Original Research

Metals and Histopathological Alterations in the Kidneys of Schizothorax niger, Heckel from the Dal Lake of Kashmir Valley

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Abstract

The study was conducted to evaluate the metal induced abnormalities in the kidneys of *Schizothorax niger,* Heckel from Dal lake seasonally for a period of two years. The varied seasonal metal concentrations for copper (64.61 ± 3.10 to 78.90 ± 3.42 ppm), zinc (88.77 ± 3.52 to 101.99 ± 4.03 ppm), iron (200.99 ± 5.04 to 292.61 ± 4.25 ppm) and manganese (0.84 ± 0.06 to 06.95 ± 0.93 ppm) were observed during the entire period of study. The highest concentration of metals was observed in the summer seasons and the lowest concentrations in the winter seasons during the study period. Further, histochemical analysis demonstrated high levels of metal ion (Cu, Fe and Zn) in the kidneys of the fish in summer seasons during the study period. Analysis of these levels demonstrated by wet digestion-based Atomic Absorption and combined with histochemical methods, showed probable relationship between these high metallic levels and kidney pathology. The general changes in the kidneys of the host included tubular atrophy with hypercellularity and hyperplasia. The other changes in the kidneys included mild congestion during winter seasons to severe tubular degeneration during summer seasons. The accumulation of different metals in the kidneys of the host can be attributed to the water pollution of Dal Lake by various metals and the subsequent histological abnormalities can be speculated to be due to the higher sensitivity of the host to different contaminants. From the present study it was concluded that the metals in the environment are polluting the water bodies and their subsequent deleterious effects harm the aquatic fauna particularly the native fish Schizothorax niger which would be one of the reasons for its decline from fresh water resources of the Kashmir Valley.

INTRODUCTION

Metals are elements found naturally in aquatic ecosystems due to various processes such as weathering and erosion [1], commercial fertilizers, sewage sludge's, urban wastes, liming material and agrochemicals and other wastes used as soil amendments [2]. Some of these metals like copper and zinc are essential to living organisms in trace amounts. Essential trace elements have a narrow optimal concentration range for growth and reproduction, and both excess and shortage can be detrimental to organisms [3], with unusually high concentrations becoming toxic to aquatic organisms [4].

The kidneys are highly metabolically active tissues, which are altered by exposure to chemicals. According to Kent [5], Kidneys besides liver are involved in the detoxification and removal of toxic substance circulating in the blood stream. Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification [6] metals might also be transported into these organs from other tissues, including gills and muscle, for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs.

Mazon, [7] and Mazon et al. [8] reported high amount accumulation of copper by kidneys during acute exposure and preliminary morphological examination showed pathological changes, even at low concentration in water. Keeping in view the declining trend of native fish in the water body of Dal Lake, the present study was designed to study the concentration of different metals in the kidneys of Schizothorax niger and their subsequent histological changes.

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.

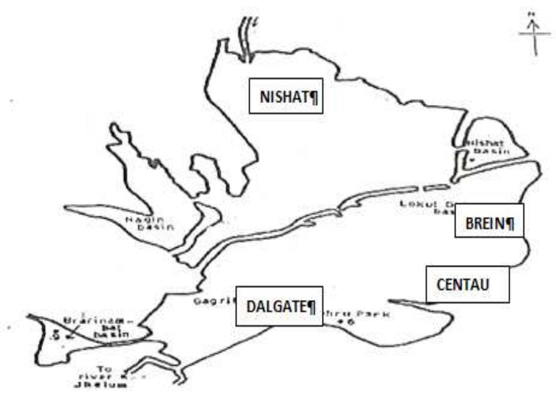

Figure 1: Map of Dal Lake showing different collection sites such as Site I (Dalgate), Site II (Centaur), Site III (Brein) and Site IV (Nishat).

Species and number of fish used:

The study was conducted on Schizothorax niger Heckel. Pooled specimens were collected from the collection 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 cm. 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 a 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 [9]. Similarly, the tissue samples of kidneys 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 like copper, iron and zinc in fish kidneys different histochemical methods such as Perl's method for Iron; Dithiooxamide method for copper; Dithizone method for zinc etc [10] were used so as to ensure metal induced toxicity.

