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Traces of Cu, Mn and Zn in Aquatic Animals, Water and Sediments from the Cris River Basin – West Romania. Part II: Distribution Study

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The paper presents the determination of Cu, Mn and Zn (TRT r.f.CCP-AES, FAAS) in water, sediment and aquatic animals (fish fillet, freshwater molluscs gills and muscles) collected in the basin of the Cris rivers (White Cris and Black Cris) in West Romania. The concentrations in water were in the range: 1–15 μg L^{-1} (Cu), 10–1500 μg L^{-1} (Mn), 3–100 μg L^{-1} (Zn). Metal contents, as dry mass fractions, $w_{dm} \times 10^6$, were: 5–380 (Cu), 225–2000 (Mn), 23–1140 (Zn) in sediment; 1–11 (Cu), 4–40 (Mn), 8–130 (Zn) in fish fillet, and 5–34 (Cu), 100–600 (Mn) and 50–130 (Zn) in mollusc muscles with higher values for the White Cris samples. Among the seven sample collection sites only one was identified as having concentrations of Mn and Zn in water higher than the admitted levels, but the limits of tolerance for aquatic organisms were not exceeded in either river. In mollusc gills, the metal contents expressed as $w_{dm} \times 10^6$ were: 8–60 (Cu), 11000–16000 (Mn), 190–1200 (Zn), and similar for both rivers in the case of Mn.

Key words: environment monitoring; biological samples; sediments; water; copper, manganese and zinc determination.

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INTRODUCTION

This article is an extension of a previous paper that provides a comparison of the analytical performance and a statistic evaluation of Cu, Mn and Zn determinations in sediment and biological samples by radiofrequency capacitively coupled plasma atomic emission spectrometry (r. f. CCP-AES) and the well-established flame atomic absorption spectrometry (FAAS).[1] The plasma source was operated in coaxial-annular geometry with two ring electrodes (TRT) spaced at different distances, with low power (275 W) and low Ar consumption (0.4 L min^{-1}). For 60 mm distances between ring electrodes in the case of Cu and Mn, and 70 mm for Zn, the real limits of detection considering the depressive effect of NaCl and $CaCl_2$ were 2 (Cu), 6 (Mn) and 3 (Zn) μg g^{-1}.

Statistical analysis based on the F-test, regression analysis and the Bland and Altman test showed satisfactory agreement between the two methods, both in accuracy and reproducibility, for Cu, Mn and Zn determinations.[2,3] This suggests that TRT r.f.CCP-AES could be an alternative to FAAS in the analysis of some metals in environmental samples. The studied plasma source offers the advantage of a low Ar consumption and the possibility of simultaneous determination compared to atomic absorption spectrometry.

This paper presents the determination of Cu, Mn and Zn in the basin of the White Cris and Black Cris rivers, which collect the rivulets from the southwest side of the Apuseni Mountains in West Romania. The aim was to investigate the pollution effect of the mining industry in this mountainous zone. In order to get a complete picture of the aquatic system under study, the present paper correlates the contents of Cu, Mn and Zn in aquatic organisms with that in the related water and sediment. In sediments and aquatic organisms, determinations were done by both r.f.CCP-AES in TRT configuration and FAAS, while in water samples Cu, Mn and Zn were determined only by FAAS.

The Cris river, an important tributary of the Tisa river, collects water in a catchment area of about 27.500 km^2 in West Romania and East Hungary. Two of the Cris tributaries, the White Cris (total length 248 km, of which 238 km in Romania) and Black Cris (total length 168 km, of which 144 km in Romania) form, together with other rivers, the Körös in Hungary. No natural pollution is present in the drainage area of the two rivers. Deterioration of the water quality might be caused by antropogenic pollution in the mining district of the upper and middle sections of the Cris rivers. Thus, ores containing Fe and Mn (black square on the map) and complex ores containing Cu and Zn (triangle on the map) are extracted in this area. These ores are processed to be concentrated at Brad and Stei (ring on the map). The entire area is rich in barytine, polychrome limestone, quartzite and clay. The mountainous zone is known for its tourist attractions, especially for its natural reservations with karst relief, water springs and protected botanical species.[4–6]

In our study, we chose molluscs (*Unionidae*) and fish to represent aquatic animals. Freshwater molluscs are relatively sedentary animals, which filter large volumes of water and are particularly known as bioaccumulators of high levels of toxic heavy metals. Hence, their use as aquatic bioindicators is recommended.[7] Freshwater molluscs play an important role in the natural purification of river water by filtration. Fish have been included into our study to establish their suitability for human consumption.

