Scientific Papers. Series D. Animal Science. Vol. LVII, 2014
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

ACCUMULATION OF LEAD (Pb) IN *SCARDINIUS ERYTHROPHTHALMUS* AND *CERATOPHYLLUM DEMERSUM* FROM FRESHWATER ECOSYSTEM BIOSPHERE RESERVE SREBARNA, BULGARIA

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Abstract

Concentration of heavy metals in Scardinius erythrophthalmus (L., 1758) found in Biosphere Reserve "Srebarna" (Northeastern Bulgaria) was examined in 25 fish individuals from every age group and season. The concentration of heavy metals was investigated by mineralization and subsequent atomic absorption spectrophotometry (according to the requirements of the methodology of the pollutants). To ensure the comparability of results the following standards were complied: 1. Sample collection and storage - ISO 5667-3/4:1995; etc. Fish sampling and analysis were carried out in compliance with National Program for Biomonitoring of Bulgaria (MEW, 1999). Particular the concentrations of Pb in Scardinius erythrophthalmus, Ceratophyllum demersum (L.), sediments and water were studied. Bioconcentration factor (BCF=C fish tissues;macrophytes/C water;sediments) for Pb in the tissues of fish and macrophytes were estimated and discussed. Biomagnification factors (BMF=C fish tissues/C macrophites) for Pb in the tissues of fish were estimated and discussed. The chemical analysis showed accumulation of Pb mainly in the fish bones as compared to the accumulation of the respective metal in the fish muscles. Biomagnification factors of Pb in Scardinius erythrophthalmus compared to Ceratophyllum demersum were evaluated. The mean concentration of Pb in the muscles of Scardinius erythrophthalmus was established to greatly exceed the reference values for lead in fish meat from Regulation № 31 of 29 July 2004 on the maximum levels of pollutants in food.

Keywords: biomagnification, fish, macrophyte, rudd, trace metal

INTRODUCTION

The circulation of heavy metals in the lake ecosystem has not been a subject of detailed and systemic studies. There is evidence of heavy metal content in sediments from 5 monitoring points of BR “Srebarna” for the period 2004 – 2005. These analyzes were carried out in close connection with the monitoring program on the territory of the reserve. The only published data are those on the content of heavy metals in sediments of Srebarna Reserve and the Bulgarian section of the Danube River (Ricking and Terytze, 1999). The content of heavy metals in the major species macrophytes (*Tipha phragmites*) of Lake Srebarna was monitored by Yurukova and Kochev (1996). These studies indicated exceeding by minimum the admissible levels of macroelements that provokes and motivates once again the interest in studying their circulation in freshwater ecosystems of Srebarna reserve.

Aquatic macrophytes are good indicators of water quality because of their exceptional abilities to accumulate chemical elements as the increased accumulation of individual elements in plant tissues may be associated with increased concentrations in the aquatic environment (Yurukova and Kochev, 1996).

The studies of Pajević et al. (2003a, b) and Borišev et al. (2006) showed *Ceratophyllum demersum* as a macrophyte with a great potential for accumulation of Pb.

Arnaudova et al. (2008) studied the accumulation of Pb, Zn and Cd in three fish species perch, rudd and bleak from Studen Kladenets and Kyrdzhali dams. In the organs and tissues of the fish species *Alburnus alburnus, Scardinius erythrophtalmus* and *Perca fluviatilis* were observed high amounts of Pb, Zn and Cd, exceeding the limit concentrations for these metals. The accumulation of heavy metals varied among the different fish species. The highest content of the three metals was detected in *S. erythrophtalmus.*

Information on the fish fauna of Srebarna Lake can be found in publications since the beginning of the XX century (Antipa, 1909;

Ivanov, 1910; Kovatchev, 1922; Morov, 1931; Bacescu, 1942; Drensky, 1951). The first detailed study of the ichthyofauna of Srebarna Lake was made by Bulgurkov (1958), which reported 31 species of fish. Assay of the content of heavy metals (Cu, Pb, Zn, Cd, Mn, Co, Ni и Fe) in the silt layer in one meter depth (Hristova, 2000) showed no reasonable contamination of the lake with trace metals.

