Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2022

59

27-35

Print ISSN: 0367-4444

Online ISSN: 2232-7266

Original Scientific Article

DOI: https://doi.org/10.35666/2232-7266.2022.59.06

UDC: 543:597(497.6 Neretva)

Bioaccumulation of metals in fish of different diets from hydro-accumulations on the Neretva River, Bosnia and Herzegovina

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Article info
Received: 30/08/2022
Accepted: 06/10/2022

Keywords:
Bioaccumulation
Metals
Fish
Neretva
Health risk assessment

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Abstract: There is a growing need to assess the level of contaminants in fish as bioindicators of the health and well-being of fish and humans as its consumers. Contamination by heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, Zn) was evaluated using atomic absorption spectrometer, flame and graphite furnace technique in the water samples and fish muscle tissues of *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* of four hydro-accumulation lakes on the Neretva River, Bosnia and Herzegovina. Samples were collected during two seasons: autumn-winter and spring-summer (2019). It has been shown that iron (Fe) was the highest accumulating metal in fish, whilst cadmium (Cd) and lead (Pb) were the lowest. Heavy metals contents were below the maximum permissible for drinking water and for fish as prescribed by national legislation. According to correlation matrix between metals content in all fish during both fishing seasons, the highest values of the Pearson coefficient were obtained in the case of essential elements (Fe, Mn, Cu and Zn) and Fe and Mn also had a statistically significant correlation with Cd and Pb. Furthermore, potential health risk assessment exposure of the adult population in B&H revealed that none of the seven heavy metals pose risk to human health, based on the estimated daily intake via consumption of these fish species as well as target hazard quotient and hazard index values less than 1.

INTRODUCTION

Fish has been used in human nutrition since ancient times, because people were able to obtain food simply and easily by fishing. With the increase of the world's population and the increase of the standard of living, especially in the developing countries, the needs for fish meat are significantly increasing (Tepe, et al. 2008). Fish catches increased 20 times from the beginning to the end of the 20th century and reached a maximum of 93 million tons per year (Baltić, Dokmanović, Bašić, et al. 2015). As fish are constantly exposed to pollutants in water, they can also be used as an excellent biological indicator of pollution in aquatic ecosystems (Benson, Essien, Akan, et al. 2007). In recent years, there has been a lot of research on the content of heavy metals in fish in different parts of the world. Most of this research has been done in the muscle tissue of fish, although, some other organs such as the liver, gills, kidneys, bones are also interesting (Karadede and Ünlü 2000, Vicente-Martorell, Galindo-Riano, Garsia-Vargas, et al. 2009, Malik, Biswas, Qureshi, et al. 2010).

Aquatic ecosystems are exposed to many substances, including heavy metals that are non-degradable and have a high capacity for bioaccumulation in water, sediment and aquatic organisms that inhabit this ecosystem. In general, the study of heavy metals can be viewed through two main aspects. The first aspect is from the point of view of health safety, ie. attention to the bioaccumulation of heavy metals that can affect human health. The second aspect is from the point of view of bioaccumulation of heavy metals in the aquatic ecosystem, which can affect the balance of the ecosystem.

Toxicity and bioaccumulation of heavy metals in aquatic ecosystems may depend on numerous factors such as: dissolved oxygen content, pH, alkalinity, and temperature (Adhikari, et al. 2006). Similarly, metals bioaccumulation in fish tissues depends on i.e., fish size, fish age, eating habits (Khalid 2004). Laboratory experiments have shown that fish that ingest heavy metals through water have a higher content of metals in their gills, while fish that ingest higher concentrations of metals through their diet have a higher content of metals in their digestive tract and muscle tissue. Also, if the concentration of heavy metals in the water is low, the main mode of metal intake is through the food that fish ingests (Clements 1997, Khalid 2004).

With the increase of man's need for electricity on the one hand, and the desire to preserve a healthy environment, on the other hand, man often takes major interventions in rivers and, thus, contrary to his aspirations and essentially long-term interests, significantly disrupts normal natural life in its surroundings. The construction of dams and the formation of hydro accumulation (HA) lakes on the Neretva River, B&H marked the second half of the 20th century and clearly showed all the negative consequences of human experimentation with nature (Škrijelj 2002). With the formation of an artificial lake on river, the affected river complexes gradually pass from the stream into the standing water ecosystem. In this way, the whole complex of ecological conditions changes, which inevitably follow the changes in the composition of living communities in the aquatic ecosystem and its immediate surroundings.

