INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 12, p. 108-116, 2015

RESEARCH PAPER OPEN ACCESS

Histopathological study of liver and kidney in common carp (*Cyprinus carpio*) exposed to different doses of potassium dichromate

Muhammad Arshad Rana[1*], Farhat Jabeen[2], Samina Shabbir[2], Aasma Naureen[2], Kishwer Sultana[2], Imran Ahmad[2], Munaza Shabnam[3]

[1]*Department of Zoology, Government Postgraduate College Okara, Pakistan*

[2]*Department of Zoology Government College University Faisalabad, Pakistan*

[3]*Department of Zoology, Wildlife and Fisheries UAF, Faisalabad, Pakistan*

Key words: *Cyprinus carpio,* Mortality, Teratogenic, Histological, Hemorrhage.

http://dx.doi.org/10.12692/ijb/6.12.108-116 Article published on June 30, 2015

Abstract

The liver plays vital role in metabolism and act, as a storage center of many substances while kidney function is to maintain homeostasis. Any damage to liver and kidney tissue will induce abnormalities in this process and ultimately can result in mortality of fish. The objective of present study was to identify the degree and damage to the histological changes of Common carp (*Cyprinus carpio*) liver and kidneys exposed to Potassium dichromate ($K_2Cr_2O_7$). Fish samples were exposed to 2 doses viz 5 and 10 mg.L^{-1} designated as P_1, P_2 while P_0 was control. 16 days exposure of Potassium dichromate ($K_2Cr_2O_7$) induced changes in liver tissue of fish were coagulative necrosis, focal area of necrosis, dilation and congestion in blood sinusoid, aggregations of inflammatory cells between the hepatocytes, and degeneration of parenchyma cell while haemosiderin and aggregations of inflammatory cells, dilation in the capillary tubes of renal tubules and hemorrhage were observed in kidney of Common carp (*Cyprinus carpio*) when exposed to sub lethal concentration of Potassium dichromate ($K_2Cr_2O_7$) with respect to control showing normal histology. Present study results showed that Potassium dichromate ($K_2Cr_2O_7$) is highly teratogenic metal and it's deteriorating affects increase with increase in its concentration.

* **Corresponding Author:** Muhammad Arshad Rana ✉ marshadrana719@gmail.com

Introduction

Fish is one of useful sources of protein food. Worldwide, people obtain 25% of their animal protein from fish and shellfish. About 35% of all fish is eaten fresh, chilled or frozen. It is also cured or canned (16$ each) or made into oil and fish meat (32%) (Stamiskiene *et al.,* 2009).Fish products are used as medicines, lubricants, varnishes, soap, margarine and cosmetics *(*Vinodhini and Narayanan, 2009).

The habitats of fish are being contaminated disturb through a various aquatic pollutants (Oner *et al.,* 2008). The major sources of heavy metal pollution in ecosystem are waste of industries, mining of metal and agriculture (Singh *et al.,* 2007). Fish are considered as the bio indicator of pollution in water quality through heavy metal, because they inhibit different trophic levels, and are among the most sensitive to the presence of toxicants in water (Alibabic *et al.,* 2007). According to many authors' fishes, which are widely being used for the assessment of the quality of the aquatic environment and cause bio indicator of pollution (Dautrempuites *et al.,* 2004).

Heavy metal considers to any metallic element that relatively greater density and is lethal or toxic at low concentrations. Examples of heavy metals are Cadmium (Cd), Chromium (Cr) and lead (Pb). Heavy metals have a specific gravity that is at least 5 times the specific gravity of water. Some highly toxic metals with a specific gravity are Chromium, 7.18; Cadmium, 8.65; Lead, 11.34 (Lide, 1992). These heavy metals are water soluble and when intake absorbs in the body. When these heavy metals tend to unite with enzymes and inhibit the functioning of particular enzymes and can have severe physiological and neurological disorders. So these heavy metals have toxic, inhibitory and mutagenic effects (Gourdon *et al.,* 1990).

