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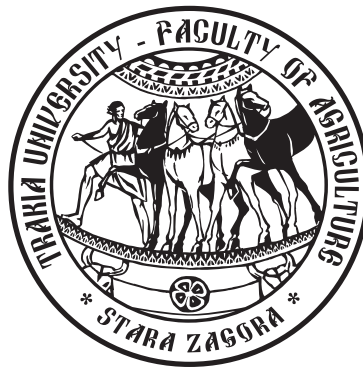
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Heavy metals in organs of gudgeon (*Gobio gobio* L.) from Vardar River, R. Macedonia

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Abstract. Ecotoxicological examinations based on seven heavy metals (Fe, Cu, Zn, Mn, Cd, Pb and Cr) concentrations in organs of gudgeon (*Gobio gobio* L.) caught by 14 monitoring points at Vardar River in Republic of Macedonia were performed. The organ's samples elements were read-out on AAS in flame and graphite cuvette. Significant increase of Pb and Cd contents in liver, gills and gonads of fish sampled downstream Skopje and the town of Veles was found. It should be pointed out that at times Cd concentrations in muscles were as high as 0.518 mg/kg which is significantly over the maximally allowed concentrations for human nutrition.

Keywords: *Gobio gobio* L., organs, heavy metals concentration

Introduction

Freshwater fish fauna biodiversity can be viewed through the vast variety of the wildlife on the Balkan Peninsula. Wildlife communities in this area have constantly been exposed to various geomorphological, climatic and anthropogenic influences. This fact has preconditioned their biodiversity. For preservation of fish species on an international level, it is a must to get insights into all the changes in the aquatic communities in the Republic of Macedonia and on the whole territory of the Balkan Peninsula.

Vardar is the biggest Macedonian river, where all the waterflows in the country flow in, except for the rivers Drim and Strumica. The first data on the river Vardar ichthyofauna was reported by Steindachner (1892) who described *Leucos macedonicus* from the Vardar River basin by the samples from Doiran Lake. Doflein (1921), in his work covering the results of the Macedonian commission surveys, quotes 11 fish species in Vardar River.

Vardar River as part of the Balkan hydroecosystems, isn't sufficiently researched from the aspect of ichthyodiversity and ecotoxicology at the ichthyofauna level. It ought to be especially mentioned that on the area of the Western Balkans many endemic species have survived, being among the eldest in Europe. Since these species are endangered, and the ichthyofauna in general, special attention to such types of research was paid in the works of Antipa (1909), Taler (1954), Drecun (1956, 1957, 1962); Janković (1965, 1977, 1978, 1979, 1980), Janković et al. (1987, 1994), Ivanović (1973), Hacker (1983), Knežević and Marić (1987), Maletin et al. (1990, 1994) and Pujin et al. (1996).

Their own contributions to the insights about the fishes in Vardar River, gave Karaman (1962), Petrovski et al. (1970) and Dimovski and Grupče (1971, 1971a, 1971b, 1972). According to their investigations, the river is inhabited by approximately 24 fish species.

The ecosystem's biodiversity includes the variety of biocenoses, habitats and ecological processes, upon which an ecosystem's functionality can be defined (Đukić et al., 1993, 1994, 1996, 1997, 1998; Maletin et al., 1994, 1997, 1998; Maletin and

Đukić, 1998). Fish fauna diversity in the upper flow of the Vardar River is still well preserved, though certain sectors of this hydroecosystem are subject to drastic changes, which presents a serious threat for the ichthyofauna structure. Some fish species in that river (*Leuciscus cephalus* L., *Barbus barbus* L., *Barbus meridionalis* Risso, *Vimba vimba* L.) are reliable bioindicators of the respective ecological conditions and their changes occurred within a shorter or longer period of time (Nastova, 2004; Nastova-Giorgioska et al., 2006).

Ichthyofauna is a part of the aquatic wildlife community and is an important component in the food chain. It has active participation in the process of self-filtration of the hydroecosystems (Đukić et al., 1991, 1992; Maletin et al., 1991, 1992, 1995; Jordanovski and Naumovski, 1998; Talevska, 1998; Talevski and Spirkovski, 1998).

