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Heavy metal contamination in two commercial fish species of a trans-Himalayan freshwater ecosystem

Mohammad Aneesul Mehmood · Humaira Qadri · Rouf Ahmad Bhat · Asmat Rashid · Sartaj Ahmad Ganie · Gowhar Hamid Dar · Shafiq-ur-Rehman

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Abstract Toxic metals have disturbed the quality of freshwater ecosystems worldwide. The concentration of heavy metals was investigated in liver, gills and muscle tissues of *Schizothorax niger* and *Cyprinus carpio* captured from river Jhelum of Kashmir Himalaya. The heavy metals displayed a wide range of disparity in studied tissues, seasons, sites and species. Cu^{2+} exhibited the highest concentration (279.6 μg/kg) in the liver tissues of *S. niger* in autumn at site 2 and the lowest (53.1 μg/kg) in the gill tissues in winter at site 1. In *C. carpio*, the Cu^{2+} was recorded highest (309.4 μg/kg) in the liver tissues in autumn at site 2. The concentration of Zn^{2+} was found highest (575.7 μg/kg) in the liver tissues at site 2 and the lowest (65.8 μg/kg) was recorded in the muscle tissues in autumn at site 1. Zn^{2+} was recorded highest (416.6 μg/kg) in the liver tissues in autumn at site 3 and lowest (51.5 μg/kg) in the gills of *C. carpio* during winter at site 1 (control). The concentration of Pb^{2+} (14.42 μg/kg) and Fe^{2+} (323.9 μg/kg) was observed in the liver tissue and gills of *S. niger* at site 3. Similar levels of Pb^{2+} and Fe^{2+} were recorded in the tissues of *C. carpio* at different sites. Four-way ANOVA (four way) indicated a statistically significant variation ($p \leq 0.05$) in heavy metals with the sites, seasons, species and organs. The study emphasises the utmost need to monitor the level of heavy metals in *S. niger* on a regular basis as this native fish species is showing a continuous decline in the freshwater ecosystems of Kashmir Valley.

M. A. Mehmood (✉) · H. Qadri · R. A. Bhat · G. H. Dar
Department of Environment and Water Management, Cluster University Srinagar, School of Sciences, Sri Pratap College Campus, Srinagar, Jammu and Kashmir, India
e-mail: aneesulmehmood@gmail.com

A. Rashid · S. A. Ganie · Shafiq-ur-Rehman
Division of Environmental Sciences, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar Campus, Srinagar, Jammu and Kashmir, India

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Introduction

The environmental concern of pollution (Bhat et al. 2017) due to heavy metals has raised pervasive worries worldwide. The surface water is more vulnerable to pollution than ground water (Aliewi and Al-Khatib 2015) due to its easy access to anthropogenic interferences (Al-Khatib et al. 2008). As consequences of human activities, this water resource gets readily polluted with numerous chemicals (Al-Khatib et al. 2003; Anayah 2006; Shomar 2011; WHO and UNICEF 2012; Tamas et al. 2013; Shamsu-Deen 2013; Hadia-e-Fatima and Ahmed 2018). These altogether give birth to a complex chain of issues leading to the deterioration of water quality, bioaccumulation of toxic chemicals and metals, encroachment and siltation of water bodies, and affects the aesthetic importance as well (Nagaprapurna and Shashikanth 2002). Surface water is suppressed to high levels of contamination (pesticides, industrial effluents, fertilisers, heavy metals, trace elements and other chemicals) by human activities (Klecka et al. 2010; Pandey et al. 2014; Skordas et al. 2015; Yadav et al. 2016). The storage, dispersal and movement of these contaminants in the aquatic ecosystem have a substantial consequence on the chemistry, ecology and biological properties of water (Jarvie et al. 1998; Ravichandran 2003; Mahvi et al. 2005; Gantidis et al. 2007; Arain et al. 2008; Liao et al. 2008). Wastewater inputs usually contribute salts, silt, oil, nutrients, pesticides, herbicides, heavy metals and other deleterious materials to fresh water bodies (Pandey et al. 2014). The impact of waste discharge could change the chemistry of concerned water body, loss of aquatic life and uptake of contaminated water by aquatic biodiversity (Jarup 2003; Bhat et al. 2018). The problem of aquatic pollution due to heavy metals has led to extensive concerns in almost all parts of the world, and observations reported by various institutions, researchers and agencies have been shocking to environmentalists (Tiwari et al. 2015). The prominent sources of heavy metal contamination to water bodies are agricultural inputs, domestic-cum-commercial sewage, untreated or partially treated effluents from small and largescale industrial units (Morais et al. 2012), metal electroplating, manufacture and disposal of batteries (Chen et al. 2012), circuit boards, car repair, motor workshops (Mandal and Sengupta 2002; Singh et al. 2018), geological quarrying (Mehmood et al. 2017), vehicular road inputs, etc. (Querol et al. 1993; Farmaki and Thomaidis 2008; Kandarp et al. 2011; Singh and Gupta 2012; Singh and Gupta 2014). Heavy metals are the most important pollutants in the lotic water bodies because of their toxic, mutagenic and carcinogenic nature (Mahurpawar 2015). Aquatic biota can get exposed and may bioaccumulate the heavy metals from several sources like overlaying water, bed sediments, settling of dust, atmospheric aerosols, etc. (Labonne et al. 2001; Goodwin et al. 2003; Nagajyoti et al. 2010; Jaishankar et al. 2014a). Once they join the aquatic system, they may get deposited or attached to sediments and might become the new source of heavy metals into the water environs, and finally accumulate and concentrate in the various tissues of aquatic biota (Oyewo and Don-Pedro 2003). Heavy metals can participate in various biochemical processes, have optimum mobility, can influence the ecosystem by means of bioaccumulation and biomagnification phenomena (Jaishankar et al. 2014b) and are potentially dangerous for human life (Trasande et al. 2005; Kumar et al. 2010). These heavy metals can cause behavioural changes and oxidative stress in animals and humans (Mehmood et al. 2017). Heavy metals can alter the normal behavioural work of fish while injuring nervous system with enhanced lipid peroxidation (Flora et al. 2008). This depicts that presence of toxic heavy metals in elevated concentrations could be one of the risk parameters for declining the fish population in lentic and lotic water bodies (Shafiq-ur-Rehman 2003; Martin and Griswold 2009).

