

ACCUMULATION OF Pb, Zn AND Cu IN SCARDINIUS ERYTHROPHthalmus AND SOME MACROPHYTES FROM FRESHWATER ECOSYSTEM BIOSPHERE RESERVE SREBARNA

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Abstract

The concentration of Pb, Zn and Cu was established in dominant herbivorous fish species *Scardinius erythrophthalmus* (Linnaeus, 1758), in macrophytes *Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Phragmites australis* (Cav.) Trin. ex Steud., water and sediments in freshwater ecosystem of Biosphere Reserve "Srebarna". The interrelationships between the content of the trace metals in rudd, its nutrients, water and sediments was examined. The current research studied the main routes of entry of Pb, Zn and Cu in the objects of the study. The Bioconcentration factor (BCF) and the Biomagnification factor (BMF) were estimated. We have established a pronounced bioindicators for monitoring the degree of contamination of freshwater ecosystems with Zn, Pb and Cu.

Key words: bioindication, fish, macrophyte, rudd, trace metal

1. INTRODUCTION

The lake Srebarna is heterotrophic lake on the bank of Danube River, surrounded by relevantly small areas of forest territory and farm land. Lake Srebarna is characterized with exceptional biodiversity. It is a habitat for 3132 taxa, of which 1123 algal species, 17 fungi species, 15 species of lichens, 280 species of higher plants, 108 species of protozoa, 1263 invertebrate species and 326 species of vertebrates. Twenty two species from the lake ecosystems are included in the European Red List. Another 8 species (*Eimeria arkutinae* Golemansky, 1978, *Eimeria micromydis* Golemansky, 1978, *Isospora talpae* Golemansky, 1978, *Hilmylepis prokopici* Genov, 1970, *Catenotaenia matovi* Genov, 1971, *Paranoplocephala aquatica* Genov, Vasileva and Georgiev, 1996, *Troglorema srebarni* Genov, 1964, *Paracrenosoma kontrimavichusi* Genov, 1978) are described for the first time in lake Srebarna, and for one of them *Eimeria micromydis* Golemansky, 1978 it is the only known habitat. Lake Srebarna is supplied with water mainly from the Danube river. Water entering from the Danube contributes both to maintain the water level and enriching with oxygen and nutrients and as well as increasing the level of pollutants, including heavy metals. (Uzunov et al., 2012).

However, the Danube watershed is influenced by various anthropogenic sources of pollutants with heavy metals and metalloids such as mining industry, discharges of domestic and industrial effluents, pesticides and fertilizers, etc. Studies on the heavy metal content are conducted in all countries (Germany, Slovakia, Hungary, Serbia, Croatia, Romania, Bulgaria, etc.), associated with the second largest river in Europe - Danube River with its tributaries and wetlands. The pool of European studies demonstrates both the relevance and importance of the issue. Most of the previous studies were focused on the content of heavy metals and only a small part discussed the aspects of the reasons for the obtained results. The majority of studies pointed out Pb, Zn and Cu as the elements with the highest content.

A large part of studies on the Danube River and in the lower part of the Danube watershed proved hazardous levels of heavy metals in sediments (Literathy and Laszlo, 1995; Gruiz et al., 1998; Guieu and Martin, 2002; JDS, 2002; Wotke et al., 2003; Bird, 2005; Milenkovic et al., 2005; Oreščanin et al., 2005; Sakan et al., 2007; Vitek et al., 2007; Yiğiterhan and Murray, 2007; Dragun et al., 2009; Sakan, 2009; Spurný et al., 2009; Makovský et al., 2010; Vuković et al., 2012), that authors associated with anthropogenic pollution and the ability of sediments to accumulate trace metals. In most cases the content of the heavy metals in the water was lower compared to the sediments. Studies of water and sediments from the Bulgarian section of the Danube River confirmed the high content of heavy metals (mostly Pb, Zn and Cu) in sediments and a little bit lower in Lake Srebarna (Yurukova and Kochev, 1996; Ricking and Terytze, 1999; Benderev, 2000; Hristova, 2000; Hiebaum et al., 2012), which could be explained with the routes of intake of heavy metals in lake ecosystems – by a flow water from the watershed of the lake, with the incoming water from the Danube River and groundwater.

The lakes have the ability to reduce heavy metal pollution mainly through deposition in the sediments (Pettersson et al., 1999). The soluble fraction of heavy metals has the highest potential of toxicity, since living organisms can directly utilize them (Morrison, 1989). Zn and Cu are essential elements for the health and development of animals (FAO/WHO, 1998), but in high concentrations they have a toxic effect on aquatic organisms.

Particularly important are the few researches relating to the transport of metals and ways to resume from the sediment-included elements in the food chain. The largest transfer of metals in macrophytes is established from the sediment to the roots of macrophytes and the smallest at the top parts of the plants. In floating plants authors established lower levels of heavy metals than in submerged ones (Yurukova and Kochev, 1994; Yurukova and Kochev, 2000; Pajević et al., 2002; Jamnická et al., 2006; Grudnik and Germ, 2009). The only studies of macrophytes from Lake Srebarna on the content of heavy metals (Pb, Zn and Cu) are two surveys of more than 15 years (Kochev and Yurukova, 1996; Yurukova and Kochev, 1996), which do not allow the prominent trends to be determined in the evolution of the relevant elements in the dynamic system of the lake.

