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RESEARCH ARTICLE

Seasonal variation in metal concentration in various tissues of the European chub (*Squalius cephalus* L.)

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Abstract

Due to the increasing industrialization, metals are discharged into all spheres of the environment, in particular, in river courses, which leads to the need for constant monitoring. Metals do not degrade into harmless end products; they are very persistent and have high potential for accumulation in biota. Metals in the fish body are accumulated in different amounts in the tissue specific matter. In relation to the biotic conditions and fish metabolism, the load of food, or the stage of the reproductive cycle, the seasonal variation of metal levels can be expected. Because of that, the objective of our present study was to analyze 15 metals and metalloids in liver, gills, muscle, and gonads of European chub (*Squalius cephalus*) throughout the 4 seasons, autumn, winter, spring, and summer. The specimens were collected from two rivers, Pestan and Beljanica at the Kolubara basin, and their concentrations were determined with inductively coupled plasma optical emission spectrometry (ICP-OES). Specimens from both rivers have shown similarities in metal accumulation like the highest accumulation of majority of elements in gills, lowest accumulation of majority of elements in muscle (except for Hg), and higher accumulation of some elements in summer (Cu, Fe, Zn). In addition, Cu and Fe showed affinity for liver, while Ba, Cr, Sr, and Zn were specific for gills. Also, Al, B, Fe, Ni, and Pb did not show significant differences in concentrations among different seasons in all investigated tissues.

Keywords ICP-OES · European chub · Metals · Seasonal variation · Freshwater · Fish tissues

Introduction

Metals are continuously released into the aquatic environment from the geological and anthropogenic sources and present a serious threat to the aquatic organisms because of their toxicity, persistence, and bioaccumulation (Bervoets et al. 2001; Duman and Kar 2012; Salem et al. 2014a, b; Sunjog et al. 2016).

Excessive release of metals into the environment due to industrialization and urbanization has posed a great problem worldwide. Unlike organic pollutants, the majority of which are susceptible to biological degradation, metal ions do not degrade into harmless end products, and many of them exhibit genotoxic properties (Gupta et al. 2001; Hegazi 2013). Metal contamination exists in aqueous wastes of many industries, such as metal plating, mining operations, tanneries, chloralkali, radiator manufacturing, smelting, alloy industries, and storage battery industries (Hegazi 2013).

Although metals are found at low levels in water, they can reach significant levels in sediment and aqueous biota (Duman and Kar 2012). Field studies on indigenous fish communities give an integrated approach to the exposure conditions and their chronic variations (Andres et al. 2000; Podrug and Raspor 2009). The uptake of metals in fish occurred by absorption across the gill surface, through the gut wall tract and the skin (Obasohan et al. 2007). They can be accumulated

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in different amounts depending on the type of uptake, deposition and excretion rates, and affinity to the tissue (Jezierska and Witeska 2006).

As many developing countries, Serbia also has a problem with processing the municipal and industrial waters, where only 5–10% of waste water is processed. More than 50% of industrial facilities do not purify wastewater, because there are no purification systems. Also, Belgrade, a city with over two million inhabitants, does not have a treatment plant for wastewaters. Untreated or purified municipal and industrial wastewaters are key sources of pollution of surface and groundwater in the Republic of Serbia.

In our previous research, the genotoxicity potential of three water bodies in Serbia—Kolubara River Basin (a region with extensive coal mining), Garasi Lake (a site with low anthropogenic impact and source of drinking water), and Uvac River "Zlatar" reservoir (a protected natural area with low anthropogenic impact)—was monitored in different tissues (blood, gills, and liver) of chub (*Squalius cephalus* L.) with the alkaline comet assay (Sunjog et al. 2014, 2016). To highlight the importance of tissue selection, concentrations of metals and metalloids were analyzed in the liver, gills, gonads, and muscle of chub during the autumn season, October and November.

In relation to the biotic conditions and fish metabolism, the load of food or the stage of the reproductive cycle, the seasonal variation of metal levels can be expected. Because of that, the objective of our present study was to analyze 15 metals and metalloids in liver, gills, muscle, and gonads of chub throughout the 4 seasons, autumn, winter, spring, and summer. The specimens were collected from two rivers, Pestan and Beljanica at the Kolubara basin, and their concentrations were determined with inductively coupled plasma optical emission spectrometry (ICP-OES). The seasonal variation in metals from the water at Pestan and water quality of both rivers, based on physico-chemical parameters, were also included in the study.

Materials and methods

Study area and fish sampling

Rivers Pestan (33 km) and Beljanica (15 km) are polluted watercourses at the Kolubara basin, surrounded by coal mines. The exploitation of lignite in this basin, as well as accompanying activities related to it, are the dominant activities that led to the degradation of hydrological conditions. In addition to that, the impact of municipal waters and agricultural runoffs should not be neglected.

A total of 65 chub specimens (3–4 specimens per season per site) were caught by an electrofishing device ELEMAX SHX 2000 (SAWAFUJI). Average weight and body length of 28 specimens at Pestan were 143.56 ± 129.46 g and 21.98 ± 6.76 cm and of 37 specimens at Beljanica were 182.99 ± 203.38 g and 23.53 ± 7.66 cm. Fish sampling at Pestan and Beljanica was performed from October 2011 to September 2012; at Pestan (8 months), at Beljanica (10 months), organized in 4 seasons, autumn and winter (2011) and spring and summer (2012).

Water sample collection

Water samples were taken at least once every season at Pestan and Beljanica. At Pestan, water was sampled for analysis of metal concentrations from 2008 to 2011. Ambient environmental factors (pH, temperature, conductivity, nitrates, phosphates, etc.) were determined at Pestan and Beljanica during 2011/2012.

Tissue sample collection and preparation

Fish are anesthetized with clove oil prior to tissue collection and dissection. The total mass (g) and the total body length (cm) of each specimen were measured. For metal analysis, samples of liver, gills, muscle, and gonads were quickly removed, washed with distilled water, and stored at − 20 °C.

