METHYL MERCURY PRODUCTION AND DISTRIBUTION IN RIVER WATER-SEDIMENT SYSTEMS INVESTIGATED THROUGH RADIOCHEMICAL TECHNIQUES

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Abstract. The toxicological consequences of Hg releases to the environment are largely governed by the conversion of inorganic Hg to the most toxic methylmercury (MeHg), that is biomagnified through aquatic food chains. To gain further insight on the biological and physico-chemical factors controlling MeHg production and distribution among freshwater sediments and water, we used a sensitive and specific radiochemical procedure, developed at the National Institute for Minamata Disease. Systems containing $^{203}Hg^{2+}$-spiked sediment cores (0.7 μg total Hg g^{-1} d.w.) and overlying water, both from a pristine mountain stream in Southern Japan, were incubated for 21–38 days in different conditions. Inorganic Hg and MeHg in sediment and water were extracted in dithizone-benzene and measured after separation by thin-layer chromatography. The conversion of added Hg to MeHg was 3.0 to 13.7% in sediments, with a tendency for higher proportions in the top layers. Surprisingly, more MeHg was found in the sediment (11.3%) and water (66.5%) of a system bubbled with air than in one bubbled with nitrogen (4.2 and 44.1%). Artificially increased levels of bioturbation reduced by half the MeHg concentrations and % of added total Hg in sediment and water. In all systems, 55–68% of total Hg and MeHg in water were associated to suspended particles >1μm. MeHg bioaccumulation factors (BFs) from water ranged 270–8100 and from sediment, 0.2–5.7 (wet weight basis). BFs in relation to water where 3 times higher for MeHg than for total Hg.

Keywords: bioturbation, freshwater systems, methylation, sediment

1. Introduction

The past and present human and environmental health impacts of Hg of natural and anthropogenic origin in different countries continue to motivate studies on the environmental cycle of mercury and on the toxicity of its many chemical forms. This interest is justified by the fact that Hg is normally released to the environment largely as inorganic Hg, while most Hg in fish is methyl mercury (MeHg), which is among the most neurotoxic compounds and one of the few that exhibit biomagnification along the aquatic food chains. MeHg is formed in the environment by biological and chemical processes in sediment (Gilmour and Riedel, 1995), water (Watras *et al*., 1995), soil (Rudd, 1995) and other sites such as the roots of floating aquatic macrophytes (Guimarães *et al*., 1998). The determination of the methyla-

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tion sites and processes and of MeHg pathways and fluxes in the environment are therefore essential in the understanding and prediction of Hg toxicological impacts. The study of the Hg cycle in aquatic systems has been limited by the difficulty to measure the very low levels of Hg and MeHg in water. With the development of atomic fluorescence spectrometry (Bloom, 1989) it is now possible to study in-situ MeHg production with additions of Hg very close to natural concentrations. However, the use of radiochemical methods to study the production and distribution of MeHg in model systems has the advantage to allow a simplification of the experimental design and avoids the need to distinguish between pre-existing and newly formed MeHg.

The aim of the present study was to evaluate the MeHg production and distribution in ^{203}Hg-spiked water-river sediment systems, using a sensitive and specific radiochemical procedure developed at NIMD, based on inorganic Hg and MeHg extraction in dithizone/benzene and ^{203}Hg and Me^{203}Hg determination after separation by thin-layer chromatography. Experiments were made in different redox regimes (air or N_2 bubbling) and degrees of bioturbation. Eh is one of the factors that control Hg methylation in water and sediment (Regnell, 1996). Burrowing insects, on the other hand, are an important pathway in the transport of MeHg formed in sediments to higher trophic levels (Tremblay *et al*., 1996) and the physical disturbance they cause promotes the exchange of Hg and MeHg between water and sediment (Guimarães *et al*., 1998).

