Arch Environ Contam Toxicol
DOI 10.1007/s00244-012-9775-2

Distribution and Accumulation of Metals in Tadpoles Inhabiting the Metalliferous Streams of Eastern Chalkidiki, Northeast Greece

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Received: 31 December 2011 / Accepted: 30 April 2012

Abstract The present study investigates the accumulation of heavy metals [copper (Cu), lead (Pb), zinc (Zn), magnesium (Mn), cadmium (Cd), nickel (Ni), and chromium (Cr)] in tadpoles inhabiting the metalliferous streams flowing within the Asprolakkas River basin (northeast Chalkidiki peninsula, Greece) and the effect of potentially harmful elements in stream water and sediment on the corresponding levels in their tissue. Animals were collected from six sampling sites influenced by a wide range of surface water and stream sediment trace element concentrations. The results of the chemical analyses showed that tadpoles accumulated significant levels of all of the examined metals. The range of whole-body mean measured concentrations were (in dry mass) as follows: Cu (46–182 mg/kg), Pb (103–4,490 mg/kg), Zn (494–11,460 mg/kg), Mn (1,620–13,310 mg/kg), Cd (1.2–82 mg/kg), Ni (57–163 mg/kg), and Cr (38–272 mg/kg). The mean concentrations of Pb, Zn, Mn, Ni, Cr, and Cd in Kokkinolakkas stream, which drains a currently active mining area, were the highest ever reported in tadpoles. Our results indicate that whole-body levels of Pb, Zn, Cu, and Cd increase with stream sediment concentrations and that these organisms tend to accumulate metals bound to Fe and Mn oxides. In addition, high dissolved concentrations and significant concentrations associated with more labile geochemical phases of sediments for specific metals were contributing factors determining whole-body levels. Given the observed bioconcentration factors, as well as the correlation with sediment concentrations, it is proposed that these organisms could be considered as bioindicators of environmental contamination and may be used for monitoring purposes within this metal-rich zone and, perhaps, within other rivers affected by metal mining.

Environmental contamination of the aquatic environment by heavy metals is of major concern and has received considerable attention during past decades. These potentially toxic elements are distributed between the dissolved phase, colloids, suspended matter, and solid phases. Determining water and sediment metal loads alone cannot evaluate the biologically available concentrations of metals and their influence and possible toxicity on the ecosystem. Bioaccumulation studies of heavy metals in aquatic biota are significant to assess the fate of these chemical elements in metal-rich contaminated systems (e.g., Niethammer et al. 1985; Smolders et al. 2003; Besser et al. 2007).

For the biomonitoring of heavy-metal aquatic contamination, a common issue is the selection of an appropriate bioindicator. Amphibians are commonly used as indicator species of ecosystem health and are well known for accumulating metals (Loumbourdis and Wray 1998). Especially, larval amphibians may accumulate metals more readily than adults, possibly due to differences in ratios of surface area to volume and skin permeability (Hall and Mullhern 1984). Tadpoles are ideal as bioindicators because they are readily available in large numbers having high local densities; they can be easily captured and handled in the laboratory; and they are good representatives of freshwater ecosystems (Burger and Snodgrass 1998;

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Hopkins et al. 1998). In addition, due to their feeding ecology, which includes the consumption of substrate and algae and the fact that they continuously process water for respiration through the skin, their tissues are exposed to a wide variety of contaminants. Their tendency to accumulate trace elements is crucial in transporting toxic elements from the aquatic to the terrestrial environment and transforming metals from less bioavailable forms to those that are available to higher trophic-level organisms (Unrine et al. 2007).

Although a considerable number of studies have reported the capability of these aquatic organisms to concentrate heavy metals in their tissue (e.g., Gale et al. 1973; Hall and Mullhern 1984; Birdsall et al. 1986; Lee and Stuebing 1990; Unrine et al. 2007), only a few field studies have linked heavy metals in tadpoles to metals in their immediate environment, i.e., sediment and water (Grillitsch and Chovanec 1995; Sparling and Lowe 1996; Hofer et al. 2005). Furthermore, there is no study that correlates trace element concentrations in tadpoles with the portions of metals that are readily liberated from the sediments. Metals in sediments are found in various geochemical forms, which determine their bioavailability, i.e., there is a restricted availability of sediment-bound metals to aquatic biota. The exchangeable, the carbonate, and the Fe–Mn oxide phases are considered to be geochemical forms that are highly to moderately available to aquatic organisms because they can interact with organic tissues more easily than sulphide–organic and residual bound metals (Pierre Stecko and Bendell-Young 2000).

The Asprolakkas catchment drains metal-rich geological formations of the eastern Chalkidiki peninsula, northeast (NE) Greece (Fig. 1). A recent study was published by Kelepertzis et al. (2012) concerning the geochemical characteristics of surface water and stream sediments in running streams from the Asprolakkas River basin. However, no attention was given to the effects of contamination on the stream biota. The geochemical conditions in the area provide a unique opportunity to study heavy-metal accumulation in the tissue of tadpoles living along various gradients of sediment and water contamination because the selected region encompasses the following: (1) sites with current intensive mining, (2) sites with past low-scale mining, and (3) sites on unmined, mineralized terrain.

In the present study, the concentrations of lead [Pb], zinc [Zn], magnesium [Mn], copper [Cu], nickel [Ni], chromium [Cr], and cadmium [Cd] were determined in water, sediments, and tissue of tadpoles inhabiting these metalliferous streams to point out differences of heavy-metal bioaccumulation according to different levels of drainage sediment and stream water contamination. In particular, we tested the hypothesis that metal levels in tadpoles correlate positively to metal concentrations in sediment and/or stream water. Data from this research will contribute (1) to the knowledge of the mechanisms responsible for the transfer of metals from abiotic to biotic components within freshwater ecosystems and (2) to environmental risk assessment methodology because information concerning bioaccessibility issues is essential to the risk-assessment framework.