Comprehensive analysis of burden on the aquatic ecosystem hasn't been carried out. The aim of the current study was to determine the concentration of Pb in the dominant species of fish fauna and the presence of Pb in the elements of the nutrient spectrum of the studied species.

MATERIALS AND METHODS

Srebarna Biosphere Reserve is situated in Northeastern Bulgaria (44°7'N, 27°5'E) and it includes the Srebarna Lake (Figure 1). The Srebarna Lake is a freshwater eutrophic lake connected through an artificial canal with Danube River and is characterized by a significant diversity of highly protected species (Michev et al., 1998; Uzunov et al., 2001; Pehlivanov et al., 2006).

For the period of the present study seasonal changes of the water level have been established. High water levels have been constituted during the spring due to the influx of water from Danube River and lower water level – during the following months.

The model fish species chosen for this study was the herbivorus fish, rudd *S. erytrophtalamus* L., 1758. The rudd is one of the most abundant fish species in the Biosphere Reserve Srebarna (Pehlivanov, 2000; Pehlivanov et al., 2005). The fishes were caught by nets in the lake.

Figure 1. Srebarna Lake (source Map Google, 2009, with reductions)

Fish material, macrophytes, water and sediments were collected within 2010 year at three times during each of the spring (22 March-22 June), summer (22 June-22 September) and fall (September 22 to December 22). For the content of Pb 9 samples of *Ceratophyllum demersum* L., 8 samples of water and 8 samples of sediments were tested. A total 225 individuals of *S. erythrophthalmus* were examined (Table 1). The fish were weighed and measured. Samples of muscles, bones and skin were collected from all individuals.

Table 1. Characteristics of the fish studied - *Scardinius erythrophthalmus*

age	number of fish	Body weight (g)				Total length (cm)				Width (cm)			
		Range	Mean	SD	SE	Range	Mean	SD	SE	Range	Mean	SD	SE
1+	75	8.00-12.50	10.60	1.33	0.34	8.80-10.80	9.93	0.66	0.17	2.00-2.30	2.15	0.10	0.03
2+	75	16.50-38.00	24.93	6.36	1.64	9.70-12.90	11.97	0.94	0.24	2.90-4.10	3.41	0.28	0.07
3+	75	48.00-164.00	66.04	31.96	8.25	13.00-16.50	13.86	1.09	0.28	4.00-6.30	4.40	0.65	0.17

Determination of the age of the studied freshwater fishes was based on a standard method (Moyseev et al., 1975). The studied fish specimens were divided into 3 groups according to their age (Tables 1 and 2).

Each fish was determined to the species using the definers by Drensky (1951) and Karapetkova and Zhivkov (1995, 2000). For the systematic determination of fish species were used additional databases: GISD, ITIS, Fauna Europaea (http://www.faunaeur.org/), FishBase (http://www.fishbase.org/).

The fish weight was measured in grams and its dimensions in centimeters. For each studied

fish specimen were examined the following parameters (Zashev and Margaritov, 1966): 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.
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.
The water samples were collected and when necessary were preserved following the hereafter mentioned standards and regulations: BSS EN 25667-2:2000; ISO 5667-3:1994; ISO 5667-4:2002. Two-liter sealed containers were used and were delivered to the laboratory for chemical analysis. Each sample was recorded and numbered in advance. Samples that could not be tested the same day were stabilized or preserved by the addition of 5 ml nitrogen acid or 1 ml sulfur acid to every 1 l of water sample. For short period preservation samples were cooled down to 4°C.
The sediment samples were isolated with the use of Bottom Sediment Grab Sampler of Ekman. They were collected in accordance with the regulations of BSS EN ISO 5667-15:2009. The sediments were kept in a temperature of 4°C until they were analyzed.
Samples of tissues from the examined fish were deeply-frozen (-25^{o}C) and posterior processed for trace element analysis. Samples of muscles, bones and skin (1 g) were dried under temperature of 110^{o}C and mineralized in the combustion mixture consisted of perchloric and nitric acid in ratio 3:2 and then burned. The concentration of heavy metal Pb was established by atomic absorption spectrophotometry (Bíreš, at al., 1995) (Table 2). Samples were analyzed in the Laboratory of atomic absorption spectrophotometry at IBER on AAS "Perkin-Elmer" - 3030 B.
The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from macrophytes. A linear correlation coefficient, r_s was used to test the associations between sediments, water, fish tissues and macrophytes.
For the calculations and analysis were used MS Excel and BioDiversity Pro Softwares.

