

Chemical Composition and Levels of Heavy Metals in Fish Meat of the Cyprinidae Family from Zhrebchevo Dam, Central Bulgaria

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Abstract. The aim of the study was to determine the chemical composition and for the first time - the content of cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn) in the meat of bream (*Abramis brama* Linnaeus, 1758), Prussian carp (*Carassius gibelio* Bloch, 1782), vimba (*Vimba vimba* Linnaeus, 1758) and chub (*Leuciscus cephalus* Linnaeus, 1758) in one of the most popular for angling dams in Central Bulgaria in order to evaluate the risk for human health. Chemical composition of fish from Zhrebchevo dam showed the highest moisture content in the meat of Prussian carp - $82.13 \pm 0.04\%$, and the lowest in that of vimba - $78.51 \pm 0.07\%$. The amount of protein and lipids was highest in vimba meat $18.41 \pm 0.23\%$ and $1.88 \pm 0.09\%$, and lowest in the Prussian carp - $16.49 \pm 0.01\%$ and $0.25 \pm 0.05\%$. The content of Cd, Ni and Pb in the meat of vimba was the lowest - 0.033 ± 0.001 mg.kg⁻¹, 0.330 ± 0.001 mg.kg⁻¹ and 0.135 ± 0.001 mg.kg⁻¹ and the highest in bream, 0.045 ± 0.004 mg.kg⁻¹, 0.457 ± 0.018 mg.kg⁻¹ and 0.177 ± 0.013 mg.kg⁻¹ respectively. The highest content of Zn was observed in the Prussian carp - 20.170 ± 0.266 mg.kg⁻¹, and lowest in bream - 15.903 ± 0.593 mg.kg⁻¹. The obtained values for the four studied heavy metals were below the maximum permissible level according to the European legislation.

Key words: *Abramis brama*, *Carassius gibelio*, *Leuciscus cephalus*, *Vimba vimba*, human health risk.

Introduction

The cyprinid family (*Cyprinidae*) comprises the highest number of fresh-water fish species inhabiting Bulgarian water pools (KARAPETKOVA & ZHIVKOV, 2010). Zhrebchevo Dam is populated by more than 15 fish species; among the most numerous and stable populations are those of the bream, Prussian carp, vimba and chub. Due to its location in central Bulgaria and the easily accessible shores, the Zhrebchevo Dam is visited by many

amateur anglers. So far, the fish inhabiting Zhrebchevo Dam water are relatively few. ZHELYAZKOV *et al.* (2014) determined some heavy metals in the meat of roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*), whereas VALKOVA *et al.* (2013; 2015a, b) studied Cd, Cr and Cu in the water, sediment, aquatic plants and muscle tissue of common carp (*Cyprinus carpio*) from Zhrebchevo Dam.

The chemical composition of the different fish species varies according to feeding mode,

environment, size, migrations etc. (BUD *et al.*, 2008). High and well-balanced dietary protein content, essential fatty acids and mineral substances make fish a valuable and beneficial human foodstuff (KATSELIS *et al.*, 2007).

The main sources for accumulation of heavy metals in the body of fish are the water, feed and sediments (YILMAZ *et al.*, 2007; ZHAO *et al.*, 2012). In addition, heavy metals are not biodegradable, and their continuous intake with feed could pose a serious health risk to both, animals and men (DAVYDOVA 2005; JAVED & USMANI 2011, 2012, 2013a, 2015). Furthermore, fish, being at the top of the aquatic foodchain, accumulate substantial heavy metal amounts in their meat, leading to a high risk for humans as end consumers (JAVED & USMANI 2013b, 2015).

The aim of the study was to determine the chemical composition and for the first time - the content of cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn) in the meat of bream (*Abramis brama* Linnaeus, 1758), Prussian carp (*Carassius gibelio* Bloch, 1782), vimba (*Vimba vimba* Linnaeus, 1758) and chub (*Leuciscus cephalus* Linnaeus, 1758) in one of the most popular for angling dams in Central Bulgaria in order to evaluate the risk for human health.

Materials and Methods

Study Area

The Zhrebchevo Dam is located in Southeastern Bulgaria, geographic coordinates (42° 36' 56" N, 25° 51' 33") (Fig. 1). The surface water area is 25 km² and maximum depth - 50.50 m. The dam water is a source of irrigation and recreation.

