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Comparative Biochemical Evaluation of *Schizothorax niger* and *Cyprinus carpio* from River Jhelum of Kashmir Valley

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ABSTRACT

The present study was conducted to evaluate the comparative biochemical profile of *Schizothorax niger* (endemic fish) and *Cyprinus carpio* (exotic fish) from the River Jhelum seasonally for a period of two years. In case of *Schizothorax niger* the varied seasonal biochemical parameters for protein (1.11±0.02 to 4.32±0.13 mg/dl), albumin (1.02 mg/dl to 0.10±0.38 mg/dl), globulin (0.09 ±0.02 mg/dl to 2.37±0.15 mg/dl), blood glucose (137.8±17.00 mg/dl to 340.1±17.00 mg/dl), urea (16.1±1.41 mg/dl to 22.8±3.13 mg/dl), creatinine (0.15±0.05mg/dl to 1.19±0.07 mg/dl), and cholesterol (37.21±2.60 mg/dl to 95.31±5.30 mg/dl) were observed during the entire period of study. In contrast, *Cyprinus carpio* showed different values seasonally with regard to protein (1.05±0.05 to 3.79±0.30 mg/dl), albumin (0.92±0.16 mg/dl to 2.66±0.13 mg/dl), globulin (0.06 ±0.05 mg/dl to 2.18±0.19 mg/dl), blood glucose (196.1±25.59 mg/dl to 352.25±24.59 mg/dl), urea (16.2±0.43 mg/dl to 23.3±2.99 mg/dl), creatinine (0.19±0.09mg/dl to 1.19±0.07 mg/dl), and cholesterol (42.66±3.82 mg/dl to 98.22±2.98 mg/dl). In both the fish hosts the higher biochemical values were recorded in the summer season and the lower values in the winter season during the study period. The seasonal differences in the biochemical parameters of the fish can be attributed to the water pollution of River Jhelum by various contaminants including metals and the comparative higher values in *Schizothorax niger* can be speculated to be due to the higher sensitivity of the host. From the present study it may be inferred that the change in biochemical parameters of the native fish would be one of the reasons for its decline from fresh water resources of the Kashmir Valley.

Key Words: *Schizothorax niger, Cyprinus carpio*, River Jhelum, Biochemistry, Kashmir Valley.

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INTRODUCTION

Jhelum- the Hydaspes of the ancient Greeks and Romans, the vetesta of the Hindus and 'Veth' in Kashmir arises from a beautiful spring called Verinag (Map-II). The river Jhelum originates from Pir Panjal and is about one kilometre ahead of Verinag. The Jhelum river is navigable from Khanabal to Baramulla, a distance of about 170 Kms. It flows in loops through the valley till it enters the Wular. Emerging from the Wular it takes a southwestern direction which it pursues up to Baramulla. It finally passes into Pakistan through the Baramulla- Uri gorge.

The oldest economic activity lined with the river Jhelum is fishing and as the home of most of the indigenous fish fauna, the Jhelum is of great economic importance. After Heckel (33, 34) who listed 16 species of fishes in Kashmir Valley and surrounding areas, Silas (78), Das and Subla (17, 18), Saxena and Koul (74), and Nath (63) summarized most of the collection and provided new checklists which contain increasing number of species. This water body serves as an important source of indigenous (Schizothorax spp.) as well as exotic fishes (*Cyprinus carpio* spp.).

Freshwater fish form one of the important food sources in both the developed as well as under-developed countries. Fish remain the major source of protein, whereas processed fishmeal plays a prominent though indirect role as an important component in the production of meat in human nutrition (7). The recreational value of angling is also important in most developed countries (49). The natives of Kashmir valley divide all types of fishes broadly into two categories of local (Kashmiri) and non-local (Punjabi) fish, zoologically known as endemic and exotic fish spp. important ichthyofauna of two water bodies under study comprises of the family Cyprinidae.

