Subterranean Biology 2: 33-41, 2004

Niphargus and *Gammarus* from karst waters: first data on heavy metal (Cd, Cu, Zn) exposure in a biospeleology laboratory

Olimpia COPPELLOTTI KRUPA[1], Vladimiro TONIELLO[2], Laura GUIDOLIN[1]

[1] Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova (Italy)
(2) INAC Project, Federazione Speleologica Veneta
olimpiak@mail.bio.unipd.it

ABSTRACT

Niphargus montellianus and *Gammarus balcanicus*, hypogean and epigean amphipods, respectively, living in karst waters of North-Eastern Italy, were exposed in a biospeleology laboratory to three heavy metals (Cd, Cu, Zn). Metal contents were checked in organisms in natural conditions and after exposure to increasing concentrations of metals in the range 0.1-25.0 μg ml^{-1} for up to 10 days. The two amphipods differed in their responses, the epigean *G. balcanicus* being more sensitive to the toxic effects of heavy metals than the hypogean *N. montellianus*. The degree of toxicity was Cu>Cd>Zn for both amphipods; in particular, Cu induces 50% mortality for *G. balcanicus* in 2 days at the concentration of 0.1 μg Cu ml^{-1} and at the concentration of 5 μg Cu ml^{-1} in 10 days for *N. montellianus*, respectively. Moreover, *G. balcanicus* accumulated higher levels of Cd and Zn than *N. montellianus,* and its mortality was always higher. The suitability of the two species as biomonitors of karst water quality is discussed.

Key words: *Niphargus, Gammarus,* karst waters, heavy metal.

INTRODUCTION

Increased contamination of the aquatic environment by metals from industrial, agricultural and domestic wastes requires detailed analysis of the dispersion of these metals in the environment. Some metals can constitute a serious public health hazard via accumulation in food chains or when released into drinking water supplies (Förstner and Wittman 1979; Crompton 1997). In many ecosystems, these human-induced changes have overwhelmed the natural biogeochemical fluxes of trace metals (Nriagu 1990). Karst aquifers are very important drinking-water reservoirs: due to the progressive impoverishment of other water resources, either superficial or underground, the exploitation of karst aquifers for drinking water supplies will increase from the present 30% to almost 80% in the near future (Forti 1999). Moreover, these aquifers have specific hydraulic and hydrogeologic characteristics that render them highly vulnerable to pollution from human activities, and they become polluted more easily and in a shorter time than waters in non-karstic aquifers (Kaçaroglu 1999).

Metals entering freshwater environments are partitioned among various compartments (water, suspended particulate matter, sediments, and biota). In particular, freshwater sediments act as an important sink for metals, their metal concentrations exceeding those in the overlying water by between three and five orders of magnitude (Campbell and Tessier 1991; Bryan and Langston 1992). This metal contamination of sediments is particularly dangerous for benthic animals and plants. Sediment-associated organisms may be exposed to chemicals whether dissolved in the pore water or overlying water or through ingestion of contaminated particles. The latter route of exposure may play the largest role in bioaccumulation of sediment–associated contaminants (Rand et al 1995). Heavy metals may be mobilised from the sediments; the free ionic form determines their bioavailability and, therefore, their toxicity, complexing with biological molecules (Bressa and Cima 1999; Mansilla-Rivera and Nriagu 1999).

The usefulness of some organisms as biological indicators for monitoring metal pollution in surface and underground freshwaters has been demonstrated, owing to their ability to accumulate metals and their degree of tolerance to them; biomonitors provide time-integrated measures of the levels of available metals in the environment, responding essentially to the fraction of the total metal load present in the ecosystem that is of direct ecotoxicological relevance (Rainbow and Phillips 1993; Amyot et al 1996). The use of bioindicators complement physico-chemical analyses and, consequently, can provide information on the quality (of a part) of its environment (Kettrup and Marth 1998). It must be noted that underground aquatic fauna seems to be endangered by any sudden changes in its environment, either natural or artificial (Sket 1999) - all the more so as many habitats of groundwater are ecologically characterized by diminished oxygen availability (Danielopol 1980).

Hypogean animals may provide available indications for monitoring karst aquifers and reaching locations where standard chemical tests are difficult to apply (Plénet 1999). This study focuses on the response of *Niphargus montellianus* and *Gammarus balcanicus* to copper and zinc, metals playing a biological role, and cadmium, a metal without a physiologically documented role, by exposure tests in a speleology laboratory. Both amphipods, the hypogean *N. montellianus* and the epigean *G. balcanicus*, are viewed as ideal comparative elements in the study of underground and surface waters (Plénet 1995).

The toxicity of the metals and metal accumulation profiles were checked, as first indications to verify the possible role of *Niphargus* and/or *Gammarus* in the biomonitoring of karst water quality, which is threatened by uncontrolled discharges. Our studies were coupled with investigations on the physico-chemical conditions of karst waters and heavy metal loads in sediments.

