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Heavy metals in two fish species, *Gobio gobio* and *Phoxinus phoxinus*, from the Skawinka River, tributary of the Vistula River, Poland

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SUMMARY

The Skawinka River is a right-bank tributary of the Vistula River, which drains from a large area near Krakow, Poland. The aim of the study was to compare the concentrations of selected heavy metals in two fish species (*Gobio gobio* and *Phoxinus phoxinus*) from the Skawinka River and its tributaries, to identify the most contaminated locations, and to assess the usefulness of gudgeon and minnow as bioindicator species. Fish and bottom sediments were sampled at selected locations, and the levels of Cd, Pb, Ni, Mn, Zn and Cu in the samples were measured using AAS. The results show that minnows accumulate higher concentrations of heavy metals in their tissue than do gudgeons. The concentrations of the metals analysed in gudgeons and minnows were positively correlated with their concentrations in the bottom sediments. The study also showed a positive association between heavy metal concentrations in fish tissue and the body weight, body length, and age of the fish, which confirms that the accumulation of the metals in fish tissue increases over time. The highest concentrations of heavy metals in the bottom sediments were found mainly at the outlet of heated wastewater from the Skawina power plant. Their concentrations in fish tissue were highest in fish from the same location and, additionally, in fish from the Cedron River in Zarzyce and from the stream Piegżówka. The results confirm that the gudgeon and Eurasian minnow are useful bioindicator species for monitoring levels of heavy metals in the environment.

KEY WORDS: Skawinka River, heavy metals, minnow, gudgeon, bottom sediments

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INTRODUCTION

The Skawinka River is a right-bank tributary of the Vistula River. It enters the Vistula near the town of Skawina. It is approximately 34 km long, and its basin has an area of about 354 km^2 (Florek, 2012). It mainly drains water from urbanized areas. The largest town located on its banks is Skawina, which has a large power plant and a number of industrial plants. The upper reaches of the Skawinka River are called the Harbutówka River. The Harbutówka begins near the town of Sułkowice, in the Babica mountain range. Its main tributaries are streams – the Gościbia, Jastrząbka, Lubianka and Piegżówka, whereas the tributaries of the Skawinka River are the rivers Cedron, Mogiłka (left-bank), Rzepnik, Włosanka, Lutówka, Pasieka and Głogoczówka (right-bank). The Głogoczówka River begins in the Maków Beskids, and its largest tributary is the Sieprawka River. The Głogoczówka River, which is 15 km long, flows through the Wielickie Foothills. The longest tributary of the Skawinka River is the Cedron River, which is 24.8 km long and begins on the side of the Chełm Mountain in the Maków Beskids (Bartnik et al., 2011). The Skawinka River is a heavily regulated water system, with seawalls, bank reinforcements and barrages in its upper reaches (the Harbutówka River). Over much of its length, the river is relatively narrow and shallow, and its banks are covered with shrub-like and woody vegetation. The river has significantly incised into its valley as a result of vertical erosion. It has both rapids, with a stony bottom, and longer meandering sections with a sandy and muddy bottom. Thus, the near-bed area of the Skawinka River and the river bed itself provide optimal habitat for fauna and flora. Due to the presence of rapids, drops in elevation become greater when the water in the river rises. Therefore, the river bed is composed of silt and sand as well as coarse stones and gravel, which are typical of mountain streams (Florek, 2012). At the turn of the 20th century, there were 23 fish species in the river's catchment area, including 14 Cyprinidae species, among which chub is the dominant species, particularly in the lower reaches of the river (Bieniarz and Epler, 1991; Bartnik et al., 2011). There are currently 20 fish species in the Skawinka River, with dominance of chub and bleak (Łuszczek-Trojnar et al. 2022). The abundant species in the upper reaches of the river include stone loaches, minnows and barbs. One fish species that can be found along the entire length of the river is the gudgeon (*Gobio gobio*). Given the industrial and urbanized nature of its catchment, the Skawinka is exposed to pollution from a variety of point and diffuse sources. The priority pollutants most harmful to the aquatic environment include heavy metals, such as cadmium, lead, mercury and nickel (Journal of Laws 2019, item 528). Heavy metals also include a number of microelements which are essential to the proper functioning of living organisms but become toxic at higher concentrations, such as zinc, copper, iron, manganese, and many others (Tchounwou et al., 2012). Heavy metals are shiny, ductile and malleable. They have relatively high boiling and melting points and very good thermal and electrical conductivity. They are naturally present in the earth's crust. However, owing to their properties, they have been used by people for centuries, causing pollution. The most common sources of heavy metal pollution include mining, metallurgy, agriculture, pharmaceutics, municipal sewage, and fumes (Tchounwou et al., 2012). Environmental pollution with heavy metals unquestionably poses a threat to human health. Their effects are usually not apparent until many years after exposure and are not fully known. The occurrence of heavy metals in the aquatic environment is variable. Once they have entered a water body, they can settle to and accumulate in bottom sediments, where their concentrations are highest. These sediments may then become a source of contamination for bottom invertebrates and the fish that feed on them (Jeong et al. 2023; Zheng et al. 2023). Once they have entered the food chain, heavy metals can easily be transferred to organisms higher up the food chain, in increasing concentrations, posing risks to humans at the top of the food chain (Ali and Khan 2018).

