

Heavy Metal Induced Histopathological Alterations in Selected Organs of the *Cyprinus carpio* L.(Common Carp)

Vinodhini, R.* and Narayanan, M.

Aquatic Biodiversity Research Centre, Dept. of Advanced Zoology and Biotechnology,
St.Xavier's College (Autonomous), Palayamkottai – 627 002, Tamilnadu, India

Received 10 March 2008;

Revised 15 Aug. 2008;

Accepted 27 Aug. 2008

ABSTRACT: The hazardous effect of heavy metals on the histopathology of selected organs of the freshwater fish common carp (*Cyprinus carpio* L.) was investigated. Gill, liver, kidney and flesh samples were collected after 32 days of exposure to a sublethal concentration of 5 mg/L of combined (Cd + Pb + Cr + Ni) metal solution containing 1.25 mg/L of each metal ion (1/10th of LC50 / 48h) was analyzed by using light microscopy. The main histopathological changes observed in the gill were edema and lifting of lamellar epithelia. The liver of control fish exhibited a normal structural pattern, while the fish exposed to heavy metals showed vacuolation, presence of hemosiderin and fibrosis. The findings in kidney exhibited the presence of macrophages with lipofuscin granules accumulated in the affected cells. In flesh samples lesions and granulomas were observed. The investigation of histological changes in organs of fish is an accurate way to assess the effects of xenobiotics compounds in experimental studies. This investigation presents a reliable indicator of the aquatic ecosystem contamination and the possible negative impact of the surrounding environment.

Key words: Environmental pollution, Heavy metals, Histopathology, Common carp, *Cyprinus carpio* L

INTRODUCTION

Marine environments near industrial and urban centers are contaminated with a wide range of chemicals that may be transformed into new potentially toxic compounds. The environmental conditions are not static and human influence has greatly stimulated the flow of environmentally deleterious changes by loading with chemicals to the aquatic system. The heavy metal and pesticide contamination of aquatic system has attracted the attention of researchers all over the world (Dutta and Dalal, 2008). In the last few decades thousands of organic trace pollutants are released into the environment (Vander Oost, *et al.*, 2003). Heavy metals cannot be destroyed through biological degradation. When exposed to higher concentrations, organs of aquatic animals may accumulate heavy metals (Pelgrom *et al.*, 1995, Grosell *et al.*, 1996, Kalay *et al.*, 1999, Mazon *et al.*, 2002 and Ashraf, 2005).

Heavy metals accumulated in the tissues of fish catalyze redox reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress and, therefore, cause biochemical and morphological alterations in fish (Varanka *et al.*, 2001 and Monteiro *et al.*, 2005). There is a positive link between the presence of certain xenobiotics in water and sediments which onset the sublethal effects in resident fish species in aquatic bodies. The xenobiotics initiate a specific enzyme that alters metabolism by cellular intoxication at cellular level and necrosis on a tissue level. Among the aquatic species the fishes are the major targets of heavy metal contamination.

Fishes come into contact with multiple metal contaminants in the aquatic environment and biomagnifies the pollutants. These pollutants built up in the food chain are responsible for adverse effects and death in the aquatic organisms (Farkas

*Corresponding author E-mail: swethasivani@yahoo.co.in

et al., 2002). Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution (Lopes et al., 2001, Whitefield and Elliott, 2002 and Dautremepuits et al., 2004).

Heavy metals undergo metabolic activation that provokes a cellular change in the affected fish. The tissue lesions and apoptosis arise from bioaccumulation, infections, diseases and parasites stimulate necrotic alterations in the fish with an inflammatory defensive reaction (Roganovic – Zafirova et al., 2003). It is imperative that histological biomarkers are the indicators of pollutants in the overall health of the entire population in the ecosystem (Velkova – Jordanoska and Kostoski, 2005). Well documented reports can be found in organs of fish exposed to heavy metals, chemical pollutants and microorganisms (Adham et al., 2002, Olojo et al., 2005, Roganovic – Zafirova and Jordanova, 1998 and Farombi et al., 2007). The freshwater fish, common carp (*Cyprinus carpio* L.) is of great commercial importance because it is the most common fish widely consumed worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. The investigation of histological changes in organs of fish is an accurate way to assess the effects of xenobiotics compounds in experimental studies. Hence, this study was undertaken to examine the effect of different heavy metals at sublethal concentrations on histology of gills, liver, kidney and flesh of common carp (*Cyprinus carpio* L.). This investigation presents a reliable indicator of the aquatic ecosystem contamination and the possible negative impact of the surrounding environment.

