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ORIGINAL PAPER

CONTENT OF Hg, Pb AND Cd IN THE MUSCLES OF GRASS CARP, BIGHEAD CARP, SIBERIAN STURGEON AND WELS CATFISH FROM EASTERN POLAND*

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Abstract

Assessment of the content of mercury (Hg), lead (Pb) and cadmium (Cd) in food fish is important for the consumer's health. The maximum acceptable amounts for these heavy metals in fish muscle are defined in the European and world legislation. The objective of the study was to determine the content of Hg, Pb and Cd in the muscles of grass carp, bighead carp, Siberian sturgeon and wels catfish, and to compare the results with the relevant international guidelines. In addition, the potential risk of any toxic effect of these elements on the consumer, estimated daily intake (EDI), target hazard quotient (THQ), and total target hazard quotient (TTHQ) were analysed. The graphite furnace atomic absorption spectrometry method (GFAAS) was used to evaluate the content Cd and Pb in fish muscles. The total Hg concentration in the same samples was determined by the cold-vapour atomic absorption spectrometry technique (CVAAS). The Hg content was significantly higher in muscle tissues of bighead carp and Siberian sturgeon than in those of grass carp and wels catfish. The level of Pb was the highest in the muscle tissues of Siberian sturgeon, being lower and comparable in the muscle tissues of grass carp, bighead carp and wels catfish. No significant differences between the fish species in the content of Cd were identified. The content of Hg, Pb and Cd in the muscles of the examined fish species was less than the threshold levels defined in the Commission Regulation (EC) 1881/2006. The EDI, THQ and TTHQ values did not indicate a risk of any toxic effect of these elements on people consuming these fish species.

Keywords: heavy metal, elements, fish, health risk.

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INTRODUCITON

Chemical elements can be classified as essential (macro- and microelements) and non-essential ones (heavy metals). Non-essential elements, such as Hg, Pb and Cd, do not perform any function in the body and are toxic even in small amounts. The thresholds levels for heavy metals in food, including fish muscles, are defined in the European and world legislation (Commission Regulation (EC) 1881/2006, Dural et al. 2007, Uysal et al. 2008, Al Sayegh Petkovšek et al. 2012, Liu et al. 2012, El-Moselhy et al. 2014, Zhang et al. 2014, Ivanović et al. 2016).

Heavy metals can reach humans via a food chain, exposing consumers to potential health risks (El-sadaawy et al. 2013). The content of Hg, Cd and Pb in the muscles of edible fish has been found to exceed acceptable levels (Agusa et al. 2004, Dural et al. 2007, Rahman et al. 2012, Squadrone et al. 2013, Leung et al. 2014, Has-Schön et al. 2015, Araújo, Cedeño-Macias 2016, Ivanović et al. 2016). Determination of the content of heavy metals in commercial fish species is very important for the health safety of consumers. Grass carp, bighead carp, Siberian sturgeon and wels catfish are commercially valuable table fish in many countries (www.fao.org/fishery/statistics/).

The objective of the present study was to determine the content of Hg, Pb and Cd in the muscles of grass carp, bighead carp, Siberian sturgeon and wels catfish, and to compare the results with the relevant international guidelines. In addition, an assessment was made of the potential risk of some toxic effect these elements could have on the consumer's health. Finally, the estimated daily intake (EDI), target hazard quotient (THQ), and total target hazard quotient (TTHQ) were estimated.

MATERIAL AND METHODS

Sampling

The material for the study consisted of grass carp (*Ctenopharyngodon idella*), n = 12, bighead carp (*Aristichthys nobilis*) n=12, Siberian sturgeon (*Acipenser baerii*), n = 12 and wels catfish (*Silurus glanis*), n = 12. All fish samples were collected in winter. The fish specimens were obtained from fish farms located in eastern Poland, in agricultural regions. The fish fed on natural food found in the ponds, and were not supplied any industrial feed. Individual fish were caught, immediately killed by a blow on the head, and transported to the laboratory in boxes at an average temperature maintained within the range of 0-4°C. For each fish, the fork length (FL in cm) and weight were recorded. The mean body weight and length of the fish were 2.04 ± 0.55 kg and 54.92 ± 5.58 cm for grass carp, 2.64 ± 0.30 kg and 50.38 ± 1.58 cm for bighead carp, 2.17 ± 0.68 kg and 80.17 ± 7.40 cm for Siberian sturgeon, and 2.68 ± 0.38 kg and 74 ± 3.72 cm for wels catfish, respectively. The fish were gutted and divided into two fillets with a ceramic knife. An amount of about 25 g of white muscle (without the skin) was taken from the dorsal musculature of each fish (both fillets) and homogenized in a vibrating ball mill to make a representative sample. Homogenized samples from each individual were collected in sterile polyethylene bags and kept in a laboratory freezer at a temperature -20°C until analysis.