The aim of the study was to compare the concentrations of selected heavy metals in fish from the Skawinka River and its tributaries, to identify the most contaminated locations, and to assess the usefulness of gudgeon and minnow as bioindicator species. This is the first study comparing the concentrations of heavy metals in fish in this region. The research hypothesis assumed that industrial areas would be more polluted with heavy metals than small tributaries of the Skawinka River. If gudgeon and minnow, as common species in the upper Vistula basin, are shown to reflect the contamination of bottom sediments and prove to be good bioindicators, they will be excellent indicator species in future studies comparing the heavy metal contamination of various tributaries of the Vistula River.

MATERIAL AND METHODS

Study area and material

The study was carried out in July 2021 at 12 locations (each 100 metres long) in the Skawinka River basin, including four on the Skawinka River (1 – Biertowice, 2 – mouth of the Głogoczówka River, 3 – Radziszów, and 4 – downstream of the outlet of heated wastewater from the Skawina power plant) and eight on its tributaries (5 – the Cedron River in Kalwaria Zebrzydowska, 6 – the Cedron in Zebrzydowice, 7 – the Cedron in Zarzyce Małe, 8 - the Głogoczówka River in Krzyszkowice, 9 – the Głogoczówka in Głogoczów, 10 – the stream Jastrząbka in Izdebnik, 11 – the stream Sieprawka, and 12 – the stream Piegżówka) (Fig. 1).

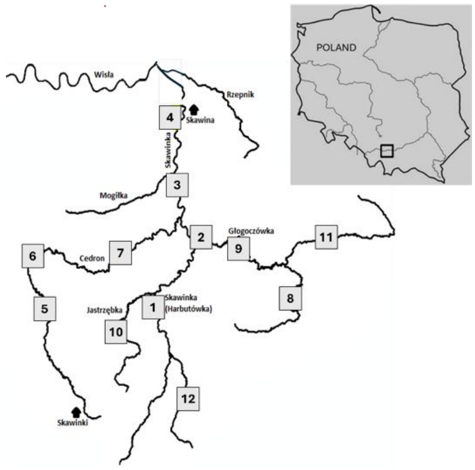

Figure 1. Map presenting the locations of sampling stations; 1 – Skawinka River in Biertowice, 2 – mouth of the Głogoczówka River, 3 – Skawinka River in Radziszów, 4 – Skawinka River at the outlet of heated wastewater from the power plant, 5 – Cedron River in Kalwaria Zebrzydowska, 6 – Cedron River in Zebrzydowice, 7 – Cedron River in Zarzyce Małe, 8 – Głogoczówka River in Krzyszkowice, 9 – Głogoczówka River in Głogoczów, 10 – the stream Jastrząbka in Izdebnik, 11 – the stream Sieprawka in Włosań, 12 – the stream Piegżówka in Rudnik (source: Michał Smoczyk (Michau Wikimedia Commons Sm), CC BY-SA 3.0).

Table 1.
Designations of locations and water parameters (temperature, conductivity and salinity) at the locations at the time of sampling.

Number	Location	GPS	Temp. [°C]	Conductivity [uS]	Salinity [ppm]
1	Skawinka in Biertowice	49.869663, 19.789011	20,6	375	180
2	Skawinka at the mouth of the Głogoczówka stream	49.90709, 19.83178	23,5	462	228
3	Skawinka in Radziszów	49.93613, 19.80905	21,0	451	222
4	Skawinka at the heated wastewater outlet	49.976986, 19.810484	29,9	3251	1659
5	Cedron in Kalwaria Zebrzydowska	49.85525, 19.69067	18,1	549	267
6	Cedron in Zebrzydowice	49.888741, 19.676252	18,9	550	266
7	Cedron in Zarzyce Małe	49.89327, 19.74899	17,2	462	221
8	Głogoczówka in Krzyszkowice	49.89245, 19.89929	15,7	430	210
9	Głogoczówka in Głogoczów	49.90152, 1986940	15,9	483	231
10	Jastrząbka in Izdebnik	49.860498, 19.754803	15,4	450	222
11	Sieprawka in Włosań	49.90295, 19.92440	15,8	466	214
12	Piegżówka in Rudnik	49.85177, 19.82286	15,6	454	233

Table 1 shows the designations of the sampling locations and the water parameters at these locations at the time of sampling. The study was carried out on gudgeons (*Gobio gobio* L. – family Gobionidae) and common minnows (*Phoxinus phoxinus* L. – family Leuciscidae) sampled by electrofishing using a Hans Grassl IG-600T stunning device, by a team licensed employees authorized by the Office of the Marshal of the Małopolska Province (permit no. RO-II.7143.1.2.2021) to use the water resource to sample fish of the two species for analysis.