In the upper zone of the White Cris and Black Cris rivers, *Unionidae* freshwater molluscs are present in small groups and their number decreases in intensively polluted sections. Water pollution as a result of the mining industry has caused a notable disappearance of the molluscs in the upper section of both rivers (Brad, Stei). In the middle zone or unpolluted sections, a significant abundance and diversity of molluscs has been noticed in the riverbed. The purpose of our study was to detect this phenomenon, by determining the heavy metal levels in the aquatic organisms collected in these areas and in downstream sections still populated with freshwater molluscs.

Seven species of *Unionidae* molluscs have been identified in the White Cris and Black Cris rivers, from which three species were selected for our determinations: *Unio crassus, Anadonta woodiana* and *Pseudanodonta complanata*. *Unio crassus* is the most abundant species throughout the rivers under study, probably owing to its resistance to the polluting effect. *Unio crassus* is a protected species in Europe. *Pseudanodonta complanata* appears sporadically, being more vulnerable to pollution, while *Anadonta woodiana* existing especially in the lower sections of the rivers is more resistant to pollution. As for fish, the species *Alburnus alburnus*, *Gobio albipinatus* and *Stizostedion lucioperca* are specific to all rivers.[8,9]

EXPERIMENTAL

Sample Collection and Digestion Procedure

Water, sediment and aquatic organisms were collected from seven sites on each river, marked on the map as riverbank localities, with a relatively uniform distribution (Figure 1). This allowed a correlation of the distribution of Cu, Mn and Zn contents in water, sediment and aquatic animals with a possible polluting effect from the ore processing centers (Fe, Mn, Cu, Zn). Freshwater molluscs were collected only at five sites about 50 km from the spring.

Water samples were collected manually into polyethylene bottles. Prior to use, all bottles were cleaned with 10% HNO_3, rinsed with distilled water and water to be analyzed. For each sampling site, three portions taken at the surface level and three

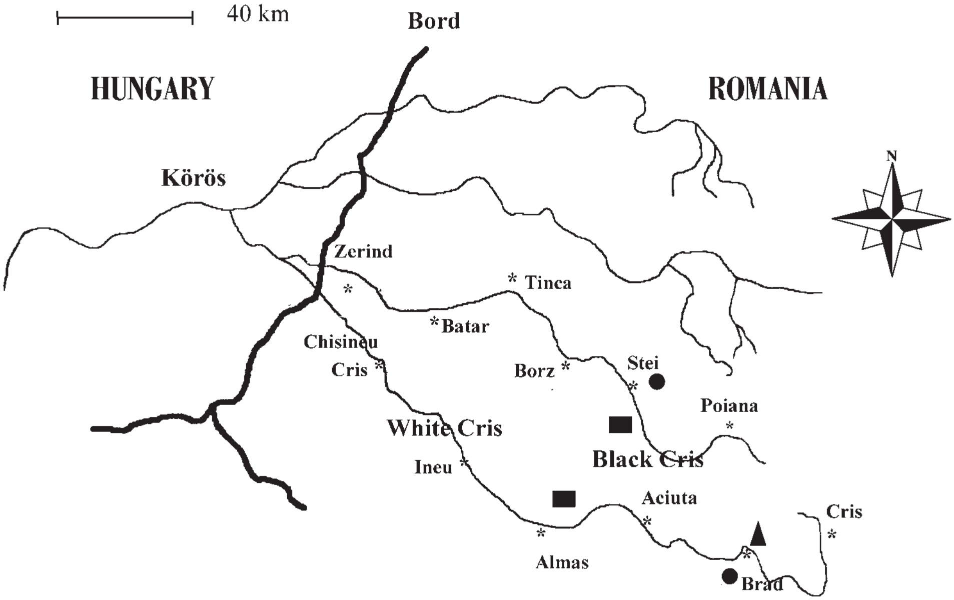

Figure 1. Sampling sites for water, sediments and aquatic organisms on the White Cris and Black Cris rivers: ■ mining centers for Fe and Mn ores; ▲ mining center for Cu and Zn complex ores; ● ore processing centers.

at mid-depth level were mixed. After sedimentation, they were transferred directly to bottles and acidified to a pH < 2 by adding concentrated nitric acid. Since the aim was to study the impact of non-filtrated water on aquatic animals, filtration was eliminated. The samples were then stored at 4 °C in polyethylene bottles.

