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Depuration processes after exposure of burrowing mayfly nymphs (*Hexagenia rigida*) to methylmercury and cadmium from water column or sediment: effects of temperature and pH

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Abstract

An experimental study, based on a complete experimental design, was set up in order to analyse depuration processes after exposure of the burrowing mayfly nymphs *Hexagenia rigida* to cadmium (Cd) and methylmercury (MeHg) from the sediment or water column as initial contamination sources. Actions and interactions of two abiotic factors (temperature and water column pH) were also quantified, from the combinations of two modalities for each parameter: temperature, 15 and 25°C; pH, 5.0 and 7.5. Marked differences were observed in depuration kinetics in relation to the metal studied and also to the exposure source during the contamination phase: results revealed a rapid and total elimination of Cd after 24 days whereas depuration of MeHg was progressive. The combined effects of temperature and water column pH were very weak on these phenomena, per opposition to their combined effects during the contamination phase.

Keywords: *Hexagenia rigida*; Depuration; Sediment source; Water source; Cadmium; Methylmercury

1. Introduction

Bioaccumulation of metals in aquatic organisms results from two concomitant processes: uptake, via transfers from the surrounding medium and/or the food ingested, and elimination or excretion. Under natural conditions, these mechanisms are extremely difficult, if not impossible, to study separately. At the laboratory scale, experimental approaches enable us to analyse separately bioaccumulation

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mechanisms from the direct or trophic route, and decontamination processes, with the organisms placed in conditions where, after an initial contamination phase, the external sources of metal are withdrawn.

We have selected the nymphs of the burrowing mayfly *Hexagenia rigida* (Mc Dunnough – Ephemeroptera) in order to study the bioaccumulation mechanisms of mercury compounds and cadmium from the water column or sediment as initial contamination sources. Our experimental approach, based on indoor microcosms, also considered the quantification of the actions and interactions between several abiotic factors (temperature, pH, photoperiod, etc.) in relation to the chemical fate of the metals in the biotopes, their bioavailability and also their bioaccumulation capacities (Odin et al., 1994, 1995a,b,c).

In this paper, we present an experimental study of decontamination processes after nymph exposure to methylmercury and cadmium from the sediment or water column source. The combined effects of temperature and pH were also investigated within a complete factorial design.

2. Materials and methods

The first step of the experiment was based on the contamination phase: *Hexagenia rigida* nymphs were exposed for a period of 10 days via the sediment or water column compartment of indoor microcosms, artificially enriched with methylmercury (MeHg) and cadmium ($CdCl_2$). The nymphs were then collected and placed in new, clean experimental systems, in order to study the decontamination dynamics over a 24-day period. For this decontamination phase a complete experimental design was drawn up, based on the two initial exposure conditions (water or sediment compartment) and including four abiotic conditions, resulting from the combination of two temperature levels (15 and 25°C) and pH levels (5.0 and 7.5). Five durations were selected (0, 1.2, 4, 10 and 24 days) in order to investigate the decontamination trends. Sixty-four experimental units were set up simultaneously (two replicates per condition).

2.1. Contamination phase

From eggs collected each summer from the banks of Lake Winnipeg (Canada), mass culture of *Hexagenia rigida* nymphs was initiated in the laboratory as described in a previously published method (Friesen, 1982; Saouter, 1990; Odin et al., 1994). Four-hundred nymphs were taken from the rearing tanks (with natural Garonne Sediment), isolated and divided into two batches, one for each of the two contamination conditions.