Currently, it is difficult to find any source of water that does not carry fingerprints of human activity (88). Acid precipitation causes leaching of metals from surrounding soils (66) and increasing numbers of synthetic organic compounds and metabolized pharmaceuticals finding their way into surface waters in unlikely places (37), and makes their identification by untargeted chemical analysis prohibitively expensive. As a result, fish have become an indispensable model system for the evaluation and/or measurement of the extent of aquatic pollution (36).

Modern agricultural practices significantly contribute towards polluting the aquatic habitat. The rapidly increasing use of pesticides, chemicals and fertilizers poses a serious threat to the fisheries, especially to the Schizothorax species. Increasing agro-chemical pollution of River Jhelum has become a matter of great concern. Pesticides are used in agricultural fields and enter the river through land drainage or with surface run-off during floods and excessive rains. These include herbicides, rodenticides, fungicides, wormicides, insecticides, etc (like chlorinated hydrocarbons, organophosphates, carbamates and phenols etc). These are lethal to fish if they exceed the tolerable limits.

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Siltation in the river Jhelum is another threat to fish life. The run-off from agricultural fields denuded forests and spent mine areas results in siltation of the riverbed. At various sites the River Jhelum is gradually becoming narrower. Siltation of the river, besides diminishing the flow of water, results in destruction of breeding grounds of fishes and the benthic fauna, migration of fishes and decline in overall productivity of the river. Metals enter the aquatic food chain through direct consumption of water or biota and through non-dietary routes such as uptake through gills in the case of fish.

On the other hand trace metals are introduced into the environment by a wide spectrum of natural and anthropogenic sources. There has been a general global increase in industrial activity over the past few decades, resulting in significant application of metals in various processes, in turn causing a great escalation of metals in the environment. Industrial activities as well as agriculture and mining make up potential source of heavy metal pollution in aquatic environment (1, 14, 30, 42, 44, 48, 52, 89). Contamination of a river with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited with the extent of contamination (81). It is well known that heavy metals accumulate in tissues of aquatic animals and their measurement in tissues of aquatic animals can reflect past exposures (11, 39, 97, 94, 98). Sub lethal effects of heavy metals are of concern as they accumulate and are transferred through food chain to humans. The impact of pollutants on aquatic ecosystems is either acute (due to exposure to immediate lethal dose) or insidious/chronic (due to gradual accumulation of lethal concentrations in body tissues) (32). Metals like lead and cadmium may present a health risk even at extremely low concentration, since they may influence enzymatic activity in living systems (9). A disease known as plumbism has been known to be caused by acute lead poisoning (79). Cadmium has also been regarded toxic at very low concentrations (10) and with hazardous effects on humans (31). Biomonitoring of trace metal pollutants has been gaining attention since different organisms can accumulate these substances and transfer them in large concentration to animals or human beings, when consumed (26).

MATERIALS AND METHODS

Collection of Fish Hosts:

Fishes were collected from the River Jhelum 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 study was conducted using two representative fish species- *Cyprinus carpio* Linnaeus and *Schizothorax niger* Heckel. Pooled specimens were collected from the collection sites of the River Jhelum so as to make a sample size of 25 fish of each species (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).

After collection of the fishes from the River Jhelum, the fishes were identified on the basis of key provided by Kullander et al. (43). The fishes were carried to the laboratory in plastic bucket. Every effort was made to keep the fish alive. The blood was collected in different labelled collection vials. The fishes were dissected midventrally and all the visceral organs were taken out in a big tray. Then various visceral organs viz. gills, liver, kidney and muscle were placed in separate petridishes containing normal saline and were later processed for the various selected parameters.

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.

Biochemical estimation of fish serum:

After collection of the fishes from the River Jhelum, the fishes were identified on the basis of key provided by Kullander et al. (43). The fishes were carried to the laboratory in plastic bucket. Every effort was made to keep the fish alive.

