Histopathological study of black molly *Poecilia latipinna* exposed to Diazinon

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Abstract

Black molly fishes exposed to (0.006, 0.012, 0.018 mg/l) concentrations of diazinon for 45 days. Liver, kidney and gills were examined for histopathological study after exposed periods. Swelling of hepatocytes, necrosis, arteriosclerosis, hyalinization, and Cirrhosis were observed in the liver tissue. Tissue in kidney suffered from degeneration and vaculation, hyperplasia in tubules, hyalinization of haemopoietic tissue, lifting of epithelial tissues, and atrophy of collecting tubules. Hypertrophy and hyperplasia of epithelial cells, fusion of secondary lamellae and oedema were found in exposed gills.

Introduction

Pesticides drained indirectly toward the aquatic ecosystem, that will effect on organisms found in water mainly on fish . Pesticides are useful tools in agriculture, but their contribution to gradual degradation of the aquatic ecosystem cannot be ignored (Konar 1975 ; Basak and Konar, 1977) . Due to the residual effect of pesticides, important organs such as liver, gill and kidney will be damage. Diazinon is highly toxic to fish (EXTOXNET, 1996). Tissue organisms exposed to sub-lethal concentrations of toxicants are functional response of organisms which provides information on the nature of toxicant (Das and Mokherjee, 2000). Wide varieties of insecticides and other toxins tend to accumulate in high concentrations with it (Meteleve *et al.*, 1971). Absorbed toxicant transported by blood circulation to liver for transformation and / or storage, and if it transformed in the liver it may be excreted through the bile or pass back into blood for possible excretion by kidney or gill (Lindstoma – seppa *et al*.., 1981).Well – documented lesions based on experimental data in liver, ovary, skeletal system and skin has been used as biomarkers so far (Hinton *et al*., 1990).

Histological changes associated with pesticides in fish have been studied by many authors (Das &Mukherjee, 2000; Macelo *et al*., 2002; Fanta *et al*., 2003; Velmurugan *et al*., 2007).Black molly fish *Poecilia latipinna* selected for this study because it is small, available and suitable for histopathological studies on the cellular level.

Material and methods

Test animals

Fishes obtained from local market (Alghazel market), healthy fish were collected and brought to the laboratory for acclimatization in aquariums for a period of two weeks (mean length 4+0.5 cm).

Test containers

Aquariums of 100 L capacity used for acclimatization. Fishes divided in to four groups depending on exposure concentrations put in aquariums (3 L capacity), each one has 8 fishes, and each group has three sets.

Chronic exposure studies

Three sublethal concentrations (0.006, 0.012, 0.018 mg/l) were used for exposure study as well as control group. Fish were fed by commercial food for tropical fishes once a day. Water exchange every couple of days intervals with fresh test solutions in

each experimental aquarium with well aerated. After 45 days test fish were killed for histopathological study.

Histopathology

Microscopic preparations were done according to (Bancroft and steven,1982).organ tissues fixed in 10% formalin, were prepared for paraffin blocks (58-60c^{o}) and sectioned at 6Mm.Stained sections examined with Olympus compound binocular microscope (LH)fitted with photomicrographic attachment.

Results and Discussion

Histopathological changes are important to identify the environmental effects of chemicals especially pesticides. One of the advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs including gills, kidney and liver that are responsible for vital infections, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish(Gernhofer *et al*.,2001).Furthermore the alternations found in these organs are normally easier to identify than functional ones(Fanta et al.,2003),and sever as warning signs of damage to animal health (Hinton &lauren,1990)The recent study shows the effect of Diazinon on liver ,kidney and gills of Black Molly fish .The alternations which found in these organs used as biomarkers .Liver in this fish consist of hepatocytes regulate as two adjacent lines the spaces between the hepatocytes called sinusoids which were raisedfrom central vein. The liver covered with capsule. Swelling of hepatocytes was observed approximately in all exposed livers fig (1) as a result of fatty degeneration, this case occurred due to catabolism process which caused by fatty infiltration and accumulate as abnormal forms in the hepatocytes (Metcalf, 1998).The cellular injury may be reversible if not too severe , impaired lipid metabolism lead to intracellular accumulation of fat in the form of Triglyceride droplets so called vacuoles (Wheater *et al* ., 1985).Liver one of the organs most affected by contaminants (Van der Oost *et al*., 2003) .The vacuoles in the cytoplasm of the hepatocytes can contain lipid and glycogen ,which are related to the normal metabolic function of the liver .Depletion of the glycogen in the hepatocytes is usually found in stressed animals(Wilhelm Filho *et al*., 2001), because the glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations(Panepucci *et al* ., 2001) and suggests metabolic damage ,possibly related to exposure to contaminated water.