Chemical analyses

The total Hg concentration was measured by the cold-vapour atomic absorption spectrometry technique in a Mercury/MA - 2000 system (NIC – Japan), where mercury was determined without sample pretreatment (Konieczka et al. 2010). The homogenized samples were weighed directly (10-100 ± 0.1 mg) into pre-cleaned combustion boats and inserted into a mercury analyzer. In order to remove any interfering substances, which can be generated during the thermal decomposition of a sample, gas washing was performed. All measurements were carried out in triplicate for each samples. The method was controlled by analyzing certified reference material (BCR – 463 tuna fish, IRMM, Geel, Belgium). Triplicate subsamples (5.0 g representative sample) were mineralized in an electric stove using final the temp. of 450°C. The ash was dissolved in 1M HNO_3 for further analysis of metals. In order to validate the method, DORM-3 (Fish Protein Certified Reference Material for Trace Metals, NRCC, Canada) was subjected to the same analytical procedure and was tested for accuracy. The content of Pb and Cd was determined in an atomic absorption spectrometer with electrothermal atomization (GFAAS) and Zeeman background correction (SpektrAA 220Z, Varian, Australia) – Szkoda, Żmudzki (2005). Palladium nitrate solution (Merck) was used as a chemical modifier for the determination of Cd, and ammonium dihydrogen phosphate solution, ADP (Merck) served for Pb determination.

Health risk assessment

The human health risk from fish consumption was estimated for each element using the following equations (Heshmati et al. 2017):

$$\mathrm{EDI} = \frac{Cm \cdot dc_{fish}}{bw},$$

$$\mathrm{THQ} = \frac{EDI}{RfD},$$

$$\mathrm{TTHQ} = \mathrm{THQ\ (Hg)} + \mathrm{THQ\ (Pb)} + \mathrm{THQ\ (Cd)},$$

where EDI is the estimated daily intake (μg kg^{-1} per day), Cm is the mean concentration of elements in the fish muscle (μg g^{-1} = mg kg^{-1}), dc_{fish} is the daily per capita consumption of freshwater fish in Poland (5.47 g per capita per day; www.fao.org/faostat/en/#data/CL) and bw is the average adult human body weight (70 kg). THQ is the target hazard quotient, TTHQ is the total target hazard quotient, and RfD is the reference dose (mg kg^{-1} per day) established by the United States Environmental Protection Agency – Regional Screening Level, Summary Table (USEPA 2016).

Statistical analysis

All data are presented as means and ± SD. Statistical analysis of the data was performed using Statistica software (version 13.1), by one-way ANOVA or the Kruskal-Wallis test (depending on whether the assumptions of analysis of variance were met). Multiple comparisons were made by *post hoc* tests (the Tukey's and Dunn's); $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The content of Hg, Pb and Cd in the muscles of grass carp, bighead carp, Siberian sturgeon and wels catfish can be found in Table 1.

Table 1

The content of elements in the muscles of the examined fish species (mg kg^{-1} WW)

Elements	Fish species		
	grass carp (n=12)		
	mean	SD	range
Hg	0.008^{a}	0.006	0.004-0.026
Pb	0.033^{ab}	0.015	0.015-0.073
Cd	0.001^{a}	0.001	0.000-0.002
bighead carp (n=12)			
Hg	0.024^{b}	0.005	0.017-0.031
Pb	0.029^{a}	0.010	0.015-0.046
Cd	0.001^{a}	0.0003	0.001-0.002
Siberian sturgeon (n=12)			
Hg	0.056^{b}	0.061	0.016-0.219
Pb	0.047^{b}	0.017	0.020-0.074
Cd	0.004^{a}	0.005	0.001-0.017
wels catfish (n=12)			
Hg	0.008^{a}	0.002	0.005-0.011
Pb	0.032^{ab}	0.020	0.014-0.072
Cd	0.001^{a}	0.001	0.001-0.003

