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RESEARCH ARTICLE

Heavy metal concentrations in fishes from Zakrzówek Reservoir and the Vistula River near Kraków: Human risk assessment

Ewa Łuszczek-Trojnar, Ewa Drąg-Kozak, Paweł Szczerbik, Artur Klaczak, Anna Lelonek, Kinga Duda, Patrycja Adamska

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Abstract. The aim of this study was to compare tissue concentrations of heavy metals (copper (Cu), manganese (Mn), iron (Fe), nickel (Ni), zinc (Zn)) in roach (*Rutilus rutilus* (L.)), chub (*Squalius cephalus* (L.)), and bleak (*Alburnus alburnus* (L.)) from two locations on the Vistula River upstream from Kraków and Zakrzówek Reservoir, a former limestone quarry flooded over 30 years ago that is located close to the center of Kraków. The findings showed that heavy metal concentrations in fishes from the Vistula River upstream from Kraków were higher compared to those in fishes from Zakrzówek Reservoir. The highest heavy metal concentrations were found in hard tissues, such as bone and scales, and the lowest was in muscle tissue. Metal pollution index (MPI), estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) analyses showed no significant non-carcinogenic risk to human health from the consumption of the muscle meat of the fishes analyzed. Cancer risk (CR) values, calculated based on maximum Ni concentrations in fish muscle tissue, did not exceed the upper limit of the acceptable cancer risk range. The findings show that the assessment of heavy metal contamination of fishes should be continued and extended to include other water bodies used for fishing and other xenobiotics that accumulate in fishes and may pose risks to human health.

Keywords: bleak, chub, flooded limestone quarry, risk to human health, roach

E. Łuszczek-Trojnar [✉], E. Drąg-Kozak, A. Klaczak, A. Lelonek, K. Duda, P. Adamska
Department of Animal Nutrition and Biotechnology, and Fisheries; University of Agriculture in Kraków, Aleja Adama Mickiewicza 24/28. 30-059 Kraków, Poland
E-mail: ewa.trojnar@urk.edu.pl

Paweł Szczerbik
Center of Experimental and Innovative Medicine; University of Agriculture in Kraków, Aleja Adama Mickiewicza 24/28. 30-059 Kraków, Poland.

Introduction

Environmental problems are currently becoming increasingly global. The need to protect the climate, the air, and open waters is widely acknowledged. To protect human health, the state of the environment should be monitored continuously to mitigate pollution in a timely manner and prevent negative impacts. Heavy metals are major pollutants due to their prevalence, wide use, and non-biodegradability. The concentrations of heavy metals released into the environment from anthropogenic sources can significantly exceed the geochemical background, posing a threat to living organisms (Ghannam 2021). Open waters, which are the lowest points in landscapes, are usually the final receiving water bodies for all types of wastewater and rainwater. Thus, it is not surprising that they may be more polluted than the air. Heavy metals quickly settle into bottom sediments of water bodies and can accumulate there following long-term exposure to human pressure (Szarek-Gwiazda et al. 2006). Since heavy metals bioaccumulate easily, they can be transferred from the water or bottom sediments to plants and aquatic invertebrates and then to organisms at higher trophic levels, such as fishes that feed on plants or invertebrates (Has-Schön 2008). Fish can also absorb heavy metals directly from water through the skin and gills (Garai et al. 2021). Heavy metals can negatively affect fish and other vertebrates, including people (Hossain et al. 2022). The consumption of fish contaminated with heavy metals can result in the accumulation of metals in consumers' bodies (Varol and Sünbül 2020). Many heavy metals, for example, lead (Pb), cadmium (Cd), and nickel (Ni), are known to be carcinogenic, mutagenic, and toxic. Some of them, including Pb, Cd, and zinc (Zn), easily cross the blood-brain barrier and can disrupt numerous physiological functions, contributing to the development of neurological disorders such as Alzheimer's and Parkinson's diseases, impair metabolism, and maturation and reproductive processes, while other metals, for example, copper (Cu) or Cd, affect the functioning of organs such as the lungs, liver, and kidneys (Türkmen et al. 2009, Saha et al. 2016, Varol et al. 2017).

Research comparing heavy metal concentrations in bottom sediments and fishes from different rivers and water bodies in Poland has shown that the Vistula River in Kraków is more contaminated than it is in Warsaw, and it is also more contaminated than the Oder River in Wroclaw, the Warta River estuary, Rożnowski and Rybnik reservoirs, Lake Łańskie, and Lake Lipczyno Wielkie (Szkoda et al. 2014). Due to its geographical location, the Vistula in Kraków is polluted by wastewater originating from Silesia, which is the most highly industrialized region of Poland. Therefore, the Vistula in Małopolska Province is exposed to the highest level of pollution. Due to self-purification processes, the concentrations of heavy metals (Cd, Pb, Ni, Cu, Zn) in the bottom sediments and organisms of the Vistula outside Małopolska Province are much lower (GIOS 2022), which is why the river in Małopolska Province should be monitored particularly closely (Helios-Rybicka 1993). One study analyzing the contamination of small Vistula tributaries running through the urbanized areas of Kraków reports that these could be a major source of pollution in the Vistula, the largest river in Poland. Furthermore, heavy metals accumulated in bottom sediments may be released thus contaminating the Vistula (Aleksander-Kwaterczak and Plenzler 2019).

There are no natural lakes in Kraków. Therefore, flooded former quarries and gravel extraction sites are often converted into recreational facilities and rest areas for residents and for fishing. One such reservoir is Zakrzówek Reservoir, which is supplied with groundwater, subcutaneous water from the Vistula, and surface infall (Motyka and Postawa 2004). The reservoir is 30–38 m deep with high water transparency (up to 17 m) and is located near the Vistula River (about 550 m) and residential areas (Galas 2003). The reservoir is popular for bathing, diving, and fishing. Its fish populations include the cyprinids roach (*Rutilus rutilus*), bleak (*Alburnus alburnus*), and chub (*Squalius cephalus*), and the predators perch (*Perca fluviatilis* L.), pike (*Esox Lucius* L.), pikeperch (*Sander lucioperca* (L.)), and European catfish (*Silurus glanis* L.) (Mikołajczyk et al. 2019). The history of the reservoir, which is a flooded former limestone quarry, and its location in an urban area suggest that it is directly exposed to pollutants that may enter it with surface runoff. Thus, the aim of the study was to compare the concentrations of heavy metals in fishes from this reservoir with those in fishes from the Vistula River near Kraków. An additional goal was to assess the non-carcinogenic and cancerogenic risk to human health resulting from the consumption of muscle meat from the fishes tested.