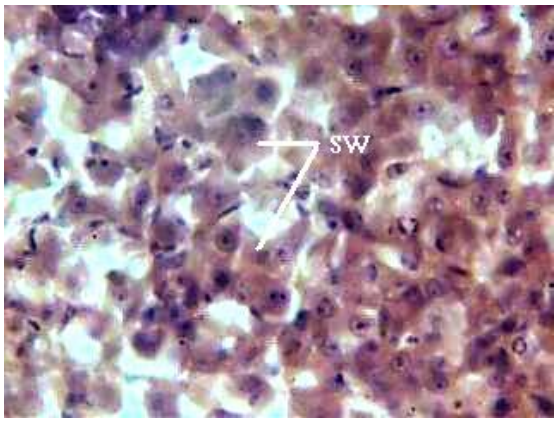

Fig 1: Showed swelling of hepatocytes(SW).

(0.006mg/l).400x.

Diazinon 0.012mg/l caused infiltration of white blood cells in liver tissue fig (2),also nuclear necrosis (Pyknotic,karyohexis and karyolysis) clearly observed in liver fig (3). At the end of these cases and due to long term exposure for diazinon,some cells were died fig (4). The dead cells have a homogenous eosinophilic cytoplasm when compare with living cells. this cytoplasmic appearance is due to loss of basophilic RNA from the damaged rough endoplasmic reticulum , disorganization of mitochondria and exposure of increase numbers of acidophilic groups from breakdown of structural protein , the nuclei of the dead cells are smaller ,more darkly stained and less well defined which is known as pyknosis, is the end result of changes in which the nuclear chromatin becomes progressively clumped, possibly due to the low pH from anaerobic metabolism.(Wheater *et al* ., 1985).The large vacuoles in the hepatocytes ,with pyknotic nuclei and necrosis of hepatocytes with enlarged sinusoid similar with (Olurin *et al* .,2006).

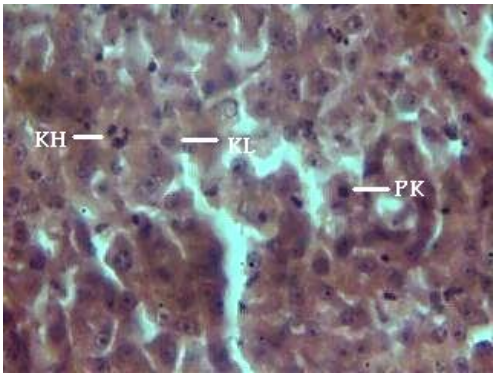

Fig 2: Showed infiltration of white blood Cells (IF).(0.012mg/l).400x.

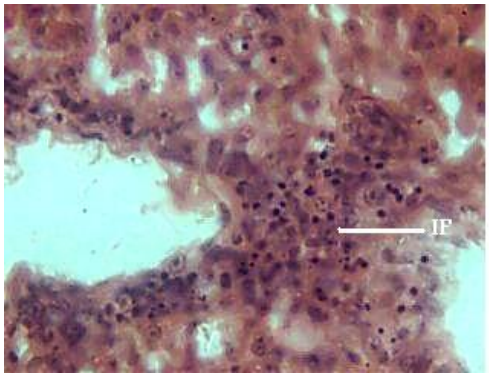

Fig 3: Showed stages of nuclear necrosis Pyknosis (PK),Karyohexis (KH), Karyolysis (KL).(0.012mg/l).400x.