Dissolution of sediments, molluscs and fish samples was carried out according to the methodology described in the first paper.[1] The reagents used to prepare 1000 μg mL^{-1} stock solutions of Cu, Mn and Zn and those used for sample dissolution (HCl, HNO_3 and H_2O_2) were of *puriss* quality (Merck, Darmstadt, Germany).

Instrumentation

The microwave system used for biological samples preparation was Milestone (MLS-1200 MEGA, Sorisole, Italy) with a Teflon vessel (MRD 1000/6/100/110).

Determinations by AAS were performed with a PHILIPS Atomic Absorption Spectrometer PU9180, equipped with an air-acetylene flame unit.

Determinations in AES were performed using a low powered Ar TRT r.f.CCP source (Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania) operated at 275 W and with low Ar consumption, 0.4 L min^{-1}.[10] The experimental set-up and other operating conditions are identical to those used in the study comparing the two techniques.[1]

RESULTS AND DISCUSSIONS

Distribution Study of Cu, Mn and Zn in Water and Sediment

The distribution content of Cu, Mn and Zn in water, sediment and biological samples from the two rivers is shown in Figures 2.a–7.a while Table I presents the concentration range of these elements. Data in Table I show that the contents of Cu, Mn and Zn in both water and sediment are higher in the White Cris than in the Black Cris but the water tolerance levels for aquatic animals are not exceeded. Figures 2a; 4a; 6a (curves 2) show the presence of a maximum of Cu, Mn and Zn contents in the White Cris water at the Brad site where there are several mining and ore processing centers. In the case of Mn, a secondary maximum is visible downstream, probably due to the presence of Fe and Mn mines in the area. According to the Romanian regulations, the admitted values in surface water are: 50 (Cu), 30 (Zn) and 100–800 (Mn) μg L^{-1}. In the case of Cu, the maximum admitted level is not exceeded throughout the White Cris, while the contents of Zn and Mn are at least three and two times higher than the admitted levels at Brad (Figures 4a, 6a, curves 2 and Table I).

Even when the Cu concentration in water at Brad is close to the upper limit of tolerance for aquatic organisms (15 μg L^{-1} Cu), there is no risk since acceptable levels of alkaline salts and dissolved oxygen act against the Cu toxicity. In the case of Mn and Zn, even when the admitted levels are exceeded, their content in water is at most equal (Mn) or far (Zn) from the upper tolerated value and there is no risk for aquatic organisms. Problems arise only in relation with the use of water in agriculture in these areas.

For all the above-mentioned elements, the maximum levels in the Black Cris water correspond to the site downstream from the Stei mining center. As compared to the White Cris, the metal contents in the Black Cris are 2–10 times lower and do not exceed the admitted levels (Table I, Figures 3a, 5a, 7a, curves 2).

Variations of Cu, Mn and Zn contents in sediment (Figures 2.a–7.a, curves 1) show the same trend as in water, but supplementary maxima appear owing to the sedimentation process. Obviously, there is an equilibrium of the three elements' distribution between the solid and liquid phases depending on chemical and physical factors. Both the temperature and the pH-level influence the equilibrium:

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$

in river water. The two parameters slightly increase downstream from the spring (pH from 6.5 to 7 and the temperature from 14 to 24 °C) and the equilibrium is shifted to the right favoring precipitation as carbonates.

TABLE I

Concentration range for Cu, Mn and Zn in water, sediment, fish fillet, freshwater mollusc muscles and gills from the White Cris and Black Cris rivers

Sample	Cu		Mn		Zn	
	White Cris	Black Cris	White Cris	Black Cris	White Cris	Black Cris
	conc. / μg L^{-1}					
Water[a,b]	2–15	1–4	20–1500	10–140	10–100	3–12
	$w_{dm} \times 10^6$					
Sediment	5–380	5–27	225–2000	235–620	30–1140	23–240
Fish fillet[c]	3–11	1–3	4–40	4–8	55–130	8–50
Mollusc muscles	6–34	5–17	100–600	200–400	70–130	50–100
Mollusc gills	43–60	8–30	13000–16000	11000–15000	800–1200	190–363

[a] Admitted values in surface water according to Romanian regulations: 50 (Cu), 30 (Zn) and 100–800 (Mn) μg L^{-1}; STAS 4706-88.

