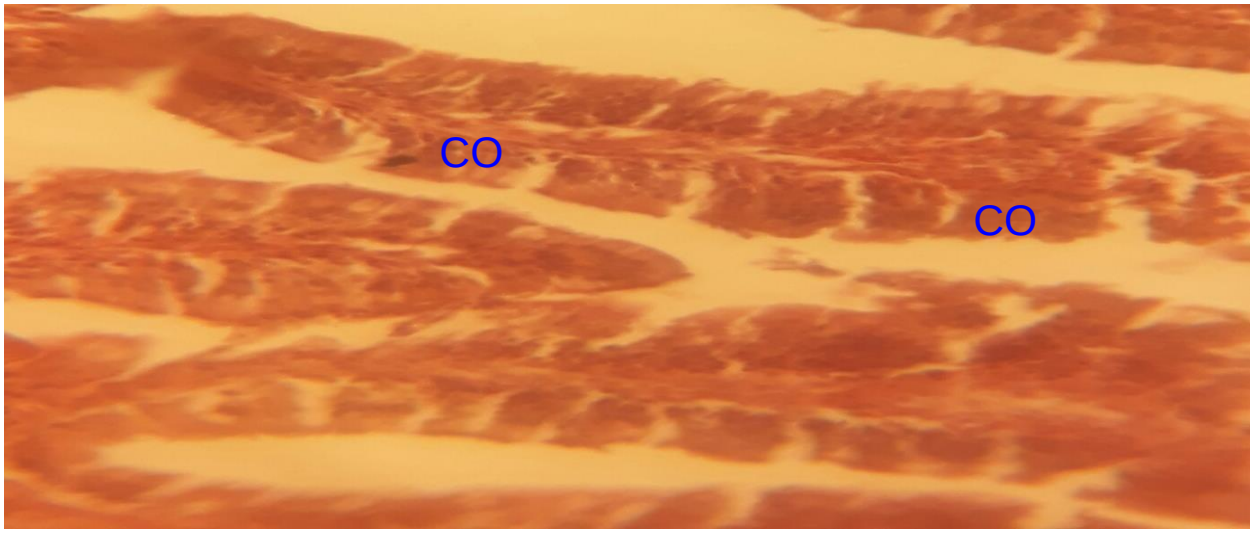

Plate 11. Section through the gills of *Oreochromis niloticus* Notice congestion (CO) in blood vessels of gill filaments and atrophy of secondary lamella H&E X 200.

