

Histopathological Effect of heavy metal on different organs of fresh water fish tissues from Garmat Ali River adjacent to Al- Najebiya Power Station

Fayhaa Salim

Pathology Department, College of Veterinary Medicine, Basra University, Iraq

Abstract

Common carp fish was caged from Shatt Al-Arab near Al- Najebiya power station to study the effect of pollution with heavy metals on different fish tissue (gills, kidney, liver, muscle, skin, intestine, spleen, gonads) sample of the water was analyzed by atomic absorption to find the concentration of heavy metal (ppm=mg/liter) which appear highest than permissible levels. The histopathological results of study were inflammatory cells in skin connective tissue, sub-cutaneous adipose tissue partly with fibrosis, muscle with vacuolation. In gills: Adhesion of some secondary lamella associated with inflammatory cells, congestion, with loss of epithelial lining only capillaries left, severe atrophy only the remnant of secondary lamella left. Small intestine showed proliferation of mucous secreting cells. Liver had small bile duct enclosed by minimal fibrosis, portal vein with some inflammatory cells. Kidney revealed glomerulus with high cellularity & tubular vacuolation, reduce hemopoietic tissue, peritubular edema, tubular degeneration with congested blood vessel. Spleen revealed central arteriolar dilatation, haemorrhage in the tissue, depletion of follicular aggregation and lymphoid tissue, hemorrhagic red pulp, atrophy of follicular white pulp. Gonads had different stages of spermatogenesis in testis and different stages of developing ova, ova with vacuoles of secondary germinal layer.

Key word: histopathology, fish, pollution, heavy metal, fresh water.

دراسة التأثير المرضي النسيجي للعناصر الثقيلة على الاعضاء المختلفة لاسماك المياه العذبة في نهر كرمة علي في المنطقة المقابلة لمحطة كهرباء النجيبية

فيحاء سالم غضبان

فرع الامراض - كلية الطب البيطري - جامعة البصرة

الخلاصة:

تم جمع اسماك الكارب الاعتيادية من شط العرب بالقرب من محطة كهرباء النجيبية لدراسة تأثير التلوث بالمعادن الثقيلة على انسجة الاسماك (الغلاصم، الكلية، الكبد، الجلد، العضلات، الامعاء، الطحال، الاعضاء التناسلية). كما تم تحليل عينات المياه بواسطة جهاز الامتصاص الذري لمعرفة تراكيز العناصر الثقيلة حيث اظهرت ارتفاع كبير عن النسب المسموح بها.

اظهرت نتائج الدراسة النسيجية المرضية وجود خلايا التهابية في الانسجة الضامة للجلد مع تليف الانسجة الدهنية تحت الجلد، تفجج الخلايا العضلية، اندماج الصفائح الثانوية للغلاصم مع وجود التهاب واحتقان وفقدان الخلايا الطلائية للغلاصم مضمور شديد في الغلاصم تاركة بقايا الصفائح الثانوية. الامعاء اظهرت تكاثر وزيادة في

الخلايا الفارزة للميوسين ، تليف حول قناة الصفراء في الكبد ،وجود خلايا التهابية في الوريد البابي الكبدي .زيادة عدد الخلايا في كبينة الكلية ،وذمة حول النبيبات ،تتكس النبيبات وتقعجها واحتقان الاوعية الدموية،طحال اظهر توسع في الشريان المركزي ،نزف ،انسلاخ الجريبات اللمفية ،نزف في اللب الاحمر وضمور في اللب الابيض ،اما الاعضاء التناسلية فظهرت مراحل مختلفة لتكوين النطف والبيوض مع وجود تقعج في الطبقة المولدة الثانوية في البيضة

Introduction

Many of Rivers in Iraq are polluted with organic trace contaminants such as aromatic hydrocarbon, acidity, pesticides, fatty acids, chlorophenols and heavy metals. The environmental exposure of aquatic organisms to trace contaminants may be determined by measuring external levels of a selected set of well-known contaminants in the surrounding water or sediments (1).

The fact that heavy metal cannot be destroyed through the biological degradation and have ability to accumulate in the environment makes these toxicants to the aquatic environment and consequently to man who depends up on aquatic product as food (2). Among the heavy metals, cadmium, lead ,mercury,copper, zinc, chromium and nickel are comparatively notorious toxicants and most of their compounds are water soluble and non –degradable(3). Heavy metals concentration in aquatic ecosystems are usually monitored by measuring their concentration in water sediments, and biota(4).Accumulated heavy metals may lead to morphological alteration in the tissues of fish (5). Histopathological studies give useful data concerning tissue change prior to external manifestation (6).

The salt of cadmium ,zinc ,copper ,lead ,and iron as well as the mercury compounds are the most toxic forms of heavy metals .A combination of zinc or cadmium with copper increases the toxic effect of copper several times ,representing a synergistic action (7).

