

Seasonal Investigation of Selected Mineral Contents in Meat, Gills, and Liver of Perch (*Perca fluviatilis* L.) from Western Poland

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Abstract

The aim of our work was to compare the concentrations of zinc (Zn), iron (Fe), and copper (Cu) in the meat, gills, and liver of perch (*Perca fluviatilis* L.) caught from lakes Góreckie, Strzeszyńskie, and Wędromierz in western Poland. The study involved 30 individual fish caught in spring, summer, and autumn. The muscle samples for analyses were taken from the large side muscle of the fish body above the lateral line. Metals concentrations were determined by atomic a Solar 969 absorption spectrophotometer (Unicam). As analysis shows, concentrations of Cu, Zn, and Fe detected in perch decreased in the following order: liver > gills > meat. The accumulation of metals decreased in the following order: Fe > Zn > Cu (in all tissues), and we observed statistically significant differences in Zn, Fe, and Cu concentrations in the same tissue collected from different reservoirs and during different seasons. Considering the analyses in terms of season, the average Cu and Zn contents were highest in the tissues taken in the spring. The highest concentrations of Fe were determined in samples collected during summer. The analyses indicated that concentrations of metals in tissues of fish caught from different reservoirs also showed great variation. Analysis of PCA allowed for a very good separation of the three reservoirs and three tissues investigated.

Keywords: minerals, meat, liver, gills, perch

Introduction

Cu, Zn, and Fe are essential elements and are carefully regulated by physiological mechanisms in most organisms.

The main roles of these elements can be described as functional and structural because of their important roles in the biological system [1]). These metals play a catalyzing role in the enzymatic systems by binding their ions to substrates, especially during oxidation-reduction

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reactions. Structurally, they stand out for their role as integrators of the body's organic compounds [2]. The content of these metals in fish body is important both with respect to nature management and human consumption of fish [3]. Copper is an essential part of several enzymes and is necessary for the synthesis of haemoglobine, and zinc is an essential micronutrient and a cofactor of nearly 300 enzymes [4].

Fish are commonly situated at the top of the food chain and they can accumulate large amounts of some metals [5, 6]. The distribution of metals in fish body varies between fish species, depending on sex, age development, and other physiological factors such as salinity, pH, hardness, and temperature [1, 2, 4, 7-11]. Concentrations of metals in fish tissues can reflect their previous exposures on to pollution. Fish can also be a reasonable indicators for public health standards, as well as indicators of animal health condition, because trace metals' accumulation in the aquatic food chain may reflect an ecological damage [12].

The aim of this work was to determine Cu, Zn, and Fe in the meat, gills, and liver tissues of perch caught from lakes Góreckie, Strzeszyńskie, and Wędromierz (Fig. 1) in western Poland during spring, summer, and autumn. Differences in the metal content in various tissues may result from their different capabilities to induce metal-binding proteins such as metallothioneins, which are low molecular weight proteins with high cysteine content [3, 11, 13]. Muscles are not always a good indicator of overall

fish metal contamination, and therefore it is important to analyze other tissues, such as the liver and gills [5]. Although the muscle is not an active tissue in accumulating metals, it is commonly analyzed in literature because it is the main fish part consumed by humans [12, 14]. Gills and liver are chosen as target organs for assessing metal accumulation. The concentration of metals in the gills reflects the content of metals in waters, and the concentration in liver represents storage of metals [15]. The use of multivariate methods (HCA and PCA) may afford a better understanding of the composition of meat according to the fish origin. PCA analysis provides information on the most meaningful parameters that describe the whole data interpretation and summarize the statistical correlation among metals [16].

Material and Methods

Study Area

Lake Góreckie is a dimictic lake in Wielkopolska National Park, with a surface area of ca. 104 ha, maximum and mean depths of 17.25 m and 8.97 m, respectively, and a shoreline of 8,300 m. The degree of antropopressure from annual nitrogen and phosphorus amounts for the eutrophic lake is estimated at $9.5 \text{ g}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ for total nitrogen and 0.36 for total phosphorus $\text{g}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ [17]. Lake



Fig. 1. Map of Poland and locations of analyzed lakes (A)Wędromierz, (B)Strzeszyńskie, (C) Góreckie.

Strzeszyńskie has a surface area of 34.9 ha and is located within the administrative boundaries of the city of Poznań. Maximum depth is 18.8 m and mean depth is 8.2 m. A shoreline of 4,500 m is mostly covered with deciduous and pine forests used mainly for recreational destination. The lake is used by the Polish Fishing Association. Lake Wędromierz is a flow reservoir in Pszczewski Landscape Park. It has a surface area of 73.8 ha, maximum and mean depths of 11.8 m and 4.9 m, respectively, and a shoreline covered with mixed forests (Fig. 1).