Three different country-specific fish species with different eating habits: *Sander lucioperca* (carnivore), *Leuciscus svallize* (omnivore)and *Tinca tinca* (herbivore), inhabit four HAs on the Neretva River. Since there is a lack of the data for these fish species, partly due to the fast-growing aquaculture sphere in B&H, the novelty as well as the primary goal of this research is to gain valuable inputs on seven heavy metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) content. In addition, to identify possible metals sources matrix correlation analysis was applied. Although official total dietary study data is scarce in B&H, potential health risk assessment exposure of the adult population in B&H was revealed based on the estimated daily intake (EDI) via consumption of these fish species. Water samples were also taken from four HAs on the Neretva River in order to analyze heavy metals content and physico-chemical parameters (water temperature, pH and electrical conductivity).

EXPERIMENTAL

Study area

The Neretva River flows through the central, southern part of B&H. The examined fish species were collected in four artificial HAs along this river. Figure 1 shows locations of fishing sites of HA Jablaničko Lake (43°41'N, 17°44'E), HA Grabovica (43°35'N, 17°43'E), HA Salakovac (43°27'N, 17°49'E) and Mostarsko Lake (43°23'N, 17°51'E).

HA Jablaničko Lake is the largest HA on the Neretva River with seasonal water leveling and a dominant influence on the regulation of the river water regime. Due to frequent and sudden oscillations of the water level during the filling and emptying of the reservoir caused by the operation of hydropower Jablanica, the coast is destroyed, and suspended sediment is brought. The emptying of the reservoir leads to the withdrawal of sand into the depths and the formation of layers of mud and silt. The maximum length is about 30 km and the surface of the lake is 1440 ha. The maximum depth is 80 m and the water level oscillations are up to 25 m. Out of a total of 13 fish species from the ichthyofauna of the Neretva accumulation lakes, the presence of seven fish species has been found in Jablaničko Lake.

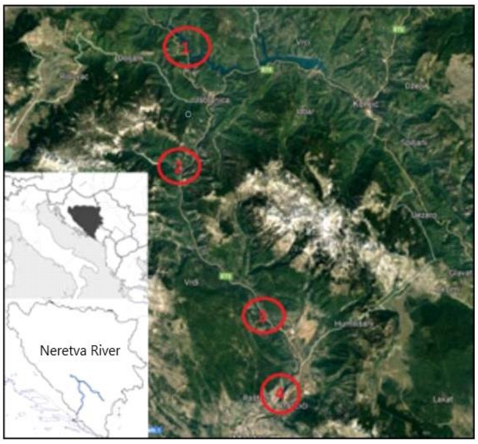

Figure 1: Sampling sites: 1 - HA Jablaničko Lake; 2 - HA Grabovica; 3 - HA Salakovac; 4 - HA Mostarsko Lake, *Google maps*).

HA Grabovica is elongated with a very small width, so it is mainly located in the canyon of the middle course of the Neretva River. The average depth of the lake is 34 m, while the water oscillations are about 4 m. During ichthyologic research of the HA Grabovica (Škrijelj 1990) the presence of six species of fish was ascertained.

HA Salakovac was formed in 1981. The maximum length of the lake is 20 km, the area is about 314 ha, and the maximum depth is about 40 m while the water oscillations are about 5 m. Studies of the ichthyic fauna of the accumulation lake Salakovac have shown that this ecosystem is inhabited by nine fish species.

HA Mostarsko Lake is the youngest HA on the Neretva River, built in 1987. The maximum length of the lake is about 10 km, and the area is about 112 ha. The greatest depth of the lake is about 20 m, while the oscillations of the water are up to 5 m. Conducted ichthyologic research of Neretva accumulation lakes included Mostarsko Lake. Research has shown that seven fish species live in this lake (Škrijelj 1990).