Fish is widely utilised as a staple food and rich supply of protein all around the world. Kashmiri people consume significant quantity of fish (Mehmood et al. 2017), and most of the fishes and fish products traded in the market are captured from the local water bodies including river Jhelum (El-Naggar et al. 2009; Malik et al. 2010; Mehmood et al. 2017). In Kashmir province of Jammu and Kashmir, Baramulla is at first rank in fish production and produces about 4714.63 tons/year (Qayoom et al. 2014) followed by the district Bandipora that adds about 3854.85 tons/year to the total fish production of the valley. However, when future fish production of the valley is taken into consideration, the statistical models have indicated that overall fish production of the valley is showing a declining trend. (Qayoom et al. 2014; Mehmood et al. 2017). There could be multiple reasons for this but the major reason can be disturbance occurred to the breeding areas of various fish species especially *Schizothorax niger* and *Cyprinus carpio* by drastic decline in water quality due to heavy metal pollution and other anthropogenic impacts (Qayoom et al. 2014; Mehmood et al. 2017). In view of all the above-mentioned facts, this study is very much vital and important to get an insight about the level of heavy metal contamination in two commercially most important edible fish species viz., *Schizothorax niger* and *Cyprinus carpio* captured from the Jhelum River of Kashmir Valley.

Materials and methods

Jhelum River is culturally the prime lotic water body of Kashmir Valley; in local language, it is known as *Veth*. Verinag Spring in south Kashmir is considered as the main source of Jhelum. From the source, Jhelum starts its 241-km-long journey through the heart of the valley and ultimately joins Pakistan in district Baramulla of Kashmir Valley (Mehmood et al. 2017). The various tributaries and streams which join Jhelum from either banks include, Sandran, Brang, Arapat kol, Lidder, Arapal, Harwan, Sindh, Erin, Mudhumati, Pohru, Vijidakil, Vishav, Rambria, Romshi, Doodhganga, Ferozpura and Ningal. The sampling zones have been selected keeping in view the geography, settlement units, agricultural areas and commercial hubs along both banks of Jhelum River (Fig. 1). The study area has been furcated into three distinct sampling location as described in Table 1. Edible status, presence at all the selected sites of the river and decline in population (*Schizothorax niger*) were the criteria followed to select the test fish species for this investigation (Mehmood et al. 2017).

Fishes were captured from all the sampling zones during four different seasons from June 2014 to March 2015. Fish 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 has been performed in the first month of each season. The sampling has been achieved between 10:30 a.m. to 03:00 p.m. (Mehmood et al. 2017). Fishes were captured from each sampling location with the help of local fishermen by using net and boat. Three replicates were captured for each fish species at each sampling site. Total fish length (cm) and weight (g) has been noted on spot (Fernandesa et al. 2007). Approximately same-sized fishes were captured in order to avoid the variability in heavy metal (Mekkawy et al. 2008) content due to weight and length of the fishes (Tables 2 and 3). Fishes were dissected by a sterile surgical blade for the extraction of gills, liver and muscle tissues (Khaled et al. 2010). Fish organs were placed in neat and clean polythene bags and were properly labelled (Youssef and Said 2011), preserved in ice box and then transported to the laboratory (Omar 2013). The exact 5 g wet weight of muscle tissue and 1 g wet weight each in case of liver and gills were taken and were oven dried to achieve a constant weight at a temperature of 105 °C (Turkmen et al. 2008). Further, 50-ml conical flasks were used to contain the tissue samples (Sharaf and Shehata 2015). Thereafter, 10 ml of concentrated nitric acid was added slowly to conical flasks containing the samples (2 ml of concentrated perchloric acid was added to the flasks containing gill tissues). All the flasks containing fish tissue samples were covered with aluminium foils and then heated at 200 °C with the help of a hot plate for a duration of 3 hours, until a small volume of solution remained at the bottom of flask (Turoczy et al. 2001). A solution of 2 ml 1 N HNO_3 was added to the flask and was again evaporated on the hot plate (Qadir and Malik 2011). By repeating the same digestion procedure multiple times, whole organic matter in the sample got digested completely (Youssef and Said 2011). After cooling, 2.5 ml of 1 N HNO_3 was transferred in the conical flask containing digested residue and was finally brought up to the volume of 50 ml with distilled water by a standard volumetric flask (Yadav et al. 2016). The samples were filtered by a 0.4-mm Whatman filter paper before sending for instrumental analysis (Alam et al. 2002). All processed samples were analysed for the heavy metals viz., Cu^{2+}, $Pb2^{+}$, Zn^{2+} 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 (Mehmood et al. 2017).

Statistical analysis

Four-way analysis of variance (ANOVA) was applied to the data to assess the statistically significant variation in heavy metals content among all sites, species and organs, and one-way (ANOVA) was adopted to get a comparative outlook of heavy metals with respect to species in a single organ. Statistical analysis indicated the significant values for the processed data ($p \leq 0.05$). All data sets were subjected and analysed for homogeneity of variances test and normality test. The data was transformed which were not found normally distributed (Fig. 2). Statistical examination was carried out with the help of SPSS 18.0 for Windows (Table 4).

Results

The concentration of the Cu^{2+} exhibited an extensive range of variation between various tissues, seasons, sites and species. The highest level in case of *S. niger* was observed in the liver tissue in autumn season with a value of 279.6 μg/kg collected from site 2 of the study area, and the lowest was recorded in the gill tissue in winter season with a value of 53.1 μg/kg collected from site 1. The highest concentration of Cu^{2+} in *Cyprinus carpio* was recorded in the liver tissue in autumn season with a value of 309.4 μg/kg collected from site 2 of the river, and the lowest was found in gill tissue in spring

