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Abstract

The Nišava River plays an important role as the source for both drinking water and agricultural irrigation dueto its hydrological and geomorphological characteristics as the largest river in the region of southeast Serbia.In this study we used the liver of the chub (*Leiciscus cephalus*) as a tool for biomonitoring heavy metalaccumulation along the river. Chub specimens were sampled from two localities (one at the border withBulgaria and a second in the city of Niš). Concentrations were estimated for six heavy metals (iron,cadmium, copper, zinc, lead and manganese) in chub liver. Low bioconcentration level was observed formost of the metals and the concentrations corresponded to the nominal concentration in livers of fishinhabiting metal unpolluted streams and rivers. However, cadmium concentration in the chub liver exceeded0.5 mg kg-1, a several hundred folds increase from nominal concentration indicating a potential toxicexposure of the fish and of the stream ecosystem to this heavy metal. Hepatosomatic indices were calculatedand tested for the impact of metal concentrations on liver size. A decrease of the hepatic index was observedin fish with higher cadmium concentration, suggesting a possible impact on the health of the chub populationin the Nišava River.

Keywords

biomonitoring, heavy metals, hepatosomatic index, Leuciscus cephalus, Nišava River

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Original Article

Assessment of heavy metal load in chub liver (Cyprinidae – *Leuciscus cephalus*) from the Nišava River (Serbia)

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The Nišava River plays an important role as the source for both drinking water and agricultural irrigation due to its hydrological and geomorphological characteristics as the largest river in the region of southeast Serbia. In this study we used the liver of the chub (*Leiciscus cephalus*) as a tool for biomonitoring heavy metal accumulation along the river. Chub specimens were sampled from two localities (one at the border with Bulgaria and a second in the city of Niš). Concentrations were estimated for six heavy metals (iron, cadmium, copper, zinc, lead and manganese) in chub liver. Low bioconcentration level was observed for most of the metals and the concentrations corresponded to the nominal concentration in livers of fish inhabiting metal unpolluted streams and rivers. However, cadmium concentration in the chub liver exceeded 0.5 mg kg^{-1}, a several hundred folds increase from nominal concentration indicating a potential toxic exposure of the fish and of the stream ecosystem to this heavy metal. Hepatosomatic indices were calculated and tested for the impact of metal concentrations on liver size. A decrease of the hepatic index was observed in fish with higher cadmium concentration, suggesting a possible impact on the health of the chub population in the Nišava River.

Key words: biomonitoring, heavy metals, hepatosomatic index, *Leuciscus cephalus,* Nišava River

Introduction

Rivers represent the most complex aquatic systems in terms of transport and interactions of heavy metals with geochemical and biological processes. Large surface to volume ratios typical of flowing waters support extensive interactions with the atmosphere, constant exchange of gases, and the introduction of material suspended in atmospheric precipitations. Oxygen saturation in the surface layer of the water allows for sustained high redox potential, causing quick degradation of organic matter and maintenance of the oxidative state of iron and manganese as suspended colloid hydroxides (Allard *et al.*, 2004; Warren & Haack 2001). Chemical assays of water and sediments have traditionally been used as tools in ecotoxicological analysis, but the variable chemistry of freshwater streams limits their application in observing cumulative effects of contaminants on the stream biota. Biomonitoring of hazardous substances in tissues of aquatic organisms have been successfully applied in recent years as indicators of heavy metal pollution (Evans *et al.*, 2000; Lamas *et al*, 2007; Teodorović, 2001; van der Oost *et al.*, 2003; Wagner & Boman 2003). Furthermore, the use of fish as bioindicators appears more time- and cost-effective when compared with the effort required for repetitive water chemistry sampling and the cost of assessing pollutants with standardized chemistry and toxicity assays (Barbour, 1999).

Water use and consumption of fish contaminated with heavy metals have deleterious effects on human health, which was widely acknowledged after a series of events in the period from 1953 to 1960 when several thousand people died in Minamata Bay in Japan as a result of poisoning caused by the consumption of mercury-contaminated fish (Smith & Smith 1975). Observed toxic effects raised public safety and human health concerns repeatedly since Minamata, prompting legislators to set limits on the lead, cadmium and mercury levels detected in the fish muscle, but other heavy metals and fish tissues were not included in the European Union (EU) regulations (European Commission, 2001; European Commission, 2002).

