

## METAL CONCENTRATIONS IN CHUB, *LEUCISCUS CEPHALUS*, FROM A SUBMONTANE RIVER

Marek KRYWULT<sup>1</sup>, Mariusz KLICH<sup>2</sup>, and Ewa SZAREK-GWIAZDA<sup>3\*</sup>

<sup>1</sup> Provincial Sanitary-Epidemiological Station, ul. Prądnicka 76, 31-202 Kraków, Poland

<sup>2</sup> Higher Vocational School in Tarnow, ul. Mickiewicza 8, 33-100 Tarnów, Poland

<sup>3</sup> Institute of Nature Conservation, Polish Academy of Sciences, Cracow, Poland

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**Background.** Fish are often used as indicators of pollution levels in water environments. The omnivorous chub inhabiting rivers of Europe, including their more polluted parts, is a potentially suitable indicator. The information published, however, on the metal accumulation in the tissues of chub living in varyingly polluted waters (both polluted and unpolluted environments) is scarce. The presently reported study was conducted to examine the metal concentrations in selected tissues of chub living in a natural submontane river.

**Materials and Methods.** Sediment samples (0–5 cm layer) and chub, *Leuciscus cephalus* (L.), were collected from the upper (stations 1–4) and lower (stations 5–8) sections of the Biała Tarnowska River in summer 2003. Fish age was determined from the otoliths. The AAS method was used to determine metal concentrations (Cd, Pb, Cu, and Zn) in sediment and chub tissues (spleen, liver, and muscle).

**Results.** According to the values of the geochemical index ( $I_{geo}$ ), the river sediments were not polluted by Pb, Cu, and Zn, while in various degrees polluted by Cd. Despite the low metal concentrations, the mean Cd and Cu concentrations were higher (c. 2× and 4×, respectively) in the upper part of the river compared to the lower section, while concentration of Pb and Zn were similar in the two areas. Chub accumulated the greatest amount of Cu in the liver, while the bulk of Cd, Pb, and Zn was found in the spleen. In the upper part of the river, elevated concentrations of trace elements in selected tissues of chub were found: Cu (spleen, muscle), Cd (spleen), Zn (muscle). The pollution status of the river, on the basis of trace element contents in the sediment, was also determined.

**Conclusions.** The obtained results indicated tissue-specific metal accumulation in chub. Selected chub tissues are sensitive to Cd and Cu contents in the river sediment.

**Keywords:** fish, chub, trace elements, sediment

### INTRODUCTION

In many aquatic environments elevated levels of trace elements occur due to anthropogenic activities. Water organisms can be precise indicators in estimating the status of pollution of water environments. Among them several species of fish have been widely used (Håkanson 1984, Chevreuil et al. 1995, Andres et al. 2000, Falandysz et al. 2000, Perkowska and Protasowicki 2000, Řehulka 2001, Wagner and Boman 2003, Polechoński 2004, Szarek-Gwiazda and Amirowicz 2006). Accumulation of trace elements in the tissues of fish living in aquatic environment depends on many factors (Jeziarska and Witeska 2001). These factors include metal contents in water and food organisms, exposure time, environment conditions (e.g., pH, alkalinity, salinity), feeding habit, age, and size of fish (Protasowicki 1991, Mason et al. 2000, Řehulka 2001,

Jeziarska et al. 2002, Witeska and Baka 2002, Dobicki and Polechoński 2003, Łuczyńska and Brucka-Jastrzębska 2005), and the differences in metallothionein (MT) gene induction by various species of fish (Olsson and Kille 1997, De Boeck et al. 2003).

Chevreuil et al. (1995) and Andres et al. (2000)—to name just a few—indicated that the omnivorous roach, which is common and often abundant in central European inland waters, is a useful species in monitoring studies. Roach can accumulate certain trace elements in considerable amounts, greater than other species inhabiting the same water bodies. Andres et al. (2000), Spurný et al. (2002), Demirak et al. (2006), and Yılmaz et al. (2007) used other omnivorous fish i.e., chub, *Leuciscus cephalus* (L.), to assess heavy metal distributions in river ecosystems. Chub inhabit rivers of central and southern Europe,

\* Correspondence: Dr Ewa Szarek-Gwiazda Instytut Ochrony Przyrody PAN, Al. Mickiewicza 33, 31-120 Kraków, Poland, phone: +48 12 632 1101, e-mail szarek@iop.krakow.pl

including the more polluted parts of these systems, and is potentially a suitable species for pollution monitoring studies. It is commonly found in the upper course of most Polish rivers. There is little information about the level of metal accumulation in chub living in varyingly polluted waters (both unpolluted and polluted environments), metal distribution in chub tissues, or the effect of intrinsic factors i.e., age and size on metal bioaccumulation.

