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Comparison of cadmium, zinc, manganese and nickel concentrations in fillets of selected species of food fish*

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The aim of the study was to compare the concentration of cadmium, zinc, manganese and nickel in fillets of four popular fish species available on the Polish market: Nile tilapia and pangasius imported from fish farms in Asia; pollock, a marine fish species living in the wild; and rainbow trout farmed domestically. The highest cadmium levels were found in the muscles of pangasius and Nile tilapia. The highest levels of zinc, manganese and nickel were observed in the muscle tissue of pollock, which supports the view that marine fish are a valuable source of micronutrients in the human diet. However, the best ratio of concentrations of the metals analysed was noted in trout, which had the lowest levels of cadmium and nickel, which are particularly harmful to the aquatic environment, and significantly higher levels of zinc and manganese than in pangasius and tilapia. The results obtained in the study indicate that trout is the safest source of micronutrients for the consumer.

KEY WORDS: cadmium / zinc / manganese / nickel / fish

Fish are a valuable source of protein in the human diet. In some countries they are the primary source of this nutrient. Fish consumption is increasing in highly developed countries, where the average resident eats as much as 70 kg of fish a year. Annual fish consumption is about 23 kg per person in the European Union and 11 kg in Poland, of which a substantial amount is imported from other continents, particularly Asia [26]. The improvement in fish price relations in 2013 has led to a 7.7% increase in demand (in the last 4 years), with consumption dominated by Alaska pollock, herring, mackerel and salmon [27].

Alaska pollock (*Gadus chalcogramma*), belonging to the cod family (Gadidae), is caught in the cold waters of the Pacific, from Alaska to the Japanese, Korean and Rus-

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sian coasts of the Bering Sea and the Sea of Okhotsk. Fish aged 4-6 years are predominant in the catches. Older individuals, even up to 16 years, occur less often. This fish is marketed in Poland almost exclusively in the form of frozen fillets. It competes mainly with fillets of European hake (*Merluccius merluccius*), but also with iridescent shark and tilapia [17, 39].

The Nile tilapia (*Oreochromis niloticus*) belongs to the African family Cichlidae. This fish adapts fairly easily to difficult environmental conditions. Owing to aquaculture, it is now present in tropical waters all over the world [18].

The iridescent shark (*Pangasianodon hypophthalmus*) is a freshwater species of the order Siluriformes, originating in the Mekong River and popularly farmed mainly in Vietnam. It is available mainly in the form of frozen fillets. In terms of sales volume its main competitors are fillets of 'white' marine fish: Alaska pollock, European hake and freshwater tilapia [39].

One of the major farmed fish in Poland, besides carp, is rainbow trout (*Oncorhynchus mykiss*), from the Pacific Coast of North America. It was brought to Europe at the end of the nineteenth century, and is currently farmed in nearly all European countries. Rainbow trout can be purchased in Europe year round. Fish in their second year of life with a body weight from 0.2 to about 0.5 kg are available for sale.

A combined 260,000 tonnes of frozen fish and fish products are imported into Poland annually, making it one of the leading European importers [17]. A few years were sufficient for the iridescent shark to become the third most frequently consumed fish species in Poland. There was a period when it was even replacing carp on the table on Christmas Eve. For Vietnamese exporters of the species Poland was one of the largest markets in the world [22]. Currently, sales of iridescent shark have fallen from nearly 2.0 to 0.7 kg per person per year, due to increased prices and to the negative opinion spread in the media regarding the conditions in which it is raised [27].