Fish can absorb heavy metals through epithelial or mucosal surfaces of the skin, gills and gastrointestinal tract. Metal ions can exert toxicity effects either directly at the gill surface (fast binding reactions), or passing through the gills and causing adverse effects in the internal organs (Simkiss & Taylor, 1989). However, the majority of heavy metal load is commonly ingested via water or feedstuff and transported to the bloodstream and liver via mucosal membranes and hepatic blood supply. Therefore, heavy metals are accumulated in the liver and subjected to a detoxication process. Being the primary target of metal deposition, the liver can be effectively used as a biomonitoring organ for heavy metal pollution of water ecosystems (ICES, 1991; Denny *et al.*, 1995; Jørgensen & Pedersen, 1994; Swaibuh Lwanga *et al.*,, 2003; Teodorović, 2001).

The presented study describes heavy metals loads in *Leuciscus cephalus* L. 1758 - Cyprinidae livers in the Nišava River, which is the major supply point for irrigation and drinking water to south-east Serbia. Research aims were to: *a)* determine the concentrations of six heavy metals (Fe, Cd, Cu, Zn, Mn and Pb) in *L. cephalus* liver; and *b)* assess the impact of the heavy metals on the size of *L. cephalus* liver as the major detoxification organ. Using fish livers as bioindicators of water quality in streams appears to be a rapid and economical method for screening waterways for the presence of heavy metals. Furthermore, collected information on water quality can be used to establish a broad categorization of the examined stream and has the potential to be used by environmental managers and policy makers to improve the management of the river, following the example of the Nišava River canyon section, "Sićevačka Gorge Nature Park", which is currently under strict State protection because of its biodiversity and for providing a habitat for a number of endemic and relict species.

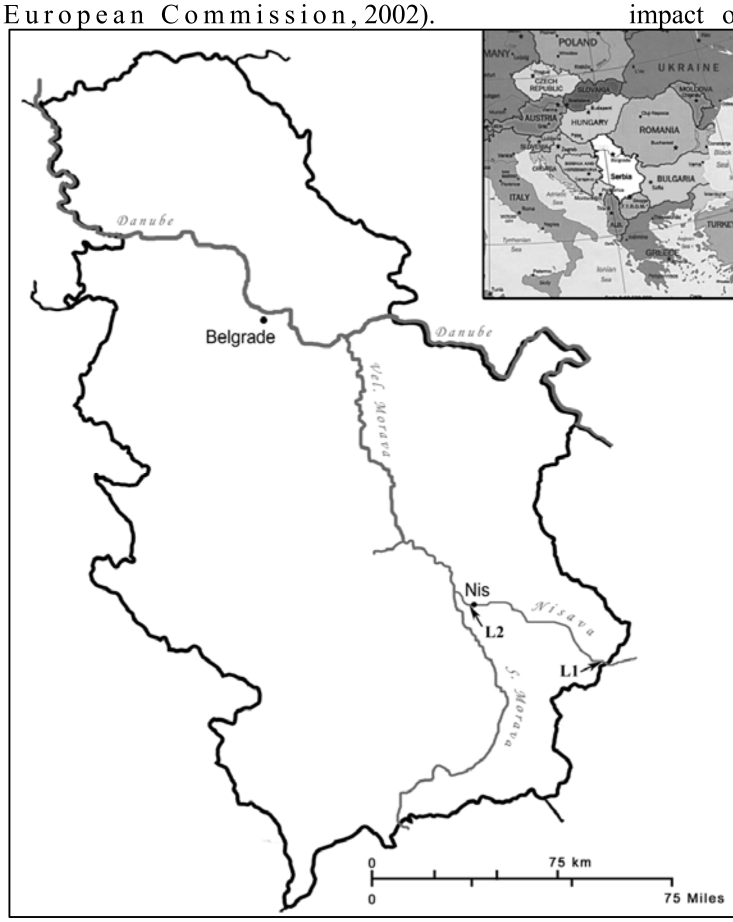


Figure 1. The geographic region of the Nišava River, The two sampling stations (L1 and L2) are indicated with arrows

