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Research Article

SEASONAL AND SPATIAL VARIATION IN BIOACCUMULATION OF HEAVY METALS IN TWO COMMERCIAL FISH SPECIES FROM RIVER JHELUM OF KASHMIR VALLEY

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A B S T R A C T

The concentrations of heavy metals copper, zinc, lead and iron were measured in the liver, gills and muscles of two fresh water fish species captured from six sampling sites in four different seasons along the course of river Jhelum, Kashmir, India. The levels of heavy metals varied significantly among fish species, seasons, sites and organs. As expected, muscle tissue was found to accumulate lowest concentrations of all heavy metals. In most of the studied fish samples, liver was found as the main target organ for heavy metal accumulation. Inter and intra specific variation of heavy metals was interpreted for the contribution of potential factors that were found to affect heavy metals uptake, size and weight, geographical distribution and species-specific factors. Generally recorded heavy metal concentrations were found well below the permissible limit of FAO/WHO, 1982. The concentration of heavy metals in fish muscle tissue was found significant but safe for human consumption.

INTRODUCTION

The problem of environmental pollution due to heavy metals has raised widespread worries in different parts of the world and results reported by various organizations have been alarming. The main sources of heavy metal pollution to water resources are agricultural runoff, sewage, discharge of untreated and semi treated effluents from several industries such as metal electroplating, manufacturing of batteries, circuit boards, car repair, motor workshops, geological quarrying and even roads are also considered as one of the major sources of heavymetals in the environment[1]. Heavy metals have long been known as one of the most vital pollutants in the river waters because of being toxic, mutagenic and carcinogenic to biotic system. Nearly all heavy metals are toxic in higher concentrations but Pb is usually considered to be the most dangerous toxicant[2]. Aquatic organisms have the capability to accumulate heavy metals from numerous sources comprising water, sediments, air depositions of dust, aerosol and discharges of waste water[3,4]. Once entered into the aquatic ecosystem, most of the heavy metals persist in sediments, from where get slowly released into the overlying water and ultimately reach the body of aquatic organisms[5]. Heavy metals can participate in various biochemical processes, have significant mobility, can affect the ecosystem through bioaccumulation and biomagnification processes and are potentially toxic for human life[6]. Aggravated concentrations of these heavy metals like arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) etc could be hazardous to riverine ecosystem wherein they can pose toxicity to fish and imbalance water chemistry as well. Heavy metals can interact with other elements and can cause severe toxicity at tissue level. Some trace elements like zinc (Zn), manganese (Mn) etc have been reported to be biologically important for human beings. However, some other heavy metals like Pb and Cr have no known significance in human physiology and biochemistry and intake of these heavy metals even at very low concentrations can be toxic. Even for those that are biologically important, dietary intake need to be within regulatory limits as excess may result in harming or toxicity[7,8].

Fish is widely consumed as a staple food source and rich supply of protein throughout the world. The per capita consumption of fish is estimated to be 5 kg/year for the whole population and it is 8 kg/ year for the non-vegetarian population of India. The monthly per capita consumption of fish varies from (<0.3 kg/month) in Haryana, Himachal Pradesh and Jammu & Kashmir to a high of 0.79 kg/month in Lakshadweep[9]. Significant amount of fish is consumed by Kashmiri people and most of the fishes traded in the market

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are caught from the local water bodies including river Jhelum. However, when future fish production of the valley is considered, the statistical models have depicted that overall fish production of the valley is showing a declining trend. There could be several reasons for this but the major reason can be damage occurred to the breeding grounds of various fish species especially *Schizothorax niger* and *Cyprinus carpio* by alarming decline in water qualitydue to heavy metal contamination and other anthropogenic disturbances[10]. In view of the above facts, the present research investigation is very vital and crucial to understand the level of heavy metalbioaccumulation in two commercially most common edible fish species viz, *Schizothorax niger* and *Cyprinus carpio* captured from the Jhelum river of Kashmir valley. The tissues selected for examination were muscle, liver, and gill. Muscle is important as it is the tissue which is mainly consumed by human beings. Liver is the major storing chamber and biotransforming organ for heavy metals and gills are the main route of exposure to trace elements from water to fish[11].

Study Area

The principal river of the Kashmir valley is the Jhelum, locally called as *Veth*. Starting from its origin at the Verinag spring, the Jhelum continues its 241 Km long journey through the valley and enters Pakistan. There are several tributaries and nallahs such as, Sandran, Brang, Arapat kol, Lidder, Arapal, Harwan, Sindh, Erin, Mudhumati, Pohru and Vijidakil, Vishav, Rambria, Romshi, Doodhganga, Ferozpura and Ningalthat contribute to river Jhelum. The sampling sites have been selected on the basis of geography, settlements, agricultural land, urban, rural and commercial areas along either banks of river Jhelum (Fig.1). The sampling sites have been divided into three regions

Upstream: [Site 1 (Chinigund Verinag), Site 2 (Zirpara Bridge Bijbehara)]

Middle Stream: **[**Site 3 (Zero Bridge Srinagar), Site 4 (Qamarwari Bridge Srinagar)]

Down Stream: [Site 5 (Ningli Sopore), Site 6 (Cement Bridge Baramulla)]