Materials and Methods

Study area

The fishes for the study were sampled at three locations. The first two were in the main current of the

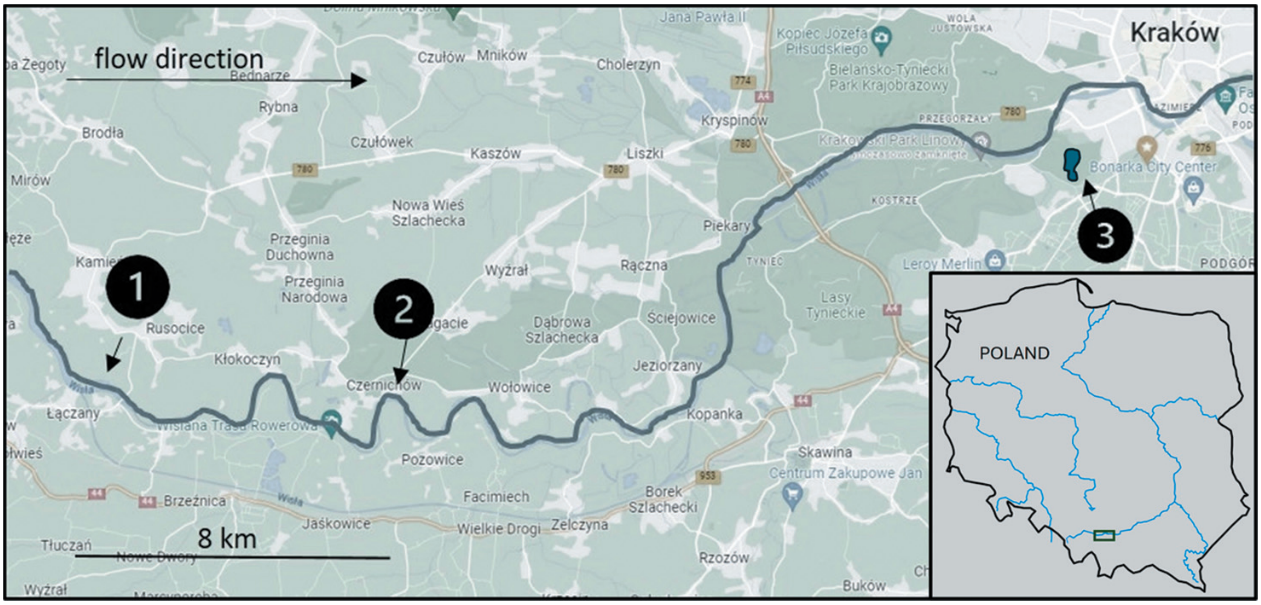

Figure 1. Map presenting the location of sampling stations, location 1 – the Vistula River in Łączany near the inlet of the Łączański channel; location 2 – the Vistula River near the village of Czernichów, location 3 – Zakrzówek Reservoir (source: Map Google©2023, and Michał Smoczyk (Michau Wikimedia Commons Sm), CC BY-SA 3.0).

Vistula River upstream from Kraków – in Łączany (site 1) and Czernichów (site 2), located approximately 8 km from each other. The third location was Zakrzówek Reservoir, a flooded limestone quarry, in Kraków. The straight line distance between locations 1 and 3 is 24 km (Fig. 1).

Fish handling

Fish sampling was done in 2019. Fishes were sampled with electrofishing using an attested electrofishing set (Hasn Grassl IG-600T) from a boat at locations 1 and 2 on the Vistula River. The Department of Animal Nutrition and Biotechnology, and Fisheries, University of Agriculture in Kraków obtained permission to catch the species mentioned above from the Marshal of Małopolska (RO-II.7143.1.6.2019.WB) and the Polish Angling Association in Kraków. At location 3 in Zakrzówek Reservoir, fishes were sampled during an ichthyofauna survey using a standard survey net set (multi-mesh NORDIC net comprising 12 panels with different mesh sizes ranging from 5mm to 55mm knot-to-knot in a standard randomized order) consisting of a multi-mesh pelagic (floating) gill net measuring 50 m in length and 5 m in height and a multi-mesh bottom gill net measuring 50 m in length and 2 m in height. The nets were placed approximately 20–30 m from the reservoir shoreline to cover the slope, i.e. the area where fishes usually congregate and feed in the reservoir. It was also the only place in the reservoir where residual littoral areas (with hydrophytes) could have occured.

Ten roach (*Rutilus rutilus*), 10 chub (*Squalius cephalus*), and 10 bleak (*Alburnus alburnus*) were sampled at locations 1 and 2 and 5 roach and 10 bleak at location 3. The fishes were euthanized using MS-222 (Sigma) at a dose of 500 mg L^{-1} and transported under refrigeration in bags to a laboratory in the Experimental Fisheries Station, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków. There, the fishes were weighed, total length (TL) was measured, and scale samples were collected for age analysis. The age of the fishes was determined using a stereoscopic microscope (Motic, Poland) by counting the number of annuli on the scales. The results of body weight and length measurements and age analysis are presented in Table 1.

Gill (with arch), bone (operculum), scale, and muscle tissue samples (about 5 g of each tissue) were

Table 1
Comparison of body weight, length, and age of fishes collected for analysis. Mean values and ranges in parentheses

Location	Body Wright (g)	Total length (cm)	Age (years)
roach			
1 – Łączany	46.8 (26.5-88.3)	15.2 (14.5-18.2)	2-3
2 – Czernichów	55.6 (28.7-111.3)	15.4 (12.0-18.5)	2-4
3 – Zakrzówek	81.0 (20.3-128.1)	19.7 (13-24)	3-5
bleak			
1 – Łączany	14.2 (10.7-22.1)	12.6 (11.5-14.0)	2
2 – Czernichów	15.9 (11.7-21.3)	13.2 (11.5-15.0)	2-3
3 – Zakrzówek	25.5 (10.4-36.4)	15.9 (14.5-17.5)	2-3
chub			
1 – Łączany	25.6 (14.6-53.1)	13.1 (10.7-17.2)	2
2 – Czernichów	24.2 (11.2-39.4)	12.7 (10.5-15.0)	2

collected from each individual for heavy metal concentration analysis. The samples were packed in bags, labelled and frozen at -20°C until analysis.

Analysis of heavy metal concentrations

After thawing, the tissue samples were mineralized with a mixture of nitric acid (65%) (Chempur, Polska) and perchloric acid (70%) (Chempur Polska) (3:1) (v/v) 10 ml per sample at a temperature of up to 180°C in a Velp 26/20 digestion system. Blank samples were prepared by digesting the 10 ml sample of deionized water in the same acid mixture. After digestion, clear liquid was diluted with deionized water into 10 ml. Cu, manganese (Mn), iron (Fe), Ni, and Zn concentrations were measured with atomic absorption spectrometry (AA Unicam 929) using standard solutions prepared based on mass concentration standards for heavy metals (Central Office of Measures – Warsaw). Blank sample analysis and calibration were repeated every 20 samples. The methods used were validated by measuring the elements concerned in reference material: DORM-4 (National Research Council Canada) with certified values of Cu, Fe, Zn, and Ni (Cu certified – 15.9 ± 0.9 mg kg^{-1}, measured – 16.15 ± 0.13 mg kg^{-1} n = 4; Fe certified – 341 ± 27 mg kg^{-1}, measured – 345 ± 11 mg kg^{-1} n = 4; Zn certified – 52.2 ± 3.2 mg kg^{-1}, measured – 51.15 ± 0.7 mg kg^{-1} n = 4; Ni certified – 1.36 ± 0.22 mg kg^{-1}, measured – 1.40 ± 0.05 mg kg^{-1} n = 4). The recovery rates of these elements were as follows: 101% for Cu, 101% for Fe, 98% for Zn, and 103% for Ni. The concentrations of Cu, Fe, Mn, Ni, and Zn in the gills, muscle tissue, bone, and scales of the fishes analyzed are expressed as mg kg^{-1} wet weight (w.w.).