Trophic levels of the lakes are based on Carlson's index [18] and calculated as an average for three seasons (spring, summer, and autumn). The basis for the calculations were the results of analyzing Secchi disk visibility, chlorophyll concentrations, and the concentrations of total phosphorus in the surface layer (2012). The obtained values of Carlson index were: Góreckie 71, Strzeszyńskie 63, and Wędromierz 83. On this basis, all the lakes were classified as eutrophic. The conditions of the Wędromierz catchment causes unfavorable changes, i.e., class III susceptibility to degradation of the water body, which has an adverse effect on the quality of water [19]. Ecological status of this reservoir is classified as moderate [20]. Water of Strzeszyńskie was estimated to be in second purity class [21, 22]. Strzeszyńskie can be classified as relatively highly resistant to human impact, and the general vulnerability of this reservoir was designated as category 2 [23, 24]. The trophic state of Góreckie was determined as hypertrophic [25]. For more than 20 years this lake had been polluted with raw waste from the nearby sanatorium in Jeziory [26].

Fish Samples

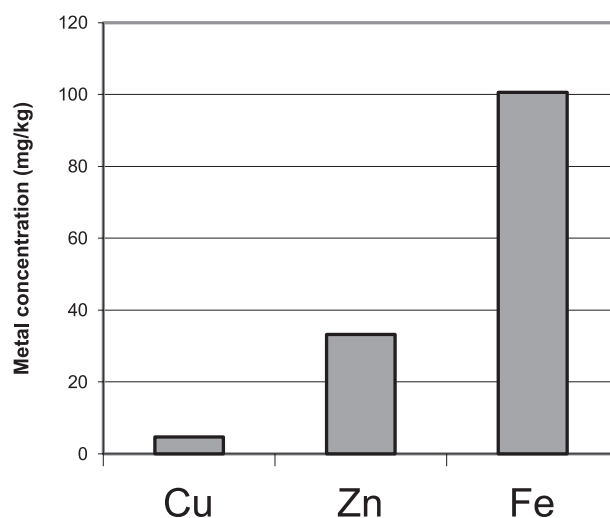
The study involved 90 individual perch (*Perca fluviatilis* L.) caught in spring (April 13-25), summer (July 9-25), and autumn (October 9-November 6). The experimental fish were obtained from their natural environment from the Wielkopolska Lake District. Measurements of the mass of the fish body (BW) (± 0.01 g), body length (Lc) (± 0.1 cm), and total length (Lt) (± 0.1 cm) were taken for each individual. The meat samples for analyses were taken from the large side muscle of fish body above the lateral line. Due to the relatively low amounts of liver and gills obtained from single individuals, the material was combined (about two pieces of each). We chose for analyses individuals with similar biometric measurements. Body weights ranged from 165 to 295 g, and body length and total length were from 17.0 to 23.5 cm and from 19.5 to 28.5, respectively.

The samples of fish organs were immediately frozen after preparation and were kept in a deep freezer before analyses. All frozen samples were freeze dried in a freeze drier (Finn-Aqua Lyovac GT2) (parameters: temperature -40°C , pressure $6 \cdot 10^{-2}$ mbar, duration at least 48 h).

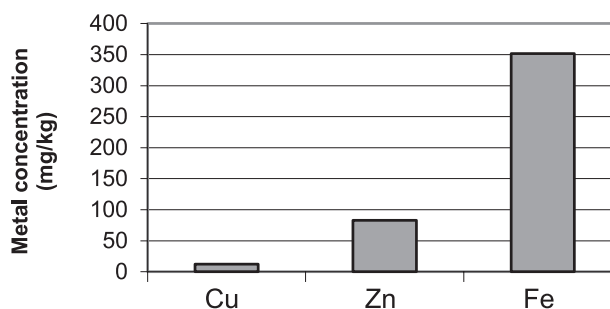
The freeze dried samples were mineralized in a microwave mineralizator (Ethos Plus, Milestone). For mineralization 0.1g of the tissue was weighted and then HNO_3 and H_2O_2 were added at a ratio of 4:1. During the

first 10 minutes, the temperature was increased to 190°C . During the next seven minutes the temperature was kept at a level of $190 \pm 5^{\circ}\text{C}$. The mineralized samples were carried quantitatively to the measuring flask with a capacity of 50 cm^3 .