Histological Procedure:

Histological examination was done after fixing the kidneys 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) [10].

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) and 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 fish tissues:

Copper accumulation

In kidney, the concentration varied in the range of 64.61 ± 3.10 to 78.90 ± 3.42 ppm, with the highest concentration observed during the summer season, (year-II) and the lowest in the winter season (year-I) (Table I).

Zinc accumulation

In Schizothorax niger collected from Dal lake, the concentration of zinc in the kidneys ranged from 88.77 ± 3.52 to 101.99 ± 4.03 ppm (Table II). In kidneys, the maximum values of 101.99 ± 4.03 ppm was observed in the summer season of year-II and the minimum value of 88.77 ±3.52 ppm was observed during the winter seasons of year-I.

Iron accumulation

In Schizothorax niger collected from Dal lake, the concentration of iron in the kidneys ranged from 200.99 ± 5.04 to 292.61 ± 4.25 (Table III). The maximum values of 292.61 ± 4.25 ppm in kidney was observed in the summer (year-II) and the minimum values of 200.99± 5.04 ppm in kidney was observed in the winter season (year-I).

Manganese accumulation

In Schizothorax niger collected from Dal lake, the concentration of manganese in the kidneys ranged between 0.84 ± 0.06 to 06.95 ± 0.93 (Table IV). The highest values of 06.95 ± 0.93 ppm in kidney was observed in summer season of year-II. The minimum value in kidney 0.84 ± 0.66 ppm in the kidneys was observed in the spring season of year-I.

Table 1. Showing Copper concentration in the kidneys of Schizothorax niger in different Season of the Study period in Dal Lake

Water resources	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	69.11 ± 3.71	73.31 ± 3.24	66.12 ± 3.52	64.61 ± 3.10
		II	25	72.18 ± 3.84	78.90 ± 3.42	70.62 ± 3.72	68.79 ± 3.18

Values are expressed as mean ±SD

Table 2. Showing zinc concentration in the kidneys of Schizothorax niger in different Season of the study period in Dal Lake.

Water resources	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	89.41± 4.12	94.32 ± 4.15	91.36 ± 2.81	88.77 ± 3.52
		II	25	96.52 ± 3.01	101.99 ± 4.03	98.05 ± 3.11	95.24 ± 3.92

Values are expressed as mean ±SD

Table 3. Showing iron concentration in the kidneys of Schizothorax niger in different Season of the study period in Dal Lake.

Water resources	Fish Host	Year	No. Observed	Iron accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	224.34 ± 6.05	280.41 ± 4.20	224.90 ± 6.24	200.99 ± 5.04
		II	25	239.44 ± 6.52	292.61 ± 4.25	238.11 ± 4.15	230.62 ± 5.55

Values are expressed as mean ±SD

Table 4. Showing manganese concentration in the kidneys of *Schizothorax niger* in different Season of the study period in Dal Lake.

Water resources	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Spring	Summer	Autumn	Winter
Dal Lake	*Schizothorax niger*	I	25	0.84 ± 0.06	05.74 ± 0.77	01.63 ± 0.55	0.99 ± 0.09
		II	25	0.95 ± 0.08	06.95 ± 0.93	02.66 ± 0.03	01.52 ± 0.02

Values are expressed as mean ±SD

Histological changes

The general changes observed in the kidneys of Schizothorax niger included prominent tubular degeneration with severe interstitial mononuclear inflammatory cell infiltration (Fig. 2). The other changes in the kidneys included mild interstitial and glomerular congestion during the winter seasons to severe tubular atrophy with endocrine type during the summer seasons (Fig. 3-4).

