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Toxicity and Bioaccumulation of Arsenic and Chromium in Epigean and Hypogean Freshwater Macroinvertebrates

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Abstract. Lethal toxicity levels of two inorganic water pollutants, chromium (Cr^{6+}) and arsenic (As^{3+}), were determined toward six freshwater macroinvertebrate species collected from a single field site. Crustaceans were represented by two amphipod species, an epigean one (*Gammarus fossarum*) and a hypogean one (*Niphargus rhenorhodanensis*), and by an isopod species (*Asellus aquaticus*). There were two insect larvae, *Heptagenia sulphurea* (Ephemeroptera) and *Hydropsiche pellucidula* (Trichoptera) and a snail, *Physa fontinalis*. Median lethal concentrations (LC50s) were determined over 96-h and 240-h periods for chromium and over a 240-h period for arsenic. Arsenic bioaccumulation was studied, too. The macroinvertebrates tested showed a wide range of sensitivity and bioaccumulation. A comparison between 96-h and 240-h experiments demonstrated that there was an increase in toxicity values following a longer time exposure for chromium. Also chromium was more toxic toward crustaceans than arsenic; conversely, arsenic was more toxic for the insect larvae and snail tested here. The lethal concentrations determined for the two metals were discussed and compared to results from other toxicity studies. The use of such macroinvertebrates, collected in the field and tested for longer exposure periods than within the standardized 96-h tests, should provide more suitable results for monitoring the general environmental quality of freshwater systems.

Monitoring the effects of pollution on aquatic organisms requires knowledge of the toxic substances involved. In recent years, interest in the factors that modify the bioavailability of environmental contaminants to aquatic organisms and toxicity analyses has grown (Hellawell 1989; Transpurger and Drews 1996). The persistence of metals and their toxic and cumulative effects on aquatic biota at low concentrations is a matter of concern in all industrial countries (Ahsanullah 1982). Many environmental laboratories are generating information on the acute and chronic toxicities of various metals; this information form the basis of water-quality criteria for the protection of aquatic ecosystems and their biota (Depledge *et al.* 1998).

The main purpose of this work was to assess the toxicity thresholds (expressed as LC50) for a set of freshwater macroinvertebrates collected from a single field site and translocated to laboratory conditions. Two metals were chosen: arsenic and chromium. Arsenic is widespread in the environment as a consequence of both anthropogenic and natural processes. It is a ubiquitous but potentially toxic trace element. Inorganic as well as organic forms of arsenic are present in the environment, and the former seem to be more toxic and slightly more accumulated in some freshwater aquatic species than the latter (Spehar *et al.* 1980). Trivalent arsenic may show an adverse effects on aquatic biota and is considered more toxic than the inorganic pentavalent form (Hall and Burton 1982). Data on the acute effects of arsenic to aquatic organisms are limited (Wageman *et al.* 1978; Forget *et al.* 1998). In contrast to other toxic elements, where the oxidation states are not distinguished, chromium presents several oxidation states, which are regulated in different ways. A distinction must be made between the two major oxidation states, chromium III and chromium VI. Cr III behaves as a hard Lewis acid and can form insoluble $Cr(OH)_3$ and $FexCr_{1-x}(OH)_3$ precipitates, contrary to Cr VI, which is highly soluble (Wang *et al.* 1997). Therefore, the U.S. Environmental Protection Agency (US EPA) classifies materials as hazardous waste if they contain leachable chromium (40CFR261.4), but excludes those materials from classification if it can be shown that the leachable chromium is not chromium VI (40CFR261.94) (Kimbrough *et al.* 1999).

Public concern with chromium is mainly related to Cr VI, since these compounds are toxic due to their oxidation of intracellular compounds. Contrary to Cr III, which is relatively nontoxic to organisms and bioaccumulated from ingested food, Cr VI bioaccumulation in aquatic organisms comes from the dissolved phase (Wang *et al.* 1997). These considerations led us to study As III and Cr VI.

To measure the environmental concentration levels bioavailability and the potential effects of pollutants, many studies have been carried out with aquatic insects (Hare 1992; Clement and Kiffney 1994). Lotic insect larvae occupy almost all tro-

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phic levels of consumers and have a variety of feeding habits, a rapid metabolism, and variable life cycles; thus they have the potential to reflect with great accuracy both the spatial and temporal aspect of the fate and effects of chemicals (Vuori and Kukkonen 1996). Trichoptera such as hydropsichids, widely distributed and abundant, fulfill the general requirements of ideal bioindicator. They tolerate great variations in water quality and react to these variations with morphological, chemical, and behavioral changes. Their ecology is sufficiently well known for any quality effect to be recognized (Vuori and Kukkonen 1996). Ephemeroptera, such as Heptageniids, are sensitive to organic pollution, but little is known about their sensitivity to metals. An important component of the food web is represented by freshwater crustaceans like Gammarids and Asellids, which are abundant and found in many temperate systems. They have been effectively used in toxicity assessment and are usually obtained from wild populations (Crane 1995; Mulliss *et al.* 1996). Until now, little attention has been paid to the fate of pollutants in water that flows through the alluvial bed sediments and the effect on hypogean crustaceans. Blind gammarid (Niphargids) constitute an important element of the subsurface food web, and they show adaptation capacities that allow them to successfully exploit the underground environment (lower metabolic rate and life history traits such as low growth, reproduction rates, and increased longevity) (Ginet 1960; Hervant *et al.* 1998). Another important group of freshwater organisms is constituted by the gastropod pulmonates (molluscs), which have already been used to test the influence of heavy metals on survival, reproduction, and bioaccumulation (Gomot 1998). Six species represented by taxonomic groups previously described have been used in this study.