Several authors examined the content of heavy metals in fish from Danube river and its wetlands (Wachs, 2000; Spurný et al., 2002; Andreji and Stráňai, 2004; Tudor et al., 2006; Alibabić, 2007; Vítek et al., 2007; Spurný et al., 2009; Makovský et al., 2010; Valová et al., 2010; Visnjic-Jeftic et al., 2010; Jankovská et al., 2011; Jarić et al., 2011; Harkabusová et al., 2012; Sunjog et al., 2012; Srđan et al., 2012; Zrnčić et al., 2013; Subotić et al., 2013), a large part of them established high concentration of various heavy metals. Till present days no systematic research of the trace metal content in tissues and organs fish from Lake Srebarna is conducted. There is no data and studies on the ways of the circulation of heavy metals in the food chain in the lake ecosystem.

The interest and need of detailed study on the content of heavy metal in Lake Srebarna is determined by range of factors such as: Insufficient knowledge on the problem of heavy metal content in the structural elements of the freshwater Lake Srebarna, the influence of the environmental factors, the lack of knowledge of the ways of transfer of heavy metals in the food chain in Lake Srebarna, the increased interest of European scientists to this kind of research, the vast biodiversity of the lake ecosystem and variety of potential biological resources and the national significance and numerous international awards of the protected area, and the other potential threats from anthropogenic pollution with heavy metals.



Figure 1. Location of the Lake Srebarna and habitats subject of the study (acc. Map Google., 2009; amended)

2. MATERIAL AND METHODS

Biosphere Reserve Srebarna BG04 (1948) is a protected area in Bulgaria with numerous international awards. BR Srebarna is a lake ecosystem and it is listed as World Heritage by the Convention on World Cultural and Natural Heritage (IN05 Srebarna, 1983) in the List of Wetlands of International Importance under the Ramsar Convention (1975). Its territory was declared a Biosphere Reserve under the program "Man and Biosphere" (IN03 Srebarna, 1977) and it is Important Bird Area, included in the Bird Life International Network (BG033 Srebarna, ID 400, 1989). The lake is part of the protected zone BG0000169 Ludogorie – Srebarna subject to Directive 92/43/EEC Habitats of protected zone BG0000241 Srebarna subject to two Directives (Directive 92/43/EEC Habitats and Directive 79/409/EEC the conservation of wild birds) (SG 21/2007) and it is also part of Corine biotope F00009900 Srebarna (1998).

The material for the study was collected from four locations of the lake ecosystem (Figure 1): 1-Central water surface of the lake, 2-Puddle "Ribarnika", 3-"Below the village" and 4-South gateway.

Habitats were visited to collect materials over the spring, summer and autumn in three consecutive years (2010-2012) of the study. The content of Pb, Zn and Cu was investigated in total of 135 samples from three species dominant macrophytes, and 108 samples from *Scardinius erythrophthalmus* (Linnaeus, 1758) divided into 4 different age groups (Table 1). Over the period of the present study the content of Pb, Zn and Cu was investigated in 9 samples each water and sediment. Each sample was previously described and numbered.

The species and taxa of the organisms collected was specified by main species guides (Drenski, 1951; Bykhovskaya-Pavlovskaya et al., 1962; Nikolyskiy, 1963; Zashev and Margaritov 1966; Pravdino 1966; Bauer Musselius Rifle, 1981; Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986; Moravec, 1994; Karapetkova and Zhivkov, 1995; Zivkov, 1996; Marcogliese et al., 1999; Karapetkova and Zhivkov, 2000; Moravec, 2001; Delipavlov et al., 2003; Karapetkova and Zhivkov, 2010). The nomenclature of macrophytes, freshwater fish and parasites is presented acc. ITIS (<http://www.itis.gov/>), Fauna Europaea (<http://www.faunaeur.org/>), FishBase (<http://www.fishbase.org/>) Zipcodezoo (<http://zipcodezoo.com/>).

The fish weight was measured in grams and its dimensions in centimeters. For each studied fish specimen were examined the following parameters: Total length (L, cm) from the tip of the snout to the end of the beams of the caudal fin; Standard length (l, cm) from the tip of the snout to the end of caudal peduncle/beginning of the caudal fin; Maximum height (H, cm), Weight (W, g), Sex and Age. Determination of the age of the studied freshwater fishes was based on a standard method (Nikolyskiy, 1963; Pravdino 1966, Mosaic et al., 1975; Zivkov, 1996). Samples of muscles, bones and skin were collected from all individuals.

The water samples were collected and when necessary were preserved following the hereafter mentioned standards and regulations: BSS EN 25667-2:2000; ISO 5667-4:2002; BSS EN ISO 5667-3:2006; ISO 5667-4:1987.