MATERIALS AND METHODS

Animals and collection sites

Specimens of *Niphargus montellianus* (Fig. 1) and *Gammarus balcanicus* (Fig. 2) were collected between March 2000 and April 2001. Assignment to one of the two species was performed according to Karaman (1993) and confirmed by F. Stoch (pers. comm.). *Niphargus* individuals were captured in two sites: the first in the Landron cave (a horizontal expansion cave, cadastral n° 1954 V TV), 30 m from the entrance, and the second 50 m away from the Landron cave in a small subterranean hollow containing a spring of deep origin (called Western Spring of Landron), at S. Pietro di Feletto (Treviso, North-East Italy). Specimens were captured in nets (10 cm diameter opening, 20 cm length) fastened by nylon threads to large stones, and in glass vessel traps. *Gammarus* individuals were collected at various sites in a small stream flowing from the Western Spring subterranean hollow, either in aquarium nets or long-handled nets. The animals were taken to the laboratory in a refrigerated container. *Gammarus* specimens were occasionally found at the entrance to the Landron cave together with *Niphargus*.

Physico-chemical analyses

Physico-chemical parameters were measured, either in the field or in the laboratory, in the waters of Landron cave, the Western Spring of Landron, and the river Meschio, belonging to the same aquifer system. Temperature was checked by a Checktemp 2 Hanna; pH using a HI 98107 Hanna pHmeter; conductivity (DIST WP3 and DIST 4 Plus Hanna conductivity meters); dissolved oxygen (HI 4810 Hanna test kit); total hardness (HI 3812 Hanna test kit); and ion (Ca^{2+}, K^+, Fe^{2+}, Cl^-, NH_4^+, NO_3^-, NO_2^-, SO_4^{2-}) concentrations by Merckacquant analytical test strips.

Metal exposure and analytical procedures

Specimens were maintained and exposed to heavy metals at the biospeleology laboratory of Villa Papadopoli (Vittorio Veneto, Treviso), located in an old air raid shelter, at 12-14 °C. The good-quality water of the river Meschio was used for tests, because no residual chemical products or chlorine are present, as shown by analyses (Table 1).

Animals were acclimatized in experimental tanks for at least one week, and then exposed to increasing concentrations of cadmium, copper, and zinc in the range 0.1-25.0 μg ml^{-1}, up to a maximum of 10 days, in separate experiments. Mortality was checked each day. Cadmium was added as $CdCl_2 \times 2H_2O$, copper as $CuSO_4 \times 5H_2O$, and zinc as $ZnSO_4 \times 7H_2O$. In order to reduce variations due to size, age and sex differences, only male adults were used, 10-14 and 14-20 mm long, for *Gammarus* and *Niphargus* respectively. For each test, performed in double, 5 animals were used in 5 l of water in a polyethylene container. Separate containers were used for each set of the two species. At the end of each treatment, the animals were washed in clean Meschio water and then in distilled water, frozen, and

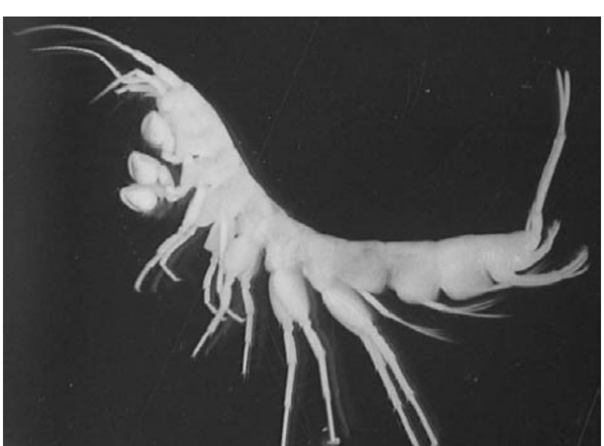

Figure 1 - Male specimen of *Niphargus montellianus* (length = 20 mm).

Figure 2 - Male specimen of *Gammarus balcanicus* (length = 12 mm).

Table 1 - Physico-chemical data on water of Landron cave, Western Spring of Landron, and river Meschio.

	Landron	Western Spring	Meschio
Temperature (°C)	9.6 - 13.9	11.9 - 12.4	10.7 - 11
pH	7.5 - 8.2	7.4 - 7.9	7.5 - 8.2
Conductivity (μS/cm)	298 - 469	380 -475	255 - 262
Dissolved oxygen (mg/l)	7.7 - 9.0	8.6 - 10.0	> 10
Total hardness (mg/ml $CaCO_3$)	195 - 240	225 - 240	150 - 165
Total alkalinity (mg/ml $CaCO_3$)	210	225	150
N-NO_3 (mg/l)	25	0 - 10	0 - 10
N-NO_2 (mg/l)	n.d.	n. d.	n. d.
S-SO_4 (mg/l)	200 - 400	200 - 400	200 - 400
NH_4^+ (mg/l)	n.d.	n. d.	n. d.
Ca^{2+} (mg/l)	50	25 - 50	25
K^+ (mg/l)	0 - 250	0 - 250	0 - 250
Cl^- (mg/l)	n.d.	n. d.	n. d.
Fe^{2+} (mg/l)	0 - 3	n. d.	0 - 3
Cu (μg/l)	0.03 - 0.67	n. d.	n. d.
Zn (μg/l)	0.005	0.002	n. d.
Cd (μg/l)	0 - 0.044	n. d.	0.029 - 0.051
Pb (μg/l)	0 - 0.02	0.02	0 - 0.010
Cr (μg/l)	n.d.	n. d.	n. d.

n. d. = not detectable

transferred to the Department of Biology (University of Padova), where metal determinations were performed. Samples were then dried, weighed and digested with 1 ml of HNO_3 AristaR in Teflon PFA vessels in a CEM MDS-2000 microwave furnace, to eliminate the organic matrix. The digestion programme consisted of four steps, each lasting 5 min, at pressures of 20, 40, 60 and 80 psi. The resulting extracts were made up to 5 ml with MilliQ water. Metal concentrations were determined on a Perkin-Elmer 4000 atomic absorption spectrophotometer.