The specific objectives of this study were to as follows: (1) document the levels of Pb, Zn, Mn, Cu, Ni, Cr, and Cd in tadpoles inhabiting the metalliferous streams of the study area and compare their concentrations with literature data on metal accumulation in tadpole tissues from other areas; and (2) relate metal tissue concentrations with the measured concentrations in the water column, the total metal content of sediment samples, and with metal portions bound to the bioaccessible fractions in sediments.

Materials and Methods

Sites of Interest

This study was conducted as part of a larger investigation for evaluating the present contamination levels of the Asprolakkas River basin, which forms part of the wider mineralized area of eastern Chalkidiki (Fig. 1). The upper reaches of the perennial streams are found in Stratonikon Mountain (northward of Stratoni), follow a general north–south direction, and discharge their water into the Aprolakakkas River, which flows toward the Gulf of Ierissos. Maximum elevations ($\leq$600 m) are found in the western part of the area and gradually decrease toward the sea. Streams are characterized by a well-developed dendritic-style drainage network and support riparian and aquatic vegetation. Hydrological and climatic conditions are typical of most Mediterranean freshwater ecosystems, i.e., rainy winters and dry, hot summers. The majority of streams in the area flow during the entire year, except for the downstream area of Kokkinolakkas, which dries out during the dry period.

The selection of sampling locations (Fig. 1) was such as to cover the three catchment basins that contribute to Asprolakkas water and sediment chemistry. Kokkinolakkas stream (sampling sites G and B) is subject to high levels of contamination by way of the weathering of the polymetallic sulphide deposits of Madem Lakkos and Mavres Petres ore bodies. Kerasia stream (sampling site C) is not related to any mining activities, whilst the upstream area of Piavitsa (sampling site I3) is influenced by the erosion of tailing piles from inactive Pb–Zn–Mn mines. Two additional sites (L2 and D) along Piavitsa creek were chosen in consideration of future potential discharges of metal

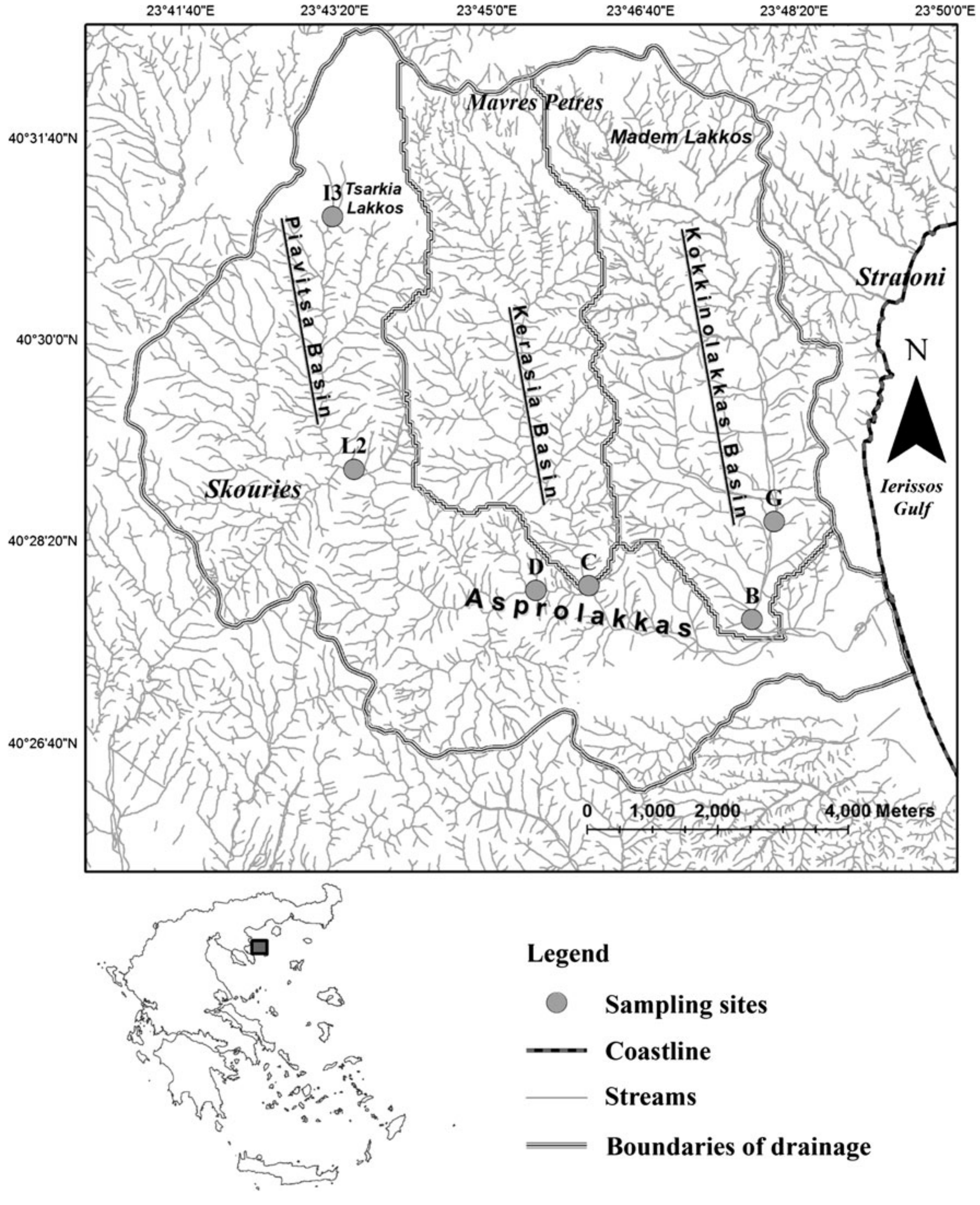

Fig. 1 Map of Asprolakkas River basin showing the sampling sites

containing effluents, resulting from the scheduled exploitation of the Skouries Cu–Au ore deposit.

