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Heavy metal concentrations in two species of fish from the Crișul Negru River, Romania

[1] Milca Petrovici, [2] Octavian Pacioglu

[1] West University of Timişoara, Faculty of Chemistry, Biology, Geography, Timişoara, Romania, EU, e-mail: milcapetrovici@yahoo.com; [2] Roehampton University, School of Human and Life Sciences, Centre for Research in Ecology, Whitelands College, London, UK, e-mail: O.Pacioglu@roehampton.ac.uk

Abstract: The aim of this paper is to establish the potential bioaccumulation of five heavy metals (Cu, Cd, Ni, Co, Zn) in two species of fish from Crişul Negru River (Romania), moderately polluted from a mining area, comparative with the fish sampled from Crişul Repede River, considered as a control site. The sampling periods extended along three campaigns/ each month, from a total of ten months, on four sites situated along the Crişul Negru River and on a control site situated on the neighbouring stream, the Crişul Repede River, same catchment. The values obtained for the five heavy metals were in accordance with values registered in the water column and the upper threshold limit concentrations for fish meat imposed by European and other international normative, but with one exception. The increased values for cobalt accumulated in the fish from the immediate locations of a mining site do make them improper for human consumption.
Keywords: heavy metals, *Phoxinus phoxinus*, *Leuciscus cephalus*, Crişul Negru River.

Sažetak. Namjera ovog rada jest odrediti potencijalnu bioakumulaciju pet teških metala (Cu, Cd, Ni, Co, Zn) dviju vrste riba iz rijeke Crişul Negru (Romanija), umjereno unočisćen zbog rudarskog kraja, usporedeno uzorcima ulovljenih riba iz rijeke Crişul Repede, kao pokusna stanica (svidok). Uzorci su prikupljeni 3 puta/mjesec, vrijeme za 10 mjeseci, četiri stanice iz Crişul Negru i jedna svidok,na Crişul Repede. Dobivene vrijednosti za onih pet teških metala su bile u skradu s vrijednosti dobivene u vodenim stupcu i sa standardnih internacionalni vrijednosti ovih pet teških metala u mesu riba za konsum, s jednom iznimkom. Za cobalt, dobivene vrijednosti u riba, u seli sta se nalaze u blizini rudarskog kraja, premašuju dopustene maximalne vrijednosti, učiniti te ribe nepravilne za potrošnju.
Key Words: teških metala, riba, *Phoxinus phoxinus*, *Leuciscus cephalus*, rijeke Crişul Negru.

Rezumat. Scopul acestei lucrări este de a stabili potențiala bioacumulare a cinci metale grele (Cu, Cd, Ni, Co, Zn) în două specii de pești din râul Crişul Negru (România), râu moderat poluat dintr-o zonă minieră, comparativ cu probele de pești colectați din râul Crișul Repede, ca stație de control (martor). Probele au fost colectate de trei ori/lună, timp de 10 luni, din patru stații de pe râul Crișul Negru și una martor, de pe râul Crișul Repede. Valorile obținute pentru cele cinci metale grele au fost în concordanță cu valorile înregistrate în coloana de apă și cu valorile standardelor internaționale ale acestor metale grele în carnea peștilor pentru consum, cu o singură excepție. Pentru cobalt, valorile acumulate în pești, la locațiile aflate în imediata apropiere a zonei miniere, depășesc valorile maxime admise, făcând acești pești să fie improprii pentru consum.
Cuvinte cheie: metale grele, pești, *Phoxinus phoxinus*, *Leuciscus cephalus*, râul Crişul Negru.

Introduction. Heavy metals are continuously released into surface waters. When present above a certain threshold (like the maximum accepted level), they do provide deleterious effects on the freshwater organisms, at the individual (e.g. histological and morphological modifications, decrease of growth and development rates, modifications of blood, enzymatic, and behavioural level or the necrosis of respiratory organs) and at the population level, as shown by the increase of death rate, the decrease of birth rate etc (Authman 2008). Their potential toxic effects are given by the presence in water solution at concentrations exceeding certain thresholds, their long persistence in the aquatic ecosystems and their bioaccumulation and biomagnification in the food webs.

