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Full Length Research paper

Metals and histopathological alterations in the liver of *Schizothorax niger,* Heckel from the Dal Lake of Kashmir Valley

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The study was conducted to evaluate the presence and subsequent effect of trace metals (copper, zinc, iron and manganese) on the histomorphology of liver of *Schizothorax niger* (endemic fish) from Dal lake of Kashmir Valley. Using Atomic Absorption Spectrophotometry, the varied seasonal metal concentrations for copper (66.77 ± 3.12 to 81.68 ± 3.51 ppm), zinc (73.81 ± 2.52 to 97.84 ± 4.62 ppm), iron (204.92 ± 5.21 to 296.51 ± 4.37 ppm) and manganese (01.13± 0.02 to 08.30 ± 1.00 ppm) were observed in the liver of hosts during the study period of two years. The highest concentration of metals was observed in the summer seasons and the lowest concentrations in the winter seasons during the entire study period. Further, histochemical analysis was done to crosscheck the presence of different metals in the tissue sections of liver of *S. niger*. The subsequent effects of metals was demonstrated histologically using haematoxylin and eosin stain that showed disruption of the hepatic cords and tubules with congestion and degenerative changes in hepatocytes that varied from mild in winter seasons to severe vascular degeneration in summer season. From the present study it may be concluded that the metals in the environment are polluting the water bodies and their subsequent deleterious effects harm the aquatic fauna particularly the sensitive native fish, *S. niger* which is one of the reasons for its decline from the fresh water resources of the Kashmir Valley.

Key words: Contamination, histological changes, *Schizothorax niger*, metals, liver.

INTRODUCTION

The varieties of human activities acting upon the natural environment result in the release of different chemicals including metals. The sources of metals include commercial fertilizers, sewage, sludge's, urban wastes, liming material and agrochemicals and other wastes used as soil amendments (Rao, 1998). Trace metals like Zn, Cu, Fe and Mn are required for metabolic activities in organisms and there lies a narrow "window" between their essentiality and toxicity. Other heavy metals like Cd, Hg, Cr and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic environments (Fatoki and Mathabatha, 2001). Analysis of fish metal burden has shown that the fish concentrated trace metals a thousand times above the levels existing in the exposure medium. Some metals were preferentially accumulated more than others and the accumulation was, in decreasing order, Pb, Fe, Zn, Cu, Mn, Cr, Ni, and Cd. Whole fish metal burden was lower in fish from which the gill, liver, and kidney had been removed, suggesting that these organs accumulated the metals more than other tissues (Onwumere and Oladimeji, 1990). The exposure of bio-organisms to metals can cause long-term and non-reversible effects (Cheng, 2003). Fish species are widely used to biologically monitor variation in environmental levels of anthropogenic pollutants (Whyte et al., 2000;

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Schmitt, 2004; Whitfield and Elliott, 2002).

The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. It is a major site of the accumulation, biotransformation and excretion of xenobiotics compounds (Meyers and Hendricks, 1985). From an environmental point of view, bioconcentration is important because metal ions usually occur in low concentrations in the aquatic environment and subtle physiological effects go unnoticed until gross chronic reactions (e.g. changes in populations structure, altered reproduction, etc.) become apparent (Carpene et al., 1990; Wicklund-Glynn, 1991). Because of its unique position and proximity to the venous drainage of the digestive tract, the liver is susceptible to damage from absorbed toxic materials (Leeson and Leeson, 1976). The high degree of metabolic activity of hepatocytes renders them vulnerable and toxins can easily affect them. The harmful effects of ingested toxic substances are primarily exerted within the liver cells (Lloyd, 1992). Subsequently, hepatocytes respond to changes in the external and internal environments by alterations in both cellular structure and function (Wheater et al., 1985). Lloyd (1992) has also reported that histopathological alterations are recognized and commonly used diagnostic tools in fish toxicological studies. The present study was therefore undertaken to prove the use of liver as an indicator of trace metals pollution in naturally occurring fresh water body of Dal Lake.

MATERIALS AND METHODS

Collection of Fish Hosts

Fishes were collected from the Dal Lake with the help of local fishermen and were brought alive in plastic buckets to the laboratory for investigating the different parameters.

Species and number of fish used

The pooled specimens of *S. niger* Heckel were collected from the different sites of the Dal Lake so as to make a sample size of 25 fish (of either sex) with an average length of 30-40 cms. The study was repeated for each season for the year-I and again during Year-II.