MATERIALS AND METHODS

Fish samples were collected from all the selected sites during four different seasons from June 2014 to March 2015. Samples were collected on seasonal basis viz, summer (June to August), autumn (September to November) winter (December to February) and spring (March to May) and the sampling have been performed in the first month of each season. The sampling has been achieved between 10.30 am to 03.00 pm. Fish samples were collected from each selected site with the help of local fishermen. Three replicates were collected for each fish sample. Total fish length (cm) and weight (g) has been noted on spot. Approximately same sized fishes were captured in order to avoid the variability in heavy metal content due to weight and length of the fishes (Tables 1 & 2). Fishes were dissected by a sterile surgical blade for the extraction of gills, liver and muscle tissues. Tissue samples were placed in clean labeled polythene bags, preserved on ice and transported to the laboratory. The exact 5 g wet weight of muscle tissue and 1g wet weight each in case of liver and gills were taken and were oven dried to constant weight at 105°C[12]. Further tissue samples were transferred into 50 ml conical flasks. Thereafter, 10 ml of concentrated nitric acid was added slowly to the samples (2ml of ultrapure concentrated perchloric acid has-been added in the case of gill tissues).The flasks were covered and heated at 200°C on a hot plate for 3 hours, until the solution evaporated slowly to near

Table 1 Length and weight of *Schizothorax niger* collected from River Jhelum (Mean ±Standard error)

Seasons		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Summer	Length (cm)	23.03 ± 0.8	21.97 ± 0.9	24.07 ± 0.8	21.5 ± 0.6	23.9 ± 0.3	22.3 ± 1.3
	Weight (g)	182.0 ± 1.40	181.2 ± 1.4	181.0 ± 2.8	181.8 ± 1.7	180.4 ± 1.6	182.6 ± 0.5
Autumn	Length (cm)	22.0 ± 0.9	23.17 ± 0.8	23.0 ± 0.4	22.2 ± 1.2	24.1 ± 0.3	22.0 ± 0.8
	Weight (g)	179.4 ± 2.1	179.4 ± 1.7	18.17 ± 1.8	179.3 ± 1.4	180.2 ± 1.2	183.8 ± 0.4
Winter	Length (cm)	20.4 ± 0.1	23.2 ± 0.9	22.2 ± 0.4	24.7 ± 0.5	23.4 ± 0.7	22.6 ± 1.0
	Weight (g)	179.40 ± 1.6	181.9 ± 1.4	180.1 ± 1.7	181.5 ± 2.6	181.7 ± 0.8	181.5 ± 1.5
Spring	Length (cm)	24.9 ± 0.7	22.4 ± 0.3	22.6 ± 0.5	24.4 ± 0.3	24.3 ± 0.3	21.6 ± 0.6
	Weight (g)	178.1 ± 2.1	182.8 ± 0.6	180.6 ± 0.6	180.7 ± 1.3	183.0 ± 0.9	179.1 ± 0.3

Table 2 length and weight of *cyprinus carpio* collected from River Jhelum (Mean ±Standard error)

Seasons		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Summer	Length (cm)	15.6 ± 0.3	17.6 ± 0.4	16.4 ± 0.5	18.3 ± 0.9	18.2 ± 0.2	17.7 ± 0.4
	Weight (g)	205.2 ± 1.8	203.2 ± 1.5	203.5 ± 0.9	207.8 ± 0.4	205.8 ± 2.2	206.7 ± 1.6
Autumn	Length (cm)	18.9 ± 0.6	16.7 ± 0.7	17.5 ± 0.6	19.0 ± 0.5	17.3 ± 0.6	16.6 ± 0.4
	Weight (g)	203.8 ± 1.4	206.1 ± 1.6	206.8 ± 1.1	206.5 ± 0.6	203.5 ± 1.2	205.3 ± 2.1
Winter	Length (cm)	15.6 ± 0.4	17.6 ± 0.6	17.8 ± 0.6	15.3 ± 0.3	16.4 ± 0.9	17.5 ± 1.1
	Weight (g)	207.0 ± 1.1	206.8 ± 1.2	208.1 ± 0.9	207.9 ± 0.7	204.3 ± 1.6	207.8 ± 1.2
Spring	Length (cm)	15.8 ± 0.4	16.5 ± 1.0	17.2 ± 1.3	19.4 ± 0.3	17.7 ± 0.5	16.9 ± 0.7
	Weight (g)	207.5 ± 0.6	202.6 ± 1.0	208.2 ± 0.9	207.0 ± 1.8	205.8 ± 1.5	208.4 ± 0.8

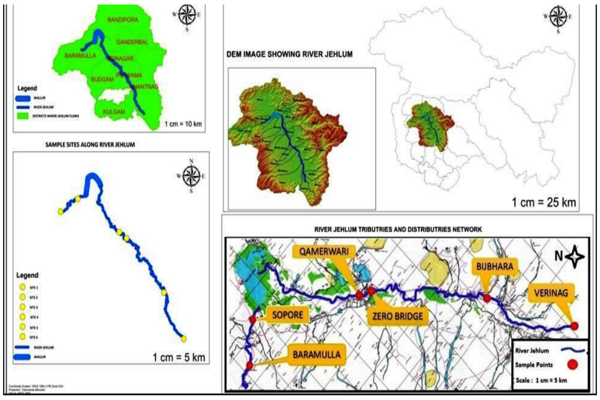

Fig 1 Study area map showing sampling sites from upstream to downstream of river Jhelum

dryness. A solution of 2 ml 1N HNO_3 was added to the residue and was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample got completely digested. After cooling, 2.5 ml of 1N HNO_3 was added to the digested residue and was transferred to 50 ml volumetric flasks and diluted upto mark with deionised water. Before analysis, the samples were filtered through a 0.4 mm Whatman filter paper[13]. All digested samples were analysed for the heavy metals viz, Cu, Pb, Zn and Fe using ICP-OES (Varian Vista MPX) at Research Centre for Residue and Quality Analysis (RCRQA), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (Shalimar Campus) Srinagar, Jammu and Kashmir India.

