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HEAVY METAL CONTENT AND ELEMENT COMPOSITION OF *ALBURNOIDES BIPUNKTATUS* BLOCH IN ISKAR RIVER, BULGARIA

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Abstract

RAIKOVA-PETROVA, G., D. ROZDINA, R. MARINOVA, I. PETROV, E. NIKOLOVA and S. ILIEVA, 2015. Heavy metal content and element composition of *Alburnoides bipunktatus* Bloch in Iskar River. Bulgaria. *Bulg. J. Agric. Sci.,* Supplement 1, 21: 196–201

Altogether 28 specimens of *Alburnoides bipunctatus* from the middle stream of Iskar River were studied to determine the element composition, distribution and deposition of heavy metals in tissues and organs of the species. An x-ray fluorescent analysis was used. Twenty one out of the 32 surveyed elements were found. The highest concentration of elements was found in the fins, gill caps and gills. The level of Pb, Zn, Cd and Cu in certain tissues and organs is higher than the maximum allowed concentrations for the same metals. The highest levels of Cd were found in the muscles (4 mg.kg^{-1}) and internal organs (3 mg.kg^{-1}) of 3–4 years old fish. The highest level of Zn was established in the fins and gill caps of 3–4 years old specimens (147 mg.kg^{-1}) while the lowest rates were found in the gills of 1–2 year olds (71 mg.kg^{-1}). The lead was accumulated mainly in the muscles (12 mg.kg^{-1} in 1–2 years old and 15 mg.kg^{-1}in 3–4 years old). The highest levels of Cu were found in the internal organs of 3–4 year olds (17 mg.kg^{-1}), and lowest in gills and muscles of 1–2 years old (2 mg.kg^{-1}). Heavy metals accumulation is age dependent and increases with the fish age. *A. bipunctatus* is "macro-concentrator" in relation to heavy metals in whole body, since bio-accumulation coefficient is higher than 2 (from 146 for cadmium to 1603 for zinc).

Key words: heavy metals, *Alburnoides bipunktatus*, Iskar River, bioaccumulation

Introduction

Over the last 20 years the living conditions in Iskar River have been altered radically. The intensive anthropogenic contamination declined sharply after the closure of almost all industrial outlets, depositing contaminators along the river bank and the building of new/reconstruction of the existing wastewater treatment plants. Though in the river's sediments there are heavy metals, petroleum products and other weakly degradable xenobiotics, accumulated for decades. There are different data about the heavy metal pollution of surface waters and sediments in the Iskar River (Stoianov, 1999; Penin and Tcholakova, 2000; Diadovski et al., 2000; Cholakova, 2002; Diadovski et al., 2003a; Diadovski et al., 2003b; Topalova et al., 2005a; Parvanov et al., 2008). Topalova et al. (2005b) have determined high concentration of heavy metals and oil products along the middle stream of the river. With highest concentration in surface waters were the ions of Mn (0.03–0.07 mg.l^{-1}), Zn (0.05–0.07 mg.l^{-1}), Cu (0.02–0.03 mg.l^{-1}), Pb (about 0.01 mg.l–1), in the sediments – Mn reaches values of 500 –700 mg.kg^{-1}, Zn – 300 mg.kg^{-1}, Cu–60 mg.kg^{-1}, Pb – 40 mg.kg^{-1}. So far there are no data available for the heavy metals and arsenic accumulation in fish inhabiting the middle stream of the Iskar River. As fish have

the ability to accumulate heavy metals and other xenobiotics they can be bio-indicators for contaminated waters (Karapetkova et al., 1995; Raikova-Petrova et al., 1996, 1997, 1998; Velcheva, 2002; Carvalho, Fernandes, 2006). According to Marcovecchio and Moreno (1993), studying the degree of bioaccumulation of heavy metals in fish reflects the bioavailability of the pollutants in the system and thus represents the true degree of pollution in the environment.

The aim of the study is to determine the element composition, the distribution and deposition of heavy metals in tissues and organs of *A. bipunctatus* from Iskar River.

