Pol. J. Environ. Stud. Vol. 21, No. 4 (2012), 1033-1038

Original Research

Content of Selected Elements in the Muscle Tissue and Gills of Perch (*Perca fluviatilis* L.) and Water From a Polish Lake

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Received: 13 June 2011
Accepted: 17 January 2012

Abstract

The aim of this work was to analyze the concentrations of select elements such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) in the water, muscle tissue, and gills of perch sampled in autumn from Lake Gopło in NW Poland. The correlations between the fish size (body length) and metal concentrations in the tissues were investigated by linear regression analysis. In addition, the bioaccumulation coefficient, as a measure of accumulation intensity of an element in an organ, was analyzed. The mean content of K, Mg, and Zn in the analyzed perch was higher in the muscle (18.90, 1.53, and 52.92, $mg \cdot kg^{-1}$) than in gills (8.88, 1.30, and 44.99 $mg \cdot kg^{-1}$), and the difference between these values (except for Zn) was statistically significant ($p \leq 0.05$). The analyses of the correlation between metal concentration in the meat and the body length of fish show that the bioaccumulation of Na, K, Mg, and Zn decreases as fish body length increases (negative correlation). The evaluation of the chemical pollution of Lake Gopło concentrations was based on the following ions ($N\text{-}NO_3$, $N\text{-}NO_2$, and $P\text{-}PO_4$) and minerals (Na, K, Ca, Mg, and Zn). The concentrations of nitrite nitrogen in the waters of Lake Gopło range from 0.012 (in September) to 0.057 mg $N\text{-}NO_2$ dm^{-3} (in November). The concentration of nitrate nitrogen ranges from 0.09 to 1.888 mg $N\text{-}NO_3$ dm^{-3}. The concentration of orthophosphate in surface waters of Lake Gopło is not very diverse (0.17-0.2 mg PO_4^{3-} dm^{-3}).

Keywords: fish, muscle tissue, gills, water, elements

Introduction

Among the products available, fish is considered to be one of the most interesting foods [1]. Fish constitutes mainly the final stages in the aquatic food chains, and the normal metabolism of fish may accumulate large amounts of certain metals (essential and non-essential) from water, food, and sediments [1, 2]. Therefore, when metals occur in aquatic environments, they pass through a food chain and, eventually, find their way into our bodies [3]. The data found in the literature indicate that the contents of macro- and microelements in fish depend on species as well as their feeding type, age, weight, and body length [4, 5]. The ecological needs, sex, age, growth rate, seasonal changes, and molting of marine animals are also found to affect metal accumulation in their tissues [6-8]. Such environmental factors as salinity, pH, hardness, and temperature of water play significant roles in metal bioaccumulation in fish tissues [1, 9].

The accumulation of metals in fish tissues and organs depends on their physiological role. For instance, the dermal route is usually a minimal contributor of exposure, due to the effective barrier provided by the external epithelium [3]. The aim of this work was to analyze the concentrations of some selected elements in the muscle tissue and gills of

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perch (*Perca fluviatilis* L.). Gills are the primary site of the metal uptake from water, especially if metals are bound to a particulate matter [10]. Muscles are not always a good indicator of the whole body fish contamination [11, 12]. And, as Rajkowska et al. [13] conclude, the muscle tissue of fish is well protected by other organs, so that only trace amounts of the metals accumulate in the muscle tissue of roach (*Rutilus rutilis* L.) from the West Pomeranian Lakes. This mechanism makes fish muscle safe for human consumption. Liver has a tendency to accumulate significantly higher metal concentrations than muscles [2, 14]. The analyses carried out by Yilmaz et al. [1] show that the highest levels of heavy metals are found in liver and the lowest in the muscles of *S. lascaris*, *L. budegassa*, and *T. lucerna*. The high accumulating ability of liver is a result of the activity of metallothioneins, the proteins that can be bound to some metals (Cu, Cd, and Zn), thus reducing their toxicity and allowing their liver to accumulate metals in a high concentration [2].