Several scientific studies have been conducted in Romania in order to determine the content of heavy metals in fresh and marine waters, in fish with economical value or fish products from the markets. Thought, the studies considering the fish as biosurvey tools on their own in lotic environments are limited (Diaconescu et al 2008; Triebskorn et al 2007; Sárkány-Kiss et al 1997). Therefore, this survey aims at reflecting the dynamic of five heavy metal concentrations (Cu, Cd, Ni, Co, Zn) in the Crişul Negru River (West of Romania) through their concentrations in fish tissues.

The studied sites are located on a bordering river between Romania and Hungary that has its springs into an area heavily mined of outcrop rocks with a high content of heavy metals. The main pollution sources are represented by the mining activities from Băița, situated at the headwaters of Crișul Negru River, and where are important outcrops of metal sulphur compounds. The exploitation of these outcrops started since the XIII century, and it is known as an important historical point for obtaining valuable metals like gold, silver, copper, lead and zinc. After the Second World War the exploitations swapped towards mainly extracting other metals like the molybdenum, bromide and other heavy metals (Stoici 1983). Therefore, the direct and chronically influences occur both like point source pollution through the mining activities and diffuse sources through the former abandoned galleries and exploitation drilling pits from where the metals are scoured in both surface and groundwater by leaching. Additionally, at the surface are present large tailing ponds, from where the mining dumps are washed away by the waters used in metals decantation and end up straight in to the river, forming diffuse sources of pollution along the catchment, very difficult to localise and quantify. Nearby the sampling sites can be found one of the biggest tailing ponds from the entire catchment (Filip et al 2009).

Fish were the chosen taxonomic group for this survey due their potential of accumulating heavy metals in their tissues, and for being usually the most frequent used taxa in the estimation of pollution with heavy metals (Chovanec et al 2003; Lamas et al 2007; Popek et al 2008). In addition, as part of the human diet, they can pass the heavy metal burden to the consumers triggering acute or chronic intoxications.

Material and Method

1. Study area. Four sampling sites were identified along the Crişul Negru River (see Figure 1): site 1, Băița, is a mining site with a population of 1.100 citizens, situated 9 km downstream the headwaters of the main stem; site 2, Fânaţe, situated 4.5 km downstream from the first site and at a distance of 27 km from the city of Beiuş (11.000 citizens); site 3, Borz, 18 km downstream the City of Beiuş and site 4, Ant, 1.2 km upstream from the Hungarian border. The fifth site (control) was chosen on the Crişul Repede River, at Bulz. The GPS coordinates are provided below (see Table 1).

Table 1

GPS coordinates at sampling sites

	Site 1 Băiţa	*Site 2 Fânaţe*	*Site 3 Borz*	*Site 4 Ant*	*Site 5 Bulz*
Latitude	46°28'55'' N	46°30'16'' N	46°40'22'' N	46°39'45'' N	46°56'49'' N
Longitude	22°36'12'' E	22°32'16'' E	22°11'32'' E	21°28'8'' E	22°40'28'' E
Altitude	483.7 m	355.6 m	210.7 m	98.5 m	366.8 m

2. Sampling frequency. The samples were collected from the aforementioned sites between August and December 2008 and March and July 2009, with a frequency of three times each month with the exception of the period between August and December 2008 when only two sampling campaigns were organised. Therefore, a total of 28 sampling dates were included into this study.