Materials and Methods

The material was collected along the middle stream of Iskar River. Fishing was done via an 8 mm wide net sack and via electro fishing with 700 A/ 60 Hz straight, pulsating current. In total 28 specimens of bleak were caught. Each specimen was measured the total length (L) in cm and the total weight (W) in g. Age was determined on the fish scales, with the use of a Carl Zeiss Jena Dokumator projector at 17.5x magnification. Gills, fins, gill caps, internal organs (liver, intestine, kidneys) and muscles were collected for studying the heavy metal accumulation. The abovementioned tissues and organs have been chosen on the basis of their physiological importance for the fishes' organism and its consumption value for humans. The samples were dried till air-dry weight.

For simultaneous qualitative and quantitative definition of all chemical elements except the lightest ($Z < 6$) an x-ray fluorescent analysis was used. For the elements with atom numbers higher than 11, the threshold sensitivity of the method was very good – 1.5 μg/g. Due to the small size of the internal organs, they were grouped according to the age – 1 and 2 years old and 3 and 4 years old fishes.

For defining the level of bio-accumulation the Perelman's (Nikanaorov et al., 1985) coefficient for bio-accumulation was used. It defines the relation between the content of metal in the organism (organ) and the content of the metal in the water.

Results

The catchments were consisted of 1, 2, 3 and 4 years old fishes (Table 1).

The element composition of *A. bipunctatus* is represented in Table 2 and Table 3. Twenty one out of the 32 surveyed elements were found. The highest concentration of elements was found in the fins and gill caps. This was mainly due to the high quantities of calcium and phosphorus (66 600 $mg.kg^{-1}$Ca and 15 400 $mg.kg^{-1}$P) accumulated in 3 and 4 years old fish. In terms of element accumulation fins and gill caps were followed by the gills.

Cadmium was found in the muscles of 3 and 4 years old fish (4 $mg.kg^{-1}$) and in the internal organs (3 $mg.kg^{-1}$). In the other tissues and organs cadmium was not found.

The zinc content in tissues and organs of *A. bipunctatus* is represented on Figure 1. Highest level of zinc was found in the fins and gill caps of 3 and 4 years old specimens (147 $mg.kg^{-1}$) and lowest rates were found in the gills of 1 and 2 years old (71 $mg.kg^{-1}$).

Table 1

Age-size composition of A. *bipunctatus* catchments in Iskar River in 2012

Age (years)	Average length (L, cm)	Average weight (W, g)	Number (n)
1	2.55	1.05	6
2	4.73	2.05	10
3	7.45	5	7
4	8.63	15.3	5

Table 2

Element composition (mg/kg) of gills and muscles of *A. bipunctatus* from the middle stream of Iskar River

Element	Gills		Muscles	
	1 and 2 years old	3 and 4 years old	1 and 2 years old	3 and 4 years old
Al	350	400	370	400
Si	3500	4000	3300	3500
P	7200	9000	7200	6600
S	6800	7800	9100	8300
Cl	3300	4700	2800	3100
K	8500	9300	13 700	12 300
Ca	16 800	21 400	18 400	17 000
Ti	9	9	9	8
Cr	15	16	7	7
Mn	6	32	12	40
Fe	700	337	266	138
Cu	2	4	2	6
Zn	71	92	116	127
Br	16	21	0	12
Rb	32	20	15	13
Sr	135	165	81	90
Cd	0	0	0	4
Sn	0	0	0	14
I	0	74	0	0
Ba	0	32	4	10
Pb	9	12	12	15

Table 3

Element composition (mg/kg) of fins, gill flaps and internal organs of *A. bipunctatus* from the middle stream of Iskar River

Element	Fins and gill flaps		Internal organs	
	1 and 2 years old	3 and 4 years old	1 and 2 years old	3 and 4 years old
Al	350	500	300	500
Si	4300	5000	3000	3100
P	13600	15400	5200	5500
S	5700	5600	6900	8000
Cl	3300	3700	3000	4400
K	5900	4700	8000	8000
Ca	50 900	66 600	10 700	3000
Ti	12	9	4	2
Cr	16	23	7	7
Mn	0	42	0	7
Fe	960	630	1140	700
Cu	6	7	11	17
Zn	115	143	96	138
Br	0	9	16	10
Rb	17	9	30	10
Sr	285	283	45	14
Sn	0	25	0	13
Ba	50	94		14
Pb	3	9	8	10

The obtained results for lead content in *A. bipunctatus* populating the middle sector of Iskar River are represented in Figure 2. The highest content was found in the muscles (12 mg.kg^{-1}in 1 and 2 years old and 15 mg.kg^{-1} in 3 and 4 years old), followed by the gills and internal organs. In the fins and gill caps were found lowest levels of the metal (3 mg.kg^{-1} in 1 and 2 year old fish and 9 mg.kg^{-1} in 3 and 4 year olds). The level of lead was rising with the age of the fish.