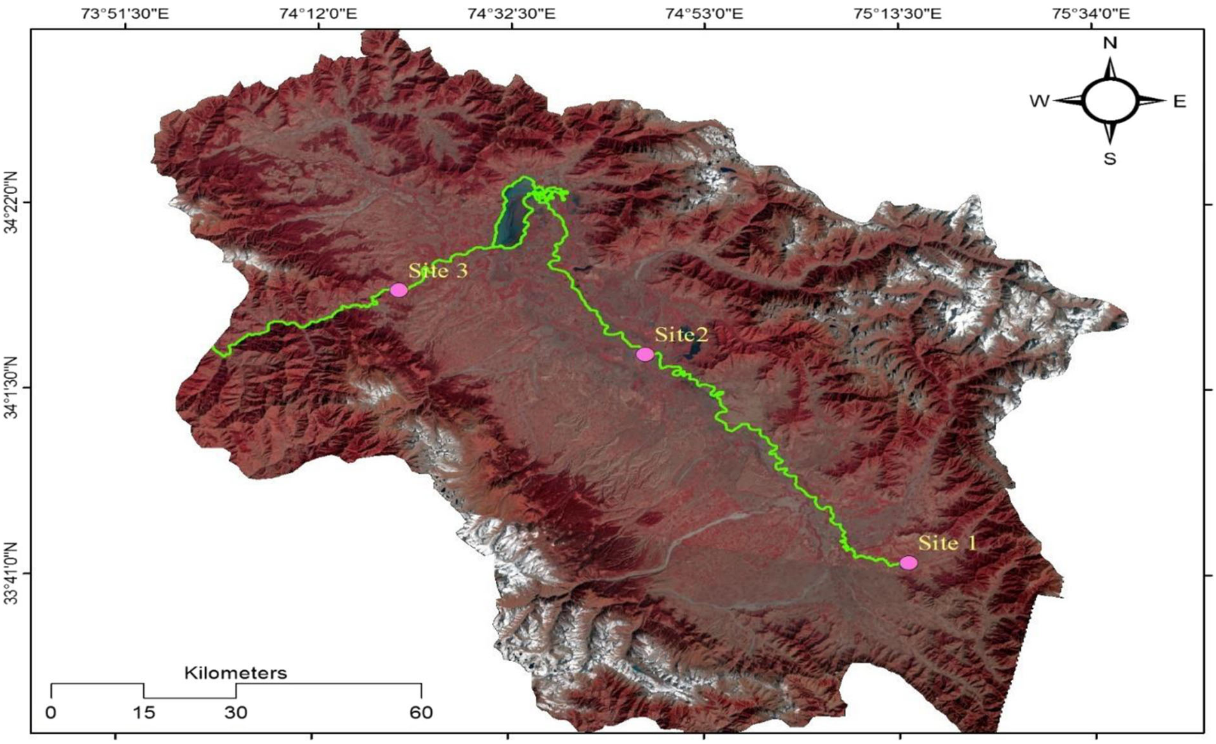

Fig. 1 Jhelum River showing sampling sites

season with a value of 52.5 μg/kg collected from site 1.The maximum concentration of Zn in *S. niger* was found in the liver tissue in autumn season with a value of 575.7 μg/kg collected from site 2 of the study area, and the lowest was recorded in the muscle tissue in autumn season with a value of 65.8 μg/kg collected from site 1. The highest concentration of Zn^{2+} in *C. carpio* was recorded in the liver tissue in autumn season with a value of 416.6 μg/kg collected from site 3 of the study area, and the lowest was recorded in the gill tissue in winter season with a value of 51.5 μg/kg collected from site 1. The highest of Pb concentration in *S. niger* was recorded in the liver tissue in winter season with a value of 14.42 μg/kg collected from site 3 of the study area, and the lowest was found in the gill tissue in autumn season with a value of 5.16 μg/kg collected from site 1. The maximum concentration of Pb^{2+} in *C. carpio* was recorded in the liver tissue in winter season with a value of 13.51 μg/kg collected from site 2 of the river, and the minimum was found in the gill tissue in winter season with a value of 5.15 μg/kg collected from site 1. The highest concentration of Fe in *S. niger* was recorded in the gill tissue in spring season with a value of 323.9 μg/kg collected from site 3 of the river, and the lowest was also found in the muscle tissue in autumn season with a value of 52.58 μg/kg collected from site 1. The highest concentration of Fe^{2+} in *C. carpio* was recorded in the liver tissue in summer season with a

Table 1 Geographical characteristics of sampling sites and study area

Study area	Sampling sites	Study area zones	Location	District	Geographical location	Altitude (meters above mean sea level
River Jhelum	Site 1	Upstream	Chinigund Verinag	Anantnag	33° 33′ 608″ N, 75° 14′ 056 E″	1828
	Site 2	Middle stream	Qamarwari	Srinagar	34° 05′ 044″, 74° 46′ 859″	1883
	Site 3	Down stream	Main Town Baramulla	Baramulla	34° 12′ 668″ N, 74° 19′ 604″ E	1590

Table 2 Seasonal and spatial variation in length and weight of *Schizothorax niger* collected from River Jhelum

Seasons		Site 1	Site 2	Site 3
Summer	Length (cm)	23.03 ± 0.8	21.5 ± 0.6	22.3 ± 1.3
	Weight (g)	182.0 ± 1.40	181.8 ± 1.7	182.6 ± 0.5
Autumn	Length (cm)	22.0 ± 0.9	22.2 ± 1.2	22.0 ± 0.8
	Weight (g)	179.4 ± 2.1	179.3 ± 1.4	183.8 ± 0.4
Winter	Length (cm)	20.4 ± 0.1	24.7 ± 0.5	22.6 ± 1.0
	Weight (g)	179.40 ± 1.6	181.5 ± 2.6	181.5 ± 1.5
Spring	Length (cm)	24.9 ± 0.7	24.4 ± 0.3	21.6 ± 0.6
	Weight (g)	178.1 ± 2.1	180.7 ± 1.3	179.1 ± 0.3

value of 491.7 μg/kg collected from site 3 of the study area, and the lowest was found in the gill tissue in autumn season with a value of 62.01 μg/kg collected from site 1.

As is evident from Fig. 3, the site wise comparison for the heavy metal accumulation followed the following patterns;

Metal	*Schizothorax niger*	*Cyprinus carpio*
Cu^{2+}	Site 2 > site 3 > site1	Site 2 > site 3 > site1
Zn^{2+}	Site 2 > site 3 > site1	Site 3 > site 2 > site1
Pb^{2+}	Site 3 > site 2 > site1	Site 3 > site 1 > site2
Fe^{2+}	Site 3 > site 2 > site1	Site 3 > site 2 > site1

It can clearly be observed in Fig. 4 that the metals preferred a certain tissue (liver) for maximum accumulation and exhibited a comparable difference between the tissues. The accumulation pattern for all the metals in both species followed the sequence as:

Metal	*Schizothorax niger*	*Cyprinus carpio*
Cu^{2+}	Liver > gills > muscle	Liver > gills > muscle
Zn^{2+}	Liver > gills > muscle	Liver > gills > muscle
Pb^{2+}	Liver > gills > muscle	Liver > muscle > gills
Fe^{2+}	Liver > muscle > gills	Liver > gills > muscle

Table 3 Seasonal andsSpatial variation in length and weight of *cyprinus carpio* collected from River Jhelum

