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Temperature and pH Effects on Cadmium and Methylmercury Bioaccumulation by Nymphs of the Burrowing Mayfly *Hexagenia rigida*, from Water Column or Sediment Source

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Abstract. An experimental approach, based on a complete experimental design, was set up in order to carry out a comparative analysis of cadmium (Cd) and methylmercury (MeHg) bioaccumulation in the burrowing mayfly nymphs of *Hexagenia rigida* (Ephemeridae) after 15 days' exposure to water column or sediment compartment as initial contamination sources. Combinations of two modalities of temperature—15 and 25°C— and pH—5.0 and 7.5—enabled us to quantify the actions of these two abiotic factors and also their interactions on the metal bioaccumulation. Whatever the initial contamination source, a high level of metal bioaccumulation was observed after exposure to MeHg; Cd transfers, on the other hand, were very low. For similar theoretical exposure conditions, differences between the bioaccumulation capacities of the two metals were between 20 and 30, in favor of MeHg. Multiple regression did not reveal significant interactions between MeHg and Cd towards their bioaccumulation in the nymphs. When the microcosms were contaminated via the sediment source, increasing the temperature from 15 to 25°C led to an increase in MeHg bioaccumulation and a decrease of Cd bioaccumulation. After exposure from the water source, no significant amounts of Cd were measured in the nymphs at 25°C even though significant metal concentrations were observed at 15°C. pH had no significant effect on the bioaccumulation processes when the two metals were initially added to the sediment compartment; acidification of the overlying water, however, gave rise to a decrease in MeHg and Cd concentrations in the nymphs, with marked interactions with temperature. The results are discussed from the data available on metal partitioning in the biotopes and their bioavailability, uptake routes and also the structural and functional properties of the biological barriers involved (gills, gut wall).

Benthic species, and especially burrowing organisms, play an important role in the biogeochemical cycle of heavy metals within freshwater ecosystems. Detritivorous, they ingest large quantities of sediment and can thus, given the often very high concentrations of metals in the superficial layers of these compartments, bioaccumulate large amounts of metals. These organisms also set up more or less constant exhanges with the water column, via the currents in the burrows, for respiratory and sometimes trophic purposes: these exchanges may be the source of metal transfers at the cutaneous barrier and especially the gill barrier level. Burrowing activity may also lead to changes in the physicochemical characteristics of the sediments, as it may amplify the metal released into the overlying water column, combined with diffusion processes from the pore water. Finally, burrowing species form an integral part of many benthic and pelagic food webs; they can thus represent a major contamination source for their various predators (fish, batracians, etc.) (Hare 1992).

Nymphs of the burrowing mayfly *Hexagenia rigida* (Mc Dunnough) were selected as biological models to investigate, at the laboratory scale, bioaccumulation and transfer of heavy metals from the water column and sediment compartments. This ephemeridae presents many advantages: mass culture can be instigated in the laboratory from large quantities of eggs collected in the field during summer emergence periods; long larval life of between six months and three years in the natural environment, depending on ecological conditions; large size, between 2.5- to 3-cm length when transformed into imago; high tolerance towards the variations in the physicochemical factors, such as temperature or pH (Friesen 1982; Saouter *et al.* 1991b).

The present study was based on a comparative analysis of cadmium (Cd) and methylmercury (MeHg) bioaccumulation in the nymphs of *H. rigida*, under the combined effects of temperature and pH. The experimental approach was based on indoor microcosms, made up of three compartments: water column, natural sediment and *H. rigida* nymphs. The two contamination sources—sediment or water column—were studied separately. Cd and MeHg were added simultaneously to these two compartments, in differing concentrations, in order to ana-

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Table 1. Nominal concentrations of methylmercury and cadmium added simultaneously to the water column and sediment as initial contamination sources

	Water column (μg/L)			Sediment (mg/kg)	
	$C1_{MeHg} = 0.2$	$C2_{MeHg} = 1$		$C1_{MeHg} = 0.2$	$C2_{MeHg} = 1$
$C1_{Cd} = 2$	$C1_{MeHg} + C1_{Cd}$	$C2_{MeHg} + C1_{Cd}$	$C1_{Cd} = 2$	$C1_{MeHg} + C1_{Cd}$	$C2_{MeHg} + C1_{Cd}$
$C2_{Cd} = 10$	$C1_{MeHg} + C2_{Cd}$	$C2_{MeHg} + C2_{Cd}$	$C2_{Cd} = 10$	$C1_{MeHg} + C2_{Cd}$	$C2_{MeHg} + C2_{Cd}$

lyse the interactions between the two metals towards their bioaccumulation by the nymphs.

Materials and Methods

Experimental Design

Two initial concentrations—C1 and C2—were retained for each metal and each contamination source, in accordance with previous studies (Odin *et al.* 1995a,b). Cd and MeHg were added simultaneously; the C1 and C2 levels were combined in order to analyse the potential interactions between the two metals, giving four exposure conditions for each contamination source (Table 1). Two levels were selected for the temperature, 15 and 25°C, and pH, 5.0 and 7.5. The factorial design was based on 32 ecotoxicological conditions, with two replicates per condition and 8 control microcosms (72 experimental units in all). The duration of the experiment was 15 days.

Preparation of Experimental Units

Each experimental unit (EU) consisted of a glass tank (12 × 12 × 30 cm), lined with a plastic bag (Plastiluz, alimentary standard) and containing natural sediment (5 cm depth–0.95 kg, ww), 2.9 L of dechlorinated tap water (general chemistry: pH = 7.5; resistivity: 2470 ohm/cm; HCO_3 = 232 mg/L; Cl = 16 mg/L; SO_4 = 37.5 mg/L; Ca = 53.5 mg/L; Mg = 12.2 mg/L; $NH_4 < 0.01$ mg/L; $NO_2 = 0.09$ mg/L; $NO_3 = 1.8$ mg/L; $PO_4 < 0.05$ mg/L) and 4 *H. rigida* nymphs.

The sediment was collected from the banks of the Garonne river, upstream from Bordeaux (France). It was a very homogeneous silt, rich in clays (75–80%), with a low total organic carbon content (2% on average). Weight conversion factors were wet weight (ww)/dry weight (dw) = 2.1 ± 0.2 (dw after 48 h desiccation at 60°C) and ww/volume = 1.96. Total Hg and Cd concentrations were 97 ± 5 and 600 ± 42 μg/g (ww), respectively. Sediment was first homogenized by mechanical mixing and was then separated in two batches: the first was used directly in the EUs contaminated by the aqueous phase (water contamination source) and also in the control units. The second was contaminated by the two metals (sediment source). Contamination was based on MeHg and Cd additions from concentrated aqueous solutions (CH_3HgCl, Merck: 500 mg/L—$CdCl_2$, Merck: 1000 mg/L). Before addition in the EUs, 5 samples (1 g, ww) were taken from each batch in order to confirm the nominal concentrations and to check the homogeneity of metal distribution. During the two weeks exposure via the sediment source, water samples were taken regularly in order to measure metal transfers and to assess the importance of this secondary contamination source.