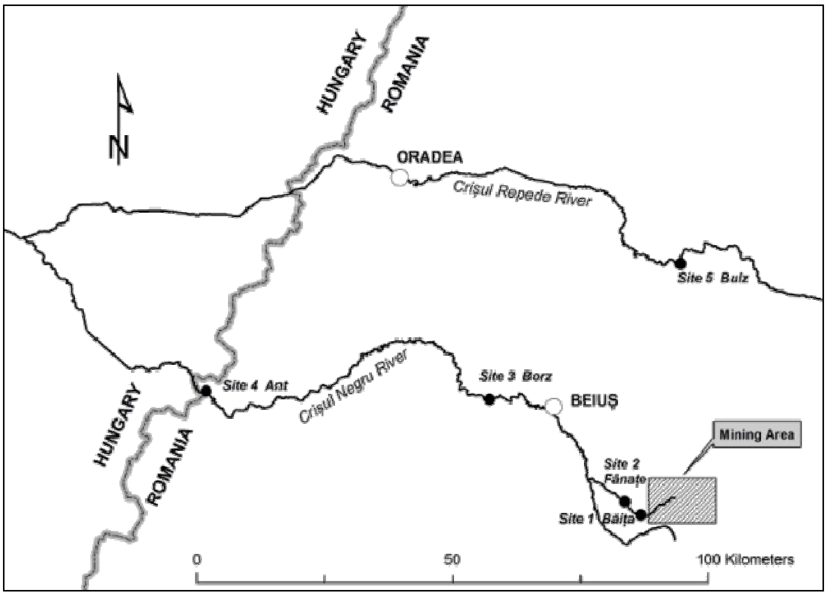

Figure 1. Location of sampling sites along the Crişul Negru River and Crişul Repede Rivers (control).

3. Fish sampling and heavy metals residual analysis of tissue. The species selected for this study were the common minnow (*Phoxinus phoxinus)* and the carnivorous European chub (*Leuciscus cephalus)*, top predators in these rivers and usually consumed by locals and anglers. The former species was sampled from two sites situated immediately downstream the mining area (sites 1 and 2) on the Crişul Negru River and the latter species further away downstream (sites 3 and 4) as well as from the control site, on the Crişul Repede River, a neighbouring and more pristine catchment.

From each sampling station were collected three fish/each sampling date, as the minimum number or replicates required in surveys of this nature and in order to establish the mean values of heavy metals. Therefore, the total volume of samples was of 84 investigated fish. The fish were caught alive, placed in an ice box, transported to the laboratory and stored in a deep freezer (-25^{0}C) until being processed for metal analysis. A slice of muscle of 0.5 g (wet weight) was sampled from the defrosted fish and analysed. For each fish were collected three samples, resulting in an average of them.

Heavy metals concentrations were measured according to the Atomic Absorption Methods for Fish (AOAC 1995). Samples were mineralized with analytical grade purity reagents: HNO_3 65% (Merck, Darmstadt, Germany) and ultrapure water (18 MW cm^{-1}, Direct Q UV 3 Millipore system).

The heavy metals (Cu, Cd, Ni, Co, Zn) were determined using the atomic absorption spectrophotometer (Varian model). The results were expressed as μgg^{-1} wet weight and are comparable without conversion with those provided in the literature or ongoing legislation (in $mgkg^{-1}$) (see Discussion and citations therein).

4. Statistical analyses. Statistical analyses were performed using Statistica version 9.0 for Windows (StatSoft, Inc.) and SPSS for Windows, version 15.0 (SPSS Inc.). The estimated parameters were: the mean, minimum and maximum values, standard deviation, and standard error. The values were log(x+1) transformed in order to meet the criteria of normality. One way ANOVA tests were applied for every chemical species in order to reveal differences between sampling sites and potential rates of bioaccumulation. Post hoc Tukey test was performed in order to reveal the significant differences amid sites. Significant p values were indicated along with the values of the F test for variance.

Results

1. Descriptive statistics. The analysis of all the mean concentrations (see Table 2), along one year of survey (August 2008 - July 2009) reveals the small concentrations for copper at the control site, Bulz (0.009±0.009 $\mu g g^{-1}$), compared to the ones at site 2, Fânaţe (0.131±0.068 $\mu g g^{-1}$); copper being a chemical species present during the entire investigated period.

The cadmium is absent from fish tissues at the control site, and has the highest concentrations at site 2, Fânaţe (0.008±0.004 $\mu g g^{-1}$). This site provides a higher cadmium concentration then the ones observed in the furthest downstream site, Ant (see Figure 4).