The bioaccumulation of heavy metal in tissue of fish is responsible of redox reaction that provides reactive species (ROS), which cause morphological and physiological changes (Monteiro *et al.,* 2005). The bioaccumulation in living organism up to toxic level depends on environmental condition such as pH and water hardness (Guven *et al.,* 1999).

Chromium is known as a heavy metal and pollutant as well as a necessary micronutrient. Wastewater pollution by chromium originating from electroplating, dyeing, tannery, hard-alloy steel and stainless steel manufacture, has affected the life on earth. Chromium is also used as a catalyst and coating material (Idachaba *et al.,* 2004).

Chromium is also a compound of biological interest, perhaps having a role in glucose and lipid metabolism as an important nutrient. Chromium (VI) compounds have been found to be mutagenic and carcinogenic (Venko 1985). Along with the heavy metal, chromium is consider as an important pollutant from industrial effluents and causes harmful effects on non-target aquatic organism resulting inequality of an ecosystem (Arunkumar *et al.,* 2004). It is also listed among 25 hazardous substances that pose threat to human health (Ali *et al.,* 2000).

Histopathological as biomarkers in the estimation of the health of fish exposed to contaminants, both in the laboratory and field studies (Schwaiger *et al.,* 1997). According to (Gernhfer *et al.,* 2001) the Great advantage of using histopathological biomarkers in ecosystem monitoring is that this category of biomarkers allows examining specific target organs including gills that are responsible for vital function such as respiration. In addition, the changes found in these organs are normally easier to identify than functional ones (Fanta *et al.,* 2003), and serve as warning signs of damage to animal health (Hinton and Lauren 1990).

Fish Kidney is an important organ and its function is to maintain the homeostasis. It is not only remove wastes from blood and it is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluid and erythropoieses (Iqbal *et al.,* 2004). The fish liver plays an important role in metabolism, excretion,

digestion, detoxification and storage of numerous substances, including some that are toxic for fish. A histopathological alteration in liver and kidney indicators of chemical toxicity and it is useful way to study the effect of aquatic animal to toxin present in aquatic environment (Loganthan *et al.,* 2006).

The fresh water Common carp (*Cyprinus carpio*) is an important culturally species of Cyprinid family all over the world. It is of excellent commercial value because it is the most usually used all over the world. Therefore it may be a good model to study the responses to various environmental contaminations (Vinodhini and Narayanan, 2009).

Aims

The present research work was aimed to monitor water pollution, determine the health of fish, observe the histopathological changes in liver and kidney of fish and to evaluate the chronic toxicity of sub lethal heavy metals.

Materials and methods

Study Area

The freshwater fish *Cyprinus carpio* (Order: Cypriniformes, Family: Cyprinidae) or common carp (Gulfam) was selected as animal model. The freshwater Common carp (*Cyprinus carpio*) was 12 to 15 cm in length and 35 to 40 g in weight was obtained from Government fish seed Hatchery, Satiana Road, Faisalabad transported live to the Paleontology Research Laboratory, Department of Zoology Government College University Faisalabad. The fish were acclimatized to Laboratory condition for 15 days in 2 glass aquaria of 80 L water capacity for each before the start of experiment. During the acclimatization, Fish were fed with maize, rice, gluten and libitum there was continuously changed every two days during the period of acclimatization.

Sampling

Analytical grade Potassium dichromate ($K_2Cr_2O_7$) M.W.=294.19g/mol, Merck Germany was used as experimental toxicant. A stock solution of $K_2Cr_2O_7$ prepared in 1000 ml distilled water containing 5.6g/L by diluting -g $K_2Cr_2O_7$. Other dilutions were made accordingly. Forty (40) fish were divided into three groups. Each group of fish was further divided into three replicate experiments that contained 6 fish each. The water and dose of metal in the replicate experiments were changed after three days to maintain the toxicant concentration in glass aquaria. Group-I was put in normal tap water in glass aquaria having 30 L water without exposure of heavy metal and is consider as control group. Group–II and Group–III were considered as experimental groups and exposed to sub lethal concentrations 5 ppm or 5mg/L and 10 ppm or 10mg/L of $K_2Cr_2O_7$ solutions for a period of 16 days respectively. All the groups of fish were fed with food throughout the experiment.

