Iranian Journal of Fisheries Sciences 19(1) 421-442 2020
DOI: 10.22092/ijfs.2019.118904

The relationship between biotic factors and the content of chosen heavy metals (Zn, Fe, Cu and Mn) in six wild freshwater fish species collected from two lakes (Łańskie and Pluszne) located in northeastern Poland

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Received: March 2017 Accepted: June 2017

Abstract

The effect of biotic factors such as species, condition factor, body weight and total length of fish on concentrations of iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) in organs of *Rutilus rutilus* (L.), *Abramis brama* (L.), *Coregonus lavaretus* (L.), *Esox lucius* (L.), *Perca fluviatilis* (L.) and *Tinca tinca* (L.) from reservoirs of Warmia and Mazury region (northeastern Poland) were determined. Differences in the content of metals were observed between species ($p \leq 0.05$). Some metals demonstrated specific affinity for particular tissues. Lower concentrations of metals were found in muscles and ranged as follows: Zn 3.427-9.950, Fe 1.297-2.550, Cu 0.133-0.279 and Mn 0.050-0.162 (expressed mg kg^{-1} wet weight). The highest levels of Fe (33.49-123.6) and Cu (3.994-27.14) (except for copper in perch) found in the liver ($p \leq 0.05$) was related to detoxification, whereas high concentrations of Mn (1.366-5.113) and Zn (15.91-135.0) (except for Zn in tench) in gills may be associated with excretion processes or uptake ($p \leq 0.05$). The two organs (gills and liver) may be used as bioindicators of metal contamination of aquatic environments. Studies on the size and condition factor dependency of heavy metal concentrations have showed that, although the relationship exists, in most cases it was not statistically significant for organs. Metal Pollution Index (MPI) in organs of fish examined was $1.503 < MPI < 3.575$. The daily per capita consumption of 34 g of fish examined showed 0.317-0.624% of Fe, 1.174-3.408% of Zn, 0.455-0.955% of Cu and 0.085-0.278% of Mn of the RDA reference dose.

Keywords: Heavy metals, Freshwater fish, Condition factor, Body weight, Total length, Mazurian Lake District

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Introduction

Fish inhabit different types of waters: streams, rivers, seas, lakes, ponds etc. (Jezierska and Witeska, 2001). Phytophagous fish belong to consumers at the first trophic level, while fish feeding on plankton and small bottom fauna constitute the 2nd order of the food chain. On the other hand, predatory fish represent the next link in the inland waters (Szczerbowski, 1995). According to Moiseenko *et al.* (2005), fish accumulate microelements in their whole lifespan, reflecting the hydrochemical conditions and contamination of water bodies. Zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) are essential micronutrients for humans and other living organisms, including fish and aquatic animals. They play a key role in the metabolism of organs and tissues and in the maintenance of cellular functions (Uauy *et al.*, 1998; Shenkin, 2006; Nadadur *et al.*, 2008; Santamaria, 2008; Angelova *et al.*, 2011; Azaman *et al.*, 2015). These elements, as well as essential elements, are also numbered among heavy metals. Sándor *et al.* (2011) showed that essentiality and toxicity of trace metals in organisms depend on the concentration of the metal; below a certain level they could be considered as essential for biochemical processes, but in the case of a high accumulation in organisms, intoxication may occur. Generally, the bioaccumulation of metals depends on biotic (species, body dimensions and mass, fish age and sex, metabolism, feeding type and position in the trophic pyramid) and abiotic factors (distribution of metals in its environment, water temperature, pH, salinity and interactions with other metals) (Jezierska and Witeska, 2006; Jakimska *et al.*, 2011; Järv *et al.*, 2013; Merciai *et al.*, 2014; Zeitoun and Mehana, 2014; Govind and Madhuri, 2014; Pokorny *et al.*, 2015; Kalisinska *et al.*, 2017). According to Jezierska and Witeska (2001), the higher the metal concentration in the environment, the more may be taken up and accumulated by fish. Pandey and Madhuri (2014) reported that heavy metals can enter from contaminated waters into the fish body by different routes. Most research confirms that fish muscles usually contain lower levels of metals than gills, liver and kidneys (Farkas *et al.*, 2000; Bochenek *et al.*, 2008; Amundsen *et al.*, 2011; Ebrahimpour *et al.*, 2011; Matasin *et al.*, 2011; Al Sayegh-Petcovšek *et al.*, 2012; Zubcov *et al.*, 2012; Bat *et al.*, 2015; Jaćimović *et al.*, 2015; Kalkan *et al.*, 2015; Đikanović *et al.*, 2016; Magu *et al.*, 2016). It is known that fish organs may be used as bioindicators of metal contamination of aquatic systems (Fatima *et al.*, 2014; Authman *et al.*, 2015; Awheda *et al.*, 2015; Salamat *et al.*, 2015; Yancheva *et al.*, 2015; Abdel-Khalek *et al.*, 2016; Nwabunike, 2016). One of the other indicators belonging to a morphological parameters is condition factor (FCF) which can indicate changes in the fish's health state caused by environmental contaminants or stress (Parente and Hauser-Davis, 2013), Yancheva *et al.* (2015) reported that fish are suitable indicators for impaired water quality as they have different size, occupy different tropic levels and are long-living and mobile. Consequently, the aim of this study was to evaluate whether the content of metals related to fish species, different organs (muscles, liver and gills) and the factor condition, body weight or total length of fish from two lakes in Mazurian Lake District (Pluszne and Łańskie) connected with to Łyna River, which are used for recreational purposes. At the same time they attempted to determine whether the fish can be a good indicator of pollution of the aquatic environment, although today the water has a good chemical status.

Materials and methods

A total of 71 specimens of freshwater fish species: roach, *Rutilus rutilus* (L.); bream, *Abramis brama* (L.); whitefish, *Coregonus lavaretus* (L.); pike, *Esox lucius* (L.); Eurasian perch, *Perca fluviatilis* (L.) and tench, *Tinca tinca* (L.) were analyzed (Table 3). The fish were caught from two lakes in Mazurian Lake District (Pluszne and Łańskie) (Fig. 1). These lakes are located next to each other and from Lake Pluszne in the south-east part flows the Poplusz River, which connects to the Łyna River and Lake Łańskie. Fishes from both lakes might migrate and populations might mix. Analysis of the results of priority substances and other polluting substances including Zn, Fe, Cu and Mn showed that none of the chemical indicators exceeded the established limits for exposure and the study found the water body in good chemical status. Therefore, the pollution of lakes examined by elements was similar. The south- western part of the basin of Lake Łańskie was dominated by fields and the forests in the north-east, whereas the total area of the basin of Lake Pluszne is surrounded by the forests. Basic morphometric data on Łańskie and Pluszne Lakes are given in Table 1. All fish samples were collected on the same day. Shortly after catching the fish were euthanized and the body weight and total length of each fish were measured (Table 3). Liver, gills and muscles were sampled from each fish. Muscle tissue was dissected from the dorsal part. The samples were kept in polypropylene bags at -18°C until analysis.

Fulton's condition factor (FCF)

The condition factor of fish was calculated using the Fulton's condition factor (FCF) (Table 3).

$FCF = 100 * W/L^3$

Where:

W is the total body weight of fish (g), L is the total length of fish (cm).

Metal Pollution Index (MPI)

The MPI was determined using the equation by Usero *et al*. (1997) and Abdel-Khalek *et al*. (2016)

$MPI= (M1\times M2 \times M3 \times \ldots.. Mn)^{1/n}$.

Where (Table 3), Mn is the concentration of metal n (mg kg^{-1} wet weight) in a certain tissue.

For analysis of Fe, Zn, Cu and Mn content, samples of muscle tissue (±0.0001 g) in duplicate were dried to

[DOR: 20.1001.1.15622916.2020.19.1.23.5]

constant weight at 105 °C, then the samples were ashed at 450 °C for 12 h. The white ash was dissolved in 1M HNO_3. (Suprapur-Merck). In the case of the liver and gills, the 2-4 g samples of (±0.0001 g) were wet-digested using a mixture of nitric and perchloric acids (3:1) (v/v) at 190°C. Then, each sample was quantitatively transferred into volumetric flasks with deionized water (MILLIPORE). Contents of Fe, Zn, Cu and Mn were determined by using flame atomic absorption spectrometry (Thermo Scientific iCE 3500Z series) with corrections made using a deuterium lamp. The parameters for the working element which were recommended by the manufactures are presented in Table 2. The methods were validated by measuring the elements in reference material: BCR CRM 422 (muscles of cod *Gadus morhua* (L.)) with a certified value of zinc, iron, copper and manganese (zinc- certified 19.6±0.5 mg kg^{-1}, measured 20.649±1.384 mg kg^{-1} n=4; iron- certified 5.46±0.30 mg kg^{-1}, measured 5.236±0.249 mg kg^{-1} n=4; copper- certified 1.05±0.07 mg kg^{-1}, measured 1.078±0.143 mg kg^{-1} n=4; and manganese- certified 0.543±0.028 mg kg^{-1}, measured 0.560±0.034 mg kg^{-1} n=4). The recovery rates of these elements were: 105% for Zn, 96% for Fe, 103% for Cu and 103% for Mn (Certified Reference Material – BCR, cod muscle, Commission of the European Communities - Brussels, March 1992) (Quevauviller *et al.*, 1993). The contents of Zn, Cu, Fe and Mn in muscles, liver and gills of fish are expressed as mg kg^{-1} wet weight (w.w.).