Analysis of metals and metalloids

Frozen tissue samples of liver, gills, muscle, and gonads were, first, measured (wet weight) and, then, dried by Freeze Dryers Rotational Vacuum Concentrator, GAMMA 1–16 LSC, Germany. After that, the samples were measured again (first dry weight), and the portions between 0.2 and 0.5 g (second dry weight) were processed in a microwave digester (speed wave MWS-3+; Bergof Products+ Instruments GmbH, Eningen, Germany), using 6 mL of 65% HNO_3 and 4 mL of 30% H_2O_2 (Merck suprapure) at a food temperature program (100–170 °C). The digested samples were diluted with distilled water to a total volume of 25 mL, and the analysis was performed by inductively coupled plasma optical spectrometry (ICP-OES) (Table 1). The following 15 metals and

Table 1 Operating conditions of Spectro Genesis II, ICP-OES, Spectro Analytical Instruments GmbH

Instrument	ICP-OES
Plasma power	1400 W
Nebulizer flow	0.9 L min^{-1}
Auxiliary flow	0.8 L min^{-1}
Coolont flow	13 L min^{-1}
Optic temperature	29–31 °C
Sample uptake rate	2 mL min^{-1}
Torch	Quartz glass

metalloids were analyzed: Al, B, As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sr, and Zn. The following wavelength lines of the ICP-OES analysis were used: Al 394.401 nm, As 189.042 nm, B 249.773 nm, Ba 233.527 nm, Cd 228.802 nm, Cr 205.552 nm, Cu 324.754 nm, Fe 259.941 nm, Hg 184.950 nm, Mn 259.373 nm, Mo 202.095 nm, Ni 231.604 nm, Pb 220.353 nm, Sr 460.733 nm, and Zn 206.1919 nm. For elements Al, As, B, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sr, and Zn, the detection limits (LOD) were as follows (μg/g): Al (0.125), As (0.223), B (0.074), Ba (0.042), Cd (0.010), Cr (0.029), Cu (0.046), Fe (0.044), Hg (0.437), Mn (0.032), Mo (0.062), Ni (0.090), Pb (0.271), Sr (0.109), and Zn (0.031). BCR-185R reference material of bovine liver as well as IAEA-336 Lichen reference material was used for the assessment of the accuracy and precision of the analysis. Analysis indicated that the concentrations were within 90–115% of the certified values for all measured elements. All metal and metalloid concentrations were expressed as μg g^{-1} dry weight (dw).

Statistical analysis

One-way ANOVA followed by Tukey's post hoc test was used to determine the differences in metal and metalloid concentration in fish tissues between seasons.

We have used the principal component analysis (PCA) in order to assess the differentiation among the analyzed fish tissues, based on the element level (Figs. 1 and 2).

The tissues were also compared through canonical discriminant analysis (CDA) to determine the degree of separation of the examined tissue relative to the levels of element accumulation (Fig. 3).

Results

The seasonal mean of metal concentrations of water samples taken from 2008 to 2011 at river Pestan was presented in Table 2. Data were taken from the Environmental Protection