Histopathological Investigation.

After the completion of experiments, the Common carp (*Cyprinus carpio*) were dissected to obtain the liver and kidney samples. The liver and kidney was fixed in 10% formalin for 15 min and then tissues were shifted to the fixative (10% formalin) for 24 hrs. The organ was dehydrated, cleared and infiltrate with 50%, 70%, 80%, 95% and 100% for overnight. After replacing the alcohol with xylene then the tissues had been dehydrated and in-filtrated with the embedding material, they were ready for embedding in paraffin wax and sectioned at 3-5 µm then stained with haematoxylin and eosin and examined microscopically.

Statistical Analysis

Statistical analysis was applied for the calculations of mean and standard error for weights and lengths of specimens as shown in table 1.

Physico-Chemical Factors

Water quality parameters viz. (1) Temperature (2) pH (3) Dissolved oxygen (4) Total hardness and (5) Electrical conductivity were determined by following APHA (1998). (Table 2).

Temperature

Temperature is an important parameter regarding its effects on the solubility of oxygen in water.

Temperature was observed in degrees Celsius (°c) by thermometer of all aquariums daily.

pH

pH is a common measure of the acidity or alkalinity of water and is indicated on a scale of 0 to 14. pH of water sample was measured by pH meter (pH 720, WTW Series Inolab).

Dissolved Oxygen

Dissolved oxygen is a necessary parameter and becomes limiting factor for survival and functioning of aquatic biota. It was measured by portable DO meter (Oxi 730, WTW Series Inolab). Dissolve oxygen concentration was measured in milligrams per liter (mg/L) or ppm.

Total Hardness

50 ml of water sample from each aquarium was collected in a conical flask and its pH was raised up to 12-13 by adding appropriate volume of buffer. 0.1ml of Erichrome Black-T (EBT) indicator was added to it and titrated against EDTA (1.0N) to reach the end point of blue color.

Electrical Conductivity

Electronic conductivity is the measure of electrolytes. Conductivity meter measures Electro conductivity of water samples. (Cond 720, WTW Series Inolab) in micro Siemens per Centimeter (μScm^{-1}).

Results

The hazardous effect of chromium on the histopathology of selected organs of the freshwater fish Common carp (*Cyprinus carpio L.)* was investigated.

Sub-lethal concentrations of chromium

Histopathological study have been proved to be a sensitive tool to indicate any change at organism and tissue levels that's why present study was designed to check the effect of inert renewal exposure of Cr concentration on Common carp (*Cyprinus carpio*) kidney and liver. This will confirm the level of declination by Cr at organ and tissue level on different sub- lethal concentration. The present study aims to investigate the histological alterations of liver and kidney of Common carp (*Cyprinus carpio*), exposed to sub- lethal concentration of chromium.

The liver of fish exposed to sub-lethal concentrations of (5 mg/L) and (10mg/L) of chromium for 16 days showed the remarkable morphological alterations as compared to control group. The microscopic examination of liver of control group showed the normal parenchyma cells arranged to form lattice network. The interspaces are sinusoid of thin strip with sparse connective tissues. Hepatocyte is normal and exhibits a homogenous cytoplasm around the centrally located spherical nucleus (Figure 1a).

Table 1. The mean and standard error for weight and length of *Cyprinus carpio*.

Fish Species	English Name	Local Name	Weight (g) Mean ± S.D	Length (cm) Mean ± S.D
Cyprinus carpio	Common Carp	Gulfam	37.3 ± 52.8	13.7 ± 19.33

Histopathological alterations

Many histopathological alterations were observed in the liver of selected fish *Cyprinus carpio*. Aggregations of inflammatory cells between the hepatocytes and also dilation and congestion in blood sinusoid were found in (Figure 1b). (Figure 1c) showed the focal area of necrosis. (Figure 1d) showed the coagulative necrosis and degeneration of parenchyma cell.