Figure 1: Location of the study area.

[DOR: 20.1001.1.15622916.2020.19.1.23.5]

Table 1: Basic data morphometric.

L.p.	Lake	Łańskie	Pluszne
1.	water Surface (ha)	1042.3	903.3
2.	maximum depth (m)	53.0	52.0
3.	volume of lake (m^3)	168 047.3	134 913.7
4.	total catchment area (km^2)	436.8	69.6
5.	average depth (m)	16.0	14.9
6.	height	134.7	140.0
7.	geographical coordinates	53˚58'60'' N, 20˚48'08'' E	53˚58'30'' N, 20˚42'06'' E
8.	cleanliness class	II	II

Table 2: Instrumental analytical conditions of heavy metals measurement.

Measurement conditions	Elements			
	Zn	Fe	Cu	Mn
absorption wavelengths (nm)	213.9	248.3	324.8	279.5
lamp current (%)	80	75	80	75
slit	100% height - 0,5			
time of measurement (second)	4.0			
Flame and gas flow rate (L min^{-1})	air-acetylene 1.0			
detection limits (mg kg^{-1})	0.1	0.5	0.05	0.05
sensivity (mg L^{-1})	0.05	0.05	0.02	0.02

Table 3: The content of heavy metals (means±SD.) and correlation coefficients between fish size (body weight and total length) and concentration of metals in muscles, liver and gills of different fish species.

Species	Weight (g) Length (cm) min- max (mean±SD)		Fe	Zn	Cu	Mn	MPI	FCR	main food
			min - max (mg/kg wet weight)						
Perch n=31	214.4 – 608.9 (415.0±109.8)	muscles	0.973 - 2.473^{c}	3.386 – 5.674□	0.115 – 0.311^{b}	0.071 – 0.187□	1.608	1.447 – 1.862 1.590±0.133	Plankton, small invertebrates – fish smaller than 10cm, Top predator – large perch
		liver	13.89 – 54.66□	18.97 – 28.61□	1.842 – 5.525□	1.231 – 1.912□	2.821		
	24.35 – 33.20 (29.44±2.45)	gills	20.32 – 32.19□	20.03 – 25.18□	0.267 – 0.313□	0.911 – 2.885□	2.669		
Pike n=11	743.4 – 1844.0 (1162.70±345.64)	muscles	0.958 – 1.801^{c}	6.542 – 13.43^{c}	0.104 – 0.219^{c}	0.070 – 0.224^{c}	1.842	0.588 – 0.807 0.693±0.071	Plankton – first food, Top predator
		liver	16.05 – 144.9□	24.45 – 61.34□	3.372 – 16.94□	0.578 – 1.358□	3.145		
	50.00 – 66.50 (54.75±5.24)	gills	15.99 – 32.74□	90.53 – 176.9□	0.172 – 0.421□	2.539 – 4.310□	3.567		
Tench n=6	472.1 – 958.4 (741.15±205.720	muscles	1.375 – 3.183^{c}	3.246 – 4.707^{c}	0.127 – 0.245^{c}	0.054 – 0.071^{c}	1.557	1.477 – 1.797 1.628±0.127	Bottom fauna, mostly crustaceans, insect larvae, worms andsnails
		liver	27.45 – 72.50□	22.62 – 27.34□	15.00 – 34.64□	0.953 – 1.311□	3.252		
	31.10 – 39.20 (35.47±3.38)	gills	25.54 – 40.44□	14.32 – 17.34^{a}	0.372 – 0.464□	1.036 – 1.718□	2.665		
Roach n=9	**329.9 – 542.8 (454.57±77.31)**	muscles	1.617 – 3.840^{c}	3.662 – 5.801^{c}	0.164 – 0.432□	0.036 – 0.263□	1.657	1.241 – 1.813 1.511±0.199	Plankton – for the first two years, molluscs and crustaceans - later
		liver	57.15 – 192.3□	19.58 – 33.89□	3.167 – 15.77□	0.917 – 2.117□	3.575		
	29.30 – 33.30 (31.07±1.31)	gills	25.12 – 37.29□	76.18 – 195.9□	0.233 – 0.487□	0.944 – 3.008□	3.464		
Whitefish n=9	275.5 – 592.7 (422.33±98.13)	muscles	0.882 – 2.024^{c}	2.589 – 4.547^{c}	0.148 – 0.321^{c}	0.041 – 0.070^{c}	1.503	0.833 – 1.124 0.962±0.095	Zooplankton and plankton, Benthic feeders - later
		liver	44.22 – 110.7□	22.87 – 39.60□	2.423 – 21.37□	1.015 – 2.015□	3.277		
	32.10 – 37.50 (35.09±1.84)	gills	21.27 – 69.38□	18.45 – 104.0□	0.261 – 0.463□	1.497 – 2.862□	3.242		
Bream n=5	423.6 – 674.0 (538.07±101.59)	muscles	0.761 – 1.761^{c}	3.649 – 4.788^{c}	0.141 – 0.232^{c}	0.079 – 0.267^{c}	1.561	0.943 – 1.216 1.110±0.108	Plankton – first food, insect larvae, crustaceans, oligochaets and chironomids - later
		liver	37.04 – 96.91□	26.89 – 35.40□	1.584 – 16.03□	0.508 – 1.358□	3.160		
	33.70 – 41.50 (36.44±3.09)	gills	28.22 – 40.44□	17.28 – 21.49□	0.481 – 0.559□	3.419 – 7.806□	2.777		

n- number of fish; MPI – Metal Pollution Index; FCF – Fulton´s Condition Factor; SD – standard deviation; a, b, c – significant differences between the organs of the same species ($p\leq0.05$) (in columns). The same letter indicates the absence of significant differences between muscles, liver and gills in the same fish species ($p>0.05$);

Statistical analysis

The results are given as means, standard deviations (±SD) and range. The statistics data were grouped according to species and organs. After testing for homogeneity of variance (test Levene's), the one-way analysis of variance ANOVA (*post-hoc* Duncan's test) was used to test significant differences in the average content of metals studied both between seven species and organs of the same species. Differences were significant at $p \leq 0.05$. The correlation coefficients between content of metal and condition factor FCF, body weight and total length of fish were calculated using STATISTICA12 program (StatSoft Polska. Sp). The significance levels of $p \leq 0.05$ were used.

Results

Interspecific differences in the content of heavy metals in muscles, liver and gills of seven freshwater fish were studied (Fig. 2). The Fe levels in muscles of roach (2.550 mg kg^{-1}) were significantly higher than other fish species ($p \leq 0.05$). The liver of roach also contained more iron (123.6 mg kg^{-1}) than that of the other fishes studied ($p \leq 0.05$). Statistically significant differences were observed between the content of iron in gills of whitefish (45.45 mg kg^{-1}) and other species ($p \leq 0.05$). The muscles, gills and liver of pike contained more zinc (9.950, 135.0 and 41.40 mg kg^{-1}, respectively) compared to the other studied fish ($p \leq 0.05$). The content of copper was significantly higher in muscles of roach (0.279 mg kg^{-1}) than in muscles of other fish examined ($p \leq 0.05$), whereas the significantly higher value of copper in liver was found for tench (27.14 mg kg^{-1}) ($p \leq 0.05$). The amount of copper in gills of perch studied was significantly lower (0.177 mg kg^{-1}), whereas the concentration of these metals in gills of bream (0.523 mg kg^{-1}) was significantly higher ($p \leq 0.05$). The muscles of bream contained significantly higher amounts of manganese (0.162 mg kg^{-1}) except in the case of perch, tench and whitefish ($p \leq 0.05$). The liver of perch (1.681 mg kg^{-1}) had a higher content of manganese compared with the other examined fish, with the exception of roach (1.567 mg kg^{-1}) ($p \leq 0.05$). In the case of manganese in gills, there were significant differences between bream (5.113 mg kg^{-1}) and other fish examined ($p \leq 0.05$).