Pollution of waterflows with bio-non-degradable substances is a complex problem the solving of which requires a comprehensive approach. In the last decade, special attention has been paid to studying the load with toxic and hazardous substances, one of which are the micropollutants. Biological parameters are some of the most reliable indicators for estimating the state of a hydroecosystem, for they are not only a reflection of the current quality of the environment, but also give a picture for a long period of time. Fish population is of exceptional ecological and economic importance. It is a certain fact that fish are members of the highest trophic levels of biocenosis and they are a reflection of the hydroecosystem metabolism. From the aspect of the quality of nutrition and protection of human health, a very important factor is the content of hazardous and toxic substances in the muscle tissues of fishes (Foerstner and Wittman, 1981; Petrović et al., 1987; Wachs, 1990, 1991; Yevtushenko and Sytnik, 1989; Pujin et al., 1990; Sytnik et al., 1991; Salanki et al., 1992; Maletin et al., 1992, 1996; Janković and Jovičić, 1994; Đukić et al., 1998; Teodorović, 1999).

According to the OECD recommendation of 1991 (State of the Environment, 1990 – quote: Jorgensen et al., 1994), heavy metal concentrations in fish tissues can be a useful indicator for the load of these pollutants in water. The implementation of the ichthyofauna in the biomonitoring process and the analyses of the trends in the hydroecosystems are also recommended by HELCOM (Helsinki

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Commission) and OSPARCOM (Oslo and Paris Commission, 1990 – quote: Jorgensen et al., 1994).

Heavy metals have negative impact on the metabolism, physiology and dynamics of fish population through the food chain in the water and may threaten human health. In this process, the most relevant for human health are the concentrations of heavy metals in the muscles, since they are used for nutrition of people. Chevreuril et al. (1995) think that, despite the high toxicity and the persistence of organic and metallic micropollutants, investigations and data about the levels of contamination of continental aquatic systems are relatively few. Determination of the contents of pollutant residuals in the tissues of aquatic organisms is used, but it hasn't been implemented widely yet within systematic biomonitoring of freshwater ecosystems, nor there are standardized methods and types for this kind of monitoring of pollution. The aforementioned authors think that fish, because of their ecological characteristics (lifetime, way of nutrition, distribution) and economic effects, consist of a group which may be a reliable parameter in monitoring aquatic ecosystems.

Gudgeon (*Gobio gobio* L.), a fish which inhabits the Vardar River in the Republic of Macedonia has not been studied enough, including the accumulation of heavy metals in the tissues and organs. Karaman (1924) found gudgeon in the smaller confluents of the Vardar River near Skopje, and later (1931) he denoted gudgeon as a frequent fish species in the running waters of Skopje round valley. It is important to know whether this fish can be used as a bioindicator for assessing the ecological status of different river zones.

Gudgeon lives in small shoals at the bottom, where it finds food. It inhabits clean waters, with good flow and sandy to clay bottom, as well as in low-flux river branches. It also lives in clean lakes. The fish spawns in portions, from May to August, on sandy or gravel substrates. It matures sexually between the second and fourth year of life, when its length is 6 to 8cm. The fertility is 1.000 to 3.000 eggs, which have a diameter of around 2mm. The hard roe is sticky, and the development of the embryos takes 7 to 10 days. The larvae and the brood feed with tiny invertebrates, while the adult units feed with larvae of chironomids, tiny molluscs, roe from other fishes, and vegetation. Gudgeon can grow up to 22cm in length and 80g in weight (Naumovski, 1995).

The aim of the study was to make an ecotoxicological examinations of the Vardar River in the Republic of Macedonia based on seven heavy metals (Fe, Cu, Zn, Mn, Cd, Pb and Cr) concentrations in gudgeon's (*Gobio gobio* L.) organs.

Material and methods

The object of the study was gudgeon (*Gobio gobio* L.) from the Vardar River, Republic of Macedonia during the period from 2014 to 2015. For the purpose of the investigation the species *Gobio gobio* L. (gudgeon) was captured in 14 of all the 16 locations (Monitoring points - MPs) of the Vardar River (Table 1, Figure 1). Samples from the species (n=376) were analyzed from each monitoring point. Five specimens of gudgeon were analyzed from each monitoring point (n=5).

