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ORIGINAL PAPER

CHANGES IN THE CONTENT OF HEAVY METALS (Pb, Cd, Hg, As, Ni, Cr) IN FRESHWATER FISH AFTER PROCESSING – THE CONSUMER'S EXPOSURE

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Abstract

Fish is a desirable component of a diet because of its high nutritive value and sensory qualities. However, fish consumption is still low in many countries. The aim of this study was to assess the content of Pb, Cd, Hg, As, Ni and Cr in the meat of freshwater fish and quantitative changes after processing (smoking or marinating). Moreover, the exposure of consumers to heavy metals by eating a portion (100 g) of the examined products was estimated as well as the safe amounts (comparing to the maximum limit - ML and as the Benchmark Dose Lover Confidence limits – BMDL) of processed fish for two population groups. The research material consisted of 3 species, i.e. common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykkis*) and northern pike (*Esox lucius*). The content of heavy metals (except for mercury) was determined in samples of fresh as well as processed fish, using inductively coupled plasma optical emission spectrometry (ICP-OES). In turn, for the determination of mercury, an AMA-254 Advanced Mercury Analyzer was used. Nickel and chromium in all the samples were below limits of detection. The study revealed a relatively safe content (below MLs) of heavy metals in almost all fish samples, except for the cadmium concentration in fresh and smoked common carp as well as in marinated rainbow trout. The calculated intakes of these contaminants through consumption of one portion of the examined fish products were all below BMDLs and Tolerable Weekly Intakes (PTWI or TWI).

Keywords: heavy metals, freshwater fish, technological processes.

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INTRODUCTION

Fish is a desirable component of a diet because of both its high nutritive value and sensory qualities. However, fish consumption is still low in many countries. In 2015, the average annual fish consumption in Poland was 12.5 kg *per capita* (almost 50% of the average *per capita* fish consumption in the EU), with pollock (among saltwater species) and carp (among freshwater species) being most popular (Ruciński 2016). Fish may contain several chemical contaminants, including heavy metals, and cause a threat to the safety of consumers (Buňka et al. 2013). Therefore, their systematic monitoring is recommended and required by the EU authorities (Efsa 2007). The presence of heavy metals in food products is the consequence of environmental contamination of soil and air (plant products), feeding animals with contaminated feed (animal products), acquisition of polluted waters (fish), and other sources (technological water, utensils, pots, etc.) (Vicarova et al. 2014). Many studies (Łuszczek-Trojnar et al. 2011, Vilizzi, Tarkan 2016) have included the determination of heavy metals in fish but they were carried out on fresh fish. Meanwhile, due to the fact that freshwater fish are not customarily eaten fresh, there is a need for determination of heavy metal levels in fish products. During thermal processing, as well as causing loss of weight and water, the application of heat accelerates protein degradation and therefore chemical contaminants may also be affected by the heat applied (Tawfik 2013). Smoking is traditionally used for fish preservation all over the world (Ayejuyo et al. 2013). Marinating involves covering fish with vinegar marinade with spices, and it guarantees good microbial quality for several months (Cieślik et al. 2017).

Heavy metals are metallic elements with relatively high density compared to water. Taking into consideration the fact that heaviness and toxicity are inter-related, heavy metals also include metalloids (arsenic), which can induce toxicity at a low level of exposure. These contaminants can cause serious human health problems (Soliman 2015). They affect the organism in two ways; first is the disruption of normal cell processes that leads to toxicity, and the second one is bioaccumulation (Vinodhini, Navayanan 2008, Vilizzi, Tarkan 2016). Among heavy metals, cadmium, lead, mercury, arsenic, nickel and chromium are of particular concern (European Communities 2008, Vilizzi, Tarkan 2016).

The aim of this study was to assess the content of heavy metals (Pb, Cd, As, Hg, Ni and Cr) in the muscle tissue of three freshwater fish (common carp, rainbow trout and northern pike), either fresh or after two different processing methods: smoking or marinating. The exposure of consumers to heavy metals by eating a portion of the examined products was also estimated as well as the safe amount of processed fish for children and adults.