The aim of this study was to examine the metal (Cd, Pb, Cu, and Zn) concentrations in selected tissues (spleen, liver, and muscle) of chub *Leuciscus cephalus* (L.) living in a natural submontane river (Biała Tarnowska River, southern Poland). The pollution status of the river, on the basis of trace element contents in the sediment, was also determined.

### MATERIALS AND METHODS

**Study area.** The Biała Tarnowska River is the main tributary of the Dunajec River. It arises in the Beskid Niski (Carpathian belt, southern Poland) (730 m above sea level). Its total length is 101.8 km. The majority of the river has unregulated banks and is in a natural state. Fields, pastures, meadows, and natural vegetation predominate in the catchment basin of the upper and middle portion of the river. Some villages and small towns (fewer than 7500 inhabitants) are located along the river. The city of Tarnów (circa 130 000 inhabitants), with among others, a nitrogen plant, a glass plant, and an electric plant, is situated by the upper part of the river. There are no large industry plants (except Tarnów) in the catchment basin of the Biała Tarnowska River. In its upper and middle portions, the river is mainly contaminated by sewage from small towns and wastewater from surrounding villages (without sewage treatment), while in the lower portion from municipal and industrial sewage from the city of Tarnów. Soils in the catchment basin of the river are unpolluted (0 class) or slightly polluted (I class) by Cd, Pb, Cu, and Zn (Tokarz and Turzański 1999).

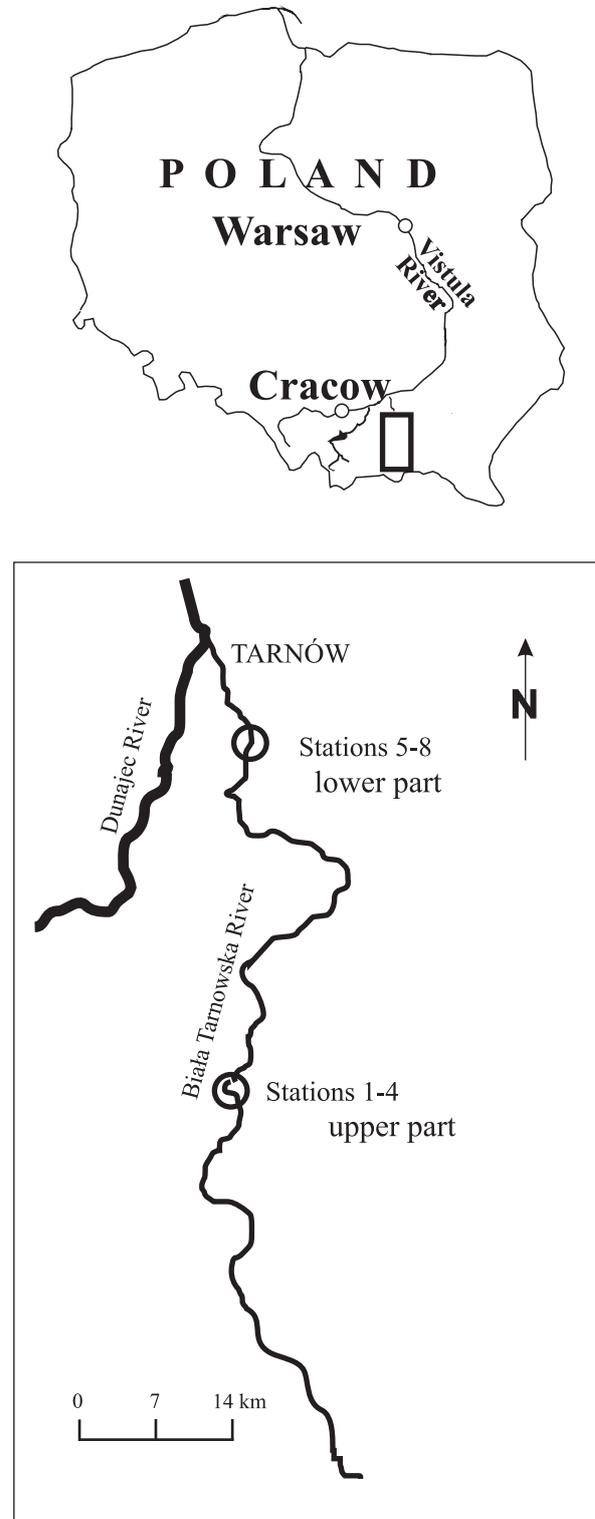
Chub *Leuciscus cephalus* (L.) usually reaches a length of 40–50 cm and a weight of 2–3 kg (Brylińska 1986). It is omnivorous and has a relatively long lifespan.