Samples of muscles, gills, liver and gonads from gudgeon (*Gobio gobio* L.) had been prepared using standard procedure – by drying at 105°C and dissolving in a mixture of three acids: HNO₃, H₂SO₄ and HClO₄ (Allen et al., 1974). The samples were read out on AAS in flame and graphite cuvette. The contents of 7 metals were

Table 1. Monitoring points at the Vardar River and number of captured specimens of *Gobio gobio* Linnaeus 1758

Locations	Number of captured specimens	%
Volkovija	52	13.83
Siricino	45	11.97
Radusa	54	14.36
Skopje	45	11.97
Aerodrom	15	3.99
Trubarevo	25	6.65
Taor I	18	4.79
Basino Selo	25	6.65
HIV	15	3.99
Nogaevci	40	10.64
Pepeliste	21	5.59
Demir Kapija	9	2.39
Miravci	5	1.33
Gevgelija	7	1.86
Total	376	100.00



Figure 1. Map of Vardar River valley with monitoring points

analysed, namely: Zn, Mn, Fe, Cu, Cr, Cd, Pb. The concentrations of metals in fish tissues and organs are expressed in mg/kg fresh mass, for compatibility with the legal regulations regarding micropollutant contents in fishes.

A complete statistical processing was performed using standard software packages: Microsoft Excel and Statistics. For establishing the distribution of heavy metals and for comparing two different locations based on the contents of metals, standard statistical methods were used:

- all the results presented are given with the arithmetic mean value (x) and standard deviation (SD).
- the significance of the difference between the concentrations of accumulated metals in individual tissues and the analysis of the variance was performed using one-way ANOVA test (Kruskal-Wallis).
- the significance of the difference between both locations was established using one-way ANOVA (Kruskal-Wallis) test, and was checked using an independent t-test.
- in all the parametric tests, a significance of the difference was allocated to p≤0.05.
- for the graphic imagery a Box-Whisker's diagram was

implemented, where, beside the arithmetic mean values, variability is also presented.

Results and discussion

The results obtained for the studied heavy metals concentrations in tissues and organs of the gudgeon (*Gobio gobio* L.) from different MPs at the Vardar River revealed the following picture:

Fe. Fe concentration values in the organs of *Gobio gobio* L. units are shown in Figures 2 and 3. There the arithmetic mean values of the samples, the corresponding standard deviations, coefficients of variations and the results of t-test and F-test are given. The maximum value for Fe concentration in gudgeon's liver in the comparison between samples from the upper and the lower parts of the Vardar River, is 315mg/kg. From the Box-Whiskers's diagram it can be seen that Fe is mostly accumulated in liver. According to t-test, the concentrations measured in liver are very significant (-3.98**).

The values for Fe concentrations in muscles from the upper and the lower part of the Vardar, are 2.28*. In Figure 3 it can be seen that

there is an overlap of the intervals, and the difference between the environments surveyed is at the threshold of the significance. By comparison of Fe concentrations in gills and gonads of dudgeon from the upper and the lower part of the river, differences have been found, which are not statistically significant.

Cu. In Figure 4 it can be seen that, according to the analysed results, there is a difference in Cu concentrations in gudgeon's organs between the units (MPs) from the upper and the lower part of the river. So, the highest maximum value for Cu in muscles is 10.5mg/kg. According to the t-test, the highest Cu concentrations in muscles are -4.21**, and the differences are statistically very significant. In gonads, liver and gills, no statistically significant differences in Cu concentrations have been measured.

Mn. The data from the monitoring of Mn concentrations in gudgeon samples from the upper and the lower part of the river are shown in Figure 5. The statistically processed data show that concentration of manganese is the highest in liver, where the difference is statistically very significant (-5.31**). In all the other organs there is no statistically significant difference in Mn concentrations for the units from both parts of the river.

Pb. The high load with Pb of Vardar's water downstream the town of Veles, is another indicator that in this part of the river there is

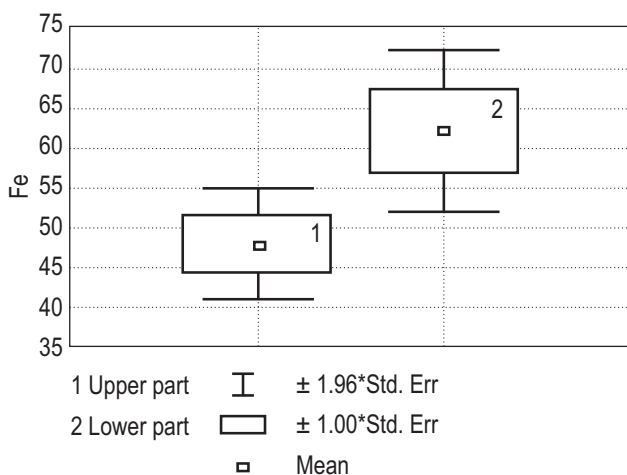


Figure 2. Fe concentrations in liver of *Gobio gobio* L. from the upper and the lower part of the Vardar River