Metal Pollution Index (MPI)

The MPI is a mathematical model that presents all heavy metal concentrations as a single value. It is an accurate, reliable index used to monitor the levels of heavy metal contamination in food and aquatic ecosystems. In the present study, MPI was calculated as the geometric mean of the concentrations of the metals analyzed in fish tissue as follows (Usero et al. 1997):

$$MPI = (C_1 \times C_2 \times .... \times C_n)^{1/n}$$

where: C – mean concentration of a given metal n (mg kg^{-1} wet weight) in tissue.

Estimated Daily Intake (EDI)

Fish consumption may pose potential health risks. Thus, the daily intake (EDI = mg kg^{-1}

body-weight/day) of the heavy metals analyzed (Cu, Fe, Mn, Ni, Zn) was calculated using the following equation as reported by Griboff et al. (2017):

$$EDI = (C_{element} \times D_{food\ intake}) / BW$$

where: $C_{element}$ is the concentration of a given heavy metal in the muscle tissue of fish (expressed as mg kg^{-1} wet weight), $D_{food\ intake}$ is the mean daily intake of food (fish) (g/person/day) – 13.3 kg per person per year in Poland (Statistical Yearbook of Agriculture 2022), and BW is the mean body weight (70 kg for adults).

Target Hazard Quotient (THQ)

THQ is an estimate of the level of non-carcinogenic risk from heavy metal exposure. It was calculated using the following equation:

$$THQ = (E_{fr}\ ED_{tot} \times FIR \times C)/(RfD_o \times BW \times AT) \times 10^{-3}$$

where: E_{fr} is the frequency of exposure (365 days a year), ED_{tot} is the duration of exposure (70 years), FIR is the fish ingestion rate (36 g per day), C is the mean concentration of a given heavy metal in fish muscle tissue (mg kg^{-1}), RfD_o is the reference oral dose (mg kg^{-1} day^{-1}), BW is the mean body weight (70 kg for adults), and AT is the mean period of exposure to non-carcinogens (365 days per year).

The threshold value for THQ is 1 (USEPA 2011), and values >1 indicate a potential non-carcinogenic risk to exposed consumers. The assessment of the risk to public health is based on the assumption that most chemicals with non-cancer effects exhibit a threshold response (Kawser Ahmed et al. 2016). THQ has recently been accepted as an important risk assessment indicator by many researchers as a valid, useful method and is used widely (Mahmoud and Abdel-Mohsein 2015, Varol et al. 2019, Töre et al. 2021).

Hazard index (HI)

HI is the total THQ for all metals analyzed (Li et al. 2013), which was calculated with the following equation:

$$HI = \Sigma^{n}_{i=1} THQ_i$$

where: HI < 1 means no hazard; it indicates that there is a health benefit from the consumption of fish and that there is no risk to consumers; HI > 1 indicates a hazard, meaning that adverse health effects in consumers are possible.

Target Cancer Risk (CR)

CR was used to indicate the carcinogenic risk of the metals analyzed. CR values for Ni were calculated using the following equation (USEPA 2000):

$$CR = (E_{fr} \times ED_{tot} \times FIR \times C \times CSF_o)/(BW \times AT) \times 10^{-3}$$

where: AT is the mean period of exposure to carcinogens (365 days a year); CSF_o is the carcinogenic slope factor for the oral route of exposure as indicated by the Integrated Risk Information System (for Ni: 1.7 mg^{-1} kg^{-1} day) (USEPA 2015).

Statistical analysis

ANOVA (one-way analysis of variance) was used to determine if there were statistically significant differences among the heavy metal concentrations in tissue samples from fishes collected at different locations ($P < 0.05$). The relationships between the concentration of heavy metals in the tissues of fish sampled and their age, length and body weight were analyzed using Pearson’s or Spearman’s correlation coefficients (depending on normal distribution analyzed with the Shapiro-Wilk’s test). Statistical analysis was performed with Statistica 12.

Results

Cu concentrations in the fishes analyzed varied among tissue types (Tables 2–5). The highest Cu concentrations were found in the gills of fishes sampled at location 2 (the Vistula in Czernichów). The relationships between the tissue concentrations of the metals in the fishes sampled and body length, body

Table 2
Comparison of average concentrations of Cu, Mn, Fe, Ni, and Zn (mg kg^{-1} w.w. ± SE) in the gills of the fish species studied from three sampling locations near Kraków. No chub was collected at location 3

		Location		
Metal	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
Cu	roach	0.80 ± 0.32^{a}	5.05 ± 1.46^{Ab}	1.31 ± 0.07^{Aa}
	bleak	0.34 ± 0.08^{a}	14.99 ± 3.53^{Bb}	1.96 ± 0.14^{Bc}
	chub	0.44 ± 0.08^{a}	5.43 ± 1.07^{Ab}	-
Mn	roach	8.27 ± 1.11^{Aa}	14.88 ± 1.98^{Ab}	2.66 ± 0.11^{Ac}
	bleak	4.82 ± 0.89^{Ba}	16.58 ± 1.61^{Ab}	5.17 ± 0.21^{Ba}
	chub	7.53 ± 1.02^{ABa}	5.02 ± 0.52^{Bb}	-
Fe	roach	51.98 ± 4.34^{Aa}	50.72 ± 4.85^{Aa}	38.24 ± 1.09^{Ab}
	bleak	103.90 ± 7.48^{Ba}	77.36 ± 14.50^{Bab}	70.47 ± 2.26^{Bb}
	chub	61.93 ± 9.40^{A}	44.81 ± 10.36^{A}	-
Ni	roach	0.90 ± 0.15^{a}	2.55 ± 0.44^{Ab}	3.18 ± 0.19^{Ab}
	bleak	0.93 ± 0.34^{a}	25.09 ± 9.79^{Bb}	6.81 ± 0.18^{Bc}
	chub	0.81 ± 0.22^{a}	8.45 ± 1.97^{Cb}	-
Zn	roach	110.00 ± 9.21^{A}	84.93 ± 8.23^{A}	98.50 ± 1.81^{A}
	bleak	44.80 ± 4.75^{B}	56.34 ± 4.11^{B}	47.00 ± 1.36^{B}
	chub	81.84 ± 4.64^{C}	84.91 ± 6.17^{A}	-

Letters ^{ABC} denote statistically significant ($P < 0.05$) differences in means among fish species, letters ^{abc} denote differences among locations

Table 3
Comparison of average concentrations of Cu, Mn, Fe, Ni, and Zn (mg kg^{-1} w.w. ± SE) in the muscle tissue of the fish species studied from three research locations near Kraków. No chub was collected at location 3 – Zakrzówek Reservoir

