



ACCUMULATION OF ZINC IN WATER, SEDIMENTS AND BLEAK FISH (*ALBURNUS ALBURNUS L.*) IN THE ECOSYSTEM OF THE DUNAJEC RIVER

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Abstract

Zinc present in water penetrates into the organism of a fish either directly, through the skin and gills, or indirectly, with food taken into the alimentary canal. The toxicity of this metal is associated with the transporting function of blood, which distributes it over the whole organism. The aim of this paper was to assess the zinc content in water and bottom sediments collected from the Dunajec River, and also in selected organs of bleak (*Alburnus alburnus L.*). The bioaccumulation factors for this element in the muscles, liver, skin and bones of the fish were calculated from the results of the research, which was carried out in 2011. Samples of water and bottom sediments were collected twice, in July and October, at 5 research points located in Szczepanowice, Janowice, Wróblowice, Lusławice, and Zakliczyn. Twenty-nine specimens of bleak were provided by members of the Polish Angling Association (PZW – Polski Związek Wędkarski), from catches performed in the second half of July. The zinc concentration in the samples was determined by inductively coupled plasma atomic emission spectrometry at a wavelength of 206.200 nm, conducted on an Optima 7600 DV spectrometer made by Perkin Elmer. The limit of detection for zinc was $5.9 \mu\text{g dm}^{-3}$. High concentrations of zinc in the water were found, indicative of anthropogenic enrichment of the river with this element. The zinc content in the analysed sediments was below the geochemical background value for bottom sediments in Poland. Zinc concentrations in the fish varied within a range from 152.6 to 352 mg kg^{-1} in the skin, from 158.8 to 271.3 mg kg^{-1} in the bones, from 27.32 to 97.35 mg kg^{-1} in the muscles, and from 82.39 to 230.7 mg kg^{-1} in the liver. The mean content of zinc in individual organs decreased in the following order: skin > skeleton > liver > muscles. Zinc concentrations in individual organs of the fish were comparable with the ones determined elsewhere in environments polluted with this element.

Keywords: zinc, the Dunajec River, bottom sediments, bleak, bioaccumulation.

INTRODUCTION

Zinc is a common element in the environment. Due to its abundance in the earth's crust as well as various applications it finds in many fields of human life, high concentrations of this element can be found in soils, surface waters and also in living organisms. The anthropogenic emission of this element is caused by a variety of human activities, for example the extraction and processing of zinc ores, combustion of fuels as well as the disposal of municipal and industrial sewage to the environment. Large amounts of zinc are found in runoffs from urbanized areas, which is the consequence of zinc leaching from surfaces protected against corrosion (roof surfaces, construction elements or car bodies). Intensive fish breeding, which involves consumption of large amounts of commercial fodder or medicines, is a source of substantial water pollution by trace elements like zinc (DEAN et al. 2007). In water, this element occurs in the dissolved, colloidal form as well as as a suspension constituent. Zinc cations are easily adsorbed on suspended solid particles. They can also create hydrated ions, ionic pairs as well as complex compounds. Zinc is an essential element for living organisms, which participates in many physiological processes, e.g. it controls ligand exchange processes, contributes to the metabolism of nucleic acids and gene expression, shows antioxidant activity, influences immunological processes of an organism and controls ionic regulation of such elements as copper, selenium, manganese and magnesium. Deficiency of this element leads to growth inhibition, delayed maturation, impaired immunity as well as neurosensory disorders. On the other hand, an excessive amount of this element causes anaemia. In fish, mortality as well as embryonic deformation can be observed in response to surplus zinc. Excess of zinc is also responsible for alimentary tract disorders. While it is an element of relatively low toxicity to humans and animals, considerable sensitivity to an excessive concentration of zinc in fodder can be observed in ruminants, in which poisoning can occur after eating plants that contain 1000 mg Zn kg⁻¹. The toxicity of zinc in an aquatic ecosystem is not high and depends on the chemical form in which this element occurs as much as on the physical and chemical properties of water. Zinc concentrations above 240 µg dm⁻³ may implicate a risk of harmful effects produced on sensitive aquatic organisms. Toxicological research results indicate that a lethal concentration of zinc for fish (LC 50) is 8.46 mg dm⁻³ (CJJI, NANDAN 2014). When introduced to the water environment, zinc does not last long in the dissolved form. In fact, by absorption, adsorption as well as biological fixation processes, it relatively quickly becomes bound to bottom sediments, which entails its temporary immobilisation. This element has strong affinity to organic matter as well as to the finest fraction of mineral particles of silt. Zinc present in water penetrates into the organism of a fish directly, through the skin and gills, or indirectly, along with the food taken into the alimentary canal. The toxic effect of the metal is associated with the trans-

