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**BOOK OF**

**PROCEEDINGS**



*X International Scientific Agriculture Symposium  
"AGROSYM 2019"  
Jahorina, October 03-06, 2019*

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## THE CONCENTRATIONS OF HEAVY METALS IN THE LIVER AND MUSCLE TISSUE OF THREE KINDS OF FISH DURING FIVE YEAR PERIOD

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### Abstract

Fish, as the top of the trophic pyramid of aquatic ecosystems, are one of the most sensitive bioindicators for the presence of heavy metals in the aquatic ecosystems that inhabit them. Concentrations of cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) were determined in the muscle tissue and liver of selected fish species from the Zapadna Morava River during 2013 and 2018, respectively. The aim of this paper was to obtain a more complete insight into the level of accumulation of heavy metals in the organism of fish, especially fish meat as an edible part that should satisfy the health safety for human consumption. Investigations of the concentration of the presence of heavy metals from *Carassius auratus*, *Abramis brama* and *Squalius cephalus* have revealed various bioaccumulation of heavy metals. Among the examined fish, the highest accumulation of heavy metals was found in *Abramis brama*, *Carassius auratus* and *Squalius cephalus*, respectively. In all fish for all four investigated heavy metals, higher concentrations were detected in the liver and less in fish muscle. Bioaccumulation of heavy metals had the following trend: Zn > Pb > Cd > Hg. The determined concentrations of the analyzed heavy metals in fish muscle were not within the permitted limits of the MAC national legislation of the Republic of Serbia. Meat of investigated fish species is not health-safe and hygienically correct for use in human nutrition (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018). The content of tested heavy metals in fish meat indicates that in this aquatic ecosystem during the five year period there was no significant pollution with these heavy metals.

**Keywords:** *Heavy metals, Bioaccumulation, Freshwater fish, Zapadna Morava River.*

### Introduction

Fish is a highly valued animal food in human nutrition. In addition to the nutritional value from the consumer's point of view, its hygienic or safety correctness is also significant. The majority of freshwater ecosystems are due to urbanization, industrialization and other forms of human activity, permanently exposed to the influence of various forms of hydrosalgia. For these reasons, many hydrobionts in them, and in particular the fish populations, suffer negative consequences, and hence the man as their consumer.

Fish come into contact with harmful pollutants through the skin, gill breathing (benthic organisms and plankton), and predator fish species bring metals into the body through other fish they feed. In this way, the metals directly reach the bloodstream in the liver, kidneys and muscle tissue, and accumulate in them more or less (Mansour *et al.*, 2002; Erdoğrul *et al.*, 2006; Has-Schön *et al.* 2006; Yilmaz *et al.*, 2007). Fish belong to the most sensitive organisms in the presence of increased concentrations of heavy metals, and the level of accumulation of certain metals in tissues and fish bodies depends on the age, size and trophic



status of fish, as well as from the concentration of metal in water (Alibabić *et al.* 2007; Ural *et al.* 2012). The main goal of this research is to determine the level of accumulation of heavy metals (lead, cadmium, mercury and zinc) in the muscle tissue and liver of different species of fish that inhabit the river Moravia. In this way, a comparison of the results obtained in 2018 with the results from 2013 will be made, as well as an assessment of the health safety of their meat for human nutrition. At the same time, information on the concentrations of metal in the liver and muscles of the fish would indicate the degree of anthropogenic water pollution in ecosystem of the Zapadna Morava, as well as the assessment of the health safety of fish fodder for human consumption. According to available literature data, the fish populations in ecosystem of the Zapadna Morava River are largely affected by numerous pollutants, especially those of anthropogenic origin such as industrial, communal water and rural wastewater (Veljović *et al.* 1992; Lazić *et al.* 2003; Marković *et al.* 2007).