Material and Methods

The study involved water and perch sampled from Lake Gopło. The experimental fish was caught in natural conditions. The fish were collected in October during one fishing day. The analyses were carried out on 10 muscle tissue samples and 10 gill samples. The measurements of the fish body mass (BW) (±0.01 g), length (Lc) (±0.1 cm), and total length (Lt) (±0.1 cm) were taken from each individual. The samples of muscles for analyses were taken from the large side muscle of the fish body above the lateral line [15]. The individuals were selected for analysis on the basis of similar biometric measurements. Body weight ranged from 120.17 to 234.69 g, and body length was from 17.0 to 21.5 cm. The water was taken from the surface layer of the lake [16] and the samples were collected in three periods: September, October, and November (during one day) near the town of Kruszwica.

The samples of fish muscle tissue were frozen immediately after preparation and kept in the deep freezer (ca -20ºC) before analysis. All the frozen samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze dryer (parameters: temperature -40ºC, pressure $6\cdot10^{-2}$ mbar, duration at least 48 h).

The freeze-dried samples were mineralized in a microwave mineralizer Ethos Plus, Milestone. 0.1g of the tissue was weighed for mineralization and HNO_3 and H_2O_2 were added in ratio 4:1. During the first 10 minutes, the temperature increased to 190ºC. During the next 7 minutes, the temperature was kept at the level of 190±5ºC. The mineralized samples were carried quantitatively to 50 ml measuring flasks.

In the muscle tissue and gills, the concentrations of Na, K, Mg, Ca, Fe, and Zn was determined, and in the water – Na, K, Mg, Ca, and Zn. The metal concentrations were determined with the use of the atomic absorption spectrophotometer Solar 969, Unicam. The analyses were carried out according to PN-EN 13805/2003 [17], PN-EN 15505/2009 [18], and PN-EN 14084/2004 [19]. The tissue concentrations of metals were reported as $g\cdot kg^{-1}$ or $mg\cdot kg^{-1}$ of dry weight ($g\cdot kg^{-1}$ and $mg\cdot kg^{-1}$ d. w.). The analyses of water were carried out according to PN ISO 9964-1/1994 [20], PN-ISO 9964-2/1994 [21], PN-EN ISO 7980/2002 [22], PN-EN 26777/1999 [23], PN-82 C-04576/08 [24], and PN-73 C-04537 [25]. The metal and ion concentrations in water were reported as $mg\cdot dm^{-3}$.

The accuracy of the analyses was controlled by adding standard solutions traceable to SRM ($NaNO_3$, KNO_3, $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Fe(NO_3)_3$, and $Zn(NO_3)_2$ in HNO_3, 0.5 $mol\cdot dm^{-3}$) (Merck, Germany). The results showed that the recovery percentage was in the range of 93 to 103%, and these values were included in the final results.

The data analyses were performed using Statistica 8.0 software (StatSoft, USA) [26]. The significance of the differences in the average content of minerals in the meat of roach and water were calculated by two-way analysis of variance. Tykey's test and t-test were applied, and statistically significant differences were evaluated as significant at $p\leq0.05$.

Table 1. The average concentrations of the selected mineral compounds in the muscle tissue and gills of perch (*Perca fluviatilis* L.) from Lake Gopło ($g\cdot kg^{-1}$ or $mg\cdot kg^{-1}$ of wet weight).