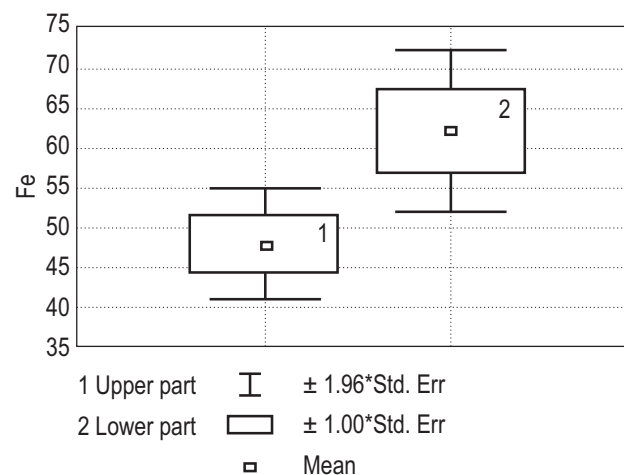


Figure 3. Fe concentrations in muscles of *Gobio gobio* L. from the upper and the lower part of the Vardar River

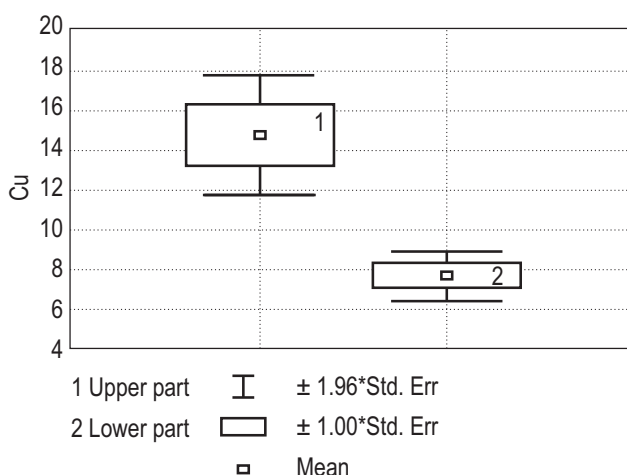


Figure 4. Cu concentrations in muscles of *Gobio gobio* L. from the upper and the lower part of the Vardar River

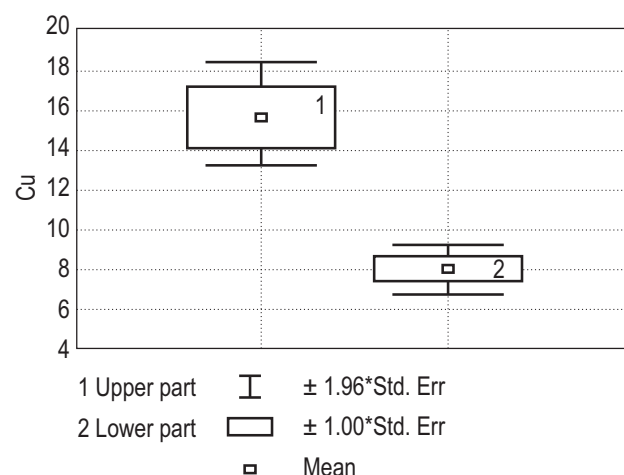


Figure 5. Mn concentrations in liver of *Gobio gobio* L. from the upper and the lower part of the Vardar River

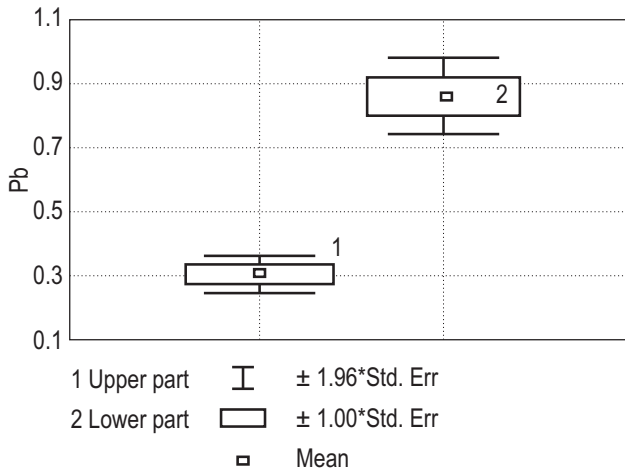


Figure 6. Pb concentrations in liver of *Gobio gobio* L. from the upper and the lower part of the Vardar River

