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Article

Effect of cypermethrin on the histoarchitecture of gills and liver of a freshwater catfish, *Pangasianodon hypophthalmus*

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Abstract: Histoarchitecture effect of cypermethrin was investigated in the gills and liver of freshwater catfish, *Pangasianodon hypophthalmus* (weight 60-70g) over an exposure period of 96 h as an endpoint of toxicity. The fish were exposed to five acute concentrations (0.00ml/L, 0.025ml/L, 0.050ml/L, 0.075ml/L and 0.10ml/L) and the 96 h LC_{50} was 0.075 ml/L. The physical reactions observed in the treated fish were erratic swimming, discolorations of the skin, loss of reflex, hyperactivities, surfacing, increasing opercula ventilation and these effects increased with increasing concentration of the toxicants and duration of exposure. The most common changes in gills histoarchitecture at all doses of cypermethrin were curl shape gill lamellae, bulged with the fusion of secondary gill lamellae, severe epithelial necrosis in gill lamellae and hypertrophy. The changes observed in the liver tissues were cloudy swelling, focal necrosis and hypertrophy of hepatocytes, degeneration of hepatocytes and cytoplasmic, extensive vacuolation of hepatocytes and pyknotic nuclei. The results of this histoarchitecture analysis of gills and liver tissues indicate a direct correlation between cypermethrin exposure and the histoarchitecture disorders.

Keywords: histoarchitecture; cypermethrin; *Pangasianodon hypophthalmus;* toxicity; LC_{50}

1. Introduction

The hazardous effect of pollutants has its impact on all living organisms. The indiscriminate use of pesticides in agriculture, animal husbandry and post-harvest technology is a threat to the natural water system, public health and welfare of mankind (Tilak *et al*., 2007). Pollution of water is any chemical, physical or biological changes in the quality of water that has a harmful effect on any living thing that drinks, uses or lives in (Lenntech, 1998). Different pesticides are used at ponds for treating fish parasitic diseases or applied to the agriculture lands are carried away by rains and floods as runoff to the water bodies and this alters the physico-chemical properties of water (Richards, 1988). Exposure of organisms to xenobiotics such pesticides, insecticides, herbicides and various kind of chemicals is a serious matter in environmental and toxicological chemistry. Aquatic environment contaminated through pesticides that show some altered behavioral patterns which may include avoidance, locomotive activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition (Gormley and Teather, 2003; Morgan *et al*., 1991). Xenobiotics usually contaminate water bodies, a number of researchers have found on the effects of different pesticides on aquatic organisms. Water quality parameters such as temperature, dissolved oxygen, pH, turbidity, alkalinity as well as conductivity are influenced by the rate of pollutants entering the water or lethal effects on the aquatic organisms (Fagbenro, 2002; Olufayo, 2009). Fish are often used as indicators of such biological impacts of pollutants as they respond to low concentrations of toxic substances (Ayas *et al.*, 2007). Alteration in the

histology of the tissue such as gill, liver, kidney or intestine that are directly related to the contaminants serve as important bio-monitoring tools or bio-markers to assess the toxicity (Thophona *et al.*, 2003).

Organophosphate pesticides have replaced the persistent chlorinated pesticide in the 1970s and at the early of 1980s the advantage of the organosphosphate pesticide was their low cumulative ability and short-term persistence in the environment; although the organophosphate pesticides have been replaced by pyrethroid based pesticides, there is still very intensive use of the organophosphate (Robert and Hutson, 1998). However, cypermethrin is a synthetic pyrethroid that commonly used for the control of ectoparasites which infest cattle, sheep, poultry and some companion animals. Presently, the cypermethrin has been widely used as a chemotherapeutic agent for the control of ectoparasite infestations in marine cage culture and freshwater aquaculture (Hart *et al*., 1997; Boxaspen and Holm, 2001; Roth *et al*., 1993; Treasurer *et al*., 2004). Fish toxicity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds. The half-lives for elimination of several pyrethroids by rainbow trout (*Oncorhynchus mykiss*) are all longer than 48 h, while elimination half-lives for birds and mammals range from 6 to 12 h (Bradbury and Coats, 1989). Considerable amount of literature is available on histopathological changes induced by organophosphorous, organochlorine and organocarbamate pesticides in fishes. Therefore, the present study reports histoarchitecture changes in the gills and liver as a result of exposing freshwater catfish, *Pangasianodon hypophthalmus* is to sublethal concentration of synthetic pyrethroids, cypermethrin.