Material and methods

Stream and sampling site description

The Nišava River with its tributary Ginska is a 202 km long with a watershed area of 4068 km^2. It is situated in south-east Serbia and Bulgaria (upper one fourth of the river) and it is the largest tributary of the Južna

Morava River, part of the southern Danube River watershed (Gavrilović & Dukić, 2002). The Nišava has two distinct parts: upstream of the town of Bela Palanka it is a narrow, rapid mountain stream ranging from 6 to 76 m in width and 0.6 to 2 m in depth with velocity from 1 to 2.5 m s^{-1} and flow of 0.3-270 m^3 s^{-1} of water. After Bela Palanka and all the way to its mouth near the village of Lalinac, the Nišava becomes a meandering stream with increased width (15-100 m), depth (1-3 m), and flow that carries 3-700 m^3 s^{-1} of water (Branković, 1997).

L. cephalus sampling was performed on two sites in May of 2005 (**Fig. 1**). The Nišava River at Ivko's watermill, 1 km upstream from the city of Dimitrovgrad was selected as site L1. This is the first section of the river as it enters Serbia and there are no larger settlements upstream to this sampling site. In this section the river has a width of 6 m, and a depth of no more than 1 m. The bottom is composed of gravel, and the banks are covered with thick vegetation. The majority of the benthofauna is composed of Ephemeroptera and Trichoptera larvae, while in the ichthyofauna the most dominant species are: *Alburnoides bipunctatus* Bloch, 1782 - Cyprinidae, *Barbus peloponnesius* Valenciennes, 1842 - Cyprinidae and *L. cephalus*.

As site L2, the Nišava River access point at the Greek consulate in the city of Niš was selected. The selected site is located on the last section of river, about 10 km before its mouth. The Greek consulate site is located downstream of the Niš city limits and other heavily populated areas (cities of Dimitrovgrad, Pirot, and Bela Palanka). The site was selected due to the high potential for heavy metal contamination from several upstream industrial areas. At this site, the Nišava River is about 80 m wide, and between 0.2 and 1.2 m deep. The bottom is composed of gravel, and both sides of the river are paved with no vegetation. The icthyofauna is rich, with the predominant species being *A. bipunctatus, Barbus barbus* L. 1758 - Cyprinidae, *B. peloponnesius, L. cephalus, Alburnus alburnus* L. 1758 - Cyprinidae, *Chondrostoma nasus* L. 1758 - Cyprinidae and *Rhodeus sericeus* Pallas, 1776 - Cyprinidae.

Fish sampling and tissue preparation

L. cephalus is one of the few species that inhabit the Nišava through the whole Serbian section of the river, can be collected with low catch per unit effort using only basic sport fishing gear, and its trophic level (omnivore – insectivore) potentially allows for bioaccumulation of heavy metals in the organs due to both water and nutritional intake. Chub sampling was performed by an angling technique using hook and line. Upon capture, specimens were immediately anesthetized in chloroform, euthanized, and stored in a field fridge at 0-4 °C. Wet weight and total length of each specimen was measured in the laboratory using digital scales (KERN 440-33, d=0.01g). The age of specimens was determined using standard scale method (APHA, 1995). To avoid contamination of samples, dissection was carried out using stainless steel instruments and latex gloves without talcum, and collected livers were weighed.

Analyses of heavy metals in *L. cephalus* livers

L. cephalus liver samples were treated using standard Nitric Acid-Perchloric Acid Digestion (APHA, 1995). Briefly, 10 ml of HNO_3 and $HClO_4$ has been added to the sample and heated until the samples dissolved. Dissolved sample was diluted to 100 ml with deionized water in volumetric flask. All digests were incubated for a minimum of five hours at room temperature, before being transferred to a conical flask, and then refrigerated for 48 h at 4 °C to allow complete settling before aspiration and analysis with a flame atomic absorption spectrometer (Varian, Spektr AA-10). A total of 10 samples were prepared for each locality. Concentrations of Fe, Cd, Cu, Zn, Mn, and Pb were expressed as mg kg^{-1} of wet weight, as well as cumulative concentrations (the summary of Fe, Cd, Cu, Zn and Mn).