Four-way analysis of variance (ANOVA) was used to indicate significant differences in heavy metals among sites, species and organs. Furthermoreone-way (ANOVA) was used to compare heavy metals between species in single organ (significant values, $p \leq 0.05$). All data values were checked, beforehand, for the homogeneity of variances and normality; the data which were not found normally distributed or not homogeneous were transformed. Statistical analysis was carried out with SPSS 18.0 for Windows**.**

RESULTS AND DISCUSSION

differences in heavy metals concentrations and physico-chemical properties of water from which fishes were sampled, ecological requirements, metabolic rate and feeding habits of fish and also the season in which investigation was carried out. In the river, fishes occupy the top most trophic level of the food chain and have the strong tendency to accumulate heavy metals from water[17]. Therefore, bioaccumulation of heavy metals in fish can be considered as an indicator of heavy metal pollution in the aquatic ecosystems[14,18,19]. That could be a useful tool to understand the biochemical role of metals present at significant levels in fish[20]. Bioaccumulation is the capability of an organism to concentrate an element or a compound from food chain and water to a level higher than that of its environment. Bioaccumulation is the consequential process of many interactions within different organs of an organism. Heavy metal uptake and their distribution and toxicity in aquatic fauna are influenced by many physico-chemical parameters such as pH, alkalinity, hardness of water, temperature, dissolved oxygen etc. Heavy metals exist in a variety of states and their toxicity depends on their nature and chemical forms as well[21]. Cu constitutes as an essential part of several enzymes and plays a vital role in the synthesis of haemoglobin[22], but can lead to adverse health effects if over-consumed. The concentration of the Cu exhibited a wide range of variation between different tissues, seasons, sites and species (Tables 3 & 4).

Table 3 Seasonal and Spatial variation of Cu (µg/kg) in different organs of *Schizothorax niger* (Mean ± SE)

Organ	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	116.5 ± 0.6	137.0 ± 5.0	94.6 ± 4.3	120.2 ± 2.6	127.8 ± 3.6	125.1 ± 5.0
	Autumn	51.6 ± 0.7	93.1 ± 2.3	160.7 ± 4.4	258.2 ± 2.0	71.5 ± 0.7	96.0 ± 2.9
	Winter	53.9 ± 1.5	73.1 ± 1.2	99.3 ± 0.5	116.9 ± 3.6	152.8 ± 0.8	148.6 ± 2.9
	Spring	54.4 ± 2.1	109.6 ± 1.6	149.4 ± 5.4	145.0 ± 3.8	164.7 ± 3.1	212.9 ± 1.5
Liver	Summer	124.9 ± 3.1	120.2 ± 5.1	117.7 ± 3.9	125.8 ± 1.9	133.8 ± 3.0	144.0 ± 1.9
	Autumn	52.9 ± 1.8	104.4 ± 1.6	179.4 ± 1.0	276.6 ± 2.5	73.4 ± 1.6	104.2 ± 1.2
	Winter	67.3 ± 2.6	88.4 ± 1.8	106.3 ± 2.0	137.8 ± 2.7	169.2 ± 1.5	168.0 ± 2.4
	Spring	69.8 ± 2.1	103.7 ± 1.5	151.1 ± 2.7	186.4 ± 1.6	174.1 ± 1.7	198.9 ± 1.5
Muscle	Summer	74.2 ± 3.5	62.4 ± 1.6	107.7 ± 3.4	108.5 ± 4.1	123.1 ± 1.3	129.7 ± 4.0
	Autumn	60.6 ± 1.8	86.6 ± 6.4	161.5 ± 1.4	254.9 ± 7.5	57.2 ± 2.7	87.8 ± 2.8
	Winter	73.1 ± 3.4	85.9 ± 5.5	116.4 ± 5.1	146.2 ± 6.7	168.7 ± 9.3	73.1 ± 3.4
	Spring	84.6 ± 3.6	125.8 ± 2.6	157.3 ± 7.4	142.4 ± 1.6	200.8 ± 1.5	84.6 ± 3.6

Table 4 Seasonal and Spatial variation of Cu (µg/kg) in *Cyprinus carpio* (Mean ± SE, BDL = Below Detection Limit)

Organs	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	117.8 ± 2.9	103.8 ± 4.9	129.7 ± 6.9	154.6 ± 3.6	175.1 ± 4.7	186.4 ± 2.1
	Autumn	61.5 ± 0.6	105.0 ± 3.5	240.9 ± 1.3	295.0 ± 3.2	362.3 ± 1.2	207.2 ± 2.7
	Winter	64.5 ± 1.4	55.5 ± 1.8	85.0 ± 2.0	104.7 ± 2.8	54.3 ± 1.5	52.5 ± 1.5
	Spring	53.5 ± 1.1	91.0 ± 3.8	105.7 ± 1.5	126.0 ± 2.1	127.2 ± 4.3	93.9 ± 1.4
Liver	Summer	140.2 ± 7.4	105.9 ± 1.5	152.5 ± 5.1	165.1 ± 1.4	196.6 ± 2.0	202.1 ± 4.1
	Autumn	75.6 ± 3.7	120.6 ± 2.4	255.6 ± 1.3	309.4 ± 3.5	381.7 ± 2.6	226.7 ± 2.7
	Winter	82.6 ± 2.0	62.8 ± 0.5	95.8 ± 2.4	86.4 ± 2.4	71.6 ± 3.7	70.3 ± 4.1
	Spring	60.8 ± 2.0	92.4 ± 4.6	115.1 ± 2.4	139.5 ± 4.1	125.0 ± 2.3	104.8 ± 2.5
Muscle	Summer	91.1 ± 0.9	76.5 ± 2.8	134.3 ± 4.3	148.7 ± 4.6	180.6 ± 4.4	192.2 ± 2.7
	Autumn	BDL	103.0 ± 3.7	224.6 ± 8.2	257.8 ± 2.5	326.5 ± 2.3	202.7 ± 4.4
	Winter	BDL	57.1 ± 3.6	76.6 ± 4.6	87.5 ± 6.4	66.5 ± 1.4	73.8 ± 5.6
	Spring	BDL	77.1 ± 3.9	95.5 ± 2.3	121.4 ± 5.2	104.2 ± 3.5	81.7 ± 8.3