The histopathological changes caused by heavy metals in gills, liver ,kidneys, gonads ,and other organs of fish have been reported by (2),(8).

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years because they are indestructible and most of them have toxic effects on organisms. Among environmental pollutants, are metals of particular concern, due to their potential toxic effect and ability to bioaccumulation in aquatic ecosystems (9).

Materials and methods:

Area of study:

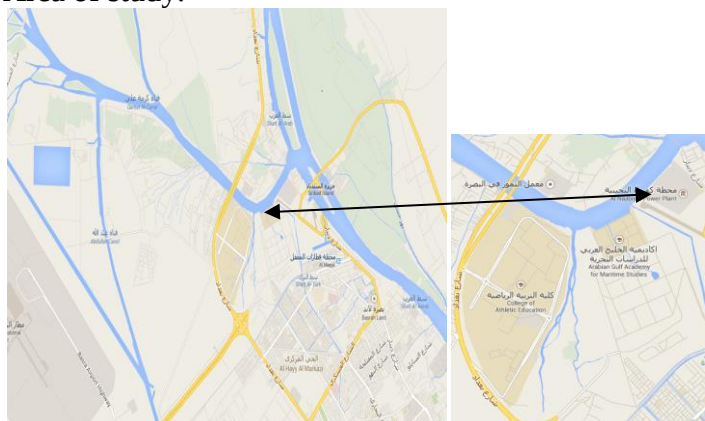


Figure (1) Maps showed the area of study.

Water sample analysis :water sample was taken from the area near to Al Najibiyia power station to the agriculture college –Basra university, to analysis by atomic absorption phoenix-986 to note the concentration of heavy metals(Zn, Ni, Cu, Fe, Mn, Cr, Pb, Cd).

Histopathology: preserved tissue samples were routinely processed and embedded in paraffin, after which thin sections ($5\mu\text{m}$)were cut and stained with hematoxylin and eosin for light microscope examination .

Result:

Water analysis for Heavy metal result: sample of the water was analyzed by atomic absorption in agriculture college to find the concentration of heavy metal which was (Zn=0.41, Ni=0.19, Cu=0.199, Fe=3.0, Mn=0.55, Cr=0.2, Pb=0.8, Cd=0.000).

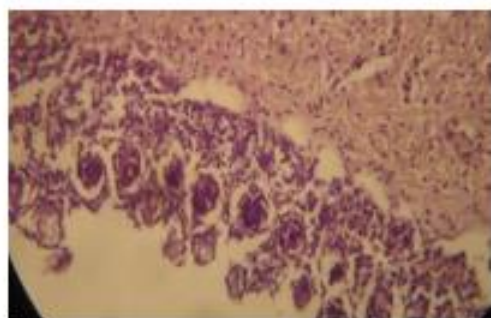
Element		Content in the polluted water sample(mg/L)	Maximum permissible limit for water (mg/L)
1	Zinc (Zn)	0.41	0.5
2	Iron (Fe)	3.0	0.3
3	Manganese (Mn)	0.55	0.1
4	Nickel (Ni)	0.19	0.10
5	Chromium (Cr)	0.2	0.05
6	Copper (Cu)	0.199	0.05
7	Lead (Pb)	0.8	0.05
8	Cadmium (Cd)	0.000	0.005

Table (1): Concentration of heavy metals in polluted water in comparison with Iraqi environmental law no 65/1967.

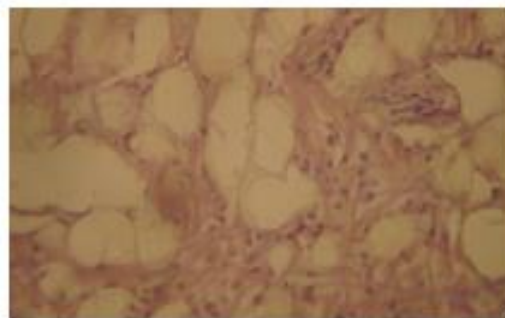
Histopathological result:

Skin:

Skin:



1

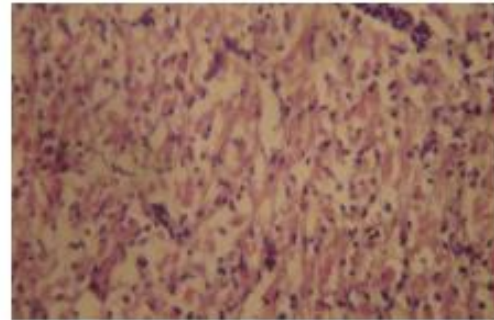
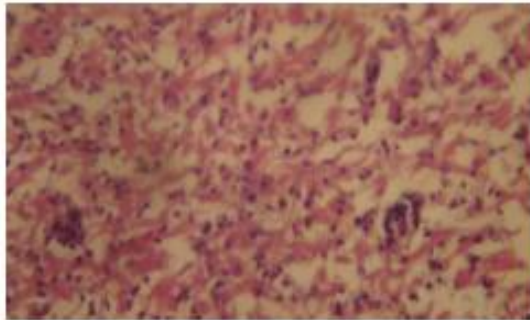


