TEMPERATURE, pH AND PHOTOPERIOD EFFECTS ON MERCURY BIOACCUMULATION BY NYMPHS OF THE BURROWING MAYFLY *Hexagenia rigida*

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Abstract: Accumulation of $HgCl_2$ and CH_3HgCl by *Hexagenia rigida* nymphs from contaminated sediment and water column was investigated experimentally, taking into account 3 abiotic factors (temperature, pH and photoperiod). When the contamination of the experimental units was based on sediment compartment, Hg concentrations at the whole organism level revealed very high bioaccumulation differences between the two chemical forms of Hg (ratio close to 20 in favour of MeHg). When Hg compounds were added to the water column, the highest Hg accumulation rates were observed for MeHg, but with a small difference between the 2 compounds (ratio close to 2.0-3.0). These bioaccumulation processes were very dependent on the 3 abiotic factors taken into account, especially temperature and water column pH.

1. Introduction

Among aquatic species, burrowing organisms are particularly exposed to Hg contamination from the sediment compartment, via substrate ingestion and/or metal transfers from the porewater, and from the water column, via permanent currents within the burrows, for respiratory and/or trophic purposes.

Two laboratory experiments were designed in order to quantify the actions and interactions of three abiotic factors - temperature, pH and photoperiod - on inorganic mercury ($HgCl_2$) and methylmercury (CH_3HgCl) bioaccumulation by nymphs of the burrowing mayfly *Hexagenia rigida* (Mc Dunnough - Ephemeroptera). The two contamination sources (sediment and water column) were studied separately, during 15 days' exposure.

The purpose of this paper is to present a synthesis of the comparative analysis of Hg bioaccumulation at the whole organism and gill levels and to assess the relative preponderance of the uptake routes, in relation to the different ecotoxicological conditions studied.

2. Materials and Methods

The experimental unit (EU, 12x12x30 cm) consisted of a three-compartment microcosm: natural sediment (homogeneous silt from the Garonne river - natural Hg level = 97 ± 5 (SD) $\mu g\ Hg.kg^{-1}$ (ww) - 5 cm deep), 2.9 L of dechlorinated tap water and 4 nymphs (151.6 ± 7.6 mg/EU). Mass culture of *Hexagenia* was initiated in the laboratory from eggs collected in the field each summer (Freshwater Institute, Winnipeg, Canada) (Friesen, 1982 ; Saouter et al., 1991).

Contamination of the water column was based on twice daily additions of identical amounts of mercury in the EUs: 2x5 ml of aqueous solutions of $HgCl_2$ or CH_3HgCl (Merck - 0.8 mg $Hg.L^{-1}$). Evolution of Hg concentrations in the dissolved and particulate phases was analyzed during the 15 days' exposure, for each experimental condition, jointly with turbidity measurements.

Contamination of the sediment compartment was based on additions of CH_3HgCl or $HgCl_2$ using aqueous solutions (500 mg $Hg.L^{-1}$), in order to obtain a final concentration of 5 mg $Hg.kg^{-1}$ (ww) for the inorganic compound and 0.5 mg $Hg.kg^{-1}$ for MeHg.

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For each contamination source, a complete experimental design was drawn up, including the combinations of the different levels for the three abiotic factors selected: 10, 18 and 26°C for the temperature; 6, 12 and 18h of light per day for the photoperiod; 5.0 and 7.5 for the water column pH. Two replicates were set up for each condition giving to 80 EUs per experiment (including controls).

Total Hg determination in the biological samples was carried out by cold vapour atomic absorption spectrometry (Varian AA 475 - detection limit = 5 ng Hg), after a digestion step (pure HNO_3, 95°C in a pressurized medium, during 3 h). Bioaccumulation in the nymphs was analyzed at whole organism level (concentrations $\mu g\ Hg.kg^{-1}$ ww, and burdens, ng Hg) and at the gills level (burdens and relative burdens, %). Natural levels of total Hg in the nymphs were $124 \pm 18\ \mu g\ Hg.kg^{-1}$ (ww; whole organism) and 1.5 ± 0.3 ng Hg (gills); these values were systematically deducted.

The data were analysed using multiple linear regression (Tomassone, 1993). An alpha risk equal to 0.01 was adopted for the statistical significance of the effects observed. F values were calculated with reference to the inter-replicate variance.