Sample collection and preparation

The research was conducted in two seasons: autumn - winter (September-March 2019) and spring - summer (April – August 2019), i.e. in high and low water levels of the HAs on the Neretva River. The catch was made for three fish species of different feeding habits (*Sander lucioperca, Leuciscus svallize* and *Tinca tinca*) on four HAs on the Neretva River. The catch was made by local fishermen using fishing nets set up in the evening. Immediately after the fish was caught, a section of fish was performed. The fish muscle tissue was separated by section, which was then washed with Milli-Q water, weighed, packed in sterile polyethylene bags and stored at −18°C, until analysis. Table 1 shows the number of fish caught in the examined seasons. Water samples were taken in September 2019, from four HAs on the Neretva River in order to analyze heavy metals content and physico-chemical parameters (temperature, pH and electrical conductivity). Samples of water were collected in sterile polyethylene bottles (previously washed by detergent, rinsed by Milli-Q water followed by 2 mol/L nitric acid (HNO_3), washed by Milli-Q water again and finally with sampled river water). The samples were preserved by the addition of concentrated HNO_3 (1 mL per 1 L of sample) and brought to the laboratory. Then, the samples were filtered through Whatman filter paper (No. 42) and kept in refrigerator at 4 ºC until analysis.

Table 1: Number of fish caught from four HAs on the Neretva River in the periods of spring-summer and autumn-winter.

Fish species/location	Jablaničko Lake	HA Grabovica	HA Salakovac	Mostarsko Lake
Season Spring-Summer (number of fish caught)				
Sander lucioperca	5	4	7	8
Leuciscus svallize	7	5	15	33
Tinca tinca	4	3	9	3
Season Autumn-Winter (number of fish caught)				
Sander lucioperca	4	2	6	5
Leuciscus svallize	3	5	13	28
Tinca inca	5	3	7	4

Sample preparation and heavy metal determination

Composite fish samples of the same species, the same catch locations as the same parts previously classified by the fish section were used. For microwave digestion (Milstone, Start D), 0.5 to 1.0 g of fish muscle tissue sample were weighed, then the samples were transferred to Teflon containers, and 7 mL of 65% (w/v) HNO_3 and 1 mL 30% (w/v) H_2O_2 were added. After digestion, the samples were cooled to room temperature and quantitatively transferred to 25 mL volumetric vessels and filled to the mark with 0.1 mol/L HNO_3.

The content of Cr, Cu, Fe, Mn, and Zn was determined in fish muscle samples using an flame atomic absorption spectrometer (FAAS), while Cd and Pb were quantified by graphite furnace technique (GFAAS) (AAS, model Varian AA240FS). The obtained results are expressed in units of mg/kg (wet weight of fish sample). In water samples, Zn was determined by FAAS and other metals by GFAAS. Field devices that were used to determine physico-chemical parameters of water were pH meter – Hanna Combo, HI98130 and Conductivity meter – Hanna Combo, HI8733N.

Quality Assurance

All reagents used were of analytical purity. For quality assurance, all samples were analyzed in triplicate along with blanks to minimize error. A blank was prepared in the same way as the samples and was analyzed after each batch of 15 samples. The mean result for all samples was reported, and repeatability standard deviations were calculated. Accuracy assessment was performed by spiking already analyzed fish samples with different concentrations of analyzed heavy metals, spiked at three different concentrations (low, medium, and high) covering the working range and the percent recovery was calculated. These standards were different from those used to prepare the calibration curves and were also from different stock standard solutions. The acceptable recoveries ranged between 81-107%.

The detection limit (LOD) was calculated as 3 times the standard deviation of blank (n = 10) absorbance signal. LODs for GFAAS were: 0.065 µg/L for Cd; 0.056 µg/L for Cr; 0.354 µg/L for Cu; 0.390 µg/L for Fe; 0.23 µg/L for Mn; 0.80 µg/L for Pb; 0.013 µg/L for Zn. LODs for FAAS were: 0.02 µg/mL for Cd; 0.16 µg/mL for Cr; 0.05 µg/mL for Cu; 0.98 µg/mL for Fe; 0.15 µg/mL for Mn; 1.12 µg/mL for Pb; 0.70 µg/mL for Zn.

Exposure and potential health risk assessment evaluation

Heavy metals exposure estimations were obtained in muscles of three fish species: *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca*. The estimated daily intake (EDI, mg kg^{-1} day^{-1}) from fish ingestion was calculated by combining the data on the consumption of fish with the determined levels (*C*, mg/kg, w.w.) of Cd, Cr, Cu, Fe, Mn, Pb and Zn for two different periods of year (spring - summer and autumn – winter). To produce exposure estimates for adult person (70 kg of body weight, BW – USEPA 2011) the fish and seafood ingestion rates (FIR) reported by Hajrić, et al. (2022) were used, since there is no available official data on dietary habits of the population in B&H. They found that the average consumption was 52.1 g day^{-1} $consumer^{-1}$ based on the conducted survey using the "Food frequency questionnaire method" (EFSA 2014) for the consumption of fish and fish products (n = 500 respondents), taking into account the proportional representation of the overall population by age, sex, education and employment status. The calculation was made according to the following expression given by Łuczyńska, et al. (2018):

$$\text{EDI (mg kg}^{-1}\text{ day}^{-1}) = \frac{C \times FIR}{BW} \quad (1)$$

The obtained EDI values of metals were compared with the oral reference dose values (Rfd) given by USEPA (2011).