Contamination modalities of the water column source were defined according to the kinetics of decrease in metal concentrations in this compartment (metal adsorption on the tank walls, transfers towards sediment and living organisms, volatilization, etc.) and to variations occurring as a result of the physico-chemical characteristics of the medium. The procedure selected was based on daily additions of Cd and MeHg to the EUs. Thus, for each of the 8 EUs corresponding to one contamination condition, the quantities of metal added at the end of the experiment were identical. Contamination of the water column was based on metal additions from aqueous solutions at 7 mg/L for MeHg and 14 mg/L for Cd. The quantities added on the first day were adapted to achieve nominal concentrations for the two metals. Throughout the exposure period, identical quantities of MeHg were daily added; for Cd, the daily additions corresponded to half of the initial volumes. Measurements of Hg and Cd concentrations in the water column were realized on samples collected at time zero and after 4, 8, 12, and 14 days. The measurements were carried out on unfiltered samples and on the dissolved phase (filtration with Nalgene nylon filters—0.45 μm), after 9 and 14 days' exposure. From these data, an index was calculated which gave the corresponding contamination pressure. This index, the "Concentration.Days" Equivalent (CDE), is based on the integration of the different Hg and Cd concentrations measured in the water column (C_i), according to the length of time between the sampling points ($t_j - t_i$):

$$\text{CDE } (\mu g/L.\text{days}) = [(C_1 + C_0)/2](t_1 - t_0) + [(C_2 + C_1)/2]\,(t_2 - t_1) + \cdots [(C_j + C_i)/2](t_j - t_i).$$

This index permitted to calculate corrected bioaccumulation values by dividing, for example, the average Hg and Cd concentrations measured in the nymphs by the corresponding CDEs. In this case, if there is a strict proportionality between metal concentrations in the water column and in the organisms, the corrected values permit to compare the bioaccumulation capacities between different experimental conditions, for similar exposure conditions (Ribeyre 1993).

The *Hexagenia* nymphs were issued from a mass culture initiated in the laboratory from eggs collected each summer from Lake Winnipeg (M.K. Friesen, Freshwater Institute, Winnipeg, Canada). Detailed culture conditions have been described earlier (Saouter *et al.* 1991b). Ten days before the beginning of the experiment, four batches of 80 nymphs were isolated from culture tanks (culture conditions: 20°C, 12 h light per day, pH 7.5 ± 0.2) and were gradually acclimatized to the four abiotic conditions selected for the experiment: 15°C/pH 5.0, 15°C/pH 7.5, 25°C/pH 5.0, 25°C/pH 7.5. After this step, the nymphs were collected, individually weighed and grouped into 4 weight classes (20–25, 25–30, 30–35, and 35–40 mg, ww). In order to obtain a similar initial biomass in each EU, one nymph from each class was introduced into each EU 5 days after the water and sediment had been added (delay sufficient to achieve a stabilisation in the physicochemical characteristics of the water column). The average nymph biomass per EU at the beginning of the experiment was 112 mg (ww), with an inter-EU variation coefficient of less than 3%. No external food supply was added during the experiment. No artificial aeration was provided in the water column: oxygen saturation varied within a range of 70 to 95% (6 mg O_2/L on average) corresponding to the nymph's tolerance zone.

At the end of the experiment, the nymphs were sieved from the sediment compartment and individually weighed (ww). As it is impossible to identify every nymph individually in each EU, quantification of ponderal growth was determined from the difference in total biomass per EU: % growth = $(W_{15} - W_0)/W_0 \times 100$.

The gut contents were not eliminated prior to metal analysis, because the procedure involved (nymphs placed in water or in uncontaminated sediment) is difficult to apply when a large number of abiotic conditions

are studied simultaneously. In fact, the depuration is temperature-dependent: 5–6 h at 25°C and more than 24 h at 15°C (Saouter *et al.* 1991b; Hare *et al.* 1989). These durations could lead to decomtamination processes and thus interfere with the bioaccumulation determinations. After the weighing stage, each nymph was placed in a glass tube and frozen.

The EUs were placed in larger tanks (140 × 65 × 30 cm), which were themselves in enclosed containers, with thermoregulation equipment (heating and cooling systems, ±0.2°C). Artificial light was produced from 2 neon tubes (Sylvania F36W/GRO) in each tank, positioned 45 cm above the surface of the EUs and operated by timer switches. The daily period of light was fixed at 12h/24h. The pH of the overlying water in the EUs was regulated by an automated system (AOIP-SAM 60). pH was measured in each unit on a scale of milliseconds and the difference between the desired pH level and the measured value triggered an injection of a controlled volume of a dilute acid solution (H_2SO_4—0.1‰), by means of an electrically operated shutter, which opened for a predetermined time (0 to 30 s). This system proved very satisfactory for regulating pH in the EUs (±0.2 pH unit).

Owing to metal transfers between the water column and sediment compartments, Cd and Hg accumulated in the superficial sediment layers could lead to a significant contamination of the burrowing organisms. Such transfers result from direct exchanges at the water/sediment interface but also from the sedimentation of metals bound to the suspended particles, a result of the bioturbation activity of the nymphs. At the end of the experiment, before the nymphs were removed from the EUs, sediment samples were collected. Four glass tubes (inner diameter 0.65 cm) were pushed into the sediment; these micro-cores were stored at −20°C. In order to assess the vertical distribution of the two metals in the sediment, the cores were sliced into 3 strata: the uppermost layer, in direct contact with the water (0–0.5 cm), and two underlying strata (0.5–1 cm and 1–5 cm). Each sediment sample was dried (60°C, 24 h) before weight measurement and metal dosage. This drying step was particularly necessary for the surface layers which contained varying amounts of water. The method used to estimate Hg and Cd stratification was based on the establishment for each core of the relationship between the metal cumulated burdens (MBc) and the corresponding cumulated dry weights (Wd) from the uppermost layer to the two underlying strata (Ribeyre 1993). For the majority of cores, the relationship between MBc and Wd (3 measurements/core) could be satisfactorily estimated with a simple linear regression model (MBc = b0 + b1Wd), where b1 corresponds to the natural metal concentration in sediment and b0 to the quantity accumulated at the sediment surface. However, for some experimental conditions, the relation between the three measured values was not linear, indicating metal transfers beyond the first sediment layer.

The evolution of the turbidity, corresponding to an indirect estimation of the burrowing activity of *H. rigida* nymphs, was analyzed in each EU on 25 ml water samples collected in the central part of the water column after 0, 3, 6, 9, and 12 days (turbidimeter ESD 800). Turbidity results were expressed as nephelometric units (NTU), which are very well correlated with the corresponding concentrations of suspended particles (r = 0.997): 10 NTU units correspond to 46.5 mg/L of suspended matter (dw) (Odin *et al.* 1995a). Periodic measurements of dissolved oxygen concentrations were also taken (Oxymeter Labo-Moderne, OXI 91).

Total Hg and Cd Determinations

Biological and sediment samples were first digested by nitric acid attack (pure HNO_3-3 ml) in a pressurized medium (borosilicate glass tubes), at 95°C for 3 h. The digests were diluted up to 20 ml with ultra-pure water (MilliQ plus). After mixing, the samples were left to stand in order to facilitate redeposition of solid materials, especially for sediment samples. Two fractions of the supernatant were used, for Hg and Cd measurements.

Total Hg determination was carried out by Flameless Atomic Absorption Spectrometry (Varian AA 475). A bromine salt treatment was applied to water samples and diluted digestates before addition of stannous chloride. The detection limit was 0.1 μg/L.

Cd determination was carried out with a Varian AA 20 spectrophotometer equipped with a model GTA 96 graphite tube atomizer and autosampler. Samples of 10 μl were taken for the metal determination and mixed before atomization with 4 μl of a mixture "50% Pd + 50% $Mg(NO_3)_2$," to facilitate removal of the matrix. The detection limit was 0.1 μg/L.

The accuracy of the two analytical procedures was monitored by periodic analyses of standard reference materials from BCR (Brussels, Belgium), KFA (Jülich, Germany), or IEAE (Monaco), together with biological samples series. Values for total Hg and Cd were consistently within the certified ranges for each element (data not shown).

Total Hg and Cd concentrations in the nymphs were 131 ± 18 and 386 ± 20 ng/g (ww). These background levels were subtracted for the final determination of the bioaccumulation criterion.