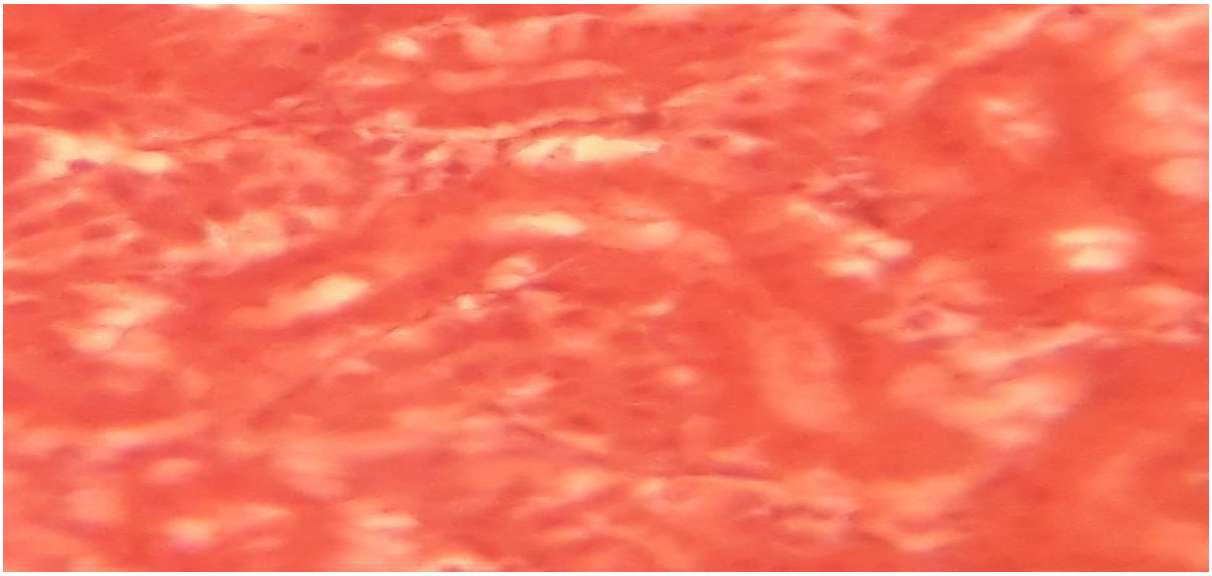

Plate 12. Section through the kidney of *Oreochromis niloticus* Notice normal tissues . H&E X 200

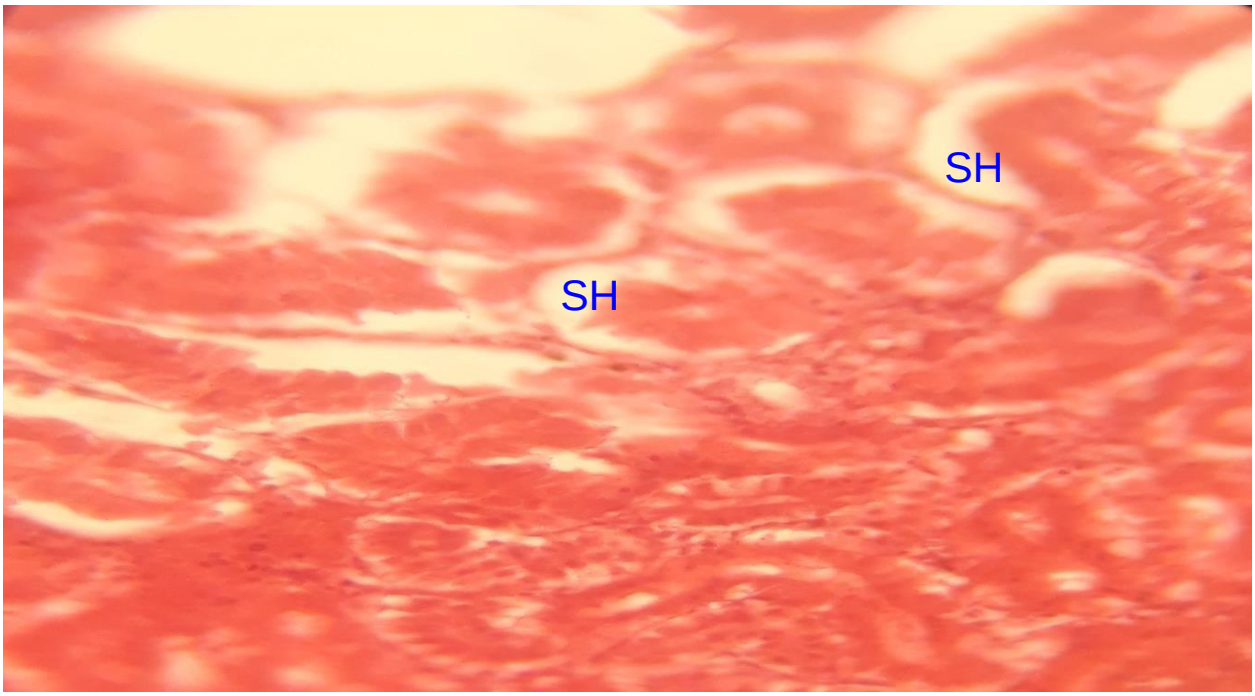

Plate 13. Section through the kidney of *Oreochromis niloticus* Notice shrinking (SH) of glomeruli H&E X 200 .