### **Material and Methods**

A field study of the sample collection in the western Morava River was conducted in June 2013 at the Stančići locality (coordinates: N 43°52'15,3" i E 20°26'17,1") and during July 2018 at the Parmenac locality (coordinates: N 43°53'44,5" i E 20°18'15,9"). Fish sampling was carried out using stackable mesh of 20 to 60 mm diameter fenders and a battery electro-picking device (AGK Kronawitter, IG200-2, battery power 12V, 20Ah). The species of fish are determined according to Simonović (Simonović, 2006), and for each fish sample, total body length and weight were measured. After fish dissection, samples of muscle tissue and liver were frozen for analysis. In the laboratory, composite samples for each fish species were prepared separately. The concentration of heavy metals in the muscular tissue and liver of the fish species of silver carp (*Carassius auratus gibelio*), as allochthonous species, bream (*Abramis brama*) and chub (*Squalius cephalus*) as autochthonous species in the ecosystem Zapadna Morava River. As for the selection of heavy metals whose content was analyzed in musculature and liver fish, we defined for cadmium and lead as very toxic metals, zinc due to the competitive relationship with cadmium and a highly toxic animal whose toxic effect on fish is present at very low concentrations in water from the 0,003 mg/l. The analysis were carried out according to the standard methodology for the analysis of heavy metals, using the necessary instrumental techniques. By the method of atomic absorption spectrometry (FAAS flame techniques), the content of cadmium, lead and zinc were determined, while the content of mercury in the tissues and organ was determined by the HGAAS atomic absorption spectrometry method, hydride technique.

### **Results and Discussion**

The concentrations obtained for all four metals in the muscles and liver of the three species of fish from different trophic levels are shown in Table 1. During this study, 15 specimens of silver carp, 9 bream and 7 fish chub were analyzed. The highest concentrations of all four tested heavy metals were found in the liver and the lowest in fish muscle. Metal accumulation had an identical trend in all three types of fish: Zn > Pb > Cd > Hg, and the total content of all four metal fish had the following trend bream > chub > silver carp. In order to determine the health correctness of the meat of the examined fish, the comparison of the obtained values of heavy metals in samples of muscle tissue of fish with the maximum allowed concentrations (MAC) established by the European Union (Commission regulation (EC) No 1881/2006) and national legislation of the Republic of Serbia (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018). According to EU regulations (European Commission Regulation, 2006), the maximum allowable amount of Cd, Hg, Pb is 0.05, 0.50 and 0.30 mg·kg<sup>-1</sup> per unit of fresh weight. According to the norms of national legislation (The Official Gazette Republic of Serbia No 22/2018 and No 90/2018) MAC za Cd, Hg, Pb is 0.05, 0.50 and 0.30 mg·kg<sup>-1</sup>,

while according to the same legal regulation, the maximum content of Zn in fish products in cans is up to 100 mg·kg<sup>-1</sup>.

Table 1. Average values (± SD) content of heavy metals in the samples of fresh fish (mg·kg<sup>-1</sup>)

2013 year, location Stančić					
Fish species	Sample	Cd	Pb	Hg	Zn
<b>Silver carp</b> n = 11	muscle tissue	0,004±0,04	0,324±0,15	0,135±0,21	17,392±0,05
	liver	0,084±0,20	0,372±0,03	0,152±0,05	18,190±0,26
<b>Bream</b> n = 8	muscle tissue	0,005±0,08	0,193±0,05	0,098±0,12	19,286±0,05
	liver	0,012±0,05	0,231±0,10	0,112±0,23	32,152±0,06
Σ		0,105±0,37	1,120±0,33	0,497±0,61	87,020±0,42
2018 year, location Parmenac					
<b>Silver carp</b> n = 5	muscle tissue	nd	0,150±0,10	0,110±0,28	19,560±0,06
	liver	0,025±0,31	0,186±0,08	0,150±0,05	21,250±0,14
<b>Chub</b> n = 7	muscle tissue	0,002±0,09	0,090±0,12	0,117±0,12	18,133±0,09
	liver	0,040±0,03	0,156±0,06	0,141±0,07	22,450±0,36
Σ		0,067±0,43	0,582±0,36	0,518±0,52	81,393±0,65

Based on the presented tabular results, it can be concluded that during 2013, the concentration of heavy metals in the musculature of the fish studied was significantly lower than their liver content. The lowest values of metal in the muscle tissue were established for cadmium and varied in the range of 0.004 mg·kg<sup>-1</sup> in silver carp to 0.005 mg·kg<sup>-1</sup> in bream. These are the permissible concentrations within the MDC regulations of the EU and national regulations of the Republic of Serbia. The reference concentrations prescribed by the national and EU legal provisions of the EU are determined in terms of the content of other analyzed metals in muscle tissue of the fish. The average lead value was from 0.193 mg·kg<sup>-1</sup> in the muscle of the bream to 0.324 mg·kg<sup>-1</sup> in silver carp (maximum of 0,3 mg·kg<sup>-1</sup> is allowed Serbian Regulation). The zinc content was also in the legal framework for fish products in cans and varied from 17.392 mg·kg<sup>-1</sup> in muscular weight of silver carp to 19.286 mg·kg<sup>-1</sup> in muscle tissue.