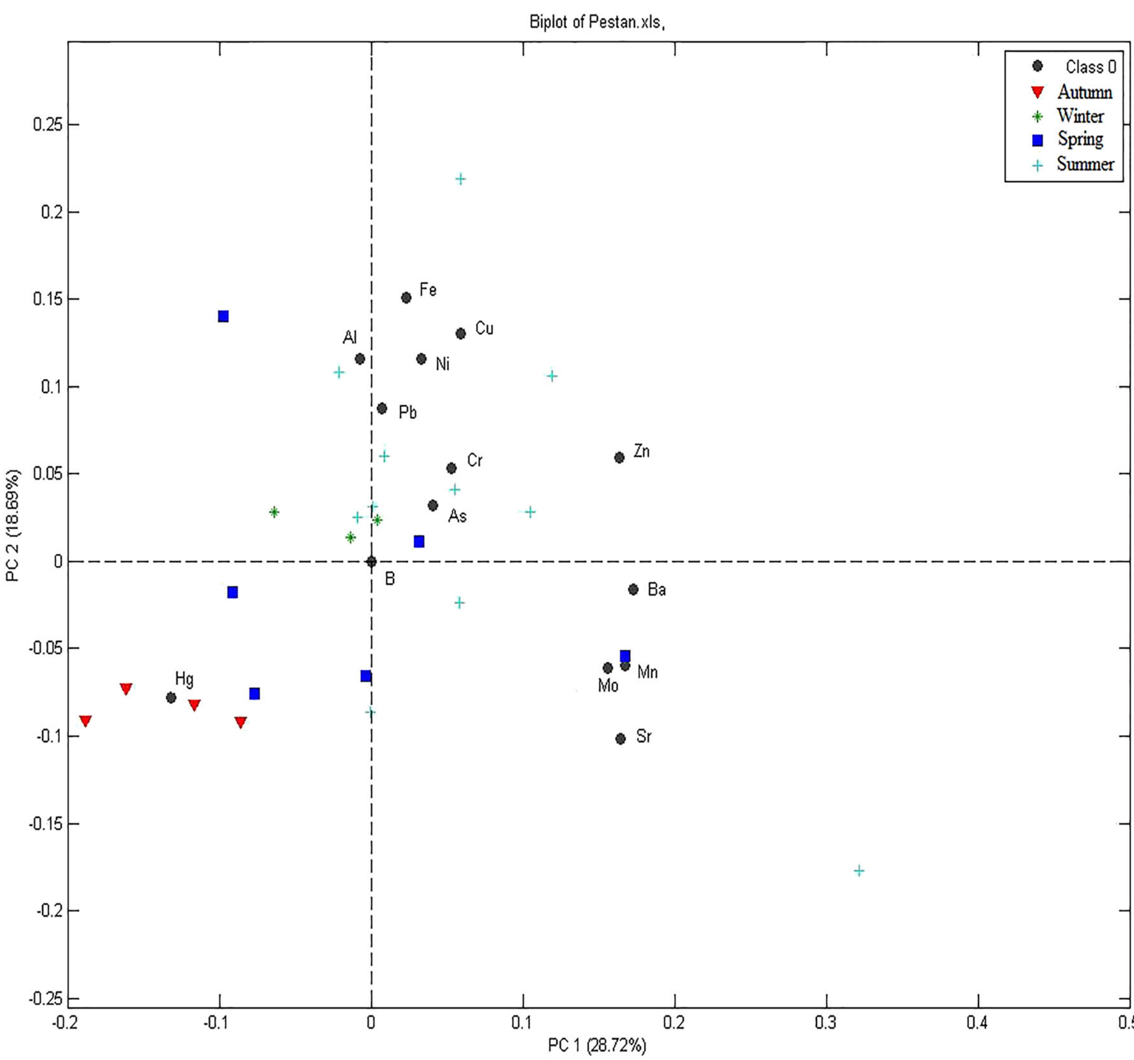

Fig. 1 The PCA plot of element concentrations in the muscle in Pestan

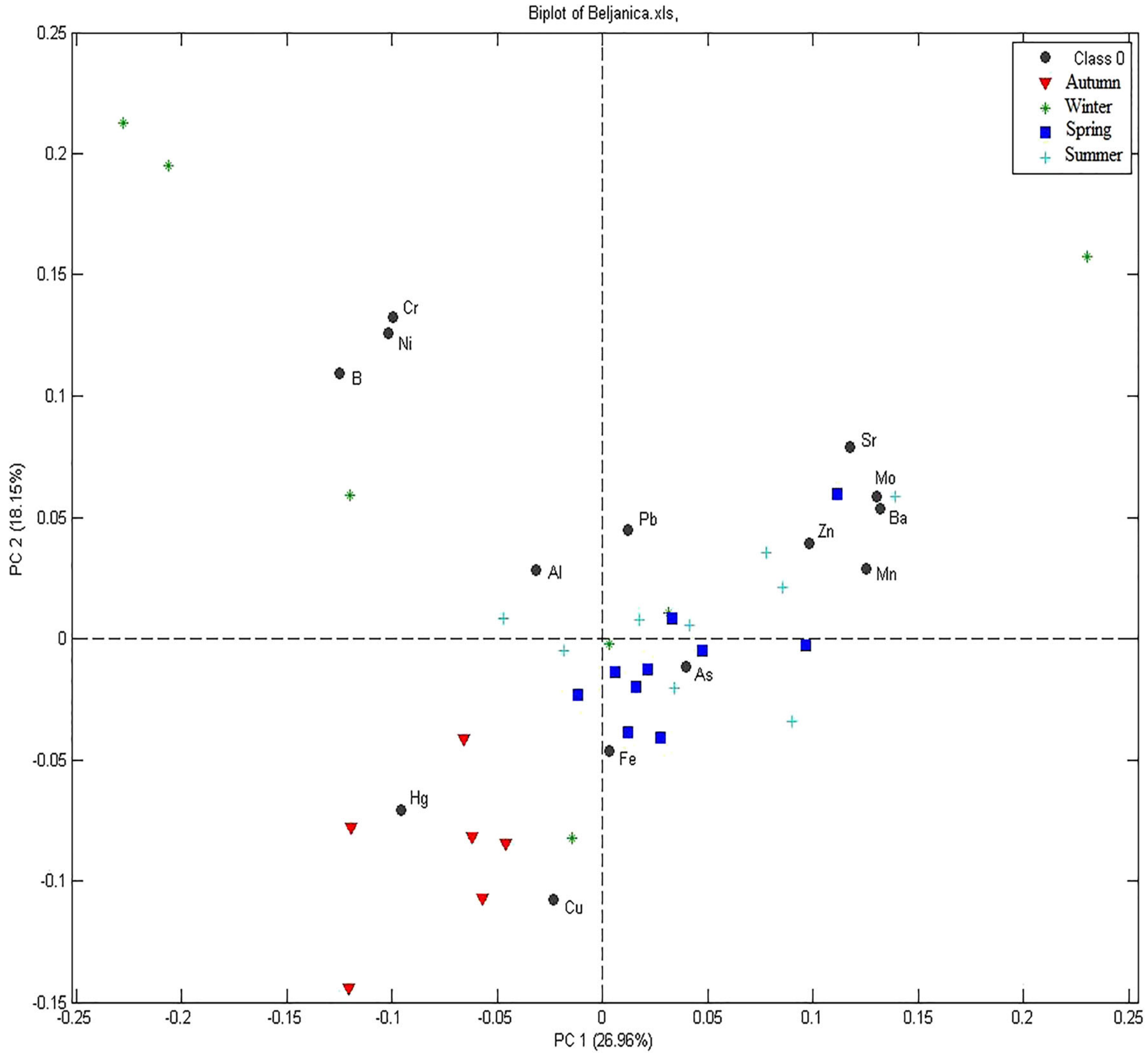

Fig. 2 The PCA plot of element concentrations in the muscle in Beljanica

Agency, Republic of Serbia. When comparing values to limit values of pollutant matter in surface waters, Cu, Zn, and Cr are in I class water quality, while As is in II/III class water quality (Official Gazette of the RS, No 50/12 n.d.). Some metals, like

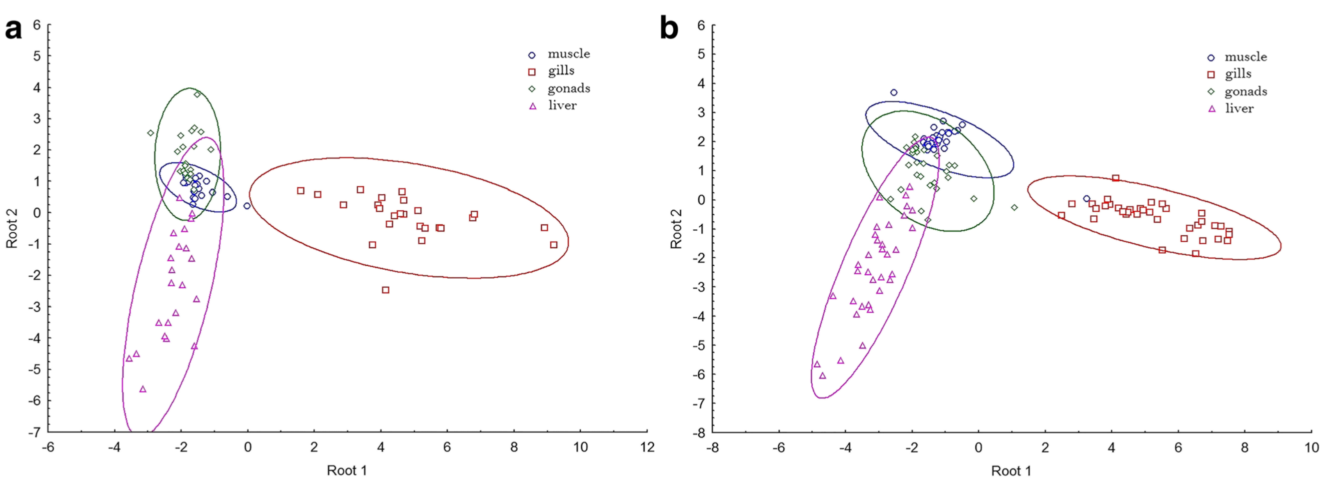

Fig. 3 CDA based on the total content of elements in the tissues in (**a**) Pestan and (**b**) Beljanica

