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Biotic and Abiotic Mercury Methylation and Demethylation in Sediments

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Inorganic mercury (Hg(II)) methylation and methylmercury (MeHg) demethylation may occur in the water column, sediment-water interface and subsurficial sediment of aquatic ecosystems. These transformations involve mainly microbial mechanisms (Mason et al. 1979; Pan-Hou and Imura 1982; Robinson and Tuovinen 1984), although abiotic methylation may play a more important role in the water compartment. The relative importance of biotic versus abiotic mechanisms of methylation has not been determined however, and abiotic demethylation remains unknown (Winfrey and Rudd 1990). Little quantitative information is available on the role of bacterial activity in mercury transformations. It has been reported that at least 16 genera of aerobic and anaerobic microorganisms are able to methylate Hg(II) (Baldi et al. 1989), and that a greater number are able to demethylate MeHg (Shariat et al. 1979). Nevertheless, not all populations of these species are capable of methyl- and demethyl-transformations (Trevors 1986; Gilmour and Henry 1991). The actual concentration of MeHg in the aquatic environment is regulated by the relative production and decomposition rates. This, in turn, depends on the availability of Hg(II), MeHg, and bacteria as well as on the physico-chemical properties of the sample (Jackson 1990).

The objective of this study was to compare mercury methylation and demethylation rates in sediment samples with and without active bacterial populations. We therefore performed experiments to follow bacterial evolution during the course of Hg(II) methylation and MeHg demethylation in sediment slurries containing both sterile and non-sterile sediments.

MATERIALS AND METHODS

Surficial floc sediment was collected in June 1991 from Lake Lusignan, a natural ecological study station situated 140 km north of Montreal, Canada. *In situ* measurements of the sample, taken at a depth of 23m, showed a temperature of 8.9°C, a pH of 6.75 and dissolved oxygen of 5.4 mg/l. Two days after sampling, the sediment slurry was prepared using laboratory nano-pure water. The solid phase was composed of the < 200 mm fraction of the sediment with a dry weight of 1.4 g per 100 ml of slurry. Sodium azide (0.1%) (Fletcher and Kaufman 1980) was added to sterilize (overnight) half of the slurry which was then used as a control.

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One series of the sterile and non-sterile slurries was enriched with 5000 ng Hg(II) (as $HgCl_2$) per 100 ml of slurry, the other with 250 ng MeHg (as CH_3HgCl).To conduct the studies under conditions similar to the *in situ* situation, we chose a temperature of 4°C, a pH of 7.0, an oxic atmosphere and dim ambient light. After incubating for different time intervals, the MeHg in the subsamples was extracted using $CuSO_4/NaBr/H_2SO_4$ and toluene (Furutani and Rudd 1980) and analyzed by gas chromatography. Simultaneously, 1 ml aliquots of each subsample were fixed with formalin (4%) and stored at 4°C for bacterial enumeration. Bacterial counts were based on 4',6'-diamidino-2-phenylindole (DAPI) coloration (Porter and Feig 1980) of protein particles. They were collected on 0.2 μm Nuclepore filters and examined under an epifluorescent microscope. A General Linear Model (GLM) procedure (SAS 1988) was performed to test statistically if the differences in bacterial populations were significant at each stop-time of the cultures.

RESULTS AND DISCUSSION

Figure 1 illustrates the experimental results of Hg(II) methylation and MeHg demethylation. In the sterile slurries (i.e., the controls in Fig. 1a) methylation (conversion of Hg(II) to MeHg) did not reach a stationary state until 48 hr of incubation. In contrast 96%, on average, of the 250 ng of MeHg added to the sterile slurries (i.e., the controls in Fig. 1b) was recovered at the end of each culture period, with an analytical variance of ± 4%.