The copper content in tissues and organs of *A. bipunctatus* is presented in Figure 3. The highest levels of copper were found in the internal organs of 3 and 4 years old (17 mg.kg^{-1}), and lowest in gills and muscles of 1 and 2 years old (2 mg.kg^{-1}).

According to the Bulgarian and European legislation the mercury is one of the hazardous heavy metals for living organisms. Concentrations of this metal were not found in *A. bipunctatus,* which was a sign that Iskar River was not contaminated with mercury.

The element with the highest concentration in all the studied tissues and organs from all the ages is the zinc. The second in concentration in the gills and muscles of all the fishes and the fins and gill caps of 3 and 4 years old is the lead. In the internal organs of all the fishes and the fins and gill caps of 1 and 2 years old the second element in concentration is the copper (Figures 4 and 5).

The bio-accumulation coefficient, determined on the basis of the average content of each of the four heavy metals (Cd, Zn, Pb and Cu) in *A. bipunctatus* is presented in Table 4. The content of these metals in the water of Iskar River is according to the "Annual bulletin on the state of the environment" for 2012, published by the Executive Environment Agency. The obtained results for the coefficient of bioaccumulation show a proven accumulation for the studied heavy metals in the fish as their content reaches 1843 in comparison to the level of the metals in the water. According to the Nikanorov's classification (1985), the researched species *A. bipunctatus* relates to the so called "macro-concentrators" in respect to heavy metals, for which values of the bio-accumulation coeffi-

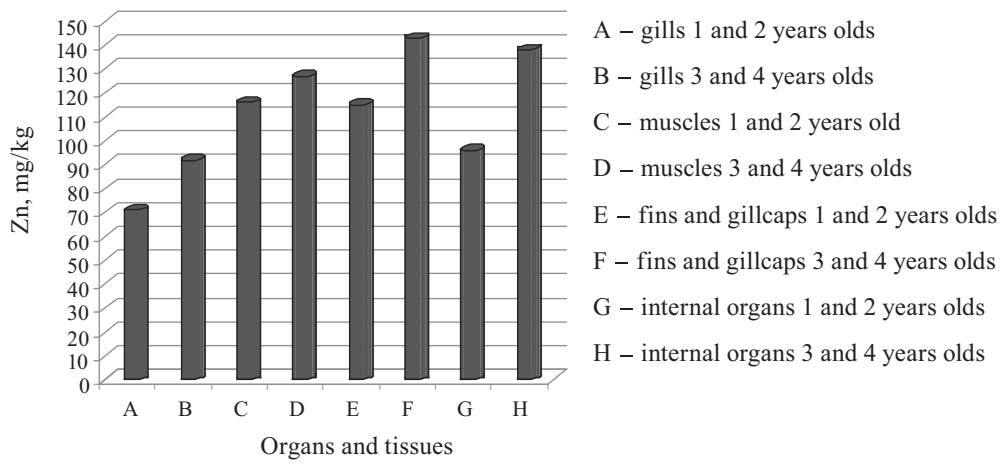

Fig. 1. Zink content in organs of *A. bipunctatus* from the middle stream of Iskar River

