METHYLMERCURY IN A BENTHIC FOOD WEB OF TWO HYDROELECTRIC RESERVOIRS AND A NATURAL LAKE OF NORTHERN QUÉBEC (CANADA)

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Abstract. Total mercury (Hg) concentrations in benthic insects from a 14 years old hydroelectric reservoir (La Grande 2) were 2 to 3 times higher than those from a reference lake and, in some groups, up to 7 times. The difference was even more pronounced for methylmercury (MeHg) concentrations, with a mean of 4 fold and a maximum of 12 fold between systems. The enrichment factors (dw) of insects, relative to the substrate in which they reside was around 3 for total Hg and 6 to 22 for MeHg. On the basis of their diet, we have classified the insects into four different trophic levels: detritivores, grazers, grazers-predators and predators. In insects collected in the reservoirs, the MeHg:Hg ratio was 20-25% in dipterans-ephemeropterans (detritivores) and 30-40% in trichopterans (grazers), but 60- 85% in heteropterans-coleopterans (grazers-predators) and 95% in odonates (predators). The pattern was similar in the lake with slightly lower values. In both systems, the proportion of MeHg increases in direct relation to our defined trophic levels, Given that insects are an important food source for many fish, our results suggest that they are a strong vector of MeHg to these fish in hydroelectric reservoirs. The bioavailability of MeHg to insect larvae appears to depend on the nature and composition of the substrate in which they reside.

Key words: mercury, methylmercury, benthic invertebrates, lakes, reservoirs

1. Introduction

Numerous researchers have reported that flooding for reservoir development results in an increase in fish mercury (Hg) levels which may be several times greater than those prescribed by government standards $(0.5-1.0 \text{ mg kg}^{-1})$; Abernathy and Cumbie, 1977; Bodaly *et al.,* 1984; Verdon *et al.,* 1991). At present, the hypothesis most often cited by researchers concerned with the accumulation of Hg in fish of reservoirs is that with an increase in bacterial activity in the flooded soils and subsequent methylation of inorganic Hg, methylmercury (MeHg) is transfered to the water column where it is made available for aquatic organisms (Hecky et al., 1991; Jackson, 1991, 1988; Morrison and Thérien, 1991). As well, both atmospheric transport and runoff from the catchment area add to the Hg released from flooded soils (Abernathy and Cumbie, 1977; Lodenius *et al.,* 1983; Bodaly *et al.,* 1984; Verta, 1990; Hecky *etal.,* 1991; Verdon *etal.,* 1991; Johnston *etal.,* 1991; Jackson, 1988, 1991). Although the uppermost sediments (0-5 cm) of a lacustrine system make up 95-98% of the total mercury pool (Verta, 1984), the diffusive flux of Hg from this compartment seems to be low, and can therefore not be used to explain the high Hg concentrations measured in fish following a flooding history

of only a few years (Montgomery *et al.,* 1995). Recents studies, however, suggest that the combined erosive effects of waves and ice in shallow zones could increase the mobility of Hg trapped in the soil, and therefore could play an important role in the transfer of Hg to the food web (Louchouarn *et al.,* 1993; Mucci *et al.,* 1995).

In addition to these processes, we propose that insects may have a strong influence on the release of biologically important quantities of Hg from the flooded soils. The larvae of aquatic insects are generally benthic, living on or within the sediments, and are therefore susceptible to the accumulation of large quantities of Hg following prolonged contact with the sediments (Bissonnette, 1977; Menzie 1980; Rossaro *et al.,* 1986; Parkman and Meili, 1993; Saouter *et al.,* 1993). These organisms, which usually constitute more than half of the diet of numerous species of benthic fish in northern Québec (Dumont, 1977; Sage, 1983; Boucher and Roy, 1985; Scott and Crossman, 1985), may then transfer the Hg along the food chain (Parkman, 1993; Tremblay *et al.,* 1995a). The investigation presented here was undertaken to quantify the Hg and MeHg concentrations of insects of natural and artificial aquatic systems of northern Ouébec in order to evaluate their potential role in the observed contamination of fish in the hydroelectric reservoirs of this region.

