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, Oreochromius niloticus

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: (وَهُوَ الَّذِي سَخَّرَ الْبَحِرَ لِتَأَكُلُواُ مِنْهُ لَحِنّا طَرِيًّا وَتَسِتَخِيرِجُونَ مِنْهُ حِلِيَةً تَلِبَسُونَ وَتَرَى الِفُلَكَ مَوَاخِرَ فِيهِ وَلِتَبِتَغُواُ مِن فَضِيلِيَ وَلَعَلَّكُمْ تَشِكُرونَ)

صدق الله العظيم

سورة النحل (الإيه 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 Abass Bakhiet 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, *Oreochromis niloticus* were exposed to different sublethal concentrations (30mg/L and 50mg/l) of chlorine to observe behavioral and histopathological alterations in gills and liver.

Methods;
Chlorine is solved (pouder) and put in different concentrations (30mg/l, 50mg/l).

Results:
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 والخلاص.

طريقة التجربة:

تم تدوير الكلور (بترتر) تم تعرضه بتراكيز مختلفة (30ملج و50ملج من الكلور لكل لتر من الماء).

النتيجة:

أظهرت الاسمك استجابة سلوكية واضحة بعد تعرضها لمحلول الكلور بمختلف تراكيزه فاظهرت سلوك السباحة السريع وفقدان التوازن بالإضافة للنشاط السطحي للأسماك. اظهرت الخياشيم تضخم في النسيج الطلائي لخيوط الثانوية واحتفاق دموي في الأوعية الدموية على الخيوط الخيشومية وضمور في الخيوط الثانوية وانسلاخ في غشاء الخييط الثانوية.

كما أظهرت الكبد تغيرات نسيجية شملت موت بعض الخلايا مما أدى إلى حدوث فجوات بالإضافة إلى تكوين بعض التخثر في الوريد المركزي وتمزق وانفجار في خلايا الكبد كما أظهرت بعض الخلايا والانوية الميت.
CHAPTER ONE

1.1 Background of the study

Fish is an excellent source of protein in human 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 Lemoset 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 Oreochromis niloticus is indigenous to Africa and introduced. It is an omnivore which feeds on plankton and aquatic plants. Oreochromis niloticus 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 outflow by gills (MAETZ, 1971).
fresh water and sea water. (Philosophical Transactions of the Royal Society of London Series B Biological Sciences 262, 209-249).

Among these pollutants the 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 times. Around 1630, chlorine gas was first synthesized in a chemical reaction, but not recognized as a fundamentally important substance.

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 chlorinated 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).

1.2 Objectives:

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

1. Behavior of the fish.
2. Histology of gill and liver of the freshwater fish *oreochromius nilotic*. 
CHAPTER TWO
LITERATURE 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.
2.3 Chlorine 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 (ElZorganiet al, 1998).

2.4 Applications of chlorine:

2.4.1 Production of industrial and consumer products

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

For example, it is used in making plastics, solvents for dry cleaning and metal degreasing, textiles, agrochemicals and pharmaceuticals, insecticides, dyestuffs, household cleaning products, etc. Many important industrial products are produced via organochlorine intermediates. Examples include polycarbonates, polyurethanes, silicones, polytetrafluoroethylene, carboxymethyl cellulose, and propylene oxide. Like the other halogens, chlorine participates in free-radical substitution reactions with hydrogen-containing organic compounds. When applied to organic substrates, reaction is often—but not invariably—non-regioselective, and, hence, may result in a mixture of isomeric products. It is often difficult to control the degree of substitution as well, so multiplesubstitutions are common. If the different reaction products are easily
separated, e.g., by distillation, substitutive free-radical chlorination (in some cases accompanied by concurrent thermal dehydrochlorination) may be a useful synthetic route. Industrial examples of this are the production of methyl chloride, methylene chloride, chloroform, and carbon tetrachloride from methane; allyl chloride from propylene, and trichloroethylene, and tetrachloroethylene from 1,2-dichloroethane.

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

2.4.2 Sanitation, disinfection, and antisepsis Water chlorination

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

The drawback of using chlorine in swimming pools is that the chlorine reacts with the proteins in human hair and skin (see Hypochlorous acid). Once the chlorine reacts with the hair and skin, it becomes chemically bonded. Even small water supplies are now routinely chlorinated. (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 accumulate at the bottom of poorly ventilated spaces. Chlorine gas is a strong oxidizer, which may react with flammable materials. Chlorine MSDS” (PDF). 1997
Chlorine is detectable with measuring devices in concentrations of as low as 0.2 parts per million (ppm), and by smell at 3 ppm. Coughing and vomiting may occur at 30 ppm and lung damage at 60 ppm. About 1000 ppm can be fatal after a few deep breaths of the gas. Winder, Chris (2001) The IDLH (immediately dangerous to life and health) concentration is 10 ppm. Chlorine MSDS” (PDF). 1997.