Seasons		Site 1	Site 4	Site 6
Summer	Length (cm)	15.6 ± 0.3	18.3 ± 0.9	17.7 ± 0.4
	Weight (g)	205.2 ± 1.8	207.8 ± 0.4	206.7 ± 1.6
Autumn	Length (cm)	18.9 ± 0.6	19.0 ± 0.5	16.6 ± 0.4
	Weight (g)	203.8 ± 1.4	206.5 ± 0.6	205.3 ± 2.1
Winter	Length (cm)	15.6 ± 0.4	15.3 ± 0.3	17.5 ± 1.1
	Weight (g)	207.0 ± 1.1	207.9 ± 0.7	207.8 ± 1.2
Spring	Length (cm)	15.8 ± 0.4	19.4 ± 0.3	16.9 ± 0.7
	Weight (g)	207.5 ± 0.6	207.0 ± 1.8	208.4 ± 0.8

The combo plot (Fig. 5) for seasonal comparison of the metal accumulation revealed that all the metals significantly varied in their accumulation with respect to seasons. *Cyprinus carpio* showed maximum accumulation in summer season for all heavy metals except for Pb^{2+} while *Schizothorax niger* showed more accumulation in winter season as compared to other seasons.

Metal	*Schizothorax niger*	*Cyprinus carpio*
Cu^{2+}	Autumn> spring> summer > winter	Summer > autumn > spring > winter
Zn^{2+}	Winter> spring> summer> autumn	Summer > autumn > spring > winter
Pb^{2+}	Winter> spring> autumn> summer	Autumn > winter > spring > summer
Fe^{2+}	Summer> spring> winter> autumn	Summer > autumn > spring > winter

Discussion

Acquaintance of heavy metal bioaccumulation in fish tissues plays a very important role for the proper management and sustainable consumption (Tunca et al. 2013). Numerous studies are available which provide the significant information about heavy metal analysis in fish tissues and have reported that the quantum of heavy metal in fishes vary considerably among various tissues and species (Javed 2005; Chattopadhyay et al.

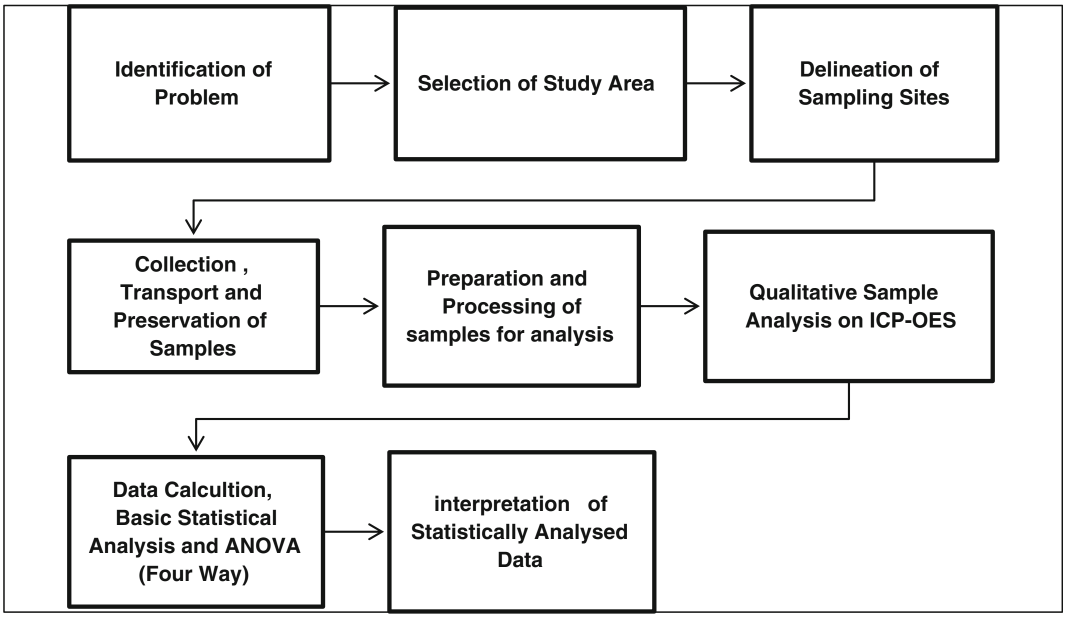

Fig. 2 Flow diagram showing stepwise methodology adopted for the study

2002; Papagiannis et al. 2004; Naggar et al. 2018). Fluctuation in bioaccumulation of heavy metals may be due to physico-chemical properties of water from which fishes were sampled, ecological preferences, age, metabolic rate, feeding ground of fishes, sampling season, etc. (Fernandesa et al. 2007; Mekkawy et al. 2008; Khaled et al. 2010; Youssef and Said 2011; Omar 2013; Sharaf and Shehata 2015). In the riverine system, fishes occupy the topmost trophic level of the food web and have accordingly more tendency to be affected by heavy metal exposure from water and sediments (Mansour and Sidky 2002; Mehmood et al. 2017). Therefore, bioaccumulation and biomagnification of heavy metals in fishes has been used extensively as a potential indicator of heavy metal pollution of aquatic environs (Javed 2005; Tawari-Fufeyin and Ekaye 2007; Karadede-Akin and Unlu 2007; Naggar et al. 2018) that could be a useful means to get an insight into the biochemical disturbance of fish (Dural et al. 2011; Youssef and Said 2011). Bioaccumulation is the capability of an organism to accumulate an element or a chemical compound from food source or any other inorganic source to a level exceeding its surrounding environmental level (Bryan and Langston 1992; Mendil and Uluözlu 2007). Process of heavy metal bioaccumulation is the consequential process of many interactions within different tissues of an organism (Goodwin et al. 2003; Mekkawy et al. 2008; Khaled et al. 2010; Nagajyoti et al. 2010; Jaishankar et al. 2014a). Uptake, toxicity and distribution of heavy metal in aquatic biota