A commonly used method of risk assessment estimation is the ratio between the measured concentration of metal and the oral reference dose, known as the target hazard quotient (THQ). This hazard quotient is mathematically expressed as follows (USEPA 2000; Liang et al. 2018):

$$THQ = \frac{EF \times ED \times FIR \times C}{Rfd \times BW \times TA} \times 10^{-3} \quad (2)$$

where the exposure frequency (EF, 365 days $year^{-1}$) is combined with the exposure duration (ED, 70 years), fish ingestion rate (FIR, 52.1 g day^{-1} $consumer^{-1}$), the mean concentration of Cd, Cr, Cu, Fe, Mn, Pb and Zn in fish (*C*, mg kg^{-1} w.w. given in Table 3), oral reference dose (Rfd, given by USEPA 2011), body weight of adult person (BW, 70 kg), and mean exposure time (*TA*, 365 days $year^{-1}$ x ED).

In addition, hazard index *(*HI) was calculated as sums of individual THQs obtained for each of seven heavy metals given by USEPA (2011) as:

$$HI = THQ_{Cd} + THQ_{Cr} + THQ_{Cu} + THQ_{Fe} + THQ_{Mn} + THQ_{Pb} + THQ_{Zn} \quad (3)$$

Statistical Analysis

In the statistical analysis, as basic statistical methods, descriptive statistical parameters were used. Two tests were used to test and determine statistically significant differences between the examined groups. The t-test was used to examine the significance of the differences between the mean values of the two groups examined. A group test, ANOVA, was used to examine significant differences between the three or more observed treatments. Significance of differences was determined at significance levels of 5%. Statistical analysis of the obtained results was done in the statistical package SPSS 17.

RESULTS AND DISCUSSION

Characterization of water samples

Natural and anthropogenic processes as well as the depth of the aquatic system affect the physico-chemical and chemical parameters in the water. For example, water temperature, at the time of fishing, is very important in terms of fish biological activities. The results of water physico-chemical and chemical analysis are shown in Table 2.

All observed physico-chemical parameters were slightly lower in the autumn-winter season. The lowest temperatures and pH values were recorded at HA Jablaničko Lake in both observed seasons, as well as the lowest value of conductivity recorded at the same location in the autumn-winter season. Comparing the obtained results with the national legislation, all the results were below limit value (Official Gazette of FB&H 43/07).

Heavy metals in fish

Fish muscle tissue is less active in terms of biotransformation and accumulation of heavy metals compared to other fish tissues (El Moselhy et al. 2014).

The content of heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, Zn) in muscle tissue of *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* fish from HAs on the river Neretva during the spring-summer and autumn-winter seasons were determined.

The results are shown in the Table 3. Concentrations of Cd and Cu were similar in the muscle tissues of all three fish species. The content of Cu in *Sander lucioperca* and *Leuciscus svallize* muscle tissue shows a statistically significant difference depending on the location of fishing ($p < 0.05$), while *Tinca tinca* caught at different locations does not show a statistically significant difference ($p > 0.05$).

The highest concentrations of Fe and Mn were found in *Leuciscus svallize* and the lowest in *Sander lucioperca* muscle tissue, in both seasons. Also, the highest Pb content was determined in *Leuciscus svallize* (HA Salakovac), while *Sander lucioperca* had lower and relatively similar concentrations of this heavy metal.

Table 2: Physico-chemical and chemical parameters of water at fishing sites on the HAs of Neretva River, autumn-winter and spring-summer seasons.