Table 1. Characteristics of the fish studied *S. erythrophthalmus*

Age group	Weight g (g)	Total length	Standard length	Width
		L (cm)	l (cm)	H (cm)
0+	6,36±1,01	8,4±0,29	6,76±0,26	1,98±0,15
1+	17,69±6,22	10,48±1,36	8,42±1,28	2,75±0,49
2+	37,9±11,93	12,72±1,18	10,1±1,07	3,84±0,43
3+	66,08±22,90	14,45±1,65	11,46±1,55	4,61±0,54

The sediment samples were isolated with the use of Bottom Sediment Grab Sampler of Ekman (range 225 cm²). They were collected in accordance with the regulations of BSS EN ISO 5667-15:2009.

The collection and study of macrophytes from the lake ecosystem were carried out in accordance with BSS EN 15460:2008. Samples of macrophytes were brought to dry state and stored in paper bags.

Fish specimens were caught with nets with 15 m in length and 1 m in width, with size of the "eye" of 15, 22 and 30 mm. Using nets with different diameter of the "eye" allowed catching fish at different age. The use of the net at the territory of the reserve was authorized by National Nature Protection Service Directorate to MOEW and after registration in the management of the reserve.

For the determination of Pb, Zn and Cu in samples of water, sediment and biological material was applied method of dry ashing and subsequent atomic absorption analysis (Weltz, 1976; ISO 8288:1986) through injection into flame oxidized filtrate sample of atomic absorption spectrophotometer. Samples were analyzed in the Laboratory of atomic absorption spectrophotometry at IBER on AAS "Perkin-Elmer" - 3030 B.

Comparing the distributions of the analyzed parameters was performed with Kruskal-Wallis test (H, Kruskal-Wallis Oneway Nonparametric AOV) and χ^2 - distribution. The tests checked the null hypothesis that comparing independent random samples arised from the same sets of distributions and parameters of localization. Conclusions achieved by the test of Kruskal-Wallis were the same, which would be attained by dispersion analysis (ANOVA). To examine the determinants of the variation was used two-dimensional dispersion analysis – Friedman test (F) (by Sokal and Rohlf, 1981).

For the calculations and analysis were used MS Excel MS Excel (Microsoft, 2010), BioDiversity Pro Softwares (McAleece, 1997) and Statistica 10 (StatSoft, Inc., 2011).

3. RESULTS AND DISCUSSIONS

Research of the contents of Pb, Zn and Cu in waters of the freshwater ecosystem of the Lake Srebarna was conducted (Table 2, Fig. 1A). The highest content in the water was established for the Zn ($167,50 \pm 72,74 \mu\text{g/l}$), followed by Pb ($47,00 \pm 25,81 \mu\text{g/l}$) and Cu ($13,50 \pm 4,73 \mu\text{g/l}$).

According to conducted statistical analysis, the results obtained for the content of Pb, Zn and Cu in the waters of the Lake Srebarna were significant (HKruskal-Wallis=6,88; $p=0,03$).

The concentration of Cu and Zn in water from Lake Srebarna exceeded the average annual rate of their content in waters regulated under the requirements of Regulation № N-4/2012 on characterization of surface waters. Standards for Pb in surface water at this stage are not regulated under the cited above Regulation.

The values obtained in the present study of the concentration of heavy metals in water were higher than the values obtained by Yurukova and Kochev (1996), Hiebaum et al. (2012) for Lake Srebarna, except for the content of Zn.

Table 21. Content of Pb, Zn, Cu in water ($\mu\text{g/l}$) and sediments (mg/kg) from Lake Srebarna

Element	Water ($\mu\text{g/l}$)		Sediments (mg/kg)	
	Mean $\pm$ SD	Range (min-max)	Mean $\pm$ SD	Range (min-max)
Pb	$47,0 \pm 25,81$	20,0-80,0	$29,19 \pm 10,43$	16,41 - 40,72
Zn	$167,50 \pm 72,74$	100,0-240,0	$92,61 \pm 82,82$	29,0 - 214,02
Cu	$13,50 \pm 4,73$	10,0-20,0	$29,91 \pm 8,61$	20,0 - 39,50

The current study established the content of Pb, Zn and Cu in the sediments from the freshwater ecosystem of Lake Srebarna. The obtained results showed a higher content of Zn, and much lower content of Cu and Pb (CCsediments= $29,90 \pm 10,43 \text{ mg/kg}$; CPbsediments = $29,20 \pm 8,61 \text{ mg/kg}$; CZnsediments= $92,61 \pm 82,82 \text{ mg/kg}$) (Table 2; Figure 2 B). For the period of this study there was established no significant annual differences in the concentration of the trace metals. The values for Cu and Pb in the sediments were very similar, while those for Zn were over 3 times higher.