2. Materials and Methods

2.1. Sediment sampling and incubations with Hg^{2+}

Surface sediments (top 2 cm) from an enbayment in a small tributary of the Minamata river, Southern Japan, were collected in February 1996, approximately 8 km upstream Minamata city, and carefully sieved through a 0.8 mm sieve to remove the debris and coarse sediment fraction and the sediment macroinvertebrates. The sieved sediment was essentially <74 μm and contained 11.8–16.5% organic matter (dry weight), determined by loss on ignition at 450 °C and 70 ng Hg g^{-1} (dry weight). Samples of fresh wet sediment of approximately 140 mL were spiked with $^{203}HgCl_2$, carefully homogenized with a spatula and slowly poured with a glass tube on the bottom of Jenkins type glass tubes (I.D. 8 cm). Total added Hg in homogenized sediment was 0.76 μg Hg g^{-1} dry weight, calculated from Hg addition and sediment water content, determined by drying sediment subsamples at 100 °C for 12 hr. One litre of unfiltered Minamata river water was added dropwise to avoid sediment ressuspension. The glass tubes were stoppered at both ends with butyl rubber stoppers covered with Teflon® sheets. Preliminary experiments had shown that unprotected rubber stoppers are efficient inorganic Hg scavengers and their use could potentially lead to an artifact, resulting in overestimated MeHg

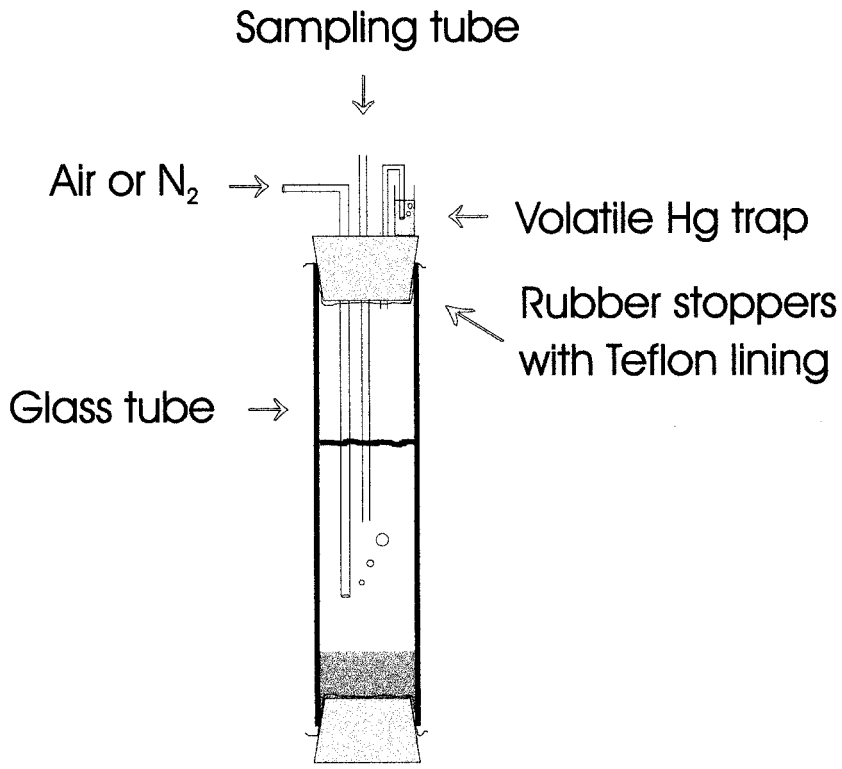


Figure 1. Schematic view of the water-sediment systems.

proportions in water. The upper stoppers were perforated by Pasteur pipettes for bubbling air or nitrogen in the water column and allowing water sampling without removing the stoppers (Figure 1). The water-sediment systems were incubated at in-situ temperature (11–13 °C) and in dim light conditions for 21–38 days. Two systems were bubbled with air, one with nitrogen, all at 25 mL min^{-1}, and sediment invertebrates (small gastropods, annelid worms and carnivore insect larvae, two of each) were added to one of the air-bubbled systems. A third oxygenated system was made with unspiked sediment and one litre of Minamata river water containing $^{203}HgCl_2$ at a concentration of 60 ng Hg mL^{-1}. In all systems, the exhaust air (or nitrogen) was bubbled in a scintillation vial containing 10 mL of $KMnO_4$ 0.5%. This solution traps both Hg° and dimethyl mercury, the main volatile Hg species. Water and sediment Eh was not measured before or during the experiments due to technical problems.