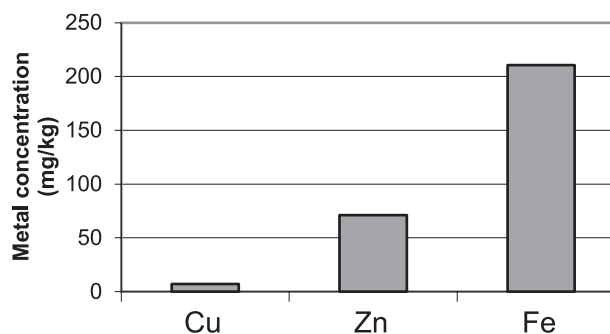
Copper, zinc, and iron concentration was determined using a Solar 969 atomic absorption spectrophotometer (Unicam). Tissue concentrations of minerals have been reported as $\text{mg} \cdot \text{kg}^{-1}$ dry weight ($\text{mg} \cdot \text{kg}^{-1} \text{ d. w.}$). Certified ICP standards of Merck were used in the calibration and validation of the standard curves. The concentrations of the metals were calculated from linear calibration plots obtained by measurement of the standard solutions. All



a) meat



b) liver



c) gills

Fig. 2. Range of the metal concentrations in different tissues of perch a) meat, b) liver, and c) gills.

Table 1. Mean concentrations of Zn, Fe, and Cu in the meat of perch (*Perca fluviatilis* L.).

Fishing period	Reservoir	Metal concentrations (mg·kg ⁻¹ dry weight)		
		Zn	Fe	Cu
Spring	Góreckie	60.84±1.34 ^a	30.41±1.13 ^a	7.11±0.22 ^a
	Strzeszyńskie	26.99±0.36 ^b	23.09±1.12 ^b	4.52±0.17 ^b
	Wędomierz	44.18±4.57 ^c	17.94±0.23 ^b	1.98±0.23 ^c
Summer	Góreckie	16.94±0.63 ^d	267.93±3.35 ^d	6.09±0.22 ^d
	Strzeszyńskie	25.50±0.96 ^b	263.87±2.53 ^d	5.19±0.28 ^e
	Wędomierz	20.50±0.51 ^e	263.02±11.13 ^d	2.67±0.32 ^f
Autumn	Góreckie	40.50±2.03 ^f	17.66±0.76 ^{b,c}	7.52±0.28 ^g
	Strzeszyńskie	30.72±2.02 ^g	9.20±0.36 ^c	4.54±0.16 ^b
	Wędomierz	33.06±2.09 ^g	12.71±0.43 ^{b,c}	2.68±0.28 ^f

The values marked with different letters in the same column are significantly different ($p \leq 0,05$, Tukey's test).

Table 2. Mean concentrations of Zn, Fe, and Cu in the liver of perch (*Perca fluviatilis* L.).

Fishing period	Reservoir	Metal concentrations (mg·kg ⁻¹ dry weight)		
		Zn	Fe	Cu
Spring	Góreckie	93.12±2.12 ^{a,b}	288.99±11.82 ^a	16.35±0.20 ^a
	Strzeszyńskie	96.19±3.82 ^a	434.53±8.41 ^b	15.87±0.33 ^a
	Wędomierz	91.91±1.10 ^{a,b}	525.02±13.32 ^c	18.73±0.75 ^b
Summer	Góreckie	49.22±1.83 ^c	472.94±11.84 ^d	8.53±0.26 ^c
	Strzeszyńskie	67.31±3.19 ^d	373.52±28.65 ^e	12.72±0.90 ^d
	Wędomierz	43.91±1.23 ^c	446.51±11.42 ^{b,d}	13.13±0.27 ^d
Autumn	Góreckie	107.39±6.24 ^e	269.99±20.91 ^a	8.25±0.14 ^c
	Strzeszyńskie	105.66±1.42 ^e	196.97±6.51 ^f	9.67±0.78 ^c
	Wędomierz	88.79±2.18 ^b	155.75±15.76 ^g	7.51±0.38 ^c

The values marked with different letters in the same column are significantly different ($p \leq 0,05$, Tukey's test).

Table 3. Mean concentrations of Zn, Fe, and Cu in the gills of perch (*Perca fluviatilis* L.).