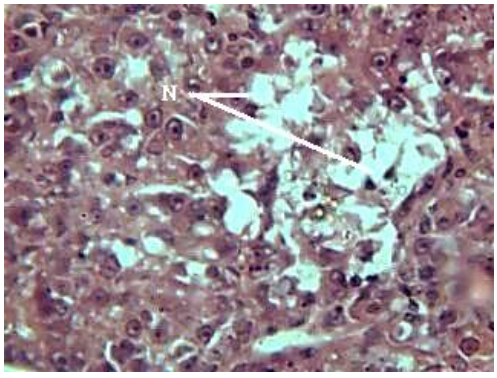

Fig 4: Showed necrosis in liver tissue (N). (0.012mg/l).400x.

Severe lesions observed in parenchyma liver as hard inflammations such as giant cells fig (5) and cirrhosis ,the high proportion of fibrotic tissue within the lobules fig (6),Arteriosclerosis fig (7),Hyalinization fig (8) and high degrees of necrosis fig (9

).All these alternation observed in liver exposed to Diazinon 0.018mg/l. The normal cords of liver cells replaced by numerous , bizarre multinucleated giant cells and the cytoplasm of these giant cells is vacuolated and often filled with bile pigment , these giant cells are an expression of faulty regeneration or fusion of cells in the periportal fields is very prominent in this phase of the disease (Sandritter and Thomas , 1977) .

Histopathological changes in the liver cause metabolic problems as well , evidence for this is the bile stagnation in liver of most fish studied .This lesion ,characterized by the remains of the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes (Pacheco&Santos,2002),indicates that the bile is not being released from the liver .The accumulation of bile indicates possible damage to the hepatic metabolism(Fanta *et al* ., 2003) .

The amyloid which is a pathological protein is deposited in the space between the walls of the sinusoid and the liver cells. at first only a narrow strip of homogenous red material, if the deposits are marked the ma cause pressure atrophy of the liver cords which may disappear completely , the lumens of the sinusoids may be greatly reduced as consequence of the infiltration (Sandritter and Thomas, 1977).

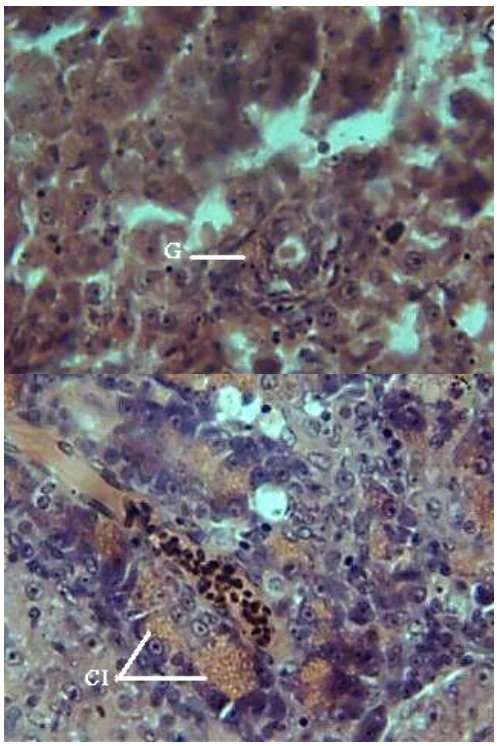

Fig 5: Showed Giant cells (G). (0.018mg/l).400x.

Fig 6: Showed Cirrhosis (CI) in liver tissue.(0.018mg/l).400x.