		Location		
Metal	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
Cu	roach	0.65 ± 0.04^{Aa}	1.23 ± 0.33^{a}	0.34 ± 0.02^{Ab}
	bleak	1.83 ± 0.22^{Ba}	0.77 ± 0.10^{b}	0.39 ± 0.02^{Bc}
	chub	1.24 ± 0.11^{Ca}	0.60 ± 0.09^{b}	-
Mn	roach	0.42 ± 0.07^{Aa}	0.65 ± 0.14^{Aa}	0.10 ± 0.00^{b}
	bleak	0.09 ± 0.02^{B}	0.10 ± 0.05^{B}	0.10 ± 0.00
	chub	0.29 ± 0.06^{Aa}	0.13 ± 0.02^{Bb}	-
Fe	roach	13.66 ± 1.61^{A}	14.41 ± 3.25	11.99 ± 0.27
	bleak	8.81 ± 0.88^{Ba}	10.29 ± 1.76^{ab}	12.72 ± 0.36^{b}
	chub	16.41 ± 2.67^{Aa}	10.28 ± 2.81^{b}	-
Ni	roach	2.20 ± 0.31	1.80 ± 0.47^{A}	2.18 ± 0.16^{A}
	bleak	1.65 ± 0.26	1.06 ± 0.57^{B}	1.13 ± 0.05^{B}
	chub	1.90 ± 0.42	1.21 ± 0.42^{AB}	-
Zn	roach	8.61 ± 0.42^{A}	8.09 ± 1.00^{A}	8.07 ± 0.19^{A}
	bleak	13.80 ± 0.69^{Ba}	15.05 ± 1.09^{Bab}	15.99 ± 0.36^{Bb}
	chub	11.71 ± 0.75^{B}	10.28 ± 1.04^{A}	-

Letters ^{ABC} denote statistically significant ($P < 0.05$) differences in means among fish species, letters ^{abc} denote differences among locations

weight, and age are presented in Tables 6–9. Cu concentrations in bone and scales of roach, chub, and bleak from the Vistula were inversely associated with fish body weight, body length, and age. Only for roach collected at station 3 in Zakrzówek Reservoir were positive correlations noted between Cu concentrations in tissues and the body weight and length of this species (Tables 8–9). Among the fish species examined, the highest Cu concentrations in tissues were noted in bleak (Tables 2–5).

As with Cu levels, Mn concentrations in the gills of rch and bleak from the Vistula in Czernichów were

Table 4

Comparison of average concentrations of Cu, Mn, Fe, Ni, and Zn (mg kg^{-1} w.w. ± SE) in the bone tissue of the fish species studied from three sampling locations near Kraków. No chub was collected at location 3 – Zakrzówek Reservoir

		Location		
Metal	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
Cu	roach	11.01 ± 3.22	6.50 ± 1.43	5.30 ± 0.15^{A}
	bleak	18.38 ± 3.06^{a}	8.29 ± 0.10^{b}	9.36 ± 0.23^{Bb}
	chub	10.74 ± 3.53	9.56 ± 2.74	-
Mn	roach	10.88 ± 0.71^{Aa}	13.71 ± 1.63^{Aa}	5.73 ± 0.17^{Ab}
	bleak	30.80 ± 2.01^{Ba}	34.77 ± 1.39^{Ba}	16.30 ± 0.47^{Bb}
	chub	12.37 ± 1.18^{Aa}	6.59 ± 0.97^{Cb}	-
Fe	roach	157.8 ± 37.1^{A}	110.5 ± 28.8^{A}	139.1 ± 5.1^{A}
	bleak	430.1 ± 61.6^{Ba}	212.2 ± 28.1^{Bb}	257.6 ± 6.8^{Bb}
	chub	158.9 ± 52.2^{C}	106.5 ± 19.5^{A}	-
Ni	roach	30.33 ± 6.99^{A}	18.89 ± 5.67	28.14 ± 1.28^{A}
	bleak	70.82 ± 12.70^{Ba}	25.37 ± 4.78^{b}	38.28 ± 0.85^{Bc}
	chub	32.99 ± 9.41^{A}	17.21 ± 3.98	-
Zn	roach	130.3 ± 12.9^{Aa}	85.7 ± 12.1^{Ab}	103.40 ± 2.73^{ab}
	bleak	151.2 ± 16.1^{Aa}	115.2 ± 7.9^{Bab}	111.10 ± 0.79^{b}
	chub	95.3 ± 14.1^{B}	89.8 ± 10.2^{A}	-

Letters ^{ABC} denote statistically significant (P < 0.05) differences in means among fish species, letters ^{abc} denote differences among locations

Table 5

Comparison of average concentrations of Cu, Mn, Fe, Ni, and Zn (mg kg^{-1} w.w. ± SE) in the scales of the fish species studied from three sampling locations near Kraków. No chub was collected at location 3 – Zakrzówek Reservoir

		Location		
Metal	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
Cu	roach	0.77 ± 0.05^{Aa}	1.08 ± 0.21^{Aa}	0.44 ± 0.02^{Ab}
	bleak	8.55 ± 0.97^{Ba}	7.92 ± 0.59^{Ba}	2.31 ± 0.09^{Bb}
	chub	2.82 ± 0.46^{C}	3.51 ± 0.50^{C}	-
Mn	roach	8.22 ± 0.72^{Aa}	10.19 ± 1.15^{Aa}	2.46 ± 0.14^{Ab}
	bleak	14.50 ± 0.66^{Ba}	9.19 ± 0.65^{Ab}	6.28 ± 0.20^{Bc}
	chub	12.22 ± 0.54^{Ca}	7.09 ± 0.29^{Bb}	-
Fe	roach	10.92 ± 0.91^{Aa}	20.68 ± 4.35^{Ab}	8.72 ± 0.25^{Ac}
	bleak	136.20 ± 17.56^{Ba}	90.65 ± 19.80^{Bab}	53.20 ± 1.44^{Bb}
	chub	32.50 ± 6.46^{C}	42.02 ± 5.53^{C}	-
Ni	roach	1.70 ± 0.18^{A}	1.57 ± 0.40^{A}	1.78 ± 0.05^{A}
	bleak	34.66 ± 4.77^{Ba}	8.57 ± 0.86^{Bb}	6.87 ± 0.13^{Bb}
	chub	13.51 ± 2.76^{Ca}	4.46 ± 0.67^{Cb}	-
Zn	roach	55.56 ± 3.50	57.10 ± 4.71^{A}	53.47 ± 0.89^{A}
	bleak	55.29 ± 2.66^{a}	100.60 ± 3.83^{Bb}	89.57 ± 0.75^{Bc}
	chub	59.26 ± 3.80^{a}	100.40 ± 6.82^{Bb}	-

Letters ^{ABC} denote statistically significant (P < 0.05) differences in means among fish species, letters ^{abc} denote differences among locations

statistically significantly higher compared to fishes from other locations (Table 2). Negative correlations were identified between tissue concentrations of Mn and fish body weight, body length, and age, similarly to other metals. Positive relationships were only found for the scales of bleak from Zakrzówek Reservoir (Table 9).