Table 2

Statistical parameters of the heavy metals from fish

	Cu ($\mu g g^{-1}$)	Cd ($\mu g g^{-1}$)	Ni ($\mu g g^{-1}$)	Co ($\mu g g^{-1}$)	Zn ($\mu g g^{-1}$)
S 1. Băiţa					
x	0.067 ±0.019	0.004 ±0.003	0.006 ±0.006	0.394 ±0.175	2.178 ±0.201
m/M	0/0.16	0/0.032	0/0.072	0/1.8	1.19/2.95
σ	0.051	0.009	0.017	0.473	0.54
S 2. Fânaţe					
x	0.131 ±0.068	0.008 ±0.004	0.024 ±0.013	0.168 ±0.069	2.423 ±0.204
m/M	0/0.604	0/0.036	0/0.116	0/0.452	1.26/3.51
σ	0.183	0.012	0.036	0.186	0.55
S 3. Borz					
x	0.017 ±0.004	0	0	0.0001 ±0.0003	2.111 ±0.267
m/M	0/0.032	0/0	0/0	0/0.004	0.8/3.96
σ	0.01	0	0	0.001	0.72
S 4. Ant					
x	0.05 ±0.026	0.0003 ±0.0004	0	0.0001 ±0.0002	0.456 ±0.223
m/M	0/0.24	0/0.004	0/0	0/0.003	0.023/1.6
σ	0.07	0.001	0	0.001	0.6
S 5. Bulz					
x	0.009 ±0.009	0.004 ±0.003	0	0	2.189 ±0.229
m/M	0/0.08	0/0.02	0/0	0/0	1.32/3.51
σ	0.021	0.006	0	0	0.62

S 1 - S 4 = sites from Crişul Negru River, S 5 = site from Crişul Repede River, x = average value 2008-2009; m/M/σ = minimum value/maximum value/standard deviation.

2. The variation of heavy metal concentrations over sites. The variation of copper mean concentrations between sites (see Figure 2) reveals an increasing tendency along an upstream-downstream gradient among the first two sites. At sites 3 and 4 the analysis reveals a tendency of decreasing concentrations, the lowest one being found at the control site, no. 5. One way ANOVA and post hoc test revealed significant differences among sites ($F_{135, 4} = 8.05$, $p < 0.001$), or more specific between the control and second site ($p < 0.001$, post hoc Tukey test). The sites 3 and 4 provide significantly lower copper concentrations then the second site ($p < 0.01$ and 0.013, post hoc Tukey test).

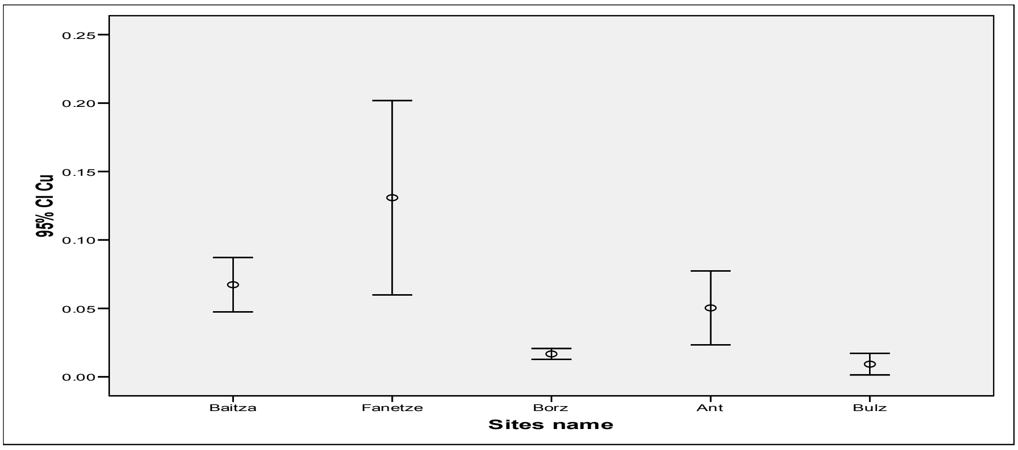

Figure 2. Variation of Cu concentrations over sampling sites (mean +/- 1 SE).

The variation of the average cadmium concentrations (see Figure 3) between sampling sites revealed a similar trend with the one observed for the copper: there is a significant difference between sites ($F_{135, 4} = 6.24$, $p < 0.001$, one way ANOVA), site 2 having significant higher levels than 3 and 4 ($p < 0.001$, post hoc Tukey test for both sites). The concentrations found at the control site although smaller that at the sites 1 and 2 are not significant different then sites 3 and 4.