The effects of pollutants have been tested using a flowthrough experimental design, which allowed tests to be performed on a range of macroinvertebrates simultaneously under identical conditions. The experimental design should give a more realistic threshold than static bioassays, which often underestimate the toxicity threshold because of adsorption. Two exposure times were chosen, one a standard exposure time of 96 h (acute toxicity test), the other a long-term test of 240 h (subacute toxicity test).

Materials and Methods

Test Organisms

The six macroinvertebrate species were collected in a single station to minimize the role of their origin as a potential source of distortion on the measured responses (Mersch and Pihan 1993). This station, located on the Ain River, which is the main tributary of the Rhône River situated 30 km upstream from the city of Lyon (France), is weakly contaminated and usually taken as a test station (Plénet 1994). Two epigean crustaceans (an amphipod and an isopod), one hypogean crustacean (amphipod), one mollusc (gastropod), and two insect larvae (Trichoptera and Ephemeroptera) (see Table 1) were collected in order to have a wide range of sensitivity with the toxicity tests. The hypogean crustacean was collected with a manual pump (Bou and Rouch 1967) at 50 cm depth in the sediment, and the other taxa were sampled using a hand net (500 μm mesh size).

Sixty organisms per species were used for each lethal toxicity experiment and thirty organisms per species per replicate for each concentration were used for bioaccumulation experiments except for Niphargids (Table 2). The organisms were transported to the laboratory in an ice-cooled container, then placed in oxygenated river water in a cooled chamber until the beginning of the experiment. They were acclimatized to laboratory conditions for 2 days before experimentation. Because of their particular sensitivity to ecotoxicological testing, the crustacean ovigerous females were excluded from the experiments. Organisms were collected in November 1998, June 1999, and July 1999 for the chromium 96-h (1), (2) and chromium 240-h (3) experiments, respectively, and in April 1997 for the arsenic experiments. Only last-instar larvae stages were kept for the experiments. The experiments were realized in summer and in winter. *H. pellucidula* is a bivoltine species (Roux *et al.* 1992). *H. sulphurea* is univoltine, but there is usually a fast-growing cohort that emerges in May/June and a slower-growing cohort that emerges in September/October. The growth rates vary with water temperature, and the period over which adults emerge vary from year to year and locality to locality (Elliot *et al.* 1988). For crustaceans and snail the size was the criteria and juveniles were excluded from the experiments.

Experimental Designs

Mortality tests were conducted with a flowthrough exposure system consisting of a multichannel toxicant injection system, which delivered the toxicant at three concentrations and a control. Tests were carried out with filtered river water collected with the organisms. River water and contaminant were continuously mixed and went through compartmentalized glass tubes containing the invertebrates. To prevent predation or competition behaviors, each organism was separated from the others and placed in an individual compartment (Figure 1). Tests were performed in a room with a constant temperature of 12 ± 2°C, and light was controlled on a 14 h light/10 h dark cycle. Only the hypogean species *N. rhenorhodanensis* was kept in total darkness. Three replicates of five organisms per species were used for each concentration. The flow rate to each replicate was 50 ml/min for a 3-L water volume (9 L per concentration). Before all experiments, dishes were washed with 10% nitric acid and rinsed a few times with deionized water. Two kinds of tests were carried out: short-term acute toxicity tests (96 h) and subacute toxicity tests (10 days).

Bioaccumulation was studied in glass aquariums (5 L) for 10-day experiments. Arsenic solutions were continuously renewed with a peristaltic pump, and water samples were collected for analyses every 24 h during the test. The experimental design was set up also in the cooled chamber (temperature: 12 ± 2°C), with a 14/10 photoperiod. Three replicates of 30 organisms were performed for each species, except for Niphargids (Table 2).

Toxicant Solution

Stock solutions of metals were freshly prepared by dissolving the appropriate salts in deionized water in 1-L glass volumetric flasks. The sodium arsenite stock solution was prepared with deionized water (AsIII). For the mortality test, three concentrations were made using a peristaltic pump, which continuously mixed the sodium arsenite solution with river water. Every 24 h 25-ml samples were taken for water analysis and acidified with nitric acid to verify the effective concentration (Table 3). Analyses were carried out with an atomic absorption spectrophotometer with a graphite furnace (Perkin Elmer Zeemer 3030). The detection limit for this procedure was 1 $\mu g\ L^{-1}$. The concentration used in each replicate of the bioaccumulation experiment was 100 $\mu g\ L^{-1}$.

In the same way, a potassium chromium stock solution was prepared, and three concentrations were made for the 96-h experiment and

Table 1. Characteristics of invertebrates tested

Phylum (Class)	Family	Genus Species	Current Preference	Feeding Group	Diet
Molluscs (Gateropods)	Physidae	*Physa fontinalis*	Lotic	Grazer	Herbivorous
Arthropods (Crustaceans)	Asellidae	*Asellus aguaticus*	Lentic	Shredder	Detritivorous
	Gammaridae	*Gammarus fossarum*	Lotic	Shredder	Omnivorous
	Niphargidae	*Niphargus rhenorhodanensis*	Lentic	Shredder	Omnivorous
	Hydropsychidae				
(Insects)	(Trichoptera)	*Hydropsyche pellucidula*	Lotic	Filterer	Carnivorous Detritivorous
	Heptageniidae				
	(Ephemeroptera)	*Heptagenia sulphurea*	Lotic	Scraper	Detritivorous

Table 2. Experimental conditions of arsenic exposure to various species of macroinvertebrates and means ($\mu g\ g^{-1}$ weight and standard deviation) of arsenic concentrations after exposure for 10 days to 100 $\mu g\ L^{-1}$