Fishing period	Reservoir	Metal concentrations (mg·kg ⁻¹ dry weight)		
		Zn	Fe	Cu
Spring	Góreckie	101.32 ± 2.96 ^a	179.87 ± 6.37 ^a	14.80 ± 0.09 ^a
	Strzeszyńskie	76.07 ± 1.08 ^b	71.23 ± 1.63 ^b	7.68 ± 0.24 ^b
	Wędomierz	94.98 ± 1.88 ^c	155.64 ± 27.49 ^c	9.40 ± 0.46 ^c
Summer	Góreckie	37.01 ± 0.21 ^d	337.61 ± 17.92 ^d	4.90 ± 0.20 ^d
	Strzeszyńskie	67.36 ± 2.29 ^e	499.20 ± 6.25 ^e	4.16 ± 0.51 ^e
	Wędomierz	42.86 ± 0.41 ^f	298.56 ± 6.29 ^f	5.99 ± 0.13 ^f
Autumn	Góreckie	79.12 ± 3.33 ^b	118.64 ± 4.36 ^g	5.00 ± 0.28 ^d
	Strzeszyńskie	75.69 ± 2.19 ^b	107.24 ± 5.39 ^g	6.35 ± 0.17 ^f
	Wędomierz	66.23 ± 2.97 ^c	128.21 ± 4.02 ^g	5.93 ± 0.33 ^f

The values marked with different letters in the same column are significantly different ($p \leq 0,05$, Tukey's test).

determinations were made in triplicate and the data for samples of the meat were corrected to oven-dry (105°C) moisture content.

Statistical Analyses

Data analyses were performed using Statistica 8.0 software (StatSoft, USA). Significance of differences in the average contents of zinc, copper, and iron in the meat, gills, and liver of perch were statistically analysed using Tukey's test. In this case, a significance of differences in the average content of metals (between the lakes and seasons within a single tissue) were calculated by two-way analysis of variance (ANOVA). The normality of data was tested using Shapiro-Wilk's test, and the homogeneity of variance was tested with Levene's test. The analysis of correlations between metals concentrations were done. Pearson rank correlation coefficient (r) was calculated. The classifications and discriminations of different seasons and tissues were achieved by cluster analysis (CA) and principal component analysis (PCA).

Results and Discussion

The analyses that are presented in Fig. 2 revealed the accumulation of metals in the following order: Fe > Zn > Cu (in all tissues) and we observed statistically significant differences in Zn, Fe, and Cu concentrations in the same tissue collected from different reservoirs and during different seasons.

The same tendency was observed by Klavins et al. [27] in perch tissues collected from the lakes in Latvia, by Radwan et al. [28] in pike from Lake Piaseczno, and in tissues of fish from Iran's Anzali Wetland [29]. In fish organs collected from northwestern and northeastern Poland, metals were ordered as follows: Zn > Fe > Mn > Cu [30, 31]. As the analyses indicated, concentrations of metals were higher in the liver than in any other tissues (Fig. 3). It appears that the concentrations of all metals behave in a similar way in the examined tissues. The liver is the major organ involved in xenobiotic metabolism in fish through specific binding proteins known as metallothioneins [3, 11, 13]. It is well known that a large amount of metallothioneins occurs in the liver cells of fish because metals mainly accumulate in metabolically active tissues.

The mean values of Zn in the liver and gills were very similar (during spring and summer) and the concentrations of Fe and Cu were significantly higher in the liver (Tables 1-3).

The gills play active and passive roles in exchanges between the animal and its aquatic environment [32], and as Brooks and Rumsey [33] observed, the gills are the major storage sites for Zn in several species of fish in New Zealand. Increased levels of Zn in gills and liver are the consequence of increased Zn concentrations in plankton and sediments, which was confirmed by the analyses of Petkovšek et al. [34]. Metal absorption in fish is carried out

via two uptake routes: the digestive tract and gill surface [31]. The highest concentrations of metals in the gills indicates that the element was absorbed via the respiratory tract [7]. As Fig. 3 shows, Cu, Zn, and Fe concentrations detected in perch increased in the following order: liver > gills > meat.

These results were in agreement with data obtained by Bochenek et al. [7], Brucka-Jastrzębska et al. [35], Canli and Atli [8], Drag-Kozak et al. [36], Dural et al. [32], Jarić et al. [5], Papagiannis et al. [3], Petkovšek et al. [34],

