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HISTOPATHOLOGICAL CHANGES OBSERVED IN THE KIDNEY OF FRESHWATER FISH, *CIRRHINUS MRIGALA* (HAMILTON) EXPOSED TO CYPERMETHRIN

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Abstract

The present study is aimed to assess the histological damage caused to the fish *Cirrhinus mrigala* exposed lethal (5.13 µg/l) and sublethal (1.026 µg/l) to pyrethroid derivative cypermethrin. Light microscopic studies exhibited sever histopathological changes in the kidney. The first sign of morphological changes in the kidney after injection of cypermethrin was found in the proximal tubule. Initial changes of these tubules included: deformation of brush border, gradual atrophy of basal cytoplasm and condensation of nuclear material. Following these initial changes, there was focal necrosis of tubular cells and pyknosis of nuclei. Degenerated cells were frequently seen extruding into the lumina of tubules, which were filled, with fragments of cellular components. Focal degeneration of tubular cells was usually followed by more extensive necrosis of the whole nephron. As the focal areas of necrosis became more widespread, more and more leucocytes and macrophages surrounded the tubules. Thus the area of interstitial tissue containing leucocytes and macrophages seemed to be increased as the tubules became reduced.

Keywords: Cypermethrin, histopathology, *Cirrhinus mrigala*, kidney

Introduction

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of pests. But the extensive use of pesticides pose a constant threat to the aquatic life by altering the habitat behavior pattern, growth and reproductive potential (Jarvinen, 1977). Although there are considerable research activities in the field of pesticides, there is wide variation in the amount of information available concerning the effect of particular pesticides on selected non-target organisms. Among the organisms studied, fishes have drawn more attention due to their economic importance (Ganesan et al. 1989).

The extent of severity of tissue damage of a particular compound as toxicant depends on the toxic potentiality of it in the tissues of organisms (Tilak et al. 2001). Susceptibility to chemical injury varies greatly in the tissues and cells of the same animal. It is even greater in different animal groups. However, the location of the major damage may be determined by the mode of action of the chemical. The mode of action of each poison and the pattern of tissue vulnerability has been well defined and the toxic level of each agent at which a fairly standard distinctive pattern of tissue damage has been studied.

Histopathology is mainly directed to study the effect of chemicals on the structural components of the living system and the ways in which cells and tissues respond to injury. A chemical or a derivative acting directly on the cell or most frequently causes chemical cytotoxicity by altering its environment. The cells in turn respond histopathologically by degeneration, proliferation, inflammation and repair. The chemical affecting the cell by altering the external environment, oxygen and nutrient transport system or the endocrine and immune system. Kumar and Pant (1984) have stated that histopathological studies are useful to evaluate the pollution potential of pesticides since trace levels of pesticides, which do not cause animal mortality over a given period, are capable of producing considerable original damage. Hence, it is useful to have an insight into histological analysis regarding the extent of damage of the tissue, kidney when cypermethrin enters the body of *Cirrhinus mrigala*.

Material and Methods

Collection and maintenance of fish

Freshwater fish, *Cirrhinus mrigala* (length 15±1 cm; weight 10±1 g) was obtained from Karnataka State Fisheries Department Fish Farms, B.R. Project, India. The fish species were reared in large cement tank. During acclimation, the fish were fed with rice bran and

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oil cakes in the ratio of 2:1 on every alternate day. Water of the tank was changed daily to avoid fungal and bacterial contaminations, if any.

Physico-chemical characterisation of water

The physico-chemical characteristics of the water used for fish bioassay were determined according to the procedures described in Standard methods (APHA 1998). The water quality parameters were as follows: pH 8, temperature 28°C, DO 6.7 to 7.2 mg/l, salinity 108 mg/l, Cl 46.3 mg/l, Na 12.2 mg/l, K 30.5 mg/l, Ca 17 mg/l, Mn 1 mg/l, CO_2 9.0 mg/l, Hardness 115 mg/l, $CaCO_3$ 57 mg/l and specific gravity 1.00374.