Location	**HA Jablaničko Lake**		**HA Grabovica**		**HA Salakovac**		**HA Mostarsko Lake**	
Season/ parameter	**autumn/ winter**	**spring/ summer**	**autumn/ winter**	**spring/ summer**	**autumn/ winter**	**spring/ summer**	**autumn/ winter**	**spring/ summer**
Temp. (°C)	14	17	15	18	16	18	16	18
pH	6.63	6.78	6.93	6.80	6.80	6.84	6.94	6.97
Conduct. (µS/cm)	397	412	344	371	330	352	313	366
Metals (µg/L)								
Mn	3.492±0.05		9.122±0.12		<LOD*		0.690±0.02	
Cd	<LOD*		0.086±0.002		<LOD*		<LOD*	
Cr	0.360±0.14		0.821±0.10		<LOD*		0.063±0.002	
Pb	0.820±0.20		2.513±0.24		8.061±0.32		<LOD*	
Fe	9.264±0.12		45.92±2.08		1.132±0.08		0.654±0.08	
Cu	13.46±0.21		24.63±0.150		20.79±0.18		4.020±0.08	
Zn	0.075±0.005		0.067±0.002		0.060±0.002		0.069±0.004	

*LOD – limit of detection

There are a large number of fish farms on HA Salakovac, where fish food is used in large quantities. Also, the highest Pb concentration was found in water from HA Salakovac. According to the diet, *Leuciscus svallize* is an omnivore and feeds on everything it can find, from mollusks, larvae, insects, to organic waste, fruit and moss. Larger *Leuciscus svallizes* feed largely on various species of smaller fish (Škrijelj 2002). The highest concentrations of Cd, Cu, Fe and Mn were determined in *Leuciscus svallize* at the locations of HA Jablaničko Lake and HA Grabovica. The highest concentrations of these metals were also found in water samples at the HA Grabovica compared to other locations. Zrnčić, Oraić, Ćaleta, et al. (2013) point out that omnivorous fish are better biological indicators of environmental contamination, i.e., they provide a more reliable assessment of the state of the environment. *Leuciscus svallize* likes to live at the bottom of the lake, and these two sites are exposed to a number of natural and anthropogenic sources of heavy metal pollution. HA Jablaničko Lake is near the industrial town of Konjic, where a large number of metal industries that discharge their wastewater into the Neretva exist. Also, these two sites are exposed to the M-17 highway, which is used by a large number of vehicles. The order of heavy metals presence of in *Leuciscus svallize* muscle tissue was: Fe>Zn>Mn>Cu>Cr>Pb>Cd. The highest Cr content was determined in *Sander lucioperca* and the highest Zn content in *Tinca tinca* fish in both seasons both in fish from Mostarsko Lake, in both seasons. On the other hand, at the Mostarsko Lake, the lowest concentrations of all analyzed metals were found in water samples. The average Zn content in *Sander lucioperca* was statistically significantly higher in the spring-summer period ($p < 0.05$). Young *Sander lucioperca* feed on plankton and invertebrates, larvae, worms, insects. Adult *Sander lucioperca* mainly consume small fish whose size is limited by the narrowness of its esophagus. As it gets older, *Sander lucioperca* become more and more interested in dead, sick or wounded fish, mening they prefer to catch prey easily (Škrijelj 2002). Low values of all metals in *Sander lucioperca* muscle tissue can be associated with a number of factors present in the observed areas. *Sander lucioperca* feeds primarily on small, sick, and vulnerable fish, which did not have enough time for bioaccumulation of heavy metals. The order of metals presence in *Sander lucioperca* muscle tissue was as follows: Fe>Zn>Cr>Mn>Cu>Pb>Cd. *Tinca tinca* is an herbivore by diet; it feeds on different types of plants depending on the place where it lives. Plant foods contain smaller amounts of nutrients, i.e. has a lower caloric value, so *Tinca tinca* belonging to herbivores must consume larger amounts of plant food that in its composition, in addition to other ingredients, always contains a certain amount of heavy metals, or whose content varies depending on the environmental conditions in which plants grow (Škrijelj 2002). As *Tinca tinca* feeds on plants and algae, it is constantly in contact with sediment, i.e. as it likes to live in muddy parts of the lake, so is constantly exposed to heavy metals that can bioacumulate. The order of metals presence in *Tinca tinca* muscle tissue was: Zn>Fe>Mn>Cu>Cr>Pb>Cd. For Cd and Zn, in contrast to Cr, Fe, Mn and Pb, it was found that there is no statistically significant difference between fishing sites and for Cr, Fe and Pb, unlike other metals, a statistically significant difference was found depending on the fishing season ($p < 0.05$). The metal content in fish muscle tissue shows that fish caught in the autumn-winter season show a significantly smaller statistical difference compared to the spring-summer catch period ($p <0.05$).