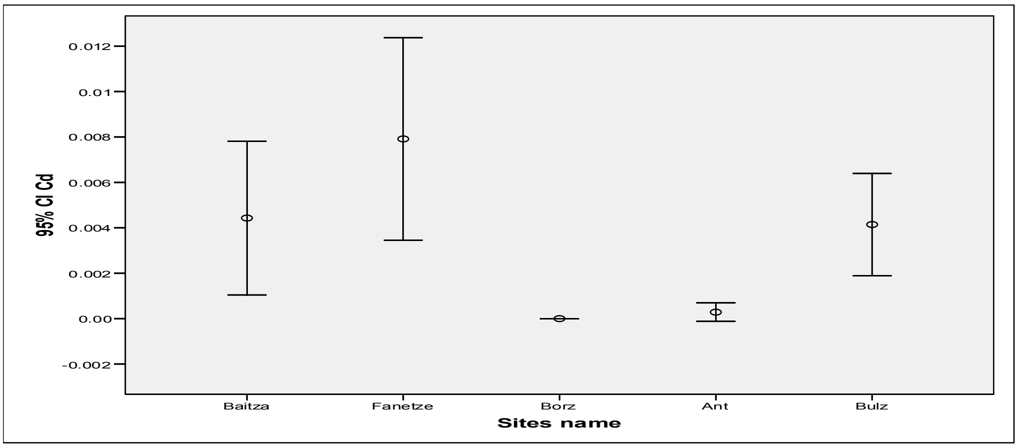

Figure 3. Variation of Cd concentrations over sampling sites (mean +/- 1SE).

The variation of nickel is revealing a somehow different pattern then copper and cadmium (see Figure 4), being found at its highest values at the upper sites, just downstream the mine exploitation and decreasing to non detectable levels to the other downstream sites, including the reference site ($F_{135, 4} = 9.26$, $p < 0.001$, one way ANOVA).

The cobalt has a similar pattern with the one noticed for nickel (see Figure 5), showing highly decreased values on a longitudinal transect (upstream-downstream direction), with significant differences ($F_{135, 4} = 23.26$, $p < 0.001$, one way ANOVA) between the first and the second stations ($p = 0.002$, post hoc Tukey test).

The zinc concentrations displayed a different pattern compared with the other heavy metals, being found in significantly higher levels (in comparison with the ones of the other chemical species) in all sites (see Figure 6), the only one having a smaller significant concentration being site 4 ($p < 0.001$, post hoc test, one way ANOVA). The differences between all the other stations were not statistically significant.

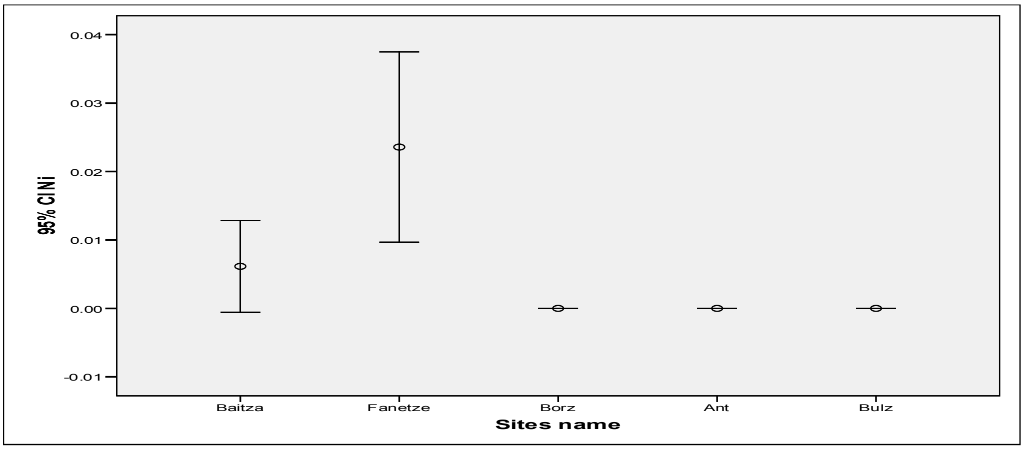

Figure 4. Variation of Ni concentrations over sampling sites (mean +/- 1 SE).