Toxicant Selected and Preparation of stock solution

Technical grade of cypermethrin (95%) was obtained from Rallis India Ltd, Bangalore. The pesticide stock solution was prepared by dissolving 10 mg of cypermethrin in 10 ml of analytical grade acetone. For experiment purpose, the pesticide was drawn from the stock solution. Maximum amount of acetone present in the highest concentration tested was less than 0.1 ml/l and the same quantity of acetone was added to the controls. Acetone was found to be non-toxic to fish (Pickering et al. 1962).

Fixation of exposure periods and Toxicity Evaluation

In order to understand the influence of time over the toxic effect of cypermethrin at lethal concentration on *Cirrhinus mrigala* at different periods of exposure. Before experimentation, healthy fishes were collected from the large cement tank with the help of big nylon net and hand net. They were acclimatized to laboratory conditions in glass troughs for fifteen days. Each trough contained 15 litres of water with uniform sized (length 15 cm; weight 10 g) fishes. During the acclimatization fishes were fed with commercial fish food pellets. After 15 days, fishes with normal behavioral activity and good health conditions were selected for further experiment purpose. The fishes were divided into two groups. Group I fishes were not exposed to pesticide and served as control. Whereas, group II fishes were exposed to lethal and sublethal concentrations of cypermethrin for 1st, 2nd, 3rd and 4th day. And fishes were chosen on 1st, 7th, 14th and 21st day to observe the short-term and long term effects respectively. Water was renewed after every 24 h to maintain the pesticide concentration. The LC50 value for 96 h was determined by probit analysis method (Finney 1971) and was found to be 5.13 μg/l. And sublethal concentration, one-fifth of LC50 value (1.026 μg/l) was considered for experimentation.

Histopathological studies

The histopathological studies were observed in liver, *Cirrhinus mrigala*, exposed to lethal and sublethal concentrations of cypermethrin. To study the histopathology of tissues, the method described by Humason (1972) was followed. The liver of treated and control fish were isolated and fixed in boun's fluid for 24 hours at room temperature. Tissue is repeatedly washed with 70% alcohol till all the traces of boun's fluid were removed. Dehydration process was carried out by washing the tissue with alcohol (90% and 100%), alcohol-benzene in different ratios (3:1, 1:1 and 1:3) followed by pure benzene and benzene-paraffin wax (1:1). After the process, the organs were embedded in paraffin (58-60°C). Sections were taken (5 micron thickness) and stained with Mayers haematoxylin (Mayers and Hendricks, 1985) and counter stained with eosin. All sections were mounted with DPX and histopathological changes were observed under light microscope.

Results

Histology of Controlled Kidney

The basic unit of kidney in fish consists of a renal corpuscle, Bowman's capsule and glomerulus and various segment of the renal tubules, namely proximal tubule, intermediate segment, distal tubule and collecting duct. Proximal tubules have prominent brush borders (Microrilli) bathed in the vascular bed in the interstitial tissues. Distal tubules and collecting ducts, both devoid of brush borders, and are sparsely distributed. The intermediate segments between proximal and distal tubules are rarely seen. The renal corpuscles are located in close vicinity of renal tubules and blood vessels in the interstitial tissue. Pigments and leucocytes are very common in the interstitial tissue (Plate 1 Fig 1, 2 & 3).

Histological changes in the Kidney of exposed fish

In lethal concentration of cypermethrin, the kidney showed reduction in renal cell number in the proximal and distal collecting tubules, which have resulted in narrowness of lumen. The tubular cells have undergone hypertrophy and some of the renal tubules have lost their normal shape. Vacuolation due to degeneration of cytoplasm is quite obvious. The nuclei of epithelial cells have become quite dominant and are found infiltrating into the surrounding tissue. The perforation of kidney tubules is commonly observed. The kidney demonstrated hyperplasia, vacuolation, degeneration and necrosis leading to the complete necrosis. Cubiodal epithelial cells lining the tubules showed complete vascuolation with degenerating cytoplasm and more nuclear division and their disorderly scattering nature. The hemopoietic tissue was fully studded with lymphatic cells at the highest rate of nuclear division. The lumen of the tubules was

found to be dilated. Kidney tubules were also found to be perforated (Plate 2 Fig 1-6).

