Ecotoxicology (2017) 26:261–270
DOI 10.1007/s10646-017-1760-0

Toxic and heavy metals as a cause of crayfish mass mortality from acidified headwater streams

Jitka Svobodová[1] · Karel Douda[2] · David Fischer[3] · Natalia Lapšanská[4] · Pavel Vlach[5]

Accepted: 10 January 2017 / Published online: 21 January 2017

Abstract Mining activities are responsible for high concentrations of metals in river networks in many parts of the world. Mining activities and the resulting high loads of heavy metals interact with intensive acid rain, and often have great consequences for biodiversity. However, considering the frequently episodic nature of these heavy acid rains, there is little detailed evidence of direct impacts. In 2011 we observed a massive mortality of noble crayfish and stone crayfish in Padrťsko Special Area of Conservation (SAC) in the Brdy Mountain region of the Czech Republic. Based on concentrations of metals (Al, Fe, As, Cd, Pb, Cu, Zn and Hg) in various tissues (gills, hepatopancreas, muscle) of both dead and live crayfish in this locality compared to reference populations, these crayfish had experienced long-term exposure to increased levels of these metals. Here we give detailed documentation of crayfish mortality associated with high metal concentrations in the gills and other tissues of these endangered invertebrates.

Keywords Water quality · Toxic metal · Invertebrates · *Austropotamobius torrentium* · *Astacus astacus*

✉ Jitka Svobodová
jitka_svobodova@vuv.cz

[1] T.G. Masaryk Water Research Institute, Podbabská 30, Prague 6 160 62, Czech Republic

[2] Department of Zoology and Fisheries, Faculty of Agrobiology Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague, CZ 165 21, Czech Republic

[3] Mining Museum Příbram, Hynka Kličky Place No. 293, Příbram, VI 261 01, Czech Republic

[4] Povodí Vltavy, State Enterprise, Holečkova 8, Prague 5 150 24, Czech Republic

[5] Centre of Biology, Geoscience and Environmental Education, Department of Education of the University of West Bohemia, Klatovská 51, Plzeň 306 19, Czech Republic

Introduction

Acidification and high concentrations of metals (Al, Fe, As, Cd, Pb, Cu, Zn and Hg) both have wide negative impacts on biodiversity at a global scale (Lampert and Sommer 2007). Both factors often act simultaneously, with heavy acid rains lowering water pH and therefore increasing the availability of toxic heavy metals for organisms from stream sediments (Singh and Agrawal 2008), often leading to vast consequences for biodiversity. Despite the seriousness of this problem, information on how heavy or toxic metals influence individual animal groups in natural aquatic ecosystems is varied. The situation in fish has been relatively well documented, with many studies demonstrating an affinity of metals for various fish tissues (Dalzell and MacFarlane 1999; Peuranen et al. 1994; Poléo et al. 1994; Wauer and Teien 2010). Some studies have also investigated the varying accumulation of metals in various organs in crayfish, reviewed by Kouba et al. (2010). These studies have mostly been focused on the accumulation of metals in North American crayfish species (Al Kaddissi et al. 2012; Alcorlo et al. 2006; Alexopoulos et al. 2003; Antòn et al. 2000; Ward et al. 2006), although the accumulation (and/or depuration) of metals in native European crayfish species has also been investigated (Antòn et al. 2000; Fjeld et al.1988; Guner 2010; Meyer et al. 1991; Simon et al. 2000).

The bioaccumulation of metals is a process involving metals from a polluted environment becoming deposited in some tissue. Crayfish readily accumulate metals in various tissues, and this accumulation is dose- and time-dependent (Antòn et al. 2000). However, crayfish are considered relatively resistant animals to environmental contamination, and common concentrations of metals are usually not responsible for crayfish mortality (Kouba et al. 2010). On the other hand, individual metals can lead to many problems. For instance, Al accumulation can cause behavioral changes (Alexopoulos et al. 2003), morphological changes in gills leading to a disruption of ion transport between the environment and the crayfish body (Fjeld et al. 1988), or destruction of the immune system (Ward et al. 2006).

Another important process in the relationship between crayfish and heavy metals is depuration. In addition to a high concentration of metals in water leading to bioaccumulation, decreasing amounts of metals in a water ecosystem can cause depuration of the accumulated metals from tissues; e.g., Zn, Cu and Cd can be depurated in only a few weeks (Guner 2010, Kouba et al. 2010). Therefore, when evaluating the influence of heavy and toxic metals it is of great importance to gather data on the dynamics of metal gradients in the examined stream or to know the approximate time the pollution occurred.

In the spring of 2011 (April to June), there was a massive mortality of stone crayfish *Austropotamobius torrentium* and noble crayfish *Astacus astacus* in the Klabava stream in Padrťsko Special Area of Conservation (SAC), in the Czech Republic. Hundreds of dead or dying individuals were found along a six-kilometer-long stretch, which is the only stretch of the stream inhabited by both crayfish species. Before dying, affected individuals left their shelters and acted apathetically, and displayed no flight response. This atypical behavior (compared to the standard crayfish behavioral elements described by Lundberg 2004) and further mortality lasted until the summer of 2011.

The Klabava stream is acidified as a result of three factors: (1) atmospheric acidification (from sulfur oxides and nitrogen emissions); despite a decline in sulfur emissions in Central Europe in the late 80's (Kopáček et al. 1998), acidification continues at some sites; (2) accumulated sulfur in soil horizons leaching out from the locally-devastated landscape after mining activities; and (3) a bedrock with exhausted neutralizing capacity (Psenner and Catalan 1994). Moreover, in January 2011, there was a rapid snowmelt accompanied by intense rainfall, whereas water levels were low in the spring.

The aim of this study was to assess the influence of all these circumstances as possible factors in this mass crayfish mortality, i.e., stream acidification, heavy and toxic metals originating from the surrounding landscape, historic pollution by toxic heavy metals from mining activities, and weather conditions.