The concentration of the studied elements in sediments of Lake Srebarna decreased in the order $\text{Zn} > \text{Pb} = \text{Cu}$. Similar results are reported by Ricking and Tertyze (1999), Hiebaum et al. (2000) and Vasilev (2005) on the concentration of heavy metals in sediments from Lake Srebarna, as well as several studies of the Danube River watershed (Milenkovic et al., 2005; Vignati et al., 2013; Vitek et al., 2012)

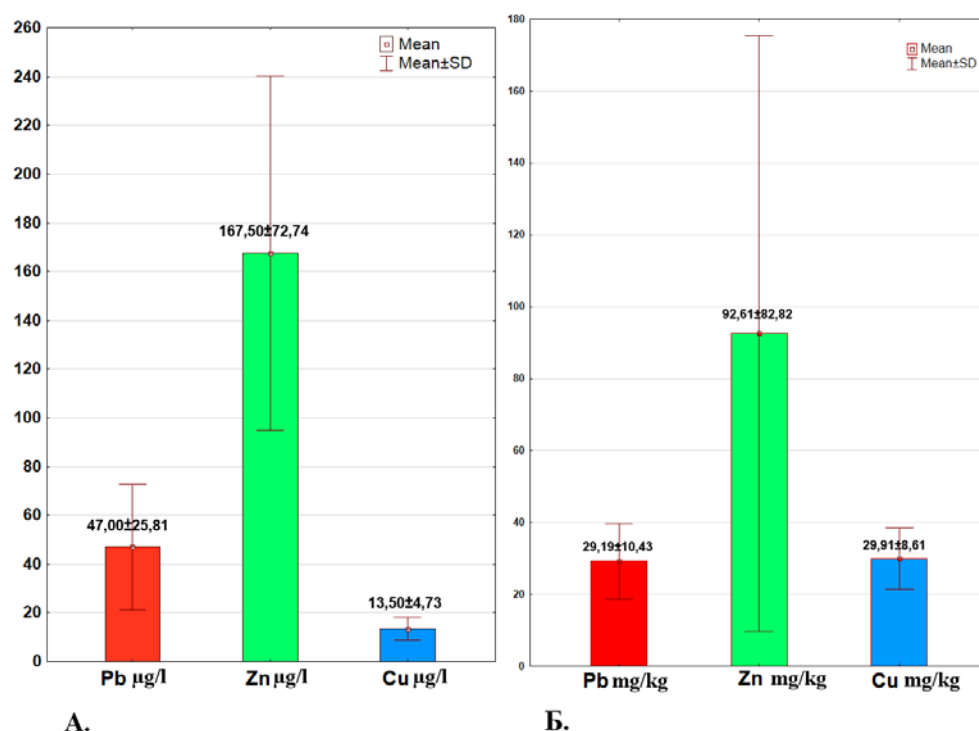


Figure 2. The content of Pb, Zn and Cu in A. water (µg/l) and B. sediments (mg/kg) from Lake Srebarna

When comparing the content of heavy metals in sediment samples from Lake Srebarna by Hiebaum et al. (2000) with the results obtained from this study a minor increase in the content of Pb through the years was established. The values of the content of Cu were higher in the present study. Regarding the content of Zn in this study values greatly exceeding those from the research of Hiebaum et al. (2000) were obtained. According to Hristova (2000), the results of analyzes of heavy metals in the one-meter layer of sediments of Lake Srebarna (Cu, Pb, Zn, Cd, Mn, Co, Ni and Fe) evidence of increased concentrations over a period of 10 years. Yurukova and Kochev (1996) also determined the content of Pb, Zn and Cu in sediments of lake ecosystem. The values obtained from them were similar and slightly lower than those in the present study. In all studies (Hiebaum et al., 2012; Ricking and Terytze, 1999) it was emphasized on the presence of technogenic pollution of the upper sediment layer of the lake.

The higher values of the studied elements in the waters and sediments of Lake Srebarna are thought to be due to the passage of contaminated water from the Danube River. The problem with the burden of the ecosystem could be related to the improper use of different fertilizers and pesticides, and improper agricultural activities.

It is assumed that with the introduction of several of legislative documents in recent years will substantially contribute to the reduction of the negative impacts and contamination of land and soil from the development of agriculture. Such strict measures are National standards for maintaining land in good agricultural and environmental condition (Minister of Agriculture and Food № RD 09-616/2010), Good Agricultural Practice (Order № RD 09-799/2010 Minister of Agriculture and Forestry), Program of measures to reduce and prevent pollution caused by nitrates from agricultural sources in vulnerable areas (Order № RD-141/2011 MOEW and the Order № RD 09-189/2011 the Minister of Agriculture and Food) introduced in accordance with Regulation № 2 for the protection of waters against pollution caused by nitrates from agricultural sources and others. Another model of action is subjecting the adjacent to the reserve territories to stricter regime of use and support farms in these areas, a strict requirement to observe a number of measures and requirements and compliance for their implementation.

As a result of the current study the concentration of Pb, Zn and Cu in *Phragmites australis* was established (Figure 3). The values of the content of trace metals in *P. australis* were highest for Zn ($33,27 \pm 16,01$ mg / kg), followed by those for Pb and Cu (respectively $11,19 \pm 1,96$ mg / kg and $4,93 \pm 3,01$ mg / kg). The highest content of Zn in *P. australis* was determined also by other authors (Cardwell et al., 2002; Stoltz and Greger, 2002; Vymazal et al., 2009).

