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Heavy metals concentration in tissues of the Eurasian minnow *Phoxinus phoxinus* from the Czarna Orawa River system, Poland

Włodzimierz Popek, Michał Nowak, Jakub Popek, Stanisław Deptuła, and Piotr Epler

Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU. Corresponding author: W. Popek, e-mail: rzpopek@cyf-kr.edu.pl

Abstract. Concentration of the four heavy metals (Cu, Zn, Pb, Cd) in tissues of the Eurasian minnow *Phoxinus phoxinus* from Polish part of the Czarna Orawa River system was investigated using the AAS method. Significant differences among sampling sites were recorded, and visible trend was observed, i.e. concentrations of these metals arose from the upper most to the lower most site. Anthropogenic pollution of the river is inferred from these data. Urgent water treatment and prevention are needed to protect environment of this submontane river.
Key Words: Cadmium, copper, Czarna Orawa, lead, zinc.

Streszczenie. Metodą AAS oznaczono stężenie czterech metali ciężkich (Cu, Zn, Pb, Cd) w tkankach strzebli potokowej *Phoxinus phoxinus* z polskiego fragmentu dorzecza Czarnej Orawy. Wykazano istotne różnice w stężeniach tych metali pomiędzy stanowiskami. Uwidocznił się wyraźny trend: stężenie wszystkich metali wzrastało wraz ze spadkiem rzeki. Wywnioskowano silne antropogeniczne zanieczyszczenie tego cieku. Natychmiastowe prace oczyszczeniowe i zapobiegawcze są niezbędne dla zachowania środowiska tej podgórskiej rzeki.
Key Words: Cynk, Czarna Orawa, kadm, miedź, ołów.

Rezumat. Concentrația a patru metale grele (Cu, Zn, Pb, Cd) în țesuturile boișteanului (*Phoxinus phoxinus*) din partea poloneză a rețelei hidrografice Czarna Orawa a fost investigată prin metoda AAS. Rezultatele arată diferențe semnificative între locurile de prelevare a probelor și o tendință de creștere a concentrației tuturor celor patru metale din amonte în aval. Poluarea a fost catalogată ca fiind de natură antropică, iar tratarea ei urgentă precum și măsurile de prevenire a poluării se impun în vederea protecției mediului acvatic al acestui râu submontan.
Cuvinte cheie: cadmiu, cupru, Czarna Orawa, plumb, zinc.

Introduction. Upper part of Czarna Orawa River is one of the two rivers that drain to Danube River from the territory of Poland. Before 1960s this river was a well-known spawning place of native population of the huchen *Hucho hucho* (L.). However, due to incorrect melioration, poaching and water pollution this species has extinct in Czarna Orawa in the mid-1960s (Witkowski 2001; Witkowski et al 2007). The last adult specimens were caught in 1963 (Witkowski et al 2007). Starting from that period populations of some fishes in the Czarna Orawa River has depressed, and for now 10 species has most probably completely extinct, i.e. *Zingel zingel*, *Carassius gibelio* and *Rhodeus amarus* (all three not recorded since 1931), *Hucho hucho* (beside the later reintroductions, the species was not found in 2006 and 2007), *Lampetra planeri*, *Romanogobio kesslerii*, *Sander lucioperca* and *Cobitis taenia* (since 1989). In addition 9 following species, *Abramis brama*, *Alburnoides bipunctatus*, *Barbus barbus*, *Cyprinus carpio*, *Gobio gobio*, *Vimba vimba*, *Esox lucius*, *Anguilla anguilla* and *Lota lota*, have not been recorded since 2002 (Kulmatycki 1931; Kołder 1964; Skóra & Włodek 1989; Przybylski et al 2002; the authors, 2006-2007, unpubl.). During the last investigations a total number of 14 fish species were found in the Czarna Orawa River drainage (the authors, 2006-2007, unpubl.).

