

## Zinc Content in the Organs and Tissues of Freshwater Fish from the Kardjali and Studen Kladenets Dam Lakes in Bulgaria

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**Abstract:** A study was conducted to estimate zinc loads in the organs and tissues of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* inhabiting 2 dam lakes Kardjali and Studen Kladenets, located along the Arda river (South Bulgaria). The zinc content was determined by atomic absorption spectrophotometry using a Perkin Elmer 380 B apparatus. The effect of the temporal factor was proved over a 3-year study. The highest zinc concentrations in fish organs and tissues were detected in summer and autumn. The highest zinc loads were found in the kidney and liver, and the lowest in the muscles of the 3 fish species tested. A correlation was found between the zinc levels in the water and those in the fish.

**Key Words:** Fish, zinc, biomonitoring

### Introduction

According to Odum (1986), environmental pollution is becoming one of the most important limiting ecological factors. Heavy metals are the main group of pollutants, with deleterious effects on organisms. Linik (1986) noted that, unlike all other toxicants of organic nature that more or less dissolve in natural waters, heavy metal ions should be considered persistent components in the aquatic environment. An important ecological specificity of these pollutants is also the fact that there are practically no self-cleaning mechanisms known for them. When present in the water, they pass through the trophic chain of aquatic communities.

The effect and distribution of heavy metals are studied at all levels of aquatic ecosystems contaminated with them. Golubev (1973) showed that heavy metals penetrated through the skin and were distributed irregularly in organisms, forming depots. Komarovski and Polishtuk (1981) detected larger metal loads in the tissues of predatory fish species. According to Tinisli (1982), there are 2 ways for penetrating into the organism – via direct water adsorption or fish feed.

Carnivores at the top of the food-chain obtain most of their heavy metal burden by way of their food, especially where fish are present, and so there exists the potential for considerable biomagnification ( Mance, 1987; Langston, 1990; Cumbie, 1975 ). Similar studies were

conducted by Badsha and Sainsbura (1977), Komarovskii et al. (1988), Wachs (1998), Velcheva (1998), Storelli and Marcotrigiano (2001), Fent (2003), and considering fish species as heavy-metal bioindicators.

Since the toxic effects of metals have been recognised, heavy metal levels in the tissues of aquatic animals are occasionally monitored. Because the heavy metal concentration in tissues reflects past exposure via water and or food, it can demonstrate the current situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment (Canli and Kalay, 1998).

Mackay et al. (1975) and Brooks et al. (1974) showed the organ specificity of zinc distribution in fish.

Kroupa and Hartvich (1990) detected high zinc levels in the fish kidney and liver. Camusso et al. (1995) showed that zinc accumulated mostly in the gills of freshwater fish from the River Po. Kovekovdova and Simokon (2002) studied the heavy metal load of fish migrating from the Gulf of Amur and the highest zinc levels were found in the skin.

According to Cosson (1994), the zinc ions of metallothioneine molecules were replaced by those of cadmium, when both metals were combined in the organism.

River systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents (Leland. et al, 1978; Mance, 1987). Contamination of a river with heavy metals may have devastating effects on the ecological balance of the aquatic environment, and the diversity of aquatic organisms becomes limited with the extent of contamination (Suziki et al., 1988).

Arda is a large Bulgarian river passing through Greece and Turkey and flowing into the Aegean Sea. Along its valley on the territory of Bulgaria there are some anthropogenic sources of heavy metal contamination – the Non-Ferrous-Metal Works in Kardjali and ore mines. The latter cause permanent water pollution for aquatic organisms.

The aim of the present study was to monitor the zinc content in the organs and tissues in 3 freshwater fish species from 2 dam lakes on the Arda river, depending on the zinc content in the water and the temporal factor.

**Materials and Methods**

The study was carried out with 3 fish species, - *Alburnus alburnus* and *Cyprinus carpio* from the family *Cyprinidae* and *Perca fluviatilis* from the family *Percidae* which are widespread in European rivers.

The fish were chosen to same-size groups, i.e.: *Alburnus alburnus* –average body length of 14 cm; *Cyprinus carpio* – average body length of 27 cm; *Perca fluviatilis* - average body length of 15 cm.