The contamination phase was carried out in two tanks ($40 \times 55 \times 25$ cm) lined with plastic bags (Plastiluz, alimentary standard) and containing 15 kg (ww) of natural sediment and 25 l of dechlorinated tap water (general chemistry of the tap water: pH 7.5; resistivity 2.470 ohm cm^{-1}; HCO_3 231.8 mg l^{-1}; Cl 16 mg l^{-1}; SO_4 37.5 mg l^{-1}; Ca 53.5 mg l^{-1}; Mg 12.2 mg l^{-1}; $NH_4 < 0.01$ mg l^{-1}; NO_2

0.09 mg l^{-1}; NO_3 1.8 mg l^{-1}; $PO_4 < 0.05$ mg l^{-1}). The sediment was collected from the banks of the Garonne river, upstream from Bordeaux (France). It is an homogeneous silt, rich in clays (75–80%) and with 2% of total organic carbon. Weight conversion factors are: wet weight (ww)/dry weight (dw) = 2.1 ± 0.2 (dw after 48 h desiccation at 60°C). Background metal levels were 97 ± 5 ng g^{-1} (ww) for total mercury and 420 ± 32 ng g^{-1} (ww) for cadmium.

The sediment in one tank was simultaneously enriched with cadmium and methylmercury ([Cd] = 3.20 ± 0.21 mg kg^{-1} (ww) and [CH_3HgCl] = 0.74 ± 0.05 mg kg^{-1} (ww)) using concentrated aqueous solutions ($CdCl_2$: 1000 mg l^{-1} – Merck; CH_3HgCl: 500 mg l^{-1} – Merck). Mechanical mixing ensured an even distribution of the metals throughout the sediment. The second tank was devoted to the water column source. The contamination procedure consisted of daily additions of Cd and MeHg from aqueous solutions ($CdCl_2$, 3 mg l^{-1}; CH_3HgCl, 1 mg l^{-1}). The initial volumes added to the water column were 26.8 ml of Cd and 18.5 ml of Hg, in order to obtain nominal concentrations of 3.22 and 0.74 μg l^{-1}, respectively. The daily additions of Cd and MeHg were after adapted according to dosages carried out periodically on samples collected from the water column, in order to maintain a constant contamination pressure.

The decision to add Cd and MeHg to the sediment or the water compartment simultaneously was justified by the absence of any significant interaction between these two metals in our experimental conditions, with respect to their bioaccumulation in the nymphs (Odin et al., 1995c).

During the 10-day exposure, no external food supply was added: the nymphs fed on the organic matter in the sediment. Diffusers placed in the upper layers of the water column and connected to air pumps (Rena 301) produced aeration in the two tanks twice a day, for a period of 3 h. The temperature was held at 20 ± 0.2°C. The photoperiod was 12:12 h light:dark; light was artificially produced by two neon tubes (Sylvania F36W/GRO) and operated by timer switches. The pH of the overlying water was checked periodically (pHmeter, Labo Moderne) and presented small variations during the contamination phase: 7.5 ± 0.3.

2.2. Decontamination phase

At the end of the 10-day exposure period, the nymphs were sieved from the sediment compartments and weighed individually (ww). For each contamination source, seven organisms were collected to determine the Hg and Cd bioaccumulated in the whole organisms. The other nymphs were introduced into new experimental units (EU: glass tank, 12 × 12 × 30 cm), with four nymphs per EU. In order to obtain a similar biomass in each unit, the nymphs from each contamination tank were grouped into four weight classes: 25–35, 35–45, 45–55, 55–65 mg (ww). One nymph from each class was then introduced into each EU (random assignment). The average biomass per EU was 41 ± 2 mg (ww).

Each EU contained 5 cm depth of natural sediment from the Garonne sampling site and 2.9 l of dechlorinated tap water. The EUs were set up 5 days before the

introduction of the nymphs, this delay being necessary to allow the physico-chemical conditions to stabilize (Odin et al., 1994, 1995b).