According to the Ordinance on maximum levels for certain contaminants in foodstuff (Official Gazette of B&H, No. 68/14, 79/ 16, 9/17) the maximum allowable concentrations (MAC) in B&H for certain metals in fish muscle tissue are defined as: 0.30 mg/kg for Pb, 0.050 for Cd, 30.0 for Cu, 30.0 for Fe; (values for Cu and Fe refer to whole fish). In all cases the MAC for these metals was not exceeded in all three fish species.

Regarding the metal content in the studied fish, the following descending order can be given: Fe>Zn>Cr>Mn>Cu>Pb>Cd and according to the content of analyzed metals in them, fish can be arranged in the following descending order: *Leuciscus svallize* (omnivorous) > *Tinca tinca* (herbivore) > *Sander lucioperca* (carnivore). Similar results were obtained by Abdelmoneim, et al. (1992), Chale (2002), Mendil, Uluözlü, Hasdemir, et al. (2005) and El Moselhy, et al. (2014).

Table 3: Heavy metals content (mg/kg) in muscle tissue of fish *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* from HAs on the river Neretva during the spring-summer and autumn-winter seasons

Fish / Metals	Cd	Cr	Cu	Fe	Mn	Pb	Zn
	Spring – Summer (mean concentration / range)						
Sander lucioperca	0.0015	0.926	0.411	8.282	0.769	0.015	3.581
	0.001-0.002	0.427-1.933	0.338-0.523	4.276-11.25	0.628-0.933	0.008-0.028	2.281-4.171
Leuciscus svallize	0.002	0.283	0.549	19.60	0.983	0.033	4.242
	0.001-0.004	0.245-0.321	0.438-0.670	15.44-22.83	0.512-1.378	0.005-0.115	3.143-5.906
Tinca tinca	0.002	0.338	0.518	14.06	0.845	0.007	14.60
	0.001-0.002	0.218-0.521	0.356-0.678	13.12-15.89	0.725-0.906	0.007-0.008	5.011-25.12
	Autumn – Winter (mean concentration / range)						
Sander lucioperca	0.001	0.853	0.424	6.780	0.659	0.011	3.500
	0.001-0.001	0.215-2.314	0.347-0.491	4.325-9.604	0.412-0.897	0.005-0.022	2.344-3.904
Leuciscus svallize	0.002	0.219	0.536	13.29	0.940	0.029	4.276
	0.001-0.003	0.194-0.261	0.439-0.620	5.117-21.74	0.453-1.322	0.003-0.103	2.968-6.764
Tinca tinca	0.002	0.292	0.479	11.95	0.780	0.007	13.58
	0.001-0.003	0.222-0.444	0.342-0.622	9.435-17.05	0.708-0.828	0.005-0.011	4.634-23.81

Matrix correlation analysis

The possible metals sources can be indicated by the correlation matrix by analyzing the value which represents the linear correlation coefficient between metals. Results on all metals content in all analyzed fish at all locations during both fishing seasons were subjected to matrix correlation analysis (Pearson correlation coefficient). Correlation analysis showed that significant correlations ($p < 0.05$, $N = 72$) were obtained between the following metals: Fe-Cd (0.517), Fe-Cu (0.526), Fe-Mn (0.745), Mn-Cd (0.426), Mn-Cu (0.651), Mn-Pb (-0.509), Zn-Cu (0.558). For other metals the correlations were below 0.400 at $p < 0.05$. A graphical presentation of the most significant correlations is given in Figure 2.

According to Yılmaz, Sangün, Yağlıoğlu,et al. (2010) essential elements in fish are Fe, Zn, Cu and Mn, and non-essential Cd and Pb, later ones reflecting an exogenous influence that can be related to pollution of environment. From the correlation analysis, the highest values of the Pearson coefficient were obtained in the case of essential elements, while Fe and Mn additionally had a statistically significant correlation with Cd and Pb. Significant correlation for the metal pair Fe-Cd was also found in fish from north-west African coast, Morocco (Afandi, Talba, Benhra, et al. 2018). Similar to our results, there was a strong positive correlation between Fe and Mn as well as negative correlation between Mn and Pb for the fish samples from Nile Delta (Talab, Goher, Ghannam, et al. 2016). In the study of Bhuyan, Bakar, Islam, et al. (2016) correlation between Cd and Mn was similar to our findings but there were weak negative correlations between Cd and Fe in fish from Meghna River, Bangladesh.