Ten gudgeons and ten minnows were sampled by electrofishing at each location (Table 2). At locations where there were no minnows (the Głogoczówka River in Krzyszkowice and the outlet of heated wastewater from the Skawina power plant) or gudgeons (the Cedron River in Zebrzydowice and the streams Jastrząbka and Piegżówka), only one species was sampled. The fish were euthanized using MS-222 and then transported under refrigeration to a laboratory at the Experimental Fisheries Station of the Department of Animal Nutrition and Fisheries, University of Agriculture in Krakow, where they were frozen at −20°C until analysis of heavy metal concentrations.

In addition, samples of bottom sediments from the 5-cm surface layer (two samples at mid-channel and two samples near the banks) were collected at each location.

Table 2.
Body weight, body length, and age of fish collected at sampling locations.

Site	n	Body weight [g] average (range)	Body length [cm] average (range)	Age [years] range
		G. gobio		
1	10	11,2 (8,3–16,0)	9,8 (8,5–11,5)	1 – 2
2	10	9,0 (6,7–12,8)	9,4 (8,5–10,5)	1 – 2
3	10	8,2 (5,4–11,9)	8,9 (8,0–10,0)	1 – 2
4	10	11,5 (9,1–15,2)	10,2 (10,5–11,3)	2 – 3
5	10	8,3 (6,9–11,3)	8,9 (8,5–10,0)	1 – 2
7	10	12,9 (10,6–17,8)	10,3 (9,5–11,3)	1 – 2
8	10	6,2 (4,6–8,3)	8,1 (7,5–8,7)	1 – 2
9	10	12,6 (5,3–18,8)	9,9 (7,0–12,0)	1 – 2
11	10	6,2 (4,1–11,4)	8,0 (7,0–10,0)	1 – 2
		P. phoxinus		
1	10	4,1 (2,4–7,1)	6,9 (6,0–9,0)	1-2
2	10	2,8 (1,9–4,2)	6,6 (6,0–7,5)	1-2
3	10	2,2 (1,0–3,5)	6,0 (4,5–7,2)	1
5	10	2,7 (1,5–5,0)	6,2 (5,0–7,5)	1-2
6	10	2,7 (1,5–8,1)	6,1 (5,2–8,5)	1-2
7	10	2,2 (1,6–3,5)	6,0 (5,5–7,0)	1
9	10	3,3 (2,4–3,7)	6,5 (5,6–7,4)	1-2
10	10	4,9 (3.5–10,0)	7,3 (6,5–9,0)	1-2
11	10	2,7 (1,4–4,9)	5,8 (5,0–7,0)	1-2
12	10	5,3 (2,3–7,6)	7,6 (6,0–8,5)	1-2

Analysis of heavy metal concentrations

After thawing, the fish were weighed and then homogenized. Weighted samples suitable for digestion (up to 5 g) were taken from the resulting homogenous mixture. The samples were digested, and the concentrations of heavy metals (Pb, Cd, Ni, Mn, Zn and Cu) were measured using methods described by Łuszczek-Trojnar et al. (2024).

Samples of bottom sediments were dried at 160°C and then passed through a sieve. The finest grain-size fraction (less than 0.5 mm) was subjected to preliminary digestion with nitric acid at room temperature and then digested for approximately 10 hours at 110°C using a Velp 20/26 digester. Clear liquid was separated, and the samples were diluted to 25 ml by washing the sediments several times with deionized water. Then the concentrations of the heavy metals were analysed in the resulting sample using a Unicam 929 spectrometer, as for the fish tissue samples. The results were presented in milligrams of metal per kilogram of wet body weight (mg/kg w.w.) with standard errors (±SE).

The results were used to calculate the correlations between the concentrations of heavy metals in fish and their concentrations in the bottom sediments at a given location.

Analysis of the age of fish

During the weighing of the fish, between five and ten scales were collected from each fish using tweezers, to determine their age. Once the scales had been washed with water and dried, slides of the scales were prepared and then analysed using a stereoscopic microscope to calculate the number of annular rings on the scales. This made it possible to determine the age of each of the fish sampled at each location (Psuty et al., 2014).