Table 4 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

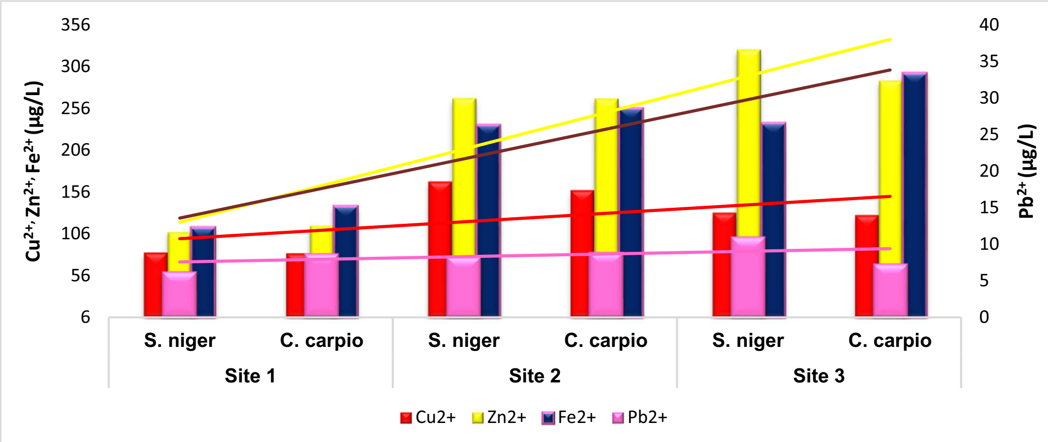

Fig. 3 Combo plot for site wise comparison of heavy metal bioaccumulation in the study area, Trend line showing the variation in metal concentration with respect to sampling sites

are influenced by several physico-chemical factors like pH, alkalinity, hardness of water, temperature, dissolved oxygen, etc. (Pandey et al. 2014; Skordas et al. 2015; Yadav et al. 2016). Oxidation states of heavy metals play a very crucial role in their toxic activity, which in turn depends on their nature, concentration and chemical forms (Bryan et al. 1995; Mehmood et al. 2017).

Cu^{2+} constitutes as an essential and integral part of many enzymes and has a pivotal role in haemoglobin production (Yacoub 2007; Matasin et al. 2011), but can lead to detrimental health effects if over-consumed (Czédli et al. 2012). The Cu^{2+} revealed a wide range of variation in tissues, seasons, sites and species (Table 5). Significant concentration of Cu^{2+} in fish organs could be due to raw sewage discharged from industries, built-up and unplanned urbanisation (Shaw and Handy 1976; Antal et al. 2013). Furthermore, this may be attributed to enhanced complexity in the synthesis of metal-binding proteins (Yacoub 2007). Cu^{2+} exposure to fish can be directly from the surrounding water through gills (WHO 1994). Consequence of high levels of Cu^{2+} in fish tissues are not well investigated; however, there are studies available which confirm that high concentrations of Cu^{2+} in fish can be toxic and dangerous (Woodward et al. 1996; Tapia et al. 2012). Cu^{2+} can actively combine with other chemicals and elements like ammonia (NH_3, Hg and Zn^{2+}) to produce synergistic detrimental effects on fish (Yacoub 2007; Qadir and Malik 2011). The Cu^{2+} in gill tissues was

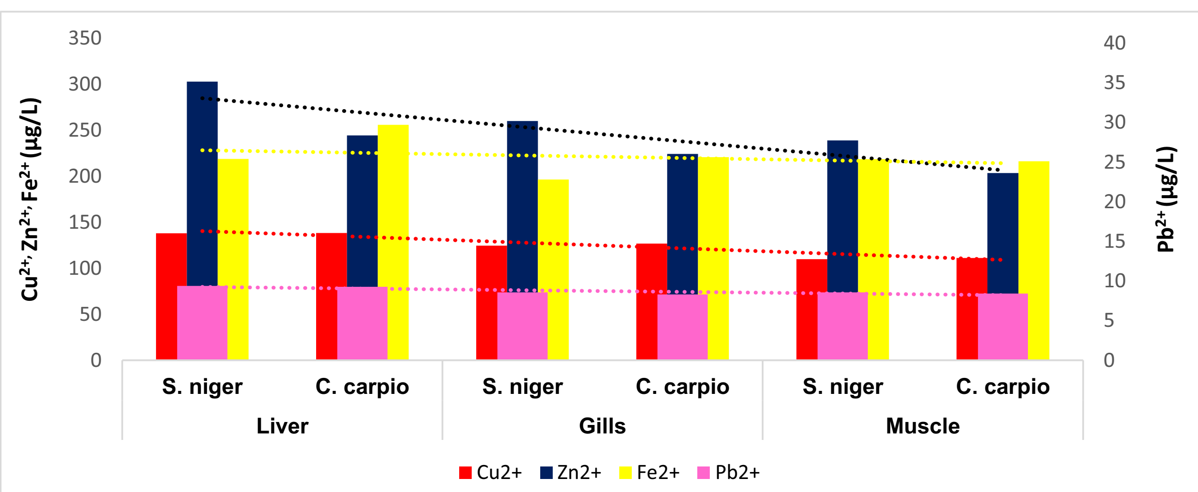

Fig. 4 Combo plot for tissue wise comparison of heavy metal bioaccumulation in the study area, Trend line showing the variation in metal concentration with respect to test organs

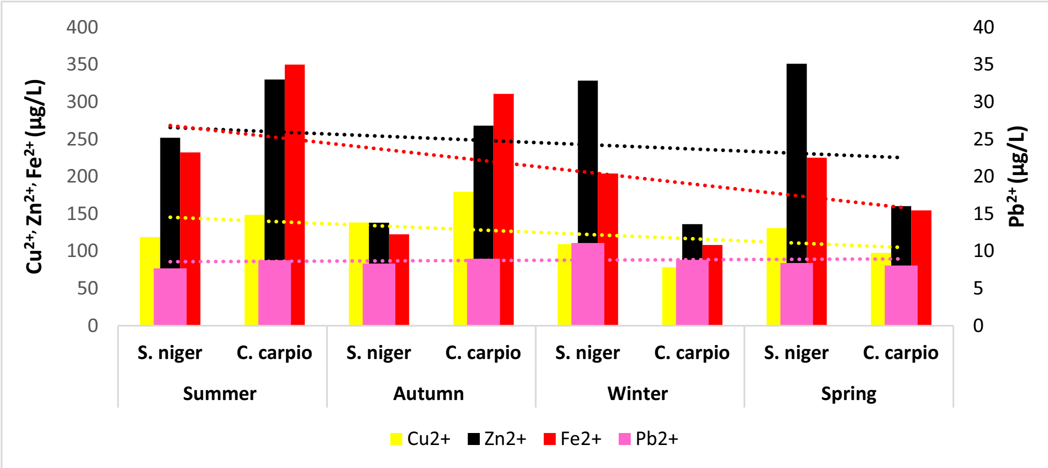

Fig. 5 Combo plot for seasonal comparison of heavy metal bioaccumulation in the study area, Trend line showing the variation in metal concentration with respect to seasons

