

Determination of Cadmium in Muscles and Liver of Freshwater Fish Species from Mazurian Lake District, and Risk Assessment of Fish Consumption (Poland)

Joanna Łuczyńska^{1,*} , Beata Paszczyk¹, Marek J. Łuczyński²

¹ University of Warmia and Mazury in Olsztyn, Chair of Commodity and Food Analysis, ul. Plac Cieszyński 1, 10-726 Olsztyn, Poland.

² The Stanisław Sakowicz Inland Fisheries Institute, Department of Ichthyology, Olsztyn, Poland.

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Corresponding Author

Tel.: +89.5234165

E-mail: jlucz@uwm.edu.pl

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Abstract

The aim of the study was to determine the cadmium content in muscle and liver of fish from Mazurian Lake District: *Rutilus rutilus* L. (roach), *Abramis brama* L. (bream), *Coregonus lavaretus* L. (whitefish), *Tinca tinca* L. (tench), *Perca fluviatilis* L. (European perch) and *Esox lucius* L. (Northern pike) and *Oncorhynchus mykiss* Walb. (rainbow trout) from the ponds. The content of cadmium in muscles ranged between 0.0006 mg/kg and 0.0104 mg/kg, whereas in liver varied from 0.0015 mg/kg to 0.0331 mg/kg. Cadmium content in muscles and liver differed between various species. The muscles contained significantly lower values of cadmium than liver ($P < 0.05$), except bream and rainbow trout. The statistically significant negative correlations were found only between cadmium content in muscles and liver of perch and body weight and total length. The Metal Pollution Index (MPI) was lower and ranged between 0.001 and 0.019. Target Hazard Quotient (THQ) was below 1, which means that these fish are safe for consumer.

Introduction

Heavy metals i.e. Cd, Pb, Hg and As are considered most toxic to humans, animals, including fish, and environment (Govind & Madhuri, 2014). They belong to elements with an unknown physiopathologic role (Krzywy, Krzywy, Peregud-Pogorzelski, Łuksza, & Brodkiewicz, 2011), because is not considered essential for biological function in humans (Mahurpawar, 2015) and has adverse and devastating effects on most organ systems and belongs to metals of considerable toxicity (Annabi, Said, & Messaoudi, 2013, Bernhoft, 2013). According to Mahurpawar (2015), these metals are released into the environment by both natural and anthropogenic means, especially mining and industrial activities, and automobile exhausts. It is known that cadmium gets into the aquatic environment through discharge of industrial waste and surface run (Dokmeci,

Ongen, & Dagdeviren, 2009). Aquatic organisms absorb the pollutants directly from water and indirectly from food chains (Khayatzadeh & Abbasi, 2010). Cadmium accumulation depends on concentration, time of exposure, way of uptake, environmental conditions, intrinsic factors, but primarily depends on waterborne and dietary pathways (Perera, Kodithuwakku, Sundarabarathy, & Edirisinghe, 2015). This metal can easily enter into the body by food chain, drinking water, smoking a cigarette or even breathing the air (Dokmeci et al., 2009). The fish consumption is recommended because it is a good source of n-3 polyunsaturated fatty acids, which have been associated with health benefits due to its cardioprotective effects. However, the content of heavy metals such as cadmium observed in some fish makes it difficult to establish clearly the role of fish consumption on a healthy diet (Castro-González & Mendéz-Armenta, 2008). Kumar and Singh (2010)

reported that monitoring toxic metals level in aquatic ecosystem, especially fish, is the need of the awareness from public health point of view. Fish, as do all aquatic life forms, also serve as “sentinel” species, alerting people that water quality is changing (Helfrich & Neves 2009). Especially fish liver is more often recommended as an environmental indicator for water pollution than other fish organs (Ebrahimpour, Pourkhabbaz, Baramaki, Babaei, & Rezaei, 2011). The bioaccumulation of metals in an animal depends on a multitude of factors: biotic ones, like its body dimensions and mass, age, sex, diet, metabolism, and position in the trophic web; and abiotic ones, such as the distribution of metals in its environment, salinity, temperature, and pH of the water, habitat type, and interactions with other metals. But it is diet that has the greatest influence on the accumulation of metals in animal tissues (Jakimska, Konieczka, Skóra, & Namieśnik, 2011). Consequently, the aims of this study were:

- to determine the differences between cadmium content in seven freshwater fish species from Mazurian Lake District,
- to determine differences between cadmium content in muscles and liver of the same species,
- to determine the impact of biometric parameters (body weight and total length) and Fulton’s condition factor (FCF) on the content of cadmium in muscles and liver of fish,

- to determine the health risk assessment with cadmium using Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ),

- to determine Metal Pollution Index (MPI) based on the cadmium content of the two organs.