Heavy metals are well known to be widespread pollutants of water environment. They are cumulated in fish tissues, and therefore their concentration can exceed the surrounding water environment up to 20 000 times (Popek et al 2006). It is now established that heavy metals can negatively affect both quality and quantity of offspring, due to disorders in gametogenesis, disrupting the endocrine system and directly decrease of quantity and quality of gamets (Jezierska & Witeska 2001; Szczerbik et al 2006; Popek et al 2004, 2006). For example, lead disturbs pituitary LH secretion (Buettner 1993), cadmium affects the ovaries production and quality (Szczerbik et al 2006, 2008; Popek et al 2004), as well as copper (Miś et al 1995; Popek et al 2003, 2006), and zinc influences development of fish at different stages (Miś et al 1996; Popek et al 2003). All those effects, and these are only exemplifications of a wide spectrum of heavy metals influences, can lead to a serious disorganization in fish reproducing system, and in consequence to depressing of their populations. Maybe even more anxiety is fact that under the experimental conditions heavy metals significantly disrupt hatching effectiveness even in concentrations often found in nature (Popek et al 2006; Szczerbik et al 2008).

In that context, consecutive depressing of riverine fishes, poor water quality, and degradation of the river itself were the reasons of our investigation on concentration of heavy metals in tissues of the most abundant fish species in the Czarna Orawa River drainage, the Eurasian minnow *Phoxinus phoxinus*.

Plate 1. Sampling sites on the Czarna Orawa River: a, site 1, upper stretch; b, site 2, middle stretch; c, site 3, lower stretch (by W. Popek, 2007); and: d, site 4, Piekielnik Stream (by M. Patrzałek, 2007).

Material and Method. Three sampling sites were chosen along the Polish part of the Czarna Orawa River: in upper stretch near the village Podwilk (site 1), where the river is 2-3 m wide, and about 0.2-0.5 m depth; in middle stretch near Orawka (site 2), 4-6 m wide, up to 0.8 m depth; and in lower stretch below of Jabłonka City (site 3), 10-15 m wide, up to 1.2 m depth. One additional site (site 4) was chosen in Piekielnik Stream, a tributary of Czarna Orawa River (about 3-4 m wide, and about 1 m depth). See the Figure 1 for the topography of studied area, and Plate 1 for general appearance of the sampling sites. From each site 15 specimens of *P. phoxinus* were caught using a lift net. Fish were over-anaesthetised by immersion in water solution of Propiscin (Etomidate, 2 mL/L), weighed to the nearest 0.1 g, and deep frozen.

In the laboratory minnows were homogenised and mineralised in the mixture of HNO_3 and $HClO_4$ (3:1 vol.) in the temperature of 200°C (Tecator), according to standard procedures (e.g. Szczerbik et al 2006). Concentration of certain heavy metals was measured with atomic absorption spectrometry (ATI UNICAM model 929, Great Britain) using a wavelength of 213.9 nm for zinc, 324.8 nm for copper, 228.8 nm for cadmium, and 217.0 nm for lead. Fish were homogenised *in toto*, without preparation and separation of certain tissues, due to their small size (about 50-80 mm in total length; cf. Fig. 2).

Results were explored using analysis of variance (ANOVA) followed by Tukey (HSD) post-hoc test. All calculations were performed using SPSS SYSTAT (ver. 10.2) software.