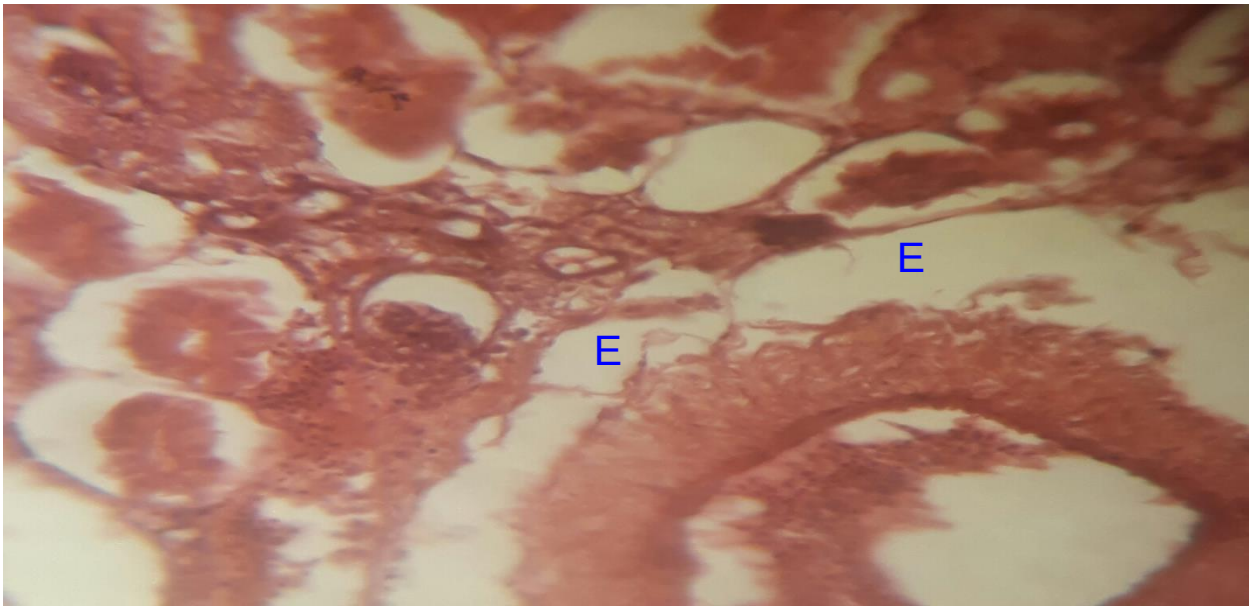

Plate 14. Section through the kidney of *Oreochromis niloticus* Notice edema (E) in Bowman's capsuleand Blood congestion. H&E X 200