Sample Preparation

The study was carried out in March-October 2016. Bream, Prussian carp, vimba and chub were caught by recreational anglers by the fishing rod from the shore. A total of 8 specimens from each studied species were collected. The total length of selected specimens was longer than permitted by the Fisheries and Aquaculture Act (2001). The weight of each fish

specimen was measured with an electronic scales (up to 0.01 g). The mean weight of the bream, Prussian carp, vimba and chub was 427.46±34.74 g, 432.68±45.37 g, 397.51±43.73 g and 352.84±54.73 g respectively. The fishes were transported at 0–4°C to the Central laboratory of Trakia University, Stara Zagora for further analysis. The fish samples were filleted and the meat was minced and homogenized for chemical analyzing.

Chemical Analysis

Meat samples were prepared according to AOAC (2006; method 983.18) and determination of water content (%) was by air drying (AOAC, 1997; method 950.46). Crude protein content (%) was calculated by converting the nitrogen content, quantified by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content (%) was investigated by incineration in a muffle furnace (MLW, Germany) at 550°C for 8 h. Crucibles were brought about the room temperature and weighed.

Heavy Metal Content

The sample preparation for heavy metals analysis was made by wet digestion in microwave (Perkin Elmer Multiwave 3000). The content of Cd, Ni, Pb and Zn (mg.kg⁻¹) in fish muscles was determined by atomic absorption spectrophotometer (Perkin-Elmer Analyst 800).

Human Health Risk Assessment

Human health risk assessment was performed by calculations of estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI). Estimated daily intake (EDI) and Target hazard quotient (THQ) were calculated as described by YABANLI & ALPARSLAN (2015):

$$EDI = \frac{FIR \times C}{BWa}$$

where EDI is estimated daily intake (μg analysed element/kg body weight/day), FIR – average daily consumption of bream, Prussian carp, vimba and chub (kg/person), C – average concentration of the heavy metals in the samples (mg/kg) and BWa – average body weight (kg).

Body weight of 70 kg was used for adults (YABANLI & ALPARSLAN, 2015).

EDI values should be compared to the oral reference dose (RfDo) (USEPA, 2016) and provisional tolerable weekly intake (PTWI) (FAO/WHO, 1993; 2004) of each analyzed element to determine whether recommended levels are exceeded or not.

$$\text{THQ} = \frac{\text{Efr} \times \text{EDtot} \times \text{FIR} \times \text{C}}{\text{RfDo} \times \text{BWA} \times \text{ATn}} \times 10^{-3}$$

where THQ is target hazard quotient, Efr – exposure frequency (365 days/year), EDtot – exposure duration (70 years), FIR – average daily consumption of bream, Prussian carp, vimba and chub (g/day), C – average concentration of the heavy metals in the samples (mg/kg), RfDo – oral reference dose (mg/kg/day), BWa – average body weight (kg) and ATn – average exposure for non-carcinogens in year (365 days/year x 70 years).

Hazard index (HI) is a sum of the THQ values of each analyzed element (JAVED & USMANI, 2016):

$$\text{HI} = \text{THQPb} + \text{THQCd} + \text{THQHg}$$

Statistical Analysis

Statistical analyses were performed using STATISTICA 6 (StatSoft Inc., 2002). The accuracy of the measurements was assessed by standard deviation (SD) for n=8.

Results and Discussion

Chemical Composition

The highest water content was found out in the meat of Prussian carps – $82.13 \pm 0.04\%$, while the lowest was that of vimba meat – $78.51 \pm 0.07\%$ (Table 1). According to

FAO/WHO (2011) the average water content of fish meat is about 80%. The dry matter content for both species was characterized with the opposite tendency – $17.88 \pm 0.04\%$ and $21.50 \pm 0.07\%$ respectively (Table 1).

Protein content was the highest in vimba meat: $18.41 \pm 0.23\%$, followed by bream, Prussian carp and chub meat (Table 1). Data correspond to those reported by ŻMIJEWSKI *et al.* (2006) in *Cyprinids*. The protein content in different fish species is from 15% to 20% with a tendency for very little within-species variations (FAO/WHO, 2011).

Fat content values were the highest in vimba meat – $1.88 \pm 0.09\%$, followed by chub, bream and Prussian carp meat. ŻMIJEWSKI *et al.* (2006) reported a higher meat fat content in *Cyprinids* (2.52-3.63%) than values in our study – 0.25-1.88%, which could be probably due to the season. According to FAO/WHO (2011), fat content of fish meat varied a lot according to the species and the catch season.