Species	Theoretical Concentration ($\mu g\ L^{-1}$) in Water	Mean of Measured Concentration ($\mu g\ L^{-1}$) in water (SD)	Initial Number of Animals per Samples	% Mortality after 10 Days (SD)	Mean of Measured Concentration in Animals ($\mu g\ g^{-1}$) (SD)
G. fossarum	Controls = 0	1 (0.1)	30	3 (1.7)	ND*
	tests (3) = 100	97.3 (0.6)	30	17 (1.9)	20.3 (1)
H. sulphurea	Controls = 0	1 (0.1)	30	6.7 (3.3)	ND
	tests (3) = 100	98 (1)	30	23 (3.3)	0.12 (0.01)
P. fontinalis	Controls = 0	1 (0.1)	30	0	ND
	tests (3) = 100	96.7 (0.5)	30	0	1.9 (0.3)
A. aquaticus	Controls = 0	1 (0.1)	30	3 (1.7)	ND
	tests (3) = 100	97.3 (0.6)	30	11 (1.9)	26.5 (1.2)
N. rhenorhodanensis	Controls = 0	1 (0.1)	100	0	ND
	tests (3) = 100	95.7 (0.5)	100	6.7 (3.3)	22.6 (2.6)
H. pellucidula	Controls = 0	1 (0.1)	30	2.2 (1.9)	ND
	tests (3) = 100	98 (1)	30	11 (1.9)	107.2 (5.2)

*ND = not detectable: concentrations in control animals were below method detection limit.

for the 240-h experiment. Every 24 h a water sample was collected and immediately analyzed to verify the actual concentration of the Cr^{6+} ion (Table 3). This analysis was carried out using a HACH colorimetric method. This is the 1,5-diphenylcarbohydrazide method, US EPA accepted for reporting wastewater analysis (Hach 1986; Kimbrough *et al.* 1999). Total chromium was measured in order to control the absence of chromium III in our samples. Preliminary tests were conducted to obtain a mortality range of 0 to 100%.

Biological Procedures

Four physicochemical parameters were measured each 24 h during the experiments (over 240 h for arsenic and for chromium over 96 h and 240 h). Organism survival was controlled every 24 h, and the dead individuals taken out. The dead point was taken as immobilization, determined by gentle probing of each animal. Postexposure observations in clean water revealed the fact that immobilized organisms did not recover. As we have an hypogean species in our experiment, toxicity tests with lethality as endpoint was chosen (Mösslacher 2000). The organisms were fed every 2 days with fish food (Tetramin); any food remaining from the previous feeding was removed after 24 h.

For bioaccumulation experiments, after 10 days' exposure, tested animals were rinsed with deionized water to minimize the contribution of surface-bound metals to the overall larval metal concentration, then frozen for residue analysis. Bioaccumulation analysis were performed owing to the nitric acid–perchloric acid digestion method (3030 H; APHA 1992). The efficiency of the method is 91%. The organisms were dried (60°C for 24 h) and then placed for 3 h in a dessication device. Then they were weighted (the precision of the weighing measurements was 0.001 mg) before mineralization. The organisms were digested with 5 ml of suprapur water, 5 ml nitric acid suprapur, and 2 ml perchloric acid suprapur, added with a calibrated pipette, for 12 h at room temperature. After that, samples were placed in a 250°C bath for 4 h. The samples were filtered, and potassium iodure was added before analysis with the atomic absorption spectrophotometer with a graphite furnace (Perkin-Elmer 2100). The method detection limit was 1 $\mu g\ L^{-1}$.

Statistical Analysis

According to the Spearman-Karber method (Hamilton *et al.* 1977), the 96-h LC50 and the 240-h LC50 were calculated from measured concentrations of metal ions. The LC50 data represent the concentration inducing 50% mortality in a population exposed for 96 h or 240 h to the contaminated medium. Only nonparametric statistics were used here because of the small number of samples (five animals per species per replicate). A Kruskall-Wallis test (one-way analysis of variance by ranks) was used to compare physicochemical parameters between the different concentrations. Differences were considered significant at $p < 0.05$. Chi-square tests were performed to test the homogeneity of mortality between replicates.

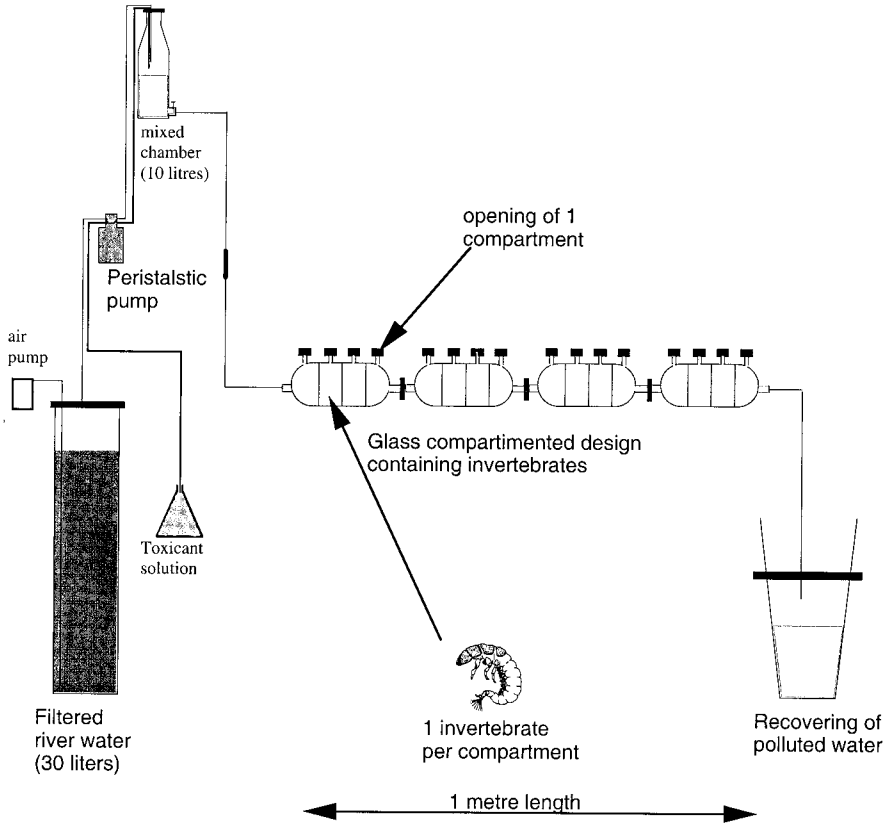

Fig. 1. Flowthrough system used to realized the toxicity experiments simultaneously on a range of macroinvertebrates