Breathing lower concentrations can aggravate the respiratory system, and exposure to the gas can irritate the eyes. Winder, Chris (2001) the toxicity of chlorine comes from its oxidizing power. When chlorine is inhaled at concentrations above 30 ppm, it begins to react with water and cells, which change it into hydrochloric acid (HCl) and hypochlorous acid (HClO).

When used at specified levels for water disinfection, the reaction of chlorine with water is not a major concern for human health. Other materials present in the water may generate disinfection by-products that are associated with negative 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.

2.6 Generalized behavioral response of fish to chlorine

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

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

When fish become lethargic, they may appear dead; they have shallow respiration rates and often are immobile. Fish that succumb to chlorine sink to the bottom without convulsive movements. Their respiration slows and finally stops. Fish killed due to chlorine exposure usually assume a natural posture. However, some fish, such as sauger (Stizostedion canadense), die with the mouth gaping and the gills flared (Seegert et al., 1979). High chlorine concentrations
also may result in the loss of large amounts of mucous, particularly for common carp (Cyprinus carpio) (Seegert et al., 1979).

The equilibrium loss response (EL50) seems particularly important for minimizing sublethal effects of chlorine exposure and reducing losses by predation. Chinook salmon (Oncorhynchus tshawytscha) exposed to elevated temperature and chlorine required a 55% reduction in the lethal time (LT50) to ensure that equilibrium loss did not occur (Stober and Hanson, 1974). Salmon generally did not recover from chlorine exposure once equilibrium loss occurred.

2.7 Generalized physiological effects of chlorine

Zeitoun (1977, 1978a,b) provided insights into the biochemical responses of rainbow trout (Oncorhynchus mykiss) to chlorine. Hematological analyses suggested that chlorine was not the primary cause of observed stress. Of parameters measured, only hematocrit and hemoglobin concentrations differed between caged and control fish. The generalized response of increases in the proportion of red blood cells in the blood of test fish, and the subsequent increase in hemoglobin could be the result of various stress factors and are not specifically due to chlorine.

Sprague (1971) reported the same problem with nonspecificity. Rainbow trout exposed to sublethal concentrations of total residual chlorine had less hepatic ribosomal translational ability (Orvos et al., 1986). These results may provide an explanation for the altered plasma proteins noted in other studies.

The ventilation rates of bluegill (Lepomis macrochirus) exposed to chlorine decrease initially, at low concentrations of chlorine, but increase at higher levels of chlorine (Miller et al., 1979).

Some investigators also have reported that the coughing rate of fish increases 15-fold in response to intermittent chlorine concentrations of 0.4 to 0.5 mg/l (Bass and Heath, 1977). These authors also reported that the arterial PO2 declined
by about 60% during the first pulse peak. Work by Block (1977) has shown that the gill is the chief target organ for chlorine toxicity.

The authors surmised that chlorine kills fish by internal hypoxia induced by gill damage. Histopathological studies (Bass et al., 1977) have indicated that sublethal concentrations of chlorine cause moderate gill hyperplasia and swelling of the lamellar epithelial cells. These studies highlight the numerous sublethal effects of chlorine that may alter the fitness of the fish. Studies investigating the long-term effects of chlorine exposure are nonexistent.

Wedemeyer and Ross (1973) suggest that the stress response and susceptibility of fish to chlorine may be related to diet. However, fish living in thermal discharge canals usually have abundant food resources (Coutant, 1970), which may negate diet as a major cofactor in the ecological effects of chlorine. Additional evidence presented by Marking et al. (1984) suggests that diet appears to have little influence on the sensitivity 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 of industrial wastes. Besides polluting waters, in many cases these waters kill the fish and other aquatic organisms.

Freshwaters are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of accidental discharges or criminal negligence (Vutukuru, S.S., 2005). Primary effects such as fish kills can be detected but secondary effects may go unnoticed (Simon, D., S. Helliwell and K. Robards, 1998).

The ability to detect, identify and properly respond to natural chemical stimuli is an important component of the environmental physiology of fishes.
The classic ecotoxicology approach to testing aquatic toxicity is to measure the direct effect in simple experiments using death, more often than not, as the endpoint (Cairns, J.J., 1983). In situations where no toxicity is available, bioassays become extremely important. Development of acute toxicity bioassay data must be viewed as an essential step for providing comparative toxicity information on different toxicants and species of organisms (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) with aerated tap water and were fed with commercially pellets. Fish were acclimatized for 2 days and an average temperature of 29°C. The tap water used for the experiment had a pH value of 6-7 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 5 fish
The second group was 50 mg/l with two replicates each 5 fish
The third was the control group
Plate: group fish

Plate: water quality parameters
3.3 Preparation 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.

3.5 Water quality parameters:

Physical measurements:
Temperature ($^\circ$C): 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

3.6 Behavioural Manifestation:

The observations of the fish behavior were recorded during the experiment.
3.7 Histopathological 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 was 50mg/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

<table>
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<th>Concentration cl₂⁻ (mg/l)²</th>
<th>Cumulativemortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Timeofexposition</td>
</tr>
<tr>
<td></td>
<td>5 minute</td>
</tr>
<tr>
<td>0.0(controlgroup)</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>

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 complete mortality (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 Tilapia tissues revealed that there are several histopathological changes 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), *(Oreochromis niloticus)* 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 *Oreochromis niloticus* showing the normal tissues of liver. H&E X 200.