Values are shown as means+/-standard error (n=10, for control animals; n=5, for metal exposed individuals, for each metal dose) and were submitted to one-way analysis of variance (ANOVA). The Student-Newman-Keuls multiple comparison of means test was used to assess which of the groups were significantly different from each other ($p<0.05$). Metal concentrations (Cd, Cu, Zn, Cr, Pb) were also determined in the sediments and waters of the collection sites of animals. Metal contents in waters were determined by a Perkin-Elmer 5100 PC with a Zeeman graphite furnace; sensitivity of instrument was 0.01 (Cd), 0.05 (Cu), 0.5 (Pb), 0.5 (Cr) μg l^{-1}, respectively. Metal contents in sediments were determined on a Perkin-Elmer 4000 atomic absorption spectrophotometer, after mineralization of aliquots of 0.5 cm^3 of sediments, as described above.

RESULTS

Physico-chemical characteristics of collection sites

Waters and sediments of collection sites of amphipod specimens were characteristic of unpolluted sites, as reported in Tables 1 and 2. However, Zn contents were 92.40 μg g^{-1}dry wt in the sediments of the Western Spring of Landron, and Cr contents were 28.65 and 70.60 μg g^{-1}dry wt in the sediments of the Landron cave and Western Spring of Landron, respectively, exceeding the natural threshold values (Prater and Anderson 1977).

Toxicity of metals

Upon exposure, the two amphipods exhibited different responses towards the three metals tested: *G. balcanicus* was much more sensitive than *N. montellianus* to toxic effects. Table 3 reporting the mortality percentage up to day 10 of exposure to increasing concentrations of metals shows that both organisms are more sensitive to Cu than to the other two metals. However, a diversified response to copper exposure was exhibited by the two organisms, although Cu was the most toxic metal for both. Mortality in *G. balcanicus* was very high at the lowest Cu concentration used (0.1 μg ml^{-1}), with a 50% value at day 2. In *N. montellianus,* survival was also inhibited

Table 2 - Heavy metal contents ($\mu g\ g^{-1}$ dry wt) in sediments of collection sites: mean values.

	Landron cave	Western Spring of Landron	* Natural threshold values
Cd	1.46	1.00	<1.00
Cu	0.00	23.70	<20.00
Zn	56.87	92.40	<70.00
Cr	28.65	70.60	<20.00
Pb	0.00	0.11	<25.00

* Data from Prater and Anderson (1977).

Table 3 - *N. montellianus* and *G. balcanicus* mortality (%) up to 10-day exposures to cadmium, copper and zinc. Total number of specimens exposed for each experiment: 10.

	Mortality (%)								
	N. montellianus					*G. balcanicus*			
	days					days			
Concentrations	1	2	4	6	10	1	2	4	6
Cd ($\mu g\ ml^{-1}$)									
0.1	0	0	0	0	0	20	50	80	100
1.0	0	0	0	0	0	50	100		
2.5	0	0	0	0	0	100			
5.0	0	0	0	20	40	100			
10.0	0	0	20	20	100	100			
25.0	0	0	40	100		100			
Cu ($\mu g\ ml^{-1}$)									
0.1	0	0	10	10	10	0	50	100	
1.0	0	0	20	20	20	50	100		
2.5	0	20	20	40	40	100			
5.0	0	20	20	40	50	100			
10.0	0	20	20	40	50	100			
25.0	0	20	20	40	90	100			
Zn ($\mu g\ ml^{-1}$)									
2.5	0	0	0	0	0	20	80	100	
5.0	0	0	0	0	0	30	90	100	
10.0	0	0	0	0	0	40	100		
25.0	0	0	0	0	0	100			

Table 4 - Natural metal concentrations ($\mu g\ g^{-1}$ dry wt) in *N. montellianus* and *G. balcanicus* specimens.

	Cd	Cu	Zn
N. montellianus	2.93 ± 0.28	86.69 ± 5.11	113.15 ± 5.72
G. balcanicus	5.97 ± 0.69	87.00 ± 4.42	98.17 ± 5.09

from the lowest Cu concentration used, but 50% mortality was reached with 5.0 μg Cu ml^{-1} at day 10.

In Cd-exposed *Gammarus,* 50% mortality was observed at day 1 with 1 $\mu g\ ml^{-1}$, whereas in Cd-exposed *Niphargus* toxicity was noted at higher doses, from 5.0 $\mu g\ ml^{-1}$, and 100% mortality appeared with 10 $\mu g\ ml^{-1}$ exposure at day 10.

As regards exposure to zinc in *Niphargus*, no mortality was observed throughout the experiments, at all concentrations tested. High mortality was observed from 2.5 $\mu g\ ml^{-1}$ in exposed *Gammarus* specimens.