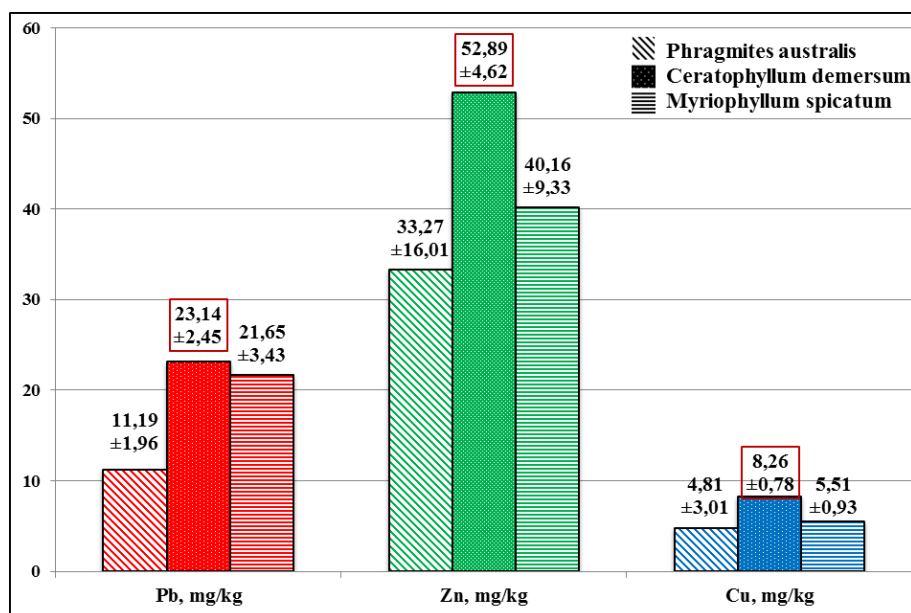


Figure 3. Content (mg/kg) of Pb, Zn and Cu in *P. australis*, *M. spicatum* and *C. demersum* from Lake Srebarna

Significant differences between the values of the three analyzed elements in samples of cane were determined (HKruskal-Wallis=18,61, $p=0,0001$). The content of the heavy metal followed the trend $Zn > Pb > Cu$, similar to the study of Senze and Kowalska-Górska (2008). Comparing the concentration of trace metals in water and the sediment with the content in the macrophytes (Table 2, Figure 2, Figure 3) showed that content in plants depends on the presence of the metals in water and sediments.

The concentration of Pb, Zn and Cu was established in *Myriophyllum spicatum* – a free floating macrophyte. Highest content was established for the Zn (40,16 mg/kg $\pm 9,33$) (Figure 3), followed by Pb (21,65 mg/kg $\pm 3,43$) and Cu (5,51 mg/kg $\pm 0,93$). Significant differences between the values of the three analyzed elements in samples of *M. spicatum* were determined (HKruskal-Wallis=6,49; $p=0,04$). The content of the heavy metal followed the trend $Zn > Pb > Cu$, similar to *C. demersum* and *P. australis*.

Comparing the average concentration of heavy metal in *M. spicatum* and *P. australis* was established similar values for Cu and Zn and nearly 2 times higher for Pb in *M. spicatum*. This could be explained by the difference in the uptake of heavy metals by the two species macrophytes. While *P. australis* uptakes the elements mainly through the roots, *M. spicatum* absorbs elements in addition to the root, and through the whole surface of its body. *M. spicatum* has roots, but occupies the upper layers, and the body of the plant does not reach the bottom, but it is totally immersed in contrast to *P. australis*. This suggests the possibility of a greater uptake of the elements by the whole body plant. The higher content of the trace metal in water is a prerequisite for the higher levels of the metals established in *M. spicatum*.

The average concentration of Zn in *M. spicatum* were 1,5 times higher than those obtained for Danube River by Yurukova (2004). Jamnická et al. (2006) established similar values of Zn and Pb in *M. spicatum*. Similar to other studies, (Grudnik and Germ, 2009, 2010; Grudnik, 2010) the macrophyte *M. spicatum* is among the key hydrophytes accumulating Pb. In the current study *M. spicatum* showed a great potential to accumulate Cu Zn and Pb, and simultaneously to cleanse the environment. Similar results obtained Yan et al. (2005).

The concentration of Pb, Zn and Cu was established in *Ceratophyllum demersum*. The highest concentration was established for Zn (52,89 $\pm 4,62$ mg/kg), followed by the content of Pb and Cu (respectively 23,14 $\pm 2,45$ mg/kg and 8,26 $\pm 0,78$ mg/kg). Significant differences between the values of the three analyzed elements in samples of *C. demersum* were determined (HKruskal-Wallis=7,20; $p=0,03$).

The average content of Pb in *C. demersum* is twice as high compared to the established value in *P. australis*. The fact could be explained with *Ceratophyllum demersum* absorbing nutrients through the whole body directly from water (Mjelde and Faafeng, 1997) where the content of Zn, Cu and particularly Pb is high (Figure 2, Figure 3). *C. demersum* hasn't got roots and inhabits the upper layers of water. *C. demersum* is more tolerant species to Pb pollution compared to *P. australis*. *C. demersum* accumulates trace metal in much bigger extent compared to both *M. spicatum* and *P. australis*.

In the current study were found lower values for Zn and Cu (52,89 and 8,26 mg/kg) in *C. demersum* from Lake Srebarna compared to the concentrations determined by Yurukova (2000) for the elements in *C. demersum* of the Bulgarian section of the Danube River (235,00 and 62,00 mg/kg). Average content of Pb in both studies is similar (21,00 mg/kg to 23,14 mg/kg $\pm$ 2,45 for *C. demersum* from Lake Srebarna).