The kidney samples of the untreated fish showed normal arenal Tubules and Glomerulus in (Figure 2a). Haemolysis in kidney was observed in (Figure 2b). Reduction in pyknotic nuclei and dilation in blood vessels were seen in (Figure 2c).

Effects of chromium

During the experimental study chromium exposed fish showed the abnormal behavior like quick swimming, jerk movement, agitation, loss of stability,

and also secretion of mucus from whole body. The exposed fish swam to the surface other than control group.

Discussion

The central aim of present research work is to determine histopathology of selected organs in freshwater Common carp (*Cyprinus carpio*) after chromium exposure. It is usually recognized that metal toxicity is more accurately measured in freshwater than sea water, because metal appears to large amount as complex compounds in sea water and this reduces the toxicity of metal ions. The reaction and survival of aquatic organisms depends not only the biological state of animals and physio-chemical characteristic of water but also on kind, toxicity type and duration of exposure (Mays, 1996).

Table 2. Physico-chemical parameters of tap water used in experimental glass aquariums.

Sr. No.	Water Quality Parameter	*Mean ± S.D*
1.	Temperature	30.06±0.29
2.	pH	7.35±0.32
3.	Dissolved Oxygen	5.03±0.11
4.	Total Hardness	445.00±14.00
5.	Electrical Conductivity	2.63±0.02

S.D = Standard Deviation.

Degenerative changes in fish

Investigation of water quality parameter such as; dissolved oxygen, temperature, pH, electrical conductivity and hardness is essential as they provide significant information about the variations caused by biological and environmental factors, leading to differences in metal bioaccumulation between different species, different organs (Esteves, 1998) and degree of toxicity of different toxicants (Al-Akeel1996). The present study aims to investigate the histological alterations of liver and kidney of Common carp (*Cyprinus carpio*), exposed to sub-lethal concentration of chromium. Heavy metal induced interactive changes have been measured as sensitive indicators of stress in aquatic organisms (Sharma and Shukla, 1990). Abnormal swimming, restlessness and surfacing studied in present research work may be an avoiding reaction to metal toxicity as reported by authors (Svecevieus, 2001). The severity of damage depends on the toxic effects of a particular compound or toxicant accumulated in the tissue and therefore, exposure to this heavy metal may adversely affect various system in fish which ultimately could lead to overall toxic impact on organs like gills, kidney and liver may seriously affect the metabolic as well as physiologic activities and could impair the growth and behavior of fish. The chromium exposure exhibited marked degenerative change in histology of liver and gills tissues. Similar kinds of toxic effects were noticed in various fish exposed to other heavy metals (Randi *et al.,* 1996; Olojo *et al.,* 2005; Figueiredo-fernandas *et al.,* 2007).

Histopathological modifications

The histopathological modifications in the liver of *Cyprinus carpio* after the exposure of chromium (5mg/L) and (10mg/L) in the present study including, dilation and congestion in blood sinusoid, aggregations of inflammatory cells between the hepatocytes, coagulative necrosis, degeneration of parenchyma cell and necrosis and focal area of necrosis. These finding are also agreement with (Soufy *et al.,* 2007; Mohamed 2001; Fanta *et al.,* 2003; Olojo *et al.,* 2005).

The histopathological modifications in the kidney of *Cyprinus carpio* after the exposure of chromium (5mg/L) and (10mg/L) in the present study including,

haemosiderin and aggregation of inflammatory cells, dilation in the capillary tubes of renal tubules, haemolysis. These finding are also agreement with (Thophon *et al.,* 2003; Camargo and Martinez 2007; Mohamed 2009).