Heavy metal accumulation in organisms

Natural metal concentrations in *N. montellianus* and *G. balcanicus* specimens are listed in Table 4 and

Table 5 – Cd, Cu, Zn contents (μg g^{-1}dry wt +/- SE) in *N. montellianus* and *G. balcanicus* exposed to Cd.

N. montellianus

Treatment (μg Cd ml^{-1})	Cd	Cu	Zn
0.1	10.55 +/- 0.93	97.31 +/- 7.51	94.98 +/- 5.73
1.0	115.34 +/- 30.49	97.31 +/- 7.51	97.60 +/- 4.05
2.5	167.68 +/- 51.85	73.77 +/- 5.77	82.26 +/- 5.98
5.0	512.75 +/- 124.88 *	81.77 +/- 6.58	81.06 +/- 4.05
10.0	756.64 +/- 172.48 *	65.97 +/- 5.99	67.81 +/- 11.57

G. balcanicus

Treatment (μg Cd ml^{-1})	Cd	Cu	Zn
0.1	150.46 +/- 14.48	55.00 +/- 1.30	69.03 +/- 6.91
1.0	248.06 +/- 20.59	46.59 +/- 1.84	86.55 +/- 15.48
2.5	282.60 +/- 58.99	74.92 +/- 14.22	50.55 +/- 14.80
5.0	606.24 +/- 114.59 *	67.31 +/- 5.10	47.18 +/- 12.56
10.0	1508.28 +/- 230.56 *	82.43 +/- 2.36	62.78 +/- 3.46

* Values significantly different ($p<0.05$) from natural metal concentrations in the two species, see Table 4.

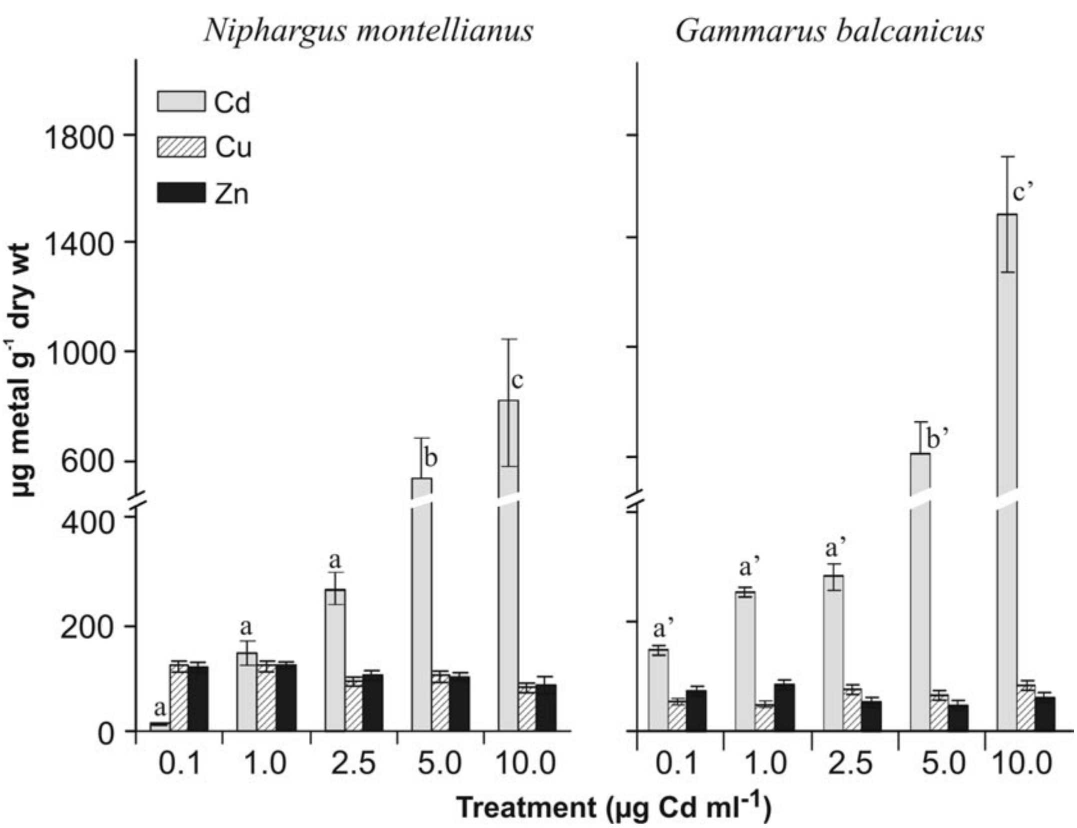

Figure 3 - Cd, Cu, Zn contents (μg g^{-1} dry wt) in *N. montellianus* (a) and *G. balcanicus* (b) exposed to Cd. Bars not sharing a common superscript letter are significantly different ($p<0.05$) in the multiple comparison of values in unexposed and exposed specimens.

accumulation levels of the three metals measured after exposure in the laboratory in Tables 5 - 7.

As regards exposure to cadmium (Fig. 3; Table 5), in *Gammarus* this metal accumulated to double the amount found in *Niphargus* (756.64 ± 172.48 μg g^{-1}dry wt) up to a value of 1508.28 ± 230.56 μg g^{-1}dry wt, after exposure

Table 6 – Cd, Cu, Zn contents (μg g^{-1}dry wt+/- SE) in *N. montellianus* and *G. balcanicus* exposed to Cu.