Differences in the chemical composition of the meat of these fish species are undoubtedly due to their varied origin. Fish farming conditions differ diametrically depending on the species' sensitivity to water parameters. Trout requires clean, cold, well-oxygenated water in a state of constant flow, while the iridescent shark and tilapia, which come from tropical climates, are more resistant to higher temperatures, oxygen deficits and the water pollution that frequently accompanies them. For this reason farming of iridescent shark or tilapia can be conducted at considerably higher stocking densities than in the case of trout. Higher stocking density results in greater accumulation of contaminants. Moreover, feeding of fish ponds with polluted water from the Mekong (in the case of iridescent shark) may increase the concentration of heavy metals in the fish meat. On the other hand, the relatively short rearing period, completed within 6-9 months, is not conducive to long-term bioaccumulation of harmful substances such as heavy metals, whose ultimate concentrations in meat depend in part on the length of exposure. Consumption of fish from polluted environments, including those contaminated with heavy metals, may lead to serious consequences for human health [19, 30]. Although food products are subject to regular control before they can be sold in shops, species differences and the origin and farming environments of individual species may affect the ultimate composition of the meat. Therefore the aim of this study was to compare the concentrations of selected heavy metals in fillets of popular species of food fish available on the Polish market: fish imported from Asia, i.e. Nile tilapia and iridescent shark; Alaska pollock, a popular marine fish species; and the domestically farmed rainbow trout. One of the metals selected for testing was cadmium, as one of the particularly toxic heavy metals, performing no physiological function in living organisms, so that any amount is superfluous. The others were zinc, manganese and nickel, which are included among heavy metals but as micronutrients are a valuable and beneficial component of fish meat.

Material and methods

The study was conducted at the Department of Ichthyology and Fisheries of the University of Agriculture in Krakow. The material for the study consisted of 10 frozen fillets each of iridescent shark, tilapia and Alaska pollock, purchased in a popular Polish supermarket chain, and 10 fresh fillets of rainbow trout from a fishery in the Podkarpackie region. Before samples were taken from the fillets they were thawed at a temperature of 4°C for about 20 hours and then dried with paper towels. From each fillet, samples weighing 5 g were taken from the anterior dorsal part of the carcass and placed individually in the glass tubes of a Velp 20/26 mineralizer for mineralization in the presence of 10 ml of a mixture of nitrous acid (HNO_2) and chloric acid ($HClO_3$) (at a 3:1 ratio). Following 20 hours of mineralization at room temperature and 5 hours at 180°C, the samples took on the form of a clear, colourless liquid, which was then transferred to volumetric flasks and diluted to 25 ml with deionized water. Each flask was sealed, labelled and stored at 4°C until analysis.

Concentrations of cadmium, zinc, manganese and nickel in the samples were determined by the atomic absorption method, using a UNICAM 929 spectrometer [1]. The standards for the standard curve were based on atomic absorption standards produced in the Central Office of Measures in Warsaw. The results are presented in milligrams of metal per kilogram of wet weight of fillet.

The results were analysed by analysis of variance (ANOVA). A t test (GraphPad Prosm 5) was used to determine significance of differences between concentrations of metals in the fillets of rainbow trout, Alaska pollock, tilapia and iridescent shark. In addition, Pearson's linear correlation coefficients were determined for concentrations between particular metals.

Results and discussion

Analysis of the cadmium concentration in the fish fillets showed the lowest level of this metal in trout—0.007 ±0.001 $mg\cdot kg^{-1}$, which was statistically significantly lower ($p<0.05$) than in all the other species: iridescent shark—0.065 ±0,004 $mg\cdot kg^{-1}$, Alaska pollock—0.043 ±0.007 $mg\cdot kg^{-1}$ and tilapia—0.058±0.004 $mg\cdot kg^{-1}$ (Tab. 1).

The level of Cd noted in the trout muscles is comparable to the concentration shown in other studies, e.g. by Drąg-Kozak et al. [8] or Tkaczewska and Migdał [35], who reported cadmium concentrations from 0 to 0.014 mg·kg^{-1} in the muscles of rainbow trout from different fisheries in Malopolska and Silesia. Similarly, in brown trout farmed in the Loučka River in the Czech Republic, cadmium was observed at concentrations from 0.003 to 0.026 mg·kg^{-1} [37]. This indicates that the farmed trout tested do not pose a risk of cadmium contamination for consumers. The same can be said of the Alaska pollock fillets used in the study, in which the level of cadmium was low (0.043 ±0.007 mg·kg^{-1}; Tab. 1) and consistent with results reported by other authors [13, 15], suggesting that marine fish of the northern Pacific are not contaminated with this metal.