Compared to the structure of the liver of control fish, exposed to sublethal concentration of cypermethrin initially exhibited few changes like slight disarray of liver lobes, mild degree of degeneration of cytoplasm, occasional blood clots and congregation of nuclei at day 1 (Plate 3 Fig 3 & 4) and cloudy swelling of hepatocytes, granulization of cytoplasm, hypertrophic and pyknotic nuclei on day 7 (Plate 3 Fig 5 & 6). However, on further exposure to day 14 certain degree of reorganization in the structure of liver cords was observed. The nuclei appeared normal, with a very little degree of cytoplasmic vacuolization (Plate 4 Fig 1 & 2). At 21 days of exposure, no significant changes were seen different from controls, except a slight degree of hyperchromatic condition of the nuclei (Plate 4 Fig 3 & 4).

Discussion

Cypermethrin exposure induced marked abnormalities in the kidney initiated with disruption of tubular organization. Thereafter degeneration of tubular epithelial cells and lympocytic infiltration was evident. Most of these pathological changes persisted with vacuolation, clotting of blood in some sinusoids and glomerular degeneration.

Cypermethrin accumulates preferentially in the kidney tissues when the body burden of cypermethrin increases, new proteins such as metallothionein are synthesized in the liver and kidney (Ooi and Law, 1989). The membranous organelles, such as mitochondria, endoplasmic reticulum and nuclear envelope, are most easily affected by cypermethrin in which disorganization, rearrangement and malfunction may occur. Thus, the proximal tubules which posses numerous mitochondria rather than the distal tubules are easily damaged by cypermethrin. The collecting ducts are usually more resistant to cypermethrin exposure. The injuries to collecting ducts are only obvious in the fish exposed to higher concentration of cypermethrin (Plate 2 Fig 1-6).

The appearance of atrophic or pyknotic nuclei in fish kidney increases with the increase of time course. The phenomenon of nuclear changes in fish is probably similar to that found in other animals (Copius-Peereboom and Copius-Peereboom-Stegeman, 1981). It has been suggested that a nuclear and nuclealar changes are induced preceding a trophy and necrosis of cells in other animals. At the beginning, the change may probably form part of a defuse mechanism, leading to defuse an activation of synthetic or other activities in the cell, such as synthesis of metallothionein. However, during prolonged treatment, further accumulation of cypermethrin causes a condensation of nuclear material to form darvely stained pyknotic nuclei (Plate 2 Fig 3-4).

Leucocytes are common in the interstitial space of control fish, but they are rarely aggregated so densely and abundantly as in cypermethrin treated renal tissue. The increase of leucocytes may have been an inflammatory response to cypermethrin. Leucocytes may either remove or engulf injured and non functional cells. The dilation of the lumen of the kidney tubules, degeneration in the hemopoitic tissue rupture in the collecting tubules and necrosis as observed in the present investigation after chlorpyrifos treatment have also been reported in various fish exposed to pollutants (Kumar and Pant, 1984; Sukumar and Karpagaganapathy, 1986; Gill et al. 1988 and Vardhani and Gowri, 2002). According to Dubale and Shah (1981) the process of destruction is a function of dosage and period of exposure and they opined that the renal tubules of kidney are the first to be affected by pesticide stress. Rashtwar and Ilyas (1984) reported the histolopathological changes in kidney to lead to cloudy swelling of renal tubules in *Nemachellus denisoni* acutely exposed to phosphamidon. In the present study also the swelling of renal tubules in acute exposure was evident. Changes like vacuolation of epithelial cells of renal tubules and pronounced enlargement of the tubules were observed at higher lethal concentration (Plate 2 Fig 1-6) and prolonged exposure to cypermethrin (Plate 3 & 4).

The proximal tubule in mammals and fishes is involved in reabsorption and lysosomal degradation of macromolecules (Hickman and Trump, 1969). After reabsorption, more macromolecules may from intracellular droplets or dense bodies in higher vertebrates (Rollason and Brewer, 1984). During this process the pesticide are excreted through kidney and appears to cause considerable damage.