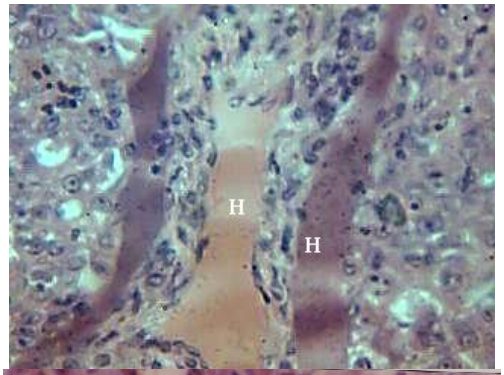

Fig 7: Showed Hyalinization (H).in liver Tissue.(0.018mg/l).400x.

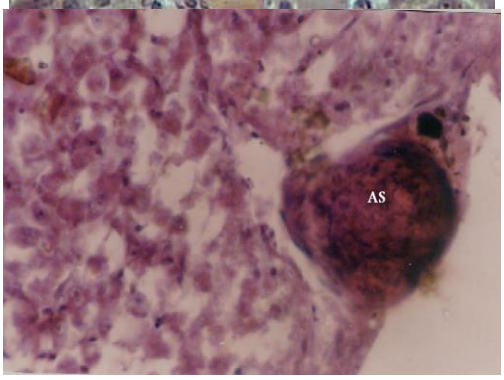

Fig 8: Showed Arteriosclerosis (AS). (0.018mg/l).400x.

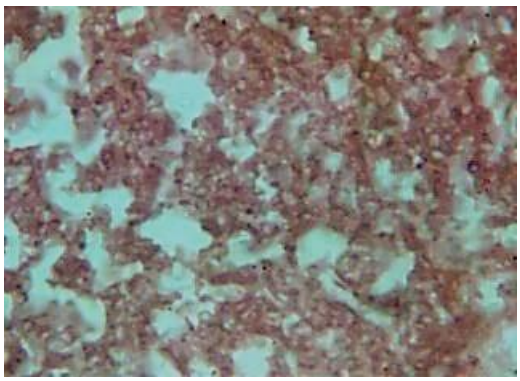

Fig 9: Showed sever necrosis in liver Exposed by diazinon 0.018mg/l.400x

Kidney in fish consist of cortex and medulla ,cortex has nephrons (Glomerulus and Bowman's capsule),whereas medulla has collecting tubules. Kidney of *Poecilia latipinna* the diazinon caused degeneration and vaculation in tubular cells particularly in proximal tubules in fish exposed to 0.0006mg/L Diazinon fig (10).

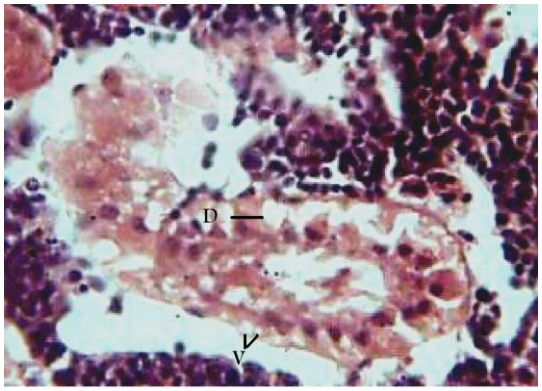

Fig 10: Showed Degeneration (D) and Vaculation (V) in the epithelial tubules. (0.006mg/l).400x.

While Diazinon 0.012mg/l caused hypertrophy of the epithelial cells in tubules fig (11) with hyalinization and hemorrhage in particular capillaries fig (12), also hyperplasia in some tubules were found fig (13) .

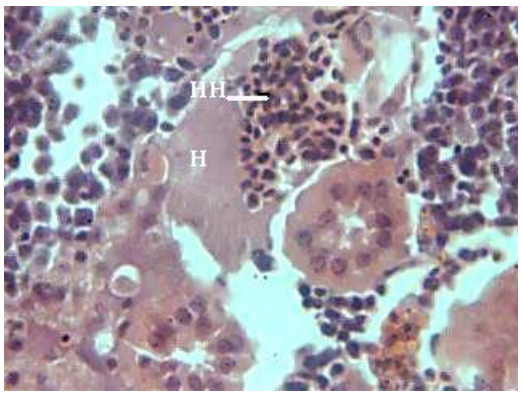

Fig 11: Showed Hyalinization (H) and Hemorrhage (HH) in haemopoetic Tissue. (0.012mgl).400x.