Table 2 Seasonal mean of metal concentrations of water samples taken from 2008 to 2011 year at the Pestan river

Sampling year	Pestan	Zn	Cu	Cr	Pb	Cd	Hg	Ni	As
2008	Spring	7.10	< 1.00	< 1.00	0.60	< 0.05	< 0.1	3.40	10.10
	Summer	2.85	8.30	< 1.00	0.55	< 0.05	< 0.1	7.10	14.60
	Autumn	1.80	1.00	< 1.00	1.20	< 0.05	< 0.1	6.80	12.40
2009	Spring	15.67	18.00	1.33	< 1	< 0.2	< 0.1	5.67	8.33
	Summer	21.35	3.50	< 1	< 0.2	< 0.2	< 0.1	10.30	8,10
	Autumn	29.45	7.05	< 0.5	0.60	0.03	0.05	10.10	6.50
	Winter	13.25	2.40	< 0.5	< 0.5	< 0.025	< 0.1	5.30	5.10
2010	Spring	14.30	6.55	0.60	0.50	0.04	< 0.1	6.70	4.10
	Summer	6.77	9.30	12.63	3.07	0.41	< 0.1	6.37	23.20
	Autumn	10.45	9.70	0.50	0.50	< 0.025	< 0.1	9,95	5.55
	Winter	7.00	9.00	0.80	0.50	0.05	< 0.1	14.20	5.30
2011	Summer	6.35	5.40	0.64	0.50	0.08	0.10	4.45	4.90
	Autumn	10.90	2.20	0.50	0.50	0.02	0.10	5.40	7.40
	Winter	30.50	4,00	1,20	0,50	0,09	0,10	4,80	3,20
Average									
2008–2011	Spring	12.36	8.18	0.64	0.37	0.01	< 0.1	5.26	7.51
	Summer	9.33	6.63	3.32	1.03	0.12	< 0.1	7.05	12.70
	Autumn	13.15	4.99	0.25	0.70	0.01	0.04	8.06	7.96
	Winter	16.92	5.13	0.67	0.33	0.05	0.03	8.10	4.53

Data were taken from Environmental Protection Agency, Republic of Serbia. Analysis was done by inductively coupled plasma-mass spectrometry by the EPA standard (http://www.sepa.gov.rs/download/Kvalitet_voda_godisnjak_2011.pdf, pp. 624–625)

As, Pb, Cr, and Cd, were elevated during summer in comparison with other seasons.

According to the physico-chemical analysis during 2011/2012, the water quality of Pestan and Beljanica belongs to the III and IV classes of water quality (Official Gazette of the RS, No 74/11 n.d.). Data were presented in the Supplementary material, Table 1.

Different metals and metalloids (15) were analyzed in chub tissues; liver, muscle, gills, and gonads and expressed in mg/g dry weight (Tables 3 and 4).

The mean values of metal and metalloid concentrations in chub tissues collected at Pestan during autumn (October and November) and winter (December) of 2011 and spring (March and April) and summer (July, August and September) of 2012 are shown in Table 3.

It was shown that there is significant difference in concentration of elements between seasons for some tissues (Table 3).

In European chub from the Pestan river during all seasons, the highest accumulation for the majority of elements was detected in the gills. During all seasons, the tested elements, except Hg, showed the lowest accumulation in muscle. In summer, in all tested tissues, the highest concentration for most elements was detected. During autumn, significantly higher values of Hg were detected. In gonads, the highest concentrations of Al, B, and Mn were detected during spring.

Regardless of the season, Cu and Fe were specific for the liver and Ba, Cr, Sr, and Zn for the gills (from 3 times up to 47 times higher concentrations when compared to other tissues).

The mean values of metal and metalloid concentrations in chub tissues collected at Beljanica during autumn (October and November) and winter (December and February) of 2011 and spring (March, April, and May) and summer (July, August, and September) of 2012 are shown in Table 4.

It was shown that there is a significant difference in concentration of elements between seasons for some tissues (Table 4).

In European chub from the Beljanica river during all seasons the highest accumulation for the majority of elements was detected in the gills. During all seasons, the tested elements, except Hg, had the lowest accumulation in muscle. During summer and spring, in all tested tissues, the highest concentration for the majority of elements was detected. During autumn and winter, in gonads, the significantly higher values of Mn and Sr were detected, respectively. During autumn, significantly higher values of Hg were detected in the muscles and lower values of Mo were detected in the gills. During winter, significantly higher values of Cr were detected in the muscles in comparison to other seasons. In addition, a number of significant differences between seasons regarding the accumulation of elements were present.

Table 3 The mean ± SD values of metal and metalloid concentrations in chub tissues collected at the Pestan river during different seasons