Materials and Methods

The roach (*Rutilus rutilus* L.), bream (*Abramis brama* L.), whitefish (*Coregonus lavaretus* L.), tench (*Tinca tinca* L.), perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.) and rainbow trout (*Oncorhynchus mykiss* Walb.) were caught from two lakes of Mazurian Lake District and the ponds of Fish Farm Szwaderki (Poland) (Figure 1). These lakes are located next to each other and from the lake Pluszne in the south-east part flows the Poplusz River, which connects them with Łyna River and Lake Łańskie. Fishes from both lakes might migrate and population might mix. Results of the studies of polluting substances, including cadmium, showed that none of the chemical indicator does not exceed exposure limits and the study showed chemical good status of the water body. Therefore, the pollution of examined lakes by elements was similar. The fish were taken to the laboratory on the same day, where the body weight (± 0.01 g) and total length (± 0.1 cm) of each fish were measured (Table 1). Muscle tissue was extracted from the dorsal part. Liver and muscles were



Figure 1. Study area was located in north-eastern Poland, near the city Olsztyn (Lake Łańskie and Pluszne geographical coordinates: 53°58'60"N, 20°48'08"E, 53°58'30"N, 20°42'06"E).

Table 1. Differences between the content of cadmium (min-max) in muscles and liver of the same fish species

Species	n	Body weight [g]	Total length [cm]	Cd	
		Min-max mean	Min-max mean	mg/kg wet weight	
				muscles	liver
Tench	6	472.07-958.43	31.10-39.20	0.0002-0.0005 b	0,002-0,0203 a
<i>Tinca tinca</i> L.		741.15	35.46		
Pike	10	743.42-1843.62	50.00-66.50	0.0005-0.0062 b	0.0009-0.0159 a
<i>Esox lucius</i> L.		1155.86	55.41		
Rainbow trout	6	467.65-1084.96	33.00-39.00	0.0001-0.0015 a	0.0001-0.0043 a
<i>Oncorhynchus mykiss</i> Walb.		774.18	36.46		
Bream	4	423.6-674.0	33.70-41.50	0.0013-0.0118 a	0.0087-0.0255 a
<i>Abramis brama</i> L.		524.72	36.28		
Whitefish	5	275.50-476.60	32.10-36.40	0.0008-0.0019 b	0.0058-0.0111 a
<i>Coregonus lavaretus</i> L.		380.20	34.20		
Roach	6	411.30-542.60	29.30-33.30	0.0005-0.0035 b	0.0089-0.0253 a
<i>Rutilus rutilus</i> L.		478.01	31.27		
Perch	9	71.70-608.90	18.65-33.20	0.0007-0.0349 b	0.0102-0.0675 a
<i>Perca fluviatilis</i> L.		314.85	25.63		

n- number of fish; a, b – significant differences between the muscles and liver of the same species ($P \leq 0.05$).

The same letter indicates the absence of significant differences between muscles and liver in the same fish species

taken from the individual fish then they were mixed and stored in polypropylene bags at -30°C prior to analysis.

Ethical permit: Fish were bought at the Fish Farm, they were already dead. According to European and Polish Law, the research done on the commercially caught fishes tissue is free to obtain permission on Local Ethical Commission.

Study Area

Lake Łańskie is located in the vast Ramuckie Forests, about 15 km south of Olsztyn. It is a tunnel valley lake with the Łyna River flowing through it. In the surrounding area there are some leisure centres. The lake is one of the largest and deepest lakes in the Olsztyn Lake District.

Lake Pluszne, like Lake Łańskie, is also one of the largest lakes in the Olsztyn Lake District. The village of Pluski is situated on the eastern shore of the lake and the village of Zielonowo is on the western shore. Near the villages are numerous leisure centres, summer houses and campsites. Many small streams flows from the surrounding area and from Lakes Staw, Niskie and Wysokie which flow into the Lake Pluszne (Planter & Łaźniewski, 2002).

Cadmium Determination

For cadmium analysis, muscle samples taken from each individual (approximately $10 \text{ g} \pm 0.0001 \text{ g}$) in duplicate were dried to constant weight at 105°C , then the samples were ashed at 450°C for 12 h using laboratory furnaces (Nabertherm P330, Germany). The white ash was dissolved in 1M HNO_3 . (Suprapur-Merck, Darmstadt, Germany). In case of liver, the 2-4 g ($\pm 0.0001 \text{ g}$) samples were wet-digested in a mixture of nitric and

perchloric acids (Merck, Darmstadt, Germany, 3:1, (v/v) at 190°C using heating block (DK 20, VELP Scientifica, Italy). Then, each sample was quantitatively transferred into 25 ml volumetric flasks with deionized water (Merck-Millipore Elix Adventage 3, USA). Cadmium were measured using flameless atomic absorption spectrometry FAAS (Thermo Scientific iCE 3500Z with a ZEEMAN background correction and atomisation in a graphite cuvette, England) under the following conditions: absorption wavelength for cadmium - 228.8 nm; lamp current – 50%; slit – 0.5 nm; sample volume – 20 μl ; modifier – $\text{Mg}(\text{NO}_3)_2$; dry - 100°C ; ash - 600°C ; atomize - 1000°C . Four blanks and samples analyzed based on calibration curves which were prepared using five solution standards (1000 $\mu\text{g/L}$) with 0.1 M HNO_3 (J.T.Baker®, Netherlands).