Fe concentrations in the gills of roach from the Vistula at locations 1 and 2 and bleak from location 1 were significantly higher compared to fishes from

Table 6

Comparison of correlation coefficient values indicating a relationship between the concentration of the heavy metals studied in the gills of the fishes tested and their age, weight, and body length. No chub was collected at location 3 – Zakrzówek Reservoir. All bleak from location 1 – Łączany were the same age, as were all chub from locations 1 – Łączany and 2 – Czernichów

		Roach			Bleak			Chub	
Metal	Location	age	length	weight	age	length	weight	length	weight
Cu	1	-0.01	-0.56	-0.39	-	-0.09	-0.10	0.31	0.31
	2	-0.47	-0.55	0.52	0.04	-0.02	-0.10	-0.40	-0.66*
	3	-0.45	-0.24	-0.22	0.36	0.41	0.39	-	-
Mn	1	-0.03	-0.49	-0.39	-	-0.15	0.23	-0.09	-0.08
	2	-0.28	-0.25	-0.16	0.19	-0.46	-0.20	-0.69*	-0.71*
	3	0.36	0.07	0.12	0.37	0.13	0.09	-	-
Fe	1	0.20	-0.48	-0.36	-	-0.11	0.20	-0.71*	-0.67*
	2	-0.78*	-0.02	-0.19	-0.01	-0.49	-0.57	-0.03	-0.36
	3	-0.03	-0.08	0.02	-0.35	0.14	-0.24	-	-
Ni	1	0.32	0.03	-0.02	-	-0.09	-0.24	-0.63*	-0.60*
	2	-0.85*	-0.87*	-0.89*	0.13	-0.49	-0.37	-0.60	-0.69*
	3	0.75*	0.76*	0.78*	-0.09	-0.06	-0.05	-	-
Zn	1	-0.19	-0.51*	-0.51	-	-0.02	0.03	0.29	-0.59*
	2	-0.59	-0.77*	-0.61	-0.01	-0.63*	-0.43	-0.80*	-0.67*
	3	0.95*	0.89*	0.93*	0.16	0.35	-0.16	-	-

*Indicates the value of correlation coefficient is statistically significant ($P < 0.05$)

Table 7

Comparison of the correlation coefficient values indicating the relationship between the concentration of heavy metals studied in the muscle tissue of the fishes tested and their age, weight, and body length. No chub was collected at location 3 – Zakrzówek Reservoir. All bleak from location 1 – Łączany were the same age, as were all chub from locations 1 – Łączany and 2 – Czernichów

		Roach			Bleak			Chub	
Metal	Location	age	length	weight	age	length	weight	length	weight
Cu	1	-0.08	-0.10	0.08	-	-0.37	-0.67*	-0.69*	-0.73*
	2	-0.24	-0.03	0.02	0.18	-0.24	-0.24	-0.59	-0.63
	3	-0.16	0.19	0.12	0.63*	0.31	0.05	-	-
Mn	1	0.10	-0.58*	-0.18	-	-0.20	-0.18	0.13	0.10
	2	-0.43	-0.67	-0.49	0.12	-0.14	0.14	0.26	0.15
	3	0.12	0.52	0.48	-0.48	-0.34	-0.46	-	-
Fe	1	0.65*	-0.13	-0.20	-	-0.28	-0.60*	-0.05	0.01
	2	-0.21	-0.56	-0.29	0.25	-0.31	0.07	-0.38	-0.56
	3	-0.35	-0.50	-0.41	0.22	0.06	0.39	-	-
Ni	1	0.31	-0.22	-0.20	-	-0.34	-0.60*	-0.02	-0.10
	2	0.03	-0.32	-0.08	0.33	-0.58*	-0.19	-0.57	-0.66*
	3	-0.62	-0.24	-0.32	0.16	0.27	-0.04	-	-
Zn	1	0.28	-0.32	-0.51*	-	-0.22	-0.51*	0.07	0.02
	2	-0.58	-0.80*	-0.66	0.25	-0.33	-0.29	-0.52	-0.70*
	3	-0.94*	-0.79*	-0.78*	-0.17	-0.53	-0.20	-	-

*Indicates the value of correlation coefficient is statistically significant ($P < 0.05$)

Zakrzówek Reservoir. An analysis of Fe levels in bleak bone and scales and roach scales showed a similar pattern (Tables 2–5). Among the fish species examined, the highest statistically significant Fe concentrations in gills, bone, and scales were found in bleak, while they were the lowest in its muscles (Tables 2–5).

Table 8

Comparison of the correlation coefficient values indicating the relationship between the concentration of heavy metals studied in the bone tissue of the fishes tested and their age, weight, and body length. No chub was collected at location 3 – Zakrzówek Reservoir. All bleak from location 1 – Łączany were the same age, as were all chub from locations 1 – Łączany and 2 – Czernichów

		Roach			Bleak			Chub	
Metal	Location	age	length	weight	age	length	weight	length	weight
Cu	1	-0.32	-0.06	-0.02	-	-0.09	0.13	-0.45	-0.36
	2	-0.68*	-0.68*	-0.73*	-0.48	-0.51	-0.66*	-0.47	-0.73*
	3	-0.25	-0.58	-0.52	-0.61*	-0.52	-0.44	-	-
Mn	1	-0.28	-0.57*	-0.38	-	0.29	0.24	-0.42	-0.39
	2	-0.48	-0.57	-0.48	0.16	0.22	0.15	0.59	0.40
	3	0.23	0.28	0.36	0.33	0.22	0.30	-	-
Fe	1	-0.19	-0.22	-0.17	-	-0.20	-0.04	-0.58*	-0.43
	2	-0.73*	-0.81*	-0.77*	-0.21	-0.50	-0.62*	-0.49	-0.61
	3	0.02	0.25	0.14	0.49	0.24	0.13	-	-
Ni	1	-0.18	-0.26	-0.22	-	-0.36	-0.27	-0.64*	-0.61*
	2	-0.71*	-0.77*	-0.76*	-0.28	-0.53	-0.69*	-0.52	0.61
	3	-0.97*	-0.90*	-0.93*	0.10	0.07	0.41	-	-
Zn	1	-0.51*	-0.30	-0.37	-	0.59	0.10	0.29	-0.10
	2	-0.54	-0.51	-0.60	-0.64*	-0.17	-0.58*	-0.25	-0.44
	3	-0.65*	-0.63*	-0.62	-0.03	0.01	-0.04	-	-

*Indicates the value of correlation coefficient is statistically significant (P < 0.05)

Table 9

Comparison of the correlation coefficient values indicating the relationship between the concentration of heavy metals studied in scales of the fishes tested and their age, weight, and body length. No chub was collected at location 3 – Zakrzówek Reservoir. All bleak from location 1 – Łączany were the same age, as were all chub from locations 1 – Łączany and 2 – Czernichów

		Roach			Bleak			Chub	
Metal	Location	age	length	weight	age	length	weight	length	weight
Cu	1	0.01	-0.29	-0.52*	-	-0.30	-0.17	-0.82	-0.92*
	2	-0.68*	-0.79*	-0.70*	-0.03	-0.30	-0.51	-0.59	-0.81*
	3	-0.84*	-0.85*	-0.89*	-0.35	-0.34	-0.68*	-	-
Mn	1	-0.05	-0.58	-0.58*	-	-0.23	0.10	0.07	0.15
	2	-0.57	-0.75*	-0.65	0.21	-0.25	0.03	-0.56	-0.46
	3	0.54	0.38	0.48	0.82*	0.58*	0.60*	-	-
Fe	1	0.25	-0.49	-0.66*	-	0.01	0.10	-0.78*	-0.74*
	2	-0.66	-0.73*	-0.68*	-0.32	-0.41	-0.69*	-0.65*	-0.74*
	3	-0.68*	-0.59	-0.68*	0.40	0.16	-0.27	-	-
Ni	1	0.05	-0.45	-0.54*	-	-0.14	-0.04	-0.78*	-0.75*
	2	-0.50	-0.61	-0.50	-0.37	-0.44	-0.68*	-0.66*	-0.88*
	3	-0.74*	-0.59*	-0.57	-0.26	-0.40	-0.33	-	-
Zn	1	0.33	-0.59*	-0.73*	-	-0.21	0.03	-0.63*	-0.70*
	2	-0.68*	-0.86*	-0.73*	0.26	-0.27	-0.21	-0.43	-0.77*
	3	0.26	-0.09	0.00	-0.01	-0.66*	-0.26	-	-