porting function of blood, which distributes it over the whole organism (FERNANDES et al. 2007). Marine organisms accumulate more of this element even though its content in sea water is significantly lower than in fresh water. The reason is the specific metabolism of marine animals stimulated by osmoregulation in the hypertonic environment (CLEARWATER et al. 2002).

The aim of this paper was to assess the zinc content in abiotic parts of an ecosystem of the Dunajec River as well as to determine the bioaccumulation factor for this element in selected organs of bleak (*Alburnus alburnus* L.), such as bones, skin, muscles and the liver.

MATERIAL AND METHODS

In order to reach the above objective, samples of water and bottom sediments from the Dunajec River were collected twice in 2011 (in July and October), at 5 research points located in Szczepanowice, Janowice, Wróblowice, Lusławice, and Zakliczyn (Figure 1). The research points were distributed along an approximately 10-kilometer course of the Dunajec River flowing through the Carpathian Mountains, in the districts of Zakliczyn and Pleśna.

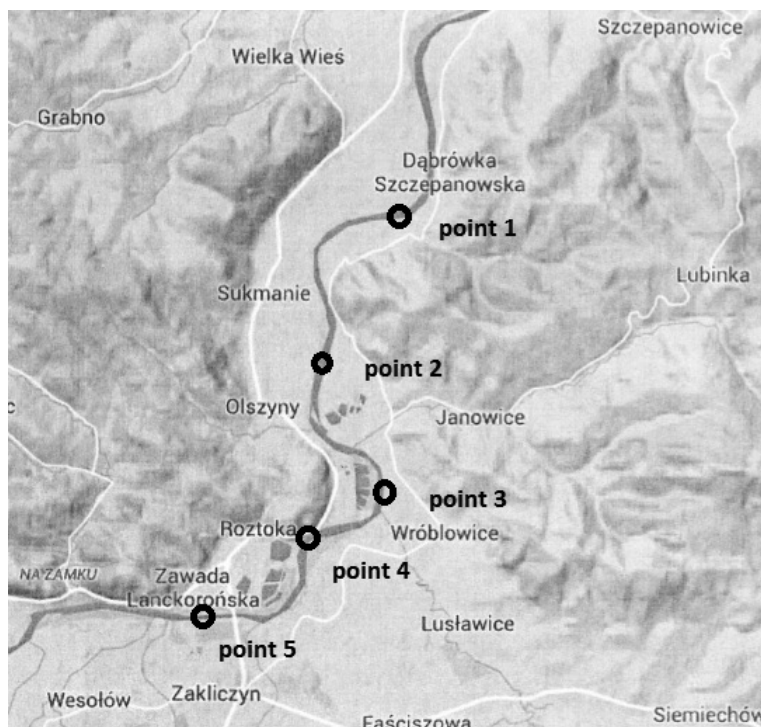


Fig. 1. Points of water and sediment sampling and the fish catching area (Source: <https://www.google.pl/maps>)