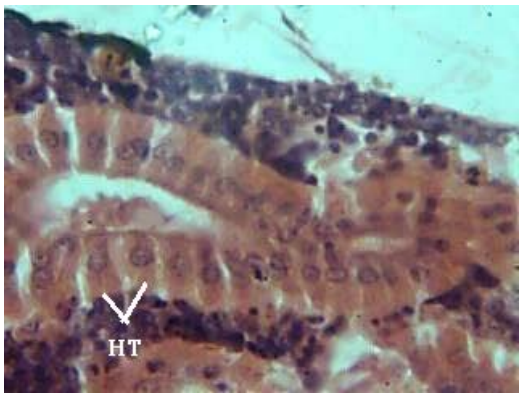

Fig 12: Showed Hypertrophied tubules epithelial (HT).)(0.012mgl).400x.

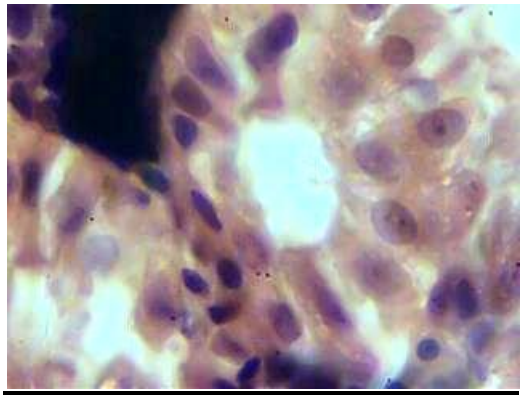

Fig 13: Showed Hyperplasia in epithelial Tubules.(0.012mg/l).1000x.

Severe lesions such as lifting of the tubular cells fig (14),and atrophied epithelial cells and oedema were observed in kidney tissue with Diazinon 0.028mg/L fig (15).

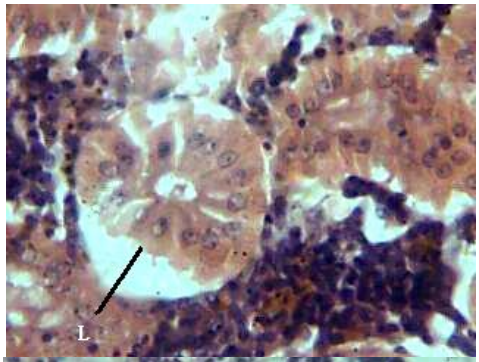

Fig 14: Showed lifting of the epithelia Cells (L).(0.018mg/l).400x.

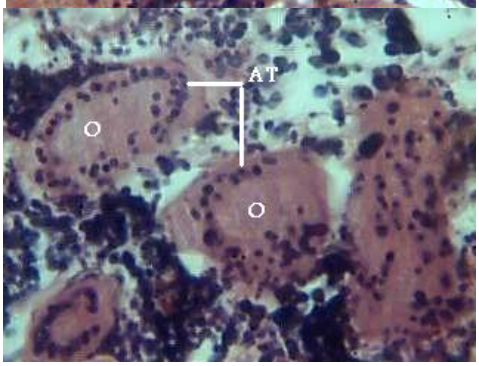

Fig 15: Showed Atrophied epithelial cells (AT) with Oedema (O). (0.018mg/l).400x.

Most common alterations found in the kidney of fished exposed to the contaminated water are tubular degeneration (cloudy swelling and hyaline droplets) and changes in corpuscles, such as dilation of capillaries in the glomerulus and reduction of Bowman's space. (Thophon *et al*., 2003) .This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm, this initial stage in the degeneration process can progress to hyaline degeneration ,characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Takashima & Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis. The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure.