Table 3. Means (mg L^{-1} and SD) of measured concentrations (control and C1, C2, and C3) realized every 24 h, for each replicate (called R1, R2, and R3) of the different experiments

Tests	Controls (3 Replicates)	C1			C2			C3		
		R1	R2	R3	R1	R2	R3	R1	R2	R3
As		0.20	0.20	0.19	1.30	1.22	1.18	4.2	4.33	4.06
240 h	< 0.002	(0.06)	(0.06)	(0.05)	(0.84)	(0.66)	(0.67)	(1.41)	(1.44)	(1.27)
Cr		0.063	0.054	0.053	2.67	2.8	2.67	19.3	16.33	18.33
240 h	< 0.003	(0.019)	(0.011)	(0.011)	(0.75)	(0.67)	(0.67)	(3.26)	(2.05)	(1.03)
Cr (1)		1.85	1.82	1.97	20.75	19.25	19.75	200	215	206.7
96 h	< 0.003	(0.17)	(0.05)	(0.17)	(4.5)	(2.22)	(1.7)	(2.83)	(7.1)	(4.04)
Cr (2)		1.83	1.20	1.23	14.25	16.25	1.5	153	160	163
96 h	< 0.003	(0.52)	(0.03)	(0.24)	(4.03)	(4.26)	(3.01)	(10)	(14)	(21)

Control was realized with filtered river water, and arsenic and chromium were not detectable. The reproducibility of the experimental design was tested by performing two 96-h tests with chromium: Cr (1) and Cr (2).

Results

Toxicant Solutions

The measured concentrations for chromium 96-h experiments ranged from 0 to 200 mg L^{-1} as chromium VI for the first experiment and from 0 to 160 mg L^{-1} as chromium VI for the second one (Table 3). There was no significant difference between the replicates (p values > 0.05). For the 240-h experiments, the concentrations ranged from 0 to 18 mg L^{-1} (Table 3) and no significant differences were observed between the replicates (p values > 0.05). The concentrations used for arsenic were lower and ranged from 0 to 4.2 mg L^{-1} (Table 3). There was no significant difference between the replicates (p values > 0.005).

The physicochemical parameters were homogeneous. Dissolved oxygen concentration was always at saturation (10 mg L^{-1} average value) without any significant difference between concentrations for each experiment. For the chromium experiments, the higher metal concentration caused a significant decrease in pH values and a significant increase in conductivity, especially for the 96-h (1) chromium experiment. However, in all cases pH values did not reach the acidic range, which could affect the organisms survival. Means of pH values ranged from 7.6 to 8.4 depending on the experiment considered and conductivity ranged from 318 $\mu s \cdot cm^{-1}$ to 423 $\mu s \cdot cm^{-1}$.

Survival

The 96-h chromium experiments were carried out to validate the experimental design. Good replicability (p value > 0.05 for the Chi-square tests) and reproducibility between the two experiments were observed with the invertebrates collected in their biota and transferred under laboratory conditions (Figure 2). Crustaceans and insect larvae reacted very differently to the toxicant. *G. fossarum* and *N. rhenorhodanensis* were very sensitive species, with LC50s of 1.37 mg L^{-1} (SD = 0.2) and of 1.18 (SD = 0.24) for the two tests and between 1.51–2.07 mg L^{-1} (SD = 0.44 and 0.42), respectively, while *A. aquaticus* was relatively resistant with LC50s of 13–15 mg L^{-1} (SD = 5 and 4.5). For the insect larvae, we noticed the great sensitivity of *H. sulphurea* (3.8 mg L^{-1} and 3.97 mg L^{-1}; SD = 0.7 and 1.15) contrary to *H. pellucidula*, which was the most tolerant species (30–32 mg L^{-1}; SD = 10.9 for the two tests). The mollusc *P. fontinalis* presented an intermediate sensitivity with LC50s of 9.46 mg L^{-1} and 9.42 mg L^{-1} (SD = 2 and 3.57).

In these experiments two groups of invertebrates were observed: the first one corresponding to the most sensitive species included *G. fossarum*, *N. rhenorhodanensis*, *H. sulphurea*, and *P. fontinalis*, and the second one with higher LC50: *A. aquaticus* and *H. pellucidula*. However, the 240-h experiment showed a different pattern (Figure 3), even though *G. fossarum* (LC50 = 0.19 mg L^{-1}; SD = 0.13) and *N. rhenorhodanensis* (LC50 = 0.23 mg L^{-1}; SD = 0.12) were always the most sensitive and *H. pellucidula* (LC50 = 4.8 mg L^{-1}; SD = 4.3) the most tolerant species. In those experiments, *A. aquaticus* and *H. sulphurea* appeared much more sensitive (LC50 = 0.51 mg L^{-1} and 0.22 mg L^{-1} and SD = 0.18 and 0.11, respectively), and *P. fontinalis* (LC50 = 4.2 mg L^{-1}; SD = 2.1) much more resistant.