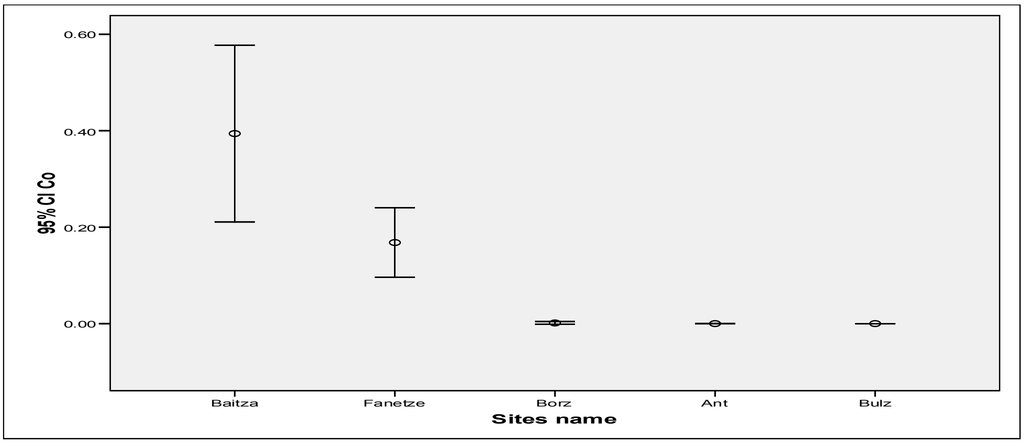

Figure 5. Variation of Co concentrations over sampling sites (mean+/- 1 SE).

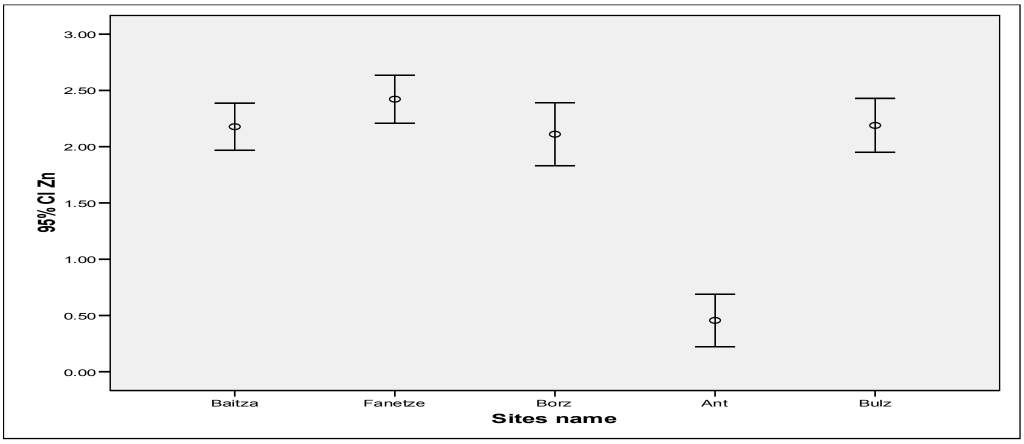

Figure 6. Variation of Zn concentrations over sampling sites (mean +/- 1SE).

Discussion

1. Fish as biosurvey tools for heavy metals pollution in sites impacted by mining activities. Because the bioaccumulation rates are determined by the specific physiology of every species but as well by the concentrations of the elements in the water column, the aforementioned presentation as a longitudinal gradient finds its place in this context. Although two different species of fish have been taken under consideration from different sites, the results were compared with the findings of river concentrations from the literature and ongoing legislation, measured in $\mu g g^{-1}$ or $mg l^{-1}$ (see below).

The copper and cadmium concentrations have a tendency of increasing in fish tissues at the first two sampling stations, indicating a possible higher concentration in the water column and the presence of additional diffuse pollution sources between these two locations. The surface water chemistry measurements suggest high values for copper in the upstream sections as a direct influence of mining activities, with mean values of 10.2 $\mu g g^{-1}$ (Josan et al 2003) upstream Site 1 and with mean values between 20-30 $\mu g g^{-1}$ for downstream sites of tailing ponds at Băița (Site 1) or with values in the range of 50-60 $\mu g g^{-1}$ for the period 2004-2006 at Fânațe (Site 2) (Filip et al 2009). For these metals the concentration at site 3 is much lower indicating a possible dilution and eventually their uptake by other ecological sectors of the river (like other species of vertebrates or invertebrates, sediments etc) then in the upstream sites, according with the literature (Josan et al 2003; Filip et al 2009). Nevertheless, the site 4 provides a higher mean concentration in fish tissue then at the third site, indicating the possible existence of very other diffuse pollution sources on this sector of the river. The diffuse pollution sources are very difficult to localise due their presence along significant distances of the catchment, and their presence can only be inferred due the lack of consistent surface water measurements. The control site indicates somehow an average position among upstream sites (1 and 2) and downstream ones (3 and 4), indicating that the copper and cadmium are not inexistent as one would expect from a control, pristine site.