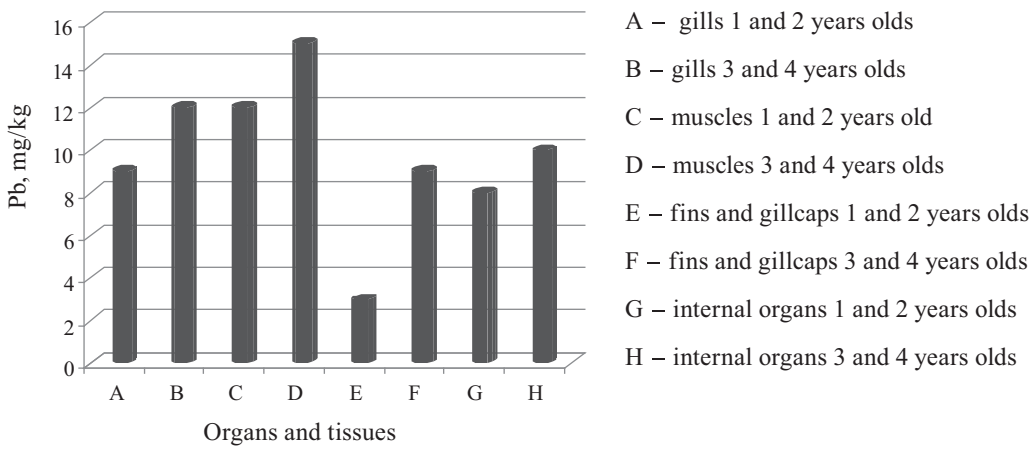

Fig. 2. Lead content in organs of *A. bipunctatus* from the middle stream of Iskar River

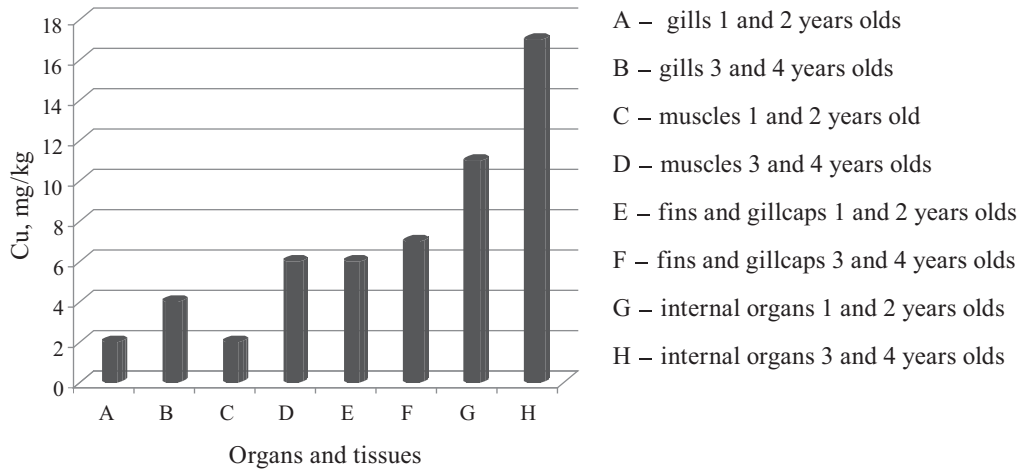

Fig. 3. Copper content in organs of *A. bipunctatus* from the middle stream of Iskar River

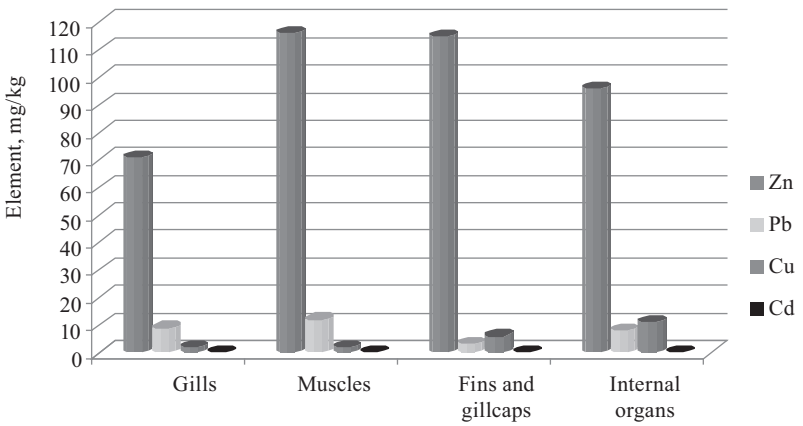

Fig. 4. Comparisson of the element content in the different organs and tissues of 1 and 2 year old specimens of *A. bipunktatus* from the middle stream of Iskar River