Mineral compounds	Muscle	Gills
	(mean±SD)	(mean±SD)
Na ($g\cdot kg^{-1}$)	0.38±0.16^a	1.10±0.13^b
K ($g\cdot kg^{-1}$)	4.11±0.29^a	1.93±0.21^b
Mg ($g\cdot kg^{-1}$)	0.33±0.03^a	0.28±0.03^b
Ca ($g\cdot kg^{-1}$)	1.39±0.63^a	8.52±1.37^b
Fe ($mg\cdot kg^{-1}$)	2.26±0.80^a	29.59±1.76^b
Zn ($mg\cdot kg^{-1}$)	11.52±4.84^a	9.79±2.67^a

Mean values denoted with various letters in the same row are statistically significantly different ($p\leq0.05$) (T- test)

Results and Discussion

The analyses indicated that higher concentrations of Na, Ca, and Fe occurred in gills, whereas lower amounts of K, Mg, and Zn were observed in gills (Table 1). Statistically significant differences in the content of all metals (except for Zn) between muscle tissue and gills were calculated.

As Malik et al. [27] indicated, heavy metals were bioaccumulated at varying levels in different tissues in *L. rohito* and *C. idella*. Zn content in *L. rohito* was lower in gills (0.233 $\mu g\cdot g^{-1}$) than in muscles (0.482 $\mu g\cdot g^{-1}$), while in *C. idella*, gills accumulated higher amounts of Zn (2.64 $\mu g\cdot g^{-1}$) than muscles (1.88 $\mu g\cdot g^{-1}$). These differences between the two species might be due to their different ecological needs, metabolic activities, and feeding habits. Gills accumulated higher levels of heavy metals than other organs because they acted as a depot tissue [1, 10, 28, 29]. Concentrations of metals were lower in muscles, because this tissue constitutes the greatest mass of the flesh that is consumed as food [27].

Zn is essential for good human nutrition. However, if its intake is too high it may cause health problems. The Food and Agricultural Organization's (FAO) limit for Zn is 30 mg·kg^{-1} [3]. The mean content of Zn in the analyzed perch was higher in the muscle tissue (52.92 mg·kg^{-1}) than in gills (44.99 mg·kg^{-1}). As Tekin-Özan and Kir [30] indicated, the mean concentration of Zn was about 2 times higher in gills (14.17 mg·kg^{-1}) than in muscles (7.01 mg·kg^{-1}) of tench (*Tinca tinca* L.). The same results were observed by Brucka-Jastrzębska et al. [31]. Uysal et al. [3] reported that the mean concentration of Zn in fish meat without skin from Beymelek Lagoon (Turkey) was lower than in gills, ranging between 4.27 and 12.28 mg·kg^{-1} in meat, and between 42.36 and 339.76 mg·kg^{-1} in gills. Gills are primary sites for the indication of tolerance mechanisms [32]. There are four mechanisms for the tolerance of gills to metals: the decreased uptake from water, detoxification by binding metals to induced metallothionenin, protection of cellular structures by induction of stress-protein, and induced excretion of metals (apical to water and basolateral to blood).

Analyses of the metal accumulation in the body of sterlet (*Acipenser ruthenus*) from the Danube River (Serbia) showed that differences in distribution of metals among different tissues were statistically significant. The muscles revealed the lowest concentrations of most of the analyzed metals [2]. The mean content of Zn in meat was 25.18 μg·g^{-1} and in gills it was 62.38 μg·g^{-1}. Several studies determined the highest Zn concentration in gills [8, 33].

As reported by Yilmaz et al. [1], Zn content in muscle samples of three demersal fish ranged from 20.8 to 28.2 μg·g^{-1}. The analyses of variance revealed Zn concentrations to differ significantly between species, suggesting that the content of this element in fish muscle increased with depth. Fe concentration in the meat of fish from Iskenderun Bay (Turkey) ranged from 37.6 to 76.7 μg·g^{-1}.