The arsenic experiment was performed as a 240-h test (Figure 4). The utmost sensitivity was found for *G. fossarum*, with an LC50 of 0.2 mg L^{-1} (SD = 0.5), whereas *N. rhenorhodanensis* appeared very resistant with highest LC50 values (3.97 mg L^{-1}; SD = 1.5). *A. aquaticus* showed a 2.31 mg L^{-1} LC50 value (SD = 0.4). Taking into account the confidence interval, there were three groups of sensitivity. The first consisted of *G. fossarum* and *H. sulphurea* (1.6 mg L^{-1}; SD = 0.4). The second one consisted of *P. fontinalis* (2.2 mg L^{-1}; SD = 0.6), *A. aquaticus* (2.3 mg L^{-1}; SD = 0.4), and *H. pellucidula* (2.4 mg L^{-1}; SD = 0.3) with no significant differences between their LC50 values; and the third one with *N. rhenorhodanensis* (3.97 mg L^{-1}; SD = 1.5).

Bioaccumulation

Mortality was relatively low with arsenic except for *H. sulphurea* where seven organisms died during the experiment. The results obtained for this species should be taken carefully. Arsenic accumulation in *G. fossarum*, *A. aquaticus*, *H. pellucidula*, and *N. rhenorhodanensis* exposed to 100 μg L^{-1} over a 10-day period was significantly higher than in the control animals (Table 2). *H. sulphurea* and *P. fontinalis* did not seem to accumulate As III. Similar bioaccumulation values were observed for the two amphipods (20 μg g^{-1} dry weight in average). They were significantly lower than those observed for *A. aquaticus* (26.5 μg g^{-1}). *H. pellucidula* showed a very high value of bioaccumulation (107 μg g^{-1} dry weight).

Discussion

Sensitivity of Invertebrate Species to Chromium

Very good reproducibility was observed for 96-h LC50 tests with chromium. Amphipods were 20 times more sensitive to chromium than Trichoptera studied. Comparison with other freshwater amphipods (Table 4) shows that *Crangonyx pseudogracilis* was much more sensitive to chromium than *G. fossarum*, in contrast with the results obtained with copper by Martin and Holdich (1986), but each species gave different responses to the different pollutants, and copper, although considered essential, was often classified as more toxic than chromium (Kangharot and Ray 1989). Comparison with marine invertebrates (Table 4) underlines the fact that amphipods were more sensitive than the marine decapod *Cancer magister*, but it has already been shown that metal toxicity is more accurately measured in fresh water than in sea water, because metals appear to a greater extent as complexes and complexation reduces the toxicity of metal ions (Martin *et al.* 1981). However the toxicity of specific chemicals can be affected by several physical or chemical factors, such as temperature, adsorption to container surfaces (which decreases the metal concentrations), complexation with organic constituents from sea water, and gaseous exchange or even loss (Glickstein 1979; Martin *et al.* 1981). For instance, *Tubifex tubifex* was 15 times more sensitive at 30°C with food than at 20°C without food.

A comparison of toxicity values for 96-h and 240-h chromium experiments demonstrated an increase in toxicity following longer exposure periods. Even if the magnitude of this increase varies between the species, *G. fossarum*, *N. rhenorhodanensis*, and *H. pellucidula* were about six times more sensitive, while *A. aquaticus* and *H. sulphurea* were 17 to 26 times more sensitive. In contrast *P. fontinalis* was just twice as sensitive. Other 240-h experiments should confirm these results, but no mortality was recorded in the experiment controls. The usefulness of longer exposure time experiments for aquatic insect larvae had already been demonstrated by Spehar *et al.* (1980) and Pontasch and Cairns (1989). These organisms tend to exhibit increased sensitivity with longer exposure times, possibly due to low resistance during molting. Toxicity threshold obtained from aquatic insects that undergo 1 or more molts during the experiment will be more representative of what happens in the environment. In this study, great variations of sensitivity were recorded with a crustacean, *A. aquaticus*, which became much more sensitive after a 240-h exposure test to chromium. Indeed *A. aquaticus* had already been used as an indicator of water quality in many short-term toxicity tests and, contrary to the results obtained with 240-h experiment, appeared to be relatively tolerant toward a range of pollutants (Naylor *et al.* 1990). Moreover, one major criticism in ecotoxicological studies is the use of field-collected macroinvertebrates, which present particular life-history traits, which could affect toxicity results. But Crane (1995) showed that, for ex-