At the beginning of the decontamination phase, temperature and pH were identical to the contamination conditions (20°C; pH 7.5). These two factors were progressively modified during the first 4 days, until the selected levels were reached: 15 and 25°C; pH 5.0 and 7.5. Temperature control involved thermoregulation equipment with heating and cooling parts. The pH of the water column in each EU was regulated by an automated equipment, based on a central control point (AOIP-SAM 60, Paris) connected to electrodes placed in each acidified unit and to systems to inject dilute acid solutions (H_2SO_4, 0.1‰), via electrically operated shutters. This system proved very satisfactory for regulating pH in the EUs (±0.2 pH unit). The photoperiod was once again fixed at 12/24 h. No aeration was provided in the EUs. Oxygen saturation in the water column varied within a range of 70 to 95%, corresponding to minimal concentrations greater than 6 mg O_2 l^{-1}, which were within the nymphs' tolerance zone (Friesen, 1982). Again, the nymphs were not fed during the decontamination period.

At each sampling point, the nymphs were collected in the corresponding EUs, weighed (ww), pooled in glass tubes (four nymphs from each EU) and frozen, before Hg and Cd determinations. As it was not possible to identify individuals in the EUs at the beginning of the decontamination phase, quantification of weight increase was determined from the difference in total biomass per EU between the beginning and the end of each decontamination period.

2.3. *Mercury and cadmium determination*

The nymphs were first digested by a nitric acid attack (pure HNO_3, 3 ml) in a pressurized medium (borosilicate glass tubes), at 95°C for 3 h. Digestates were then diluted up to 20 ml using ultra pure water (MilliQ plus). After mixing, subsamples were used for Cd and Hg determinations.

Table 1
Results from total Hg and Cd determinations on several reference materials

	n determinations	Total Hg (ng Hg g^{-1})	Cd (mg Cd kg^{-1})	Reference values (ng Hg g^{-1} and mg Cd kg^{-1})
Fish muscle	4	277.4 ± 10		268 ± 12
Rooted macrophytes	6	20.2 ± 0.7		20.5 ± 0.9
Sediment	4	316.7 ± 13		305 ± 12
Mussel tissue (CRM 278)	4	175.4 ± 12		188 ± 7
Sediment (MESS I)	4		0.63 ± 0.04	0.59 ± 0.10
Sediment (BCSS I)	4		0.22 ± 0.05	0.25 ± 0.04
Tort-2	4		26.6 ± 0.6	26.7 ± 0.6

Fish, plants and sediment from KFA, Jülich, Germany.
Mussels, sediment MESS and BCSS from BCR, Brussels, Belgium.
Lobster hepatopancreas (Tort-2) from NRC, Canada.

Cadmium was determined with a Varian AA 20 spectrophotometer equipped with a GTA 96 graphite tube atomizer and autosampler. Samples of 10 µl were taken for this determination and mixed before atomization with 4 µl of a mixture (50% Pd (0.2 g l^{-1})+50% $Mg(NO_3)_2$ (500 mg l^{-1})), to facilitate removal of the matrix. The detection limit was 0.2 µg Cd l^{-1}.

Total mercury determination was carried out by flameless atomic absorption spectrometry (Varian AA 475). A bromine salt treatment was applied to water samples (50 ml) and to the diluted digestates (50 ml) before the addition of stannous chloride (Farey et al., 1978). The detection limit was 0.1 µg Hg l^{-1}.

The accuracy of the two analytical procedures was monitored by periodical analyses of standard reference materials from BCR (Brussels, Belgium), KFA (Jülich, Germany) or IEAE (Monaco), together with the biological samples series. Values for Cd and Hg were consistently within the certified ranges for each element (Table 1).

Background metal concentrations in the nymphs from samples collected in the culture tanks were 160 ± 28 ng Cd g^{-1} and 118 ± 10 ng Hg g^{-1} (ww). These values were not subtracted for the final determination of the bioaccumulation criterion.