The water column was periodically sampled by siphoning and analyzed for total Hg and MeHg. Invertebrates and the sediment column were analyzed for total Hg and MeHg only at the end of the incubation period. The invertebrates, of 50 to 120 mg wet weight were washed in distilled water and finely chopped. The sediment was separated in top (0–7 mm), middle (7–17 mm) and bottom (17–27 mm)

layers by aspiration from the top with a glass tube and carefully homogenized. All sediment samples were analyzed in duplicate. The water samples taken at the end of the incubation period were analyzed before and after filtration through 0.45 μm Millipore® filters and 1.0 μm glass fiber Whatman GFC membranes.

2.2. TOTAL Hg AND MeHg MEASUREMENT IN WATER, SEDIMENT AND BIOTA SAMPLES

Sediment (0.2–0.7 g w. w.) or biota (50–120 mg w.w.) samples were measured for total ^{203}Hg by gamma spectrometry in glass scintillation vials and digested at room temperature in the same vials by shaking 20 min with 8 mL of 1N KOH in ethanol. After being slightly acidified by addition of 5 mL of 2.4 N HCL, the samples were shaken for 5 min with 5 mL of 0.1% dithizone in benzene (Dz:Bz), and centrifuged at 1000 rpm for 5 min. Next, 1–2 mL of the Dz:Bz layer were dried and cleaned by percolation trough a column made in a disposable Pasteur pipette, containing 0.5 g of Florisil® topped by 0.5 g of anhydrous Na_2SO_4. The eluate was collected in another vial, shaken twice with 10 mL of 2.5% ammonia to remove the excess of free dithizone and centrifuged at 1000 rpm for 5 min. A precise volume of the Dz:Bz (usually 0.5–1 mL) was collected in a scintillation vial and measured for total ^{203}Hg by gamma spectrometry to check the recovery, evaporated to dryness under vacuum or a N_2 stream, dissolved in 2–3 drops of acetone, and applied with glass capillaries on a thin-layer chromatography sheet (silica-gel Polygram Cell, Macherel-Nagel, Germany) together with similarly prepared standards of non-radioactive Hg^{2+} and MeHg. The plates were developed using 1:1 benzene-hexane as a developing agent and the strips corresponding to inorganic Hg-dithizonate and MeHg-dithizonate (respectively visualized as pink and yellow spots, with Rf values of 0.6 and 0.25) were cut and measured by gamma spectrometry, as well as those corresponding to the application point and solvent front. All radioactivity measurements were made on a gamma counter (Aloka Model ARC-2000, Japan) with a $3 \times 3'$ well-type NaI detector. Counting the TLC strips by liquid scintillation would benefit from the higher counting efficiency (close to 100%) obtained when measuring the beta emissions of ^{203}Hg, but preliminary tests showed a strong and durable chemiluminescence effect, that excluded obtaining reliable measurements of ^{203}Hg in the TLC strips with this counting method.

Total water samples of 20–75 mL were collected in 100 mL erlenmeyer flasks – aliquots were reserved for total Hg measurements – mixed with 5 drops of 0.5% $KMnO_4$ and 1 mL of HCl 2.4 N and extracted with 5 mL of 0.1% Dz:Bz by shaking for 5 min. The aqueous layer was aspirated, the benzene layer washed twice with 10 mL of 2.5% amonia and centrifuged. One mL of the Dz:Bz was counted for recovery check, evaporated to dryness, dissolved in a few drops of acetone and subjected to TLC separation as described above. All chemicals were of analytical grade.

