

Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham)

S. V. Deore* and S.B Wagh¹

* Smt. Pushpatai Hiray Arts, Science and Commerce Mahila Mahavidyalaya, Malegaon Camp, Malegaon, Nashik, India

¹Department of Zoology Dr. B. A. M. University, Aurangabad, India

Abstract

Discharge of heavy metals into aquatic environment from various sources even below permissible levels, creates health hazards in aquatic organisms. The persistence and ubiquitous nature of these pollutant compounds coupled with their tendency to accumulate in organisms ultimately produce toxic reaction in aquatic biota especially, fish. This study aims to investigate histopathological impact of lethal (1.0625 ppm, 1.4202ppm) and sublethal (0.1062ppm, 0.0531ppm and 0.1420ppm, 0.0710ppm) concentrations of mercury chloride and copper chloride in liver of fresh water teleost, *Channa gachua* (Ham). The histopathology studies revealed vacuolation in cytoplasm, degeneration of nuclei, vacuolation in stroma, cloudy swellings, pycnotic nuclei, necrosis, rupture of blood sinusoids, disarray of hepatic cords, loss of shape of hepatocytes. Severity of damage was found to be dose dependent and time of exposure.

Keywords: Histopathology, Heavy metals, necrosis, cloudy swelling, disarray, *Channa gachua*.

INTRODUCTION

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001; Voegborlo et al, 1998). The natural aquatic system may extensively be contaminated with heavy metals released from domestic, industrial and other man made activities (Velez and Montoro, 1998; Conacher et al 1993). Heavy metal contamination may have devastating effect on the ecological balance of the recipient environment and diversity of aquatic organisms (Farombi et al, 2007; Vosyliene and Jankiite, 2006; Ashraj, 2005). The chief source of contaminants are industrial waste discharge, mining, agriculture, household waste disposal and fuel combustion (Woodling et al 2001; Patra et al 2005 and Swarup et al 2006; Saxsena and Garg, 2011). It appears that problems of heavy metal accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Das and Kaviraj, 2000; Laxi, 2005; Jayakumar Paul, 2006; Kumar et al 2007, 08). Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro et al 2005).

Histopathology deals with the study of pathological changes induced in the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substance. Thus study of histopathology is of prime importance in the diagnosis, etiology and prevention of disease. In fishes, it is observed that the external organs are affected due to toxic chemicals, causing loss of equilibrium, increase in opercular movements, to and fro irregular vertical movements, finally leading to

death. This may be attributed to the significant damage to the internal organs. Histopathological study thus gives us useful data concerning tissue change prior to external manifestation.

MATERIALS AND METHODS

For studies of histopathological changes, the live test fish, *Channa gachua* were collected from Mosam river near Malegaon, brought to the laboratory, cleaned by using 0.1% KMnO₄ to avoid dermal infection. These fish were acclimatized in the laboratory for 2 week prior to the experimentation. Fish showing normal activities were selected for each test. In first set fish were exposed to 96 hours LC₅₀ concentration of mercury chloride (1.062 ppm) and copper chloride (1.4202 ppm). Above values of lethal concentrations are obtained in earlier work. At the end of acute exposure the survived fish were decapitated and immediately liver tissue was removed and fixed in aqueous Bouin's fluid for 24 hours, This tissue was dehydrated in different alcohol grades and blocks were prepared in paraffin wax (58° to 60°C). The sections of 5µ to 6µ were cut and stained with Mallory's triple stain (Mallory 1944) and mounted in DPX.

The same procedure was repeated for liver tissue of control fish as well as fish exposed to sublethal concentrations of mercury chloride and copper chloride. Sublethal concentrations were 1/10th and 1/20th of 96 hrs LC₅₀ concentrations of mercury chloride (0.1062 and 0.531 ppm) and copper chloride (0.1420 and 0.0710 ppm).

RESULTS

Metabolism of food items, their storage and detoxification are the important functions of liver. Toxic substances reach liver through blood. Hence liver is susceptible to number of toxic substances and metabolic distributions.

The histopathological changes observed in present investigation after exposure to lethal and sublethal concentrations of mercury chloride and copper chloride in the liver of test fish *Channa gachua* have been depicted in photo plate 1 (B to E). The liver of the fish exposed to heavy metal compounds exhibited marked histopathological alterations.