Fortunately, the mean concentrations of nickel and cobalt are zero in the control site, the same as in the third and forth sites, indicating the self cleaning capacity of rivers and a possible more rapid physiologic speed in eliminating these compounds of the Common minnow as compared to the European chub. This statement is supported by the high concentration of these elements in the Common minnow in both first sites (1 and 2) and their virtual absence in the European chub tissue sampled downstream. The decreasing tendency of nickel and cobalt concentrations amid the first two sites and the rest could indicate as well their elimination from the water column and their uptake by the river ecosystem through abiotic processes, by the benthic and hyporheic sediments, as it has been suggested for the river Aries (Marin et al 2005) and as well due their bioaccumulation and biotransformation by other species of the lotic biota.

The only element with similar concentrations at all sites and possible uptake rates is the zinc that does not differ significantly apart from the forth site, indicating a higher delay in the elimination rate in respect to the other metals. In what concerns the relation with the river water chemistry, it can be suggested that the average concentrations of Zn were high in the areas of Site 1, 21.3 $\mu g g^{-1}$ (Josan et al 2003) or with a mean of 10-15 $\mu g g^{-1}$ for Băița area and even higher values, between 25-30 $\mu g g^{-1}$ downstream the tailing ponds for the Site 2 (Filip et al 2009). Nevertheless, the Zn higher concentration then the other cations in both water column and fish tissue could be explained on a pure chemical basis: the Zn has a higher mobility then all the other metals under consideration, and therefore its higher concentrations in all the investigated sites, independent of the longitudinal distance from headwaters, is explicable this way (Josan et al 2003).

Therefore, one can say that the pattern depicted in the heavy metals concentrations in the tissue of these two species of fish are in accordance with the values provided for river concentration of three of the five analysed heavy metals in the same river stretches and could be interpreted as a strong support in the use of the fish in the nearby future as biosurvey tools for rivers heavily impacted by mining activities and with leaching of heavy metals in the surface waters (both point and diffuse sources). The putative rates of bioaccumulation, although established only on a qualitative basis do provide the background for further studies in the nearby future, emphasising the correlation and the ratio between the presence of heavy metals in the river and their uptake by the biota and river sediments, including their hyporheic habitat, in the same manner like it was suggested for the river Aries, similarly polluted with the same chemical species like those analysed in this survey (Marin et al 2005).

2. The heavy metals and the risks for humans. In order to properly analyse the risk assumed by the consumption of the fish species from the investigated area, comparisons with the maximum values found in the tissues of fish and the indicated values from ongoing legislations were made.

The variability of copper in animal tissues is denoted by its highest concentration at the second station, followed closely by the sites 1 and 3. The lowest values or its absence is registered for this metal in the control site. In the both rivers, at any station, the maximum values have not reached the maximum accepted levels from legislation (see Table 3).

Table 3

The maximum admitted values for fish with economic value from national and international legislation and the maximum values recorded for the species of fish from this study, from the Crişul Negru and Crişul Repede Rivers

Normative (measure unit)	*Cu*	*Cd*	*Ni*	*Co*	*Zn*
1 ($mgkg^{-1}$)	5.0	0.1	¤	¤	50
2 ($mgkg^{-1}$)	¤	0.05-0.3	¤	¤	¤
3 ($mgkg^{-1}$)	¤	0.05-0.01	¤	¤	¤
NOAEL($mgkg^{-1}day^{-1}$)	1.11 (*)	0.01 (**)	30.77 (*)	0.92 (*)	66.59 (*)
Site 1 Băiţa (μgg^{-1})	0.16	0.032	0.072	0.1.8	2.95
Site 2 Fânaţe (μgg^{-1})	0.604	0.036	0.116	0.452	3.51
Site 3 Borz (μgg^{-1})	0.032	0	0	0.004	3.96
Site 4 Ant (μgg^{-1})	0.24	0.004	0	0.003	1.6
Site 5 Bulz (μgg^{-1})	0.08	0.02	0	0	3.51

1 = The Normative 975/1998, 2 = The Normative CE 629/2008 and 1881/2006, 3 = The Normative CE 466/2001 and 78/2005. The symbol ¤, indicates the lack of values from the indicated act.