Collection of blood:

Blood samples for biochemical estimation were collected from live fishes by either severing/puncturing the caudal vessel or puncturing the heart/dorsal aorta, which ever method was convenient at the time of collection.

Handling of blood:

Blood samples for harvesting of serum were collected in different labeled vials containing no anticoagulants. Blood samples before processing for biochemical estimation were centrifuged at 3000 rpm for 10-15 minutes and the liquid fraction in the form of serum was obtained.

Total protein content of the serum was estimated using commercial estimation kit- Liquichem Total Protein (Recorders and Medicare Systems (P) Ltd., India), by the Biuret method (85). The serum albumin was estimated using commercial estimation kit- Liquichem Albumin (Recorders and Medicare Systems (P) Ltd., India), by the BCG method (51). The amount of total globulin was calculated by deducting the calculated value of albumin from calculated value of

total protein. For the analysis of blood sugar enzymatic kit method as described by Tietz (85) was employed using commercial estimation kit. Estimation of blood urea was done by Berthelot method (86) and that of serum creatinine by Alkaline Picrate Method. The serum cholesterol was estimated by Cholesterol Oxidase Method (86).

RESULTS

Metal Estimation:

The concentration of different metals in the River Jhelum estimated seasonally for a period of two recent years is presented in the tables (I & II).

Table I: Showing Metal concentration (ppm) in River Jhelum (Year-I)

Water Resource	Metal Concentration	Spring	Summer	Autumn	Winter
River Jhelum	Copper	1.003	1.004	1.003	1.002
	Iron	0.159	0.166	0.129	0.148
	Zinc	0.460	0.472	0.340	0.100
	Manganese	0.011	0.044	0.033	0.0056

Table II: Showing Metal concentration (ppm) in River Jhelum (Year-II)

Water Resource	Metal Concentration	Spring	Summer	Autumn	Winter
River Jhelum	Copper	1.004	1.006	1.005	1.003
	Iron	0.165	0.168	0.133	0.159
	Zinc	0.462	0.483	0.356	0.111
	Maganese	0.013	0.053	0.034	0.0057

Biochemical Estimation:

Protein Estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of total proteins in different seasons during the entire study period (Table III-VI).

In spring seasons the *Schizothorax niger* showed total protein values of 2.18 $\pm$ 0.09 g/dl and 2.33 $\pm$ 0.15 g/dl. In summer seasons the values recorded were 3.91 + 0.22 g/dl and 4.23 + 0.43 g/dl, in autumn seasons the values were 3.66 $\pm$ 0.15 g/dl and 2.71 $\pm$ 0.08 g/dl and in winter seasons 1.55 $\pm$ 0.18 g/dl and 1.38 $\pm$ 0.12 g/dl values were recorded. The minimum concentration of total proteins was observed in winter season (year-II) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. collected from river Jhelum showed varied total protein concentrations with lowest values during winter season (year-I) and highest values during summer season. Total protein concentrations of 1.92 $\pm$ 0.13 g/dl and 1.88 $\pm$ 0.18 g/dl in

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respective spring seasons, 2.35 ± 0.12 g/dl and 3.13 ± 0.40 g/dl in respective summer seasons, 2.26 ± 0.07 g/dl and 2.39 ± 0.22 g/dl in respective autumn seasons and 1.61 ± 0.05 g/dl and 1.86 ± 0.09 g/dl in respective winter seasons were observed.