Material and methods

Study area

This study was conducted in the Czech Republic at two sites on the Klabava stream within the Padrťsko SAC, and at the Stroupínský stream in a nearby SAC serving as a reference locality (Fig. 1). These sites had previously been documented as having sympatric populations of both noble and stone crayfish (Vlach et al. 2009). The Klabava stream has its origin in the Brdy Mountains, a region long used for iron ore mining. Demand from the local mining and glassworks industries for charcoal led to the destruction of the original beech-fir forests; since about the end of the 18th century they have been replaced by fast-growing Norway spruce (*Picea abies*) monocultures. Iron ore mining in the area ceased around the end of the 19th to beginning of the 20th centuries (Sucharová and Suchara 2004; Žák et al. 2009). Intensive forestry harvesting still occurs, and emissions containing toxic metals from a steelworks in nearby Příbram have negative effects (Hojdová et al. 2011; Sucharová and Suchara 2004; Sucharová et al. 2011). In addition, the bedrock in the watershed has an exhausted neutralization capacity (see Results). Streambeds in the affected area have been partly modified, including the Klabava, which used to flow through several fish ponds but was diverted to a channel bypassing the ponds around the year 2000. Endangered crayfish only occur in natural meanders in a section of the stream from below the fish ponds to the confluence with the Třítrubecký stream (the stream section inhabited by both crayfish species is about 6000 m long). The Třítrubecký stream is highly acidified and has high metal concentrations (e.g., Al) as well as $ANC_{4.5}$, and below this point no crayfish occur.

While the Klabava mainly flows through forests and meadows with two managed ponds, the Stroupínský stream flows through an agricultural region with several towns. Both localities can be considered as intermediately polluted, but oxygen levels are normal (Table 1). The Stroupínský stream has organic pollution year-round, while higher BOD_5 levels are only found in the Klabava during autumn when fish ponds are drained.

The average abundance of stone crayfish in the Klabava is 1.37 individuals m^{-2} and in the Stroupínský 2.13 individuals m^{-2} (Vlach et al. 2009); abundances of noble crayfish in these stretches have yet to be measured. The species are partially syntopic in both streams, generally hiding under stones; burrows, if present, are preferably inhabited by noble crayfish.

Water quality analyses

Water quality in both studied streams has been analyzed long-term, with two sampling points in each stream (K1,

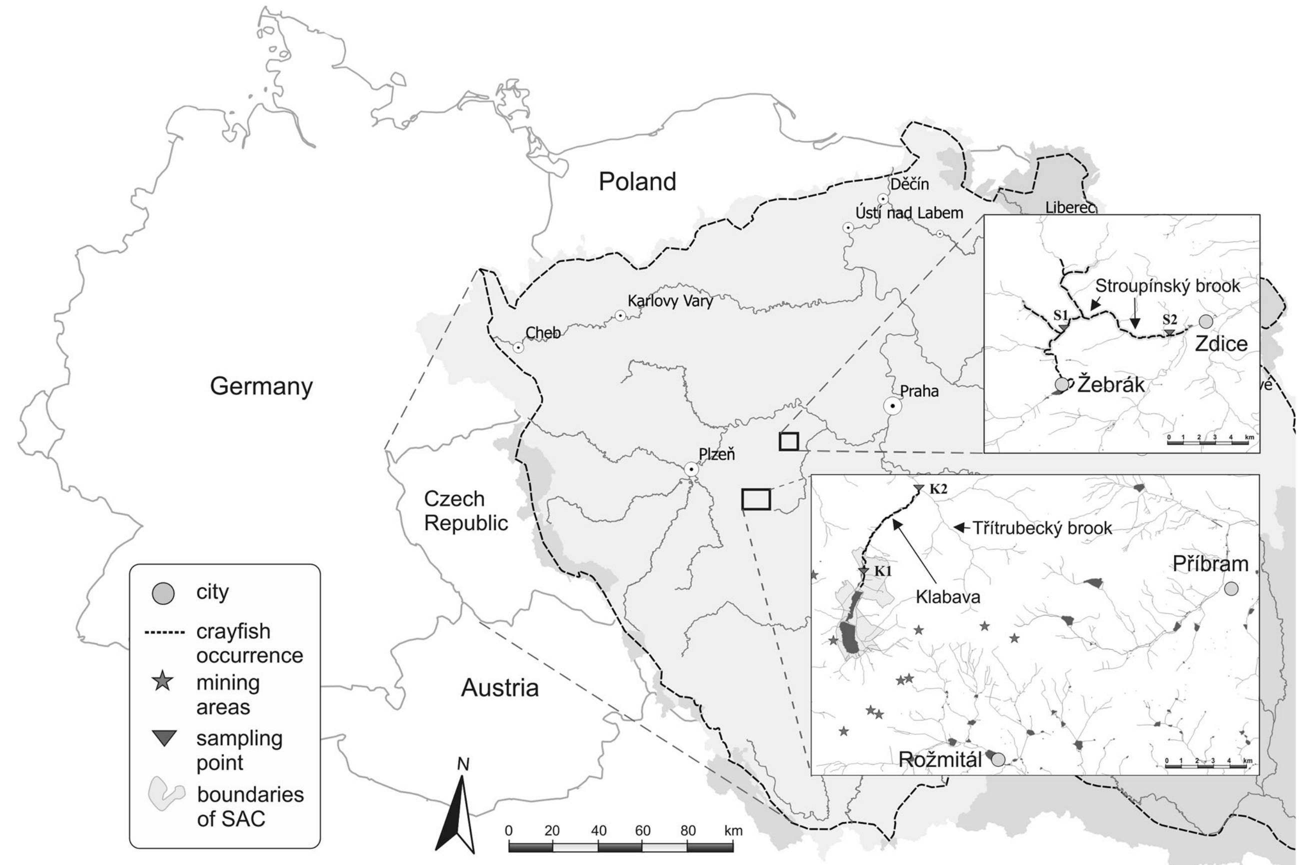

Fig. 1 Map of the observed stream: dashed lines indicate presence of sympatric occurrence of noble and stone crayfish; K1-2…sampling points on Klabava, S1-2…sampling points on the Stroupínský brook, stars indicate presence of mining input