Pooled samples consisting of 5 individuals of each species were analysed. Chemical analyses of the samples of gills, skin, bones, muscles, liver and kidney were done during the 4 seasons over a 3 year period (1998- 2000). The fish samples were prepared by dry ashing. Gills ( $0.420 \pm 0.09$  mg), skin ( $0.312 \pm 0.05$  mg), bones ( $0.453 \pm 0.19$  mg), muscles ( $0.525 \pm 0.15$  mg), liver ( $0.375 \pm 0.05$  mg) and kidney ( $0.210 \pm 0.10$  mg) tissue was put into crucibles. The crucibles were placed in chemical oven at 135 °C for 2 h. After that the samples were mineralized at 400 °C in the chemical oven for 24 h. To each crucible was added 2 ml of concentrated nitric acid ( Merck). The samples were dehydrated at 450 °C for 1 h. To each sample 10 ml of hydrochloric acid (Merck) was added. We reduced the samples to a volume of 25 ml with distilled water in graduated flasks . The content of zinc was determined by atomic absorption spectrophotometry using a Perkin Elmer 380 B apparatus and the results were given as mg/kg fresh weight.

The study was conducted in 2 dam lakes, Kardjali and Studen Kladenets, located on the Arda river (South Bulgaria).

Water samples (4 for each year) were collected from the fishing sites in parallel with the fish samples. A litre of water was taken from the depth to the surface of the dam lakes. Surface water temperature was measured (Table 1). The zinc content was analysed using atomic absorption spectrophotometry method a Perkin Elmer 380 B apparatus (Table 2).

Table 1. Temperature (°C) of water from the Kardjali- and Studen Kladenetz dam lakes.

	Kardjali dam lake				Studen kladenetz dam lake			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
First year	17.2	21.0	16.0	9.0	17.0	21.2	16.0	8.0
Second year	10.0	22.0	16.2	9.0	9.0	20.0	15.5	8.0
Third year	14.0	21.5	17.0	8.0	15.0	22.0	15.0	9.0

Table 2. Zinc content in water from the Kardjali- and Studen Kladenetz dam lakes, mg/dm<sup>3</sup>.

	Kardjali dam lake				Studen kladenetz dam lake			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
First year	0.011	0.180	0.033	0.0056	0.056	0.180	0.600	0.200
Second year	0.080	0.109	0.140	0.036	0.970	1.405	1.000	0.047
Third year	0.160	0.180	0.050	0.150	0.530	0.500	0.350	0.150

The results obtained were mathematically treated, comparing the data for the zinc content between the fish samples taken from the 2 dams, between the 3 fish species, between the organs and tissues tested, as well as between the fish and water. For this purpose we used a statistical analysis of variance to compare mean values ( $\bar{x}$ ) using Student's t-test ( $n < 30$ ), correlation analysis to prove the interrelationship between the zinc load in fish organs and water through the correlation coefficient – R xy (with R xy values  $> 0.5$  a strong positive correlation was established), and analysis of variance to establish the effect of the organ type and fish species factors on the zinc content (Lakin, 1990).

## Results

Using analysis of variance, we proved that zinc content depended on the “fish organ” but not on the fish species. This was evidenced by the fact that no significant differences in zinc content were established between the three fish species studied within a season.

The results from the first year of the study are given in Table 3. The highest zinc content in all fish samples tested from Kardjali and Studen Kladenetz dam lakes was determined in the fish kidney, compared to those in the other organs and tissues ( $P > 0.05$ ). The zinc found in liver was considerably higher than that in gills, skin, muscles and bones ( $P > 0.05$ ). The order of zinc contents of the organs was as follow kidney  $>$  liver  $>$  gills  $>$  skin  $>$  bones  $>$  muscles.

Similar trends of zinc distribution in the tissues and organs tested were determined during the second (Table 4) and third years of the study (Table 5).

The analyses of the for zinc load of fish during different seasons showed the following trends: higher values metal content were detected in summer and autumn than spring and winter. The comparison of these seasonal changes with the respective zinc levels in the water of both dam lakes revealed their direct correlation – the highest metal levels in the water were established in summer and autumn. Badsha and Sainsbura (1978) also

Table 3. Mean zinc content ( $\bar{x} \pm Sx$ ) in the organs and tissues of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* from the Kardjali and Studen Kladenetz dam lakes – mg/kg net weight (first year).