Seasonal classification

The study was conducted in four seasons annually, each with duration of 3 months. The four seasons included spring (March-May), summer (June-August), autumn (September-November) and winter (December-February).

Metal Analysis of Water

For detection of metals in water, the samples were collected in conical flasks, filtered through Whatman's filter paper and processed in Atomic Absorption Spectrophotometer (AAS) for estimation of various metal concentrations employing Lindsay and Norwell Method (1978). Similarly, the tissue samples of liver were dried at 1200C for 48 hours weighed and incinerated at 5500C for 8-10 hours. 1gm of oven dried sample was taken and further processed for metal detection using the Lindsay and Norwell Method.

Histochemical Demonstration of Metals

For detection of metals viz copper, iron and zinc in fish liver different histochemical methods such as Perl's method for Iron; Dithiooxamide method for copper; Dithizone method for zinc etc (Luna, 1968) were used so as to ensure metal induced toxicity.

Histological Procedure

Histological examination was done after fixing the liver in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 µm thick and stained with Harris haematoxylin and eosin (H&E) (Luna 1968).

RESULTS

Metal concentrations in water

In Dal lake the concentration of copper was in the range of 1.020 to 1.070 ppm, with maximum concentration found in summer season (year-II) and the minimum in winter season (year-I). The iron concentration ranged between 0.110 to 0.191ppm. The highest value was observed during summer season (year-II) and the minimum in winter season (year-I). The zinc concentration ranged between 0.150 to 0.542 ppm, with maximum value observed in summer season (year-II) and the lowest values in winter season (year-I). The manganese concentration ranged between 0.021 to 0.083 ppm with maximum value observed in summer season year-II and the lowest values in winter season (year-I).

Metal concentrations in the Liver

In *S. niger*, the mean concentration of copper in the liver ranged from 66.77 $\pm$ 3.12 to 81.68 $\pm$ 3.51 ppm (Table 1). The maximum value of 81.68 $\pm$ 3.51 ppm was observed

Table 1. Showing Copper concentration in the liver of *Schizothorax niger* in different Seasons of the study period in Dal lake.

Water resource	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	70.01 ± 2.12	76.52 ± 2.81	68.52 ± 2.12	66.77 ± 3.12
		II	25	74.54 ± 3.24	81.68 ± 3.51	72.82 ± 3.24	70.54 ± 3.12

Values are expressed as mean ±SEM

Table 2. Showing zinc concentration in the liver of *Schizothorax niger* in different Seasons of the study period in Dal lake.

Water resource	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	74.52 ± 2.24	90.61 ± 3.92	75.12 ± 4.77	73.81 ± 2.52
		II	25	81.06 ± 3.44	97.84 ± 4.62	82.52 ± 3.99	80.88 ± 4.15

Values are expressed as mean ±SEM

Table 3. Showing iron concentration in the liver of *Schizothorax niger* in different Seasons of the study period in Dal lake.

Water resource	Fish Host	Year	No. Observed	Iron accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	227.91 ± 6.52	284.31 ± 4.29	228.36 ± 6.55	204.92 ± 5.21
		II	25	241.20 ± 6.96	296.51 ± 4.37	242.54 ± 4.85	234.56 ± 5.95

Values are expressed as mean ±SEM

Table 4. Showing manganese concentration in the liver of *Schizothorax niger* in different Seasons of the study period in Dal lake.

Water resource	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	01.13 ± 0.02	07.13 ± 0.99	03.16 ± 0.98	02.81 ± 0.05
		II	25	02.74 ± 0.03	08.30 ± 1.00	04.74 ± 0.11	03.55 ± 0.07

Values are expressed as mean ±SEM

in summer (year-II) and the lowest value of 66.77 ± 3.12 was observed in winter season (year-I).

The concentration of zinc in the liver was 73.81 ± 2.52 to 97.84 ± 4.62 ppm (Table 2). The maximum value of 97.84 ± 4.62 ppm was observed in the summer season of year-II and the minimum value of 73.81 ± 2.52 ppm was observed in the winter seasons of year-I.

The concentration of iron in liver varied between 204.92 ± 5.21 to 296.51 ± 4.37 ppm (Table 3). The maximum values of 296.51 ± 4.37 ppm in liver was observed in the summer (year-II) and the minimum values of 204. 92 ± 5.21 ppm in liver was observed in the winter season (year-I).