Table 2. Mean concentration of Pb (mg/kg) in muscles, bones and skin of *Scardinius erythrophthalmus* in age groups

age	number of fish	Muscles			Bones			Sкin		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
1+	75	1.09-10.42	5.31	3.34	1.21-13.12	7.15	3.47	1.51-5.94	3.64	1.62
2+	75	0.86-7.40	3.70	1.72	0.36-11.53	4.87	2.70	0.18-3.65	2.20	0.87
3+	75	0.76-7.43	3.94	2.50	1.43-9.21	4.53	2.42	1.01-10.10	3.98	3.04

RESULTS AND DISCUSSIONS

The content of Pb in water and sediments of the freshwater ecosystem of the Lake Sreberna was established (Table 3). The results obtained in the present study for the content of Pb in water (35.00 μg/l) were higher than the values obtained by Yurukova and Kochev (1996), Hiebaum et al., (2012) for Lake Srebarna.

Table 3. Mean concentration of Pb in water (μg/l) and sediment (mg/kg) from Lake Srebarna

Elements	Water (μg/l)	Sediments (mg/kg)
Pb	35.00	28.29

The high values of the studied elements in the water of Lake Srebarna were thought to be due to the passage of contaminated water from Danube River. The problem with the burden of the ecosystem could be related to the improper use of different fertilizers and pesticides as well as the improper agricultural activities in the surrounding land used for agricultural needs. The high concentrations of Pb in *C. demersum* showed that there was a chance that they were present there from the surrounding agricultural areas indicated by Pajević et al. (2003a).
The content of Pb in sediments exceeded the element content in the water of Lake Srebarna. The values obtained from previous studies (Yurukova and Kochev, 1996) were similar and slightly lower than those in the present study. When comparing the content of heavy metals in sediment samples from Lake Srebarna by Hiebaum et al. (2000) with the results of this

study it can be seen minor increase in the content of Pb over the years (Table 4).

Table 4. Mean concentration (mg/kg) of Pb in sediments from Lake Srebarna

	Year	Pb
1	1999	35.855
	2002	41.707
	2006	15.25
2	1999	20.716
	2002	26.213
	2006	17.817
3	1999	26.056
	2002	29.438
	2006	16.527
X	**2010**	**28.29**

Legend: 1-3 points of comparison from Hiebaum and etc. (2012); X – data from the present study.

In all researches regarding the content of elements in Lake Srebarna (Hiebaum et al., 2012; Ricking and Terytze, 1999, others) the presence of technogenic pollution of the upper sediment layer of the lake was highlighted. Similar results were presented by a number of authors who studied Danube's basin (Milenkovic et al., 2005; Vignati et al., 2013; Vítek et al., 2012, etc.).

The accumulation of Pb in *Ceratophyllum demersum* was highest in spring followed by autumn and summer, with typical similar mean values respectively 23.46 mg/kg, 21.93 mg/kg and 21.52 mg/kg (Figure 2).

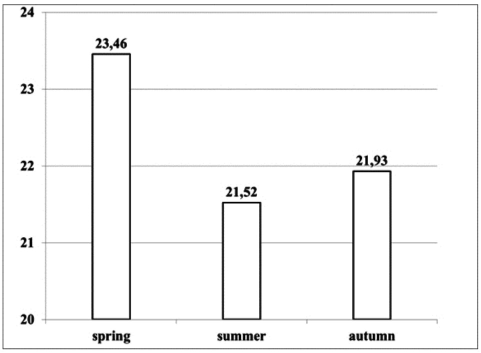

Figure 2. Seasonal variations in the concentration of Pb (mg/kg) in *Ceratophyllum demersum*

The mean values of concentration of Pb in *C. demersum* in this study (22.31 mg/kg) corresponds to the highest levels of accumulation of macrophyte survey of Predrag et al. (2006) (23.0 mg/kg) for chemical analysis of dominant aquatic macrophytes from DTD canal (Vlajkovac locality).