[b] Concentration levels considered tolerated for aquatic animals: Cu 5–15 μg L^{-1}, Mn 500–1500 μg L^{-1}, Zn 10–1000 μg L^{-1}.[7]

[c] Typical concentrations of Cu, Mn and Zn as dry mass fractions, $w_{dm} \times 10^6$, in aquatic animal tissue were reported to be 4–50, 1–60 and 6–1500, respectively.[11]

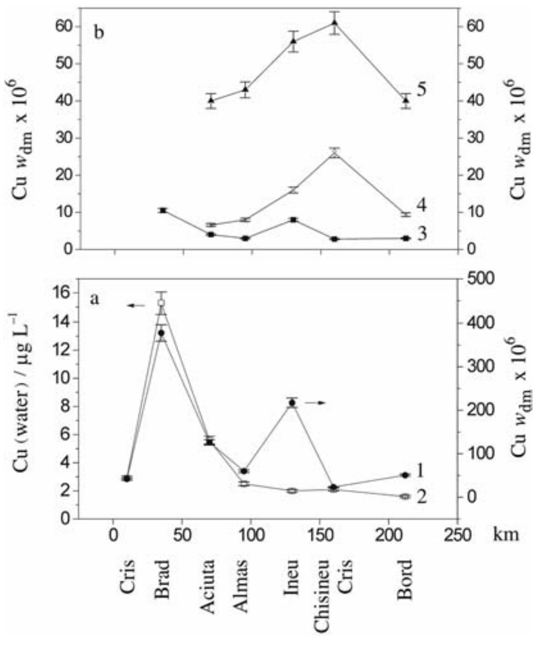

Figure 2. Variation of Cu content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the White Cris. $w_{dm} \times 10^6$, dry mass fraction.

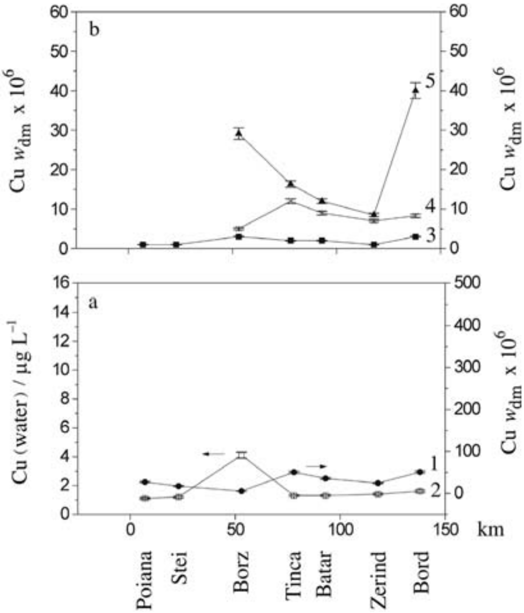

Figure 3. Variation of Cu content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the Black Cris. $w_{dm} \times 10^6$, dry mass fraction.

Manganese could also be oxidized to MnO_2 by either Mn^{VII} or other oxidants. This phenomena could explain the presence of a supplementary maximum in the middle course of the White Cris river.

Distribution Study of Cu, Mn and Zn in Aquatic Animals

The concentration ranges for Cu, Mn and Zn in fish fillet, freshwater mollusc muscles and gills collected from the White Cris and Black Cris rivers are given in Table I. Figures 2.b–7.b show their variation. The biological samples analyzed for Cu, Mn and Zn were 3 fish species (*Gobio Albipinatus, Alburnus alburnus* and *Stizostedion Lucioperca*) and 3 species of freshwater molluscs (*Unio crassus, Anodonta woodiana* and *Pseudanodonta complanata*). Variations of Cu, Mn and Zn contents in fish fillet (Figures 2.b–7.b curve 3) occur in a relatively narrow range and closely follow the trend in water and sediment with maxima generally corresponding to the major mining centers. The content of all three elements is higher in the fish fillet from the White Cris, the maximum difference being noted for Mn. All determined levels are within the normal range for aquatic animals. Typical concentrations of Cu, Mn and Zn in aquatic animal tissue expressed as dry mass fractions ($w_{dm} \times 10^6$) were 4–50, 1–60 and 6–1500, respectively.[11]