MATERIALS & METHODS

The freshwater *Cyprinus carpio* L. (Common carp) (10 – 13 cm length and 35.70 ± 0.60g) was collected from ponds of southern districts of Tamilnadu, India and was acclimated to laboratory conditions for a week. Twenty to twenty five individuals were used for the experiments. All the fish were kept in batches under constant temperature (25 ± 1°C) with a controlled photoperiod of 12:12 hour light and dark cycle and constant filtration. Analytical grade cadmium chloride, lead nitrate, potassium chromate and nickel sulphate supplied by BDH

(India) were used as metal toxicant through out the experiment. The fish used for this experiment were maintained in 200L recirculating tanks, filled with dechlorinated tap water. The physico - chemical characteristics of tap water were presented in (Table 1). The water in the control and experimental tanks was changed for every 3 days. The fish were divided into two groups, with the first group serves as control and remaining as experimental group. The experimental group was exposed to a sublethal concentration of 5 mg/L of combined (Cd + Pb + Cr + Ni) metal solution containing 1.25 mg/L of each metal ion (1/10th of LC50 / 48h) for a period of 32 days. The heavy metal concentrations were selected based on preliminary results, shown to be sublethal after a 32 day period of exposure. No fish mortality was observed during the experiment.

Table 1. Physico – chemical parameters of tap water used in the experimental ponds

Water Quality Parameters	Values (mg/L) Mean ± SD
pH	7.20 ± 0.2
Electrical conductivity (µS/cm)	1255.00 ± 0.5
Total dissolved solids	815.75 ± 0.3
Alkalinity	140.50 ± 0.2
Total hardness	280.45 ± 0.5
Calcium	120.25 ± 0.4
Magnesium	75.20 ± 0.3
Sodium	15.00 ± 0.6
Potassium	08.00 ± 0.3
Chloride	148.55 ± 0.4
Sulphate	68.65 ± 0.6
Total ammonia	10.80 ± 0.2

Note: The values were statistically significant at p < 0.05

The fish was fed with commercially available fish feeds at a daily rate of 3 – 4 % body weight through out the experiment. The fish were starved for 24 hours before experimentation. After dissection at least five sampled pieces of gills, liver, kidney and flesh were collected per fish (making a representative sample of the entire organ). A gill arch of the right side of each fish, liver, kidney and flesh samples were collected and fixed in 10% formalin for 24h, dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with hematoxylin – eosin (HE) method and approximately 2 – 4 sections of each individual fish were analyzed by light microscope.

RESULTS & DISCUSSION

The gill morphology of control common carp (*Cyprinus carpio* L.) remains an ordinary structure in which lamellae are lined by squamous epithelium composed of non differentiated cells (Fig. 1). The gill of heavy metal exposed group show some epithelial lesions when exposed to heavy metals. The filament region constitutes edema with intense lamellar vasodilatation. The gills exhibit a stratified fusion of pigments in numerous spaces are found in the gill of heavy metal exposed common carp (Fig. 2).