observed slightly lower than that of liver. Cu^{2+} has lower binding affinity on the gill surface (Antal et al. 2013; Malik et al. 2014). Gills and associated proteins are directly in contact with water and have the tendency to accumulate heavy metals due to adsorption (Mutlu et al. 2012; Zhuang et al. 2013). Bioaccumulation affinity of some organs for heavy metals were found slightly lesser due to extreme secretion of mucous and clogging of gills (Widianarko et al. 2000; Mehmood et al. 2017). Muscle tissue was observed to accumulate least quantity of Cu^{2+} and might have reached to it via blood circulation (Woodward et al. 1996; Malik et al. 2014). The weak binding potential of Cu^{2+} with proteins may be attributed to its least accumulation in muscle tissue (Matasin et al. 2011; Mutlu et al. 2012). Moreover, the weak binding capacity of Cu with muscle proteins has a significant role in the environment as muscle tissue are consumed as food worldwide (Yacoub 2007; Tapia et al. 2012; Pandey et al. 2014).

Zn is an essential micronutrient and is one of the most common contaminants in aquatic environment (Romanenko et al. 1986). The Zn^{2+} levels depicted a significant variation between tissues, seasons, sites and species (Table 6). Sewage disposal and geological quarrying are the major sources of Zn^{2+} pollution to surface water bodies (Spry et al. 1988; Stonard et al. 1976). Zn exposure to fishes takes place directly from water, especially through gills and mucous (Sultana and Rao 1998; El-Naggar et al. 2009). The elevated levels of Zn^{2+} in fish liver in both species could be due to its role in activating several liver (Yamamoto et al. 1977; Vinikour et al. 1980; Yacoub 2007). Zn^{2+} was bioaccumulated in almost all tissues of the fish samples in all seasons and sampling sites.

The Pb^{2+} content showed a discrete fluctuation between different organs, seasons, sites and species (Table 7). Lead is non-essential trace metal and eminent quanta in aquatic organisms can occur in areas situated very close to anthropogenic sources (Ryan et al. 2000; Tuzen 2009; Mohammadi et al. 2011). It is toxic and dangerous even at lower concentrations (Kojadinovic et al. 2007) and has no known role to play in biochemical processes (Karadede and Ünlü 2000; El-Naggar et al. 2009; Malik et al. 2010). Pb^{2+} has been reported to supress the impulse conductivity by impeding the activities of monoamine oxidase enzyme (Dhaneesh et al. 2012) and acetylcholine esterase (Altindag and Yigit 2005; Agarwal et al. 2007), and renders the tissue with pathological changes (Bustamante et al. 2003; Dural et al. 2011; Ali et al. 2011). The significant quantum of Pb^{2+} could be attributed to industrial effluents, agricultural inputs (Amundsen et al. 1997) and household sewage (Gorur et al. 2012) in the study area. The recorded level of Pb^{2+} could be due to sufficient Pb^{2+} content in water and sediment (Tayel et al. 2008) of the river.

Fe is an important and most abundant element, unparalleled by any other metal in the geosphere (Aucoin et al. 1999; Abdullah et al. 2007; El-Naggar et al. 2009). The significant levels of Fe^{2+} content in fish liver tissue may be attributed to increased concentration of total dissolved Fe^{2+} in Jhelum water that consequently lead to increase in its overall availability in river ecosystem

Table 5 Seasonal and spatial variation of Cu (μg/kg) in different organs of two test fish species in river Jhelum

Organ	Season	Site 1		Site 2		Site 3	
		Schizothorax niger	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*
Gills	Summer	116.5 ± 2.7	117.8 ± 1.7	120.2 ± 4.2	154.6 ± 4.7	125.1 ± 3.1	186.4 ± 2.3
	Autumn	51.6 ± 1.2	61.5 ± 2.1	258.2 ± 3.7	295.0 ± 3.7	96.0 ± 2.7	207.2 ± 1.5
	Winter	53.9 ± 2.3	64.5 ± 2.9	116.9 ± 4.1	104.7 ± 3.6	148.6 ± 1.9	58.5 ± 2.7
	Spring	54.4 ± 2.2	53.5 ± 1.9	145.0 ± 4.7	126.0 ± 3.1	212.9 ± 4.7	93.9 ± 1.9
Liver	Summer	124.9 ± 1.6	140.2 ± 3.7	125.8 ± 3.3	165.1 ± 2.4	144.0 ± 3.4	202.1 ± 3.7
	Autumn	54.9 ± 1.9	75.6 ± 3.2	276.6 ± 3.1	309.4 ± 1.3	104.2 ± 4.1	226.7 ± 2.4
	Winter	67.3 ± 2.6	82.6 ± 3.6	137.8 ± 2.8	86.4 ± 3.3	168.0 ± 2.2	70.3 ± 4.8
	Spring	69.8 ± 4.3	60.8 ± 2.2	186.4 ± 1.5	139.5 ± 1.6	198.9 ± 1.1	104.8 ± 3.7
Muscle	Summer	74.2 ± 3.3	91.1 ± 2.4	108.5 ± 2.4	148. ± 2.3	129.7 ± 2.5	132.2 ± 5.1
	Autumn	60.6 ± 2.6	BDL	254.9 ± 1.8	157.8 ± 3.1	87.8 ± 2.5	102.7 ± 3.7
	Winter	73.1 ± 1.9	BDL	146.2 ± 2.7	87.5 ± 2.2	73.1 ± 1.7	73.8 ± 2.2
	Spring	84.6 ± 2.6	BDL	142.4 ± 2.4	121.4 ± 1.9	84.6 ± 2.7	81.7 ± 1.9

BDL Below detection limit

(Koli et al. 1977; Basa et al. 2003) and finally accelerate Fe^{2+} uptake by aquatic organisms (Adami et al. 2002; Rajotte and Couture 2002; Tayel et al. 2008). Yacoub (2007) observed bioaccumulation of Fe^{2+} ligand protein (Hemosidrin) diffused in liver tissue of fish exposed to very high Fe^{2+} levels (Table 8).

