BLEAK (*ALBURNUS ALBURNUS*) AS POTENTIAL BIOINDICATOR OF HEAVY METAL POLLUTION

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UKLIJA (*ALBURNUS ALBURNUS*) KAO POTENCIJALNI BIOINDIKATOR ZAGAĐENJA TEŠKIM METALIMA

Apstrakt

Analize vode i/ili sedimenta mogu biti neefikasne u identifikovanju metala u fluvijalnim ekosistemima usled nerazdvojive varijabilnosti rečnog toka i koncentracije zagađivača. Monitoring zagađenja ribljih tkiva ima važnu ulogu ranog alarma koji ukazuje na probleme kvaliteta vode i sedimenta, a takođe omogućava detekciju toksičnih materija u ribama koje dalje mogu da imaju negativan efekat na konzumente.

Reka Sava je tipična nizijska reka i najveća desna pritoka Dunava koja protiče kroz tri zemlje: Sloveniju, Hrvatsku i Srbiju. Do 1990-ih je bila izložena zagađenju iz metalurgije, hemijske, kožne, tekstilne, prehrambene i industrije celuloze i papira, ali i usled poljoprivrednih aktivnosti. Takođe je i glavni recipijent otpadnih voda mnogih gradova i zagađenih pritoka.

Ciljevi ove studije su da se utvrde koncentracije Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn i Zn u kompostu uklije (*Alburnus alburnus*) kao potencijalne bioindikatorske vrste zagađenja teškim metalima reke Save, kao i da se izračuna Indeks zagađenja metalima (MPI-*Metal Pollution Index*) kako bi se uporedio status zagađenja različitih lokaliteta reke Save.

Terensko istraživanje je sprovedeno tokom septembra 2014. godine. Uklije su sakupljene iz reke Save na sledećim lokalitetima: Čatež u Sloveniji, Zagreb i Slavonski Brod u Hrvatskoj, Jarak i Umka u Srbiji. Koncentracije Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, i Zn su merene u kompozitnom uzorku uklije uz pomoć Thermo Scientific iCAP 6500 Duo ICP-OES (Thermo Fisher Scientific, Cambridge, United Kingdom).

Prema dobijenim rezultatima, izdvaja se Zagreb sa najvećim brojem najviših koncentracija elemenata (Cd, Co, Cr, Fe, Pb, i Se). Takođe, najviši MPI je izračunat za Zagreb (0.23) u odnosu na Umku sa najnižim MPI (0.14). Na osnovu dobijenih rezultata, možemo označiti

gornji tok reke Save kao znatno zagađeniji (posebno deo kod Zagreba u Hrvatskoj) od donjeg toka i ušća u Dunav kod Beograda (Umka).

Ključne reči: reka Sava. uklija, bioindikator, indeks zagađenja metalima
Keywords: Sava River, bleak, bioindicator, metal pollution index

INTRODUCTION

Among the various contaminants, heavy metals in rivers has become a matter of great concern, due to environmental persistence, biogeochemical recycling and ecological risks, the threat it poses to public water supplies, and also because of the hazard to human consumption of fishery resources (Terra et al., 2008).

Water and/or sediment analysis may be inefficient at identifying metal inputs to fluvial systems of the inherent variability of flow and contaminant concentrations (Ricart et al., 2010). Fish tissue contamination monitoring has an important role as an early warning indicator regarding problems related to water and sediment quality, and it also enables detection of toxic chemicals in fish, which can produce adverse effects on consumers. Thus, such monitoring allows taking appropriate and timely measures to protect public health and the environment (Lenhardt et al., 2012).

The Sava River as typical lowland and the largest right side tributary of the Danube River is located on the southern fringe of Central Europe. It flows through three countries: Slovenia, Croatia and Serbia. Until the 1990s it was affected by heavy pollution from the metallurgical, chemical, leather, textile, food, cellulose and paper industries, as well as from agricultural activities. Also Sava is the main recipient of waste water from many cities and it is impacted by the polluted water of the tributaries.

The aims of this study were to determine the concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn in the whole body composite sample of bleak (*Alburnus alburnus*) from Sava River as potential fish bioindicator of heavy metal pollution and to calculate Metal Pollution Index (MPI) in order to compare the pollution status of different locations and to establish locations with the highest or the lowest level of pollution in Sava River.