Table 1 Water quality in the Klabava and the Stroupínský streams

Variable	Klabava (Kla.)				Stroupínský (Str.) reference site				Salmonid waters (EHP 2006/44/ES)
	N	Med	Min	Max	N	Med	Min	Max	limit value
O_2 (mg L^{-1})	50	10.60	7.30	13.70	120	10.0	7.50	18.7	50% >9
pH	50	7.30*	4.70	8.33	123	8.10*	7.31	9.40	6–9
$ANC_{4.5}$	3	0.67*	0.36	0.90	3	4.66*	3.20	5.41	–
BOD_5 (mg L^{-1})	49	1.20*	0.50	12.0	119	1.60*	0.70	7.30	<3
COD_{Cr} (mg L^{-1})	48	20.00	6.30	146	102	13.5	4.40	36.0	–
NH_4^+ (mg L^{-1})	49	0.06	0.03	2.84	120	0.05	0.01	1.12	<0.04
Susp. solids (mg L^{-1})	49	4.80	1.00	647	120	5.20	1.00	111	<25
NO_2^- (mg L^{-1})	49	0.013*	0.005	0.115	120	0.071*	0.004	0.440	<0.01
t (°C)	50	8.20*	0.00	18.9	120	12.2*	0.30	22.50	2% >21.5
Conduk. (µS cm^{-1})	50	68.5*	27.0	136.5	123	723*	467	1170	–
Ca^{2+} (mg L^{-1})	16	10.0*	4.40	19.0	54	67.1*	50.6	89.60	–
Al (µg L^{-1})	16	414.*	163	4930	46	215*	40.0	2700	–
As (µg L^{-1})	11	40.1*	9.36	117	4	1.35*	1.00	1.83	–
Cd (µg L^{-1})	35	0.16	0.05	6.80	5	0.20	0.20	0.20	–
Cu (µg L^{-1})	14	5.60	1.70	20.10	16	3.60	1.76	21.80	<400
Fe (µg L^{-1})	49	600*	170	13500	15	157*	20.0	660	–
Zn (µg L^{-1})	14	11.6	5.40	340	11	11.9	5.0	32.10	<300

Asterisk (*) indicates significant differences between streams (Pairwise Wilcoxon rank sum tests with adjusted *p* values using the Bonferroni method, $p < 0.05$), *N*…number of samples evaluated, underlined values indicate exceeding the limits for salmonids water in European Union (following the rule EHP 2006/44/ES)

Table 2 Metal concentrations (dry weight) in sediment (mg kg^{-1})

Variable	Klabava	Stroupínský brook
Al	17,300	27400
As	406	12.8
Cd	<0.8	<0.8
Cu	8.71	31.3
Fe	30,900	35800
Zn	60.7	272

K2, S1, S2—see Fig. 1). A total of 48 water samples were taken from 2009–2011 in Klabava (8 samples per year and sampling point, at seven-week intervals), and 120 water samples from 2007–2011 in the Stroupínský stream (12 samples per year and sampling point, at four-week intervals). More detailed information is shown in Table 1, and sediment profiles are given in Table 2. Water quality was conducted using standard methods and equipment (APHA, AWWA and WEF 2005); sediment was sampled following European standards (ISO 5667-1:2006). At the time of the crayfish mortality event (June 8, 2011), water and sediment samples were taken from the Klabava for the measurement of standard parameters (pH, $ANC_{4.5}$, O_2, BOD_5, COD_{cr}, NH_4^+, NO_2^-, NO_3^-, Cl^-, SO_4^{2-} and P in total) and metals (Al, Fe, Cd, As, Cu, Zn), as well as being analyzed for pesticides and petroleum compounds.

Water quality was tested using standard methods and equipment (APHA, AWWA and WEF 2005); sediment was sampled following European standards (ISO 5667-1:2006). Metal analyses were carried out in accredited laboratories of the State Enterprise Povodí Vltavy (the Vltava River watershed public management organization, Prague, Czech Republic) using operating procedures according to the International Organization for Standardization (ISO) standards. Pesticides were measured by liquid chromatography-mass spectrometry using triple quadrupole instrumentation in Multiple Reaction Monitoring mode. Petroleum compounds were measured using GC/MS.

Crayfish sampling

In June 2011, five dead noble crayfish with total body length (TL) varying between 77 and 109 mm ($\bar{x} = 90.2$ mm) and two dead stone crayfish (with TL of 72 and 54 mm) with no signs of decomposition were taken from the Klabava for analysis. For comparison purposes, an additional two noble crayfish (with TL 109 and 65 mm) and 19 stone crayfish with TL between 51 and 82 mm ($\bar{x} = 65.9$ mm) were taken from Klabava in November 2011. The numbers of crayfish available for this study were constrained by: 1. the accessibility of dead crayfish without signs of decomposition; 2. the standard population density of both species in the locality; 3. the accessibility of the locality (the stream flows through a prohibited military area); and 4. the fact that both species are critically endangered.

Because of the lack of information on metal levels in various organs, especially for the stone crayfish, an additional 14 crayfish (7 stone crayfish with TL between 54 and 85 mm, $\bar{x} = 67.5$ mm, and 7 noble crayfish with TL between 61 and 95 mm, $\bar{x} = 76.5$ mm) were taken from the Stroupínský stream to serve as a reference.

Crayfish from both localities were tested for the presence of the most common pathogens: *Aphanomyces astaci* Shikora (crayfish plague) using conventional PCR according to Oidtmann et al. (2006) and Vrålstad et al. (2009), and *Thelohania contejeani* Henneguy (thelohaniasis) using the observation of sporoblasts in crayfish muscles (Bower et al. 1994).

Metal analysis

In order to measure metals from various crayfish organs, the gills, hepatopancreas, and abdomen muscle tissue were dissected. Hepatopancreas analysis was performed on just one of the dead individuals, and for crayfish taken live from both the Stroupínský and Klabava streams, the hepatopancreas was only dissected from individuals larger than 50 mm in TL. Samples were homogenized by pulverization, and then lyophilized and mineralized. Mineralization was performed on sub-samples weighing from 10 mg (for gills and hepatopancreas) to 100 mg (for muscle) using high-pressure microwave digestion in nitric acid and hydrogen peroxide. This was followed by ICP-MS analysis for Al, Fe, Cu, Zn, As, Cd, Pb, and Be. When sample amounts were sufficient, Hg was determined directly from the dry material on an AMA 254 analyzer using 20–50 mg of homogenized material.