The research area included the river's course below the complex of dam reservoirs: Rożnów-Czchów. Generally, a river ecosystem below a dam reservoir is more sensitive to pollution due to a lower amount of seston carried by waters below such a man-made structure. Water samples were collected from the top layer, down to the depth of 10 cm. The sample collection sites were selected so as to eliminate the risk of collecting stagnant water. The samples were collected approximately 2-3 m from the river bank, but in each case they came from the main stream. Every laboratory sample was 1 dm³ in volume. Samples of the bottom sediments were collected at the same sites as water, from places where seston accumulates in backwaters. Cumulative samples were created from 6-10 batches of bottom sediment weighing approximately 200 g. The mass of a laboratory sample contained approximately 500 g of dry matter. Meanwhile, in the second half of July, bleaks (*Alburnus alburnus* L.) were caught from the analysed course of the river. In total, 29 fish were obtained from members of the Polish Angling Association. The collected water samples were preserved at the collection sites by adding nitric acid in the amount of 2 cm³ per each 100 cm³ water. Afterwards, the samples were transported to a laboratory. The bottom sediments were dried, passed through a sieve with 1 mm mesh and ground in a mortar. The fish used in the research weighed from 30 to 42 g, and were between 15 and 19 cm long. They were approximately 1 year old, sexually immature and originating from the previous year's spawning. Organs such as the skeleton, muscles, liver and skin were excised from the fish. The choice of these organs was dictated by their usefulness in the evaluation of the environment using bioindication methods (SINGH et al. 1991, DURAL et al. 2006, MOUREAUX et al. 2011) and by their analytical potential. The laboratory samples were subjected to wet mineralisation in a closed system with the use of microwave energy. An ANTON PAAR Multiwave 3000 microwave system was employed for that purpose. Each analytical sample contained approximately 0.5 g of dry matter. The biological material was digested in a mixture of HNO₃ and H₂O₂, in a 5:1, v/v ratio, whereas the bottom sediments were digested in *aqua regia* in a quantitative ratio of 1:10 (the sediment to reagents). The zinc concentration in the samples was determined by inductively coupled plasma atomic emission spectrometry at a wavelength of 206.200 nm, on an Optima 7600 DV spectrometer made by Perkin Elmer. The limit of detection for zinc was 5.9 µg dm⁻³. The uncertainty of measurements achieved by the applied method was 4%. The limit of detection was 0.295 mg kg⁻¹ DM of the biological material, and 0.59 mg kg⁻¹ DM of the sediments. Certified reference material CRM 16-050 was used to check the correctness of zinc analyses (Table 1). Based on the results, bioaccumulation factors for zinc in individual fish organs were calculated by dividing the zinc concentration in dry matter of individual fish organs by the content of this element in the water or in the bottom sediments. In addition, values of the linear correlation between the zinc concentration in individual fish organs, in the water or in the bottom sediments were calculated (using a non-parametric test).

Table 1

Statistic parameters for Zn assays in reference material

Parameter	CRMO 16-050 bottom sediments	IAEA-407 fish
Mean, $n = 5$ (mg kg ⁻¹)	65.23	66.29
Standard deviation (mg kg ⁻¹)	4.07	3.61
Certified value (mg kg ⁻¹)	69.70	67.1

RESULTS AND DISCUSSION

The mean zinc concentration in the water collected at individual research sites varied from 40.80 to 63.00 $\mu\text{g Zn dm}^{-3}$, with 46.76 $\mu\text{g dm}^{-3}$ on average (Table 2). The content of this element in the water samples collected in autumn was approximately 25% higher than its concentration determined in the water collected in July. Similar relationships between the zinc content in water and the date of sample collection were found by WIŚNIEWSKA-KIELIAN and NIEMIEC (2004) in the water from the Dunajec River collected at the same sites in 2003. No statistically significant differences between the zinc content in the water collected at individual research points were observed. The natural zinc content in fresh water is approximately a dozen of $\mu\text{g dm}^{-3}$ (EASTWOOD, COUTURE 2002). The content of this element in the water collected from the middle course of the Yangtze River in anthropogenically modified regions was approximately 30% lower than obtained in the author's own research (YI, ZHANG 2012). Low levels of this element in the Yangtze River water are a result of extremely intensive processes of its binding with bottom sediments. Suspensions, both mineral and organic, intensively bind zinc dissolved in water, which leads to a decrease in its concentration in water and to its limited bioavailability (SCHMITT et al. 2007b). The zinc concentration in the Dunajec River water indicates anthropogenic enrichment, but does not pose a threat to living organisms (JONES et al. 2000). The Dunajec River is a right-bank tributary of the Vistula River. The