First species Organs	Kardjali dam lake				Studen kladenetz dam lake			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
<i>A. alburnus</i>								
Gills	57.46 ± 1.2	66.12 ± 0.8	57.55 ± 1.3	36.75 ± 0.9	67.66 ± 2.05	72.59 ± 2.3	73.00 ± 2.1	59.99 ± 2.1
Skin	42.56 ± 0.9	46.020 ± 0.9	43.775 ± 0.9	47.605 ± 1.0	60.190 ± 2.0	70.120 ± 2.4	72.021 ± 2.4	69.353 ± 1.9
Muscle	7.897 ± 0.5	23.233 ± 2.1	19.361 ± 0.5	14.858 ± 0.3	18.781 ± 0.2	20.110 ± 0.7	27.397 ± 0.9	15.422 ± 0.2
Liver	83.07 ± 2.1	558.551 ± 6.5	170.897 ± 2.8	93.923 ± 4.0	152.054 ± 3.6	145.164 ± 4.1	540.255 ± 6.4	199.897 ± 3.8
Kidney	128.409 ± 5.3	682.586 ± 9.1	245.127 ± 3.2	106.473 ± 3.9	165.279 ± 3.8	136.789 ± 3.9	713.664 ± 7.1	249.301 ± 4.0
Bones	54.709 ± 1.8	79.47 ± 1.4	39.456 ± 1.5	26.153 ± 1.2	27.315 ± 0.8	88.297 ± 3.5	80.601 ± 3.1	65.173 ± 2.1
<i>C. carpio</i>								
Gills	82.158 ± 2.1	85.011 ± 2.0	83.000 ± 2.8	74.208 ± 3.5	83.001 ± 3.5	88.213 ± 3.1	119.491 ± 3.1	112.111 ± 3.2
Skin	69.716 ± 2.0	73.600 ± 1.7	70.022 ± 2.4	70.111 ± 3.1	72.111 ± 2.1	79.179 ± 2.4	82.131 ± 2.6	75.208 ± 2.4
Muscle	15.422 ± 0.5	17.012 ± 0.5	12.441 ± 0.4	11.623 ± 0.2	14.152 ± 0.4	18.261 ± 0.3	28.484 ± 0.8	12.623 ± 0.5
Liver	117.02 ± 3.2	144.403 ± 2.5	140.255 ± 2.1	69.698 ± 3.0	124.032 ± 3.8	158.763 ± 2.4	201.211 ± 5.1	139.127 ± 3.4
Kidney	115.526 ± 3.1	207.625 ± 3.2	167.710 ± 2.4	113.664 ± 3.5	167.902 ± 3.4	444.444 ± 4.5	672.319 ± 6.4	411.121 ± 6.1
Bones	26.785 ± 0.9	80.601 ± 1.9	30.229 ± 0.9	15.787 ± 0.2	17.821 ± 0.7	27.983 ± 0.7	85.556 ± 3.6	55.145 ± 1.9
<i>P. fluviatilis</i>								
Gills	149.000 ± 2.6	155.921 ± 3.4	149.121 ± 2.5	148.714 ± 5.1	118.421 ± 2.4	162.321 ± 3.4	165.814 ± 3.4	160.021 ± 2.4
Skin	70.605 ± 1.9	83.082 ± 2.1	73.032 ± 3.1	75.341 ± 2.4	71.052 ± 2.4	85.106 ± 2.6	90.152 ± 5.1	78.191 ± 3.1
Muscle	11.038 ± 0.5	17.673 ± 0.5	11.872 ± 0.6	11.741 ± 0.2	11.381 ± 0.5	12.086 ± 0.4	21.281 ± 0.9	13.000 ± 0.2
Liver	137.011 ± 3.8	353.985 ± 2.7	169.058 ± 3.4	111.021 ± 1.8	141.580 ± 4.1	169.038 ± 3.8	705.864 ± 6.8	154.083 ± 6.1
Kidney	400.641 ± 4.0	696.311 ± 6.2	603.648 ± 6.4	115.284 ± 1.5	140.982 ± 3.1	170.861 ± 4.5	689.311 ± 5.8	202.255 ± 4.3

Table 4. Mean zinc content ( $\bar{x} \pm Sx$ ) in the organs and tissues of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* from the Kardjali and Studen Kladenetz dam lakes – mg/kg net weight (second year).