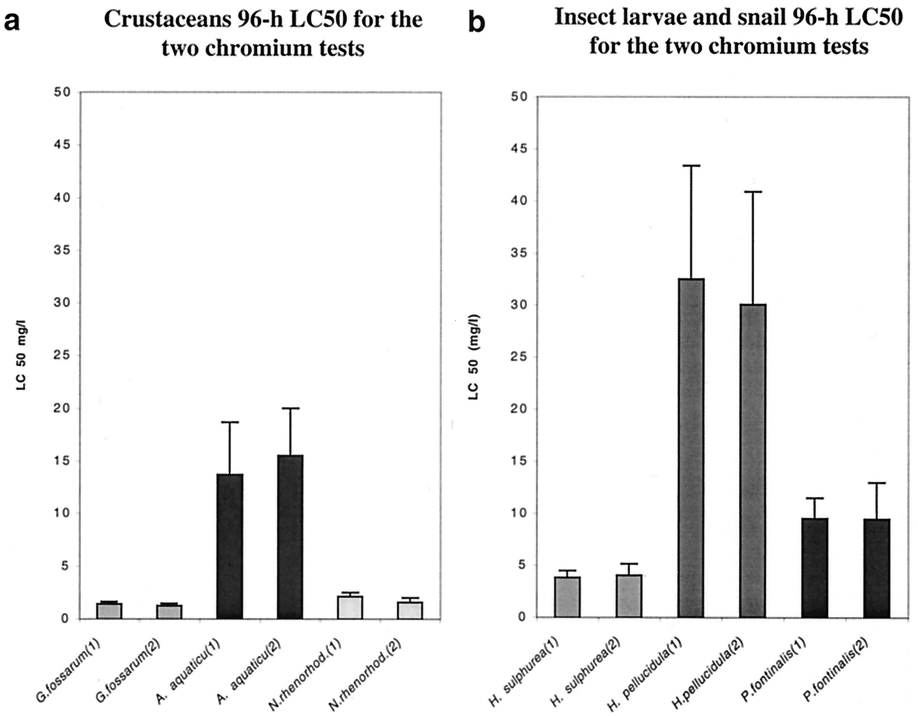

Fig. 2. Means (and standard deviation) of LC50 values (mg L^{-1}) of (a) crustaceans and (b) insect larvae and snail for the two 96-h chromium tests. The same experiment was reproduced two times: (1) in November and (2) in June

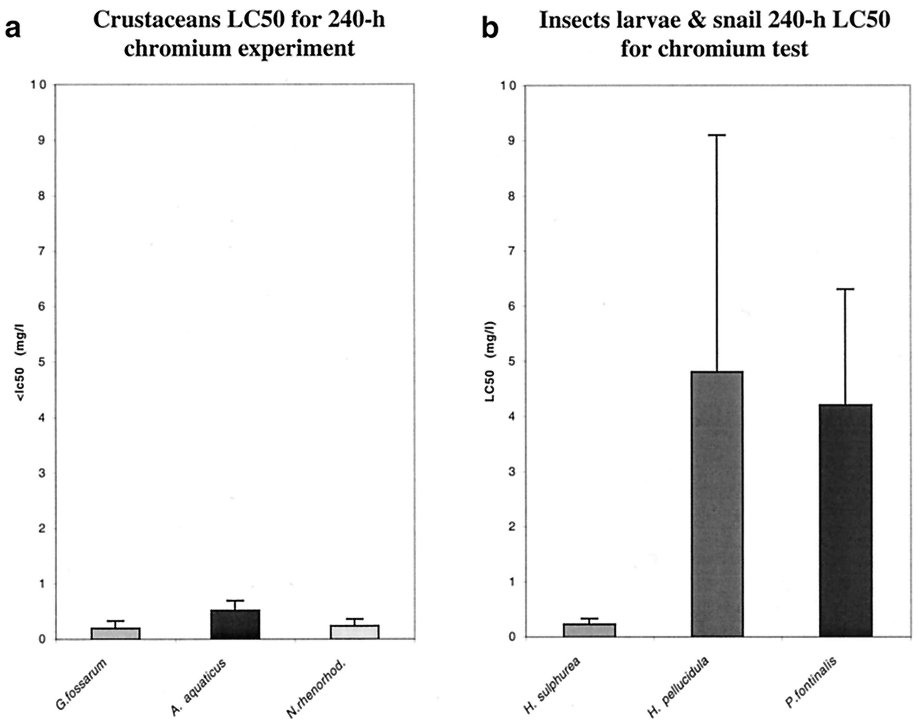

Fig. 3. Means (and standard deviation) of LC50 values (mg L^{-1}) of crustaceans (a) and insect larvae and snail (b) for 240-h chromium test

posure times greater than 6 days, such differences in the responses of some populations within a species living under different environmental conditions are avoided. This study shows the importance of long-term exposure tests that allow a comparison with results obtained in other populations of the same species.

Sensitivity of Invertebrate Species to Arsenic

For the 240-h arsenic experiment, the LC50 values ranged from 0.2 mg L^{-1} for *G. fossarum* (the most sensitive species) to 3.9 mg L^{-1} for *N. rhenorhodanensis*. The hypogean crustacean was 2 to 20 times less sensitive than the epigean crustacean

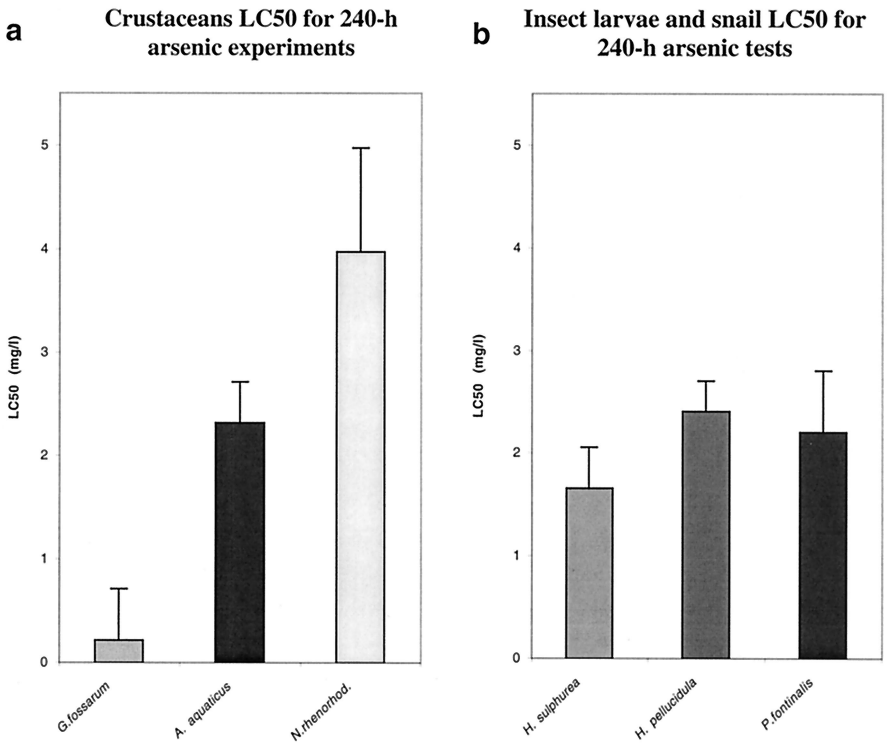

Fig. 4. Means (and standard deviations) of LC50 values of crustaceans (a) and insect larvae and snail (b) for 240-h arsenic test