Water, Sediment, and Tadpole Sampling

Sampling campaigns were performed during two periods: April 2008 and May 2010. Specifically, in April 2008, stream water and sediment samples were collected from all six sampling sites (G, B, C, I3, L2, and D). Water samples were recovered in polyethylene bottles, filtered in the field through 0.45-μm membrane filters, acidified to pH <2, and stored at 4 °C until analysis. At each sampling site, the upper layer (0–5 cm) of stream sediment was recovered by collecting material over a stream stretch of approximately

100 m. At the laboratory, they were oven dried at a constant temperature of 45 °C, sieved to <2 mm fraction, and pulverized in an agate mortar to <0.075 mm. Tadpoles of the species *Pelophylax kurtmuelleri* were collected using dip net along Kokkinolakkas stream (locations G and B) and Asprolakkas stream (location D).

In a second sampling survey (May 2010), we collected organisms of the same species from sites I3 (upstream in Piavitsa), L2 (downstream in Piavitsa), and C (downstream in Kerasia).

Approximately 50 individuals were collected at each site to ensure that sufficient tissue material would be available for analysis. Tadpoles from both sampling surveys were immediately refrigerated after collection to lower their metabolic rate and prevent them from evacuating their gut content and stored in plastic bags without water. Later, in the laboratory, they were frozen and kept at–20 °C until preparation and analysis. Samples were processed within 2 months of collection.

Instrumental Analyses

The heavy-metal levels of surface water and stream sediment samples were examined to determine their influence on tadpole metal accumulation. Dissolved concentrations of Pb, Cu, Ni, Cr, and Cd were measured by graphite furnace atomic absorption spectroscopy (GAAS), whilst Zn and Mn were measured by GAAS and by flame atomic absorption spectroscopy (FAAS) when necessary. Heavy-metal concentrations in sediment samples were determined after dissolution by aqua regia ($HCl–HNO_3–H_2O$). A 0.5-g aliquot of each pulverized sample was digested at 95 °C for 1 h, diluted to 10 ml with deionized water, and analyzed by inductively coupled plasma–optical emission spectrometry. In addition, selected sediment samples (I3, L2, C, and B) were subjected to a five-step sequential extraction procedure (Tessier et al. 1979). Based on logistical considerations during the initial survey, the sequential extraction process was applied on two samples per catchment basin within the study area (Kelepertzis et al. 2012). According to this method, metals may be fractioned in sediments as exchangeable, carbonate-bound, iron/manganese oxide-bound, organic matter/sulphide–bound, and residual-bound species. Details for the applied reagents and digestion conditions are given by Kelepertzis et al. (2012). The extracted solutions were analyzed by FAAS.

Regarding the tadpole samples, we determined trace element concentrations in the whole body of all individuals, even though it has been reported that the digestive tract contains a significant percentage of the total metal content (Burger and Snodgrass 2001). According to these investigators, tadpoles can be used to test differences in metal levels among different sites, and if the desired aim is only to assess functional metal concentrations (i.e., those that may affect their growth, physiology, and behaviour), the digestive tract should be removed before analysis.

At each sampling location, three independent subsamples were collected during the April 2008 sampling period, and eight independent subsamples were collected during the May 2010 sampling period. Samples were thawed, identified to species according to Valakos et al. (2008), and digested with a 1:1 mixture of Suprapure HNO_3 and $HClO_4$ (4 ml HNO_3 and 4 ml $HClO_4$) until digestion was complete. The resulting solutions were cooled, filtered with Whatman No. 40 filter papers, and diluted to 25 ml using deionized water.

Heavy metals (Pb, Zn, Mn, Cu, Ni, Cr. and Cd) were measured by FAAS for organisms collected in April 2008 and by inductively coupled–plasma atomic emission spectrometry for organisms collected in May 2010. All concentrations were expressed in milligrams per kilogram dry weight. For quality-control purposes, analytical method blanks and the certified reference material ERM-CE 278 [Institute for Reference Materials and Measurements (mussel tissue–trace elements)] were included in each digestion batch. Mean recovery values of chemical elements for the reference material were in good agreement with the certified values, with the percent difference being <10 %, except for Cu and Pb, which showed analytical bias of 15 and 23 %, respectively. Based on the analytical results of the reference material, no statistically significant bias ($p < 0.05$) was observed between samples analysed by AAS versus ICP-AES. This confirmed the comparability of analytical results from the two sampling periods and allowed further processing of the combined data set.

Statistical Treatment and Bioconcentration Factors

Kruskal–Wallis test, followed by post hoc analysis (Fisher's least significant difference test), was applied to test for differences in trace elements concentration of tadpole tissues among the examined populations (all tests were two-tailed). The relationships of metal concentrations between tadpoles and sediments were elucidated by Pearson's correlation analysis. For the statistical calculations described previously, we set the 95 % level of significance. Statistical analysis was performed according to Zar (1984). Principal components analysis (PCA) was also applied to analyze the structure of interrelationships among the trace elements concentrations in the tadpole tissues. The PCA factor scores were then used to complete perceptual mapping of the examined sites.

With the aim to evaluate metal bioaccumulation in tadpole tissues, bioconcentration factors (BCFs) were determined with respect to the aqua regia–extractable concentrations in sediments and the values measured in the

Table 1 Metal concentrations in tadpole tissues (mg/kg), and metal levels in water (µg/l) and sediment (mg/kg) from the study sites

Metal	Kokkonolakkas						Kerasia		
	Site G			Site B			Site C		
	Water	Sediment	Tadpoles	Water	Sediment	Tadpoles	Water	Sediment	Tadpoles
Pb	28	2,074	4,490	9	3,439	4,480	5	320	103
Zn	290	4,115	11,460	380	3,749	9,460	50	330	494
Mn	1,860	7,464	13,100	1,680	6,811	12,500	16	1,986	1,620
Cu	8	157	148	9	175	182	4	46	48
Ni	13	79	163	14	68	140	3	78	67
Cr	1	94	272	1	92	193	2	93	75
Cd	3	12.7	82	3	15.2	42	ND	1.2	3.8
Metals	Piavitsa								
	Site I3			Site L2			Site D		
	Water	Sediment	Tadpoles	Water	Sediment	Tadpoles	Water	Sediment	Tadpoles
Pb	34	1,716	550	21	271	261	2	245	310
Zn	50	1,621	2,030	14	476	609	30	405	520
Mn	55	69,398	13,310	8	7,252	2,150	15	7,400	2,450
Cu	3	70	46	3	47	61	2	86	61
Ni	ND	121	57	2	92	84	2	134	143
Cr	ND	63	38	1	107	118	ND	144	223
Cd	ND	6.5	6.7	ND	1.4	4.1	ND	1	1.2