The concentration of manganese in liver ranged between 01.13± 0.02 to 08.30 ± 1.00 ppm (Table 4). The highest values of 08.30 ± 1.00 ppm in liver was observed in summer season of year-II. The lowest value of 01.13 ± 0.02 ppm in liver was observed in the

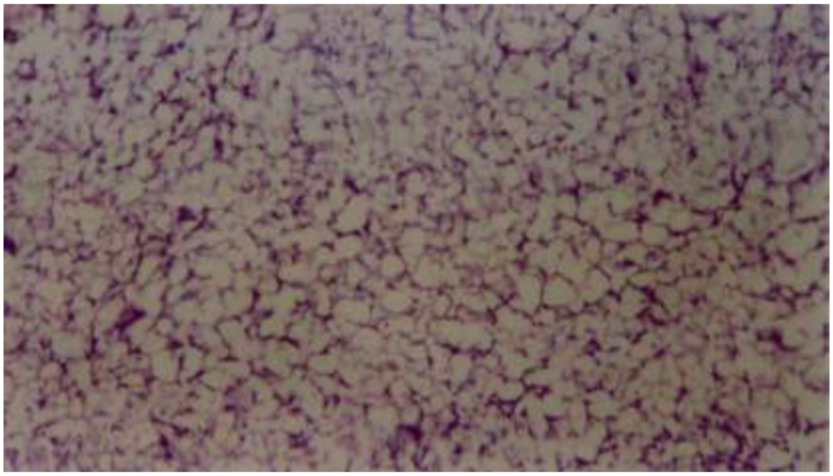

Figure 1. Histological section of fish liver showing severe congestion and significant fat accumulation in hepatocytes. H&E. 100X.

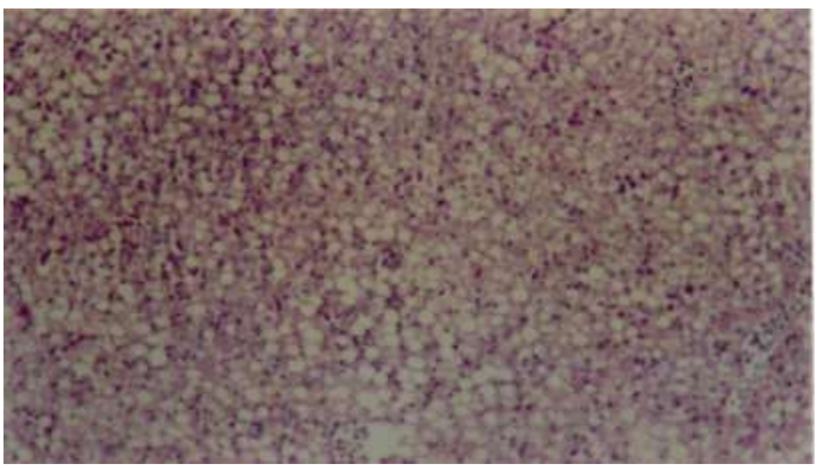

Figure 2. Histological section showing Kupffer cell hyperplasia after the damage of liver parenchyma. H&E. 100X.

spring season of year-I.

Histological Changes

The histological sections of liver showed disruption of the hepatic cords and tubules with congestion and degenerative changes in hepatocytes that varied from mild in winter seasons to severe vascular degeneration in summer season (Figure 1-2). Further, Kupffer cell hyperplasia was noticed in the liver of Schizothorax niger (Figure 2).

DISCUSSION

In Dal Lake the concentration of copper was in the range of 1.020 to 1.070 ppm; iron 0.110 to 0.191ppm; zinc 0.150 to 0.542 ppm and manganese 0.021 to 0.822 ppm. Metal accumulations can be attributed to a variety of sources-such as rocks, solids, dead and decomposing vegetation, wet and dry fallout of atmospheric particulate matter and from human activities including the discharge of various treated and untreated liquid wastes into the water bodies (Lasheen, 1987). The concentration of metals in the Dal waters can be attributed to its stagnant waters. Seasonal differences were also observed with higher concentrations during the summers, followed by spring, autumn and winter. This may be due to higher fallout of metals from the decomposing matter and increase in temperature during the hot seasons, which gradually reduce during the colder months. Further, it is generally accepted that heavy metal uptake occurs mainly from water, food and sediment (bottom feeders and burrowing animals) (Canli et al., 1998). However, the metal uptake from water is much higher than uptake from sediment (Mance, 1987; Langston, 1990; Merian, 1991). It may be emphasized that the efficiency of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism and the contamination gradients of water and food and sediment, as well as other factors such as salinity, temperature and interacting agents (Cusimano et al., 1986; Heath, 1987; Canli and Furness, 1993; Goyer, 1991; Canli and Furness, 1995). Years-wise data showed a higher heavy metal concentration in the latter year than the preceding in both water bodies. This clearly suggested an increase in pollution levels in the water body.