SD – standard deviation,
^{a,b,c,d} – means in the same row with different superscript letters were significantly different $P < 0.05$

The average Hg content in the muscles of the four analyzed fish species was 0.008-0.056 mg kg^{-1} WW. Significantly ($P < 0.05$) more Hg was found in Siberian sturgeon and bighead carp muscle than in grass carp and wels catfish. The levels of Hg (<0.02-0.03 mg kg^{-1}) in the muscles of rainbow trout and carp from different regions of Poland (Tkaczewska, Migdał 2012*a,b*) were within the range of values found in the present study. Higher Hg levels as compared to the four examined species have been noted in the muscles of yellowfin tuna (*Thunnus albacares*) and common dolphinfish (*Coryphaena hippurus*) from the Eastern Pacific Ocean caught in Manta, Ecuador (1.4 ± 1.3 and 1.6 ± 1.4 mg kg^{-1} WW) (Araújo, Cedeño-Macias 2016); yellowfin tuna (*Thunnus albacares*) caught off the Atlantic coast of South Africa (0.72-0.88 mg kg^{-1} WW) (Bosch et al. 2016); and freshwater fish from five French fishing areas, i.e. bream, pike, pikeperch and European eel (0.128-0.199 mg kg^{-1} WW) (Noël et al. 2013). Wei et al. (2014) reported a higher Hg content in the muscles of predatory fish (0.082-0.168 mg kg^{-1}) than in non-predatory fish (0.008-0.054 mg kg^{-1}). Our study did not prove it as the Hg content was similar in muscles of the predatory and herbivorous fish, i.e. catfish and grass carp as well as Siberian sturgeon and bighead carp.

The average Pb content in the muscles of the four examined fish species was 0.029-0.047 mg kg^{-1} WW. Significant differences ($P < 0.05$) in the level of Pb were observed between two fish species, namely the Pb level was higher in Siberian sturgeon than in bighead carp muscles. In comparison with previous studies, the mean concentration of Pb in the present study was lower than determined in muscles of edible fish such as *Ailia coila*, *Gagata youssoufi* and *Mastacembelus pancalus* from the Buriganga River in Bangladesh (0.47-3.17 mg kg^{-1} WW) (Ahmed et al. 2015); in black bass, mandarin fish, grass carp, snakehead, catfish, bighead carp and tilapia from China (0.15 do 8.62 mg kg^{-1} WW) (Leung et al. 2014); and in *G. giuris*, *P.chola*, *P. ticto*, *P. sophore* and *L. rohita* (1.77-6.98 mg kg^{-1} WW) collected from the Buriganga River in Bangladesh (Ahmed et al. 2016). Similar Pb levels to those found in the present study have been reported in the muscles of *Rutilus rutilus* and *B. balcanicus* (0.03-0.04 mg kg^{-1} WW) from the Šalek lakes in Slovenia (Al Sayegh Petkovšek et al. 2012) and in the muscles of rainbow trout and carp from Poland (<0.05 do 0.056 mg kg^{-1} WW) (Tkaczewska, Migdał 2012*a,b*).

In the present study, the average content of Cd in the muscle tissues of the four fish species was the lowest of all the metals analyzed, ranging between 0.001 and 0.004 mg kg^{-1} WW. The Cd levels did not differ significantly among these species ($P > 0.05$). The Cd content in the examined samples was lower than in the muscles of rainbow trout and carp from Poland (<0.010 to 0.014 mg kg^{-1}) (Tkaczewska, Migdał 2012*a,b*); in the muscles of tropical fish, i.e. *A. coila*, *G. youssoufi* and *M. pancalus* from the Buriganga River, Bangladesh (0.01-0.02 mg kg^{-1}WW) (Ahmed et al. 2015); in the muscles of tench, pike-perch and common carp (0.0784-0.1766 mg kg^{-1}WW) from Turkey (Mert et al. 2014) and in the muscles of *C. hippurus* and *T. albacares* from the Eastern Pacific Ocean landed in Manta, Ecuador (0.64-2.4 mg kg^{-1} WW) (Araújo, Cedeño-Macias 2016). A similar content of Cd has been noted in the muscles of bighead carp, bream, carp, catfish, crucian, grass carp, mandarin fish, silver carp, white semiknife carp and yellow catfish from Poyang Lake, China (0.0009-0.009 mg kg^{-1} WW) (Wei et al. 2014).