2. Materials and Methods

2.1. Collection of experimental fish

The experiment was carried out in the Fish Diseases and Health Management Laboratory, Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Healthy *P. hypophthalmus* having a weight range of 60-70 were collected from Mymensingh local fish market. Collected fishes were washed with 0.1% of potassium permagnate ($KMnO_4$) solution to avoid dermal infection. Fishes were then rinsed in water and acclimatized to the laboratory conditions up to 14 days in 75 L capacity glass aquaria. During acclimatization period, fishes were fed with artificial pellet feed and water of the aquaria was also changed once daily. Feeding was stopped 24 h prior to the commencement of the toxicity test experiment.

2.2. Experimental procedure

The cypermethrin toxicity test was carried out in fifteen (15) glass aquaria and each aquarium contained 25 L of water where six healthy fish were introduced. The definitive toxicity test was carried out using; 0.00ml/L (control), 0.025ml/L, 0.050ml/L, 0.075ml/L and 0.10ml/L concentration of cypermethrin. Each of the toxicant concentration was replicated three times. The LC_{50} of cypermethrin on the test fish was determined using probit analysis. All data obtained in both tests were analyzed using probit method and the graphical method (Finney, 1971).

2.3. Histoarchitecture examination

After the 96 h experiment, gills and liver specimens were collected from fish alive and were preserved in 10% buffered formalin. Preserved tissues were washed under tap water for 24 h to remove formalin, dehydrated, clarified with xylene and embedded in paraffin blocks. They were cut at 4-5µ thickness by using Elisa microtome and stained routinely with haematoxylin and eosin (H&E) for histoarchitecture examination. Stained histoarchitecture sections were examined under trinoculare microscope. Histoarchitecture changes observed were photographed and interpreted in comparison to the work of previous.

3. Results

3.1. Fish behaviour and mortality

The physical reactions observed in the treated fish were erratic swimming, discolorations of the skin, loss of reflex, hyperactivities, surfacing, increasing opercula ventilation and these effects increased with increasing concentration of the toxicants and duration of exposure. However, the control group fish (only tap water) was found to be normal behavior during the experimental period. Increased the experimental duration, the treated fish showed increase in weakness, motionless and gasp for air with slow opercula movement. The 96 h LC_{50} value was found with 0.075 ml/L (Figure 1) but the lowest (10%) and highest (77%) mortality was observed in 0.025 and 0.10 ml/L, respectively in the experiment.

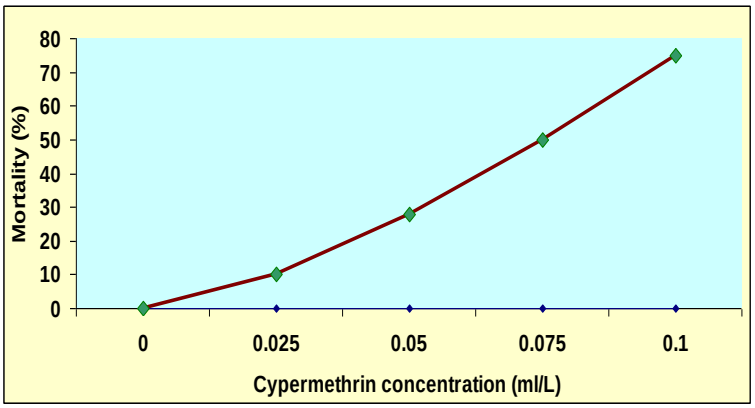

Figure 1. 96 h LC_{50} of freshwater catfish, *P. hypophthalmus* exposed to different concentrations of cypermethrin.