Statistical analyses

Water quality of both analyzed streams was compared using pairwise Wilcoxon rank sum tests (with adjusted *P* values using the Bonferroni method). Data on metal concentrations obtained from alive and dead crayfish from the Klabava stream and alive crayfish from the Stroupínský stream were compared using Kruskal—Wallis ANOVA; Dunn's Test (with adjusted *P* values using the Bonferroni method) was used to find individual differences among particular groups. These non-parametric methods were used because most of the observed parameters did not have a normal distribution as found previously by the Shapiro—Wilcox normality test. Moreover, the data on all crayfish were grouped and after log-transformation compared with data from the Stroupínský stream. Pairwise Wilcoxon rank sum tests (with adjusted P values using the Bonferroni method) were used to test for differences in all groups of metal concentrations found in particular tissues (gills, muscle, hepatopancreas) and crayfish occurrence sites (Stroupínský, Klabava). A non-parametric method was again used because normality and variance homogeneity assumptions were not satisfied (using the Shapiro—Wilk normality test and the Bartlett test of homogeneity of variances).

Results

Individuals that died in the mass mortality were tested for the presence of *Aphanomyces astaci*, but no samples tested positive. Likewise, microscopic examination revealed no presence of the microsporidian parasite *Thelohania contejeani*.

Water analysis from the Klabava at the time of the mortality did not reveal the presence of any of the tested harmful parameters (low pH, O_2, BOD_5, NH_4^+, NO_2^-, pesticides, or petroleum products). Although metal concentrations (Al, Fe, Cd, As, Cu, Zn) were also not higher in the period of the mortality, earlier monitoring (see Material and methods) had found incidents of high concentrations of Fe (up to 5.7 mg L^{-1}), Al (up to 3.55 mg L^{-1}) and As (up to 108 µg L^{-1}), and pH values as low as 4 (Table 1). The neutralization capacity of the stream was very low, with $ANC_{4.5}$ varying between 0.36 and 0.90. Before capturing live crayfish, the lower pond on the Klabava had started to be drained (Fig. 1), and extreme values for some metals were measured at this site (the maximum values for Al, Fe, Zn, and As in Table 1). At the reference Stroupínský stream, no water sample analyzed had low pH, and there were no high concentrations of metals, even though the sediments had higher concentrations of Al, Fe, Zn, and Cu (Table 2) than the Klabava did. The neutralization capacity $ANC_{4.5}$ in Stroupínský varied between 3.2 and 5.4.

When comparing data from long-term water quality monitoring in both streams, many significant ($P < 0.05$) differences were found. There were higher amounts of Al ($Z = 2.91$), As ($Z = -2,81$), Fe ($Z = -4.72$) and COD_{cr} ($Z = 4.02$) in the Klabava stream, whereas higher amounts of BOD_5 ($Z = -2.57$), Ca ($Z = -6.04$), Cl ($Z = -5.52$), NO_2 ($Z = -8.87$), NO_3 ($Z = -10.17$), P ($Z = -8.73$), pH ($Z = -9.36$), SO_4 ($Z = -4.68$) and conductivity ($Z = -10.30$) were found in the Stroupínský stream (Table 1).

Firstly, three groups of data were analyzed: dead crayfish from the Klabava, live crayfish from the Klabava, and live crayfish from the Stroupínský stream. When comparing the concentrations of Al, Fe, As, Cd and Pb in gills, no significant differences were found between the first two groups. All the crayfish from the Klabava (both live and dead) had a higher amount of these metals in their gills. When comparing the total loads of metals in gills, the

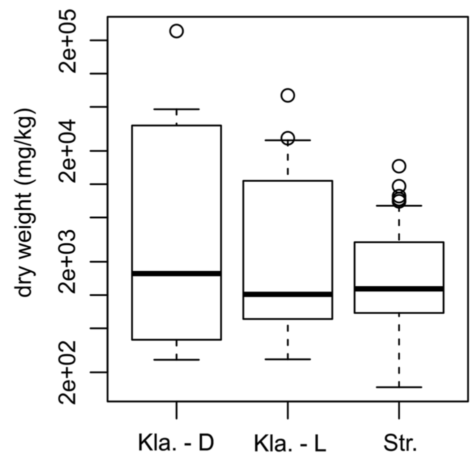

Fig. 2 The total concentration of all metals (Al, As, Cd, Fe, Pb), on the gills of live (Kla.-L) and dead (Kla.-D) crayfish from the Klabava and on the gills of live crayfish from the reference site (Str.-Stroupínský stream)

differences were similar (see Fig. 2). In contrast, dead crayfish had a significantly lower concentration of Cu and higher concentration of Zn in their gills than the two other groups (Dunn's Test, *z*-value >2.39).

The differences in individual metals in muscle between localities were not as marked as for gills. The only significantly greater ($P < 0.05$) concentrations were of As and Cd in the muscle of crayfish from Klabava compared to Stroupínský, while concentrations of Al were significantly lower from Klabava. Other metals in crayfish muscle did not show significant differences between the localities (Table 3.)

The essential elements Cu and Zn showed different patterns than the remaining metals. There were no significant differences in Cu and Zn in either gills or muscles between crayfish in the Klabava and the Stroupínský brook, while the concentration of Cu was significantly higher ($P < 0.05$) in the hepatopancreas of crayfish from the less-polluted reference locality (Table 3, Fig. 6). Higher Cu values ($P < 0.05$) were found in the gills of live crayfish at the Klabava and Stroupínský brooks compared to dead crayfish.

Total loads of all metals in each crayfish group

Based on these results, the concentrations of all metals in the tissues of all crayfish (i.e., both crayfish species, as there were no statistical differences between the species) from Klabava were compared to concentrations from the reference Stroupínský stream; significant differences ($P < 0.05$) were found between these two sites (Table 3, Figs. 3–8).