Table III: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Spring Season in River Jhelum

Water Resource	Fish Host	Year	NO	TP (g/dl)	A (g/dl)	G (g/dl)	BG (mg/dl)	SU (mg/dl)	SC (mg/dl)	TC (mg/dl)
River Jhelum	*S. niger*	I	25	2.18 ± 0.09	1.96 ± 0.18	0.22 ± 0.09	192.3 ± 18.02	17.2 ± 0.88	0.72 ± 0.11	51.66 ± 9.01
		II	25	2.33 ± 0.15	2.18 ± 0.09	0.15 ± 0.06	202.8± 29.38	17.8 ± 1.52	0.81 ± 0.06	52.44 ± 3.12
	C. carpio	I	25	1.92 ± 0.13	1.03 ± 0.01	0.89 ± 0.12	218.8 ± 39.20	20.9 ± 1.60	0.74 ± 0.02	63.20 ± 3.01
		II	25	1.88 ± 0.18	1.78 ± 0.07	0.10 ± 0.11	222.6 ± 66.52	22.2 ± 1.44	0.77 ± 0.13	63.20 ± 1.55

Where, NO= No. Observed; TP= Total Protein; A= Albumin; G= Globulin; BG= Blood Glucose; SU= Serum Urea; SC= Serum Creatinine and TC= Total Cholesterol.

Table IV: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Summer Season in River Jhelum

Water Resource	Fish Host	Year	NO	TP (g/dl)	A (g/dl)	G (g/dl)	BG (mg/dl)	SU (mg/dl)	SC (mg/dl)	TC (mg/dl)
		I	25	3.68 ± 0.33	1.50 ± 0.14	2.18 ± 0.19	348.8 ± 28.01	23.3 ± 2.99	1.19 ± 0.07	98.22 ± 2.98
River Jhelum	*S. niger*	II	25	3.91 ± 0.22	3.60 ± 0.15	0.31 ± 0.07	332.1 ± 17.14	21.6 ± 1.72	1.09 ± 1.20	99.34± 4.55
		I	25	4.23 ± 0.43	3.72 ± 0.38	0.51 ± 0.05	337.6 ± 12.12	21.7 ± 3.59	1.11± 0.06	100.01 ± 5.88
	C. carpio	II	25	2.35 ± 0.12	2.23 ± 0.21	0.02 ± 0.09	341.6 ± 25.01	23.8 ± 1.99	1.12 ± 0.12	82.77 ± 3.52
		I	25	3.13 ± 0.40	2.22 ± 0.11	0.91 ± 0.29	342.9 ± 28.77	24.0 ± 2.12	1.14 ± 0.09	92.03 ± 3.01

Where, NO= No. Observed; TP= Total Protein; A= Albumin; G= Globulin; BG= Blood Glucose; SU= Serum Urea; SC= Serum Creatinine and TC= Total Cholesterol

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Table V: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Autumn Season in River Jhelum

Water Resource	Fish Host	Year	NO	TP (g/dl)	A (g/dl)	G (g/dl)	BG (mg/dl)	SU (mg/dl)	SC (mg/dl)	TC (mg/dl)
River Jhelum	*Schizothorax niger*	I	25	3.66 ± 0.15	3.60 ± 0.28	0.06 ± 0.13	176.3 ± 19.92	18.1 ± 2.11	0.84 ± 0.04	59.77 ± 2.22
		II	25	2.71 ± 0.08	2.51 ± 0.15	0.20 ± 0.07	181.8 ± 13.22	18.3 ± 1.36	0.86 ± 0.22	65.42 ± 2.99
	Cyprinus carpio	I	25	2.26 ± 0.07	2.16 ± 0.07	0.10 ± 0.00	220.3 ± 28.88	19.2 ± 1.33	0.88 ± 0.50	60.11 ± 3.55
		II	25	2.39 ± 0.22	2.33 ± 0.16	0.06 ± 0.01	272.0 ± 27.72	19.6 ± 3.08	0.91 ± 0.09	67.30 ± 3.01

Where, NO= No. Observed; TP= Total Protein; A= Albumin; G= Globulin; BG= Blood Glucose; SU= Serum Urea; SC= Serum Creatinine and TC= Total Cholesterol

Table VI: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Winter Season in River Jhelum