Gills consist of primary lamellae each one consist of secondary lamellae arranged parallel. All primary and secondary lamellae covered by one thin layer of epithelial cells.Chlodide cells located between each secondary lamellae.Diazinon 0.006mg/L caused hypertrophy of chloride cells fig (16).While Diazinon 0.012mg/L effect in gills as Hyperplasia of epithelial cells and fusion of secondary lamellae fig (17).After long term exposure of Diazinon 0.018mg/L left many disorders such as lifting of epithelial cells from lamellae with oedema fig (18).

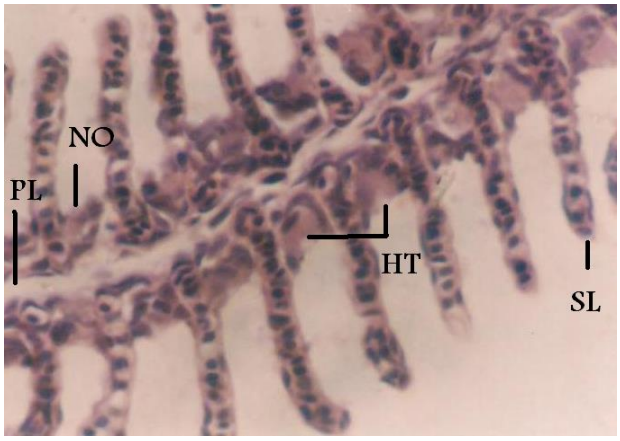

Fig 16; Showed Hypertrophy of chloride cells (HT).
(PL) Primary lamella,(SL) Secondary lamella,
(NO) Normal chloride cell.(0.006mg/l).400x.

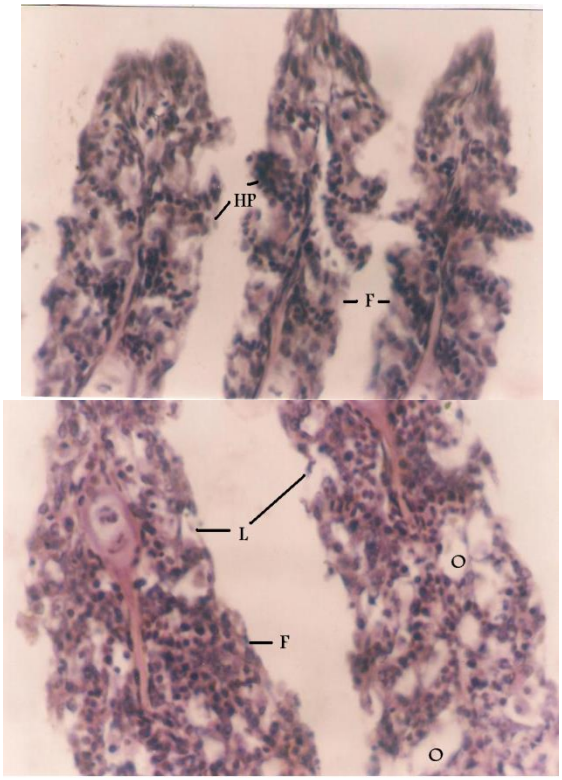

Fig 17: Showed Hyperplasia of epithelial cells (L)
Cells (HP) and Fusion of secondary secondary
Lamellae (F).(0.012mg/l).400x

Fig 18: Showed Lifting of epithelial with Oedema (O).Fusion of lamellae.(0.018mg/l).400x.

The gills, which participate in many important functions in fish ,such as respiration, osmoregulation, and excretion ,remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants.

The main histological changes of the gills in this study included hypertrophy of chloride cells ,lifting of epithelium, oedema , fusion of secondary lamellae ,and hyperplasia of epithelium , these result similar with(Cengiz &Unlu 2002) (Olurin *et al* .,2006)

The reason is that gills are very important absorption place for the toxic compounds. Epithelial necrosis and rupture of gills epithelium are direct deleterious effects of the irritants .The fish defense responses are excessive mucus secretion. Lifting the epithelium, lamellar fusion, could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta, 1989). The histopathological changes of gill result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi *et al*., 1996).