Data Treatment

All data collected were analyzed in an exhaustive graphic representation and multiple linear regression technique in order to quantify the actions and interactions of the different ecotoxicological factors taken into account on the bioaccumulation processes.

Orthogonal polynomials simplified the interpretation of the effects of each regressor because of the independence of the regression coefficients. The regressor coding was based on tables (Snedecor and Cochran 1971): $C1_{MeHg} = -1$ and $C2_{MeHg} = +1$; $C1_{Cd} = -1$, and $C2_{Cd} = +1$; sediment source = −1 and water colum source = +1; 15°C = −1 and 25°C = +1; pH5.0 = −1 and pH7.5 = +1. Depending on the variance/average relationships for each set of data, different types of transformation of the explained variables were used (logY, 1/Y, $\sqrt{Y}$,. . .). The alpha risk adopted was equal to 0.01 for the statistical significance of the effects observed; F values were calculated with reference to the inter-replicate variance.

Results

Growth of the Nymphs

The inventory of the nymphs in the 72 EUs at the end of the experiment showed a mortality rate of 7.3% (21/288 nymphs). One EU was lost at the beginning of the experiment after a malfunction in the pH regulation equipment. In fact, this mortality rate was similar to that observed in the rearing conditions, and was not related in any significant way to the different exposure conditions.

Multiple regression analysis of the average weight data measured at the end of the experiment showed a significant effect of temperature and water column pH. Average nymph biomasses were higher at 25°C; the pH effect was significant only at 15°C. The contamination modalities of the sediment and water column sources by Cd and MeHg had no significant effect on the weight data. The growth percentages calculated from the total biomasses at time zero and those measured at the end of the experiment revealed an overall increase of between 10 and 30%, according to the combined effects of temperature and pH. However, these data are very heterogeneous despite the precautions taken when allocating the nymphs to the EUs. These variations in growth are often found at the larval stages and were

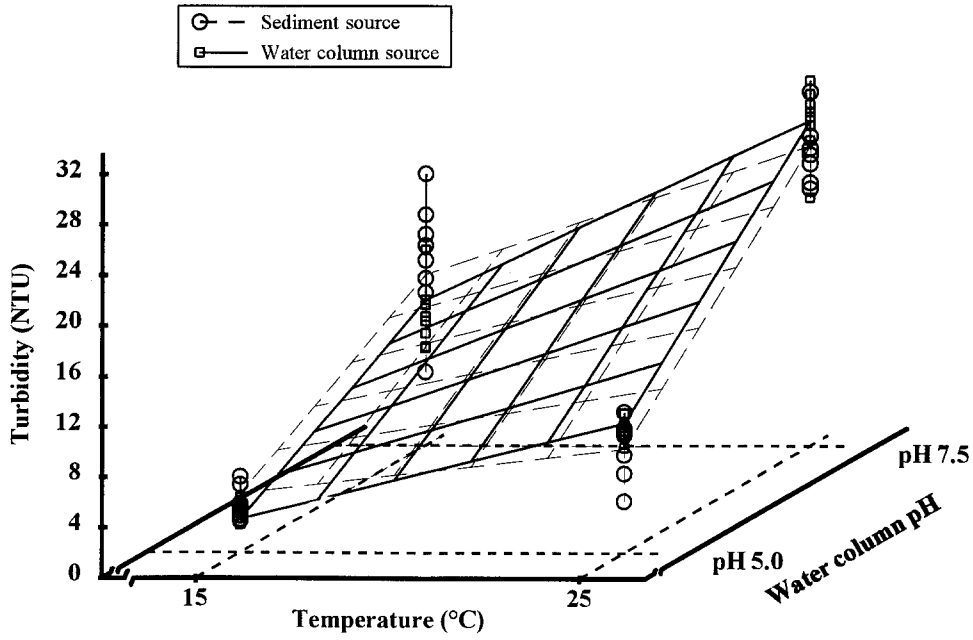

Fig. 1. Turbidity measurements in the water column of the EUs (nephelometric units - NTU), as a function of temperature, water column pH, and initial contamination source. *Regression model:* $\alpha = 0.01$; contribution = 91%. Turbidity = 12.6 + 6.1 (pH) + 4.5 (temperature) + 1.7 (temperature * pH) + 1.0 (contamination source * temperature)

observed in earlier studies carried out on this species(Saouter *et al.* 1991b, 1992; Odin *et al.* 1994).

Water Column Turbidity

The four turbidity measurements taken in the EUs during the 15 days' exposure revealed a progressive increase in the concentrations of the suspended sediment particles during the first 6 days, followed by a plateau tendency (data not shown). The quantification of the effects of the different factors taken into account was realized on the data collected after 9 days, during the plateau phase (Figure 1). pH and temperature exerted a marked effect on the bioturbation activity of the nymphs: these two factors contributed to 55 and 30%, respectively, of the total variance of the regression model. They acted both in isolation and in interaction, maximal differences being observed between the two conditions 15°C/pH 5.0 and 25°C/pH 7.5: concentrations of the particulate phase were 19 and 112 mg/L (dw), respectively. The contamination source had very little effect on turbidity and this effect was in inverse proportion to temperature. Analysis of the data revealed no significant effects of the Cd and MeHg contamination levels on this parameter.

Evolution of Cadmium and Mercury Concentrations in Water Column

Contamination by the Water Source: Results from the Cd measurements on the unfiltered samples collected in the EUs corresponding to the C1 level of contamination are shown in Figure 2A. Cd concentrations in the water column increased progressively, the tendencies being close to linearity. At the end of the experiment there were wide differences between the different conditions, with the combined actions of temperature and pH being quite marked. A factor of about 6 separated the highest and lowest concentrations, corresponding to conditions 15°C/pH 5.0 and 25°C/pH 7.5, even though identical amounts of metal were added to all the EUs. Acidification of the medium led to an increase in Cd concentrations in the water column; metal concentrations were lower at 25°C (factor of about 2.0). For the C2 contamination level of the water source, similar phenomena were observed (data not shown). The variations from the nominal concentrations defined at time zero (C2/C1 = 5) were similar to those observed at the end of the experiment (5.2 at 25°C/pH 7.5; 5.4 at 25°C/pH 5.0; 6.0 at 15°C/pH 7.5; 5.9 at 15°C/pH 5.0). The contamination pressures (CDEs), calculated for the different experimental conditions, are shown on Figure 2B. The 3D plot gives a good visualization of the effects of the factors considered towards the changes in Cd concentrations in the water column. Cd dosages on filtered water samples showed that a very large proportion of the metal is present in the dissolved fraction: at 15°C, close to 100% at pH 5.0 and 90% at pH 7.5; at 25°C, these proportions were 95% at pH 5.0 and 80% at pH 7.5.