Our results confirm those of other authors (e.g., Alcorlo et al. 2006; Williams et al. 2009) that have found that metal bioaccumulation differed between crayfish organs. The median of the sum of all analyzed metals in the gills of

Table 3 Metal concentrations in different crayfish organs (tissues)

Metal	Klabava (Kla.) gill *N*	Med	Min	Max	muscle N	Med	Min	Max	hepatopancreas N	Med	Min	Max	Stroupínský (Str.,reference site) gill N	Med	Min	Max	muscle N	Med	Min	Max	hepatopancreas N	Med	Min	Max
Al	28	6100^{A}	2000	64,000	28	140^{B}	30	420	19	33^{C}	14	98	14	1425^{D}	160	7100	14	195^{D}	10	1700	9	30^{F}	12	71
As	28	100^{A}	45.0	1500.0	28	5.2^{B}	2.5	22	19	5.5^{B}	3.7	9.7	14	1.75^{C}	1.1	3.5	14	0.885^{D}	0.6	1.5	9	2.5^{C}	1.6	3.3
Be	28	0.33	0.11	1.30	28	0.1	<0.1	<0.1	0				14	0.11	0.1	0.26	14	0.1	0.1	0.1	0			
Cd	28	5.3^{A}	1.30	42.00	28	0.41^{B}	0.065	1.7	19	13^{C}	4	38	14	0.175^{D}	0.05	0.3	14	0.05^{E}	0.05	0.11	9	1^{B}	0.48	1.6
Cu	28	320^{A}	160	3000	28	58.5^{B}	19	120	19	$190^{A,C}$	35	950	14	$330^{A,C}$	240	450	14	$68.5^{B,C}$	29	120	9	710^{D}	350	2500
Fe	28	9350^{A}	3600	160,000	28	200^{B}	58	710	19	450^{C}	130	900	14	$1970^{D,C}$	270	7000	14	$180^{B,C,E}$	50	1300	9	$220^{B,C,E}$	150	620
Hg	4	0.19	0.12	0.32	24	0.83	0.35	1.6	9	0.18	0.06	0.55	0				12	0.66	0.4	1.4	8	0.08	0.06	0.12
Pb	28	8.70	2.90	290.00	28	<0.5	<0.5	1.2	19	<0.5	<0.5	0.98	14	1.86	0.5	8.9	14	0.5	0.5	1.4	9	<0.5	<0.5	<0.5
Zn	28	110^{A}	61	14,000	28	$110^{A,B}$	73	180	19	260^{C}	120	410	14	$92^{A,B,D}$	70	160	14	$110^{A,B,D,E}$	61	170	9	$220^{A,C,F}$	160	320

Superscript letters indicate whether differences between species are significant; Pairwise Wilcoxon rank sum tests (with adjusted *P* values using the Bonferroni method) were used to test for differences in all groups of metal concentrations found in particular tissues (gills, muscle, hepatopancreas) and crayfish occurrence sites (Stroupínský, Klabava). The same letters indicate no differences between pairs marked by the same superscript, different letters in superscript (e.g., A B, A C) indicate significant differences between streams and (or) evaluated tissues, *N*…number of samples

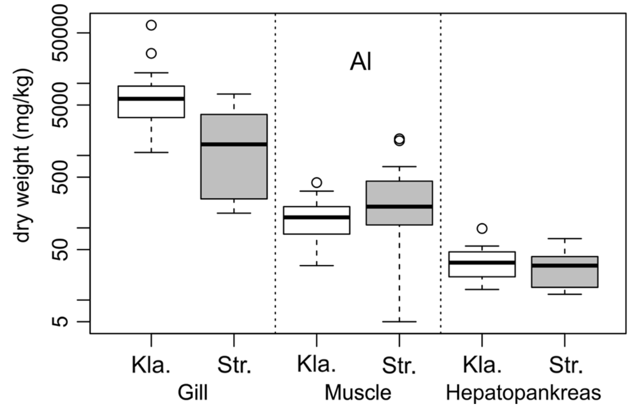

Fig. 3 Concentration of Al, in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

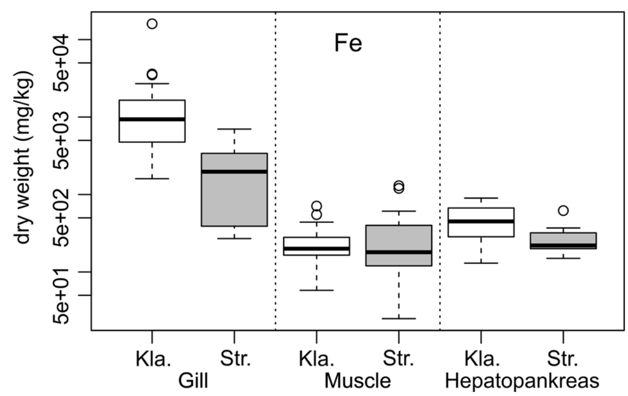

Fig. 4 Concentration of Fe, in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

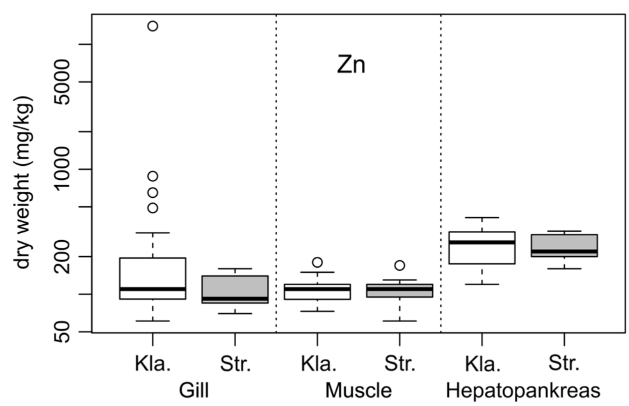

Fig. 5 Concentration of Zn, in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

crayfish from Klabava reached a value of 17,421.3 mg kg^{-1} dry weight (maximum 242,832 mg kg^{-1}), while the median from Stroupínský was only 3908.8 mg kg^{-1} (maximum 14,552 mg kg^{-1}). There were also significant differences

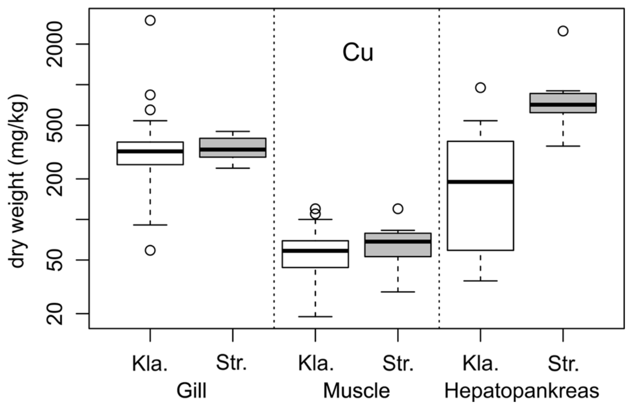

Fig. 6 Concentration of Cu, in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