In the meat of perch from Latvia, the mean concentration of Fe was about 100 μg·g^{-1}, and in gills, the content of this metal was lower. Muscle tissues had lower Zn concentration than gills [10]. As Brucka-Jastrzębska and Potasowicki [34] indicated, the highest iron concentration in 5-month-old carp (*Cyprinus carpio* L.) was found in gills (36.47 μg·g^{-1} w.w.) and kidneys (33.94 μg·g^{-1} w.w.), the lowest being recorded in muscles (2.79 μg·g^{-1} w.w.). Klavins et al. [10] reported that in gills of perch, the pattern of metal concentration was Fe<Zn, and in muscles, it was Zn<Fe. The same results were obtained in this study for perch sampled from Lake Gopło and for other freshwater fish from the Polish West Pomeranian Province, where Brucka-Jastrzębska et al. [31] reported higher Fe levels in gills (28.9-54.7 mg·kg^{-1} w.w.) than in muscles (4.6-9.1 mg·kg^{-1}) as well as higher levels of Zn in muscles (6.1-8.1 mg·kg^{-1}) than in gills (3.1-3.6 mg·kg^{-1}). In the meat of freshwater fish from Lake Dhanmonodi (Bangladesh), the mean concentration of Fe was higher than of Zn in all samples [35]. Klavins et al. [10] concluded that the mean content of the studied metals was low, and that these values could be taken as background values for uncontaminated sites.

As the analyses indicated, metal concentrations in fish depended on the feeding type. Perch is a predatory fish that feeds on other, smaller fish [4, 10, 14]. In the meat of perch from the Great Mazurian Lakes, Zn concentrations ranged from 20.2 to 24.5 mg·kg^{-1} d.w., and was higher than in the tissue of un-predatory fish [5]. The mean content of Zn in the muscles of pike (*Esox lucius* L.) from Anzali wetland was 25.4 mg·kg^{-1} [9]. The meat of perch from the Great Mazurian Lakes had lower Fe content than un-predatory fish from this region and these values ranged from 4.6 to 6.8 mg·kg^{-1}. The mean level of Na, K, Ca, and Mg in the meat of perch was 306.78, 2076.28, 65.7, and 98.52 mg·100 g^{-1}, respectively [5]. As reported by Yilmaz et al. [1], the mean value of major elements in the meat of fish from Iskenderun Bay (Turkey) was 29.16 μg·g^{-1} for Na, 3.34 μg·g^{-1} for K, 242 μg·g^{-1} for Ca, and 16.18 μg·g^{-1} for Mg. Na, K and Ca were the most abundant essential elements in these studies. The highest concentrations of these elements were in the muscles of *S. lascaris*. The analyses indicated that these fish species seemed to be more contaminated than other fish and it showed that fish were a potential bioindicator of pollution of deep sea ecosystems [1].

Table 2. The value of the bioaccumulation coefficient (k) in the muscle tissue of perch (*Perca fluviatilis* L.) from Lake Gopło.

Mineral compounds	k
Na (g·kg^{-1})	0.039
K (g·kg^{-1})	0.834
Mg (g·kg^{-1})	0.012
Ca (g·kg^{-1})	0.023
Zn (mg·kg^{-1})	288

Iron is not toxic to fish, but long-term exposure to a high iron level is dangerous [31]. Fe is found to reach the maximum concentration in liver [1, 3, 33]. Fe is bound to proteins or occurs as an iron phosphate that is deposited in various tissues, mainly in liver. In their analyses, Jarić et al. [2] found that Fe concentration in sterlet revealed similar levels in liver (379.44 μg·g^{-1}) as in gills (380.32 μg·g^{-1}).

The bioaccumulation coefficient, as a measure of intensity of accumulation of an element in an organ, was analyzed in the perch from Lake Gopło. The results are presented in Table 2. The same results were observed by Szulkowska-Wojaczek et al. [36]. The bioaccumulation coefficient of Zn in the meat of carp from fisheries was 240. In gills, this coefficient was 869, and was higher than in the meat and gills of perch from Lake Gopło. The bioaccumulation coefficient of metals in the muscle of perch from Lake Żnin Duże was 288.67 for Zn, 4.38 for Ca, 10.79 for Mg, 421.24 for K, and 12.36 for Na [29].