In summer 2003, sediment samples were collected from eight stations located in river pools, where the most intensive accumulation of trace elements occurs (Ciszewski 1998). Stations 1, 2, 3, and 4 were located in the upper section of the river, while stations 5, 6, 7, and 8 were located in the lower part of the river, below city of Tarnów (Fig. 1). The upper layer (0–5 cm) of the sediment was taken using a polyethylene corer (diameter 4 cm). Collected sediment samples were placed into plastic containers that had been previously rinsed with double-distilled water.

The chub were collected by electrofishing from the upper (between stations 1 and 4) and lower (between stations 5–8) sections of the river (Fig. 1). In each section, 16 specimens of chub were caught, in total 32 specimens. The fish caught were placed individually in polyethylene bags and chilled on ice during transport to the laboratory.

As much as possible, individuals of similar size were selected for trace element determination.

**Analytical methods.** Sediment samples were dried at 105°C for  $\geq 24$  h, and then ground to a fine powder using the Planetary Mill “Pulverisette 5” (Fritsch, Germany). A detailed description of the mineralization condition of



**Fig. 1.** Location of the Biała Tarnowska River and the sampling stations

sediment samples was described by Szarek-Gwiazda and Amirowicz (2006). The reference material NCS DC 73308 (stream sediment) was used to check the precision of analytical procedure.

In the lab, fish were measured, and samples of dorsal muscle, spleen, and liver were taken. Fish age was determined using otoliths. Samples of tissues were dried at the temperature of 60°C for 60 h to obtain dry mass, and digested with a mixture of pure (MERCK) nitric- and perchloric acid (4 : 1, 15 mL) on the heated block of Tecator Digestion System 12, in conjunction with an Autostep 2000 controller. Analysis of studied elements was performed with flame atomic absorption spectrophotometer (FS 220 Varian Techtron, Australia) and using a graphite tube atomizer (GTA 110 Varian Techtron, Australia) following the manual method. The detection limits for the studied elements (in  $\mu\text{g} \cdot \text{L}^{-1}$ ) determined by using the GFAAS technique were as follows: Cd 0.01, Pb 0.28, and Cu 0.3, while using the FAAS technique: Cd 2, Pb 10, Cu 3, and Zn 4. All results were reported as  $\mu\text{g} \cdot \text{g}^{-1}$  dry weight.

**Statistics.** To establish pollution status of the Biała Tarnowska River a geochemical index ( $I_{\text{geo}}$ ) was calculated according to Müller's (1981) formula:

$$I_{\text{geo}} = \log_2 C_n \cdot 1.5B_n^{-1}$$

where:

$C_n$  = trace element concentration in the sediment at the particular station,

$B_n$  = geochemical background of the element given by Turiekian and Wedepohl (1961).

The significance levels of differences in concentrations of trace elements between studied organs of fish were assessed using Student's *t*-test for pair comparison, whereas the difference between the sections of the river were assessed using the Mann–Whitney test (Sokal and Rohlf 1987).