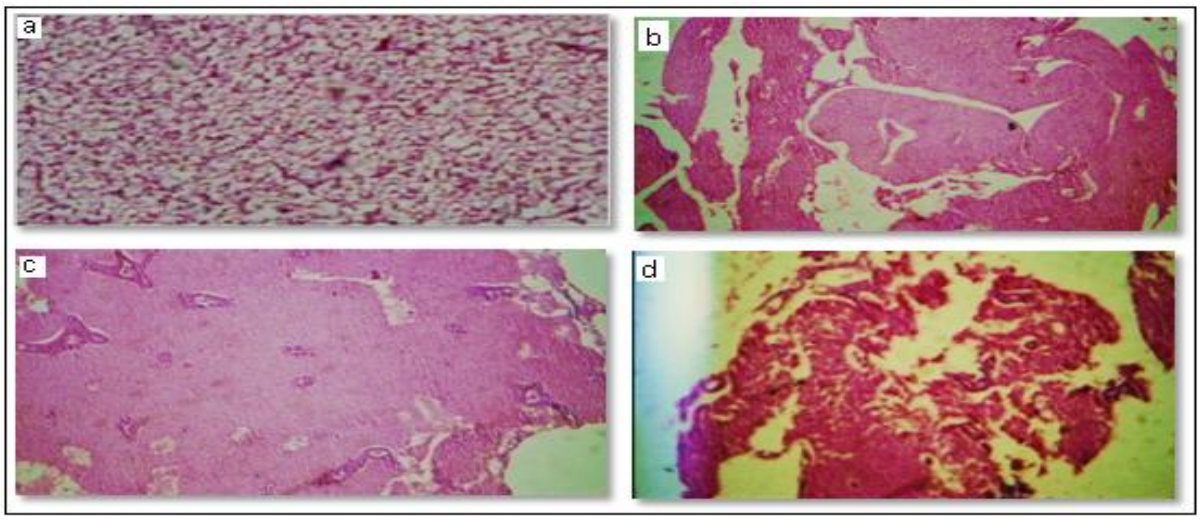

Fig. 1. Photomicrograph of hematoxylin-eosin stained section (a) showing the normal structure of liver have normal network of Parenchyma cells, convergence of sinusoid into large central vein.(b) showing dilation and congestion in blood sinusoid aggregations of inflammatory cells between the hepatocytes on the exposure of 5 ppm Cr in *Cyprinus carpio* (600x). (c) showing focal area of necrosis on the exposure of 10 ppm Cr in *Cyprinus carpio* (600x). coagulative necrosis.(d) degeneration of parenchyma cell and necrosis on the exposure of 10 ppm Cr in *Cyprinus carpio*(600x).

Histological assessments in Common carp (*Cyprinus carpio*) under the impact of heavy metal chromium can be used as a sensitive biomarker to monitor the aquatic pollution. The present study shows that the heavy metal contamination definitely affected the vital organs of fish such as liver and kidney (flesh). Hence a scientific approach to minimize the toxicity effect is essential to enhance the health of this economically important freshwater fish.