Season-wise higher tissue concentrations of heavy metals were observed in summer with decline in their levels during spring, autumn and winter in a decreasing order. Obviously, the progressive increase in the metal levels in the tissues coincides with the period of rising temperatures during summer. It is generally accepted that metal accumulation in living organisms is largely controlled by specific uptake, detoxification and elimination mechanisms and therefore depends significantly on the season (Cogun et al., 2006). Seasonal differences in the heavy metal accumulation in fish can be related to their metabolic rate, which determines the physiological condition of fish (Farkas et al., 2003). Laboratory experiments have shown that changes in temperature can affect the increase or decrease of heavy metal concentrations because of changes in metabolic and excretion rates (Hilmy et al., 1987; Yang and Chen, 1996). The copper was found to be greater in amount in the fish tissues during the present research study and can be attributed to the fact that it has a tendency to accumulate to a greater extent than other essential elements, such as zinc and iron (Heath, 1987; Roesijadi and Robinson, 1994). Fish are naturally exposed to a variety of metals including both essential and non-essential elements. Copper is one of the essential metals that after absorption from gills and intestines is transported by metallothionein into the blood circulation and some of it accumulates in different internal organs especially liver and kidneys (Peyghan et al., 2003).

It is generally accepted that metal accumulation in tissues of aquatic animals is dependent upon exposure concentration and period as well as some other factor such as salinity, temperature, interacting agents and metabolic activity of tissue. Similarly, it is also known that metal accumulation in tissues of fish is dependent upon the rate of uptake, storage and elimination (Health, 1987; Langston, 1990; Roesijadi and Robisnson, 1994).

In terms of zinc toxicity, the concentrations of the metal within certain tissue may be associated with mortalities (Zitko, 1979) and sub lethal effects such as behavioral and physiological disruptions (Buikema et al., 1982). The analysis of the zinc in different tissues of fish hosts observed in the present study during different seasons showed higher concentration in summer. These observations are similar to findings of Velcheva (2006), who reported higher zinc content in summer and autumn than spring and winter in the water and fish tissue of both Kardjali and Studen Kladenets dam lakes in Bulgaria. Other studies have shown that zinc possesses affinity to protein sulfhydryl groups and its increased load in the kidneys and liver lead to a release of a specific metal protein, metallothioneine from these organs (Cosson, 1994; Vilella et al., 1999).

Fish acquire iron predominantly from the diet and its uptake varies in different organs (Andersen, 1997; Bury et al., 2001). The highest concentration of iron in liver observed in the present study can be attributed to the fact that liver is the main storage pool for iron in fish (Van Dijk et al., 1975; Walker and Fromm, 1976). Further studies have shown that liver, which is a major producer of metal-binding proteins, show high concentration of metals (Roesijadi and Robinson, 1994; Allen, 1994).

Manganese, which is required in trace amounts by the fish hosts, was found to be predominant in summer followed by autumn, spring and winter in both fishes. Excess external concentration of manganese in the medium could lead to high internal levels and thereby interfering with enzymatic activity or other metabolic functions (Gonzalez et al., 1990). However, its concentration was found to be lower than other observed metals viz. copper, iron and zinc. This can be attributed to the fact that fish can regulate the amount of manganese in their body (Kwasnik et al., 1978).

The study confirms the presence of trace metals in the fresh water body of Kashmir Valley. The native fish thereby, accumulates trace metals from the polluted waters and develop histomorphological alterations. These metal-induced histological changes may also be responsible for the mortality of the hosts. Thus trace metal pollution seems to be one of the obvious reasons of a drastic decline of the sensitive native fish from the Dal Lake of Kashmir Valley.

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