species, contrary to the results obtained with chromium. Such observations have been realized with other species: epigean crustaceans (such as *Nitocra spinipes*, *Gammarus pulex*, *Allorchestes compressa*) were more sensitive than hypogean crustaceans (such as *Parastenocaris germanica*, *Niphargus aquilex*, and *Proasellus cavaticus*), respectively (Bengtsson and Bergström 1987; Bosnak and Morgan 1981; Notenboom *et al.* 1992). In the present study *N. rhenorhodanensis* was not always the most resistant species, which suggested that data on the toxicity of pollutants on hypogean or groundwater species are too scarce.

Spehar *et al.* (1980) showed that *G. pseudolimneus* was much more sensitive to As III than other invertebrates, since 96 $\mu g\ g^{-1}$ As III killed all the organisms after 14 days' exposure, whereas no mortality was observed for stonefly or snail. This result is consistent with our study, where *G. fossarum* was the most sensitive species with arsenic. Insect larvae and the gastropod *P. fontinalis* thresholds were closer to *A. aquaticus* LC50 values with a greater sensitivity of *H. sulphurea*. Up to now, very few data are available on arsenic toxicity to freshwater invertebrates (see Table 4).

Crustaceans, insect larvae, and snail species studied here presented difference in sensitivity to chromium and arsenic. In fact, crustaceans seemed to be much more sensitive than the insect larvae and snail tested here to chromium (Figure 3) and conversely for arsenic (Figure 4). Such results confirmed previous observations (Khangarot and Ray 1989).

Patterns of Bioaccumulation of Arsenic

Despite their differences in survival, the two amphipod species (epigean and hypogean) had the same rate of bioaccumulation for a concentration of 100 $\mu g\ L^{-1}$ of As III in water. But the two crustaceans had different metabolic rates, and when the concentration of As increased *G. fossarum* bioaccumulated more As and died more rapidly than the hypogean crustacean. Plénet (1999) showed that Zn bioaccumulation rates of *G. fossarum* were different from *N. rhenorhodanensis* after 12-day experiments.

The isopod *A. aquaticus* bioaccumulated more arsenic than *G. fossarum*, and its LC50 value was intermediate between the LC50 of the two amphipod species. The greater tolerance of *A. aquaticus* compared to the *Gammarus* species had already been demonstrated with short-term toxicity tests (Martin and Holdich 1986; Naylor *et al.* 1990), but there is no clue to such differences in tolerance. Brown (1977) showed that trace amounts of arsenic were recorded in *Asellus meridianus* hepatopancreas (in lipid inclusions). This small organ, which constitutes only 4–6% of the total dry weight, contained 30–60% of the total body burden of metals like cadmium or copper (Van Hattum *et al.* 1996). It has been suggested that this organ allowed high concentration of metals to be taken out of circulation, but only between molts, since during the molt metals are released in the body of the invertebrates (Wieser and Klima 1969). Some decapods, like lobsters, accumulated primary organic and inorganic arsenic in their hepatopancreas (Cooney and Benson 1980), but other decapods, such as the marine shrimp *Lysmata seticaudata*, accumulated arsenic to a higher degree in muscle tissue (Fowler and Unlu 1978). These results suggest that the mode of action of arsenic varies a lot among crustaceans and could explain the differences between amphipod and isopod LC50s and the greater sensitivity of *A. aquaticus* exposed for longer time periods to chromium, for example.

Considerable molting was observed during the experiments for *H. sulphurea* (twice as many molts as in the control).

Table 4. Comparison of LC50 values from the present study and results obtained for other aquatic marine or freshwater invertebrates (*240 h exposure time; LC50s without any indication are 96-h LC50)

Species	Arsenic	Chromium	Laboratory Conditions	Authors
Marine Invertebrates				
Tigriopus brevicornis			20°C pH = 7.7–8.14	Forget *et al.* (1998)
Nauplii	10.9 μg L^{-1}		12/12 dark/light	
Copeodids	19.8 μg L^{-1}		Salinity 34.5–35%	
Ovigerous fem.	27.5 μg L^{-1}			
Scylla serrata (intertidal crab)	1,700 μg L^{-1} (As_2O_3)		26.5–29.5°C pH = 7/7.2 Static bioassay	Krishnaja *et al.* (1987)
Cancer magister	232 μg L^{-1} (As_2O_3)	3.44 mg L^{-1}	15°C pH = 8.1 ± 0.2 Salinity: 33.8%	Martin *et al.* (1981)
Nitocra spinipes	3.5 μg L^{-1}		20–22°C Salinity 3%-static bioassay feeding	Bentsson and Bergström (1987)
Tisbe battagliai		5.9 mg L^{-1}	20°C pH 7.7–8.1 Salinity 35% 8/16 Dark/light feeding	Hutchinson *et al.* (1994)
Nereis virens		2 mg L^{-1}	20°C	Eisler and Henneckey (1977)
Pagurus longicarpus		10 mg L^{-1}	Salinity 20%	
Neanthes arenaceodentata		3.1 mg L^{-1} 1.63 (168 h)	20°C Salinity 33.5%	Mearns *et al.* (1977)
Allorchetes compressa (amphipod)		5.56 mg L^{-1} 6.34 mg L^{-1}	20°C pH = 8 Salinity: 33% 10/14 dark/light Flowing seawater system	Ahsanullah (1982)
Freshwater Invertebrates				
Daphnia obtusa		0.06 1 mg L^{-1} (48 h)	20°C pH = ? 12/12 dark/light	Coniglio and Baudo (1989)
Daphnia magna		1.79 mg L^{-1} (48 h)	13°C pH = 7.6	Kangharot and Ray 1987
	6.23 mg L^{-1} (48 h)		Static bioassay	Kangharot and Fargasova (1994)
Daphnia magna	44.66 mg L^{-1}* (As^{5+})	0.162 mg L^{-1}	20°C pH = 6.15–7.9	Fargasova (1994)
Tubifex tubifex	127.36 mg L^{-1}	2.91 mg L^{-1}	No direct sunlight Static bioassay feeding	
Tubifex tubifex	8.87 mg L^{-1} (Na_3AsO_3)	0.19 mg L^{-1}	30°C No food pH = 7.6	Khangarot (1991)
Limnea luteola		3.88 mg L^{-1}	Static bioassay	Khangarot and Ray (1988)
Crangonyx pseudogracilis		2.20 mg L^{-1} (48 h)	13°C pH = 6.75 Static bioassays	Martin and Holdich (1986)
Gammarus fossarum	0.2 mg L^{-1}	1.4–1.2/0.19* mg L^{-1}	12°C pH = 8	Present study
Heptagenia sulfurea	1.65 mg L^{-1}	3.8–3.9/0.22*	10/14 dark/light Flowthrough syst.	
Physa fontinalis	2.2 mg L^{-1}	9.5–9.4/4.2*	Effective	
Asellus aquaticus	2.3 mg L^{-1}	13–15.3/0.5*	Concentration used for LC50 feeding for 240-h test	
Niphargus rhenorhodanensis	3.9 mg L^{-1}	2.1–1.5/0.23*		
Hydropsychidae pellucidula	2.4 mg L^{-1}	30.4–32/4.8*		