## RESULTS

The total trace element concentrations in the sediments of the Biała Tarnowska River at studied stations are presented in Fig. 2, while the values of geochemical index ( $I_{\text{geo}}$ ), calculated for the particular element and the station, are presented in Table 1. The concentrations of trace elements in the sediment ranged between (in  $\mu\text{g} \cdot \text{g}^{-1}$ ): Cd

0.4–10.2, Pb 1.7–10.8, Cu 4.4–28.4, and Zn 5.3–61.2. The mean concentration of Cd was c. 2× and Cu c. 4× higher in the upper part of the river (stations 1–4) compared to the lower part (stations 5–8), while those of Pb and Zn were similar in both sections of the river. The highest concentrations of Cd, Cu, and Zn were found at station 3, situated in the upper part of the river. Because of great variation in the trace element concentrations in the sediment among the stations, the result of Mann–Whitney test indicated a lack of significant differences between the upper and lower parts of the river. In general, all concentrations of Pb, Cu, and Zn determined in the sediments of the Biała Tarnowska River were rather low. According to  $I_{\text{geo}}$  the sediments were unpolluted by those elements. Studied sediments were in varying degrees polluted by Cd. According to  $I_{\text{geo}}$  sediments at stations 1, 4, and 7 were strongly polluted, station 3 extremely polluted, stations 2, 5, and 6 moderately polluted, while station 8 was unpolluted by Cd.

Physical characteristics of the collected fish are presented in Table 2. The maximum total length of the fish was 33.5 and 39 cm, weight 560 and 650 g, while age ranged from 8+ to 10+ and from 6+ to 12+, respectively in the upper and lower parts of the river.

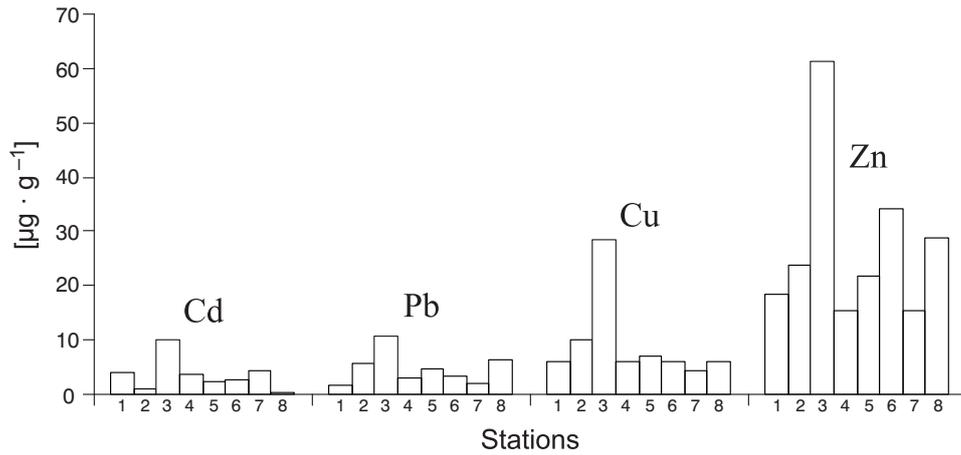
Mean concentrations ( $\mu\text{g} \cdot \text{g}^{-1}$  dry weight) of the trace elements in the tissues of the chub *Leuciscus cephalus* (L.) in different parts of the River Biała Tarnowska were as follows: Cd: 0.001–0.160, Pb: 0.06–2.53, Cu: 1.3–75.8, Zn: 65.4–330.9 (Fig. 3). High values of standard deviation indicated considerable variation in trace element concentration in particular tissues of chub. Trace elements show different affinities to various chub tissues: spleen (S), liver (L), muscle (M). In general, the elements were mainly accumulated in the liver or spleen and in the lowest amounts in the muscle of chub. The concentration of studied elements in the spleen of chub was similar (Cd) or even higher (Zn, Pb) than those found in liver. Statistical calculation show the following order of element accumulation in chub tissues at both parts of the river: Cd: S, L > M; Pb, Zn: S > L > M; Cu: L > S > M (Table 3).

In general, the differences in the metal concentrations in chub tissues between the two sites were small. However, statistical calculation (Table 4) shows higher concentrations of Cd (spleen), Cu (spleen, muscle), and

**Table 1**

The values of  $I_{\text{geo}}$  calculated for trace elements in the sediment of the Biała Tarnowska River in 2002

Station	Part of the river	Cd	Pb	Cu	Zn
1	upper	3.1	−4.2	−0.2	−3.0
2	upper	1.3	−2.4	−3.5	−2.6
3	upper	4.5	−1.5	−2.7	−1.2
4	upper	3.1	−3.4	−1.3	−3.2
5	lower	2.3	−2.6	−3.5	−2.7
6	lower	2.7	−3.2	−3.3	−2.1
7	lower	3.3	−3.8	−3.5	−3.2
8	lower	−0.2	−2.2	−3.9	−2.3