Statistical analysis

Statistical analysis of the results was carried out using analysis of variance (ANOVA) and Student's t-test to determine whether there were significant differences in the concentrations of heavy metals in the fish and bottom sediments between locations. In addition, Pearson's r or Spearman's correlation analysis (depending on normal distribution, determined using the Shapiro–Wilk test) were carried out to determine the relationships between the heavy metal concentrations in the fish and their body weight, body length and age, as well as correlations between the concentrations of metals in fish tissue and their concentrations in the bottom sediment samples. Pearson's test was used for the Zn concentration, and Spearman's test for all other metals. Statistical significance was set at the alpha value of 0.05. The analysis was performed using Statistica 12.

RESULTS

The highest concentrations of Pb were found in gudgeons and minnows sampled at location 9 on the Głogoczówka River in Głogoczów (0.23 and 0.76 mg/kg, respectively). The Pb concentrations in minnows were higher than the concentrations in gudgeons from the same locations (Table 3). The highest Pb concentration in bottom sediments was found at location 4, directly downstream of the outlet of heated wastewater from the Skawina power plant (Table 4). There were significant positive Spearman's correlations between the Pb concentration in gudgeons and in bottom sediments ($r = 0.25$) (Table 5) as well as between the Pb concentration in gudgeons and the weight and body length of the fish ($r = 0.21$, $r = 0.22$) (Table 6).

Cd concentrations in the fish were highest in minnows from the Skawinka River in Biertowice and the Cedron River in Kalwaria Zebrzydowska and Zarzyce Małe (locations 1, 5 and 7). The Cd concentrations in gudgeons were lower (Table 3). In this species, the Cd concentrations were highest in fish sampled directly downstream of the outlet of heated wastewater from the Skawina power plant, which was also the location with the highest Cd concentrations in the bottom sediments (Table 4). The statistical analysis showed a significant positive relationship between the Cd concentration in minnows and in the bottom sediments ($r = 0.29$) (Table 5).

Table 3.
Comparison of average Pb, Cd and Ni concentrations [mg/kg w.w.] (±SE) in *G. gobio* and *P. phoxinus* from the sampling locations in the Skawinka River basin. At each site n = 10 for each fish species, if present. Fish samples were analysed in duplicate. An empty cell means there were no fish of this species at the location.

Site	Pb		Cd		Ni	
	Gobio gobio	***Phoxinus phoxinus***	***Gobio gobio***	***Phoxinus phoxinus***	***Gobio gobio***	***Phoxinus phoxinus***
1	0,10 ±0,03^{ac}	0,25 ± 0,03^{x}	0,01 ± 0,00^{a}	0,14 ± 0,01^{x***}	0,37 ± 0,05^{a}	0,18 ± 0,06^{x*}
2	0,17 ±0,03^{ab}	0,12 ± 0,03^{x}	0,03 ± 0,00^{b}	0,07 ± 0,02^{xy}	0,82 ± 0,08^{a}	0,26 ± 0,10^{x***}
3	0,07 ±0,03^{c}	0,19 ± 0,05^{x}	0,03 ± 0,00^{b}	0,06 ± 0,02^{y}	0,25 ± 0,04^{a}	0,07 ± 0,02^{x***}
4	0,22 ± 0,04^{ab}	-	0,06 ± 0,00^{c}	-	2,18 ± 0,14^{b}	-
5	0,08 ± 0,02^{c}	0,33 ± 0,07^{xy**}	0,0 ± 0,00^{a}	0,11 ± 0,04^{xy**}	0,63 ± 0,07^{a}	1,84 ± 0,37^{y*}
6	-	0,21 ± 0,08^{x}	-	0,02 ± 0,00^{z}	-	0,23 ± 0,05^{x}
7	0,12 ± 0,02^{ab}	0,21 ± 0,07^{x}	0,03 ± 0,00^{b}	0,12 ± 0,01^{x***}	1,65 ± 0,23^{b}	0,07 ± 0,03^{x***}
8	0,02 ± 0,00^{c}	-	0,03 ± 0,00^{b}	-	0,42 ± 0,14^{a}	-
9	0,23 ± 0,03^{b}	0,76 ± 0,17^{y*}	0,00 ±0,00^{a}	0,07±0,01^{xy***}	0,79 ± 0,09^{a}	0,27 ± 0,04^{x***}
10	-	0,40 ± 0,09^{xy}	-	0,04 ± 0,00^{yz}	-	1,28 ± 0,19^{y}
11	0,19 ± 0,03^{ab}	0,34 ± 0,10^{xy}	0,03 ± 0,00^{b}	0,05 ± 0,01^{xyz*}	0,76 ± 0,19^{a}	2,35 ± 0,37^{y***}
12	-	0,55 ± 0,16^{xy}	-	0,07 ± 0,02^{xyz}	-	1,94 ± 0,45^{y}

Different letters indicate statistical differences between groups of fish of the same species collected at different sites; asterisks indicate statistical differences between fish species from the same site (*p < 0.05, **p < 0.01, ***p < 0.001).