2

1-Skin:at the periphery inflammatory cells with some aggregate of glomeruli like under its connective tissue with infiltration of inflammatory cells(x10)

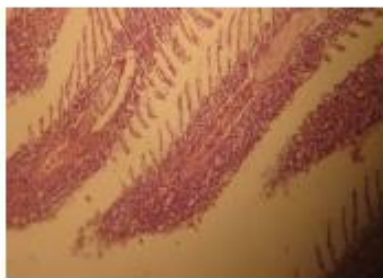
2-Skin :Sub cutaneous adipose tissue partly with fibrosis (x40).

MUSCLE



Muscle with vacuolation, in between was small blood vesseles(x40,x40)

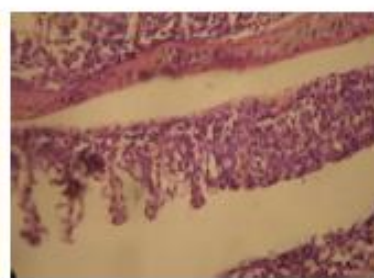
Gills :



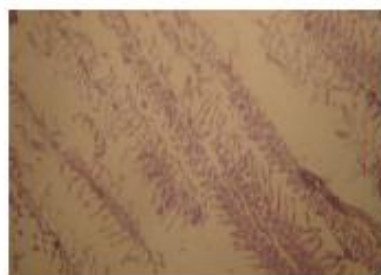
1



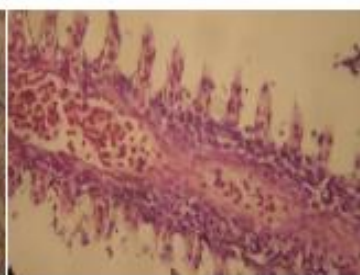
2



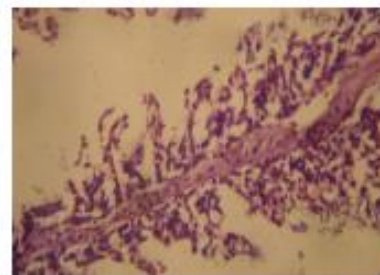
3



4



5



6

1-Gills :primary lamella with part showing loss of secondary lamella and other part showed secondary lamella with sloughing epithelial lining and congestion (x10).

2-Loss of secondary lamella in the top with inflammatory cells ,the other sides lamellae with only capillary and sloughing of the outer epithelial lining (x10).

3-Area of complete loss of secondary lamella (x10,x40)

4-Variation in morphology of secondary lamellae,some congested other swelling ,some short other congested (x10)

5-Short atrophied secondary lamella (x40)

6-Sever atrophy only the remenant of secondary lamella left(x40)