Yurukova and Kochev (2000) in a study of the content of Zn, Pb and Cu in *C. demersum* from Malak Preslavets a dam situated along the Danube River established higher values (72,00 mg/kg) of the content of Zn in *C. demersum* to current results from Lake Srebarna (52,89 mg/kg $\pm$ 4,62). Comparing the average content of Pb in the two basins also revealed differences (2,70 mg/kg), that were 8 times higher in *C. demersum* (23,14 mg/kg $\pm$ 2,45) from Lake Srebarna. However, the content of Cu in the present study (8,26 mg/kg $\pm$ 0,78) was lower than that obtained from Yurukova and Kochev for Malak Preslavets (13,00 mg/kg). The content of Zn in *C. demersum* was found about 3 times lower than the studied *C. demersum* from the Danube from Yurukova (2004). Tudor et al. (2006) reported an accumulation of Pb, Zn and Cu in *C. demersum* from the Danube in the order Zn > Cu > Pb. The fact that *C. demersum* is an indicator species for heavy metals in aquatic ecosystems and the higher values of metal in Danube determines the possibility of entry of the elements from the river. Another hypothesis for the high concentrations of elements in *C. demersum* and the lake respectively is the possibility of their entry from the surrounding agricultural areas indicated also by Pajević et al. (2003).

The accumulation of Pb, Zn and Cu was established at highest values in *C. demersum*, followed by *M. spicatum* and the lowest values were determined for *P. australis*. The reason for the difference in their accumulation by the macrophytes is due to the specificity in the environment and their development. *P. australis* absorbs nutrients mostly through its roots from upper part of the sediments and water. *M. spicatum* has roots, nevertheless absorbs nutrient through its body, too. *C. demersum* is submerged, free floating aquatic plant that has no root system. The main root of accumulation of trace metals in *C. demersum* is absorption by its whole body surface.

The accumulation of trace metal in tissues and organs of *S. erythrophthalmus* was established. The content of Pb and Zn followed the trend bones > skin > muscles (respectively for Pb 12,43 $\pm$ 12,09 mg/kg; 9,33 $\pm$ 8,36 mg/kg and 6,77 $\pm$ 4,51 mg/kg, and for Zn 137,20 $\pm$ 72,57 mg/kg; 132,10 $\pm$ 81,15 mg/kg and 38,24 $\pm$ 24,72 mg/kg). The content of Zn in the muscles was 3,5 times less than in bones. Zn values varied in much larger range than for Pb, as the variation was expressed most strongly in skin and bones and least in muscles (Table 3, Figure 4).

Table 3. Content (mg/kg) of Pb, Zn and Cu in muscles, bones and skin in *S. erythrophthalmus* from Lake Srebarna

<i>S. erythrophthalmus</i>	Element	Mean $\pm$ SD	Range (min-max)
Muscles	Pb	6,77 $\pm$ 4,51	0,76 - 20,00
	Zn	38,24 $\pm$ 24,72	11,00 - 129,71
	Cu	1,56 $\pm$ 0,81	0,15 - 3,61
Bones	Pb	12,43 $\pm$ 12,09	0,36 - 42,90
	Zn	137,20 $\pm$ 72,57	14,44 - 350,75
	Cu	3,98 $\pm$ 2,54	0,33 - 17,92
Skin	Pb	9,33 $\pm$ 8,36	0,18 - 32,01
	Zn	132,10 $\pm$ 81,15	14,25 - 317,38
	Cu	6,02 $\pm$ 6,71	1,24 - 37,62

The values for of Cu in the skin, bones and muscles of *S. erythrophthalmus* were different from those of the content of Pb and Zn. The highest values were obtained for the content of Cu in the skin of rudd (6,02 $\pm$ 6,71 mg/kg), followed by those in the bones (3,98 $\pm$ 2,54 mg/kg) and muscles (1,56 $\pm$ 0,81 mg/kg). The values of Cu varied in broad range in skin and bones, and at smaller range in the muscles of the rudd (Table 3, Figure 4).

Differences in values of the content of Pb, Zn and Cu in the tissues of *S. erythrophthalmus* were not significant (HKruskal-Wallis test, $p > 0,05$). The type of the tissue of the rudd does not determine the absorption of the three elements of the environment (FFriedman test, $p > 0,05$). The content of the studied elements in the skin, bones and

muscles in rudd decreased in the order: Zn > Pb > Cu. Similar results have been reported by other authors (Arnaudova et al., 2008; Çiçek et al., 2009; Al Sayegh Petkovšek et al., 2012; Sevcikova et al., 2013). The minimum and the average value of the content of Pb in the muscles of rudd ($0,76 \text{ mg/kg}$ and $6,77 \pm 4,51 \text{ mg/kg}$) exceeded 4 and respectively 34 times the standards for Pb in Regulation 31/2004 ($0,20 \text{ mg/kg}$). The average values of the content of Zn in the muscle at the time were in the norm of 50 mg/kg . The average and the maximum values of the content of Cu in the muscles ($1,56 \pm 0,81 \text{ mg/kg}$; $3,61 \text{ mg/kg}$) did not exceeded the standards in Regulation 31/2004 (10 mg/kg).