The highest concentrations of Ni were found in gudgeons sampled at location 4, directly downstream of the outlet of heated wastewater, and location 7, the Cedron River in Zarzyce Małe (2.18 and 1.65 mg/kg, respectively), and in minnows from locations 11 – the Sieprawka River, 12 – the stream Piegżówka and 5 – the Cedron River in Kalwaria Zebrzydowska (2.35, 1.94 and 1.84

mg/kg, respectively) (Table 3). The statistical analysis showed a significant positive relationship between the Ni concentration in gudgeons and their body weight, body length, and age ($r = 0.46$, $r = 0.49$ and $r = 0.50$; $p < 0.0001$, respectively) and between the Ni concentration in minnows and their body weight and age ($r = 0.24$ and $r = 0.21$; $p < 0.05$, respectively) (Table 6).

The highest Mn concentrations were found in gudgeons from location 7 – the Cedron River in Zarzyce Małe – and 8 – the Głogoczówka River in Krzyszkowice (7.14 and 7.09 mg/kg, respectively) and in minnows from locations 10 and 12 – the streams Jastrząbka and Piegżówka (26.41 and 9.37 mg/kg, respectively) (Table 7). The Mn concentrations in gudgeons were significantly negatively correlated with the Mn concentrations in the bottom sediments, whereas statistically significant positive correlations were demonstrated in the case of minnows ($r = -0.30$ $p < 0.05$ and $r = 0.43$ $p < 0.0001$, respectively) (Table 5). The Mn concentrations in minnows were also significantly associated with the body weight, body length, and age of the fish ($r = 0.24$, $r = 0.34$ and $r = 0.30$, respectively) (Table 6).

Table 4.
Comparison of average concentrations of heavy metals [mg/kg d.w.] (±SE) in bottom sediment samples from locations 1–9. For each site n = 4. Each sediment sample was analysed in triplicate.

Metal	1	2	3	4	6	7	8	9
Cd	0,14[a] ±	0,05[a]	0,05[a]	2,40[b]	0,11[a]	0,08[a]	0,05[a]	0,13[a]
	0,03	±0,02	±0,02	±1,41	±0,02	±0,03	±0,03	±0,01
Pb	8,76[a]	11,79[ab]	11,77[ab]	23,10[b]	10,72[ab]	2,73[a]	4,76[a]	5,99[a]
	±1,26	±4,81	±5,73	±7,94	±3,82	±1,44	±0,96	±1,42
Ni	12,49[a]	6,65[ab]	4,44[b]	9,08[ab]	11,71[ab]	5,00[ab]	3,93[b]	8,11[ab]
	±0,68	±1,69	±1,21	±1,61	±3,38	±3,34	±1,22	±2,24
Mn	215,2[a]	153,4[ab]	135,7[b]	331,0[ab]	172,7[abc]	61,2[abc]	46,57[c]	73,57[abc]
	±7,76	±28,52	±7,43	±85,98	±50,91	±24,25	±19,23	±19,88
Zn	44,8[ab]	28,31[ab]	18,47[a]	164,9[b]	37,19[ab]	20,11[ab]	21,62[ab]	37,14[ab]
	±2,00	±8,15	±4,84	±71,53	±10,36	±10,08	±8,30	±9,09
Cu	12,63[a]	4,81[ab]	3,37[b]	16,65[a]	9,61[a]	3,84[ab]	2,43[b]	4,92[ab]
	±0,48	±1,16	±0,71	±6,72	±3,19	±2,70	±1,03	±1,48

Different letters indicate statistically significant differences between sampling locations.

Table 5.
Comparison of correlation coefficients between heavy metal concentrations in fish and in bottom sediments collected at the same locations.

Fish species	Pb	Cd	Ni	Mn	Zn	Cu
Gobio gobio	0,248*	ns	0,278*	-0,297*	ns	0,257*
Phoxinus phoxinus	ns	0,285*	0,276*	-0,430***	ns	ns

Asterisks indicate the significance level of the correlation (*p < 0.05, **p < 0.01, ***p < 0.001), ns – not significant.

Table 6.
Comparison of correlation coefficients between heavy metal concentrations in fish and their body weight, body length, and age.

Parameter	Pb	Cd	Ni	Mn	Zn	Cu
			Gobio gobio			
Weight	0,211*	ns	0,458***	ns	0,264*	ns
Length	0,210*	ns	0,487***	ns	0,338**	ns
Age	ns	ns	0,497***	ns	0,315**	0,262*
			Phoxinus phoxinus			
Weight	ns	ns	0,241*	0,243*	0,199*	ns
Length	ns	ns	ns	0,335***	0,305**	ns
Age	ns	ns	0,305**	0,303**	ns	ns

Asterisks indicate the significance level of the correlation (*p < 0.05, **p < 0.01, ***p < 0.001), ns – not significant.