Mean and standard error values of the tested heavy metals in three different tissues of *Schizothorax niger* and *Cyprinus carpio* are given in Tables 3 to 10. Knowledge of heavy metal accumulation in fish tissues is very important with respect to management and human consumption. Several studies have been carried out in order to assess the heavy metals in fish tissues and the concentration of heavy metals have been found to vary considerably among different tissues and species[14,15,16]. Variation in bioaccumulation may be due to

The highest concentration in case of *Schizothorax niger* was recorded in the liver tissue in autumn season with a value of 279.6 µg/kg collected from Site 4 of the study area and the lowest was recorded in the gill tissue in winter season with a value of 53.9 µg/kg collected from Site 1. The distribution pattern of Cu in *Schizothorax niger* was found in the order of Liver > Gills > Muscle. Site wise distribution of Cu accumulation has been found as Site4> Site 3> Site 6>Site 5>Site 2> Site1. Seasonal trend of bioaccumulation of Cu for

Schizothorax niger has been found as spring> autumn> summer> winter. The highest concentration of Cu in

Table 5 Seasonal and Spatial variation of Zn (μg/kg) in different organs of *Schizothorax niger* (Mean ± SE)

Organs	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	233.8 ± 2.2	245.1 ± 1.6	194.4 ± 4.5	253.0 ± 4.0	322.0 ± 1.5	309.6 ± 4.8
	Autumn	78.3 ± 1.16	238.8 ± 1.5	331.5 ± 3.7	129.6 ± 1.6	62.7 ± 3.7	54.6± 1.6
	Winter	84.35 ± 2.0	201.6 ± 1.9	405.5 ± 2.4	448.6 ± 1.2	298.4 ± 1.1	473.6 ± 1.4
	Spring	91.1 ± 2.7	228.7 ± 4.6	454.4 ± 4.4	491.9 ± 3.2	355.0 ± 1.7	475.9 ± 5.5
Liver	Summer	204.9 ± 6.2	318.9 ± 9.2	228.9 ± 4.7	268.5 ± 4.0	349.8 ± 4.0	334.8 ± 2.9
	Autumn	92.6 ± 3.1	256.4 ± 3.6	345.9 ± 3.6	145.7 ± 3.2	87.8 ± 7.4	63.3 ± 1.6
	Winter	89.91 ± 1.1	221.7 ± 5.0	416.9 ± 3.2	464.6 ± 1.8	324.4 ± 4.1	456.8 ± 3.2
	Spring	106.2 ± 2.1	257.7 ± 2.0	472.3 ± 5.0	506.4 ± 3.5	369.7 ± 5.3	472.8 ± 1.3
Muscle	Summer	103.7 ± 3.2	202.1 ± 3.6	209.2 ± 1.5	247.5 ± 4.8	308.9 ± 2.7	312.9 ± 6.8
	Autumn	65.8 ± 2.5	222.9 ± 5.9	321.4 ± 6.7	124.7 ± 3.4	322.5 ± 1.2	59.11 ± 0.2
	Winter	75.41 ± 5.0	207.9 ± 1.8	366.2 ± 4.7	434.5 ± 3.9	232.5 ± 5.7	430.9 ± 1.3
	Spring	77.5 ± 7.9	229.3 ± 8.6	429.2 ± 1.9	473.9 ± 6.4	356.0 ± 3.8	466.7 ± 7.7

Table 6 Seasonal and Spatial variation of Zn (μg/kg) in different organs of *Schizothorax niger* (Mean ± SE)

Organ	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	222.4 ± 5.0	226.9 ± 2.5	205.3 ± 3.7	367.3 ± 3.1	298.0 ± 4.9	401.7 ± 2.3
	Autumn	109.0 ± 1.5	209.4 ± 2.3	271.6 ± 2.4	378.2 ± 3.3	411.6 ± 6.3	308.6 ± 4.0
	Winter	55.6 ± 2.3	90.3 ± 0.6	103.3 ± 1.8	146.2 ± 1.9	228.0 ± 4.4	234.6 ± 2.1
	Spring	64.6 ± 2.3	110.6 ± 1.5	130.0 ± 3.2	139.1 ± 1.9	280.4 ± 3.5	270.8 ± 1.9
Liver	Summer	296.7 ± 9.5	262.3 ± 1.2	374.8 ± 7.5	383.8 ± 2.3	299.1 ± 0.8	416.6 ± 4.0
	Autumn	130.7 ± 3.3	220.3 ± 4.2	304.4 ± 3.5	399.6 ± 3.8	434.6 ± 2.4	331.9 ± 3.6
	Winter	65.8 ± 2.0	93.0 ± 1.5	116.8 ± 2.7	159.8 ± 1.8	243.0 ± 6.0	241.4 ± 2.0
	Spring	73.9 ± 1.0	122.6 ± 8.7	145.4 ± 2.2	154.1 ± 1.8	275.4 ± 2.8	279.5 ± 2.2
Muscle	Summer	117.8 ± 4.3	132.6 ± 3.5	336.5 ± 1.6	365.9 ± 7.0	290.7 ± 6.4	399.9 ± 3.9
	Autumn	123.0 ± 6.3	223.0 ± 8.1	284.9 ± 2.8	371.9 ± 5.6	405.6 ± 2.1	261.1 ± 8.2
	Winter	69.1 ± 2.3	108.1 ± 3.1	131.1 ± 8.0	201.3 ± 1.8	191.1 ± 3.1	74.64 ± 6.2
	Spring	64.3 ± 3.9	94.4 ± 4.7	120.0 ± 6.4	147.7 ± 8.7	258.7 ± 6.4	248.0 ± 5.0