2. Materials and Methods

2.1. STUDY AREA

The La Grande complex is located on the Canadian Shield, about 1000 km north of Montréal (55° N). It is made up of 7 main dams and 8 reservoirs. The flooded area covers 13 672 km². Sampling was conducted in June 1992 in the LG-2 reservoir at two sampling stations in the northern and southern arms of a bay near Dike 24, and at one station near Dike 14 (Figure 1). The LG-2 complex had been flooded for 14 years. A small bay flooded for one year during the construction of the LG-1 reservoir was also sampled. The shallow zone of Duncan Lake was sampled for the determination of background conditions.

The catchments of the reservoirs and of lake Duncan are dominated by coniferous forests, shallow podzolic soils, and igneous bedrock. Lake Duncan is an oligotrophic, shallow clear water lake (the color is around 68 Pt units) which has a surface area of 100 km^2 . Additional information describing the study area and the lake can be found in Tremblay *et al.* (1995b).

2.2. SAMPLING

Insects were collected from littoral zones (less than 2 m deep), using a handheld net with a 500 μ m mesh. Surface sediments collected with the net were immediately sorted, and all of the insects visible to the naked eye were removed. Upon return to the field laboratory, the insect samples were sorted by family and frozen. Although

Figure 1. Location of the sampling stations in the La Grande hydroelectric complex, James Bay territory, Canada.

there are problems inherent to the use of handheld nets, this method was chosen because of the difficulty of sampling in the presence of broken trees, root systems and high density of the flooded soils.

From the same area that the insect samples were collected and at depths not exceeding 4 m, sediment and flooded soil cores were collected by SCUBA divers using 15 cm diameter PVC tubes. All cores were sectioned at 1 cm intervals and frozen until analysis.

2.3. ANALYSIS

The insects were thawed and separated according to taxonomic groups (Tables I) and II) generally by genus, and when possible by species. Taxonomic verification of reference sets of insects was conducted by L. Cloutier (Department of Biology, University of Montréal). For each station, insects of the same genus or species were counted and then placed in separate scintillation vials for freeze-drying (3 days). Insects were then homogenized using a glass pestle and mortar.

Total Hg concentrations in the insect samples as well the flooded soils and lacustrine sediments were determined using atomic fluorescence spectrometry (AFS), following the methodology described by Louchouarn *et al.* (1993). In brief, 0.5 to 1 g (dw) aliquots of sediment or flooded soil and 1 to 10 mg (dw) insect aliquots were transferred to glass tubes and digested in $10:1 \text{ HNO}_3$:HCl for 6 hours at 120 °C. MeHg in the insect aggregates was concentrated into a 1 mL solution (0.7 mL water, 0.2 mL HCl, and 0.1 mL CuBr) at a constant temperature of 90 $^{\circ}$ C for about 90 minutes, using a distillation process (Pichet *et al.,* in prep.), in the presence of a nitrogen gas stream. The distillates were then injected with a potassium persulfate solution and subjected to ultraviolet oxidation for 25 minutes in a photochemical reactor, prior to analysis as total Hg by AFS. The MeHg recovery rate for the distillation process is between 85 and 95%. MeHg in the flooded soils and lacustrine sediments was separated by gas chromatography and then quantified using AFS. The separation was preceded by a copper bromide digestion and a toluene extraction as described by Ma (1994). The detection limit (3 standard deviations) for a 1 mg (dw) sample is 5 ng g^{-1} and the analytical variability (<3%, coefficient of variation) was verified regularly using standards. The total Hg and MeHg concentrations in the insects, are expressed per dry weight. To evaluate the homogeneity of the aggregates, every tenth sample was analysed in triplicate. The variability between analyses of the same aggregate was always less then 10% (coefficient of variation). The use of homogenized samples was decided upon since this procedure allows all analyses (Hg, MeHg, C/N) to be conducted on the same sample. Nitrogen and carbon concentrations in the insect aggregates and sediments were determined using a Carlo-Erba autoanalyser, with a reproducibility of about 1% (coefficient of variation).