Alteration like epithelial lifting, hyperplasia, hypertrophy of the epithelial cell, fusion of some secondary lamellae are examples of defense mechanisms, in general, these result increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants.(Fernandes&Mazon,2003) These alterations were most common found in the gills of fish caged (Coutinho&Gokhale,2000) .As a consequence of the increased distance between water and blood due to epithelial lifting ,the oxygen uptake is impaired .However ,fishes have the capacity to increase their ventilation rate, compensate low oxygen uptake(Fernandes&Mazon,2003).

Winkaler et al.,2001 mention there is a hypothesis around hyperplasia and hypertrophy is result of contaminated and exposure water make structural damage to the fish gill. In the other hand mention the hyperplasia in some situation represents adaptations by the organism to protect underlying tissues from any irritant (Meissner &Diamandopoulous,1977) and fusion of secondary lamellae as a result of hyperplasia would not only decrease the surface area available for oxygen extraction, but also would increase the oxygen diffusion distance between water and blood (Skidmore andTovell ,1972),while hyperplasia may indeed be having a protective function it may also inhibit the respiratory, secretory and excretory functions of the gills. . This study present oedema in the lamellae, there were apparent lamellae oedematous changes probably due to increase capillary permeability (Roberts, 1980).When severe this could lead to difficulty in respiration and osmoregulation stress (Skidmore and Tovell, 1972)

References

Alazemi, BM., Lewis ,JW., and Anderws , EB (1996) Gill damage in the freshwater fish *Gnathonemus ptersii* (Family: Mormyridae) exposed to selected pollutants: an ultrastructural study .Environ .Technol **17** :225-238

Bancroft, J & Stevens, A. (1982). Theory & practice of histological technique. (2nd Ed). Churchill Livingston, London.

Basak,P,K and Konar,S,K.(1977). Estimation of save concentration of insecticides , a new method tested on DDT and BHC .J.Inl.Fish.Soc.,India **9:**9-29.

Cengiz, E .I and Unlu, E (2002) Histopathological changes in the gills of Mosquitofish, *Gambusia affinis* exposed to endosulfan .Bull Environ. Contan . Toxicol. **68** :290-296

Coutinho , C. and Gokhale, K.S . (2000) .Selected oxidative enzymes and histopathological changes in the gills of *Cyprinus carpio* and *Oreochromis mossambicus* in secondary sewage effluent. Water Research**, 34**:2997-3004

Das B K &Mukherjee S Ch.(2000). A histological study of carp *Labio rohita* exposed to hexachlorocyclohexane ,VETERINARSKI ARHIV **70**(4):169-180.

Fanta ,E.,Rios,F.S.,Romao,S.,Vianna,A.C.C.and Freiberger,S.(2003).Histopathology of the fish *paleatus* contaminated with sublethal levels of organophosphorus in water and food .Ecotoxicology and Environmental Safely, **54**:119-130

Fernandes ,M. N. and Mazon,A.F.(2003).Environmental pollution and fish gill morphology .In :Van ,A.L.&B.G.Kapoor(eds).Fish adaptation. Enfield, Science Publishers,203-231Gernhofer ,M;M.Powet;M.Schramm;E.Muller &R.Triebskorn.(2001).Ultrastructural biomarkers as tools to characterize the health status of fish in streams contaminated.Journal of Aquatic Ecosystem,Stress and Recovery,**8**:241-260.

Hinton,D.E. and Lauren,D.J.(1990).Liver structural alterations accompanying chronic

Konar,S,K.(1975). Pesticides and aquatic ecosystems.India.J.Fish **22**(1 and 2): 80-85. Lindstoma-Seppa,P;V,Koivussri;D,O,Hanninen.(1981).Extrahepatic xenobiotic metabolism in north European freshwater fish. Comp. Biochem. Physiol.69:291.

Macelo L ; Rodreguez E ;Pacheco F J and Ranzani-Piva M J T.(2002) Histopathologic changes in the kidney tissue of *Prochilodus lineatus* Vaenciennes,1836 (Characiformes,Prochilodontidae) Induced by sublethal concentration of Trichlorfon Exposure.—.Brazilian Archives of Biology and Technology. **45**:171-175.