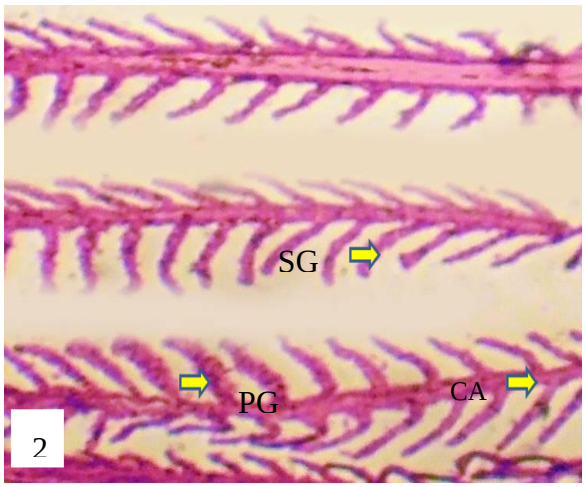

Figure 2. Gills of *P. hypophthalmus* in the control aquarium shows normal structure. Primary gill lamellae (PGL), secondary gill lamellae (SGL) and central axis (CA), H&E x 400.

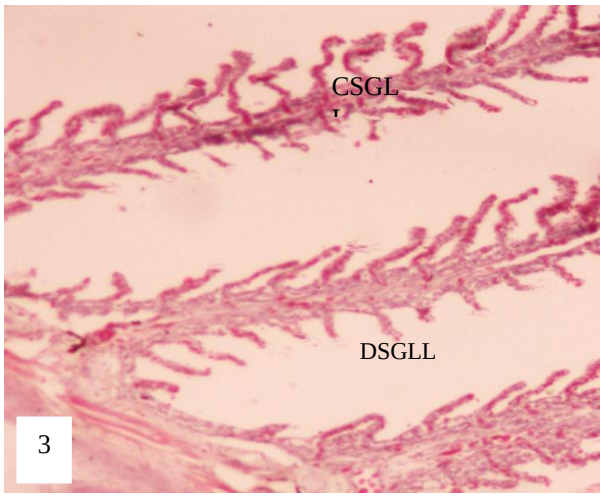

Figure 3. Gills of *P. hypophthalmus* exposed to 0.025 ml/L of cypermethrin shows curled shape gill lamellae (CSGL) and damages the secondary gill lamellae (DSGL), H&E x 400.

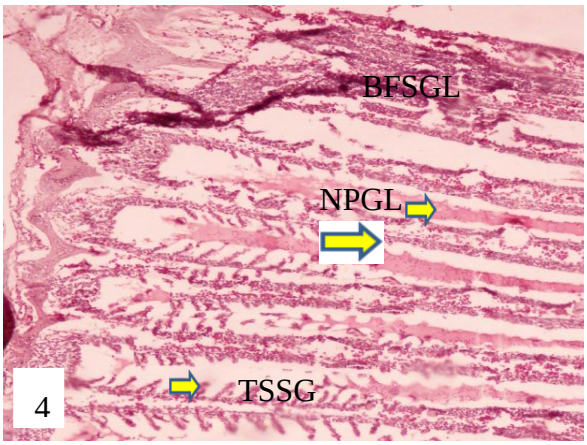

Figure 4. Gills of *P. hypophthalmus* exposed to 0.050 ml/L of cypermethrin shows bulging with fusion (BFSGL), thickening and shortening of secondary gill lamellae necrosis of primary gill lamellae (NPGL), H&E x 400.

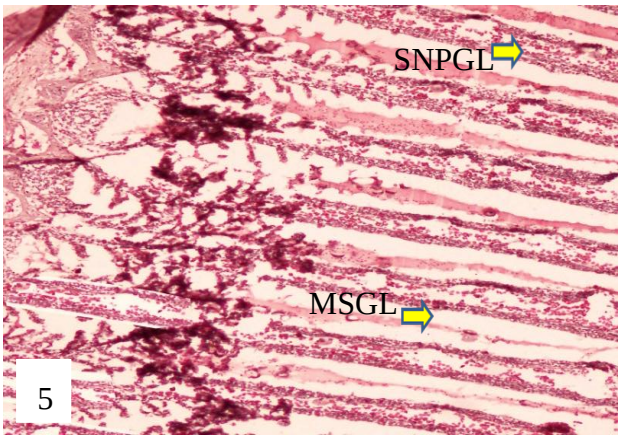

Figure 5. Gills of *P. hypophthalmus* exposed to 0.075 ml/L of cypermethrin shows severally necrosis in primary gill lamellae (SNGL) and almost missing the secondary gill lamellae (MSGL), H&E x 400.

