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HISTOPATHOLOGICAL EFFECT OF DICHLORVOS ON INTESTINE OF MAJOR CARP *LABEO ROHITA* (HAMILTON).

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ABSTRACT

The present study is aimed to assess the histological damage caused to the fish Labeo rohita exposed acute (4 days) and chronic (30 days) sub lethal concentration of 1/10 and 1/15 of 96hrs LC_{50}. In a short term (96 hr) study Labeo rohita were exposed to ($1/10^{th}$ and $1/15^{th}$) 1.671ppm and 1.114ppm of 96hr LC_{50} for 4 days of dichlorvos. Exposed intestine showed atropy in the muscularis, pycnosis of nuclei, vacuolation in the muscularis, erosion of mucosal layer and necrosis. Fishes exposed to sublethal concentration at 1.671ppm and 1.114ppm (1/10 & 1/15) of Dichlorvos for 30 days showed pathological changes. The most common changes in the intestine of studied fish were erosion of brush border and lamina propia, degeneration of villi with severe necrosis in the absorptive columnar epithelial cells including brush border, aggregation of necrotized cells in the intestinal lumen, clumping of cytoplasm, infiltration of lymphocytes, broken villi tips, increase in intervilli space and catarrhal extrude. Also, pycnosis of nuclei, necrosis, vacuolation, shortening of villi, necrosis in the mucosal layer and atropy in muscularis were prominent in the treated fishes. From the above studies it is evident that Dichlorvos induced severe histopathological changes in intestine of fish Labeo rohita.

Key words: Dichlorvos, *Labeo rohita*, sublethal, intestine, necrosis, vacuolation, atropy.

INTRODUCTION:

Pesticides of various categories viz., organophosphates, organochlorines, pyrethroids and carbamates are used against a number of pests, to increase the crop production. Besides their use against agricultural pests, the pesticides are generously used to control the population of mosquitoes, houseflies, termites, other household and stored grain pests. The short- sighted approach to eradicate harmful insects has led to indiscriminate use of pesticides, unmindful of their consequences to the environment. The remains of pesticides either enter into the atmosphere as aerosol or into the aquatic system through runoff water, inadvertently exposing the non-target organisms and finally finding their way to the food chain threatening the ecological balance and the biodiversity of the nature. Aquatic contamination of pesticides causes acute and chronic poisoning of fish and cause severe damage to vital organs (Omitoyin et al., 2006, Velmurugan et al., 2007).

Among different classes of pesticides, organophosphate pesticides are finding increasing use in recent years since they are biodegradable and, therefore, persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies (Jyothi and Narayan 1999). Histopathological study gives useful data of concerning tissue changes prior to external manifestation. It is the study of the structure of diseased or injured cells or a study pathological change in the microanatomy of tissues is known as histopathology. Histological investigation appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Dutta, 1996).

Organophosphates are highly toxic to fish and non-target aquatic organisms and are powerful nerve poisons, since they inhibit AChE activity (Coppage *et al.*, 1975; Klaverkamp and Hobden, 1980). 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. Therefore, in this study we aimed to evaluate the toxic histopathological effects in the intestine of *Labeo rohita* exposed to synthetic pesticide dichlorvos.

MATERIALS AND METHODS:

Collection and acclimatization of experimental fish:

For the study of histopathological effects, live specimens of Healthy and active adult *Labeo rohita* were obtained from Patra and Bhadbhade fish farms barkhedi and bhadbhada Bhopal M.P respectively. They weighed 55±3gm and their length was in the range 14cm±1. They were brought to laboratory carefully in oxygen filled polythene bags in card board boxes to avoid any injury and disinfected by giving a bath for five minutes in $KMno_4$ solution. Thereafter, they were transferred to glass aquariums filled with dechlorinated water. The fishes were acclimated to the laboratory conditions for at least 20 days prior to the experiment. During acclimatization fishes were fed daily with commercial fish food which was given at morning hours. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish during acclimation and experimental period. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic wastes. Dead fishes whenever located were removed immediately to avoid fouling of the water.

Pesticide:

Dichlorvos manufactured by Sygneta India Ltd. 14, J. Tata road, Mumbai-400 020 was purchased from the local market and was used for evaluation of its toxicity to the fish.

Histopathological studies:

The fishes were exposed to acute exposure (4 days) 1/10 1.671ppm and 1/15 1.114ppm sublethal concentrations of Dichlorvos (96hrs LC_{50}). Simultaneously a control aquarium was also maintained. At the end of acute exposure for 96h the survived fishes were killed by decapitation and liver were removed and fixed in Bouins fluid for 24h.