First species Organs	Kardjali dam lake				Studen kladenetz dam lake			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
<i>A. alburnus</i>								
Gills	45.39 ± 1.2	48.42 ± 2.5	47.35 ± 2.0	32.95 ± 1.2	52.50 ± 2.01	78.18 ± 4.1	56.07 ± 2.1	48.00 ± 2.1
Skin	33.78 ± 1.1	36.90 ± 1.1	36.21 ± 1.6	24.17 ± 0.9	42.64 ± 1.2	46.15 ± 3.0	45.14 ± 1.3	35.00 ± 1.0
Muscle	14.65 ± 0.5	18.17 ± 0.6	16.85 ± 0.9	10.19 ± 0.2	19.72 ± 0.7	27.45 ± 2.1	12.50 ± 0.6	13.89 ± 0.4
Liver	70.95 ± 2.1	45.81 ± 1.4	147.05 ± 2.8	75.51 ± 3.6	130.40 ± 3.2	1071.42 ± 8.9	197.36 ± 2.8	152.70 ± 3.1
Kidney	66.05 ± 2.2	73.33 ± 3.2	187.50 ± 3.1	65.60 ± 3.2	218.75 ± 2.9	1250.00 ± 9.6	300.00 ± 3.6	238.23 ± 3.5
Bones	23.68 ± 1.4	24.17 ± 0.9	26.95 ± 0.9	23.46 ± 0.7	30.25 ± 1.6	45.34 ± 2.3	33.58 ± 1.2	29.59 ± 0.9
<i>C. carpio</i>								
Gills	50.60 ± 2.5	53.82 ± 2.1	55.62 ± 2.9	52.72 ± 3.2	70.17 ± 3.2	77.33 ± 3.9	71.59 ± 3.2	59.50 ± 1.7
Skin	42.01 ± 1.9	45.15 ± 1.1	46.16 ± 2.1	33.23 ± 1.0	40.60 ± 2.1	68.32 ± 3.2	58.96 ± 2.5	50.90 ± 1.1
Muscle	12.73 ± 0.5	11.54 ± 0.1	11.06 ± 0.5	10.04 ± 0.3	14.04 ± 0.4	28.97 ± 1.0	22.00 ± 0.9	12.00 ± 0.4
Liver	75.83 ± 3.1	94.63 ± 2.7	127.99 ± 3.1	84.50 ± 3.5	150.32 ± 3.6	458.70 ± 4.8	165.89 ± 3.5	151.81 ± 2.4
Kidney	107.98 ± 3.9	506.25 ± 3.6	199.21 ± 3.2	148.60 ± 2.1	331.63 ± 4.8	675.33 ± 5.7	352.19 ± 4.1	165.21 ± 2.5
Bones	19.27 ± 0.6	24.55 ± 0.9	26.50 ± 1.0	19.20 ± 0.7	30.00 ± 2.1	46.13 ± 2.2	38.70 ± 1.2	31.13 ± 1.1
<i>P. fluviatilis</i>								
Gills	41.36 ± 1.4	49.05 ± 1.1	64.28 ± 3.2	48.66 ± 2.4	91.46 ± 5.6	133.67 ± 3.6	101.99 ± 3.6	80.92 ± 3.4
Skin	40.10 ± 2.1	48.75 ± 1.2	59.46 ± 2.8	44.73 ± 2.1	85.75 ± 4.7	97.95 ± 6.0	89.62 ± 4.1	78.21 ± 2.4
Muscle	18.08 ± 0.6	19.06 ± 0.5	18.20 ± 0.4	17.67 ± 0.9	18.75 ± 0.4	41.17 ± 2.1	20.20 ± 0.9	16.48 ± 0.7
Liver	210.12 ± 3.9	287.35 ± 4.2	336.44 ± 4.1	165.30 ± 4.2	195.00 ± 2.5	597.08 ± 6.3	460.23 ± 3.5	170.19 ± 3.1
Kidney	463.25 ± 4.6	1121 ± 9.4	2656 ± 3.5	529.31 ± 6.8	1216.2 ± 8.9	1500.33 ± 0.6	1041.6 ± 9.7	972.00 ± 9.1
Bones	23.08 ± 0.4	23.86 ± 0.2	31.48 ± 0.9	44.64 ± 1.1	50.23 ± 2.6	57.94 ± 2.3	51.12 ± 2.4	48.30 ± 1.2

established the seasonal dynamics of zinc load in fish organs. In parallel to our results they, detected the highest metal levels in summer and autumn.