The intensity of bioaccumulation, expressed as a bioaccumulation coefficient, is inversely proportional to the metal concentration in water. This means that even very small concentrations of metals in water may lead to dangerous amounts of certain elements in fish organs [36]. The ability of fish to accumulate metals in muscle tissues was for the fish of Wielkopolski National Park. The bioaccumulation coefficient in the meat of perch was 277 for Zn, with a lower value - 52 for Cu [37].

Table 3. The correlation coefficient (r) at the level of significance $p \leq 0.05$ determined for the relationship between element content in meat and the body length of fish.

Mineral compounds	r
Na ($g \cdot kg^{-1}$)	-0.55
K ($g \cdot kg^{-1}$)	-0.03
Mg ($g \cdot kg^{-1}$)	-0.32
Ca ($g \cdot kg^{-1}$)	0.08
Fe ($mg \cdot kg^{-1}$)	0.04
Zn ($mg \cdot kg^{-1}$)	-0.26

The analyses of the correlation between the metal concentration in the meat and body length of fish show that bioaccumulation of Na, K, Mg, and Zn decreased as fish body length increased (negative correlation) (Table 3). The same results were observed for Zn and Mn [29] and for Cr, Mn, Ni, and Pb [38]. The primary explanation of the negative correlation between the concentration of metals and the size of fish is a difference in the ability to metabolize compounds, higher for young fish than for older individuals. The other explanation is the fact that in young animals, the mechanisms of the neutralization of harmful compounds are not developed sufficiently. Therefore, larger amounts of toxins can accumulate in their bodies [39]. The decreasing content of metals in the organisms of older fish can also result from lower amounts of proteins responsible for binding these compounds in tissues [40].

The quality of water in Lake Gopło is influenced by a variety of factors, one of them being the management of the catchment areas, which in total covers 1,408.21 km^2. Predominantly, the land in this region is used as arable land, because of highly developed agriculture. The agricultural use of the catchments provides considerable supplies of organic matter and minerals. Residues of fertilizers as well as various types of wastewater (domestic, municipal and industrial) have a high effect on surface water quality. Table 4 shows the results of the analyses. The evaluation of chemical pollution of Lake Gopło concentrations was based on the following ions: $N\text{-}NO_3$, $N\text{-}NO_2$, and $P\text{-}PO_4$, and minerals: Na, K, Ca, Mg, and Zn.

The increase in eutrophication is associated with higher levels of nitrogen compounds, particularly nitrites. Nitrites are considered to be hazardous compounds, because after they enter the fish bloodstream, methemoglobin is formed, which causes hypoxia and cyanosis. Furthermore, high concentrations of these ions in water lead to bacterial diseases in fish, affecting their gills and creating lesions [41]. Concentrations of nitrite nitrogen in the water from Lake Gopło ranged from 0.012 (in September) to 0.057 mg $N\text{-}NO_2 \cdot dm^{-3}$ (in November). The values obtained were similar to those published by Sroka [42] who examined the water quality of Lake Świdwie (0.026-0.066 $mg \cdot dm^{-3}$) and by Sojka et al. [43], who analyzed the water in the Mała Wełna River (0.02-0.04 $mg \cdot dm^{-3}$).

The three-month study of the water from Lake Gopło indicated that the value of nitrate nitrogen concentrations changed greatly (0.09-1.888 mg $N\text{-}NO_3 \cdot dm^{-3}$). A slightly higher range (0.118-3.453 $mg \cdot dm^{-3}$) was determined in the water of Lake Świdwie, analyzed by Sroka [42]. An increased concentration of $N\text{-}NO_2$ and $N\text{-}NO_3$ observed in October and November can be explained by the reduced intake of nitrogen by plants and the decomposition of organic matter (leaves), which is an additional source of nitrogen.