MATERIAL AND METHODS

Research material

The research material consisted of 3 fish species, farmed in the Carp Valley (Zator, Malopolska, Poland), i.e. common carp (*Cyprinus carpio*) – Carp of Zator, rainbow trout (*Oncorhynchus mykkis*), and northern pike (*Esox lucius*). A total of 6 fish from each species were collected. The age of common carp was 2 years, and its weight was 1.5-2.0 kg; the age of rainbow trout was 0.5 years and its weight was 0.5-1.0 kg; the pike's age was 3 years and its weight was 2-3 kg. The specimens were killed, washed, gutted, cleaned and divided into parts. The samples were stored at -80°C until analyzed. The fish were also used to prepare smoked (common carp, rainbow trout, northern pike) and marinated (common carp, rainbow trout) products, according to traditional recipes. Prior to smoking, the samples were cured in spices and stored at 4-8°C for 12 hours. Next, they were subjected to warm smoking (4-6 h, smoke from fruit tree wood and alder wood in the last stage). In turn, the marinating process (vinegar and water in the ratio 1:3, sugar, mustard seed, bay leaf, allspice and slices of vegetables) was preceded by deep-frying in rapeseed oil (until golden brown). Fish smoking as well as preparation of marinated products were described in detail previously (Cieślik et al. 2017).

Chemical analyses

A total of 18 samples of fresh fish (3 species) and 15 samples of fish products (smoked or marinated) were determined. The content of Pb, Cd, As, Ni and Cr was determined by the spectrometric method according to internal laboratory procedures. Prior to analysis, material was freeze-dried using an Alpha 1-2 LD Plus freeze dryer (Martin Christ, Germany). The process was carried out at a temp. of -40°C and pressure of 0.2 mbar until constant mass was obtained. A freeze-dried sample in the amount of 0.5 g was added to 5 ml of nitric acid (65%, Suprapur, Merck, Germany) and 1 ml hydrochloric (30% Suprapur, Merck, Germany). Wet mineralization was performed in a microwave system Multiwave 3000 (Anton Paar, Graz, Austria) under following conditions: power of 800 W, reaching time of 10 min, holding time of 20 min, ventilation time of 15 min. Next, the heavy metal levels were measured using a spectrometer ICP-OES 7300 DualView (Perkin Elmer, USA), at the following wavelengths: Pb 220.353 nm, Cd 228.802 nm, As 188.979 nm, Ni 231.604 nm and Cr 267.716 nm. The detection limits were: Pb – 0.042 mg L^{-1}, Cd – 0.0027 mg L^{-1}, As – 86 μg L^{-1}, Ni – 0.0105 μg L^{-1} and Cr – 0.0071 mg L^{-1}. Quality of analyses was ensured by making a parallel analysis of Certified Reference Material IAEA-407 (Fish Homogenate). The recovery for the applied method was: Pb – 104.59%, Cd – 94.71%, As – 98.55%, Ni – 94.56% and Cr – 97.26%. The analysis of Hg was performed on an AMA-254 Advanced Mercury Analyzer (Spectro-Lab, Łomianki, Poland) at 254 nm wavelength, according to Costley et al. (2000). The limit of detection for the Hg content was 0.01 ng. All chemical analyses were performed in triple replications.

Calculations

The results were compared with the current limit content of heavy metals in fish and fish products, included in Commission Regulation (EC) No 629/2008, of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuff (European Communities 2008).

Additionally, the content of examined heavy metals was compared with:

- Provisional Tolerable Weekly Intake (PTWI) of 25 μg kg^{-1} b.w./week for lead, 7 μg kg^{-1} b.w./week for cadmium and 1.6 μg kg^{-1} b.w./week for total mercury;
- Tolerable Weekly Intake (TWI) of 2,5 μg kg^{-1} b.w. for cadmium;
- Benchmark Dose Lower Confidence Limit (BMDL) for lead:
  - ✓ $BMDL_{01}$ for children (neurotoxicity) – 0.50 mg kg^{-1} b.w./day;
  - ✓ $BMDL_{10}$ for adults (nephrotoxicity) – 0.63 mg kg^{-1} b.w./day;
  - ✓ $BMDL_{01}$ for adults (cardiovascular disorders) – 1.50 mg kg^{-1} b.w./day;
- Benchmark Dose Lower Confidence Limit BMDL for mercury:
  - ✓ $BMDL_{0.5}$ for adults (imunotoxicity) – 0.01 μg kg^{-1} b.w./day;
  - ✓ $BMDL_{10}$ for adults (nephrotoxicity) – 0.06 μg kg^{-1} b.w./day;
- Benchmark Dose Lower Confidence Limit BMDL for arsenic:
  - ✓ $BMDL_{0.5}$ for adults (carcinogenic) – 3.00 μg kg^{-1} b.w./day.