As was the case for the Cd, the procedure selected for the contamination of the water source by MeHg led to a progressive increase of Hg concentrations in the unfiltered water samples, with the experimental conditions having a marked effect. For the C1 contamination level, maximal Hg concentrations were observed at 15°C/pH 5.0 (1.4 μgHg/L); on the contrary, concentrations were about 0.3 μgHg/L at 25°C/pH 7.5 (data not shown). These differences in contamination pressures are comparable to those observed for Cd (Figure 3). However, the daily additions of MeHg in the EUs were identical to those at time zero, whereas for the Cd they were only half the initial amount: the decrease in MeHg concentrations was therefore much greater. These observations were in agreement with data obtained during similar experimental conditions: the decrease in MeHg concentrations in the water column after 24 h corresponded to 30–35% of the initial concentrations, whereas it was close to 10% for Cd (Odin *et al.* 1995a). A study of Hg partitioning in the overlying water, from measurements taken from filtered water samples collected at the end of the experi-

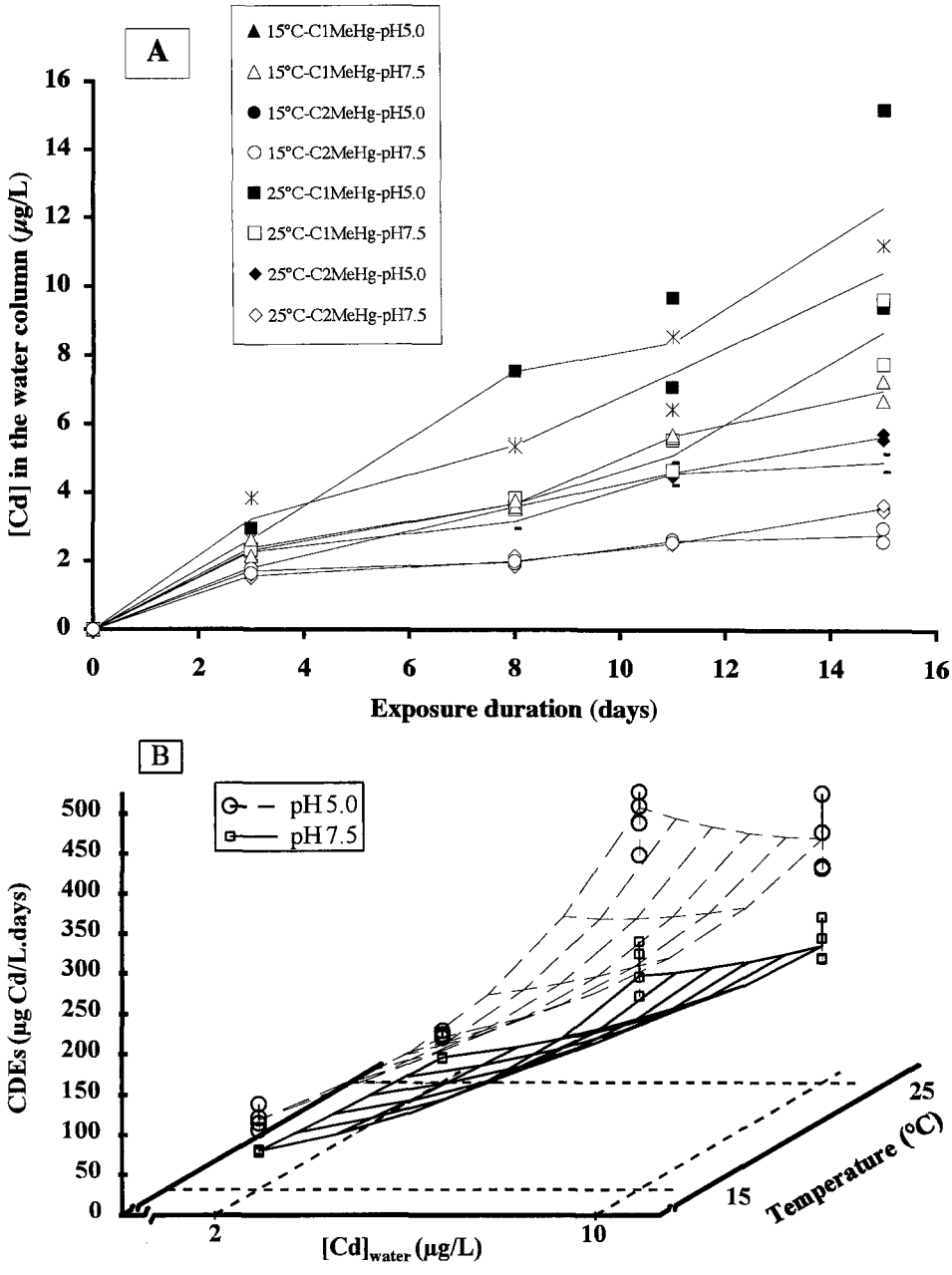

Fig. 2. (A) Evolution of Cd concentrations (µg/L) in the water column throughout the experiment, function of water column pH, temperature, and [MeHg] in the water column. Only data from EUs contaminated with the C1 nominal concentration (2 µg/L) are represented on this graph. (B) Cadmium contamination pressure from the water column at 15 days' exposure expressed by CDEs index (µg Cd/L . days), function of the metal concentration levels in the water column, temperature, and water column pH. *Regression model:* $\alpha = 0.01$; contribution = 99%. $Log_{10}(CDEs_{Cd}) = 2.1 + 0.4([Cd]_{water}) - 0.1\ (pH) - 0.1(temperature)$

ment, showed a great affinity of the metal for the particles in suspension, with the temperature and pH having a marked influence (data not shown). The proportion of Hg in the dissolved phase decreased when temperature increased: for $C1_{MeHg}$, average values at 15°C were close to 20% and between 10 and 15% at 25°C. As for Cd, acidification induced an increase in Hg concentrations in the dissolved fraction in EUs contaminated with the $C2_{MeHg}$ level ($\%Hg_{pH5.0}/\%Hg_{pH7.5} = 1.2$ at 15°C and 1.3 at 25°C). An inverse tendency was observed in units corresponding to the $C1_{MeHg}$ level (factor of 1.8 at 15°C and 1.2 at 25°C in favor of pH 7.5).

This comparative analysis of the exposure conditions from the water column shows that MeHg and Cd when added daily to this compartment have a similar fate globally, in relation to the effects of the different factors taken into account (Figures 2B and 3). Nevertheless, partitioning of the two metals between the particulate and dissolved phases was markedly different: the relative burdens of Hg bound to the suspended matter were higher than those of cadmium. The affinity of Hg for complexation sites at the surface of sedimentary particles is much greater than that of Cd. Cd nominal concentrations in the water were 2 to 50 times higher than those of mercury and the molar weight of Cd is approximately half that of mercury. This partitioning may play an important role in relation to bioavailability; it may also contribute, via the depositing of particles in suspension at the surface of the sediments, to the much greater decrease in Hg concentrations in the water column between daily additions of the metal, together with adsorption on the tank walls, volatilization (production of Hg^0 and Me_2Hg) and bioaccumulation in the nymphs.

Contamination by the Sediment Source: For the EUs contaminated by MeHg, Hg concentrations measured in the water column were always under the detection limit for the method of determi-

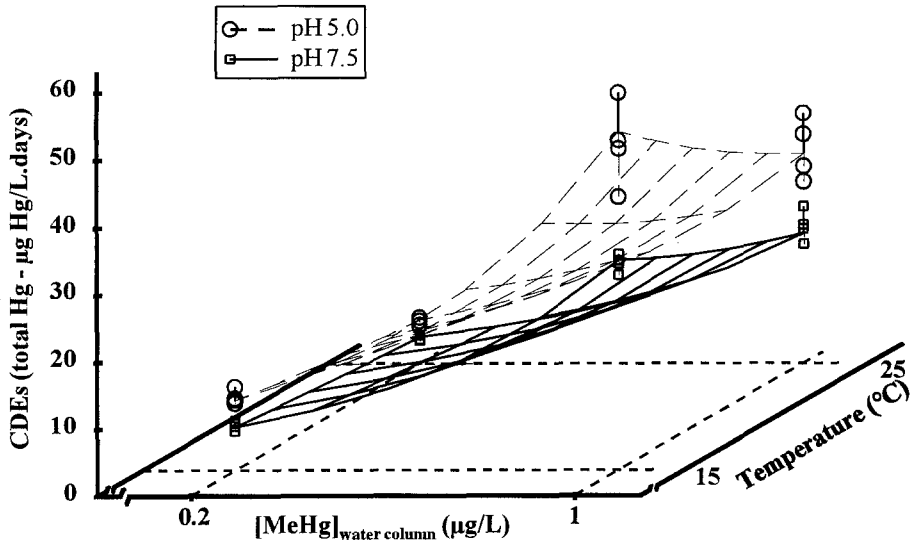

Fig. 3. Mercury contamination pressure from the water column at 15 days' exposure expressed by CDEs index (µg Hg/L.days), function of [MeHg] in the water column, temperature, and water column pH. *Regression model:* $\alpha = 0.01$; contribution = 99%. $Log_{10}(CDEs_{Hg}) = 1.2 + 0.3([MeHg]_{water}) - 0.1(temperature) - 0.1(pH)$

nation used (0.1 µg/L). Earlier studies showed that metaltransfers were very much dependent on the level of contamination in the sediment: for 2 and 4 mgHg/kg in this compartment, Hg concentrations in the water column were close to 0.1 and 0.4 µgHg/L, respectively. Moreover, sediment/water exchanges took place mainly during the first hours after the water was put into the EUs; the introduction of the nymphs did not give rise to any significant modifications in the flux of mercury into the water column, despite their bioturbation activity (Odin *et al.* 1994).