The reliability of the analytical methods was examined by measuring the elements in reference material: CRM 422 – cod muscle tissue (lyophilized sample) with a certified concentration of Cd (Certified Reference Material – BCR, lyophilized sample of cod, *Gadus morhua* L., muscle). The percent recovery rate was 102.9% and variability coefficients $V(\%)$ – 3.30 (Cd–certified $0.017 \pm 0.002 \text{ mg/kg}$, obtained $0.018 \pm 0.001 \text{ mg/kg}$; $n=4$). The cadmium content in the muscles and liver of fish are expressed in mg/kg wet weight.

1. Fulton's condition factor (FCF):

The condition of fish was calculated as follows:

$$\text{FCF} = 100 \times W/L^3$$

Where: W – total body weight of fish (g), L – total length of fish (cm).

2. Metal Pollution Index (MPI):

The MPI was determined using the equation by Usero, Regalado, and Gracia (1997) and Abdel-Khalek, Elhaddad, Mamdouh, and Saed Marie (2016).

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

Where, Mn is the concentration of metal n (mg/kg wet wt.) in a certain tissue.

3. Human health risk assessment

3.1. Estimated daily intake of heavy metals (EDI)

EDI - the estimated daily intake ($\mu\text{g}/\text{kg}$ body weight/day)

$$\text{EDI} = C \times \text{IR} / \text{BW}$$

TWI – Tolerable weekly intake

$$\text{TWI} = \text{EDI} \times 7$$

C - the average concentration of heavy metals in food stuffs ($\mu\text{g}/\text{g}$ wet weight)

IR - the daily ingestion rate (g/day)

BW - the average body weight (60 kg)

3.2. Target Hazard Quotient (THQ)

The THQ assessed the non-carcinogenic health risk of consumers due to intake of heavy metal polluted fish use Oral reference dose (Ahmed, Baki, Kundu, Islam, & Islam, 2016, US EPA, 2017). When $\text{THQ} < 1$ then it means the health benefit from fish consumption and the consumers is safe, whereas $\text{THQ} > 1$ suggested a high probability of adverse risk of human health.

$$\text{THQ} = (\text{Efr} \times \text{ED} \times \text{FiR} \times \text{C} / \text{RfD} \times \text{BW} \times \text{TA}) \times 10^{-3}$$

Efr - the Exposure Frequency (365 days/year)

ED - the Exposure Duration (70 years)

FiR - the fish ingestion rate (g/person/day)

C - the average concentration of heavy metals in food stuffs ($\mu\text{g}/\text{g}$ wet weight)

RfD - the Oral reference dose (mg/kg/day) (USEPA 2017), Rfd for cadmium (diet) – 1.0×10^{-3}

BW - the average body weight (60 kg)

TA - the average exposure time (365 days/year \times ED)

Statistical analysis

The statistical analysis were performed using the computer software STATISTICA 12 (StatSoft Kraków, Poland). Bartlett's test showed that the variances were heterogeneous therefore mean values in particular groups were transformed ($\log \bar{x}$). The one-way analysis of variance (ANOVA) was used to test significant differences in the content of cadmium, both between species and organs of the same species. The correlation coefficients between content of cadmium and body weight, total length and Fulton's condition factor of fish were calculated using STATISTICA 12 (StatSoft Kraków, Poland). The significance level of $P < 0.05$ was used.

Results and Discussion

The cadmium content of the muscles of selected fish species is presented in Table 1. The content of cadmium in muscles ranged from 0.0006 mg/kg (tench) to 0.0104 mg/kg (perch) (Figure 2), whereas in liver varied between 0.0015 mg/kg (rainbow trout) and 0.0331 mg/kg (perch) (Figure 3). The mean concentrations of cadmium in the liver of examined fish gave rise to the following sequence: perch > roach \approx