N. montellianus

Treatment (μg Cu ml^{-1})	Cd	Cu	Zn
0.1	2.43 +/- 0.22	100.88 +/- 4.34	98.48 +/- 8.06
1.0	1.79 +/- 0.27	190.90 +/- 13.22	103.13 +/- 2.23
2.5	3.2 +/- 1.13	187.17 +/- 16.14	77.52 +/- 3.66
5.0	1.81 +/- 0.18	506.55 +/- 97.27 *	81.16 +/- 6.12
10.0	1.51 +/- 0.18	848.43 +/- 182.47 *	81.18 +/- 4.41

G. balcanicus

Treatment (μg Cu ml^{-1})	Cd	Cu	Zn
0.1	2.51 +/- 0.39	45.03 +/- 14.04	43.32 +/- 11.35
1.0	3.00 +/- 0.16	64.69 +/- 13.77	62.15 +/- 6.45
2.5	2.92 +/- 1.55	205.66 +/- 30.94 *	80.86 +/- 29.86
5.0	2.63 +/- 0.96	549.85 +/- 80.70 *	85.26 +/- 20.74
10.0	2.46 +/- 1.03	443.70 +/- 17.35 *	97.06 +/- 26.90

* Values significantly different (p<0.05) from natural metal concentrations in the two species, see Table 4.

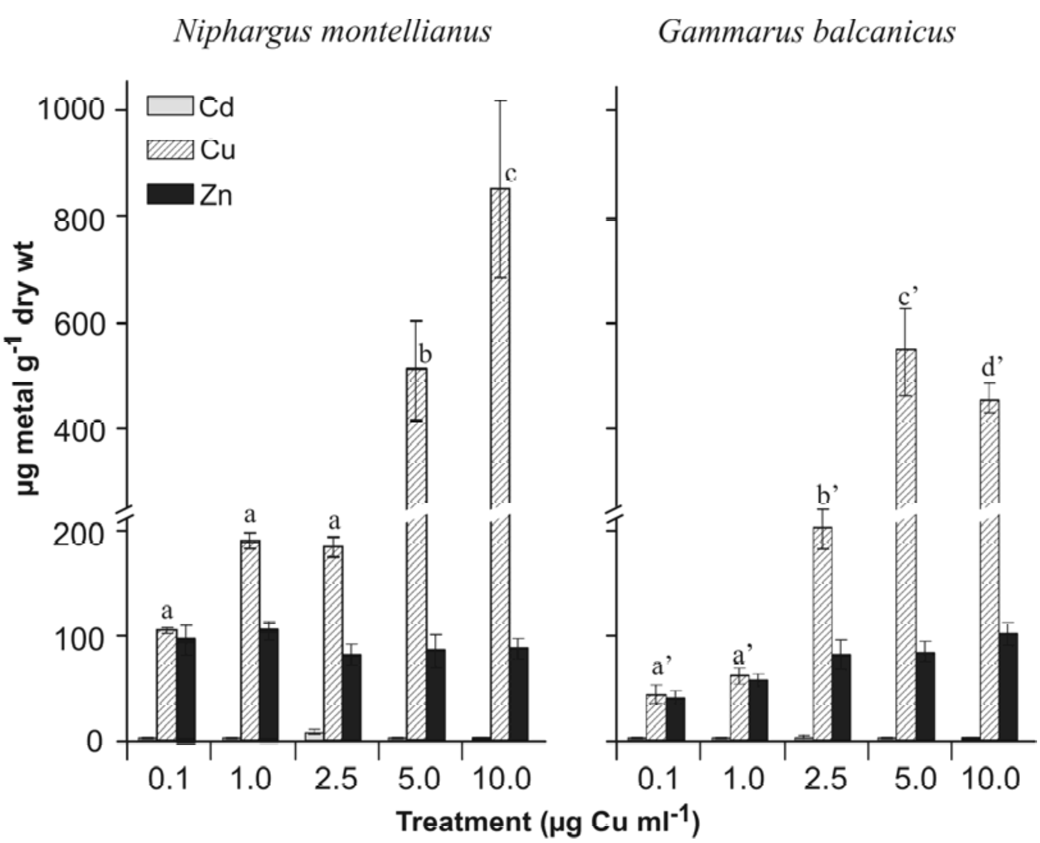

Figure 4 - Cd, Cu, Zn contents (μg g^{-1} dry wt) in *N. montellianus* (a) and *G. balcanicus* (b) exposed to Cu. Bars not sharing a common superscript letter are significantly different (p<0.05) in the multiple comparison of values in unexposed and exposed specimens.

to 10 μg ml^{-1}. Cd content values were significantly different (p<0.05) from a dose of 5 μg ml^{-1}, in both organisms, in multiple comparisons with unexposed and lower concentration exposed specimens.

Upon Cu exposure, the extent of Cu accumulation was up to 848.43 ± 182.47 and 549.85 ± 80.7 μg g^{-1}dry

Table 7 – Cd, Cu, Zn contents (μg g^{-1}dry wt+/- SE) in *N. montellianus* and *G. balcanicus* exposed to Zn.