Pestan	2011		2012	
	Autumn	Winter	Spring	Summer
Al				
Muscle	0.09 ± 0.03[a]	15.2 ± 11.56[a]	13.77 ± 17.79[a]	15.77 ± 21.75[a]
Gills	22.03 ± 30.47[a]	25.71 ± 17.89[a]	22.91 ± 30.37[a]	50.86 ± 59.18[a]
Gonads	1.06 ± 1.4[a]	–	29.23 ± 59.71[a]	7.04 ± 10.36[a]
Liver	0.11 ± 0.05[a]	–	15.19 ± 11.41[a]	20.41 ± 25.69[a]
B				
Muscle	ND	ND	ND	ND
Gills	0.36 ± 0.14	ND	ND	1.02; 0.27**
Gonads	0.17 ± 0.07[a]	–	29.23 ± 59.71[a]	7.04 ± 10.36[a]
Liver	0.31 ± 0.36[a]	–	ND	0.33 ± 1.08[a]
As				
Muscle	ND	1 ± 1.07[a]	0.13 ± 0.08[a]	0.46 ± 0.39[a]
Gills	ND	1.81 ± 2.78[a]	0.19 ± 0.05[a]	0.67 ± 0.67[a]
Gonads	**0.33 ± 0.31[a,b]**	–	**0.48 ± 0.54[a]**	**3.79 ± 2.3[b]**
Liver	ND	–	0.51 ± 0.64[a]	0.89 ± 0.87[a]
Ba				
Muscle	0.62 ± 0.29[a]	0.63 ± 0.10[a]	1.13 ± 0.59[a]	1.3 ± 0.76[a]
Gills	18.55 ± 5.76[a]	15.16 ± 3.39[a]	23.27 ± 8.73[a]	23.62 ± 8.46[a]
Gonads	**1.8 ± 0.93[a,b]**	–	**2.06 ± 1.88[a]**	**12.09 ± 7.43[b]**
Liver	ND	–	0.05*	0.12 ± 0.25
Cr				
Muscle	0.02 ± 0.01[a]	0.05 ± 0.01[a]	0.1 ± 0.07[a]	0.18 ± 0.25[a]
Gills	**0.14 ± 0.09[a]**	**0.94 ± 0.56[b]**	**0.64 ± 0.16[b]**	**0.79 ± 0.28[b]**
Gonads	0.01 ± 0.01[a]	–	0.11 ± 0.13[b]	0.24 ± 0.24[b]
Liver	**0.03 ± 0.01[a]**	–	**0.20 ± 0.1[a,b]**	**0.42 ± 0.28[b]**
Cu				
Muscle	0.89 ± 0.19[a]	1.45 ± 0.04[a]	0.98 ± 0.21[a]	1.75 ± 1.27[a]
Gills	**1.96 ± 0.95[a,b]**	**2.64 ± 0.87[a,b]**	**1.99 ± 0.61[a]**	**3.56 ± 1.45[b]**
Gonads	4.38 ± 2.12[a]	–	2.23 ± 0.85[a]	4.5 ± 3.09[a]
Liver	18.65 ± 16.62[a]	–	24.71 ± 16.3[a]	34.48 ± 15.45[a]
Fe				
Muscle	7.06 ± 1.74[a]	10.21 ± 1.02[a]	8.34 ± 4.00[a]	10.99 ± 4.46[a]
Gills	90.73 ± 30.15[a]	101.7 ± 4.06[a]	91.69 ± 20.77[a]	203.03 ± 175.77[a]
Gonads	58.72 ± 17.41[a]	–	44.84 ± 46.24[a]	59.1 ± 26.37[a]
Liver	128.05 ± 102.69[a]	–	302.98 ± 248.46[a]	316.85 ± 220.42[a]
Hg				
Muscle	**1.78 ± 0.29[a]**	**0.94 ± 0.21[b]**	**0.99 ± 0.36[b]**	**0.88 ± 0.26[b]**
Gills	**1.28 ± 0.27[a]**	**0.85 ± 0.52[a,b]**	**0.57 ± 0.12[b]**	**0.85 ± 0.26[a,b]**
Gonads	**1.29 ± 0.34[a]**	–	**0.47 ± 0.08[b]**	**1.00 ± 0.35[a]**
Liver	**1.01 ± 0.28[a,b]**	–	**0.69 ± 0.18[a]**	**1.15 ± 0.23[b]**
Mn				
Muscle	0.79 ± 0.29[a]	0.89 ± 0.21[a]	0.83 ± 0.46[a]	1.04 ± 0.34[a]
Gills	22.66[a]	14.92 ± 3.86[a]	19.27 ± 8.46[a]	25.22 ± 11.37[a]
Gonads	10.05 ± 10.44[a]	–	14.79 ± 23.82[a]	2.61 ± 1.88[a]
Liver	**2.60 ± 0.84[a,b]**	–	**5.62 ± 1.51[a]**	**7.41 ± 2.65[b]**
Mo				
Muscle	0.03 ± 0.03[a]	0.14 ± 0.08[a]	0.25 ± 0.12[a]	0.33 ± 0.28[a]

Table 3 (continued)

Pestan	2011		2012	
	Autumn	Winter	Spring	Summer
Gills	**0.03 ± 0.03^{a}**	**2.26 ± 1.96^{b}**	**1.60 ± 0.4^{b}**	**2.05 ± 0.68^{b}**
Gonads	0.16 ± 0.01^{a}	–	0.10 ± 0.18^{a}	0.10 ± 0.10^{a}
Liver	0.71 ± 0.42 ^{a}	–	0.40 ± 0.41 ^{a}	0.76 ± 0.35 ^{a}
Ni				
Muscle	ND	0.07 ± 0.05^{a}	0.04 ± 0.01^{a}	0.37 ± 0.91^{a}
Gills	0.84 ± 1.65^{a}	0.16 ± 0.13^{a}	0.10 ± 0.05^{a}	0.54 ± 0.73^{a}
Gonads	0.04 ± 0.01^{a}	–	0.13 ± 0.24^{a}	0.15 ± 0.09^{a}
Liver	0.07 ± 0.13^{a}	–	0.08 ± 0.04^{a}	0.22 ± 0.17^{a}
Pb				
Muscle	ND	0.35*	0.21 ± 0.32^{a}	0.10 ± 0.17^{a}
Gills	ND	ND	ND	0.39 ± 0.79
Gonads	ND	–	0.27 ± 0.28	6.15*
Liver	ND	–	0.31 ± 0.25	1.14*
Sr				
Muscle	1.61 ± 0.42^{a}	2.11 ± 1.12^{a}	2.03 ± 1.94^{a}	3.06 ± 2.78^{a}
Gills	66.42 ± 14.75^{a}	57.98 ± 16.62^{a}	95.95 ± 29.8^{a}	86.46 ± 22.01^{a}
Gonads	0.41 ± 0.08^{a}	–	0.62 ± 0.63^{a}	1.34 ± 1.88^{a}
Liver	**0.15 ± 0.07^{a}**	–	**0.53 ± 0.32^{a,b}**	**0.60 ± 0.33^{b}**
Zn				
Muscle	**32.57 ± 8.89^{a}**	**60.56 ± 9.24^{b}**	**63.08 ± 12.77^{b}**	**74.36 ± 13.85^{b}**
Gills	**197.67 ± 43.29^{a,b}**	**246.23 ± 58.7^{a,b}**	**210.57 ± 46.19^{a}**	**307.24 ± 86.60^{b}**
Gonads	**194.46 ± 51.7^{a,b}**	–	**93.50 ± 35.77^{a}**	**481.16 ± 257.48^{b}**
Liver	**51.36 ± 21.35^{a}**	–	**100.14 ± 26.27^{a,b}**	**117.93 ± 39.71^{b}**

Different letters indicate significant difference between seasons within the tissue (values marked in bold)

Cd was below the detection limit in all tissues during all seasons

Regardless of the season, Cu and Fe were specific for the liver and Ba, Cr, Sr, and Zn for the gills (from 3 times up to 400 times higher concentrations when compared to other tissues).