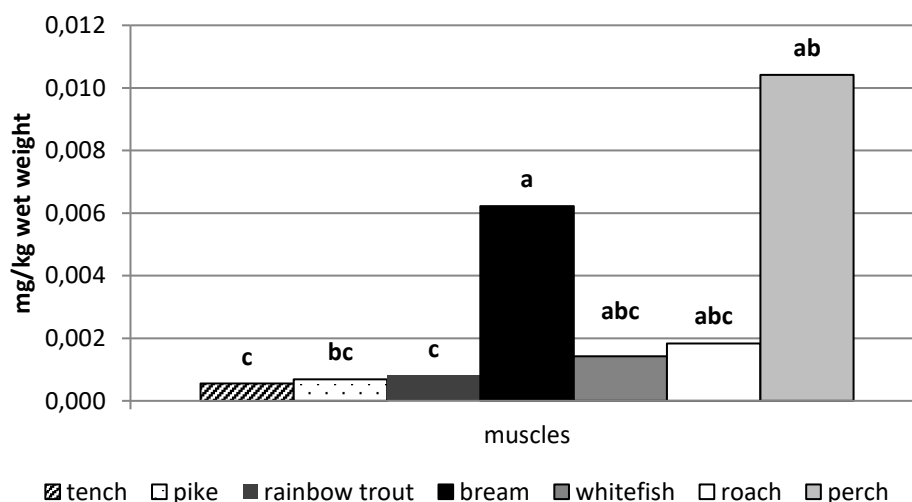


Figure 2. Interspecific differences in the content of cadmium in muscles of fish examined a, b, c – significant differences between the same organs of the species ($P < 0.05$). The same letter indicates the absence of significant differences between fish species.

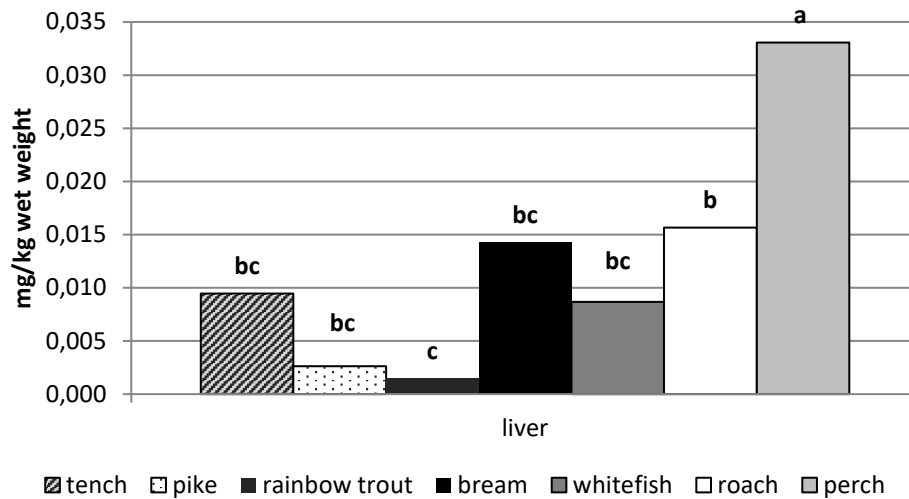


Figure 3. Interspecific differences in the content of cadmium in liver of fish examined a, b, c – significant differences between the same organs of the species ($P \leq 0.05$). The same letter indicates the absence of significant differences between fish species.

(bream \approx tench \approx whitefish \approx pike) \approx rainbow trout ($P \leq 0.05$).

Significant higher values of cadmium were observed in liver of examined fish species than in muscles of these species, except with rainbow trout and bream, because there were no significant differences between cadmium content in organs of these fish (Table 1).

Significant negative correlation coefficients were found only between cadmium content in the muscle of perch and the body weight and length ($r = -0.704$ and $r = -0.743$, $P \leq 0.05$) (Table 2). The concentration of cadmium in the liver of perch also negatively correlated with body weight ($r = -0.882$, $P \leq 0.01$) and total length ($r = -0.863$, $P \leq 0.01$) of these species. No significant correlations were found, both muscles and liver in other examined fish ($P > 0.05$). In most cases, there were no significant correlations between the content of cadmium and fish condition (Table 3). Only the negative correlation coefficient between the condition factor and cadmium level was at $r = -0.824$ for liver of perch ($P = 0.006$).

Jeziarska and Witeska (2001) reported that accumulation of metals in the liver is related with exposure duration and deposition of them is also related with its detoxifying ability and in particular with presence of metal-binding proteins. May be it had an impact on the larger amount of cadmium in liver of perch. These authors also noted that accumulation of metals in various organs depends on species, feeding habit and size of fish. The results presented by Hosseini, Nabavi, Nabavi, and Pour (2015) 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. No significant difference between the content of cadmium in the

muscles of fish species (roach, perch, river trout *Salmo trutta fario* and gudgeon *Gobio gobio*) from the rivers of Lithuania was found by Virbickas and Sakalauskiene (2006).

According Jeziarska and Witeska (2001) cadmium in larger quantities accumulates in the liver than the muscles, which has been confirmed both in our (Table 1) and other studies (Table 4). Moreover, the highest content of cadmium was observed in the liver of bream from Reservoir Želivka (Czech Republic) (Sevcikova *et al.*, 2013) and the muscles of roach from River Nitra (Slovakia) (Andreji, Stránai, Massányi, & Valent, 2006). Cadmium content was also higher in liver than muscles of both species (tench and perch) from the southwest of the Caspian Sea (Iran) (Eslami, Sattari, Namin, & Ashrafi, 2014). Yazdi, Ebrahimpour, Mansouri, Rezaei and Babaei (2012) reported that the content of cadmium was different between the tissues of perch (intestine > liver > gills > muscles). There were significant differences between the content of Cd in organs of pike (muscle, gill, kidney, intestine and liver) from Anzali Wetland (Iran), because the cadmium content in fish decreased in the following order: liver > kidney > gills > intestine > muscles (Ebrahimpour, Pourkhabbaz, Baramaki, Babaei, & Rezaei, 2011). The highest cadmium content in liver and the lowest in muscle tissue of perch from lakes in Latvia was found by Klavins, Potapovits, and Rodinov (2009). Whereas the content of cadmium in organs of rainbow trout from Iran decreased as follows: gills (0.004 mg/kg) > liver (0.002 mg/kg) > muscles (0.001 mg/kg) (Mert, Alaş, Bulut, & Özcan, 2014). Farkas, Salánki, Specziár, and Varanka (2001) observed the highest cadmium concentration in the liver followed by gills and muscle of bream.