Dosages of cadmium in the water column revealed a gradual increase in concentrations and a marked effect of pH on metal transfers from the sediments. For contamination level C1 (2 mg/kg), mean concentrations measured at the end of the experiment were nearly 0.15 µg/L at pH 7.5, and 0.4 µg/L at pH 5.0 (Figure 4). For level C2 (data not shown), they were 0.9 and 1.5 µg/L, respectively. These results are in agreement with several other field and laboratory studies on the effects of acidification on the releasing of Cd from lacustrine or running water sediments (Curtis and Walker 1994). These concentrations in the water may represent a secondary source of contamination for burrowing organisms, via the water currents inside their burrows, but nevertheless, they represent only very small quantities of metal compared with the burdens introduced initially into the sediments, being less than 0.06%. The temperature factor had no significant effect on Cd transfers. Nymph introduction in the EUs did not modify the evolution tendencies of Cd concentrations in the water column: the burrowing activity, which is much greater at pH 7.5 and 25°C, does not lead to any significant increase in metal concentrations in the water column (Figure 4).

Mercury and Cadmium Accumulation in the Sediment Compartment after Contamination by the Water Source

For the EUs exposed to MeHg, the mean burdens measured in the cores for the two contamination levels in the water column were 13 and 36 ng Hg per core. The corresponding amounts of Hg accumulated in the sediment compartment were 4.3 and 12 µg Hg (330 cores/EU) respectively: this represents 50 and 28% of the Hg added to the water column throughout the 15 days of the experiment. Increasing the temperature from 15 to 25°C results in an increase in Hg accumulated in the sediment: the differences between readings for the different conditions were between 2 (C1) and 5 (C2). pH had no significant effect on these Hg transfers. The vertical Hg distribution in the sediments indicated a preferential accumulation in the first layer (0-0.5 cm). Average concentrations, expressed as dry weight, were 600 (level C1) and 950 ng/g (level C2) at 15°C; at 25°C they reached 1,400 and 2,500 ng/g, respectively. For some of the cores, the regression models revealed a non-linearity between the three measurement points, suggesting diffusion of the metal into the underlying layers. A comparative analysis of the b1 coefficients from these regression models shows that the Hg diffusion into the underlying sediment layers increases as the temperature increases (data not shown).

Cadmium dosages of the sediment cores revealed a high level of transfers from the water column: after 15 days exposure, estimated burdens in sediment were between 7 and 43 µg Cd for contamination level C1 and between 13 and 99 µg Cd for level C2. Extreme values among the different experimental conditions represented between 15 and 43% of the total amounts of Cd added to the water column. Temperature and pH effects on these transfers were relatively similar to those observed for mercury, although in this case pH had a significant effect, with a reduction in amounts of Cd transferred to the sediments under acidic conditions (data not shown). As with mercury, the vertical distribution of Cd in the cores revealed a preferential accumulation in the upper sediment layer: concentrations were between 1,500 and 6,000 ngCd/g (dw). It is important to stress that there was a wide scattering of the data. Cadmium diffusion into the lower layers was also observed; as was the case with MeHg, these transfers were enhanced by an increase in temperature.

Mercury and Cadmium Bioaccumulation in Hexagenia rigida Nymphs

Contamination from the Sediment Source: Total Hg concentrations in the nymphs were quasi-proportional to those corre-

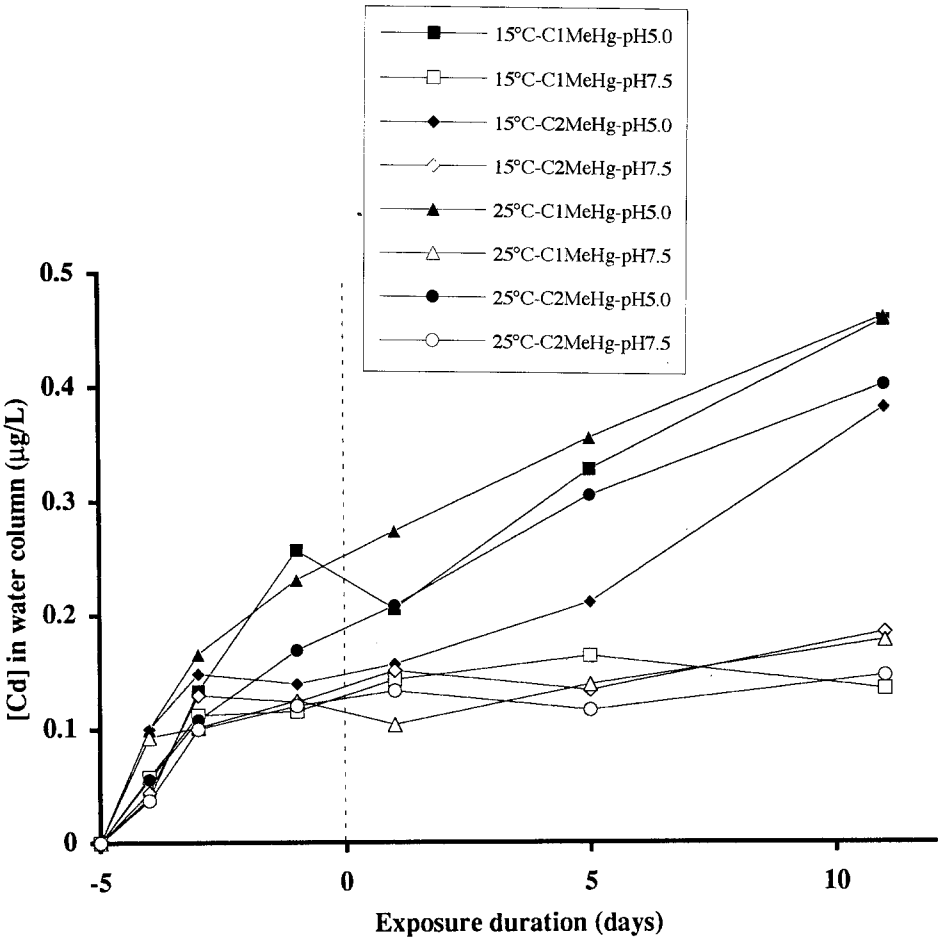

Fig. 4. Evolution of Cd concentrations (µg/L - average values) in the water column of EUs contaminated via the sediment source (C1 level = 2 mg/kg), during the two weeks' exposure period (time zero: nymph introduction), function of temperature, water column pH, and [MeHg] in the medium

sponding to the initial contamination levels of the sediment (C2/C1 = 5): the ratios between the average Hg concentrations in the nymphs were close to 5.6 at 15°C and 5.2 at 25°C. Hg determination was carried out on organisms whose gut had not been cleared. Our own estimates and the data from literature (Hare *et al.* 1989) indicate that the weight of the sediment inside the gut represents about 12% of the total biomass of the nymphs (dw), corresponding to 0.94 mg or about 1.9 mg (ww), in our experimental conditions. If we consider that the metal concentration in the sediment within the nymph gut is identical to the concentration in the surrounding sediment, Hg content represents only 1.3 and 1.8% of the total amounts accumulated in the whole organisms. Only temperature had a significant effect on Hg bioaccumulation: an increase from 15 to 25°C led to an increase in the metal concentrations in the nymphs of about 1.3, for the two exposure conditions (C1 and C2 levels).