The calculations were performed for adults (assuming 70 kg b.w.) and children (assuming 35 kg b.w.) (Staszowska et al. 2013).

Statistical analysis of the results

The statistical analysis was carried out using analysis of variance (one -way ANOVA). Normality of the results and homogeneity of variance were calculated using the Shapiro-Wilk test. The *post-hoc* Tukey's Honestly Significant Difference test was used for determining the statistically significant differences between fresh fish (in dependence of a species) and after the process (for each fish species). Differences with the significance of $\alpha = 0.05$ were considered statistically significant. All calculations and figures were made using Statistica ver. 12.0 software.

RESULTS AND DISCUSSION

The content of heavy metals in fish and fish products

The concentration of lead in fresh common carp was 0.031 mg kg^{-1} on average (Figure 1*a*). According to the literature, many factors can influence

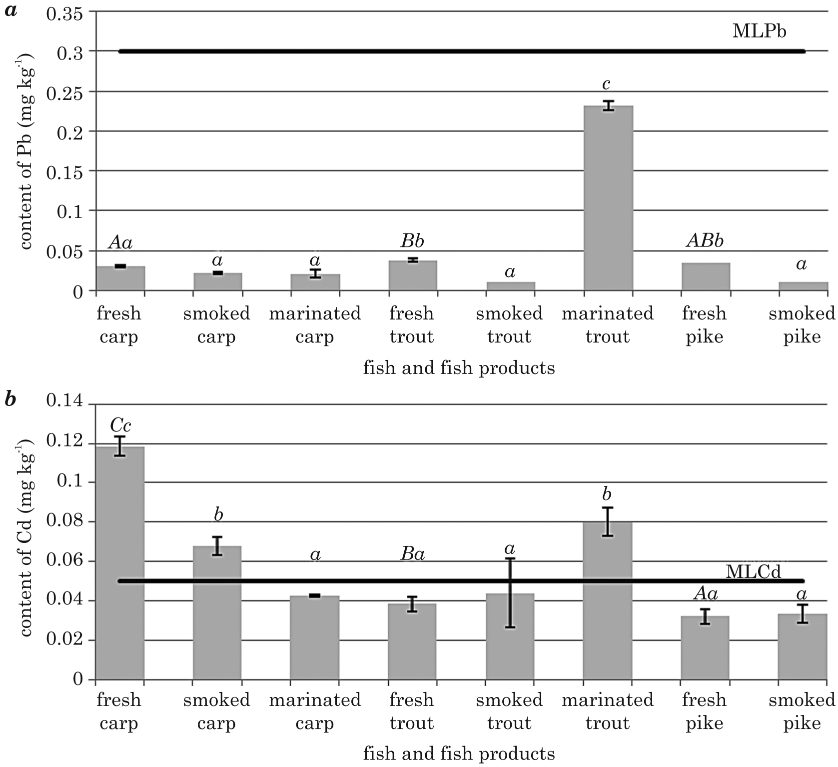

Fig. 1. The content of Pb (*a*) and Cd (*b*) in fish and fish products (mg kg^{-1}) in comparison to the Maximum Levels (ML): ML_{Pb} – 0.300 (mg kg^{-1}), ML_{Cd} – 0.050 (mg kg^{-1}); *A*, *B*, *C* – mean values followed by the same letter are not significantly different (α = 0.05) – the differentiating factor: fish species; *a b c* – mean values followed by the same letter are not significantly different (α = 0.05) – the differentiating factor: technological process

fish metal uptake, i.e. age, size, feeding behavior and living environment (El -Moselhy et al. 2014). The levels of Pb measured in fish products varied from 0.011 mg kg^{-1} (in smoked pike and trout) to 0.232 mg kg^{-1} (in marinated trout), but no sample exceeded the maximum level (ML) of 0.30 mg kg^{-1} set by Commission Regulation No 629/2008 (Figure 1*a*). A drop in the concentration of heavy metals in samples after thermal treatment, attributed to the heat effect, was observed in fish meat by Eboh et al. (2006) as well as Kobia et al. (2016). Heavy metals could be converted to other compounds (Kobia et al. 2016).