According to the PCA analyses of metal concentrations in muscles, specimens sampled in autumn from both rivers grouped around higher values for Hg (Figs. 1 and 2).

Taking into account the proximity of the rivers, as well as the naturally expressed physiological regularity during the seasons in fish tissues, specimens from both sites have shown similarities in metal accumulation: (1) the highest accumulation for the majority of elements in the gills; (2) the lowest accumulation in the muscle; (3) higher accumulation of elements in summer; (4) Cu and Fe were specific for liver; and (5) Ba, Cr, Sr, and Zn were specific for gills.

Also, Al, B, Fe, Ni, and Pb did not show significant differences in concentrations among different seasons in all investigated tissues. Hg and Zn concentrations showed significant differences among seasons in all tissues. Only concentrations of Cr, Hg, and Zn were different among seasons in the muscle while more elements (7 in gills, 6 in gonads and liver) showed different concentrations in other investigated tissues.

The difference in specific accumulation of elements in the gills, liver, muscle, and gonads at Pestan and Beljanica was analyzed with CDA (Fig. 3). Based on the total content of elements, the gills showed a high degree of differentiation compared to other tissues. Also, partial overlap of liver with the muscles and gonads can be seen.

Concentrations of metals that are found in chub specimens during these surveys were compared with the prescribed MAC (Cd 0.05, Pb 0.3, Hg 0.5–1, As 2; Official Gazette of the RS, No 28/11 n.d.); so, all concentration values per unit dry weight (dw) were translated into the concentration per unit fresh weight (ww) (Table 5). In all analyzed samples of chub, concentrations of Pb, Cd, Hg, and As (in muscle tissue) were below the prescribed MAC values. Based on these results, we can conclude that chub meat can be safely used in human nutrition. Nevertheless, these concentrations could have been genotoxic (Sunjog et al. 2014). So, even if they are safe for consumption, they could have had mutagenic properties which are potentially dangerous

Table 4 The mean ± SD values of metal and metalloid concentrations of chub tissues collected at the Beljanica river during different seasons

Beljanica	2011		2012	
	Autumn	Winter	Spring	Summer
Al				
Muscle	1.11 ± 2.25[a]	9.35 ± 10.93[a]	7.76 ± 11.24[a]	8.77 ± 10.23[a]
Gills	17.31 ± 23.53[a]	15.62 ± 15.49[a]	31.88 ± 21.10[a]	40.45 ± 65.62[a]
Gonads	7.23 ± 5.29[a]	11.74 ± 22.19[a]	20.40 ± 18.06[a]	10.09 ± 12.78[a]
Liver	1.23 ± 1.65 [a]	3.94 ± 2.61 [a]	17.90 ± 23.18 [a]	2.25 ± 5.40 [a]
B				
Muscle	0.05 ± 0.04[a]	0.18 ± 0.26[a]	ND	ND
Gills	0.63 ± 0.13[a]	0.07 ± 0.19[a]	ND	ND
Gonads	0.23 ± 0.03[a]	0.16 ± 0.26[a]	0.04 ± 0.12[a]	0.08 ± 0.22[a]
Liver	0.71 ± 0.53	0.03 ± 0.08	ND	ND
As				
Muscle	0.26 ± 0.26[a]	0.20 ± 0.19[a]	0.59 ± 1.21[a]	0.45 ± 0.63[a]
Gills	0.08 ± 0.22[a]	0.22 ± 0.29[a]	0.35 ± 0.42[a]	0.29 ± 0.13[a]
Gonads	1.00 ± 0.67[a]	0.82 ± 1.19[a]	2.30 ± 2.36[a]	1.52 ± 2.20[a]
Liver	0.05 ± 0.11[a]	0.13 ± 0.07[a]	0.71 ± 0.83[a]	0.86 ± 0.82[a]
Ba				
Muscle	0.18 ± 0.14[a]	1.69 ± 3.22[a]	0.7 ± 0.59[a]	0.62 ± 0.26[a]
Gills	**10.14 ± 2.03[a]**	**15.97 ± 2.59[b]**	**11.80 ± 3.54[a,c]**	**14.69 ± 3.47[b,c]**
Gonads	1.31 ± 0.62[a]	1.40 ± 1.20[a]	2.31 ± 2.45[a]	2.44 ± 2.46[a]
Liver	0.02 ± 0.02[a]	0.04 ± 0.11[a]	0.17 ± 0.34[a]	0.05 ± 0.11 [a]
Cr				
Muscle	**0.02 ± 0.02[a]**	**0.26 ± 0.20[b]**	**0.07 ± 0.07[a]**	**0.06 ± 0.08[a]**
Gills	0.44 ± 0.43[a]	0.96 ± 0.82[a]	0.64 ± 0.22 [a]	0.69 ± 0.33 [a]
Gonads	0.07 ± 0.03 [a]	0.24 ± 0.15 [a]	0.08 ± 0.07 [a]	0.26 ± 0.54 [a]
Liver	0.03 ± 0.02 [a]	0.18 ± 0.18 [a]	0.14 ± 0.12 [a]	0.15 ± 0.16 [a]
Cu				
Muscle	1.64 ± 0.54 [a]	1.43 ± 0.70 [a]	2.21 ± 2.27 [a]	1.19 ± 0.33 [a]
Gills	**2.42 ± 0.44[a,b]**	**2.24 ± 0.52[a]**	**2.51 ± 0.35[a,b]**	**2.98 ± 0.79[b]**
Gonads	6.87 ± 1.03 [a]	5.23 ± 3.63 [a]	2.94 ± 1.37 [a]	8.64 ± 5.98 [a]
Liver	**22.01 ± 9.09[a]**	**32.03 ± 16.63[a,b]**	**28.72 ± 11.69[a]**	**45.46 ± 14.09[b]**
Fe				
Muscle	7.62 ± 3.16 [a]	17.62 ± 23.91 [a]	11.50 ± 8.32 [a]	11.13 ± 6.42 [a]
Gills	83.97 ± 19.36 [a]	99.40 ± 19.22 [a]	106.64 ± 32.92 [a]	115.74 ± 50.97 [a]
Gonads	54.64 ± 4.52 [a]	51.88 ± 12.24 [a]	51.76 ± 47.18 [a]	65.51 ± 28.51 [a]
Liver	198.35 ± 159.19 [a]	190.85 ± 103.24 [a]	202.18 ± 96.37 [a]	390.69 ± 235.29 [a]
Hg				
Muscle	**1.03 ± 0.19[a]**	**0.53 ± 0.18[b]**	**0.54 ± 0.15[b]**	**0.64 ± 0.16[b]**
Gills	0.75 ± 0.52 [a]	0.48 ± 0.18 [a]	0.64 ± 0.21 [a]	0.75 ± 0.26 [a]
Gonads	0.77 ± 0.05 [a]	0.44 ± 0.21 [a]	0.58 ± 0.40 [a]	0.48 ± 0.22 [a]
Liver	**0.46 ± 0.41[a]**	**0.41 ± 0.25[a]**	**0.62 ± 0.22[a]**	**1.03 ± 0.30[b]**
Mn				
Muscle	0.77 ± 0.39 [a]	1.97 ± 3.17 [a]	1.12 ± 0.33 [a]	1.01 ± 0.23 [a]
Gills	26.70 ± 9.11 [a]	18.84 ± 6.98 [a]	25.00 ± 7.54 [a]	31.46 ± 14.62 [a]
Gonads	**40.06 ± 7.53[a]**	**17.23 ± 21.39[a,b]**	**2.39 ± 1.52[b]**	**17.11 ± 16.90[a,b]**
Liver	4.71 ± 1.14 [a]	4.71 ± 1.07 [a]	9.21 ± 4.91 [a]	9.21 ± 4.32 [a]
Mo				
Muscle	0.07 ± 0.08 [a]	0.18 ± 0.23 [a]	0.15 ± 0.12 [a]	0.19 ± 0.14 [a]