Data analysis

Prior to any further analysis, all data were subjected to tests of normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's), and no deviations were detected. Analysis of variance (ANOVA; (StatSoft, 1996)) was used to determine differences in metal loads between localities. To determine if the size of specimens was associated with the concentrations of metals in the liver, Spearman's rank correlation tests (StatSoft, 1996) were conducted between metal concentrations from chub liver and total length of fish specimen (Swaibuh Lwanga, 2003).

The hepatosomatic index was calculated according to the formula: HSI=m/M*100, where m is the mass of the liver; and M is the mass of the fish specimen. Spearman's rank correlation test (StatSoft, 1996) was also used to explore the correlation level between the hepatosomatic index and concentrations of metals in the liver, as well as to test hypotheses as to the possible negative impacts to fish due to high metal concentrations.

Results

Total length of fish specimens ranged from 13.6 to 20.5 cm, with the age ranging from 2 years to 3 years. The smallest specimen weighed 24.6 g, while the largest one weighed 86.1 g. No correlations between fish total length and metal concentration in the liver were observed, neither with single metal concentrations nor with cumulative concentrations (Spearman's rank correlation, $P > 0.05$).

Mean concentrations of heavy metals in chub liver at the L1 site for Fe, Cd, Cu, Zn and Mn are 76.73, 0.98, 2.98, 38.83 and 3.47 mg kg^{-1} wet weight; while at the L2 site, concentrations were of the same order of magnitude: 93.95, 0.57, 6.94, 31.81 and 2.82 mg kg^{-1} wet weight, respectively (**Fig. 2**). In both sites, Pb was below the detection limit of the instrument. The comparison of data from the two sites revealed a significantly increased concentration of cadmium and zinc (**Fig. 3**) at site L1 (ANOVA: $F = 5.2$, $P < 0.05$; $F = 4.7$, $P < 0.05$ respectively). There were no differences in Fe, Cu and Mn concentrations.

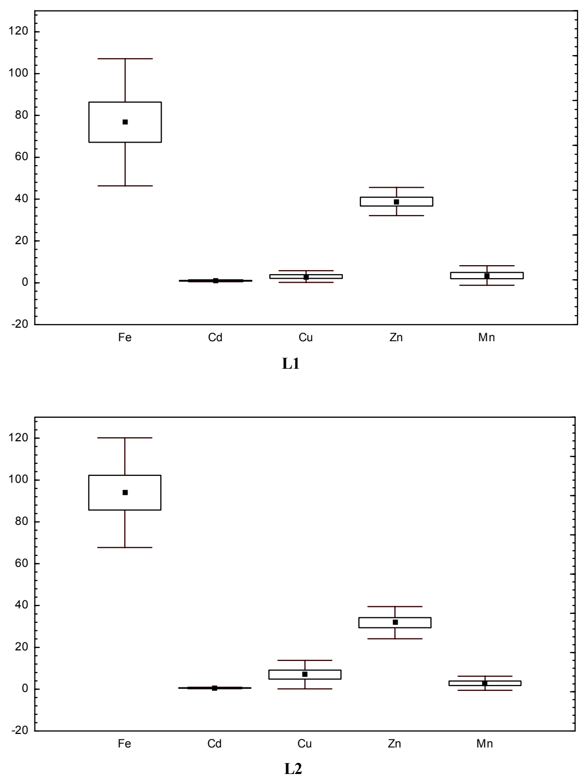


Figure 2. Heavy metal concentration in chub liver at site L1 and site L2. Values on the y-axis represent mg kg^{-1} wet weight. Boxes refer to standard errors and whiskers refer to standard deviations

Correlation tests between hepatosomatic index and metal concentration in liver can be used to infer potential deleterious effects of heavy metal bioaccumulation. The concentration of cadmium in fish collected at site L1 is strongly and negatively correlated with liver size in chub (Spearman's rank correlation, $R = -0.87$, $P < 0.001$), which suggests potential influence of a metal on fish metabolism (**Fig. 4**). All other concentrations of metals, singular and cumulative, from either site did not show any significant correlation. Hepatosomatic index was 1.36 $\pm$ 0.11 (standard error of the mean) for site L1 and 1.57 $\pm$ 0.12 for site L2.