In our study only the significant negative correlations between size and cadmium content was found in muscles and liver of perch (Table 2). This is accordance with results of Klavins *et al.* (2009), who

Table 2. Correlation coefficient between the content of cadmium in muscles and liver (mg/kg wet weight) and body weight, total length

Species	n	Correlation coefficient			
		Body weight		Total length	
		muscles	liver	muscles	liver
Tench <i>Tinca tinca</i> L.	6	0.025	0.646	0.198	0.597
Pike <i>Esox lucius</i> L.	10	0.045	-0.087	-0.031	-0.213
Rainbow trout <i>Oncorhynchus mykiss</i> Walb.	6	0.150	-0.577	-0.051	-0.525
Bream <i>Abramis brama</i> L.	4	-0.286	0.947	-0.465	0.937
Whitefish <i>Coregonus lavaretus</i> L.	5	0.092	0.012	-0.018	0.196
Roach <i>Rutilus rutilus</i> L.	6	0.322	-0.504	0.554	-0.804
Perch <i>Perca fluviatilis</i> L.	9	-0.704*	-0.882**	-0.743*	-0.863**

* - $P \leq 0.05$; ** - $P \leq 0.01$ **Table 3.** Regression equations and linear correlation coefficients (r) between content of cadmium (mean x) (mg/kg wet weight) in organs of fish and Fulton's condition factor (mean y)

Species	n	FCF (r)	P	Regression equations
muscles				
Tench <i>Tinca tinca</i> L.	6	-0.800	0.056	$Y = 1.9098 - 512.761x$
Pike <i>Esox lucius</i> L.	10	0.333	0.346	$Y = 0.6721 + 14.0347x$
Rainbow trout <i>Oncorhynchus mykiss</i> Walb.	6	0.157	0.766	$Y = 1.5112 + 65.6319x$
Bream <i>Abramis brama</i> L.	4	0.801	0.199	$Y = 0.9957 + 16.6261x$
Whitefish <i>Coregonus lavaretus</i> L.	5	0.281	0.646	$Y = 0.8609 + 56.1747x$
Roach <i>Rutilus rutilus</i> L.	6	-0.315	0.543	$Y = 1.6658 - 54.1429x$
Perch <i>Perca fluviatilis</i> L.	9	-0.659	0.054	$Y = 1.5751 - 15.2489x$
liver				
Tench <i>Tinca tinca</i> L.	6	0.243	0.643	$Y = 1.5825 + 4.7853x$
Pike <i>Esox lucius</i> L.	10	0.483	0.157	$Y = 0.6505 + 8.2613x$
Rainbow trout <i>Oncorhynchus mykiss</i> Walb.	6	-0.567	0.241	$Y = 1.6956 - 90.8989x$
Bream <i>Abramis brama</i> L.	4	-0.751	0.249	$Y = 1.2559 - 10.9527x$
Whitefish <i>Coregonus lavaretus</i> L.	5	-0.374	0.535	$Y = 1.0717 - 15.1052x$
Roach <i>Rutilus rutilus</i> L.	6	0.508	0.303	$Y = 1.3498 + 13.8371x$
Perch <i>Perca fluviatilis</i> L.	9	-0.824	0.0063	$Y = 1.7829 - 11.1598x$

P – significance level

Table 4. The content of cadmium in muscles and liver of freshwater fish (mg/kg wet weight)