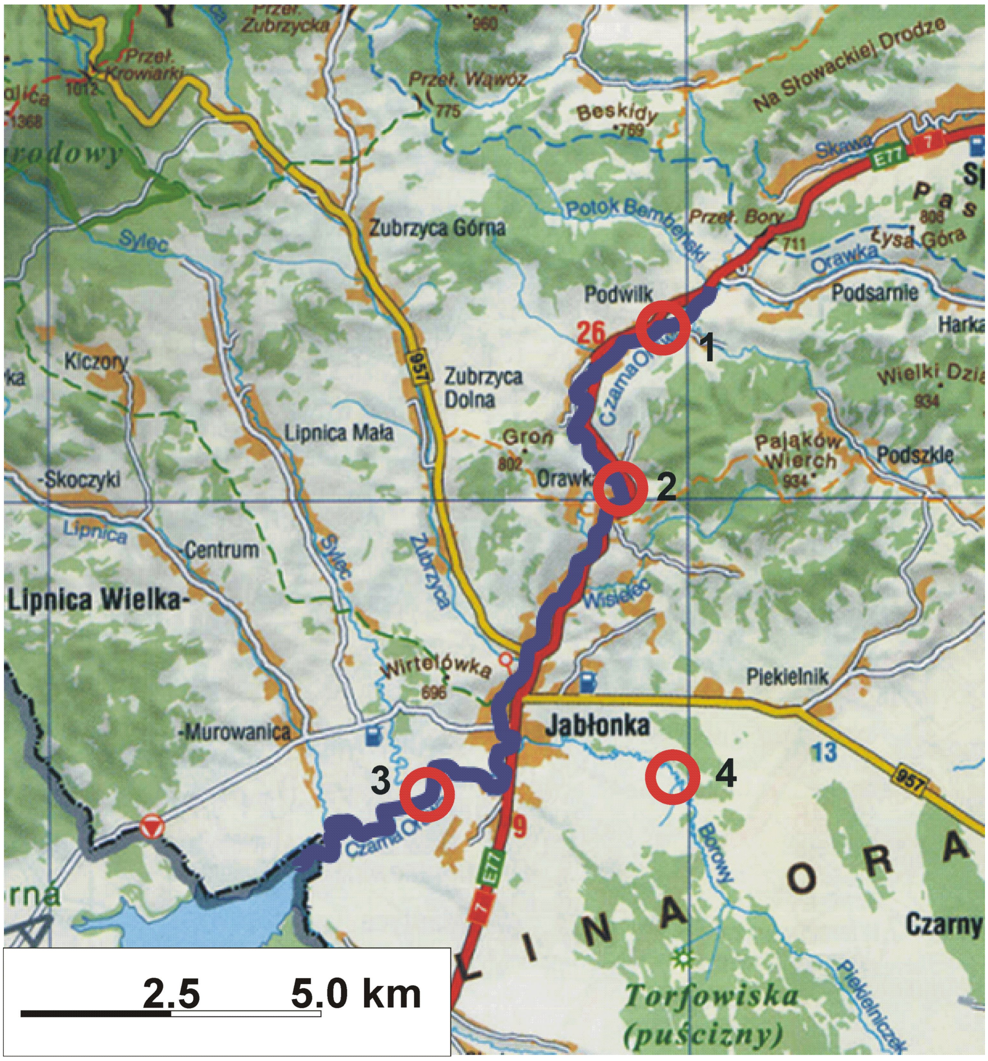

Figure 1. Area of the study with marked sampling sites: 1, Podwilk; 2, Orawka; 3, below of Jabłonka; 4, Piekielnik.

Figure 2. The Eurasian minnow *Phoxinus phoxinus* from Piekielnik Stream: female (above) and male (below) (by M. Patrzałek, 2007).

Results and Discussion. All results were summarised in Fig. 1-2. Concentration of Zn was about 100 times higher than of the other metals. Its average concentration in tissues varied from 40.1720 µg/g in minnows from Pikielnik Stream (site 4) to 53.3633 µg/g in the fish from site 3. These two were, consecutively, significantly the lowest and the highest value ($P < 0.001$). Sites 1 and 3 differed significantly ($P < 0.05$). Sites 1 and 2, and 2 and 3 did not differ significantly from each other.

Not surprisingly, concentrations of the other metals were much lower than Zn. The second was Cu, which varied from 0.6244 µg/g in site 1 to 0.7719 in site 4 ($P < 0.001$). Sites 1-2, 1-3, and 2-3 did not differ significantly, however they all differed from site 4 ($P < 0.05$).

Concentrations of Cd and Pb were much lower. The lowest concentration of Cd was observed in site 1 (0.1538 µg/g), and the highest in site 4 (0.5171 µg/g). The concentration of Cd in site 1 differed significantly from all other sites ($P < 0.001$). Other three sites did not differ significantly each other. The concentration of Pb in site 1 (the lowest value, 0.2147 µg/g) was significantly different from sites 2 and 3 (consecutively, $P < 0.05$ and $P < 0.01$). In sites 2-4 concentration of Pb was somehow higher, however without significant differences among groups, and amounted from 0.2947 µg/g in site 4 to 0.3081 µg/g in site 2. The lowest concentrations of Cd and Pb in fish from all sites were not surprising due to their highest potential toxicity (Jezierska & Witeska 2001; Szczerbik et al 2006, 2008; Popek et al 2006). Also the lowest concentrations of the metals (beside of Zn) in the site 1, the uppermost places, were understandable, because in that region sources of pollutants were relatively rare.