In second set of experiment, the test fishes *Labeo rohita* were exposed to two sublethal concentrations 1/10 1.671ppm and 1/15 1.114ppm of Dichlorvos for 30 days simultaneously, a control aquarium was also maintained. At the end of experiment, surviving fishes were utilized for histopathological study. All the tissues were immediately fixed in Bouins fluid for 24h and processed according to standard procedure of routine micro technique. The blocks were prepared in paraffin wax and sections were cut on rotatory microtome to a thickness of 6 to 8µ. For staining the double staining method was followed by using Haematoxyline and Eosin as a stains and mounting was done in DPX.

RESULTS:

Histology of controlled intestine

The intestine of fishes consists of structures similar to those found in terrestrial vertebrates. The histological patterns are also similar, and the same nomenclature can be applied in most cases. It composed of the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The tunica mucosa usually consists of a mucosal epithelium overlying a layer of loose connective tissue or lamina propria that is vascularized and contains nerves and leukocytes. The three layers of the tunica mucosa are supported on a connective tissue tunica submucosa. The tunica muscularis usually consists of inner circular and outer longitudinal layers of either striated or smooth muscle. The tunica serosa is only present within the coelomic cavity and corresponds to mesothelial cells and loose connective tissue containing blood vessels (Fig.1 Control).

Histological changes in the intestine of exposed fish

96 hours exposure:

Fishes exposed to sublethal concentrations ($1/10^{th}$ and $1/15^{th}$) 1.671ppm and 1.114ppm of 96hr LC_{50} for 4 days of Dichlorvos showed atropy in the muscularis, pycnosis of nuclei, vacuolation in the muscularis, erosion of mucosal layer and necrosis.

Chronic exposure 30 days:

1.671ppm Dichlorvos (chronic exposure 30 days):

Fishes exposed to sublethal concentration at 1.671ppm (1/10) of Dichlorvos for 30 days showed pathological changes. The most common changes in the intestine of studied fish were erosion of brush border and lamina propia, degeneration of villi with severe necrosis in the absorptive columnar epithelial cells including brush border, aggregation of necrotized cells in the intestinal lumen, clumping of cytoplasm, infiltration of lymphocytes, broken villi tips, increase in intervilli space and catarrhal extrude. Also, pycnosis of nuclei, necrosis, vacuolation and atropy in muscularis were prominent in the treated fishes.

1.114 ppm Dichlorvos (chronic exposure 30 days):

After 30 days of exposure to sublethal concentration 1.114ppm (1/15) of Dichlorvos, histopathological examination of the intestine of *Labeo rohita* showed fusion of villi, infiltration of lymphocytes, shortening of villi and increased lumen & necrosis in the mucosal layer. Also, vacuolation, pycnosis of nuclei and necrosis were seen in the dichlorvos exposed fishes.

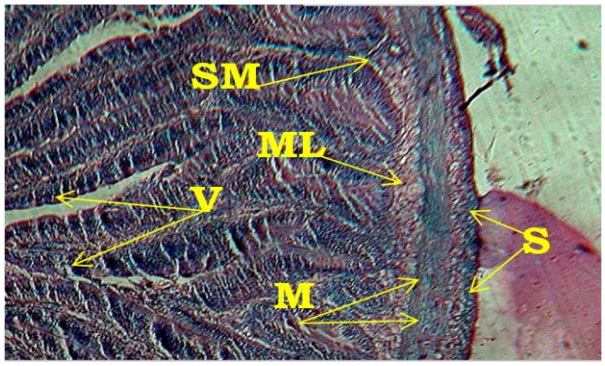

Fig. 1. Photomicrograph of intestine (×100) control Showing intestinal villi **(v)**, mucosal layer **(ML)**,muscularis **(M)**, serosa **(S)** and submucosa **(SM).**

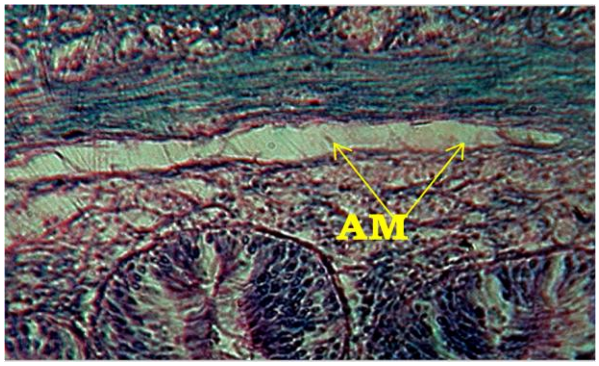

Fig. 2. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 4 days Showing atropy in the muscularis **(AM).**