2.4. Data treatment

Data obtained during the decontamination phase were analysed by simple and multiple linear regression techniques which enabled us to quantify the actions and interactions between the different factors being taken into account (Tomassone et al., 1993). Complete factorial design and orthogonal polynomials simplified the interpretation of the effects of each regressor, as the regression coefficients were independent. Regressor coding was based on Snedecor and Cochran tables (Snedecor and Cochran, 1971): contamination source, sediment $= -1$ and water $= +1$; temperature, 15°C $= -1$ and 25°C $= +1$; water column pH, pH 5.0 $= -1$ and pH 7.5 $= +1$; decontamination time, $0_{days} = -2$, $1.2_{days} = -1$, $4_{days} = 0$, $10_{days} = +1$ and $24_{days} = +2$; square terms for five modalities, +2, −1, +2, −1, +2. An 'R^2' value is assigned to each regression model indicating the percentage of explained variance. Explained variables were tested with different types of transformation ($\sqrt{Y}$, $1/Y$, $1/\sqrt{Y}$ and in particular $\log_{10} Y$). We adopted an alpha risk equal to 0.01 for the statistical significance of the effects observed.

3. Results and discussion

The mortality rate increased with the decontamination duration (0%, 3.1%, 6.3% and 14% after 1.2, 4, 10 and 24 days, respectively) but was not significantly affected by the experimental conditions. These percentages were similar to those observed in earlier experiments (Odin et al., 1994, 1995a,b,c).

Ponderal growth of the nymphs was small but significant at the threshold 0.01: the average weight ratio between the end of the decontamination phase and Day 0 was about 1.2. Data were characterized by a wide scattering, despite precautions

taken when preparing the batches of nymphs and allocating them to the EUs. This is traditionally observed in the larval stages (Odin et al., 1994) and contributes to mask the effects of the factors being considered: thus, the nymph growth was not significantly influenced by temperature and pH (data not shown).

3.1. Cadmium and mercury bioaccumulation in the nymphs at the end of the contamination phase

After 10 days of exposure, average Cd concentrations in the nymphs were 615 ± 64 ng g^{-1} (ww) for the sediment source and 455 ± 72 ng g^{-1} (ww) for the water source. Total Hg concentrations were 3477 ± 238 ng g^{-1} (ww) and 241 ± 30 ng g^{-1} (ww), respectively. These average values were in agreement with data previously obtained in similar experimental conditions (Odin et al., 1995b,c). They indicate the low Cd bioaccumulation capacity by this species: the average ratio between the two metal concentrations in the nymphs is close to 20, if the data were extrapolated to identical levels of contamination from the sediment source. As regards the water source, MeHg bioaccumulation is very low: it is nevertheless 2.3 times greater than levels for cadmium, if the contamination pressures are brought to the same level. Extrapolating the results in this way presupposes a strict proportionality between concentrations of the metals in the medium and the bioaccumulation capacities in the nymphs. In fact, results obtained from a wide range of concentrations of methylmercury in the water column or in the sediments showed that the relation between these two variables was very close to linear (Saouter et al., 1991a, 1992; Odin et al., 1995b).

These metal concentrations in the nymphs correspond to the untreated dosage values: as well as the fraction of metal really accumulated, they include both the background levels of Cd and Hg in the organisms and the quantities of metals contained in the sediment within the nymph gut. Although the background level is negligible in comparison with the quantities of mercury bioaccumulated after exposure via the sediment source ($< 3\%$), it does represent about 50% of the mean concentrations measured after exposure via the water source. For the cadmium, these values are 26 and 35%, respectively. Determination of metal bioaccumulated in benthic species can often be overestimated because of the presence of contaminated sediment in their digestive tract. In our experimental conditions, this affected particularly the nymphs contaminated by the sediment source. Based on data published by Hare et al. (1989) and on our own measurements, we were able to estimate the quantity of sediment contained in the gut and also, by assuming that the metal concentrations were identical to those in the surrounding sediment compartment, the corresponding Cd and Hg burdens. The dry weight of the sediment represented on average 12% of the nymph biomass, expressed in dry weight (dw/ww = 0.23). Thus the estimated weight of the sediment is 1.21 mg (dw) or 2.5 mg (ww) per individual. The Cd and Hg concentrations in the sediment were 3.20 and 0.74 mg kg^{-1} (ww), respectively; the metal contents in the gut are 8.0 ng Cd and 1.8 ng Hg. Average burdens in the whole organisms were 27 and 153 ng for Cd and Hg, respectively. Thus, the amounts of Cd and Hg in the gut represent 30% and

1.2% of the metals accumulated in the nymphs at the end of the 10-day exposure via the sediment source.