Cyprinus carpio was recorded in the liver tissue in autumn season with a value of 381.7 μg/kg collected from Site 5 of the river and the lowest was found in muscle tissue in winter season with a value of 57.1 μg/kg collected from Site 2. The distribution pattern of Cu was found in the order of Liver > Gills > Muscle. Spatial sequence of Cu accumulation for *Cyprinus carpio* has been recorded as Site5> Site 4> Site 3>Site 6>Site 2> Site1. Seasonal trend of accumulation for *Cyprinus carpio* has been recorded as summer>autumn>spring>winter.

Significant level of Cu in fish tissue may be due to industrial, domestic and municipal sewage wastes. Also, it may be due to elevated metal–binding protein synthesis[22]. Cu exposure to fish can be directly from the water via gills. Effects of high levels of Cuin fish tissues are not well established; however, there are evidences that high concentrations of Cu in fish can pose toxicity[23]. Cu can actively combine with other contaminants like ammonia (NH_3, mercury (Hg) and Zn to produce synergistic effects on fish[22]. The lower concentration of Cu in the gill than that of liver may be due to lower binding affinity of Cu on the gill surface. Gills which are in direct contact with water accumulate some amount of heavy metals due to adsorption to the gill surfaces and may also depend on the availability of proteins to which these heavy metals may bind. The lower affinity towards accumulation of heavy metals by some tissues may be due to development of some defensive mechanism such as excessive mucous secretion and clogging of gills. Muscle tissue was found to accumulate least amount of Cu and might have received it through circulation of blood. It is also suggested that the low accumulation of heavy metals in muscle tissue may be due to lack of binding affinity of Cu with the muscle proteins. This is particularly important because muscles contribute the greatest mass of the flesh that is consumed as food by human beings. Zn is an essential trace element and is a common contaminant as well. Sewage disposal, geological quarrying is major source of Zn pollution to surface water bodies. Fish are exposed to it directly from water, especially by mucous and gills[24]. The relatively higher Zn concentration in the liver compared to other tissues in both the species may be due to the role of Zn as an activator of several enzymes present in the liver[22]. Zn was accumulated in almost all tissues of the fish samples in all seasons and sites (Tables 5 and 6).The highest concentration of Zn in *Schizothorax niger* was found in the liver tissue in autumn season with a value of 506.4 μg/kg collected from Site 4 of the study area and the lowest was recorded in the muscle tissue in autumn season with a value of 65.80 μg/kg collected from Site 1. The distribution pattern of Zn *Schizothorax niger* was in the order of Liver > Gills > Muscle. Site wise distribution of Zn accumulation has been found as Site3> Site 4>Site 6>Site 5>Site 2> Site1. Seasonal trend of accumulation of Zn for *Schizothorax niger* has been found as spring> winter> summer> autumn. The highest concentration of Zn in *Cyprinus carpio* was recorded in the liver tissue in autumn season with a value of 434.6 μg/kg collected from Site 5 of the study area and the lowest was recorded in the gill tissue in autumn season with a value of 55.6 μg/kg collected from Site 1. The distribution pattern of Zn was in the order of Liver > Gills > Muscle. Spatial distribution of Zn accumulation for *Cyprinus carpio* has been found as Site6> Site 5> Site 4>Site 3>Site 2> Site1. Seasonal trend forbioaccumulation of Zn for *Cyprinus carpio* has been recorded as summer>autumn>spring>winter.

Pb is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes[24]. The distribution of Pb in both the species is given in Tables 7 and 8. The highest of Pbconcentration in *Schizothorax niger* was recorded in the liver tissue in winter season with a value of 14.42 μg/kg collected from Site 6 of the study area and the lowest was found in the gill tissue in autumn season with a value of 5.14 µg/kg collected from Site 1. The distribution pattern of Pb for *Schizothorax niger* was in the order of Liver > Gills > Muscle. Site wise distribution of Pbaccumulation has been found as Site4> Site 6> Site 5>Site 3>Site 2> Site1. Seasonal trend of accumulation of Pb for *Schizothorax niger* has been found as winter> spring> summer> autumn. The maximum concentration of Pb in *Cyprinus carpio* was recorded in the liver tissue in winter season with a value of 13.51 µg/kg collected from Site 4 of the river and the minimum was found in the muscle tissue in spring season with a value of 5.04 µg/kg collected from Site 2.