Species	n	Area	Cd		References
			muscles	liver	
Bream, <i>Abramis brama</i> (L.)	9		0.0023	1.0592	
Roach, <i>Rutilus rutilus</i> (L.)	6	Reservoir Želivka/Czech Republic	0.0024	0.2867	Sevcikova et al. (2013)
Perch, <i>Perca fluviatilis</i> (L.)	7		0.0018	0.3559	
Pike, <i>Esox lucius</i> (L.)	7		<0.0016	0.0591	
Bream, <i>Abramis brama</i> (L.)	10	Danube River/Serbia	0.004	-	Milošković et al. (2016)
Bream, <i>Abramis brama</i> (L.)	-	Danube River/Serbia	0.021	-	Jovanović et al. (2017)
Perch, <i>Perca fluviatilis</i> (L.)	79	Baltic Sea/Estonia	0.03	0.23	Järv, Kotta, & Simm (2013)
Perch, <i>Perca fluviatilis</i> (L.)	10	Lake Miedwie/Poland	0.001	-	Lidwin-Kaźmierkiewicz, Pokorska, Protasowicki, Rajkowska, & Wechterowicz (2009)
Bream, <i>Abramis brama</i> (L.)	62	Lake Ińsko/Poland	0.002	-	
Pike, <i>Esox lucius</i> (L.)	64		0.003	-	
Perch, <i>Perca fluviatilis</i> (L.)	20		<0.01	0.10	
Bream, <i>Abramis brama danubii</i>	16	Lake Velenjsko/ Slovenia	<0.01	0.06	Mazej, Al Sayegh-Petkovšek S., & Pokorny (2010)
Roach, <i>Rutilus rutilus</i> (L.)	13		<0.01	0.02	
Roach, <i>Rutilus rutilus</i> (L.)	10	River Nitra/Slovakia	0.41		Andreji, Stránai, Massányi, & Valent (2006)
Roach, <i>Rutilus rutilus</i> (L.)		River Dyje/ Czech Republic	0.01		Dvorak, Andreji, Dvořáková-Líšková, & Vejsada (2014)
Tench, <i>Tinca tinca</i> (L.)	12	River Neretva/Croatia	0.067	0.133	Has-Schön, Bogut & Strelec (2006)
Roach, <i>Rutilus rutilus</i> (L.)	10	Lake Zhrabchevo Dam/ Bulgaria	0.01	-	Zhelyazkov, Georgiev, Dospatliev, & Staykov (2014)
Bream, <i>Abramis brama</i> (L.)	-		<0.05	-	
Perch, <i>Perca fluviatilis</i> (L.)	-	Reservoir Boskovice/ Czech Republic	<0.05	-	Kuklina, Kouba, Buřič, Horká, Ďuriš, & Kozák (2014)
Roach, <i>Rutilus rutilus</i> (L.)	-		<0.05	-	
Tench, <i>Tinca tinca</i> (L.)	-		<0.05	-	
Perch, <i>Perca fluviatilis</i> (L.)	20	Lake Šalek/ Slovenia	<0.01	0.10	Al. Sayegh Petkovšek, Mazej Grudnik, & Pokorny (2012)
Roach, <i>Rutilus rutilus</i> (L.)	13		<0.01	0.02	
Bream, <i>Abramis brama</i> (L.)	5		0.083	-	
Perch, <i>Perca fluviatilis</i> (L.)	4	River Vistula/ Toruń, Poland	<0.05	-	Wyrzykowska, Falandysz, & Jarzyńska (2012)
Roach, <i>Rutilus rutilus</i> (L.)	10		<0.05	-	
Tench, <i>Tinca tinca</i> (L.)	7		<0.05	-	
Rainbow trout <i>Oncorhynchus mykiss</i> Walb.	-	Market in Khorramabad City. Iran	0.123		Mortazavi, Hatamikia, Bahmani, & Hassanzadazar (2016)
Rainbow trout <i>Oncorhynchus mykiss</i> Walb.	10	Fish farm / Poland	0.007		Łuszczek-Trojnar, Błoniarz, Winiarski, Drag-kozak, & Popek (2015)
Tench, <i>Tinca tinca</i> (L.)	-	Damsa dam lake/Turkey	0.0784-0.1766		Mert, Alaş, Bulut, & Özcan (2014)

found significant negative correlations between cadmium content in muscles of perch and weight and length ($r=-0.20$ and $r=-0.17$, respectively). There was significant negative relationship between length of perch and cadmium of liver this species ($P<0.05$) (Eslami, Sattari, Namin, & Ashrafi, 2014). Significant negative correlation was also found between length and weight of tench and cadmium content in its liver. Whereas Szefer, Domagała-Wieloszewska, Warzocha, Garbacik-Wesołowska and Ciesielski (2003) found statistically significant positive correlation between length of perch (from the Pomeranian Bay and Szczecin Lagoon, southern Baltic, Poland) and cadmium concentration both in muscles ($P<0.05$) and liver ($P<0.01$), and between weight and cadmium content its muscles and liver ($P<0.01$). Generally, level of metals such as Hg, Zn, Pb, Cd and Cr increased with roach size from the Dije river basin (Czech Republic) (Dvořák, Andreji, Dvořáková-Líšková, & Vejsada 2014). Sirizi et al. (2012)

showed significant negative correlation between Cd content in muscles of pike from Anzali Wetland (Iran) with body length ($r= -0.88$, $P<0.05$) and weight ($r=-0.90$, $P<0.05$). Correlation between cadmium content in muscle tissue of pike in Anzali Wetland (Iran) and length ($r=0.326$), body weight ($r=0.215$) and condition factor ($r=0.118$) was no significantly positive ($P>0.05$), whereas concentration of this metal in muscles as size and condition factor of bream significantly increased ($r= 0.819$, 0.959 , 0.578 , respectively) (Mansouri, Khorasani, Karbassi, Riazi, & Panahandeh 2013). Farkas, Salánki, Specziár, and Varanka (2001) found significant negative correlation between cadmium content in liver of bream and the condition factor.