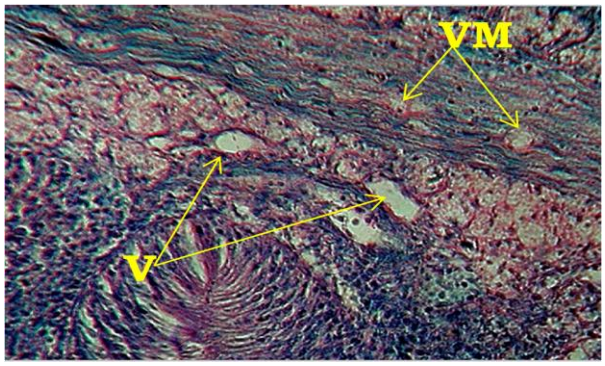

Fig. 3. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 4 days Showing vacuolation in the muscularis **(V)** and mucosa layer **(VM).**

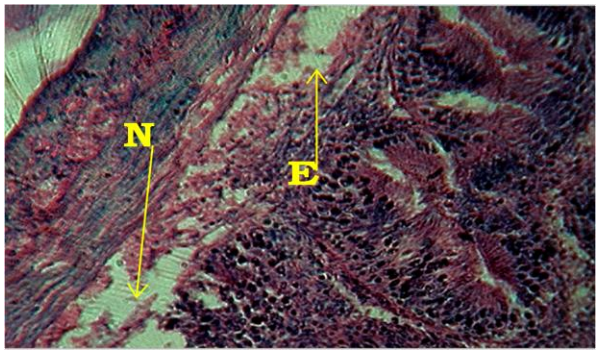

Fig. 4. Photomicrograph of intestine (×400) exposed to 1.114ppm dichlorvos 4 days erosion **(E)** and necrosis **(N)** in the mucosal Layer.

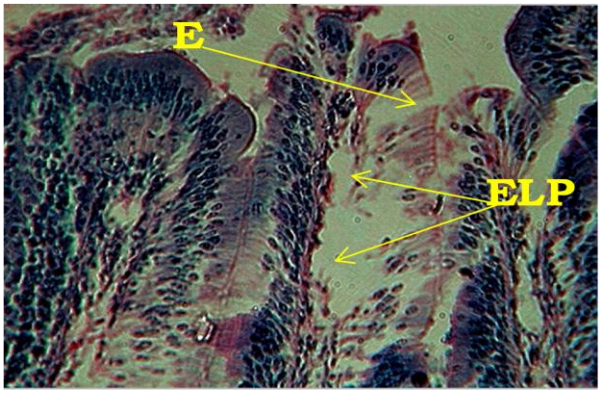

Fig. 5. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 30 days showing erosion of brush border **(E)** and severe lamina propia **(ELP).**

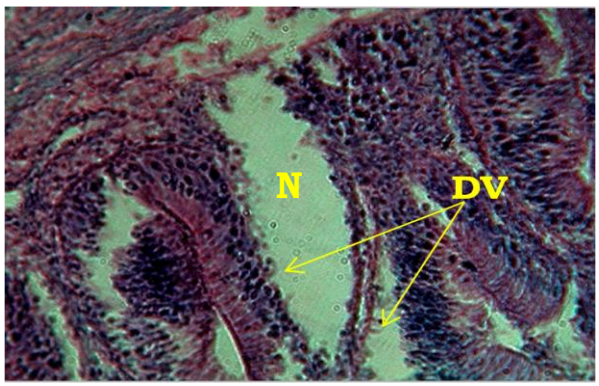

Fig. 6. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 30 days showing degeneration of villi **(DV)** with necrosis **(N).**

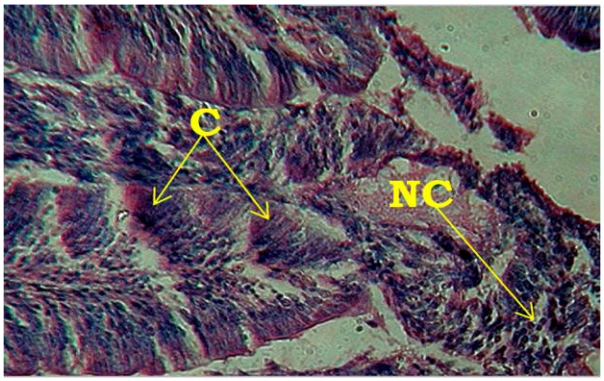

Fig. 7. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 30 days showing necrotized cells in the intestinal lumen **(NC)** and clumping of cytoplasm **(C).**