Studies on heavy metal content in macrophytes (Pajević et al., 2006) showed approximately 4-times lower levels of accumulation compared to the present study. The studies of Pajević et al. (2003a, b, 2005) and Borišev et al. (2006) showed the macrophyte *C. demersum* as a great potential for accumulation of Pb. The current study showed 4.5-times higher accumulation of Pb when compared to Borišev et al. (2006). The study of Jamnická et al. (2006) confirmed the high levels of accumulation of Pb in *Ceratophyllum demersum* determined in this study.

The content of Pb in *C. demersum* from Lake Srebarna were up to 8 times higher than the values obtained by Yurukova and Kochev (2000) at the same location.

Mean concentrations of Pb in *C. demersum* were very close to the studies of Branković et al. (2009) for the accumulation of heavy metals in different macrophytes of lake ecosystems.

Differences in the accumulation of Pb in the tissue of the *S. erythrophthalmus* were established. In general the accumulation of Pb in rudd followed the trend bones>muscles>skin (Figure 3).

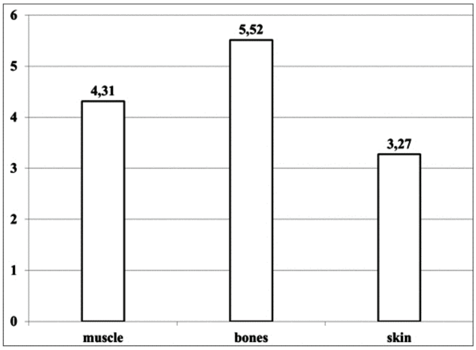

Figure 3. Mean concentration of Pb (mg/kg) in the tissues of *Scardinius erythrophthalmus*

In contrast to the results of the present study Arnaudova et al. (2008), Çiçek et al. (2009) and Sevcikova et al. (2013) established lowest concentrations of Pb in the muscles of *S. erythrophthalmus*. However, in the current study were found much higher mean values of Pb compared to study of Çiçek et al. (2009) (0.32 mg/kg).

We found that the accumulation of Pb differed in distinct age groups. Most Pb was accumulated in the youngest species (Figure 4), they were followed by 3+ and 2+ age groups where it was detected the lowest accumulation of Pb.

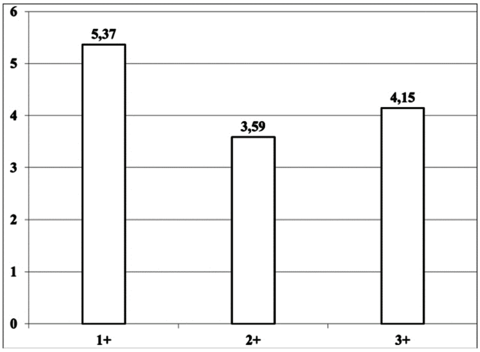

Figure 4. Mean concentration of Pb (mg/kg) at different age of *Scardinius erythrophthalmus*

The concentration of Pb in muscles varied between age groups. Age group 1+ had the highest measured values of accumulation. There was at least accumulation of Pb in age group 2+. The concentration of Pb in bones followed the same trend and it was highest in age group 1+, but the lowest concentration was in age group 3+ (the largest one). Overall, accumulation of Pb in bones was 2 times greater than in muscles (Table 2, Figure 5).

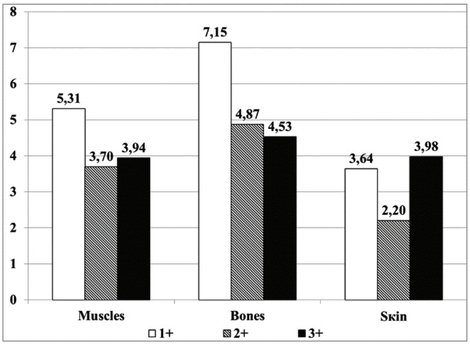

Figure 5. Mean concentrations of Pb (mg/kg) in tissues and organs in different age groups *S. erythrophthalmus*

Evaluation of the concentrations of Pb in different tissues and organs with respect to the age of the fishes showed that highest accumulation of Pb was in 1+ age group in the bones (7.15 mg/kg), followed by accumulation of Pb in 1+ age group in muscles (5.31 mg/kg). However, in the skin was detected higher level of Pb in 3+ age group than in 1+ age group.