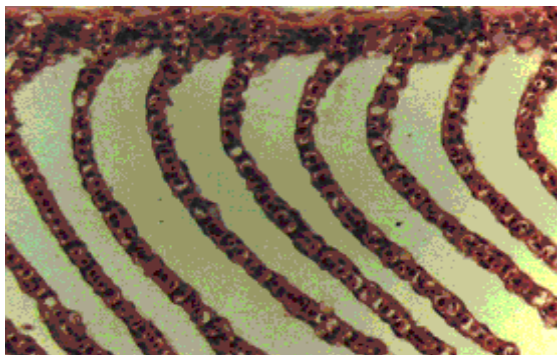


Fig. 1. Gill structure of control fish

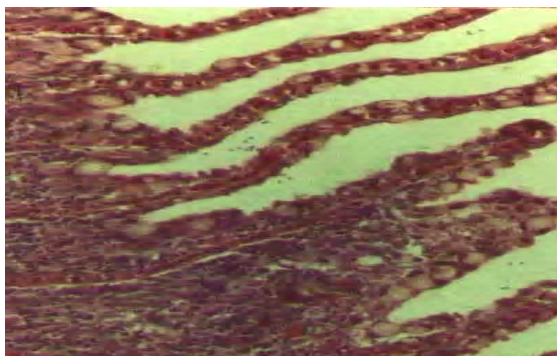


Fig. 2. Status of gill after heavy metal exposure

The liver sections of control hepatocytes exhibit a normal structure with no abnormalities in the hepatocytes. It exhibits a homogenous cytoplasm around the centrally located spherical nucleus (Fig. 3). The hepatic tissues of common carp pronounced pathological changes after 32 days of exposure exhibit a marked cytoplasmic vacuolization. The presence of hemosiderin a product of hemoglobin degradation (Khan *et al.*, 1994) results in internal bleeding in the liver tissues of common carp. The interruption of blood sinusoids separates the hepatic cords and the cells lost its normal size, number and structure. Liver parenchyma cells depict the accumulation of

macrophages contained lipofuscin pigments indicate the reaction of malondialdehyde in oxidative stress developed by heavy metals. The cells show an intrusion of fatty degeneration with liver fibrosis caused by infiltration of collagen fibers in the hepatocytes of common carp (Fig. 4).

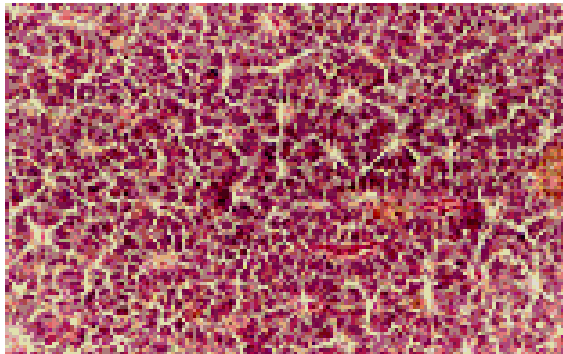


Fig. 3. Liver tissue of control fish

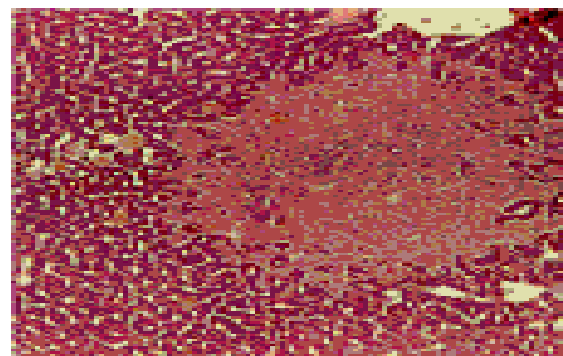


Fig. 4. Status of liver tissue after heavy metal exposure

The section of control kidney tissues exhibits an ordinary pattern with no abnormal changes in the cells (Fig. 5). There was an increased activity of connective tissues, particularly near the kidney tract. The circulatory disorders and numerous macrophages and inflammatory cells develop necrosis around the border of tissues changed the normal shape of kidney with a dislocation of epithelial cells were found in fish from a chronic toxicity of heavy metals. A selective dystrophic change in kidney tubules together with hyper secretion of mucous cells in the affected region showing atrophy in the underlying tissue was observed in heavy metal intoxicated groups. The pronounced activity of macrophages and large eosinophilous cells with granulated cytoplasm in the sub mucosa of gut wall in the kidney tubules showed a diminished appearance revealed the long term effect of heavy metals in the kidney of common carp tissues (Fig. 6).