The highest concentrations of Zn were present in gudgeons from location 4, directly downstream of the outlet of heated wastewater, location 7, the Cedron River in Zarzyce Małe, and location 2, the Skawinka River at the mouth of the Głogoczówka River (24.47, 23.30 and 21.44 mg/kg, respectively), and in minnows from locations 12 – the stream Piegżówka, 2 – the mouth of the Głogoczówka River, and 10 – the stream Jastrząbka (77.22, 56.65 and 45.92 mg/kg, respectively) (Table 7). No significant correlations were found between these concentrations and the Zn concentrations in the bottom sediments at the same sites. However, significant relationships were found for the Zn concentrations in gudgeons and their body weight, body length, and age (r = 0.26, r = 0.34 and r = 0.32, respectively), as well as between the Zn concentration in minnows and their body weight and body length (r = 0.20 and r = 0.30, respectively) (Table 6).

Table 7.
Comparison of average Mn, Zn and Cu concentrations [mg/kg w.w.] (±SE) in *G. gobio* and *P. phoxinus* collected at the sampling locations in the Skawinka River basin. At each site n = 10 for each fish species, if present. Fish samples were analysed in duplicate. An empty cell means there were no fish of this species at the location.

Site	Mn		Zn		Cu	
	Gobio gobio	***Phoxinus phoxinus***	***Gobio gobio***	***Phoxinus phoxinus***	***Gobio gobio***	***Phoxinus phoxinus***
1	4,35 ±0,28	2,62 ± 0,22^{x**}	17,67 ±0,89^{a}	44,79 ±5,24^{x***}	0,93 ±0,08^{ab}	2,17 ±0,66**
2	5,97 ±0,53	7,20 ± 1,32^{x}	21,44 ±1,20^{ab}	56,65 ±10,4^{x***}	0,77 ±0,05^{abc}	1,87 ±0,44***
3	5,77 ±0,38	5,76 ± 0,53^{x}	16,75 ±0,77^{a}	39,89 ±4,20^{x***}	0,73 ±0,14^{bc}	1,87 ±0,70**
4	5,29 ± 0,54	-	24,47 ±1,57^{b}	-	0,61 ±0,05^{bc}	-
5	4,00 ± 0,22	2,03 ± 0,13^{x***}	18,51 ±0,72^{a}	37,96 ±2,51^{x***}	1,11 ±0,14^{a}	1,03 ±0,04
6	-	4,85 ± 1,06^{x}	-	40,25 ±3,05^{x}	-	1,23 ±0,15
7	7,14 ± 0,66	5,60 ± 0,83^{x}	23,30 ±1,01^{b}	35,96 ±1,84^{x***}	0,57 ±0,03^{c}	0,93 ±0,05***
8	7,09 ± 1,82	-	17,98 ±0,65^{a}	-	0,55 ±0,03^{c}	-
9	6,24 ± 0,68	5,16 ± 0,79^{x}	19,83 ±1,00^{ab}	34,20 ±1,10^{x***}	0,58 ±0,04^{bc}	1,07 ±0,15**
10	-	26,41 ± 7,03^{y}	-	45,92 ±6,87^{x}	-	1,31 ±0,14
11	4,46 ± 0,48	3,38 ± 0,29^{x*}	19,80 ±1,36^{ab}	31,25 ±1,59^{x***}	0,48 ±0,02^{c}	0,94 ±0,09 ***
12	-	9,37 ± 2,01^{x}	-	77,22 ±12,11^{y}	-	1,32 ±0,29

Different letters indicate statistical differences between groups of fish of the same species collected at different sites; asterisks indicate statistical differences between fish species from the same site (*p < 0.05, **p < 0.01, ***p < 0.001).

The concentration of Cu was highest in gudgeons from location 5, the Cedron River in Kalwaria Zebrzydowska, and location 1, the Skawinka River in Biertowice (1.11 and 0.93 mg/kg, respectively). In the case of minnows, Cu concentrations were highest in fish from location 1, the

Skawinka River in Biertowice, location 2, the mouth of the Głogoczówka River, and location 3, Radziszów (2.17, 1.87 and 1.87 mg/kg, respectively) (Table 7). There was a significant relationship between the concentration of Cu in gudgeons and its concentration in the bottom sediments (r = 0.26) (Table 5). In the same species, a positive correlation was confirmed between the Cu concentration in the fish and the age of the fish (r = 0.26) (Table 6).