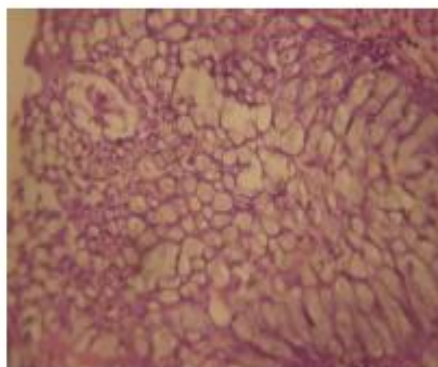
Small intestine



1

2

3



4

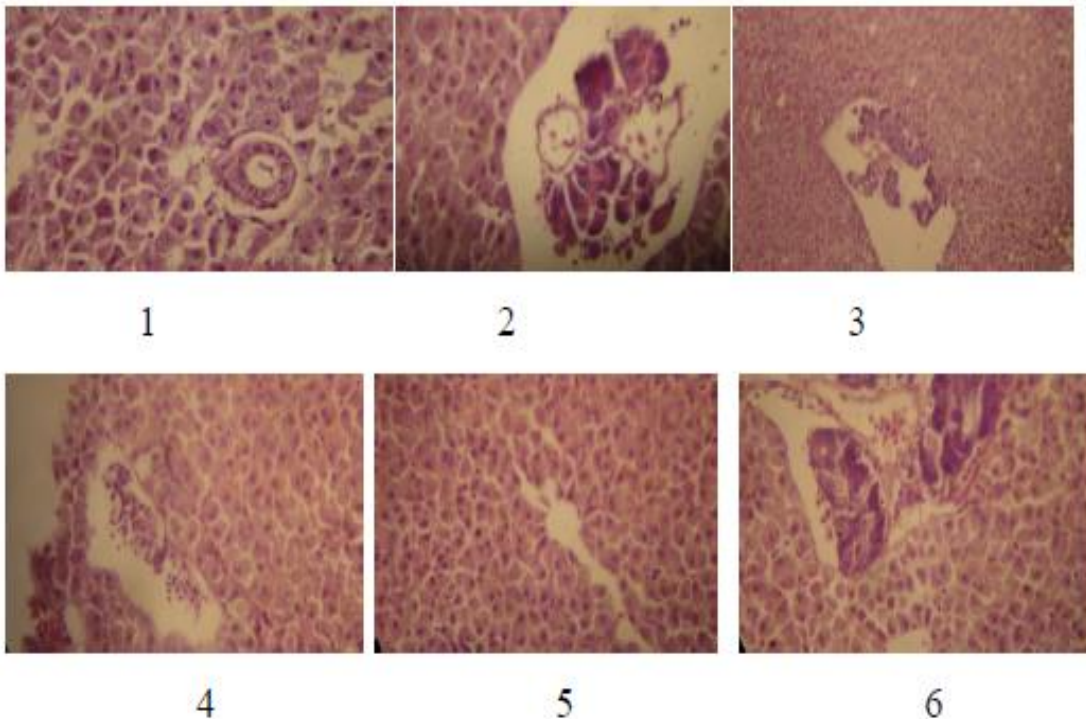
5

(1-2) Small intestine : proliferation of mucous secreting cells (x10,x40).

(3-4) Small intestine: Proliferation of foamy /vacuolated goblet cells like in the lamina properia(x40) .

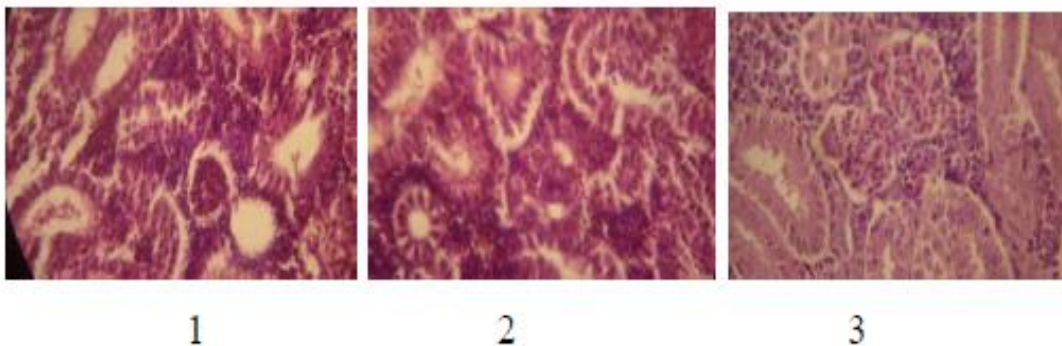
(5) Small intestine :vacuolated mucous epithelial cells(x10)

Liver:



- 1-Liver :small bile duct enclosed by minimal fibrosis (x40)
- 2-Ectopic pancreatic exocrine tissuein periportal region and liver cell not highly dissociated (x40)
- 3-Ectopic pancreatic exocrine tissuein periportal region(x4)
- 4-Portal vein with some inflammatory cells &RBC(x40)
- 5- central vein with dissociated hepatocyte(x40)
- 6- ectopic exocrine pancreatic tissue (x40)

Kidney





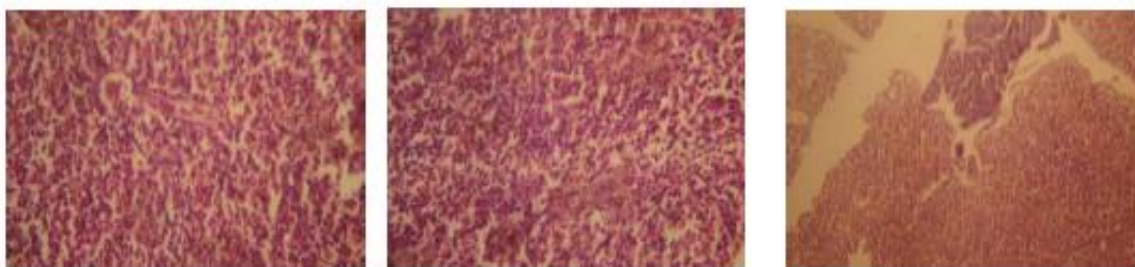
4

5

6

- 1- kidney:glomerulus with high cellularity &tubular vaculation(X40).
- 2- reduce hemopoietic tissue,vacuolation &peritubular odema(X40).
- 3-Glumeruli with high cellularity and proliferating tubule (x40)
- 4-Kidney :Perirenal adipose tissue with inflammatory cells (x40,x10)
- 5-kidney:thyroid follicle and vaculation of corticaltubules(x40).
- 6-Kidney:sever vaculation of cortical tubule with congested blood vessel (x40)