Fig. 5. Kidney tissue of control fish

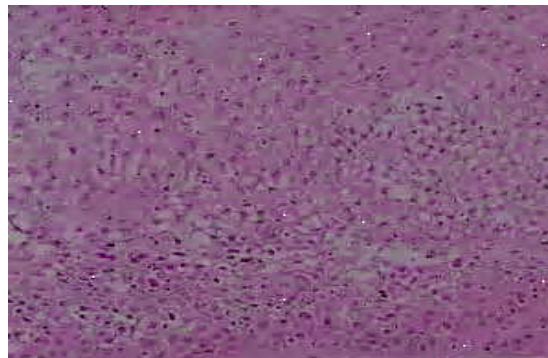


Fig. 7. Flesh structure of control flesh



Fig. 6. Kidney after heavy metal exposure

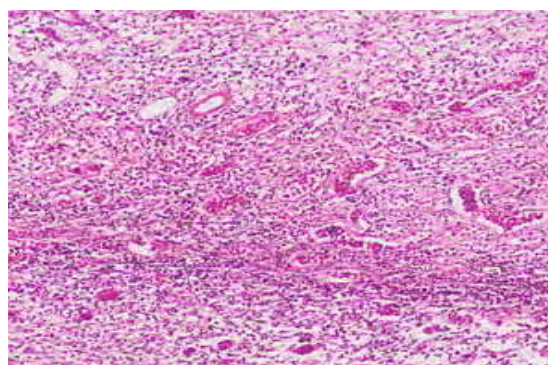


Fig. 8. Status of flesh tissue after heavy metal exposure

The flesh samples of control fish shows a normal appearance (Fig. 7). In case experimental fish, a severe regressive changes in flesh samples was accompanied with the spread of tissue injury. The toxicity in the flesh samples are characterized by disappearance of striations. The tissues involved in necrosis with a homogenous liquid appearance and loss of staining properties. Enlarge lesions in epidermis completely changed the architecture of external cell layer. A granulomatous lesion observed in the tissues was totally damaged proceeding to hyperplasia in cells (Fig. 8). The fish exposed to heavy metals reflect several histological alterations. The gills of control fish show a regular structural organization of the lamellae. The fish exposed to heavy metals dispute changes in histology with proliferation of epithelial cells, fusion and degenerative changes in the lamellae. Lesions are found in the target cells with the accumulation of edema. Several earlier studies reported that edema with frequent lesions in gill epithelium of fish exposed to heavy metals (Mallatt, 1985) specifically cadmium (Reid and Mc Donald, 1988) and nickel (Pane *et al.*, 2004). The higher accumulation of lead, cadmium, chromium and nickel induce lipid peroxidation and biochemical alteration in the antioxidant enzymes

could be toxic mechanism in the organs of exposed fish. The gills serve as a respiratory organ in fishes that has direct contact with water. The metals penetrate the epithelium of gills and develop oxidative stress in cells. It was found that heavy metals accumulated in the liver, kidney, heart and gills induce oxidative stress in fish (Farombi, 2007 and Oakes and Vander Kraak, 2003). The lesions found in gill structure can change the normal structure and function of cells. The present findings reflect similar trend in cadmium exposure to gills induce lesions in gill epithelium (Thophon *et al.*, 2003).

The liver of control fish provides normal structure with no pathomorphological abnormalities. A homogenous cytoplasm with a large central spherical nucleus is the characteristic nature of control hepatocytes. The histology of heavy metal exposed liver caused a reduction in size and shape of nucleus with degenerative changes in parenchyma cells with necrosis and apoptosis. The decrease number of nucleus in the hepatic tissues was reported in copper exposed to *Oreochromis niloticus* (Figueiredo, 2007). The concentration of heavy metals create an adduct in the liver cells due to their metal chelating proteins that target the

cells to release lipofuscin an end product of lipid peroxidation and pigment hemosiderin as a result of internal bleeding in the hepatic tissue of *Cyprinus carpio*. The higher accumulation of combined heavy metals in liver support other work of lead accumulation in the liver (Kargin and Cogun, 1999) and cadmium in liver (Kalay *et al.*, 1999). The characteristic appearance of liver fibrosis in the heavy metal exposed fish was supported by report of sunfish in Texas reservoir contaminated with selenium enriched power plant (Sorenson, 1988). The higher bioaccumulation of heavy metals in the kidney could be on specific metabolic process. Macrophages are key cells present in kidney dealing with foreign material and cellular debris (Blazer *et al.*, 1944 and Evans, 1998). The macrophages constitute lipofuscin, melanin and hemosiderin pigments. The present study revealed the presence of hemosiderin pigment in the heavy metal intoxicated kidney tissues supported by exogenous environmental factors like contaminants influence macrophage pigment composition (Blazer *et al.*, 1994 and Kruger *et al.*, 1996). The tissues in flesh samples of intoxicated group show damaged structure with the invasion of cytoplasm. The lesions on the exposed group in turn cause muscle destruction. The affected flesh region arise giant granulomas that undergone necrotic changes in the tissues of heavy metal exposed fish. The present investigation was supported by Kabata arthuri infection in muscle fibres of sutchi cat fish, *Pfangasius sutchi* (Dykova, 2000).