Based on the general feeding regime ascribed by Merritt and Cummins (1985), we arbitrarily defined 4 trophic levels for the insects collected during this study: (1) detritus feeders (dipterans, ephemeroterans), (2) detritivores-grazers-filterers (trichopterans), (3) grazers-predators (heteropterans, coleopterans), and (4) predators (odonates).

3. Results

3.1. NATURAL LAKES

The mean concentration of total Hg in insects from the natural lake (Table I) ranged from around 129 ng g^{-1} (dw) in the ephemeropterans, 120–143 ng g^{-1} in the trichopterans, 136 ng g^{-1} in the odonates, 175 ng g^{-1} in the coleopterans to 256 ng g^{-1} in the heteropterans. The MeHg concentration in these insects varied from around 13 ng g⁻¹ (dw), 28–61 ng g⁻¹, 102 ng g⁻¹, 107 ng g⁻¹ and 124 ng g⁻¹ respectively. The fraction of MeHg to total Hg varied with feeding type from 11% in the detritivores, 29-46% in the detritivores-grazers, 46-49% in the predatorsgrazers to 75% in the predators. The nitrogen content of insects was around 11% with the exception of the *Asynarchus spp.* with 5.8%.

The surficial sediment Hg concentrations varied from 36 to 59 ng g^{-1} (dw), with MeHg concentrations varying from 0.11 to 0.64 ng g^{-1} (dw) and carbon contents varying from 2% to 4% (dw) (Table I).

3.2. HYDROELECTRIC RESERVOIRS

The concentration of total Hg in insects from the hydroelectric reservoirs (Table II) ranged from around 139–224 ng g^{-1} (dw) in the ephemeropterans, 285–1020 ng g^{-1} in the dipterans, 292–544 ng g^{-1} in the trichopterans, 283–615 ng g^{-1} in the odonates, 621 ng g⁻¹ in the coleopterans to 514-1675 ng g⁻¹ in the heteropterans. The MeHg concentration of these insects varied from 68-81 ng g^{-1} (dw), 63-75 ng g⁻¹, 59–106 ng g⁻¹, 211–615 ng g⁻¹, 473 ng g⁻¹ to 1043–1519 ng g⁻¹, respectively. The Hg levels in these insects are high given their low trophic levels, in comparison to top predators of natural lakes (pike 250–900 ng g^{-1} wet weight, walleye 320-1260 ng g^{-1} wet weight) and reservoirs from the same area (pike 810-2900 ng g⁻¹ wet weight, walleye 1200-2800 ng g⁻¹ wet weight) (Brouard *et al.,* 1990). As in the natural environment, the percentage of MeHg increased with trophic level from 6-35% in the detritivores, 19-25% in the detritivores-grazers, 76-90% in the predators-grazers to 75-100% in the predators. Similar to the natural lake, the nitrogen content of the insects was around 11% with the exception of the *Asynarchus spp.* with 6.9%.

The Hg concentrations of the flooded soils varied from 33–47 ng g^{-1} *(dw)* for LG-1, to 175-275 ng g^{-1} *(dw)* for the D-24 south arm (Table II). The MeHg concentrations, on the other hand varied from 2-12 ng g^{-1} (dw) for LG-1 to 6-21 ng g^{-1} for the D-24 south arm. The carbon content of the flooded soils ranged from 35-42% (dw).

4. Discussion

4.1. METHYLMERCURY IN THE INSECT FOOD WEB

Two possible pathways for the accumulation of Hg in aquatic organisms can be considered: direct absorption of dissolved Hg (from sediment pore waters or the water column) by the gills during respiration, or indirectly through the ingestion of contaminated food (Cope *et aI.,* 1990; Meili, 1991a; Spry and Wiener, 1991). The relative importance of these two pathways of Hg has not yet, however, been fully established. In some studies, where water column MeHg concentrations were 0.01 to 2 ng/1, direct absorption by the gills could account for, at most, 10% of the mercury load of fish (Spry and Wiener, 1991; Meili, 1991a). The ingestion pathway would therefore predominate for accumulation in fish, as demonstrated by Hall *et al.* (1994). Accordingly, numerous researchers have noted strong positive