Water and sediment concentrations measured by Kelepertzis et al. (2012)

ND not detected

exchangeable, carbonate, and Fe-Mn oxide phases of the sequential extraction scheme, which are considered to be the most bioavailable to aquatic organisms. The bioconcentration factors were calculated as follows (Santoro et al. 2009; Pradit et al. 2010) (Eq. 1):

$$BCF = M_{\mathrm{tissue}} / M_{\mathrm{sed}}, \qquad (1)$$

where M_{tissue} is the metal concentration in tadpole tissues, and M_{sed} is the metal amount in the sediment.

Results

Trace Element Concentrations in Water and Sediment

As listed in Table 1, dissolved concentrations of Zn, Mn, Ni, and Cd in stream water from Kokkinolakkas (samples G and B) are greater when compared with those in Piavitsa and Kerasia samples. All water samples had Cu and Cr levels that were either low or lower than the detection limit of the analytical technique. The highest Pb concentrations were recorded in samples G, I3, and L2 and showed no systematic trend within the studied basins.

The highest concentrations of Pb, Zn, Cu, and Cd were determined in sediment samples from Kokkinolakkas (Table 1). These samples are also characterized by high Mn concentrations. Site I3, located at the headwaters of Piavitsa stream, also exhibits anomalous values in Pb, Zn, and Mn compared with the local geochemical background (Kelepertzis et al. 2010, 2012). In contrast, Ni and Cr levels are within the local background range for all of the sediment samples (Kelepertzis et al. 2010, 2012), indicating that there are no significant sources for these elements. The surface water and stream sediment data highlight that Kokkinolakkas is the most contaminated stream within the studied basin.

Table 2 lists the metal concentrations bound to the three labile geochemical phases of selected sediment samples. It is evident that significant amounts of the studied metals are associated with the Fe–Mn oxides, which mainly exist as coatings on mineral surfaces, or as fine distinct particles, and are excellent trace element scavengers (Filgueiras et al. 2002). High concentrations of Pb, Zn, and Mn were also measured in the carbonate phase in Kokkinolakkas sediment material. The exchangeable phase is of minor importance for all of the elements except for Cd, especially in the Kokkinolakkas stream sediment sample.

Table 2 Metal concentrations bound to the exchangeable, carbonate, and Fe–Mn oxide phases of selected sediment samples (mg/kg)

Basin	Sampling site	I	II	III	I	II	III
		Pb			Zn		
Piavitsa	I3	3	9	501	21	73	1,228
	L2	2	6	188	6	14	306
Kerasia	C	2	6	218	3	5	208
Kokkinolakkas	B	4	170	1,590	24	221	1,976
		Mn			Cd		
Piavitsa	I3	30	82	48,796	1	1	4
	L2	12	28	6,888	1	1	ND
Kerasia	C	11	24	1,876	1	1	ND
Kokkinolakkas	B	29	290	6,010	3	2	7
		Cu			Ni		
Piavitsa	I3	2	2	33	3	4	72
	L2	2	2	19	4	3	39
Kerasia	C	2	2	12	3	3	28
Kokkinolakkas	B	2	7	18	3	3	35
		Cr					
Piavitsa	I3	2	ND	18			
	L2	2	1	29			
Kerasia	C	2	ND	26			
Kokkinolakkas	B	2	1	33			

As presented by Kelepertzis et al. (2012)

ND not detected, *I* exchangeable phase, *II* carbonate phase, *III* Fe and Mn oxides phase

Table 3 Results of Kruskall–Wallis test and post hoc similarities among the sampling sites

Metal	Kruskal–Wallis test	Post hoc similarities among sites ($p > 0.05$)
Pb	$x^2 = 31.0$, df = 6, $p = 0.00001$	G and B; D and L2
Zn	$x^2 = 35.5$, df = 6, $p = 0.00001$	C and D and L2
Mn	$x^2 = 31.0$, df = 6, $p = 0.00001$	G and B; D and L2
Cu	$x^2 = 23.0$, df = 6, $p = 0.0008$	C and I3; D and L2
Ni	$x^2 = 27.0$, df = 6, $p = 0.0001$	G and B and D; C and L2 and I3
Cr	$x^2 = 28.8$, df = 6, $p = 0.0001$	–
Cd	$x^2 = 21.5$, df = 6, $p = 0.0015$	C and L2

Table 4 Results of the first principal component from PCA of heavy-metal concentrations in tadpoles inhabiting streams within a mineralized river basin in Chalkidiki, NE Greece: eigenvalues, percent of variance, and cumulative percentage

Component	Eigevalues	% Variance	Cumulative %
1	5.25	75.05	75.05
2	1.30	18.7	93.75

Metal Content in Tadpole Tissues

Average concentrations for the seven heavy metals determined in tadpole tissues are listed in Table 1. Whole-body levels of Pb, Zn, Mn, Cu, and Cd in tadpoles from sites B and G along Kokkinolakkas stream appear to be several orders of magnitude greater than the concentrations determined in organisms from the other sampling locations. Manganese in site I3, upstream in Piavitsa, presents similar levels as those found in Kokkinolakkas. Greater concentrations of Pb, Zn, and Cd were also recorded for tadpoles living upstream in Piavitsa (site I3) compared with those measured at downstream sites L2, D, and C. Locations L2 and D tend to show similar levels for Pb, Zn, Mn, and Cu. Ni and Cr present their highest concentrations in tadpoles from Kokkinolakkas and Asprolakkas (site D). The lowest values for these elements were measured at site I3, whereas the lowest values for Pb, Zn, and Mn were determined at site C (Kerasia).