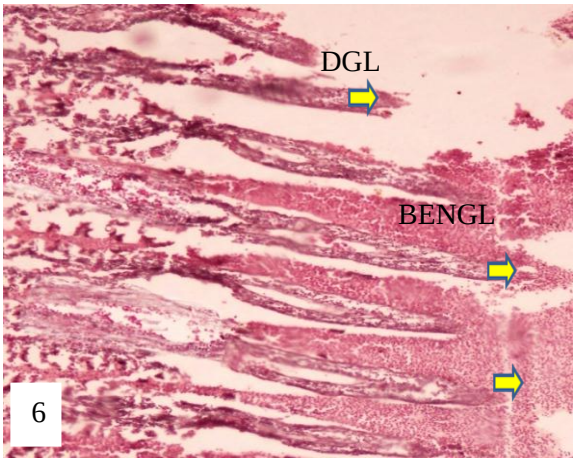

Figure 6. Gills of *P. hypophthalmus* exposed to 0.1 ml/L of cypermethrin shows bulged and severe epithelial necrosis in gill lamellae (BENGL) and degeneration of gill lamellae (DGL), H&E x 400.

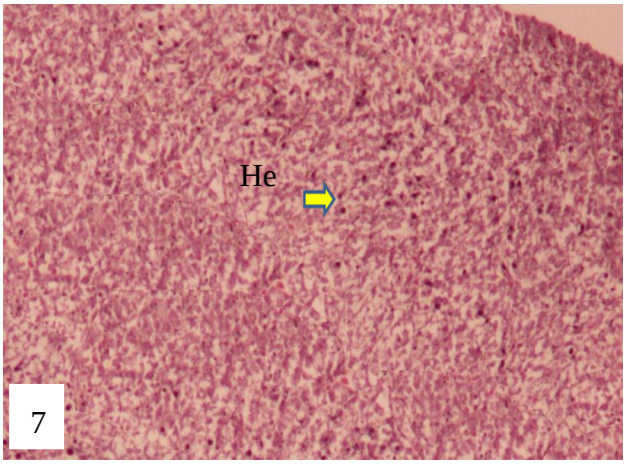

Figure 7. Liver of *P. hypophthalmus* in the control aquarium shows normal structure, Hepatocytes (He), H&E x 400.

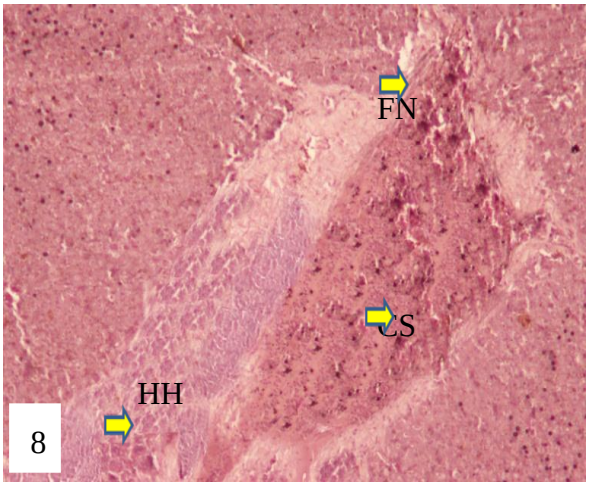

Figure 8. Liver of *P. hypophthalmus* exposed to 0.025 ml/L of cypermethrin shows cloudy swelling (CS), focal necrosis (FN) and hypertrophy of hepatocytes (HH), H&E x 400.