The comparison between the Kardjali and Studen Kladenetz dam lakes in terms of zinc loads in the tissues and organs of the 3 fish species tested showed the dominance of the latter ( $P < 0.01$ ).

## Discussion

The analysis of the zinc load of the organs and tissues in the fish species tested from both dam lakes showed similar trends. The mode of metal deposition and distribution depended on the specific organ.

The same result was observed over the whole 3 year period of the study, valid for *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* populating both the Kardjali and the Studen Kladenetz dam lakes. The results obtained suggested that the kidney and liver were the main depots-organs for zinc in fish. Our opinion was similar to that of

other authors, such as Moor and Ramamurti (1987), Komarovski et al. (1988) and Kroupa and Hartvich (1990), who thought that zinc showed affinity to parenchyma organs. Organs such as the liver, gills and kidney are metabolically active and accumulate heavy metals in higher levels, as was observed in experimental studies (Allen, 1994; Allen, 1995; Kalay and Edrem, 1995) and field studies (Langston, 1990; Camusso et al., 1995; Noris and Lake, 1995; Spehar et al., 1998). This metal also showed affinity to protein SH groups and its increased load in the kidney and liver led to a release of a specific metal protein, metallothioneine, from these organs (Cosson, 1994; Shleng et al., 1995; Vilella et al., 1999). Our results showed that the zinc load in the kidney and liver varied. It increased rapidly with the increase on zinc content in the water. Our study established a high degree of correlation between the zinc contents in the kidney and water and those of the liver and water (i.e. -  $R_{xy} = 0.772$  for *Alburnus alburnus*;  $R_{xy} = 0.556$  for *Cyprinus carpio*;  $R_{xy} = 0.830$  for *Perca fluviatilis*). The significance of these correlations was high ( $P < 0.05$ ). During the third

Table 5. Mean zinc content ( $\bar{x} \pm Sx$ ) in the organs and tissues of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* from the Kardjali and Studen Kladenetz dam lakes – mg/kg net weight (third year).