Table 7 Seasonal and Spatial variation of Pb (µg/kg) in *Schizothorax niger*(BDL= Below detection limit)

Organs	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	6.32 ± 0.09	5.33 ± 0.11	7.43 ± 0.61	12.2 ± 0.30	10.5 ± 0.27	6.43 ± 0.15
	Autumn	5.16 ± 0.04	6.35 ± 0.25	10.07 ± 0.2	7.98 ± 0.43	5.62 ± 0.19	10.09 ± 0.1
	Winter	7.14 ± 0.07	7.50 ± 0.20	8.26 ± 0.03	10.55 ± 0.3	10.15 ± 0.2	12.95 ± 0.2
	Spring	5.93 ± 0.16	6.93 ± 0.43	6.30 ± 0.58	8.69 ± 0.19	10.03 ± 0.3	9.67 ± 0.28
Liver	Summer	5.67 ± 0.26	8.75 ± 0.15	7.21 ± 0.23	10.21 ± 0.2	12.83 ± 0.1	7.39 ± 0.22
	Autumn	6.06 ± 0.34	7.26 ± 0.05	10.71 ± 0.2	9.63 ± 0.55	6.59 ± 0.22	11.72 ± 0.2
	Winter	7.77 ± 0.09	8.69 ± 0.09	9.50 ± 0.23	12.60 ± 0.1	10.88 ± 0.1	14.42 ± 0.1
	Spring	6.48 ± 0.13	6.62 ± 0.10	8.36 ± 0.12	10.37 ± 0.5	10.82 ± 0.2	10.28 ± 0.3
Muscle	Summer	BDL	5.78 ± 0.27	8.75 ± 0.26	7.59 ± 0.31	6.00 ± 0.19	5.69 ± 0.34
	Autumn	BDL	BDL	8.46 ± 0.49	6.60 ± 0.69	BDL	9.57 ± 0.56
	Winter	BDL	7.15 ± 0.39	8.30 ± 0.14	10.84 ± 0.5	9.19 ± 0.43	12.50 ± 1.1
	Spring	BDL	5.63 ± 0.28	6.36 ± 0.47	8.02 ± 0.25	7.80 ± 0.82	7.73 ± 0.65

Table 8 Seasonal and Spatial variation of Pb (µg/kg) in *Cyprinus carpio*(BDL= Below detection limit)

Organ	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	7.87 ± 0.23	7.15 ± 0.06	7.66 ± 0.58	11.14 ± 0.1	11.85 ± 0.5	8.28 ± 0.15
	Autumn	6.27 ± 0.06	5.66 ± 0.08	10.68 ± 0.2	10.45 ± 0.3	6.25 ± 0.15	7.42 ± 0.24
	Winter	5.75 ± 0.14	5.42 ± 0.20	7.49 ± 0.23	12.65 ± 0.1	8.48 ± 0.09	6.67 ± 0.39
	Spring	6.33 ± 0.21	7.82 ± 0.24	8.12 ± 0.09	10.45 ± 0.1	7.50 ± 0.12	7.15 ± 0.07
Liver	Summer	6.48 ± 0.24	10.45 ± 0.2	9.71 ± 0.19	12.89 ± 0.3	14.30 ± 0.5	9.41 ± 0.16
	Autumn	7.57 ± 0.21	5.44 ± 0.16	13.03 ± 0.3	12.66 ± 0.3	8.20 ± 0.24	8.43 ± 0.21
	Winter	6.44 ± 0.26	6.53 ± 0.15	7.47 ± 0.16	13.51 ± 0.2	9.84 ± 0.06	7.90 ± 0.15
	Spring	7.61 ± 0.19	7.72 ± 0.16	6.80 ± 0.35	11.28 ± 0.2	8.23 ± 0.06	7.52 ± 0.25
Muscle	Summer	6.93 ± 0.21	8.14 ± 0.13	10.83 ± 0.2	9.11 ± 0.30	8.20 ± 0.35	6.92 ± 0.26
	Autumn	BDL	BDL	10.45 ± 0.5	12.29 ± 0.4	6.11 ± 0.26	6.70 ± 0.42
	Winter	BDL	BDL	5.49 ± 0.18	11.62 ± 0.5	8.10 ± 0.40	6.54 ± 0.47
	Spring	BDL	5.04 ± 0.17	6.19 ± 0.19	8.29 ± 0.54	5.83 ± 0.46	5.72 ± 0.35

The distribution pattern of Pb was in the order of Liver > Gills > Muscle. Spatial distribution of Pb accumulation for *Cyprinus carpio* has been found as Site4> Site 5> Site 3>Site 6>Site 2> Site1. Seasonal trend of accumulation for *Cyprinus carpio* has been recorded as summer>autumn>winter>spring. Pb was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase and leads to pathological changes in tissues and organs.

Table 9 Seasonal and Spatial variation of Fe (µg/kg) in *Schizothora xniger* (BDL= Below detection limit

Organs	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	254.7 ± 4.8	187.3 ± 5.7	170.8 ± 3.9	238.7 ± 1.6	218.7 ± 4.4	248.3 ± 1.5
	Autumn	56.46 ± 2.6	74.24 ± 2.8	208.3 ± 1.6	205.1 ± 2.6	59.59 ± 1.6	97.56 ± 3.5
	Winter	75.3 ± 1.9	105.8 ± 1.9	106.7 ± 2.9	232.5 ± 3.7	343.4 ± 1.3	295.6 ± 2.8
	Spring	99.74 ± 3.6	117.7 ± 7.1	155.6 ± 2.0	233.3 ± 5.1	384.1 ± 2.0	323.9 ± 4.3
Liver	Summer	318.7 ± 4.7	217.6 ± 1.9	219.6 ± 1.7	254.8 ± 3.6	236.8 ± 3.9	271.5 ± 7.2
	Autumn	62.6 ± 1.34	85.1 ± 2.29	227.0 ± 2.9	231.0 ± 1.8	72.08 ± 5.8	115.9 ± 5.3
	Winter	84.24 ± 3.0	96.81 ± 2.2	129.6 ± 3.8	256.7 ± 3.8	352.0 ± 1.6	310.6 ± 4.5
	Spring	109.2 ± 1.5	117.5 ± 1.0	166.1 ± 1.8	290.8 ± 5.1	392.4 ± 0.7	320.7 ± 6.4
Muscle	Summer	88.6 ±8.68	134.7 ± 5.5	168.5 ± 1.7	201.0 ± 1.0	210.2 ± 3.4	216.3 ± 9.9
	Autumn	52.58 ± 0.8	86.5 ± 5.35	209.6 ± 4.9	202.9 ± 6.2	54.8 ± 1.20	80.6 ± 3.11
	Winter	67.07 ± 3.9	78.7 ± 10.0	108.8 ± 4.1	230.3 ± 7.9	337.3 ± 5.6	282.6 ± 4.8
	Spring	94.6 ± 4.97	101.4 ± 4.9	147.5 ± 5.8	257.5 ± 7.0	354.6 ± 5.1	295.7 ± 5.9