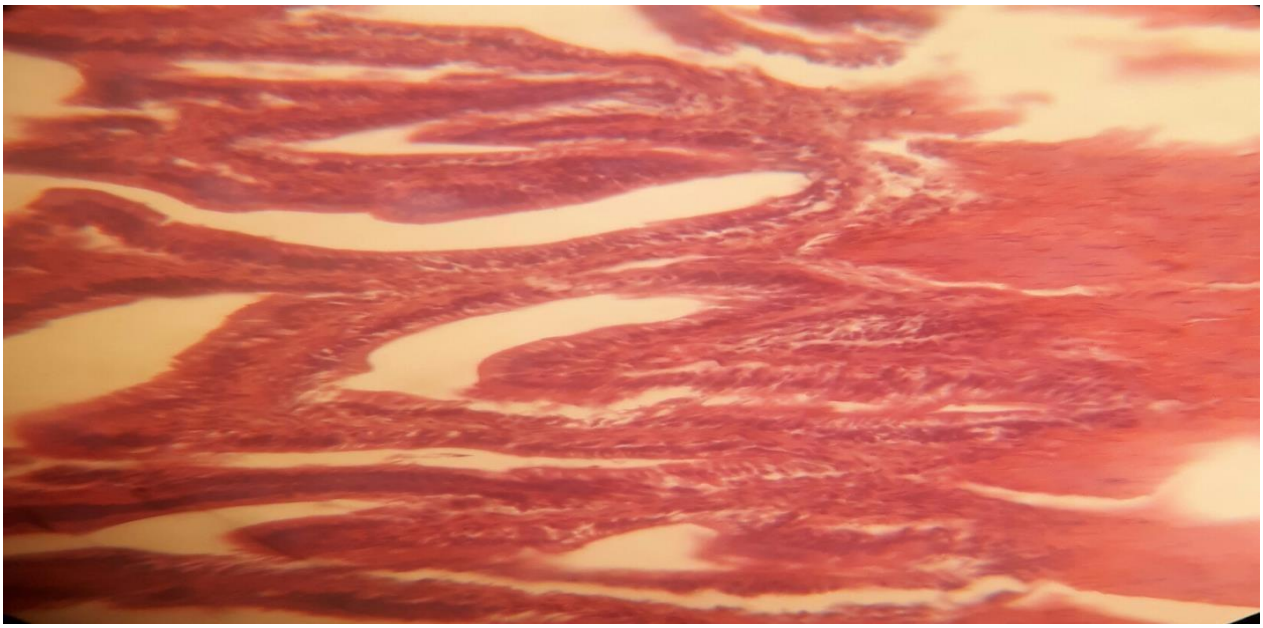

Plate 15. Section through the intestine of *Oreochromis niloticus* Notice normal tissues . H&E X 200

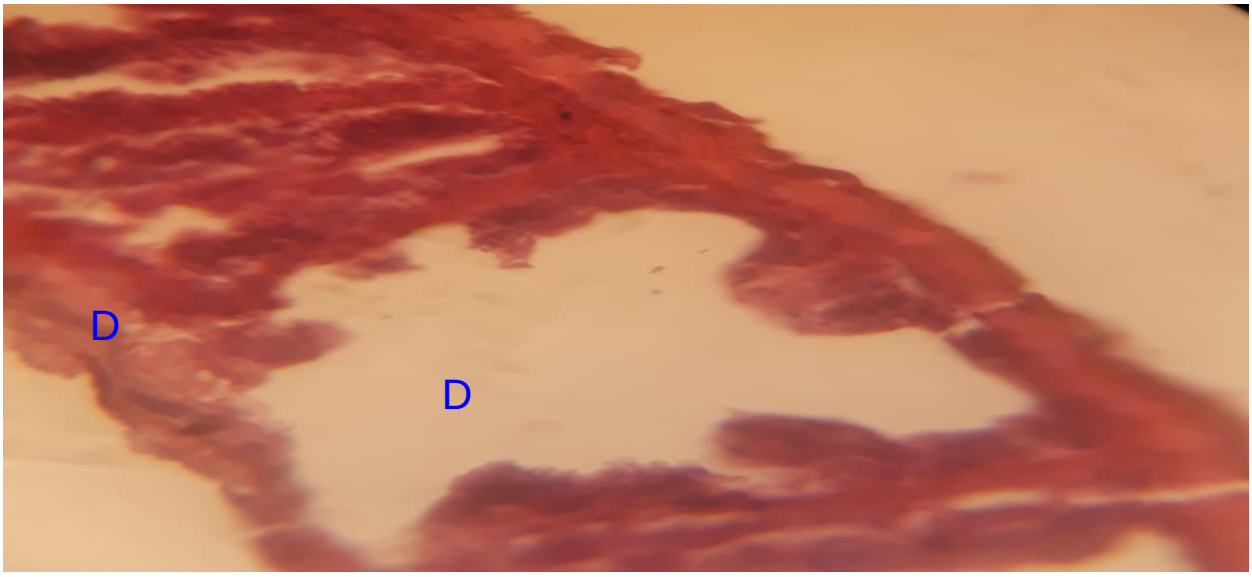

Plate 16. Section through the intestine of *Oreochromis niloticus* Notice mucosa (M), submucosa (SM) damage (D) H&E X 200 .