Ash content was the highest in bream meat $1.24 \pm 0.05\%$. According to FAO/WHO (2011) ash content of fish meat does not exceed 2% as also confirmed by our results and those of ŻMIJEWSKI *et al.* (2006) in *Cyprinids* – 0.99-1.01%.

Heavy Metal Content

The highest Cd content was observed in bream meat – $0.045 \pm 0.004 \text{ mg.kg}^{-1}$, it exceeded that in Prussian carp, chub and vimba, by 8.99%, 24.45% and 26.67%, respectively (Table 2). The results corresponded to data of PEYCHEVA *et al.* (2017) $0.020\text{-}0.046 \text{ mg.kg}^{-1}$ in *Cyprinids* from the Mandra Lake and to these reported by YANCHEVA *et al.* (2014) for the Topolnitsa reservoir. Substantially lower Cd concentrations (0.01 mg.kg^{-1}) were reported in roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*) from the Zhrebchevo Dam in both species (ZHELYAZKOV *et al.*, 2014). Similar values were reported by VALKOVA *et al.* (2015a) in common carp from the same water basin. The maximum Cd level above which fish consumption is prohibited by the EC and Bulgarian legislation is 0.05 mg.kg^{-1} Commission Regulation 1881 (EC, 2006) and Regulation 31 (2004).

The Ni content was the highest in bream meat - $0.457 \pm 0.018 \text{ mg.kg}^{-1}$, higher than that in Prussian carp, chub and vimba by 9.85%, 23.86% and 27.79% respectively (Table 2).

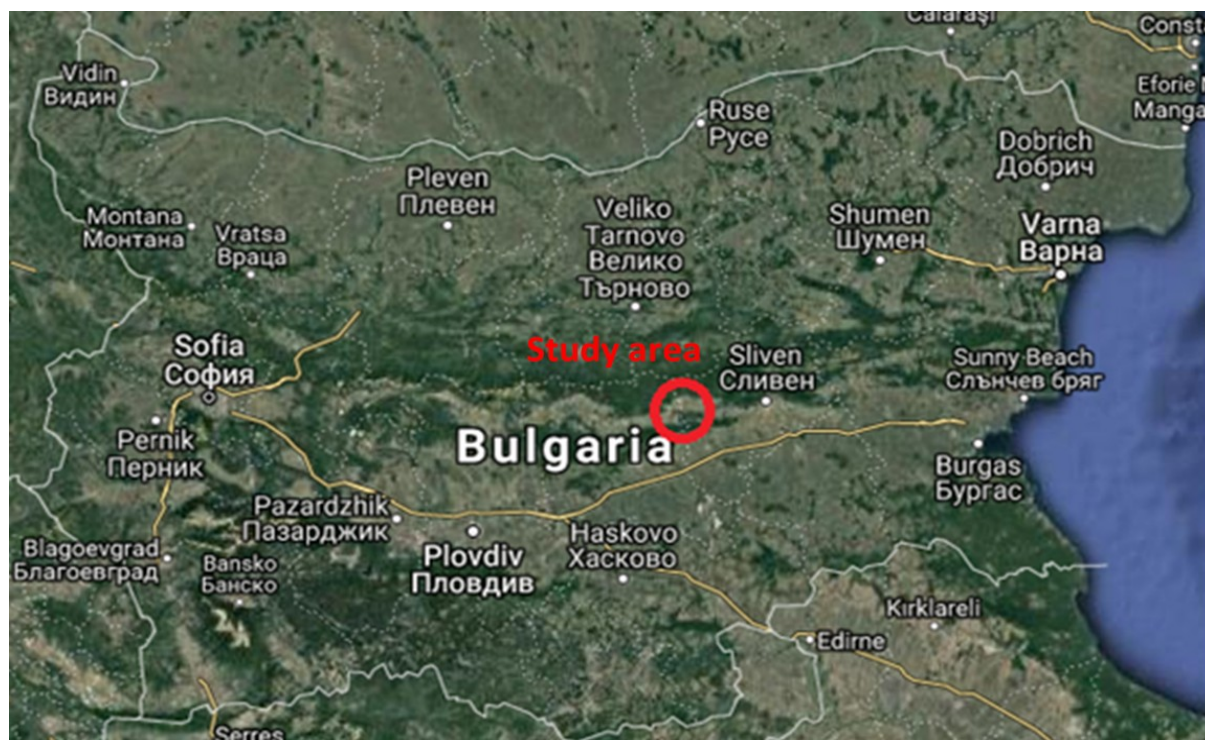


Fig. 1. Indicative map of the study area.