Although net methylation in the sterile slurries showed a definite increase with time, the non-sterile slurries showed their own capacity for producing net MeHg. This was measured by the difference in MeHg production between the sterile and non-sterile systems. This difference was not statistically significant ($p = 0.055$), possibly because of bacterial inhibition caused by the low temperature (4°C) at which the experiments were carried out (Winfrey and Rudd 1990). The maximal rate of microbial methylation appeared in the first few hours. At the end of an 8-hr incubation (Fig. 2a), about 1 ng of MeHg was produced from the 5 μg Hg(II) added. Net MeHg demethylation was more significant ($p = 0.0013$) and rapid, with 8.8% of the added MeHg (250 ng) degraded in 4 hr (Fig. 2b).

Production of MeHg in the sterile slurries thus provided experimental evidence for the existence of an abiotic methylating mechanism (Nagase 1982). Since about 30.5% (by weight) of the sediment was organic matter, it is possible that an abiotic methylation process occurred by the transfer of methyl carbanions (Craig and Moreton 1985) to the Hg(II) added to the slurries. In our study (Fig. 1a) the MeHg produced by abiotic processes was two to three times higher than that resulting from biotic processes. The demethylation of MeHg (Fig. 1b), however, showed a different pattern. Contrary to the non-sterile slurries, there was no significant MeHg decomposition in the sterile slurries. This suggests that MeHg demethylation in aquatic systems may be more dependent on bacterial activity (Oremland et al. 1991) than Hg(II) methylation.

Bacterial enumeration produced some interesting results (Fig. 3). At the start of the experiments the sterile slurries (controls) had half the number of bacteria/g dry sediment than that found in the non-sterile samples, and this number remained constant throughout the experiment. Thus no bacterial reproduction seems to have occurred in those sediments sterilized with sodium azide. Sodium azide is an enzyme inhibitor which completely inhibits microbial growth (Skipper and Westermann 1973). Previous studies have demonstrated that azide is a respiratory