3. Results and Discussion

Figure 1 shows the results obtained at the whole organism level. In our experimental conditions, the two Hg compounds were more accumulated when introduced in the water column, according to the differences between the order of magnitude in initial Hg concentrations in the sediment and water compartments - respectively mg $Hg.kg^{-1}$ and $\mu g\ Hg.L^{-1}$ -, in relation to the metal partitioning and bioavailability. However, greatest concentrations of Hg were observed in the nymphs when EUs were contaminated with the organic form, but the differences are strongly linked to the contamination source: a factor close to 20 between the two mercury compounds for the sediment source, if the difference between the initial Hg concentrations is taken into account, and a factor close to 2.5 for the water column source.

Analysis of Hg bioaccumulation at the gills level clearly showed that metal burdens in this organ were small when contamination of the EUs occured via the sediment source (relative average burdens < 6%) (Odin et al., 1994b). In contrast, contamination via the water column gave rise to an important accumulation in this organ: relative burdens were close to 40 to 50% after $HgCl_2$ exposure and 30 to 40% after CH_3HgCl exposure (Odin et al., 1994a). These differences between the two contamination sources could be directly linked to the uptake routes: direct uptake from the water is predominant when EUs are contaminated via the water column, whereas the trophic route is predominant when Hg is added to the sediment. These results are in agreement with data obtained previously under similar exposure conditions, at the gut level: an important accumulation of Hg was measured in the digestive tract when nymphs were exposed to contaminated sediment (43% and 25% of the metal bioaccumulated in the nymphs were located in the gut after contamination by inorganic Hg and CH_3HgCl respectively). A reverse trend was observed when Hg compounds were initially introduced in the water column, average relative burdens being close to 8 and 20% respectively (Saouter et al., 1991, 1992). The structural and functional properties of the biological barriers at the interface between the nymphs and their surrounding medium play an important role at this level: for example, the gut wall is relatively impermeable to inorganic Hg, but is characterized by a great capacity of fixation during the contamination phase; MeHg, on the other hand has a very high capacity to cross the gut barrier and to be transferred to the other tissues via the haemolymph (Boudou et al., 1991).

Impacts of the three abiotic factors studied are very important on Hg bioaccumulation by *H. rigida* nymphs, with strong interactions, especially between temperature and pH, and marked differences between the two contamination sources.