Water Resource	Fish Host	Year	NO	TP (g/dl)	A (g/dl)	G (g/dl)	BG (mg/dl)	SU (mg/dl)	SC (mg/dl)	TC (mg/dl)
River Jhelum	*Schizothorax niger*	I	25	1.55 ± 0.18	1.01 ± 0.02	0.54 ± 0.16	125.5 ± 17.11	17.0 ± 0.59	0.09 ± 0.22	36.42 ± 2.71
		II	25	1.38 ± 0.12	1.02 ± 0.03	0.36 ± 0.09	133.1 ± 13.01	17.1 ± 2.33	0.11 ± 0.04	51.42 ± 4.20
	Cyprinus carpio	I	25	1.61 ± 0.05	1.01 ± 0.02	0.60 ± 0.03	136.1 ± 25.01	16.1 ± 1.50	0.14 ± 0.10	40.11 ± 3.22
		II	25	1.86 ± 0.09	1.21 ± 0.07	0.65 ± 0.02	143.2 ± 33.00	15.2 ± 1.44	0.16 ± 0.09	48.76 ± 3.14

Where, NO= No. Observed; TP= Total Protein; A= Albumin; G= Globulin; BG= Blood Glucose; SU= Serum Urea; SC= Serum Creatinine and TC= Total Cholesterol

Albumin Estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of total proteins in different seasons during the entire study period (Table III-VI).

In spring seasons in *Schizothorax niger* albumin values varied from 1.96 ± 0.18 g/dl and 2.18 ± 0.09 g/dl. In summer seasons the values recorded were 3.60 + 0.15 g/dl and 3.72 + 0.38 g/dl, in autumn seasons the values were 3.60 ± 0.28 g/dl and 2.51 ± 0.15 g/dl and in winter the values 1.01 ± 0.02 g/dl and 1.02 ± 0.03 g/dl. The minimum concentration of albumin were

observed in winter season (year-I) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. collected from river Jhelum showed varied albumin concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II). Albumin concentrations of 1.92 ± 0.13 g/dl and 1.88 ± 0.18 g/dl in respective spring seasons, 2.23 ± 0.21 g/dl and 2.22 ± 0.11 g/dl in respective summer seasons, 2.16 ± 0.07 g/dl and 2.33 ± 0.16 g/dl in respective autumn seasons and 1.01 ± 0.02 g/dl and 1.21 ± 0.07 g/dl in respective winter seasons (2005-2007) were observed.

Globulin Estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of globulin in different seasons during the entire study period (Table III-VI).

In spring seasons the *Schizothorax niger* showed globulin values of 0.22 ± 0.09 g/dl and 0.15 ± 0.06 g/dl respectively. In summer seasons the values recorded were 0.31 + 0.07 g/dl and 0.51 + 0.05 g/dl, in autumn seasons the values were 0.06 ± 0.13 g/dl and 0.20 ± 0.07 g/dl and in winter seasons the values 0.54 ± 0.16 g/dl and 0.36 ± 0.09 were recorded. The minimum concentration of globulin was observed in winter season (year-II) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. collected from river Jhelum showed varied globulin concentrations with lowest values during winter season (year-I) and highest values during spring season (year-II). Globulin concentrations of 0.89 ± 0.12 g/dl and 0.10 ± 0.11 g/dl in respective spring seasons, 0.02 ± 0.09 g/dl and 0.91 ± 0.29 g/dl in respective summer seasons, 0.10 ± 0.00 g/dl and 0.06 ± 0.01 g/dl in respective autumn seasons and 0.60 ± 0.03 g/dl and 0.65 ±0.02 g/dl in respective winter seasons were observed.

Glucose Estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of glucose in different seasons during the entire study period (Table III-VI).