First species Organs	Kardjali dam lake				Studen kladenetz dam lake			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
<i>A. alburnus</i>								
Gills	67.06 ± 2.1	67.13 ± 3.0	56.69 ± 2.3	62.87 ± 3.2	69.64 ± 3.1	69.89 ± 3.5	63.12 ± 3.2	65.93 ± 3.9
Skin	48.00 ± 1.1	50.21 ± 2.1	45.17 ± 1.9	50.00 ± 2.3	61.23 ± 3.2	63.14 ± 2.1	60.99 ± 2.8	60.00 ± 3.4
Muscle	8.03 ± 0.02	10.71 ± 0.04	7.81 ± 0.09	9.21 ± 0.09	15.00 ± 0.9	15.31 ± 0.1	14.68 ± 0.3	14.47 ± 0.7
Liver	89.39 ± 3.1	102.34 ± 2.1	89.36 ± 3.8	82.89 ± 4.2	126.18 ± 3.5	98.57 ± 2.1	97.22 ± 3.1	90.25 ± 6.1
Kidney	108.55 ± 3.0	157.00 ± 3.2	99.65 ± 3.7	104.00 ± 2.8	148.50 ± 4.1	231.90 ± 3.8	228.32 ± 3.7	90.65 ± 5.4
Bones	22.83 ± 0.9	22.72 ± 0.7	20.51 ± 1.1	22.54 ± 0.7	25.87 ± 1.1	26.61 ± 0.5	23.94 ± 2.2	29.39 ± 1.1
<i>C. carpio</i>								
Gills	56.25 ± 2.1	59.09 ± 2.9	52.13 ± 2.1	53.10 ± 3.2	70.00 ± 2.2	68.12 ± 3.8	63.57 ± 3.5	59.90 ± 4.1
Skin	46.93 ± 2.0	49.99 ± 2.1	37.50 ± 1.9	43.99 ± 2.8	50.10 ± 3.1	51.33 ± 2.4	48.71 ± 2.1	46.99 ± 3.0
Muscle	5.84 ± 0.02	9.12 ± 0.05	8.10 ± 0.1	5.79 ± 0.1	15.00 ± 0.8	15.77 ± 0.8	7.400 ± 0.04	8.15 ± 0.02
Liver	99.15 ± 3.1	120.46 ± 2.1	95.58 ± 3.8	112.08 ± 3.4	85.06 ± 3.2	89.42 ± 4.1	80.44 ± 3.4	78.43 ± 3.4
Kidney	125.76 ± 3.9	135.24 ± 3.4	99.47 ± 3.7	120.48 ± 3.8	82.15 ± 3.1	88.13 ± 3.8	87.32 ± 3.5	82.92 ± 3.5
Bones	19.06 ± 0.4	18.18 ± 0.5	14.12 ± 0.2	14.32 ± 0.6	14.12 ± 0.8	22.14 ± 0.7	13.24 ± 0.4	12.75 ± 0.7
<i>P. fluviatilis</i>								
Gills	77.50 ± 3.0	81.84 ± 3.9	71.98 ± 3.9	73.30 ± 4.8	95.12 ± 3.8	90.13 ± 4.8	78.85 ± 3.5	76.81 ± 3.8
Skin	61.11 ± 3.1	69.92 ± 3.4	48.71 ± 2.1	60.00 ± 3.1	87.43 ± 2.9	89.10 ± 3.8	77.68 ± 3.1	73.30 ± 3.4
Muscle	12.82 ± 0.2	13.81 ± 0.6	9.10 ± 0.7	12.57 ± 0.9	16.54 ± 0.9	16.78 ± 0.9	16.66 ± 0.3	16.23 ± 0.8
Liver	108.63 ± 3.2	167.06 ± 3.7	90.83 ± 3.9	107.61 ± 4.1	252.45 ± 4.2	281.25 ± 3.9	256.66 ± 6.9	233.64 ± 5.1
Kidney	149.52 ± 3.4	178.03 ± 3.5	127.25 ± 4.0	153.50 ± 5.1	325.00 ± 4.9	500.00 ± 5.2	417.50 ± 8.1	282.14 ± 6.2
Bones	18.58 ± 0.7	21.42 ± 0.9	19.73 ± 0.2	17.85 ± 0.9	30.48 ± 1.2	31.91 ± 1.1	25.00 ± 1.6	25.19 ± 1.2

year of the study, we established the lowest zinc loads in these organs. In our opinion, this was due to the rapid metabolism of the metal associated also with its faster release.

Tracing the interrelationship between the zinc load in the kidney and liver, we established a positive correlation between them ( $P < 0.05$ ).

According to Moor and Ramamurti (1987), zinc induced negative changes when penetrating fish gills. Gills and skin were the main route for metal penetration from the water. Our results supported their opinion. For the period of our study, we established a positive correlation between zinc loads of gills and skin ( $P < 0.05$ ).

In the gills, followed by the skin of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* both from the Kardjali and Studen Kladenetz dam lakes high zinc loads were found. They ranked immediately after the kidney and liver.

In our opinion, these results supported the suggestion that the main route of zinc entry into the fish was via water.

During the whole period of the study, the lowest zinc loads were found in the muscles. In their studies, Brow and Chow (1977) and Kroupa and Hartvich (1990) also reported low zinc levels in muscles.

The information available on the effect of the seasonal factor upon the zinc load of fish is relatively limited. The results obtained from our 3 year study showed that the seasonal factor did have a statistically significant effect. Like Badsha and Sainsbura (1978) and in contrast to Bobori and Economidis (1996), who found the highest heavy metal load in *Perca fluviatilis* in early spring, we established the highest metal concentrations in the tissues and organs of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* from both the Kardjali and Studen Kladenetz dam lakes in summer and autumn. In our opinion this fact was associated with the higher water temperatures and physiological activity of fish in summer and autumn.

The comparison of zinc load in fish for the whole period of the study between samples from Kardjali and

Studen Kladenetz dam lakes showed the greater statistical significance of the latter ( $P < 0.05$ ). Higher metal levels were also detected in the water of the Studen Kladenetz dam lake, which confirmed our aforementioned suggestion for the zinc route from water to fish organs.

## Conclusions

For the 3-year period of the study, the zinc load of *Alburnus alburnus*, *Cyprinus carpio* and *Perca fluviatilis* populating the Kardjali and Studen Kladenetz dam lakes decreased in the following order: kidney > liver > gills > skin > bones > muscles.

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