**Fig. 2.** Concentrations of Cd, Pb, Cu, and Zn in the bottom sediment at stations 1–8 of the Biała Tarnowska River in summer 2002

**Table 2**

Chub, *Leuciscus cephalus*, collected for analyses of the content of trace elements in the upper- (stations 1, 2, 3, and 4) and lower (stations 5, 6, 7, and 8) sections of the Biała Tarnowska River in 2003

Part of the river	n	TL [cm]		SL [cm]		Weigh [g]		Age [yr]	
		min.	max.	min.	max.	min.	max.	min.	max.
upper	16	21	33.5	17.5	29	90	560	8	10
lower	16	21.5	39	18	34	110	650	6	12

TL, total length; SL, standard length.

**Table 3**

Significant differences in concentrations of trace elements between studied tissues of chub, *Leuciscus cephalus*, collected in the Biała Tarnowska River in 2002; the presented values were obtained using Student's *t*-test for pair comparison

Tissues	Cd		Pb		Cu		Zn	
	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df
S–M	3.1*	30	4.5**	29	3.0*	27	5.9**	31
L–M	3.6*	30	4.5**	30	5.0**	27	4.6**	30
S–L	1.7	29	3.4*	28	4.5**	25	5.5**	30

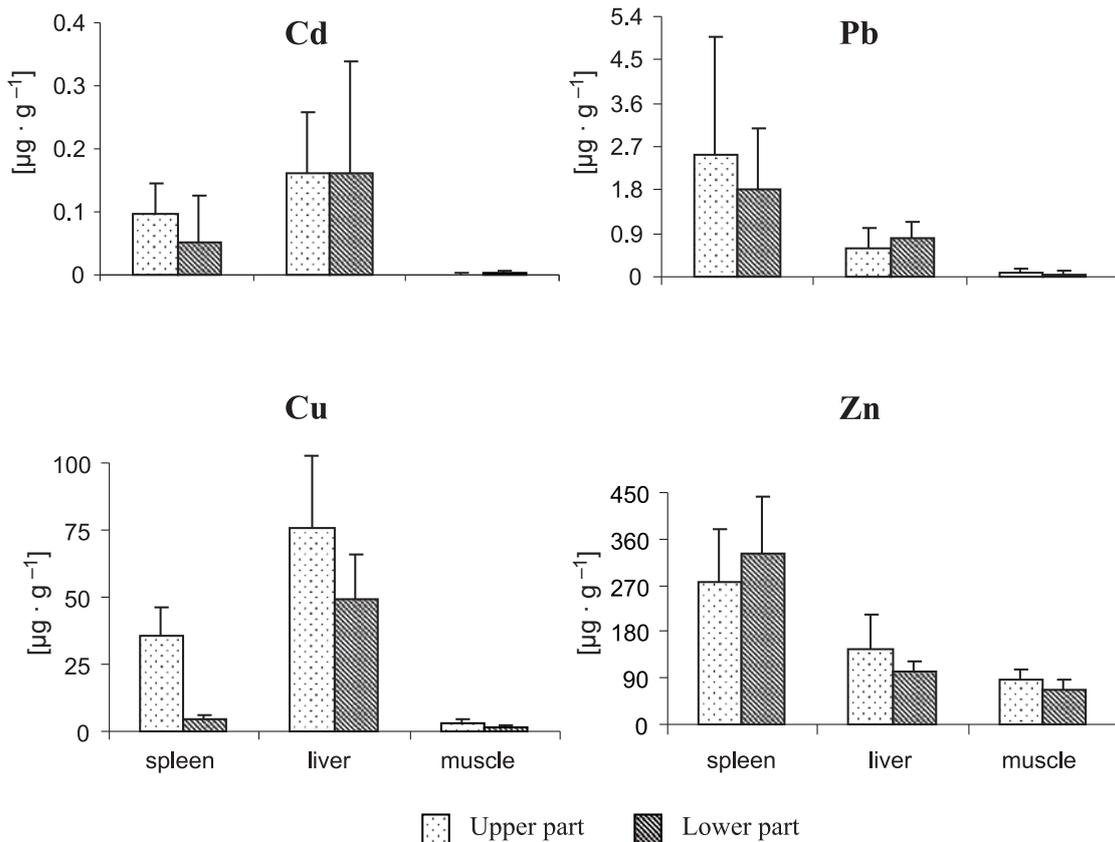
S, spleen; L, liver; M, muscle; \*  $P < 0.01$ ; \*\*  $P < 0.001$ ; df, degree of freedom; *t*, Student's *t*-test statistic.