In general, quite well visible trend was observed. Concentration of all four metals (Zn, Cu, Cd, Pb) was growing from site 1 (the uppermost) to site 3 (the lowest one). In Piekielnik Stream (site 4) concentration of Zn was somehow lower than in the main river (sites 1-3), however these differences were not significant ($P > 0.05$). Irrespective, concentration of Cu in that tributary was significantly ($P < 0.01$) higher than in all other sites. Concentration of Pb in site 4 was higher than in site 1 ($P < 0.05$), however slightly lower (not significant) than in sites 2 and 3.

The degree of bioaccumulation of heavy metals depends on the behavior and diet of a fish, as well as varies among different tissues. It is established and rather expected that liver is always the most contaminated organ, and the muscles accumulate relatively small amount of metals (e.g. Popek et al 2006; Krywult et al 2008; the authors, in prep.). Cyprinids, as being mainly omnivorous dwellers, are thought to be more exposed on heavy metals contamination than predatory fish. And so is the Eurasian minnow, omnivorous, eurythypic species.

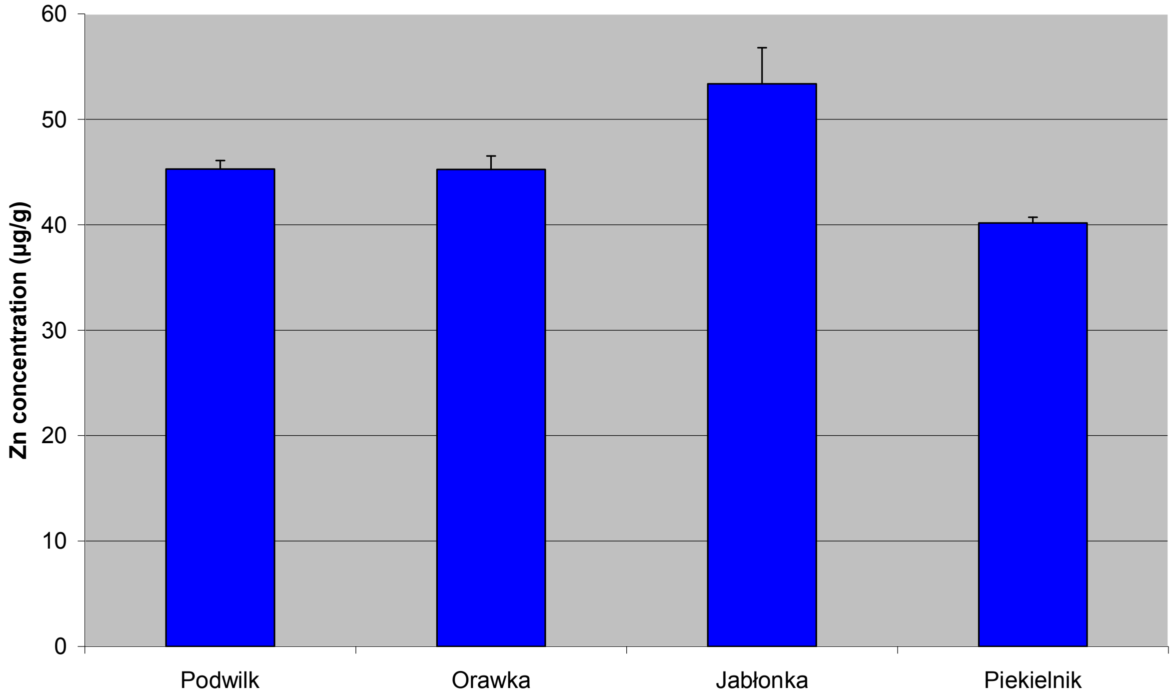

Figure 3. Zn concentration in the tissues of *P. phoxinus*. Arithmetic mean of Zn concentration (μg/g) and its SE are given.