Table 2

Statistic parameters of the zinc content in elements of the aquatic ecosystem

Statistic parameters	Water	Sediments	Organs of fish			
			skin	skeleton	muscle	liver
	$\mu\text{g dm}^{-3}$	(mg kg ⁻¹ DM)				
Minimum	40.80	40.88	152.6	158.8	27.32	82.39
Maksimum	63.00	83.75	352.0	271.3	97.35	230.7
Mean	46.76	60.03	237.1	201.3	68.43	144.1
Standard deviation	7.096	16.33	47.79	32.12	20.09	41.10
Relative standard deviation (%)	15.18	27.20	20.16	15.96	29.36	28.51

main sources of pollution to the river are municipal sewage, domestic sewage discharged from Zakopane, Nowy Targ, Nowy Sącz and Tarnów, tannery waste generated in Nowy Targ and Bukowina Tatrzańska and industrial wastewater from Nowy Sącz and Tarnów. Apart from the sewage discharged into the river in a controlled manner, some domestic sewage reaches the river and its tributaries uncontrollably, which creates a grave problem. The catchment of the Dunajec River, which constitutes the research area, is densely developed and has an unregulated water supply and sewage disposal system, which considerably aggravates the water pollution.

Zinc accumulation in bottom sediments depends on the concentration of this element in water and, perhaps more importantly, on the amount of organic matter in sediment and the sediment grain size. The more organic matter in an aquatic ecosystem, the more intensive the binding of this element to bottom sediments. Many authors draw attention to large amounts of zinc immobilised in bottom sediments even when the zinc concentration in water does not indicate anthropogenic enrichment. On the other hand, a high concentration of zinc dissolved in water can be observed in aquatic ecosystems poor in organic and mineral seston with fine granulation, even though little amounts of this element have been introduced to the environment (YI et al. 2011). Mobilisation of zinc bound to bottom sediments may occur as a result of changes in water reaction, in the redox potential of supernatant water or in the content of oxygen in water (YUVANATEMIYA, BOYD 2006). This creates the risk of excessive zinc inclusion in a food chain.

The zinc content in the sediments collected from the Dunajec River bed was between 40.88 and 83.75 mg Zn kg⁻¹ DM, and on average amounted to 60.03 mg Zn kg⁻¹ DM (Tab. 2). According to LIS and PASIECZNA (1995), zinc amounts below 100 mg Zn kg⁻¹ can be regarded as natural. Thus, the zinc content in the sediments of the Dunajec River does not indicate anthropogenic enrichment, despite high zinc concentrations in the water. On average, the coefficient of sediment enrichment in zinc was almost 1.300. YI and ZHANG (2012) reported a coefficient of sediment enrichment equal 4.354 for sediments of the middle course of the Yangtze River. The higher value achieved by these authors can be explained by the fact that waters of the Yangtze River carry considerable amounts of seston, which encourages fast binding of zinc to sediments.

Bioindication is a popular tool in the evaluation of environmental risks posed by trace elements (BERMEJO et al. 2012, SCHMITT et al. 2007a). Having determined the accumulation of harmful elements in organisms dwelling in certain environmental conditions, we are able to assess the degree of pollution and estimate changes in structures of biocoenoses (in terms of both quantities and quality). As a rule, biomonitoring research results become valuable input data when designing systems of natural resources management. Depending on the existing trend of using environmental resources as well as the levels and specificity of pollutants, organisms from different links of the trophic chain are used for biomonitoring research (BRITO et al. 2012, ZUYKOV et al. 2013, CHAKRABORTY et al. 2014). However, in order to obtain

reliable data, organisms of sufficient affinity to a specific xenobiotic and simultaneously occurring in sufficient amounts in the examined ecosystem should be selected for research (MOISEENKO et al. 2008). Trace elements are absorbed into fish organisms through the alimentary canal and through the gills. Ratios of an element penetrating into a fish organism through particular paths vary and depend on the type of an element, its concentration in water and its content in food (CLEARWATER et al. 2002). The ichthyofauna of the Dunajec River comprise 30 fish species, which belong to 9 families. Most of these species are the fish which do not forage actively. They include bleak (*Alburnus alburnus* L.), which represents as much as 29.81% of the fish stock in the river. Such a high quantitative share of this species makes it a good indicator of water pollution by trace elements. In terms of foraging, this species belongs to the group of inventivores, i.e. organisms that eat insects and nematodes. The high position it occupies in the trophic pyramid makes this species particularly useful in biomonitoring tests.