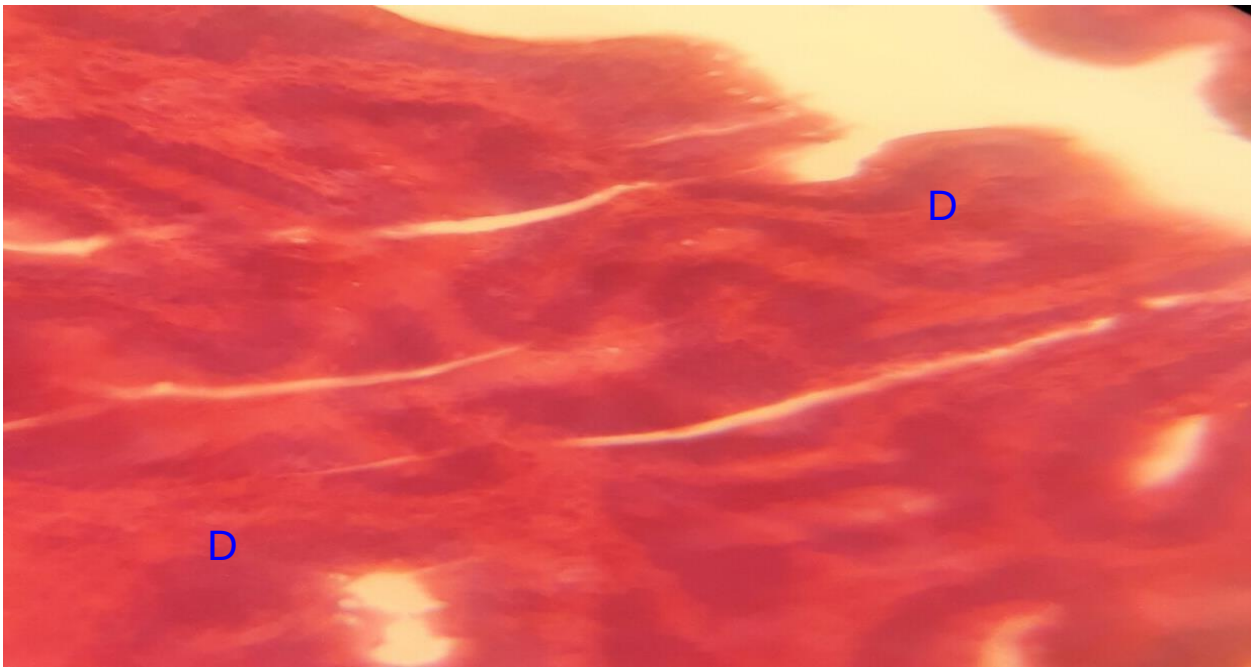

Plate 17. Section through the intestine of *Oreochromis niloticus* Notice mucosa (M), submucosa (SM) damage (D) H&E X 200 .

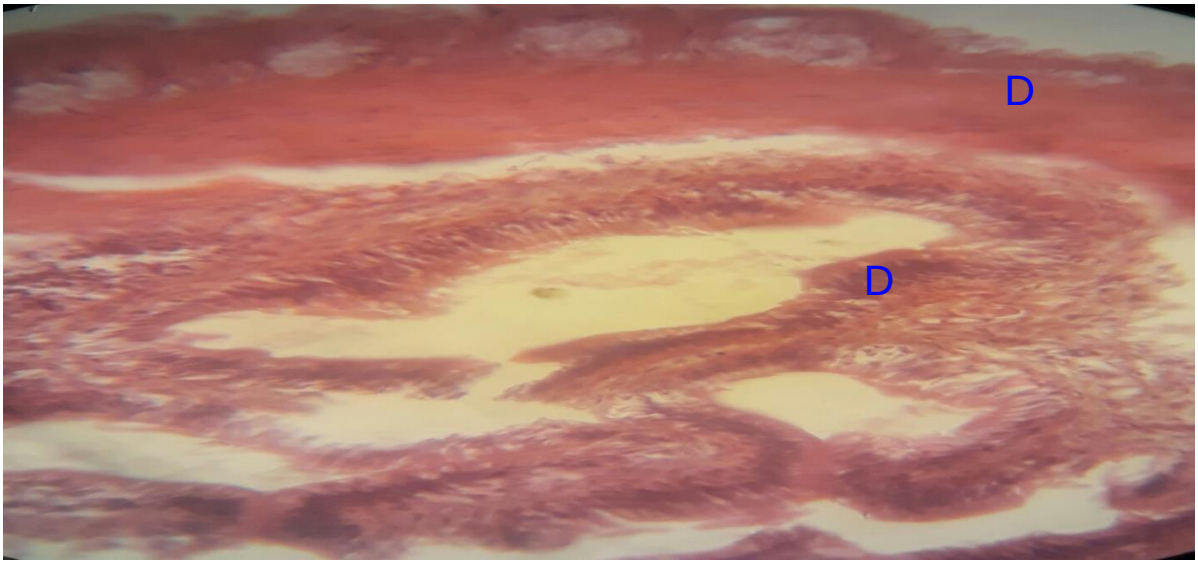

Plate 18. Section through the intestine of *Oreochromis niloticus* Notice mucosa (M), submucosa (SM) damage (D) H&E X 200 .