Necrosis and vascuolation were observed by Dhanapakiam and Premalatha (1994) in *Cyprinus carpio* exposed to malathion. Sastry and Sharma, (1979) observed a number of striking changes in the histological structure of the kidney of *Channa punctatus* exposed to sub lethal concentration of 0.01 ppm of endrin for a span of 30 days and found that the shrinkage of glomerulus was the visible sign of intoxication. Konar, (1979) observed shrinkage and degeneration of glomerulus and valcuolation of tubules in carp chronically treated with hepatochlor. Vinod Ghanathay, (1989) studied histopathological changes in the kidney of *Channa puntatus*, exposed to BHC. He observed that in kidney tissues, after 5 days of exposure to BHC, the glomeruli were shrunken, but some of them were slightly vacuolated on the 10th day and there was a cloudy swelling and hydropic degeneration of interstitial tissues. On the 15th day, they reported that most of the glomeruli were completely necrosis and the tubular epithelium was

fibrosed. Histopathological effects of insecticides on intestine of fish have been studied by several authors (Tilak et al. 2005; Prashanth 2003; Tilak and Yacobu, 2002; Tilak et al. 2001; Thorat, 2001).

In view of the literature cited above, it is apparent that in the present investigation, cypermethrin at lethal and sublethal concentration caused considerable histological damages to the organs studied and extend support to the earlier mentioned alterations in hematological aspects. It is concluded that more or less similar pathological changes are induced in the kidney of different fishes by different biocides but the extent of damage varies depending upon the dose of biocide, duration damage varies depending upon the dose of biocide, duration of exposure, toxicity of biocide and susceptibility of fish.

Plate 1

Fig. 1 and 2: Section of kidney of control fish, *Cirrhinus mrigala* showing normal structure P- Proximal tubule, G -Glomerulus, BV- Blood vessels, DT- Distal tubule and IT-Interstitial tissue, R- Renal Tubule. H and E. X 400

Fig. 3: Section of kidney of control fish, *Cirrhinus mrigala* showing a enlarged Proximal tubules (P) with Blood vessels (BV) and Interstitial tissue (IT). H & E. X 1000

Fig. 4 & 5: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (5.13 μg/l) for 24 hr showing enlargement of tubular lumen, Vacuolization (VZ), Necrotic martial (N) and damage of Proximal tubule (P) & Renal Tubule (R). H & E X 400. (H & E = Hematoxylin and Eosin)