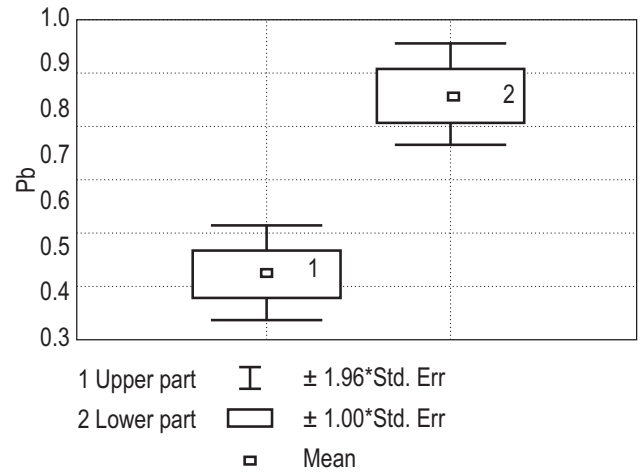


Figure 7. Pb concentrations in gills of *Gobio gobio* L. from the upper and the lower part of the Vardar River

presence of heavy metals. Effluents flow into this part of the Vardar River, originating from the industrial plants in the town. The highest value of the difference between Pb concentrations from the upper and the lower part of the Vardar River, is 1.18mg/kg. From Figure 6 it can be seen that Pb concentration is the highest in gudgeon's liver, and amounts to 8.29**. In the diagram it can be seen that there is no overlap of intervals, meaning that the difference between the river parts surveyed is very significant. A statistically very significant Pb concentration is present in the gills, too (5.08**). This is clearly shown in Figure 7.

Cd. The analysis of Cd concentrations in particular tissues of gudgeon samples from the upper and the lower part of the Vardar River, is shown in Figures 8, 9, 10 and 11. The highest Cd concentrations in gudgeon's liver from the lower part of the Vardar were 1.87mg/kg (Figure 8). By comparison of Cd concentrations in the samples from the upper and the lower part of the river using t-test, it was found that Cd is mostly accumulated in liver (26.59**). The difference is very significant. In gudgeon units from the lower part, maximum Cd concentration in gills was 1.56mg/kg, and 0.16mg/kg in gonads (Figures 9 and 10). The values from the t-test for Cd concentrations in gills were 7.40** in gills and 4.45** in gonads. This means that there is a very significant difference

between the amounts of accumulated Cd in the gills and the gonads of gudgeon units from both parts (Figure 10). However, by comparison of Cd concentrations in muscles which are greatly less than those in liver, gonads and gills, values have been obtained indicating that the differences in this respect are not statistically proven (Figure 11).

Cr. In Figures 12, 13, 14 and 15, the intervals of Box-Whisker's diagrams are far from one another. This means that the differences between Cr concentrations in liver (Figure 12), muscles (Figure 13) and gonads (Figure 14) of gudgeons from the upper and the lower part of the Vardar River are statistically very significant. Only in Figure 15 there is some overlap of the intervals, and this is for concentrations of chromium in gills. Independent t-test between the samples from both parts has shown that the values for Cr in the gills are at the threshold of significance, and are equal to 2.36*. The values for Cr concentrations in liver are 28.22**, in muscles – 4.88**, and in gonads – 7.33**.

The results obtained indicate that in gudgeons from both upper and lower parts of the Vardar, the liver is the end organ of accumulation of all the examined metals, especially Pb and Cd which in high concentrations accumulate in the gills too. Unlike the liver, muscles are tissues with very low contents of the examined

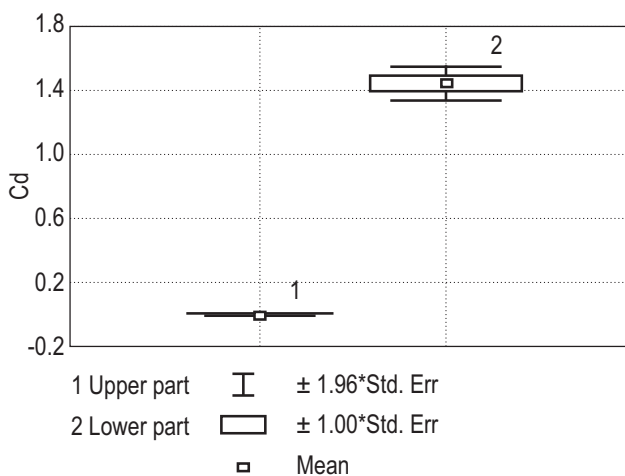


Figure 8. Cd concentrations in liver of *Gobio gobio* L. from the upper and the lower part of the Vardar River