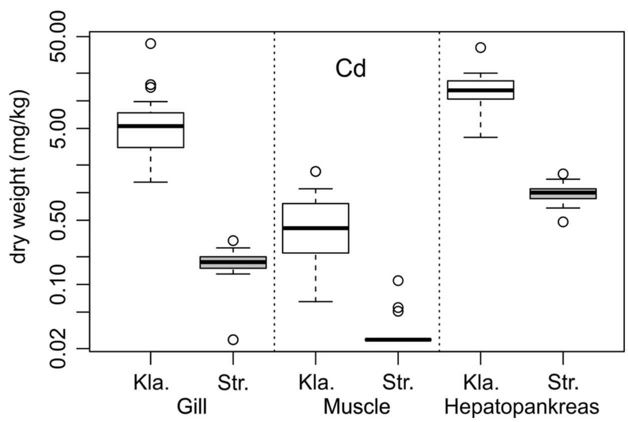

Fig. 7 Concentration of Cd, in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

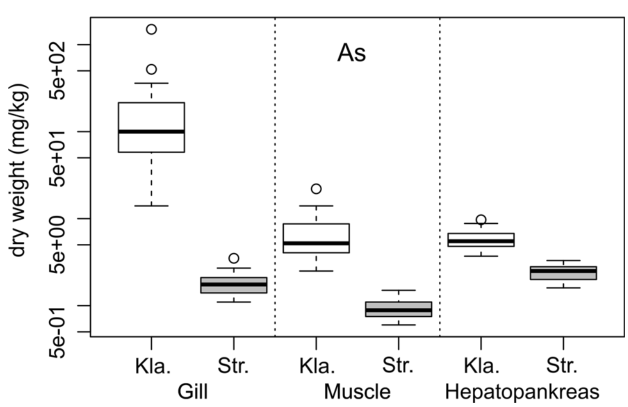

Fig. 8 Concentration of As in gills, muscle and hepatopancreas of crayfish (*Astacus astacus*, *Austropotamobius torrentium*) from Klabava (Kla.) and from the reference site, Stroupínský brook (Str.)

($P < 0.05$) not just in the total loads of metals in gills but also for individual metals except for the essential elements Zn and Cu (Table 3). Concentrations of metals on a kg dry weight basis were significantly greater ($P < 0.05$) in gills than in muscle except for Zn and Hg. Mercury accumulation was significant lower in gills than in muscle. Because of insufficient sample material, Hg in gills was only analyzed from four crayfish (mean 0.2 ± 0.073 mg kg^{-1}), but when compared to the amount of Hg in the muscle of these same crayfish, the concentrations were 4× to 13× higher (mean 1.3 ± 0.19 mg kg^{-1}).

Discussion

Because of a mass mortality of noble and stone crayfish, we compared the accumulation of metals in crayfish tissues from the affected stream and a reference locality. In total, 28 crayfish from the affected Klabava stream and 14 from the reference Stroupínský stream were studied (of both sexes). Differences between sexes were not evaluated, since there is no intersexual difference in the accumulation of metals in tissues (e.g., Alcorlo et al. 2006; Naghshbandi et al. 2007).

Our analysis found significant differences between crayfish from the two localities in the accumulation of metals in the gills. Concentrations of Al, Fe, and other metals were many times higher in both dead and live crayfish from the Klabava compared to the reference site. As in the study of Naghshbandi et al. (2007), the highest concentration of Fe was found in the gills of Klabava crayfish. According to a study by Svobodová et al. (2012), there is a negative correlation between the presence of crayfish and Fe and Al concentrations in water. Maximal concentrations of Al in the gills of dead crayfish from the Klabava were 50× higher (64,000 mg kg^{-1}) than that found by Alexopoulos et al. (2003) in the American signal crayfish *Pacifastacus leniusculus* (1294 mg kg^{-1}). In that study, crayfish were exposed to sub-lethal Al concentrations in the water (0.5 mg L^{-1}) compared to the Klabava stream where there was a higher amount of heavy and toxic metals. In addition to Al concentrations in various crayfish tissues, Alexopoulos et al. (2003) studied changes in behavior associated with length of exposure to this metal (0–20 days). After 15 days of exposure to Al, crayfish stopped moving and did not respond to prodding with a probe (Alexopoulos et al. 2003). Similar behavior was observed in Klabava crayfish, which left their shelters even during the day, lay on the bottom of the stream without moving, and occasionally tried to leave the current or lay apathetically in the shallows or completely out of the water.

The median concentration of carcinogenic and teratogenic Cd in the water of the Klabava was 0.16 µg L^{-1}, but Cd concentrations in the muscle of living crayfish sometimes reached up to 1.7 mg kg^{-1} and even 38 mg kg^{-1} in the hepatopancreas. This shows that even under relatively low concentrations in the water, with only one outlying high value of 6.8 µg L^{-1} measured during our study, toxic Cd can accumulate in high concentrations in some tissues, especially the hepatopancreas. This high concentration of Cd in the hepatopancreas indicates repeated high episodes, which were not recorded; however, during our monthly water quality monitoring. Moreover, Cd is rapidly depurated from crayfish tissues (Guner 2010) and the lasting high amounts of Cd also supports these supposed episodes with high stream levels of Cd. Limits of Cd in muscle for biota (0.016 mg kg^{-1}, IRIS) were exceeded in 22 out of 28 crayfish from the Klabava.