Cd concentrations in the nymphs after 15 days exposure from the sediment source showed a significant effect of the contamination level of this source and also of the temperature. In contrast to Hg, Cd proportion in the nymph gut was higher in comparison with the burdens bioaccumulated in the whole organisms. Indeed, estimates gave relative contents of 20% (condition 15°C/C1), 31% (15°C/C2), 40% (25°C/C1), and 36% (25°C/C2). These values were determined on the basis of Cd burdens accumulated during the experiment, as the background level estimated from the control nymphs had already been deducted. Cd bioaccumulation, unlike that of mercury, was greater at 15°C than at 25°C: ratios between the average concentrations were 2.0 (C1 level) and 1.2 (C2 level). pH does not have any significant effect on the bioaccumulation of Hg and Cd from the sediment source. It is important to stress that the presence of both MeHg and Cd together in the sediment at levels C1 and C2 did not modify in any significant way the bioaccumulation of the two metals.

Contamination from the Water Source: Multiple regression analysis of the average Hg concentrations in the nymphs showed that the four factors studied—MeHg and Cd concentrations in the water column, temperature and pH—exerted a significant influence on Hg bioaccumulation (Figure 5).

After 15 days' exposure, Hg concentrations measured in the organisms varied between 130 and 1450 ng/g (ww). The difference between these two extremes—factor of almost 10—is greater than that which separates the maximal and minimal concentrations measured in the water column at the end of the experiment (ratio of about 6). A similar experimental study based on a wide range of MeHg concentrations in the water column (0 to 1.8 µg/L) drew similar conclusions (Odin *et al.* 1995a).

pH had a marked effect on Hg bioaccumulation, in interaction with temperature: at 15°C, Hg concentrations measured in the nymphs were higher at pH 5.0; at 25°C, the pH effect is reversed. The presence of Cd in the water column had a significant effect on Hg bioaccumulation in interaction with the temperature and pH factors. This interaction between the two metals is of little

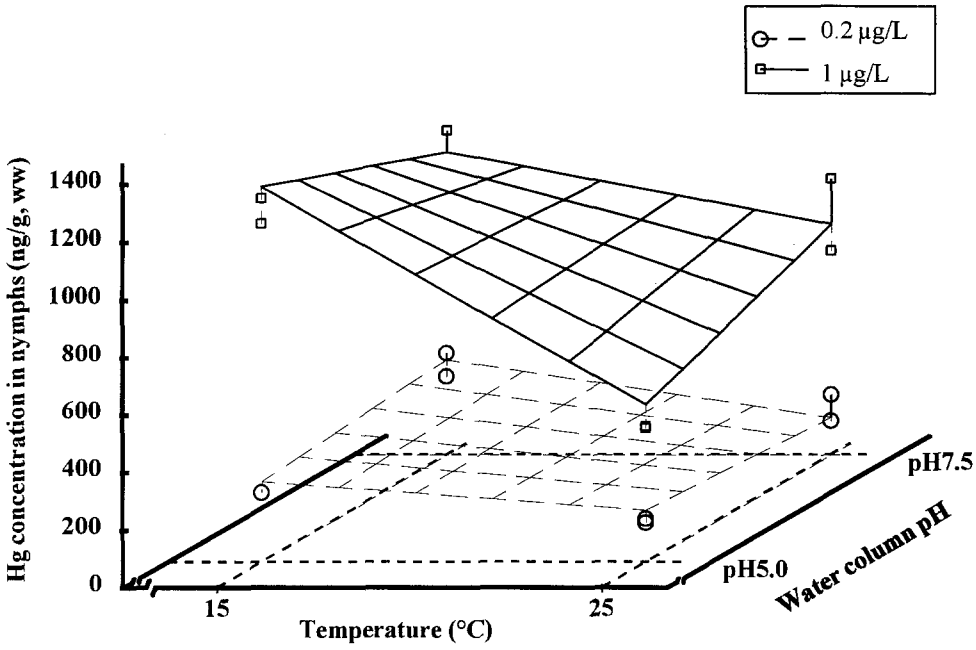

Fig. 5. Total Hg concentration in *Hexagenia rigida* nymphs (whole organism) after 15 days' exposure via the water column source, function of initial [MeHg] and [Cd] levels in the water column, temperature, and water column pH. Data on this graph correspond to the $C2_{Cd}$ contamination condition. *Regression model:* $\alpha = 0.01$; contribution = 97%. $[Hg]_{nymphs} = 578 + 347([MeHg]_{water}) - 163$ (temperature) + 108(temperature ∗pH) − 88($[MeHg]_{water}$ ∗ temperature) + 76($[MeHg]_{water}$ ∗ temperature ∗ pH) − 57($[Cd]_{water}$ ∗ temperature∗pH)

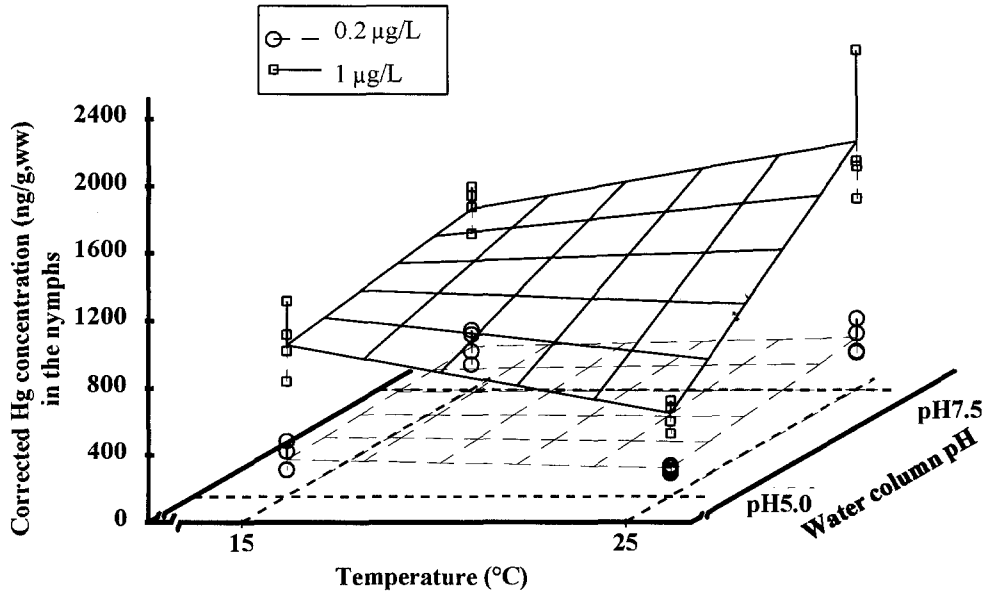

Fig. 6. Corrected Hg concentrations in *Hexagenia rigida* nymphs, as a function of the initial [MeHg] levels in the water column, temperature, and water column pH. *Regression model:* $\alpha = 0.01$; contribution = 90%. Corrected $[Hg]_{nymphs} = 613 + 374([MeHg]_{water}) + 164(pH) + 121([MeHg]_{water} * pH) + 114$(temperature ∗ pH) + 87 ($[MeHg]_{water}$ ∗ temperature ∗ pH)

importance, representing only 1.8% of the total variance of the regression model.