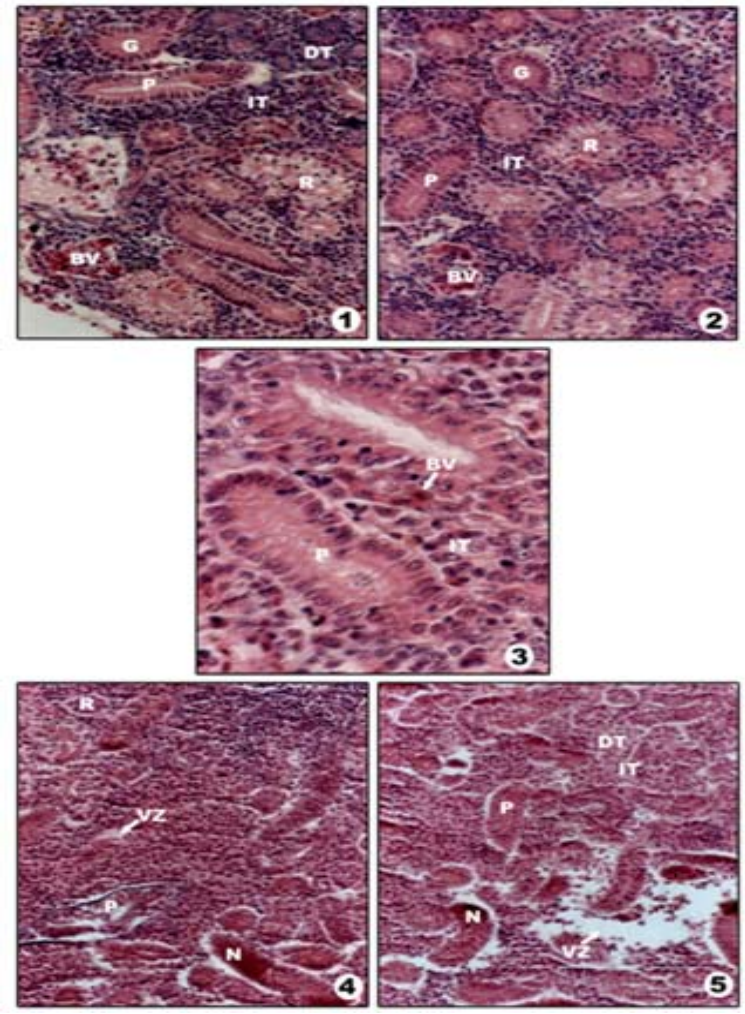


Plate 2

Fig. 1 & 2: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (5.13 μg/l) for 48 hr showing Necrosis of proximal tubule (N), Glumerular shrinkage (G), Vacuolization (VZ) and tubular degeneration. H & E. X 400

Fig. 3 & 4: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (5.13 μg/l) for 72 hr showing maximum damages like Necrosis of proximal tubule (N), Glomerular shrinkage (G), Vacuolization (VZ), Interstitial tissue (IT) and tubular degeneration of Renal tubule (R) & proximal tubule (P). H & E. X 400

Fig. 5 & 6: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (5.13 μg/l) for 96 hr showing degeneration of Interstitial tissue & Renal tubules (IT & R), tubular degeneration, Necrosis of proximal tubule (P), damage of Blood Vessels (BV) and Vacuolization (VZ). H & E. X 400

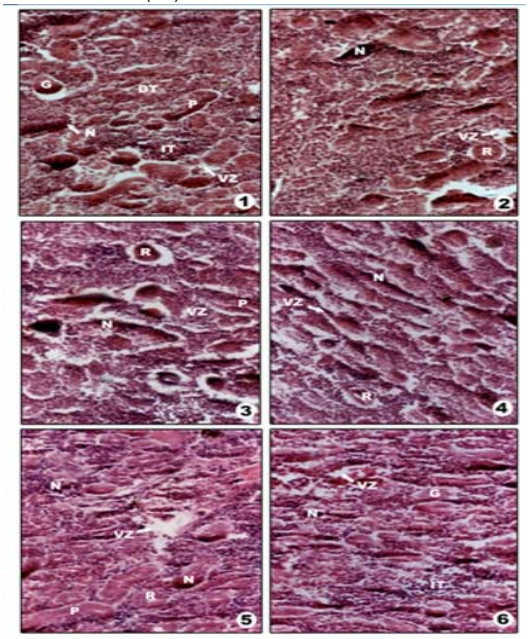


Plate 3

Fig. 1 & 2: Section of kidney of control fish, *Cirrhinus mrigala* showing normal structure. P-Proximal tubule, G-Glomerulus, IT-Interstitial tissue, BV- Blood vessels and DT-Distal tubule. H & E. X 400

Fig. 3 & 4: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (1.02 μg/l) for 1 day showing desquamation and degeneration of tubules, necrosis (N) and glomerular shrinkage (G). H & E. X 400

Fig. 5 & 6: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (1.02 μg/l) for 7 day showing degeneration tubular epithelial cells, lymphocyte infiltration in interstitial space, tubular degeneration, Necrosis of proximal tubule (N) and Vacuolization (VZ). H & E. X 400. (H & E = Hematoxylin and Eosin)