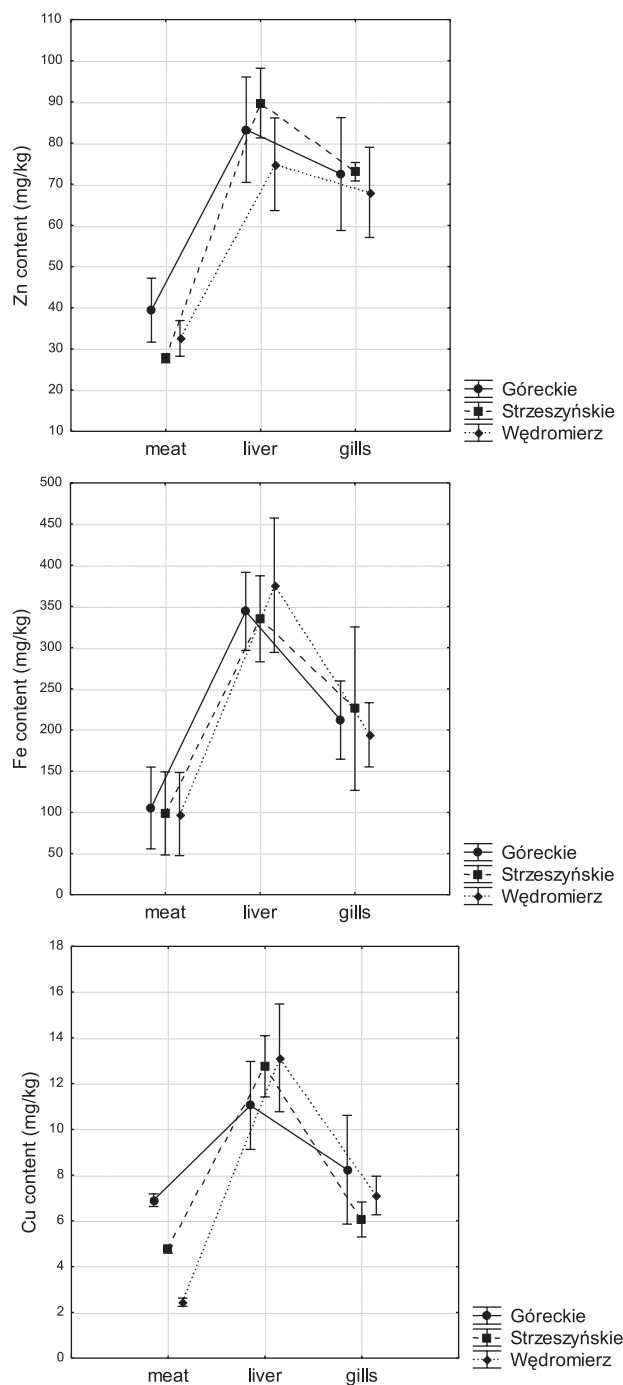


Fig. 3. The mean concentrations of Zn, Fe, and Cu (mg/kg) in various tissues collected from Lakes Góreckie, Strzeszyńskie, and Wędomierz.

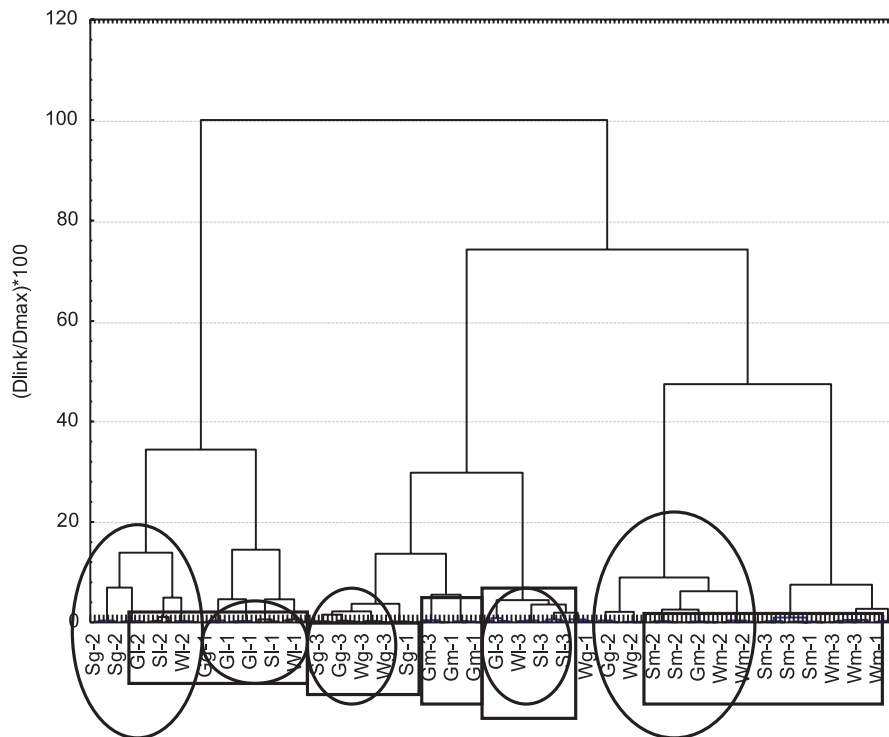


Fig. 4. The dendrogram produced by hierarchical cluster analysis (HCA) using Ward's method and squared Euclidean distance. Abbreviations: G – Góreckie Lake, S – Strzeszyńskie Lake, W – Wędomierz Lake, g – gills, l - liver, m - meat, 1 - spring, 2 – summer, and 3 – autumn.