DISCUSSION

We measured the concentrations of heavy metals in the bottom sediments at selected locations. The highest concentrations of Pb, Cd and Zn were found at location 4, the outlet of wastewater from the Skawina power plant into the Skawinka River. The highest concentrations of Cu were found at locations 1 and 4, i.e. in Biertowice and at the outlet of heated wastewater from the Skawina power plant. The differences between locations in the concentrations of Ni and Mn were smaller. However, their concentrations at location 4 were higher than those at other locations. The concentrations of the heavy metals analysed were lowest in the bottom sediments at locations 8 and 3, i.e. on the Głogoczówka River in Krzyszkowice and on the Skawinka River in Radziszów. According to the ecotoxicological criterion currently used in Poland to assess the quality of bottom sediments under the State Environmental Monitoring system (MacDonald et al., 2000), the sediment samples analysed can be classified, based on their concentrations of heavy metals, as level I (Pb, Ni, Cu, Mn) or level II (Cd and Zn) sediments, which means that they are not polluted and do not have a negative impact on living organisms. Similar concentrations of heavy metals in bottom sediments have been reported by other authors for other rivers in Poland. For example, in the Ełk River, the reported concentrations of heavy metals ranged from 3.82 to 15.99 mg/kg for Pb, from 0.21 to 0.75 mg/kg for Cd, and from 2.58 to 8.50 mg/kg for Ni (Skorbiłowicz et al., 2018 a), while in the Biała River in Białystok, the reported concentrations of Pb ranged from 11.2 to 16.7 mg/kg, while the concentrations of Zn ranged from 29.97 to 88.54 mg/kg (Skorbiłowicz et al., 2018 b). For the Krzna River, the reported concentrations of heavy metals were as follows: 9.7–16.3 mg/kg for Pb, 0.4–0.8 mg/kg for Cd, 4.6–6.1 mg/kg for Ni, 16.2–23.5 mg/kg for Zn, and 1.8–6.9 mg/kg for Cu (Kluska and Jabłońska, 2024). That study also showed that the concentrations of heavy metals vary over the year and are slightly higher in August than in May. The concentration of heavy metals in bottom sediments also depends on the sediment grain size (Zhao et al. 1999) and is one of the indicators of environmental pollution. The concentrations of the heavy metals analysed in the bottom sediments of the Skawinka River and its tributaries are within the normal range and do not indicate the presence of dangerous sources of contamination. The highest concentrations of the elements were found at the location where wastewater from the Skawina power plant enters the Skawinka River, which indicates that industry may have the greatest impact on heavy metal pollution of the river. Since our analysis showed differences in the concentrations of the heavy metals analysed between locations, we examined whether their concentrations in fish were correlated with their concentrations in bottom sediments. Heavy metals are known to settle to the bottom sediments, where their concentrations are highest, and are then taken up by bottom invertebrates and aquatic plants. They can also be released into the water during erosion of the bed, leading to their accumulation in aquatic animals, including fish. Our analyses showed that the concentrations of Pb, Ni, Mn and Cu in gudgeons and the concentrations of Cd, Ni and Mn in minnows were associated with the concentrations of these metals in the bottom sediments at the same locations. This demonstrates that these species are useful and effective bioindicators of river pollution with metals. Similar correlations have been reported by other authors. In roach, Bochenek et al. (2008) found that the Cd concentration in the muscle tissue and liver, the Zn concentration in the kidneys, muscle tissue, gills and liver, and the Cu concentration in the muscle tissue and liver were positively correlated with the total concentration of these metals in the bottom sediments. The same authors also found a significant negative correlation between the concentration of Pb in the muscle tissue of roach and its concentration in the bottom sediments. Positive relationships between the concentrations of Pb, Cd and Cu in fish and their concentrations in bottom sediments have been reported by Miao et al. (2021), who analysed fish and bottom sediments sampled from rivers in the Liuzhou region in southern China. According to the authors, these positive correlations indicate that these metals present in bottom sediments are bioavailable for uptake by fish. In contrast, negative correlation coefficients show that the metals present in bottom sediments are poorly bioavailable for uptake by fish. The present study found a negative correlation between the concentration of Mn in fish and its concentration in bottom sediments, which may indicate that Mn is less bioavailable for uptake by fish, despite its high concentration in bottom sediments.