Spatial variation revealed no constant increase of heavy metal content at a specific location except site 2, which depicted more content of heavy metals as compared to other two sites. Remarkable variations were observed among seasons, sites and species for all tested heavy metals. However, it has been observed that, different organs showed different heavy metal bioaccumulation patterns. Fish samples from the same species collected from different sites and seasons also depicted a significant variation in metal bioaccumulation (ANOVA; $p < 0.001$, Table 4). Current investigation recorded the lowest concentration of heavy metals in muscle tissue and the maximum content of heavy metals in liver tissue. The maximum bioaccumulation of heavy

Table 6 Seasonal and spatial variation of Zn (μg/kg) in different organs of two test fish species in river Jhelum

Organ	Season	Site 1		Site 2		Site 3	
		Schizothorax niger	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*
Gills	Summer	233.8 ± 2.2	222.4 ± 4.2	253.0 ± 2.1	367.3 ± 1.8	309.6 ± 4.1	401.7 ± 4.8
	Autumn	78.3 ± 1.2	109.0 ± 3.2	129.6 ± 5.5	378.2 ± 3.3	54.6 ± 3.1	308.6 ± 5.6
	Winter	84.35 ± 2.3	51.5 ± 4.1	448.6 ± 3.7	146.2 ± 2.1	473.6 ± 2.7	234.6 ± 3.8
	Spring	91.1 ± 3.01	64.6 ± 2.2	491.9 ± 3.21	139.1 ± 2.6	475.9 ± 4.3	270.8 ± 3.7
Liver	Summer	204.9 ± 1.02	296.7 ± 5.4	268.5 ± 2.9	383.8 ± 5.1	334.8 ± 6.6	416.6 ± 5.6
	Autumn	92.6 ± 3.03	130.7 ± 3.8	575.7 ± 3.3	399.6 ± 5.6	63.3 ± 4.4	331.9 ± 3.1
	Winter	89.91 ± 1.6	65.8 ± 2.12	464.6 ± 3.2	159.8 ± 2.7	456.8 ± 4.8	241.4 ± 2.6
	Spring	106.2 ± 1.52	73.9 ± 2.7	506.4 ± 1.1	154.1 ± 3.6	472.8 ± 3.6	279.5 ± 2.9
Muscle	Summer	103.7 ± 3.2	117.8 ± 3.9	247.5 ± 1.4	365.9 ± 1.9	312.9 ± 3.6	399.9 ± 3.3
	Autumn	65.8 ± 1.2	123.0 ± 4.2	124.7 ± 4.1	371.9 ± 4.1	59.11 ± 4.9	261.1 ± 3.1
	Winter	75.41 ± 3.2	69.1 ± 3.2	434.5 ± 2.1	201.3 ± 3.6	430.9 ± 2.2	74.64 ± 1.8
	Spring	77.5 ± 1.2	64.3 ± 2.2	473.9 ± 1.8	147.7 ± 2.2	466.7 ± 3.2	248.0 ± 1.6

Table 7 Seasonal and spatial variation of Pb (μg/kg) in different organs of two test fish species in river Jhelum

Organ	Season	Site 1		Site 2		Site 3	
		Schizothorax niger	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*
Gills	Summer	6.32 ± 3.8	7.87 ± 2.6	12.2 ± 1.8	11.14 ± 1.8	6.43 ± 3.3	8.28 ± 2.2
	Autumn	5.16 ± 2.3	6.27 ± 2.3	7.98 ± 3.9	10.4 ± 2.7	10.09 ± 2.7	7.42 ± 2.8
	Winter	7.14 ± 1.8	5.15 ± 2.6	10.55 ± 3.1	12.65 ± 2.3	12.95 ± 2.6	6.67 ± 3.8
	Spring	5.93 ± 3.3	6.33 ± 3.9	8.69 ± 3.8	10.45 ± 2.2	9.67 ± 3.9	7.15 ± 1.8
Liver	Summer	5.67 ± 3.8	6.48 ± 3.8	10.21 ± 0.5	12.89 ± 2.7	7.39 ± 0.5	9.41 ± 2.3
	Autumn	6.06 ± 2.6	7.57 ± 3.3	9.63 ± 2.2	12.66 ± 3.3	11.72 ± 3.8	8.43 ± 1.7
	Winter	7.77 ± 2.3	6.44 ± 0.5	12.60 ± 4.2	13.51 ± 2.6	14.42 ± 1.8	7.90 ± 2.6
	Spring	6.48 ± 3.3	7.61 ± 3.1	10.37 ± 3.8	11.28 ± 4.2	10.28 ± 1.2	7.52 ± 2.7
Muscle	Summer	BDL	6.93 ± 1.8	7.59 ± 2.7	9.11 ± 3.1	5.69 ± 2.2	6.92 ± 3.8
	Autumn	BDL	BDL	6.60 ± 2.6	12.29 ± 2.3	9.57 ± 2.5	6.70 ± 0.5
	Winter	BDL	BDL	10.84 ± 3.8	11.62 ± 2.3	12.50 ± 3.8	6.54 ± 4.2
	Spring	BDL	BDL	8.02 ± 1.9	8.29 ± 3.1	7.73 ± 3.1	5.92 ± 3.9

BDL Below detection limit

metals in the liver tissue may be due to its role in body metabolism (Malik et al. 2010; Zhao et al. 2012). Remarkable amount of heavy metals in liver tissues could be attributed to organ binding specific proteins such as metallothioneins (MT) (Yacoub 2007; Gorur et al. 2012) which act as storing units for heavy metals (Roesijadi 1996; Amiard et al. 2006). Similarly, remarkable Fe^{2+} bioaccumulation in liver may be attributed to the role of liver in the synthesis of blood cells and haemoglobin (Aucoin et al. 1999; Amiard et al. 2006; Abdullah et al. 2007). Elevated levels of Pb^{2+} and Cu^{2+} could be attributed to the ability of these heavy metals to replace the MT-combined essential trace elements in the hepatic tissue (Qadir and Malik 2011; Mehmood et al. 2017). The fishes bioaccumulated some heavy metals in their gills, as this tissue contacts the water directly (Qadir and Malik 2011; Antal et al. 2013; Malik et al. 2014) and their large surface area facilitates rapid exposure of heavy metals inside the fishes (Yacoub 2007; Matasin et al. 2011; Dhaneesh et al. 2012). Therefore, it can be

Table 8 Seasonal and spatial variation of Fe (μg/kg) in different organs of two test fish species in river Jhelum