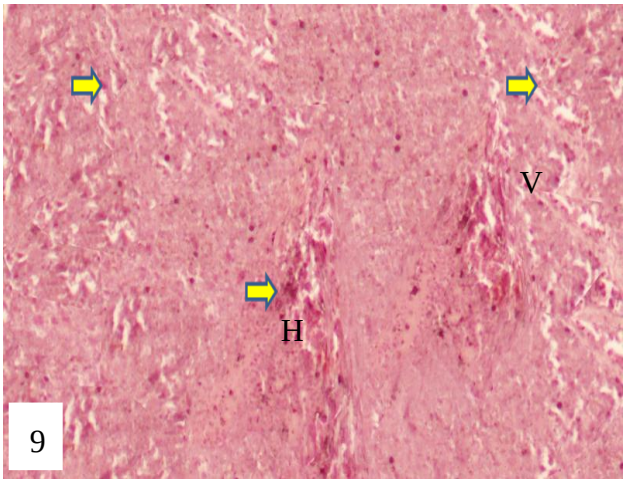

Figure 9. Liver of *P. hypophthalmus* exposed to 0.050 ml/L of cypermethrin shows severe diffuse vacuolation of hepatocytes (VH) and hemorrhages (H), H&E x 400.

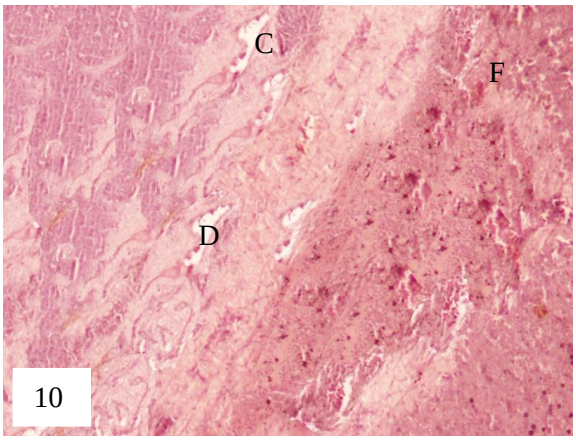

Figure 10. Liver of *P. hypophthalmus* exposed to 0.075 ml/L of cypermethrin shows degeneration of hepatocytes (DH) and cytoplasmic (CD), focal necrosis (FN) of hepatic tissue, H&E x 400.

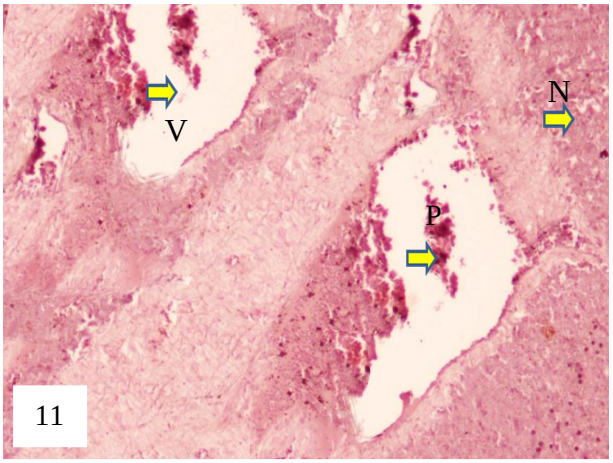

Figure 11. Liver of *P. hypophthalmus* exposed to 0.10 ml/L of cypermethrin shows extensive vacuolation of hepatocytes (VH), pyknotic nuclei (P) and necrosis (N), H&E x 400.