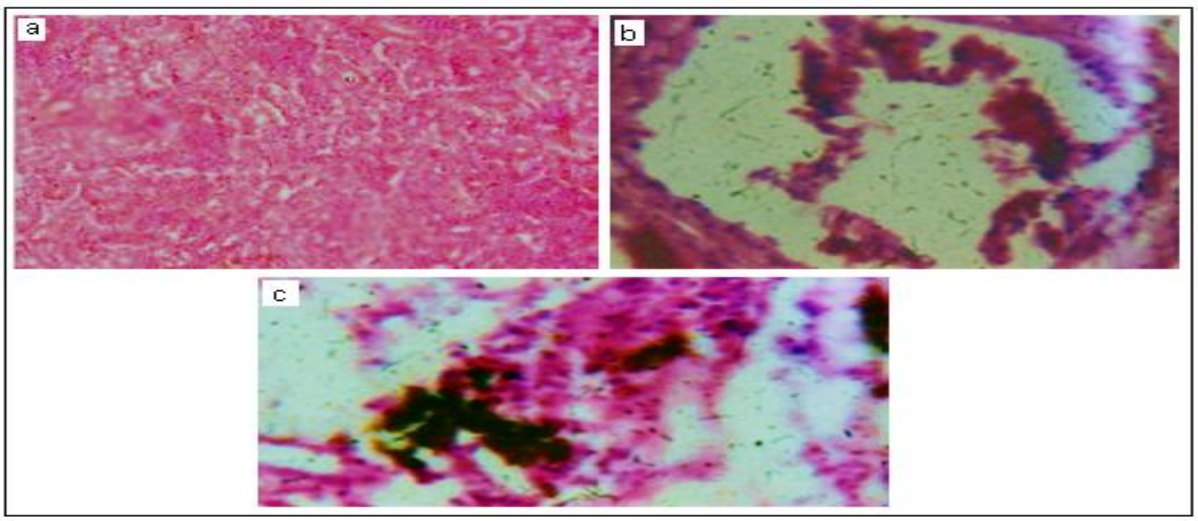

Fig. 2. Photomicrograph of hematoxylin-eosin stained section showing the normal structure of kidney normal arenal Tubules and Glumerulus (a). (b) showing Haemorrhage, haemolysis on the exposure of 5 ppm Cr in *Cyprinus carpio*(600x). (c) showing reduction in pyknotic nuclei and dilation in blood vessels.

Conclusion

Histopathological alterations in Common carp (*Cyprinus carpio*), under the influence of chromium can be used as a sensitive model to monitor the aquatic pollution. The current result indicates that the heavy metal contamination definitely affects the liver, and kidney of fish exposed to chromium toxicity. Hence, a scientific method of detoxification is essentials to improve the health of these economic fish. The present research served as experimental tools for the estimation of environmental pollution.

References

Al-akel AS. 1996. Chromium toxicity and its impact on behavioral responses in freshwater carp (*Cyrinus carpio*) from Saudi Arabia. Pakistan Journal of Zoology **28,** 361.

Ali SS, Hussain A, Shakoori SD. 2000. Hematological effects of hexavalent chromium in fresh water teleost, *Oreochromis mossambicus*. Journal of Eco-toxicology **10,** 3-10.

Alibabic V, Vahcic N, Bajramovic M. 2007. Bioaccumulation of metals in fish of *Salmonidae* family and impact on fish meat quality. Environmental Monitoring and Assessment **131,** 349-364.

Arun Kumar Sing, Rana KS. 2004. Bioconcentration of chromium, copper andiron in an Indian major carp *Labeo rohita* (Ham). Indian. Journal of Environment and Ecoplan **8(1),** 217-219.

Camargo M, Martinez R. 2007. Histopathology of gills, kidney and liver of Neotropical fish caged in an urban stream. Neotropical Ichthyology **5(3),** 327-336.

Dautremepuits C, Paris-Palacios S, Betoulle S, Vernet G. 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. Comprehensive Biochemical Physiology **137,** 325-33.

Fanta E, Rios FS, Rom OS, Vianna ACC, Freiberger S. 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sub lethal levels of organo posphorus in water and food. Eco-toxicology and Environmental Safety **54,** 119-130.

Figueiredo-Fernandas, A, Ferreira- Cardoso, JV, Garcia-Santos S, Monterio SM, Carrola J, Matos P, Fontai- Nhas- Fernandes A. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromism niloticus*, exposed to water borne copper. Turk Journal of Biology **27,** 103 – 109.

Forstner U, Wittaman GTU. 1979. Metal pollution in aquatic environment. Springer Verlag, New York, 486-532 P.

Gernhofer M, Pawet M, Schramm M, Müller E, Triebskorn R. 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. Journal of Aquatic Ecosystem **8,** 241-260.

Gourdon R, Bhende S, Rus E, Sofer SS. 1990. Comparison of cadmium bio absorption by Gram positive and Gram-negative bacteria from activated sludge. Biotechnology Lett **12,** 839-842.

Guven K, Ozbay C, Unlu E, Satar A. 1999. Acute lethal toxicity and accumulation of copper in *Gammanus pulex*. Turk Journal of Biology **23,** 513 - 521.