The process of marinating led to a 5-fold increase in the Pb concentration in samples of rainbow trout (Figure 1*a*). This increase could be due to the ingredients used in marinating. According to TAWFIK (2013), spices may contain high levels of lead. KREJPCIO et al. (2007) reported a Pb content in black grainy pepper at 0.32 mg kg^{-1}, and in dried garlic – at 0.53 mg kg^{-1}. These author also determined the concentration of lead in dried onion (0.44 mg kg^{-1}) as well as in allspice (0.29 mg kg^{-1}). In the study of DARKO et al. (2014), garlic contained 1.0 mg kg^{-1} of lead. All the spices and seasonings mentioned above were present in the recipes for smoked or marinated fish products examined in this study. Therefore, the increase in the Pb content might have resulted partially from the accumulation of Pb in spices through air pollution, inclusion or absorption at a mill during grinding, etc. (INAM et al. 2013). It was also reported that cooking methods (i.e. frying) can change the levels of toxic metals through various means, including the evaporation of water and volatile components, solubilization of the element and also by metal binding to other macronutrients present in the food (KOBIA et al. 2016).

The cadmium content in the examined fresh fish differed significantly and ranged from 0.032 mg kg^{-1} (in northern pike) to 0.119 mg kg^{-1} (in common carp) – Figure 1*b*. It is to emphasize that the concentration of cadmium in common carp was higher than ML set by Commission Regulation No 629/2008. The differences between the fish species might be due to feeding habits or place of habitation (open water or bottom of a pond) of the examined fish species. The processing method had a significant impact on the Cd concentration in the examined fish species, except for northern pike and rainbow trout (smoking) – Figure 1*b*. It was observed that the processing of common carp (both smoking and marinating) led to a decrease in cadmium, whereas marinating of rainbow trout caused a 2-fold rise ($P < 0.05$) in the concentration of this heavy metal, above ML. In the opinion of DIACONESCU et al. (2012), this increase may be related to evaporation that occurs during frying process. A higher level of Cd in fried and then marinated trout (as compared to the fresh material) could be also an effect of the addition of spices, breading (breadcrumbs and flour), frying in rapeseed oil, and also the adding of vinegar marinade (sugar, spices and slices of vegetables). IBRAHIM et al. (2012) reported a cadmium concentration in black pepper at 0.046 mg kg^{-1}. In the study of KREJPCIO et al. (2007), dried garlic contained 0.04 mg kg^{-1}, whereas dried onion – 0.05 mg kg^{-1} and allspice – 0.03 mg kg^{-1} of Cd. As regards smoking, ŞIRELLI et al. (2006) reported lower (0.011 mg kg^{-1}) cadmium concentrations in vacuum packaged smoked rainbow trout.

As shown in Figure 2*a*, the average concentration of mercury in the muscles of fresh fish was the highest ($P < 0.05$) in common carp (0.055 mg kg^{-1}). Processing of fish caused significant changes in the Hg content. Smoking led to a significant increase in mercury in rainbow trout (almost 2-fold) and pike (almost 3-fold) – Figure 2*a*. This might be attributed to fat drainage or water loss during smoking (AYEJUYO et al. 2013). In turn, a marked decrease in the Hg content was observed only in marinated carp samples (from 0.06 mg kg^{-1} to 0.011 mg kg^{-1}) – Figure 2*a*. POLAK-JUSZCZAK (2010) examined the content of mercury in different fish products and reported the Hg content (0.05-0.07 mg kg^{-1})