According to Regulation of the Minister of Health on foodstuff intended for particular nutritional uses, Recommended Daily Allowances (RDA) (mg $capita^{-1}$ day^{-1}, for consumers weighing 70 kg) for Fe, Zn, Cu and Mn was 14, 10, 1 and 2, respectively (Table 7). If fish consumption in 2015 amounted to 12.5 kg per capita (adults of body weight 70 kg) (Statistical Yearbook of Agriculture, 2016) it daily consumed 0.044-0.087 mg of Fe, 0.117-0.341 mg of Zn, 0.005-0.010 mg of Cu and 0.002-0.006 mg of Mn that corresponded to 0.317-0.624% of Fe, 1.174-3.408% of Zn, 0.455-0.955% of Cu and 0.085-

[DOR: 20.1001.1.15622916.2020.19.1.23.5]

0.278% of Mn, of the RDA reference dose. (http://www.ecolex.org/details/legislation/regulation-on-foodstuffs-intended-for-particular-nutritional-uses-lex-faoc113738/;http://stat.gov.pl/en/topics/statistical-yearbooks/statistical-yearbooks/statistical-yearbook-of-agriculture-2016,6,11.html, (in Polish).

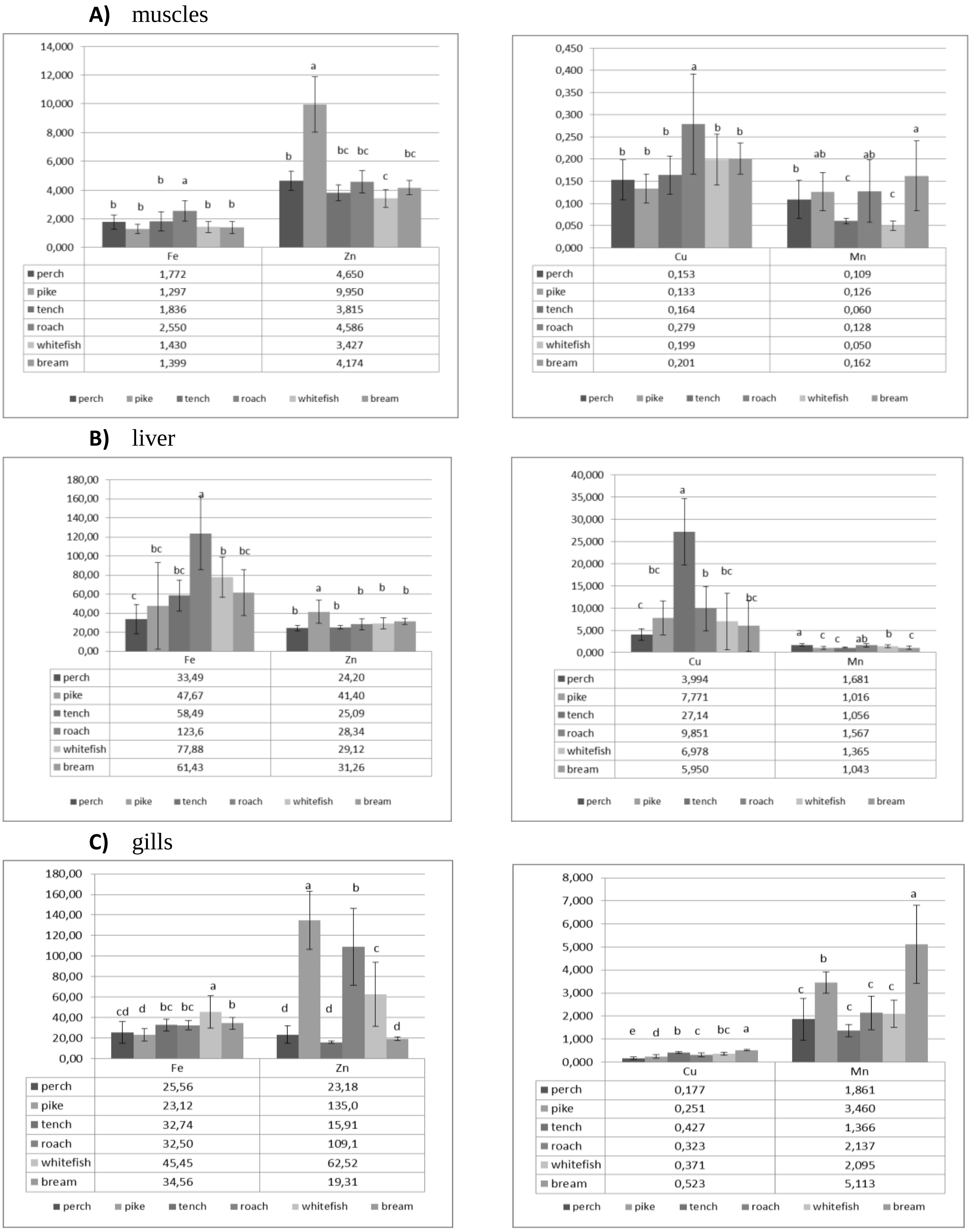

Figure 2: Interspecific differences (mean±SD) in the content of heavy metals in the same organs of fish, a) muscles, b) liver, c) gills.
a, b, c, d, e – significant differences between the same organs of the different species ($p\leq0.05$). The same letter indicates the absence of significant differences ($p>0.05$).

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The content of Fe, Zn, Cu and Mn varied between selected organs (muscles, liver and gills) (Table 3). Generally, the liver of the examined fish was characterized by significantly high contents of Fe and Cu (with the exception of copper in perch) ($p \leq 0.05$). Whereas, the concentration of Mn and Zn (with the exception of Zn in tench) was significant higher in gills ($p \leq 0.05$). In almost all cases (with the exception of copper in roach), the muscles were characterized by a lower content of Zn, Cu, Fe and Mn than the liver and gills ($p \leq 0.05$). There were no statistically significant differences in the content of Cu in muscles and gills of roach ($p > 0.05$). Metal Pollution Index (MPI) was lower in muscles of each fish species and below 2 (Table 3). The higher MPI was found in liver of fish (with the exception of pike), because in the case of pike, the gills were characterized by higher values of MPI. The pollution of these metals in each tissue varied from not impacted contamination to very low contamination.

In most cases, the correlation between the concentration of metals studied in the muscles, liver and gills of fish and fish size (body weight and total length) was not statistically significant ($p > 0.05$) (Table 4 and 5). There were a negative correlations between the levels of Fe in muscles of pike (r=-0.601, p=0.05). The contents of copper in muscles of bream were negatively correlated with total length of these fish (r=-0.932, p=0.021). Positive correlation coefficients were observed between Zn level in muscles of whitefish (r=0.744, p=0.034) and body weight. Negative correlation coefficients were found between Mn content in gills of perch and length or weight body (r=-0.694, p=0.006 and r=-0.754, p=0.002, respectively). Similarly, for liver of bream and roach there was a negative correlation between the length and zinc concentration (r = -0.886, p=0.045 and r=-0.698, p=0.037, respectively). In most cases, there were no significant correlations between the content of metals and fish condition. The positive correlation coefficient between the condition factor and Zn level was at r=0.902 for muscles of whitefish (p=0.002). The content of Mn grew linearly with condition factor and was r=0.761 (muscles of roach, p=0.017) and 0.936 (liver of bream, p=0.019). The Fe levels in gills of pike (r=-0.835, p=0.001) decreased as condition factor increased. A similar correlation was found for Zn and Cu in liver of pike (r=-0.767, p=0.006 and r=-0.741, p=0.009, respectively), for Cu in gills of roach (r=-0.723, p=0.028).

The heavy metals content in muscles of all fish examined was identified to have the following decreasing sequence (Fig. 2): Zn>Fe>Cu>Mn. In the case of fish liver, the concentration of these elements followed the pattern Fe>Zn> Cu>Mn (with the exception of tench). The content of metals in liver of tench included in this study showed the following sequence: Fe>Cu>Zn>Mn.

The metal values in gills of perch, tench, and bream were in a descending order of Fe>Zn>Mn>Cu, whereas in gills of Pike, Roach and Whitefish, it was Zn>Fe>Mn>Cu. Significant positive correlation coefficient were noted between the following metals pairs (Table 6): Fe-Zn (in liver and gills of perch, muscles of pike), Zn-Cu (in muscles and liver of pike, muscles and gills of tench, liver of whitefish), Cu-Mn (in gills of perch, muscles of roach), Fe-Mn (in gills of pike, muscles of roach).