spleen



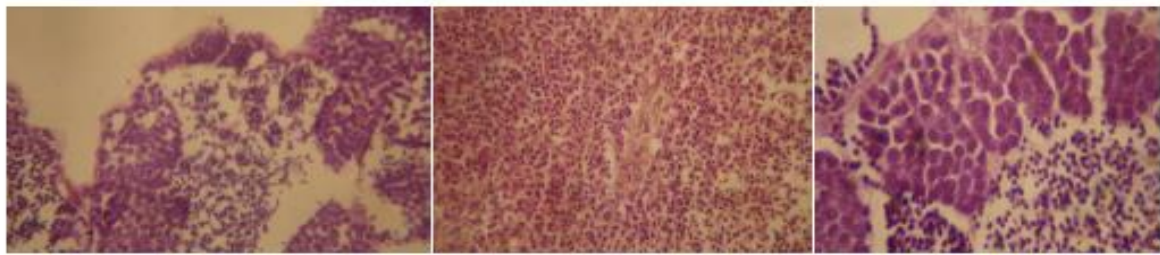
1

2

3

- 1-Spleen :central arteriol diletation&lymphoid tissue (x40)
- 2-spleen:deplitation of follicular aggregation & lymphoid tissue, haemorrhagic red pulp,Atrophy of follicular white pulp (x40).
- 3-spleen:ectopic pancreatic tissue in spleen(X10)

Testis



1

2

3

1-Testis :different stages of spermatogenesis&in the center spermatozoa (X100)

2-Testis:different stages of spermatogenesis in the end stage of spermatozoa(x40)

3--Testes:varying stages of spermatogenesis (x100).

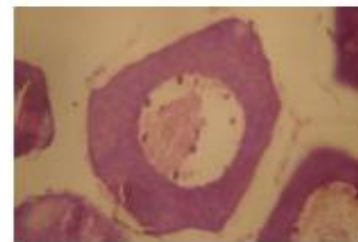
Ovary



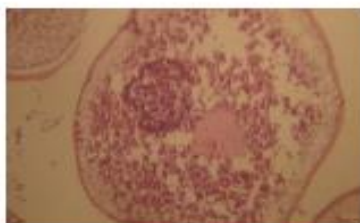
1



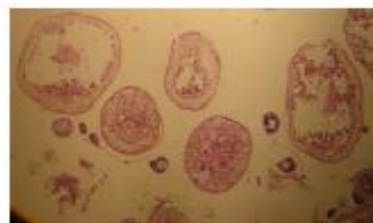
2



3



4



5

1-Neomerus fat droplet in ova(x40)

2- Degenerative ova(x10)

3-perinucleolar oocyte(x40).

4-Secondary ova with beginning of degeneration,vaculation in secondary germinal layer(x10).

5-Ovary: Varying stages of development of ova(x10)

Discussion:

Heavy metal pollution is regarded as a severe problem because it injures the biological functions of the aquatic organisms and their accumulation in fish organs and flesh leading to serious healthy hazardous to the consumers (10).

Regarding to water chemistry table 1 represents the means of heavy metals ((Zn=0.41, Ni=0.19, Cu=0.199, Fe=3.0, Mn=0.55, Cr=0.2, Pb=0.8, Cd=not sens) were drastically elevated. According to Iraqi environmental law no 65/1967 (11) state that the maximum concentration of heavy metals were as the following : (Zn=0.5, Ni=0.1, Cu=0.05, Fe=0.3, Mn=0.1

, Cr=0.05, Pb=0.05, Cd=0.005). The comparison between the present results and the previous recommended permissible level indicated a higher concentration recorded at Al Najebiya power station. The source of Pb in this station water may be resulted from gasoline contains Pb from the fishery boats. The Cd concentrations in the water are still below the permissible level (0.005mg/l) recommended by the Iraqi Organization for Standardization .

In this study the result showed that Zn, Cu, Fe, Mn, Cr and Pb had a high value which were greater than the recommended benchmark by World Health Organization WHO (12). (13) noticed that heavy material concentration in Shatt Al-Arab were Cd < Cu < Pb < Zn, these result were resemble the present study unless Pb Which was greater than Zn and this may be due to type of material used in this station. The concentration levels of these metals would impair the portability of the water.