There were significant differences between Hg concentrations in the water column of the EUs for the different experimental conditions studied, producing a very wide variation in contamination pressures (Figure 3). It is therefore important to take these differences into account and to make a comparative analysis of the corrected data using the corresponding CDEs (Figure 6). Analysis of these corrected concentrations leads to conclusions which are fairly different from those initially reached on the basis of raw data (Figure 5), except for the differences observed between the two contamination levels, C1 and C2, of the water source. Hence, the corrected Hg concentrations are greater than at the basic pH level, with this factor having a more marked effect at the C2 level. Temperature clearly acts in interaction with pH and the contamination level of the water source: for level C1, temperature has little effect; for level C2 however, raising the temperature from 15 to 25°C leads to a marked decrease in Hg concentration at pH 5.0, and to an increase at pH 7.5.

Cadmium determinations in the nymphs after contamination of the EUs from the water source reveal no significant bioaccumulation at 25°C. These results confirm those obtained during an earlier experiment, based on a comparative study of six levels of contamination of the water source (control, 0.6, 1.6, 3.2, 5.9, and 10 µg Cd/L), after two weeks' exposure at 24°C (Odin *et al.* 1995b). However, at 15°C, there is a small amount of bioaccumulation: average concentrations were 400 ng/g (ww) for level C1 and 700 ng/g for level C2 (BF subtracted). The combined presence of MeHg and Cd in the water column does not have any significant effect on Cd bioaccumulation. When the contamination pressure is taken into account, acidification

has a very marked effect on the corrected values, inducing an important decrease in Cd bioaccumulation, differences being almost of a factor of 3.

Discussion

Comparative Study of Methylmercury and Cadmium Bioaccumulation in the Nymphs of Hexagenia rigida

For the sediment source and identical exposure conditions for the two metals, differences between the average concentrations in the nymphs would be close to 20, in favor of methylmercury. A comparative study of wide ranges of sediment enrichment by the two metals ($[MeHg]_{sediment}$:0 to 2.98 mg/kg: $[Cd]_{sediment}$: 0 to 10 mg/kg), for similar experimental conditions, have shown a linear tendency between these two criteria, after 2 weeks' exposure (Odin *et al.* 1995b). If the Cd burdens in the gut are deducted, differences between the two metals are close to 30. The behavior of Cd is similar to that of inorganic Hg in relation to the bioaccumulation capacities of *H. rigida* nymphs, when the EUs are initially contaminated by the sediment source (Odin *et al.* 1994).

From the water column source, Cd transfers were not significant at 25°C. At 15°C, similar estimations to those made for the sediment source produced a ratio of about 20 between Hg and Cd concentrations in the organisms, always in favor of MeHg.

The high bioaccumulation of MeHg, from both the water column and the sediment sources, is mainly due to its physicochemical properties, its partitioning and bioavailability within the aquatic biotopes and its ability to cross the biological barriers of the nymphs. Hg distribution in the principal organs of *H. rigida* after exposure to sediment enriched with MeHg revealed a high absorption capacity through the gut barrier, with estimated transfer efficiencies from ingested sediment of over 90% (Saouter *et al.* 1991a; Odin *et al.* 1994). These results are similar to those obtained with other freshwater species, such as zooplankton or carnivorous fish (Boudou and Ribeyre 1983). When nymph contamination was via the water column, Hg was distributed throughout the various organs and tissues of the nymphs, indicating a high permeability of the gill barrier (Saouter *et al.* 1992). Biophysical studies on membrane models have demonstrated that the ability of MeHg to cross these barriers is not really due to its liposolubility ($P_{octanol/water} = 0.2$ for CH_3HgOH and 1.7 for CH_3HgCl) (Major *et al.* 1991). Rapid diffusion rather than lipid affinity is responsible for MeHg transport across the membranes (Boudou *et al.* 1991; Delnomdedieu *et al.* 1992).

Under our experimental conditions, notably due to the presence of bacterial populations in the water column and in the oxic and anoxic layers of the sediment, MeHg could be demethylated ($MeHg \rightarrow HgII \rightarrow Hg^0$ volatile form), and/or dimethylated (Me_2Hg, volatile form) (Winfrey and Rudd 1990; Gilmour *et al.* 1992). In our experimental conditions, no direct measurements of these chemical transformations are available, apart from the presence of resistant bacterial strains in the water column which are able to volatilize mercury (Baldi *et al.* 1992). Given the procedure selected for the contamination of the two sources, these processes are able to play a more important role in the case of the sediment source (single and initial enrichment of the sediment by MeHg; daily additions of MeHg into the water column). In any event, these potential chemical transformations can only lead to a reduction and thus an underestimation of Hg bioaccumulation in the nymphs, since the transformation products are much less bioavailable and thus less bioaccumulable than MeHg.

Very little data are currently published on the bioaccumulation of cadmium by freshwater burrowing insects, according to the different uptake routes (Hare 1992). Cd concentrations in the gut of *H. rigida* nymphs after an exposure to natural lake sediments enriched with trace amounts of ^{109}Cd, together with lead and zine, suggested that the predominant route for Cd uptake was via sediment consumed as food; bioaccumulation from water via the gills was negligible (Hare *et al.* 1991). Experimental studies on *Chironomus riparius* larvae indicated that very small quantities of Cd were bound to the chitineous outer surface of the body (Timmermans *et al.* 1992). Structural and ultrastructural studies of the gut barrier of *H. rigida* defined three fundamental parts: the foregut or stomodeum, the midgut or mesenteron and the hindgut or proctodeum. Microvilli on the apical face of epithelial cells (mesenteron and proctodeum) represent a large surface area for nutrient absorption and potentially for toxic metal uptake. Cd analysis in the different parts of the gut of *H. rigida* nymphs, after microdissection, indicated that Cd was found largely in the anterior region of the mesenteron (Hare *et al.* 1991).

Among the different hypotheses offered to explain the very small amounts, or even the absence, of Cd bioaccumulation when the EUs were contaminated via the water column, the chitinous layer of the biological barriers involved (gills, cutaneous coating) appears to play an important role. For example, the several hundred secondary ramifications of the six pairs of external filamentous gills present a thick external chitin envelope (Saouter *et al.* 1991a), and this protective layer could represent an impermeable barrier to Cd absorption from the surrounding medium, jointly with a low adsorptive capacity.

Bioaccumulation is a global process, resulting from the differences between metal uptake and elimination. An experimental approach to the decontamination processes after exposure of *H. rigida* nymphs to the water column or sediment contamination sources enriched with MeHg and Cd shows that the decrease of Hg concentrations in the whole body is slow and gradual; for cadmium, however, it is much faster, with almost the entire metal burden eliminated after only 5 days, at 15 and 25°C (Odin *et al.* 1996).

The fact that the two metals are present in the water column or sediment simultaneously does not give rise to any significant interactions in relation to the bioaccumulation of Cd and MeHg. Cd and Hg have common chemical affinities for many ligands at the cell level, notably the thiol groups of membrane and cytosol proteins. When used in combination they may therefore give rise to synergic or antagonistic reactions with regard to their bioaccumulation and any toxic effects produced. However, the majority of comparative studies have dealt with inorganic Hg and cadmium. As is the case, for example, in the induction of metallothioneins: there are very little data available on mercury, the fixation capacities of MeHg on the thiol sites of the two MT clusters being much less than those of HgII and Cd (Roesijadi 1992).