Table II

Insects collected from reservoirs, June 1992. The following parameters have been measured on the homogenized aggregates: Lev. = Trophic level; Num. = number of individuals in aggregate; Hg = total mercury, ng g^{-1} dw; M % N = weight fraction of nitrogen, dw; Hg R/L = Hg concentration in an insect aggregate from a reservoir station/Hg concentration in an insect aggregate from Duncan Lake; MeHg R/L = MeHg concentration in an insect aggregate from a reservoir station/ MeHg concentration in an insect aggregate from Duncan Lake

^a Range of values measured in the flooded soils of the station.

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correlations between fish Hg levels and Hg concentrations in organisms of lower trophic levels (Philips and Gregory, 1979; Mathers and Johansen, 1985; Allard and Stokes, 1989; Meili, 1991a; Cope *et al.,* 1990). However, little is known about the relationship within invertebrate food web.

The concentration of Hg in animals generally increases as a function of the trophic level. Within a given ichthyological community, piscivorous species contain the highest Hg levels (Wren *et al.,* 1983; Cabana *et al.,* 1994). Similarly, we might expect the Hg concentration of predatory invertebrates to surpass that of detritivorous or grazing species. However, this was not the case in our study. Both detritivorous and detritivorous-herbivorous taxa (e.g. dipterans and trichopterans) have similar or higher Hg concentrations when compared to predatory taxa (odonates) (Tables I and 1I). A similar observation was made by Verta *et al.* (1986) who found trichopteran Hg levels to be on the order of 760 ng g^{-1} (dw) while those of odonates were around 420 ng g^{-1} (dw). Parkman and Meili (1993) found both concentrations and variations of Hg to be much greater in detritivorous species (200 to 5000 ng g^{-1} dw) than in predatory species (100 to 600 ng g^{-1} dw). These results were explained by the fact that detritivores, through their feeding regime, are exposed to high concentrations of Hg. That is, the organo-terrigenous detritic matter of lacustrine sediments, flooded soils or suspended particulates, which is an important food source for bacteria and invertebrates (Tranvik, 1989, 1990; Hessen, 1992), is characterized by high Hg concentrations but a low nutritive value (Mucci *et al.,* 1995). This necessitates considerable ingestion rates in detritivores and hence greater exposure of the organisms to Hg. Similar results of high Hg exposure related to a poor quality of food have been obtained for zooplankton by Plourde *et al.* (1995).

The high Hg levels observed in detritivores as compared to predators may be associated with a greater proportion of inorganic Hg in detritivores, as suggested by Parkman and Meili (1993). With MeHg being more efficiently accumulated, only a very small fraction of inorganic Hg would be transferred from the detritivores to the predators. Indeed, our MeHg analyses for insect aggregates appear to confirm this hypothesis since we observe an increase in the proportion of total Hg that is methylated when moving from the first trophic level (the detritivores) to the last (the predators) (Tables I and II, Figure 2). These results confirm the biomagnification of MeHg along food chains and highlight the need to fully comprehend the mechanisms regulating the formation of MeHg and its subsequent availability and bioaccumulation in organisms at the base of the food web.

In Table I, considerable variation in the MeHg concentrations in insects of some trophic levels can be seen. For example, variations of 28 to 61 ng g^{-1} are shown for the three trichopteran taxa in Duncan Lake. These variations can be related to the ecology of the species, mainly the difference in diet between species, their physiology and their degree of exposure to MeHg. Among the insects, the concentrations of MeHg were high in predators, lower in grazers and much lower in detritivorous species. These comparisons illustrate the importance of considering feeding habits

Figure 2. Fraction of methylmercury to total mercury in five arbitrarily defined trophic levels (level) 0: lacustrine sediments or flooded soil, level 1: detritivores, level 2: detritivores-grazers, level 3: grazers-predators, level 4: predators).