CONCLUSION

Histopathological alterations in common carp under the influence of heavy metals can be used as a sensitive model to monitor the aquatic pollution. The current result indicates that the heavy metal contamination definitely affect the gills by proliferation of epithelial cells and accumulation of edema in the target cells. Liver tissue generates hemosiderin and cytoplasmic vacuolization as a result of metal toxicity. Aggressive invasion of macrophages and giant granulomas observed in the kidney and flesh tissues were the characteristic changes at the cellular level of fresh water fish. Hence, a scientific method of detoxification is essential to improve the health of these economic fish. The present research work served as an experimental

tools and bioindicators for the first line evaluation of environmental pollution.

ACKNOWLEDGEMENT

The authors wish to thank our college Principal Rev. Dr. Alphonse Manickam SJ and Prof. M.Thomas Punithan, Head of the Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamilnadu, India, for their kind encouragement and their laboratory facilities. This paper forms a part of the Ph.D thesis submitted by R.Vinodhini to M.S. University, Tirunelveli, Tamilnadu, India.

REFERENCES

- Ashraf, W., (2005). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environ. Monit. Assess.*, **101**, 311.
- Blazer, V.S., Fournie, J.W. and Weeks – Perkins, B.A., (1997). Macrophage aggregates: Biomarker for immune function in fishes? In: *Environmental Toxicology and Risk Assessment: Modeling and risk assessment (Sixth Volume)*, ASTM STP 1317, Ed: Dwyer, J.F., Doane, T.R., Hinman, M.L., American society of testing and materials, 360 – 375.
- Blazer, V.S., Facey, D.E., Fournie, J.W., Courtney, L.A. and Summers, J.K., (1994). Macrophage aggregates as indicators of environmental stress. In: *Modulators of fish immune responses*, SOS Publications, Ed: Stolen, J.S., Fletcher, Fair Haven, New Jersey. **1**, 169 – 185.
- Dautremepuits, C., Paris – Palacios, S., Betoulle, S. and Vernet, G., (2004). Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. *Com. Biochem. Physiol. C. Toxicol. Pharmacol.*, **137**, 325 – 333.
- Dutta, H. M. and Dalal, R., (2008) The Effect of Endosulfan on the Ovary of Bluegill Sunfish: A Histopathological Study (*Lepomis macrochirus* sp). *Int. J. Environ. Res.*, **2(3)**, 215-224.
- Farkas, A., Salanki, J and Specziar, A., (2002). Relation between growth and the heavy metal concentration in organs of bream, *Abramis brama* L. populating lake Balaton. *Arch. Environ. Contam. Toxicol.*, **43(2)**, 236 – 243.
- Farombi, E. O., Adelowo, O.A. and Ajimoko, Y. R., (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun river. *Int. J. Environ. Res. Public Health.*, **4(2)**, 158 – 165.
- Figueiredo – Fernandes, A., Ferreira – Cardoso, J.V., Garcia – Santos, S., Monteiro, S.M., Carrola, J., Matos, P., and Fontainhas – Fernandes, A., (2007).

- Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesq. Vet. Bras.*, **27**(3), 103 – 109.
- Grosell, M., Boetius I., Hansen, H.J.M. and Rosenkilde, P., (1996). Influence of preexposure to sublethal levels of copper on Cu – 64 uptake and distribution among tissues of the European eel (*Anguilla anguilla*). *Comp. Biochem. Physiol. C.*, **114**, 229 – 235.
- Iva Dykova and Jiri Lom., (2000). Histopathology of *Kabatana arthuri* (Microspora) infection in sutchi catfish, *Pangasius sutchi*. *Folia Parasitologica.*, **47**, 161 – 166.
- Kalay, M., Ay, P. and Canil, M., (1999). Heavy metal concentration in fish tissues from the northeast Mediterranean Sea. *Bull. Environ. Contam. Toxicol.*, **36**, 671 – 673.
- Kalay, M., Ay, P., Canil, M., (1999). Heavy metal concentration in fish tissues from the northeast Mediterranean Sea. *Bull. Environ. Contam. Toxicol.*, **63**, 671 – 673.
- Khan, R.A., Barker, D.E., Hooper, R., Lee, E.M., Ryan, K. and Nag, K., (1994). Histopathology in Winter Flounder (*Pleuronectes americanus*) living adjacent to a pulp and paper mill. *Arch. Environ. Contam. Toxicol.*, **26**, 95 – 102.
- Kargin, F. and Cogun, H.Y., (1999). Metal interactions during accumulation and elimination of zinc and cadmium in tissues of the fresh water fish, *Tilapia nilotica*. *Bull. Environ. Contam. Toxicol.*, **63**, 511 – 519.
- Kruger, R., Pietrock, M., Meinneit, T., Yoshida, T., Steffens, W. and Steinberg, Ch., (1996). Distribution of macrophage centres in bream (*Abramis brama* L.) liver from the Oder river (Germany / Poland) within the nature reserve “Unteres Odertal” near the town of Schwedt. *Int. Rev. Ges. Hydrobiol.*, **81**(4), 635 – 644.
- Lopes, P.A., Pinheiro, T., Santos, M.C., da Luz Mathias, M., Collares – Pereira, M.J. and Viegas – Crespo, A.M., (2001). Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. *Sci. Total. Environ.*, **280**, 153 – 163.
- Mallat, J., (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Can. J. Fish. Aquat. Sci.*, **42**, 630 – 648.
- Mazon, A.F., Cerqueira, C.C.C. and Fernandes, M.N., (2002). Gill cellular changes induced by copper exposure in the South American tropical freshwater fish *Prochilodus scrofa*. *Environ. Res.*, **88**, 52 – 63.
- Monteiro, S.M., Mancera, J.M., Fontainhas – Fernandes, A. and Sousa, M., (2005). Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. *Comp. Biochem. Physiol. C.*, **141**, 375 – 383.
- Oakes, K.D. and Vander Kraak, G.J., (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquat. Toxicol.*, **63**(4), 447 – 463.
- Pane, E.F., Haque, A. and Wood, C.M., (2004). Mechanistic analysis of acute, Ni- induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon. *Aquat. Toxicol.*, **69**, 11 – 24.
- Pelgrom, S., Lamers, L., Lock, R., Balm, P. and Wendelaar Bonga, S., (1995). Integrated physiological response of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. *Aquat. Toxicol.*, **32**, 303 – 320.
- Reid, S.D. and Mc Donald, D.G., (1988). Effects of cadmium, copper and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. *Can. J. Fish. Aquat. Sci.*, **45**, 244 – 253.
- Roganovic – Zafirova, D. and Jordanova, M., (1998). Histopathological analysis of liver from Ohrid roach (*Rutilus rubilio ohridanus*) collected in Gransnica, a contaminated lake site of lake Ohrid. *Special Issues of Macedonian Ecological. Society.*, **5**(1-2), 530 – 544.
- Roganovic – Zafirova, D., Jordanova, M., Panov, S. and Velkova – Jordanoska, L., (2003). Hepatic capillaritis in the Mediterranean barbell (*Barbus meridionalis petenyi* heck.) from lake Ohrid. *Folia Veterinaria.*, **47**(1), 35 – 37.
- Sorenson, E.M.B., (1988). Selenium accumulation, reproductive status and histopathological changes in environmentally exposed redear sunfish. *Arch. Toxicol.*, **61**, 324 – 329.
- Thophon, S, K., Kruatrachue, M., Upathan, E. S., Pokhithiyook, P., Sahaphong, S. and Jaritkhuan, S., (2003). Histopathological alterations of white sea bass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environ. Pollut.* **121**, 307 – 320.
- Vander Oost, R., Beyer, J. and Vermeulen, N.P.E., (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.*, **13**(2), 57 – 149.
- Varanka, Z., Rojik, I., Varanka, I., Nemcsok, J and Abraham, M., (2001). Biochemical and morphological changes in carp (*Cyprinus carpio* L.) liver following exposure to copper sulphate and tannic acid. *Comp. Biochem. Physiol. C.*, **128**, 467 – 478.
- Velkova – Jordanoska, L. and Kostoski, G., (2005). Histopathological analysis of liver in fish (*Barbus meridionalis petenyi* heckle) in reservoir Trebenista. *Nat. Croat.*, **14**(2), 147 – 153.
- Whitefield, A.K. and Elliott, M., (2002). Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. *J. Fish. Biol.*, **61**(1), 220 – 250.