Plate 19. Section through the intestine of *Oreochromis niloticus* Notice mucosa (M), submucosa (SM) hemorrhages (H) H&E X 200 .

Table (3). Showing the main types of histopathological changes detected

In *Oreochromis niloticus* exposed to Cypermethrin**.**

organ	***Histopathological changes***	***Number of fish in which the effect was detected***			
		0.0001	*0.0002 ppm*	***0.0003ppm***	*0.0004pp*
liver	Focal areas of necrosis	**1**	***1***	***2***	***5***
	Dilation and thrombosis formation in central vein	***2***	***2***	***5***	***5***
	Ruptured hepatocytes and vacuolation	***3***	***4***	***5***	***5***
	pyknosis (PY) and hemorrhages	***2***	***3***	***3***	***5***
gills	sloughing of secondary lamellae	**1**	***1***	***4***	***5***
	proliferation and damage in the epithelium of gill fila	***3***	***4***	***5***	***5***
	congestion in blood vessels of gill filaments and secondary lamella	***3***	***4***	***5***	***5***
Kidney	shrinking of glomeruli	***1***	***2***	***3***	***5***
	edema in Bowman's capsule and Blood congestions	***3***	***3***	***5***	***5***
Intestine	mucosa (M), submucosa (SM) damage,	***2***	***4***	***5***	***5***
	mucosa (M), submucosa (SM) hemorrhages	***3***	***5***	***5***	***5***

CHAPTER FIVE

DISCUSSION

From the study results, The observations of fish behaviors provide information of toxic effects of Cypermethrin, Swimming performance is considered as behavior parameters to assess the physiological status of fish, behavior alteration can be related to physiological biomarker and water pollutants in aquatic species **(Sabullah, M. K et al, 2015).**

Behavioral results were agreed with many authors, **Vieira et al. (2009).** Who study the effects of copper exposure on fish behavior, his result clearly showed that the dependence on the concentration of toxic causes the loss of resistance in the fish swims.

Histopathological alterations consider as important measurement to study the water pollution and toxic effects of pollutants.

Histopathological results indicated that gill was the primary target tissue affected by Cypermethrin.

Gills are generally considered good indicator of water quality, being models for studies of environmental impact ,since the gills are the primary route for the entry of pesticide. In fish, gills are critical organs for their respiratory, osmoregulatory and excretory functions. **(Babu Velmurugan et al 2009).**

Several other studies have shown similar effects of pesticides on fish gills ,Many investigators have reported the histopathological changes in gills of different fish species exposed to pesticides. Mucus extrusion, lamellar swelling, fused and

reduced microridges, were observed in bluegill sunfish, Lepomis macrochirus to different sublethal concentrations of diazinon **(Babu Velmurugan et al 2009).**

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 **(Dr: Haram Hassan AbassBakhiet et al 2016).**

The liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to pesticides ,Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. This results were agree with the observed histopathological alteration like cloudy swelling, focal necrosis, atropy and vacuolization in the Corydoras paleatus exposed tomethyl parathion **(Babu Velmurugan et al 2009).**

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 **(Dr: Haram Hassan AbassBakhiet et al 2016).**

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids ,The kidney is one of the first organs to be affected by contaminants in the water **(Bakhiet,2015)**

The present results are in agreement with those observed in P. lineatus exposed trichlorfon **(Authman, 2008).**

Intetine is final target of the toxicant and also was affected with many shapes like mucosa (M), submucosa (SM) damage and hemorrhages ,that may be according to the direct toxic effects of Cypermethrin,

CONCLUTION:

- All the behavioral and histopathological observation indicated that exposure to lethal concentrations of Cypermethrin, caused direct physiological effects

- Destructive effect in the gill, liver, kidney and intestine tissues of of *Oreochromis niloticus* exposure to lethal concentrations of *Cypermethrin* in all experiment dosages leading to the death of fish

- Histological investigations demonstrated a direct correlation between pesticide exposure and histopathological disorders observed in several tissues.