Even though these crayfish species are protected in the Czech Republic and their consumption is prohibited, another indicator of acceptable levels of Cd loads is European Commission Regulation No 629/2008 for certain contaminants in foodstuffs for crayfish meat, which sets the highest acceptable level at 0.5 mg kg^{-1} wet weight. Although Simon et al. (2000) did not consider the bioaccumulation of Cd in crayfish tissues to be a risk to crayfish predators (including humans), our study shows that half of the crayfish from the Klabava exceeded the acceptable limit for consumption. Dalzell and MacFarlane (1999) demonstrated unsafe high concentrations of metals in the gill filaments of aquatic organisms, and found non-dissolved Fe compounds in the III oxidation state covering the gill filaments of fish, which decreases the respiratory surface area of the gill and can lead to death by asphyxiation. According to the studies of Fjeld et al. (1988) and Alexopoulos et al. (2003), the toxicity of freshly neutralized Al to the signal crayfish is largely due to its extracellular interaction with the gill, resulting in asphyxiation and osmoregulatory dysfunction. Al-hydroxides may easily bind to the gill surface (Wilkinson and Campbell 1993), where negatively charged sites act as Al polymerization nuclei. Repeated episodes of such anomalies on the gills of crayfish caused by sub-lethal Al concentrations (0.5 mg L^{-1}) can lead to hypoxia and weaken the immune system, and can thus be even more harmful than direct Al toxicity (Ward et al. 2006). Alexopoulos et al. (2003) showed that exposure to sub-lethal Al concentrations for 15 days led to a complete lack of a flight response in signal crayfish. Other studies on toxic metals have shown that chronic loads can appear in high concentrations in the hepatopancreas of crayfish even though during acute episodes there are higher concentrations in gills, for instance Cd (Al Kaddissi et al. 2012; Meyer et al. 1991).

Crayfish in the Klabava are repeatedly exposed to high metal concentrations, which mainly occur in runoff during heavy rains or snowmelt from acidification-damaged spruce monoculture forests with highly degraded soils or from old mine drainage. For instance, in January 2011, there was a rapid snowmelt accompanied by intense rainfall, whereas water levels were low in the spring. Crayfish are also endangered here when fish ponds are drained for harvesting because their sediments are contaminated with toxic metals. For these reasons, there were no significant differences between crayfish dying in the spring and live crayfish caught after pond draining in autumn. These expositions are presumably recurring because the level accumulation of Cd was high in both dead and live crayfish from Klabava and Cd is rapidly depurated from crayfish tissues (Guner 2010).

The different patterns in the essential elements Cu and Zn (there were no difference in these metals in gills between the polluted and the reference stream) could also be associated with the rapid depuration of these metals (Kouba et al. 2010).

On the other hand, the presence of ponds in the watershed is partly responsible for the continued occurrence of crayfish. The acidification processes mentioned above occasionally decrease stream pH level below 5; this pH level is usually considered as a limiting factor for the presence of crayfish (Nyström 2002). Moreover, lower pH affects reproduction processes (Appelberg 1984) as well as disturbed Ca metabolism leading to exoskeleton defects (France 1983). However, the ponds in the watershed are managed, with liming performed to prevent lower Ca levels. In addition, the accumulation of water plays an undisputable role in buffering lower pH. Unfortunately, a bypass was recently built around the biggest pond, which has resulted in the acidified precipitation from tributaries flowing directly into the Klabava without buffering. Moreover, crayfish don't occur below the confluence with the Třítrubecký stream, a highly acidified tributary.

Crayfish from the Klabava had extremely high concentrations of Al and Fe in the gills and of As and Cd in muscle (Figs 3, 4, 7, and 8) compared to concentrations in the gills and muscles of crayfish from the reference Stroupínský stream. The mass crayfish mortality, lasting more than three months, was thus probably due to a combination of the negative influence of extracellular interactions of individual metals (Al, Fe, As, Cd and Pb) with the gill epithelia, resulting in hypoxia and osmoregulatory stress, as well as the chronic effects of toxic metals and bioaccumulation of these metals in crayfish tissues (As and Cd). Both factors can lead to a weakening of the immune system, making crayfish more susceptible to lethal infections from commonly occurring bacteria in the water. However, our data did not allow us to determine whether low concentrations of Cu in dead crayfish from polluted localities contributed to weakening their immune systems and thus if this played a role in the mortality.

Acknowledgements The authors would like to thank the Nature Conservation Agency of the Czech Republic for providing data, and both to David Hardekopf and Mark Sixsmith for improving our English. This study was financially supported by the Ministry of Environment of the Czech Republic within the framework of project MZP 000 2071101.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable national guidelines for the care and use of animals were followed.

References

Al Kaddissi S, Legeay A, Elia A, Gonzalez P, Floriani M, Cavalie I, Simon O (2012) Mitochondrial gene expression, antioxidant responses, and histopathology after cadmium exposure. Environ Toxicol 29:893–907. doi:10.1002/tox.21817

Alcorlo P, Otero M, Crehuet M, Baltanás A, Montes C (2006) The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). Sci Total Environ 366:380–390. doi:10.1016/j.scitotenv.2006.02.023

Alexopoulos E, McCrohan CR, Powell JJ, Jugdaohsingh R, White KN (2003) Bioavailability and toxicity of freshly neutralised aluminium to the freshwater crayfish *Pacifasticus leniusculus*. Arch Environ Contam Toxicol 45:509–514. doi:10.1007/s00244-003-0228-9

Antòn A, Serrano T, Angulo E, Ferrero G, Rallo A (2000) The use of two species of crayfish as environmental quality sentinels: the relationship between heavy metal content, cell and tissue biomarkers and physico-chemical characteristics of the environment. Sci Total Environ 247:239–251. doi:10.1016/S0048-9697(99)00493-3

APHA, AWWA, WEF (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington, DC

Appelberg M (1984) Early development of the crayfish *Astacus astacus* L. in acid waters. Reports of the Institute of Freshwater Research. Drottnigholm 61:48–59

Bower SM, McGladdery SE, Price IM (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu Rev Fish Dis 4:1–199. doi:10.1016/0959-8030(94)90028-0

Dalzell DJB, MacFarlane NAA (1999) The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. J Fish Biol 55:301–315. doi:10.1111/j.1095-8649.1999.tb00680.x

Fjeld E, Hessen DO, Roos N, Taugbøl T (1988) Changes in gill ultrastructure and haemolymph chloride concentrations in the crayfish, *Astacus astacus*, exposed to de-acidified aluminium-rich water. Aquaculture 72:139–150. doi:10.1016/0044-8486(88)90154-8