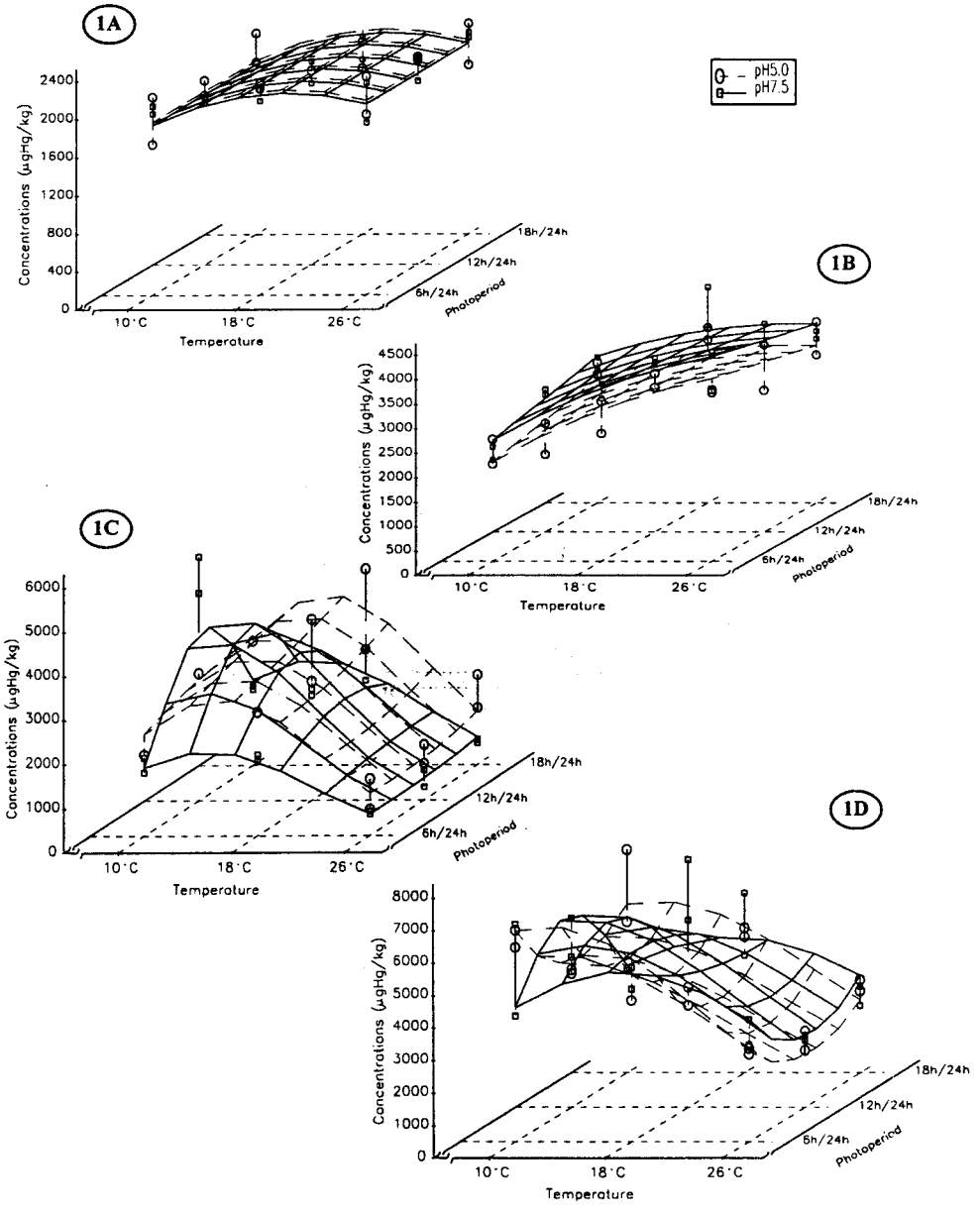

Fig. 1: Total Hg concentrations in *Hexagenia rigida* nymphs (whole organism), as a function of the initial contamination source (1A and 1B: sediment source; 1C and 1D: water source), Hg chemical form initially added (1A and 1C: $HgCl_2$; 1B and 1D: CH_3HgCl), temperature, photoperiod and water column pH. Symbols: average values/EU; 2 replicates/condition. Plans on the 3D plots correspond to the multilinear regression models.

An increase in temperature gave rise to an increase in Hg quantities bioaccumulated at the whole organism level, when nymphs were contaminated via the

sediment compartment (Figures 1A and 1B). An inverse trend was observed after 15 days exposure via the water column source (Figures 1C and 1D). Nymph activity was directly affected by this factor. It increased with the temperature, thus contributing to an increase in the amounts of sediment ingested and in the amounts of metal bioaccumulated from the sediment. However, it also led to an increase in bioturbation (average turbidity values in the water column: 5 NTU at 10°C and around 60 at 26°C), which modified metal partitioning in the water column considerably, leading to a marked decrease in bioavailability. If the differences between Hg concentrations in the dissolved phase (50-85% at 10°C and 20% at 26°C) are taken into account, in order to simulate identical exposure conditions via the direct route, corrected bioaccumulation values revealed an important increase when the temperature moved from 10 to 26°C (data not shown).

The pH factor did not influence to any great extent Hg concentrations in *H. rigida* nymphs contaminated via the sediment source (Figures 1A and 1B). However, when exposure was via the water column, there were important and similar effects at whole organism and gills levels, but with complex interactions with the other two controlled factors. So, when EUs were contaminated by $HgCl_2$, acidification gave rise to a global increase in Hg concentrations. For the organic form, this trend was inversed when temperature was in the range of 18 to 26°C; between 10 and 18°C, effects also varied in relation to the photoperiod. Acidification of the water column involved a modification in the chemistry of the overlying water and in the partitioning of Hg, which may explain several differences observed according to the initial contamination source (Gilmour and Henry, 1991); for example, a decrease in pH gave rise to an increase in metal concentrations in the dissolved phase (data not shown), as the metal is more bioavailable at the gill interface (Odin et al., 1994b).

The third factor, photoperiod, played a small but significant role in Hg transfers via the sediment source. When Hg compounds were added to the water column, its effects on bioaccumulation appeared to be more important but also more complex, due to the interactions with the other factors considered and to the non-linear trends among the three modalities of the photoperiod. Burrowing organisms are characterized by a negative phototropism; they are protected by the substrate from light rays, which minimize the direct influence of the daily period of light. For example, turbidity measurements in the water column were not significantly affected by the 3 photoperiod durations studied. Further experiments are currently being set up in order to analyze the direct and indirect effects of this factor.

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