After exposure via the water source, metal transfers into the sediment compartment are basically limited to the upper layers of sediment (0–0.5 cm). They derive mainly from deposition of particles in suspension in the water column, due to the bioturbation activity of the nymphs; the binding capacity of these particles, especially for mercury, is very efficient (Odin et al., 1995a). The nymphs live buried at the bottom of their burrows and ingest sediment from the underlying layers, thus reducing the possibilities of contamination by indirect transfers between water column and sediment.

3.2. Dynamics of mercury and cadmium concentrations in the nymphs during the decontamination phase

We have selected the concentration criterion to present the bioaccumulation results obtained after exposure to mercury and cadmium, as growth dilution phenomena were negligible.

3.2.1. Cadmium

Fig. 1 shows the evolution of the Cd concentrations in the nymphs during the decontamination phase, as a function of the different factors taken into account. For the two contamination conditions (sediment source, Fig. 1(A), or water column, Fig. 1(B)), the general tendency is similar: the regression model showed a marked decrease in concentrations during the first 4 days, followed by an asymptotic pattern until the end of the experiment, which was close to the background level in the nymphs.

When the burrowing organisms were previously exposed to the sediment source, Cd in the sediment inside the gut accounted for about 50% of the decrease in metal concentration observed between time zero and +1.2 days. Hence, it is clear that along with this depuration effect which occurred in the first hours after the nymphs were placed into the new EUs, a large proportion of bioaccumulated Cd was eliminated very rapidly. The estimated half-life in these experimental conditions is close to 12 h. The regression model indicates a small but significant effect of temperature on the decontamination processes, the Cd decrease being slightly greater at 25°C. pH, on the other hand, had no significant effect on the metal efflux.

The results obtained after contamination via the water column source reveal similar phenomena: the estimated half-life is very short, about 12–14 h; the effect of temperature is small, but a significant increase of the decontamination rate is observed at 25°C; no significant differences were observed between the two pH conditions.

The fact that the abiotic factors had very little effect, and in the case of pH virtually no significant effect at all, on decontamination may be attributed to the very rapid nature of these phenomena, which achieve the elimination of virtually all the Cd bioaccumulated in the nymphs before the modalities for temperature