**Table 4**

The significance of differences in concentrations of selected trace elements in tissues of chub, *Leuciscus cephalus*, between the upper and lower sections of the Biała Tarnowska River; the presented values were obtained using the Mann–Whitney test

Tissue	Cd			Pb			Cu			Zn		
	U	N <sub>1</sub>	N <sub>2</sub>									
S	39*	15	15	102	15	15	0*	15	14	92	16	16
L	122	16	16	86	16	15	78	16	13	76	16	15
M	87	16	16	91	16	16	19*	16	15	59*	16	16

S, spleen; L, liver; M, muscle; \*  $P < 0.05$ ; N<sub>1</sub>, N<sub>2</sub>, sample sizes; U, the Mann–Whitney statistic.



**Fig. 3.** The mean concentrations (dry weight) and standard deviation of Cd, Pb, Cu, and Zn in the spleen, liver, and muscle of chub, *Leuciscus cephalus*, in the upper and lower ( $n = 16$ ) parts of the Biała Tarnowska River in 2002

Zn (muscle) in chub from the upper part of the river compared to the lower part. Concentrations of Cd, Pb, Cu, and Zn in the liver were similar in both sections of the river.

## DISCUSSION

The concentrations of Pb, Cu, and Zn in the sediments of the Biała Tarnowska River were rather low (Fig. 2, Table 1) and characteristic of slightly polluted water environments. Obtained Pb, Cu and Zn contents in the sediments of Biała Tarnowska River were in accordance with concentrations found in the sediments of sections of rivers slightly polluted by trace elements (Aleksander-Kwaterczuk et al. 2004, Wiśniowska-Kielan and Niemiec 2005). They were much lower compared to concentrations found in sediments in polluted parts of rivers (van den Berg 1998, Ciszewski 2001, Helios-Rybicka et al. 2001, Boszke et al. 2004). In the sediment of the Mała Panew River, one of the most polluted rivers in the Odra River drainage, the Cd and Pb concentrations reach values of 92 and 418  $\mu\text{g} \cdot \text{g}^{-1}$ , respectively (Helios-Rybicka et al. 2001). The sediment of the Biała Przemsza River, which is situated in the Silesian Upland and receives polluted water from zinc and lead mines, contained extremely high concentrations of Cd (172  $\mu\text{g} \cdot \text{g}^{-1}$ ), Pb (335  $\mu\text{g} \cdot \text{g}^{-1}$ ), and Zn (42 100  $\mu\text{g} \cdot \text{g}^{-1}$ ) (Ciszewski 2001). The sediment of the

polluted section of the Meuse River (Netherlands) contained up to 232  $\mu\text{g} \cdot \text{g}^{-1}$  of Pb, 105  $\mu\text{g} \cdot \text{g}^{-1}$  of Cu, and 1083  $\mu\text{g} \cdot \text{g}^{-1}$  of Zn (van den Berg 1998). According to the classification given by Bojakowska (2001) the sediments of the Biała Tarnowska River were unpolluted by Pb, Zn, and Cu (except station 3—slightly contaminated), which confirmed the pollution status established on the basis of  $I_{\text{geo}}$  (Table 2). The sediments were unpolluted by Cd at station 8, slightly polluted at stations 2, 5, and 6, moderately polluted at station 1, 4, and 7, and strongly polluted at station 3. Elevated Cd contents in the sediments of the Biała Tarnowska River were probably caused by phosphate fertilizers, which play a particularly important role in soil contamination (Sager 1997). Elevated contents of Cd and Cu in the upper part of the river were probably caused by sewage from surrounding villages and municipal sewage from the town (c. 7500 inhabitants) located below. The influence of the city of Tarnów on trace element contents in the sediments at stations situated in the lower part of the river was not mentioned.