Temperature and pH Effects on Methylmercury and Cadmium Bioaccumulation in the nymphs

Temperature Effects: An increase in temperature from 15 to 25°C has a significant effect on aquatic poïkilothermic organisms. *H. rigida* nymphs increase their metabolic activity and likewise their exchanges with the surrounding medium. Given the direct and indirect relationships between motor activity by the

nymphs and the potential for metal transfers from the surrounding medium, an increase in temperature should normally produce an increase in metal uptake from the bioavailable fractions in the water and/or ingested sediment. The increase in MeHg bioaccumulation from the sediment source may be associated with the increase in the amount of sediment ingested. In this case, the ability of the Hg organic form to cross the nymphs' intestinal barrier may explain the differences in metal concentrations observed after 2 weeks' exposure at 15 and at 25°C.

For the water source, the increased level of exchanges in the burrows when the temperature rises does not produce a similar increase in the amounts of mercury bioaccumulated, so other processes must necessarily be involved. At 25°C the estimated contamination pressure from this compartment was much less than at 15°C (Figure 5); thus, correction of the bioaccumulation data using the CDEs, in order to simulate similar exposure conditions, reduces, or even reverses, the effect of temperature (Figure 6). Moreover, at 25°C, the large quantity of suspended matter in the water column considerably reduces MeHg concentrations in the dissolved phase, which are considered to be the most bioavailable, especially in exchanges at the gill barrier level. Hg dosages in the sediment layers at the end of the experiment showed an increase in metal transfers at 25°C, but metal distribution was essentially limited to the superficial layer (0–0.5 cm). Given the lifestyle of the nymphs in their burrows, this upper layer is probably not ingested nearly as much as the other deeper sediment layers. Finally, if we consider that MeHg added to the microcosms can be partially transformed by demethylation or conversely by dimethylation reactions, available data in the literature shows that these reactions generally increase with an increase in temperature, due in particular to the effects of this factor on bacterial populations and the resulting enzyme activities (organomercurial lyase, mercuric reductase—Moore *et al.* 1990). In this case, as described earlier, these chemical transformations should lead to a decrease in bioaccumulation of the metal in the nymphs.

For the Cd added to the sediment source, the increase in the amounts of metal measured in the nymphs when the temperature decreases is more difficult to interpret. If we agree that the quantities of ingested sediment are much less at 15°C than at 25°C, again in relation to bioturbation activity, metal bioavailability and/or bioaccumulation mechanisms may have a marked influence. Among the hypotheses suggested in relation to the effects of temperature on the partitioning of Cd in the sediments, the burrowing activity of the nymphs brings about major modifications in the physicochemical conditions within the sediment, especially in the anoxic layers. Geochemical studies on these anoxic layers show that Cd is present largely in its complex form with the acid volatile sulfides (AVS—Di Toro *et al.* 1992). Additions of oxygen, via the burrowing activity of the nymphs may produce oxidation of the sediments on the walls of the burrows, which may in turn lead to a rapid transformation of the AVS, liberation of Cd and fixation of the metal on new ligands, like iron or manganese oxides and carbonates. Such modifications in the Cd partitioning could therefore reduce the Cd bioavailability, as has been demonstrated with oligochaetes (Carlson *et al.* 1991). A second reason for the decrease in Cd bioaccumulation when the temperature increases may be an increase in decontamination via the instars. An increase in temperature is usually accompanied by an increased frequency of instars in the larval stages of insects; to our knowledge, no estimate has been published of the number and frequency of instars in *H. rigida.*

Temperature plays an important role in respect of Cd bioaccumulation when the EUs are contaminated from the water source (absence of net bioaccumulation at 25°C). These results are in agreement with those obtained for this biological model after direct exposure to a wide range of Cd concentrations in the water column at 24°C (Odin *et al.* 1995b). From the data collected during this experiment, evolution of Cd concentrations and partitioning in the water column indicate that, as was the case for mercury, metal bioavailability is amplified at 15°C (Figure 2B); similarly, the proportion of Cd in the dissolved fraction decreases at 25°C. As mentioned earlier for the sediment source, instars may also contribute to minimise, or even suppress, Cd bioaccumulation when the temperature is raised. Complementary studies are necessary in order to be more precise about the location of the Cd in the organisms after exposure from the water column source and to confirm the hypothesis relating to the vital role of the cuticle barriers in relation to the fixation and absorption of the metal.

pH Effects: The pH factor, like temperature, acts simultaneously on the chemical fate of the metals in the biotopes (chemical speciation), and on the structural and functional properties of the organisms. Although no direct measurements of pH were made inside the burrows, effects observed via the turbidity suggest that the environment immediately surrounding the nymphs is very much modified when the water column of the EUs is acidified, with bioturbation activity being very much reduced at pH 5.0 (Figure 1), and jointly sediment ingested. In order to explain the no significant pH effect on the bioaccumulation of the two metals from the sediment source, it is necessary to take into account the major reduction in motor activity by the nymphs in the sediment and, likewise, in the quantities of sediment ingested. Among such mechanisms, the pH effects on Hg and Cd partitioning in the sediment can be considered, especially at the areas of interface inside the burrows, microenvironments from where exchanges with the nymphs via direct and trophic routes are established. In fact, acidification of the water in the burrows, via the water currents, may lead, especially in the ingested sediment, to an increase in the bioavailability of the metals and also to a greater bioaccumulation at pH 5.0. Such an hypothesis is very difficult to prove, as the techniques available for assessing metal partitioning in the sediments—porewater sampling, sequential extraction procedures, dialysis cells (peepers), etc,—can only provide information on a macroscopic scale, whereas these phenomena are probably located in the sediment microlayers at the burrow wall surface. It is possible, however, to use micromethods for sampling the sediments at the burrow wall surfaces, based on aluminum probes cooled in liquid nitrogen to which sediment adheres temporarily (Gerould and Gloss 1986).

As the effects of pH on the bioturbation activity of the nymphs are similar to those of temperature, the phenomena described above, in association with the oxygenation of the sediment layers and the modification of the chemical speciation of the metals, may also be involved in differences observed between amounts of metal bioaccumulated at pH 5.0 and at 7.5. Acidification effects on MeHg and Cd bioaccumulation can be also accounted for by structural and functional perturbations of the biological barriers in contact with the surrounding medium. Several biophysical studies on membrane models and biological membranes show that pH can very much modify the fixation of the two metals on the membrane sites, especially on the polar heads of the phospholipids; it can also modify the transport processes through the membrane barriers via passive diffusion or, in the case of Cd,

via carrier systems (voltage-operated Ca channels) (Hinkle *et al.* 1992). Among the hypotheses put forward in the literature, Cd in competition with the H^+ ions at the membrane level, would be likely to restrict the entrance of metal into the organisms when the medium is acid (Campbell *et al.* 1988).

When the EUs are contaminated by the water source, the acidification effects can be directly associated with the decrease in exchanges between the organisms and the water column. However, temperature has a similar effect to pH on the bioturbation activity of the nymphs (extreme turbidity values for 15°C/pH 5.0 and 25°C/pH 7.5), yet temperature has the opposite effect on mercury bioaccumulation to pH. The relationship between pH and the contamination pressures exerted by the water source shows that metal concentrations in the water column, or the corresponding CDEs, are much higher at pH 5.0, both for MeHg and Cd (Figures 2 and 3). Thus, by correcting the concentrations measured in the nymphs in order to achieve uniform contamination pressures, differences between the two pH conditions were also increased, always in favor of the non-acidified condition (Figures 5 and 6). This pH effect is the inverse of the temperature effect. As shown earlier for the sediment source, the chemical speciation in the aqueous phase may play an important role in metal accessibility to the biological barriers at the interfaces with the surrounding medium, especially the gill barrier, in their binding capacity to the membrane ligands and transport through these barriers.

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