Evaluation of the concentrations of Pb in the different tissues and organs with respect to the season showed that most Pb was accumulated in bones in spring (Figure 6). The second high value was again in bones but in autumn. In skin was detected the lowest content of Pb in autumn. The muscles accumulate Pb mostly in autumn.

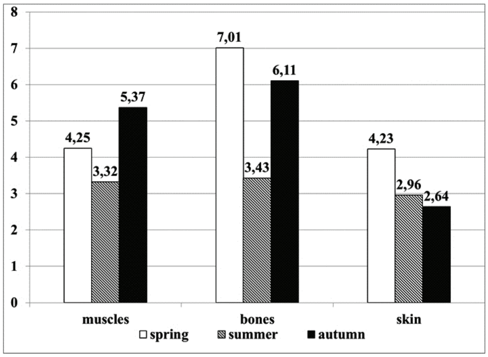

Figure 6. Seasonal variations in the concentration of Pb (mg/kg) in *Scardinius erythrophthalmus*

The concentration of Pb in *S. erythrophthalmus* from Biosphere Reserve Srebarna was higher than several other surveys on the accumulation of heavy metals in rudd (Kalyoncu et al., 2012; Özparlak et al., 2012).

Al Sayegh Petkovšek et al. (2012) who studied the accumulation of heavy metals in fish from the Šalek lakes, found repeatedly less accumulation of Pb in the muscle (0.02 mg/kg) compared to our study (4.31 mg/kg). The study of Sevcikova et al. (2013) also determined that the muscles accumulate in the least Pb to other organs, the average concentrations of Pb in muscles *S. erythrophthalmus* from Skalka reservoir are several times lower (0.05 mg/kg) than those of rudd from Srebarna .

The correlation (correlation coefficient of Spearman, r_s) between the content of Pb in the environment - water and sediments and dominant taxa of the biota of the lake ecosystem *S. erythrophthalmus* and *C. demersum*, as well as the factors of bioconcentration and biomagnification were all studied.

Positive correlations (r_s) to the content of Pb in macrophytes, water and sediments were found (Table 5).

Table 5. Correlation coefficient Spearman (r_s) and the levels of importance of the content of Pb in *S. erythrophthalmus, C. demersum,* water and sediments

x-y	r_s	p
S. erythrophthalmus - *C. demersum*	0.65***	<0.001
S. erythrophthalmus – water	0.21*	<0.05
S. erythrophthalmus – sediments	0.12*	<0.05
C. demersum – water	0.47**	<0.01
C. demersum - sediments	0.22*	<0.05

Note: *significant correlation p<0.5;
**highly significant correlation p<0.01;
***very significant correlation p<0.001;
^{ns} non-significant correlation p>0.05.

The calculated values of bioconcentration factor (BCF) in *S. erythrophthalmus* and *C. demersum* were high compared with those for water (Table 6).

Table 6. Values of bioconcentration factor (BCF=C tissue /C water, sediments) for Pb to *S. erythrophthalmus* and *C. demersum* compared to their content in the water and sediment

BCF	Water	Sediments
S. erythrophthalmus	124.00	0.15
C. demersum	637.14	0.79

The mean values of BMF were the highest for bones and range as follows BMFPbbones>> BMFPbmuscle >>BMFPbskin (Table 7).

Table 7. Mean values of Biomagnification factor (BMF=C host tissues/C macrophytes) of Pb in *S. erythrophthalmus* compared to *C. demersum.*

Srebarna Lake	Fish tissues	BMF Pb
Scardinius erythrophthalmus	muscle	0.194
	bones	0.248
	skin	0.147

CONCLUSIONS

Increase of the content of Pb in sediments compared to previous studies of Lake Srebarna was found. The content of Pb in waters was up to 10 times higher than previous studies of the lake ecosystem. Seasonal fluctuations in the content of Pb in *Ceratophyllum demersum*, related to vegetation and development of plants were found. High values of BCF in *C. demersum* compared to the water and sediments were due to the high sensitivity of the species to Pb in that area. *Ceratophyllum demersum* is a reliable biomarker for monitoring the degree of contamination of aquatic ecosystems with Pb. The data obtained testify to the application of the studied macrophytes as a good indicator for biomonitoring of pollution with Pb.