3.2. Effects of cypermethrin on the histoarchitecture of gills

The gill arches of *Pangasianodon hypophthalmus* in the control group fish (without using cypermethrin) showed almost normal structure. The arches contain primary gill lamella (Figure 2) projecting on the lateral sides of the primary and secondary lamella (respiratory lamella). However, this gill histoarchitecture showed almost normal without any alteration or damages. But, gill histoarchitecture examination of *P. hypophthalmus* was found significant indication of toxicity of cypermethrin (Figures 3 to 6). The observation of experimental fish that exposed to 0.025 ml/L of cypermethrin showed curl shaped gill lamellae (CSGL) and damaged in the secondary gill lamellae (DSGL) (Figure 3). The gills histoarchitecture changes were more prominent for using higher concentration of the cypermethrin. The gill epithelium bulged with the fusion of secondary gill lamellae (FSGL), necrosis in the primary gill lamellae (NPGL) and severe necrosis in the secondary gill lamellae (SNGL), those fish treated with 0.050 ml/L (Figure 4) and fish exposed to 0.075 ml/L severally necrosis in primary gill lamellae (SNGL) and almost missing the secondary gill lamellae (MSGL) (Figure 5) was noticed. In higher concentration 0.10 ml/L of cypermethrin, bulged and severe epithelial necrosis in gill lamellae (BEN), fully missing primary and secondary gill lamellae in most of the part of the gill samples (Figure 6).

3.3. Effect of cypermethrin on the histoarchitecture of liver

The liver of untreated fish was exhibited normal histoarchitecture that was characterized by polygonal shaped hepatocytes with granular cytoplasm and centrally placed round nuclei. Hepatocytes were arranged in well-organized hepatic cords and separated by narrow blood sinusoids (Figure 7). The degree of fish liver histoarchitecture changes was related to the increasing concentration of cypermethrin in the experimental fish. The changes of fish liver that treated with 0.025 ml/L of cypermethrin showed cloudy swelling (CS), focal necrosis (FC) and hypertrophy of hepatocytes (HH) (Figure 8). Liver of fishes exposed to 0.050 ml/L that resulted severe diffuse vacuolation of hepatocytes (VH) and hemorrhages (H) (Figure 9). The liver section of fish treated to 0.075 ml/L showed degeneration of hepatocytes (DH) and cytoplasmic (CD), focal necrosis (FN) of hepatic tissue (Figure 10). In higher concentration 0.10 ml/L, extensive vacuolation of hepatocytes (VH), pyknotic nuclei (P) and necrosis (N) in liver was found (Figure 11)

4. Discussion

Cypermethrin are commonly used in agriculture sector that continuously pollute the inland fishery water. Furthermore, it has been widely used as a chemotherapeutic agent for the control of ectoparasite infestations in marine cage culture of and freshwater aquaculture (Hart *et al*., 1997; Boxaspen and Holm, 2001; Treasurer *et al*., 2004). The present study revealed that the toxic effect of cypermethrin on the survival rate of the *P. hypophthalmus* was assessed by the LC_{50} value calculated as 0.075ml/L at 96 h exposure. This result indicated that the fish was unable to withstand the exposure of cypermethrin with time and thereby the toxicity of the insecticide was possible on long exposure. The stressful behavior such as erratic movement, loss of reflex, discoloration, changes in behavior and increased opercula ventilation, resting at the bottom of the treated fish due to the toxic effect of cypermethrin on the gills was similar with the findings of Omitoyin *et al.*, 2006 and Omoniyi *et al*., 2002. The changes in behavioral patterns exhibited by the treated fish were possibly to counteract aquatic hypoxia condition possibly due to the cypermethrin. When there is impossibility of escape from hypoxic stress, physiological alterations may be evoked to compensate for low oxygen supply (Graham and Iwama, 2003). The present findings were also similar with that of Jiraungkoorskul *et al*., (2003), where report that lamellar cell hyperplasia, lamellar fusion and epithelial lifting were observed in gill filaments of *Oreochromis niloticus* when exposed for 3 months to sublethal concentrations of the commercial glyphosate herbicide Roundup. On the gills of common carp, *Cyprinus carpio* L. exposed to 5.0 mg/l glyphosate concentration, epithelial hyperplasia and subepithelial edema were found by Neskovic *et al*., 1996. Furthermore, Edwards *et al.* (1986) reported that rainbow trout exposed to 10 µg/L cypermethrin exhibited toxin signs of bulged with the fusion of secondary gill lamellae, severe epithelial necrosis in gill lamellae and hypertrophy. Histological changes in the gills of fishes due to pesticides and other contaminates have been reported by several authors (Mallatt, 1985) as gill is the primary rout for entry of pesticides. However, these degenerated lamellar or severe damaged of the gills are thereby unable to absorb the dissolved oxygen from the water to diffuse through the membrane to the underlying capillaries.