Similar results, but with a somewhat reduced concentration of metal in fish muscle, were also found during the research in 2018. The concentration of Cd was up to 0.002 mg·kg<sup>-1</sup> in chub, while Pb concentrations ranged from 0.090 mg·kg<sup>-1</sup> in chub muscles to 0.150 mg·kg<sup>-1</sup> in silver carp muscularity. The smallest Hg value was 0.110 mgkg<sup>-1</sup> in silver carp, while slightly higher values of 0.117 mg·kg<sup>-1</sup> were recorded in chub. The smallest Zn concentration of 18,133 mg·kg<sup>-1</sup> was determined in the cluster musculature and the highest of 19,560 mg·kg<sup>-1</sup> in silver carp. The metal concentrations in muscle analyzed fish did not exceed the permitted values prescribed by national and EU regulations.

By comparing the obtained average values of the content of the tested metals in the muscle tissue of the studied fish from 2018 with the data from 2013 year, it can be concluded that the accumulation of metals in fish from the Zapadna Morava River is significantly lower than the previous period. The total concentration of cadmium in musculature and liver in all types of fish in 2018 compared to 2013 was lower by 36%, lead by 48%, zinc by 6.4%, while the content of mercury was by 4.2% higher. The metal concentrations in the fish organism depend on their type, age, physiological state, type of tissue and feeding (Storelli *et al.* 2006; Szarek-Gwiazda *et al.* 2006). According to Oloy *et al.* (2005) due to the biodegradability and ability to accumulate along the food chain, heavy metals are considered to be one of the primary contaminants of the internal organs of fish and the entire aquatic ecosystem.

The obtained results of the concentration of heavy metals in the musculature and liver of the analyzed fish species clearly show that the aquatic ecosystem of the Zapadna Morava River is not encircled by pollutants from industrial waters, although this ecosystem is their basic recipient. The reason for this is the reduced level of industrialization and the cessation of work of a large number of factories. Determined concentrations of heavy metals in the liver all three types of fish varied within the limits of normal physiological concentrations for all three chemical elements and were below the level of human food tolerance (Biro *et al.*, 1991). Lead (Pb), content in silver carp in 2013.year of 0,324 and 0,372 mgkg-1 is above the permitted values. This means that the Zapadna Morava River waterway on the studied profiles can be considered relatively unpolluted in terms of the loads of the tested heavy metals. The results of the research indicate the necessity of continuous sampling and analysis of this water course in order to prevent accidental situations and maintain the health correctness of the meat of fish that inhabit this ecosystem. In order to be able to make a final assessment of the hygienic quality of fish meat for human consumption and the ecological status of this ecosystem, it is necessary to carry out a complex analysis, which would include the determination of the content of heavy metals in water, sediment and commercially important fish species from the ihtiofauna composition. Other authors also point to the necessity of this kind of research (Đukić *et al.*, 1998; Farkas *et al.*, 2002).

### **Conclusions**

Taking into account that fish are usually located at the top of the food chain in the aquatic environment, they often accumulate large quantities of certain heavy metals, which is why they are considered one of the most sensitive aquatic organisms for the presence of toxic substances. Since fish meat is one of the basic components of human nutrition, excessive accumulation of heavy metals in fish muscle makes this food unsafe for human consumption. The content of heavy metals (Cd, Hg and Zn) in the muscle tissue of three types of fish silver carp, bream and chub from the Zapadna Morava River was within the boundaries of the maximum perm fish chub issible concentration MAC and in accordance with the prescribed national and EU legal acts on the content of metals and other poisonous substances that can be found in freshwater fish. Lead (Pb), content in silver carp in 2013.year of 0,324 and 0,372 mgkg-1 is above the permitted values. Based on the conducted surveys during 2013 and 2018, it can be concluded that ecosystem of the Zapadna Morava River shows a trend of improving the quality in terms of anthropogenic pollution, because the registered quantities of stored heavy metals in the musculature and liver of the investigated fish varied within normal limits for maximum permissible concentrations.

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