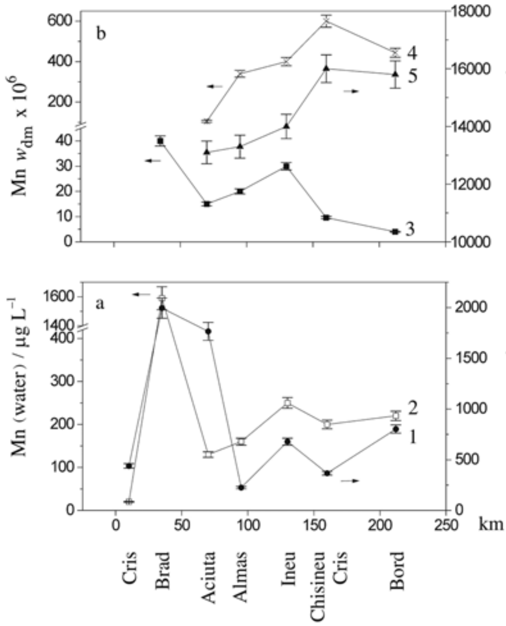

Figure 4. Variation of Mn content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the White Cris. $w_{dm} \times 10^6$, dry mass fraction.

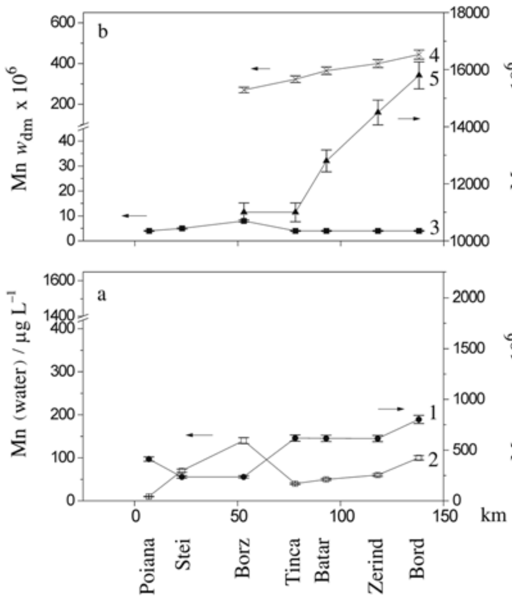

Figure 5. Variation of Mn content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the Black Cris. $w_{dm} \times 10^6$, dry mass fraction.

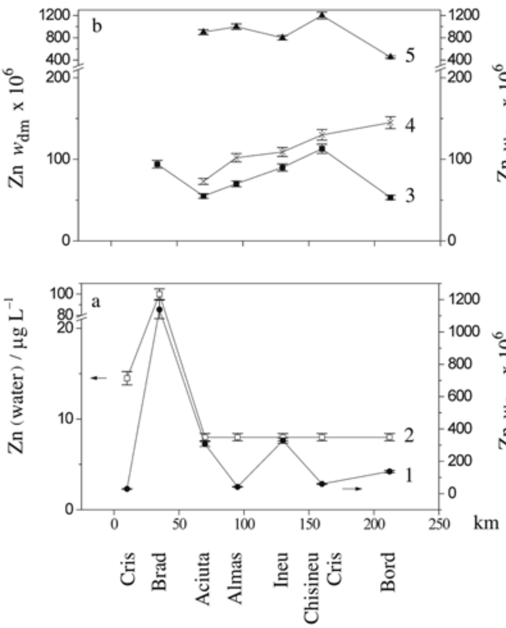

Figure 6. Variation of Zn content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the White Cris. $w_{dm} \times 10^6$, dry mass fraction.

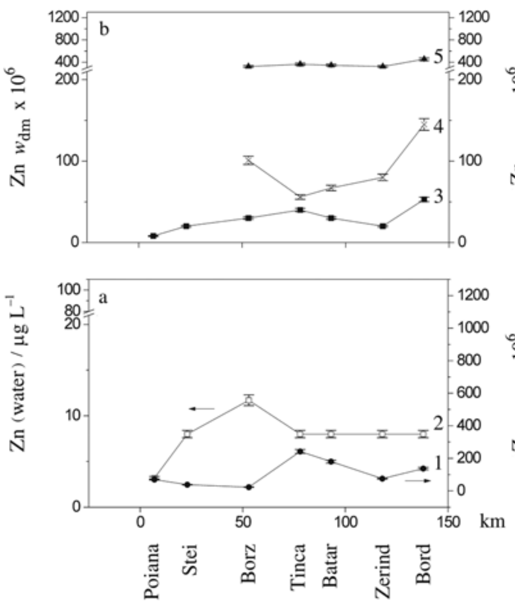

Figure 7. Variation of Zn content: a. sediment (1), water (2); b. fish fillet (3), mollusc muscles (4), mollusc gills (5), collected in the Black Cris. $w_{dm} \times 10^6$, dry mass fraction.