Fish has been reported to accumulate metals from water by

diffusion via skin and gills as well as oral consumption /drinking of water (4), so that many histopathological changes could be noticed in different tissues as in skin showed at the periphery inflammatory cells with some aggregate of glomeruli like under it connective tissue with infiltration of inflammatory cells, sub cutaneous adipose tissue partly with fibrosis. Muscle with vaculation, small blood vesseles was in between, this results agree with (14) who found alteration of skin and muscles of *Tilapia zillii* fish summarized in hemorrhage, haemosiderin, Fatty degeneration and necrosis in connective tissue of hypodermal layer as well as degeneration, necrosis and edema in muscle fiber layer, these results agree with those obtained by (15). These alterations in skin and muscles may be attributed to inorganic fertilizers (16), ammonia (17), heavy metals, parasitic infection (18) and changes in water quality, (19).

The histopathological alteration of gills in present study were resulted in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (20). (36) treated groups with (Cd & Pb) showed degeneration of gills, hyperplasia led to adjacent secondary lamellar fusion. Degeneration, necrosis, hemorrhage, haemosiderin, and separation in primary and secondary lamellae were noticed by (14) due to increase of ammonia and heavy metals, pH change, oxygen depletion occurrence of bacteria, micro-organisms and parasites with increasing turbidity in water polluted by sewage and agricultural discharge in lake Qarun.

In the present study, kidney of the fishes often showed glomerular

degeneration in Bowman's spaces, reduce hemopoietic tissue, peritubular edema, perirenal adipose tissue with inflammatory cells, thyroid follicle and vaculation of corticall tubules of this study are similar to that's find by (22). Similar changes were reported in case of fish chronically exposed to Zinc (23), and Pb (8).

One of the most important functions of liver is to clean pollutants from the blood coming from the intestine, so it is considered as indicator of aquatic environmental pollution (24). Liver in present study had similar changes which found by (13). The imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation causes vacuolization of hepatocytes, edema, and degeneration, while the imbalance in flow and capillaries pressure may be causes of hemorrhage state (25). The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (26). While the necrosis of the hepatocytes may be due to accumulative effect of chemical organs (27). (21) noticed that group of fish treated with Cd & Pb showed various degrees of hepatic degeneration, focal necrosis and severe sinusoidal congestion. (28) observed that tissue alterations could be observed even with low concentrations of trace metals. (29) showed that the liver in African catfish showed degeneration in the hepatocytes, necrosis and aggregation of inflammatory cells, dilatation and congestion in blood sinusoid and fibrosis and these changes may be

attributed to the direct toxic effects of pollutants on hepatocytes

Small intestine : proliferation of mucous secreting cells, Proliferation of foamy /vaculated goblet cells like in the lamina propria, vaculated mucous epithelial cells. This result agree with (22). (30) found increases in number of goblet (mucosal) cells, width of the lamina propria and degeneration of villi after 60 days of an exposure. According to (31), metals may be in high concentrations in the gills, intestine and digestive glands. These organs have relatively high potential for metal accumulation. High levels of heavy metals were found in intestine of fish. Although fish intestines are seldom consumed, it usually accumulated more heavy metals in this study and might represent good biomonitors of metals present in the surrounding environment.

Spleen in present study revealed central arteriolar dilatation, haemorrhage in the tissue, depletion of follicular aggregation & lymphoid tissue, red pulp, atrophy of follicular white pulp, ectopic pancreatic tissue in spleen, these were agree with (14) who found that spleen of the controlled *Tilapia zillii* fish almost enclosed within a capsule and consisted of lymphatic laden area, white pulp. The surrounding pink areas were the red pulp. Red blood cells were broken in the red pulp. Macrophages may contain pigments from broken down erythrocytes (Haemosiderin). Exposure of fish to lead for up to 183 days was reported to produce a reduction in spleen size but an increase in leukocyte number of thrombocytes (32).

Spleen of *Tilapia zillii* fish inhabiting the water of lake Qarun suffered from hemorrhage, hemolysis, hemosiderin, necrosis and degeneration in spleen

tissues (14). These alterations may be due to viral and parasitic microbes and trace metals which were accumulated in the spleen from polluted water. Also the hemosiderin formation (accumulation) may be attributed to the increase in iron content as reported by (33).

Gonads: different stages of spermatogenesis in the end stage of spermatozoa, epididymus like tube in which all 3 stages were found. ovary showed numerous fat droplet in ova, degenerative ova, perinucleolar oocyte, secondary ova with beginning of degeneration, varying stages of development of ova, vacuolation of secondary germinal layer.