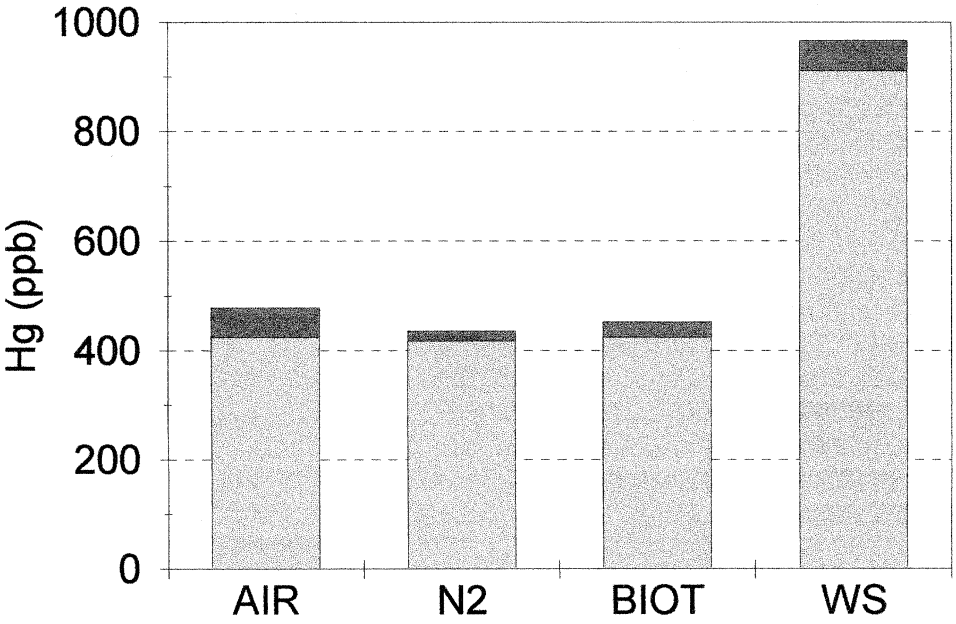


Figure 2. Inorganic Hg (light grey) and methylmercury (dark grey) in sediment at the end of the incubation period. AIR: system bubbled with air; N2: system bubbled with nitrogen; BIOT: system with increased bioturbation; WS: system with Hg spiking in water only.

The comparison of total Hg in initial samples and Dz:Bz extracts indicated recoveries close to 100% or higher, the recoveries over 100% being attributed to the evaporation of some benzene during extraction, which concentrates MeHg in the benzene extract and leads to a higher Hg concentration in the final extracts when compared to the initial sample. As the activity of the strips corresponding to the application point and the solvent front were negligible, the proportions of inorganic Hg and MeHg in each sample was calculated from the total activity of the corresponding TLC strips.

$^{203}HgCl_2$, lot number 102 A, was purchased from Amersham International Plc, UK, presented a specific activity of 1.14 mg Hg. mCi^{-1} (30.8 ng Hg MBq^{-1}) and was diluted in distilled water prior to use. Desintegrations per minute from ^{203}Hg were converted to total Hg and Hg as MeHg by means of the specific activity and appropriate decay corrections. Detection limits calculated with 99% confidence levels were equivalent to 2.2 ng g^{-1} dry weight for sediment and 3.2 ng L^{-1} for water, assuming extraction of samples of 0.1 g and 70 mL respectively. All radioactive samples were manipulated in well-ventilated fume hoods. Counting error was kept under 1% and data were corrected for decay.

3. Results and Discussion

3.1. Hg METHYLATION IN SEDIMENT

The distribution of inorganic Hg and MeHg in the sediment layers at the end of the incubation period is shown in Figure 2. The higher MeHg production, ranging 9.9–13.8% of added Hg, was found in the air-bubbled system. The lower MeHg production was found in the system bubbled with N_2 (3–6.3%) while MeHg production in the sediment of the system with increased bioturbation levels was intermediate (4 to 7.9%). As expected, total Hg varied little among the top, middle and bottom sediment layers while for MeHg a clear gradient was found, MeHg concentrations being 40 to 111% higher in the top than in the bottom layer. Higher MeHg concentrations and potential methylation rates are commonly found in the top layer of river and lake sediments (Korthals and Winfrey, 1987; Gilmour and Riedel, 1995), which is attributed to factors such as higher organic matter concentrations and bacterial activity and mildly reducing conditions, but it is noteworthy that in the present study a vertical gradient developed in sediment that was initially homogenized. As mentionned earlier, it was not possible to measure Eh in sediment layers to verify its influence on Hg methylation.

In the system with Hg spiking in water only, 5.7% of added Hg was found as MeHg in the top sediment layer. Traces of inorganic Hg and MeHg were also found in the middle and bottom layers but this is probably a result of contamination of these layers by Hg and MeHg from the surface layer, due to the sampling method, rather than a suggestion of water Hg penetration to that depth of the sediment. In the experimental conditions used herein, Hg from a water spike is expected to penetrate only a few millimeters in the sediment, and the Hg spike being higher in this system, Hg must have reached higher concentrations in this thin top sediment layer than reported in Figure 2, where the Hg concentrations in the top layer represent averages for the first 7 mm. This may explain the lower MeHg% in the sediment of this system, when compared with the sediment-spiked system incubated under the same redox conditions, as a lower proportion of added Hg is converted to MeHg at higher total Hg concentrations (Jensen and Jernelov, 1969; Guimarães *et al.*, 1995).