Meissner,M,A and Diamandopoulos,G,Th.1977.Neoplasia. In. Pathology (WAD Anderson,J M Kissane,Eds),**1**:640-691.

Meletev,V,V;A,S Kanaev and N,G,Dzasokhova.1971.Water toxicology.American Publishing Co.Pvt.Ltd.216.

Metkalf,C,D.(1998). Toxopathic responses to organic compounds.From Fish Diseases and Disorder Vol **2**:Non infections disorders Edited by J F Leather land and P T K Woo University of Guelph,Canda,p 133-162.

Olurin ,K.B.,Olojo,E.A.A.,Mbaka,G.O. and Akindele,A.T.(2006).Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. J of Biotechnology .**5**:(24) 2480-2487.

Pacheco,M.& M,A,Santos.2002.Biotransformation,genotoxic and histopathological effects of environmental contaminations in Europian eel *Anguilla anguila* L.Ecotoxicoloy and Environmental Safety,**53**:331-347.

Panepucci,R.A;L.Panepucci;M.NFernandes;J.R.Sanchez & F.T Rantin.2001. The effect of hypoxia and recuperation on carbohydrate metabolism in pacu *Piaractus mesopotamicus* Brazilian Journal of Biology,**61:**547-554.

Richmonds, C. and Dutta, HM (1989). Histopathological changes induced by malathion in the gills of bluegill *Lepomis macrochirus* .Bull.Environ.Contam.Toxicol.**45**:123-130

Roberts,RJ.(1980).Fish Pathlogy . Cassell Books Ltd. (cited by Olurin et al.,2006)Sandritter ,W and Thomas,C.(1977).Color Atlas and Textbook of Histopathology.(6th ed).William,B.Wartman,M.D.by Year Book Medical Publishers,INC.Chicago,London.p 375.

Skidmore,JF and Tovell,PWA.(1972).Toxic effects of zinc sulphate on the gills of rainbow trout.Water Res.6:217-230.(cited by Olurin et al.,2006).

Takashima F and T Hibya.1995.An atlas of fish histology :normal and pathological features ,2nd ed.Tokyo,Kodansha.

Thophon,S;M.Kruatrachue;E.S.Upathan;P.Pokethitiyook;S.Sahaphong;S.Jarikhua n.2003.Histo logical alterations of white seabass Lates calcarifer in acute and subchronic cadmium exposure .Environmental Pollution,**121**:307-320 toxicity in fishes:potentioal biomarkers of exposure.pp 51-65 .In

McCarthy,J.F.&L.R.Shugart(eds). Biomarkers of Environmental Contamination.Boca. Raton,Lewis publishers.(cited by Marina,et al., 2007)

Van der ,Oost,R;Beber,J and Vermeulen,N,P,E.(2003).Fish bioacummulation and biomarkers in environmental risk assessment:a review.Environmental,Toxicology and Pharmacology.**13:**57-149**.**

Velmurugan B;Selvanayagam M;Cengiz E I and Unlu E.(2007). The effect of monocrotophos to different tissue of fresh water fish Cirrhinus mrigala. Bulletin of environmental contamination and Toxicology.**78**(6)pp450-454.

Wheater,P.R.;Burkitt, H.G.; Stevens, A. and Lowe, J.S. (1985). Basic Histopathology. A colour Atlas and text. Foreword by Dawson, I.M., Churchill livingstone, Edinburgh, London Melbourne and NewYork.

Wilhelm Filho,D;Torres,M,A;Tribess,T,B;Pedrosa,R,C and Soares,C,H,L.(2001).Influence of season and pollution on the antioxidant defenses of the

Winkaler,E,U;A,G,Silva;H,C,Galindo & C,B,R,Martinez.(2001).Biomarcadores histologicos e fisiologicos para o monitoramento da saude de peixes ribeiroes de Londrina,Estado do Parana.Acta Scientiarum,**23**:507-514.