Table 4: The correlation coefficients between fish size (body weight and total length) or FCR and concentration of Fe and Zn in muscles, liver and gills of different fish species.

		weight	length	FCF	muscles	liver	weight	length	FCF	muscles	liver
				Fe					**Zn**		
perch	muscles	0,428 P=0,126	0,383 P=0,176	0,183 P=0,531			0,181 P=0,536	0,149 P=0,611	0,005 P=0,986		
	liver	-0,230 P=0,429	-0,249 P=0,390	-0,200 P=0,493	0,057 P=0,848		-0,092 P=0,753	-0,082 P=0,781	0,117 P=0,691	-0,244 P=0,401	
	gills	-0,245 P=0,398	-0,163 P=0,578	-0,462 P=0,096	-0,379 P=0,181	0,401 P=0,156	-0,186 P=,524	-0,148 P=,615	-0,092 P=0,755	-0,216 P=0,458	-0,130 P=0,659
pike	muscles	**-0,601 P=0,050**	-0,545 P=,083	-0,216 P=0,524			0,123 P=0,718	0,098 P=0,774	0,0991 P=0,772		
	liver	-0,112 P=0,742	-0,175 P=0,606	0,234 P=0,489	0,150 P=0,660		-0,510 P=0,109	-0,326 P=0,327	**-0,767 P=0,006**	-0,013 P=,970	
	gills	-0,228 P=0,500	-0,021 P=0,951	**-0,835 P=0,001**	0,248 P=0,463	-0,197 P=0,561	-0,262 P=0,437	-0,229 P=0,498	-0,172 P=0,613	**0,653 P=,029**	0,452 P=0,163
tench	muscles	0,382 P=0,455	0,416 P=0,411	-0,211 P=0,688			-0,060 P=0,911	0,025 P=0,962	-0,435 P=0,389		
	Liver	-0,694 P=0,126	-0,688 P=0,131	0,002 P=0,997	**-0,899 P=,015**		0,090 P=0,865	0,165 P=0,755	-0,060 P=0,910	-0,595 P=0,213	
	gills	0,765 P=0,076	0,774 P=0,071	-0,061 P=0,908	-0,113 P=0,832	-0,158 P=0,765	0,726 P=0,103	0,801 P=0,056	-0,174 P=0,742	0,268 P=0,608	0,350 P=0,496
roach	Muscles	0,581 P=0,101	0,109 P=0,780	0,656 P=0,055			0,294 P=0,443	-0,215 P=0,579	0,631 P=0,068		
	Liver	-0,659 P=0,054	-0,485 P=0,185	-0,390 P=0,300	-0,596 P=0,090		-0,517 P=0,154	**-0,698 P=0,037**	-0,012 P=0,975	-0,226 P=0,559	
	gills	0,208 P=0,592	0,628 P=0,070	-0,374 P=0,321	0,098 P=0,803	-0,345 P=0,363	0,062 P=0,875	-0,390 P=0,299	0,489 P=0,181	**0,851 P=0,004**	-0,215 P=0,579
whitefish	Muscles	-0,272 P=0,515	-0,498 P=0,210	0,143 P=0,736			**0,744 P=0,034**	0,505 P=0,202	**0,902 P=,002**		
	Liver	0,500 P=0,207	0,472 P=0,238	0,334 P=0,419	-0,460 P=0,252		0,185 P=0,661	0,066 P=0,876	0,200 P=0,636	0,175 P=0,679	
	gills	-0,033 P=0,938	0,053 P=0,901	-0,045 P=0,916	-0,384 P=0,348	0,135 P=0,750	-0,100 P=,814	-0,174 P=0,681	-0,048 P=0,910	-0,037 P=0,931	-0,075 P=0,859
bream	Muscles	-0,577 P=0,309	-0,782 P=0,118	0,848 P=0,070			-0,820 P=0,089	-0,797 P=0,106	0,467 P=0,428		
	Liver	-0,250 P=0,685	-0,079 P=0,899	-0,312 P=0,610	0,127 P=0,839		-0,788 P=0,113	**-0,886 P=0,045**	0,747 P=0,147	**0,920 P=0,027**	
	gills	0,010 P=0,987	-0,261 P=0,672	0,710 P=0,179	0,324 P=0,5 95	**-0,888 P=0,044**	-0,748 P=0,146	-0,727 P=0,164	0,424 P=0,477	0,764 P=0,133	0,640 P=0,245

P- significant level

Table 5: The correlation coefficients between fish size (body weight and total length) or FCR and concentration of Cu and Mn in muscles, liver and gills of different fish species.

		weight	length	FCF	muscles	liver	weight	length	FCF	muscles	liver
				Cu					**Mn**		
perch	Muscles	0,384 P=0,175	0,214 P=0,463	0,347 P=0,224			0,272 P=0,346	0,163 P=0,579	0,219 P=0,452		
	Liver	-0,024 P=0,935	-0,098 P=0,739	0,119 P=0,685	0,392 P=0,166		-0,334 P=0,242	-0,349 P=0,221	-0,019 P=0,948	-0,463 P=0,095	
	gills	-0,324 P=0,258	-0,256 P=0,378	-0,2650 P=0,360	0,037 P=0,900	0,459 P=0,099	**-0,694 P=0,006**	**-0,754 P=0,002**	0,034 P=0,909	-0,144 P=0,624	0,440 P=0,116
pike	Muscles	-0,168 P=,622	-0,172 P=0,614	0,005 P=0,987			-0,005 P=0,989	-0,028 P=0,936	0,134 P=0,695		
	Liver	-0,512 P=0,107	-0,325 P=0,329	**-0,741 P=0,009**	0,064 P=0,852		0,086 P=0,802	0,236 P=0,485	-0,600 P=0,051	0,248 P=0,462	
	gills	-0,183 P=0,591	-0,326 P=0,327	0,550 P=0,080	0,389 P=0,237	-0,402 P=0,220	-0,544 P=0,084	-0,410 P=0,211	-0,534 P=0,091	0,061 P=0,858	0,134 P=0,693
tench	Muscles	0,3004 P=0,563	0,400 P=0,432	-0,379 P=0,459			0,608 P=0,201	0,604 P=0,204	-0,122 P=0,817		

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Table 5 continued:

	Liver	0,534 P=0,275	0,337 P=0,514	0,791 P=0,061	-0,096 P=0,857		-0,394 P=0,440	-0,498 P=0,314	0,517 P=0,292	-0,328 P=0,525	
	gills	0,368 P=0,472	0,433 P=0,391	-0,075 P=0,888	0,5682P =0,239	0,337 P=0,514	0,265 P=0,612	0,264 P=0,613	-0,213 P=0,685	**0,837 P=0,038**	-0,516 P=0,294
roach	Muscles	0,459 P=0,214	0,255 P=0,509	0,402 P=0,284			0,530 P=0,142	0,001 P=0,998	**0,761 P=0,017**		
	Liver	0,005 P=0,990	-0,395 P=0,293	0,366 P=0,333	0,300 P=0,432		0,225 P=0,560	0,178 P=0,646	0,216 P=0,577	0,609 P=0,082	
	gills	-0,648 P=0,060	-0,152 P=0,697	**-0,723 P=0,028**	-0,274 P=0,475	-0,079 P=0,840	0,416 P=0,265	0,247 P=0,521	0,384 P=0,308	**0,810 P=0,008**	**0,773 P=0,015**
whitefish	Muscles	0,121 P=0,775	-0,084 P=0,844	0,500 P=0,207			0,535 P=0,171	0,634 P=0,091	0,297 P=0,476		
	Liver	0,506 P=0,201	0,360 P=0,381	0,512 P=0,194	0,059 P=0,890		0,127 P=0,764	0,184 P=0,662	0,090 P=0,832	0,569 P=0,141	
	gills	-0,387 P=0,344	-0,368 P=0,370	-0,181 P=0,667	0,618 P=0,102	**-0,706 P=0,050**	-0,255 P=0,543	-0,084 P=0,844	-0,536 P=0,171	0,064 P=0,881	0,203 P=0,630
bream	Muscles	-0,837 P=0,077	**-0,932 P=,021**	0,718 P=0,172			0,200 P=0,747	-0,131 P=0,834	0,747 P=0,147		
	Liver	-0,263 P=0,669	-0,457 P=0,439	0,715 P=0,174	0,221 P=0,721		-0,503 P=0,387	-0,743 P=0,151	**0,936 P=0,019**	0,734 P=0,158	
	gills	0,047 P=0,940	0,341 P=0,574	-0,768 P=0,130	-0,560 P=0,326	-0,204 P=0,742	-0,415 P=0,487	-0,410 P=0,493	0,216 P=0,726	0,245 P=0,691	0,481 P=0,412

P- significant level

Table 6: Correlation coefficients (r) of dependence between contents of metal in fish.