N. montellianus

Treatment (μg Zn ml^{-1})	Cd	Cu	Zn
2.5	1.18 +/- 0.63 *	83.83 +/- 2.03	136.16 +/- 24.39
5.0	1.10 +/- 0.53 *	83.42 +/- 5.02	247.90 +/- 79.09
10.0	1.23 +/- 0.32 *	90.56 +/- 1.4	525.67 +/- 155.59 *

G. balcanicus

Treatment (μg Zn ml^{-1})	Cd	Cu	Zn
2.5	3.5 +/- 0.56 *	113.09 +/- 14.25 *	259.84 +/- 35.54
5.0	2.46 +/- 0.89 *	68.8 +/- 9.36	586.26 +/- 268.42 *
10.0	1.41 +/- 0.26 *	57.28 +/- 7.09 *	958.35 +/- 311.66 *

* Values significantly different (p<0.05) from the natural metal concentrations in the two species, see Table 4.

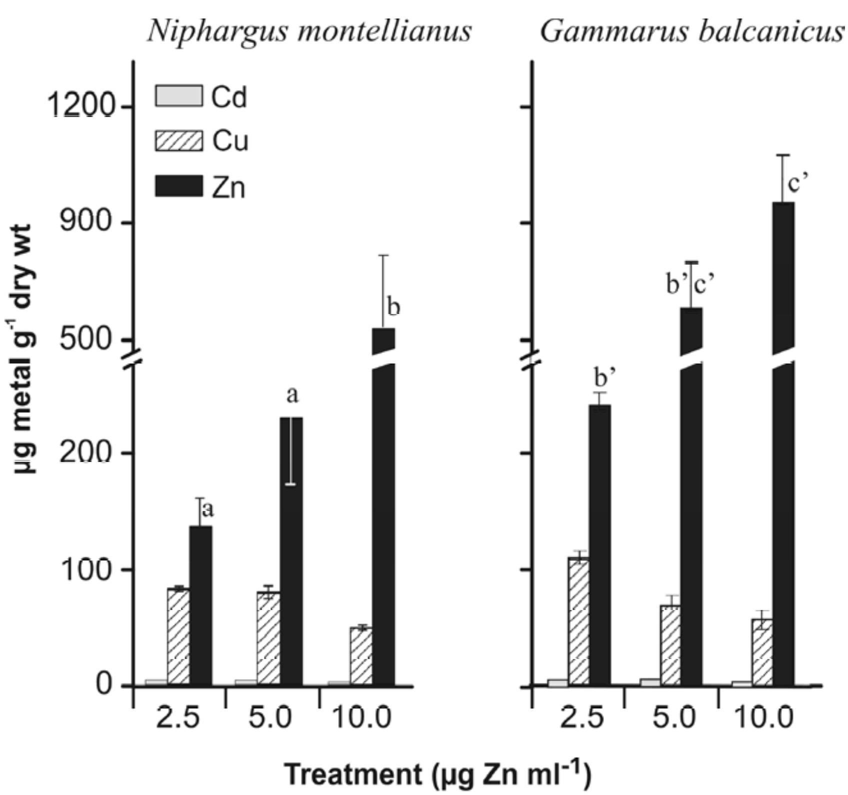

Figure 5 - Cd, Cu, Zn contents (μg g^{-1} dry wt) in *N. montellianus* (a) and *G. balcanicus* (b) exposed to Zn. Bars not sharing a common superscript letter are significantly different (p<0.05) in the multiple comparison of values in unexposed and exposed specimens.

wt, in *Niphargus* and *Gammarus*, respectively (Fig. 4; Table 6), values not significantly different. Significant differences (p<0.05) in Cu content values were observed from a dose of 2.5 μg Cu ml^{-1} in *Gammarus* and 5.0 μg Cu ml^{-1} in *Niphargus*, in comparison with unexposed specimens and animals exposed to lower Cu doses.

Upon Zn exposure, (Fig. 5; Table 7) Zn contents were significantly different (p<0.05) in *Niphargus* specimens exposed only to 10 μg ml^{-1}(525.67 ± 155.59 μg g^{-1}dry wt), in comparison with unexposed animals (113.15 ± 5.71μg g^{-1}dry wt) and with specimens exposed to 2.5 and 5 μg Zn ml^{-1}. Zn was not significantly accumulated at exposure doses lower than 5 μg Zn ml^{-1}, even in *Gammarus*. In the *Gammarus* control specimens, the Zn content was 98.17 ± 5.09 μg g^{-1}dry wt, whereas in specimens exposed to 10 μg ml^{-1} it reached the value of 958.35 ± 311.66 μg g^{-1}dry wt. In both species, significant differences (p<0.05) were recorded in the Cd values in specimens exposed to zinc (even at the lowest dose) in comparison with controls (Tables 3 and 7). A decrease in Cd content was recorded up to 1.10 +/- 0.53 μg g^{1}dry wt in *Niphargus* and 1.41 ± 0.26 μg g^{-1} dry wt in *Gammarus*.