Health Risk

Cadmium content in muscles of fish examined did not exceed the permissible level declined as 0.05 mg/kg

(Table 1). The fish consumption was 12.1 kg per capita/year (an adult of the body weight 60 kg) (Statistical Yearbook of Agriculture 2013), therefore the estimated daily intake (EDI) of cadmium from the 33.16 g portions of fish was: 0.0003 µg/body weight (tench), 0.0004 (pike), 0.0004 (rainbow trout), 0.0034 (bream), 0.0008 (whitefish), 0.0010 (roach) and 0.0058 µg/body weight (perch) (Table 5). The weekly intake of cadmium (232.12 g of fish portion) accounts for 0.085%, 0.106%, 0.124%, 0.963%, 0.220%, 0.284% and 1.611% of the TWI (as 2.5 µg/kg body weight). The Target Hazard Quotient (THQ) was below 1 (0.0003-0.0058) which shows that there is no non-carcinogenic health risk for to the consumer consumption of fish examined (Table 5), whereas the Metal Pollution Index (MPI) was lower and ranged between 0.001 and 0.019.

According to Jamil et al. (2014), when MPI in the tissues ranged from 5 to 10 we are dealing with low contamination. The MPI index values varied between 2 and 5, we observe very low contamination, while MPI below 2 it means – not impacted. The MPI = (As x B x Ba x Cd x Co x Cr x Cu x Fe x Hg x Li x Mn x Mo x Ni x Pb x Sr x Zn)^{1/16} value was highest in the liver of perch from the Međuvršje Reservoir (West Morava River Basin, Serbia) than muscles and gills, whereas this factor was higher in gills of pike and roach (Đikanović, Skorić, & Gačić, 2016).

Hazard Quotient (HQ)<1 for Cd suggests that consumption limit of fish in Anzali Wetland (Iran) was acceptable dairy intake of human (Mansouri, Khorasani, Karbassi, Riazi, & Panahandeh, 2013). Experts from FAO/WHO have estimated a temporary tolerable weekly cadmium (PTWI) of 7 µg/kg body weight (WHO-ICPS, 2000, WHO, 2001). EFSA (2009) defined tolerable weekly intake (TWI) for cadmium at 2.5 µg/kg body

weight. According to Commission Regulation (EU) 488/2014 (Commission Regulation, 2014) the admissible cadmium level for fish is 0.05 mg/kg, except for fish such as mackerel (*Scomber* species), tuna (*Thunnus* species, *Katsuwonus pelamis*, *Euthynnus* species), *Sicyopterus lagpcephalus*, for which the level is 0.10 mg/kg and tazar marun (species *Auxis*) – 0.15 mg/kg and anchovy (*Engraulis* species), swordfish (*Xiphias gladius*) or sardine (*Sardina pilchardus*) – 0.25 mg/kg. Cadmium concentrations in the edible muscle and liver tissues of fish from Lake Balik, Kizilirmak Delta (Samsun, Turkey) ranged from 0.03 (*C. carpio*) to 0.04 (*M. cephalus* and *S. lucioperca*) and 0.054 (*M. cephalus*) to 0.085 (*P. fluviatilis*) mg/kg in wet weight, respectively (Bat, Arıcı, Sezgin, & Sahin, 2015). The maximum Cd concentrations in liver tissues were considerably higher than the maximum level (0.05 µg/g wet weight) in food of the Turkish Food Codex and Commission Regulation (EC). Cd levels in the muscle tissues were below the guideline levels. The observed metal levels were compared with the maximum permitted levels in the results of this study showed that estimated daily and weekly intakes of selected metals via consumption of fish were below the permissible tolerable daily intake and provisional tolerable weekly intake values established by Food and Agriculture Organization/World Health Organization. The consumers do not routinely consume the liver of fish, therefore, the fishes from Balik Lake are healthy for consumption. The cadmium content of tench and perch from the southwest of the Caspian Sea (Iran) was also below the guidelines for food summarized by MAFF (0.2 mg/kg wet wt.) and EEC (0.05 mg/kg wet wt.) (Eslami, Sattari, Namin, & Ashrafi, 2014). The cadmium values in muscles of roach from five French fishing areas not

Table 4. The Hazard quotient calculated for cadmium content in the muscle tissue of fish