Table 4 (continued)

Beljanica	2011		2012	
	Autumn	Winter	Spring	Summer
Gills	**0.24 ± 0.11[a]**	**1.09 ± 0.19[b]**	**1.51 ± 0.28[b]**	**2.09 ± 0.67[c]**
Gonads	0.18 ± 0.08 [a]	0.30 ± 0.34 [a]	0.06 ± 0.05 [a]	0.23 ± 0.26 [a]
Liver	0.94 ± 0.30 [a]	0.53 ± 0.38 [a]	0.16 ± 0.18 [a]	0.83 ± 1.38 [a]
Ni				
Muscle	0.01 ± 0.03 [a]	0.37 ± 0.63 [a]	0.25 ± 0.71 [a]	0.05 ± 0.04 [a]
Gills	0.10 ± 0.10 [a]	0.12 ± 0.11 [a]	0.10 ± 0.04 [a]	0.18 ± 0.16 [a]
Gonads	0.03 ± 0.02 [a]	0.20 ± 0.36 [a]	0.07 ± 0.04 [a]	0.12 ± 0.17 [a]
Liver	0.09 ± 0.16 [a]	0.07 ± 0.05 [a]	0.11 ± 0.04 [a]	0.09 ± 0.07 [a]
Pb				
Muscle	ND	0.23 ± 0.13[a]	0.22 ± 0.22[a]	0.18 ± 0.12[a]
Gills	ND	0.68 ± 0.43[a]	0.25 ± 0.42[a]	0.37 ± 0.43[a]
Gonads	ND	0.51 ± 0.38[a]	0.65 ± 0.35[a]	0.44 ± 0.24[a]
Liver	ND	0.69 ± 1.07 [a]	0.74 ± 0.24 [a]	0.68 ± 0.61 [a]
Sr				
Muscle	1.80 ± 0.81 [a]	17.96 ± 36.51 [a]	2.94 ± 1.36 [a]	3.77 ± 2.38 [a]
Gills	**108.94 ± 16.46[a]**	**170.89 ± 26.80[b]**	**118.12 ± 26.36[a]**	**160.89 ± 45.02[b]**
Gonads	**1.01 ± 0.35[a,b]**	**17.10 ± 38.68[a]**	**0.64 ± 0.37[b]**	**1.19 ± 0.70[b]**
Liver	0.48 ± 0.17 [a]	2.45 ± 3.82 [a]	1.01 ± 0.57 [a]	1.87 ± 2.40 [a]
Zn				
Muscle	40.18 ± 19.53 [a]	68.89 ± 45.81 [a]	67.42 ± 10.28 [a]	72.39 ± 12.07 [a]
Gills	**206.68 ± 64.38[a]**	**236.11 ± 54.92[a,b]**	**221.16 ± 39.31[a]**	**313.94 ± 80.37[b]**
Gonads	162.39 ± 22.75 [a]	188.30 ± 97.89 [a]	188.80 ± 135.30 [a]	296.16 ± 204.19 [a]
Liver	74.36 ± 15.77 [a]	146.98 ± 75.61 [a]	165.86 ± 87.20 [a]	161.36 ± 48.96 [a]

Different letters indicate significant difference between seasons within the tissue (values marked in bold)

Cd was below the detection limit in all tissues during all seasons

for both fish species and human consummators as well. Exposure limits for most of the other elements have not been established neither by the European Union (EU) nor the Republic of Serbia.

Discussion

Several factors might be the cause of seasonal variability in metal accumulation, such as the differences in growth and reproductive cycles and changes in water quality parameters. After the spawning period, fish exhibit specific feeding patterns which reach their peak at autumn, which is the end of the main feeding period. So, the variations in the heavy metal accumulation in fish could be related to the seasonal differences in their metabolic rate, changes in the feeding rate, as well as the dynamics of the growth of fish, which all determine the physiological condition of fish (Farkas et al. 2003). Farkas et al. (2003) found significant seasonal variations of the heavy metals, with higher concentration of Cd, Cu, Pb, and Zn in the autumn season. At Pestan and Beljanica, Hg showed a greater degree of accumulation in autumn especially in the muscle tissue. Rašković et al. (2018) showed the higher degree of accumulation of Hg in muscle in *Squalius cephalus*. Other studies also showed a tendency of Hg to bind in muscle tissues in various fish species (Kenšová et al. 2010; Heidary et al. 2012; Harley et al. 2015). Havelková et al. (2008) showed that the target organ for Hg accumulation in fish from heavily contaminated localities was the liver and that the main target organ for Hg accumulation in fish from lightly contaminated localities was the muscle.