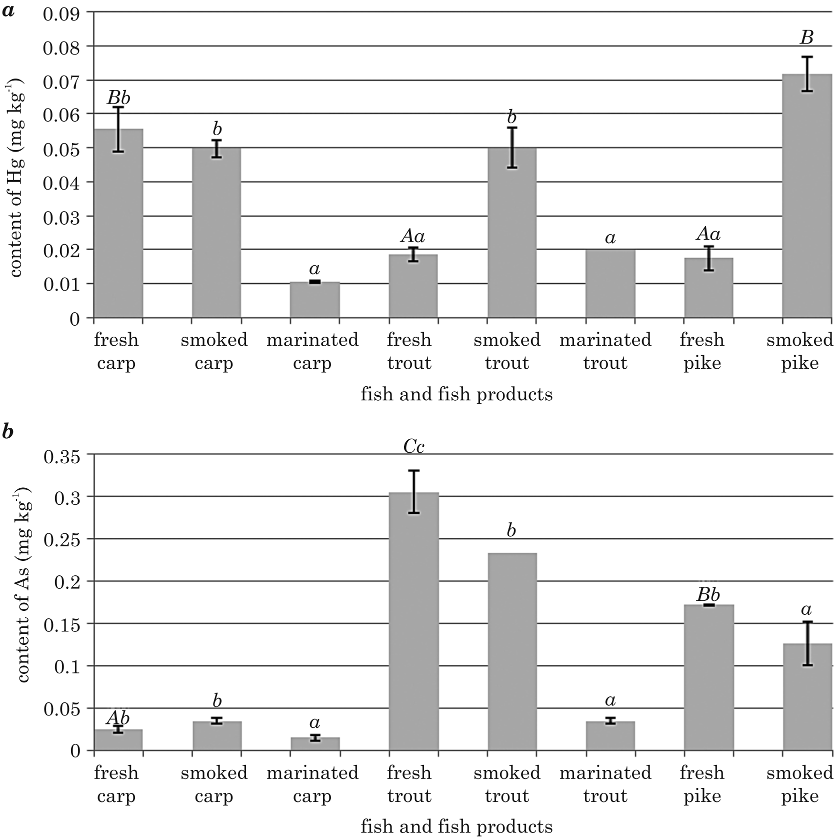

Fig. 2. The content of Hg (*a*) and As (*b*) in fresh fish and fish products (mg kg^{-1}): *A*, *B*, *C* – mean values followed by the same letter are not significantly different (α = 0.05) the differentiating factor: fish species; *a b c* – mean values followed by the same letter are not significantly different (α = 0.05) – the differentiating factor: technological process

in smoked as well as in marinated varieties. TKACZEWSKA and MIGDAŁ (2012) showed that a mercury level in rainbow trout from Malopolska province was 0.02 mg kg^{-1}.

The arsenic concentration in fresh fish was found to differ significantly between the species, in the range from 0.025 mg kg^{-1} (common carp) to 0.305 mg kg^{-1} (rainbow trout) – Figure 2*b*. Processing of fish led to changes in the As content. Smoking decreased As levels in all the samples except for carp. The lowest values of arsenic were found in marinated fish, with the biggest decrease (10-fold) noticed for rainbow trout (Figure 2*b*).

To sum up, heavy metal concentrations were found to decrease in common carp samples in the sequence Cd>Hg>Pb>As in fresh fish, Cd>Hg>As>Pb in smoked fish and Cd>Pb>As>Hg in carp. In the study of MAJNONI et al. (2013) on metal concentrations in tissues of common carp and silver carp from the Zarival Wetland, this sequence for fresh fish was Pb>Hg>Ni>Cd.

Meanwhile, the concentrations of the examined metals in rainbow trout followed the sequence of As>Cd>Pb>Hg (for fresh fish), As>Hg>Cd>Pb (for smoked fish) and Pb>Cd>As>Hg (for marinated fish). The sequence of heavy metals with respect to their levels in northern pike samples was As>Pb>Cd>Hg and As>Hg> Cd>Pb, for fresh and smoked fish, respectively.

Simultaneously, nickel and chromium was below the limit of detection (LOD) - both in fresh and processed fishes. Meanwhile, Kenšová et al. (2010) determined higher chromium concentrations in non-predatory fish, and the highest concentrations were found in bream and carp (muscle, liver and gills).