The Cd level in the muscles of tilapia and iridescent shark (0.058 and 0.065 mg·kg^{-1}) slightly exceeded the maximum permissible concentration of this element in foodstuffs, which is 0.05 mg·kg^{-1} [31]. Most literature data indicate that the concentration of Cd in the muscles of tilapia is low and does not exceed norms [3, 21, 28, 33, 34], but some results demonstrate the occurrence of much higher Cd levels than in the present study—even 10.36 mg kg^{-1} DW [32]. Daily intake of cadmium with food by adults in various countries ranges from 25 to 200 µg. In Poland it is 11-30 µg. The tolerable weekly intake of cadmium, which takes into account safety conditions and the degree of environmental contamination with cadmium, has been set at 7 µg/kg body weight/week. According to FAO/WHO recommendations, the tolerable intake of cadmium for an adult is about 0.4-0.5 mg/week, and the permissible intake level is 60-70 µg per day [6]. Symptoms of cadmium contamination immediately after consumption of fish meat are unlikely, even if the concentration of this metal exceeds limits, because its bioavailability is about 10%. As a heavy metal, however, cadmium is capable of bioaccumulation, e.g. in the kidneys or liver. Repeated and long-term ingestion of low doses may result in its storage in human kidneys after years of chronic exposure to toxic levels, especially since the biological half-life of this metal in humans is estimated at 16-38 years. The effects of cadmium contamination include functional disturbances in the entire body, such as itai-itai disease, osteomalacia, osteoporosis, or cancer [30]. The literature also contains reports of an elevated level of this metal in the muscles of iridescent shark. Amin [4], for example, noted a cadmium concentration of 0.11 mg·kg^{-1} in frozen fillets of iridescent shark, which was twice as high as the current European limit and seems to confirm that fish farmed in conditions of greater density, as less sensitive to environmental pollution, may present elevated levels of toxic metals. However, studies conducted in Poland thus far have not demonstrated a risk of contamination of the meat of these fish with cadmium or other toxic metals [28, 33].

Zinc is a key element for all living organisms. As a microelement it combines with proteins to form metalloproteins, which are part of numerous enzymes essential to the proper function of vertebrate organisms. Zinc deficiencies are rare in aquatic ecosystems, but excessive amounts of zinc may become a problem [25], because it is then accumulated in the kidneys and liver, leading to anaemia, which is linked to reduced assimilation of other elements, such as iron, phosphorus, copper and calcium. In the present study the highest level of Zn was noted in the muscles of Alaska pollock, at 31.88 ±5.03 mg·kg^{-1} (Tab. 1). This result was within the acceptable range of daily intake of zinc for adults (10-40 mg/day, according to WHO [38]), and consumption of even a kilogram of Alaska pollock fillets a day does not pose a risk to potential consumers. A nearly twofold lower level of Zn, at 15.63 ±1.892 mg·kg^{-1}, was noted in the rainbow trout fillets (Tab. 1). Even lower zinc concentrations in the muscles of rainbow trout from various fisheries in southern Poland have been noted by other authors [8, 29]. The lowest zinc concentrations were shown in the muscles of iridescent shark and tilapia—4.38 ±0.6 and 6.28 ±0.395 mg·kg^{-1} (Tab. 1). The results of studies by other authors on these two Asian fish species indicate varied Zn concentrations, ranging from 1.9 to 212.44 mg·kg^{-1} [3, 10, 19, 21, 23, 32, 33].