Table 10 Seasonal and Spatial variation of Fe (µg/kg) in *Cyprinus carpio* (BDL= Below detection limit)

Organs	Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Gills	Summer	133.4 ± 2.9	139.6 ± 2.9	315.3 ± 7.4	376.2 ± 6.6	313.8 ± 6.4	465.8 ± 1.2
	Autumn	62.01 ± 0.9	105.5 ± 3.0	383.7 ± 2.9	403.1 ± 4.1	201.2 ± 2.3	432.8 ± 1.2
	Winter	108.6 ± 1.8	170.3 ± 3.0	122.1± 1.7	102.1 ± 2.7	223.5 ± 1.4	105.0 ± 2.3
	Spring	142.1 ± 1.6	185.3 ± 3.8	171.4 ± 2.1	127.7 ± 2.4	272.9 ± 3.4	192.4 ± 2.7
Liver	Summer	343.4 ± 3.3	293.0 ± 4.4	410.4 ± 3.4	388.8 ± 3.6	406.8 ± 3.3	491.7 ± 1.1
	Autumn	77.07 ± 3.0	119.7 ± 2.0	400.1 ± 4.0	435.6 ± 2.6	234.4 ± 1.3	475.3 ± 2.9
	Winter	118.8 ± 3.0	193.8 ± 5.4	139.8 ± 2.3	117.7 ± 4.5	235.7 ± 1.8	117.8 ± 3.5
	Spring	158.4 ± 1.4	176.4 ± 4.2	192.6 ± 2.6	143.1 ± 2.0	290.9 ± 2.7	204.7 ± 2.7
Muscle	Summer	208.7 ± 7.9	228.9 ± 4.0	336.5 ± 6.9	356.0 ± 2.7	385.1 ± 3.4	385.5 ± 9.2
	Autumn	61.30 ± 4.1	105.0 ± 3.5	376.6 ± 4.0	398.1 ± 8.6	221.1 ± 4.0	452.6 ± 5.0
	Winter	120.3 ± 5.2	161.1 ± 8.7	125.0 ± 8.5	87.0 ± 5.78	217.0 ± 4.9	98.0 ± 4.71
	Spring	133.9 ± 5.6	161.4 ± 9.9	157.6 ± 5.8	134.3 ± 6.5	254.9 ± 4.8	156.3 ± 9.0

The increase of Pb level is due to the discharge of industrial, agricultural and sewage wastes in the investigated area. The significant level of Pb may be attributed to sufficient Pb concentration in water and sediment of the river [25]. liver tissues are usually related to within organ binding proteins such as metallothioneins (MT)[27] which act as an essential metal store for heavy metals[28,29].

Table 11 Four-way ANOVA showing variation in heavy metals between different locations, organs, species and seasons

	Source	df	F value	P value		Source	df	F value	P value
Zn	Site	5	252.227	<0.001	Pb	Site	5	727.276	<0.001
	Species	1	45.690	<0.001		Species	1	37.014	<0.001
	Organ	2	29.843	<0.001		Organ	2	1.479	<0.001
	Season	3	2.373	<0.001		Season	3	13.440	<0.001
Cu	Site	5	273.056	<0.001	Fe	Site	5	246.394	<0.001
	Species	1	35.751	<0.001		Species	1	36.565	<0.001
	Organ	2	5.163	<0.001		Organ	2	24.380	<0.001
	Season	3	4.264	<0.001		Season	3	4.269	<0.001

With different magnitude the Fe accumulated mainly in the liver of fishes collected from all sites in all seasons. The distribution of Fe in both the species is given in Tables 7 and 8. The highest concentration of Fein *Schizothorax niger* was recorded in the gill tissue in spring season with a value of 392.4 µg/kg collected from Site 5 of the river and the lowest was also found in the gill tissue in autumn season with a value of 52.58 µg/kg collected from Site 1. The distribution pattern of Fe for *Schizothorax niger* was in the order of Liver > Gills > Muscle. Site wise distribution of Fe accumulation has been found as Site5> Site 4> Site 6>Site 3>Site 2> Site1. Seasonal trend of accumulation of Fe for *Schizothorax niger* has been found as summer> spring> winter> autumn. The highest concentration of Fe in *Cyprinus carpio* was recorded in the liver tissue in summer season with a value of 491.7 µg/kg collected from Site 6 of the study area and the lowest was found in the muscle tissue in autumn season with a value of 62.01 µg/kg collected from Site 1. The distribution pattern of Fe was recorded in the order of Liver > Muscle > Gills. Spatial distribution of Fe accumulation for *Cyprinus carpio* has been found as Site6> Site 5> Site 3>Site 4>Site 2> Site1. Seasonal trend of accumulation for *Cyprinus carpio* has been recorded as summer>autumn>spring>winter.