*Indicates the value of correlation coefficient is statistically significant (P < 0.05)

Ni concentrations in the gills of roach, chub, and bleak from the Vistula in Czernichów were significantly higher compared to fishes from the Vistula in Łączany. Moreover, Ni concentrations in the gills of roach and bleak from Zakrzówek Reservoir were significantly higher compared to fishes from Łączany. Ni concentrations in bleak bone and the scales of bleak and chub from Łączany were significantly higher compared to fishes from other locations. Statistical analysis showed many significant negative

Table 10
Comparison of metal pollution index (MPI) values in the tissues of fishes collected from three locations

		Location		
Tissue	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
Muscle	roach	2.3	2.8	1.5
	bleak	2.0	1.7	1.5
	chub	2.6	1.6	-
Gills	roach	8.1	8.6	8.4
	bleak	5.9	34.1	11.8
	chub	6.7	15.4	-
Bone	roach	37.6	27.6	26.2
	bleak	73.4	44.7	44.1
	chub	36.7	25.3	-
Scales	roach	5.8	7.3	3.9
	bleak	31.8	22.4	13.6
	chub	15.5	13.7	-

Table 11
Comparison of values of EDI (mg/kg/individual) and THQ calculated for fishes collected from three locations. No chub was collected at location 3 – Zakrzówek Reservoir

		1 – Łączany		2 – Czernichów		3 – Zakrzówek	
Metal/	Fish species	EDI	THQ	EDI	THQ	EDI	THQ
Cu	roach	2.8×10^{-4}	4.9×10^{-4}	5.7×10^{-4}	9.2×10^{-4}	1.4×10^{-4}	2.3×10^{-4}
	bleak	7.8×10^{-4}	1.3×10^{-3}	3.3×10^{-4}	5.8×10^{-4}	1.6×10^{-4}	8.3×10^{-5}
	chub	5.3×10^{-4}	9.3×10^{-4}	2.5×10^{-4}	4.5×10^{-4}	-	-
Mn	roach	1.8×10^{-4}	9.1×10^{-5}	2.8×10^{-4}	1.4×10^{-4}	4.2×10^{-5}	2.1×10^{-5}
	bleak	4.1×10^{-5}	2.2×10^{-5}	4.3×10^{-5}	4.7×10^{-5}	4.1×10^{-5}	2.3×10^{-5}
	chub	1.2×10^{-4}	6.2×10^{-5}	5.4×10^{-5}	2.7×10^{-5}	-	-
Fe	roach	5.8×10^{-3}	5.8×10^{-4}	6.1×10^{-3}	6.2×10^{-4}	5.1×10^{-3}	5.1×10^{-4}
	bleak	3.8×10^{-3}	3.8×10^{-4}	4.4×10^{-3}	4.4×10^{-4}	5.4×10^{-3}	5.4×10^{-4}
	chub	7.0×10^{-3}	7.0×10^{-4}	4.4×10^{-3}	4.4×10^{-4}	-	-
Ni	roach	9.4×10^{-4}	3.3×10^{-3}	7.7×10^{-4}	2.7×10^{-3}	9.3×10^{-4}	3.3×10^{-3}
	bleak	7.1×10^{-4}	2.4×10^{-3}	4.5×10^{-4}	1.6×10^{-3}	4.8×10^{-4}	1.7×10^{-3}
	chub	8.1×10^{-4}	2.8×10^{-3}	5.2×10^{-4}	1.8×10^{-3}	-	-
Zn	roach	3.7×10^{-3}	8.6×10^{-4}	3.5×10^{-3}	8.1×10^{-4}	3.5×10^{-3}	8.1×10^{-4}
	bleak	5.0×10^{-3}	1.4×10^{-3}	6.4×10^{-3}	1.5×10^{-3}	6.8×10^{-3}	1.6×10^{-3}
	chub	5.0×10^{-3}	1.2×10^{-3}	4.4×10^{-3}	1.0×10^{-3}	-	-

EDI – Estimated Daily Intake, THQ – Target Hazard Quotient

relationships between tissue concentrations of Ni and the age, length, and weight of the fishes analyzed. Positive correlations were found between Ni concentrations in the gills of roach from Zakrzówek Reservoir and age, body length, and body weight (Tables 6–9). Among the fish species examined, the highest statistically significant Ni concentrations in gills, bone, and scales were found in bleak, while the highest level of this metal in muscle was found in roach (Tables 2–5).

Zn concentrations in roach bone from the Vistula in Łączany were significantly higher compared to

Table 12
Comparison of values of HI and CR calculated for fishes collected from three locations. No chub was collected at location 3

		Location		
Index	Fish species	1 – Łączany	2 – Czernichów	3 – Zakrzówek
HI	roach	5.3×10^{-3}	5.2×10^{-3}	4.9×10^{-3}
	bleak	5.6×10^{-3}	4.1×10^{-3}	3.9×10^{-3}
	chub	5.7×10^{-3}	3.8×10^{-3}	-
CR	roach	1.1×10^{-4}	9.1×10^{-5}	1.1×10^{-4}
	bleak	8.4×10^{-5}	5.4×10^{-5}	5.7×10^{-5}
	chub	9.7×10^{-5}	6.2×10^{-5}	-

HI – hazard index, CR – target cancer risk

fishes from the Vistula in Czernichów, whereas Zn concentrations in the bone of bleak from Łączany were significantly higher compared to fishes from Zakrzówek Reservoir. Significantly higher Zn levels were noted in the scales of chub and bleak from Czernichów. Zn concentrations in the scales and muscle tissue of bleak from Zakrzówek Reservoir were higher compared to fishes from location 1 (Tables 2–5). Statistical analysis showed some negative relationships between Zn concentrations in the tissue samples analyzed and the weight, length, and age of the fishes (Tables 6–9). Among the fish species examined, bleak had the highest statistically significant Zn concentrations in muscle, bone, and scales, while the Zn levels in their gills were significantly lower than in other species (Tables 2-5).

The MPI values for the muscle tissue of the fishes analyzed ranged between 1.5 for roach and bleak from Zakrzówek Reservoir and 2.8 for roach from the Vistula in Czernichów (Table 10), while those for the gills of the fishes ranged between 5.9 for bleak from Łączany and 34.1 for bleak from Czernichów. The MPI values for bone were significantly higher ranging from 25.3 for chub from Czernichów to 73.4 for bleak from Łączany. The MPI values for scales ranged between 3.9 for roach from Zakrzówek Reservoir and 31.8 for bleak from Łączany (Table 10). A comparison of EDI, THQ, HI, and CR values for fishes from the locations analyzed are presented in Tables 11 and 12.