Table 1. Proximate analysis of the meat from analyzed fish species, caught in Zhrebchevo Dam (%).

Parameter	n	Bream	Prussian carp	Vimba	Chub
		(<i>Abramis brama</i>)	(<i>Carassius gibelio</i>)	(<i>Vimba vimba</i>)	(<i>Leuciscus cephalus</i>)
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Water	8	80.48±0.0	82.13±0.04	78.51±0.07	81.03±0.04
Protein	8	17.82±0.10	16.49±0.01	18.41±0.23	16.49±0.05
Lipids	8	0.47±0.06	0.25±0.05	1.88±0.09	1.30±0.03
Dry matter	8	19.52±0.01	17.88±0.04	21.50±0.07	18.98±0.04
Ash	8	1.24±0.05	1.14±0.00	1.21±0.08	1.19±0.04

Similar results for Ni content in the meat of *Cyprinids* from the Topolnitsa reservoir were reported (YANCHEVA *et al.*, 2014). Again, ZHELYAZKOV *et al.* (2014) detected lower Ni concentrations in the meat of roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*) caught from Zhrebchevo Dam - 0.04 mg.kg^{-1} and 0.025 mg.kg^{-1} , respectively. Lower values ($0.055\text{-}0.11 \text{ mg.kg}^{-1}$) of Ni were also reported by PEYCHEVA

et al. (2017) in *Cyprinids* from the Mandra Lake. FAO/WHO (1983) recommendations for daily Ni intake are $100\text{-}300 \text{ }\mu\text{g.kg}^{-1}$ body weight. Nevertheless, the toxicity of Ni for men is not high as its absorption is very low (ÖZDEN, 2008). The established maximum Ni level as per Bulgarian legislation, above which fish consumption is not allowed, is 0.50 mg.kg^{-1} (Regulation 31, 2004).

The highest Pb content was that of bream meat - $0.177 \pm 0.013 \text{ mg.kg}^{-1}$, higher than Pb in Prussian carp, chub and vimba by 6.22%, 21.47% and 23.73%, respectively (Table 2). Significantly lower Pb concentrations were demonstrated in roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*) from the Zhrebchevo Dam - 0.07 mg.kg^{-1} and 0.06 mg.kg^{-1} , respectively (ZHELYAZKOV *et al.*, 2014). Similar values ($0.075\text{-}0.086 \text{ mg.kg}^{-1}$) were reported by STANEK *et al.* (2015) in *Abramis brama* L., *Blicca bjoerkna* L. and *Alburnus alburnus* L. from the Vistula River, whereas PEYCHEVA *et al.* (2017) established a higher lead content in Cyprinids from the Mandra Lake ($0.15\text{-}0.27 \text{ mg.kg}^{-1}$). The maximum allowed daily intake of Pb for adults specified by FAO/WHO (1983) is $25 \mu\text{g/kg}$ body weight. The established maximum Pb level as per Bulgarian legislation, above which fish consumption is not allowed, is 0.20 mg.kg^{-1} (Regulation 31, 2004), and even higher according to EC regulations - 0.30 mg.kg^{-1} (EC, 2006).

The highest Zn content was observed in Prussian carp meat - $20.170 \pm 0.266 \text{ mg.kg}^{-1}$ which was higher than Zn concentrations in vimba, chub and bream samples by 16.26%, 19.34% and 21.16% respectively (Table 2). Considerably lower Zn concentrations were found out in the meat of roach (*Rutilus rutilus*)

and bleak (*Alburnus alburnus*) - 5.46 mg.kg^{-1} and 4.05 mg.kg^{-1} respectively (ZHELYAZKOV *et al.*, 2014). Similar low Zn concentrations were reported by YANCHEVA *et al.* (2014) and by PEYCHEVA *et al.* (2017) in Cyprinids from the Topolnitsa reservoir and from the Mandra Lake, respectively. FAO/WHO (1983) specify a daily intake limit of 30 mg.kg^{-1} Zn per capita. The Bulgarian legislation sets the maximum allowed Zn level in fish for consumption to 50 mg.kg^{-1} (Regulation 31, 2004).