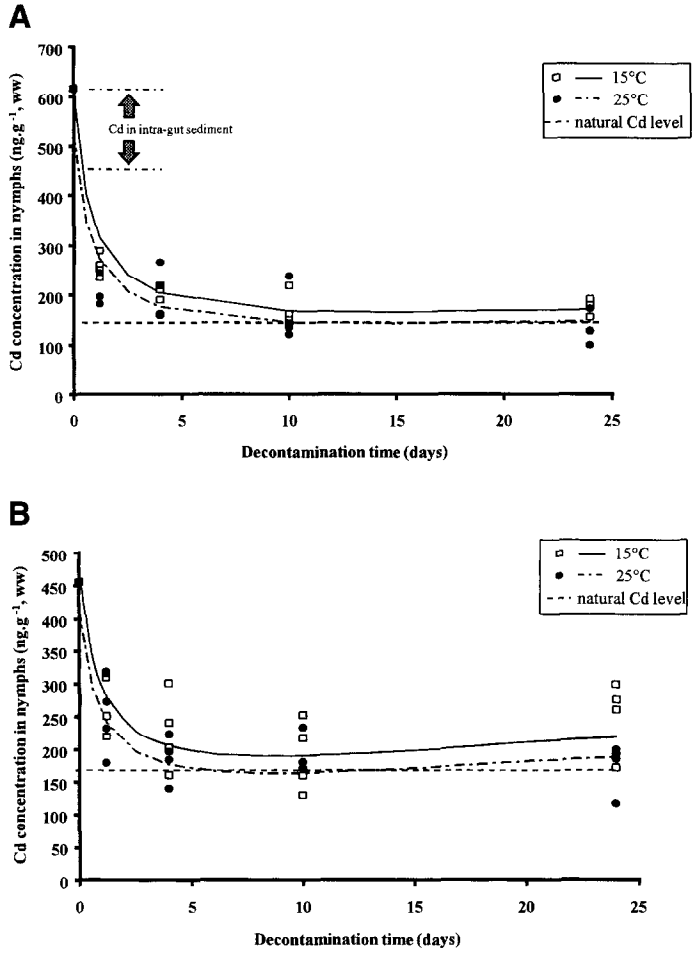

Fig. 1. Evolution of Cd concentrations in *Hexagenia rigida* nymphs (whole organism) after contamination by Cd via the sediment source (A) and the water column source (B), as a function of time and temperature. Regression model: $\alpha = 0.01$, contribution = 80.6%. $\text{Log}_{10}[\text{Cd}]_{\text{nymphs}} = 2.4 - (0.1 \times D_{\text{time}}) + (0.05 \times D_{\text{time}}^2) + (0.03 \times S_{\text{contamination}} \times D_{\text{time}}) - (0.03 \times \text{temperature})$. ($D_{\text{time}}$ = decontamination time.)

and pH have been reached (delay of 4 days). During the contamination phases, important combined effects of temperature and pH were observed on Cd bioaccumulation in the nymphs, especially when the microcosms were contaminated via the water column source (Odin et al., 1995c).

Very few data are available in the literature on the depuration processes in aquatic insects after exposure to cadmium. In other species, in particular in mussels and fish, it has been established that depuration of this metal is very slow (Giesy et al., 1980; Cossa, 1989; Norey et al., 1990). This lack of elimination is generally attributed to the intra-cellular sequestration mechanisms of Cd, espe-

cially as a result of induction of the biosynthesis of the Cd-binding proteins or metallothioneins (Giesy et al., 1980). Several authors have observed variable elimination rates according to exposure levels. Nevertheless, an experimental study of Cd excretion by the predatory beetle (*Pterostichus niger*) fed with contaminated *Tenebrio molitor* showed that metal loss was rapid during the first 10 days and thereafter occurred at a slow rate until the 30th day (Linqvist et al., 1996); these authors put forward the hypothesis that the initial rapid decrease was partly due to faecal excretion of Cd and discharge from the gut epithelium, which represents the biological barrier involved in the ad- and absorption of the metal via the contaminated ingested prey.

An experimental study of the exchange rates (influx and efflux) of trace metals (Cd, Pb and Zn) between *H. rigida* nymphs and their environment showed that biological half-lives were about 1 week for Cd and between 2 weeks and 1 month for Zn and Pb (Hare et al., 1991). Cd bioaccumulation in the nymphs was mainly based on adsorption and possibly also uptake at the gut wall level. Despite the large volumes of water passing over the gills, Cd absorption through this barrier appeared to be negligible, even when the nymphs were exposed via the water column source (Hare, 1992). The cutaneous coating may also represent a compartment for metal fixation: an autoradiography study on a predatory beetle (*Pterostichus niger*), contaminated via the trophic route, had revealed significant amounts of Cd localized in the integument (Linqvist et al., 1996). The decontamination phenomena observed during this study can be compared with the preferential accumulation of cadmium in the biological barriers which separate the nymphs from the surrounding environment, with the gut wall playing a preponderant role. In this case, the Cd losses would be linked to the lability of the bonds established with the surface ligands, such as the cell coat of the midgut enterocytes (Saouter et al., 1991b), and/or to the rapid turnover of these epithelial structures. Instars may also play an important role in the context of decontamination phenomena. No precise estimate of the number and frequency of instars has been produced for *H. rigida*. For other species of Ephemeridae, estimates range from 30 to 45 throughout the entire larval lifespan (Fink, 1980; Friesen, 1982; Tessier et al., 1994).