DISCUSSION

The *N. montellianus* and *G. balcanicus* specimens studied in this work were collected from sites in which both waters and sediments may be considered unpolluted, according to differing sources (Prater and Anderson 1977; Bryan and Langston 1992). However, Zn contents in the sediments of the Western spring of Landron (92.40 μg g^{-1}dry wt) and Cr contents in the sediments of both sites (28.65 and 70.60 μg g^{-1}dry wt) are typical of moderately polluted sediments, exceeding 70.0 μg g^{-1}dry wt for Zn and 20.0 μg g^{-1}dry wt for Cr, respectively, considered as natural threshold values (Prater and Anderson 1977). The amphipods accumulated Cd, Cu and Zn to a greater extent than the surrounding sediments. In particular, natural metal concentrations in the animals were similar to those recorded in amphipods collected from unpolluted sites, as reported by Amyot et al (1994) and Plénet (1999). However, higher Cd contents in the epigean *G. balcanicus* (5.97 ± 0.69 μg g^{-1}dry wt) were significantly different ($p<0.05$) from those in the hypogean *N. montellianus* (2.93 ± 0.28 μg g^{-1}dry wt), indicating more active accumulation. Otherwise, Dickson et al. (1979) reported that the tissue concentrations of non-essential metals, such as Cd and Pb, were significantly greater in the troglobitic crayfish *Orconectes australis australis* in comparison with the troglophilic species *Cambarus tenebrosus*, while the concentrations of essential metals did not exhibit significant differences in the two species. The bioaccumulation of non-essential metals and the greater longevity of *O. a. australis* would explain the significantly higher concentration of Cd and Pb (Dickson et al. 1979).

The toxicity of metals against organisms exposed in the laboratory was Cu>Cd>Zn in both species; however, *N. montellianus* showed greater resistance to the toxic effects than *G. balcanicus*, as may be seen by the mortality percentage. It is well-known that essential metals such as Cu and Zn become toxic if internal concentrations exceed the detoxification ability of physiological processes (Albergoni and Piccinni 1983). The increase in mortality in the presence of increased metal contents suggests that detoxification mechanisms can no longer regulate metals, because threshold concentrations were reached in the target tissues. In particular, copper was found to be more toxic than zinc, because gill tissues appeared seriously damaged, inducing death by hypoxia and loss of ion regulation, with Cu accumulation. Indeed, gills are a major site of aqueous metal uptake in crustaceans and are sensitive to metal-induced damage, which may result in respiratory and osmoregulatory impairment (Maltby 1999 and references therein). The non-essential metal Cd was highly accumulated in both organisms, indicating that this metal is sequestered in different organs, such as the digestive gland, as reported in various Crustacea (Coombs 1979; Amyot et al 1996). An interesting correlation was found between Cd contents in organisms of both species exposed to Zn; Cd contents fell significantly ($p<0.05$) with increasing exposure. This datum confirms the antagonistic function of Zn against Cd (Webb 1979). This discrepancy is probably related to the relative concentration of the two metals with respect to the concentration of chelating -SH groups in detoxifying compounds induced by metal exposure.

Our experiments also indicate that a hypogean species, such as *Niphargus montellianus*, is less sensitive to the toxic effects of heavy metals than epigean ones, such as *Gammarus balcanicus*, perhaps due to the long life-cycle and low metabolic rate of troglobic animals (Hervant 1996), allowing a slower rate of metal uptake, as also demonstrated by Plénet (1999) in *N. rhenorhodanensis* and *G. fossarum*, respectively. Other data on the toxicity of heavy metals, such as cadmium and zinc, on isopod and copepod crustaceans, either epigean or hypogean, indicate that the toxicity of heavy metals to aquatic organisms depends to a great extent on certain water quality characteristics, such as water hardness, acidity and temperature, and indicates that caution should be used in performing interspecific comparisons (Notenboom et al 1992).

The high sensitivity of *Gammarus* to metals suggests that possible pollution of freshwaters by the highest concentrations of copper (1 mg l^{-1}) and zinc (5 mg l^{-1}) permitted by Italian laws (see Decreto Legislativo no. 152 of May 11 1999, and Decreto Legislativo no. 31 of February 2 2001), which are equal to those used in our experiments, would be capable of eliminating *Gammarus* populations from this environment. Metal accumulation in *N. montellianus* may be useful especially in verifying contamination by Cu and Zn exceeding 5 μg ml^{-1}. According to Kettrup and Marth (1998), *Niphargus* may be considered a promising biological indicator for monitoring groundwater pollution by heavy metals, fulfilling the requirement for representative species of the groundwater ecosystem that the species should be sedentary enough and easy to identify, and should accumulate the pollutant without being killed or rendered incapable of reproduction. However, parallel experiments of exposure in the laboratory to increasing concentrations of heavy metals using *Glaucoma scintillans*, a ubiquitous ciliate in caves, indicating 0.40 μg ml^{-1}, 0.84 μg ml^{-1}and 0.2 μg ml^{-}1, for Cd, Cu and Cr as end point IC_{50} (Coppellotti Krupa and Guidolin 2003), suggest that more than one species must be considered as a "sentinel" in monitoring heavy metal karst water pollution. Future works will examine the effects of other metals, such as Cr and Pb, and the effects of long-term exposure to heavy metals; the levels of metal binding compounds, such as glutathione and metallothionein, in the tissues of organisms exposed will be checked as biomarkers in monitoring environmental quality.

ACKNOWLEDGMENTS

Grateful thanks are due to Gabriel Walton for correcting the English text.

REFERENCES

Albergoni, V., E. Piccinni. 1983. Biological response to trace metals and their biochemical effects. Pp. 159-175 *in* G.G. Leppard, ed. Trace elements speciation in surface waters and its ecological implications. New York, London: Plenum Publishing Corporation.