In the sediment spiked systems, average Hg methylation rates ranged 0.62–2.92 ng g^{-1} dry weight. day^{-1}, or 0.06 to 0.66% day^{-1}. These rates are relatively low when compared with the ones compiled by Gilmour and Henry (1991), from different studies in lakes, rivers and reservoirs. It should be pointed out, however, that methylation rates show marked spatial and temporal variations and are strongly influenced by factors such as added Hg concentration and duration of the incubation, which is a difficulty when comparing data on Hg methylation obtained in different studies or aquatic systems. Curiously, less MeHg was formed in the N_2-bubbled system than in any of the air-bubbled ones. Anoxia would be expected to stimulate MeHg formation in lake water and sediment, as observed by many authors (Watras *et al.*, 1995; Regnell *et al.*, 1996). Sediment and water Eh were not

recorded during or after the experiment. The sediment of the N_2-bubbled system did not show any noticeable color change during the 38 days of the experiment. It can not be excluded that the duration of the incubations was insufficient to cause anoxia in the sediments of the system bubbled with nitrogen.

In the system with increased bioturbation activity, less MeHg was found in the sediment than in the other system incubated in similar redox conditions but without addition of invertebrates. This difference in MeHg concentration is far from being accounted for by MeHg uptake by the added invertebrates. The activity of the introduced invertebrates was restricted to the top sediment layer, while low MeHg proportions were found in all sediment layers, suggesting that other variables than bioturbation could also be involved. Sediment invertebrates that build galleries, like chironomid larvae and others, were shown to have an important role in the inorganic and MeHg exchange between water and sediment in the Pantanal floodplain (Guimarães *et al*., 1998). The invertebrates included in the present study, however, were not gallery builders and reworked the top sediment with sufficient intensity to cause a high water turbidity during all the experiment. This may partially explain the lower MeHg formation in the system containing invertebrates: Guimarães *et al*. (1995) found that slow stirring of river sediment during incubation reduced the methylation of added Hg.

3.2. MeHg AND INORGANIC Hg IN WATER

The distribution of MeHg and inorganic Hg in total water at the end of the experiments is shown in Figure 3. Since total Hg and MeHg in the water of the different systems was not sampled and analyzed during all the incubation period, it is not known if the reported concentrations of inorganic Hg and MeHg in water at the end of the experiments are equilibrium concentrations or not.

In the sediment-spiked systems, total Hg in water ranged 44.7–158 ng L^{-1}, and the proportion of MeHg to total Hg was 9.3% in the bioturbated system, 44% in the N_2-bubbled system and 66% in the air-bubbled system. Inorganic Hg and MeHg release from the sediment were respectively 2 and 5 times higher in the air-bubbled system than in the N_2-bubbled one. Inorganic Hg was highest in the water of the bioturbated system, probably as an effect of sediment ressuspension by the activity of the invertebrates, while MeHg in water was lowest in this system.

In the water-spiked system, only 1.1% of initial activity remained in total water at the end of the incubation period. The MeHg proportion in total water was 43%, comparable to the one found in the N_2-bubbled system.

Assuming that all MeHg was formed in the sediment, it is estimated – based on total Hg and MeHg in sediment (average values) and water, at the end of the experiment – that approximately 8% of MeHg formed in the sediment of the air-bubbled system was released to the water, while for the N_2-bubbled system this release was of only 0.5%, resulting in average fluxes of respectively 0.38 and 1.3×10^{-2}% d^{-1}

TABLE I

Fraction (%) of total Hg and MeHg in total water associated to suspended particles greater than 1.0 and 0.45 μm

System	Total Hg (1 μm)	MeHg (1 μm)	MeHg (0.45 μm)
Air-bubbled	NA	NA	NA
N_2-bubbled	50.7	63.4	84.8
Bioturbation	60.0	68.2	92.7
Water spike only	69.1	55.7	NA

NA: not analyzed.

over the experimental period. Longer experiments and replication are needed to evaluate the variations of these fluxes among different systems and along time.