Lake ecosystem is loaded with high content of Pb, caused by technogenic pollution of the upper layer of the lake sediment mainly from waters entering from the Danube River.

The mean concentration of Pb in the muscles of *Scardinius erythrophthalmus* was established to greatly exceed the reference values for lead in fish meat from Regulation № 31 of 29 July 2004 on the maximum levels of pollutants in food.

The ways in which Pb enters in *S. erythrophthalmus* are from the food sources, water and sediment.

Considering our results from the study of accumulation of Pb in *Scardinius erythrophthalmus* and *Ceratophyllum demersum* in Srebarna Biosphere Reserve we can conclude that the lake is burdened with heavy metals compared to other freshwater ecosystem.

ACKNOWLEDGMENTS

The author would like to thank Prof. B. Georgiev and Prof. L. Pehlivanov (Institute of Biodiversity and Ecosystem Research (IBER) at the Bulgarian Academy of Sciences) for their useful guidance during this study and to thank R. Hristova (Institute of Biodiversity and Ecosystem Research (IBER), at the Bulgarian Academy of Sciences) for her technical help for chemical analyses. The study was financed by PhD thesis №D65/01.03.2009 from the Agricultural University – Plovdiv and the Ministry of Education, Youth and Science, Bulgaria and bilateral Slovak-Bulgarian scientific project BG-SK01-4/2011.

REFERENCES

Al Sayegh Petkovšek S., Mazej Grudnik Z., Pokorny B., 2012. Heavy metals and arsenic concentrations in ten fish species from the Šalek lakes (Slovenia): assessment of potential human health risk due to fish consumption. Environ Monit Assess. 2012 May,184(5), p.2647-2662.