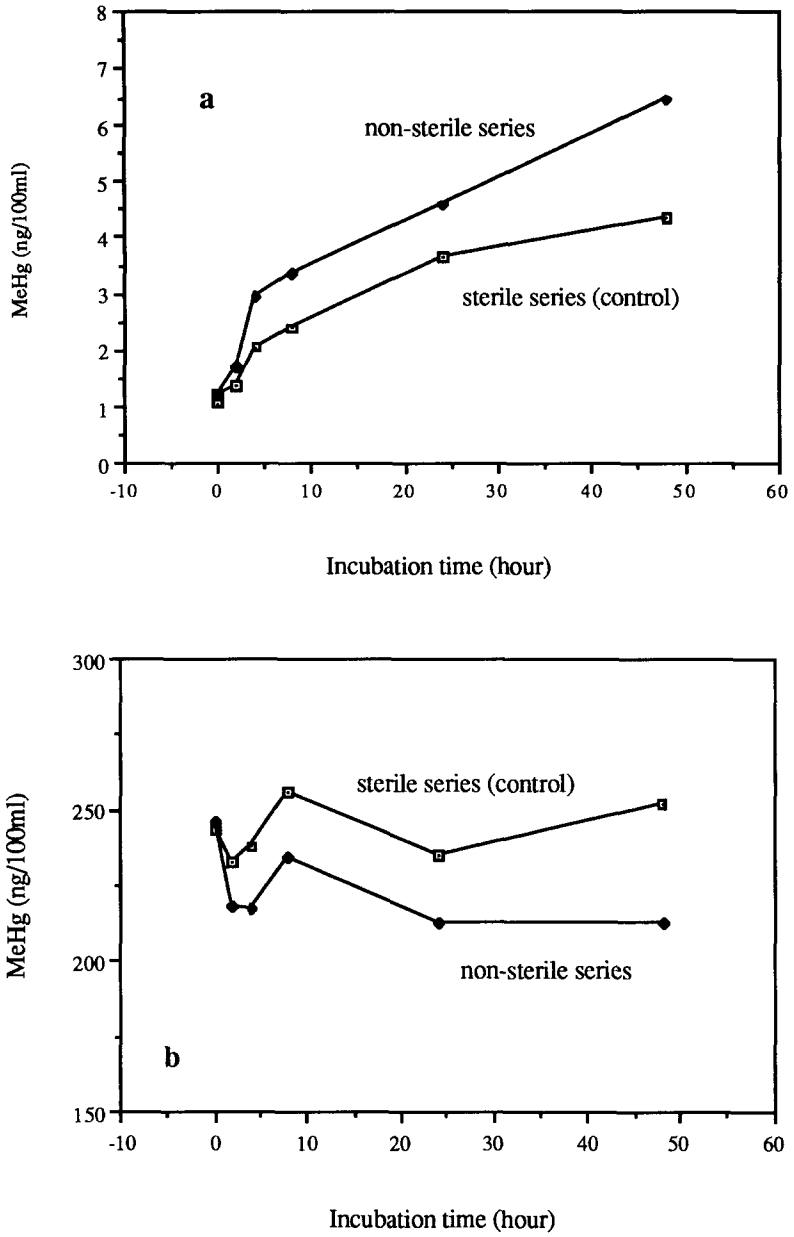

Figure 1. Hg(II) methylation (a) and MeHg demethylation (b) in the prepared slurries after different time intervals of incubation.

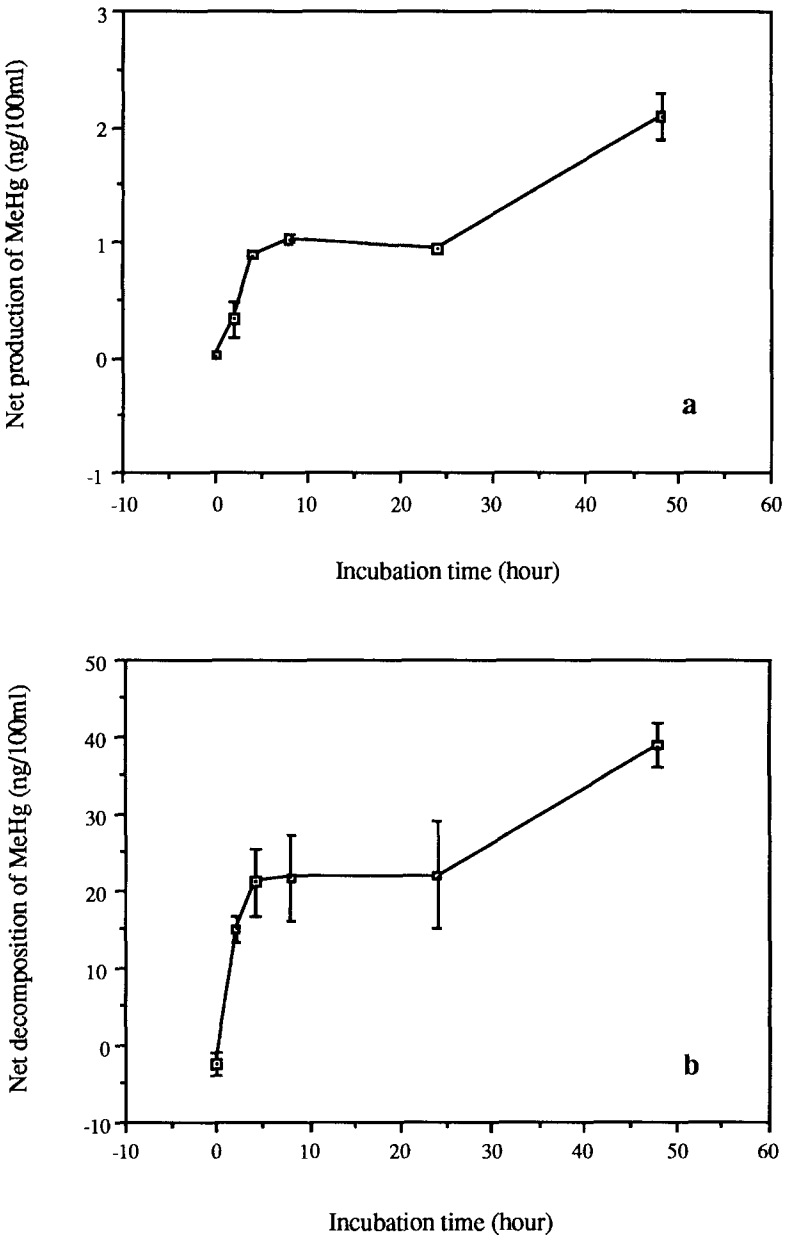

Figure 2. Net biological production (a) and decomposition (b) of MeHg in the non-sterile slurries.