Discussion

The findings from the present study clearly indicated that there is a relationship between concentrations of Cu, Mn, Ni, Fe, and Zn in fishes and species, habitat, and tissue type, which is consistent with findings from other studies (Gandhewar and Zade 2019, Łuczyńska et al. 2020). Fishes are considered to be good bioindicators of the health of aquatic environments. Changes in fish biodiversity and the accumulation of various xenobiotics in fishes from water may confirm anthropogenic impacts on aquatic ecosystems (Plessl et al. 2017). Differences in fish species biology could also influence the bioaccumulation of heavy metals present in environments. The fishes sampled for the present study were three species of minnows (*Leuciscidae*): roach, which preys predominantly on benthic invertebrates, zooplankton, plant material, and detritus; bleak, which feeds mainly on plankton, including crustaceans and insects; and chub, which feeds on a wide variety of aquatic and terrestrial animals and plant material. Large chub preys predominantly on fishes (Kottelat and Freyhof 2007). All the species studied are fished recreationally and consumed by humans, although their importance in human nutrition is not very important.

The analysis of Cu concentrations in the gills of the fish studied indicated statistically significantly higher Cu levels in all fish species sampled at location 2 on the Vistula River in Czernichów compared with those caught at the other locations. Gills, which are used by fishes for gas exchange, are constantly exposed to water and the substances dissolved in it. The presence of increased concentrations of heavy metals in the gills of fishes may indicate that these metals are in the water the fishes inhabit (Łuszczek-Trojnar et al. 2019). Cu concentrations in the gills of roach, bleak and chub from location 2 were between four to 19 times higher than those in muscle tissue. Cu concentrations in the gills and muscle tissue were comparable in fishes from location 1, with the exception of bleak, in which Cu concentrations in muscle tissue were higher than in the gills. Similar Cu concentrations in the gills and muscle tissue of fishes have been reported in many other studies (Stanek et al. 2005, Łuczyńska et al. 2020). While the findings of some studies are similar to ours, Szarek-Gwiazda and Amirowicz (2006) reported different results. These authors analyzed fishes from a flooded opencast sulphur mine in Piaseczno and report that CU concentrations in gills were significantly higher compared with those in the muscle tissue of the fishes (Szarek-Gwiazda and Amirowicz 2006).

In the present study, the fact that Cu concentrations in the gills of bleak from Zakrzówek Reservoir were significantly higher compared with the same species from the Vistula in Łączany suggests that fishes from Zakrzówek Reservoir may have been exposed to higher Cu levels than those from the Vistula. However, the comparison of Cu concentrations in muscle tissue, bone, and scales of roach and bleak from all sampling locations indicated that fishes from the Vistula had been exposed to this metal in higher concentrations or for longer periods of time. This is because Cu also accumulated in their hard tissues, which do not respond to changes in environmental Cu levels as fast as gills (Łuszczek-Trojnar et al. 2015, Łuszczek-Trojnar and Nowacki 2021). Such proportions of Cu concentrations in the tissues of the fishes analyzed in the present study could indicate short-term water contamination in Czernichów, as the metal easily accumulated in the gills of fish at this location but did not manage to accumulate at significantly higher concentrations in other tissue types. Cu concentrations in the bone and muscle tissues of bleak from the Vistula in Łączany was significantly higher compared with fishes from other locations, which may indicate that, in the long term, fishes from Łączany are exposed more intensively to Cu in the water. In hard tissues, such as bone and scales, it is a slow, long-lasting process for metals to accumulate to certain concentrations, and this adequately reflects chronic exposure of fishes to metals (Łuszczek-Trojnar et al. 2015). Cu concentrations in scales and muscle tissue of roach and bleak from the Vistula were significantly higher compared with fishes from Zakrzówek Reservoir. This may indicate that the water in Zakrzówek Reservoir, which is a flooded former limestone quarry, is cleaner than that of the Vistula. This seems likely because the reservoir is not directly exposed to inflows of pollutants. However, roach and bleak from Zakrzówek Reservoir were larger compared to fishes of the same age from the Vistula. Statistical analysis results showed many significant negative correlations between fish body weight, body length, and age and Cu concentrations in their gills, muscle, bone, and scales. The larger the size of the fish, the lower the Cu concentration in their tissues. Tissue metal concentrations in fishes decrease with growth, which is a phenomenon described by other authors (Canli and Atli 2003). Naturally, this phenomenon is only observed in clean environments where healthy fishes can systematically eliminate excessive heavy metals absorbed from the environment. When the amounts of heavy metals in the environment are too high, the amounts absorbed are greater than those eliminated, which means fishes constantly accumulate metals. Consequently, concentrations increase with fish age and growth (Łuszczek-Trojnar et al. 2015, Drąg-Kozak et al. 2021). In our study, negative correlations were noted between Cu concentrations in fish tissues and fish size, irrespective of location, which confirmed that their aquatic environments were not contaminated with Cu.

The concentration of Mn in the gills of roach and bleak from the Vistula in Czernichów was significantly higher compared with fishes from other locations. As in the case of Cu, this may indicate short-term contamination in this part of the Vistula with Mn from local sources. Indeed, the concentration of Mn in other tissues in fishes from this location was lower compared with fish from the Vistula in Łączany. We also found that tissue concentrations of Mn in fishes from the Vistula (locations 1 and 2) were significantly higher compared with fishes from Zakrzówek Reservoir, which indicated that the concentration of Mn in the reservoir was lower. Mn concentrations in the muscle tissue and gills of fishes from the Vistula were significantly higher compared with different fish species from Lake Łańskie and Lake Pluszne, which are located in northern Poland. In those fishes, Mn levels in muscle tissue and gills did not exceed 0.26 mg kg^{-1} or 7.8 mg kg^{-1}, respectively (Łuczyńska et al. 2020). However, Mn concentrations in the muscle tissue and gills of fishes from the Vistula were similar to or lower than, for instance, mean Mn concentrations in the muscle tissue of *Cyprinodon macrostomus* from the Euphrates (10.29 mg kg^{-1}) and the mean Mn concentration in the gills of fishes of the same species (14.32 mg kg^{-1}) (Töre et al. 2021). The negative correlations between the tissue concentration of Mn in roach from two locations on the Vistula and the body weight and body length of the fish indicated that the fish had not been chronically exposed to pollution. A positive correlation was found in bleak between Mn concentrations in scales and fish body weight. However, Mn concentrations in the scales of these fish were significantly lower compared with Mn levels in the scales of bleak from other locations. Negative correlations between the tissue concentrations of Mn in fishes and fish length are also reported by other authors (Kostecki 2000, Łuczyńska et al. 2020). The high affinity of Mn with scales may indicate their usefulness as a bioindicator for the contamination of fishes with this metal. The great advantage of scales as a bioindicator tissue is that they can be collected non-lethally (Łuszczek-Trojnar and Nowacki 2021, Łuszczek-Trojnar et al. 2022).

In the present study, the tissue concentration of Fe in fishes from both stations located at the Vistula River was significantly higher compared with those of fishes from Zakrzówek Reservoir. The exception was muscle tissue as it had the lowest levels of Fe compared with other tissues, which is confirmed by the results of other studies (Ateş et al. 2015, Łuczyńska et al. 2020). In our study, the highest Fe levels were found in bone. The numerous negative correlations between Fe concentrations and the body weight and body length of the fishes analyzed indicated that the larger the sizes of the fishes were, the lower Fe concentrations were in their bodies. This also indicated that the fishes analyzed had not been exposed chronically to Fe in their environments.