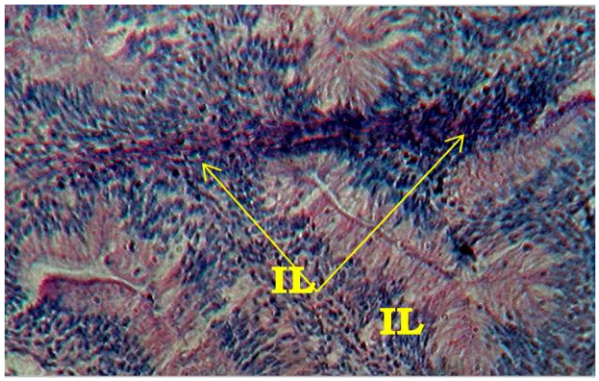

Fig. 8. Photomicrograph of intestine (×400) exposed to 1.671ppm dichlorvos 30 days showing infiltration of lymphocytes **(IL).**

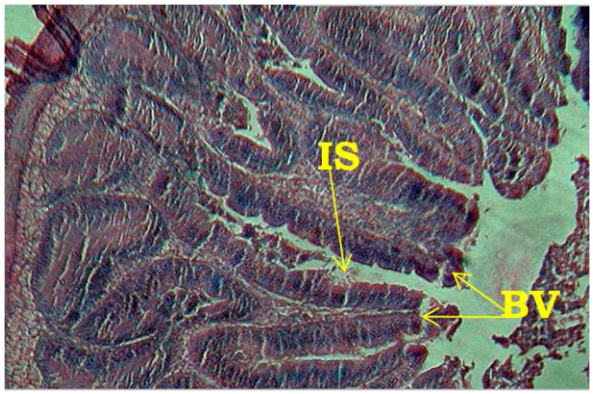

Fig. 9. Photomicrograph of intestine (×100) (×400)exposed to 1.671ppm dichlorvos 30 days showing broken villi tips **(BV)**, increase in intervilli space **(IS).**

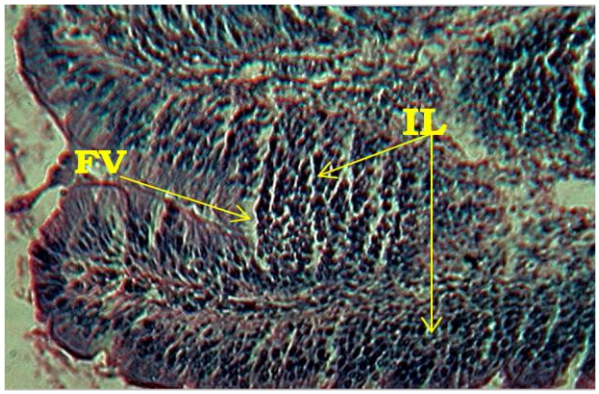

Fig. 10. Photomicrograph of intestine exposed to 1.114ppm dichlorvos 30 days showing fusion of villi **(FV)** and infiltration of lymphocytes in the intestine **(IL).**

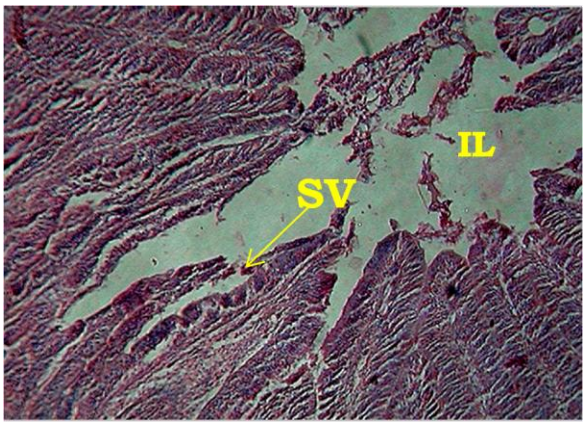

Fig. 11. Photomicrograph of intestine (×100) (×400) exposed to 1.114ppm dichlorvos 30 days showing shortening of villi **(SV)** and increased lumen **(IL).**