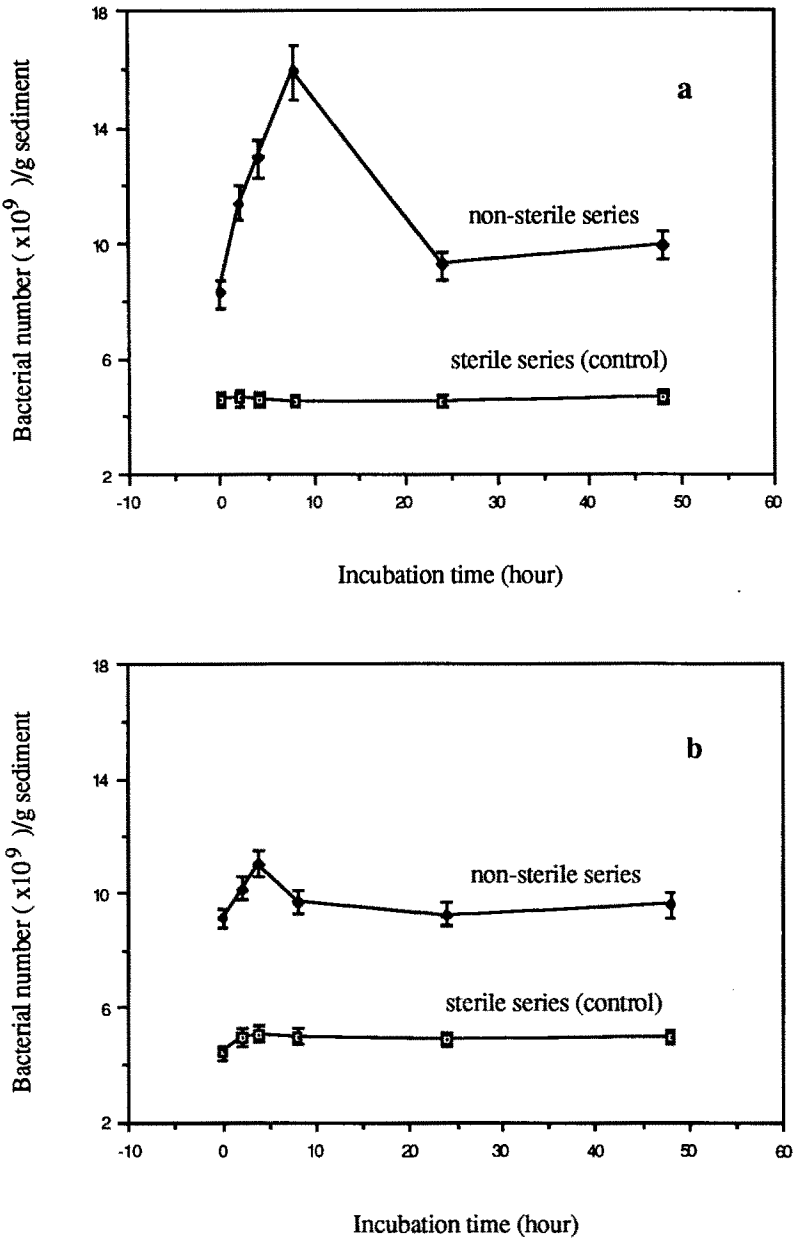

Figure 3. The occurrence of transforming bacteria within the methylation (a) and demethylation (b) processes in the experiments.

poison (Pramer and Bartha 1972; Rozycki and Bartha 1981) which specifically inhibits active but not resting respiration (Wilson and Chance 1967). Similarly to our observations, a recent study on phosphorus release from sediments (*DeMontigny*, Dép. des Sciences Biologiques, Univ. du Québec à Montréal, personal communication) also showed that sodium azide depresses the activity (i.e., proliferation) but not the initial numbers of bacteria. Sodium azide was used as a bacterial inhibitor rather than steam sterilization because we considered that to verify the microbial mechanisms involved in mercury methylation and demethylation it was preferable to avoid alterations in the sediment composition of nutrient elements and, particularly, of Fe-Mn oxides (Skipper and Westermann 1973).