	Zn	**Cu**	**Mn**	**Zn**	**Cu**	**Mn**	**Zn**	**Cu**	**Mn**
Perch (n=31)		**muscles**			**Liver**			**gills**	
Fe	0.174	0.350	0.437	0.776***	-0.153	-0.326	0.935***	0.418	0.413
Zn		0.393	-0.183		0.068	-0.472		0.455	0.369
Cu			0.424			-0.051			0.588*
Pike (n=11)		muscles			Liver			gills	
Fe	0.194	0.658*	-0.234	-0.107	-0.096	-0.079	0.215	-0.587	0.659*
Zn		0.751**	0.366		0.776**	0.533		0.006	0.230
Cu			0.015			0.685*			-0.117
Tench (n=6)		muscles			Liver			gills	
Fe	0.881*	0.923**	0.835*	0.322	-0.274	0.181	0.280	-0.067	0.163
Zn		0.836*	0.662		0.074	0.247		0.844*	0.029
Cu			0.629			0.422			-0.326
Roach (n=9)		muscles			Liver			gills	
Fe	0.659	0.365	0.668*	0.518	-0.220	0.031	-0.455	-0.107	0.190
Zn		0.432	0.730*		0.587	-0.396		-0.615	0.305
Cu			0.762*			-0.377			-0.609
Whitefish (n=9)		muscles			Liver			gills	
Fe	0.319	0.228	-0.636	0.178	0.269	0.459	0.397	0.660	0.336
Zn		0.269	0.086		0.902**	-0.656		0.332	0.331
Cu			0.033			-0.598			0.056
Bream (n=5)		muscles			Liver			gills	
Fe	0.409	0.841	0.409	0.177	-0.110	-0.520	0.405	-0.872	0.549
Zn		0.526	-0.116		0.784	0.712		-0.152	0.642
Cu			0.247			0.667			-0.496

n - number of fish; *significant correlation ($p \leq 0.05$), ** highly significant correlation ($p \leq 0.01$), *** very highly significant correlation ($p \leq 0.001$).

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Table 7: Coverage of the recommended daily allowances of metals (%).

Species	Fe	Zn	Cu	Mn	References
Perch	0.433	1.592	0.524	0.187	This study
Pike	0.317	3.408	0.455	0.217	This study
Tench	0.449	1.307	0.561	0.103	This study
Whitefish	0.350	1.174	0.680	0.085	This study
Roach	0.624	1.570	0.955	0.219	This study
Bream	0.342	1.429	0.690	0.278	This study
RDA	14	10	1	2	(Regulation of the Minister of Health, 2010)
AI			1.6* 1.3**	3	(EFSA, 2013; EFSA, 2015)
ARs	6.2-10.2*** 7.5-12.7****				(EFSA, 2014)

RDA – Recommended Daily Allowances (mg capita^{-1} day^{-1}. for consumers with weight 70 kg)
AI – Adequate Intake in adults (mg capita^{-1} day^{-1})
ARs – Average Requirements (mg capita^{-1} day^{-1})
*-for adults men; ** - for adults women; *** - for women with a reference weight of 58.5 kg; **** - for men with a reference weight of 68.1 kg

Discussion

This study of chosen heavy metals content in freshwater fish showed both differences between some species, as well as among their organs (Fig. 2 and Table 3). El-Moselhy *et al.* (2014) reported that metal accumulation varied between organs and species depending on species-specific factors like feeding behavior, swimming patterns and genetic tendency, and/or other factors like age and geographical distribution that caused variation in metals accumulations between fish even from the same species. Jakimska *et al.* (2011) noted that the bioaccumulation of metals in tissues of animals depended on biotic factors like diet and position in the trophic web. Jezierska and Witeska (2001) found that the differences in body metal concentration may result from different feeding rates, food composition and feeding site. Mazej *et al.* (2010) also found that zinc and other metals (Hg, Pb and Cd) in organs of fish varied considerably both between species and tissues. According to our previous study, the content of Fe in fish muscles was affected by the feeding habits (vendace>roach>bream ≈ whitefish>perch≈pike) ($p\leq0.05$) (Łuczyńska *et al.*, 2006). The same authors reported that the concentration of Zn, Cu and Mn in muscles of fish gave rise to the following sequence: pike>vendace≈roach>perch>whitefish ≈bream; vendace>roach≈bream> whitefish≈pike≈perch; vendace>roach ≈whitefish≈bream and pike>perch ($p\leq0.05$), respectively. Interspecific differences in the content of Fe and Cu ($p\leq0.05$) in muscles of bream (3.94 and 0.79 mg kg^{-1}, respectively) and pike (5.01 and 1.16 mg kg^{-1}, respectively) could be due to their different feeding habits (i.e., benthophagous – bream, piscivorous – pike) (Grela *et al.*, 2010). The results presented by Hosseini *et al*.

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(2015) also showed that the concentration of heavy metals (Cd, Co, Cu, Ni, Pb, Fe and Hg) in fish from Khuzestan shore (northwest of the Persian Gulf) was strongly affected by habitat and feeding habit and increased in the following order: benthic omnivorous fish>zooplanktivore fish> phytoplanktivore fish>piscivore fish. Lidwin-Kaźmierkiewicz *et al.* (2009) found the lowest content of Mn in muscles of pike, whereas Zn level was significantly higher ($p \leq 0.05$) in pike and perch than in bream and carp, *Cyprinus carpio* L. This is in accordance with the results of our study (Fig. 2). The data indicated by Kenšová *et al.* (2010) showed that Zn concentration in non-predatory fish species was higher than in predatory fish. The same authors observed that Cu concentration in all tissues (with the exception of liver) was comparable in all the fish species. In the case of liver, the concentration of Cu could be ranked as follows: asp>carp>bream>pike>pikeperch (*Sander lucioperca* L.). Szarek-Gwiazda and Amirowicz (2006) found a relationship between the concentrations of metals (Cd, Pb, Cu, Mn, Fe and Sr) in some fish tissues and their trophic habits. According to Łuczyńska *et al.* (2009), muscles of the benthophagous species (roach and bream) had more Fe than piscivorous species (pike and perch) ($p \leq 0.01$), whereas there were no clear differences in the concentrations of Mn, Cu and Zn between groups of non-predatory and predatory fish ($p > 0.01$). In turn, Svecevičius *et al.* (2014) showed that benthophagous fish (gibel carp, *Carassius gibelio* and roach) accumulated more Zn and Cu than predatory fish (perch and pike).

Accumulation of metals in the fish organs is a function of uptake and depuration rates (Jezierska and Witeska, 2001). According to these authors, concentrations of metals in various organs may change during exposure, according to various patterns. Lenhardt *et al.* (2012) observed that the content of Cu was higher in liver, Mn in gills, whereas Fe and Zn was higher in both liver and gills. The lowest contents of most elements were found in muscles. Similar findings were published by Rajkowska and Protasowicki (2013). These results are in good agreement with those of Zubcov *et al.* (2012), Yancheva *et al.* (2014), Milošković and Simić (2015) and Arantes *et al.* (2016). According to the above authors, the content of Cu and Zn for all species and locations was the lowest in muscles. Therefore, the liver is often considered a good monitor of water pollution with metals since their concentrations are proportional to those present in the environment (Jezierska and Witeska, 2001). Shinn *et al.* (2009) observed that contents of Cu, Zn were significantly higher in the liver of bream, perch and roach than in muscles. Khaled *et al.* (2016) comparing concentration of metals in *Claries lazera* collected from El Ebrahimia canal (Egypt) showed the following accumulation ranking: liver>gills> kidney>blood>muscles. The concentration of copper in all studied organs of carp from the Indus river (Pakistan) was recorded in the order of liver>kidney>muscles>gills (Mahboob *et al.,* 2016). Differences in Zn, Fe, Cu and Mn between muscles, liver and gills of perch were also observed by Klavins *et al.* (2009). A similar observation was made by Farkas *et al.* (2001) for Cu and Zn in bream. Namin *et al.* (2011) showed that the Zn content in muscle tissue of pike was slightly higher than in the liver, while the level of Cu was significantly higher in the liver than in muscles. Zinc accumulates in the body tissues of bentophagous and predatory fishes in the following order: gills>liver>muscle (Pilecka *et al*., 2015). Jezierska and Witeska (2006) showed that at the beginning of waterborne exposure metal concentrations in the gills rapidly increase, and then usually decline, while after the end of exposure metals are rapidly removed from the gills. Rajkowska *et al.* (2008) observed that Fe and Cu accumulated in the liver of roach, Mn accumulated mostly in the gills, whereas Zn accumulation was similar in the gills and kidney. The same author also found that the lowest content of those metals was in muscles. According Jezierska and Witeska (2006), levels of metal in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared.