MATERIALS AND METHODS

The field work was conducted during the September of 2014. Bleak specimens were collected on different locations from Sava River: Čatež (Slovenia), Zagreb and Slavonski Brod (Croatia), Jarak and Umka (Serbia). In the field, all samples were washed with distilled water and in plastic bags transferred to the laboratory. In the laboratory, all samples are measured, length (to the nearest cm), and weigh (to the nearest g), grinded in a Laboratory homogenizer Sterilmixer (International P.B.I. S.p.A.) and whole body composite (wbc) sample stored at -20°C prior to analysis. In the laboratory, fish samples (~1.5 g) were dried in a lyophilizer (Christ Alpha 2-4 LD, Harz, Germany), and then digested in an Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using a mixture of 65% nitric acid and 30% hydrogen peroxide (Merck, Darmstadt, Germany, 10:2 v/v) at 220°C for 20 min. After cooling to room temperature and without filtration, the solution was diluted to

a fixed volume (volumetric flask, 25 mL) with deionized water. Concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn were measured in wbc of bleak using a Thermo Scientific iCAP 6500 Duo ICP-OES instrument (Thermo Fisher Scientific, Cambridge, United Kingdom). The potential presence of trace elements in chemicals used in sample preparation was resolved by using a number of blank samples. Standards for the instrument calibration were prepared on the basis of multi-element (SS-Low Level Elements ICV Stock, 10 mg/L) and mono-element (Hg Calibration Stock, 10 mg/L Hg; Sn LSN-100, 10 mg/L Sn) certified reference solution ICP Standard (VHG Labs, Inc-Part of LGC Standards, Manchester, NH 03103 USA). The detection limits for Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Se were: 0.1, 0.00022, 0.00047, 0.00005, 0.003, 0.0028, 0.0018, 0.00027, 0.0025, 0.007, 0.0093, 0.0001, 0.00027, 0.109 $mgkg^{-1}$, respectively.

The analytical process quality control, performed by the use of fish protein certified reference material for trace metals DORM 4 (NRCC, Canada), indicated that the resulting concentrations were within 85.8-116.25%. There were no certified values for Al, Mn, and Co. Concentrations of all metals were expressed as mg kg^{-1} wet weight basis (ww).

In order to assess significant differences between the levels of elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn) at five different locations, non-parametric Kruskal-Wallis test was applied. Post hoc inter-group comparisons of element levels (between pairs of locations) were performed by the non-parametric Mann-Whitney test for two independent samples. All statistical analysis of data was carried out using SPSS 16.0 statistical package programs for Windows (SPSS Inc., Chicago, IL, USA).

The metal pollution index (MPI) was calculated to compare the total metal content in the different sampling sites using the following equation (Usero et al., 1997):

$$MPI = (Cf_1 \times Cf_{2\ldots} Cf_n)^{1/n} \text{ where } Cf_n = \text{concentration of the metal } n \text{ in the sample.}$$

RESULTS AND DISCUSSION

The main characteristics (number of individuals, total body length, and weight) and average element concentrations ($mgkg^{-1}$) determined on the wet weight basis in the wbc of bleak are summarized in Table 1.

Muscles are often a major tissue of interest for routine environmental monitoring. However, since they are not always the best indicators of element contamination present in

fish, the analysis of other tissues is recommended as well (Has-Schön et al., 2006). Gills are the primary site of metal uptake from water (Dogru et al., 2011), especially if metals are bound to particulate matters (Klavins et al., 2009), while the liver as metabolically active tissue is the accumulation place of metals (Yilmaz et al., 2007). The accumulation in muscle tissue is, except Hg, usually lower or the lowest (Jarić et al., 2011; Poleksić et al., 2010; Višnjić-Jeftić et al., 2010).

Table 1. The main characteristics of bleak and average element concentrations (mean ± SD) determined on the wet weight basis in the wbc of bleak from Sava River

Ecosystem/ Metal	Čatež (n* = 10)	Zagreb (n = 10)	Slavonski Brod (n = 10)	Jarak (n = 10)	Umka (n = 10)
total body length (cm)	12.33 ± 0.65	11.68 ± 0.96	10.04 ± 1.55	12.01 ± 1.31	11.21 ± 1.64
weight (g)	12.6 ± 2.27	10.16 ± 3.2	6.8 ± 3.03	12 ± 4.92	9.4 ± 5.38
Al	3.31 ± 0.18[a]	2.92 ± 2.42	2.08 ± 0.88	1.55 ± 0.43	1.79 ± 0.93
As	0.15 ± 0.06	0.12 ± 0.025	0.13 ± 0.09	0.12 ± 0.04	0.1 ± 0.086
Cd	0.008 ± 0.002	0.1 ± 0.002[a]	0.016 ± 0.008	0.008 ± 0.002	0.013 ± 0.008
Co	0.0007 ± 0.0006	0.001 ± 0.0007	0.0008 ± 0.0004	0.0005 ± 0.0004	0.0006 ± 0.0004
Cr	0.17 ±0.022	0.18 ± 0.045	0.154 ± 0.024	0.18 ± 0.03	0.18 ± 0.02
Cu	0.48 ± 0.05	0.67 ± 0.12[a]	0.68 ± 0.34[a]	0.43 ± 0.12	0.44 ± 0.14
Fe	9.74 ± 3.2	14.5 ± 8.27	11.44 ± 6.67	9.21 ± 2.71	8.76 ± 3.44
Hg	0.02 ± 0.004[a]	0.013 ± 0.003	0.011 ± 0.003	0.013 ± 0.004	0.011 ± 0.004
Mn	1.37 ± 0.48	1.55 ± 0.71	1.2 ± 0.47	1.6 ± 0.63	1.73 ± 0.72
Ni	0.02 ± 0.004	0.032 ± 0.033	0.034 ± 0.01	0.047 ± 0.064	0.025 ± 0.015
Pb	0.11 ± 0.02	0.17 ± 0.03	0.1 ± 0.01	0.12 ± 0.02	0.11 ± 0.26
Se	0.24 ±0.06	0.34 ± 0.066[a]	0.11 ± 0.025	0.08 ± 0.033	0.085 ± 0.04
Sn	0.016 ± 0.0007	0.016 ± 0.0008	0.015 ± 0.002	0.017 ± 0.002	0.016 ± 0.002
Zn	24.74 ± 7.2	24.47 ± 6.6	23.48 ± 4.82	23.1 ± 4.07	23.09 ± 6.65