The higher bacterial numbers found in the non-sterile slurries at the start of the experiment (Fig. 3) represent the bacterial proliferation which occurred overnight after the sterilization of the control series. Thereafter, as methylation proceeded, the amount of bacteria in the non-sterile slurries increased as a function of time. The mean proliferation rate was 8.18×10^7/h•g during the first 8 hr of incubation. This rate was more than double that of the pre-enrichment average of 3.68×10^7/h•g (overnight average), indicating that the presence of 5 µg of Hg(II) played a role in stimulating bacterial growth during this short incubation period. The bacterial population then sharply decreased to close to the initial level (Fig. 3a). In the demethylation experiment, the proliferation rate of bacteria appeared to increase (from 3.90×10^7 to 4.67×10^7/h•g) in the presence of MeHg (Fig. 3b) during the first 4 hr, then decreased. Although this increase seemed small when compared to that observed in the methylation experiment, statistical analysis showed that the bacterial count at 4 hr was significantly different from those at 0 and 8 hr ($p = 0.004$).

In regard to the relationship between bacterial activity and mercury transformations, we found that biological methylation occurred simultaneously to demethylation (Fig. 2) although it was not proportional to MeHg production or decomposition. Transformation between the chemical species of mercury coincided with bacterial population growth (Fig. 3). It has been recorded that microbial growth in pure cultures does not always lead to MeHg production (Pan-Hou and Imura 1982; Baldi et al. 1989), however biological methylation is related primarily to microbial activity. Whether biological methylation is characterized by an involvement of methylcobalamin (Pan-Hou and Imura 1982), cobalt-containing protein (Gilmour and Henry 1991) or some other material is not important. These materials are all a result of bacterial activity, the sulfate-reducing bacteria included. Our experiment provides an indirect and qualitative demonstration of biological methylation, by which one can further examine the physiological mechanisms of mercury transformation. It would be interesting to verify, for example, if the obvious increase in net biological methylation and demethylation which was observed in our experiments after 24 hr, concomitant to the cessation of bacterial proliferation, was a result of abiotic transformation mediated by the above-mentioned materials or by the degradation of naturally present organic matter (Winfrey and Rudd 1990).

It is well known that Hg(II) and MeHg are generally toxic, however, at the levels used in the tests they were not expected to affect bacterial metabolic activity. At 4°C, $HgCl_2$ is not considered to be inhibitory at sediment concentrations below 10 µg/g (Trevors 1983). The survival of the bacterial communities along with their active proliferation rate confirmed the resistance or tolerance of the bacteria to the

mercury species. Two hypotheses may explain why the bacterial population reproduced only in the first few hours and then reverted to its initial level. Firstly, although the quantities added were not high enough to immediately kill the bacteria or inhibit their activity, Hg(II) and MeHg are toxic products. It is possible that the resisitance capacity of the bacterial community may have reached its limit at that particular point in time. When the bacteria could no longer support the bioaccumulation of mercury, they stopped their activity and waited for the surrounding environment to change favorably.

Another possiblity is that since the experimental slurry was prepared using laboratory nano-pure water, certain mineral elements (e.g., nutrients), organic matter, and/or other adsorbed chemicals present in the sediment may have dissolved into the water. This may have favored bacterial activity, and, thereby, mercury transformations (Winfrey and Rudd 1990; Gilmour and Henry 1991). Also, the addition of Hg(II)/MeHg to the system could have favored some substrate release which, indirectly, provoked bacterial proliferation. After the first flourishing, the nutrient resource may have become limiting and, consequently, the proliferation ceased.

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