The smaller and younger fish accumulate greater amounts of metals examined than larger, older fish, which could be attributable to the higher metabolic rate (Jezierska and Witeska, 2001). In the present study, there were only a few significant correlations between the levels of metals in organs of fish and body weight or total length ($p \leq 0.05$) (Table 4 and Table 5). Szarek-Gwiazda and Amirowicz (2006) found very weak correlations between fish length or weight and metal contents (Cu, Fe, Mn) in tissues of roach and perch, whereas level of metals such as Hg, Zn, Pb, Cd and Cr increased with roach size from the Dije River basin (Czech Republic) (Dvořák *et al*., 2014). Negative correlations were reported between the content of Cu, Mn and Fe and a positive correlation between Zn and size of perch, although they were not statistically significant ($p>0.05$) (Klavins *et al.*, 2009). A positive correlation for Cu and Fe and a negative correlation for Zn and Mn in muscles of fish from Żnin Duże Lake (Poland) and the body length were observed by Stanek *et al.* (2005). The content of Cu in the liver of bream positively related to size (length and weight), whereas in the case of muscles and gills the content of Cu and Zn, as well as Zn for liver, negatively related to size (Farkas *et al.*, 2003). According to Rajkowska and Protasowicki (2013), the concentrations of Zn, Cu and Fe were also correlated with body weight or length. Milošković and Símić (2015) found a few significant correlations

[DOR: 20.1001.1.15622916.2020.19.1.23.5]

between the element accumulation (As, Sn, Ni, Co, Al, Se and Fe) and fish size and weight, while they observed the most correlations between element accumulation and fish size and weight in the tissues of pike, which could probably be explained by life histories, as well as by habitat of this species. Kostecki (2000) found that as opposed to roach, the content of Zn and Mn in muscles of tench decreased as fish weight increased. The results of this author are not consistent with the results obtained in this work (Table 4 and 5). Kasimoglu (2014) showed that the correlations between the trace metal concentrations of muscles (Co, Cr, Cu, Fe, Mn, Ni and Zn) and the condition factor (FCF) of eel, *Anguilla anguilla* L. varied with characteristic opposite trends compared to those related to, length, weight and age. Whereas Farkas *et al.* (2001) did not find any significant correlation between contents of Cu and Zn in organs and the condition factor of bream ($p>0.05$). Similarly, Hama *et al.* (2015) observed that there was no significant correlation between heavy metals (Cd, Zn, Cr, Cu and Pb) and condition factor of fish from Lake Ranya (Iraq), while Alkan *et al*. (2016) found different correlations between metals, including Cu and Zn and FCF of the fish species *Trachurus mediterraneus*, *Engraulis encrasicolus ponticus*, and *Sprattus sprattus*.

The decreasing sequence (Table 6): Zn>Fe>Cu>Mn in muscles of all fish examined is in good agreement with the previous studies reported by Łuczyńska *et al.* (2009). Ebrahimpour *et al.* (2011) showed that the muscles, gills and liver of pike accumulated more zinc than copper. These patterns were found for perch by Yazdi *et al.* (2012). According to Brázová *et al.* (2012), the content of heavy metals in organs of perch decreased in the order Zn>Cu>Mn. These results are consistent with the present study (except for gills of all fish and liver of tench and rainbow trout) (Table 6). Klavins *et al.* (2009) noted that in gills of perch, the pattern of metal content was: Zn>Fe>Mn>Cu; whereas in the liver and muscles it was Fe>Zn>Cu>Mn. On the other hand, Staniskiene *et al.* (2006) found the following sequences: Zn>Fe>Cu>Mn (in fish flesh) and Fe>Zn>Mn>Cu (in fish liver and gills), whereas Andreji *et al.* (2006) noted that in muscles of roach the order of the studied elements was Fe>Zn>Mn>Cu. Iron and then zinc were predominant in muscles of perch from the Ob River basin (Osipova *et al.*, 2015).

The obtained results showed interspecific differences between the concentration of heavy metals in fish species belonging to the food chain of freshwater aquatic ecosystems and having different feeding habits (piscivorous and bentophagous). In most cases, the highest contents of iron and copper were found in the liver, whereas it was zinc and manganese in gills for tench. Therefore, these organs may be good indicators of freshwater pollution. Generally, lower concentrations of these metals were found in muscles. A few significant correlations were also observed between the levels of metals in organs and condition factor, body weight or total length. Existing small differences require further investigation. Otherwise, the fish species is safe for human consumption but the levels of these metals should be controlled to avoid excessive intake of elements.

Acknowledgements

This research was partially financed by Chair of Commodity and Food Analysis (statutory research No. 17.610.008-300)

References

Abdel-Khalek, A.A., Elhaddad, E., Mamdouh, S. and Saed Marie, M.A., 2016. Assessment of metal pollution around Sabal Drainage in River Nile and its impacts on bioaccumulation level, metals correlation and human risk hazard using *Oreochromis niloticus* as a bioindicator. *Turkish Journal of Fisheries and Aquatic Sciences*, 16, 227-239. DOI:10.4194/1303-2712-v16_2_02

Alkan, N., **Alkan, A., Gedik, K. and Fisher, A., 2016**. Assessment of metal concentrations in commercially important fish species in Black Sea. *Toxicology and Industrial Health*, 32(**3**), 447-456. DOI:10.1177/0748233713502840

Al Sayegh-Petkovšek, S., Mazej Grudnik, Z. and 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. *Environmental Monitoring and Assessment*, 184(**5**), 2647-2662. DOI:10.1007/s10661-011-2141-4

Amundsen, P.-A., Kashulin, N.A., Terentjev, P., Gjelland, K.Ø., Koroleva, I.M., Dauvalter, V.A., Sandimirov, S., Kashulin, A. and Knudsen, R., 2011. Heavy metal contents in whitefish (*Coregonus lavaretus*) along a pollution gradient in a subarctic watercourse. *Environmental Monitoring and Assessment*, 182(**1-4**), 301-316. DOI:10.1007/s10661-011-1877-1

Andreji, J., Stránai, I., Massányi, P. and Valent, M., 2006. Accumulation of some metals in muscles of five fish species from lower Nitra River. *Journal of Environmental Science and Health*, Part A, 41(**11**), 2607-2622. DOI:10.1080/10934520600928003

Angelova, M., Asenova, S., Nedkova, V. and Koleva-Kolarova, R., 2011. Copper in the human organism. *Trakia Journal of Sciences*, 9, 88-98.

Arantes, F.P., Savassi, L.A., Santos, H.B., Gomes, M.V.T. and Bazzoli, N., 2016. Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil. *Annals of the Brazilian Academy of Sciences,* 88(**1**), 137-147. DOI:org/10.1590/0001-3765201620140434

Authman, M.M.N., Zaki, M.S., Khallaf, E.A. and Abbas, H.H., 2015. Use of fish as bio-indicator of the effects of heavy metals pollution. Journal Aquaculture Research and Development, 6(**4**), 1-13. DOI:10.4172/2155-9546.1000328

Awheda, I., Ahmed, A.Y., Fahej, M.A.S., Elwahaishi, S.S. and Smida, F.A., 2015. Fish as bioindicators of heavy metals pollution in marine environments: a review. *Indian Journal of Applied Research*, 5(**8**), 379-384.

Azaman, F., Juahir, H., Yunus, K., Azid, A., Kamarudin, M.K.A., Tariman, E.M., Mustafa, A.D., Amran, M.A., Hasnam, Ch.N.Ch. and Saudi, A.S.M., 2015. Heavy metal in fish: Analysis and human health-a review. *Jurnal Teknologi*, 77(**1**), 61-69.

Bat, L., Anci, E., Sezgin, M. and Şahin, F., 2015. Heavy metal levels in the liver and muscle tissues of the four commercial fishes from Lake Balik, Kizilirmak Delta (Samsun, Turkey). *Journal of Coastal Life Medicine*, 3(**12**), 950-955. DOI:10.12980/jclm.3.2015j5-224

Bochenek, I., Protasowicki, M. and Brucka-Jastrzębska, E., 2008. Concentrations of Cd, Pb, Zn, and Cu in roach, *Rutilus rutilus* (L.) from the lower reaches of the Oder River, and their correlation with concentrations of heavy metals in bottom sediments collected in the same area. *Archives of Polish Fisheries*, 16(**1**), 21-36. DOI:10.2478/s10086-008-0002-8

Brázová, T., Torres, J., Eira, C., Hanzelová, V., Miklisová, D. and Šalamún, P., 2012. Perch and its parasites as heavy metal biomonitors in a freshwater environment: The case study of the Ružín water reservoir, Slovakia. *Sensors*, 12(**3**), 3068-3081. DOI:10.3390/s120303068

Đikanović, V., Skorić, S. and Gačić, Z., 2016. Concentrations of metals and trace elements in different tissues of nine fish species from the Meduršje Reservoir (West Morava River Basin, Serbia). *Archives of Biological Sciences*, 68(**4**), 811-819. DOI:10.2298/AB151104069D

Dvořak, P., Andreji, J., Dvořáková-Líšková, Z. and Vejsada, P., 2014. Assessment of selected heavy metals pollution in water, sediments and fish in the basin Dyje, Czech Republic. *Neuroendocrinology Letters*, 35(**2**), 101-109.