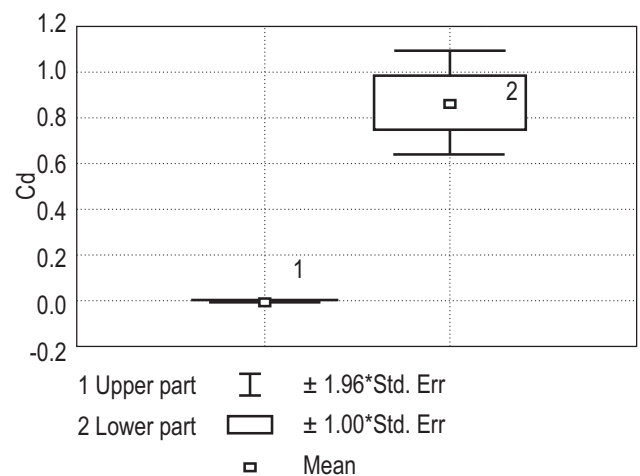


Figure 9. Cd concentrations in gills of *Gobio gobio* L. from the upper and the lower part of the Vardar River



Figure 10. Cd concentrations in gonads of *Gobio gobio* L. from the upper and the lower part of the Vardar River

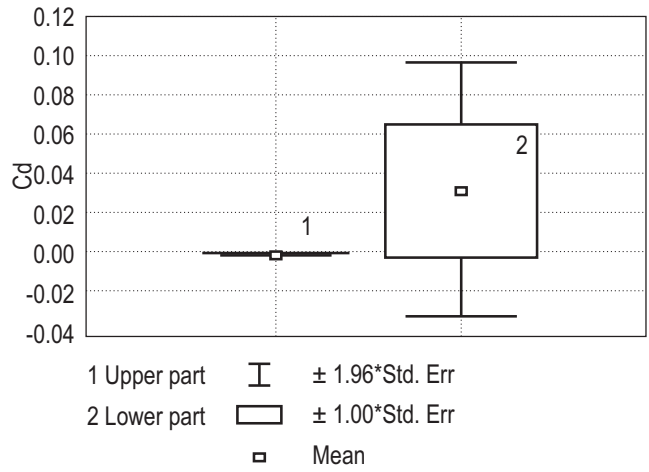


Figure 11. Cd concentrations in muscles of *Gobio gobio* L. from the upper and the lower part of the Vardar River

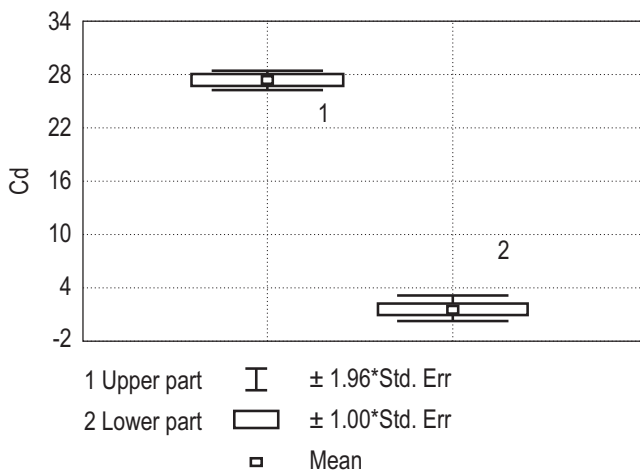


Figure 12. Cr concentrations in the liver of *Gobio gobio* L. from the upper and the lower part of the Vardar River