Significant differences in trace element concentrations were observed among animals from all sampling sites for every element (Kruskal–Wallis test, $p < 0.05$, Table 3). Post hoc analysis (Table 3) showed that sites G and B along Kokkinolakkas have similar concentrations for Pb, Mn, and Ni. Other significant similarities were observed between sites L2 and D for Pb, Zn, Mn, and Cu; between sites C, L2, and D for Zn; between sites C and I3 for Cu; between sites C, L2, and I3 for Ni; and between sites C and L2 for Cd.

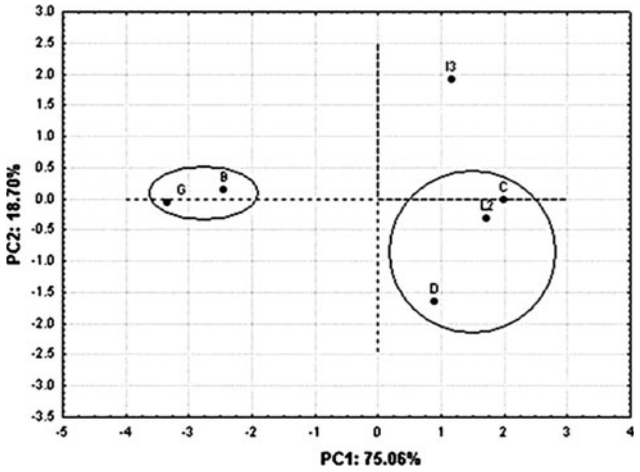

Fig. 2 Differentiation of the sampling sites based on their tadpole heavy-metal concentrations according to the PCA ordination plot. Kerasia *site C*, Piavitsa *sites I3*, *L2*, and *D*, and Kokkinolakkas *sites G* and *B*

Table 5 Results of the first principal component from PCA of heavy-metal concentrations in tadpoles inhabiting streams within a mineralized river basin in Chalkidiki, NE Greece: component matrix (eigenvectors)

Variable	PC1	PC2
Pb	0.18	0.003
Zn	0.19	0.01
Mn	0.1	0.31
Cu	0.16	0.002
Ni	0.12	0.26
Cr	0.11	0.30
Cd	0.14	0.12

The results of the PCA are listed in Table 4 and Fig. 2. Components 1 and 2 accounted for 93.75 % of the total variation (Table 4). Component 1 consists of Pb, Zn, and Cu with high loadings, whereas component 2 comprises Mn, Ni, and Cr (Table 5). In the corresponding ordination plot (Fig. 2), there is a clear separation between the two sampling sites from Kokkinolakkas and the rest of the sites for PC1. Regarding PC2, there is a separation between site I3 and locations L2, D, and C. Such results indicate that the sampling sites can be grouped into three distinct groups: (1) the first group (sites G and B) is related to the stream that drains the mined areas of Mavres Petres and Madem Lakkos (Pb–Zn) ore deposits, i.e., an area of intense anthropogenic activity; (2) the second group (site I3) is associated with the erosion of tailing piles from inactive Mn mines, *i.e.*, an area that is slightly influenced by past mining operations; and (3) the third group (sites L2, D, and C) reflects a pristine mineralized area.

Levels of Pb, Zn, Cu, and Cd in tadpoles inhabiting the aquatic environment of the study area were positively correlated with their respective levels in the sediment samples (Fig. 3). Statistical correlations with sediment levels were significant for these chemical elements (Fig. 3). For Mn and Cr, the Pearson correlations were positive (0.50 and 0.55, respectively), but they were statistically insignificant ($p = 0.31$ and $p = 0.26$, respectively). Nickel concentrations were negatively correlated with the levels in sediments ($r = -0.16$).

Correlation coefficients between dissolved concentrations and concentrations in tadpoles were not calculated for following two reasons:

1. The levels of metal values in tadpoles are comparable with the order of magnitude of metal levels measured in sediment rather than water samples. Especially for some elements, such as Ni, Cr, Cd, and Cu, water concentrations measured at several sites were either low or close to the detection limit of the analytical technique, but tadpole samples showed considerable greater concentrations of these metals.
2. Dissolved metal concentrations are subject to great fluctuations according to stream water flow, so the lack of water data for May 2010 prohibited the application of Pearson correlation coefficient between metal concentrations of waters sampled in April 2008 and tadpoles collected in May 2010.

In contrast, stream sediments provide a stable base for routine application of geochemical and environmental impact surveys (Chork 1977; Jain et al. 2005). Drainage sediments may display a slight seasonal variation in metal concentrations; however, year-to-year differences in the fine-grained portion of sediment, which is characterized by the presence of stable mineralogical phases, are not significant unless contaminant load influx, water pH, or redox conditions are changed (Rose et al. 1979; Axtmann and Luoma 1991). No significant changes of such parameters were observed during the 2-year period of our study. As a result, sediment composition was considered to be unaltered between 2008 and 2010.

BCFs between metal concentrations in tadpoles and sediments are presented in Fig. 4. The highest BCFs Pb ($\leq$2.2), Zn ($\leq$2.8), Mn ($\leq$1.8), Ni ($\leq$2.1), Cr ($\leq$2.9), and Cd ($\leq$6.5) were observed for organisms living in Kokkinolakkas stream (sites B and G). Regarding Cu, similar factors, ranging from 0.7 to 1.3, were calculated for organisms from all of the sampling locations. Zn and Cd are the only elements for which residues in whole body of tadpoles systematically exceed the sediment concentrations by factors $\geq$1 (range 1.3–2.8 and 1–6.5, respectively).