Organ	Season	Site 1		Site 2		Site 3	
		Schizothorax niger	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*	*Schizothorax niger*	*Cyprinus carpio*
Gills	Summer	254.7 ± 5.1	133.4 ± 5.1	238.7 ± 1.5	376.2 ± 4.2	248.3 ± 5.1	465.8 ± 2.8
	Autumn	56.46 ± 3.6	62.01 ± 4.5	205.1 ± 4.7	403.1 ± 3.3	97.56 ± 4.4	432.8 ± 3.3
	Winter	75.3 ± 1.5	108.6 ± 1.1	232.5 ± 2.2	102.1 ± 2.2	295.6 ± 2.2	105.0 ± 4.5
	Spring	99.74 ± 3.3	142.1 ± 4.2	233.3 ± 4.4	127.7 ± 3.6	323.9 ± 3.3	192.4 ± 3.3
Liver	Summer	318.7 ± 2.8	343.4 ± 3.6	254.8 ± 3.6	388.8 ± 2.8	271.5 ± 1.5	491.7 ± 1.1
	Autumn	62.6 ± 2.2	77.07 ± 4.4	231.0 ± 3.3	435.6 ± 3.3	115.9 ± 1.1	475.3 ± 4.2
	Winter	84.24 ± 4.5	118.8 ± 2.8	256.7 ± 1.1	117.7 ± 1.5	310.6 ± 3.4	117.8 ± 4.4
	Spring	109.2 ± 4.2	158.4 ± 3.3	290.8 ± 4.2	143.1 ± 4.4	320.7 ± 4.7	204.7 ± 4.7
Muscle	Summer	88.6 ± 1.1	208.7 ± 3.4	201.0 ± 3.4	356.0 ± 3.4	216.3 ± 4.5	385.5 ± 2.2
	Autumn	52.58 ± 3.3	61.30 ± 2.2	202.9 ± 4.5	398.1 ± 4.7	80.6 ± 3.3	452.6 ± 1.5
	Winter	67.07 ± 2.8	120.3 ± 3.3	230.3 ± 3.3	87.0 ± 4.5	282.6 ± 3.6	98.0 ± 3.6
	Spring	94.6 ± 5.1	133.9 ± 1.5	257.5 ± 5.1	134.3 ± 5.1	295.7 ± 4.2	156.3 ± 5.1

understood that overlaying water containing heavy metals enter mainly through gills into the fish body (Moore and Ramamoorthy 1984; Mekkawy et al. 2008; Khaled et al. 2010; Youssef and Said 2011; Omar 2013; Sharaf and Shehata 2015).

Significant seasonal effect on the heavy metal uptake of fish species was also recorded which might reflect the variation in metabolic activity of the species (Sultana and Rao 1998). The highest levels of heavy metals were found during summer season of the year due to the elevated anthropogenic pressure of industrial, commercial, domestic and agricultural practices along the river (Saad et al. 1985) followed by autumn season is mainly due to decrease in overall water discharge in the river (Mzimela et al. 2003) which in turn concentrate the heavy metals in the river (Long et al. 1995), and least content of heavy metals and anthropogenic inputs have been recorded in spring season as the discharge of the river in this season increases drastically (Hamza-Chffai et al. 1996; Karadede and Ünlü 2000; Kucuksezgin et al. 2001). Seasonal heavy metal variation has been well studied by researchers all around the world (Foster et al. 2000; Kargin et al. 2001; Eastwood and Couture 2002; Canli and Atli 2003). It has been reported that due to different seasonal growth rate, varying reproductive cycle, water salinity fluctuations and change in temperature are season dependent. Larson et al. (1985) also reported that fish physiological parameters are affected by seasonal variations.

Fishes are mostly in continuous movement and seldom stay at a single place. Heavy metal bioaccumulation in fish tissues provides an index of contaminated aquatic environment of the area (Qadir and Malik 2011). In the present study, spatial distribution of heavy metal bioaccumulation in fish tissues showed significant concentration of Cu^{2+}, Zn^{2+}, Pb^{2+} and Fe^{2+} at site 2 of the study area. This phenomenon can be attributed mainly with commercialisation, urbanisation, industrialisation and other anthropogenic activities in Srinagar City and its adjoining areas (Khaled et al. 2010). Least content of heavy metals has been observed at site 1 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 a least polluted site for being at high altitude with least anthropogenic pressure (Amiard et al. 2006; Abdullah et al. 2007). Muscle tissue is an inactive destination for heavy metal bioaccumulation (Elnabris et al. 2013). But in polluted aquatic systems, significant quantum of heavy metals can be found in muscles as can lead to adverse health effects (Rajotte and Couture 2002; Tayel et al. 2008). To get an insight into public health risk in our study, heavy metal levels were compared with the maximum permissible limits for human consumption (Skordas et al. 2015; Yadav et al. 2016). The tested heavy metal contents were well below the maximum permissible limit for human consumption suggested by FAO and WHO (Asgedom et al. 2012).

Field and laboratory results from many studies showed that heavy metal bioaccumulation depends on water contamination level and duration of exposure (Jeffree et al. 2006). Some other significant parameters include salinity (Jeffree et al. 2006), pH (Quan et al. 2007), hardness (Singh et al. 2007), temperature (Has-Schon et al. 2007), etc. Furthermore, ecological needs, fish age and size (Rurangwa et al. 1998), fish life cycle (Basa et al. 2003), fish feeding grounds (Kime et al. 1996) and the capturing season also affect the observed results significantly (Yadav et al. 2016). Low heavy metal concentration may not show any apparent detrimental effect on fish (Gorur et al. 2012) but may lead to decrease in fish fertility (Tuzen 2009; Mohammadi et al. 2011), which in turn may lead to decline in population of some sensitive fishes like *Schzothoraz niger*, etc. (Mehmood et al. 2017; Krishnani et al. 2003; Burger and Gochfeld 2005). Reproduction effects like sperm or gamete formation gets badly affected even by traces of heavy metals in water (Omar 2013; Sharaf and Shehata 2015). Effect of heavy metals on fish reproduction is a complicated process regulating multiple factors and slight heavy metal concentration could affect this pathway at any stage (Youssef and Said 2011; Dural et al. 2011). 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 (Rurangwa et al. 1998; Krishnani et al. 2003; Yousuf et al. 2013; Mehmood et al. 2017).

Conclusion

Quantum of heavy metals in three 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 various factors like geographical location, climate, genetic tendency, feeding behaviour, swimming patterns, etc. leading to fluctuation in heavy metal accumulation between the two fish species and samples within the same species. Health risk date indicated that fishes are safe for human consumption. However, heavy metal content in *Schizothorax niger* species must be monitored on a regular basis in Jhelum River since population of this fish is showing a continuous declining trend in Kashmir waters.

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