Ebrahimpour, M., Pourkhabbaz, A., Baramaki, R., Babaei, H. and Rezaei, M., 2011. Bioaccumulation of heavy metals in freshwater fish species, Anzali, Iran. *Bulletin of Environmental Contamination and Toxicology*, 87(**4**), 386-392. DOI: 10.1007/s00128-011-0376-y

EFSA Journal, 2013. Scientific Opinion on Dietary Reference Values for manganese. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). European Food Safety Authority (EFSA), Parma, Italy, 11(**11**), 3419-3444. DOI:10.2903/j.efsa.2013.3419

EFSA Journal, 2014. Scientific Opinion on Dietary Reference Values for zinc. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). European Food Safety Authority (EFSA), Parma, Italy, 12(**10**), 3844-3876. DOI:10.2903/j.efsa.2014.3844

EFSA Journal, 2015. Scientific Opinion on Dietary Reference Values for copper. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). European Food Safety Authority (EFSA), Parma, Italy, 13(**10**), 4251-4253. DOI:10.2903/j.efsa.2015.4253

El-Moselhy, K.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A., 2014. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian Journal of Basic and Applied Sciences*, 1(**2**), 97-105. DOI:org/10.1016/j.ejbas.2014.06.001

Farkas, A., Salánki, J. and Varanka, I., 2000. Heavy metal concentrations in fish of Lake Balaton. Lakes and Reservoirs: *Research and Management*, 5(**4**), 271-279. DOI:10.1046/j.1440-1770.2000.00127.x

Farkas, A., Salánki, J., Specziár, A. and Varanka, I., 2001. Metal pollution as health indicator of lake ecosystems. *International Journal of Occupational Medicine and Environmental Health*, 14, 163-170.

Farkas, A., Salánki, J. and Specziár, A., 2003. Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Research*, 37(**5**), 959-964. DOI:10.1016/SOO43-1354 (02)00447-5

Fatima, M., Usmani, N. and Hossain, M.M., 2014. Heavy metal in aquatic ecosystem emphasizing its effect on tissue bioaccumulation and histopathology: A review. *Journal of Environmental Science and Technology*, 7(**1**), 1-15, DOI:103923/jest.2014.1.15

Govind, P. and Madhuri, S., 2014. Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, 2(**2**), 17-23.

Grela, E.R., Pisarski R., Kowalczuk-Vasilev, E. and Rudnicka, A., 2010. Content of nutrients and minerals, and fatty acid profile in some fish flesh depending on fishing period. *Food. Science. Technology. Quality*, 71, 63-72 (in Polish).

Hama, S.R., Abdulrahman, N.M. and Wahab, N.K., 2015. Correlation between heavy metals in water and some health parameters. *International Journal of Plant, Animal and Environmental Sciences*, 5(**4**), 257-260.

Hosseini, M., Nabavi, S.M.B., Nabavi, S.N. and Pour, N.A., 2015. Heavy metals (Cd, Co, Cu, Ni, Pb, Fe, and Hg) content in four fish commonly consumed in Iran: risk assessment for the consumers. *Environmental Monitoring and Assessment*, 187, 237, 1-7. DOI:10.1007/s10661-015-4464-z

Jaćimowić, M., Lenhardt, M., Višnjić-Jeftić, Ž., Jarić, I., Gačić, Z., Hegediš, A. and Krpo-Ćetković, J., 2015. Elemental concentrations in different tissues of European perch and black bullhead from Sava Lake (Serbia). *Slovenian Veterinary Research*, 52(**2**), 57-65.

Jakimska, A., Konieczka, P., Skóra, K. and Namieśnik, J., 2011. Bioaccumulation of metals in tissues of marine animals, Part II: Metal concentrations in animal tissues. *Polish Journal of Environmental Studies*, 20, 1127-1146.

Järv, L., Kotta, J. and Simm, M., 2013. Relationship between biological characteristics of fish and their contamination with trace metals: a case study of perch *Perca fluviatilis* L. in the Baltic Sea. *Proceedings of the Estonian Academy of Sciences*, 62(**3**), 193-201. DOI:103176proc.2013.3.05

Jezierska, B. and Witeska, M., 2001. Metal toxicity to fish. Monographs No 42. Siedlce, Poland. 318 P.

Jezierska, B. and Witeska, M., 2006. The metal uptake and accumulation in fish living in polluted waters. *Soil Water Pollution Monitoring, Protection and Remediation*, 23, 107-114.

Kalisinska, E., Lanocha-Arendarczyk, N., Kosik-Bogacka, D., Budis, H., Pilarczyk, B., Tomza-Marciniak, A., Podlasinska, J., Cieslik, L., Popiolek, M., Pirog, A. and Jedrzejewska, E., 2017. Muscle mercury and selenium in fishes and semiaquatic mammals from a selenium-deficient area. *Ecotoxicology and Environmental Safety*, 136, 24-30. DOI: 10.1016/j.ecoenv.2016.10.028

Kalkan, H., Şişman, T. and Kiliç, D., 2015. Assessment of heavy metal bioaccumulation in some tissues of *Leuciscus cephalus* from Karasu River, Erzurum-Turkey. *Austin Journal of Environmental Toxicology*, 1(**1**), 1-6.

Kasimoglu, C., 2014. The Effect of fish size, age and condition factor on the contents of seven essential elements in *Anguilla anguilla* from Tersakan Stream Mugla (Turkey). *Journal of Pollution Effects & Control*, 2(**2**), 1-6. DOI:org/10.4172/2375-4397.1000123

Kenšová, R., Čelechovská, O., Doubravová, J. and Svobodová, Z., 2010. Concentrations of metals in tissues of fish from the Věstonice reservoir. *Acta Veterinario Brno*, 79, 335-345. DOI:10.2754/avb201079020335

Khaled, A.A., Khadiga, A.I., Shehata, M.A.S. and Manal, H.S., 2016. Distributions of metals (cadmium, lead, iron, manganese, zinc and copper) in water, aquatic plant and fish. *Journal of Research in*

Environmental Science and Toxicology, 5(**2**), 026-038, DOI:org/10.14303/jrest.2016.011

Klavins, M., Potapovics, O. and Rodinov, V., 2009. Heavy metals in fish from lakes in Latvia: Concentrations and trends of changes. *Bulletin of Environmental Contamination and Toxicology*, 82(**1**), 96-100. DOI:10.1007/s00128-008-9510-x

Kostecki, M., 2000. Heavy metals in flesh and liver of some fish species in Dzierżno Duże dam-reservoir (upper Silesia). *Archives of Environmental Protection*, 26, 109-125 (in Polish).

Lenhardt, M., Jarić, I., Vinjić-Jeftić, Z., Skorić, S., Gačić, Z., Pucar, M. and Hegediš, A., 2012. Concentrations of 17 elements in muscle, gills, liver and gonads of five economically important fish species from the Danube River. *Knowledge and Management of Aquatic Ecosystems*, 407(**02**), 1-10. DOI:org/10.1051/kmae/2012028

Lidwin-Kaźmierkiewicz, M., Pokorska, K., Protasowicki, M., Rajkowska, M. and Wechterowicz, Z., 2009. Content of selected essential and toxic metals in meat of freshwater fish from West Pomerania, Poland. *Polish Journal of Food and Nutrition Sciences*, 59, 219-224.

Łuczyńska, J., Markiewicz, K. and Jaworski, J., 2006. Interspecific differences in the contents of macro- and microelements in the muscle of six fish species from lakes of the Olsztyn Lake District (north-east of Poland). *Polish Journal of Food and Nutrition Sciences*, 15/56, 29-35.

Łuczyńska, J., Tońska, E. and Łuczyński, M.J., 2009. Essential mineral components in the muscles of six freshwater fish from the Mazurian Great Lakes (northeastern Poland). *Archives of Polish Fisheries*, 17, 171-178. DOI:10.2478/v10086-009-0015-y

Magu, M.M., Keriko, J.M., Kareru, P.G. and Chege, C.W., 2016. 'Burdens' of selected heavy metals in common fish species from specific Kenyan freshwaters. *International Journal of Fisheries and Aquatic Studies*, 4(**3**), 173-179.