Amyot, M., B. Pinel-Alloul, P.G.C. Campbell, J.C. Désy. 1994. Abiotic and seasonal factors influencing trace metal levels (Cd, Cu, Ni, Pb, and Zn) in the freshwater amphipod *Gammarus fasciatus* in two fluvial lakes of the St. Lawrence river. Canadian Journal of Fisheries and Aquatic Sciences 51: 2003-2016.

Amyot, M., B. Pinel-Alloul, P.G.C. Campbell, J.C. Désy. 1996. Total metal burdens in the freshwater amphipod *Gammarus fasciatus*: contribution of various body parts and influence of gut contents. Freshwater Biology 33: 363-373.

Bressa, G., F. Cima. 1999. Il rischio in Italia da sostanze inorganiche. Fondo naturale incontaminato e contaminato. Roma: ANPA, Agenzia Nazionale per la Protezione dell'Ambiente.

Bryan, G.W., W.J. Langston. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environmental Pollution 76: 89-131.

Campbell, P.G.C., A. Tessier. 1991. Biological availability of metals in sediments: analytical approaches. Pp. 161-173 *in* J.-P. Vernet, ed. Heavy metals in the environment. Amsterdam, The Netherlands: Elsevier Science Publishers B.V.

Coombs, T.L. 1979. Cadmium in aquatic organisms. Pp. 93-139 *in* M. Webb, ed. The chemistry, biochemistry and biology of cadmium. Amsterdam: Elsevier, North-Holland Biomedical Press.

Coppellotti Krupa, O., L. Guidolin. 2003. Taxonomy and ecology of ciliate fauna (Protozoa, Ciliophora) from karst caves in North-East Italy. Subterranean Biology 1: 3-11.

Crompton, T.R. 1997. Toxicants in the aqueous ecosystem. Chichester: John Wiley & Sons Ltd.

Danielopol, D.L. 1980. The role of the limnologist in ground water studies. Internationale Revue der Gesamten Hydrobiologie 65: 777-791.

Dickson, G.W., L.A. Briese, J.P.Jr. Giesy. 1979. Tissue metal concentrations in two crayfish species cohabiting a Tennessee cave stream. Oecologia 44: 8-12.

Forti, P. 1999. "Gli acquiferi carsici: problematiche per il loro uso ed utilizzo". Pp. 13-39 *in* Inquinamento delle grotte e degli acquiferi carsici e possibili ricadute sulla collettività, Padova 26-27 September 1998, M. Crema and G. Ferrarese, eds. Padova, Italy: Imprimitur.

Förstner, U., G.T.W. Wittman. 1979. Metal pollution in the aquatic environment. Berlin: Springer-Verlag.

Hervant, F. 1996. The activities of enzymes associated with the intermediary and energy metabolism in hypogean and epigean crustaceans. Comptes Rendues de l'Académie des Sciences, Paris, Sciences de la vie 319: 1071-1077.

Kaçaroglu, F. 1999. Review of groundwater pollution and protection in karst areas. Water, Air, and Soil Pollution 113**:** 337-356.

Karaman, G.S.1993. Crustacea Amphipoda di acqua dolce. Fauna d'Italia, Vol. 31. Bologna, Italy: Calderini.

Ketrupp, A., P. Marth. 1998. Specimen banking as an environmental surveillance tool. Pp. 413-436 *in* G. Schüürmann, B. Markett, eds., Ecotoxicology. New York: John Wiley & Sons, Inc. and Spektrum Akademischer Verlag.

Maltby, L. 1999. Studying stress: the importance of organism-level responses. Ecological Applications 9: 431-440.

Mansilla-Rivera, I., J.O. Nriagu. 1999. Copper chemistry in freshwater ecosystems: an overview. Journal of Great Lakes Research 25: 599-610.

Notenboom, J., K. Cruys, J. Hoekstra, P. van Beelen. 1992. Effect of ambient oxygen concentration upon the acute toxicity of chlorophenols and heavy metals to the groundwater copepod *Parastenocaris germanica* (Crustacea). Ecotoxicology and Environmental Safety 24: 131-143.

Nriagu, J. O. 1990. Global metal pollution. Environment 32 : 7-33.

Plénet, S. 1995. Freshwater amphipods as biomonitors of metal pollution in surface and interstitial aquatic systems. Freshwater Biology 33: 127-137.

Plénet, S. 1999. Metal accumulation by an epigean and a hypogean freshwater amphipod: considerations for water quality assessment. Water Environonmental Research 71: 1298-1309.

Prater, B.L., M.A. Anderson. 1977. A 96-hour bioassay of Otter Creek, Ohio. Journal of the Water Pollution Control Federation 49**:** 2099-2106.

Rand, G.M., P.G. Wells, L.S. McCarty. 1995. Introduction to aquatic toxicology. Pp. 3-66 *in* G.M., Rand ed., Fundamentals of Aquatic Toxicology, Second Edition. Washington, D.C.: Taylor & Francis.

Sket, B. 1999. The nature of biodiversity in hypogean waters and how it is endangered. Biodiversity and Conservation 8: 1319-1338.

Webb, M. 1979. Interactions of cadmium with cellular components. Pp. 285-330 *in* M. Webb, ed. The chemistry, biochemistry and biology of cadmium. Amsterdam: Elsevier, North-Holland Biomedical Press.