Health risk assessment

EDI of Cd, Cr, Cu, Fe, Mn, Pb and Zn in adult person who consumed *Sander lucioperca*, *Leuciscus svallize* or *Tinca tinca* fish muscles from the Neretva River is shown in Table 4.

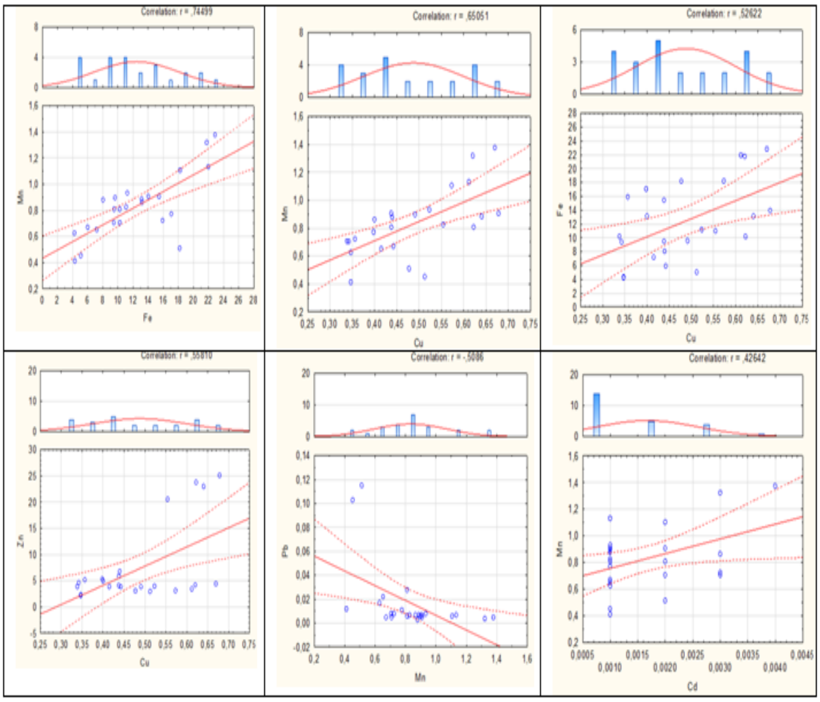

Figure 2: The most significant Pearson correlations between metals in fish from four HAs at Neretva River

Table 4: EDI (mg kg^{-1} day^{-1}) of heavy metals in *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* fish muscles for adults.

Fish / Metals	Cd	Cr	Cu	Fe	Mn	Pb	Zn
	Spring - Summer EDI (mg kg^{-1} day^{-1})						
Sander lucioperca	1.12E-06	6.89E-04	3.06E-04	6.16E-03	5.72E-04	1.12E-05	2.67E-03
Leuciscus svallize	1.49E-06	2.11E-04	4.09E-04	1.46E-02	7.32E-04	2.46E-05	3.16E-03
Tinca tinca	1.49E-06	2.52E-04	3.86E-04	1.05E-02	6.29E-04	5.21E-06	1.09E-02
	Autumn - Winter EDI (mg kg^{-1} day^{-1})						
Sander lucioperca	7.44E-07	6.35E-04	3.16E-04	5.05E-03	4.90E-04	8.19E-06	2.61E-03
Leuciscus svallize	1.49E-06	1.63E-04	3.99E-04	9.89E-03	7.00E-04	2.16E-05	3.18E-03
Tinca tinca	1.49E-06	2.17E-04	3.57E-04	8.89E-03	5.81E-04	5.21E-06	1.01E-02
Rfd	**0.001**	**0.003**	**0.04**	**0.7**	**0.14**	**0.004**	**0.3**

For the metals examined, the calculated EDI values were in the following order: Fe≥Zn>Mn≥Cu ≥Cr>Pb>Cd for all three fish species. In general, obtained trends for EDI values for each of all of seven metals were identical in spring – summer as well as autumn – winter periods of year, indicating that season has no impact at all on final EDI value. Furthermore, none of the seven heavy metals pose risk to human health, since all obtained EDI values were significantly less than the Rfd levels (Table 4). However, heavy metal content must be monitored regularly since these pollutants can accumulate to lethal levels in fish tissue.

The THQ values for Cd, Cr, Cu, Fe, Mn, Pb and Zn as well as the hazard index per season via the consumption of the examined three fish species are shown in Table 5.