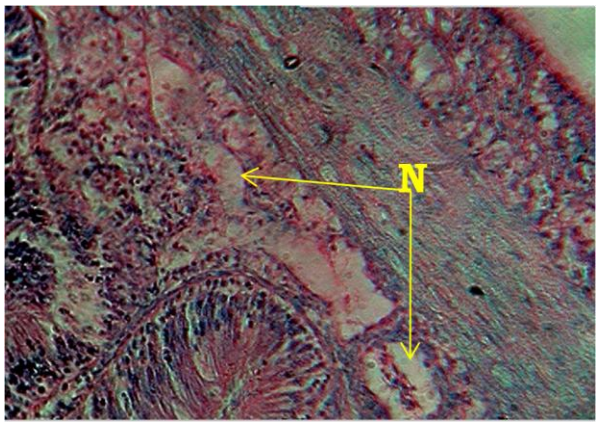

Fig. 12. Photomicrograph of intestine exposed to 1.114ppm dichlorvos 30 days showing necrosis in the mucosal layer **(N).**

DISCUSSION:

Histopathological study gives useful data of concerning tissue changes prior to external manifestation. 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.

Fishes exposed to sublethal concentrations ($1/10^{th}$ and $1/15^{th}$) 1.671ppm and 1.114ppm of 96hr LC_{50} for 4 days of Dichlorvos showed atropy in the muscularis, pycnosis of nuclei, vacuolation in the muscularis, erosion of mucosal layer and necrosis. Fishes exposed to sublethal concentration at 1.671ppm and 1.114ppm (1/10 & 1/15) of Dichlorvos for 30 days showed pathological changes. The most common changes in the intestine of studied fish were erosion of brush border and lamina propia, degeneration of villi with severe necrosis in the absorptive columnar epithelial cells including brush border, aggregation of necrotized cells in the intestinal lumen, clumping of cytoplasm, infiltration of lymphocytes, broken villi tips, increase in intervilli space and catarrhal extrude. Also, pycnosis of nuclei, necrosis, vacuolation, shortening of villi, necrosis in the mucosal layer and atropy in muscularis were prominent in the treated fishes.

Fish intestine plays a vital role in digestion and absorption of dietary nutrients. The anatomical and histological characteristics of fish intestine are expected to be helpful for understanding the related functional mechanisms and feeding habits, which can further be helpful for diagnosing some intestinal diseases and formulating suitable feeds. The histological characteristics of fish intestine can be affected by abiotic and biotic factors. The histological study of the intestine is important in establishing the status of structural integrity. According to Bhatnagar *et al.* 2007, the observed irritation and destruction of the mucosa membrane of the intestine, hamper in absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine (Hanna *et al.,* 2005; Cengiz *et al.,* 2006). Epithelial degeneration, inflammatory cells infiltration in the submucosa as well as Submucosal edema was seen in the intestine of tilapia fish exposed to carbofuran (Soufy *et al., 2007*). Histopathology of intestine in caged and feral freshwater fish revealed lifting of columnar epithelium of villi and hyperplasia as responses constituting the defence mechanisms of fish when exposed to the toxicants (Tuvikene *et al*., 1999). The lymphocytic infiltration showed various intensities with the treated fish. This

indicates signs of irritability, inflammation and hypersensitivity to the chemical used.

According to Desai *et al.* (1984) and Mohamed (2004), the degenerative and necrotic changes observed in the different intestinal layers of the studied fish may be due to a direct effect of the detected pesticides on the cells, to an accumulation of acetylcholine in the tissues or to a reduction in oxygen supply. The increase in the goblet cell population observed in the intestine of the fish may act to immobilize pesticides by binding it to mucus (Naidu *et al,* 1983). The present pathological results in the intestine of studied fish are also in agreement with those observed by many investigators about the effects of different pesticides on fish intestine (El-Elaimy *et al,* 1990; Sakr, 1993; Braunbeck and Appelbaum, 1999; Mohamed, 2006 and Dezfuli *et al,* 2006). Moreover, Cengiz *et al* (2006) observed edema, degeneration, accumulation of lymphocytes and disintegration of villi in the intestine of *G. affinis* subjected to thiodan. Velmurugan *et al* (2007) observed atrophy of epithelial cells, necrosis of epithelial cells, desquamation of mucosal epithelium and infiltration of lymphocytes into the lamina propria in the intestine of *C. mrigala* exposed to fenvalerate.