In spring seasons the *Schizothorax niger* showed glucose values of 192.3 ± 18.02 g/dl and 202.8 ± 29.38 g/dl respectively. In summer seasons the values recorded were 332.1 + 17.14 g/dl and 337.6 + 12.12 g/dl, in autumn seasons the values were 176.3 ± 19.92 g/dl and 181.8 ± 13.22 g/dl and in winter seasons the values 125.5 ± 17.11 g/dl and 133.1 ± 13.01 g/dl were recorded. The minimum concentrations of glucose were observed in winter season (year-I) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. collected from river Jhelum showed varied glucose concentrations with lowest values during winter season (year-I) and highest values during summer season

(year-II). Glucose concentrations of 218.8 ± 39.20 g/dl and 222.6 ± 66.52 g/dl in respective spring seasons, 341.6 ± 25.01 g/dl and 342.9 ± 28.77 g/dl in respective summer seasons, 220.3 ± 28.88 g/dl and 272.0 ± 27.72 g/dl in respective autumn seasons and 136.1 ± 25.01 g/dl and 143.2 ± 23.00 g/dl in respective winter seasons (2005-2007) were observed.

Urea and Creatinine Estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of urea and creatinine in different seasons during the entire study period (Table III-VI).

In spring seasons, the *Schizothorax niger* showed urea values of 17.2 ± 0.88 g/dl and 17.8 ± 1.52 g/dl respectively. In summer seasons the values recorded were 21.6 + 1.72 g/dl and 21.7 + 3.59 g/dl, in autumn seasons the values were 18.1 ± 2.11 g/dl and 18.3 ± 1.36 g/dl and in winter the values were 17.0 ± 0.59 g/dl and 17.1 ± 2.33 g/dl recorded. The minimum concentrations of urea were observed in winter season (year-I) and maximum values were recorded during summer season (year-II). *Cyprinus carpio* spp. collected from river Jhelum showed varied urea concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 90). Urea concentrations of 20.9 ± 1.60 g/dl and 22.2 ± 1.44 g/dl in respective spring seasons, 23.8 ± 1.99 g/dl and 24.0 ± 2.12 g/dl in respective summer seasons, 19.2 ± 1.33 g/dl and 19.6 ± 3.08 g/dl in respective autumn seasons and 16.1 ± 1.50 g/dl and 15.2 ± 1.44 g/dl in respective winter seasons were observed.

However, the concentration of creatinine in *Schizothorax niger* varied from 0.09 ± 0.22 to 1.11 ± 0.06 g/dl respectively. A minimum value of creatinine was observed in winter season of year-I and maximum value in summer season of year-I. Further, the creatinine estimation during rest of the seasons showed different concentrations. A concentration of 0.72 ± 0.11 g/dl and 0.81 ± 0.06 g/dl in respective spring seasons; 1.09 ± 1.20 g/dl and 1.11 ± 0.06 g/dl in summer season; 0.84 ± 0.04 g/dl and 0.86 ± 0.22 g/dl in autumn seasons and 0.09 ± 0.22 g/dl and 0.11 ± 0.04 g/dl in winter seasons during the entire study period was observed.

Cyprinus carpio spp. collected from river Jhelum showed varied creatinine concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II). Creatinine concentrations of 0.74 ± 0.02 g/dl and 0.77 ± 0.13 g/dl in respective spring seasons, 1.12 ± 0.12 g/dl and 1.14 ± 0.09 g/dl in respective summer seasons, 0.88 ± 0.50 g/dl and 0.91 ± 0.09 g/dl in respective autumn seasons and 0.14 ± 0.10 g/dl and 0.16 ± 0.09 g/dl in respective winter seasons were observed.

Cholesterol estimation

Schizothorax niger and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of cholesterol in different seasons during the entire study period (Table III-VI).