The zinc content in the skin of the fish was high and ranged between 152.6 and 352.0 mg Zn kg⁻¹ DM. The mean for all the samples was 237.1 mg Zn kg⁻¹. An insignificant variation in the accumulation of this element was found (20% for all the samples studied). In the research of QIAO-QIAO et al. (2007), the zinc content in the skin of different fish species from the Yangtze River varied from 141 to 773 mg kg⁻¹ DM. These authors drew attention to a strong correlation between the zinc content in the skin and in abiotic parts of the ecosystem. The content of this element in the skin of the *Parapimelodus valenciennes* L. fish, a member of the family of catfishes, from Chascomus Lake in Argentina was 110 mg Zn kg⁻¹ DM. The content of this element in the *Prochilodus lineatus* L. fish was twice as high (SCHENONE et al. 2014). The bioaccumulation factor for this element in the skin reached 5.071 and 3.950 in relation to its amount in the water and sediments, respectively. SCHENONE et al. (2014) obtained similar results. They recorded values of the bioaccumulation factor for zinc in the skin of fish from Chascomus Lake within the range of approximately 4.000 to almost 9.000. The cited researchers underlined the significant impact of fish species on the value of this parameter. On the other hand, the content of zinc in the skin of several species of fish from the Yangtze River estuary did not differ significantly and were within the range of approximately 30 to almost 40 mg kg⁻¹ FM. (ZHAO et al. 2012). AL-YOUSUF et al. (2000) provide similar results concerning the zinc content in the skin of *Lethrinus lentjan* fish from the Arabian Sea, between 31 and 41 mg kg⁻¹ FM. Converted to dry matter, the above results are lower than the ones reported herein.

In many aquatic organisms, the osseous tissue plays the role of a deactivator of toxic elements. Elements such as zinc, lead or copper are embedded in the osseous tissue, which protects an animal against the toxic effect of these elements but may lead to disorders of skeletal development (SCHENONE et al. 2014). The mean zinc content in the osseous tissue of the analysed fish was 201.3 mg Zn kg⁻¹ DM, and varied between 158.8 and 271.3 mg Zn kg⁻¹ DM. The bioaccumulation factor for this element in the bones was 4.306 and

3.354 (Table 3), respectively, in relation to its amount in the water and in the bottom sediments.

The zinc content in muscles of fish is not regarded to be a good indicator of environmental pollution by this element. Low levels of this element in muscles are associated with the physiological specificity of this element. An increase in zinc accumulation in muscles is usually observed at its very high concentrations in a biotop that cause toxicological effect. The gills, liver, kid-

Table 3

Bioaccumulation coefficient of zinc

Element of ecosystem	Skin	Skeleton	Muscle	Liver
Water	5071	4306	1464	3082
Sediment	3.950	3.354	1.140	2.401

neys and gonads are the target organs of zinc accumulation in fish organisms (QIAO-QIAO et al. 2007). BRUCKA-JASTRZEBSKA and PROTASOWICKI (2010) state that the natural zinc content in the muscles of rainbow trout is approximately 6.7 mg kg⁻¹ FM, whereas in the muscles of grass carp it is about 20.1 mg kg⁻¹ FM. In the current research, the zinc content in the muscles of bleak from the Dunajec River was between 27.32 and 97.35 mg kg⁻¹ DM and its mean value reached 68.43 mg kg⁻¹ DM (Table 2). ARASIMOWICZ et al. (2012) found zinc concentrations in muscles of carps bred in ponds similar to the ones reported herein, despite the fact that the content of this element in the water was five-fold higher than determined in this research, and twice as high in the sediment. QIAO-QIAO et al. (2007) demonstrated that the zinc content in muscles of common carp and silver carp from the Yangtze River was 25 mg kg⁻¹ and 21 mg kg⁻¹ FM, respectively. Despite the considerable concentration of this element in the water, no accumulation of this element in muscles was detected that would suggest a threat to living organisms. UYSAL et al. (2008) reported contents of this element in muscles of various fish species that migrate from the area of Beymelek Lagoon in Turkey to range between 4.27 and 12.28 mg Zn kg⁻¹ FM, which in terms of dry matter yields values close to the ones reported in this paper. JONES et al. (2000) found a very low zinc content (approximately 4.8 mg kg⁻¹ FM) in muscles of various fish species inhabiting Cleveland Bay, despite a high concentration of this element in the environment. In the same region, the zinc content in oysters was 2.5 g kg⁻¹ DM. RAHMAN et al. (2012) stated that the zinc content in muscles of various species of river fish from polluted regions in Bangladesh was within the range from 53.18 to 418.05 mg Zn kg⁻¹ DM, whereas VISNJIC-JEFTIC et al. (2010) concluded that the levels of this element in muscles of the Black Sea herrings caught in polluted regions of the Dunajec River were within the range of 68-76 mg Zn kg⁻¹ DM. In relation to the zinc content in the water and sediments, the bioaccumulation factor for zinc in the water and sediments was 1.464 and 1.140, respectively (Table 3). YI and ZHANG (2012) de-