RECOMMENDATIONS

- Several studied are needed in the field of aquatic environment toxicology.

- further investigation on bioaccumulation and the impacts of Cypermethrin and different pesticides in different organs and different fish species is needed.

- The use of Cypermethrin in agricultural field must be done under experts supervision.

CHAPTER SIX
REFERENCES

Abowei and sikoki ,2005 Abowei, j.f.n and f.d sikoki, 2005 Water pollution Management and Control, Double Trust Publication Company, Port Harcourt,ISBN :978303802016,pp:236.

Alaa g. m. Osman1 Abd-El –Baset m. Abd El Reheem1, Khalid y. AbuelFadl2, Ali G. GadEl-Rab1,(2010) Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes, *Natural Science*, **Vol.2, No.11, 1302-1311.**

Ali Muhammad Yousafzai and A.R. Shakoori 2011, Hepatic Responses of A

Freshwater Fish Against Aquatic Pollution**,** *Pakistan J. Zool., vol. 43(2), pp. 209-221.*

Authman mmn, Bayoumy em, Kenawy am (2008) Heavy metal concentrations and liver histopathology of Oreochromis niloticus in relation to aquatic pollution. *Global Vet* **1** (2): 110-116.

Babu Velmurugan1, Mariadoss Selvanayagam1, Elif Ipek Cengiz2 and Erhan Unlu2 2009. Histopathological Changes in the Gill and Liver Tissues of Freshwater Fish, *Cirrhinus mrigala* Exposed to Dichlorvos , BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY, Vol.52, n. 5: pp. 1291-1296, September-October 2009 ISSN 1516-8913 Printed in Brazil.

Bradbury, S.P. and J.R. Coats. 1989 .Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. Environ .Toxicol. Chem .

Campagna, a. f., Fracácio, r., Rodrigues, b. k., Eler, m. n., Fenerich- Verani, N. and Espíndola, e. l. g. 2008. Effects of the copper in the survival, growth and gill morphology of *Danio rerio* (Cypriniformes, Cyprinidae). Acta Limnologica Brasiliensia 20: 253- 259.

Carolyn Randall (ed.), et al.2013, *National Pesticide Applicator Certification Core Manual (2013) National Association of State Research Foundation ,*

Washington , DC, Ch.1.

El-Toukhy, m.a. and r.s. Girgis .1993 . In vivo and in vitro studies on the effect of larvin and cypermethrin on adenosine triphosphatase activity of male rats. J. Environ. Sci. Health B28:599-619. .1518-12:1513.

fewtrell and colford, 2004 Water Sanitation and hygiene :Intervention and diarrhea A systematic review and meta. analysis water supply and sanitation board the world Bank .Retrieved from :tp ://www1.world bank. Org /hnp /pubs __Discussion /Fe wtrell& colford july.

Carolyn Randall (ed.), et al.2013, *National Pesticide Applicator Certification Core Manual (2013) National Association of State Research Foundation , Washington , DC, Ch.1.*

Campagna, a. f., Fracácio, r., Rodrigues, b. k., Eler, m. n., Fenerich- Verani, N. and Espíndola, E. L. G. 2008. Effects of the copper in the survival, growth and gill morphology of *Danio rerio* (Cypriniformes, Cyprinidae). Acta Limnologica Brasiliensia 20: 253- 259.

GRACE ,2017*. Check date values in: |accessdate= Communications. Retrieved February (help).*

Graham R. Scott and Katherine A. Sloman, 2004. the effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity, *Aquatic Toxicology* 68 (2004) 369–392.

Haram hassan abbs bakhiet 2015, Bioaccumulation and Histopathological Alterations of Heavy Metals in the Liver and Kidney of Oreochromis niloticus Fish Collected from the Blue Nile, Agricultural and Biological Sciences Journ.

Helfrich, l a, Weigmann, d l, Hipkins, p, and Stinson, e r (June1996)*, Pesticides and aquatic animals: A guide to reducing impacts on aquatic systems Archived 2009-03-05 at the Wayback Machine.. Virginia Cooperative Extension. Retrieved*

on2007-10-14.