One of the main indicators of eutrophication in lake waters is phosphorus. Even small amounts of this element (from the continuous release of dead and living plant and animal organisms) can act as building blocks of a new organic matter. However, the more phosphorus in the surface water, the more biomass from dying plants and animals that can cause an oxygen deficit, which in turn can lead to loss of life in the biotope [42]. The concentration of orthophosphate in the water surface layer of Lake Gopło was not very diverse (0.17-0.2 mg $PO_4^{-3} \cdot dm^{-3}$). Similar values of phosphate ions

Table 4. Average content of select chemical parameters obtained in the water of Lake Gopło.

Parameters ($mg \cdot dm^{-3}$)	Data of collecting samples		
	September	October	November
	Mean±SD	Mean±SD	Mean±SD
Sodium	$29.05^a \pm 0.87$	$28.34^b \pm 0.56$	27.16c±0.66
Potassium	$4.23^a \pm 0.21$	$4.35^a \pm 0.17$	$6.20^b \pm 0.92$
Magnesium	$20.86^a \pm 1.52$	$20.77^a \pm 0.49$	$26.79^b \pm 0.49$
Calcium	$54.88^a \pm 4.52$	53.75a±2.18	$70.38^b \pm 3.69$
Zinc	0.04±0.01	0.04±0.00	0.05±0.001
$N\text{-}NO_2$	$0.012^a \pm 0.005$	$0.049^b \pm 0.004$	$0.057^{b,c} \pm 0.003$
$N\text{-}NO_3$	$0.090^a \pm 0.004$	$0.202^b \pm 0.13$	$1.888^c \pm 0.11$
$P\text{-}PO_4^{-3}$	0.17±0.03	0.20±0.07	0.17±0.04

were obtained by Deryło et al. [44], who carried out research in the hydrobiological dam reservoir in Przeczyce. The content of PO_4^{-3} in the water was 0.178 to 0.23 mg·dm^{-3}. A slightly lower content of orthophosphate was determined by Brucka-Jastrzębska et al. [31] in the waters from the Polish West Pomeranian Province. Lake Gopło is situated near Kruszwica, which influences the level of phosphates in its waters. There are two large factories in the town (producing fat and sugar). As the analyses indicated, urban, agricultural, and industrial development cause pollution in waters, which is linked with the increase of phosphorus concentrations [45].

In the waters of Lake Gopło, the concentration of minerals was analyzed. Large amounts of sodium were determined in the first month of the study (September) (29.05 mg·dm^{-3}), and the lowest were recorded in the last month (27.16 mg·dm^{-3}). The highest concentration of potassium was determined in the water sampled in November. A slightly higher range of both minerals was determined by Sojka et al. [43], 21.8-38.7 for Na, and 8.6-12.3 mg·dm^{-3} for K. The course of changes in calcium and magnesium in the analyzed period from September to November was similar. The highest and statistically significant contents of both ions were obtained in the last month of research. The concentration of magnesium did not exceed 26.79 mg·dm^{-3}, and the concentration of calcium did not exceed 70.38 mg·dm^{-3}. These values were higher than those obtained in the waters from the Polish West Pomeranian Province [31] and in the water from Lake Giłwa [46].

Other compounds occurring in surface waters are heavy metals. Most of them are essential for the proper functioning of the body, while others are toxic. The relationship between the accumulated amount of metal and its toxicity depends not only on the physico-chemical properties, but also on the individual defensive mechanisms of the body. The average concentrations of zinc in the waters from Lake Gopło ranged from 0.04 to 0.05 mg·dm^{-3}). These values did not exceed the requirements for inland waters in which wild fish live [47].

Conclusions

1. Statistically significant differences in the content of all metals (except for Zn) between muscle tissue and gills were calculated. Higher concentrations of Na, Ca, and Fe occurred in gills, whereas lower amounts of K, Mg, and Zn were observed in gills.
2. The analyses of the correlation between the metal concentration in the meat and body length of fish show that bioaccumulation of Na, K, Mg, and Zn decreased as fish body length increased.
3. The values of analyzed metals in surface waters did not exceed the requirements for inland waters in which wild fish live.

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