terminated the respective values of this parameter in pelagic fish of the middle course of the Yangtze River to be approximately 980 and 0.222. The lower values reported from China may be explained by the higher water pollution in the Yangtze than in the Dunajec River.

The liver is predisposed to accumulate zinc in fish. Zinc metabolism is closely connected with functions of this organ, which translates into high hepatic concentrations of this element (MORMEDE, DAVIES 2011). Nonetheless, VISNJIC-JEFTIC et al. (2010) did not find a higher content of this element in the liver of the Black Sea herrings compared with their muscles. On average, the zinc content in the liver of the fish they studied was 144.4, and varied between 82.39 and 230.7 mg Zn kg⁻¹ DM (Table 2). MORMEDE and DAVIES (2011) stated that the mean zinc content in the liver of fish from the Atlantic Ocean and intended for human consumption approximated 62.35 mg kg⁻¹ FM. Such zinc concentrations in the liver are not indicative of environmental pollution by this element. In terms of dry matter, these values are close to the ones obtained during the current study. SCHMITT et al. (2007a) stated that 733 mg Zn kg⁻¹ DM was a mean zinc content in the liver of fish from areas contaminated due to zinc extraction, whereas its hepatic content in fish living outside a contaminated zone was 74 mg kg⁻¹ DM. The authors highlighted a statistically significant relation between the distance from the source of pollution and the zinc content in fish liver. HEIER et al. (2009) noticed a similar significant dependence between the zinc content in water and in fish liver.

In the author's research, the bioaccumulation factor for zinc in the liver of bleak compared to the content of this metal in the water and sediments was 3.082 and 2.40, respectively (Table 3).

The zinc concentrations determined during this study in individual fish organs suggest considerable variation in the accumulation of this element in different specimens. Regardless of the concentration of trace elements in water or the conditions that shape and their assimilability by fish, their total bioaccumulated amounts depend on individual traits of an organism, related to the age, gender, physiological condition, access to food at individual developmental stages, and other specific characteristics (AL-YOUSUF et al. 2000). The calculated values of the correlation coefficient suggest a statistically significant (at a level of $p = 0.01$) correlation between the zinc content in the skin ($r = 0.635$) – Table 4. Relationships for the zinc content in the muscles as well as in the skin and skeleton were statistically significant at $p = 0.05$.

Table 4
Value of correlation coefficient

Organs	Skeleton	Muscle	Liver
Skin	0.653**	0.394*	0.335
Skeleton	-	0.491*	0.259
Muscle	0.491	-	0.291

* the significance of the correlation coefficient $p = 0.05$

** the significance of the correlation coefficient $p = 0.01$

CONCLUSIONS

1. Zinc concentrations in the Dunajec River water suggest its enrichment with this element. The content of this element in the sediments was below the geochemical background of Poland. A low value of the coefficient of sediment zinc enrichment was found.

2. The zinc content in individual fish organs decreased in the following order: skin > skeleton > liver > muscles.

3. The zinc content in the skin of the fish was high, yet characteristic for ecosystems that are not polluted by this element, which is confirmed by literature data indicating that the skin is the organ with the highest potential for zinc accumulation.

4. Values of the bioaccumulation factors calculated for individual organs of bleak in relation to the concentration of this element in the water varied from 1.464 to 5.071.

5. Values of bioaccumulation factors achieved for the analysed fish organisms in relation to the content of this element in the bottom sediments were within the range of 1.140 to 3.950.

6. No statistically significant correlation (at $p = 0.01$) between the zinc content in individual organs of the fish, except for the content in the bones and skin, was detected.

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