Heavy metal toxicity in natural water is the major source of contamination which have adverse effects on the hypothalamic –pituitary –gonadal (HPG) relationship of fish and disturbs the aquatic biodiversity which is responsible for maintaining and supporting overall environmental health. Heavy metals pollution cause greater loss to advance stages of oogenesis (34). (35) found the groups were exposed to higher concentrations of lead acetate showed increased number of atretic follicles. These studies suggest a direct action of heavy metals on the gonads.

Increasing the concentration of Cd and Pb pollution causing severe testicular atrophy with arrested spermatogenesis, necrotic spermatogenic cells, and vacuolization in the interstitial tissue (36). Permanent testicular damage resulted from increasing degree levels of heavy metal accumulation (37).

Conclusion: histopathological alterations in fresh water fish under the influence of heavy metals can be used as a sensitive model to monitor the

aquatic pollution. the current result indicates that the heavy metal contamination definitely affect different fish organs. Hence a scientific method of detoxication is essential to improve the health of these economic fish.

References:

- 1-Van Welie, R., Van Dijk, R., Vermeulen, N., Van Sittert, N. 1992. Mercapturic acids, protein adducts and DNA adducts as biomarkers of electrophilic chemical. Crit. Rev. Toxicol. 22:271-306.
- 2-Desphande, A., Zade, S. and Sitre, S. 2011. Histopathological changes in the gills architecture of *Labeo rohita* from the pond adjacent to thermal power station, Koradi, Nagpur, India. Journal of Applied and Natural Science 3(2):284-286.
- 3-Bose, M., Ilavazhahan, M., Tamilselvi, R. and Viswanathan, M. 2013. Effect of heavy metals on the histopathology of gills and brain of fresh water fish *Catla catla*. Biomedical & pharmacology journal. vol. 6(1), 99-105.
- 4- Oguzie, F. 2003. Heavy Metals in Water and Sediments of the Lower Ikpoba River, Benin City, Nigeria. Pak. J. Sci. Ind. Res. Vol. 46(3) pp.156-160.
- 5- Monteiro, S., Mancera, J.; Fontainhas Fernandes, A. and Sousa, M. 2005. Copper induced alterations of biochemical parameter in the gill and plasma of *Oreochromis niloticus*. Comp. Biochem. Physiol. C. 141, 375-383.
- 6- Deore, S. & Wagh, S. 2012. Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham). Journal of Experimental Sciences 2012, 3(3): 35-38.
- 7- Meteleev, V.; Kanev, A. and Dzsoka Hova, N. 1994. "Water Toxicology.", 2nd edition. CABI publishing Canada.

- 8- Sexana. M, Sexana. H. 2007. Histopathological changes in lymphoid organs of fish after exposure to water polluted with heavy metals .the internet journal of veterinary medicine .2007 vol.5.number 1.
- 9- Gledhill M., Nimmo M, Hill S., and Brown M. 1997. *Journal of Phycology*, 33, 2-11.
- 10- Daoud J., Amin, A and Abd El-Khalek, M. 1999. Residual analysis of some heavy metals in water and *Oreochromis niloticus* fish from polluted areas. *Vet. Med. J.*, 74: 351-365.
- 11- Act on maintenance of rivers and water pollution sheet of No. 25 of 1967.
- 12- World Health Organization (WHO). 2003. Guidelines for drinking water quality .WHO, Geneva, 249pp.
- 13- Majdy, F. .2015. Assessment Some of Biochemical, Cytogenetic and Histological Biomarkers In Fish, gibel carp, *Carassius gibelio* As Early Detection of Environmental Pollution Risk In Shatt Al-Arab River-Basrah-Iraq. *Iraqi J. Aquacult.* .In press.
- 14- Tayel, I.; Seham A. Ibrahim and Soaad A. Mahmoud 2013. Histopathological and muscle composition studies on *Tilapia zillii* in relation to water quality of Lake Qarun, Egypt. *Journal of Applied Sciences Research*, 9(6): 3857-3872.
- 15- Ahmed, N., 2007. Effect of River Nile pollution on *Clarias gariepinus* located between El-Kanater El-Khayria and Helwan. M.Sc. Thesis, Faculty of Agriculture, Zagazig Univ.
- 16- Mahmoud, S., 2002. Evaluation of toxicity effect of some pollutants on histological feature and biochemical composition of *Oreochromis niloticus* L. living in River Nile (Damietta branch) Ph.D. Thesis Fac. Sci. Zagazig Univ. Egypt.
- 17- Tayel, S., 2003. Histopathological, biochemical and hematological studies on *Tilapia zillii* and *Clarias gariepinus* in relation to water quality criteria at different localities in Delta Barrage. Ph. D. Thesis, Fac. Sci., Benha branch, Zagazig Univ.
- 18- Mahmoud, S. and El-Naggar, A. 2007. Alterations in *Clarias gariepinus* caused by pollutants at El-Rahawy area, Rosetta branch, River Nile, Egypt. *J. Egypt. Acad. Environ., Develop.*, 8(2): 61-70.
- 19- Abou El-Gheit, E., Abdo, M and Mahmoud, S. 2012. Impacts of Blooming Phenomenon on Water Quality and Fishes in Qarun Lake, Egypt *International Journal of Environmental Science and Engineering (IJESE)*, 3: 11- 24.
- 20- Fernandes, N. and Mazon, A. 2003. Environmental pollution and fish gill morphology. In: val, A.L. and B.G. Kapoor (Eds). *Fish adaptation*. Enfield, Science Publishers, pp: 203-231.
- 21- Hanan S. 2007. Impact of certain heavy metals on the gill and liver of the Nile tilapia (*Oreochromis niloticus*) *Egypt. J. Aquat. Biol. & Fish.*, Vol .11, No. 2: 79-100.
- 22- Salim, F., Kadhim, S., 2014. Survey on histopathological changes in different organs of local fresh water in Basra Province . *Journal of International Academic Research For Multidisciplinary* . 2: 10.
- 23- Rougier F, Troutaud D, Ndoye A and Deschaux P. 1994. Non –specific immune response of zebrafish, *Branchyodanio rerio* (Hamilton – Buchanan) following copper and zinc exposure. *Fish Shellfish immunol.* 4: 115.
- 24- Soufy, H., Soliman, M., El-Manakhly, E. and Gaafa, A. (2007). Some biochemical and pathological investigations on monosex *Tilapia* following chronic exposure to carbofuran pesticides. *Global Veterinaria*, 1: 45-5