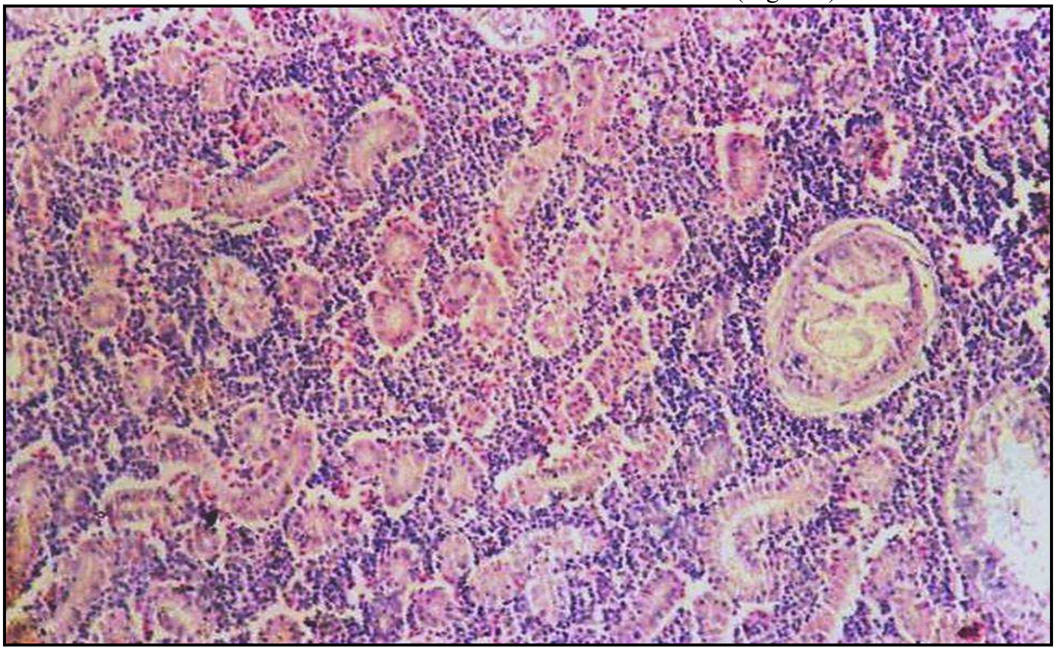

Figure 2. Prominent tubular degeneration and severe interstitial mononuclear inflammatory cell infiltration in the kidneys (H&E, x100).