BCFs between metal values in tadpoles and the exchangeable, carbonate, and Fe–Mn oxide phases were also calculated. Although the factors between metal concentrations in tadpoles and metals bound to the

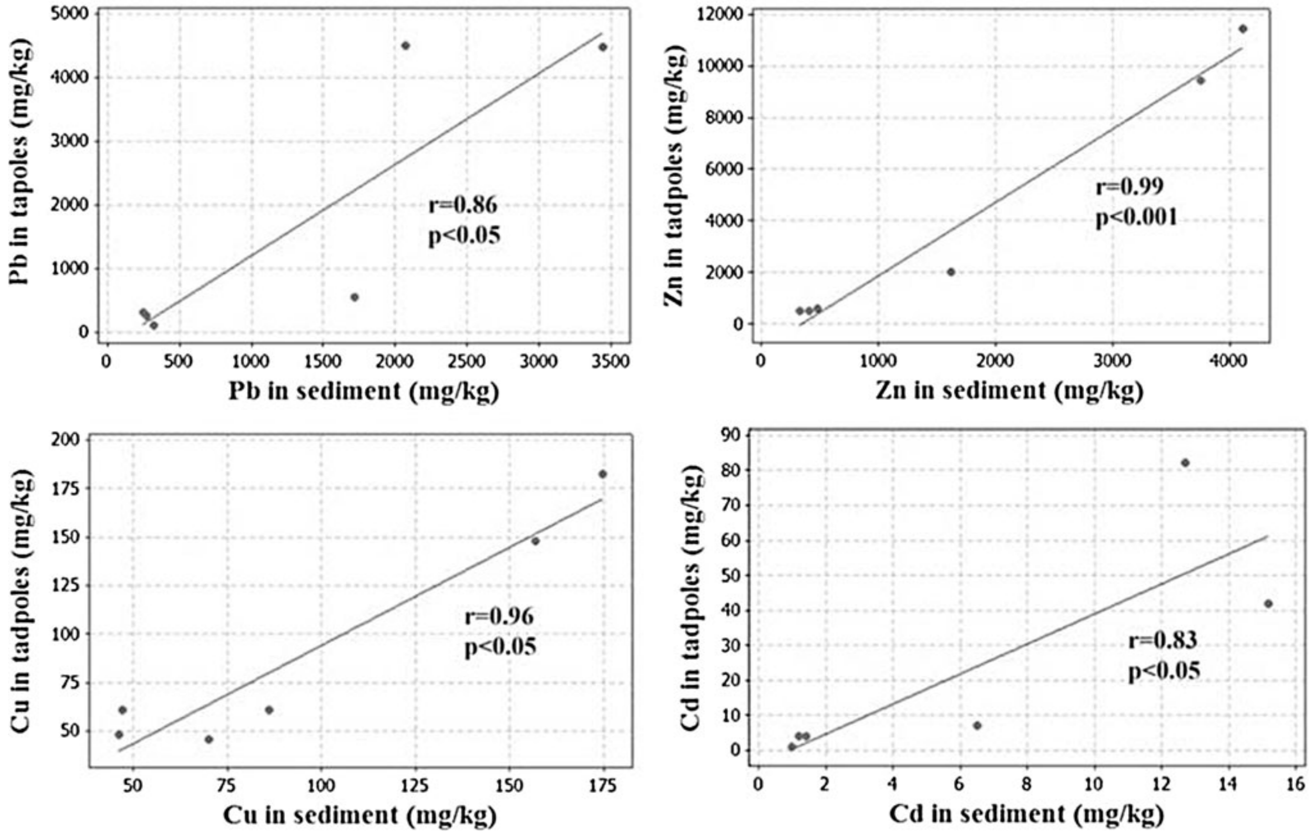

Fig. 3 Correlation between Pb (*upper left panel*), Zn (*upper right panel*), Cu (*lower left panel*), and Cd (*lower right panel*) concentrations in tadpoles and the corresponding concentrations in sediments (r = correlation coefficient, p = significance level). Only metals that have at least one significant ($p < 0.05$) correlation are shown

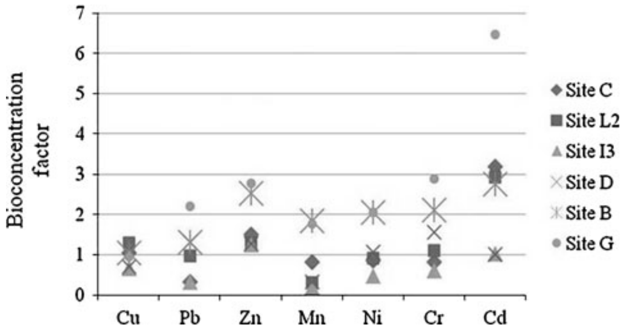

Fig. 4 BCFs between metal concentrations in tadpoles and sediments at each sampling site. Kerasia *site C*, Piavitsa *sites I3*, *L2*, and *D*, and Kokkinolakkas *sites G* and *B*

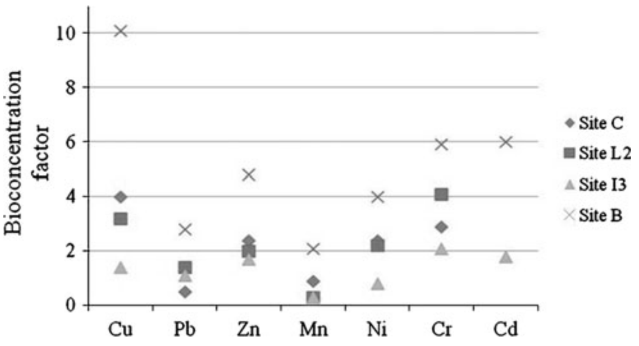

Fig. 5 BCFs between metal concentrations in tadpoles and the Fe–Mn oxide phase in sediments (regarding Cd, factors were not calculated for sites *L2* and *C* because the concentrations measured in the Fe–Mn oxide phase were lower than the detection limit). Kerasia *site C*, Piavitsa *sites I3* and *L2*; and Kokkinolakkas *site B*

exchangeable and carbonate fractions showed an unclear pattern, a systematic trend was observed for metals associated with Fe and Mn oxides. In particular, BCFs for all studied metals were greater for the Kokkinolakkas site (Fig. 5). In addition, tadpole metal concentrations were several times greater than the concentrations identified in the Fe–Mn oxide phase, with the exception of Mn, for sites I3, L2, and C.