Fe is an abundant and important element, unparalleled by any other heavy metals in the earth's crust[24]. The increase of Fe accumulation in fish liver in this study may be related to the increase of total dissolved Fe in Jhelum water and consequently increase the free metal Fe concentration and thereby lead to an increase in Fe uptake by different organs[25]. Yacoub (2007)[22] observed accumulation of Fe ligand protein (Hemosidrin) scattered in liver section of fish exposed to very high Fe concentration.

Regarding the spatial variation, there was no consistent increase of all heavy metals in all fish species from one site. However, statistically, Site 4 showed significantly high concentrations of heavy metals as compared to rest of the sites. The accumulation of heavy metals showed significant variations among seasons, sites and species for all heavy metals. However it has been found that, different organs exhibited different patterns in heavy metals accumulation. However, some fish samples from the same species collected from different sites and seasons also significantly accumulated different concentrations of heavy metals (ANOVA: $p < 0.001$, Table, 11). Present study revealed the lowest concentration of heavy metals in muscle tissue and the maximum concentration of heavy metals in hepatic tissue. The bioaccumulation of heavy metals in the liver may be due to its role in metabolism[26]. High levels of heavy metals in

Similarly Fe tends to bio-accumulate in liver tissue due to the vital role of the liver in the formation of blood cells and haemoglobin synthesis[29]. Presence of high levels of Pb and Cu could be due to the ability of these heavy metals to displace the normally MT-combined essential trace elements in the liver[30].The test fish species tend to bio-accumulate some heavy metals in their gills. Gills are the main route of heavy metal exchange from water[30] as they possess a very large surface area which facilitates rapid diffusion of heavy metals[31]. Therefore, it can be suggested that heavy metals accumulated in gills are mainly transported from water[32].

There is a seasonal effect on the heavy metal uptake of fish species which might reflect the variation in metabolic activity of the species. Seasonal differences for all heavy metals in liver, gills and muscle of two species were observed in this study and the highest levels were found during summer season of the year due to the reason that industrial, commercial, domestic and agricultural practices are at peak in this season so maximum anthropogenic pollution to river and the concentration of heavy metals in fish tissues was followed further by autumn season mainly due to decrease in overall water quantity in the river in this dry season which in turn concentrate the heavy metals in the river and least content of heavy metals has been recorded in spring season as the discharge of the river in this season increases and least anthropogenic inputs[33]. Variation in metal concentrations with season has been well-documented in several studies from freshwater environment [34,35,36]. It has been found due to varying seasonal growth rate, reproductive cycle, water salinity and temperature. Larson *et al*. (1985)[37] also found that most of the physiological parameters in fish are subjected to seasonal fluctuations.

Fishes are mostly in continuous movement and seldom stay at a single place, heavy metal bioaccumulation in fish tissues provide an index of contaminated aquatic environment of the area[30]. In the present study, spatial distribution of heavy metal bioaccumulation in fish tissues showed significant concentration of Cu, Zn, Pb, and Fe at Site 4 of the study area. This is mainly due to the commercial, urban, industrial and other anthropogenic input of heavy metals from Srinagar City and its adjoining areas. On the other hand, least content of heavy metals has been recorded at Site1 of the study area. The reason might be that Site 1 is located near to the source of Jhelum River i.e. Verinag, which is least polluted site for being at high altitude and least anthropogenic pressure. It is well known fact that muscle tissue is not an active site for heavy metal biotransformation and accumulation[38]. But in contaminated aquatic environments, significant amount of heavy metals can be found in muscles as can lead to severe health hazards. To asses public health risk of the fishes captured from River Jhelum for human consumption, we compared heavy metal levels in muscles of the current study (Tables 3-10) with the maximum permissible limits for human consumption (MPL) established by many different organizations The metal concentrations in the examined fish species from the Jhelum river fall below the maximum permissible limit for human consumption recommended by FAO and WHO[39].

Studies from the field and laboratory experiments revealed that bioaccumulation of heavy metals in a tissue is mainly dependent on water concentrations of heavy metals and exposure period; although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in heavy metal accumulation[40,41]. Environmental needs, size and age of fishes, their life history and life cycle, feeding habits and the season of capture were found to affect experimental results significantly from the tissues[42]. Low levels of contamination may have no apparent impact on the fish, which might not show signs of illness, but may lead to decrease in fecundity of fish populations, which inturn accelerate a long-term decline and eventual extinction of some important sensitive fishes like *Schzothorazniger* etc[43]. Such low-level contamination could have a remarkable impact on reproduction, either indirectly via bioaccumulation in reproductive organs, or directly on gametes (sperm or ovum) which are released into the water. Effect on reproduction of fish is very complex phenomena and is regulated by a numerous factors and low-level contamination could also affect any part of this pathway. On conclusion it can be suggested that decline of *Schizothorax* population in Kashmir waters can be due to several reasons but the presence of significant amount of heavy metals inside the tissues might be one of the potential reasons[42,43,44].

CONCLUSION

Heavy metal concentrations in the six studied locations were found below the permissible limit set by international authorities. The findings also revealed that heavy metal bioaccumulation significantly varied between sites, seasons, species and organs depending on species specific factors like geographical location, climate, feeding behaviour, swimming patterns and genetic tendency that caused variation in heavy metal accumulation between fish even from the same species. Health risk analysis of heavy metals in the fish tissues indicated safe levels for human consumption. However, the levels of heavy metals in *Schizothorax niger* species should be continuously monitored in Jhelum river since population of this fish is showing a continuous declining trend in Kashmir waters.

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