Current knowledge of temperature effects on Cd decontamination in freshwater species is extremely limited and often contradictory. Cadmium depuration rates estimated for the short-lived gastropod *Viviparus georgianus* and the pelecypod *Elliptio complanata* were not correlated with an increase of temperature (Tessier et al., 1994). After 60 days' exposure from contaminated sediments at three temperatures (5, 10 and 20°C), elimination of Cd by the freshwater isopod *Asellus aquaticus* appeared significant only at the lowest temperature (Van Hattum et al., 1993).

In our experimental conditions, results did not reveal any significant effect of pH on Cd decontamination processes. pH did, however, have a significant action on Cd bioaccumulation after the nymphs were exposed via the water column source: Cd concentrations in the nymphs at pH 7.5 were about three times greater than those measured at pH 5.0, after 15 days' exposure (Odin et al., 1995c).

3.2.2. Mercury

Evolutions of Hg concentrations in *H. rigida* nymphs over the 24 days of the decontamination phase are shown in Fig. 2.

The decrease in concentrations after contamination via the sediment source reveals a slow rate of decontamination (Fig. 2(A)), with values in the nymphs measured at the end of the experiment representing about 50% of the average initial concentration. It is not possible to estimate the biological half-life of mercury in these conditions. Similar results were obtained with the rainbow trout (*Oncorhynchus mykiss*) after direct contamination by MeHg and inorganic Hg, when the decontamination phenomena were monitored for 256 days (Ribeyre and Boudou, 1984).

When the nymphs were contaminated via the water source (Fig. 2(B)), the small quantities of mercury bioaccumulated after 10 days' exposure were eliminated more rapidly, with the half-life estimated at about 6–7 days.

In both cases temperature and pH did not have any significant effect on the decontamination phenomena. Although the kinetics of the decrease in mercury concentrations in the nymphs were gradual and therefore more likely to reveal the effects of the two abiotic factors than was the case for Cd, they were not significant beyond the 4 days necessary to obtain the final levels selected for the temperature and pH. When these factors were considered during the contamination phases, they had a major effect on mercury bioaccumulation. They produced nevertheless a very complex set of results, associated with the actions and interactions between pH and temperature, which varied greatly depending on the initial contamination source and the chemical form of mercury. For example, an increase in temperature from 10 to 26°C gave rise to an increase in Hg bioaccumulation when the nymphs were contaminated via the sediment source (Odin et al., 1994); after exposure via the water column, an increase in Hg quantities in the nymphs was observed between 10 and 18°C, followed by a decrease between 18 and 26°C (Odin et al., 1995a). Note also that effects of acidification of the water column were dependent on the chemical form of the metal introduced in the aqueous phase: for inorganic Hg, an increase in Hg bioaccumulation by *Hexagenia rigida* was observed at pH 5.0, whereas contamination by methylmercury led to the opposite effect (Odin et al., 1995a).

Earlier studies have shown that after contamination of the nymphs via the sediment source enriched with methylmercury, burdens measured in the gills were very low and a large proportion of the metal had crossed the intestinal barrier then accumulated in the internal tissue compartments (Saouter et al., 1992; Odin et al., 1994). Studies on fish have revealed high levels of mercury transfers between the organs during the decontamination phases: for example, the skeletal muscle acts as a receiver organ in respect of quantities of metal liberated by donor organs, such as the gills, kidneys or liver (Ribeyre and Boudou, 1984). If such mechanisms occur within *H. rigida* nymphs, a preferential storage of MeHg in the muscle tissue may contribute to limiting efflux towards the external medium, since metal stored within the organisms could be considered as being less easy to eliminate than that fixed on the interface structures.