Discussion

Comparison with Literature Data

It is of interest to compare the maximum heavy-metal concentrations in tadpoles from our field study with published concentrations found in tadpoles at other locations

(Table 6). Observed levels of Pb in tadpoles inhabiting the Kokkinolakkas stream (sites G and B) were similar to those in tadpoles from regions directly influenced by lead mining and milling operations at southeastern Missouri (Jennet et al. 1977). Zn in Kokkinolakkas and Mn concentrations at site I3 were at least two orders of magnitude greater than levels reported for tadpoles living in drainages that receive effluents from the above-mentioned lead mines (Gale et al. 1973; Jennet et al. 1977) and several orders of magnitude greater than the corresponding levels in ponds contaminated by acidic deposition in central Maryland (Sparling and Lowe 1996). In contrast, Cu values were considerably lower than the concentrations determined in species of *Bufo juxtasper* at an open-pit Cu mine area, which is polluted by effluents deriving from the flotation process in East Malaysia (Lee and Stuebing 1990). The highest reported concentrations of Ni and Cd were manifested in tadpoles from East Malaysia, which are substantially lower than the values measured in animals from Kokkinolakkas. Finally, Cr also exceeded the highest published concentrations, as observed in tadpoles from an area in Texas contaminated by production of calcium arsenate and an arsenic-based compound used to defoliate cotton (Clark et al. 1998). To the best of our knowledge, mean concentrations of Pb, Mn, Zn, Cd, Ni, and Cr in Kokkinolakkas were the highest ever reported in tadpoles. Even the organisms with the minimum measured concentrations of Pb, Zn, and Mn (site C), Ni and Cr (site I3), and Cd (site D) appeared to contain a significant metal load, approaching or exceeding those identified in tadpoles from other contaminated ecosystems (Table 6). Our data were consistent with previous studies and demonstrate that tadpoles are highly susceptible to bioaccumulation of a great number of elements under the various gradients of contamination levels found in the study area.

Correlation of Tadpole Metal Concentrations with Sediment Geochemistry

The significant correlations found between Pb, Zn, Cu, and Cd in organisms and the respective levels in the sediments indicate that sediments provided the bulk of these metals for tadpoles. The observed correlations are attributed to the feeding habits of tadpoles, which are omnivorous, microphageous filter feeders and ingest everything from the sediment. As a result, they are highly exposed to substances concentrating in sediments (Grillitsch and Chovanec 1995). Various researchers have reported strong positive correlations between Pb concentrations in sediments and those in tadpoles (Birdsall et al. 1986; Sparling and Lowe 1996). Karasov et al. (2005) exposed tadpoles along a pollution gradient in the Fox River and Green Bay

Table 6 Compiled literature data on published heavy-metal concentrations in tadpoles (concentrations based on mg/kg dry weight)

Species	Cu	Pb	Zn	Mn	Ni	Cr	Cd	Compartment	Reference
Rana clamitans melanota and *R. pipiens*	4–6.9	ND				0.5–0.7	0.05	Whole body	Karasov et al. (2005)
Bufo bufo	15.6–28.2	10.5–34.4	48.3–117.7				0.2–1.3	Whole body	Grillitsch and Chovanec (1995)
R. dalmatina	7.2–19.6	3.9–13	37.4–216.9				0.1–0.4		
R. ridibunda	17.7–23.4	9.5–25.3	54.5–69.1				0.3–0.8		
R. temporaria		10–95					0.3–0.7	Intestine	Hofer et al. (2005)
R. catesbeiana and *R. clamitans*		20–250						Whole body	Birdsall et al. (1986)
R. catesbeiana	≈50	≈19	≈490	≈80			≈4	Without gut	Unrine et al. (2007)
A. crepitans	9.8–15.7	6.7–19.7	59.0–103.1	252–2,335	2.4–10	12–18		Whole body	Sparling and Lowe (1996)
H. versicolor	7.4–12.6	1.1–26.2	59.1–71.9	112–4,618	2–7.1	4–14.8			
A. crepitans			2,170			52.2			Clark et al. (1998)
R. clamitans			734			36.9			
B. juxtasper	80.7–1,020	6.9–15.8	122.4–206.9	2.0–12.9	13–49	0–2.4	1.4–5.2	Liver	Lee and Stuebing (1990)
R. catesbeiana		5.43		25.9		6.59	0.405	Whole body	Burger and Snodgrass (1998)
Unspecified species (tadpoles)	17–48	36–1,590	160–1,090	500–5,650			1.4–3.0	Whole body	Gale et al. (1973)
Unspecified species (tadpoles)		4,100	2,808						Jennet al. (1977)
Pelophylax kurtmuelleri	46–182	103–4,490	494–11,460	1,620–13,310	57–163	38–272	1.2–82	Whole body	This study

ecosystem in Wisconsin and found a positive correlation between levels in sediments and tadpoles for Cd, Cr, and Pb. Snodgrass et al. (2005) also cited that accumulation patterns for Pb and Zn in species of *Rana clamitans* agreed well with the concentrations of elements encountered in three different types of sediments: clean sand (control), sediment from an abandoned surface mine, and sediment contaminated with coal-combustion waste.