- 25- Gingerich, W. (1982). Hepatic Toxicology of Fishes. In: Weber, L.J. Ed., Aquatic Toxicology, Raven Press, New York, 55-105.
- 26- Mohamed, F. (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*. J. Egypt. Acad. Soc. Environ. Develop., 2: 25-42.
- 27- Authman, M. and Abbas, H. (2007). Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of Lake Qarun, Fayoum Province, Egypt. Pakistan J. Bio. Sci., 10: 2106-2122.
- 28- Jeantet, A, Y.; Ballan-Du Francais, C. and Anglo, A. (1997). Pollution par les métaux et atteintes cytologiques chez les bivalves marins. In Biomarqueurs en ecotoxicologie. Aspects fondamentaux. Lagdic, Caquet, T., Amiard, J. & Ramade, F. (eds.) . Paris: Masson: pp.315-353.
- 29- El-Naggar, A., Mahmoud, S. and Tayel, S. 2009. Bioaccumulation of some heavy metals and histopathological alterations in liver of *Oreochromis niloticus* in relation to water quality at different localities along the river Nile ,Egypt. World J. Fish & Marine Sci., 1(2): 105-114.
- 30- Begum. A, Mustafai, A, Nurul Amini, MD., Nasrin and Chowdhury, T. 2013. Accumulation and histopathological effects of Arsenic in tissues of Shingi fish (Stinging Catfish) *Heteropneustes Fossilis* (BLOCH, 1794) J. Asiat. Soc. Bangladesh, Sci. 39(2): 221-230, June 2013.
- 31- Deb, S., Fukushima, T. 1999. Metal in aquatic ecosystems : mechanisms of uptake accumulation and release. Int. J. Environ. Stud. 56 (3), 385.
- 32- Robohm, J. 1986. Paradoxical effect of cadmium exposure on antibacterial antibody responses in two fish species : inhibition in cunners (*Tautoglabrus adspersus*) and enhancement in striped bass (*Morone saxatilis*). Vet Immunol Immunopathol. 12:251.
- 33- Haggag, A., Marie, M. Zaghloul, K. and Eissa, S. 1993. Treatment of underground water for fish culture in Abbassa Farm, Sharkia. Bull. Fac. Sci., Cairo Univ., 61: 43-69.
- 34- Raksha modi & Sharma. 2012. Histopathological study of effect of heavy metal pollutant (CuSO₄) on neurohypophyseal complex and spawning period of female *Anabas Testudineus* . Bionano Frontier .vol.5(2-1).
- 35- Doaa, M. and Hanan, H. 2013. Histological Changes in Selected Organs of *Oreochromis niloticus* Exposed to Doses of Lead Acetate. J. Life Sci. Biomed. 3(3): 256-263.
- 36- Koizumi T, Li ZG .1992. Role of oxidative stress in single-dose, cadmium induced testicular cancer. J. Toxicol Environ Health 37: 25-36.
- 37- Hanna, M.; Shaheed, L. and Elias, N. 2008. A contribution on chromium and lead toxicity in cultured *Oreochromis niloticus*. Egypt. J. Aquat. Biol. Fish., 9: 177-209.