Heavy metal concentrations in the tissue of freshwater fish may vary considerably across different studies, possibly due to differences in metal concentrations and chemical characteristics of water from which the fish were sampled, ecological needs, metabolism and feeding patterns of fish, and also the season when the studies were carried out. What is particularly important is that a food product can become contaminated during a thermal treatment, which occurs in the processes of food preparation and manufacture, like roasting, baking or frying (Ayejuyo et al. 2013). Thus, the awareness of consumers is crucial.

Human exposure

The threat posed by the examined heavy metals to consumer health was analyzed based on their Provisional Tolerable Weekly Intakes. With regard to PTWI (Table 1), one can conclude that in the presence of lead as a contaminant, an adult weighing 70 kg can consume 87.50 kg of smoked and marinated common carp, 175.0 kg of smoked rainbow trout and 7.609 kg of marinated rainbow trout, 175.0 kg of smoked northern pike, on a weekly basis. In the case of cadmium, an adult can consume 7.000 kg of smoked common carp and 12.25 kg of marinated common carp, 9.800 kg of smoked rainbow

Table 1

Amount of fish products (kg) containing the PTWI[a] (in male adult weighing 70 kg) of the examined heavy metals

Heavy metal	Common carp			Rainbow trout			Northern pike		PTWI[a] (μg kg^{-1})	PTWI[b] (μg)
	fresh*	smoked**	marinated**	fresh *	smoked**	marinated**	fresh *	smoked**		
Pb	58.33	87.50	87.50	43.75	175.0	7.609	43.75	175.0	25.00	1750
Cd	4.083	7.000	12.25	12.25	9.800	6.125	16.33	16.33	7.000	490.0
Hg	1.867	2.240	11.20	5.600	2.240	5.600	5.600	1.600	1.600	112.0[c]

[a] PTWI – Provisional Tolerable Weekly Intake, [b] in a male adult weighing 70 kg, [c] methylmercury content – assumed that total mercury occurred as methylmercury

trout and 6.125 kg of marinated rainbow trout, as well as 16.33 kg of smoked northern pike. Whereas if mercury is the contaminant, the consumption of just 2.240 kg of smoked carp, 11.2 kg of marinated carp, 2.240 kg of smoked rainbow trout, 5.600 kg of marinated trout, as well as 1.600 kg of smoked northern pike weekly is permitted (Table 1). For comparison, USYDUS et al. (2009) in the study on fish products available on the Polish market, showed more than a six-fold larger allowable portion of smoked trout (62.8 kg) with regard to cadmium. In the case of mercury, the values for smoked trout were similar (2.5 kg) to those obtained in this study. As regards marinated fish, these authors (USYDUS et al. 2009) estimated that an allowed weekly amount of fried mackerel in vinegar is 2.1 kg, whereas a permitted portion of marinated common carp and rainbow trout is 11.2 kg and 5.6 kg, respectively.

Taking into consideration the fact that the European Food Safety Authority's Panel on contaminants in the food chain has set a reduced tolerable weekly intake for cadmium of 2.5 μg kg^{-1} b.w. (based on an analysis of new data; EFSA 2009), %TWIs were calculated for all processed fish, and they were (in the decreasing order): 4.57% (marinated trout), 3.89% (smoked carp), 2.51% (smoked trout), 2.43% (marinated carp) and 1.91% (smoked pike).

In order to estimate the risk associated with the supply of heavy metals in 1 portion (100 g) of fish, percentages of the Benchmark Dose Lower Confidence Limits for a child (when appropriate) and an adult were also presented (Table 2). In the case of Pb, a 100 g portion of fish product will cover the 6.080% (smoked pike) to 132.3% (marinated trout) of $BMDL_{01}$ for child and from 2.413% to 52.52% (as regards $BMDL_{10}$) as well as from 1.013% to 22.06% (considering $BMDL_{01}$). For a child, a safe amount of marinated trout should not exceed 75 g, and for an adult – 190 g ($BMDL_{10}$) or 450 g ($BMDL_{01}$). In the case of smoked carp, a safe portion size is 795 g, 2000 g and 4772 g, respectively. In the case of mercury, a portion weighing 100 g will cover from 0.143% (marinated carp) to 1.000% (smoked pike) of $BMDL_{0.5}$ as well as from 0.024% to 0.167% $BMDL_{10}$ (Table 3). As regards arsenic, a 100 g portion will cover from 0.710% (marinated carp) to 11.09% (smoked trout) of $BMDL_{0.5}$ for adult (Table 3). Thus, a safe portion of the products mentioned above is 14 000 g and 900 g, respectively.