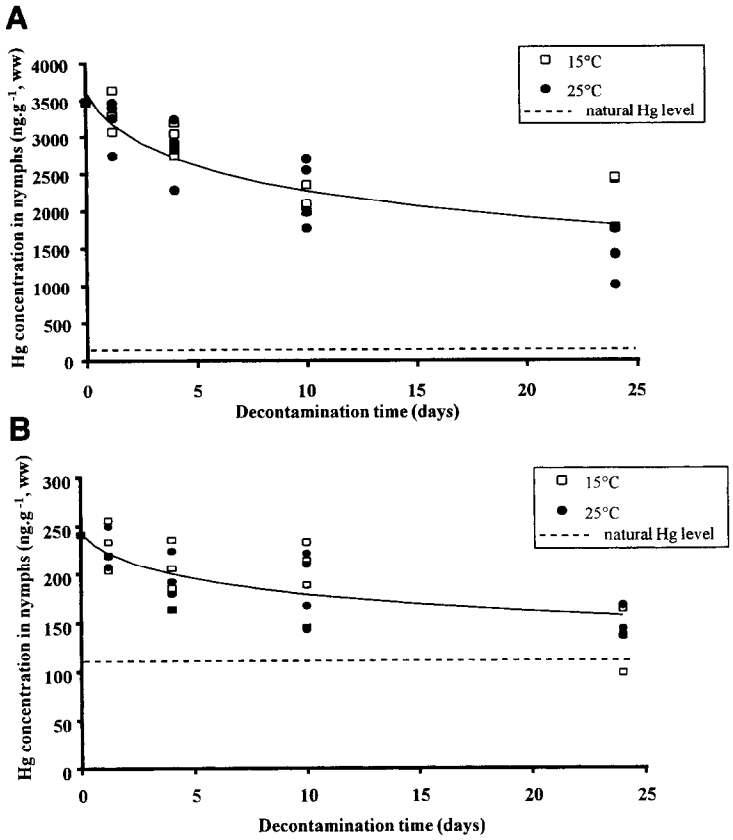

Fig. 2. Evolution of Hg concentrations in *Hexagenia rigida* nymphs (whole organism) after contamination by MeHg via the sediment source (A) and the water source (B), as a function of time and temperature. (A) regression model: $\alpha = 0.01$, contribution = 80%. [Hg]nymphs = $2720-(451 \times D_{time})$. (B) regression model: $\alpha = 0.01$, contribution = 56.2%. [Hg]nymphs = $200-(21 \times D_{time})$.

When the nymphs were contaminated via the water column, on the other hand, the relative Hg burdens in the gills were much greater and the metal adsorbed on the cuticular coating can represent a large proportion of the burdens measured in the rest of the body (62% of the total burden). Moreover, the amounts of metal bioaccumulated are very small. Decontamination would appear to be linked mainly to losses of mercury stored in the biological barriers through direct transfers into the surrounding environment and/or destruction of the epithelial structures during instars.

Studies are currently being undertaken to analyse in more detail metal distribution in the nymphs after exposure via the direct and trophic routes, using autoradiography (^{203}Hg and ^{109}Cd) after cryopreservation of the samples, which will tend to reduce losses of metal adsorbed on the interfaces during sample preparation.

4. Conclusion

Under our ecotoxicological conditions, kinetics of cadmium and methylmercury decontamination from the burrowing mayfly nymphs of *Hexagenia rigida* show marked differences according to the metal studied and also to the exposure source during the contamination phase: Cd was rapidly and totally eliminated, the estimated half-lives being close to 12 h, whereas Hg decontamination was progressive, producing some very slow decreases in concentrations in the nymphs after 24 days of decontamination, when the organisms had previously been exposed via the sediment source.

In contrast to phenomena observed during the contamination phases, in this case the combined effects of temperature and pH on Cd and Hg effluxes were very weak, even insignificant, despite the wide differences in the modalities of these two factors selected for this experiment.

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