Cadmium and lead concentrations obtained in the muscle and liver of chub from the Biała Tarnowska River show low contamination levels (Fig. 3). They were similar to those found in chub from a slightly polluted portion of the Tiber River, Italy (Mancini et al. 2005) and a Turkish river (Yılmaz et al. 2007). Concentrations of Cu

and Zn in chub (muscle, liver) from the Biała Tarnowska River were slightly higher compared to those in chub from a polluted part of the river in Turkey (Yılmaz et al. 2007), which may be caused by their better bioavailability in the first river. Concentrations of Cd, Pb, Cu, and Zn in the muscle of chub from the Biała Tarnowska River were lower compared to those from the heavily polluted Jihlava River, Czech Republic (Spurný et al. 2002), while Cd and Zn in the liver and Cd in the muscle were lower compared to those from polluted sections of the contaminated Lot River (France) (Andres et al. 2000). According to Polish law, the level of Cd in fish muscles cannot exceed  $0.05 \mu\text{g} \cdot \text{g}^{-1}$  and Pb  $0.2 \mu\text{g} \cdot \text{g}^{-1}$  wet weight (i.e., Cd 0.25 and Pb  $1.0 \mu\text{g} \cdot \text{g}^{-1}$  dry weight). Cd and Pb contents in chub from the Biała Tarnowska River were below permissible limits for human consumption.

In general, the elements in lowest concentrations were accumulated in the muscle of chub from the Biała Tarnowska River (Fig. 3, Table 3), which is in accordance with the results of Andres et al. (2000), Spurný et al. (2002), Demirak et al. (2006), and Yılmaz et al. (2007). Andres et al. (2000) found Cd concentration in the chub tissues in the following order: kidney > intestine > liver > gill > muscle, while Zn: kidney > intestine = gill > liver > muscle in the polluted Lot River (France). The kidney is known as a storage organ for Cd and Pb in different fish species (Andres et al. 2000). The obtained results indicated high concentrations of the studied elements in the spleen of chub from the Biała Tarnowska River. High concentrations of Cd in fish spleens were also recorded by Allen (1995), Pb by Somero et al. (1977), and Cd, Pb, and Zn by Camusso et al. (1995). According to Somero et al. (1977) lead concentrations in the spleen may be an indicator of lead pollution. The spleen is a site of synthesis, storage and cleansing for red blood cells, which are known for their lead binding ability. There is little data about trace element concentrations in this organ.

Elevated Cd (spleen) and Cu (spleen, muscle) contents in chub in the upper part of the Biała Tarnowska River (Table 4) probably reflect the contents of these elements in the sediment (higher c. 2× for Cd and c. 4× for Cu in the upper part). They may also reflect the better bioavailability of these elements to organisms in this section of the river. Unexpectedly, the concentrations of Cd, Pb, Cu and Zn in the liver, which is regarded as a storage organ and suitable in monitoring studies, were similar in chub from both parts of the Biała Tarnowska River. The differences in metal contents between localities were pronounced in muscle of chub, although muscle usually shows low levels of accumulation of trace elements and is recognised as a poor indicator of the pollution status of water environments. Similarly, differences in trace element concentrations in chub muscle in various polluted sections of the river were also stated by other authors (Andres et al. 2000, Spurný et al. 2002, Yılmaz et al. 2007).

## CONCLUSIONS

Bioconcentrations of trace metals (Cd, Pb, Cu, and

Zn) in chub, *Leuciscus cephalus* (L.), living in the natural, submontane Biała Tarnowska River (southern Poland) and the pollution status of the river, based on the trace element contents in the sediments, were studied. The sediment samples and fish were collected from the upper (stations 1, 2, 3, and 4) and lower (stations 5, 6, 7, and 8) parts of the river. The values of  $I_{\text{geo}}$  indicated that the sediments of the river were unpolluted by Pb, Cu, and Zn, while in various degrees polluted by Cd. Despite low metal concentrations, the mean Cd (c. 2×) and Cu (c. 4×) concentrations were higher in the upper portion of the river (stations 1–4) compared to the lower portion (stations 5–8). The concentrations of Pb and Zn were similar in both sections of the river. Chub accumulated the greatest amount of Cu in the liver; Cd in the liver and spleen, while it accumulated Pb and Zn mostly in the spleen. In the upper part of the river, elevated concentrations of trace elements in selected tissues of chub (Cu - spleen, muscle; Cd - spleen; Zn - muscle) were found. Therefore, it seems that metal (Cd and Cu) concentrations in chub tissues reflect the contents of these elements in the river sediment.

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