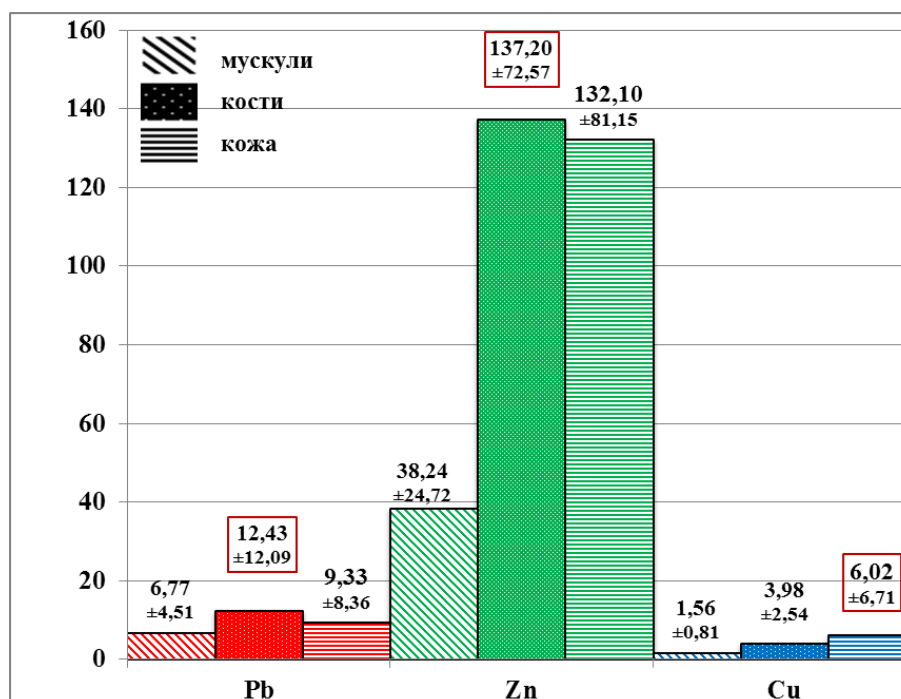


Figure 41. Content (mg/kg) of Pb, Zn and Cu in muscles, bones and skin in *S. erythrophthalmus* from Lake Srebarina

In the current study the correlations between the content of Pb, Zn and Cu in *Scardinius erythrophthalmus*, macrophytes, water and sediments (Table 4) were determined. With high levels of correlation were distinguished the content of Pb in *S. erythrophthalmus* and *C. demersum* ($r_s \text{Pb}=0,22$, $p<0,001$). Highly significant correlations ($p<0,01$) were recorded for the content of Pb between *S. erythrophthalmus* and *P. australis*; for Zn between *S. erythrophthalmus* and *C. demersum*; for Cu between *S. erythrophthalmus* and sediments (Table 4, Figure 5). Significant correlations were established for Zn between *S. erythrophthalmus* - *P. australis*; for Cu between *S. erythrophthalmus* and *C. demersum*; for Cu between *S. erythrophthalmus* and water; for Pb between *S. erythrophthalmus* and sediments.

Table 4. Correlation coefficient Spearman (r_s) and degree of significance between the content of Pb, Zn and Cu in *S. erythrophthalmus*, macrophytes, water and sediments

x-y	Element	r_s
<i>S. erythrophthalmus</i> - <i>P. australis</i>	Pb	0,35**
	Zn	0,28*
	Cu	0,48**
<i>S. erythrophthalmus</i> - <i>M. spicatum</i>	Pb	0,34**
	Zn	0,40ns
	Cu	-0,25ns

<i>S. erythrophthalmus</i> - <i>C. demersum</i>	Pb	0,22***
	Zn	0,89**
	Cu	0,26*
<i>S. erythrophthalmus</i> – water	Pb	0,31ns
	Zn	-0,23ns
	Cu	0,34*
<i>S. erythrophthalmus</i> – sediments	Pb	0,12*
	Zn	0,14ns
	Cu	0,67**

Legend: * significant correlation $p < 0,05$; ** very significant correlation $p < 0,01$; ***exceptional significant correlation $p < 0,001$; ns insignificant correlation

The main routes of entry of Pb, Zn and Cu in *S. erythrophthalmus* are related to food and food chains. Secondary routes for entering trace metals in rudd are related to sediments and water.