Antipa Gr., 1909. Fauna ichthyologica a Rominiei. Bucuresti, Publ. fond. Adamachi, p.294.
Arnaudova D., Tomova E., Velcheva I., Arnaudov A., 2008. A study on the Lead, Zink and Cadmium content in various organs in fishes from Cyprinidae and Percidae families in "Studen Kladenets" and "Kardzhali" dam lakes. Proceedings of the anniversary scientific conference of ecology Eds. Iliana G. Velcheva, Angel G. Tsekov. Plovdiv, November 1st 2008, p. 327-335.
Bacescu M., 1942. Eupomotis gibbosus L. Studiu entozoologie, zoogeographie si morphologie. Ann. Ac. Rom., Mem. Sect. Stintifice Acad. Rom., ser. VI, T. XVII, p.547-560.
Bíreš J., Dianovský J., Bartko P., Juhásová Z., 1995. Effects on enzymes and the genetiv apparatus of sheep after administration of samples from industrial emissions. BioMetals, 8, p.53-58.
Borišević M., Pajević S., Stanković Ž., Krstić B., 2006. Macrophytes as phytoindicators and potential phytoremediators in aquatic ecosystems. In: Proceedings 36th International Conference of IAD. Austrian Committee Danube Research / IAD, Vienna. ISBN 13: 978-3-9500723-2-7, p.76-80.
Branković S., Pavlović-Muratspahić D., Topizović M., Milivojević J., 2009. Concentration of metals (Fe, Mn, Cu and Pb) in some aquatic macrophytes of lakes Gruža, Grošnica, Memorial Park-Kragujevac and Bubanj. Kragujevac J. Sci. 31, p.91-101.
Bulgurkov K., 1958. Hydrological peculiarities of the Srebarna Reserve and composition of its fish fauna. Izvestiya na Zoologicheskiya institut s Muzey 7: 252-263
Çiçek A., Emiroğlu Ö., Arslan N., 2009. Heavy Metal Concentration in Fish of Lake Manyas. Proceedings of 13th Conference, Wuhan, Hubei Province, China - World Lake Conference, http://wldb.ilec.or.jp/data/ilec/WLC13_Papers/others/18.pdf (10 May, 2013 date last accessed).
Drensky P., 1951. The fishes of Bulgaria. Fauna of Bulgaria, Volume 2, p.268.
EN 14011, 2003. Water quality. Sampling of fish with electricity.
Fauna Europaea, http://www.faunaeur.org/.
FishBase, http://www.fishbase.org.
GISD, 2006. Global Invasive Species database.
Hiebaum G., Mitchev T., Vassilev V., Uzunov Y., 2000. Management Plan for Biosphere Reserve Silver. Academy of Sciences, Central Laboratory of General Ecology, Sofia, 157.
Hristova R., 2000. Water quality: Hiebaum, G., T. Mitchev, V. Vassilev, Y. Uzunov (eds.) 2000. Management plan for the biosphere reserve "Srebarna" CLGE-BAS, Sofia, 11.
BSS EN 25667-2:2000. Water quality - Sampling - Part 2: Guidance on sampling techniques (ISO 5667-2:1991).
ISO 5667-3, 1994. Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.
ISO 5667-4:2002. Water quality — Sampling — Part 4: Guidance on sampling from lakes and reservoirs.
BSS EN ISO 5667-15:2009. Water quality – Sampling - Guidance on the preservation and handling of sludge and sediment samples.
ITIS – (Integrated Taxonomic Information System), 2005. Online Database, http://www.itis.gov/.
Ivanov D., 1910. Our fishing in the Danube and its marshes. A Russe's Chamber of commerce, Russe, 32.
Jamnická G., Hrivnák R., Oťaheľová H., Skoršepa M., Valachovič M., 2006. Heavy metals content in aquatic plant species from some aquatic biotopes in Slovakia. In: Proceedings 36th International Conference of IAD. Austrian Committee Danube Research / IAD, Vienna. ISBN 13: 978-3-9500723-2-7, p.366-370.
Kalyoncu L., Kalyoncu H., Arslan G., 2012. Determination of heavy metals and metals levels in five fish species from Işıklı Dam Lake and Karacaören Dam Lake (Turkey). Environmental Monitoring & Assessment; Vol. 184 Issue 4, p.22-31.
Karapetkova M., Zhivkov M., 1995. The Fishes of Bulgaria. Sofia, Gea Libris Publ. House - Nature, BAS.
Karapetkova M., Zhivkov M., 2000. The Fishes of Bulgaria. Sofia, Gea Libris.
Kir I., Tekin-Ozan S., Barlas M., 2006. Heavy metal concentrations in organs of rudd, *Scardinus erythrophthalmus* L., 1758. Fresenius environmental bulletin. Volume 15, p.25-29.
Kovatchev V., 1922. Fauna of freshwater fishes of Bulgaria. Archives du Ministere de l'Agriculture 3, p.163
McAleece N., Gage J., Lambshead P., Paterson G., 1997. BioDiversity Professional statistics analysis software.
Michev T., Georgiev B., Petrova A., Stoyneva M., 1998. Biodiversity of the Srebarna Biosphere Reserve, Checklist and Biblography. Sofia: Co-published by Context & Pensoft, ivx + 130.
Microsoft, 2010. Microsoft Excel [computer software]. Redmond, Washington: Microsoft.
Milenkovic, N., Damjanovic M., Ristic M., 2005. Study of Heavy Metal Pollution in Sediments from the Iron Gate (Danube River), Serbia and Montenegro. Polish Journal of Environmental Studies;2005, Vol. 14 Issue 6, p.781-787.
Morov T., 1931. The Freshwater Fishes in Bulgaria. Union of Hunters and Fishermen of Bulgaria, Sofia, 93.
Moyseev P., Vasilkin A. and Kuranova I., 1975. Ihtiologia i ribovadstvo. M., Pishtevaya promishlenost., p.279.
Özparlak H., Arslan G., Arslan E., 2012. Determination of Some Metal Levels in Muscle Tissue of Nine Fish Species from Beyşehir Lake, Turkey. Turkish Journal of Fisheries and Aquatic Sciences 12, p.761-770.
Pajević S., Vučković M., Kevrešan Ž., Matavulj M., Radulović S., Radnović D., 2003a. Aquatic macrophytes as indicators of heavy metal pollution of water in DTD canal system. Zbornik Matice Srpske za Prirodne Nauke. Proceedings for Natural Sciences, Matica Srpska, Novi Sad, № 104, p.51-60.
Pajević S., Kevrešan Ž., Radulović S., Radnović D., Vučković M., Matavulj M., 2003b. Aquatic macrophytes – role in monitoring and remediation of nutrients and heavy metals. ISIRR 2003. Section III. Hunedoara, Romania, p.95-100.
Pajević S., Igić R., Krstić B., Vukov D., Borišev M., Nikolić N., 2005. Chemical compositions of aquatic macrophytes from the Danube – role in biomonitoring and bioremediation. Proceedings of the Balkan scientific conference of biology in Plovdiv (Bulgaria) from 19th till 21st of May 2005 (eds B. Gruev, M. Nikolova and A. Donev), p.485–498.