CONCLUSIONS

Regarding fresh material, the statistical analysis showed significant differences in lead, cadmium, mercury and arsenic content, dependently on a fish species. In turn, among processed fish, marinating led to a 5-fold increase in Pb and a 2-fold enhancement of the Cd concentration as well as a 10-fold drop noticed for As in the samples of rainbow trout. Smoking led to a significant rise in the mercury level of rainbow trout (almost 2-fold) and pike

Table 2

BMDL values for lead, as the intake of Pb and Cd with one portion (100 g) of fish

Sample	Mean dose of Pb in 100 g of meat (µg 100 g^{-1})	$BDML_{01}$ for child of body weight 35 kg (µg/child/day)	Intake of Pb from a portion of 100 g of fish by child of body weight 35 kg (% $BMDL_{01}$)	$BMDL_{10}$ for adult of body weight 70 kg (µg/adult/day)	Intake of Pb with a portion of 100 g of fish by adult of body weight 70 kg (% $BMDL_{10}$)	$BMDL_{01}$ for adult (µg/person/day)	Intake of Pb with a portion of 100 g of fish by adult of body weight 70 kg (% $BMDL_{01}$)	Mean dose of Cd in 100 g of meat (µg 100 g^{-1})
Fresh carp	3.077		17.58		6.977		2.930	11.85
Smoked carp	2.197		12.56		4.982		2.092	6.801
Marinated carp	2.111		12.06		4.787		2.010	4.261
Fresh trout	3.821	17.50	21.83	44.10	8.664	105.0	3.639	3.805
Smoked trout	1.100		6.286		2.494		1.048	4.402
Marinated trout	23.16		132.3		52.52		22.06	8.000
Fresh pike	3.533		20.19		8.001		3.365	3.203
Smoked pike	1.064		6.080		2.413		1.013	3.334

Table 3

BMDL values for Hg and As with one portion (100 g) of fish

Sample	Mean dose of Hg in 100 g of meat (mg 100 g^{-1})	$BMDL_{0.5}$ for adult of body weight 70 kg (mg/adult/day)	Intake of Hg from a portion of 100 g of fish by child of body weight 35 kg (% $BMDL_{0.5}$)	$BMDL_{10}$ for adult of body weight 70 kg (mg/adult/day)	Intake of Hg from a portion of 100 g of fish by child of body weight 35 kg (% $BMDL_{10}$)	Mean dose of As in 100 g of meat (µg 100 g^{-1})	$BMDL_{0.5}$ for adult of body weight 70 kg (µg/adult/day)	Intake of As with a portion of 100 g of fish by adult of body weight 70 kg (% $BMDL_{0.5}$)
Fresh carp	0.006		0.856		0.143	2.483		1.182
Smoked carp	0.005		0.714		0.119	3.474		1.654
Marinated carp	0.001		0.143		0.024	1.490		0.710
Fresh trout	0.002	0.700	0.286	4.200	0.048	30.46	210.0	14.50
Smoked trout	0.005		0.714		0.119	23.29		11.09
Marinated trout	0.002		0.286		0.048	3.461		1.648
Fresh pike	0.002		0.286		0.048	17.14		8.162
Smoked pike	0.007		1.000		0.167	12.60		6.000

(almost 3-fold) and to a decrease in the As levels in all the samples except for carp. It was stated that the examined processing methods caused a significant increase in the concentrations of most metals compared to those of the fresh samples. However, these technological practices also caused a decrease in the concentrations of heavy metals in some fish types. This finding could be attributed to the inter-play between the size of a processed fish, oil uptake, water loss, and metal evaporation during the processing.

The calculated intakes of the examined heavy metals (consumption of 100 g fish portion) were below the BMDLs and Tolerable Weekly Intakes (PTWI or TWI). Simultaneously, nickel and chromium were below limits of detection in all samples.

The practical application of this study is to enhance the consumer's knowledge about heavy metals content in freshwater fish species after different ways of processing, both more popular like smoking and less common like marinating.

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