The liver of the treated fish compared to the control showed cloudy swelling, focal necrosis and hypertrophy of hepatocytes, degeneration of hepatocytes and cytoplasmic, extensive vacuolation of hepatocytes and pyknotic nuclei; these alterations were dose-dependent. Furthermore, similar findings also have been reported in *Heteropneustes fossilis* exposed to cypermethrin (Joshi *et al*., 2007), in *Heterobranchus bidorsalis* exposed to

different doses of cypermethrin (Olufayo and Alade, 2012), in *Channa punctatus* exposed to hexavalent chromium (Ashish *et al*., 2008) and in *Clarias batrachus* exposed to 4 ppm and 8 ppm cadmium chloride for 90 days (Bilal *et al.*, 2011). However, Cengiz and Balci, (2001) also observed similar histological changes when different concentrations of thiodanR were applied to mosquito fish (*Gambusia affinis*).

Mandal and Kulshrestha, (1980) studied the effects of sublethal concentration of sumithion on liver, kidney and intestine of Magur (*Clarias batachus*). They found liver necrosis, vacuolization and breakdown of the cell boundaries. Couch (1975) revealed that perivascular lesions in liver of fishes exposed to organic contaminants and pesticides. According to Gingerich (1982) the vacuolization of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in hepatocytes. Changes such as hyperplasia, disintegration of hepatic mass and focal coagulative necrosis were found in Rhou (*Labeo rohita)* exposed to cypermethrin Jee *et al*., 2005. However, liver is the detoxification place of toxicants. The hepatic changes suggested mobilization of same kind of defensive mechanism in an endeavour to detoxify the toxicant cypermethrin.

5. Conclusions

The freshwater catfish, *P. hypophthalmus* exposed to various concentration of cypermethrin resulted that this synthetic pyrethroid was highly toxic to the *P. hypophthalmus* with lethal concentration (LC_{50}) of 0.075ml/L. The present histpathological investigation demonstrates a direct correlation between cypermethrin exposure and histoarchitecture changes observed in gills and liver. Therefore, cypermethrin which are used in aquaculture as treatment and disinfectant should be used very carefully.

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Conflict of interest

None to declare.

References

Ayas Z, G Ekmekci, M Ozmen and SV Yerli, 2007. Histopathological changes in the livers and kidneys of fish in Sariyer Reservoir. Turkey Environmental Toxicology and Pharmacology, 23:242-249.

Bilal A, 2011. Bioaccumulation and effect of cadmium chloride on certain organs of *Clarias batrachus*, Ph.D thesis, Barkatullah University Bhopal, India. pp. 1-130.

Boxaspen K and JC Holm, 2001. The development of pyrethrum-based treatments against the ectoparasitic salmon lice *Lepeophtheirus salmonis* in sea cage rearing of Atlantic salmon *Salmo salar L.* Aquaculture Research, 32:701–707.

Bradbury SP and JR Coats, 1989. Comparative toxicology of the pyrethroid insecticides. Bulletin of Environmental Contamination and Toxicology, 108:134–177.

Cengiz EI and K Balci, 2001. The histopathological effects of ThiodanR on the liver and Gut of mosquito fish, Gambusiaaffinis. Journal of Environmental Science Health, 1:75-85.

Edwards R, P Milburn and DH Huston, 1986. Comparative Toxicity of cypermethrin in rainbow trout, frog, mouse, and quail. Toxicol. Appl. Pharmacol., 84:512-522.

Fagbenro OA, 2002. Tilapia:Fish for Thought, Inaugural Lecture Series 32. Delivered at Federal Univ. Technol. Akure, P. 77.

Finney DJ, 1971. Probit Analysis. 3rd *Edn*. Cambidge Univ. Press, NY. P. 328.

Gingerich WH, 1982. Hepatic toxicology of fishes. In: Aquatic toxicology. (Eds.: L.J. Weber). H Raven Press, NewYork, 55-105.