On the basis of the obtained results, daily consumption of examined three fish species will not cause any significant adverse health effects since THQ and HI levels were less than 1 (Yi, Tang, Yang, et al. 2017; Łuczyńska, et al. 2018). Therefore, all seven heavy metals examined in this study were found not to be potential health hazard for consumers.

CONCLUSION

In this research, the content of seven heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, Zn) in water and three different country-specific fish species *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* with different eating habits, during spring-summer and autumn-winter seasons, were analyzed. Heavy metals were found in the tested fish muscle tissue samples. The metal content was in all cases higher in the spring-summer season, which is probably due to the fact that the tested fish species are more active during this period of year. However, the maximum allowed concentrations prescribed by national legislation were not exceeded. From the matrix correlation analysis, the statistically significant correlations were obtained mostly in the case of essential elements (Fe, Mn, Cu and Zn). Daily consumption of examined fish species will not cause any significant adverse health effects for consumers since all calculated EDI values were lower than Rfds, as well as THQ and HI levels were less than 1.

People consume mostly large amounts of fish around the world where certain amounts of heavy metals can be detected. However, the potential benefit of consuming fish is very important due to its rich nutritional value. It is necessary that heavy metal levels do not exceed allowable amounts through fish intake.

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Table 5: Estimated target THQ and HI of seven metals in muscles for *Sander lucioperca*, *Leuciscus svallize* and *Tinca tinca* fishes for adults.

Fish / Metals	Cd	Cr	Cu	Fe	Mn	Pb	Zn	HI
	Spring - Summer THQ							Spring - Summer
(*Sander luciopera*)	1.12E-03	2.30E-01	7.65E-03	8.81E-03	4.09E-03	2.79E-03	8.88E-03	2.63E-01
(*Leuciscus svallize*)	1.49E-03	7.02E-02	1.02E-02	2.08E-02	5.23E-03	6.14E-03	1.05E-02	1.25E-01
(*Tinca tinca*)	1.49E-03	8.39E-02	9.64E-03	1.49E-02	4.49E-03	1.30E-03	3.62E-02	1.52E-01
	Autumn - Winter THQ							Autumn - Winter
(*Sander luciopera*)	7.44E-04	2.12E-01	7.89E-03	7.21E-03	3.50E-03	2.05E-03	8.68E-03	2.42E-01
(*Leuciscus svallize*)	1.49E-03	5.43E-02	9.97E-03	1.41E-02	5.00E-03	5.40E-03	1.06E-02	1.01E-01
(*Tinca tinca*)	1.49E-03	7.24E-02	8.91E-03	1.27E-02	4.15E-03	1.30E-03	3.37E-02	1.35E-01

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Summary/Sažetak

Sve je veća potreba za procjenom nivoa kontaminanata u ribi kao bioindikatora zdravlja i dobrobiti ribe kao i njenih potrošača. Kontaminacija teškim metalima (Cd, Cr, Cu, Fe, Mn, Pb, Zn) procijenjena je atomskim apsorpcionim spektrometrom, plamenom i grafitnom tehnikom u uzorcima vode i mišićnog tkiva riba *Sander lucioperca*, *Leuciscus svallize* i *Tinca tincana* četiri hidroakumulacijska jezera na rijeci Neretvi, Bosna i Hercegovina, sakupljena tokom dvije sezone: jesen-zima i proljeće-ljeto (2019.). Pokazalo se da je Fe najviše akumulirani metal u ribama, dok su Cd i Pb najmanje akumulirani metali u ribama. Sadržaj teških metala bio je ispod maksimalno dozvoljenog nivoa koji je propisan nacionalnim zakonodavstvom za vodu za piće i ribu. Prema korelacionoj matriks analizi sadržaja metala u svim ribama tokom obje ribolovne sezone, najveće vrijednosti Pirsonovog koeficijenta dobijene su u slučaju esencijalnih elemenata (Fe, Mn, Cu i Zn), a Fe i Mn su takođe imali statistički značajnu korelaciju sa Cd i Pb. Nadalje, procjena potencijalne izloženosti zdravstvenom riziku kod odrasle populacije u BiH pokazala je da nijedan od sedam teških metala ne predstavlja rizik za zdravlje ljudi, na osnovu procijenjenog dnevnog unosa konzumiranjem ovih vrsta riba, kao i ciljanog koeficijenta opasnosti i vrijednosti indeksa opasnosti koji su iznosili manje od 1.