Plate 2. Section through the liver of *Oreochromis niloticus* showing Focal areas of necrosis (FN). H&E X 200
Plate 3. Section through the liver of *Oreochromis niloticus* showing Dilation and thrombosis formation in central vein (CV). H&E X200

Plate 4. Section through the liver of *Oreochromis niloticus* showing Dilation and thrombosis formation in central vein (CV). H&E X200
Plate 5. Section through the liver of *Oreochromis niloticus* showing ruptured hepatocytes (RH) and vacuolation (V) H&E X200

Plate 6. Section through the liver of *Oreochromis niloticus* showing ruptured hepatocytes (RH) and vacuolation (V) H&E X200
Plate 7. Section through the liver of *Oreochromis niloticus* showing cell damage (CD). H&E X200

Plate 8. Section through the liver of *Oreochromis niloticus* showing pyknosis (PY) and hemorrhage. H&E X400
Plate 9. Section through the liver of Oreochromis niloticus showing pyknosis (PY) and hemorrhage. H&E X400

Plate 10. Section through the liver of Oreochromis niloticus showing pyknosis (PY) and hemorrhage. H&E X400
Plate 11. Section through the control gills of Oreochromis niloticus. H&E X200

Plate 12. Section through the gills of Oreochromis niloticus showing sloughing of secondary lamellae. H&E X400
Plate 13. Section through the gills of *Oreochromis niloticus* showing sloughing of secondary lamellae. H&E X400

Plate 14. Section through the gills of *Oreochromis niloticus* showing sloughing of secondary lamellae. H&E X400
Plate 15. Section through the gills of *Oreochromis niloticus* showing sloughing of secondary lamellae. H&E X400

Plate 16. Section through the gills of *Oreochromis niloticus* showing sloughing of secondary lamellae. H&E X400
Plate 17. Section through the gills of *Oreochromis niloticus* showing sloughing of secondary lamellae. H&E X400

Plate 18. Section through the gills of *Oreochromis niloticus* showing proliferation and damage in the epithelium of gill filaments (EPC) H&E X400
Plate 19. Section through the gills of *Oreochromis niloticus* showing proliferation and damage in the epithelium of gill filaments (EPC) H&E X400

Plate 20. Section through the gills of *Oreochromis niloticus* 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 *Oreochromis niloticus* exposed to chlorine.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histopathological changes</th>
<th>Number of fish in which the effect was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 ppm</td>
</tr>
<tr>
<td>Liver</td>
<td>Focal areas of necrosis</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Dilation and thrombosis formation in central vein</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Ruptured hepatocytes and vacuolation</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Cell damage</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pyknosis (PY) and hemorrhages</td>
<td>9</td>
</tr>
<tr>
<td>Gills</td>
<td>Sloughing of secondary lamellae</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Proliferation and damage in the epithelium of gill filaments</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Congestion in blood vessels of gill filaments and secondary lamella</td>
<td>10</td>
</tr>
</tbody>
</table>
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 fishes' behavioral 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 the treated fish indicates the loss of equilibrium. chlorine has profound effect on the central nervous system.

The histopathological alterations attributed to the prolonged exposure to chlorine resulted in respiratory, 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 that produces epithelial thickening of the filament epithelium enhances migration of chloride cells up to the edge of the secondary lamellae and provokes the hypertrophy and fusion of secondary lamellae. These could be considered as unspecific biomarker responses of chloride exposure and disturbed health of fish. Gills showed edema of the primary lamellae; severe edema, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary
lamellae were observed. According to Mallatt (1985), the edema of the gill epithelium is one of the main structural changes caused by the exposure to chlorine. Our results confirm this lesion of chlorine exposure. These alterations have been reported for other species exposed to heavy metals particularly Cd (Gardner and Yevich 1970; Karlsson-Norrgren et al. 1985; Pratap and Wendelaar Bonga 1993; Thophon et al. 2003) and sometimes referred as a first sign of pathology (Thophon et al. 2003). Cellular proliferation in the gill epithelium is also observed in fish exposed to different pollutants as described by Gardner and Yevich 1970 and Thophon et al. 2003. Lifting, swelling, and hyperplasia of the gill epithelium could serve as a defense function. The liver showed congestion of central vein and nuclear pyknosis in the majority of hepatic cells. These findings were apparent as the liver considered the organ of detoxification, excretion and binding proteins such as metallothionein (MTs).

The cell damages. Similar results were observed by Van Dyk (2003) and Mela et al. (2007).

Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment or other Organs (Heath 1995). Pandey et al., (1994) described the alterations in liver 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 Salvelinus alpinus exposed to 0.15 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 fish species 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 contain 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

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