Szefer et al. [37], and Zubcov et al. [14]. The same results were observed by Yazdi et al. [29] for Cu determined in the tissues of *Ctenopharyngo idella* and *Perca fluviatilis*, and for Zn determined in the tissues of *Perca fluviatilis*. Muscle proved to have the lowest accumulation capacity of the investigated metals. This is important because muscle constitutes the greatest mass of the fish that is consumed. Unless fish muscle cannot be considered to be a metal-accumulating tissue, it is the ideal tissue for use in monitoring programs [27]. The recommended daily allowance of Zn for girls and adult women and for boys and men ranges 13-21 and 16 mg per person, respectively. The average recommended daily allowance of Fe and Cu ranges 13-26 and 1.5-2.5, respectively [11]. Therefore, consumption of 100 g of an edible portion of analyzed fish would satisfy about 17.4 percent of human daily demand for Zn, about 51.6 for Fe, and 23.5 for Cu.

Considering the analyses in terms of seasons, average Cu and Zn contents were the highest in the tissues taken in the spring. The highest concentration of Fe was determined in the samples collected during summer (Tables 1-3). The seasonal changes of the metal concentrations in fish may result from the intrinsic factors such as growth and reproductive cycle, and changes in water temperature [32]. The seasonal variations in the concentrations of the selected metals are probably reflected by different metal bioavailabilities, depending on the ligands present in the biotopes and the chemical speciations between the dissolved and particulate phases [37]. As Yilmaz et al. [4] reported, Fe content depends on the species, individuals, and sampling period. This suggest that the highest con-

centrations of this metal could be a resultsresult of individual diet.

The observed seasonal variations in the contents of Cu, Zn, and Fe in fish body may be reflected by different metal bioavailabilities, depending on the ligands present in the biotopes [36]. As the authors have concluded, the great differences presented in Fig. 3 may be due to physiological roles of the different metals in fish metabolism. Mendil et al. [2] observed that concentrations of the most frequently

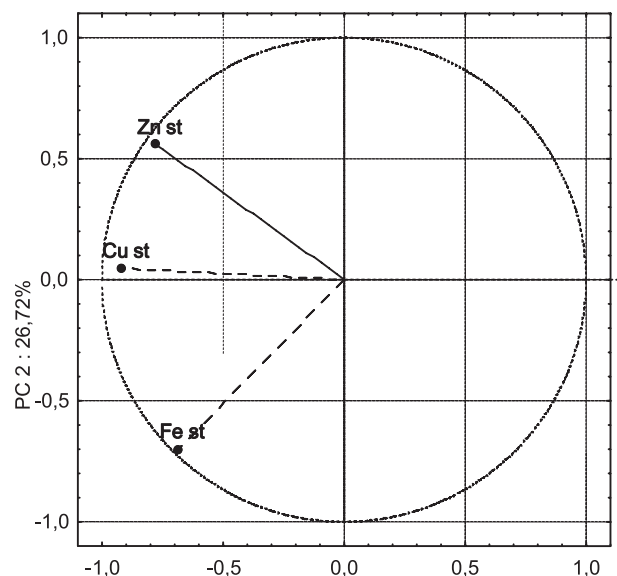
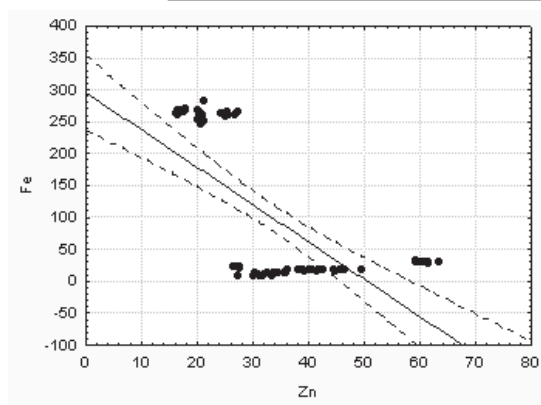
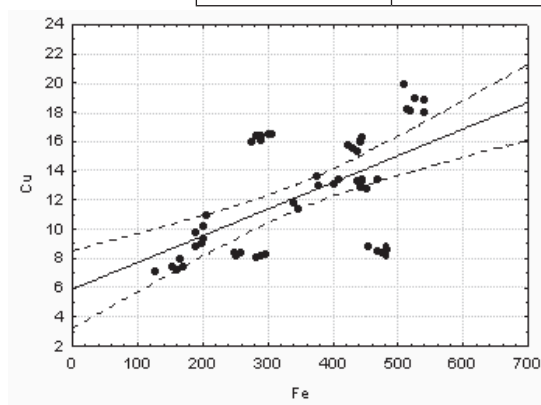