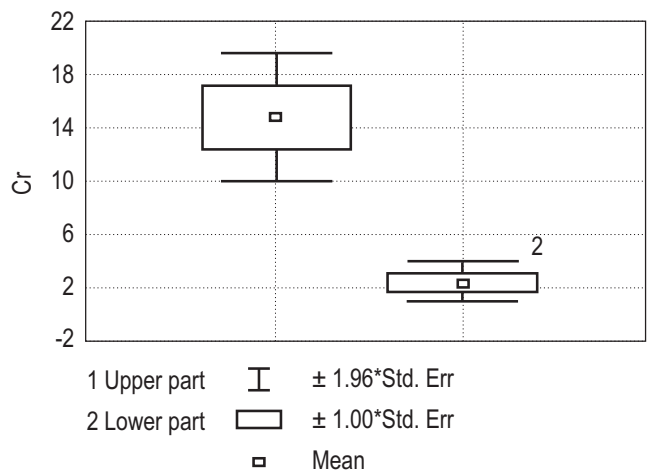


Figure 13. Cr concentrations in muscles of *Gobio gobio* L. from the upper and the lower part of the Vardar River

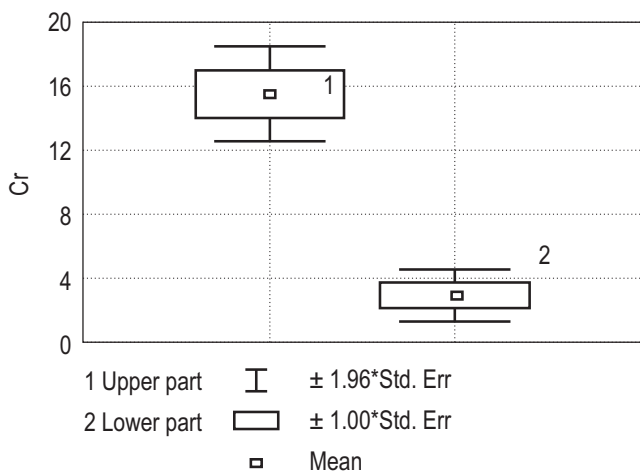


Figure 14. Cr concentrations in gonads of *Gobio gobio* L. from the upper and the lower part of the Vardar River

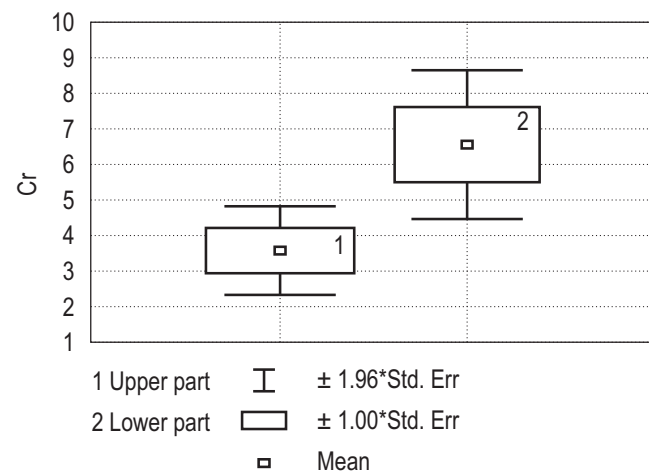


Figure 15. Cr concentrations in gills of *Gobio gobio* L. from the upper and the lower part of the Vardar River

metals, which is in line with the results of many authors who were studying the issue of their distribution in tissues. According to Wickund et al. (1988), cadmium accumulates in liver and in kidneys, and can be eliminated very slowly, while the zinc, present in the water, even increases its period of retention. The authors came to

the conclusion that in waters loaded with zinc and cadmium, increased concentrations of zinc may lead to an increase of accumulation of cadmium in liver and in kidneys. One of the more interesting explanations for the significantly lower Cd and Pb accumulations in the muscles compared to those in kidneys and

liver, is given by Allen-Gill and Martynov (1995). Although without any physiological function, the organisms retain both these metals through specific metal-binding proteins. Namely, in kidneys and in liver the concentrations of metalloprotein and lead-binding proteins are significantly higher than in muscles, so it is logical the total Pb and Cd contents in these tissues to be higher.

Pujin et al. (1990) found that in Planktivora and Bentivora the muscles are the tissues with the lowest accumulations of Zn, Mn, Fe, Cu, Cr, Co, Ni, Pb, Cd, Al and Sr, while Maletin et al. (1996) spread this claim to all other trophic categories of fish. Đukić et al. (1998a, b), demonstrated that the accumulated amounts of all these metals are statistically quite low in muscles, and the highest in liver and kidneys. According to the investigations performed by Salanki et al. (1982), the concentrations of all the metals (Hg, Cd, Pb, Zn, Fe, Cu and Mn) were the lowest in the muscles and the highest in the kidneys of both bentivore and piscivore species from Lake Balaton.