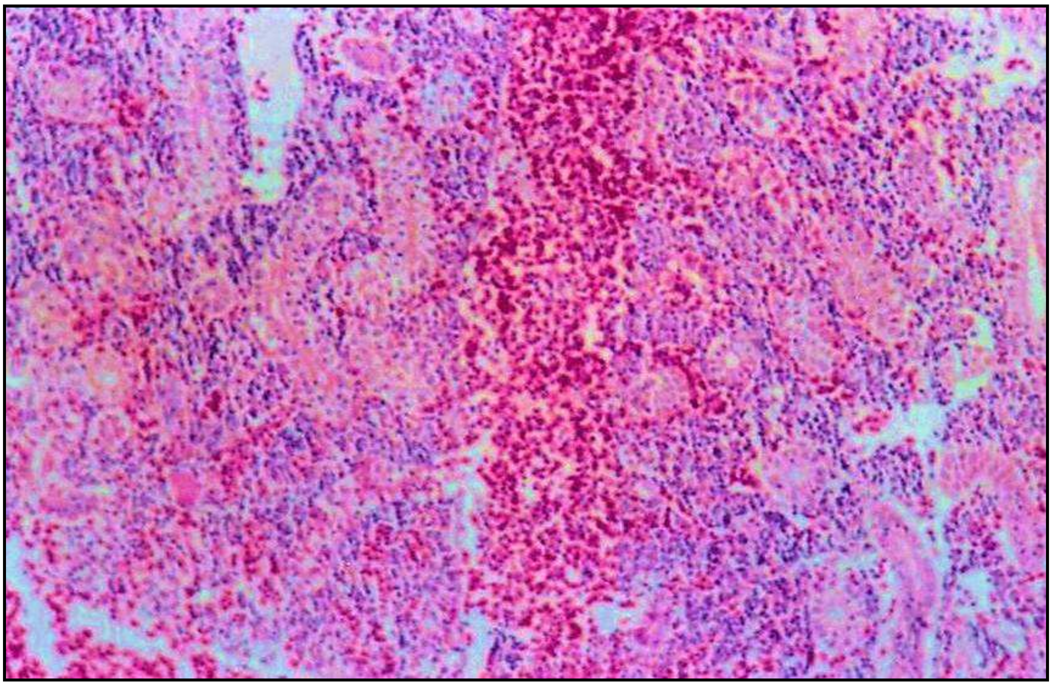

Fig 3. Severe congestion, tubular degeneration, and interstitial inflammation in the kidneys (H&E, x100).

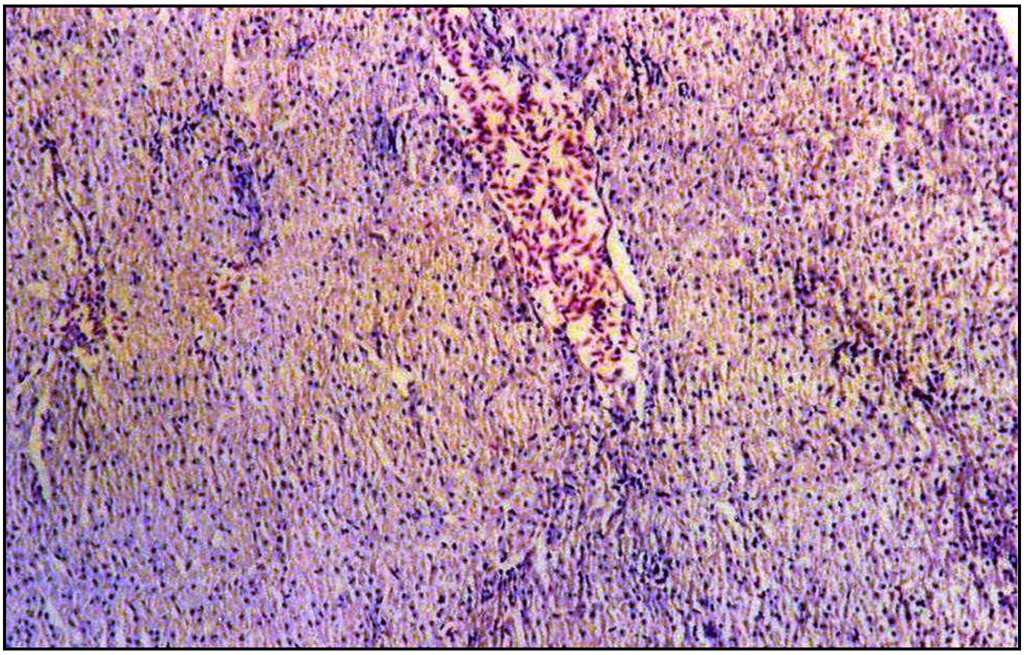

Fig 4. Severe tubular atrophy with endocrine type in kidneys (H&E, x100).

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 from 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 [11]. 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) [12]. However, the metal uptake from water is much higher than uptake from sediment [13-15]. It may be emphasised, 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 [16-21]. 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 [22]. Seasonal differences in the heavy metal accumulation in fish can be related to their metabolic rate, which determines the physiological condition of fish [23]. 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 [24-25]. 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 [18; 26]. 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 [27].

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 in concern. Similarly, it is also known that metal accumulation in tissues of fish is dependent upon the rate of uptake, storage and elimination [14, 18, 26].

In terms of zinc toxicity, the concentrations of the metal within certain tissue may be associated with mortalities [28] and sublethal effects such as behavioral and physiological disruptions [29]. 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 [30], 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 [31-32].

Fish acquire iron predominantly from the diet and its uptake varies in different organs [33-34].

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 [35]. However, its concentration was found to be lower than other observed metals like copper, iron and zinc. This can be attributed to the fact that fish can regulate the amount of manganese in their body [36].

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