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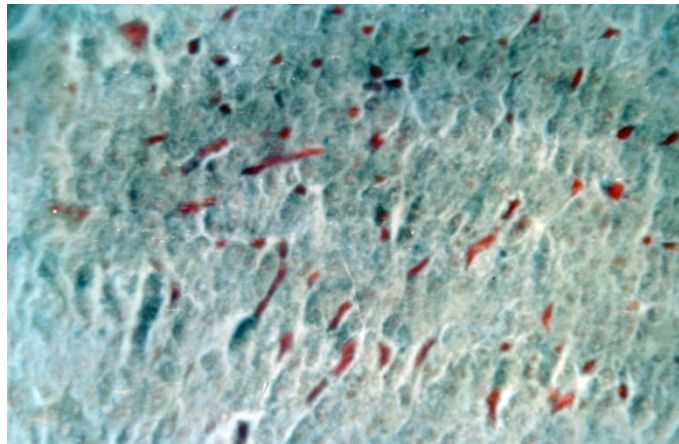
*Corresponding Author

S. V. Deore
Smt. Pushpatai Hiray Arts, Science and Commerce Mahila Mahavidyalaya,
Malegaon Camp, Malegaon, Nashik, India

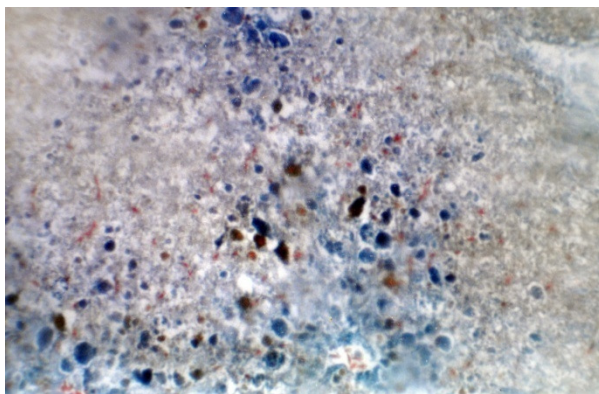
Tel: + 917709909741
Email: svdeore1@gmail.com

Liver of test fish *Channa gachua* exposed to lethal concentrations (96 hrs LC50 concentrations) of mercury chloride and copper chloride showed vacuolation in the cytoplasm, degeneration of nuclei, vacuolation in stroma. The alterations in liver of fish exposed to sublethal concentration of mercury chloride were, cloudy swellings of the cells with large vacuoles, degeneration of nuclei, vacuolation in stroma, pycnotic nuclei, shifting of nuclei on one side

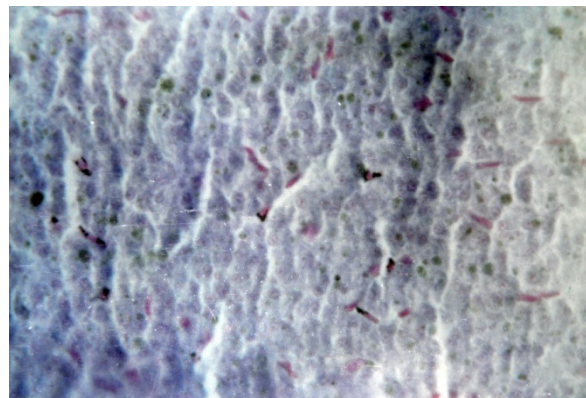
of the cell, prominent necrosis. The changes observed due to exposure to sublethal concentrations of copper chloride include rupture of blood sinusoids, disorganised (disarray) hepatic cords, loss of shape of hepatocytes. Severity of damage was more in mercury chloride exposed fish than copper chloride exposed fish. It was also found to be dose dependent and time of exposure.