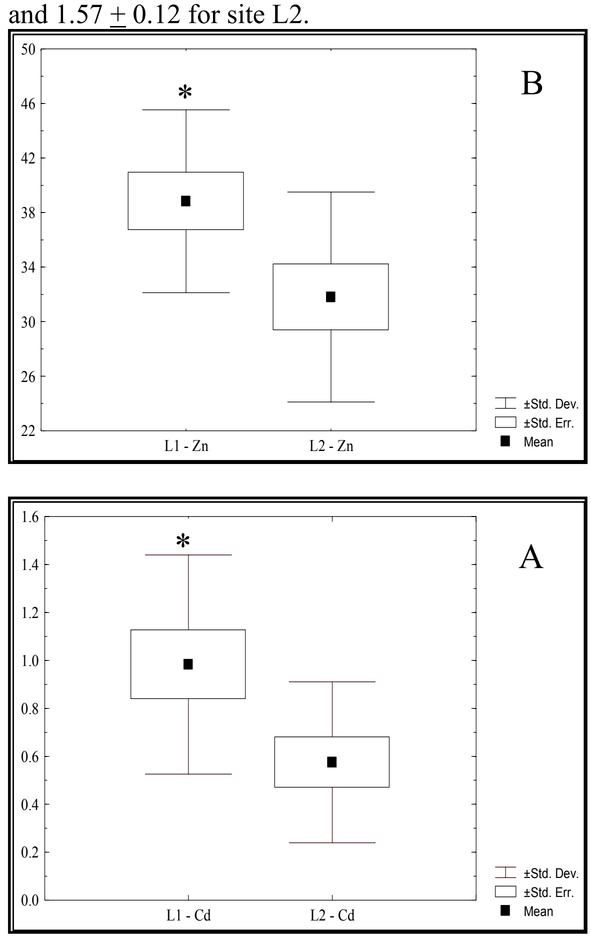
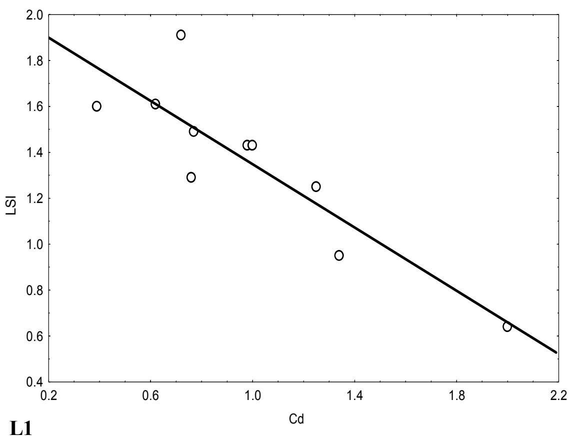


Figure 3. Differences in concentration of cadmium and zinc at localities L1 (3A) and L2 (3B). Values on the y-axis represent mg kg^{-1} wet weight. Boxes refer to standard errors and whiskers refer to standard deviations. * - indicates that the effect is statistically significant (ANOVA $P<0.05$)

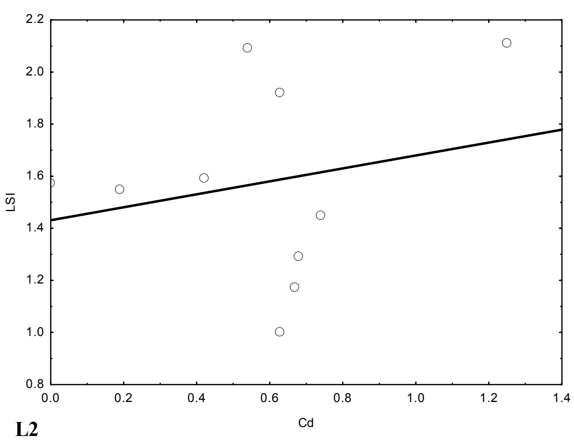
Discussion

Progressive accumulation of metal residues during the aging process in fish has been discussed recently, and results presented in this study are in accordance with published data (Swaibuh

L w a n g a *et al.*, 2003; Vinagre *et al.*, 2004). It has been demonstrated that in relatively young fish progressive accumulation is not significant (except for mercury), and it was proposed that only fish from 1+ to 4+ years old should be used for such analysis (T e o d o r o v i ć , 2001). Most of the concentrations of heavy metals in *L. cephalus* liver were close to the nominal concentrations (T e o d o r o v i ć , 2001) that are present in livers of fish inhabiting metal unpolluted streams and rivers. However, concentrations of Cd were several hundreds folds greater than the nominal concentrations (T e o d o r o v i ć , 2001).