In spring seasons the *Schizothorax niger* showed cholesterol values of 51.66 ± 9.01 g/dl and 52.44 ± 3.12 g/dl respectively. In summer seasons the values were 99.34 ± 4.55 g/dl and 100.01 ± 5.88 g/dl, in autumn seasons the values were 59.77 ± 2.22 g/dl and 65.42 ± 2.99 g/dl and in winter seasons the values were 36.42 ± 2.71 g/dl and 51.42 ± 4.20 g/dl. The minimum concentrations of cholesterol were observed in winter season (year-I) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. from river Jhelum showed cholesterol concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II). Cholesterol concentrations of 63.20 ± 3.01 g/dl and 63.20 ± 1.55 g/dl in respective spring seasons, 82.77 ± 3.52 g/dl and 92.03 ± 3.01 g/dl in respective summer seasons, 60.11 ± 3.55 g/dl and 67.30 ± 3.01 g/dl in respective autumn seasons and 40.11 ± 3.22 g/dl and 48.76 ± 3.14 g/dl in respective winter seasons were observed.

DISCUSSION

The biochemical data of fish hosts in both the water bodies indicated alterations particularly in blood glucose in the present study. The use of biochemical approach has been advocated to provide an early warning of potentially damaging changes or health status of fish (19). According to Luskova (50); Bottcher (6); Edsall (24) biochemical characterization of fish blood is an index of the state of internal milieu. Fish are particularly sensitive to water-borne environmental contamination, and are recognized as a useful model for indicating water quality (53). Pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (61, 83). In the present study variations in blood chemistry of fish hosts was observed seasonally. Seasonal variations in blood biochemistry of fish have been reported by Terasawa et al. (84) which are influenced by toxins (12, 40), herbicides (64, 72), diet (29), temperature (73), pH (95), photoperiod (41), reproduction cycle (2, 82) and pollutants (61, 83). In the present study however, biochemical changes in fish hosts with regards to metal exposure in naturally occurring water bodies was a purpose so that this evidence could be used to determine the possible adverse effects of metals. Alterations in biochemical and hematological parameters following metal exposure have been reported earlier by Dhanapakram and Ramasamy (21) and Monteiro et al. (58).

The plasma or serum proteins are known to play a vital role in the maintenance of osmolarity, buffer capacity and pH of blood besides acting as carrier of various nutrients, metabolites and metal ions. Plasma proteins also have great importance in the defense mechanism of the body with respect to their globulin fraction. Alterations in the concentrations of such proteins therefore, reflect changes in several important physiological processes in the organism. The plasma protein concentration, though relatively low as compared to other vertebrates, show great variations from species to species with recorded values varying from 0.9 to 9.1 gm % (25, 70, 71, 75, 80). In the present study, total protein levels ranged from 1.11 ± 0.02 g/dl to 4.32 ± 0.13 g/dl with albumin ranging from 1.01 ± 0.02 g/dl to 3.72 ± 0.38 and globulin ranging from 0.06 ± 0.13 g/dl to 2.37 ± 0.15 g/dl in *Schizothorax niger*. However, in

carp species the total protein levels ranged from 1.05 ± 0.05 g/dl to 3.79 ± 0.30 g/dl with albumin values ranging from 0.92 ± 0.16 g/dl to 2.33 ± 0.16 g/dl and globulins ranging from 0.06 ± 0.05 g/dl to 2.18 + 0.19 g/dl. Changes in total protein concentrations of various magnitudes and duration in fish due to exposure of metals have also been reported by Coello and Khan (13). Further studies suggest that exposure of fish to metals not only alters the total protein concentrations and hemoglobin of the host (46) but also serves as the criterion of stress reaction (45, 46, 47). Variations in total proteins were found throughout the year, generally the lowest values were determined during winter and the highest during summer. Seasonal variations in the blood biochemistry of fish are known to occur and to be significant with regard to their health and well being (19, 29, 41, 73, 95). The changes in total protein concentrations may be attributed to the relative changes in the mobilization of proteins and increased production of metallotheionein which is a sequestering agent (15). Other studies have reported that variation in the level of plasma proteins in fish can be related to cold acclimation, condition of the gonads and helminth infection (65, 68, 77). Contrary to the present investigation, an increase in the plasma proteins in cold seasons due to higher synthesis of proteins have been reported (65, 68, 77). However, Helmy et al. (35) reported essential differences in the amount of total proteins in summer months, thus supporting the present findings. High albumin /globulin (A:G) ratio across seasons could reflect a decrease in globulin's in the period of higher nutritional constraints and gluconeogenesis might result in decreased levels of proteins and might be used to assess dietary inadequacies (22). In the present study the highest A:G ratio in summer was found corresponding to the levels of protein. Albumin is one of the major compounds of protein (28). Therefore, it is likely that for *Schizothorax niger* and *Cyprinus carpio* spp. higher nutritional constraints and high serum protein levels exist in summer.