(Tables I and II) when comparing animals within a habitat. Additional evidence of the impact of feeding habit on the MeHg accumulation is provided by the trichopterans of Duncan Lake. In these animals, the lowest MeHg concentrations were found in *Asynarchus spp.* (detritivores-grazers), and the highest in *Agrypnia spp.* The feeding behaviour of the latter is unknown, but as they have a high percentage of MeHg which is similar to other grazers-predators, it may be speculated that they belonged to one of the known predatory species of the genus (Merritt and Cummins, 1985).

According to Huckabee *et al.* (1979), the large differences among Hg concentrations obtained in macroinvertebrates living on or in the sediments may be explained by the Hg contribution of the absorption surface or the stomach contents of the invertebrates. As mentioned by Parkman and Meili (1993), these factors could be important in the case of contaminated sediments, whereas in systems not affected by point source contamination, the quantity of Hg present in the intestinal tract will contribute very little to the total Hg load of the organism or even dilute it. As a means of minimizing the influence of intestinal Hg, some researchers allow insects to purge their stomach contents (Bissonnette, 1977; Ward and Williams, 1986).

The total Hg and MeHg concentrations for the homogenized aggregates shown in Tables I and II represent nonpurged insects may be slightly underestimated since the Hg concentrations in animals are higher than in the sediment or the flooded soils.

The wide range of variation in MeHg concentrations may also be explained by the method of standardization between the various taxa. The traditional method of relating Hg (or other contaminant) concentrations to the biomass in terms of volume or weight does not take into account the significance of constituents such as the chitin of invertebrates or ingested sediment, which both are important contributors to the mass, but not to the Hg burden (Wright *et al.,* 1991). This may be accounted for by considering the concentration ratio of MeHg to nitrogen, in accordance with the concept of Hg/biomass developed by Meili (1991c): Since Hg has a strong affinity for organic matter, the cycling of Hg from the point of its emission until its accumulation in the food chain may be described by comparison with elements that characterize biotic matter (C, N, P, S). Nitrogen makes up a significant portion of biotic matter and can be used as a better measure of biomass than dry weight. By applying the MeHg/N ratio to our results, we slightly reduced the variations within trophic levels. The ratio between the MeHg/N values of adjacent trophic levels of Duncan Lake varied from 1.4 to 3.4 fold, which is similar to 12 other natural lakes (Tremblay *et al.,* 1995a). These ratios are slightly lower than those reported by Meili (1991a) for a food chain ranging from particulates in the water column up to predatory fish. It is important to note, however, that the trophic levels described in our study were arbitrarily defined, and are therefore not necessarily representative of a true food chain.

5. Hg and MeHg concentrations in insects from natural and flooded systems

Mercury and MeHg concentrations of the homogenized aggregates, as well as the proportion of Hg that is MeHg, are generally higher in insects sampled from the reservoirs than the lake. We consistently find 2 to 3 times more Hg in the reservoir insects than in those of the lake, although the observed difference can be up to 7 times greater for some groups (heteropterans, odonates). The difference is even more pronounced for MeHg concentrations, on average about 4 fold and up to a maximum of 12 fold (heteropterans) in reservoir samples. Although Duncan lake has a low sediment Hg concentrations in comparison to the flooded soils, the observed patterns in invertebrates between the lake and the reservoirs are similar to the ones observed in 5 lakes and 12 stations in reservoirs in northern Qurbec (Tremblay *et al.,* 1995b). Surma-Aho *et al.* (1986) report a range of total Hg values of 20-620 ng g^{-1} (dw) for trichopterans and odonates from lakes, and 70-1600 ng g^{-1} (dw) for the same groups of animals in Finnish hydroelectric reservoirs. The mean values determined by these authors corresponds to a Hg enrichment of 2 to

Figure 3. The methylmercury/nitrogen ratio (μ gMeHg gN⁻¹) in five arbitrarily defined trophic levels (level 0: lacustrine sediments or flooded soil, level l: detritivores, level 2: detritivores-grazers, level 3: grazers-predators, level 4: predators).

2.5 times for reservoir larvae as compared to those of lakes, which corroborates our observations.