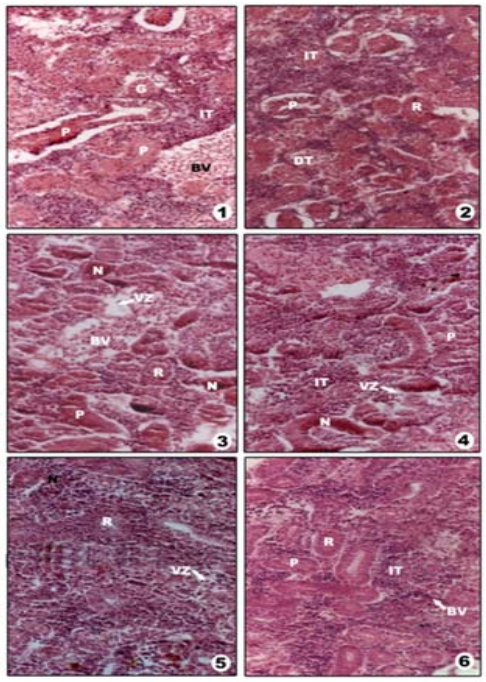
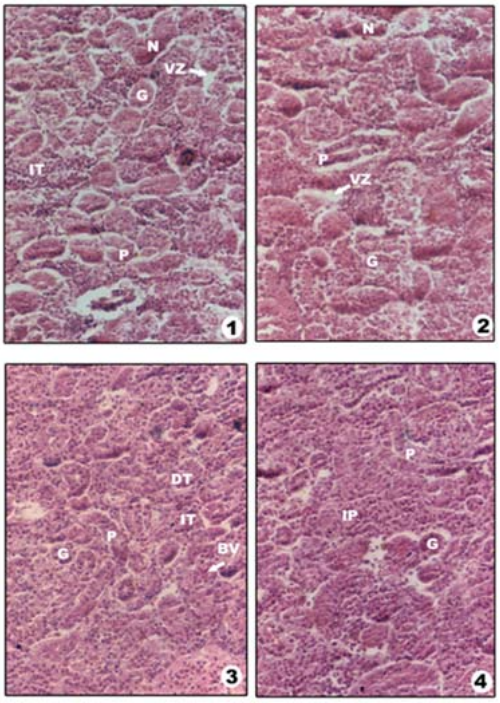


Plate 4

Fig. 1 & 2: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (1.02 μg/l) for 14 day showing enlargement of lobular lumen, necrosis of Renal tubules (R), damage of proximal tubules (P) and vacuolization (VZ). H & E. X 400

Fig. 3 & 4: Section of kidney of fish, *Cirrhinus mrigala* exposed to cypermethrin (1.02 μg/l) for 21 day showing recovery structure, cellular damages were reduced. P- Proximal tubule, G -Glomerulus, BV- Blood vessels, DT- Distal tubule and IT-Interstitial tissue. H & E. X 400



Reference

APHA., 2000. Ammerican Standard methods for the examination of water wastewater. 20th Edn. Amer. Publ. Hlth. Assoc., Washington, DC. 20005-2605.

Carpenter, P.L., 1975. In: Immunology and Serology, 3rd W.B. Sounders Campany Philadelphia, London. Toronto. P. 254.

Copius-Peereboom, J.W. and Copius-Peereboom-Stegeman, 1981. Exposure and heath effects of cadmium to animals and man. Tooxicol. Environ. Chem. Reviews. 4, 67-178.

Dhanapakiam, P. and Juliet Premalatha, 1994. Histopathological changes in the Kidney *Cyprinus carpio* to malathion and sevin. J. Environ. Biol. 15(4), 283-287.

Dubale, M.S. and Shah. P., 1981. Histopathology of the kidney of the fish, *Channa punctatus* exposed to cadium. J. Anim. Morphol. Phyxiol. 28(1-2), 166-171.

Finney, D.J., 1971. Probit Analysis, 3rd Edition, Cambridge University, Press, London, p, 333.

Ganesan, R.M., Tebakumar, S.R.D., Jayaraman, J., 1989. Sub lethal effects of organochlorine insecticides (endosulfan) on protein, carbohydrate and lipid contents in liver tissue of *Orechromis mossambicus*. Proc. Indian Acad. Sci. (Anim Sci). 98(1), 51-55.

Gill, T.S., Pant, S.C. and Pant. J., 1988. Gill, liver and kidney lesions associated with experimental exposure to carbaryl and diemethoate in the fish, *Puntius conchonius*. Ham. Bull. Environ. Contam. Toxicol. 41, 71-78.