A. Micro photograph showing T.S liver of *C.gachua* Control MTx400

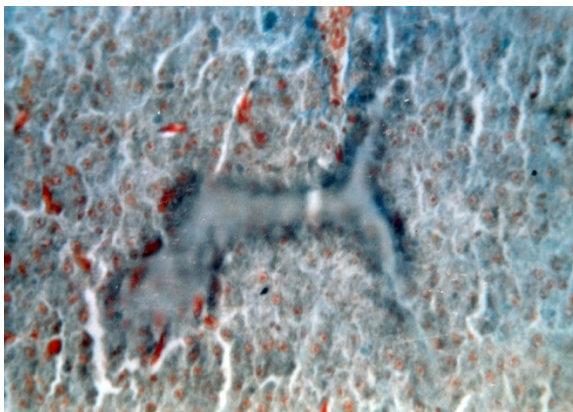


B. Micro photograph showing T.S liver of *C.gachua* 96 hrs exposure to mercury chloride (1.06 ppm)

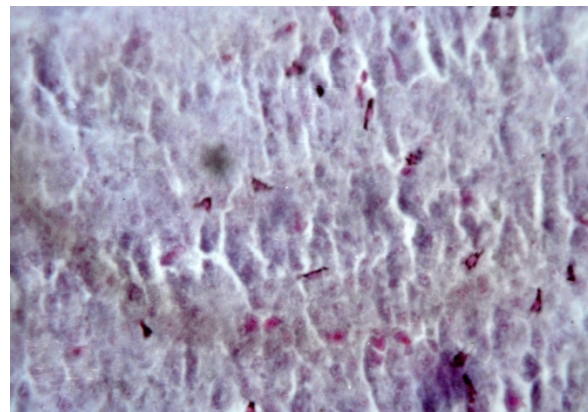


C. Micro photograph showing T.S liver of *C.gachua* 96 hrs exposure to copper chloride (1.47 ppm)

MTx400



D. Micro photograph showing T.S liver of *C.gachua* 45 days exposure to mercury chloride (0.106 ppm)



E. Micro photograph showing T.S liver of *C.gachua* 45 days exposure to copper chloride (0.147ppm)

MTx400

DISCUSSION

The toxicity effect of heavy metals and pesticides on liver have been studied by many workers. Sastry and Gupta (1978) reported liver cord disarray, shrinkage in the liver cells, degenerated nuclei and focal necrosis in *Channa punctatus* due to lead intoxication. Benedetti et al (1981) reported cytoplasmic vacuolation in hepatocytes of liver of *Ictalurus nebulosus* due to copper pollution. Kumar and pant (1981) studied vacuolation within and outside the hepatocytes, severe necrotic changes in liver, breakdown of cellular boundary, vacuolation in liver of *Puntius conchoniensis* induced by copper and zinc intoxication. Singh (1983) reported vacuolation and necrosis in liver of *Colisa fasciatus* exposed to copper sulphate. Dalela et al (1984) reported necrosis, hypertrophy and atrophy in the liver tissues, loss of polygonal shape of liver cells, splitting of the cells and formation of spaces in the tissues after exposure of *Cyprinus carpio* to lethal and sublethal concentration of copper and cadmium. Bakre (1985) reported cellular damage, nuclear hypertrophy of hepatocytes, vacuolation and necrosis leading to lysis, increase in blood sinuses, bile canaliculi and bile pigments become prominent in *Gambusia affinis* on exposure to mercury chloride. Ram and Sathyanesen (1987) reported hyperplasia, nuclear pycnosis, fatty necrosis, degeneration of hepatocytes leading to tumor and Sycytium formation, blood vessel congestion, oedema, marked reduction in hypatosomatic index in *Channa punctatus* exposed to mercurial fungicide. Ramlingam (1988) reported necrosis, fatty degeneration, red blood cell occlusion in portal vessels, engorged blood vessel congestion, vacuolar degeneration of hepatocytes, in the liver of *Sartherodon mossambicus*. Khangarot (1992) reported cellular necrosis, clumping of chromatin and its aggregation at the centre, loss of nuclear membrane of hepatocytes after exposure to copper in *Channa punctatus*. Roncero et al (1992) reported intense hemolysis, massive necrosis liver parenchyma after acute exposure to copper sulphate in *Tinca tinca* L. Similar histopathological alterations were observed by Figueiredo et al (2007) in liver of *Oreochromis niloticus* exposed to copper, Grosell M et al (1996) in tissues of *Anguilla anguilla* on exposed to copper, Roganovic et al (1998) in liver of *Rutilus rubiliohridanus* from heavy metal contaminated lake, Roganovic et al (2003) in hepatic capillaries in *Barbus meridionalis* (Petenyiheck), Varanka et al (2001) in liver of *Cyprinus carpio* L on exposure to copper sulphate.

CONCLUSION

Heavy metals like mercury and copper enter the aquatic ecosystem through a wide spectrum of natural source such as volcanic activities, erosion and anthropogenic ones including industrial wastes as well as a leakage and get further biomagnified in the food chain. Histopathological alterations in fresh water test fish 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 liver showing vacuolation in cytoplasm, in stroma, degeneration of nuclei cloudy swelling of the cells, pycnotic nuclei shifting of nuclei on one side of the cell. Prominent necrosis, rupture of blood sinusoids, disarray of hepatic cords, loss of shape of hepatocytes. Hence a scientific method of detoxication 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.

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