The proportion of MeHg to total Hg in insects varies from 3-4% to 100% (Tables I and II), which is in agreement with the findings of Surma-Aho *et al.* (1986) for benthic organisms and zooplankton of Finnish reservoirs and natural lakes, as well as with a literature review done by Huckabee *et al.* (1979). Although Hg and MeHg concentrations differ between the natural lake and the reservoirs, our results clearly demonstrate that the increase in the fraction of methylated Hg with trophic level is independent of the type of environment, whether it be a natural lake or a hydroelectric reservoir (Figures 2 and 4). Abiotic-biotic transfer between substrate and the detritivorous insects (insect MeHg of Hg concentration/substrate MeHg or Hg concentration) are similar between the lake and the reservoirs for the total Hg, with respectively 2.8 and 1.9-2.4, but are different for MeHg, being higher in the lake with a ratio of 22.6 and lower in reservoirs with a ratio of 5.2. However, the ratio of MeHg between adjacent trophic levels are similar for both environments with a mean ratio of 2.4 (Figure 4), thus stressing the difference between lakes and reservoirs in the bioavailability of MeHg at the base of the food chain. From an investigation on Southern Indian lake, Jackson (1988) reported no significant correlation between total Hg in zoobenthos (chironomids and

Figure 4. Mean total Hg concentration (ng g^{-1} , dry weight) (A) and mean MeHg concentration (ng g^{-1} , dry weight) (B) in five arbitrarily defined trophic levels (level 0: lacustrine sediments or flooded soil, level 1: detritivores, level 2: detritivores-grazers, level 3: grazers-predators, level 4: predators).

oligochaetes) and MeHg or methylating activity in the sediment. Jackson (1988) concluded that the net rates of Hg accumulation by benthic invertebrates were influenced by environmental and biological processes rather than by the concentration of MeHg in sediments. Tremblay *et al.* (1995a) came to the same conclusions when comparing MeHg in sediments to total Hg in chironomids. However, a significant correlation between MeHg in chironomids and sediment MeHg ($r = 0.78$, p<0.005) was observed by Tremblay *et al.* (1995a), since MeHg is more efficiently bioaccumulated (see above). The observed relationship between chironomids and sediment MeHg concentration, strongly suggest that the nature of substrate and its intrinsic geochemistry play an important role in the MeHg bioavailability to benthic invertebrates (Figure 4). Many studies have demonstrated that inundation of forest soils stimulate microbial activity and methylation (Bodaly *et al.,* 1984; Hecky *et al.*, 1987; Jackson, 1988, 1991; Hecky *et al.*, 1991; Morrison and Thérien, 1991). Therefore, the enhance microbial methylation in carbon-rich flooded soils (%C = 35-50%) would rapidly (within a year, see LG-1 Table II) favor the accumulation of MeHg in the inundated soils (%MeHg content = 18-25%) (Begin *et al.,* in preparation). This process is likely to increase the biotransfer, of MeHg from flooded soils, to fish via the injestion of contaminated insect larvae.

6. **Conclusions**

Our results demonstrate that insects are efficient bioaccumulators in comparison to the substrate in which they reside, having enrichment factors of 3-22, and strongly suggests that feeding behaviour is of primary importance in controlling the MeHg concentrations in the animals. The elevated MeHg levels determined for insects in reservoirs suggest that, following extensive flooding, they would be an important source of MeHg available for incorporation into the higher trophic levels (up to fish). In addition, the results obtained for the LG-2 reservoir (flooded for 14 years) and for the small bay near the LG-1 reservoir (flooded for one year), suggest that insects would represent a long term transfer mechanism for sedimentary Hg toward the food chain. That is, even though the significant increase in fish Hg levels observed during the first 4 to 5 years following inundation (Verdon *et al.,* 1991) can be linked to a rapid degradation of the labile organic matter, to erosion and resuspension of the flooded soils (Mucci *et al.,* 1995), the presence of burrowing insects (to depths of 12-16 cm) in the flooded soils may represent an important source of Hg, thereby prolonging its transfer to higher level aquatic organisms. Future research should be focused on the factors determining the bioavailability of MeHg in the sediments and its bioaccumulation in the lower trophic levels of the benthic invertebrates.

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