Mahboob, S., Kausar, S., Jabeen, F., Sultana, S., Sultana, T., Al-Ghanim, K.A., Hussain, B., Al-Misned, F. and Ahmed, Z., 2016. Effect of heavy metals on liver, kidney, gills and muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus. *Brazilian Archives of Biology and Technology*, 59, 1-10. DOI:org/10.1590/1678-4324-2016150275

Matasin, Z., Ivanusic, M., Orescanin, V., Nejedli, S. and Tlak Gajger, I., 2011. Heavy metal concentration in predator fish. *Journal of Animal and Veterinary Advances*, 10(**90**), 1214-1218.
DOI:10.3923/javaa.2011.1214.1218

Mazej, Z., Al Sayegh-Petkovšek, S. and Pokorny, B., 2010. Heavy metal concentrations in food chain of

Lake Velenjsko jezero, Slovenia: An artificial lake from mining. *Archives of Environmental Contamination and Toxicology*, 58(**40**), 998-1007. DOI:10.1007/s00244-009-9417-5

Merciai, R., Guasch, H., Kumar, A., Sabater, S. and Garcia-Berthou, E., 2014. Trace metal concentration and fish size: variation among fish species in Mediterranean river. *Ecotoxicology and Environmental Safety*, 107, 154-161. DOI:org/10.1016/j.econv.2014.05.006

Milošković, A. and Simić, V., 2015. Arsenic and other trace elements in five edible fish species in relation to fish size and weight and potential health risks for human consumption. *Polish Journal of Environmental Studies*, 24(**1**), 199-206. DOI:10.15244/pjoes/24929

Moiseenko, T.I., Kudryavtseva, L.P. and Gashkina, N.A., 2005. Assessment of the geochemical background and anthropogenic load by bioaccumulation of microelements in fish. *Water Resources*, 32, 640-652.

Nadadur, S.S., Srirama, K. and Mudipalli, A., 2008. Iron transport and homeostasis mechanisms; Their role in health and disease. *Indian Journal of Medical Research*, 128, 533-544.

Namin, J.I., Mohammadi, M., Heydari, S. and Rad, F.M., 2011. Heavy metals Cu, Zn, Cd and Pb in tissue, liver of *Esox lucius* and sediment from the Anzali international lagoon-Iran. *Caspian Journal of Environmental Sciences*, 9, 1-8.

Nwabunike, M.O., 2016. The effects of bioaccumulation of heavy metals on fish fin over two years. *Global Journal of Fisheries and Aquaculture*, 4(**1**), 281-289.

Osipova, N.A., Stepanova, K.D. and Matveenko, I.A., 2015. Evaluation of metal content in perch of the Ob River basin. *IOPscience*, 27, 1-5. DOI:10.1088/1755-1315/27/1/012041

Pandey, G. and Madhuri, S., 2014. Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal Veterinary and Fishery Sciences*, 2(**2**), 17-23.

Parente, T.E.M. and Hauser-Davis, R.A., 2013. The use of fish biomarkers in the evaluation of water pollution. *Pollution and Fish Health in Tropical Ecosystems. Chapter*, 7, 164-181.

Pilecka, J., Bradulienė, J. and Paliulis, D., 2015. Bioaccumulation of lead and zinc in fishes from Kairiai Landfill aquatic ecosystems. *Environmental Protection Engineering*, 7, 418-423. DOI:10.3846/mla.2015.809

Pokorny, P., Dobicki, W., Tarbaj, K., Kowalska-Góralska, M., Polechoński, R. and Senze, M., 2015. Age-dependent heavy metal content in muscle tissue of brown bullhead (*Ictalurus nebulosus*, Le Suerur, 1819). *Polish Journal of*

Environmental Studies, 24(**5**), 2139-2146. DOI:10.15244/pjoes/39549

Quevauviller, P.H., Imbert, J.L., Wagstaffe, P.J., Kramer, G.N. and Griepink, B., 1993. Commission of the European Communities BCR Information – Reference materials, ECSC EEC-EAEC Report EUR 14557 EN, Brussels – Luxembourg. pp. 1–64.

Rajkowska, M. and Protasowicki, M., 2013. Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. *Environmental Monitoring and Assessment*, 185(**4**), 3493-3502. DOI:10.1007/s10661-012-2805-8

Rajkowska, M., Wechterowicz, Z., Lidwin-Kaźmierkiewicz, M., Pokorska, K. and Protasowicki, M., 2008. Accumulation of selected metals in roach (*Rutilus rutilus* L.) from West Pomeranian lakes. *Ecological Chemistry and Engineering A*, 15, 119-123.

Regulation of the Minister of Health on foods intended for particular nutritional uses (16 September 2010), http://www.ecolex.org/details/legislation/regulation-on-foodstuffs-intended-for-particular-nutritional-uses-lex-faoc113738/.

Salamat, N., Movahedinia, A., Etemadi-Deylami, E. and Mohammadi, Y., 2015. Pike (*Esox Lucius*) bio-indicator of heavy metals pollution in Anzali wetland. *Water Quality Exposure and Health*, 7, 251-254. DOI:10.1007/s12403-014-0138-2

Sándor, Z., Gy. Papp, Z., Csengeri, I. and Jeney, Z., 2011. Fish meat quality and safety. *Technologija Mesa*, 52, 97-105.

Santamaria, A.B., 2008. Manganese exposure, essentiality and toxicity. *Indian Journal of Medical Research*, 128, 484-500.

Shenkin, A., 2006. The key role of micronutrients. *Clinical Nutrition*, 25(**1**), 1-13. DOI:10.1016/j.clnu.2005.11.006

Shinn, C., Dauba, F., Grenouillet, G., Guenard, G. and Lek, S., 2009. Temporal variation of heavy metal contamination in fish of the river lot in southern France. *Ecotoxicology and Environmental Safety*, 72(**7**), 1957-1965. DOI:10.1016/j.ecoenv.2009.06.007

Stanek, M., Janicki, B. and Kupcewicz, B., 2005. Content of selected heavy metals in the organs of fish from Żnin Duże Lake. *Folia Biologica (Kraków)*, 53(**suppl**),115-119.

Staniskiene, B., Matusevicius, P., Budreckiene, R. and Skibniewska, K.A., 2006. Distribution of heavy metals in tissues of freshwater fish in Lithuania. *Polish Journal of Environmental Studies*, 15, 585-591.

Statistical Yearbook of Agriculture, 2016. Food economy, consumption. 460 P. http://stat.gov.pl/en/topics/statistical-yearbooks/statistical-yearbooks/statistical-yearbook-of-agriculture-2016,6,11.html.

Svecevičius, G., Kazlauskienė, N., Kesminas, V., Staponkus, R., Taujanskis, E. and Saulituė, G., 2014. Heavy metal accumulation in fishes of different ecological groups from Kairiai landfill regional aquatic ecosystem. The 9th International Conference "ENVIRONMENTAL ENGINEERING". Vilnius. Lithuania. 22-23 May. 2014.

Szarek-Gwiazda, E. and Amirowicz, A., 2006. Bioaccumulation of trace elements in roach, silver bream, rudd, and perch living in an inundated opencast sulphur mine. *Aquatic Ecology*, 40, 221-236. DOI:10.1007/s10452-004-7341-3

Szczerbowski, J.A., 1995. Ecological bases of inland fisheries. Pre-conditions of fish production. In: J.A. Szczerbowski (Ed.), *Inland fisheries in Poland (1st ed)* (pp.153-178). Olsztyn, IRS, Poland, 544 P.

Uauy, R., Olivares, M. and Gonzalez, M., 1998. Essentiality of copper in humans. The American *Journal of Clinical Nutrition*, 67(**suppl**), 952-959.

Usero, J., Regalado, E.G. and Gracia, I., 1997. Trace metals in bivalve molluscs *Ruditapes decussatus* and *Ruditapes philippinarum* from the Atlantic coast of southern Spain. *Environment International*, 23, 291–298. DOI:10.1016/S0160-4120 (97)00030-5

Yancheva, V., Stoyanova, S., Velcheva, I., Petrova, S. and Georgieva, E., 2014. Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, Bulgaria. *Archives of Industrial Hygiene and Toxicology*, 65, 57-66. DOI:10.2478/10004-1254-2014-2451

Yancheva, V., Velcheva, I., Stoyanova, S. and Georgieva, E., 2015. Fish in ecotoxicological studies. *Ecologia Balkanica*, 7(**1**), 149-169.

Yazdi, R.B., Ebrahinpour, M., Mansouri, B., Rezaei, M.R. and Babaei, H., 2012. Contamination of metals in tissues of *Ctenopharyngodon idella* and *Perca fluviatilis*, from Anzali wetland, Iran. *Bulletin of Environmental Contamination and Toxicology*, 89(**4**), 831-835. DOI:10.1007/s00128-012-0795-4

Zeitoun, M.M. and Mehana, El-Sayed E., 2014. Impact of water pollution with heavy metals on fish health: Overview and updates. *Global Veterinaria*, 12, 219-231, DOI:10.5829/idosi.gv.2014.12.02.82 219

Zubcov, E., Zubcov, N., Ene, A. and Biletchi, L., 2012. Assessment of copper and zinc levels in fish from freshwater ecosystems of Moldova. *Environmental Science and Pollution Research*, 19(**6**), 2238-2247. DOI:10.1007/s11356-011-0728-5