	Cd					References
RfD (mg/kg/day)	1.00E-03					US EPA, 2017
TWI	2.5					EFSA 2009
	EDI	THQ	EWI	%TWI	MPI	
Tench <i>Tinca tinca</i> L. (n=6)	0.0003	0.0003	0.0021	0.085	0.002	This study
Pike <i>Esox lucius</i> L. (n=10)	0.0004	0.0004	0.0026	0.106	0.001	This study
Rainbow trout <i>Oncorhynchus mykiss</i> Walb. (n=6)	0.0004	0.0004	0.0031	0.124	0.001	This study
Bream <i>Abramis brama</i> L. (n=4)	0.0034	0.0034	0.0241	0.963	0.009	This study
Whitefish <i>Coregonus lavaretus</i> L. (n=5)	0.0008	0.0008	0.0055	0.220	0.004	This study
Roach <i>Rutilus rutilus</i> L. (n=6)	0.0010	0.0010	0.0071	0.284	0.005	This study
Perch <i>Perca fluviatilis</i> L.(n=9)	0.0058	0.0058	0.0403	1.611	0.019	This study

n – number of fish; EDI - is the estimated daily intake (µg/kg body weight/day); THQ - Target Hazard Quotient; RfD - Oral reference dose (mg/kg/day) (USEPA 2017); TWI – Tolerable Weekly Intake (µg/kg body weight); MPI – Metal Pollution Index

exceeded the maximum level set by Commission Regulation (EC) (0.05 mg/kg wet weight for muscle meat of fish) (Noël *et al.*, 2013). Cadmium in rainbow trout muscles (0.123 mg/kg) from marketed in Khorramabad City Iran is lower than maximum recommended levels according to WHO (0.2 mg/kg) and FDA guidelines (1.0 mg/kg) but higher than recommended levels to EC (0.05 mg/kg) (Mortazavi, Hatamikia, Bahmani, & Hassanzadazar, 2016). Whereas omnivorous (Prussian carp *Carassius auratus gibelio*, barbel *Barbus barbus*, bream *Abramis brama*, carp *Cyprinus carpio*) and carnivorous (pike perch *Stizostedion lucioperca* and catfish *Silurus glanis*) fish muscles from both locations of Danube River (Belgrade) contained a higher amount of cadmium than is specified in the Official Gazette of the Republic of Serbia and in the European Union Regulation (0.05 mg/kg), with the exception of bream and pike perch muscles (Jovanović *et al.* 2017). Similarly, cadmium concentration in muscles of pike from fish ponds in Belgrade area varied from 0.063 to 0.079 mg/kg and higher than maximum level according to EU legislation (Janjić *et al.* 2015). The Cd concentration in the muscle of pike from Anzali Wetland is below levels of concern for human consumption as defined by the FAO in 1983 year (0.5 mg/kg) (Ebrahimpour, Pourkhabbaz, Baramaki, Babaei, & Rezaei, 2011). Cadmium concentration in muscles of fish (pike, bream, perch and carp) from river of West Pomerania (Poland) ranged within 0.001-0.003 mg/kg and was below the maximum allowable level of 0.05 mg/kg (Lidwin-Kaźmierkiewicz, Pokorska, Protasowicki, Rajkowska, & Wechterowicz, 2009). The concentrations of cadmium in muscles of common nase (*Chondrostoma nasus*), roach (*Rutilus rutilus*), freshwater bream (*Abramis brama*), barbel (*Barbus barbus*), Prussian carp (*Carassius gibelio*), chub (*Squalius cephalus*), European perch (*Perca fluviatilis*), wels catfish (*Silurus glanis*) and northern pike (*Esox lucius*) did not exceed the MAC prescribed by the EU Regulations (0.05 mg/kg), international standards FAO (2.00 mg/kg) and the Regulation of the Republic of Serbia (0.05 mg/kg) (Đikanović *et al.* 2016). The levels of Cd in muscles of pike from Anzali Wetland (Iran) exceeded the standards of WHO and EPA, therefore these results can be a warning for consumers of these fish (Sirizi *et al.* 2012). The Cd concentration in muscle of fish from lakes in Mazurian District (Poland) not exceeded the Polish safety limit of 0.05 mg/kg (Łuczyńska & Brucka-Jastrzębska 2006). The contents of cadmium in roach and chub (*Squalius cephalus*) muscles from Dyje river were low and did not exceed the values of limits admissible in the Czech Republic and defined as 0.05 mg/kg (Dvořák, Andreji, Dvořáková-Líšková, & Vejsada, 2014).

Conclusion

Generally, liver contained more cadmium than muscles, but there were still very low levels, and because it was not part of the bodies consumed by

humans, it was not a threat. Biotic factors as species size and Fulton's condition factor in a few cases have affected the content of cadmium in organs of these fish. Due to the properties of cadmium, further investigation of fish from these reservoirs should be continued. From the human health point of view, the THQ which was calculated to evaluate the non-carcinogenic health risk was below 1 and showed that consumption of examined fish does not pose a health risk to the consumers due to the content of cadmium in them. The content of cadmium in muscles of fish examined did not exceed the permissible level declined as 0.05 mg/kg which also indicates that these fish are safe. The current results also demonstrated that metal pollution index (MPI) was low and indicate very low contamination of the aquatic environment.

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