According to Hare *et al.* (1991) molting may allow arsenic loss in several stream-dwelling insects, which could explain the results obtained here. Nevertheless in the field study of Mason *et al.* (2000), Heptageniids bioaccumulated much more arsenic than Hydropsichids did, and the authors explained that this is probably due to the dependence on the surface/volume ratio during the process of adsorption directly from water.

H. pellucidula accumulated 100 times more inorganic arsenic than the controls. The potentially large quantities of trace metals (Cd, Cu, and Zn) found in the gut in both aquatic and terrestrial insects could be stored in granules and/or lysosomes (Hare 1992). The rate at which such storage structures are excreted could determine the capacity of a given species to resist the toxic effects of metals (Hare *et al.* 1991). It is possible

that the Hydropsychid species could store and excrete arsenic after accumulating certain quantities. In the study realized by Leslie *et al.* (1999) *H. pellucidula* exposed to high concentration of chromium in its natural biota presented discoloration of anal papilla. These ion-exchange organs are responsible for taking up ions from the aquatic environment and ensuring that the hypertonic body fluids of the animal remain hypertonic with respect to the surrounding water. For this reason, the authors would expect these organs to be easy targets for Cr VI. After absorption by the organisms, chromium VI is reduced to Cr II, which has the ability to bond to biological macromolecules, which became difficult to excrete again and may accumulate. We could hypothesize that the same phenomenon occurs with arsenic in our experiment.

Spehar and Fiandt (1986) showed that the snail *H. campanulata* accumulated about 2.7 $\mu g\ g^{-1}$ dry weight after 14 days' arsenic exposure (100 $\mu g\ L^{-1}$ As III). During our experiment, we observed that the freshwater pulmonate snail *P. fontinalis* did not accumulate much more arsenic.

Conclusion

In the present study, crustaceans were more sensitive to hexavalent chromium than to trivalent arsenic, and conversely for the aquatic insects and the snail species tested here. The greater sensitivity of crustaceans to chromium compared to insect larvae and a better tolerance to arsenic by crustaceans compared to insect larvae are consistent with other studies (Martin *et al.* 1981; Kangharot and Ray 1989; Fargasova 1994; Adler-Ivanbrook and Breslin 1999). Every species tested in these experiments reacted differently to the metals tested, even *N. rhenorhodanensis*, which was one of the most sensitive species with chromium. Up to now, it was generally observed a greater tolerance of the hypogean species compared to the epigean one. With chromium the hypogean species was as sensitive than the epigean one, which suggests the need for more experiments with a lot of pollutants (metals, mixtures, pesticids, herbicids. . .) with hypogean species to confirm such results.

Experiments carried out with arsenic and chromium on freshwater macroinvertebrates were; we found in the literature arsenic LC50s for just two freshwater species and for chromium for three freshwater species. The advantage of our study is that toxicity thresholds are more realistic than those obtained with laboratory organisms (*Daphnia magna*, for example) under short-term toxicity testing. Presently almost all of the standards and criteria established to protect freshwater systems are based on this type of information, and their ultimate goal is to predict the effects of "pollutants" on long-term health of communities and ecosystem.

The change in LC50 of chromium with increased exposure time and with the species tested are also of interest and indicate that caution must be exercised in setting water quality standards with short-term acute toxicity tests, mostly carried out with laboratory organisms (*A. aquaticus*, which is generally considered as tolerant species, became very sensitive with the 240-h experiment).

Biological monitoring with carefully selected organisms may be an important instrument for evaluating the bioavailability and effects of contaminants, even when all biotic and abiotic processes are not fully understood. Most of the six species used in our experiments have already shown their potential as bioindicators of trace elements (Hydropsychids, Gammarids, Asellids, and Gastropods) in previous works (Cain *et al.* 1992; Maund *et al.* 1992; Martin and Holdich 1986; Gomot 1998, respectively). The use of an hypogean species and the possibility of comparing the sensitivity of the six species submitted to the same conditions should provide more suitable results in monitoring the general environmental quality of freshwater systems.

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