Pajević S., Matavulj M., Borišev M., Ilić P., Krstić B., 2006. Macrophytic nutrient and heavy metal accumulation ability as a parameter of pollutant remediation in aquatic ecosystems. Proceedings 36th International Conference of IAD. Austrian Committee Danube Research/IAD, Vienna. ISBN 13: 978-3-9500723-2-7, p.382-387.

Pehlivanov L., 2000. Ichthyofauna in the Srebarna Lake, the Danube Basin: state and significance of the management and conservation strategies of this wetland. Internet Assoc. Danube Res. 33, p. 317 – 322.

Pehlivanov L., Vassilev V., Vassilev M., 2005. Changes of ichthyofauna of Srebarna Lake during the pastsixty years. In Proceedings of First National Scientific Conference of Ecology, Sofia, p.265 – 270.

Pehlivanov L., Tzavkova V., Vassilev V., 2006. Development of the zooplankton community in the Srebarna Lake (north-eastern Bulgaria) along the process of ecosystem rehabilitation. Proceedings of the 36th International Conference of IAD, Vienna, p.280-284.

Predrag I., Slobodanka P., Sanja T., 2006. The content of heavy metals in tissue of *Ceratophyllum demersum* L. from Danube-Tisza-Danube canal in Banat region of Vojvodina (Serbia and Montenegro). 36th International Conference of IAD, Vienna, p.362-365.

Regulation № 31/29.07.2004. The maximum levels of pollutants in food. Issued by Ministry of health.

Ricking M., Terytze K., 1999. Trace metals and organic compounds in sediment samples from the River Danube in Russe and Lake Srebarna (Bulgaria). Environmental Geology 37 (1-2), p. 40-46.

Sevcikova M., Modra H., Kruzikova K., Zitka O., Hynek D., Adam V., Celechovska O., Kizek R., Svobodova Z., 2013. Effect of metals on metallothionein content in fish from Skalka and Želivka Reservoirs. International Journal of Electrochemical Science, Vol. 8 Issue 2, p.1650-1663.

Uzunov Y., Tzavkova V., Todorov I., Varadinova E., 2001. The macrozoobenthic fauna of the Biosphere reserve Srebarna Lake in North-Eastern Bulgaria. Lauterbornia, 40, p.43-53.

Velcheva I., 1998. Ecological study of cadmium (Cd), lead (Pb), and zinc (Zn) loads of bleak (*Alburnus alburnus* L), common carp (*Cyprinus carpio* L.) and perch (*Perca fluviatilis* L.) populating the "Kardjali" and the "Studen Kladenetz" dam lakes. Author's Summary of a Ph.D. dissertation. Plovdiv University.

Vignati D., Secrieru D., Bogatova Y., Berlinsky N., Stanica A., Céréghino R., 2013. Trace element contamination in the arms of the Danube Delta (Romania/Ukraine): Current state of knowledge and future needs. Journal of Environmental Management 125, p.169-178.

Vítek T., Hedbávný J., Mareš J., Spurný P., 2012. Trace metal contamination of the Dyje river stretch between Znojmo and Nové Mlýny, Czech republic. Acta universitatis agriculturae et silviculturae mendelianae brunensis. Volume LX, p.217 - 224.

Yurukova L., Kochev H., 1996. Heavy metal concentrations in main macrophytes from the Srebarna Lake along the Danube - Bulgaria: 31. Konferenz der IAD, Baja-Ungarn, Wissenschaftliche Referate, Limnologishe Berichte Donau, Band I, p.195-200.

Yurukova L., Kochev H., 2000. Element accumulation in macrophytes from Bulgarian swamps along the Danube. - In: Horvatić, J. (ed.), Proceedings of the 33rd Conference of IAD (International Association for Danube Research), Osijek, Croatia, 33, p. 409-413.

Zashev, Margaritov, 1966. Fish diseases. Nauka i izkustvo, Sofia.