France RL (1983) Response of the crayfish *Orconectes virilis* to experimental acidification of a lake with special reference to the importance of calcium. In: Goldman CR (ed) Freshwater Crayfish V. AVI Publishing, Sestpot, Connnecticut, pp 98–111

Guner U (2010) Cadmium bioaccumulation and depuration by freshwater crayfish, *Astacus leptodactylus*. Ekoloji 19:23–28. doi:10.5053/ekoloji.2010.774

Hojdová M, Navrátil T, Rohovec J, Žák K, Vaněk A, Chrastný V, Svoboda M (2011) Changes in mercury deposition in a mining and smelting region as recorded in tree rings. Water Air Soil Poll 2016:73–82. doi:10.1007/s11270-010-0515-9

Kopáček J, Hejzlar J, Stuchlík E, Fott J, Veselý J (1998) Reversibility of acidification of mountain lakes after reduction in nitrogen and sulphur emissions in Central Europe. Limnol Oceanogr 43:357–361. doi:10.4319/lo.1998.43.2.0357

Kouba A, Buřič M, Kozák P (2010) Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Pollut 211:5–16. doi:10.1007/s11270-009-0273-8

Lampert W, Sommer U (2007) Limnology: the ecology of lakes and streams, 2nd edn. Oxford University Press, New York

Lundberg U (2004) Behavioural elements of the noble crayfish, *Astacus astacus* (Linnaeus, 1758). Crustaceana 77:137–162. doi:10.1163/156854004774003510

Meyer W, Kretschmer M, Hoffman A, Harisch G (1991) Biochemical and histochemical observations on effects of low level heavy metal load (lead, cadmium) in different organ systems of freshwater crayfish, *Astacus astacus* L. (Crustecea Decapoda). Ecotoxicol Environ Saf 21:137–156. doi:10.1016/0147-6513(91)90016-I

Naghshbandi N, Zare S, Heidari R, Razzaghzadeh S (2007) Concentration of heavy metals in different tissues of *Astacus leptodactylus* from Aras dam of Iran. Pak J Biol Sci 10:956–3959

Nyström P (2002) Ecology. In: Holdich DM (ed) Biology of freshwater crayfish. Blackwell Science, Oxford, p 192–235

Oidtmann B, Geiger S, Steinbauer P, Culas A, Hoffmann RW (2006) Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. Dis Aquat Organ 72:53–64. doi:10.3354/dao072053

Peuranen S, Vuorinen PJ, Vuorinen M, Hollender A (1994) The effects of iron, humic acids and low pH on the gills and physiology of brown trout (*Salmo trutta*). Ann Zool Fenn 31:389–396

Poléo ABS, Lydersen E, Rosseland BO, Kroglund F, Salbu B, Vogt RD, Kvellestad A (1994) Increased mortality of fish due to changing Al-chemistry of mixing zones between limed streams and acidic tributaries. Water Air Soil Poll 75:339–351. doi:10.1007/BF00482945

Psenner R, Catalan J (1994) Chemical composition of lakes in crystaline basins: a combination of atmospheric deposition, geologic background, biological activity and human action. In: Margalef R (ed) Limnology now: a paradigm of planetary problems. Elsevier, Amsterdam, pp 255–314

Simon O, Ribeyre F, Boudou A (2000) Comparative experimental study of cadmium and methylmercury trophic transfers between the Asiatic clam *Corbicula fluminea* and the crayfish *Astacus astacus*. Arch Environ Con Tox 38:317–326. doi:10.1007/s002449910042

Singh A, Agrawal A (2008) Acid rain and its ecological consequences. J Environ Biol 29:15–24

Sucharová J, Suchara I (2004) Distribution of 36 element deposition rates in a historic mining and smelting area as determined through fine-scale biomonitoring techniques. Water Air Soil Poll 153:205–228. doi:10.1023/B:WATE.0000019944.33209.83

Sucharová J, Suchara I, Reimann C, Boyd R, Filzmoser P, Englmaier P (2011) Spatial distribution of lead and lead isotopes in soil B-horizon, forest-floor humus, grass (*Avenella flexuosa*) and spruce (*Picea abies*) needles across the Czech Republic. Appl Geochem 26:1205–1214. doi:10.1016/j.apgeochem.2011.04.009

Svobodová J, Douda K, Štambergová M, Picek J, Vlach P, Fischer D (2012) The relationship between water quality and indigenous and alien crayfish distribution in the Czech Republic: patterns and conservation implications. Aquatic Conserv: Mar Freshw Ecosyst 22:776–786. doi:10.1002/aqc.2262

Vlach P, Hulec L, Fischer D (2009) Recent distribution, population densities and ecological requirements of the stone crayfish (*Austropotamobius torrentium*) in the Czech Republic. Knowl Managt Aquatic Ecosyst 384–395:13. doi:10.1051/kmae/2010005

Vrålstad T, Knutsen AK, Tengs T, Holst-Jensen A (2009) A quantitative TaqMan MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. Vet Microbiol 137:146–155. doi:10.1016/j.vetmic.2008.12.022

Ward RJ, McCrohan CR, White KN (2006) Influence of aqueous aluminium on the immune system of the freshwater crayfish *Pacifasticus leniusculus*. Aquat Toxicol 77:222–228. doi:10.1016/j.aquatox.2005.12.006

Wauer G, Teien HC (2010) Risk of acute toxicity for fish during aluminium application to hardwater lakes. Sci Total Environ 408:4020–4025. doi:10.1016/j.scitotenv.2010.05.033

Wilkinson KJ, Campbell PG (1993) Aluminum bioconcentration at the gill surface of juvenile Atlantic salmon in acidic media. Environ Toxicol Chem 12:2083–2095. doi:10.1002/etc.5620121116

Williams G, West JM, Koch I, Reimer KJ, Snow ET (2009) Arsenic speciation in the freshwater crayfish, Cherax destructor Clark. Sci Total Environ 407:2650–2658. doi:10.1016/j.scitotenv.2008.12.065

Žák K, Rohovec J, Navrátil T (2009) Fluxes of heavy metals from a highly polluted watershed during flood events: a case study of the Litavka River, Czech Republic. Water Air Soil Poll 203:343–358. doi:10.1007/s11270-009-0017-9