Our analysis of heavy metal concentrations in fish tissue showed that they were higher in minnows than in gudgeons living in the same parts of the river. This may be due to differences in feeding behaviour between the two species. Gudgeons depend on near-bottom habitats and prefer to feed on bottom invertebrates, with plankton and plants forming a small part of their diet (Kennedy and Fitzmaurice, 1972; Oscoz et al., 2003). Oscoz et al. (2006) compared the diets of *P. phoxinus* and *Gobio lozanoi* from the Larraun River and found a significant diet overlap between the two species. Both these species showed a preference for Chironomidae larvae, Trichoptera larvae, and terrestrial invertebrates. However, trophic diversity was significantly higher for *P. phoxinus*. The differences in heavy metal concentrations between the two species may also be due to differences in the size of the fish, as concentrations of heavy metals are higher in smaller fish. According to some authors, this is because tissue metal concentrations in fish become diluted as a result of their growth (Canli and Atli, 2003). This is due to metabolic rates, which in larger fish are associated with the consumption of more food (Merciai et al., 2014). However, the present study found positive relationships with fish size only for the concentrations of Pb, Zn and Ni in gudgeons and the concentrations of Ni, Mn and Zn in minnows. Positive correlations were also shown between Ni, Zn and Cu concentrations in gudgeons and the age of the fish and between Ni and Mn concentrations in minnows and the age of the fish. These correlations indicate that the older the fish from the basin of the Skawinka River and the greater their body size, the higher the concentration of metals in their tissues. Dobicki and Polechoński (2003) investigated the relationship between heavy metal concentrations in fish and the size of the fish and found that the relationship was species-specific. Positive correlations were shown between the Cd concentration in zander and bream and the age and length of the fish; the Pb concentration in bream and the age, length and weight of the fish; and the Ni concentration in roach and the weight of the fish. All correlations for perch were negative. This may indicate that metals in the living environment of the fish are bioavailable and accumulate faster than they are eliminated. The two species analysed in the present study probably take up heavy metals from bottom sediments through the bottom invertebrates they feed on. The similarity of the diets of minnows and gudgeons suggests that the higher concentrations of heavy meals in *P. phoxinus* are due to differences in metabolic rates between the two species. Minnows live in shoals (groups), which, according to some authors, results in lower metabolic rates (Plath et al., 2013). They are smaller than gudgeons and thus grow more slowly. The fact that the elimination rate of metals taken up with food is lower in minnows than in gudgeons can likely be explained by the lower metabolic rates of minnows. Minnows are also more sensitive to environmental conditions. Due to their water temperature requirements, they do not live in the Skawinka River downstream of the outlet of heated wastewater from the Skawina power plant, where the water temperature is higher. In contrast, gudgeons tolerate such conditions well.

The differences in heavy metal concentrations between fish sampled at different locations may indicate that the river water at these locations is contaminated with forms of metals that are bioavailable for uptake by living organisms. Our results indicate that fish living in the location where wastewater from the Skawina power plant enters the Skawinka River are at the highest risk of accumulating harmful heavy metals. This is also confirmed by the fact that the highest concentrations of heavy metals in bottom sediments were found at this location. Due to the high water temperature, the heavy metals here are more bioavailable, and the fish that live there have a faster metabolism. There are no minnows at this site due to the high water temperature. The gudgeons sampled at this location had higher concentrations of Cd, Ni and Zn than gudgeons sampled at other sites. Higher concentrations of heavy metals were also found in the Głogoczówka River upstream of the river mouth (Pb in gudgeons and minnows) and in the Skawinka River at the mouth of the Głogoczówka River, which confirms that this tributary is a source of pollution of the Skawinka River with Pb and Zn. This may be due to line-source pollution on the intensively used motorway to the popular city of Zakopane, which crosses the Głogoczówka River and may be a source of contamination. The present study showed increased concentrations of metals in tributaries of the Skawinka River – the Jastrząbka, Cedron and Piegżówka, which drain from areas where towns famous for furniture production are located. The furniture industry requires the use of various chemical agents, varnishes and finishing products, which may contain metals or other substances (not analysed in the present study) that may have a negative impact on the environment. Our results confirm that gudgeon and minnow can be used as bioindicator species of environmental contamination with heavy metals. While the concentrations of the heavy metals analysed in the bottom sediments of the Skawinka River did not exceed established limits, their levels in fish reflected differences in their concentrations in the environment. This confirms that the fish species used in the study are sensitive to low environmental concentrations of these metals. Due to the varied environmental conditions along rivers such as the Skawinka, not all fish species are present along their entire length. Therefore, it is advisable to select two or three robust species that best reflect environmental conditions and are not threatened with extinction, as these would serve well as bioindicators of water quality.

CONCLUSIONS

Minnows accumulate higher concentrations of heavy metals than gudgeons. The concentrations of the metals analysed in gudgeons and minnows were positively correlated with their concentrations in the bottom sediments. The study also showed positive relationships between heavy metal concentrations in fish tissue and the body weight, body length, and age of fish, which confirms that the accumulation of these metals in fish tissue increases over time. The highest concentrations of heavy metals in the bottom sediments were found at the outlet of heated wastewater from the Skawina power plant, whereas the highest concentrations of the metals in fish tissue were found in fish from that same location as well as in fish from the Cedron River in Zarzyce and the stream Piegżówka.

The results confirm that gudgeon and Eurasian minnow are useful bioindicator species for monitoring heavy metal concentrations in the environment.

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