Gormley KL and KL Teather, 2003. Developmental, behavioural, and reproductive effects experienced by Japanese medaka (*Oryzias latipes*) in response to short-term exposure to endosulfan. Ecotoxicol. Environ. Saf., 54:330-338.

Graham M and GK Iwama, 2003. The physiological effects of anaesthetic ketamine hydrochloride on two *Salmorid* species. Aquaculture, 90: 323-331.

Hart JL, JRM Thacker, JC Braidwood, NR Fraser and JE Mattews, 1997. Novel cypermethrin formulationfor the control of sea lice on salmon (*Salmo salar*). Veterinary Records, 140:179–181.

Jee LH, F Masroor and JC Kang, 2005. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Research, 36:898–905.

Jiraungkoorskul W, ES Upatham, M Kruatrachue, S Sahaphong, S Vichasri-Grams and P Pokethitiyook, 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). Environ. Toxicol., 19: 260- 267.

Joshi N, Dharmlata and AP Sahu, 2007. Histopathological changes in liver of *Heteropneustes fossilis* exposed to cypermethrin. Journal of Environmental Biology, 28: 35-37.

Lenntech I, 1998. Water pollution, Available: www.lenntech.com/water pollution/water pollution.; FAO.htm.

Mallat, J, 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci., 42:630–648.

Mandel PK and AK Kulshrestha, 1980. Histopathological changes induced by the sublethal sumithion in *Clarias batrachus* (L). Ind. J. Exp. Biol., 18: 547-552.

Mishara A and Mohanty B, 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). Environmental Toxicology and Pharmacology, 26:136-141.

Morgan JD, GA Vigers, AP Farrell, DM Janz and JF Maniville, 1991. Acute avoidance reactions and behavioural responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to Garlon 4, Garlon 3A and vision herbicides. Environ. Toxicol. Chem., 10:73-39.

Neskovic NK, V Poleksic, I Elezovic, V Karan and M Budimir, 1996. Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L**.** Bull. Environ. Contam. Toxicol., 56: 295-302.

Olufayo MO and OH Alade, 2012. Acute toxicity and histological changes in gills, liver and kidney of catfish, *Heterobranchus bidorsalis e*xposed to cypermethrin concentration. African Journal of Agricultural Research, 7: 4453-4459.

Olufayo MO, 2009. Haematological Characteristics of *C. gariepinus* juveniles exposed to Derris root powder. Afr. J. Food Agric. Nutr. Dev., 9:921-933.

Omitoyin BO, EK Ajani, BT Adesina and CNF Okuagu, 2006. Toxicity of lindane (Gamma Hexachloro-cyclohexane) to *Clarias gariepinus* (Burchell, 1822). World J. Zool., 1:57-63.

Omoniyi I, AO Agbon and SA Sodunke, 2002. Effect of lethal and sublethal concentrations of Tobacco (*Nicotianato baccum*) leaf dustextract on weight and haematological changes in *Clarias gariepinus* (Burchell). Journal of Applied Sciences and Environmental Management, 6:37-41.

Richards RH, 1988. Diseases of farmed fish: Salmonids. Veterinary Records,112:124–126.

Robert and Hutson, 1998. Metabolic Pathways of Agrochemical Parts 2; Insecticides and Fungicides. The Royal Society of Chemistry, Cambridge, 1475 p.

Roth M, RH Richards and C Sommerville, 1993. Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. Journal of Fish Diseases, 16:1–26.

Thophona, M Kruatrachuea, ES Upathama, P Pokethitiyooka, S Sahaphongb and S Jaritkhuanc, 2003. Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environmental Pollution, 121 : 307-320.

Tilak KS, K Veeraiah and MS Butchiram, 2007. Effect of phenol on haematological components of India major carps *Catla catla, Labeo rohita and Cirrhinus mrigala*. J. Environ. Biol., 28:177-179.

Treasurer JW and SL Wadsworth, 2004. Interspecific comparison of experimental and natural routes of *Lepeophtheirus salmonis* and *Caligus elongatus* challenge and consequences for distribution of chalimus on salmonids and therapeutant screening. Aquaculture Research, 35:773–783.