Reports on degenerative changes and rupture in tip of villi, loss of structural integrity of mucosal folds and degeneration & necrosis of submucosa in the intestine of *Channa punctatus* after the exposure to carbofuran are found in literature (Muley *et al.,* 1996). Report on vacuolization in submucosa & circular muscles and dilation of columnar & goblet cells of mucosal folds were observed by (Sastry *et al.,* 1979). Destruction of columnar epithelium, submucosa fused with muscles and serosa was found in broken condition after 10 days exposure to Malathion (Srivastava *et al.,* 1995). These histopathological changes were observed in the intestine of *Labeo rohita* subjected to pesticides in the present studies. Necrosis, degeneration, and accumulation of lymphocyte in lamina propia were observed in the intestine of Cirrhinus *mrigala* treated with lambda-cyhalothrin (Velmurugan *et al*., 2007). This result is similar to the observations by Glover *et al*. (2007) in Atlantic salmon (*S. salar*) to dietary endosulfan exposure. All the different groups of pesticides or even the different pesticides of the same group do not have the same effect on fishes. Moreover, the mode and site of action of different pesticides differ Mathur D. S. (1965).

Histopathological effects of pesticides on organs of fish have been studied by several authors, in view of the studies cited above, it is apparent that in the present investigation, dichlorvos at acute and chronic concentrations caused considerable histopathological damages to

the organ studied. It is concluded that more or less similar pathological changes are induced in the intestine of different fishes by different toxicants but the extent of damage varies depending upon the dose of toxicants, duration damage varies depending upon the dose of chemical, duration of exposure, toxicity of chemical and susceptibility of the fish. The results obtained during this study, it can be concluded that the intestine histopathology of *Labeo rohita* appears to be sensitive monitoring tool to aquatic health. Fish histopathology could therefore make valuable contribution in the monitoring of aquatic ecosystem and should from an important part of environmental management process.

REFERENCES:

Bhatnagar, C., Bhatnagar, M., Regar, B. C. 2007. Fluoride-induced histopathological changes in gill, kidney, and intestine of fresh water teleost, *Labeo rohita*. Research report Fluoride, Vol. 40 (1): 55–61.

Cengiz, E. and Unlu, E. 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquito fish, *Gambusia affinis*: A microscopic study. Environ. Toxicol. Pharmacol., Vol. 21: 246- 253.

Coppage, D.L., Mathew, G., Cook, G.H., Knight, J. 1975. Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by Malathion, o, o–dimethyl s-(1.2 dicarbetaoxyethyl) Phosphoro–dithoate. Pesticide Biochemistry and Physiology, 5 (6):536-542.

Hanna, M., Shaheed, V and Elias, N. 2005. A contribution on chromium and lead toxicity in cultured *Oreochromis niloticus*. Egypt. J. Aquat. Biol. Fish., Vol (9): 177-209.

Jyothi, B., Narayan, G., 1999. Certain pesticide induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). *Food. Chem. Toxicol.,* 37, 417-421.

Klaverkamp, J.F., Hobden, B.R., 1980. Brain–acetyl cholinesterase inhibition and hepatic activation of acephote and Fenitrothion in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences, 37:1450-1453

Mathur, S. M. and Singh, J. K. 2006. Chemical spraying in agriculture and its environmental impact. All India seminar on environmental Impact assessment. The Institution of Engineers (I), Udaipur.

Muley, D. V., Kamble, G. B & Gaikwad, P. T. 1996. Endosulfan toxicity in the freshwater fish *Tilapia mossambica.* Proc. Acad. Environ. Biol. Vol 5 (1):49-55.

Omitoyin, B.O., Ajani, E.K., Adesina, B.T and Okuagu, C.N.F. 2006. Toxicity of Lindane (Gamma (Burchell 1822). World J. Zool., 1(1): 57-63.

Sastry K. V. & Sharma S. K. 1979. Toxic effects of endrin on liver and kidney of a freshwater fish. Proc. Symp. Environ. Biol.: 337 - 342.

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, Vol (1): 45-52.

Srivastava A. K. & Srivastava A. K. 1995. Histopathological changes in the liver associated with selenium exposure in the freshwater Indian catfish *Heteropneustes fossilis.* J. Adv. Zool. Vol 16 (1): 30 - 33.

Tuvikene, A., Huuskonen, S., Koponen, K., Ritola, O and Mauer, U. 1999. Oil shale processing as a source of aquatic pollution: monitoring of the biological effects in caged and feral freshwater fish. Environ. Health Perspective, Vol 107 (9), 745-752

Velmurgan, B., Selvanayagam, M., Cenigz, E.I., Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. J. Environ. Sci. Health (B), Vol (42): 157-163.