A greater and significant accumulation of the majority of elements was visible during summer, especially at Pestan. Duman and Kar (2012) showed increased accumulation of Pb, Ni, and Cd in the chub liver during summer, while Cr, Mn, and Zn were highest during winter. The same authors also showed increased accumulation of Pb in chub gills during summer, as well as increased concentrations of Cr, Mn, and Cu in chub gills during winter. Omar and Ahmed (2013) showed greater accumulation of Fe, Cu, Pb, Zn, Cr, and Mn in muscle of African catfish during the warmer months. Şaşi et al. (2018) also showed higher accumulation of Cr and Cu in

Table 5 Mean ± SD concentration of muscle elements (expressed in µg/g wet weight) in chub samples from the (a) Pestan and (b) Beljanica. ND, below the detection limit, *, detected in one specimen

	Cd	Pb	Hg	As
(a)				
October	ND	ND	0.32*	ND
November	ND	ND	0.44 ± 0.05	ND
December	ND	0.09*	0.22 ± 0.03	0.22 ± 0.22
March	ND	0.11 ± 0.09	0.18 ± 0.07	0.04 ± 0.02
April	ND	ND	0.33 ± 0.05	0.02 ± 0.00
July	ND	0.06 ± 0.04	0.16 ± 0.04	0.17 ± 0.10
August	ND	ND	0.20 ± 0.04	0.05 ± 0.08
September	ND	ND	0.23 ± 0.11	0.08 ± 0.05
(b)				
October	ND	ND	0.21 ± 0.02	0.05*
November	ND	ND	0.25 ± 0.03	0.09 ± 0.06
December	ND	0.06 ± 0.03	0.16 ± 0.05	0.04 ± 0.05
February	ND	0.06 ± 0.02	0.11 ± 0.02	0.06 ± 0.06
March	ND	0.05 ± 0.02	0.11 ± 0.03	0.04 ± 0.03
April	ND	0.17*	0.15 ± 0.04	0.29 ± 0.42
May	ND	0.05 ± 0.01	0.12 ± 0.02	0.04 ± 0.05
July	ND	0.05 ± 0.03	0.14 ± 0.04	0.11 ± 0.12
August	ND	0.05 ± 0.00	0.17 ± 0.04	0.17 ± 0.26
September	ND	0.05*	0.13 ± 0.03	0.04 ± 0.03

the muscle of *Squalius fellowesii* during summer. Increased accumulation of metals in the tissues of fish during the warmer seasons was probably the result of increased rates of metabolism. It has been shown that higher temperatures favor the accumulation of Cd, especially in the most stressed tissues, such as liver and kidneys (Jezierska and Witeska 2006). The emergence of greater accumulation of metals in the tissues during the warmer months was also noticed by Salem et al. (2014a) in the various tissues of *Rutilus rutilus* and by Pereira et al. (2010) in gills of *Liza aurata*.

Analysis of the accumulation of metals and metalloids in different chub tissues showed a high level of differentiation, as well as significant differences in the distribution of elements in the tissues at the studied sites. Looking generally for both sites, the largest concentration for most of the analyzed metals and metalloids was found in gills, liver, and gonads, and least in the muscles. Muscles are generally considered as a tissue with poor potential for accumulation (Canli and Atli 2003; Jarić et al. 2011; Akan et al. 2012; Sunjog et al. 2012; Jaćimović et al. 2015; Jordanova et al. 2018). This principle of tissue-specific accumulation, where gills and liver accumulate higher metal concentrations in relation to muscles, has proved to be very common in both chub and other fish species (Bervoets et al. 2001; Duman and Kar 2012; Harkabusová et al. 2012; Rajkowska and Protasowicki 2013; Salem et al. 2014b; Rašković et al. 2018). Common to all sites is that Cu accumulates in the liver in the highest concentrations, which is consistent with the literature (Lenhardt et al. 2012; Rajkowska and Protasowicki 2013; Ahmed et al. 2014). As Cu is essential for many enzymatic processes that occur in the liver, as an integral part of the enzyme, accumulation of copper can be interpreted as the physiological need of an organism. As stated by Pyle et al. (2005), the concentration of Cu in the liver is usually regulated by homeostatic mechanisms and maintained at a level below 50 mg/gdw which is also showed in our case. Only the values for a couple of specimens from July and August at Beljanica have been close to it with 48.15 g/gdw and 54.03 g/gdw, while the other values ranged mainly from 10 to 20 mg/gdw. A higher accumulation of Fe in the liver and gills was observed at all locations. Because Fe accumulates in the liver in the form of ferritin and hemosiderin, the highest concentration in the liver can also be interpreted as a physiological response (Ahmed et al. 2014).

The gills have shown a pattern of accumulation characterized by the highest concentrations of Zn, Sr, Cr, and Ba at both rivers. The occurrence that some of these metals have a higher concentration in gills than in other organs has been noted in species *Hypophthalmichthys molitrix*, *Abramis brama*, *Cyprinus carpio*, *Silurus glanis*, *Sander lucioperca*, and *Lota lota* (Lenhardt et al. 2012; Subotić et al. 2013).

The gonads from all localities accumulated significant concentrations of Zn, which would also be linked to their role in the development of the gonads (Olsson et al. 1990; Zubcov et al. 2012). Higher concentrations of this element in the gonads is very common (Papagiannis et al. 2004; Sunjog et al. 2012), especially before the spawning season (Olsson et al. 1990; Podrug and Raspor 2009).

Interestingly enough, when it comes to nonspecific elements, which do not produce a clear pattern of accumulation in a particular tissue, we can also see the similarity in accumulation between Pestan and Beljanica, which tells us not only that the proximity of these rivers leads to similar responses in fish but also the general pattern of accumulation of some elements in fish. When it comes to Al, B, Fe, Ni, and Pb, it was shown that they do not vary between seasons. Beside the fact that these elements were not required for essential metabolic processes (except Fe), and could be highly genotoxic, we can expect their higher accumulation in specific seasons to be due to extensive pollution incidents and not a consequence of physiological causes or changes in metal speciation in the water during the year (Jezierska and Witeska 2006). According to Jezierska and Witeska (2006), in terms of extensive contamination of the environment, metals and metalloids do not show a specific tendency towards certain organs. On the other hand, Hg, Mn, and Mo have been fluctuating between seasons, which can be linked to some physiological needs of fish.

Conclusion

Field study on indigenous European chub from Pestan and Beljanica rivers gave an insight into the seasonal variations of metal concentrations. Both rivers gave similar results which can be explained by similar vicinity from the main polluters and by similar anthropogenic stress, along with a strong physiological affinity of some elements for the investigated tissues.

Our results have shown high specific accumulation of elements in fish tissues especially in the case of gills. We obtained the highest accumulation of elements in gills and the lowest in muscle. We have shown that some elements, like Al, B, Fe, Ni, and Pb, do not show seasonally depended accumulation, probably because they are not physiologically essential.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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