The content of the elements under study in mollusc muscles (Figures 2.b–7.b, curve 4) and mollusc gills (Figures 2.b–7.b, curve 5) is influenced by the metal concentration both in water and sediment and the maximum level is generally identified downstream compared to the maximum in sediment. The variation trend and data in Table I show an accumulation of Cu, Mn and Zn in molluscs in comparison with fish, different for the three metals. Thus, the Zn content in mollusc muscles (Figures 6.b and 7.b, curve 4) reaches a level close to that in fish fillet (Figures 6.b and 7.b, curve 3) with a maximum factor of concentration of 4 (Figure 7.b, Zerind). Copper is 2–9 times more concentrated (Figure 2.b, curves 4 and 3; Aciuta and Chisineu Cris) while the level of Mn is 10–30 times higher (Figure 4.b, curves 4 and 3; Aciuta and Almas). The absolute contents of the considered metals in mollusc muscles from the White Cris are higher than those from the Black Cris, following the water and sediment contents. The concentration ratio of metals is much higher in mollusc gills and they act as natural depolluting factors. The level of Cu is 7–20 times higher than that in fish fillet (Figure 2.b, curves 5 and 3; Ineu and Chisineu Cris) and Zn is 8–16 times more concentrated (Figure 7.b, curves 5 and 3) than in fish. The case of Mn is different since it shows a very high tendency to concentration within the range of 600–5000 in comparison with fish fillet (Figure 4.b, curves 3 and 5; Ineu and Almas). The Mn content in mollusc gills from the two rivers is similar, even though the concentrations in water and sediment are quite different.

CONCLUSIONS

Determinations of Cu, Mn and Zn contents in water, sediment and aquatic animals existing in the White Cris and Black Cris rivers in Romania were carried out with the aim to point to the possible polluting effect of the mining and processing centers. The metal levels were higher in the samples from the White Cris. Among the sample collection sites, only one was found to have concentrations of Mn and Zn in water higher than the admitted levels (Brad on White Cris). However, the data showed that the levels of these elements in water were not higher than those considered as "without effect" for aquatic animals. The analysis allowed also estimation of the contents of Cu, Mn and Zn in fish and molluscs from these two rivers. According to the concentrations in water and sediment, the Cu, Mn and Zn contents in fish fillet in the two rivers are different, but within the range reported as typical of aquatic animals. For mollusc gills, data confirmed the tendency to highly concentrate Mn and a moderate tendency to concentrate Cu and Zn.

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SAŽETAK

Tragovi Cu, Mn i Zn u vodenim organizmima, vodi i sedimentu iz korita rijeke Cris – zapadna Rumunjska. Dio II: Raspodjela

Michaela Ponta, Tiberiu Frentiu, Andrei Sarkany-Kiss i Emil A. Cordos

Prikazano je određivanje Cu, Mn i Zn (TRT r.f. CCP-AES, FAAS) u vodi, sedimentu i vodenim životinjama (riblji filet, škrge i mišići slatkovodne školjke) sakupljenima u koritu rijeke Cris (Bijeli Cris i Crni Cris) u Zapadnoj Rumunjskoj. Masene koncentracije u vodi / $\mu g\ L^{-1}$ bile su u području: 1–15 (Cu), 10–1500 (Mn), 3–100 (Zn). Sadržaji metala kao udjel u suhoj masi ($w_{dm} \times 10^6$) bili su: 5–380 (Cu), 225–2000 (Mn), 23–1140 (Zn) u sedimentu, te 1–11 (Cu), 4–40 (Mn), 8–130 (Zn) u ribljem filetu i 5–34 (Cu), 100–600 (Mn) i 50–130 (Zn) u mišićima školjke, pri čemu su vrijednosti za uzorke iz Bijelog Crisa bile veće. Samo za jedno mjesto od sedam mjesta sakupljanja uzoraka nađene koncentracije Mn i Zn u vodi bile su iznad dopuštenih granica, dok su u obje rijeke koncentracije za vodene organizme bile u granicama dopuštenih. U škrgama školjke, sadržaji metala izraženi kao $w_{dm} \times 10^6$ bili su 8–60 (Cu), 11000–16000 (Mn), 190–1200 (Zn), slično za obje rijeke u slučaju Mn.