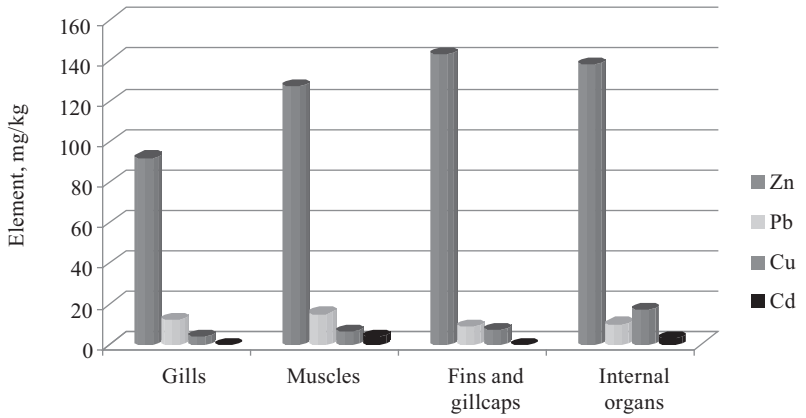

Fig. 5. Comparisson of the element content in the different organs and tissues of 3 and 4 year old specimens of *A. bipunktatus* from the middle stream of Iskar River

Table 4

Bio-accumulation coefficient of *A. bipunctatus* from the middle stream of Iskar River

Element	Whole body	Gills	Fins and gill caps	Internal organs	Muscels
Cd	146	0	0	250	333
Zn	1603	1164	1843	1671	1736
Pb	390	420	240	360	540
Cu	229	100	217	467	133

cient higher than 2 are specific. In relation to cadmium, gills, fins and gill caps are related to "deconcentrators" (bio-accumulation coefficient < 1).

Discussion

The cadmium levels in the muscles and organs of *A. bipunctatus* were much higher than the maximum permitted levels (0.05 mg.kg^{-1} fresh weights) by Ordinance No 12, Ordinance 31 and Regulation (EO) No 1881/2006. These higher levels that we received were probably due to the fact that such were defined on the basis of dry weight of the samples.

The cadmium content in different fresh water fish species (carp, silver carp, grass carp and trout) from 22 reservoirs (fish ponds and dams) in 7 regions of Southern Bulgaria and Sofia region was many times lower (0.001 to 0.040 mg.kg^{-1}), (Peneva et al., 1990; Stoyanov, 1999). Age and species difference in the level of Cd was not found.

The quantity of Zn is much higher than that, determined by Stoyanov (1999) in fresh water fish from Southern Bulgaria and Sofia region (1.0 to 34.89 mg.kg^{-1}).

The lead levels in *A. bipunctatus* are much higher than the maximum permitted, defined by the Ordinance No12 and the Ordinance No 31 – 0.2 mg.kg^{-1}, as well as Regulation (EO) No 1881/2006 (0.30 mg.kg^{-1} fresh weight). Much higher levels of lead in the samples of *A. bipunctatus* from the middle sector of Iskar River were observed, compared to the results for different fish species from other reservoirs. In the studied samples, the increased levels of lead are also due to the method of observation – RFA, since, there is lead in the detector of the system, which raises the levels of the results when tested.

Conclusions

The catchments were consisted of 1, 2, 3 and 4 years old fishes. The following elements were found: Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Br, Rb, Sr, Cd, Sn, I, Ba, Pb. Cadmium was established in the muscles of 3 and 4 years old fish (4 mg.kg^{-1}) and in the internal organs (3 mg.kg^{-1}). The element with the highest concentration in all the studied tissues and organs from all the ages is the zinc. Highest level of zinc was found in the fins and gill caps of 3 and 4 years old specimens (147 mg.kg^{-1}) and lowest rates were found in the gills of 1 and 2 years old (71 mg.kg^{-1}). The highest content of lead was found in the muscles, followed by the gills and internal organs. The level of lead was found to rise with the age of the fish. The highest levels of copper were found in the internal organs of 3 and 4 years old (17 mg.kg^{-1}), and lowest in gills and muscles of 1 and 2 years old (2 mg.kg^{-1}). Concentrations of mercury were not found in *A. bipunctatus*, which was a sign that Iskar River was not contaminated with

this metal. The researched species relates to the so called ”macro-concentrators” in respect to heavy metals.

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