The NOAEL values (No Observable Adverse Effect Level) that denotes the level of exposure of an organism, found by experiment or observation, at which there is no biologically or statistically significant for copper is of 1.11 $mgkg^{-1}day^{-1}$ of consumed meat (*). On the other hand, neither the values from the literature have not been reached: 0.099 μgg^{-1} (Awadallah et al 1985) or 0.27 1 μgg^{-1} (Rashed 2001) found for the species *Tilapia nilotica,* from Nasser Lake (Africa). This species is as well a top predator in the same way like the present investigated species.

Khallaf et al (1994) have found variations between 0.87 – 2.49 μgg^{-1} for the same species in the Nile River and between 0.36 – 1.13 μgg^{-1} in the fish farms. The highest value found in the literature for copper is 41 μgg^{-1} (Kalfakakon & Akrida-Demetrai 2000), in Ioannina Lake (Greece), concentration that was making improper the consumption of this species, being much higher than the NOAEL.

For cadmium, according to the data form Table 3, the highest value was that of 0.036 μgg^{-1}, in a minnow sampled in the second site, and still being under the maximum admitted concentrations provided by the national and international legislation. The NOAEL value for cadmium is 0.01 $mgkg^{-1}day^{-1}$ of ingested fish.

For nickel significant concentrations have been found only in the upper reaches, immediately downstream the mines. In these two sites the maximum values was the one of 0.116 μgg^{-1}, for a Minnow sampled in the second site. The Romanian and international legislation do not state the maximum admitted values for this chemical species in the fish meet, but the NOAEL indicated value is of 30.77 $mgkg^{-1}day^{-1}$. According to these data, one can say that in both investigated rivers, the top predator meet is consumable form the point of view of nickel presence. Other values for this metal from the literature indicate between 0.92 - 4.04 μgg^{-1} for *Tilapia nilotica* or between 0.07 - 4.2 μgg^{-1} for the same species in farms (Khallaf et al 1994).

For the cobalt, higher values were obtained from the first site and they were compared with the NOAEL values of 0.92 $mgkg^{-1}day^{-1}$. This value is the threshold after adverse reactions occurs, if 1 kg of fish is consumed / day and it was surpassed in three cases, for minnows sampled between 2008-2009, with concentrations of 1.43 μgg^{-1}, 1.8

μgg^{-1} and 1.7 μgg^{-1}. These values make improper the consumption of Common minnow immediately downstream the mining sites.

The zinc had high concentrations in all the sites, with a maximum value of 3.9596 μgg^{-1} on site 4, but nevertheless beyond the NOAEL values of 66.59 mgkg^{-1}day^{-1}. Other values registered in the literature are: 1.32 – 5.08 μgg^{-1} for *Tilapia nilotica* on the Nile and between 0.39 – 1.79 μgg^{-1} in the fish farms (Khallaf et al 1994). A mean value of 0.63 μgg^{-1} was found for fish sampled in the Ioannina Lake (Greece) (Kalfakakon & Akrida-Demetrai 2000).

Therefore, at any station during the investigation period (August 2008-July 2009), the maximum admitted values for copper, cadmium, nickel and zinc have not been reached, according to the national and international legislation, in the Crişul Repede and Crişul Negru Rivers. The only exception was found for cobalt in site 1, just downstream the mine sites, with concentrations higher than the one accepted for fish with economic value. Due the fact that these high concentrations were registered three times, during different sampling periods, we consider improper the consume of the common minnow at this point of the river.

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Authors:
Milca Petrovici, West University of Timişoara, Faculty of Chemistry, Biology, Geography, Pestalozzi Street no. 16, Timişoara, Romania, EU, e-mail: milcapetrovici@yahoo.com;
Octavian Pacioglu, Roehampton University, School of Human and Life Sciences, Centre for Research in Ecology, Whitelands College, Holybourne avenue, SW15 4JD, London, UK, e-mail: O.Pacioglu@roehampton.ac.uk
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