L1



L2

Figure 4. Linear regression (Spearman's rank correlation, R = - 0.87, $P < 0.001$) of hepatosomatic index (y-axis) and concentration of cadmium in chub liver mg kg^{-1} wet weight from locality L1 (x-axis) and R= - 0.07, $P >> 0.05$ from locality L2

A recent comparable study was conducted on the Nišava River using *B. peloponnesius* as a model on four sites, including site L2 of the present study (B r a n k o v i ć , 2003), and a significantly lower concentration of lead (nil vs. 0.76 mg kg^{-1}) and cadmium (0.57 vs 1.82 mg kg^{-1}) were detected in the *L. cephalus* liver compared to *B. peloponnesius*. Lower concentrations of Pb and Cd detected after two years could suggest that the water quality had improved, but also can reflect different ecotoxicological effects of heavy metals on two analyzed species. *B. peloponnesius* is a strict benthivore species and could be more likely to accumulate heavy metals when compared to *L. cephalus*, which also feed in the upper water column.

Cadmium concentration in chub liver was higher in locality L1, which was expected to be the less polluted site. It is unclear why this phenomenon occurred, as there are no known pollution sources upstream of the site on the Serbian side of the border, unless Cd is found in the waters flowing in from nearby Bulgaria. Cadmium at observed concentrations has the potential to affect the metabolism of the fish (Fig. 4) and could account for the increase of zinc concentration in the livers on this locality (Fig. 3). Zinc is an antagonist to cadmium and adaptation has been observed that in the presence of toxic concentrations of Cd, zinc can be more rapidly absorbed by receptor spots in the gills in order to lower Cd toxicity (W i c k l u n d *et al.*, 1988).

Although the concentration of the heavy metals in the liver can be used to estimate the overall heavy metal burden in the stream that the aquatic biota can uptake, it is unrelated to food safety of the fish used in human consumption. Therefore, it is strongly advisable to compare all suspicious metal concentrations with the proposed maximal allowed concentration cited in the regulations before fish is used for this purpose. The Cd concentration in the liver was converted to muscle concentration using the bioconcentration index for carp, in order to check if there are any possible threats for human health, (C i n i e r d e C o n t o *et al.*, 1999). The maximal possible Cd concentration was used as output corresponding to 53 µg L^{-1} exposure of Cd for 127 days as described (C i n i e r d e C o n t o *et al.*, 1999). A calculated estimate placed Cd concentration in fish muscle at 0.07 mg kg^{-1} wet weight at site L1 and 0.04 mg kg^{-1} wet weight at L2. According to current EU legislation (European C o m m i s s i o n , 2002), the L1 values could represent potential threats to human health as the maximum allowed concentrations (MAC) for cadmium in freshwater fish musculature are 0.05 mg kg^{-1} wet weight. However, Serbian laws are much looser and the MAC for cadmium is 0.1 mg kg^{-1} wet weight (A N O N , 1992). Nevertheless, the observed concentrations of Cd exceed European standards on locality L1 and suggest a pressing need for frequent monitoring and a stronger environmental policy in order to maintain the water quality in the Nišava River.

In conclusion, the concentrations of cadmium and zinc are increased in *L. cephalus* livers from the upper part of the river, and Cd concentration is high enough to suggest a certain degree of effects on the metabolism of *L. cephalus*, also involving a possible buffer-like interaction with zinc. Historical information on the presence of cadmium in the upper parts of the river system is not available, and we therefore propose that further investigations should also include the Bulgarian river section. A potential risk to human health is observed due to increased Cd concentration in fish tissues collected from the upper Nišava River system.

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