Analysis of manganese concentrations in the fish fillets showed the highest levels in Alaska pollock (1.8 ±0.3 mg·kg^{-1}) and rainbow trout (1.4 ±0.1 mg·kg^{-1}), where it was statistically significantly higher ($p<0.0001$) than in tilapia (0.4 ±0.02 mg·kg^{-1}) and iridescent shark (0.3 ±0.02 mg·kg^{-1}). Statistically significantly differences were also noted between the Mn concentration in tilapia and iridescent shark, but no statistically significantly differences were found between the mean concentration of this metal in Alaska pollock and rainbow trout (Tab. 1). According to WHO [38], the daily requirement for manganese in the human diet ranges from 2 to 9 mg. The meat of the species analysed seems to be a valuable source of this element, although according to numerous literature studies tilapia muscles can have much higher manganese concentrations, from 0.11 to 48.87 µg·g^{-1} [2, 7, 9, 11, 16, 19, 20, 21, 24], which is 12-30 times higher than in the tilapia fillets tested in the present study.

Analysis of the nickel concentration in the fish fillets revealed the lowest level, 2.3 ±0.2 mg·kg^{-1} in rainbow trout, which was statistically significantly lower ($p<0.05$) than in all other species tested: Alaska pollock—8.5 ±1.3 mg·kg^{-1}, tilapia—5.8 ±1.0 mg·kg^{-1} and iridescent shark—5.2 ±1.2 mg·kg^{-1} (Tab. 1). Even lower nickel concentrations, from 0.058 to 0.102 mg·kg^{-1}, were reported by Vitek et al. [37] in brown trout during monitoring of the Louãka River ecosystem in the Czech Republic in 2006. Due to insufficient data, the WHO has not established a daily requirement for nickel for adults, but a suggested level of 0.2 mg is based on conversion from the nutritional requirements for monogastric animals, while the maximum intake should not exceed 0.6 mg, due to the risk of skin allergies [38]. Given these data, the nickel concentrations in the muscles of all the fish analysed appear to be high. The results obtained in our study, however, are consistent with data presented by other authors, who observed nickel concentrations in tilapia ranging from 2.6 to 15.9 µg·g^{-1} [16, 19]. Much lower nickel concentrations, ranging from 0.11 to 3.97 mg·kg^{-1}, were noted by Ekeanyanwu et al. [9] in Nile tilapia of the Okumeshi River delta in Nigeria, by Ntiforo et al. [24] and Mokhtar et al. [21] in tilapia from Malaysia, by Tawell et al. [34] in Nile tilapia available for sale in Malaysia, and by Asgedom et al. [5] in Nile tilapia in the waters of Ethiopia and in *Tilapia zillii* in Nigerian rivers [2].

Marine fish are often named as a valuable source of nickel in the human diet. This is confirmed by the results of the present study, in which the highest concentration of this metal, 8.534 mg·kg^{-1}, was observed in Alaska pollock (Tab. 1). The results obtained differ from observations made in 2008 in a study on the heavy metal concentrations in Alaska

Table 1

Comparision of concentrations of selected heavy metals (±SE) in the fish species studied

	Oncorhynchus mykiss	*Gadus chalcogramma*	*Oreochromis niloticus*	*Pangasianodon hypophthalmus*
Cd	0.007^{a} ±0.001	0.042^{b} ±0.007	0.051^{c} ±0.004	0.065^{c} ±0.004
Zn	15.629^{a} ±1.892	31.883^{b} ±5.030	6.279^{c} ±0.395	4.376^{d} ±0.639
Mn	1.366^{a} ±0.118	1.856^{a} ±0.386	0.399^{b} ±0.024	0.298^{c} ±0.026
Ni	2.380^{a} ±0.276	8.534^{b} ±1.327	5.746^{bc} ±1.036	5.204^{c} ±1.254