House, W.A. et al. 1991. The occurrence of synthetic pyrethroid and selected organochlorine pesticides in river sediments. In Walker, A. (ed.). Pesticides in soils and water: Current perspectives. Farnham, Surrey, U.K.: British Crop Protection Council.

Khalid Abdullah Al-Ghanim,2014, Effect of cypermethrin toxicity on enzyme activities in the freshwater fish Cyprinus carpio, *African Journal of Biotechnology*, Vol. **13**(10), pp. 1169-1173.

Louis A. Helfrich 2009, Pesticides and Aquatic Animals: A Guide to Reducing Impacts on Aquatic Systems, Produced by Communications and Marketing, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Publication 420-013.

Merriam Webster https://www.merriam _Webster.com/dictionary /pollution

M.F. Legrand et al. 1991. Occurrence of 38 pesticides in various French surface and ground waters. Environ. Technol.996-12:985.

OTI, Egwu Emmanuel and 2Nwani, Christopher Didigwu, impact of lamda cyhalothrin pyrethroid insecticide on the uptake of cations and anions by the gills of freshwater catfish hybrid juvenile , *Animal Research International* (2007) 4(1): 591 – 596.

Palmar 1980 ,laliberte1994 kamarudin2015 Palmer 1980 Algae and water pollution the identification sigrificance and Control of algae in water supplies and in polluted water" castle House publication Laliberte1994.

P. G. Rasgele1*, m. Oktay2, m. Kekecoglu3 and f. d. g. Muranl4, 2015 , the histopathological investigation of liver in experimental animals after short term exposures to pesticides Bulgarian journal of agricultural science 21 (no 2) 2015 446-453..

Ramadan, a.a. et al. 1988. Action of pyrethroids on GABAA receptor function. Pest. Biochem. Physiol . .105-32:97.

Rao gv, Rupela op, Rao vr, Reddy yv(2007). *"Role of biopesticides in crop protection: present status and future prospects" (PDF). Indian Journal of Plant Protection.* ***35*** *(1): 1–9. 91. Miller.*

Sabullah, m. k., 1,Ahmad, s. a., 1shukor, m. y., 2Gansau, a. j., 1Syed, m. a., and Sulaiman, m. r. and 3Shamaan,n. a.,(2015) Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches, *International Food Research Journal* ,22(2): 435-454.

Siegfried, b.d. 1993. Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects

Taylor, k.s. et al. 1987. Impairment of a classical conditioned response of the honey bee (Apis mellifera L.) by sublethal doses of synthetic pyrethroid insecticides. Apidol. 18:243-252.

U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economics Research Service.1996. Agricultural chemical usage: 1995 field crops summary. Washington, D.C. (March).

Vijverberg, h.p.m. and j. van den Bercken. 1990. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit. Rev. Toxicol. 21:105-126.

Vieira, l. r., Gravato, c., Soares, a. m. v. m., Morgado, f. and Guilhermino,

l. 2009. Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: Linking biomarkers to behaviour. Chemosphere 76: 1416-1427.

World Health Organization (who). 1989 .Cypermethrin. Environmental Health Criteria 82. Geneva, Switzerland: United Nations Environment Programme, International Labor Organization, and WHO.

Yang and Yongguan2004 Yang, g.,c.Gangcai and c.Yangguan 2004.The

econometric assessment of losses by water pollution in chongging. South West China, chineseJ. Geochemical.,23(1):94-100.

Shankar KM, Kiran BR, Venkateshwarlu M, "A review on toxicity of pesticides in fish", International Journal of Open Scientific Research, 1(1), 15-36, 2013.

Dhasarathan P, Palaniappen R, Ranjit SJA, Pazhanisamy K, Indra N "Effect of endosulfan and butachloron on the digestive enzyme and proximate composition of the fish, Cyprinus carpio", Indian J.Environ.and Ecoplan., 3(3):611-614, 2000. Toxic effects of arsenic on protein content in the fish, labeorhita (Hamilton)", Nature Environment and pollution Technology 6(1): 113-116, 2007.

Dr: Haram Hassan AbassBakhiet Abdelbain AlaaHashim Onnor Rayan Omer

Behavioural and Some histopathological effects of chlorine on tilapia fish, *Oreochromius niloticus.*