Hinton DE, Laurén DJ. 1990. Integrative histopathological effects of environmental stressors on fishes. Journal of Science Research **8(2),** 51-65.

Idachaba MA, Nyavor K, Egiebor NO. 2004. The leaching of chromium from cement based waste form via a predominantly biological mechanism. Adverse Environment Science Research **8,** 483 – 491.

Iqbal F, Qureshi IZ, Ali M. 2004. A Histopathological changes in the kidney of common

carp, *Cyprinus carpio* following nitrate exposure. Journal of Science Research **15,** 411-418.

Kakuta I, Murachi S. 1997. Physiological response of carp, *Cyprinus carpio,* exposed to raw sewage containing fish processing waste water. Environmental Toxicology **12,** 1-9.

Kumar Singh R, Chavan SL, Sapkale PH. 2007. Heavy metal concentrations in waste, sediments and body tissues of red worm *(Tubifex spp.)* collected from natural habitats in Mumbai, India. Environmental Monitoring and Assessment **129,** 471-481.

Loganathan K, Velmurugan B, Hongray-Howrelia J, Selvanayagam M, Bhusan-Patnaik B. 2006. Zinc induced histological changes in brain and liver of *Labeo rohita* (Ham.). Journal of Environmental Biology **27,** 107-10.

Mohamed FA. 2009. Histopathological studies on *Tilapia zilli* and *Solea vulgaris* from Lake Qarun, Egypt. Journal of Marine Sciences **1(1),** 29-39.

Mohamed FA. 2001. Impacts of environmental pollution in the southern region of Lake Manzalah, Egypt, on the histological structures of the liver and intestine of *Oreochromis niloticus* and *Tilapia zillii*. Journal of Environmental Development **2,** 25-42.

Monteiro SM, Mancera JM, Fontainhas–fernandes A, Sousa M. 2005. Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. Comprehensive Biochemical Physiology **141,** 375 – 383.

Olojo EA, Olurin KB, Mbaka G, Oluwemimo A. 2005. Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. African Journal of. Biotechnology **4,** 117-122.

Oner M, Atli G, Canli M. 2008. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposure. Environmental Toxicology **27,** 360- 366.

Randi AS, Monserrat JM, Rodriguez EM, Romano LA. 1996. Histopathlogical effects of cadmium on gills of the freshwater fish, *Macropsobryconuru guayanae* Eigenmann (Pisces, Atherinidae). Journal of Fish Diseases **19,** 311- 322.

Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W, Triebskorn R. 1997. The use of histopatological indicators to evaluate contaminant-related stress in fish. Journal of Aquatic Ecosystem **6,** 75-86.

Sharma UD, Shukla S. 1990. Biological effects of heavy metals. An over view. Journal of Environmental Biology **26(2),** 301 – 313.

Soufy H, Soliman M, El-Manakhly E, Gaafa A. 2007. Some biochemical and pathological investigations on monosex Tilapia following exposure to carbofuran pesticides. Global Veterinarian **1,** 45-52.

Staniskiene B, Matusevicius P, Urbonavicius A. 2009. Distribution of heavy metals in muscles of fish: concentrations and change tendencies. Environmental Research Management **2(48),** 35-41.

Svecevieus G. 2001. Avoidance response of rainbow trout *Oncorhyncus mykiss* to heavy metal model mixtures. A comparison with acute toxicity tests. Bull. Environmental Toxicology **67,** 680-687.

Thopon S, Kruatachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S. 2004. Histopathological alterations of White Sea bass (*Latescal carifer*) in acute and sub chronic cadmium exposure. Environmental Pollution Management **121,** 307-20.

Velmurugan B, Selvanayagam M, Cengiz E, Unlu E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. Journal

of Environmental Science **42,** 157-163.

Venko V. 1985. Chromium: A review of environmental and occupational toxicology. Journal of Microbiology and Immunology **29,** 26-37.

Vinodhmi R, Narayan M. 2009. Heavy metal induced histopathological alternations in selected organs of *Cyprinus carpio* (Common carp). International Journal of Environment **3(1),** 95 -100.