Different letters indicate statistically significant differences between means at $p<0.05$

pollock and four other marine fish species (bluefin tuna, cod, salmon and mackerel), as well as in tinned tuna available for sale in Gwangju, South Korea. Manganese and nickel concentrations in the marine fish were found to range from 0.32 to 0.6 mg Mn kg^{-1} and 0.12 to 1.025 mg Ni kg^{-1}, and in Alaska pollock from 0.45 to 12.38 mg Mn kg^{-1} and 0.39 to 0.59 mg Ni kg^{-1} [15]. The levels of nickel in the fillets of all the fish tested seem to be high, substantially exceeding requirements for humans, but there have never been any observations of nickel poisoning by ingestion, and probably for this reason no maximum acceptable concentrations in meat have been established for this metal.

The results obtained indicate correlations between the concentrations of different metals. In all the fish tested the cadmium level was correlated with the levels of zinc and nickel, but the correlation was negative in iridescent shark and positive in the other species (Tab. 2). During bioaccumulation cadmium and zinc are known to compete for the same transporters, and thus a higher proportion of other metals in the environment usually reduces the final cadmium concentration. In tilapia and iridescent shark the strongest correlation was noted between the concentrations of zinc and nickel ($r=0.81$ and $r=0.93$, respectively), which was not observed in the case of trout or Alaska pollock (Tab. 2).

Table 2

Comparison of correlation coefficients of heavy metal concentrations in the muscles of the fish species

	Oncorhynchus mykiss			*Gadus chalcogramma*			*Oreochromis niloticus*			*Pangasianodon hypophthalmus*		
	Cd	Zn	Mn	Cd	Zn	Mn	Cd	Zn	Mn	Cd	Zn	Mn
Zn	0.45*	–	–	0.78***	–	–	0.55*	–	–	–0.51*	–	–
Mn	ns	ns	–	ns	ns	–	0.63**	ns	–	ns	0.46*	–
Ni	ns	0.44*	0.58**	0.46*	ns	0.53*	0.55*	0.81***	ns	–0.45*	0.93***	0.54*

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ns – no statistically significant correlation

Aquaculture in which iridescent shark or tilapia are raised is characterized by very high density and poor sanitary conditions, due to an insufficient amount of clean water [14]. This may cause excessive accumulation of heavy metals in the tissues of these fish, including cadmium, of which the elevated concentrations shown in our study slightly exceeded permissible levels [31]. Alaska pollock is caught in the salt water of the Pacific [12]. In its natural environment this fish does not accumulate cadmium in its muscles in concentrations exceeding permissible levels, while it is a valuable source of micronutrients, such as zinc, manganese or nickel, whose highest concentrations were noted in this species. Trout requires appropriate conditions for rapid growth, such as suitably oxygenated water and a specific water flow. This fish is sensitive to changes in water properties, and satisfying the strict requirements for farming it probably contributes to the lower concentrations of cadmium and nickel observed in trout in comparison with the other species analysed. Trout also contains a much higher percentage of protein and polyunsaturated fatty acids [36] than any of the other species tested, which in conjunction with the results of the analysis of heavy metal concentrations suggests that it is the most valuable of these species in terms of nutrition and health.

The results of the study indicate that the variation in the origin of the food fish significantly influences their composition, including the content of heavy metals. Even when their concentrations in the muscles do not exceed established limits, they differ significantly between species. The cadmium concentrations noted in Alaska pollock, tilapia and iridescent shark were 5 to 8 times higher than in trout, while the level of micronutrients, Zn, Mn and Ni, were highest in the Alaska pollock fillets. Rainbow trout had the lowest level of cadmium, a metal considered toxic and completely unnecessary for the body, as well as relatively high levels of zinc and manganese, and thus had the most beneficial proportions of these metals among all the fish tested. As the other fish species were not available in fresh form, it could not be determined whether the ultimate composition of the fillet was influenced by its processing, preservation and transport; only the final form of the product available to the consumer was compared.

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