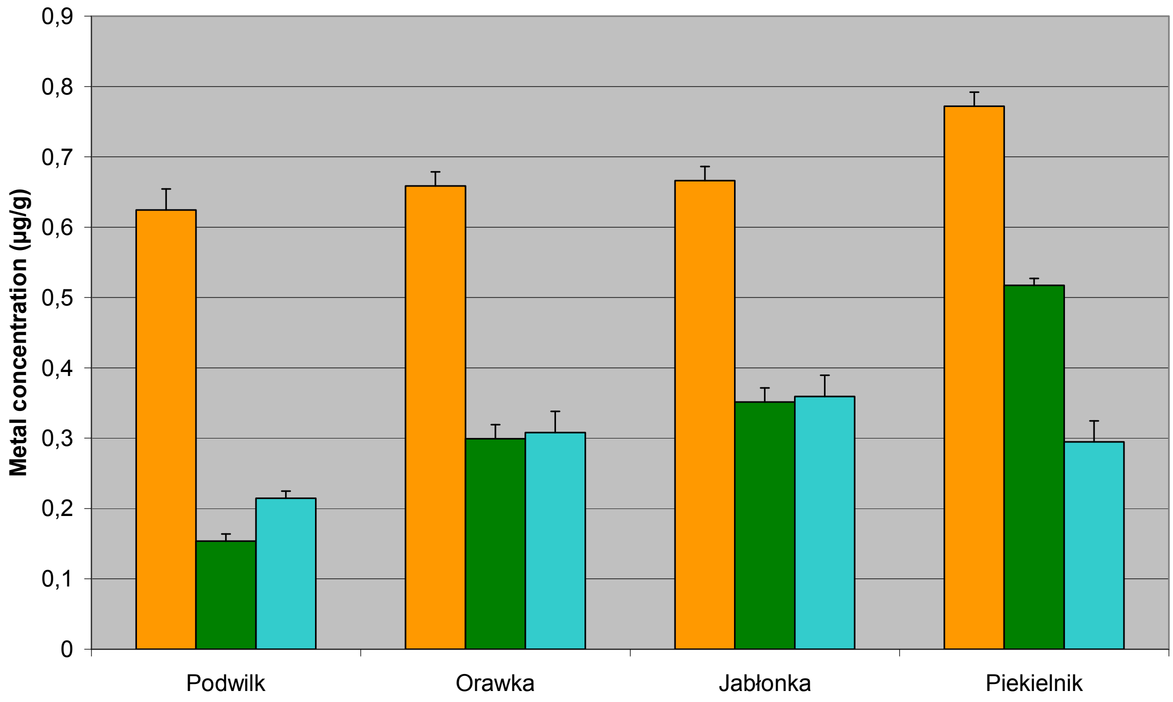

Figure 4. Cu (orange), Cd (green) and Pb (blue) concentration in the tissues of *P. phoxinus*. Arithmetic mean of each metal concentration (μg/g) and its SE are given.

In European Union maximum allowed concentrations in fish meat are: Cd 50 μg/kg wet weight, and Pb 200 μg/kg wet weight (The Commission of the European Communities 2001). Concentrations of these two metals in fish from the Czarna Orawa River system were significantly ($P < 0.01$) higher, moreover some of these norms were exceeded even more than 10 times (in Piekielnik stream concentration of Cd amounted 517.13 μg/kg).

Detected concentrations of heavy metals in tissues of *P. phoxinus* from the Czarna Orawa River system imply a conclusion about poor quality of the water. In fact, during the field expedition we observed visible pollutions (as, for example, in Plate 1a), as well as sources of these pollutants, i.e. sewer pipes emptying directly to the river. In the Orawa Region a serious problem arose together with successive urbanization of the floodplain, which was however conducted without simultaneous building of an appropriate sanitation system connected with effluent treatment plants. In that context all efforts concentrated on reintroduction of *H. hucho* in the Czarna Orawa River system seem questionable. It is also the case of conservation of other rare or rare-to-be species. Moreover, the dam that was built in 1960s in the territory of Slovakia inhibits the processes of river self-purification, so future of other fauna that inhabit the river does not rather seem optimistic.

It must be stretched herein that intensified efforts in order to increase water quality, together with other conservatory actions, are definitely expected and necessary. It is especially important in the context of conservation status of the river. Czarna Orawa River is recorded in NATURE 2000 network, according to the Habitats Directive (The Council of the European Communities 1992), as PLH120002.

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Authors:
Włodzimierz Popek, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU, e-mail: rzpopek@cyf-kr.edu.pl
Michał Nowak, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU, e-mail: mikhael.nowak@gmail.com
Jakub Popek, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU, e-mail: rzbienia@cyf-kr.edu.pl
Stanisław Deptuła, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU, e-mail: rzbienia@cyf-kr.edu.pl
Piotr Epler, Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, ul. T. Spiczakowa 6, 30-199 Kraków, Poland, EU, e-mail: rzbienia@cyf-kr.edu.pl
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