Hickman, C.P. and Trump, B.F., 1969. The kidney. In fish physiology. Vol. I. Hoar. W.S, Rondall. D.J. (Ed). P. 91.

Humason, G.L., 1972. Animal technique III ed. W.H. Freeman and Co. San. Franancisco.

Jarvinen, A.W., Haffman, M. J., Thorslund, T. W., 1977. Long-term toxic effects of DDT food and water exposure on fat head minnows (*Pimephales promelas*). J. Fish. Res. Broadcas. 34, 2089.

Konar, S.K., 1979. Hazards of water pollution by pesticides in: Proceeding of international Symposium on environmental pollution and toxicology. Nov, 28-30. 1977. Hissar Campus, Haryana. India.

Kumar, S. and Pant, S.C., 1984. Organal damage caused by aldicarb to freshwater teleost, *Barbus conchonius* Hamilton. Bull. Environ. Contam. Toxicol. 33, 50-53.

Mayers, T.R. and Hendricks, J.D., 1985. Histopathology: In fundamental of aquatic toxicology: Eds Rand. G. M. and Petrocelli. S. R. Hemisphere publishing Corp. USA.

Ooi, V.K. and F.K. Law., 1989. Changes induced by cadium in kindny of black Sea Bream, *Mylio macrocephalus* (Teleostei). Bull. Environ. Contam. Toxicol. 43, 769-775.

Prashanth, M.S., 2003. Cypermethrin induced physiological, biochemical and histopathological changes in freshwater fish, C*irrhinus mrigala* (Hamilton). Ph.d thesis, Karanataka University, Dharwad, India

Rashatwar, S.S. and Ilyas, R., 1984. Effect phosphamidon in freshwater teleost fish *Nemacheilus denisoni* (Day). Histopathological and biochemical Studies. J. Environ. Biol. 5(1), 1-18.

Rollason, T.P. and Brewer, D.B., 1984. A. Study of glomerutar Basement membrane anionic sites and glomerular visceral, epithelial Cell Coat in Protein Overload. Protenuria in rats. J. Pathol. 142, 306-316.

Sastry, K.V. and Sharma, S.K., 1979. The effect of endrin on the histopathologicl changes in the liver of *Channa punctatus.* Bull. Environ. Contam. Toxicol. 20, 674-677.

Sukumar, A. Karapagaganapathy, P.R., 1986. Renal dysfunction in a freshwater fish, *Colisa lalia* after carbofuran intoxication. In. "Environmental and Ecotoxicology". (eds. Dalela Rc. Shashi Y.N. and Gupta). Acad. Environ. Biol. India. 243-247.

Tilak, K.S., Veeraiah K. and Yacobu. K., 2001. Studies on histopathological changes in the gill, liver, and kidney of *Ctenophrayngodon idellus* (valenciennes) exposed to technical fenvalerate and EC 20%. Poll. Res. 20(3), 387-393.

Tilak, K.S, Koteswara Rao and Veeraiah, 2005. Effect of chlorpyrifos on histopathology of the fish *Catla catla*. J. Ecotoxicol. Environ. Moint. 15(2), 127-140.

Tilak, K.S. and Yacobu, K., 2002. Toxicity and effect of fenva;erate on fish Ctenophargodon idellu. J. Ecotoxicol. Environ. Moint. 12(1), 09-15.

Thorat, S.R., 2001. Histopathological Changes in the Intestine of Freshwater fish, *Channa gachua* exposed to tannery effluent. Poll. Res. 20(2), 267-270.

Vardhani, V.V. and Gowri, 2002. Antigen induced histopathological changes in liver and kidney of *Leabeo rohita*. J. Ecotoxicol. Environ. Moint. 12(1), 209-213.

Vinod, V. Ghanathay, 1989. *In vivo* effects of BHC on some aspects of physiology and histopathology of fish, Channa Punctatus (Bloch). Ph.D. Thesis Osmania University, Hyderabad, India.