Human Health Risk Assessment

Fishes consumption rate in Bulgaria was $2.60 \text{ kg/capita/year}$ or 7.11g/capita/day in 2013 (FAO, 2017). EDI values were lower than published RfDo and PTWI (Table 3). According to USEPA (2016), RfDo of Cd, Ni, Pb and Zn are 1×10^{-3} , 2×10^{-2} , 2×10^{-3} , 3×10^{-1} and mg/kg/day , respectively. FAO/WHO (1993 and 2004) recommended PTWI values of Cd ($7 \mu\text{g/kg}$), Ni ($35 \mu\text{g/kg}$), Pb ($25 \mu\text{g/kg}$) and Zn ($100 \mu\text{g/kg}$).

THQ and HI were lower than 1, which, according to the norms set by FAO/WHO (1993 and 2004) signifies that the meat of the studied fish species is safe for consumption as the content of the four heavy metals is concerned. Our data are comparable to those reported by PEYCHEVA *et al.* (2017) about Cyprinids from the Mandra Lake.

Table 2. Heavy metal content in the meat of studied fish species from the Zhrebchevo Dam (mg.kg^{-1}).

Metal	n	Bream (<i>Abramis brama</i>) Mean±SD	Prussian carp (<i>Carassius gibelio</i>) Mean±SD	Vimba (<i>Vimba vimba</i>) Mean±SD	Chub (<i>Leuciscus cephalus</i>) Mean±SD	Reference (Commission Regulation 1881 (EC, 2006)	Reference (Regulation 31, 2004)
Cd	8	0.045 ± 0.004	0.041 ± 0.000	0.033 ± 0.001	0.034 ± 0.000	0.05	0.05
Ni	8	0.457 ± 0.018	0.412 ± 0.013	0.330 ± 0.001	0.348 ± 0.001	-	0.50
Pb	8	0.177 ± 0.013	0.166 ± 0.003	0.135 ± 0.001	0.139 ± 0.001	0.30	0.20
Zn	8	15.903 ± 0.593	20.170 ± 0.266	16.270 ± 0.233	16.890 ± 0.094	-	50.00

Table 3. EDI, THQ and HI values for adults consuming four fish species from Zhrebchevo Dam (assuming 70 kg person).

Metal		Bream (<i>Abramis brama</i>)	Prussian carp (<i>Carassius gibelio</i>)	Vimba (<i>Vimba vimba</i>)	Chub (<i>Leuciscus cephalus</i>)	*RfDo ($\mu\text{g}/\text{kg}/\text{day}$)	**PTWI ($\mu\text{g}/\text{kg}$)
Cd	EDI ($\mu\text{g}/\text{kg}/\text{day}$)	0.0017	0.0015	0.0012	0.0013		
Ni	EDI ($\mu\text{g}/\text{kg}/\text{day}$)	0.017	0.015	0.012	0.013		
Pb	EDI ($\mu\text{g}/\text{kg}/\text{day}$)	0.007	0.006	0.005	0.005		
Zn	EDI ($\mu\text{g}/\text{kg}/\text{day}$)	0.59	0.75	0.6	0.63		
Cd	THQ ($\mu\text{g}/\text{kg}/\text{day}$)	0.012	0.011	0.009	0.009	1	7
Ni	THQ ($\mu\text{g}/\text{kg}/\text{day}$)	0.006	0.006	0.004	0.005	5	35
Pb	THQ ($\mu\text{g}/\text{kg}/\text{day}$)	0.012	0.011	0.009	0.009	2	25
Zn	THQ ($\mu\text{g}/\text{kg}/\text{day}$)	0.014	0.018	0.015	0.015	1	100
	HI	0.044	0.046	0.037	0.038		

*RfDo - Oral reference dose, [USEPA \(2016\)](#); **PTWI - Provisional tolerable weekly intake (PTWI), [FAO/WHO \(1993 and 2004\)](#)

Conclusions

The chemical composition of the four studied fish species (bream, Prussian carp, vimba and chub) from the Zhrebchevo Dam is appropriate for consumers from a dietetic viewpoint. The content of Cd, Ni, Pb and Zn in these species did not exceed the maximum allowable concentrations as per Commission Regulation 1881/2006 ([EC, 2006](#)) and [Regulation 31 \(2004\)](#) of the Bulgarian legislation. Therefore, the consumption of Bream, Prussian carp, Vimba and Chub, caught from the Zhrebchevo Dam, did not pose any risk for the health of adults.

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Compliance with ethical standards. Conflict of interest: The authors declare that they have no conflict of interest. *Ethical approval:* All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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