Fig. 5. Loading plot of PC1-PC2.

meat			
Zn	-0.6489		
Cu	0.0042	0.2670	
	Fe		Zn



liver			
Zn	-0.5126		
Cu	0.5824	0.0644	
	Fe		Zn



gills			
Zn	-0.4869		
Cu	-0.3543	0.7015	
	Fe		Zn

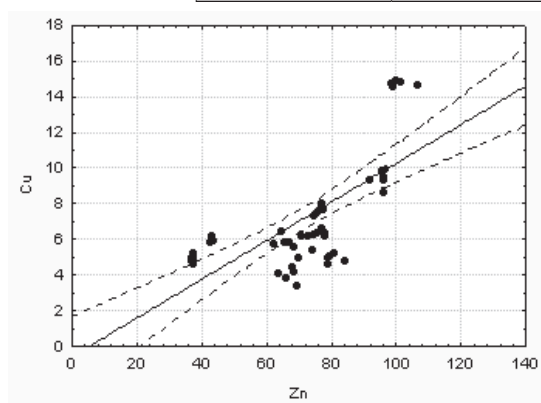


Fig. 6. Values of Pearson's rank correlations coefficient for selected metals in the meat, liver, and gills of perch (*Perca fluviatilis* L.) from Lakes Góreckie, Strzeszyńskie, and Wędomierz.

analyzed metals (for example: Fe and Cu) in the fish samples collected in summer, have been reported at the highest level. Most probably, the dynamics of trace element accumulation is determined by the growth rate of fish, which is the highest during summer, and by changes in the feeding rate and dynamics of lipid metabolism in the tissues [14]. Additionally, the differences noted in the metal concentrations in the different tissues between seasons could result from local pollution [32, 38].

The analyses indicated that the concentrations of metals in tissues of fishes collected from different reservoirs showed a great variations and statistically important differences (Tables 1-3), but similar trends in metal accumulation in various organs were observed (Fig. 3). These results were generally in the agreement with data published in literature. This is related to environmental conditions, changes in water temperature, and swimming activity [8]. It is a difficult to compare metal concentrations between the same tissue of two different species – or even the same species living in different reservoirs – because of different feeding habits, differences in an aquatic environmental states, growing rates of the species, types of the analyzed tissues, and other factors [4]. As Brucka-Jastrzębska and Protasowicki [39] indicated, levels of the bio-elements and harmful metals in the carp body depended on the culture method, water quality, and type of the feed. The rate at which metals affect the organism is related to the metal transportation function of the blood.

Chemometric Analysis

Euclidean distance was used as a similarity measurement and Ward's method as an amalgamation rule. The resulting dendrogram described in Fig. 4 shows six different clusters at $(D_{link}/D_{max}) \times 100$ under 20. The clusters marked with an ellipses aggregate samples collected in the same season, and the clusters marked with a rectangle aggregate samples of the same tissue.

The data matrix of variables (Cu, Zn, and Fe) and scores (180 fish samples) was subjected to PCA in order to decrease the number of descriptors associated with the data set. PCA is mostly used for data compression and visualization. The main goal of this method is to explain the information contained in the data by a set of the so-called principal components.

Fig. 5 illustrates the factor-loading plot for the metals. All three variables have negative loadings in PC1 and gathered on the left side of PC1×PC2. PC1 showed a low negative loading from Zn, Fe and Cu. The highest positive loadings in PC2 have Zn (0.56).

The loadings plot can explain correlations between variables. Variables closest to each other in two-dimensional space and far from the plot origin are positively correlated, and variables opposite to each other are negatively correlated. Results of the analysis of correlation are presented in Fig. 6 and show a positive – but not high – correlation between Zn and Cu. Furthermore, we observed a high and statistically significant correlations between Cu and Fe in the liver.