Conclusion

Ecotoxicological examinations were performed based on studying the presence of seven heavy metals (Fe, Cu, Zn, Mn, Cd, Pb and Cr) in gudgeon's (*Gobio gobio* L.) organs. Increased concentrations of these hazardous substances, especially Pb and Cd, in water and sediment from the lower part of the Vardar River, influenced higher accumulations of metals in liver, gills and gonads of *Gobio gobio* L. With the implementation of t-test, very significant differences in this respect were found between both surveyed parts of the river (the upper part and the lower part). It was found that the water of the Vardar River downstream the town of Veles is under permanent influence of the effluents which have very unfavourable impact on the wildlife of this ecosystem. These are the reasons for which it is necessary, based on the results obtained, to undertake steps for wastewater treatment, so the river would be able to recuperate and serve its purpose again. Simultaneously with the introduction to wastewater treatment plants, further monitoring of water quality is necessary if we want to achieve the goal of preservation and improvement of this hydroecosystem.

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Genetics and Breeding

- Variation in the agronomic and morphological traits in spring barley** 263
N. Dyulgerov, B. Dyulgerova
- Study on the loss of accuracy of AC method for milk yield control in sheep** 268
D. Dimov, P. Zhelyazkova, A. Vuchkov
- Hordein polymorphism between spring barley cultivars by SDS-PAGE electrophoresis** 273
N. Neykov, S. Doneva

Nutrition and Physiology

- Comparative study of rapeseed, monofloral types and multifloral honey by some physico-chemical parameters** 277
I. Zhelyazkova, S. Lazarov
- Body condition score, nonesterified fatty acids and beta-hydroxybutyrate concentrations in goats with subclinical ketosis** 282
V. Marutsova, R. Binev

Production Systems

- Lucrative status of improved dual purpose cowpea (*Vigna unguiculata* L., Walp) in Damboa, Borno State, North-Eastern Nigeria** 286
B.H. Gabdo
- Study on the emptying time of grain harvester hoppers** 291
K. Trendafilov, N. Delchev, B. Kolev, G. Tihanov
- Length of the growing season and yield in *Triticum monococcum* L., in accordance with the growing conditions** 296
S. Stamatov, E. Valchinova, G. Desheva, K. Uzundzhalieva, P. Chavdarov, T. Cholakov, B. Kyosev, R. Ruseva, N. Velcheva
- Productivity of durum wheat cultivar Predel at nitrogen-phosphorous fertilization** 301
L. Plescuta
- Effect of the herbicide treatment dose on the weed infestation in common winter wheat** 306
Z. Petrova
- Evaluation of some technological properties of Caucasian ram wool** 311
D. Pamukova, G. Staykova, N. Stancheva, D. Panayotov

Agriculture and Environment

- Saved CO₂ emissions by using renewable sources for hot water yield in Bulgarian dairy farms** 315
R. Georgiev, R. Slavov, K. Peychev, D. Georgiev, S. Apostolov, J. Ellingsen, J. Tønnesen
- Inventory of the legal base for reclamation of lands disturbed by open-cast mining in Bulgaria** 320
M. Banov, V. Tzolova, I. Kirilov
- Taxonomic composition of phytoplankton in Black Sea area in front of the Cape Galata (2008-2016)** 326
D. Klisarova, D. Gerdzhirov
- Biodiversity of the macrozoobenthos in some protected marine areas along Bulgarian Black Sea coast** 336
E. Petrova, S. Stoykov
- Heavy metals in organs of gudgeon (*Gobio gobio* L.) from Vardar River, R. Macedonia** 340
R. Nastova, V. Kostov, I. Uslinowska

Product Quality and Safety

- Mathematical methods for assessment and analysis of honey yield data for Bulgaria and the European Union for the period 1961-2014** 347
N. Keranova
- Carcass traits and meat quality of different slow growing and fast growing broiler chickens** 351
M. Oblakova, N. Mincheva, P. Hristakieva, I. Ivanova, M. Lalev, Sv. Georgieva
- Role and importance of the awareness for whey in dairy sector at an international level** 358
M. Yilmaz, H. Celik, A.D. Karaman, K. Celik

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows. IXth International Conference on Production Diseases in Farm Animals, September 11-14, Berlin, Germany.

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