In the present study an increase in blood glucose in *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum was observed during summer seasons. The increase in glucose was inconsistent with higher levels of metals during summer. These results are in agreement with the finding of Mourad and Wahby (59) who recorded a significant hyperglycemia after exposure of fish to a wastewater containing copper. The increase in blood glucose during summer observed in the present study indicated that fish were subjected to some sort of hypertoxic stress (90, 92). It is well known that stressful stimuli elicit rapid secretion of both glucocorticoides (90) and catecholamines (62) from the adrenal tissues of fish and both of these hormones produced hyperglycemia (67). The obtained results are also in agreement with Dange (16) and Benson et al. (4) who recorded an increase in plasma glucose levels after exposure to heavy metals. According to Wendelaar Bonga (93) the determination of glucose concentration in blood serum is widely used as an indicator of stress in fish, and hyperglycemia is associated with stressful conditions (91). Seasonal variation of blood glucose were observed in fish hosts in the present study which might be significant with regard to their health and well being (19, 29, 41, 73, 95). Contrary to our findings, Bayir et al. (3) have recorded an increase in glucose concentration during winter and decrease during summer in Siraz (Capoeta capoeta umbla) and have correlated it with water temperature. However, there is no correlation between glucose levels and water temperature as observed in tench (Tinca tinca) (19, 82).

Further, the biochemical parameters with regard to blood urea, serum creatinine and blood cholesterol showed seasonal variations during the entire period of study. Urea is present in all fish, the liver being the primary organ of production and the gills appearing to be the main organ of excretion. Therefore, elevated levels of urea during summer might be associated with the increased concentration of metals and their subsequent deleterious effects on gills and liver. The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some situations materials may accumulate in liver to toxic levels and cause pathological alteration as also reported by Meyers and Hendrick (57), Ferguson (27), Braunbeck *et al.* (8). The type of liver injury is often dependent upon not only on the particular agent and its mechanism of action but also on the length of exposure (38).

The increase in cholesterol level observed during summer in fish hosts is inconsistent with earlier observations of Desia *et al.* (20) who reported an increase in cholesterol level due to exposure of nickel in freshwater fish Channa punctatus and attributed it to the stressful condition of the fish. Further, the author attributed high cholesterol level due to hepatic dysfunction and accumulation in brain. Although cholesterol is an important component of cell membranes and functions as a precursor for the synthesis of sexual hormones (56) but too much cholesterol can be lethal because of atherosclerosis that results from the deposition of plaques of cholesterol esters (76). Therefore, an increase in cholesterol level in fish hosts during summer can be attributed to the toxic effects of metals on liver and the subsequent variations in biochemical parameters. Previous studies have reported that as a consequence of negative environmental factors (stress and pollution) the same fish species exhibited hypercholesterolema (5, 54, 69) and also decrease of cholesterol level (23, 55). Amount of urea-nitrogen in the blood serum is an indicator of protein metabolism. Creatinine content may be regarded as measure of glomerular filtrations rate (60). Increase in urea during summer can be attributed as an indicator of failing gill osmoregulatory capability (56). Osmoregulatory failure has often being demonstrated to be an important contributor to death in fish (96). Further, variation in creatinine levels in fish hosts observed in the present study can be attributed to the unique excretory system of fish, in which most of the nitrogenous wastes are excreted via the gills with only a small fraction is excreted by the kidney (87).

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