Ni is a priority substance that is considered to be carcinogenic, and its presence in the environment and organisms may be harmful (Prueitt et al. 2020). Our analysis showed that in the fishes from the Vistula, the tissue concentration of Ni was the highest in the gills and bone. Ni concentrations in gills were significantly higher in fishes from Czernichów, and in bleak it was almost 27 times higher than in fishes collected upstream from this location in Łączany. This observation may indicate that the source of the Vistula Ni contamination could be located between Łączany and Czernichów; however, no significant differences were found in Ni concentrations in muscle tissues among fishes from different locations. Our findings regarding Ni levels in fish muscle tissue are consistent with those reported by other authors (Ateş et al. 2015). However, Ni concentrations in muscle tissue in fishes inhabiting clean waters are usually significantly lower (Vitek et al. 2007). The highest Ni concentrations were found in bleak and chub scales, in which the level of this metal was up to 20 and six times higher, respectively, compared with muscle tissue; however, this was not found for roach. Since scales accumulate Ni, this makes them good bioindicators of fish Ni contamination, which is corroborated by the findings of earlier studies (Łuszczek-Trojnar and Nowacki 2021). But the lower Ni level in scales of roach, similar to its concentration in muscle tissue, may indicate that this fish species, which occupies a slightly different habitat in ecosystems, accumulates Ni in its scales differently. The numerous negative correlations between the concentration of Ni and the weight and length of the fishes analyzed indicated that the fishes had not been exposed chronically to Ni in their environments, and, thus, had not accumulated it continuously in their tissues.

The lowest Zn concentrations were noted in muscle tissue (8–16 mg kg^{-1}) and the highest in bone (80–150 mg kg^{-1}). Other studies confirm that Zn levels in fish muscle tissue are the lowest compared with other tissues (Łuczyńska et al. 2020, Garai et al. 2021). The numerous negative correlations found in the present study between Zn tissue concentrations and fish body weight and length confirmed the hypothesis that tissue metal concentrations in fishes decrease with growth and that the fishes analyzed had not been exposed chronically to increased Zn concentrations in their environments.

To conclude, the results of our metal concentration analysis in fishes from different locations demonstrated clearly that tissue levels of the metals analyzed in fishes from the Vistula were higher compared with the other fishes analyzed. This stems from the fact that the river is contaminated with industrial effluents and numerous point-source inflows of sewage. Among the fish species examined, the highest concentrations of heavy metals in the tissues studied were found mainly in bleak. The differences in heavy metal concentrations among different fish species from the same location can result from varied feeding behaviors, habitats in ecosystems, contact with bottom sediments, and physiological differences in metabolism (Varol et al. 2019). The higher concentrations of the metals analyzed in bleak tissues may also have been due to their smaller body size, because, according to the phenomenon of metal levels decreasing with growth, small fishes present with higher metal levels (Canli and Atli 2003).

In the literature, MPI is usually reported for fish muscle tissue, gills, and liver. There are no studies in the literature that report MPI values for fish bone or scales, even though scales as a bioindicator tissue have a broad scope of application as they can be collected non-lethally. This study is the first to report MPI values for the bone and scales of fishes. The highest MPI values were found for bone, followed by gills, and then scales. The lowest MPI values were found for muscle tissue. Furthermore, the highest MPI values were found for each type of tissue in fishes from the Vistula (location 1- Łączany or 2 – Czernichów). Higher MPI values are considered to indicate a higher levels of contamination in fishes (Ju et al. 2017). The MPI values in our study differ from those reported by other authors for the muscle tissue of fishes from different water bodies around the world that are lower in fishes from the Tigris in Turkey at 0.29–1.33 (Töre et al. 2021), similar in fishes from Lake Łańskie and Lake Pluszne in Poland at 1.5–1.8 (Łuczyńska et al. 2020) and in fishes from a fish market in Saudi Arabia at 0.41–1.82 (Shehawy et al. 2016), and higher in fishes from the Meghna River in Bangladesh at 9.8–20.7 (Hossain et al. 2022). The MPI values calculated based on the concentration of metals in the gills of the fishes analyzed in the present study are higher compared with those reported for fishes from Lake Pluszne in Poland (Łuczyńska et al. 2018) and from the Tigris (Töre et al. 2021), which may indicate the presence of increased levels of the metals in the Vistula.

In the present study, we assessed human health risk from the consumption of the muscle meat of the fish analyzed by calculating the estimated daily intake (EDI), target hazard quotients (THQ), and hazard index (HI). None of the EDI values for the metals analyzed exceeded the RfD recommended by the US EPA (USEPA 2011). In a study by Somparn et al. (2020), which investigated health risks to consumers from the consumption of Nile tilapia (*Oreochromis niloticus* (L.)) from the Huai Luang River Basin in Thailand, THQ and HI values for Cd and Pb were higher than 1.0, whereas THQ and HI values for Zn, Cr, and Cu did not exceed 1.0.

In the present study, THQ values for the metals analyzed in the muscle tissue of roach, bleak, and chub sampled near Kraków did not exceed 1.0, indicating there was no non-carcinogenic risk to the health of consumers. HI values were also lower than 1.0, indicating no risk of Cu, Mn, Fe, Ni, or Zn contamination to consumers. CR values for each fish species from the Vistula and Zakrzówek Reservoir ranged between 6.2×10^{-5} and 1.0×10^{-4}. According to US EPA guidelines, CR values below 10^{-6} indicate a negligible cancer risk, whereas those greater than 10^{-4} indicate an unacceptable cancer risk. CR values between 10^{-6} and 10^{-4} indicate an acceptable level of cancer risk (USEPA 2015). In our study, the concentrations of Ni in the muscle tissue of the fish analyzed were within the acceptable range, indicating no risk of carcinogenic effects. However, CR values for roach from locations 1 and 3 were close to the upper limit of the acceptable cancer risk range.

Our findings show that fishes from the Vistula near Kraków are more contaminated with heavy metals compared with fishes from the water reservoir close to the center of Kraków, which is a flooded former limestone quarry. The highest concentrations of the metals analyzed were found in bone and scales, i.e., hard tissues, followed by gills, whereas the lowest levels were found in muscle tissue, which is consistent with the findings of other authors. We found numerous negative correlations between tissue concentrations of heavy metals and fish body weight and body length, which confirms that metal concentrations in fishes decrease with growth. However, this is only true for fish inhabiting unpolluted environments. Nevertheless, when comparing MPI values from our study with those published by other authors, the fishes analyzed in the present study exhibited a higher level of contamination. An analysis of the health risk to potential consumers indicated that the consumption of roach, chub, or bleak from the study locations did not pose a non-carcinogenic risk; however, CR values for roach from the Vistula and Zakrzówek Reservoir were close to the upper limit of the acceptable cancer risk range. It should be noted that open waters can also be contaminated with other metals and priority substances that are particularly harmful to the aquatic environment that were not analyzed in the present study. Therefore, the quality of the muscle meat of fishes inhabiting open waters should also be monitored for other xenobiotics.

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ORCID iD

Ewa Łuszczek-Trojnar https://orcid.org/0000-0002-7929-267X
Ewa Drąg-Kozak https://orcid.org/0000-0002-2572-0034
Paweł Szczerbik https://orcid.org/0000-0002-3207-0383

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