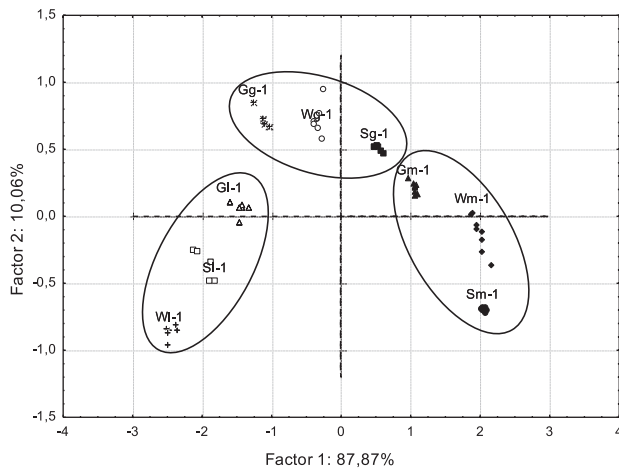


Fig. 7. Score plot for PC1 and PC2 for spring (1). Abbreviations: G – Góreckie Lake, S – Strzeszyńskie Lake, W – Wędomierz Lake, g – gills, l – liver, m – meat.

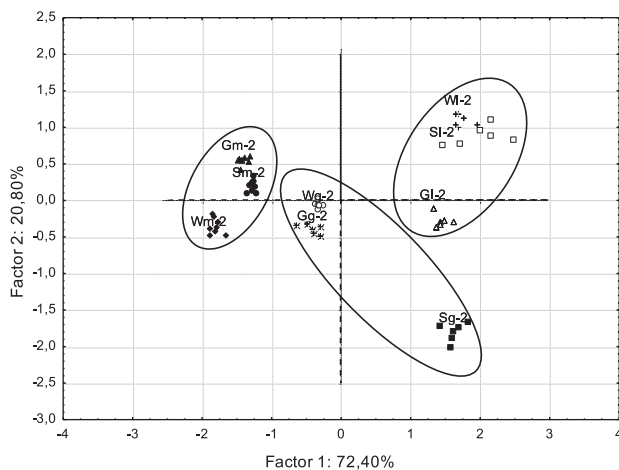


Fig. 8. Score plot for PC1 and PC2 for summer (2). Abbreviations: G – Góreckie Lake, S – Strzeszyńskie Lake, W – Wędomierz Lake, g – gills, l – liver, m – meat.

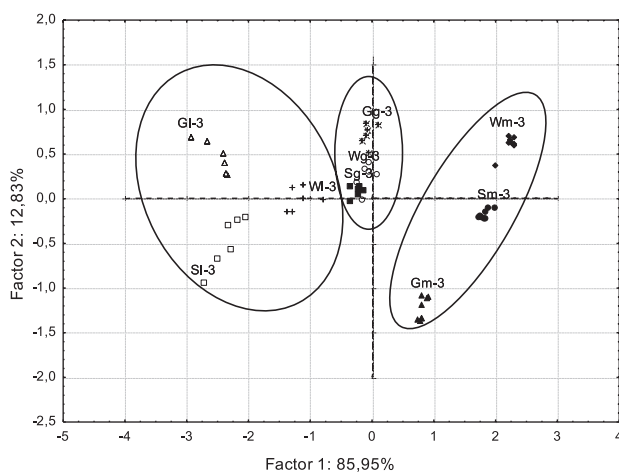


Fig. 9. Score plot for PC1 and PC2 for autumn (3). Abbreviations: G – Góreckie Lake, S – Strzeszyńskie Lake, W – Wędomierz Lake, g – gills, l – liver, m – meat.

As Brucka-Jastrzębska et al. [35] observed, absorption and metabolic function of Fe are dependent on influences of the other elements. For example, the relationship between Fe and Cu often has a synergetic character, because both of these metals are involved in reduction-oxidation processes.

Figs 7-9 show that PC1 and PC2 scores allow for good separation of three reservoirs and three tissues investigated.

Conclusions

1. The concentrations of Zn, Fe, and Cu in meat were similar to the values obtained by the other authors and did not exceed the acceptable levels in food intended for human consumption.
2. Seasonal variabilities were found in the analyzed samples, with the highest concentrations of Zn and Cu occurring in spring and Fe in the summer. The highest concentrations of Fe that were determined in samples collected during summer suggest greater elution of iron from sediments at higher temperatures.
3. There were observed differences in the content of elements depending on lake type. Analysis of PCA allowed for very good separation of the three reservoirs and three tissues investigated, because there were statistically significant differences in the accumulation of metals in the organs of fish caught from different reservoirs. But the tendency of metals accumulation in the organs increased in the following order: liver > gills > meat.

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