We found that differences in tadpole concentrations for the majority of analyzed metals were due to different metal levels in the sediments where they develop and breed. Although tadpoles from all of the sampling locations have the ability to accumulate heavy metals to high concentrations, significant biological magnification for Pb, Zn, Mn, Ni, and Cr only occur for organisms that inhabit the Kokkinolakkas aquatic community (Fig. 4). Regarding Pb, the extremely high concentrations measured in tadpole tissues, especially at site G, could be attributed to the metal's propensity to extensively accumulate in suspended and particulate colloidal particles by way of adsorption processes. These particles represent a significant transport pathway for this element in Kokkinolakkas stream (Kelepertzis et al. 2012). Tadpoles are known to accumulate substances concentrating in suspended matter (Hall and Mullhern 1984). For Zn, Mn, and Ni, it is suggested that the high dissolved concentrations exhibited in Kokkinolakkas results in a significantly greater accumulation of these elements to tadpole tissues compared with organisms from the other sites. The water-soluble form of a metal is considered to be the most bioavailable and toxic to aquatic organisms, leading to an accelerated uptake of metal ions that are in the solution (Gerhardt 1993). Amphibian larvae have been reported to be sensitive to increased aqueous concentrations of several metals (Freda 1991). Furthermore, sequential extraction data indicated increased concentrations of Pb, Zn, and Mn in the carbonate phase of stream sediment material at Kokkinolakkas, which may indicate preferential uptake of these metals by tadpoles. However, the greater BCFs of Cr in tadpoles from Konnikolakkas are not explained because all of the study sites are characterized by low Cr water levels. One explanation that could be given is that potential differences in some parameters, such as alkalinity, temperature, and dissolved oxygen, may enhance Cr accumulation in Kokkinolakkas in relation to organisms living in the rest of the streams. For example, Hofer et al. (2005) reported that Pb and Cd accumulation in tadpoles increases with the ratio of metal to HCO_3. Cu is the only element that presents similar BCFs for all of the tadpole samples (Fig. 4). This contrasting behaviour of Cu in relation to the other metals may be attributed to kinetic characteristics and physiological mechanisms specific to this metal involving assimilation, distribution, storage, and elimination.

It is also evident from Fig. 4 that all tadpole samples tend to concentrate Zn and Cd to a greater extent than the other metals. The selective assimilation of Zn and Cd by tadpoles has previously been reported (Sparling and Lowe 1996). Zn is an essential element and may have been taken up selectively (Clark et al. 1998), whereas Cd is a very toxic metal, even in low ambient concentrations. Substantial proportions of Cd in the studied sediments are associated with the more labile fractions, facilitating its accumulation by tadpoles, which live in close proximity to sediments. Dissolved Cd concentrations in site G might have contributed to the abnormally high Cd levels determined in tadpoles inhabiting this site.

Observing the relation of tadpole concentrations with the metal concentrations determined in the Fe–Mn oxide phase of sediments (Fig. 5), we suggest that these organisms tend to concentrate and assimilate considerable portions of metals bound to Fe and Mn oxides. These precipitates comprise an important fraction of the studied sediment material and have been proposed to provide tadpoles with a variety of elements, such as Pb, Zn, and Mn, which are commonly scavenged by their surfaces (Unrine et al. 2007). The fact that metal concentrations in tadpole samples are several times greater than the concentrations determined in the Fe–Mn oxide phase of sediments indicates that these organisms seem to preferentially assimilate metals bound to these oxides. The only exception is Mn, for which organisms that live in sites I3, L2, and C present lower concentrations than the levels measured in this fraction.

Implications for the Health of the Studied Aquatic Ecosystem

The results of study showed that increased heavy-metal concentrations in stream water and sediments of the study area do not seem to have a marked effect on the survival of tadpoles, even under levels of severe water and sediment contamination found in Kokkinolakkas and Piavitsa headwaters. This observation is in accordance with the study by Lazaridou-Dimitriadou et al. (2004), who reported that rich and diverse macroinvertebrate fauna may coexist with increased concentrations of some trace metals in the same streams. However, these investigators mentioned that the total number of collected macroinvertebrate was low, comprising only tolerant taxonomic groups, in a site upstream of locations G and B along Kokkinolakkas. In addition, and in accordance with our findings for preferential Zn and Cd assimilation, they recorded significant Zn and Cd accumulation in liver and muscle of *Barbus cyclolepis* living in Asprolakkas and Piavitsa waters, indicating the transfer of these metals to higher trophic levels, such as fish.

The observed resistance of tadpoles to various degrees of heavy-metal load may be related to various mechanisms of tolerance, possibly through the storage of metals in an inert form. As illustrated in several studies (Sparling and Lowe 1996; Burger and Snodgrass 2001), the digestive tract contains significantly greater concentrations of metals than body tissues. The potentially high metal content sequestered in the digestive tract seems to be the main reason for tadpole survival, even under the Kokkinolakkas' extremely high ambient contamination levels. However, the exact effects of such high metal concentrations on their growth, physiology, and behaviour need further investigation. According to literature data, there are several problems, such as delayed development, decreased fright response, morphological deformities, limitation of the type of food they can consume, and decreased growth and size at metamorphosis (Lefcort et al. 1998; Rowe et al. 1996; Snodgrass et al. 2005, Sparling et al. 2006). It is worthwhile to mention, however, that considerable variation may exist in sensitivity among species exposed to contaminated sediments (Snodgrass et al. 2004).

This study showed that tadpoles inhabiting the metalliferous streams of eastern Chalkidiki, Greece, accumulate significant amounts of heavy metals according to the contamination levels of sediments where they develop and breed. Our study, which extended over a wide range of ambient metal concentrations, suggests that these organisms tend to concentrate metals bound to the Fe and Mn oxides that comprise a significant constituent of the studied sediment material. High dissolved concentrations and significant concentrations associated with the exchangeable and the carbonate geochemical phases of sediments appear to be contributing factors determining whole-body levels.

Conclusively, tadpoles could be considered as biondicators of environmental contamination within this metal-rich zone by estimating the bioavailability of metals to the freshwater biota. It is therefore proposed that these organisms are included in monitoring programmes within the wider mining area of Stratoni and, perhaps, in other rivers affected by metal mining. A methodology according to the model source (sediment), pathway (water), and target (tadpoles) is established, which may also have predictive value for other mining areas around the world. The proposed methodology, which relies on determination of metal ions in solution, metals that may by be liberated from sediments, and metals in tadpole tissues, is considered significant in light of concerns regarding the decline of amphibian populations.

Acknowledgments We gratefully acknowledge the staff of Hellas Gold S.A. and, particularly, N. Adam, D. Apostolidis, and M. Gliniadakis for invaluable help during tadpole collection. Many thanks are given to N. Skoulikidis and J. Karaouzas for providing a thorough review on an earlier version of the manuscript and to two anonymous reviewers for constructive comments. Data presented in this study are a revised part of the first author's PhD thesis.

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