The data obtained on the distribution of total Hg and MeHg between the dissolved and particulate water phases at the end of the experiments are shown in Table I. Similar proportions of MeHg (55.7 to 68.4%) and total Hg (50.7 to 69.1%) were associated to particles >1.0 μm in the N_2-bubbled, bioturbated and water-spiked systems. In the N_2-bubbled and in the bioturbated systems, respectively 84.8 and 92.7% of MeHg in water was associated to particles >0.45 μm. Rask and Verta (1995) and Verta and Matilainen (1995) found similarly high proportions of MeHg in water and particulates in Finnish lakes.

3.3. TOTAL Hg AND MeHg WATER-SEDIMENT PARTITION

The water-sediment distribution coefficients (Kd's) for total Hg and MeHg are presented in Table II. Higher Kd's were found for total Hg than for MeHg in the air-bubbled and N_2-bubbled systems, while in the system containing added invertebrates the Kd's were similar for total Hg and MeHg.

The figures in Table II for the water-spiked system are probably very underestimated, since as mentioned before, Hg probably penetrated very little in the sediment but all Kd's were calculated from the MeHg and total Hg concentrations in the first top 7 mm of sediment. Some data on intesticial water, limited to the air-bubbled system, were also obtained. The different sediment layers were centrifuged at 3000 rpm for 10 min, immediately after sampling. The distribution coefficients for total Hg in interstitial water obtained this way were 207, 450 and 385, respectively, in the top, middle and bottom sediment layers.

3.4. Hg VOLATILIZATION IN THE AIR-BUBBLED SYSTEM

At the end of the incubation period, <1% of added Hg was found in the Hg traps of all systems, except in the water-spiked system where 7% of added Hg was

TABLE II

Total Hg and MeHg distribution coefficients (ng Hg g^{-1} of sediment dry weight/ng Hg mL^{-1} of water), based on Hg and MeHg concentrations in top sediment layer and in total water. In brackets, values calculated for dissolved (<0.45 μm) total Hg and MeHg

System	Total Hg	MeHg
Air-bubbled	2820 (NA)	580 (NA)
N_2-bubbled	9500 (28500)	1350 (9000)
Bioturbation	4100 (56200)	3450 (47500)
Water spike only	4250 (13600[a])	560 (1270[a])

[a] Water filtered in 1.0 μm pore membranes.

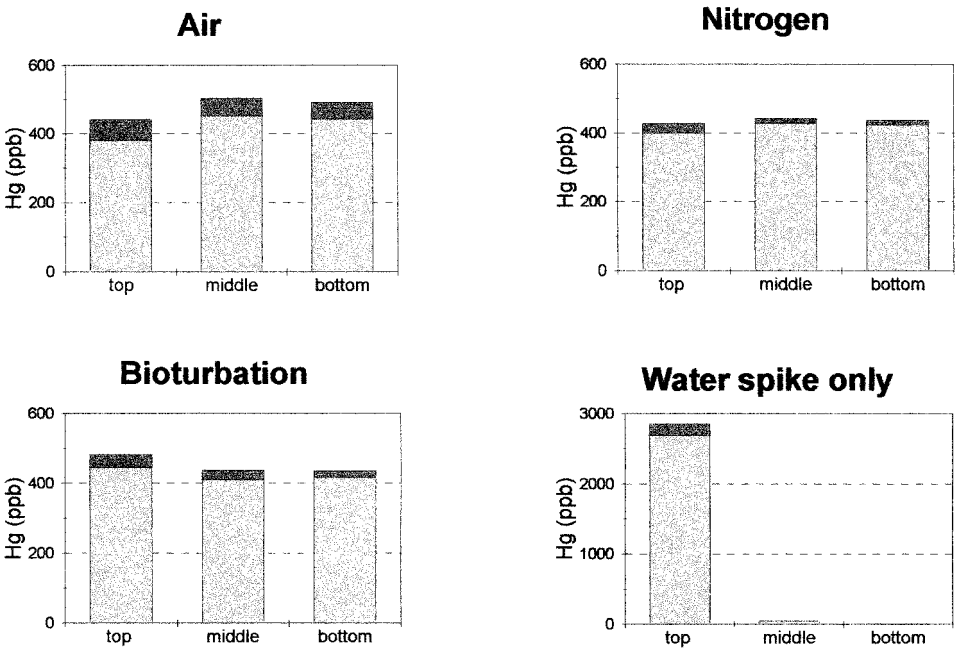


Figure 3. Inorganic Hg and methylmercury in the different sediment layers at the end of the incubation period. Keys same as Figure 2.