The Hg, Pb and Cd content in all muscle samples from grass carp, bighead carp, Siberian sturgeon and wels catfish was below the maximum thresholds defined in the Commission Regulation (EC) 1881/2006, i.e. for Hg: 0.50 mg kg^{-1} WW for grass carp, bighead carp and wels catfish and 1.0 mg kg^{-1} WW for sturgeon, and for Pb and Cd: 0.30 and 0.050 mg kg^{-1} WW, respectively, for all species. The levels of Hg, Pb and Cd in the muscles of grass carp from China and of trout and carp from Poland, as well as Pb and Cd levels in the muscles of five sturgeon species from the Caspian Sea and European catfish from Italian rivers were below the maximum acceptable limits (Liu et al. 2012, Tkaczewska, Migdał 2012*a*,*b*, Pourang et al. 2005, Squadrone et al. 2013). In contrast, in the muscles of European catfish from the Buško Blato reservoir (Bosnia and Herzegovina), the content of Hg, Pb and Cd exceeded the Maximal Admissible Concentrations in Croatia (Has-Schön et al. 2015). The Hg content in the muscles of European catfish from Italian rivers also exceeded the maximum levels defined by the European regulations (18% of samples) (Squadrone et al. 2013). The differences in the content of elements in the muscles of fish may arise from differences between the species and sizes of fish, the geographical region where they live, or the season when they are caught (Cheung et al. 2008, Yi, Zhang 2012, Mert et al. 2014).

The EDI (estimated daily intake), THQ (target hazard quotient) and TTHQ (total target hazard quotient) determined for Hg, Pb and Cd are presented in Table 2.

Quantities of elements supplied to the body with food depend on both their content in food and the amount of food consumed. The estimated EDI values were based on the assumption that a person weighing 70 kg will consume 5.47 g of fish per day. The results showed that the EDI values were lower than the reference dose (RfD) values defined by the USEPA (2016), indicating that there was no health risk associated with the intake of the elements tested through the consumption of grass carp, bighead carp, Siberian sturgeon and wels catfish analyzed in our study. The acceptable value for THQ and TTHQ is 1 (USEPA 2016). The THQ and TTHQ values were less than 1, which means that the elements Hg, Pb and Cd posed no threat to the health of consumers of these four fish species, either individually (THQ) or in combination (TTHQ). Similarly, THQ values were less than 1 for Pb, Cd and Hg in the muscles of the commercial fish species *Engraulis encrasicolus*, *Sardina pilchardus* and *Mullus barbatus* from various Sicilian areas (Copat et al. 2012).

Table 2

The estimated daily intakes (EDI) of elements by the consumption of the examined fish and the hazard quotient (THQ and TTHQ)

Fish species and trace elements	Average concentration (μg g^{-1})	EDI (μg kg^{-1} per day)	RfD (μg kg^{-1} per day)	THQ and TTHQ
Grass carp				
Hg	0.008	6.0 E-04	0.1	6.0 E-03
Pb	0.033	2.6 E-03	4*	7 E-04
Cd	0.001	1.0 E-04	1	1.0 E-04
				TTHQ = 6.8 E-03
Bighead carp				
Hg	0.024	1.9 E-03	0.1	1.9 E-02
Pb	0.029	2.3 E-03	4*	6 E-04
Cd	0.001	1.0 E-04	1	1.0 E-04
				TTHQ = 19.7 E-03
Siberian sturgeon				
Hg	0.056	4.4 E-03	0.1	4.4 E-0.2
Pb	0.047	3.7 E-03	4*	1.0 E-03
Cd	0.004	3.0 E-04	1	3.0 E-04
				TTHQ = 45.3 E-03
Wels catfish				
Hg	0.008	7.0 E-04	0.1	7.0 E-03
Pb	0.032	2.6 E-03	4*	7 E-04
Cd	0.001	1.0 E-04	1	1.0 E-04
				TTHQ = 7.8 E-0.3

* RfD for Pb according to Heshmati et al. (2017)

CONCLUSIONS

The content of Hg, Pb and Cd in the muscles of grass carp, bighead carp, Siberian sturgeon and wels catfish were below the maximum acceptable levels as defined in the Commission Regulation (EC) 1881/2006. The EDI, THQ and TTHQ values did not indicate a risk of any toxic effect of Hg, Pb and Cd on people consuming these four fish species.

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Conflict of interest

The authors declare that they have no conflict of interest.

Animal Rights Statement: None required.