[*] the number of sampled bleak per location

[a] the value with this letter is significantly different ($p < 0.05$)

Although literature data indicate that different tissues of the fishes showed significant difference for heavy metal accumulation, we used wbc to assume water pollution with heavy metals. The Kruskal-Wallis test revealed significant differences between locations in regard to Al, Cd, Cu, Hg, and Se concentration ($p < 0.05$). The post hoc Mann-Whitney test showed that concentrations of Cd, Cu, and Se were significantly higher ($p < 0.05$) in Sava River near Zagreb in Croatia, while concentrations of Al and Fe were significantly ($p < 0.05$) higher in Sava River near Čatež in Slovenia (Table 1).

MPI has been calculated to enable presentation of all results from the element concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn) as one value, with both application and understanding of demanding statistical analysis. The highest MPI was calculated for Zagreb; followed by Čatež, while the lowest was calculated for Umka (Figure 1). It follows that Zagreb site is the most polluted area, while Umka site is the least polluted compared to other areas. According to MPI values, it can be seen that the highest MPI values were recorded for the Zagreb location. To conclude, on the basis of these results, we can mark upper stream of Sava River as more polluted than lower reaches of Sava River.

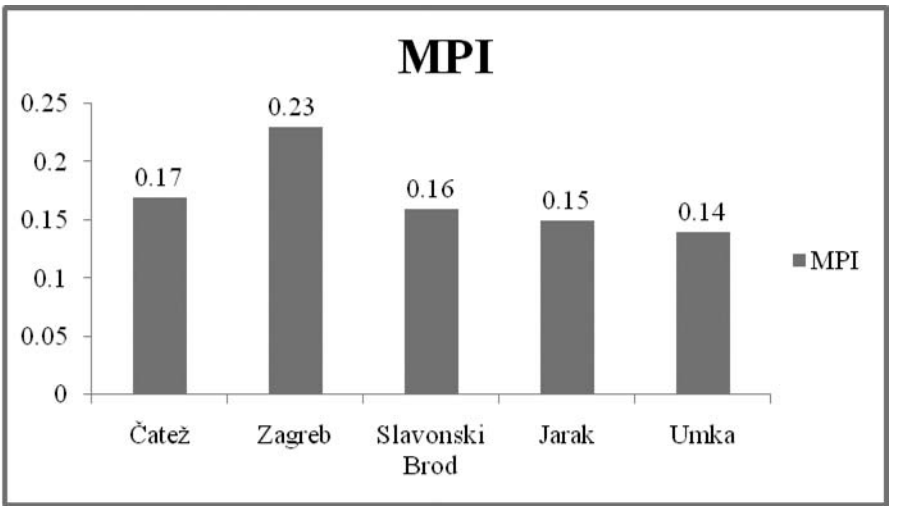

Figure 1. MPI of each examined location from Sava River

The maximum permitted levels (MPC) prescribed by the National Regulation of the Republic of Serbia (28/2011) for Pb, Cd, Hg, Cu, and Zn are 0.3 $mgkg^{-1}$, 0.05 $mgkg^{-1}$, 0.5 $mgkg^{-1}$, 30 $mgkg^{-1}$ (in tin containers), and 100 $mgkg^{-1}$ (in tin containers), respectively (Official Gazette of FRY, No 28/2011) and those prescribed by the EU Regulation (1881/2006) for Pb, Cd, and Hg are 0.3 $mgkg^{-1}$, 0.05 $mgkg^{-1}$, 0.5 $mgkg^{-1}$. This indicates that the bleak samples, except samples from Zagreb where elevated concentration of Cd (0.1 $mgkg^{-1}$) were found, should be safe for utilization in human diet.

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