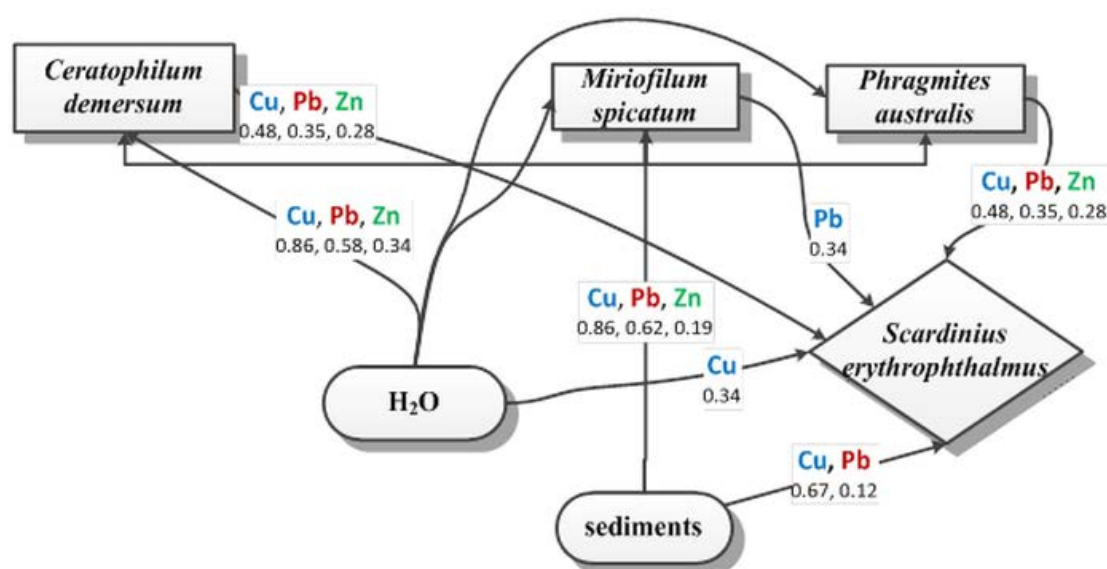


Figure 4. Relationships between the content of Pb, Zn and Cu in the dominant taxa of freshwater organisms, sediments and water in freshwater ecosystems of the Lake Srebarna

The Bioconcentration factor (BCF) (Table 5) of the studied elements in *S. erythrophthalmus* and water were highest for Zn, followed by those of Cu and Pb in the order: $BCF_{Zn} > BCF_{Cu} > BCF_{Pb}$. The Bioconcentration factor of Pb and Cu relative to their content in the sediments were similar, respectively $BCF_{Pb} = 0,33$ and $BCF_{Cu} = 0,32$.

Table 5. Bioconcentration factor (BCF=C_{tissues}/C_{sediments}) of Pb, Zn and Cu for *S. erythrophthalmus* relative to their content in the water and sediments

BCF	Element	Water	Sediments
<i>S. erythrophthalmus</i>	Pb	202,35	0,33
	Zn	612,01	0,10
	Cu	285,58	0,32

The variation in the Biomagnification factor (BMF=C_{fish tissues}/C_{macrophytes}) of the studied heavy metals in the tissues of *S. erythrophthalmus* was examined (Table 6). The highest values of the Biomagnification factor were established for *P. australis* for the all three elements. The Biomagnification factor of Pb, Zn and Cu followed the order: BMF_{Zn}>>BMF_{Cu}>BMF_{Pb}.

Table 6. Biomagnification factor (BMF=C_{fish}/C_{macrophyte}) of Pb, Zn and Cu for *S. erythrophthalmus* relative to macrophytes

Macrophytes	Pb	Zn	Cu
<i>P. australis</i>	0,86	3,06	0,91
<i>C. demersum</i>	0,41	1,94	0,47
<i>M. spicatum</i>	0,44	2,55	0,70

The choice of objects for the evaluation of the circulation of the elements in *S. erythrophthalmus* depends on the fish food source and the environment. The young rudds feed with zooplankton, whereas the adults are herbivorous (Karapetkova and Jivkov, 1995). These specifics in feeding suggest differences in the way of absorption of substances and pollutants, including Pb, Zn, Cu in fish. Pehlivanov and Pavlova (2012) determined that the food of rudd in Lake Srebarna was represented by detritus.

The absorption of Cu by *S. erythrophthalmus* depends on the concentration of the metal in water and sediments, while the absorption of Pb depends on its concentration in sediments. The water and sediments are recognized as secondary routes of entry of trace metals.

4. CONCLUSION

The content of Pb, Zn and Cu in water followed the trend: Zn>Pb>Cu, whereas in sediments the trend was Zn>Pb=Cu. The concentration of Cu and Zn in water of Lake Srebarna exceeded (respectively 13 and 24 times) the average annual values for the elements in waters under Regulation № N-4/2012 on characterization of surface waters.

The dominant species of macrophytes accumulated Pb, Zn and Cu in the order: *C. demersum* > *M. spicatum* > *P. australis*. The three macrophytes had pronounced clear trend for decreasing the content of the studied elements in the order: Zn> Pb> Cu. *C. demersum*, *M. spicatum* and *P. australis* are reliable bioindicators to monitor the degree of contamination of freshwater ecosystems with Zn, Pb and Cu.

The dominant fish species accumulated trace elements in the order Zn> Pb> Cu. The values for the content of Pb and Zn in *S. erythrophthalmus* decreased in the order bones> skin> muscle, while the content of Cu decreased in the order skin> bones> muscles. The average concentration of Pb and Zn in muscles in *S. erythrophthalmus* exceeded the standards in accordance with Regulation 31/2004.

The type of trace element, its chemical properties, concentration and mechanisms of incorporating into food chains are determining factors for absorption in the body of the fish from the environment. Main routes of uptake of Pb, Zn and Cu in *S. erythrophthalmus* are by the food - macrophytes, followed by water and sediments.

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