volatilized. In all systems, approximately half of total Hg in the traps was in the $KMnO_4$ solution and half in the Mn oxides coating the bubbling glass tip and vial walls. ^{203}Hg was near background in the upper and lower Teflon® sheets lining the rubber stoppers as well as in rubber chips cut from the surface of the upper stopper facing the core head space. A certain share of added Hg is usually unaccounted for at the end of experiments like described herein. Volatilization is usually modest, as

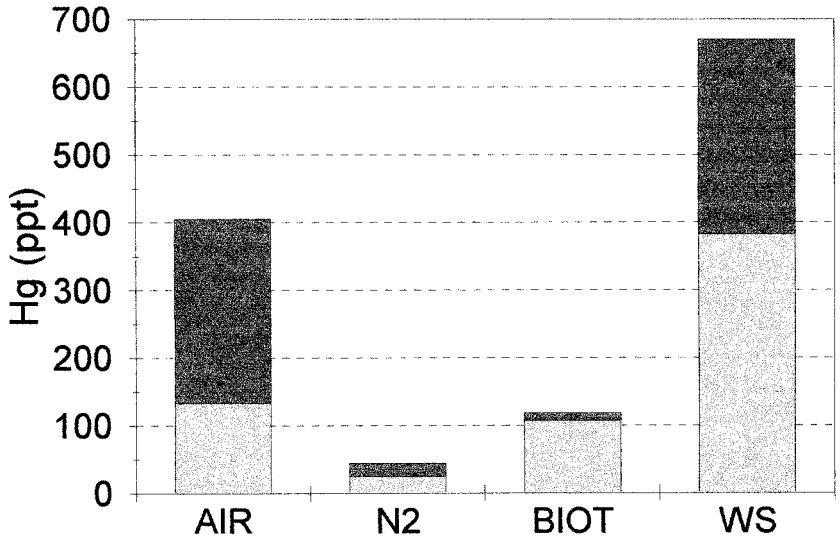


Figure 4. Inorganic Hg and methylmercury in total water at the end of the incubation period. Keys same as in Figure 2.

TABLE III

Average and range of bioaccumulation factors for total Hg and MeHg in sediment biota[a] (ng Hg g^{-1} of wet weight ng^{-1} Hg mL^{-1} of total water or g wet weight of top sediment), calculated for total water and sediment

	Water	Sediment
Total Hg	620 (220–1940)	0.37 (0.13–1.15)
MeHg	1970 (270–8100)	1.38 (0.19–5.67)

[a] Snails, worms and carnivore insect larvae.

reported here and in Regnell (1990) and Regnell *et al*. (1996), but it should always be controlled, since it may be a relevant Hg sink, besides representing a potential occupational hazard.

3.5. Hg AND MeHg IN INVERTEBRATES

The bioaccumulation factors (BFs) for total Hg and MeHg in sediment biota are shown in Table III. A high variability for total Hg and MeHg was found among and between invertebrate types, resulting in the large ranges observed. As expected, higher BFs were found for MeHg than total Hg, from water as well as from sediment. These BFs can be considered low, probably as an effect of the relatively short period of the experiment, the high biomass created in the experimental system, and

the relatively low water temperature, that are all factors that tend to decrease the MeHg BFs. Becker and Bigham (1995) found MeHg BFs of 8.3×10^4 in natural benthic invertebrates in Onondaga lake, NY, U.S.A.

4. Concluding Remarks

Though not conclusive in many aspects, this work illustrates the applicability of the described radiochemical technique to the study of Hg and MeHg behavior in model aquatic systems. The quantification of ^{203}Hg and Me^{203}Hg in the different types of samples was hardly a limiting factor during this work and it could have been done with total Hg additions as low as 300 ng g^{-1} dry weight, a concentration that is within the range found in natural soils and sediments, and certainly common in contaminated areas.

The separation of MeHg and inorganic Hg by TLC and measurement by gamma spectrometry allows a direct and simple quantification of these Hg forms. The simplification in the experimental design it allows and the modest Hg addition it requires are additional advantages, while the disturbance of the system by mixing after addition of significant Hg amounts is one of its main drawbacks. It adds to the available techniques for testing hypotheses on the behavior of Hg species in aquatic systems and may be useful, together with measurements of natural methyl mercury, to gain further insight on this important environmental issue.

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